

Light and temperature requirements for growth and reproduction in gametophytes of *Ecklonia maxima* (Alariaceae: Laminariales)

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Abstract

The morphological and reproductive development of gametophytes of *Ecklonia maxima* (Osbeck) Papenf. has been studied *in vitro* under a wide range of light and temperature conditions, and the results related to the natural environment over the range of the distribution of the species. Initial vegetative growth was light saturated at $20 \mu\text{E m}^{-2} \text{s}^{-1}$, and maximal at 17.5° and 20°C . Most rapid egg production corresponded to low cell number of female gametophytes, and this process was light saturated at $60 \mu\text{E m}^{-2} \text{s}^{-1}$, and optimised at 15° and 17.5°C . Cell number was also low at lower temperatures, with reduced fertility rates. Sub-saturating irradiances and supra-optimal temperatures caused females to become filamentous, producing many more cells, and reducing reproductive rates. The final egg production per female was, however, greater in these “sub-optimal” conditions, and this phenomenon is interpreted as an ecological adaptation which improves survival prospects in conditions prevailing at the fringes of the depth and geographical distribution of the species. The optimal, and maximum (22.5°C) temperatures for reproduction are far higher than those of northern hemisphere Laminarians. *E. maxima* is very much a warm temperate organism, and this could have implications for marine phytogeographical studies on the west coast of southern Africa.

Introduction

Ecklonia maxima is a species of kelp (Alariaceae: Laminariales) endemic to southern Africa where it occurs from the vicinity of Cape Agulhas (Lat. $34^\circ 50'\text{S}$; Long. $20^\circ 0'\text{E}$) to an as yet undetermined point on the coast of South West Africa/Namibia (Simons and Jarman, 1981). The species forms extensive kelp beds in the shallow sublittoral of these regions. The west coast region of southern Africa has an extraordinary set of environmental condi-

tions for seaweed growth, primarily due to the upwelling conditions which prevail during the summer months (Andrews and Hutchings, 1980; Field *et al.*, 1980, 1981). This causes irregularities in the seasonal temperature regime in the nearshore (Diekmann, 1980). The period of the Austral summer (September to April) is marked by large temperature fluctuations which include both the annual minimum (ca 8°C) and the annual maximum (ca 16°C). Winter (May to August) is characterized by relatively stable temperatures in the range of 13° to 16°C . Upwelling also greatly affects submarine light levels during the summer, with recorded irradiances being much less in downwelling than in upwelling conditions (Anderson, 1982).

Limiting factors for growth and reproduction of gametophytes of *Ecklonia maxima* were investigated by Branch (1974), and attempts were made to relate gametophyte tolerances to the natural distribution of the species. These data are difficult to compare with other literature on gametophyte tolerances since environmental factors other than those tested were not optimal, and also because Branch did not investigate the effects of different irradiances. Growth and reproduction rates were much slower than more recent data on other Laminarian species (e.g. Lüning and Neushul, 1978; Lüning, 1980) due to insufficient light and nutrient concentrations. Lüning and Neushul (1978) showed that Laminarian gametophyte temperature tolerances in California vary on a geographical basis, with southern Californian species having higher temperature optima for vegetative growth and reproduction than central Californian species. The west coast upwelling region of southern Africa is currently designated as having a cold temperate biota (Stephenson, 1948; Brown and Jarman, 1978); a major criterion for this designation being the presence of extensive kelp beds in this region, and not on the warm temperate south coast. A detailed comparison of *E. maxima* gametophyte tolerances with those of other kelps would be extremely interesting phytogeographically.

The aims of this investigation are thus threefold:

(1) to study the light and temperature requirements for growth and reproduction of *Ecklonia maxima* in culture, under nutrient conditions allowing direct comparison with published data on other kelp species;

(2) to investigate those factors in greater detail than previous studies in order to ascertain the effects of sub-optimal conditions which may be encountered in nature; and

(3) to compare the tolerances of *E. maxima* gametophytes with those of other kelps, in order to provide evidence towards an understanding of the phytogeographical position of southern African west coast seaweeds.

