

RF Project 2410
Final Report

THE OHIO STATE UNIVERSITY

AD 738885



RESEARCH FOUNDATION

1314 KINNEAR ROAD COLUMBUS, OHIO 43212

RESEARCH ON AND CULTURE OF CALCAREOUS GREEN ALGAE

Dr. Llewellyn Hillis-Colinvaux

Department of Botany



DEPARTMENT OF THE NAVY
Office of Naval Research
Washington, D.C.

Contract No. N00014-67-C-0262

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
Springfield, Va 22151

DISTRIBUTION STATEMENT A

Approved for public release;
Distribution Unlimited

20 R

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) The Ohio State University Research Foundation 1314 Kinnear Rd., Columbus, Ohio		2a. REPORT SECURITY CLASSIFICATION Unclassified	
		2b. GROUP N/A	
3. REPORT TITLE RESEARCH ON AND CULTURE OF CALCAREOUS GREEN ALGAE			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Final March 1, 1967 to December 31, 1970			
5. AUTHOR(S) (First name, middle initial, last name) Llewellyn Hillis-Colinvaux			
6. REPORT DATE January 11, 1972	7a. TOTAL NO. OF PAGES 26	7b. NO. OF REFS 27	
8a. CONTRACT OR GRANT NO. N00014-67-C-0262	8b. ORIGINATOR'S REPORT NUMBER(S)		
8c. PROJECT NO.	8d. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)		
10. DISTRIBUTION STATEMENT 1 - Distribution of this document is unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY Department of the Navy Office of Naval Research Washington D.C.	
13. ABSTRACT Four genera of coral reef Siphonales, <u>Halimeda</u> , <u>Penicillus</u> , <u>Rhipocephalus</u> and <u>Udotea</u> were grown in laboratory aquaria under light intensities of 650, 200-375 and 125-200 ft-candles. The commercial preparation "Instant Ocean" was tested for use with plants. Penicillin and Lindane were tested for their effect on epiphytes and other nuisances. The growth and development of all 4 genera from tiny protuberances above the sand to white, dying and disintegrating individuals was followed. All produced new individuals from rhizoidal-like filaments that extended outwards through the sand from the holdfast of an older plant. I demonstrated how vegetative reproduction might occur with partial burying of individuals by shifting sands in a reef, or from portions of plant broken off by grazing or other natural activities. Plants produced vegetatively are not initially epiphytized and so may be a source of clean plants for laboratory experiment. The first swarmer-producing <u>Penicillus</u> plants so far known were produced in my aquaria. The rather undistinguished structures associated with them, the release of swarmers from <u>Halimeda</u> , and associated observations are described. Insight into the role of calcareous plants in a reef was obtained by a productivity study which combined a census of such plants in a reef and a laboratory measure of oxygen changes in an entire aquarium. Some <u>Halimeda</u> reef populations appeared to be grazed by the urchin <u>Diadema</u> . A conservative figure for the productivity of laboratory <u>Halimeda</u> plants is 2.5 mg C/plant/day (net) or 4.5 mg C/plant/day (gross).			

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Coral reef plants <u>Halimeda</u> Productivity Culture Calcareous algae Siphonales <u>Penicillus</u> Growth Reproduction <u>Rhipocephalus</u> <u>Udotea</u>						

RF Project... 2410

Report No.....

.....
FINAL
.....

REPORT

By

THE OHIO STATE UNIVERSITY
RESEARCH FOUNDATION

1314 KINNEAR RD.
COLUMBUS, OHIO 43212

To..... DEPARTMENT OF THE NAVY
Office of Naval Research
Washington, D.C. 20360
.....
Contract No. N00014-67-C-0262

On..... RESEARCH ON AND CULTURE OF CALCAREOUS GREEN
.....
ALGAE

For the period March 1, 1967 - December 31, 1970

Submitted by Dr. Llewellyn Hillis-Colinvaux
.....
Department of Botany

Date February 11, 1972

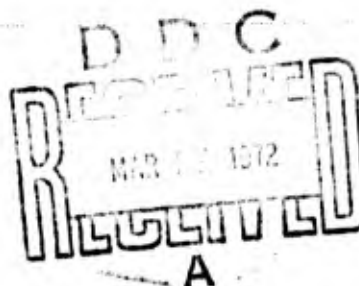


TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT	iii
INTRODUCTION AND ACKNOWLEDGMENTS	1
LABORATORY MAINTENANCE	2
Basic Procedure	2
Variations on Method and Comment	2
Epiphytes	3
REPRODUCTION, REGENERATION AND GROWTH	4
Introduction and Methods	4
Growth and Asexual Reproduction	5
Regeneration	7
Swarmer Production	8
Discussion and Conclusions	10
PRODUCTIVITY	11
Introduction	11
Field Work	12
Summary and Comment on Field Work	16
Laboratory Productivity Studies--Methods	17
Discussion and Results	18
REFERENCES	21

RESEARCH ON AND CULTURE OF CALCAREOUS GREEN ALGAE

ABSTRACT

Few large marine algae have been grown in the laboratory. In this project the calcareous green coral reef plants Halimeda, Penicillus, Rhizocephalus and Udotea were maintained for several months and some Halimeda plants for over 2 years. Three light intensities were used: 650, 200-375, and 125-200 ft - candles. Good growth was obtained with both the first two, but the intermediate intensity was preferred for most growth work since the calcareous algae were more quickly overgrown by epiphytes and contaminating blue-green algae at the highest intensity. At the lowest intensity range growth of both calcareous Siphonales and epiphytes was very slow. Millepore-filtered natural seawater was used entirely at the beginning of the work, but natural seawater is awkward and expensive to obtain in inland Ohio. The commercial preparation "Instant Ocean" at that time had not been satisfactorily tested on sea plants, but with experiment I found it satisfactory for work of at least 6 months duration, and thereafter used it in short-term culture and productivity experiments. This information should be helpful in subsequent inland culture work involving marine macrophytes. In experiments on the control of weed plants (commonly epiphytes), I found that a 15 ppm solution of the invertebrate killer Lindane did not kill Halimeda which were immersed in it for 15 minutes, but additional experiments are needed to determine its effect on epiphytes. Penicillin at a concentration of 3000 units per ml was initially effective in controlling the weeds, but within 2 months they were abundant again and penicillin was thereafter ineffective.

At the outset of this work these 4 calcareous genera were mostly known as static mature individuals, the material seen in the "typical" specimens available on herbarium sheets. How young were produced and to a certain extent their appearance and change from young to old stages was not known. In the laboratory aquaria I was able to observe growth stages from young to disintegrating plants, and to show how the many new plants developed from certain rhizoidal-like filaments that extended outwards through the sand or gravel from the holdfast of an older plant. I also demonstrated, with experiments involving regeneration and burying, that asexual reproduction could occur by the development of new individuals from partially buried plants or from small pieces of plant that were broken off by various natural activities such as grazing. Many of the observations made in the laboratory were seen amid coral reefs as well, which indicates that much of this development might also occur in the wild. The plants produced by these vegetative methods do not have epiphytes, so now a source of clean plants exists for at least some experimental work.

Some Halimeda plants in the aquaria produced the characteristic grape-like structures which have been assumed by many workers to be sexual and I was able to observe phases in the development and release of the associated swarmer. I found, among other things, that light seemed important in the release of these swarmer which emerged from the "gametangia" in a series of short puffs, and were held together with a sort of mucilage. The swarmer appeared to be thrust out through a small opening shared with a cluster of gametangia, rather than through individual pores on each gametangium. Other workers have suggested that some chemical factor induces the production of clumps of fertile Halimeda in reefs. Evidence from my aquaria indicates that some other factor must be active, at least in the laboratory.

