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THE LIFE HISTORY OF *LAMINARIA BRASILIENSIS* (PHAEOPHYTA) IN CULTURE

O CICLO DE VIDA DE *LAMINARIA BRASILIENSIS* (PHAEOPHYTA) NO LABORATÓRIO

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SUMMARY – Fertile sporophytes of *Laminaria brasiliensis* Joly & Oliveira Filho dredged off the coast of Espírito Santo State (Brazil) were put in filtered seawater at 15ºC, 1000 lux and 12:12 photoperiod. Zoospores were liberated, attached to glass slides and developed into male and female gametophytes. Male plants exhibited a ramified filamentous thallus with a few dozen cells, bearing terminal antheridia. Female plants were unicellular with one oogonium each. Fertilized oospheres soon divided to produce a short (3-4 cells) filament, which later divided longitudinaly producing a small monostromatic blade, fixed to the substratum through filamentous rhyzoids; later on the branches became plutistratified and a stipe became conspicuous. The same results were obtained with plants maintained at room temperature, varying between 4-25ºC with maximum illumination of 2,800 lux (diffuse sun-light). When the sporophytes attained the size of 2-3 mm they were transferred to Provasoli ES medium. Bleaching of the thallus followed by marginal decay occured and the plants did not grow longer than 2.5 cm in the conditions employed. German dioxide proved to be deleterious to sporelings at concentrations higher than 0.50 mg/l causing progressive bleaching of the thallus border. The importance of light and temperature is discussed in relation to the distribution of the Brazilian plants.

RESUMO – Porções férteis de esporófitos de *Laminaria brasiliensis* Joly & Oliveira Fº dragadas nas costas do Estado do Espírito Santo foram transportadas para o Laboratório em S. Paulo e mantidas em água do mar filtrada à 15ºC e fotoperíodo de 12:12 (1000 lux, luz fluorescente). Os zoósporos liberados germinaram dando origem a gametófitos masculinos filamentosos e ramificados com número variável de células e gametófitos femininos unicelulares. Após a fecundação das oosferas os zigotos sofreram divisões perpendiculares ao seu maior eixo, formando inicialmente esporófitos filamentosos com 3-4 células que, posteriormente, por divisões longitudinais, formaram talos laminares, monostromáticos. Em estágios posteriores observou-se a formação de estipes e diferenciação de tecidos ficando o talo multiestratificado em sua porção mediana inferior. O mesmo processo de desenvolvimento foi constatado tanto nas plantas mantidas nas condições descritas acima, como em condições ambientais, onde a temperatura variou de 4-25ºC e a intensidade luminosa máxima esteve em torno de 2.800 lux (luz natural difusa). Ao atingirem o tamanho de 2-3 mm de comprimento os jóvens esporófitos foram transferidos para meio de cultura Provasoli (ES) onde cresceram até cerca de 2,5 cm, quando começaram a sofrer um processo de destruição dos pigmentos, seguido de degeneração das porções marginais do talo. Para controlar o desenvolvimento excessivo de diatomáceas utilizou-se diferentes concentrações de GeO_2, o qual mostrou-se prejudicial ao desenvolvimento dos esporófitos jovens em concentrações superiores a 0,50 mg/l. Discute-se a importância da luz e temperatura no controle da distribuição da espécie no litoral brasileiro.

INTRODUCTION

Until recently the genus *Laminaria* Lamour., a well known cold water boreal genus was know in the southern hemisphere by a single occurrence, *L. pallida* J. Ag. from the Atlantic Coast of South Africa and Saint Paul Island in the Indian Ocean (Fritish 1945). Later on Joly and Oliveira Fº (1967) described two other species, *L. brasiliensis* Joly and Oliveira Fº and *L. abyssalis* Joly and Oliveira Fº, dedged off the coast

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of Brazil (Rio de Janeiro State, from 71-75 m deep). The distribution of these species on the Brazilian coast was extended further by Oliveira Fº and Quége (1978). Up to now, however, only the sporophytes of the Brazilian plants were known. During a cruise of the "Prof. Besnard" Oceanographic Ship, of the University of S. Paulo, we had the opportunity to bring fertile sporophytes of *L. brasiliensis* to the laboratory and hence to cultivate them "in vitro".

This paper describeds the morphology and development of the gametophytes and young sporophytes of *L. brasiliensis* in the laboratory.

