Constant short-day treatment of outdoor-cultivated *Laminaria digitata* prevents summer drop in growth rate

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Previous laboratory studies in species of the Laminariales have revealed that both the onset of growth in early winter and the summer drop in growth rate are controlled by the annual course of daylength synchronizing endogenous, circannual clocks within the thallus. Moreover, it is known for some laminarian species that cultivation in the laboratory in constant short days (SD) leads to arhythmic, continuous growth activity of the blade throughout the year. Such a prolonged SD treatment has now been performed for the first time in outdoor-cultivated *Laminaria digitata*. Field-grown sporophytes were collected in May from the sea near Helgoland (North Sea) and cultivated on the island of Sylt (North Sea) in temperature-controlled outdoor tanks (300 l) at 10 °C for 1·5 years either in a constant 8 h of light per day, controlled by an automatic blind on top of the tank, or in ambient daylengths. In constant SD, the growth rate remained at a steady-state level of approximately 0·4 cm day^{-1} from the end of June until mid-October, whereas growth rate in ambient daylengths declined steadily after June to half the rate in SD in October. Growth became light-limited between October and February in both treatments and, in the second year from July onwards, higher growth rates were again observed in constant SD than in ambient daylengths. Further work is required to find out whether SD treatment in summer would also prevent the summer drop in growth rate in other perennial seaweed species, e.g. commercially valuable red algae. This would potentially increase the chance of more constant biomass production throughout the year, and decrease the danger of the cultivated perennial algae being overgrown by annual epiphytes in summer.

Key words: cultivation, daylength, growth rhythm, kelp, Laminariales, *Laminaria digitata*, photoperiod, seasonality, short days

Introduction

Species of *Laminaria* exhibit a period of rapid growth from January to June (in the Northern Hemisphere) and a period of slow growth from July to December (Parke, 1948; Kain, 1979). A more detailed look at this seasonal pattern reveals two cardinal time points per year, namely December with the start of new blade growth activity and May/June with the start of growth rate decline. Concerning the start month, Printz (1926) first noted in his detailed study of the algal vegetation of the Trondhjem Fjord that, during the first half of December, the first signs of the new blade became visible at the base as a light-brown, 1–2 cm long zone in both *Laminaria hyperborea* and *L. digitata*. Later it became clear that this happens in December throughout the whole geographical range of *L. hyperborea*, from northern Norway to Portugal (Kain, 1971). Pérez (1969, 1971) also demonstrated the increase in blade growth rate from December onwards in a 3 year study with *L. digitata* from the coast of northern France. Similarly, by excising basal blade discs of *L. digitata* from the coast of Scotland repeatedly throughout the year, Conolly & Drew (1985*b*) noted the sudden increase in cell division rate in December and the re-establishment of a new surface meristodem in the dormant winter tissue.

After the often-observed April/May maximum of blade growth in laminarian species, growth rate starts to decline in May and becomes almost zero in summer in *L. hyperborea* or is strongly reduced in *L. saccharina* and *L. digitata* (Kain, 1979). The physiological factors causing the 'summer drop' in the growth rate of these species were first sought in obvious features of primary environmental conditions such as high summer temperatures (e.g. Sundene, 1962, 1964) or low nutrients (e.g. Conolly & Drew, 1985*a*). Later it was found that experimental sporophytes of *Pterygophora californica*, *Laminaria setchellii* and *L. hyperborea* cultivated in the laboratory in a constant daylength (⩾ 12 h light per day) were able to produce autonomously the seasonal sequence of growth starts and stops as a circannual rhythm (Lüning, 1991, 1993; tom Dieck,

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1991; Schaffelke & Lüning, 1994). The period of this free-running circannual growth rhythm was found to be shorter than a year and was synchronized to the 12 month period of the natural year by the annual course of daylength, which acted as the main *zeitgeber*, as in many animal species (Gwinner, 1986). In all three laminarian species, it was also found that cultivation in constant short days (SD) in the laboratory resulted in continuous, arhythmic growth of the blade throughout the year (Lüning, 1991, 1993; tom Dieck, 1991; Schaffelke & Lüning, 1994). A similar result with another circannual system had been obtained in *Sturnus vulgaris*, the European starling, which exhibited continuously high reproductive activity in constant SD, and this is thought to indicate that a strong *zeitgeber* signal such as constant SD arrests the circannual clock (Gwinner, 1986).

Achieving continuous growth throughout the year by constant SD treatment would offer two major benefits for the aquaculture of perennial macroalgae. Firstly, high biomass production could be obtained throughout the year and, secondly, contamination with epiphytes would be reduced because actively growing macroalgal thalli are not as easily overgrown as those which are dormant through the summer. The objective of the present investigation was to find out whether the high level of blade growth activity typical of spring in *Laminaria digitata* could be maintained throughout the summer in an outdoor cultivation system by manipulating the daylength.