The first swarmer-producing Penicillus plants so far known were produced in my aquaria, and I have described the rather undistinguished structures associated with them.

Although the productivity section of this research was interrupted by a change of research funding, much progress was made on both field and laboratory portions. To study the contribution of these plants to a coral reef system, I made a survey and census of the calcareous Siphonales in the inner portion of a fringing reef in Jamaica, and a laboratory determination of their productivity. In the survey-census the major areas of the reef were delimited up to the dropoff area which occurred at about 18 m depth, and an indication of the numbers of calcareous Siphonales in the more populous regions obtained by quadrat counts, mapping and general observation. I found that cover by the calcareous Siphonales ranged from 0% on the tidal sandy beach to 90% on the inshore, partially exposed opuntia rocks, and that although Udotea and Rhipocephalus might be locally common, over the entire reef their numbers were low. I also found that Penicillus was more abundant than anticipated in sand areas, and was more common over sizeable portions of the reef than was Halimeda. Some Halimeda populations appeared to be grazed by the spiny black sea urchin Diadema which sometimes occurred in a density of 20/m², and there were sizeable barren patches on some parts of the reef which seemed to have been produced by grazing.

Calculations for laboratory productivity were based on the amount of oxygen produced by these plants. A special aquarium cover was devised which permitted the measurement of oxygen changes in an entire aquarium, thereby making it unnecessary to pull up or otherwise disturb the growing plants. Oxygen changes were usually monitored for 36 hours but could be recorded for longer intervals. Productivity of the Halimeda plants under the laboratory conditions imposed, was calculated as 2.5 mg C/plant/day (net) or 4.5 mg C/plant/day (gross). These figures represent the first determination of productivity of Halimeda in laboratory or in field, and although possibly somewhat on the low side because of limitations in plant material available and lighting conditions, they compare favorably with the very, very few productivity measurements made on marine macrophytes.

INTRODUCTION AND ACKNOWLEDGMENTS

Living calcareous green algae, particularly Halimeda and Penicillus (order Siphonales), are prominent plants of many coral reef areas. Fragments of their dead bodies become conspicuous components of some of the surrounding sands, and in certain regions contribute a sizeable percentage of the calcareous sediments. These facts have been known since early collecting expeditions and the examination of cores from the atolls of Funafuti in the Ellice Isles. More indications of their contribution to sediments came from "Operation Crossroads" in the Bikini Islands. Yet apart from the taxonomy of these plants and their obvious distribution in coral reef areas around the world, very little is known. Growth, reproduction, calcification, ecology and productivity are all essentially unworked topics.

In this project my aim was to investigate the culture, growth, reproduction, and productivity of these plants, to obtain some insight into their contribution to the coral reef community. Some of the work was carried out in the field, the rest in the laboratory, where to do so it was first essential to establish and maintain the plants in healthy condition, and to develop methods of transporting them healthily from field to laboratory. Very few sizeable sea plants have been grown in the laboratory, but certain methods for maintaining these particular plants had been developed in the work of Colinvaux, Wilbur and Watabe (1965), so laboratory-based work was known to be feasible. It was not possible, however, to complete all of the field and laboratory work planned for an effect of the Mansfield Amendment was the earlier termination of support of these aspects of the project. A sizeable proportion of the aims were accomplished nonetheless, and are presented herein.

Part of the work is also included in a Symposium Volume on the Indian Ocean being published by the Marine Biological Society of India. It was presented and discussed in Cochin, India in January 1971 at an International Symposium. The work of the project also formed the bases of two lectures sponsored by the environmental training program of the University of Miami, Florida, in April 1971. I am now holder of the Founder's Fellowship of the American Association of University Women, one of their two most senior fellowships, and am continuing my work on Halimeda while based at the British Museum (Natural History) until the latter part of 1972.

This work was supported by contracts from the Office of Naval Research, Nos. N00014-67-C-0262 and 313-3018 under project NR 104-873. I am very grateful for this support, and for the spur they provided these aspects of research. I thank Professor John D. Costlow for his encouragement of the project, and Miss Marion Simmons for providing access to the "Glory Be" reef as well as various thoughtful touches to our field existence. The laboratory research was carried out in the Botany Department of the Ohio State University with the help of some very dedicated assistants.

LABORATORY MAINTENANCE

Basic Procedure

The equipment and method are similar to those used by Colinvaux, Wilbur and Watabe (1965). Plants, as clean of epiphytes and other contaminants as feasible were planted in small aquaria arranged under fluorescent lights in a room with a temperature suitable for maintaining the water temperature of the aquaria at about 27°C. A mixture of coarse gravel and calcareous material such as aquarium filtrant were the substrate of sand-growing species, autoclaved fossil coral rock of the other species. Millipore-filtered natural seawater was used initially as medium, but commercial artificial seawater was used later, as is described below. Filtered air was bubbled into the aquaria through standard aquaria bubblers, and in this project the long "Millionaire" type was the most satisfactory. Cool-white fluorescent lights provided intensities of 650, 200-375, and 125-200 ft -candles, on a 12-hour light:12-hour dark cycle.

Halimeda incrassata, a species which grows fairly readily and can usually be obtained even by inexperienced collectors, was used for most of this work, and some plants of it were maintained for more than 2 years using these methods. Other Siphonales used were Halimeda species discoidea, goreauii, macroloba, monile, opuntia, simulans, and tuna, Penicillus capitatus and P. dumentosus, Rhipocephalus phoenix, Udotea flabellum, Bryopsis sp., and several species of Caulerpa. All the culture work in this project was carried out in controlled environment chambers.

Variations on Method and Comment

A fair range of variation could probably be introduced into the basic procedure which should let the plants grow as well. Other types or sizes of aquaria, different light qualities or intensities, as well as special media and substrates might indeed be preferable in other studies. The straight-sided, all-glass aquaria with length-depth-width measurements of 14", 10", and 8", respectively, which I preferred for my growth study, were chosen for photographic, handling and culture characteristics. A good photographic record of growth changes could be made because the aquaria sides were straight and clear, and all the plants could, if necessary, be photographed since there were no inner rows in such relatively narrow aquaria. Glass was particularly desirable since it does not absorb elements from, or contribute them to, the basic seawater environment as readily as do other aquarium materials.

Because no literature existed on the use of commercial seawaters in algal culturing, and because the suppliers were hesitant to recommend their products for plants since they, too, lacked data, natural seawater was used in the early phases of this work, although inconvenient because

of difficulties with transport. As soon as sufficient aquaria and plants became available, I established aquaria with different commercial seawaters as media. From this research I found the preparation "Instant Ocean" (available from Aquarium Systems Inc., Wickliffe, Ohio) to be satisfactory for short-term work, and for maintaining plants at least 6 months -- a discovery which makes research on marine macrophytes at inland sites more feasible in many ways.

Light intensities for this work had also to be chosen with relatively little to guide. There are measurements of light intensity at noon in the tropics from various land elevations which indicate the maximum light available, and for plankton work there are numerous measurements of light intensity at various depths in the open ocean, but there are very few measurements taken in situ at various depths and at various slopes of substrate where macrophytes grow, and indeed no commercial equipment exists which enables a free diver to readily measure this parameter. This project was not of long enough duration to experiment extensively with light, but for this work I found the Halimeda plants grew slowly or not at all at the lowest of the three light ranges used, and best at the highest intensity -- where many of their epiphytes also grew best. Epiphytes were less of a problem at the intermediate range, and since the various Siphonales grew satisfactorily at this intensity, this intensity range was used most often for routine observations.

