8 PUBLICATIONS
ON SOME GREEN ALGAE FROM THE CHANNELS AND RESERVOIRS OF SAURASHTRA SALT WORKS, PORBANDAR.

SUDHA KALE

ON SOME GREEN ALGAE FROM THE CHANNELS AND RESERVOIRS OF SAURASHTRA SALT WORKS, PORBANDAR

The mile long channel at the Saurashtra Salt Works is 7-5 m. in width and 0-6-0-9 m. in depth. The channel is connected by estuarine streamlets with the sea; and the density of water in the channel is about 5' Baume, in January. There is inflow to the channel mainly during spring tides. Cladophora crystallina, C. expansa and Enteromorpha plumosa were found growing luxuriantly on soft silt at the bottom of the channel. C. expansa was found in the month of December 1963 and the other two species in January, 1964. E. clathrata was collected in January, 1964 from the edge of a large reservoir in the Salt Works. These species are described below, the descriptions being based on the present materials from Porbandar.

1. Cladophora crystallina (Roth) Kuetzing Pl. II, Figs. 1-4; Tab. Phyc., 1854, t. 19, Fig. 2; De Toni, 1889, pp. 318-19; Taylor, 1957, p. 86; 1960, p. 58.

Plants faint green, soft but slightly coarse at the basal region, forming entangled masses; branching dichotomous in basal region, unilateral or sometimes whorled in the upper region; main branchlets 70-110 μm in diameter; ultimate branchlets 20-42 μm in diameter; cells 4-11 μm diameters long, non-constricted at nodes.


Geogr. Distrib.—Atlantic Coasts of Europe and America, Mediterranean Sea, Canary Islands, West Indies.


Tab. Phyc., 1853, t. 99, Fig. 1; De Toni, 1889, p. 319; Boergesen, 1925, p. 68; Taylor, 1957, p. 85, pl. 5, Fig. 5; 1960, p. 85.

Plants light green with dark green tips, forming soft entangled spongy masses of about 0-6 m. in diameter and consisting of densely branched cylindrical filaments; branching alternate or divericate in the lower region, unilateral in the apical region; main branchlets angular-filiform; apical cells becoming longer than the other cells; cells of the larger axes 120-140 μm in diameter with length 3-5 times their diameters; cells in basal region with thick stratified walls.

Reproductive bodies in the cells grading from zoospores to aplanospores, spherical, with diameter 9-12 μm.

Specimen.—S. Kale No. 1, 20-12-1963, in Herbarium C.S. & M.C.R.I., Bhavnagar.

Geogr. Distrib.—Atlantic Coasts of Europe and America.


De Toni, 1889, p. 32; Boergesen, 1925, p. 10; Taylor, 1957, p. 63; 1960, p. 58.

Plants solitary, attached, 7-25 cm. tall, yellowish-green, soft, branching repeatedly; branches delicate and irregular, in the basal region opposite or whorled, cylindrical, with long uniseriate tips; cells sub-rectangular, in branch tips 9-12 μm diameter, in longitudinal as well as transverse series, in main axis 12-21 μm.
ENTEROMORPHA GUJARATENSIS, A NEW SPECIES FROM GUJARAT, INDIA

SUDHA R. KALE

Reprinted from "Phykos", 1967, 6(1&2), 29-31
EFFECT OF DIFFERENT MEDIA ON THE GERMLINGS OF
ULVA LACTUCA VAR. RIGIDA

SUDHA R. KALE and V. KRISHNAMURTHY

Reprinted from "Phykos" 1967, 6(1 & 2), 32-35
**Enteromorpha gujaratensis, a new species from Gujarat, India**

SUDHA R. KALE
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**Enteromorpha gujaratensis** n. sp.

Plants tufted, when fully grown not attached, submerged, yellowish green, slimy to touch, up to 28 cm. tall; filaments cylindrical, subsolid at the base; main axis 0.2-0.5 mm. in diam. with longer branches 70-140 μ in diameter, irregularly branched with lesser branches stout and terminating in a number of monosiphonous filaments; cells rectangular to polyhedral, with one pyrenoid (2-3 μ) in each cell, arranged in longitudinal series, but getting slightly displaced in older region of the main axis, 15-39 μ in diameter, 12-39 (-40) μ long, in the branches and the monosiphonous filaments distinctly broader than long, in basal region of main axis sometimes longer than broad (Text-Figs. 1-8).

Type: S. R. Kale No. 5 in Herb. Central Salt & Marine Chemicals Research Institute, Bhavnagar, collected in the channel of Saurashtra Salt Works, Porbandar, Gujarat on 29-1-1964.