Material and methods

Fertile sporophylls of *Ecklonia maxima* (Osbeck) Papenf. were collected at Kommetjie (Lat. 34°8'S; Long. 18°19'E) and Bakoven (Lat. 33°58'S; Long. 18°22'E), sites on the Cape Peninsula, Republic of South Africa. Zoospore release was stimulated by first wiping the fertile area clean, and then placing the sporophylls together with a moistened paper towel in closed crystallizing dishes at 10°C in the dark overnight. Zoospores were released on placing the plants in freshly enriched seawater medium (PES; Provasoli, 1968) the following morning. One ml of spore suspension was added to 200-ml crystallizing dishes containing glass coverslips on which the spores could settle, and also PES medium. These dishes were then placed in the experimental conditions. To inhibit diatom contamination, germanium dioxide (1 ml of an aqueous saturated solution per litre of PES) was added to the experimental medium. The medium was renewed weekly thereafter.

The dishes were incubated in controlled temperature rooms at 3°, 5°, 10° and 15°C. Other temperatures were obtained in 10° and 15°C controlled temperature rooms using water baths in which the dishes were immersed to the level of the medium. A temperature range of 3° to 28°C (each $\pm 1^\circ\text{C}$) was thus achieved. Light was provided by cool white fluorescent lamps arranged in banks. A range of irradiances was achieved by positioning the dishes at various distances from the light banks (light was measured in quanta using a LICOR LI-188 integrating quantum meter). All experiments were repeated at least twice, and similar results were obtained.

The effects of the ten experimental temperatures (3°; 5°; 10°; 12.5°; 15°; 17.5°; 20°; 22.5°; 25° and 28°C – all $\pm 1^\circ\text{C}$) and five irradiances (20, 40, 60, 80 and 100 $\mu\text{E m}^{-2} \text{s}^{-1}$ – all $\pm 5 \mu\text{E m}^{-2} \text{s}^{-1}$) were assessed by counting the number of vegetative cells and the number of released eggs of 100 female gametophytes in each treatment (50 in each of two treatment replicates). In addition, the length of 100 female gametophytes was measured at the age of one week for comparison with published data on other Laminarian species. The growth and reproduction of male

gametophytes were not studied in detail, but did not obviously differ from that of the females under all experimental conditions. Degree of gametophyte survival under conditions of complete darkness was followed by placing coverslips with settled spores in 30-ml petri dishes in a light-proof box for periods of up to 60 d, with weekly medium renewal. Dishes were removed at intervals and the gametophytes cultured at 60 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 15°C, and subsequent development was observed.

Results

Light

The vegetative and reproductive responses of female gametophytes to different irradiances are shown in Fig. 1. In the range 60 to 100 $\mu\text{E m}^{-2} \text{s}^{-1}$, similar responses were observed, i.e.:

- initial growth up to an average of ca 3 cells per female gametophyte at Day 10;
- egg production commencing between Days 10 and 14;
- at Day 18 virtually all cells having been released as eggs. (n.b. Counts of eggs in later stages tend to be lower than expected from the number of vegetative cells observed because of the disintegration of empty cells following egg production).

At 40 $\mu\text{E m}^{-2} \text{s}^{-1}$ the pattern was slightly different, i.e.:

- a slightly greater average number of cells was produced (3.78) a little more slowly (peak at 12 d);
- reproduction was also slowed, with a few vegetative cells remaining (0.84 per female) at 18 d.

At 20 $\mu\text{E m}^{-2} \text{s}^{-1}$ these trends were much accentuated, with:

- a much greater maximum number of vegetative cells (average 6.51 per female) again with a later peak (at 16 d);
- delayed egg production with an average of 2.5 cells per female still vegetative at 25 d.

Initial vegetative growth, measured as the average length of females at age one week, was light saturated at 20 $\mu\text{E m}^{-2} \text{s}^{-1}$. The actual saturating irradiance may be lower than this figure, as no intermediate treatments were tested between dark and 20 $\mu\text{E m}^{-2} \text{s}^{-1}$.