Epiphytes

Epiphytes are perhaps the greatest problem for satisfactory laboratory maintenance, and they are difficult to exclude because one must usually start with whole plants -- with all their "contaminants". When this project was begun no method for reproduction of these plants had been worked out whereby one could circumvent starting with such an inoculum. Some preventative control can, however, be carried out, first of all in the field (if one collects one's own plants), by selecting as vigorous and as clean individuals as exist at that time. At certain seasons this may be exceedingly difficult, for sometimes I have found the bulk of plants heavily epiphytized, even more heavily overgrown than most plants I have cared for in the laboratory. Further control is possible if the individuals are stripped of obvious epiphytes before planting, and they can be made very clean if a microscope is used. The latter is much less feasible however, if large populations of 500 or so await planting.

In one small-scale experiment, Halimeda were immersed for 15 minutes before planting in a 15 ppm solution of the invertebrate killer Lindane, which Strand et al (1966) found effective in Ulva culture. The treated Halimeda were not killed by this concentration, but more tests with Lindane will have to be carried out before its effectiveness on contaminating organisms can be reasonably gauged.

Once Halimeda or the other genera are planted, epiphytes and other contaminants can be partially held in abeyance by gently rubbing, scraping or picking them off. Penicillin, in a concentration of 3000 units per ml, was fairly effective shortly after treatment, but within 2 months the unwanted epiphytic plants were abundant again in all the treated aquaria, and we were unable henceforth to control these particular contaminants.

Reasonable long-term contaminant control was achieved in many aquaria by installing dynaflo filters, which were found to be considerably more satisfactory than the simple internal or external filters. Bottom filters, which are apparently useful in some animal aquaria work, were not tested, for at least in most set-ups they would involve aerating the substrate, a condition to which macrophytes are not normally exposed.

With the discovery, during the course of this project, of a method of vegetative reproduction in the 4 calcareous genera of Siphonales grown, and with some investigation of the ability of these plants to regenerate from smallish portions (cf. the next sections of this report), the mechanism perhaps exists for obtaining clean, possibly contaminant-free young plants from healthy, but epiphytized original stock. So far, most of the plants I have grown from aquarium babies or cuttings have remained fairly small, but, with more extensive experiment conditions for producing, a more typical habit might be achieved.

REPRODUCTION, REGENERATION AND GROWTH

Introduction and Methods

In 1966, Dawson wrote of an extraordinary lack of information on reproduction in the Codiacea (order Siphonales), a family containing many sizeable and common coral reef plants. Of its more than fifteen genera, the sexual cycle has been completely worked out only for Codium. Sexual or apparent sexual stages have been reported for Chlorodesmis (Ducker, 1965), Halimeda (Hillis, 1959, literature review included), and Udotea (Nizamuddin, 1963); and aplanospore-like or akinete-like structures reported for Avrainvillea (Howe, 1907) and Boodleopsis (Taylor et al, 1953). Fritsch (1948) states that "vegetative propagation is no doubt widespread," but there is little documented evidence of how this actually takes place in the various genera. The meagre literature on vegetative reproduction mentions detachable propagules (Fritsch, 1948), and fragmentation of juvenile filamentous stages (Borden and Stein, 1969) for some species of Codium. Protonemal-like stages are suggested for Penicillus and Udotea (Taylor 1960), but not further defined, and rhizoidal-like filaments are reported for Halimeda (Colinvaux, 1968). Duchassaing (1850) very briefly describes reproduction by "propagules" in Nesea (= Penicillus), and figures a sort of scaly and thick rhizome terminating in a scaly young plant. A similar rhizomatous-like connection is shown between plants of Udotea Desfontainii (= U. petiolata),

although a thinner, simple filament is shown for U. minima (Ernst, 1904). Data on growth -- either rate or form, and longevity are yet more meagrely recorded in the literature.

For this phase of the project I studied the growth of various Siphonales and observed development from them of numerous new plants, both in laboratory aquaria and among coral reefs. The plants for the laboratory study were grown as already described. For the study of regeneration, pieces of mature Halimeda and Penicillus plants were separated from the whole by cutting with a razor blade. The cut ends of each portion were then pressed together firmly for about a minute to control loss of cytoplasm (Jacobs, 1964). Loose portions were planted in gravel, or occasionally placed prostrate in an aquarium without gravel. For Halimeda, cuts were most commonly made at substrate level, thereby producing an above-sand piece, and a holdfast piece which might also contain one or so segments of the upright system which had been buried prior to cutting. Sometimes the cut was made near the top of the plant, thereby removing a small portion of but a few segments. Penicillus plants were also cut into two portions, but at three levels on the plant -- at substrate level, about midway along the stalk, and at the apex of the stalk, that is, the base of the capitulum.

Field observations for this phase of the project were made on the north shore of Jamaica in connection with my productivity investigation there, in Bermuda, on the southernmost tip of the Florida peninsula, Guam, and in various reef areas of the eastern Indian Ocean from southern Thailand to Java, as a scientific member on Te Vega cruise A of the International Indian Ocean Expedition.

Growth and Asexual Reproduction

Halimeda and Penicillus are dominant plants in many reef areas of the Caribbean. Their vigorous, healthy thalli are what the casual swimmer-biologist often sees as he swims his way through the reef. With careful probing he may perhaps become aware of numerous turgid, greenish, immature thalli of these 2 genera projecting above the sand. He may also see numbers of epiphytized plants (nearly always full-sized), and whitish plants falling apart in bits, their segments amassed nearby or else shifted by the sea. These observations of young, mature and old plants (which are usually passed over or ignored) give insight into the probable growth pattern of Halimeda and Penicillus. The relative abundance of these plants in numerous reef areas indicates that these plants succeed in maintaining their numbers very satisfactorily, that they are successfully reproducing in some way.

In the months that Halimeda grew in my laboratory aquaria I was able to follow a pattern of development from young bright green plants into mature plants which eventually became covered with epiphytes, whitened, and disintegrated. Many new plants appeared too, usually in the vicinity of a fully mature plant or near one that was white.

Since Halimeda is known to produce flagellated swimmers from structures often called gametangia, and several gametangia-bearing plants had developed in various aquaria, it seemed possible that the new plants I had observed in aquaria might have been produced by such swimmers. Some data on this was contributed by observations made on twenty aquaria, each containing 12 Halimeda, over a 4-month period. Out of a total of approximately 240 sand-growing Halimeda in these aquaria, 17 became fertile, released their flagellated swimmers, and were dead about a month later, death of the plant apparently being the consequence of swimmer production. During this same time, over 160 new Halimeda sprouted up through the gravel, increasing the population by about 67%. But over three-quarters of these new Halimeda developed in aquaria in which there had been no fertile plants. I, therefore, concluded that the plants were reproducing in some way other than by swimmers.

When I removed some of the new plants for various experiments other plants moved slightly, suggesting physical attachment. Careful lifting and dissecting showed that young and old plants were connected by delicate thread-like strands which sometimes branch, and which may be at least 20 cm long. I also found tiny plantlets, not yet emergent from the sand, attached to the holdfast of older plants by similar threads. These connecting threads resemble individual rhizoidal filaments of the holdfast, but may be somewhat thicker, with a wall more pigmented and regularly constricted, so that the thread sometimes appears like a string of beads, particularly in the immediate vicinity of the plants. The Halimeda plants in these aquaria were thus reproducing themselves by a method similar to the underground runners of higher plants. Subsequently I found similar connections between young and old plants of Penicillus, Udotea and Rhipocephalus.

Whenever possible I made field searches for similar inter-connections in the wild, but the searches had to be very limited because of the exceedingly short time available for such aspects of the work. They were also somewhat hampered by the natural movements of swimmer, plants and sea. Even with SCUBA the surges encountered made delicate work difficult, and I was unable to pull plants up connected for any considerable distance. I did, however, find very young plants coming from the holdfast of a mature plant as they do in the laboratory, and other workers have reported to me finding plants attached over a longer distance. Wild plants, also, are often patchily distributed, a distribution that could be produced with reproduction by rhizoidal "runners".