**DISCUSSION**

The present plant comes near to *Enteromorpha salina* Kuetz. (1856, Tab. Phyc. 6, p. 13, t. 36, no. 1712, fig. 2; as *Enteromorpha polyelodas* Kuetz.; Taylor, 1960, p. 56-57) and also near to *E. corniculata* Kuetz. (I.c., p. 13, t. 37, no. 1713, Fig. 1; De Toni, 1889, p. 136) but it is distinct from both of them. As given in De Toni, *E. salina* has a height of 1-2 cm. only with main axis 20-60 μ broad and cells 8-12 μ in diam. The cell size given by Taylor (1960, p. 57) for var. *polyelodas* Kuetz., is 15-23 μ diam., 1-1.5 diameters long and as seen in Kuetzing's figure, cited above, the cells are longer than broad in the spine-like branchlets also. In the present plant the height of the plant is up to 28 cm. and the cells of the larger branches are broader than long, with diam. of 15-39 μ. Thus the present plant is distinct from *E.
Enteromorpha gujaratensis sp. nov.

18 μ; and the cells are broader than long in both cases. One difference between the present plant and *E. corniculata*, is that the main axis in the present plant is thicker.

**SUMMARY**

At the bottom of the channel, Saurashtra Salt Works, Porbandar, *Enteromorpha gujaratensis* forms a luxuriant growth. This species differs from *E. salina* Kuetz., in having cells which are broader than long and in being a much taller plant. It also differs from *E. corniculata* Kuetz. in having a much thicker main axis.

The author wishes to express her deep sense of gratitude to Dr. D. S. Datar, Director, Central Salt & Marine Chemicals Research Institute, Bhavnagar for his kind encouragement and facilities. Thanks are also expressed to Dr. (Mrs.) F. Thivy for giving the valuable guidance and in the study.

**REFERENCES**


THE GROWTH OF EXCISED PIECES OF THALLUS OF ULVA LACTUCA
VAR. RIGIDA IN LABORATORY CULTURES

SUDHA R. KALE and V. KRISHNAMURTHY

Reprinted from the Proceedings of the Seminar on Sea,
Salt and Plants, pages 234-239
GAMETES AND GERMLINGS OF ULVA FASCIATA DELILE

K. SUBBARAMAIAH, SUDHA R. KALE
and
V. KRISHNAMURTHY

Reprinted from "Current Science", March, 5, 1967,
36, No. 5, 128-129
GAMETES AND GERMLINGS OF *ULVA FASCIATA* DELILE

The green seaweed *Ulva fasciata* grows commonly on the Jalleshwar Reef, Veraval (Saurashtra), on the margins of pools situated in the intertidal region and attains the maximum length of 1—2 m. during October—December. However, full growth is not necessary for swarmer formation as this has been observed frequently in earlier months. The plants shed swarmers during exposure in the mornings in very large numbers and these impart a yellow-green colour to the surrounding sea-water. A fortnightly rhythm in their formation associated with spring tides was also noted. A study of swarmer output and the early growth of germlings was undertaken.

A small plant (16-9 cm. long) with one prominent blade was chosen and six fragments were removed. Each fragment was placed over a slide immersed in sea-water in a petri dish. The fragments, marked serially, were placed under constant temperature (22° C.) and light (800 lux) conditions. Entire fragments taken from the apical portion and in the other fragments, the part corresponding to the margins of the thallus showed change in pigmentation from green to yellow-green an hour prior to the division of the cells. The formation and liberation of the swarmers form the yellow-green part of the thallus took three hours.

The swarmer output in the shedding was estimated by measuring the area of the blade over which swarmer formation took place, and from this the approximate number of reproductive cells determined. The number of swarmers formed per cell was determined by direct microscopic counting. The swarmer output was computed from these data and this numbered 11,634,400 in the plant observed. The swarmer formation took place at fortnightly intervals, and in course of time the entire thallus formed swarmers. The above estimate is, however, for the first shedding only.

The swarmers which were estimated in this instance proved to be gametes. The gametes were ovoid with tapering anterior end which may sometimes be slightly drawn out (Figs. 1, 5, 6, 10), from which two flagella of equal size arose. The gametes were of unequal sizes and measured 6-4-9-6 μ long and 3-2-6-4 μ broad. Gametes of different sizes usually met by their anterior ends and fusion occurred either anteriorly or laterally (Figs. 2-4, 7-9).

The flagella were retained for some time, but were usually shed before fusion of the gametes completed. Although aflagony has been reported in *Ulva Spp.*, the report for *Ulva fasciata* appears to be the first.

The germlings were kept growing attached or in a free-floating condition in the petri dishes in sterile sea-water which was changed once a week. In two months the germlings differentiated into cylindrical plants with 2-3 branches arising from the basal cells. The floating plants were longer 0-75 to 1-75 mm. and produced branches, while the attached ones were shorter 0-75 to 0-83 mm. and unbranched. The growth of germlings did not advance beyond the cylindrical form during these two months as has been observed by other workers.

The authors wish to thank Dr. D. S. Datar, Director, for his kind encouragement.

