

## REPRODUCTION IN THE CALCAREOUS GREEN ALGAE OF CORAL REEFS\*

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### ABSTRACT

Reproduction in *Halimeda* is often summarised in text books as 'sexual'; for *Penicillus* the existence of any sexual stage has been questioned. In this paper I present information on physiological and morphological aspects of sexual stages in the two genera, based on material from the International Indian Ocean Expedition [IIOE] and my Laboratory cultures.

Although Halimeda with the so-called 'gametangia' appear in culture relatively frequently, the life cycle is not completed. The many new plants in my cultures, and possibly those in the field, arise vegetatively in several ways. The commonest method, the development of new plants at the end of certain rhizoids, occurs in *Halimeda*, *Penicillus*, *Rhipocephalus*, and *Udotea*. New plants may also develop at cut surfaces, and by fragmentation.

The distributional pattern of some species has been extended considerably, based on the large amount of *Halimeda* material from the 1963-65 IIOE which filled the existing void. Sixteen species are now known for the Indian Ocean, two *macrophysa* and *velasquezi* for the first time. *Halimeda micronesica* is known from many sites throughout the Indian Ocean rather than from the Seychelles only; *H. copiosa* is new; and *H. remschii* reinstated. Particularly exciting is the extension for *H. cuneata*, the only distinctly subtropical species in the genus. It had seemed to be restricted to the southern hemisphere, but during the IIOE was collected in the northern subtropics too. The vertical distribution of certain species is extended also.

### INTRODUCTION

IN 1966 Dawson wrote of the extraordinary lack of knowledge about reproduction in the Codiaceae (order Siphonales), a family of many sizeable and very common coral reef plants. Of its more than fifteen genera, the sexual cycle has been worked out for but one—*Codium*. Sexual or apparent sexual stages have been reported for *Chlorodesmis* (Ducker, 1965), *Halimeda* (Hillis, 1959, includes literature review), and *Udotea* (Nizamuddin, 1963), and aplanospore-like or akinete-like structures reported for *Ayrainvillea* (Howe, 1907) and *Boodleopsis* (Taylor *et al.*, 1953). Fritsch (1948) states that 'vegetative propagation is no doubt widespread', but there appears to be little documented evidence of how it occurs. Detachable propagules (Fritsch, 1948), and fragmentation of juvenile filamentous stages (Borden and Stein, 1969) have been mentioned for some species of *Codium*, protonemal-like stages suggested for *Penicillus* and *Udotea* (Taylor 1960), and rhizoidal-like filaments for *Halimeda* (Colinvaux 1968). Duchassaing (1850) very briefly describes reproduction by 'propagules' in *Nesea* (= *Penicillus*), and figures a sort of rhizome terminating in a

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scaly young plant. A similar rhizomatous-like connection is also shown between plants of *Udotea desfontainii* (Ernst, 1904).

In laboratory aquaria and in the field, I have observed stages in the growth of various members of the Siphonales and the development from them of many new plants. Many of the observations on reproduction are reported herein.

#### METHODS

The plants are grown in small aquaria arranged under fluorescent lights in a controlled environment room, essentially as described by Colinvaux, Wilbur and Watabe (1965). A mixture of coarse gravel and calcareous aquarium filtrant is used as substrate for sand-growing species; autoclaved fossil coral rock for other species. Millipore-filtered natural sea water was used in the early work in which some plants were maintained for over two years, but the plants have done well also, for at least six months, in aquaria containing water constructed from the commercial preparation 'Instant Ocean'.<sup>2</sup> The Siphonales which have been grown are the *Halimeda* species *discoidea*, *goreauii*, *incrassata*, *macroloba*, *monile*, *opuntia*, *simulans*, and *tuna*, *Penicillus capitatus* and *P. dumentosus*, *Rhypocephalus phoenix*, *Udotea flabellum*, *Bryopsis* sp., and several species of *Caulerpa*, but I have concentrated on the first four genera, which, with *Tydemania*, are the calcareous members of the group.

Field observations have been made in tropical reef areas of the eastern Indian Ocean from southern Thailand to Java, as a member of the International Indian Ocean Expedition, *Te Vega* cruise A, in Bermuda, and on the north shore of Jamaica in connection with my productivity work. The Jamaican work was encouraged and funded by contract N00014-67-C-0262 from the Office of Naval Research. The United States Program in Biology, International Indian Ocean Expedition was supported by National Science Foundation Grant No. G 17465.