Materials and methods

Growth experiment in outdoor tanks

Adult sporophytes of *Laminaria digitata* (Huds.) Lamour were collected on 5 May 1999 by SCUBA diving at 1 m below mean low water of spring tides in the sublittoral zone of Helgoland (North Sea). The experimental algae selected had stipe or blade lengths of 30–50 cm or 50–80 cm, respectively, and were presumed to be in their second year of growth, as judged from their size (Pérez, 1969; Lüning, 1979). The algae were placed in a plastic container filled with 30 l of seawater, transported by ship to the island of Sylt (North Sea) and subsequently cultivated in an outdoor cultivation system (Fig. 1*A*).

The cultivation system consisted of 300 l tanks filled with filtered seawater which, for constant SD treatment, were fitted with an automatic blind (adapted from commercial window blinds; Isitec, Bremerhaven, Germany), which was opened and closed in response to a timer-switch. Each alga was fixed to a numbered PVC plate by silicone tubing that was wound around the haptera, and the PVC plates were mounted on a PVC ground plate in the bottom of the tank (Fig. 1*B*). Ten individual plants were kept either at ambient daylengths or at constant 8 h light per day (SD; light from 0800 to 1600 hours). SD treatment started on 24 May 1999, 18 days after the transfer of the experimental algae into the tank system. In

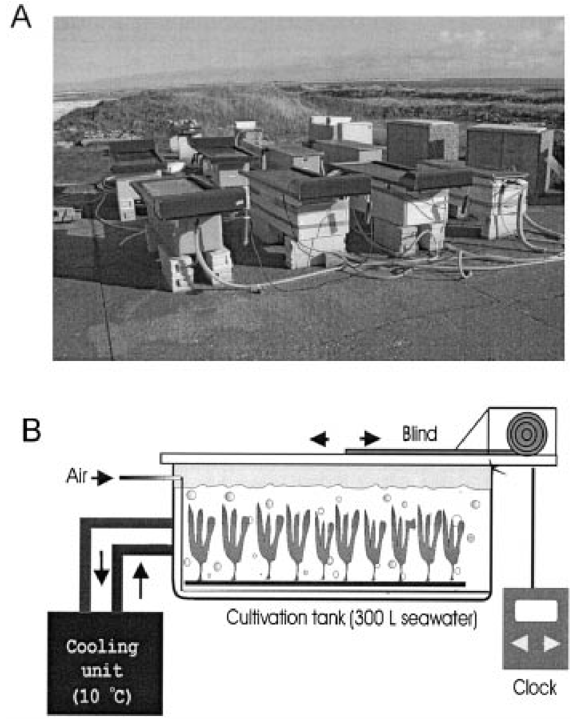

Fig. 1. (*A*) Photograph of the experimental outdoor tank system at List (island of Sylt, North Sea). (*B*) Schematic representation of the experimental tank (not to scale), in which daylength was controlled by an automatic blind and temperature by a flow-through cooling unit and an immersed electrical heater (not shown). A semi-transparent PVC screen on top of the tank reduced incident radiation by 50 % (see text).

order to avoid photoinhibition of the plants, a light shading screen (two layers of grey, semi-transparent PVC, each 6 mm thick), which reduced the incident irradiance by 50 %, was placed on top of the tank.

The seawater was maintained at a temperature of $10 \pm 1{\cdot}0$ °C using a thermostat-controlled cooling unit (model SK 5, AquaMedic, Melle, Germany). The flow of the seawater through the tanks was achieved with a centrifugal pump (type 1060, Eheim, Deizisau, Germany). During the cold season, the seawater inside the tanks was heated by a waterproof insulated aquarium tube heater (type SH 10-300; Jäger, Wüstenrot, Germany). Vigorous aeration was provided by an air compressor (type Elmo-G, Siemens, Neustadt an der Saale, Germany). The seawater in the tanks was renewed weekly and nutrients were added every 3 days to give concentrations equivalent to 10 % of those in Provasoli enriched seawater (Starr & Zeikus, 1987).

Measurement of growth rate and source of daylength data

Blade elongation was measured every 2 weeks using the conventional method of following the movement of a hole punched initially at 10 cm from the transition between the stipe and the blade (Parke, 1948; Sundene, 1964). To maintain constant biomass in the tank, the blades of the experimental algae were pruned to a total length of 40 cm every 2 weeks, at the time of the elongation measurement. Punched holes that had been displaced by more than 30 cm from the transition zone were replaced by new measuring holes. Daylength data were obtained from the online photoperiod calculator using the Internet address http://www.netti.fi/~jjlammi//sun.html.

Light measurements

PAR (photosynthetically active radiation) was monitored outdoors about 50 m away from the experimental tanks at 10 min intervals from May 1999 to March 2000 using a watertight quantum sensor (type LI-192 SA; LI-COR, Lincoln, Nebraska, USA). Light-signal conversion and data acquisition were achieved by a 14 bit AC/DA card (Decision Computer Int., Taipei, Taiwan) and the software program Medealab 4·0 (Medea/AV, Erlangen, Germany). From April 2000 to the end of the growth experiment in September 2000, PAR was monitored at 1 min intervals using the automatic, computer-aided measuring system ELDONET (Real-Time Computer, Möhrendorf, Germany; Häder *et al.*, 1999). This system measured PAR in terms of W m^{-2}, and these irradiance values were multiplied by a factor of 4·6 to obtain approximate values of quantum irradiance in μmol photons m^{-2} s^{-1} (Lüning & Dring, 1979).