Dark

In the dark experiment, germination of spores occurred, but no development beyond the unicellular “dumbbell” stage was observed. These germinated spores survived even the longest dark treatment (60 d), and on return to the light produced large filamentous gametophytes which began to produce eggs 15 to 20 d after exposure to an irradiance of 60 $\mu\text{E m}^{-2} \text{s}^{-1}$.

Temperature

The vegetative and reproductive responses of female gametophytes to different temperatures are shown in

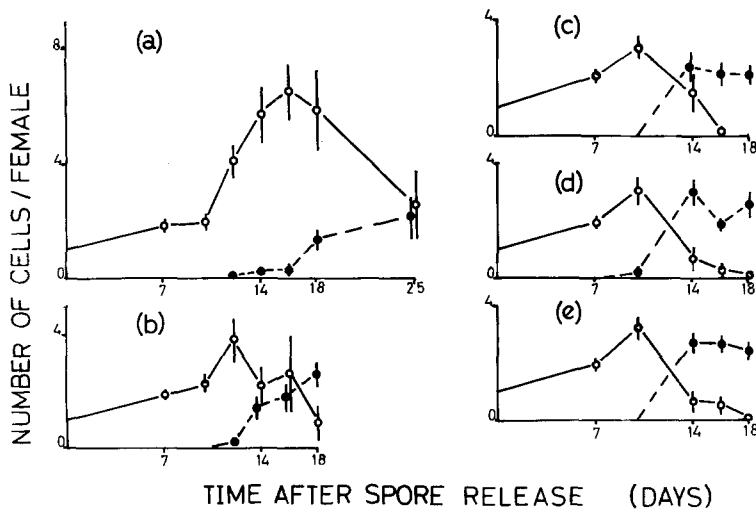


Fig. 1. *Ecklonia maxima*. Vegetative growth and reproduction of female gametophytes at 15°C, and the following irradiances; (a) $20 \mu\text{E m}^{-2} \text{s}^{-1}$, (b) $40 \mu\text{E m}^{-2} \text{s}^{-1}$, (c) $60 \mu\text{E m}^{-2} \text{s}^{-1}$, (d) $80 \mu\text{E m}^{-2} \text{s}^{-1}$, and (e) $100 \mu\text{E m}^{-2} \text{s}^{-1}$. Solid lines: number of vegetative cells; dotted lines: number of eggs. Bars indicate 95% confidence limits