#### OBSERVATIONS

##### *Asexual Reproduction*

*Halimeda* and *Penicillus* are dominant plants in many reef areas of the Caribbean. Their vigorous, healthy thalli are what the swimmer-biologist often sees, as he works his way through the reef. A closer probing, and he becomes aware, frequently, of many turgid, immature thalli of these same genera projecting above the sand. He may also see numbers of epiphytized plants, and whitish plants, falling apart in bits. Taken together, these observations illustrate the growth cycle of *Halimeda* and *Penicillus*, and indicate that these plants maintain their numbers very satisfactorily. For *Penicillus* this growth cycle may be repeated several times a year. The cycle is repeated less frequently for *Halimeda*.

In the laboratory, I have many times seen *Halimeda*, *Penicillus*, *Rhypocephalus* and *Udotea* go through this cycle: young bright green plants; mature plants which eventually become covered with epiphytes; whitening; and eventual disintegration; with new plants often appearing when the plant is mature or beginning to whiten. These observations were made first with *Halimeda*, a genus known to produce flagel-

<sup>2</sup> Available from Aquarium Systems, Inc., 1450 E. 289 Street, Wickliffe, Ohio.

lated swarmers, or, what are often referred to as gametes, since fusion has been reported by Nasr (1947) and Chihara (1956) for *H. tuna*. However, since development of the zygote has not been observed, the use of the word 'gamete' should perhaps be a bit restrained. Nevertheless, it was conceivable that the swarmers had produced the new plants observed in field and aquaria. A study of twenty aquaria over a four month period gave some data on this production of young plants. Out of about 240 sand-growing *Halimeda* plants, 17 became fertile, released their flagellated swarmers, and were dead about a month later. During the same time, over 160 new *Halimeda* sprouted up through the gravel—increasing the population by about 67%. Significantly, over three-quarters of these new *Halimeda* developed in aquaria in which there had been no fertile plants. It, therefore, seemed that the new plants, or most of them, could not have developed from the swarmers, but had somehow been produced vegetatively.

When I removed some of these 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 thread-like strands (Plate IC) which sometimes branch, and are 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 the 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.

In the field, I have examined *Halimeda* communities on sandy patches for indications that this sort of reproduction, by rhizoidal-like extension, is common in the natural habitat. SCUBA has been useful, and I have observed that the plants are often patchily distributed, the sort of distribution one would expect with reproduction by rhizoidal 'runners'. I have also found very young, essentially undeveloped plants coming from the holdfast, just as I have in the laboratory. But the surges on a coral reef make the delicate work hard, even with SCUBA, so that I have been unable to be sure that I am not breaking any rhizoidal-like connections.

In the laboratory, I have been able to bring about multiplication of *Halimeda* from cuttings. Isolated tips, the entire above-gravel portion of the plant, and the holdfast system obtained by cutting through the basal segment of the photosynthetic portion are able to regenerate. The cut portions were sometimes planted in gravel; sometimes placed prostrate in an aquarium without gravel, so that rhizoidal growth and the vegetative production of new plants could be observed. The cut ends were pressed firmly, immediately after cutting, for about a minute (Jacobs 1964), to control loss of cytoplasm. Part of the cut material subsequently became overgrown by epiphytes, and produced no new growth. But some of the isolated tips, when planted, produced new apical segments, regenerated a holdfast system, had occasionally produced yet another plant by growth from the holdfast system. The entire above-gravel portion or photosynthetic portion which had been produced by a cut through a basal segment, usually regenerated a new holdfast system, often with young plants amid the filaments. Sometimes it formed new green segments on the upper branches of the original material. If the regenerating portion were horizontal, the new segments eventually assumed a vertical position, while most of the rhizoids developed from the ventral cut surface of the lowermost segment. The severed holdfast produced a new top of green segments and branches.

For *Penicillus*, *Rhipocephalus*, and *Udotea*, I have observed a similar development of young plants in laboratory aquaria, and have pulled them up, still attached

to an older plant by a fine thread. In limited regeneration work on *Penicillus*, a new capitulum has regenerated when the old one is cut off. A rather similar result was reported by Kupfer (1907). 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.