MATERIAL AND METHODS

Fertile plants of *Laminaria brasiliensis* Joly and Oliveira Fº were dredged at 19º35' N – 039º25'W, off the Espírito Santo coast from 55m deep. Pieces of the blades were kept in running seawater for 3 days during the trip to S. Paulo. In the laboratory pieces (5 x 10 cm) with ripe sori were put on microscopic slides in flasks with filtered seawater (Whatman 1) and kept in an incubator at 15ºC (± 1), under a 12 h light, 12 h dark photoperiod illuminated by 40 w fluorescent tubes (1000 lux).

After liberation of the zoospores and their attachment to the slides the sporophyte lamina were removed and the seawater changed to Provasoli Es medium (Provasoli 1968). Duplicates were kept at room temperature, which varied between 4-25ºC, with a maximum illumination of 2,800 lux from a north-facing window. The photoperiod was about 11 h light, 13 h dark. After the young sporophytes reached a size of 2-3 mm, various concentrations of GeO_2 were tried, to inhibit diatom growht (Lewin 1966).

RESULTS

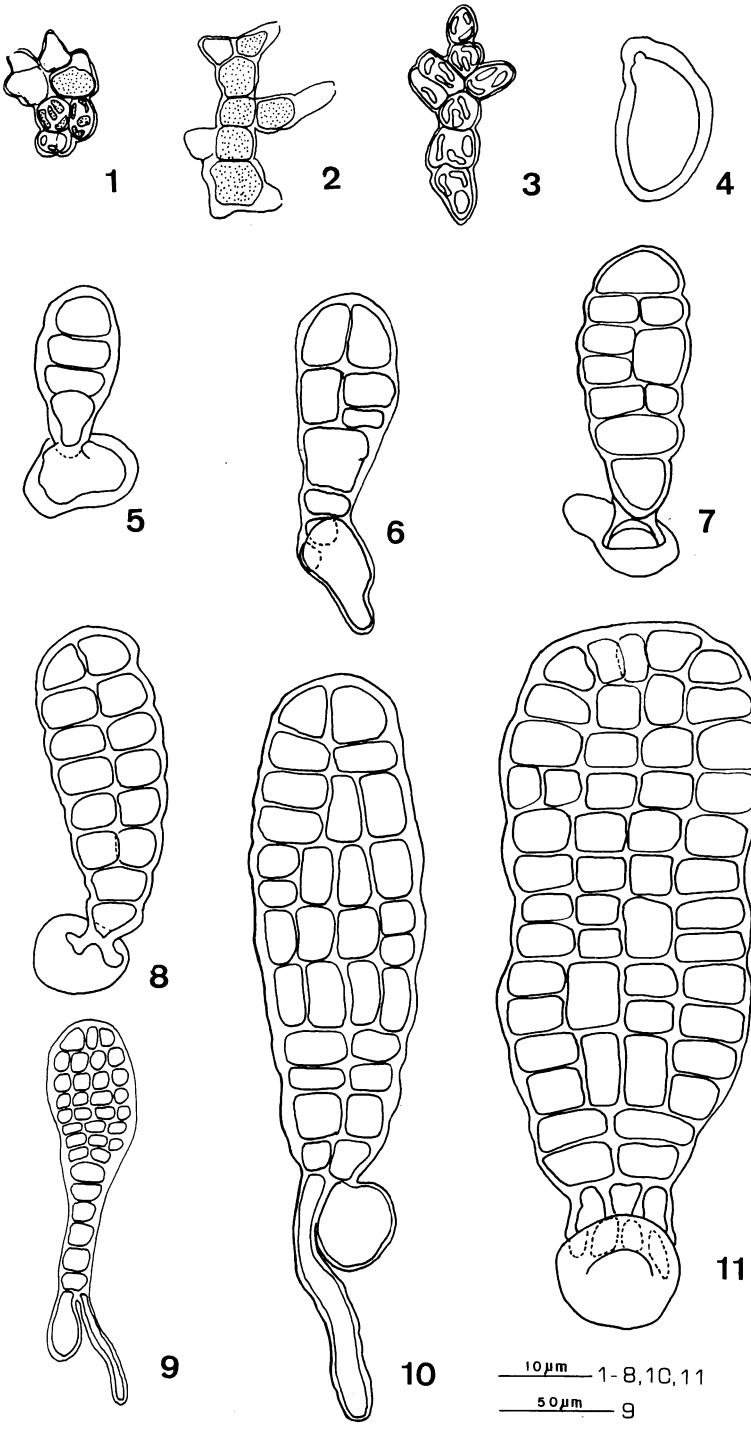
Zoospores liberated from the fertile blade fixed to the substratum, swelled, and produced a tube into which all the cell content passed.