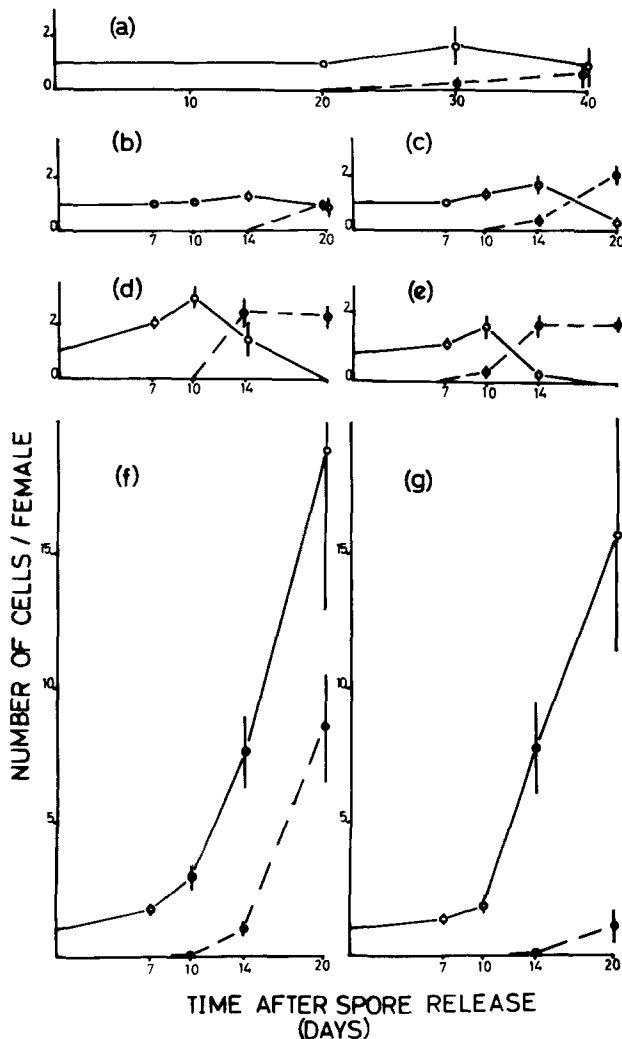


Fig. 2. *Ecklonia maxima*. Vegetative growth and reproduction of female gametophytes at $60 \mu\text{E m}^{-2} \text{s}^{-1}$, and the following temperatures: (a) 5°C, (b) 10°C, (c) 12.5°C, (d) 15°C, (e) 17.5°C, (f) 20°C, (g) 22.5°C. Solid lines: number of vegetative cells; dotted lines: number of eggs. Bars indicate 95% confidence limits

Fig. 2. At 3° and 25 °C, gametophytes survived the experimental period, but did not develop beyond the unicellular stage. At 5° and 10 °C a comparable pattern of development was observed over a progressively shorter time span, with a maximum number of vegetative cells (ca 1.5 per female) at 30 and 14 d respectively; egg release commencing at 30 and 20 d; and about half the cells being released as eggs by 40 and 20 d. The process is further speeded up at 12.5° and 15 °C, with very few vegetative cells remaining after 20 d at 12.5 °C, and all cells being released as eggs at 15 °C after the same period. At these temperatures the maximum number of vegetative cells was also higher (1.75 at 12.5 °C; 3.0 at 15 °C). At 17.5 °C, egg production was most rapid with almost no vegetative cells remaining after 14 d, compared with ca one vegetative cell per female at 15 °C at this stage (n.b. the reduction in the total eggs plus vegetative cells is again due to deterioration of empty cells after egg release, causing them to be omitted in the following count).

An extremely different growth pattern was observed at 20 °C, with rapid vegetative growth and cell division producing an average of 27.5 cells per female (total, eggs plus vegetative) after 20 d. Egg production commenced at 10 d, and at 20 d an average of 8.5 eggs per female had been produced, and a large potential for egg production remained in the form of vegetative cells.

The pattern at 22.5 °C was similar to that at 20 °C, with rapid growth and cell division, although at a slower rate in the former. Egg production also commenced later (14 d), and at 20 d only a small proportion of the vegetative cells had been released as eggs.

Data from the same experiments are condensed, and represented in a different manner in Fig. 3 for comparison in the discussion with similar published accounts. This figure represents initial vegetative growth and reproduction measured as average length of females at one week, and percentage of females reproductive (i.e. with at least one egg) at two weeks after spore release. The optimum

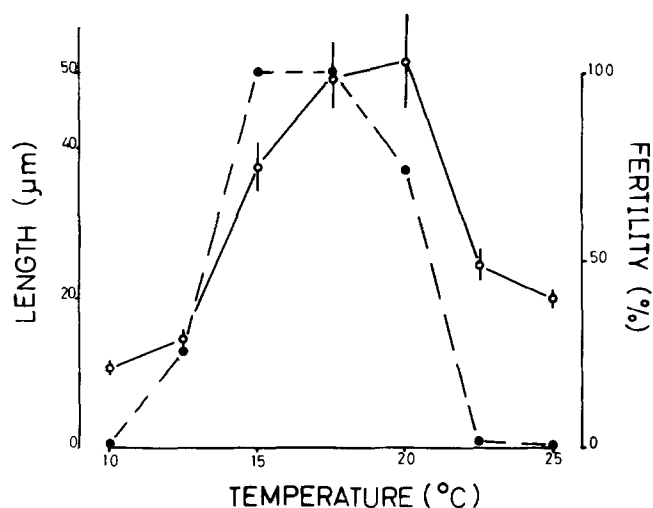


Fig. 3. *Ecklonia maxima*. Solid lines: average length of female gametophytes at 7 d at various temperatures. Dotted lines: percentage of female gametophytes with at least one egg at 14 d at various temperatures. All at $60 \mu\text{E m}^{-2} \text{ s}^{-1}$; bars indicate 95% confidence limits