Central Salt and Marine Chemicals Research Institute, V. Krishnamurthy, Bhavnagar, August 19, 1966.

EFFECT OF IAA ON THE EXCISED PIECES FROM DIFFERENT REGIONS OF ULVA LACTUCA VAR. RIGIDA

SUDHA R. KALE and V. KRISHNAMURTHY

Reprinted from "Review Algology" 1969, 9(3), 273-281
Effect of I.A.A. on the excised pieces from different regions of Ulva lactuca var. rigida

SUDHA KALE & KRISHNAMURTHY V.

In the previous paper (Kale & Krishnamurthy, 1964) it was shown that in Ulva lactuca var. rigida there is a definite pattern of growth. The apical region of the thallus constitutes the region of elongation while the middle, midbasal and marginal regions constitute regions of areal expansion. Whether it is due to some bound growth substance already present in the plant or due to some other factor is not clearly known. So it was thought worthwhile to see the effect of external application of growth substance on the different regions of the thallus.

With this in mind, the present study was undertaken to determine the effect of a definite concentration of indole-acetic acid on the rate of growth of different regions of the thallus.

Materials & Methods.

Plants of Ulva lactuca var. rigida, 10-15 cm in length, showing vigorous growth were collected from a tide pool from Gopnath and were brought to the laboratory for further treatment. Strips of thalli 12 mm in length and 6 mm in breadth were cut out of the thallus from different regions. The regions selected were designated (1) basal region, (2) midbasal region, (3) middle region, (4) apical region, (5) marginal region.

The culture medium used was Foyn's Erd-Schreiber seawater and the indole acetic acid was used in a concentration of 5 µg %. The strips were treated with an antibiotic mixture to get rid of bacterial contamination and after treatment overnight, were washed thoroughly with sterile sea water and then transferred to indole-acetic acid solution prepared in sterile seawater and left in this solution for 24 hours, then transferred to sterile petridishes containing 15 ml of Foyn's Erd-Schreiber seawater. The culture vessels were kept under continuous artificial illumination supplied by daylight fluorescent tubes of intensity varying from 300-600 lux and temperature fluctuating from 25-28°C during the experimen-
tal period. At the end of the experiments all the strips were • growing well and no abnormality or reproduction was found. The experiment was carried out in triplicate. Untreated strips were maintained as control.

The growth was measured by recording the changes in area at intervals of 5 days. Initial and final weights were recorded.

Results.

The results are summarized in Table 1 & 2 and graphically represented in Fig. 1 & 2.

In the basal region the treated strips were showing increase both in fresh weight as well as area over the control. The increase in fresh weight was greatest in this region than in all the other regions.

In the midbasal region the IAA treatment retards the growth both in fresh weight and area. From the graph it is clear that the treated strips show an increase in area over control for the first 15 days after which there is a decrease.

In the middle region the treated strips show much faster growth, in fresh weight as well as in area when compared with the control.

In the apical region, the treated strips show an increase in growth, both in area and fresh weight as compared with the control. However, the percentage increase in growth over the control is less than that of the middle region.

In the marginal region the treated strips show a sudden increase in area after 15 days but in fresh weight there is a fall as compared to the control.

Discussion.

The effects of exogenous application of different concentrations of IAA on marine algae have been studied by several workers (Prat, 1938; Branon, Melvin & Bartsch, 1939; Algeus, 1946; Williams, 1949; Davidson, 1950; Provasoli, 1958; Conard, Saltman & Effley, 1959; Dycus, 1962). But most of these workers were concerned with planktonic and unicellular algae and hence the effects described by them would not be strictly comparable to those on larger seaweeds such as Ulva. Williams (1949 & 1952) has reported differential responses to applied IAA in Laminaria and Codium decorticatum. While distal parts of the frond in Laminaria show an increased growth in response to low concentration of IAA, the meristematic region between stipe and lamina disintegrates quickly. In Codium, apical segments show greater increase
in growth than in intercalary segments. Davidson (1950), studied the effect of IAA on the fronds of Ascophyllum & Fucus. Both respond well to lower concentrations, 10⁻⁵ to 10⁻⁸ M., unaffected by concentration lower than 10⁻⁶, while the growth retards in concentrations higher than 10⁻⁴. Provasoli (1958) has studied the effect of IAA and other growth substances on Ulva germlings and has shown that indole-acetic acid is effective only in a narrow range of concentration, 1 μg % IAA is barely active, 5 μg % is optimal and 10 μg % induces rapid growth followed by rapid death. Conard et al. (1959), while studying the effect of auxin on growth of Ulothrix, concluded that the optimal growth is obtained at 3 μg/l of IAA in sun light. Other miscellaneous observations on the effect of exogenous auxin on algae have been reviewed by Lang (1965).
Similar results have been obtained by the present authors
(unpublished). As a result of this experience, a low concentration,
5 μg % of IAA was chosen as the dose of treatment in the present
experiment. The object was to stimulate vegetative growth in the
fragments under observation and avoid any swarmer formation.

