Helgoländer wiss. Meeresunters. 20, 216–228 (1970)

Experimental culture of *Alaria* in a sub-arctic, free-flowing sea water system*

G. R. SOUTH

Department of Biology and Marine Sciences Research Laboratory, Memorial University of Newfoundland; St. John's, Newfoundland, Canada

KURZFASSUNG: **Experimentelle Kultur von *Alaria* in einem sub-arktischen, freifließenden Meerwassersystem.** Zur Zeit kennt man in Neufundland drei Arten der Braunalgen-Gattung *Alaria: A. esculenta* (L.) GREV., *A. grandifolia* J. AG. und *A. pylaii* (BORY) J. AG. Da neuere Untersuchungen jedoch gezeigt haben, daß viele der neufundländischen *Alaria*-Exemplare nicht mit Sicherheit zu identifizieren sind, muß die Artbestimmung experimentell erfolgen. Es wird eine Tankkultur von Sporophyten der *Alaria esculenta* in fließendem und filtriertem Meerwasser bei künstlicher Beleuchtung beschrieben. Die Algen, die als Zoosporen in die Kulturbecken eingebracht wurden, wuchsen innerhalb von 6 Monaten zu Sporophyten von einer Länge bis zu ca. 30 cm heran; sie blieben jedoch steril und bildeten keine Sporophylle. Für weitere experimentelle-taxonomische Untersuchungen ist es notwendig, Sporophyten zu kultivieren, welche die für die Artbestimmung wichtigen Sporophylle tragen. Möglicherweise ist das Licht ein wichtiger Faktor bei der Induktion und/oder Differenzierung der Sporophyllentwicklung.

INTRODUCTION

According to information given in a recent study of variation in the genus *Alaria* by WIDDOWSON (1964), three species are represented in Newfoundland, namely *A. esculenta* (L.) GREV., *A. grandifolia* J. AG. and *A. pylaii* (BORY) J. AG. During recent studies in Newfoundland by the author, however, it has proved impossible in many instances to satisfactorily identify examples of *Alaria* collected at various times of the year in a wide selection of localities. As a consequence, the need for further investigation into variation and speciation within the genus, particularly from a local standpoint, has been realised. An experimental approach is required, essentially one where plants can be grown to maturity in the laboratory. The aim of the present investigation was, therefore, to study the possibility of culturing *Alaria*, in this instance *A. esculenta*, in the laboratory using a free-flowing sea water system.

The development of *Alaria* ist best known from the works of SAUVAGEAU (1918), YENDO (1919), PRINTZ (1922), and SUNDENE (1962b). Studies by the last two authors were based principally, or in part, on laboratory culture observations. For the most

* Studies in Biology from Memorial University of Newfoundland, No. 165; Contribution No. 36 from the Marine Sciences Research Laboratory. Supported by N.R.C. grant A-4648.

part, however, cultivation studies of *Alaria* have been conducted in the laboratory in small vessels containing enriched sea water or Erdschreiber medium; under these conditions sporophytes have generally reached little more than a few mm in length. SUNDENE (1962b) used such laboratory cultures as a basis for experimental work from which he drew important conclusions concerning the growth and distribution of *A. esculenta*. SUNDENE (1962a, b, 1963, 1964) utilized, however, one method of surmounting some of the obvious difficulties of experimentation in artificial culture media by transplanting both natural and cultured populations of various members of the Laminariales and studying them under different field conditions. Others have attempted to overcome the difficulties of artificial culture media by utilizing continuously flowing or re-circulating natural sea water systems. Under these conditions some factors, notably light, can be manipulated with ease experimentally in the laboratory. Cultivation of *Macrocystis* (NEUSHUL & HAXO 1963, NEUSHUL 1963, ANDERSON 1965), *Laminaria* and *Undaria* (MACFARLANE 1968) has been carried out in this way; as far as is known to the author, no attempt has yet been made to apply these techniques to cultures of any North Atlantic representative of *Alaria*.