Within 2 weeks a large number of gametophytes were already growing on the slides. Male plants exhibited the form of small branched filaments (Figures 1-3), without a fixed morphology, sometimes more aggregated and massive, bearing one or a few antheridia each at the apex. Liberation of gametes occured through small apical openings (Figures 1-2). Female gametophytes were unicellular (Figure 4).

The sporophytes started as a short single filament up to the stage of 4-6 cells (Figure 5), after which longitudinal divisions took place usually at the subterminal cells, followed by divisions of the terminal one (Figure 6-8). The basal cell remained uniseriate for a long period (Figures 7-9). The young sporophytes attached tremselves to the substratum through the fertilised cells of the gametophytes that appeared empty and with thickened wall (Figures 5-11). These cells remained clearly recognizable until several rhyzoids were produced. The first rhyzoid was produced from the basal cell of the sporeling and the others from its daughter cells (Figure 9-10). The number of thallus cells increased rapidly through divisions of all cells and soon a one-layered lamina was produced (Figure 11). Later on, a stipe was differentiated and the central-basal

Figs. 1-11 – *Laminaria brasiliensis*. 1-3 male gametophytes. 4 female gametophyte. 5-11 sequential development of young sporophytes.

Fig. 1-11 – Laminaria brasiliensis. *1-3 gametófitos masculinos. 4 gametófito feminino. 5-11 seqüência do desenvolvimento de esporófitos.*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11
10µm 1-8,10,11
50µm 9

portion of the sporeling became multi-layered (Figures 12-15). Sporophytes with only 2-3 mm long had already a well differenciated stipe, initially flattened (Figure 17), and in the basal median portion, a multistratified lamina, with a recognizable medulla and cortex (Figure 19). Later on a discoid holdfast developed at the base of the stipe. Muscilage ducts were not found up to this stage of development.

Fertilization and development of sporophytes occured also in the material maintained at room temperature and natural light, being kept in filtered seawater, weekly changed, or in the Provasoli medium as well. However increase in size was much more noticeble in Provasoli than in seawater.

Due to an intense proliferation of *Navicula* sp., german dioxide was added to the cultures in several concentrations to stop diatom growht. Table 1 show that concentrations of GeO_2 higher than 0.5 mg/l were deleterious to young sporophytes at least under the conditions here described. With concentrations of 4-6 mg/l the thallus started to bleach within a week and to disagregate in small cell clumps within 2 weeks. German dioxide at concentrations of 0.25 mg/l was enough to inhibit diatom growth under the conditions of the experiment.

All plants kept in culture reached a maximum size of 2.5 cm beggining to bleach after that, initially at the edge and then in other pontions of the thalli.

DISCUSSION

The morphology of the gametophytes of *L. brasiliensis* obtained in culture is similar to the one known for other species of Laminarilaes (Fritisch 1945, Yabu 1964). The existence of 1-celled female gametophyte was already referred to other species of *Laminaria* (Kaneko 1973). The pattern of sporophyte development from fertilization of oospheres to sporelings up to 2.5 cm long also showed a similar general course to the ones described in the literature for this genus (Fritisch 1945, Yabu 1964, Kaneko 1973).

Control of diatoms through addition of GeO_2 (Lewin 1966) should be kept at its minimum efective concentration since it seems to be deleterious to the sporeling

TABLE 1. Influence of GeO_2 concentration on development of sporelings of *Laminaria brasiliensis* (5 plants, 1-2 mm long). Experiment initiated on Aug. 31, 1978. Culture medium ES Provasoli, 15°C 12/12 photoperiod. Diatoms +, bact. = extensive growth of bacteria.

GeO_2 (mg/l)	Sept. 06	Sept. 15	Oct. 24
0.00	normal growth	normal growth, + +	bleaching, + + +
0.12	normal growth	normal growth, +	bleaching, +
0.25	normal growth	normal growth	bleaching
0.50	normal growth	local bleaching	bleaching
1.00	local bleaching	bleaching, bact.	bleaching, bact.
2.00	less pigmentation	bleaching, bact.	bleaching, twisting
4.00	bleaching	desagretation	
6.00	bleaching	desagregation	

as was also noted for some Fucales (McLachlan *et al.* 1971, for *Fucus;* Fletcher & Fletcher 1975, for *Sargassum*).

It was interesting to see that production of gametophytes and normal development of young sporophytes took place even at temperatures varying from 4-25°C, showing that at least for this process temperature seems not be a limiting factor for explaining the non occurrence of this species in warmer water. However it is important to remark that no experiment was carried out at constant temperature over 15°C.

So, despite the fact that the gametophytes and young sporophytes could stand temperatures as high as 25°C (during the period of the experiment), temperatures dropped as low as 4°C in the laboratory, indicating that perhaps low temperatures could be essential for the development of these plants.