The results of this experiment show that the exogenous appli­
cation of the hormone stimulates the growth in middle, marginal,
apical and basal regions, growth being maximum in marginal and
middle region. While it retards the growth in midbasal region.
The above phenomena need to be explained in terms of distri­
bution of growth hormones in different parts of the thallus. It has
been shown in an earlier paper (Sudha R. Kale & V. Krishna­
murthy, 1965) that the growth rate of fragments from different
parts of the thallus is not uniform but show a definite pattern of
variation. The apical region of the thallus constitutes the region
of elongation while the middle, midbasal and marginal regions
constitute the regions of areal expansion.

It is to be presumed that such variation in rate of growth should
be associated with the level of growth substances present in the
concerned regions of the thallus. The present experiment is aimed
at ascertaining the effect of increasing the level of growth sub­
stances in these different regions. The effect of exogenous applica­
tion of growth substances depend on their concentration in the
growing tissue. The effect of such application will be to increase
the level of endogenous auxins (Shanmugavelu & Rangaswamy,
1967). An optimal level is required for the maximum stimulation
of growth. Any increase over the optimum may not only have no
effect on further growth but may actually retard the rate of growth
(cf. Audus, 1956, p. 455). If we examine the results of the above
experiment with this principle in view, it will become clear that in
the control, the level in growth substance is already fairly high
in the apical and marginal region though not optimum. Hence an
increase in the level of growth substances by the addition of 5 μg %
of IAA, results in a further increase in rate of growth in these
fragments, though this increase is not very high. In the middle
fragments of the control there is a comparatively less growth than,
in the marginal and apical fragments. In the treated middle frag­
ments the growth is increased greatly whereby indicating that the
level of growth substance in the middle fragment is distinctly
lower than in marginal and apical fragments so that any addition
of IAA increases markedly the growth rate in this region. The
basal fragments show a comparatively small growth rate in the
control which is enhanced slightly by addition of IAA. The mid-
EFFECT OF IAA CONCENTRATION 5 μg% ON DIFFERENT REGIONS OF ULVA LACTUCA VAR. RIGIDA

- TREATED
- CONTROL

B - BASAL
MB - MIDBASEL
M - MIDDLE
MG - MARGINAL
A - APICAL
basal fragment in the control shows nearly as much growth as the middle fragment. But when IAA is added the growth of this fragment is actually retarded. This probably indicates that the level of growth substances in this region is already more than the optimum required for maximum growth. Any addition of IAA, therefore, results in further retardation of growth.

So we can summarise in two points. 1° There is a distinct pattern of distribution of auxin in Ulva lactuca var. rigida. 2° It is probable that an optimum level of growth substance exists for a maximum growth of thallus and any increase over the optimum level will actually retard the growth rate.

The authors take the opportunity to thank Dr. D. S. Datar, Director, C.S.M.C.R.I., Bhavnagar, for his kind encouragement and facilities given.

REFERENCES


Table 1. — Increase in area (sq.mm) of the strips from different regions of the thallus of *Ulva lactuca* var. *rigida* during the experimental period (Mean of 3 sets).

<table>
<thead>
<tr>
<th>Regions</th>
<th>TREATED</th>
<th>CONTROL</th>
<th>% Increase over control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Increase</td>
</tr>
<tr>
<td>Basal</td>
<td>72</td>
<td>182</td>
<td>110</td>
</tr>
<tr>
<td>Midbasal</td>
<td>72</td>
<td>197</td>
<td>125</td>
</tr>
<tr>
<td>Middle</td>
<td>72</td>
<td>383</td>
<td>311</td>
</tr>
<tr>
<td>Marginal</td>
<td>72</td>
<td>549</td>
<td>477</td>
</tr>
<tr>
<td>Apical</td>
<td>72</td>
<td>507</td>
<td>435</td>
</tr>
</tbody>
</table>

Table 2. — Increase in fresh weight (mg) of the strips from different regions of the thallus of *Ulva lactuca* var. *rigida* during the experimental period (Mean of 3 sets).

<table>
<thead>
<tr>
<th>Regions</th>
<th>TREATED</th>
<th>CONTROL</th>
<th>% Increase over control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Increase</td>
</tr>
<tr>
<td>Basal</td>
<td>64.8</td>
<td>77.8</td>
<td>13.0</td>
</tr>
<tr>
<td>Midbasal</td>
<td>63.9</td>
<td>75.8</td>
<td>11.9</td>
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<tr>
<td>Middle</td>
<td>47.9</td>
<td>74.7</td>
<td>26.8</td>
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<tr>
<td>Marginal</td>
<td>43.4</td>
<td>85.5</td>
<td>42.1</td>
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<tr>
<td>Apical</td>
<td>37.2</td>
<td>96.5</td>
<td>59.3</td>
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</table>
Effect of different media on the germlings of *Ulva lactuca* var. *rigida*

Sudha R. Kale and V. Krishnamurthy

Central Salt and Marine Chemicals Research Institute, Bhavnagar

In trying to cultivate species of *Ulva* for protein production two possible methods were available. One method was to grow fragments of the thallus with or without pre-treatment of hormones, the other to start with germlings of the alga raised in the laboratory in a suitable culture medium and later transfer these germlings to field conditions.