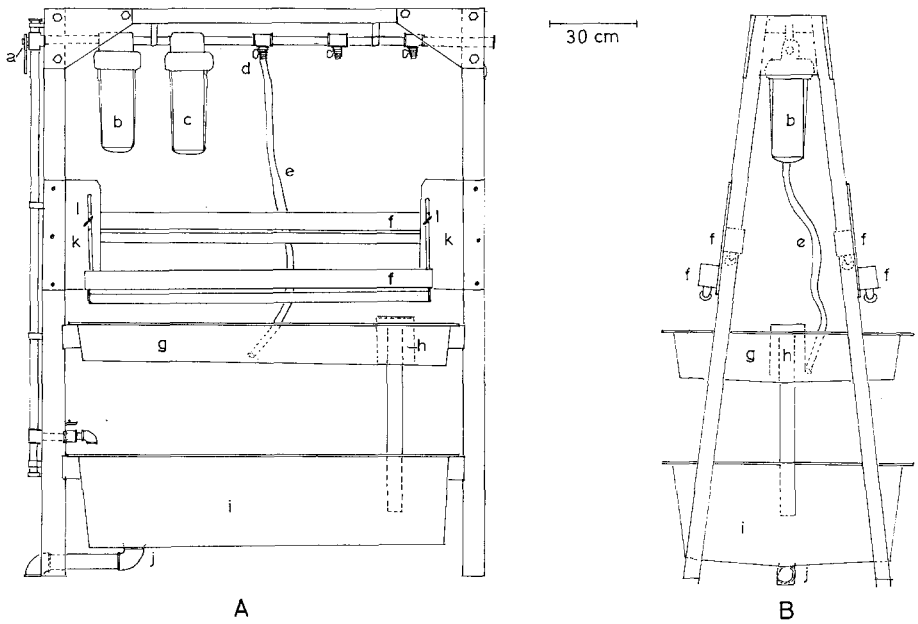


Fig. 1: Side view (A) and end view (B) of main culture tank for cultivation of *Alaria esculenta*. For detailed explanation consult text

MATERIALS AND METHODS

Culture work was conducted at the Marine Sciences Research Laboratory, Logy Bay, Newfoundland, Canada (47° 37′ N, 52° 39′ W). Situated on the East coast of

the Avalon Peninsula, the waters of Logy Bay are unpolluted and under the direct influence of the cold waters of the Labrador Current.

The laboratory is provided with a continuous supply of fresh sea water conducted throughout by PVC piping. Water enters the system from a man-made cave 30 feet below low tide level, is pumped up into a storage tank and through supply pipes into the laboratory building.

Culture apparatus

The main apparatus (fourth level of the laboratory building) consisted of a specially modified water table enclosed in a light-tight room (Fig. 1); this apparatus is referred to here as the main culture tank. Side and end views of the main culture tank are shown in Figure 1. Plants were grown in the upper tank *(g)*. Water enters the apparatus via a faucet *(a)* and passes through filters *(b, c)* designed to filter out particles down to 15 μ and 5 μ respectively (Commercial Filters Corporation Honeycomb filter tubes, types W17 R10-AV and W19X 10-AV in H15-10 Hypur filter holders). Filters were replaced when necessary, usually at 3 to 10 day intervals, depending on the particle load. Debris and larger organisms were successfully removed from the culture area in this way. Water enters the culture tank through a faucet *(d)* and a plastic tube *(e)*, at a flow rate adjusted to 1½ l/min, although fluctuations in flow rate were at times unavoidable owing to changes in water pressure. Water overflows from the tank through a nylon-filtered plug *(h)*, into a lower tank *(i)* and then out through a further plug *(j)*. Four fluorescent tubes (*f*; Westinghouse 40 Watt daylight tubes) were attached to plates *(k)* on a wooden groove, fixed by nuts *(l)*. The tubes gave out a total intensity of 2,500 lux at the culture level, with a spectral composition as shown in Figure 2. A 12 h light/12 h dark sequence was used, with the lights wired in series to a single time clock.

Some additional culture observations were made on plants kept on microscope slides on a sea water table in a glass domed room on top of the main laboratory building. This room is referred to here as the phycorium. Cultures in the phycorium were under natural conditions of daylength and daylight and were supplied with flowing, unfiltered sea water.

Records of temperatures were kept regularly throughout the culture period.

Establishment of cultures

Fertile plants of *Alaria esculenta* were collected from Ferryland, Newfoundland, Canada (47° 01′ N, 52° 52′ W) on December 4, 1968 and transferred to a refrigerator. Each plant was stored in a separate plastic bag and a single individual selected for culture work. Ripe sporophylls were removed from the parent plant, thoroughly washed in sterile sea water, wiped with tissue and stored overnight in a plastic bag in a refrigerator. The following day sporophylls were placed over cleaned microscope slides in a shallow tray of filtered sea water maintained at 5° C, where

zoospores were allowed to settle. After 24 hs a heavy spore release had been obtained and the slides were transferred to the main culture tank and the phycorium.