All the plants discussed so far, Penicillus, Udotea, Rhipocephalus, and sand-growing Halimeda typically have large holdfasts. This is not so for rock-growing species of Halimeda such as tuna and discoidea, in which the holdfast is typically less than 1 cm long. Thus far, I have not followed in detail the growth or multiplication of rock-growing Halimeda, but can comment on three -- micronesica, tuna, and opuntia. In H. tuna, plants are often closely attached to rock with a definite but usually inconspicuous holdfast. Collecting the plants means breaking the

rock, which would destroy any rhizoidal connections to other plants and I have not so far been able to find connections between plants, either in or over the rock although some rhizoidal filaments have seemed to penetrate it.

Halimeda micronesica has a peculiar basal segment which is much larger than the other segments on the plant and which usually supports numerous smaller segments (Hillis, 1959). Some of these smaller segments may sometimes be replaced by rope-like extensions consisting of intertwined rhizoidal-like filaments, or else certain branches terminate with these twisted strands. These strands may attach the plant to the rock at places other than the main holdfast area, and remain uncovered or are very loosely covered by sand. They also seem to be involved in reproduction, for I have collected fairly mature specimens with smaller plants at the end of one or more of these "ropes".

The picture is again different for opuntia, a species which is typically attached at several places rather than only one, with predominant direction of growth often apparently horizontal -- thereby producing a spreading habit, rather than an erect, or pendant one. From field and laboratory observation of this species, I have obtained the impression of new plants commonly developing by a breaking or attrition of other plants, the separate parts then remaining attached and continuing to grow.

The same result is brought about in at least some of the sand-growing species of Halimeda, when the plant becomes partially buried by sand. What may then be visible are the several branch tips of a single plant. If the burying is fairly permanent, the buried portion becomes whitish and starts disintegrating, while the exposed tips continue to grow. In the laboratory I have buried all but the tips of some plants. The tips have added new segments, and when the plants were later excavated, the buried portions were seen to be whitish and softer. Adventitious holdfast filaments had sometimes developed between segments near the substrate surface. With the decay of the older parts, the younger tip portions would exist as several separate individuals. Before this process is completed, however, such plants, should they have been in a quadrat chosen for a population count, would probably have been considered and counted as individuals.

Regeneration

Some of the cut material eventually became overgrown by epiphytes, and produced no new growth, but this was not typical. Of that which survived, isolated tips regenerated a holdfast system, produced new apical segments, and occasionally produced yet another plant by horizontal development from the holdfast system. In Halimeda which had been severed at substrate level, the above-gravel or photosynthetic portion regenerated a new holdfast system, and sometimes also produced new green

segments on the upper branches of the original material. Severed holdfasts produced a new top of green segments and branches. Both regenerating portions sometimes produced new plants amid the holdfast filaments. If the regenerating material was horizontal, the new segments eventually became vertical, while most new rhizoids developed from the ventral surface of the lowermost segment.

In the limited work on Penicillus, a new capitulum nearly always regenerated when the old one was cut off -- a result rather similar to that reported by Kupfer (1907). Only occasionally, however, did a holdfast develop from a cap and stalk portion, and so far, a new stalk portion has never developed; so if the cut is made at the base of the stalk, the plant developing from the holdfast is stalkless.

Swarmer Production

In field work and herbarium studies, I have now found fertile plants of Halimeda species cuneata, cylindracea, discoidea, favulosa, gracilis, incrassata, macroloba, macrophysa, micronesica, monile, opuntia, scabra, simulans, tuna and velasquezii. The list represents about half the described species of Halimeda, with cylindracea, macrophysa, micronesica, opuntia, velasquezii and possibly cuneata (the exact identity of the material cited in 1959 is still in doubt) being added to the species on which I reported in 1959 (Hillis, pp. 331, 371). These fertile plants were far from common in the field, but they do seem less rare than indicated in the earlier literature which had to be based on materials gathered mostly by grabbing from above, or by dredging. Seen in the field, fertile plants sometimes appeared to be isolated from others, but frequently several plants of a large population were fertile. Near them might be white, disintegrating plants, the color and disintegrating character suggesting that they might have been fertile earlier, although they might also represent dying vegetative plants.

Fertile Halimeda plants developed in the laboratory too. In one planting of approximately 500 Halimeda in 44 aquaria, with 10-12 plants per aquarium, 24 plants became fertile within the six months that these aquaria were maintained. Most commonly only one plant in an aquarium became fertile, but 3 aquaria had 2 fertile plants, one had 3, and one had 4. The dates on which the plants were observed fertile are given in Table I.

TABLE I

Aquarium number	No. plants fertile	Approx. date plants fertile
45	4	8 ix 69, 8 x 69(2), 13 x 69
55	2	11 viii 69, 10 xi 69
64	2	11 xi 69(2)
69	2	18 ix 69(2)
77	3	16 ix 69, 30 ix 69, 20 x 69
11 separate	1 each	12 viii 69 to 6 xi 69

When a Halimeda became fertile in the laboratory, it changed from green to white overnight, or during the dark phase of growth. Stalked grape-like clusters of the so-called gametangia developed from filaments protruding beyond the surface of the segment, and appeared on the segment's surface, or along its margins. At first the developing structures were white, but soon they became pale green, darkening as they matured. Most of the cytoplasmic contents of the segment migrated into them as reported by Feldmann (1951). Swarmers were usually released from the "gametangia" within a few hours after the lights came on the next "day", or about 36 hours after the first sign of gametangial development. The quantity emitted from even a single healthy plant soon imparted to the water of the aquarium the same transparency as does a good pea soup, and it did not clear for many hours. The swarmers were released in a series of puffs, with the swarmers of each puff more or less held together by a mucilage. Discharge, as observed in the aquaria, appeared to be from one or two points within the grape-like clusters, rather than from individual gametangia, and the gametangia themselves seemed to have no natural pores. But detailed viewing was difficult because of the developing murkiness. Microscopical viewing of natural discharge also proved awkward, because of the absence of cross walls in the filaments. The portions I examined microscopically showed swarmers being readily released from the central siphon, similar to what Ducker (1965) found for Chlorodesmis. This may represent the normal release. But there was also a backing up and escape of contents from the broken ends of the medullary and cortical filaments inside the segment; so conditions were not completely natural. Not all the fertile plants that developed released their swarmers, and the thousands of swarmers released seemed not to develop further. The parts of the plants which became white and fertile gradually fell apart within about a month. Most commonly this meant the demise of the entire plant, although sometimes only a branch was involved.

At least two Penicillus plants appear to have become fertile in my aquaria. They were discovered when the water in two aquaria became a cloudy green a few hours after the lights came on. There were no fertile Halimeda plants in the aquaria. Instead, the lone Penicillus plant in each aquarium had become white, except for a brownish fuzz, making a sort of halo around the capitulum of the plants. This fuzz, when examined microscopically, appeared as soft, easily broken, noncalcified extensions of the filaments of the capitulum. These extensions were about the same diameter as the regular filaments, and were sometimes dichotomously branched, without obvious cross walls between calcified and uncalcified portions. The tube extensions and main filaments of the capitulum were essentially empty of contents, indicating discharge, and the apical ends of the soft filaments were open. The green gelatinous scum which eventually formed on the surface of the water consisted largely of biflagellated swarmers which were fairly similar in size and appearance to those observed for Halimeda. Most of the fuzz on the plants disappeared in about a week, and the entire capitulum, which seemed especially brittle, disintegrated within a month. This sort of death of a Penicillus is unusual in my aquaria. A Penicillus plant usually malingers for several months, and generally produces, before it dies, new Penicillus plants by rhizoidal development. No new plants developed from the Penicillus plants described above.