The earlier work in this laboratory concerned the growth of fragments in specific culture media. However, it was felt, that cultivation by the use of germlings would be more productive. One problem in such a method of cultivation would be to transfer the germlings from laboratory culture to field condition. This should be done at a convenient stage of development of the germling. Therefore, it is necessary to grow the germlings in the laboratory for sometime before transplantation.

Quick growth of the germling with normal morphology is most essential. Previous attempts at culture of *Ulva* indicate that different culture media have specific effects on germling growth and development and so it was decided to study the effects of different media on the germlings of a species of *Ulva* growing commonly on the Gujarat Coast, viz., *Ulva lactuca* var. *rigida*.

MATERIAL AND METHODS

The following media were used

(1) Plain, filtered autoclaved sea water
(2) Föyn's Erd-Schreiber sea water
(3) Modified version of ASP 6*

The germlings were cultured in sterile pyrex conical flasks containing 15 ml of the culture medium. The flasks were kept in the culture room maintained at a temperature of 24±2°C. Illumination was effected by a white fluorescent tube giving out an intensity of 560-600 lux.

The medium was changed once a week just after taking measurements. Growth was measured as linear extension of the thallus with the help of a microscope. The mean value of 10 measurements was recorded.

Six replicates were maintained in each culture medium. The germlings in one of the flasks in each batch were used for measurement and the flask was then discarded.

The results are presented in Table 1.

*The modification of ASP 6 is in the composition of the vitamin mixture: 1 ml of vitamin mixture contains: Ca-pantothenate, 0.1 mg.; nicotinic acid, 0.1 mg.; pyridoxine 2HCl, 0.04 mg.; orotic acid, 0.26 mg.; B12, 0.05 µg; folic acid, 2.5 µg.
Effect of media on germlings of Ulva lactuca

Table 1. Linear growth measurement of the germlings (mean of 10)

<table>
<thead>
<tr>
<th>Date of observation</th>
<th>Plain sea water (control)</th>
<th>Erd-Schreiber sea water</th>
<th>ASP 6 (modified)</th>
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</thead>
<tbody>
<tr>
<td>31-8-65</td>
<td>530 μ</td>
<td>630 μ</td>
<td>474 μ</td>
</tr>
<tr>
<td>Initial measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-9-65</td>
<td>538 μ</td>
<td>702 μ</td>
<td>534 μ</td>
</tr>
<tr>
<td>14-9-65</td>
<td>731 μ</td>
<td>1029 μ</td>
<td>738 μ</td>
</tr>
<tr>
<td>21-9-65</td>
<td>1012 μ</td>
<td>1400 μ</td>
<td>618 μ</td>
</tr>
<tr>
<td>28-9-65</td>
<td>948 μ</td>
<td>2020 μ</td>
<td>—</td>
</tr>
<tr>
<td>6-10-65</td>
<td>985 μ</td>
<td>3024 μ</td>
<td>—</td>
</tr>
</tbody>
</table>

OBSERVATIONS ON THE CULTURE

Plain sea water (Control):

The germlings were green and healthy in the initial stages. Within 15 days, the germlings showed the characteristic leaf-like growth, though some of the marginal cells developed into hair-like outgrowths (Text-Fig. 1). By the end of a month the germlings faded in colour and bleaching took place in the apical region causing breakdown of the thallus in this region. However, numerous hairy outgrowths were seen from the marginal cells. After three months' time many germlings were dead. Some of the surviving individuals showed a development of rhizcids from the persistent basal portion (Text-Fig. 2).

Erd-Schreiber sea water:

The immediate effect of transfer into the culture solution on some of the germlings was a fading in colour, while the rest were green and healthy. The germlings later responded well to this solution by growing both in width as well as in length. After a fortnight, branching was observed in the thalli. Within one month, the germlings developed into well defined blades with a minute colourless holdfast (Text-Fig. 3). Microscopic observations revealed two regions, (1) a lower half containing a number of isolated dark green cells scattered among closely packed light green round cells and (2) an upper half consisting uniformly of small light green cells (Text-Figs. 4, 5).

After two months, a well developed young plant with a solid cylindrical stalk and a definite attaching disc and an expanded green blade was observed (Text-Fig. 6). Both linear growth as well as growth in width were seen. At a later stage, bleaching was observed in the apical region and this gradually extended downwards. Further growth was arrested and new off-shoots were developed from the basal region just near the holdfast (Text-Fig. 7).