#### Sexual Reproduction

From field work and herbarium studies, I have now observed fertile plants of *H. cuneata*, *H. cylindracea* (hb. G. Valet), *H. discoidea*, *H. favulosa* (Hillis 1959, p. 371, pl. 8, figs. 2-4), *H. gracilis*, *H. incrassata*, *H. macroloba*, *H. macrophysa* (LH 28, East Indies, 3 xii 63, hb. LH-C), *H. micronesica* (LH 27a, East Indies, 2 xii 63, hb. LH-C), *H. monile*, *H. opuntia* (LH 29, East Indies, 4-7 xii 63, hb. LH-C), *H. scabra*, *H. simulans*, *H. tuna* and *H. velasquezii* (hb. R. Tsuda). The list represents about half the described species of *Halimeda*. These fertile plants are 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. Fertile plants sometimes appear to be isolated from others, but frequently several plants in a large cluster are fertile. Nearby there may be white, disintegrating plants, an indicator that they may have been fertile a short time ago, but they might also represent dying vegetative plants.

Fertile *Halimeda* plants have developed in the laboratory too. In one planting of approximately 500 *Halimeda*e in 44 aquaria, 24 plants became fertile within the six months that all aquaria were maintained. Between 10-12 plants were in an aquarium, and most commonly only one plant in an aquarium became fertile. At some time 16 aquaria had a fertile *Halimeda*, three had two, one had three, and one four. Data on the dates the plants were observed fertile is given below:

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 aquaria	1 each	12 viii 69 to 6 xi 69

When a *Halimeda* becomes fertile, it changes from green to white overnight, or during the dark growth phase, and gradually falls apart within about a month. Stalked clusters of gametangia which develop from filaments protruding beyond the surface of the segment, appear on the segment's surface, and along its margins. At first the developing structures are white, but they soon become pale green, and darken as they mature. Most of the cytoplasmic contents of the segment migrate into them as reported by Feldmann (1951). The swimmers in my cultures have usually been released within a few hours after lights go on the next 'day', or about 36 hours after the first sign of gametangial development. The quantity emitted from even a single plant soon turns the water of the aquarium into a sort of pea soup, which does not clear for many hours. The swimmers are released in a series of puffs, with the swimmers of each puff often held together by a mucilaginous substance. Discharge,

as observed in the aquaria, appears to be from one or two points in a gametangial cluster, rather than from individual gametangia. A microscope view has proved difficult, however, because of the absence of cross walls in these plants. The portions I have prepared for microscopical study at release time have shown contents being readily released from the central siphon as Ducker (1965) found for *Chlorodesmis*, and this may represent the normal release, but there has also been a backing up and release of contents from the broken ends of the medullary and cortical filaments inside the segment. I have found no special pores on the gametangia themselves. Not all the fertile plants developed released their swarmers, and the thousands of swarmers released seem not to have developed further.

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 (Plate IA). This fuzz, when examined microscopically, appeared as soft, easily broken, non-calcified 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 (Plate IB). 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 generally indicate that vegetative reproduction in *Penicillus*, *Rhipocephalus*, *Udotea* and *Halimeda* is commonplace and successful. The commonest method observed for the first three genera and sand-growing species of *Halimeda* involves the proliferation 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 separate plants of each genus, when they are interconnected by these rhizoidal-like filaments, show a certain similarity in growth pattern to *Caulerpa*, a prominent but non-calcareous siphonaceous genus of coral reefs. In *Caulerpa*, however, the erect photosynthetic portions are connected by a stolon which persists for much of the life of the plant. In the four calcareous genera being discussed, the filament-like connections are more delicate, meandering, and do not appear to persist. The filamentous connections observed in the species grown are also more delicate than the rhizome-like connections illustrated for *Penicillus* by Duchassaing (1850) and the Mediterranean *Udotea desfontainii* by Ernst (1904).

This extension of rhizoidal-type filaments away from the holdfast area, with eventual differentiation and development into a new plant, may also account for