This then would explain why in nature they are restricted to the region bathed by the deep cold current (Oliveira Fº & Quëge 1978). Yabu (1964) concluded from his experiments with *Laminaria japonica* that a certain period of low temperature is necessary for the maturation of the gametophytes, as was already established by other researchers for other Laminariales (cf. Fritisch 1945). Kain (1964) showed that the survival of gametophytes of *Laminaria hyperborea* at different temperatures was identical over range of 5-17°C, but much reduced at 20°C. Yabu (1964) working with gametophytes of *L. japonica* at temperatures of 20, 16, 12, and 8°C found that gametophytes kept at a constant temperature of 20°C showed the poorest growth, while growth was best in the plants kept at 20°C during day time but subjected to a lower temperature during the night. The same result was obtained by Perez (1971) for *Laminaria digitata*; he verified optimum growth at 11-13°C and growth inhibition at 20°C.

The reason why the sporophytes did not grow bigger than 2.5 cm and bleached in our cultures could be due to excessive light or to inadequate culture medium; this should be cleared up in further experiments. Seger and Kida (1958) found that a light intensity of 4800 lux was inhibitory to the sporophyte of another Laminariales, *Undaria undarioides*. Similar results were obtained by Yabu (1964) who verified that gametophytes of *L. japonica* grew well under the medium light intensities of 400-2500 lux but not well under the higher, 4100 lux or lower, 50 lux, light intensities. For L. *hyperborea* Kain (1965) showed that up to an intensity of 3600 lux (fluorescent light) was not inhibitory though saturation was attained at 1000 lux (10°C). However, the Brazilian plants grow at greater depth than *L. hyperborea* and could be more sensitive to light. Thallus bleaching of sporelings of *L. digitata* was verified by Perez (1971) at light intensities higher than 4000 lux.

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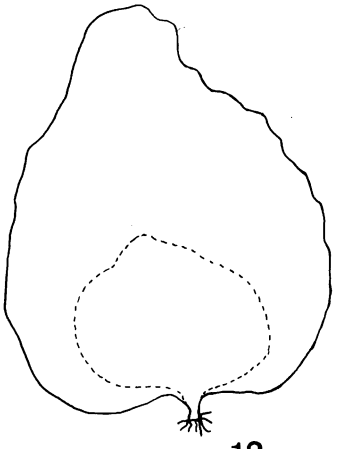
REFERENCES

FLETCHER, R.L. & FLETCHER, S.M. 1975. Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. I Ecoloty and Reproduction. *Botanica Marina* 18: 149-156.

FRITSCH, F.E. 1945. *Structure and reproduction of the algae.* vol. II Cambridge Univ. Press.

JOLY, A.B. & OLIVEIRA Fº, E.C. de 1967. Two Brazilian Laminarias. *Inst. Pesq. Marinha* (Min. Marinha, Rio de Janeiro, Brasil). 4:1-13 + 3 pls. + 1 map.

KAIN, J.M. 1964. Aspects of the biology of *Laminaria hyperborea* III. Survival and growth of the gametophytes. *J. mar. biol. Ass. U. K.* 44: 415-433.



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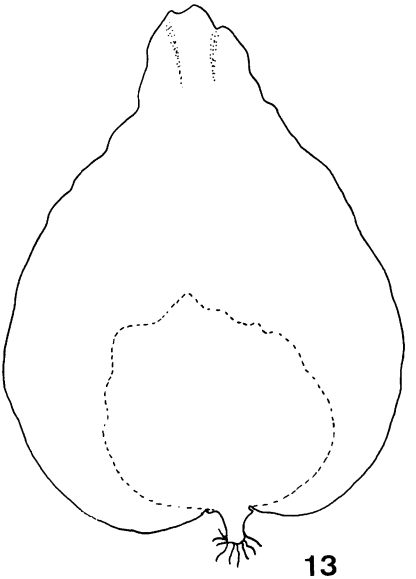

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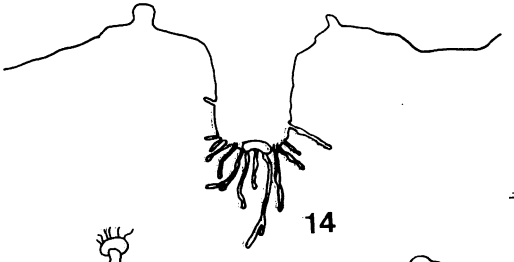

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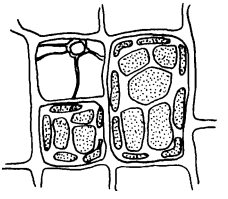