Development of gametophytes and young sporophytes was followed from observations of the microscope slides; later stages of development of sporophytes were followed from free-floating plants maintained in the main culture tank. Ten sporophytes were tagged and their growth recorded at regular intervals.

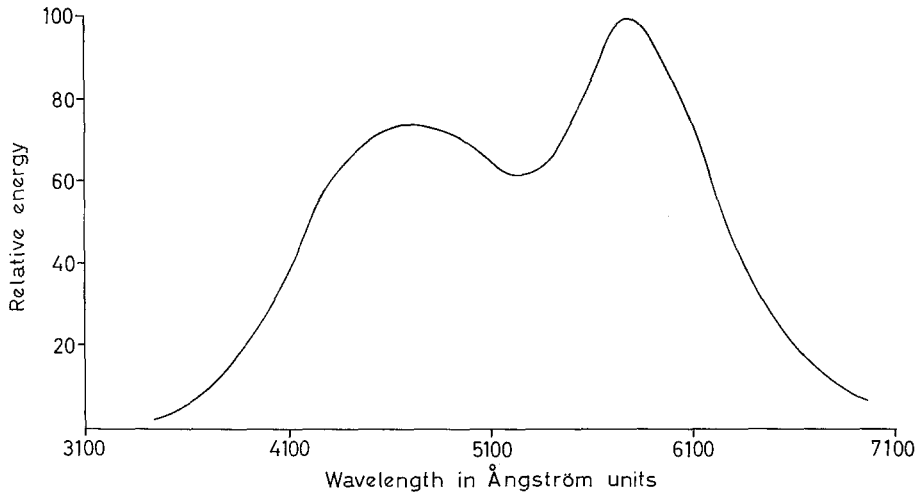


Fig. 2: Spectral distribution curve, 40 Watt daylight fluorescent lamp. (Reproduced from data supplied by Westinghouse Electric Corporation)

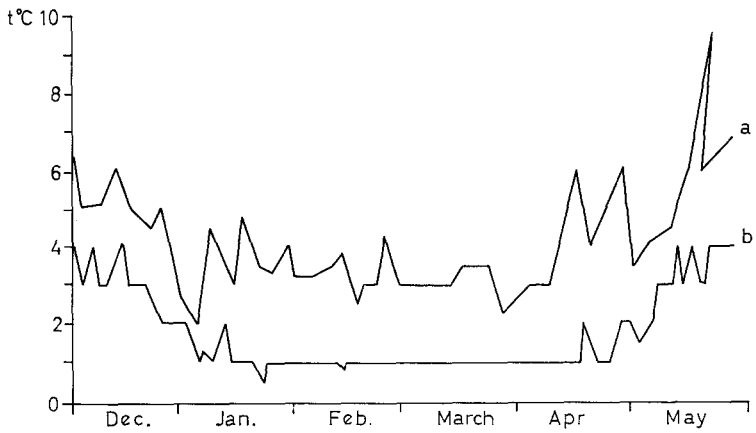
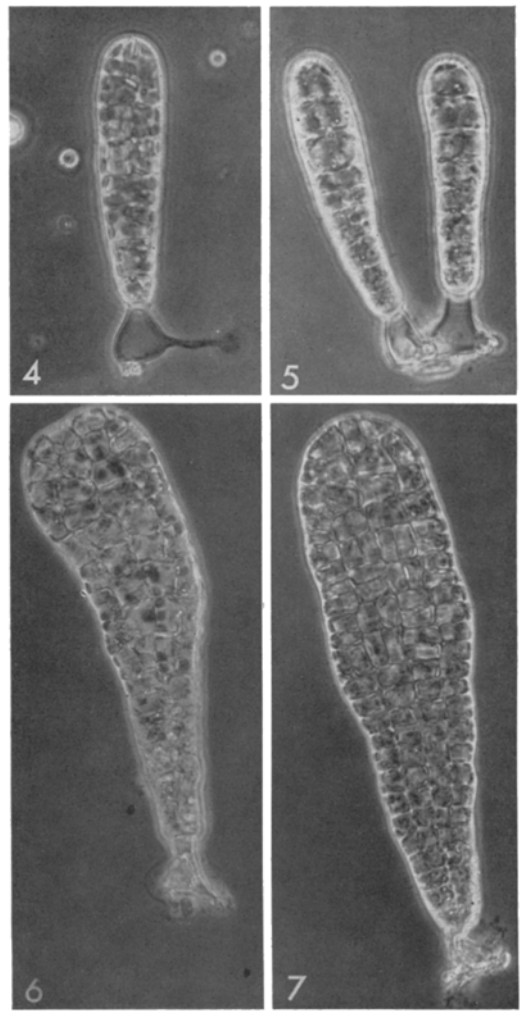