Discussion and Conclusions

My observations in aquarium and, to a lesser extent, in the field indicate that vegetative reproduction in Penicillus, Rhipocephalus, Udotea and Halimeda is commonplace and successful. The most common method observed for the first three genera and sand-growing species of Halimeda involves the horizontal growth and differentiation of rhizoidal-like filaments, the filament(s) eventually becoming concentrated within the substrate into a small plantlet, often cone-shaped in Halimeda, which subsequently protrudes above the substrate and forms the photosynthetic portion. The appearance of the community which results -- erect portions with at least temporary horizontal connections, is similar in general style to the habit of Caulerpa, a prominent but noncalcareous siphonaceous genus of coral reefs. For in Caulerpa erect photosynthetic portions are connected by a stolon. This stolon, however, is considerably thicker, and persists for much or all of the life of the plant. In the four calcareous genera discussed in this report, interconnections are more delicate, meandering, possibly more numerous between erect portions, and they do not appear to persist. The filamentous connections observed for the plants grown are also more delicate than the rhizome-like connections illustrated for Penicillus by Duchassaing (1850) and the Mediterranean Udotea Desfontainii by Ernst (1904), but are perhaps rather similar to the connection shown for U. minima by Ernst.

This extension of rhizoidal-type filaments away from the holdfast area, with eventual differentiation and development into a new plant, may also bring about the multiplication of various rock-growing Halimeda species such as H. tuna. Although so far I have been unable to find, in field or laboratory, any external filament connecting discrete plants of such species, there are various indications that reproductive filaments may penetrate the rock. Such behaviour would indeed seem very reasonable, for filaments of other algae seem able to grow in dead coral fairly readily.

In the rock-growing species H. micronesica, the strands connecting young and older plants are thicker than the inter-sand connections on sand-growing species, and are composed of intertwined rhizoidal-like filaments. Perhaps they are similar to the thick rhizome-like structures illustrated by Duchassaing (1850) and Ernst (1904), but I have not yet found such material. The rope-like strands of micronesica differ also in that they originate from a segment rather than from the holdfast. Such extensions from segments also occur in H. opuntia, and new plants may originate from them, but the new opuntia plants I have observed in aquaria so far seem to have arisen by fragmentation.

In regions of moderate shifting sands it seems possible that another sort of vegetative multiplication, at least for Halimeda, may occur, brought about by a burying of the lower portions of the plant, the continued growth of exposed portions, and the development of adventitious rhizoidal filaments from the nodal regions of segments near the surface.

Various stages of this were induced in the laboratory. This multiplication is fairly similar to that also brought about in the laboratory by various regeneration experiments on Halimeda and Penicillus, in which various sized portions of these plants were shown to be able to develop entire plants, with new plants sometimes developing vegetatively, by extension of rhizoidal filaments, from the regenerated plant. In nature, bits of plant may be produced by surge, storm, perhaps by animals such as fish removing some delicacy from the plant's surface, or by grazers -- possibly the black urchin Diadema or cryptically colored slugs. Some of the resulting fragments may be redistributed by current or surge, and perhaps a few survive, to develop as separate plants.

All the reproduction of Halimeda, Penicillus, Udotea, and Rhipocephalus plants in laboratory aquaria seems to have occurred vegetatively, which may also be true for natural populations. I have been able to add information to that already known about swarmer production in these genera, but the data are still too scant to allow general conclusions on the importance of sexual reproduction in these plants. Zygote development has not been followed in Halimeda and Penicillus, and "sexual" plants have not been reported for Rhipocephalus, Tydemania, or for many species of the other genera. A few conclusions may be reached, however, based on the evidence from this aquarium work. The presumably fertile plants described for Penicillus have gametangia similar to those reported for Udotea (Nizamuddin, 1963). In both genera they are essentially unmodified filaments. Swarmer release in the laboratory, for both Halimeda and Penicillus, seems to be induced, at least partly, by light. Although several Halimeda became fertile at about the same time in the laboratory, the Halimeda involved were usually in separate aquaria. Their fertile condition, then, could hardly be chemically induced. The occurrence of clumps of fertile Halimeda in the field may thus have another, or an additional explanation to that of chemical induction which has been put forth previously.

PRODUCTIVITY

Introduction

This phase of the work was undertaken to provide data on the contribution of the four calcareous genera -- Halimeda, Penicillus, Rhipocephalus, and Udotea to a reef. An extensive literature exists on productivity studies with plankton, but very few measurements have been made on attached water plants such as those that fringe the shores of the continents. The productivity of an entire reef has been determined by a few workers (cf. Odum and Odum, 1955), and from such work we have come to think of many coral reefs as areas of richness in an impoverished sea. In such a nutrient-poor setting it thus seems that these reefs must provide much of their own energy, and it is becoming of increasing interest to know the role of its various components.

The experimental work was planned in two parts -- a census of calcareous Siphonales in a reef, and a laboratory determination of their productivity. Neither was completed when the work was interrupted by the change in research funding, but much progress had been made on both.

Field Work

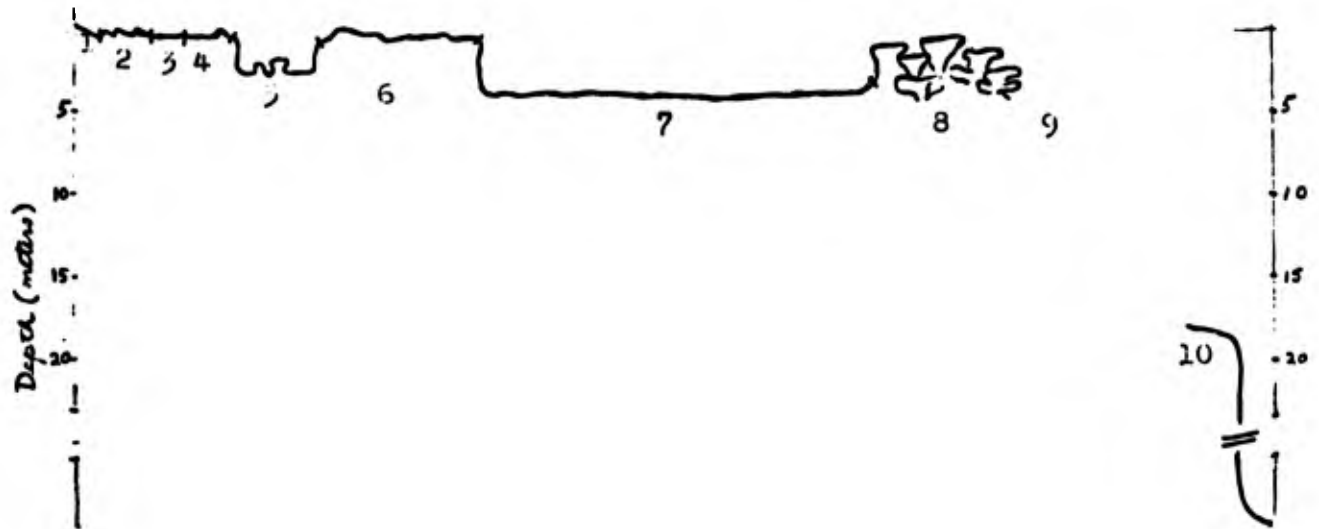
The reef chosen for most of this work belongs to the house "Glory Be" on the north shore of Jamaica, between Ocho Rios and Port Antonio. It is a fringing reef, probably reasonably representative of the north shore reefs of Jamaica, and has two special advantages. It is overhung by a cliff of fossil limestone which provided a useful vantage point of the reef and the regions of the inner reef itself are compressed so that the main zones are included in a small area.