Artificial sea water medium (Modified ASP 6):

When the germlings were transferred to this medium a few germlings were completely bleached while others remained green. After a fortnight the germlings showed unhealthy development in the form of curved filaments, a few showing scanty branching. The growth was generally poor. After a month, bleaching in the apical region was observed but leaving a few scattered green cells. These green cells directly developed into new germlings. After 2-3-celled filamentous stage, longitudinal division took place in these secondary germlings. These gave the appearance
Text-Figs. 1-9. 1-2. Germlings raised in plain sea water. 1. 15 days old, with marginal hair-like outgrowths; 2. Three months old showing rhizoids from basal portion; 3-7. Germlings raised in Red-Schreiber sea water; 3. One month old, with well developed blade and holdfast; 4. Lower portion of blade with isolated dark green cells scattered among closely packed light green cells; 5. Same germling, upper half showing uniformly small light green cells; 6. Two months old, with cylindrical stalk and attaching disc; 7. Basal region of well grown germling with new offshoots; 8-9. Germlings in ASP 6 (modified) showing secondary germlings arising from scattered green cells.
Effect of media on germlings of Ulva lactuca of cluster of branches arising from the primary germlings (Figs. 8, 9). After another month, the growth of these secondary germlings stopped, bleaching except for a few scattered green cells took place and from these green cells, tertiary germlings arose.

DISCUSSION

In artificial sea water the germlings do not grow beyond a certain filamentous stage. They fail to attain normal development, but bleach out, leaving some green scattered cells which directly develop into new germlings without forming spores. So these green cells are arrested sporangia functioning as aplanospores. Again, the same cycle is repeated after sometime. The development of such aplanospores renders the reproductive capacity of germlings rather limited. Continued growth in the artificial sea water medium merely repeats the phenomenon as far as it has been observed. These observations would seem to indicate that the artificial sea water medium so far developed is not adequate to raise thalli of normal morphology in Ulva.

Erd-Schreiber sea water, on the other hand, gives normal morphology and constitutes an improvement over plain sterile sea water as a medium for growth of germlings.

The rate of growth is much higher in Erd-Schreiber sea water than in the other two media, thereby showing that this culture medium has all the factors required for normal thallus development at a quick rate.

Since Erd-Schreiber sea water contains besides the unknown constituents of sea water, enrichments in the form of NaNO₃ and Na₂HPO₄ and certain unknown factors derived from soil extract, it becomes obvious that nutrients for rapid growth as well as factors governing the development of normal morphology are both present in this medium.

The high rate of growth may be attributed to the presence of nutritive elements in the form of nitrates and phosphates. These are also present in the artificial medium. One main difference between Erd-Schreiber sea water and ASP 6 (modified) is that, ASP 6 contains a number of known factors in definite proportions while Erd-Schreiber contains unknown factors in unknown quantities in the soil extract. The known factors in ASP 6 (modified) are found inadequate to develop normal morphology and therefore we should look for some unknown factor. It is present either in the sea water or soil extract, and is essential for normal development of Ulva thallus.

It is probable that this unknown factor is some growth regulating substance which may be present in either sea water or soil or both.

The authors express their gratitude to Dr. D. S. Datar, Director, for kind encouragement and facilities.

REFERENCES

[References cited in the text]
significant results have been seen in linear growth and increase in net fresh weight. These would seem to indicate vigorous cell multiplication along the length of the thallus and a high rate of metabolism. Growth is also significant in the middle region and in the marginal region. One point of interest is that there is greater increase in breadth in strips obtained from the middle and marginal (parallel) regions. In the strips cut perpendicular to the edge along the margin, by contrast, growth in breadth is insignificant. These results indicate that in the middle and marginal regions there is greater increase in breadth than in length of the thallus. In the midbasal region, similarly, there is significant increase in breadth and area. When these are compared with the pattern of growth in the apical region—one can safely conclude that the apical region of the thallus constitutes the region of elongation, while the middle, midbasal and marginal (parallel) regions constitute regions of areal expansions.

Since all the strips showed healthy growth up to the end of the experiments, it is assumed that these strips are capable of growing continuously provided proper conditions are maintained. The absence of any abnormality in these strips indicates that the best medium for growth of vegetative thalli is an enriched sea-water medium rather than an artificial sea water such as used by Provasoli (1958). This conclusion is also in conformity with the observations made by Leving (1946).

References

Fomy, B. 1934 Arch Protistenk., 83: 154-177.