Fig. 3: Temperature records from the main culture tank *(a)* and the first level of the laboratory building *(b)*, Marine Sciences Research Laboratory, Logy Bay, Newfoundland. December 1968 to May 1969

RESULTS

Records of temperatures are shown in Figure 3; temperatures in the main culture tank were generally 1° to 2° C higher than temperatures on the first level of the laboratory building. Records of actual sea temperatures in Logy Bay have shown that



Figs 4–7: Young sporophytes of *Alaria esculenta* from the main culture tank, January 2nd, 1969, (27 days from initiation of culture). Phase contrast. Fig. 4 single sporophyte arising from a single oogonium on a female gametophyte (X 1480). Figs 5–7 progressively larger sporophytes, remaining attached to the empty oogonia (925:1)

the levels recorded on the first laboratory floor are generally 1° to 2° C higher than those in the ocean. The temperatures in the main culture tank are, therefore, between 2° to 4° C higher than the actual sea temperature at any one time. The temperature

rise occurs during transport of the water from the reservoir to the actual experimental area and the degree of the rise depends on the actual amount of water being used in the whole laboratory building.

Germination

Zoospore germination occurred immediately in both the main culture tank and the phycorium; within ten days gametophytes were fertile under both sets of conditions. Male and female gametophytes consisted of only one to a few cells; phycorium gametophytes of both sexes were noticeably smaller than those in the main culture tank, and most female gametophytes consisted of a single cell which produced a single oogonium (Fig. 9). By December 22 (16 days from initiation of cultures) sporophytes were present in both sets of conditions.

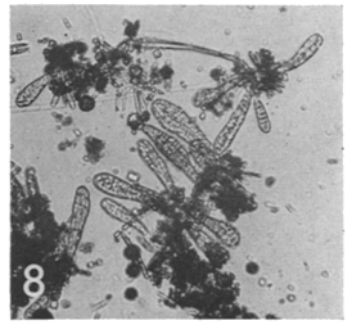


Fig. 8: Young sporophytes of *Alaria esculenta* from the phycorium, January 2nd, 1969, (27 days from initiation of culture). Considerable contamination from debris and diatoms is evident (252:1)

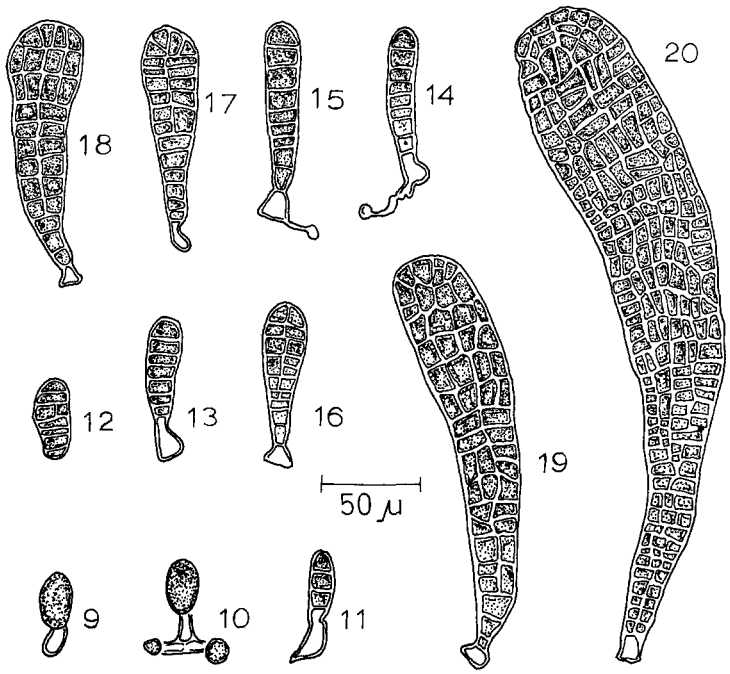
Sporophyte development

On January 2 (27 days from initiation of cultures) sporophytes in the main culture tank were clearly visible to the naked eye. Microscopic examination revealed a whole range of developmental stages, from oogonia to young sporophytes (Figs 4–7). In Figure 4 a female gametophyte consisting of an empty zoospore, germination tube, single oogonium and single sporophyte is shown. Figures 5 to 7 show later stages of early sporophyte development. Plants in the phycorium were less advanced on this date than those in the main culture tank and, furthermore, contamination by diatoms was considerable. Sporophytes in the phycorium are shown in Figure 8. Owing to the considerable contamination, development of phycorium sporophytes was not followed further.