The work on this reef included a general survey of the entire reef, quadrat counts of entire isolated coral heads, and of areas of one square meter, $\frac{1}{4}$ m², and $\frac{1}{20}$ m², plant mapping, estimation of percentage cover by eye, measurements of plant spacing, distance measurements so that area of the reef could be calculated, collection of specimens, and photography. For all of this, my field associate and I had about three weeks of diving time, that is, about 30 hours SCUBA time, most of which was in June 1969. Our equipment, in addition to the usual skin and SCUBA diving and photographic apparatus, consisted of two rubber boats which were used principally as diving tenders but also in survey work, a Precision Scientific galvanic cell analyser with thermistor probe and 100-foot lead for measuring water temperatures, and a Gossen tri-lux foot-candle meter in underwater case to measure the quantity of light available throughout the day at sites where Halimeda grows.

From the general survey we obtained a picture of the components of the Glory Be reef (Fig. 1). There was a narrow tidal sandy beach (0-3 m) passing into sand with emergent flat rocks (3-8 m), sea grass beds at 8-13 m in very shallow water, and shore reef (13-20 m), followed by channel (or lagoon, 10 m wide) with isolated and submerged coral heads. This was followed by a steepish rise to what I call the "breaker reef" (where the waves break, and about 20 m wide), then a steep drop to an "outer channel" about 50 m wide. The next zone seemed a veritable forest of elk-horn coral Acropora palmata, its orangish antler-like projections approaching the surface. This region passed rather abruptly into a buttress zone which in aerial view looks like a row of finger-like projections -- the buttresses alternating with canyons, and oriented perpendicularly to the shore. Then began a gentle (seaward) slope to a sheer and dramatic drop-off at about 18 m.

Most of our diving time on this reef was spent in the inner 100 m, that is, up to the Acropora zone, where there was a reasonably heavy and conspicuous cover of plants, and where, of the zones surveyed, there was the greatest population of calcareous green algae. Within this region, the percentage cover by calcareous green algae, as present in June 1969,

FIGURE I
 PROFILE OF GLORY BE REEF



- 1 Tidal sandy beach
- 2 Sand with emergent flat rocks
- 3 Sea grass beds in very shallow water
- 4 Shore reef
- 5 Channel (or lagoon)
- 6 Breaker reef
- 7 Outer channel
- 8 Acropora palmata region
- 9 Buttress and canyon region
- 10 Seaward slope to drop-off

varied from 0 to 90%, being highest on the seaward edges of the emergent rocks at about 6-8 m from shore, where H. opuntia, with occasional dense populations of H. goreauii, provided the very dense cover. It was lowest on the tidal sandy beach.

The shallow water, almost emergent sea grass beds within sandy patches of the shore reef had sizeable populations of H. monile and H. incrassata, Penicillus (mostly capitatus) and Udotea flabellum in addition to the conspicuous monocot, Thalassia testudinum. The cover these calcareous green algae provided was estimated at about 40%.

Over the inshore reef the conspicuous cover was mostly of reds and browns, which formed a spongy turf of about 16 cm thickness. The most common calcareous greens among them were Halimedae, species monile and incrassata, with compressed growth form, and with the portion of thallus buried by the turf, white. They were fairly dense; there were about 300 of these plants per square meter, and provided a cover of about 30%. H. opuntia was not conspicuous there except near the edge, where at times it formed a cover about as dense as on the emergent opuntia rocks. The outer edge of this inshore reef, however, was conspicuously barren of turf plants and of Halimedae, both in June 1969 and September 1968, and appeared as a whitish border about 2/3 m wide. The coral rock of this barren fringe was pitted, and scattered over it were numerous hollows, possibly rasped out, which contained the spiny black urchin Diadema antillarum. With the urchins, sometimes, were white fragments of H. opuntia, and certain nearby cushions of H. opuntia showed clear signs of being eaten for they had curved bites out of them and were floored by bitten segments. I noticed too that the opuntia plants in this vicinity had a rounded habit, devoid of any loosely projecting branches such as were immediately produced in the laboratory by similar opuntia cushions I took back, and the streamlining of the plants was such that one could picture an urchin passing over, eating as it went. The densest H. opuntia cover on this urchin border was 5%, but overall cover did not seem to be > 1% for the entire band. A green-grey fuzz on the rock was the conspicuous plant growth.

The remainder of the reef up to the Acropora zone generally had much lower densities of the calcareous greens. In taking the reef census, I treated as one unit -- for convenience, the vertical rock face of the shore reef, the coral outcrops from the sandy floor of the channel (av. depth 2.5 m), and the vertical shoreward face of the breaker reef. I swam along much of the rock surface included in this area, counting the individual plants or clumps. Plant cover was generally very thin, with H. opuntia, H. goreauii and encrusting reds appearing to be the dominants. They were accompanied by isolated thalli or clusters of plants such as Valonia -- 2 sp., leafy reds and Anadomene. Halimedae were present usually only on the lower portion of the vertical walls of the reefs, the barrenness of the upper regions perhaps being accounted for by urchin grazing. On the vertical face of the shore reef (N. facing) only H. opuntia was observed, but on the opposite rock face (south wall of the

breaker reef), both H. opuntia and H. goreauii were present. The distribution along both walls was patchy and sparse, and this contrasted dramatically with the roughly 90% cover at places near the edge of the horizontal surface of the shore reef. A 1% cover was estimated for the coral rock sides and outcrops.

In the inner channel of the Glory Be reef, the densities of all plants were also reduced. Patchiness of vegetation was characteristic, and wherever there was prominent vegetation either the calcareous Siphonales or Thalassia dominated. One plant, Udotea ad spinulosa occupied a conspicuously distinctive site. It frequently occurred in clumps of about 40 in sand under overhangs of coral rock. Sample quadrat counts are shown in Table II. These counts are based on a square meter, and the letters H, P, U, R, and Th represent the genera Halimeda, Penicillus, Udotea, Rhipocephalus and Thalassia, respectively. The Thalassia counts represent leaf clusters.

TABLE II

R	H	P	U	Th	Comment
---	441	194, both P&U		2	High density for channel
---	131	65	5	42	--
---	---	60	---	---	--
120	---	20	---	120	--
---	---	---	---	300	Highest density found
---	---	---	---	144	--
---	---	---	---	100	Thinly spaced leaf clusters
---	---	---	---	---	Sand barren of obvious plants, particularly noticeable by coral walls

Using all the quadrat counts made, and considering the size of the area, I have arrived at an estimate of 5% cover of the channel area of this reef by calcareous Siphonales. I consider this a generous estimate.

In both the breaker reef and outer channel I found many aspects of shore reef and inner channel, respectively. About 2/3 of the breaker reef seemed to be an urchin barren (at least in June 1969), and 1/3 had almost closed vegetation rather similar to that of the shore reef. H. opuntia seemed the most prominent Siphonales, and again I had the impression of its being grazed. The outer vertical face appeared almost barren of calcareous Siphonales. I estimated the percentage cover of the surface by calcareous greens as roughly 1/3 that of the shore reef, or about 10-15%.

In the outer channel (50-100 m from shore) there were few calcareous greens in June 1969, and the greater part of it (outermost 37 m) contained a Diadema population that averaged 6/m², in places reaching a density as high as 12/m², with 20/m² in a few areas. Such densities

indicate heavy production. Large algae such as Halimeda occupied only a tiny fraction of the grazed surface of the channel floor which contained much rock and largish pebbles. Instead of Siphonales, a green fuzz of filamentous green algae covered much of the floor, and seemed to be maintained by the grazing of urchins. About 20% of the channel contained reasonably dense stands (ca. 300/m²) of Thalassia on cobble and pebble-covered sand with white urchins and heart urchins (density of each estimated as < 1/m²), alternating with grassless barren areas. Among the seagrasses were occasional small plants of sand-growing Halimeda (ca. 4/m²), while a greenish-grey fuzz seemed to cover the pebbles and cobbles. These Thalassia stands gave the impression of advancing and retreating, with the heart urchins and fuzz remaining in the new community. This entire outer channel needs further study, especially later in the growing season. The best estimate of cover by calcareous greens that can be made at present is 1-5%.