temperature for initial vegetative growth is higher than that for reproduction measured in this way (viz. $17.5^{\circ}\text{--}20^{\circ}\text{C}$ for growth, $15^{\circ}\text{--}17.5^{\circ}\text{C}$ for reproduction).

Discussion

At 15°C the number of cells produced per female gametophyte and the rate of egg production both reach a maximum at $60 \mu\text{E m}^{-2} \text{ s}^{-1}$ and are not increased by any further increase in irradiance. However, light of less than $60 \mu\text{E m}^{-2} \text{ s}^{-1}$ had two effects, namely that the number of vegetative cells per female increases and the rate of reproduction decreases. It is important to note that the total number of eggs produced for a given number of females in 14 d at $60 \mu\text{E m}^{-2} \text{ s}^{-1}$ is equalled in 18 d at $40 \mu\text{E m}^{-2} \text{ s}^{-1}$ and 25 d at $20 \mu\text{E m}^{-2} \text{ s}^{-1}$. Furthermore, vegetative cells remain in these lower irradiances which, when released as eggs, will result in a higher overall egg production per female at the lower light intensities. Fig. 2 shows that the initial increase in size of female gametophytes is light saturated at a lower irradiance ($20 \mu\text{E m}^{-2} \text{ s}^{-1}$) than later developmental processes, i.e. cell division and egg production ($60 \mu\text{E m}^{-2} \text{ s}^{-1}$). These values are of the same order as those obtained with various Laminarians by Lüning and Neushul (1978). These authors, and also Lüning and Dring (1972, 1975) and Lüning (1980), have demonstrated with other Laminarians that it is the number of light quanta received in the blue range (400–512 nm) which determines the developmental pathway from a filamentous, infertile state (as in the case of three *Laminaria* species; Lüning, 1980) to the suggested optimum reproductive state of a one-celled vegetative female producing one egg. It is evident from the present study that this is not (for *Ecklonia maxima*) an on/off

mechanism depending on the receipt of a threshold level of blue light quanta, but rather that, at irradiances sub-optimal for rapid egg production, the less light the more cells per female, the slower the egg production and the more eggs finally produced per female. Data on natural underwater light conditions in the study area are sparse. Anderson (1982) provided data for a depth of 6 m at the edge of a kelp bed for 2 d, representing extreme conditions in the summer, i.e. a sunny day under active upwelling conditions, and a cloudy day with downwelling. In upwelling conditions underwater light was around 500 to $600 \mu\text{E m}^{-2} \text{ s}^{-1}$ for an 8-h period. In the absence of upwelling, the maximum irradiance at 6 m was $200 \mu\text{E m}^{-2} \text{ s}^{-1}$, and for most of the day was less than $100 \mu\text{E m}^{-2} \text{ s}^{-1}$. Data for mean monthly global radiation on the Cape Peninsula shows a maximum of $800 \text{ deka-watt m}^{-2}$ in December–January, and a minimum of around $200 \text{ deka-watt m}^{-2}$ in June–July (Diekmann, 1980). Fertile *E. maxima* sporophytes can be found all year-round, although any possible seasonality of fertility and spore production remains to be investigated. It is thus evident that zoospores settling in the water towards the lower depth limits of the species distribution will experience irradiances less than the $60 \mu\text{E m}^{-2} \text{ s}^{-1}$ required for the most rapid development. Many-celled female gametophytes will result, which at low-light levels (e.g. $20 \mu\text{E m}^{-2} \text{ s}^{-1}$) will release a number of eggs over a period of weeks. With even lower light, insufficient for growth and reproduction at all (even in the dark in this experiment), the gametophytes will survive for long periods and, when light conditions improve, will again become many-celled, resulting in a large number of eggs being produced per female.