Progressive developmental stages from oogonia to sporophytes growing in the main culture tank are shown in Figures 9 to 20. Mature oogonia are shown in Figures 9 and 10. Young sporophytes generally remained attached to the empty oogo-

nium (Figs 11–13; 15–20) although occasionally became detached (Fig. 12). It should be noted here that developmental stages described under the present culture conditions are comparable with the observations for *Alaria esculenta* made by SAUVAGEAU (1918) and PRINTZ (1922).

Further development of sporophytes continued through stages resulting in the characteristic appearance of *Alaria*. First an undifferentiated, monostromatic blade

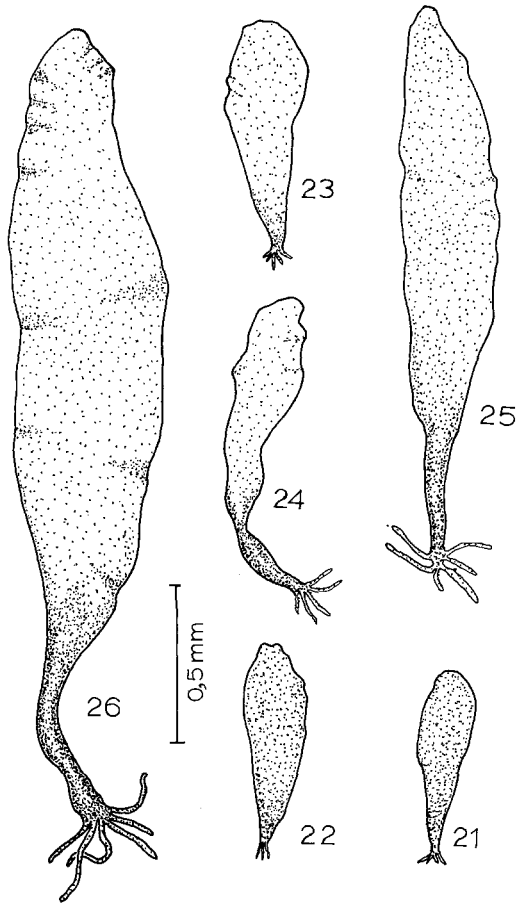


Figs 9–20: Progressive developmental stages of *Alaria esculenta* in the main culture tank, drawn with the aid of a camera lucida on various dates. Figs 9 and 10 gametophytes with eggs extruded from oogonia. Figs 11–20 progressive developmental stages of sporophytes prior to formation of rhizoids. The empty oogonium is evident in Figs 11 and 13 to 20, while a sporophyte detached from the oogonium is shown in Fig. 12. In Figs 14 and 15 the entire gametophyte and young sporophyte can be seen

with basal rhizoids was produced (Figs 21–23). A stipe was developed at the base of the blade (Figs 24–26) and the rhizoids later became compacted together during differentiation of the haptera. Appearance of a stipe, midrib and blade structure quickly followed (Figs 27–29). By January 22nd, 1969 (47 days from initiation of cultures) the majority of the sporophytes in the main culture tanks had reached the stage shown in Figures 27 to 29; the largest plants measured 4.0 mm.

Ten sporophytes were tagged on January 22 and a further five on March 5. Only three survived until May 19, two of these from the first tagging (plants *b* and *c*) and one from the second tagging (plant *a*). The increase in total length and maximum lamina width in plants *a* to *c* is shown in Figures 30 and 31, respectively. In Figure