DISCUSSION

Dr M. N. Sarin pointed out that the pattern of growth observed by the authors may be due to the distribution of plant growth hormones and suggested that this can easily be verified by a simple method, viz. treating these pieces with known concentrations of a growth hormone such as indole acetic acid,
### Table 3 — Net Increase in Area (sq mm) of Excised Pieces of *Ulva lactuca* var. *rigida* (30 days after treatment)

<table>
<thead>
<tr>
<th>Sampling sites of the thallus</th>
<th>Basal region</th>
<th>Midbasal region</th>
<th>Middle region</th>
<th>Apexal region</th>
<th>Marginal (parallel)</th>
<th>Marginal (perpendicular)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net increase in area (sq mm)</td>
<td>54.8</td>
<td>105.2</td>
<td>152.6</td>
<td>180.8</td>
<td>180.3</td>
<td>77.8</td>
</tr>
<tr>
<td>Standard deviation derived from mean</td>
<td>±14.7</td>
<td>±27.3</td>
<td>±33.5</td>
<td>±34.2</td>
<td>±33.3</td>
<td>±25.5</td>
</tr>
<tr>
<td>% increase over initial</td>
<td>7.6%</td>
<td>14.1%</td>
<td>21.1%</td>
<td>23.5%</td>
<td>22.6%</td>
<td>10.8%</td>
</tr>
<tr>
<td>C.D. at %</td>
<td>0.5</td>
<td>29-00</td>
<td>at 1%</td>
<td>39-24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4 — Net Increase in Fresh Weight (mg) of Excised Pieces of *Ulva lactuca* var. *rigida* (30 days after treatment)

<table>
<thead>
<tr>
<th>Sampling sites of the thallus</th>
<th>Basal region</th>
<th>Midbasal region</th>
<th>Middle region</th>
<th>Apexal region</th>
<th>Marginal (parallel)</th>
<th>Marginal (perpendicular)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net increase in fresh weight (mg)</td>
<td>5.08</td>
<td>8.36</td>
<td>8.40</td>
<td>20.50</td>
<td>10.88</td>
<td>7.6</td>
</tr>
<tr>
<td>Standard deviation derived from mean</td>
<td>±2.61</td>
<td>±1.66</td>
<td>±2.23</td>
<td>±4.20</td>
<td>±4.88</td>
<td>±1.85</td>
</tr>
<tr>
<td>% increase over initial</td>
<td>52.9%</td>
<td>105.4</td>
<td>142.3</td>
<td>399.6</td>
<td>205.3</td>
<td>131.0</td>
</tr>
<tr>
<td>C.D. at %</td>
<td>0.5</td>
<td>3.36</td>
<td>at 1%</td>
<td>4.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1 — Net Increase in Linear Growth (mm) of Excised Pieces of *Ulua lactuca* var. *rigida*  
(30 days after treatment)

<table>
<thead>
<tr>
<th>Sampling sites of the thallus</th>
<th>Basal region</th>
<th>Midbasal region</th>
<th>Middle region</th>
<th>Apical region</th>
<th>Marginal (parallel)</th>
<th>Marginal (perpendicular)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net increase in linear growth (mm)</td>
<td>4 ± 1.41</td>
<td>5.5 ± 1.71</td>
<td>9.0 ± 1.70</td>
<td>17.0 ± 4.34</td>
<td>± 1.17</td>
<td>± 3.62</td>
</tr>
<tr>
<td>Standard deviation derived from mean</td>
<td>± 0.45</td>
<td>± 0.45</td>
<td>± 1.14</td>
<td>± 0.35</td>
<td>± 0.35</td>
<td>± 0.71</td>
</tr>
<tr>
<td>% increase over initial</td>
<td>33.33</td>
<td>45.83</td>
<td>75.0</td>
<td>141.67</td>
<td>51.67</td>
<td>30.0</td>
</tr>
<tr>
<td>C.D.</td>
<td>at 0.5 %</td>
<td>3.14</td>
<td>at 1 %</td>
<td>4.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 — Net Increase in Breadth (mm) of Excised Pieces of *Ulua lactuca* var. *rigida*  
(30 days after treatment)

<table>
<thead>
<tr>
<th>Sampling sites of the thallus</th>
<th>Basal region</th>
<th>Midbasal region</th>
<th>Middle region</th>
<th>Apical region</th>
<th>Marginal (parallel)</th>
<th>Marginal (perpendicular)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net increase in breadth (mm)</td>
<td>1.8</td>
<td>3.8</td>
<td>5.4</td>
<td>4.4</td>
<td>5.6</td>
<td>2.4</td>
</tr>
<tr>
<td>Standard deviation derived from mean</td>
<td>± 0.45</td>
<td>± 0.45</td>
<td>± 1.14</td>
<td>± 0.35</td>
<td>± 0.35</td>
<td>± 0.71</td>
</tr>
<tr>
<td>% increase over initial</td>
<td>30.0</td>
<td>63.33</td>
<td>90.0</td>
<td>73.33</td>
<td>93.33</td>
<td>40</td>
</tr>
<tr>
<td>C.D.</td>
<td>at 0.5 %</td>
<td>0.942</td>
<td>at 1 %</td>
<td>1.285</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The results have been statistically analysed and summarized below.