Summary and Comment on Field Work

For the inner reef system (up to the Acropora zone) of the Glory Be reef on the north shore of Jamaica, the percentage cover by calcareous green algae varied from zero on the tidal sandy beach to about 90% over portions of inshore and partially exposed rocks bearing Halimeda opuntia during June 1969. Grazing by sea urchins appeared to affect it locally. Of the 4 calcareous genera present, Udotea and Rhipocephalus were locally common, but over the reef as a whole their numbers were low. On the rocky portions of the reef H. opuntia is the commonest representative, but H. goreauii may be locally very abundant. In sand Penicillus capitatus and lamourouxii are more abundant than anticipated, and over sizeable portions of the reef are more common than the sand species of Halimeda. The estimated percentage cover for the regions delimited in the pre-Acropora region of the Glory Be reef are: opuntia rocks 3-6 m from high tide line, 5%; opuntia rocks 6-8 m out, 90%; Thalassia beds amid shore reef, 8-13 m, 40%; shore reef, 13-20 m, 35%; coral rock sides and outcrops, 1%; inner channel, 20-30 m, 5%; breaker reef, 30-50 m, 10-15%; outer channel, 50-100 m, 1-5%.

Beyond the Acropora zone we made exploratory dives up to, and over the drop-off region (approx. 18 m depth) of the reef. On the sheer vertical wall below this drop-off we observed relatively few populations of Halimeda. Large populations have, however, been reported (Goreau laboratory, personal communication) from below about 25 m depth. It had been planned, in the next field season, to continue the survey of calcareous green algae into these depths, and to study populations of the shallower parts of the reef system at other seasons of their growth. Although it proved not possible to do so under this contract, I plan to continue some aspects of this work.

Laboratory Productivity Studies--Methods

Productivity may be defined as the rate of conversion of radiant energy into chemical energy, and can be measured by determining the input or output of any of the components of the photosynthetic equation. In this project, I concentrated on measuring the amount of oxygen evolved. An extensive literature exists on methods for this, with the two principal ones being C^{14} , and the light and dark bottle technique. The organisms that have been used in nearly all the studies are small -- the plankton of lakes and sea. Oxygen production by largish plants such as Halimeda or Penicillus has essentially not been measured in field or laboratory. In the short time I had available for this work I did not feel that methods using C^{14} could be readily adapted to largish coenocytic plants, and it is perhaps not surprising that, by experiment, I found the standard light and dark bottle method which entails uprooting plants also unsatisfactory. I set about, therefore, to devise a closed salt-water aquarium system in which the day and night changes in oxygen production by the plants in the aquarium could be monitored. My assistant for this phase of the project, Mr. Dwayne Maxwell, designed of 3/4-inch plexiglass, a suitable lid which was machined to fit the tops of the all-glass aquaria. The lid was held firmly to the aquarium by turnbuckles, and an O-ring type seal was provided by a neoprene rubber cord placed in a groove around its edge. Holes in the top accepted rubber stoppers containing a lead of a dissolved oxygen probe, glass tubing for filling the aquarium, for overflow, for pumping in and out, and a thermometer.

The procedure was as follows. The probe of the dissolved oxygen meter (Yellowsprings model 54RC) and the various glass fittings were positioned in the plexiglass top, and the top secured to the test aquarium by turnbuckles. The aquarium was then filled by siphoning seawater from a glass carboy. Before the tank was full, white silicone rubber tubing was threaded through a peristaltic pump, and the pump turned on. This allowed the tubing to be filled with water and displaced air to escape. Filling proceeded until 4-6 inches of water rose in the overflow tube, then the siphon tube and the overflow tube were clamped off. Clamps were placed between the tank and the top of the water in the tubes so that no air spaces could be in contact with the water column.

The oxygen meter and the recorder (Yellowsprings model 80) were joined. Both instruments were turned on to check zero points and to calibrate the recorder with the oxygen meter, the oxygen meter already being calibrated (at outset of each experiment) as is described later. The equipment was plugged into the timed circuits of the controlled temperature room after the lights went out. When the lights came on in the morning all instruments were automatically turned on. Later in the morning the instruments were plugged into an electrical line not controlled by the time clocks so that the probe, recorder and pump would not be turned off when the lights went off after 12 hours. A water flow of about one foot per second past the probe was maintained by the pump.

This flow was necessary for accurate readings, and its adequacy was confirmed by a Yellowsprings consultant.

The dissolved oxygen meter was calibrated before each experiment using a seawater sample of the same salinity as the water in the experimental aquarium. First, the concentration of dissolved oxygen in 300 ml of the sample (a B.O.D. bottle) was determined using the modified Winkler titration described by Strickland and Parsons (1968). By immersing the oxygen probe in the remainder of the sample of now known dissolved oxygen concentration (500 ml is sufficient), the scale on the meter was adjusted as necessary. A magnetic stirrer provided the necessary circulation past the probe.

With these arrangements the changes in dissolved oxygen concentration within the aquarium over about 18 hours were recorded, the time limit being set by the tubing which fatigued where grasped by the pump. The life of the system could, however, be extended up to 36 hours by using a longer tube, and moving it through the pump. The tygon tubing which was initially recommended for our pump broke after but a few hours, so white silicon rubber tubing was subsequently used for the pump. The tubing used elsewhere in the system was tygon.

Test aquaria were planted with about 40 freshly collected, cleaned plants of Halimeda incrassata or Penicillus capitatus which were allowed to establish themselves for about a week (ample time as indicated by my growth studies) before oxygen measurements were made. Three different light intensities were used, 650, 375 and 200 ft-candles. At the end of experiments, whenever feasible, the plants were cut off at substrate level and the cut portions removed. Oxygen production by any remaining organisms on or in the essentially undisturbed gravel was then monitored to obtain the contribution to oxygen production by the "contaminating" organisms of the aquarium other than those on the test plants.

Discussion and Results

The number of experiments carried out was severely limited by the short time available for this phase of the work, the material obtainable, and the university conditions of unrest during part of this time. However, all the recordings obtained for aquaria of Penicilli or Halimeda (ppm plotted against time) were sine-shaped, and showed an increase in oxygen production during the 12-hour light phase (0700-1900 hrs.), followed by an immediate decrease when the lights were turned off and photosynthesis ceased. The peak appeared to be reached somewhat before the lights went out when the plants were grown under the highest light intensity, whereas under the two lower intensities oxygen concentration continued to increase until the lights went out. With lights out the amount of oxygen in the water steadily decreased, consumed by the respiration of Penicilli or Halimeda plus any other organisms (this respiration being continuous over the 24-hour day) until the lights came on again, when the pattern was repeated.

From an analysis of the curves obtained, smallish Halimeda plants (av. height 7 cm excluding holdfast) appear to have a net productivity of about 2.5 mg C/plant/day, and a gross productivity of about 4.5 mg C/plant/day under the laboratory conditions described. If one allows a similar productivity for plants of the reef, and considers a dense population of 1000 such plants/m² (approximately 100% cover on the Glory Be reef), these rates would be 2.5 g C/m²/day (net) and 4.5 g C/m²/day (gross) or 914 g C/m²/yr and 1700 g C/m²/yr.

There are very few figures in the literature with which to compare these data, since the bulk of the determinations are for plankton. The information on coral reef communities would be somewhat more appropriate, but the most apt data are the few existing figures for other macrophytes. The table below gives these figures, recalculated to the units indicated as necessary.