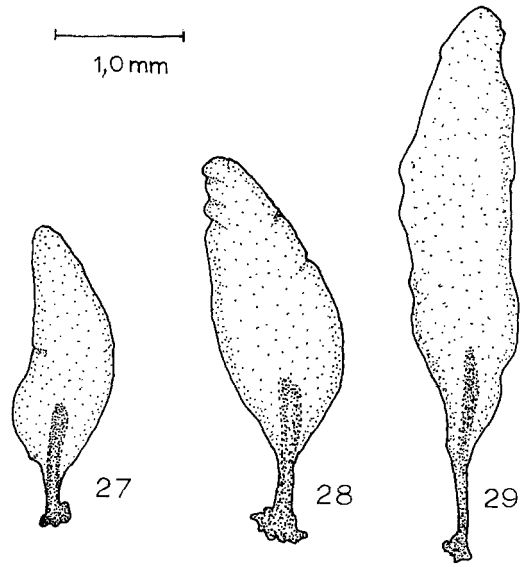
32, plants are shown at the beginning of tagging and again on May 5. Increase in length and width of all three sporophytes was comparable, and plants were proportionately narrow. An examination of untagged plants in the main culture tank on May 5 showed that all had grown with similarly narrow proportions. Terminal



Figs 21–26: Progressive developmental stages of sporophytes of *Alaria esculenta* in the main culture tank. Figs 21–23 sporophytes with rhizoids and no differentiation of the stipe region. Figs 24–26 sporophytes with a developing stipe

shedding of tissue occurred in most plants from May 5, and accounted for the decrease in length of sporophytes *a* and *c* between the dates of May 5 and 19. On May 5, both plants *a* and *c* still retained the terminal, undifferentiated young portion of the blade (Fig. 32).

Cultures were maintained until June 9, at which time sporophytes measured over 30 cm in some instances; plants were six months old. No sporophytes had shown any initiation or development of sporophylls.



Figs 27–29: Sporophytes of *Alaria esculenta* from the main culture tank showing, on various dates, development of the holdfast, stipe, midrib and blade structure

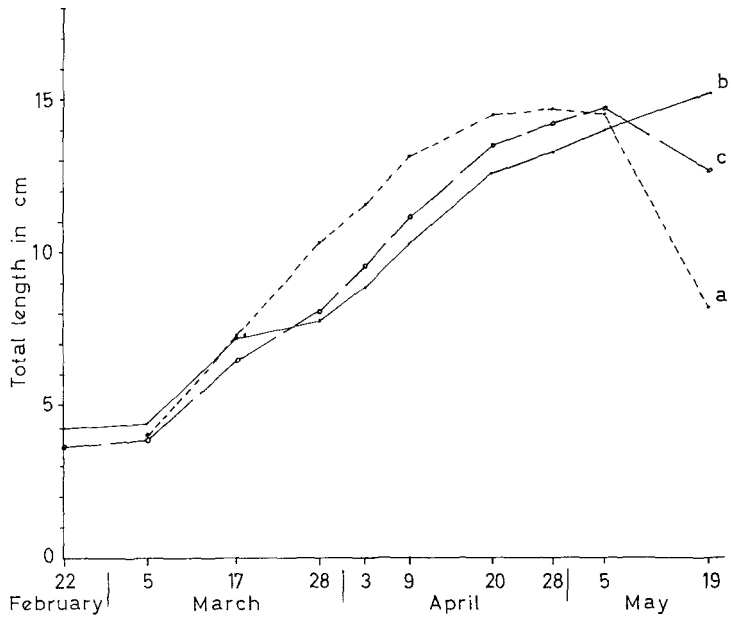


Fig. 30: Length increase of tagged *Alaria esculenta* sporophytes *a*, *b* and *c* in the main culture tank during the period from February 22nd (plants *b* and *c*) or March 5th (plant *a*) until May 19th, 1969

DISCUSSION

The present study has shown that *Alaria esculenta* can be grown in a free-flowing natural sea water system of the type described. Development of the sporophytes progressed far beyond the culture stages reported by earlier workers with the species. In his cultures, SAUVAGEAU (1918, Fig. 82, p. 226) produced plants only to the stage shown in Figures 21 to 23 of the present paper; no differentation beyond the