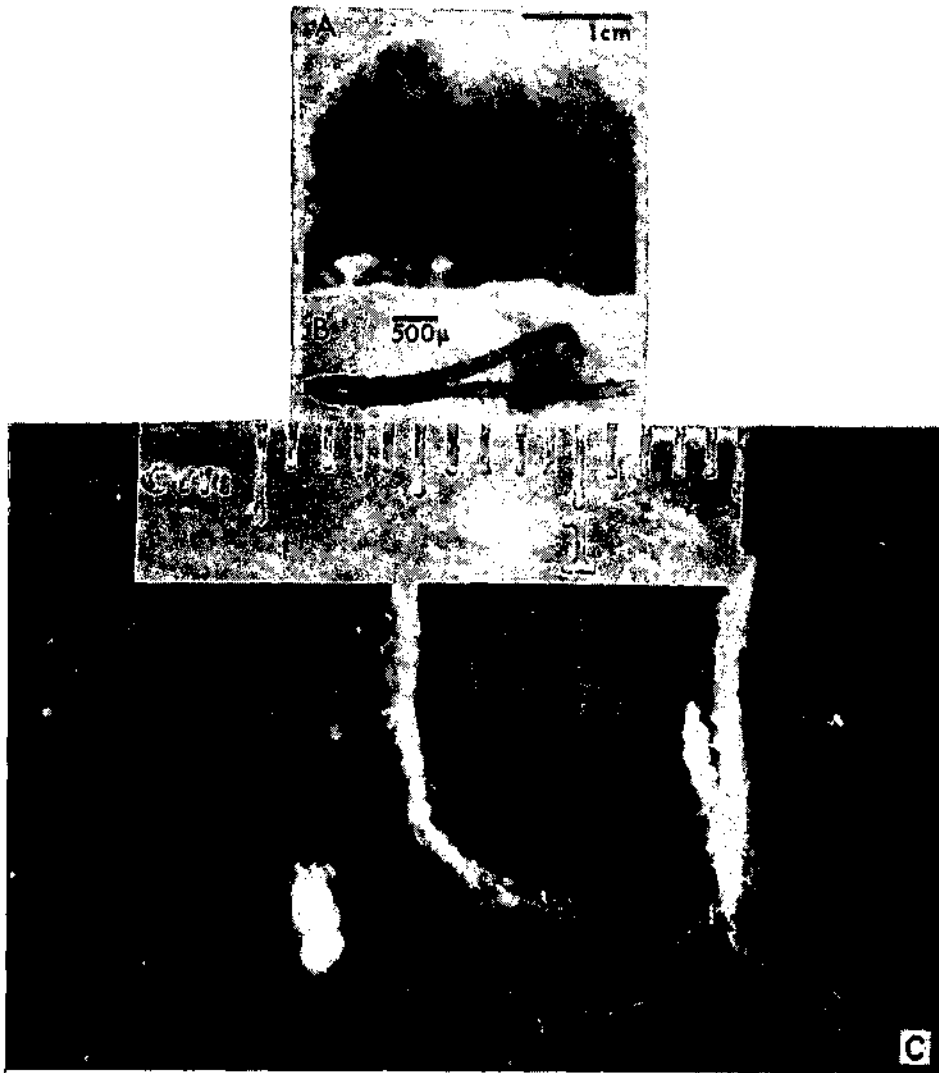


Plate IA. *Penicillus capitatus* in aquarium showing conspicuous capitulum or brush. Capitulum and large particles of substrate obscure stalk portion of thallus. Darker parts of capitulum are uncalcified filamentous extensions of regular capitulum filaments; lighter portions are regular capitulum filaments that have turned white; B. Filaments from the capitulum showing calcified (left) and uncalcified (right) portions. Swimmers were observed about three days before picture was taken. Fine lines approximately at right angles to filaments are epiphytes; and C. Four young *Halimeda* connected by thread-like intra-sand connections.

multiplication of 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 on a rock. However, the filaments of other algae grow in dead coral fairly readily; so the filaments of these plants, if they occur, could probably grow through dead coral too.

My experience with regeneration of *Halimeda* and *Penicillus* indicates that various sized portions of these plants are viable and able to develop a new plant which in turn produces new plants vegetatively. This sort of reproduction was artificially induced in the laboratory, but in nature bits of plant may be broken off by surge, by storm, perhaps by animals such as fish removing some delicacy from their surface, or by grazers. The black-spined urchin, *Diadema*, and some cryptically coloured slugs seem to graze on *Halimeda*. Some of the broken portions may be redistributed by current or surge, and a few perhaps survive eventually to grow.

A sort of multiplication may occur, at least among certain 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 to disintegrate. I have seen this in plants in the field, and in some of my regenerating *Halimeda* tops, which were placed essentially horizontally in the gravel, than buried except for certain growing tips. I have not observed the complete dissolution of the older portions of such buried plants, but the stages leading up to it were present. If death and decay of the older parts occurred, the younger portions would exist as several separate individuals. Such plants would probably have been counted as several individuals, perhaps long before the dissolution of connecting segments, were a quadrat count made.

The evidence is still too scant to come to 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.

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