An optimum temperature for rapid egg production with the minimum cell number per gametophyte occurred at 17.5°C . This is higher than the 15.8°C growth and reproductive optimum found under sub-optimal light and nutrient conditions by Branch (1974). Egg release was almost as rapid at 15°C , and number of cells per gametophyte only slightly greater. Sub-optimal temperatures had different effects than low irradiances, with few cells per gametophyte. The main effect of sub-optimal temperatures was thus a progressive slowing of the rate of egg release. Supra-optimal temperatures, however, showed somewhat similar effects to sub-optimal irradiance, with slower egg release coupled with a greatly increased cell number per gametophyte, again resulting in a greater egg production per female than in optimal conditions. This phenomenon has been previously reported for gametophytes of the closely related *Ecklonia radiata* from New Zealand, (Novaczek, 1984).

Novaczek (1984), working with New Zealand *Ecklonia radiata*, demonstrated ecotypic variation in temperature tolerances of gametophytes, with isolates from Goat Island Bay (range of water temperature 10.7° to 23.9°C) reproducing at 24°C , but not surviving at 26°C , whereas plants from Houghton Bay (water temperatures from 10° to 19°C) reproduced at 15°C , but not at 21.5°C . The

reproductive maximum for *E. maxima* (22.5 °C) is higher than that of Houghton Bay *E. radiata*, despite the cooler temperature regime in the Benguela upwelling region. The ecological consequences of these findings are interesting. Under sub-optimal light and supra-optimal temperature conditions, *E. maxima* gametophytes survive and grow filamentously, and produce a greater number of eggs over an extended period. This has obvious benefits in a fluctuating environment, where zoospores will often settle in areas with temporarily unfavourable conditions for sporophyte growth on the fringes of the depth or geographical distribution of the species.

Figure 3 can be compared with Figs. 2 and 3 in Lüning & Neushul (1978), showing the temperature responses for gametophyte growth and fertility in nine Laminarians from southern and central California. Most of these species correspond with the pattern in *Ecklonia maxima*, with vegetative growth having a higher optimum range (17.5–20 °C) than reproduction (15–17.5 °C). The *E. maxima* female gametophytes were much larger than those of any of the Californian species, despite growing under a 16 hL:8 hD regime (Lüning and Neushul used continuous light). There were differences between central and southern California species in temperature optimum for growth (12 °C in the former, 17 °C in the latter) and upper temperature limit for reproduction (17 °C in the former, 20 °C in the latter). *E. maxima* is seen to be tolerant of even higher temperatures than southern California species, with a growth optimum range of 17.5° to 20 °C, and an upper temperature limit for reproduction of 22.5 °C.

Van den Hoek (1982) placed marine algal species into phytogeographical groupings on the basis of temperature regulation of their life histories. In comparison, both *Ecklonia* species thus far tested (*E. maxima*, this study, and *E. radiata*, both populations of Novacek, 1984) would fit into Van den Hoek's warm temperate grouping. The limiting factor in the distribution of *E. maxima* would appear to be the upper temperature limit for growth and/or reproduction of the sporophyte, as maximum temperatures at the distribution limits seldom exceed 22.5 °C (Branch, 1974), at which temperature the gametophytes are capable of growth and reproduction.

The southern African west coast biota has been regarded as cold-temperate since the work of Stephenson (1948; see also Brown and Jarman, 1978). From Stephenson's discussion, it emerges that the temperature range in this region "verges upon the cold-temperate" as compared with other world regions. Stephenson's main reason for describing this region as cold-temperate, however, stresses the presence of "great beds of giant Laminarians so characteristic of colder seas". If, as clearly demonstrated in these results, one of the two major west coast kelps can be described as very much a warm-temperate organism (and probably also the other, *Laminaria pallida*, judging from the results of Branch, 1974), was Stephenson correct in describing the region as cold-temperate? Further studies

on comparative floristics and tolerance experiments with selected species are needed to resolve this question.

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