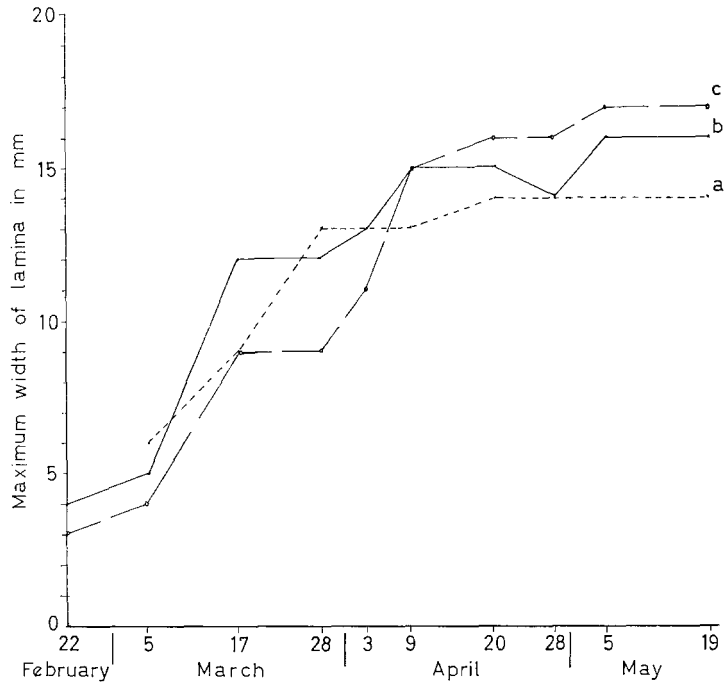


Fig. 31: Increase in maximum width of lamina of tagged *Alaria esculenta* sporophytes *a*, *b* and *c* in the main culture tank. (Dates as in Fig. 30)

simple blade with rhizoids was obtained. The culture sporophytes of PRINTZ (1922, Fig. 13 and 14) developed no further than those of SAUVAGEAU. Neither of these workers, therefore, was able to grow sporophytes in which development of the stipe, haptera, midrib and blade had occurred.

For taxonomic studies of *Alaria* in culture developmental stages of sporophytes even further advanced than those reached by the present cultures would be required in the laboratory. Sporophyll size, shape and arrangement are well known characters of taxonomic significance in the genus (YENDO 1919, WIDDOWSON 1964), yet plants grown here did not at any time produce them. The difficulty of obtaining fertile sporophytes of members of the Laminariales in culture is well known and, as far as the author is aware, the only documented instance is that of *Chorda filum* (L.)

STACKH., where plants, grown entirely in culture by SOUTH & BURROWS (1967), developed sporangia.

It was clear from observations of local populations of *Alaria esculenta* that young sporophytes established on the shore in December to January produced sporophylls by May (SOUTH, unpublished data), a period covered entirely by that of the present culture investigation. Growth and development of naturally occurring plants are, therefore, apparently more rapid than in the culture conditions used here. Both the factor of the slightly higher sea water temperature and the artificial illumination of the culture conditions were considered as possibly influencing the suppression of

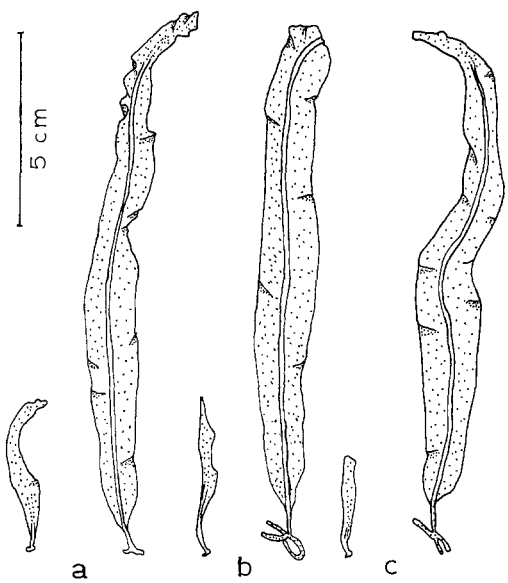


Fig. 32: Tagged *Alaria esculenta* sporophytes *a*, *b* and *c* at the beginning of measurements and as on May 5th, 1969. Undifferentiated terminal portions of the blades still remain on plants *a* and *c* on May 5th

sporophyll initiation in the cultured plants. Small plants transferred from the field to the laboratory on a number of occasions during the experimental period, for example, produced sporophylls in a short period of time; cultivation conditions in the culture tanks thus did not inhibit sporophyll development. This might indicate that sporophyll initiation in *A. esculenta* depends on field-induced factors absent from the artificial illumination and fixed photoperiod conditions in the laboratory, pinpointing light as an obvious factor for further experimentation.