TABLE III

	PRODUCTIVITY	
	Gross g C/m ² /day	Net g C/m ² /day
Coral reef communities		
Odum and Odum, 1955	9.6	--
Sargent and Austin, 1954	4.1	--
Kohn and Helfrich, 1957	8.0	--
<u>Thalassia</u> population		
Odum, 1957	10.6	--
Odum, Burkholder & Rivers, 1959	1.8-4.5	--
Westlake, 1963	--	3.8
<u>Ascophyllum</u> pop., Westlake, 1963	--	2.1-2.8
<u>Laminaria</u> pop., Westlake, 1963	--	3.9
<u>Macrocystis</u> pop., McFarland & Prescott, 1959	9.3-10.8	--
<u>Halimeda</u> pop., Colinvaux	4.5	2.5

Detailed critical comparison using these data is difficult because different methods were used in obtaining the results, and the densities of the populations of macrophytes were generally not indicated. I would, however, expect the population density of the wild plant to be very high, particularly the stands of Thalassia. If this assumption is granted, then the laboratory Halimeda seem capable of the same sort of production as the seagrass Thalassia, particularly as found by Odum in 1959, although if such pure and dense stands of Halimeda exist, their productivity probably would be lowered by the shading by fronds.

The Halimeda productivity figures I obtained, per plant, in this work are, I believe, lower, and perhaps rather considerably so, than those that would have been obtained had field methods been readily applicable to the plants of the Glory Be reef. Although most of the plants used appeared to settle in and grow satisfactorily, in their recent history they had all been transplanted from reef to laboratory with 2-3 days in transit, since I was unable to collect this particular batch. In addition, the selection of plants available from the field when these experiments were carried out seemed very limited. Lighting in the field would have been "normal" and considerably higher than it was practical to use in the laboratory. The highest intensity I used, about 650 ft-candles, was ideal for some of the growth studies, but is not likely to be optimum for photosynthesis. Higher intensities in the productivity measurements would have overheated the water in the closed system and were not feasible for the short time, and the materials available for these experiments.

A logical extension of this productivity and growth study is an investigation of the contributions made by these plants to the carbonates of the reef system, so that the role of calcareous green plants in reef building can be more completely understood. Some aspects of this carbonate production are being considered during my current fellowship year, and it is planned to continue various productivity investigations in subsequent years.

REFERENCES

- Borden, C. A. and J. R. Stein, 1969. Reproduction and early development in Codium fragile (Suringar) Hariot: Chlorophyceae. *Phycologia*, 8(2): 91-99.
- Chihara, M., 1956. Studies on the life-history of the green algae in the warm seas around Japan. 4. On the life-history of Halimeda cuneata Hering (1). *J. Jap. Bot.*, 31(4): 102-110.
- Colinvaux, L. Hillis, 1968. Vegetative reproduction of Halimeda and related Siphonales in laboratory culture. *J. Phyc.*, 4(supplement): 4.
- Colinvaux, L. Hillis, K. M. Wilbur, and N. Watabe, 1965. Tropical marine algae: growth in laboratory culture. *J. Phyc.*, 1(2): 69-78.
- Dawson, E. Y., 1966. *Marine Botany*. Holt, Rinehart and Winston, Inc. xii + 371 pp.
- Duchassaing, P., 1850. *Animaux radiaires des Antilles*. Paris. pp. 27-8 and pl. 1.
- Ducker, S. C., 1965. The structure and reproduction of the green alga Chlorodesmis bulbosa. *Phycologia*, 4(3): 149-162.
- Ernst, A., 1904. Siphoneen-Studien. II. Beiträge zur Kenntnis der Codiaceen. Beihefte zum Bot. Centralblatt, 16(104): 199-236 + pls. 7-9.
- Feldmann, J., 1951. Sur la reproduction sexuée de l'Halimeda tuna (Ell. et Sol.) Lamour. f. platydisca (Decaisne) Barton. *C. R. Acad. Sci.*, Paris, 233(14): 1309-10.
- Fritsch, F. E., 1948. *The structure and reproduction of the algae*. Vol. I. Cambridge Univ. Press. xviii + 791 pp.
- Hillis, L. W. (Colinvaux, L. H.), 1959. A revision of the genus Halimeda (order Siphonales). *Publ. Inst. Mar. Sci. Univ. Texas*, 6: 321-403.
- Howe, M. A., 1907. *Phycological studies*. III. Further notes on Halimeda and Avrainvillea. *Bull. Torrey Bot. Cl.*, 34: 491-516.
- Jacobs, W. P., 1964. Rhizoid-production and regeneration of Caulerpa prolifera. *Pubbl. staz. zool. Napoli*, 34: 185-196.
- Kohn, A. J. and P. Helfrich, 1957. Primary organic productivity of a Hawaiian coral reef. *Limnol. & Oceanogr.* 2(3): 241-251.
- Kupfer, E., 1907. Studies in plant regeneration. *Mem. Torrey Bot. Cl.*, 12(3): 195-241.

- McFarland, W. N. and J. Prescott, 1959. Standing crop, chlorophyll content and in situ metabolism of a giant kelp community in southern California. *Publ. Inst. Mar. Sci. Univ. Texas*, 6: 109-132.
- Nasr, A. H., 1947. Synopsis of the marine algae of the Egyptian Red Sea coast. *Bull. Fac. Sci. Egypt Univ.*, no. 26: 1-155.
- Nizamuddin, M., 1963. Studies on the green alga, Udotea indica A. & E. S. Gepp, 1911. *Pacific Sci.*, 17(2): 243-245.
- Odum, H. E., 1957. Primary production measurements in eleven Florida springs and a marine turtle-grass community. *Limnol. & Oceanogr.* 2(2): 85-97.
- Odum, H. T., P. R. Burkholder and J. Rivero, 1959. Measurements of productivity of turtle grass flats, reefs, and the Bahia Fosforescente of southern Puerto Rico. *Publ. Inst. Mar. Sci. Univ. Texas*, 6: 159-170.
- Odum, H. T. and E. P. Odum, 1955. Tropic structure and productivity of a windward coral reef community on Eniwetok atoll. *Ecol. Monogr.*, 25(3): 291-320.
- Sargent, M. C. and T. S. Austin, 1954. Biologic economy of coral reefs. U. S. Geol. Surv. Prof. Paper 260-E: 293-300. In professional paper series on Bikini and nearby atolls. Part 2. Oceanography (biologic).
- Strand, J. A., J. T. Cummins and B. E. Vaughan, 1966. Artificial culture of marine seaweeds in recirculation aquarium systems. *Biol. Bull.* 131(3): 487-500.
- Strickland, J. D. H. and T. R. Parsons, 1968. A practical handbook of seawater analysis. Fisheries Research Board of Canada Bull. No. 167, Ottawa. x + 311 pp.
- Taylor, W. R., 1960. Marine algae of the eastern tropical and subtropical coasts of the Americas. Univ. of Mich. Press, Ann Arbor. xii + 870 pp.
- Taylor, W. R., A. B. Joly and A. J. Bernatowicz, 1953. The relation of Dichotomosiphon pusillus to the algal genus Boodleopsis. *Pap. Mich. Acad. Sci.*, 38: 97-107, 3 pls.
- Westlake, D. F., 1963. Comparisons of plant productivity. *Biol. Rev.* 38: 385-425.

Signature Page
for
Final Report
on
Contract No. N00014-67-C-0262
(RF Project 2410)

Investigator..... Date.....

Supervisor..... L. Hillis - Coleman..... Date 3/6/72

For The Ohio State University Research Foundation

Executive Director..... Robert C. Stephenson..... Date 7/18/72
#3 #2