The results of the experiment indicate that there is a significant increase in linear growth (Table 1) in the middle and apical regions, the increase in the middle region being 75 per cent over the initial length and in the apical region, 141-7 per cent over the initial length. These values are significant at 1 per cent level. The growth in breadth (Table 2) was more extensive, since this was significant in the midbasal, middle, apical and marginal (parallel) regions. Percentage increase in breadth in different regions is: in midbasal region, 63-33; middle region, 90; apical region, 73-33; marginal (parallel), 93-33.

Significant increase in area is also found in the midbasal, middle, apical and marginal regions (Table 3). The percentage increase in area over initial area is: in midbasal, 146-1; middle, 211-9; apical, 250-7; and marginal (parallel), 222-6. Thus the apical and marginal (parallel) regions are regions of the greatest areal expansion.

The weights of the excised pieces at the beginning of the experiment and at the end of 30 days were recorded and the net increase in weight for each piece was calculated. The results of statistical analysis of net increase in fresh weight of the excised pieces are summarized in Table 4. It is seen from the table that increase in weight is significant in the middle, apical and marginal (parallel) regions. The percentage increase in weight is most marked in the apical region than in the rest of the thallus.

Discussion

The experiments detailed above were conducted using strips from vigorously growing vegetative thalli. These were transferred from its natural habitat to sea water in the laboratory. These strips showed purely vegetative growth throughout and were still growing at the end of the experiments. There was no bleaching or malformation of the strips, thereby showing potentiality of strips of thalli for continued growth provided proper nutrients are available. The cultures were not bacteria-free and the culture medium was not a defined medium, but uniformity of cultural conditions was maintained throughout the experiments.

A consideration of results of these experiments shows that strips from the apical region of thalli show significant growth in length, breadth and surface area and increase in fresh weight. The most
breadth, were cut out of the thallus from different regions. The regions selected have been designated (A) basal region, (B) midbasal region, (C) middle region, (D) apical region, and (E) and (F) marginal regions. One of the marginal strips was cut with the length parallel to the edge of the thallus (E), while the other was cut with its length perpendicular to the edge (Fig. 1).

The strips were accurately weighed and immediately transferred to sterile petri dishes containing 15 ml of Föyn's 'Erdschreiber' medium. The medium was changed every alternate day. The culture vessels were kept under artificial illumination supplied by fluorescent tubes of intensity varying from 300 to 600 lux and under temperature fluctuating from 25°C to 28°C during the experimental period. Six replicates were used for each experiment. At the end of 30 days all the strips were growing well and no abnormality was observed.

The growth was measured by recording the changes in length, breadth, area and fresh weight of the strips after 30 days in culture.

Fig. 1 — Diagram of Ulva lactuca var. rigida showing regions from which experimental pieces were obtained.
THE GROWTH OF EXCISED PIECES OF THALLUS OF *ULVA LACTUCA* VAR. *RIGIDA* IN LABORATORY CULTURES

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Although in recent years vast strides have been made in the culture of marine phytoplankton organisms, the culture in artificial media, of larger seaweeds, is yet to be successfully achieved. One of the seaweeds attempted to be brought into culture is *Ulva*.

Levring (1946) successfully germinated the zygotes of *Ulva lactuca* and zoospores of *Ulva lima* in artificial sea-water media containing Na\(^+\), K\(^+\), Mg\(^+\), Ca\(^+\), Cl\(^-\), SO\(_4\)\(^-\), NO\(_3\)\(^-\) and PO\(_4\)\(^-\) and trace elements. Provasoli (1958) tried to grow excised pieces from the median part of the thallus of *Ulva lactuca*, both in artificial sea-water media and Föyn's 'Erdschreiber' solution. He found that the excised pieces are sooner or later bleached. On examination the bleached pieces showed patches of green cells scattered over their area. When these were transferred to fresh media, the green cells developed directly into germlings and further into normal plants with the addition of adenin and kinetin. He concluded that these resistant cells in the foliaceous part of the thallus may be important ecologically in multiplication and spreading of the thallus. Bernatowicz (1961) suggested that a careful mapping of reproductive and vegetative power of different parts of the thallus in *Ulva* should prove extremely interesting.

Some of the early experiments in this institute showed that the excised pieces of *Ulva* can be grown easily in enriched sea water. It was thought worth while to investigate the pattern of distribution of actively growing regions in the thallus of *Ulva lactuca* var. *rigida*, so that the pieces from these regions can be used in an attempt at large-scale vegetative multiplication of the alga. The present paper summarizes the results of such an investigation.

Plants of *Ulva lactuca* var. *rigida*, 10-12 cm in length and showing vigorous vegetative growth, were gathered from a tide-pool from Gopnath and these were brought to the laboratory for further treatment. Strips of the thallus, 12 mm in length and 6 mm in