Both male and female gametophytes grown in the present culture conditions were less filamentous than those obtained by previous investigators using enriched sea water or Erdschreiber. The reduced form of the gametophytes in the phycorium could, perhaps, be considered as nearest to the appearance of naturally occurring gametophytes. As far as the author is aware, gametophytes of *Alaria esculenta* have never been

found or described from the field. The gametophytes of *A. crassifolia* described by YENDO (1919) are certainly doubtful since he did not find sporophytes originating from them.

It may be of some significance to note here that sporophytes grown in the relatively still water conditions of the main culture tank bore laminae narrow in proportion to length. YENDO (1919) and SUNDENE (1962b) have both suggested, the latter using supporting experimental evidence, that lamina width in *Alaria* is directly influenced by the degree of wave action, with the narrowest forms occurring in the most exposed conditions. The narrow form of the plants produced here in "sheltered" conditions would appear to contradict such a suggestion, since broad laminae would have been expected. A factor other than or in addition to that of the environment is, therefore, influential in determining lamina breadth in *A. esculenta.*

SUMMARY

1. In Newfoundland (Canada) the Phaeophycean genus *Alaria* presents difficult taxonomic problems, best approached by an experimental culture technique in which plants can be grown to maturity in the laboratory.
2. A method is described for growing *Alaria esculenta* (L.) GREV. in a tank supplied with filtered, free-flowing sea water and artificial illumination.
3. Starting from zoospores, sporophytes up to 30 cm long were obtained in a six months' period of growth. Although completely developed vegetatively, plants did not produce sporophylls and remained sterile.
4. Until fertile sporophytes bearing the taxonomically important sporophylls can be grown in the laboratory, experimental taxonomic work with the genus will be hampered.
5. Evidence did accumulate to indicate that sporophyll initiation, once it occurs in the field, will continue in culture. Light is considered an important factor possibly controlling sporophyll production.

Acknowledgements. The author is grateful to members of staff at the Marine Sciences Research Laboratory for valuable assistance and advice during the course of this work. Mr. R. D. HILL, Mrs. P. C. YORKE and Mr. R. FICKEN contributed considerably with technical assistance. Dr. E. M. BURROWS kindly read the manuscript.

LITERATURE CITED

ANDERSON, E. K., 1965. Culturing gametophytes and sporophytes. *In:* Annual Report, W. M. Keck Laboratory of Environmental Health Engineering, California Institute of Technology, **1964–1965**, 42–48.

MACFARLANE, C. I., 1968. The cultivation of seaweeds in Japan and its possible application in the Atlantic Provinces of Canada. *Proj. Rep. ind. Dev. Serv. Dep. Fish. Can.* **20**, 1–96.

NEUSHUL, M., 1963. Studies on the giant kelp, *Macrocystis.* II. Reproduction. *Am. J. Bot.* **50**, 354–359.

— & HAXO, F. T., 1963. Studies on the giant kelp, *Macrocystis.* I. Growth of young plants. *Am. J. Bot.* **50**, 349–353.

PRINTZ, H., 1922. Über den Generationswechsel bei den Alarien der norwegischen Westküste. *K. norske Vidensk. Selsk. Skr.* **1**, 1–27.

SAUVAGEAU, C., 1918. Recherches sur les Laminaires des côtes de France. *Mém. Acad. Sci. Inst. Fr.* **65**, 1–240.

SOUTH, G. R. & BURROWS, E. M., 1967. Studies on marine algae of the British Isles, 5. *Chorda filum* (L.) STACKH. *Br. phycol. Bull.* **3**, 379–402.

SUNDENE, O., 1962a. Growth in the sea of *Laminaria digitata* sporophytes from culture. *Nytt Mag. Bot.* **9**, 5–24.

— 1962b. The implications of transplant and culture experiments on the growth and distribution of *Alaria esculenta*. *Nytt Mag. Bot.* **9**, 155–174.

— 1963. Reproduction and ecology of *Chorda tomentosa*. *Nytt Mag. Bot.* **10**, 159–167.

— 1964. The ecology of *Laminaria digitata* in Norway in view of transplant experiments. *Nytt Mag. Bot.* **11**, 83–107.

WIDDOWSON, T. B., 1964. A study of variation in the genus *Alaria*, Greville. Ph. D. Thesis. Univ. of Br. Columbia, Vancouver, 229 pp.

YENDO, K., 1919. A monograph of the genus *Alaria*. *J. Coll. Sci. imp. Univ. Tokyo* **43** (1), 145 pp.

Author's address: Dr. G. R. SOUTH
Department of Biology
Memorial University of Newfoundland
St. John's, Newfoundland, Canada