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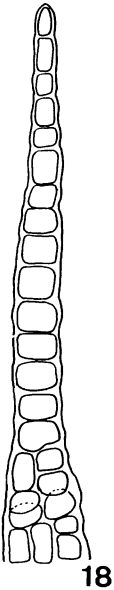

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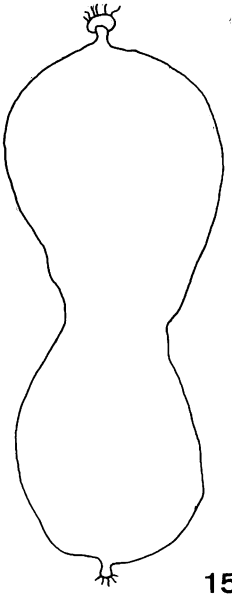

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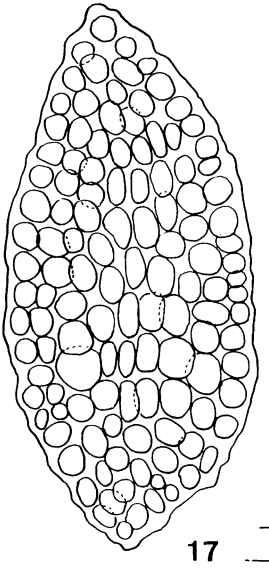

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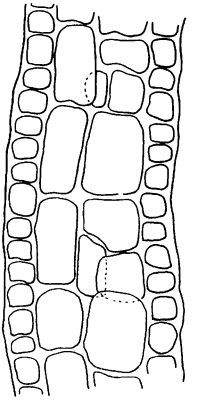

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10 µm 16

1 mm 12,13,15

400 µm 14

50 µm 17 - 19

KAIN, J.M. 1965. Aspects of the bilogy of *Laminaria hyperborea* IV Growth of early sporophytes. *J. mar. biol. Ass. U. K.* 45: 129-143.

KANEKO, T. 1973. Morphology of the female gametophyte and the young sporophyte of *Laminaria japonica* var. *ochotensis* Okamura in nature. *Scient. Rep. Hokkaido Fish. Exper. Stat.* 15: 1-18.

LEWIN, J. 1966. Silicon metabolism in Diatoms. V. German Dioxide, a specific inhibitor of Diatom growth. *Phycologia* 6: 1-12.

McLACHLAN, J. CHEN, C.M. & EDELSTEIN, T. 1971. The culture of four species of *Fucus* under laboratory conditions. *Can. J. Bot.* 49: 1643-1649.

OLIVEIRA Fº, E.C. de & QUÉGE, N. 1978. O gênero *Laminaria* (Phaeophyta) no Brasil. Ocorrência e potencialidade econômica. *Inst. Pesq. Tec. Est. S. Paulo, Pes. & Desenv.* 1: 1-18.

PEREZ, R. 1971. Influence de quelques facteurs physiques sur le développmentt de *Laminaria digitata* (L.) Lamour. Bull. *Soc. Phycol. France* 16: 89-105.

PROVASOLI, L. 1968. Media and prospects for the cultivation of marine algae. In Watanabe, A. & Hattori, A. (eds.) *Cultures and Collections of Algae.* Proc. U.S. Japan Conf. Hakone. Jap. Soc. Plant Phys. 63: 75.

SEGI, T. & KIDA, W. 1958. Studies on the development of *Undaria undarioides* (Yendo) Okamura. II. On the development of the sporophytes and influence of light on it. *Rep. Fac. Fish. Pref. Univ. Mie.* 3: 236-246.

YABU, H. 1964. Early development of several species of Laminariales in Hokkaido. *Mem. Fac. Fish. Hokkaido. Univ.* 12: 1-72.

Figs. 12-19 – *Laminaria brasiliensis*. 12-13 Young sporophytes. 14 detail of the basal portion of a sporophyte showing the stipe and rhyzoids. 15 abnormal sporophyte with 2 stipes. 16 detail of thc sporophyte cells, showing plastides and nucleus. 17 cross-section of the stipe of a young sporophyte. 18-19 cross-section of the blade of a young sporophyte.

Fig. 12-19 – Laminaria brasiliensis. *12-13 Esporófitos jovens. 14 detalhe da porção basal de um esporófito mostrando o estipe e rizóides. 15 Esporófito anormal com dois estipes. 16 detalhe das células do esporófito mostrando os plastos e núcleo. 17 corte transversal ao estipe de um esporófito jovem. 18-19 corte transversal à lâmina de um esporófito jovem.*