Irradiance was occasionally measured among and below the experimental algae by a spherical LI-COR underwater quantum sensor (type LI-193SA) and revealed a greater reduction of irradiance than could be attributed to the shading screen on top of the tank. The final irradiance reaching the experimental algae was estimated to be approximately 25% of the irradiance monitored continuously in open air.

Results

In constant SD, the growth rate of the *Laminaria digitata* sporophytes transferred from the sea into the experimental tanks in May remained at a steady-state level of approximately 0·4 cm day^{-1} from the end of June until mid-October (Fig. 2). In ambient daylengths, however, growth rate declined steadily after June, and the first significant difference (at $p =$ 0·05; ANOVA) between the two treatments was recorded in early August (Fig. 2).

The steady-state growth level of approximately 0·4 cm day^{-1} in constant SD continued until the end of October, when daily PAR doses became less than 3 mol m^{-2} day^{-1}, equivalent to a mean daily irradiance of approximately 80 μmol m^{-2} s^{-1}. The same growth rate was reached again in mid-March, when PAR had increased again to 3 mol m^{-2} day^{-1} (Fig. 2). No significant differences between the growth rates in the two daylength treatments were observed during the season of low light but, from July to August of the second year of the experiment, higher growth rates were again observed in constant SD, although the steady-state level of 0·3 cm day^{-1} was lower than in the previous year (Fig. 2). Thallus deterioration and/or massive epiphytism were observed in the older, distal blade parts during the second year and towards the end of the experiment, with no obvious differences between the two treatments.

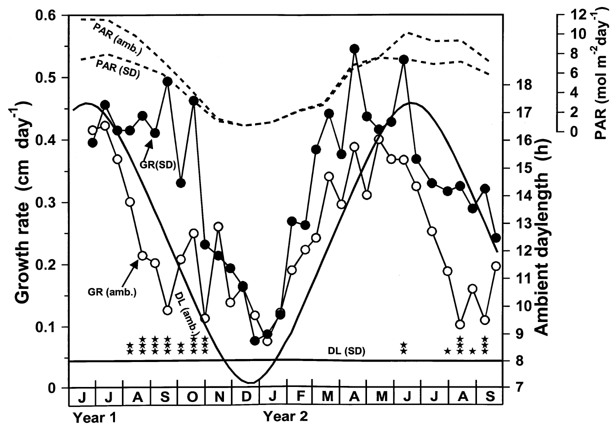

Fig. 2. Growth rate (GR) of experimental individuals of *Laminaria digitata* cultivated in outdoor tanks in constant short days (8 h light per day; SD, filled circles) or in ambient daylengths (amb., open circles). Stars indicate significance of differences between the daylength treatments on each sampling date (ANOVA; ***$p < 0.001$; **$p < 0.01$, *$p < 0.05$). Growth rate symbols refer to the mid-point of each measuring interval of 2 weeks. PAR values are monthly means of irradiance received at the algal surface, estimated as 25% of the irradiance above tanks.

Discussion

The experimental results showed that an elevated level of blade growth activity could be maintained in outdoor cultivation in *Laminaria digitata* throughout the summer until October by constant SD treatment. This confirms results of previous laboratory studies showing arhythmicity of the circannual system in species of the Laminariales, and their continuous growth activity in constant SD (Lüning, 1991, 1993; tom Dieck, 1991; Schaffelke & Lüning, 1994). In contrast, the growth rate of the experimental sporophytes in the outdoor tank declined in ambient daylengths, and this was most probably due to the annual course of daylength entraining a putative, circannual oscillator within *Laminaria digitata*.

The seasonal course of growth activity of the experimental sporophytes in ambient daylength conditions was quite similar to the seasonal course of growth in *Laminaria digitata* as measured by Pérez (1971) in a natural population on the coast of northern France, even in quantitative terms, with initial, high growth rates of 0·7 cm day^{-1} in May during the first year and a reduced growth rate of around 0·2 cm day^{-1} from August to November. The initial, high growth rate was not observed, however, during the second year in ambient daylengths in the tank, and the steady-state rate of growth in constant SD throughout the summer during the first year was only about 0·4 cm day^{-1} and was even lower during the second year. These restrictions may be due, firstly, to the less than ideal growing conditions for the relatively voluminous algae in the experimental tanks, compared with the sea. Growth may have been restricted by the batch type of culture, with replacement of the tank seawater only once a week, and the massive epiphytism of older parts of the blade, particularly towards the end of the experiment. Secondly, the blades of the experimental sporophytes were pruned to a total length of 40 cm every 2 weeks in order to prevent overcrowding in the tank and, consequently, less translocate from distal blade portions was available to support the growth activity of the basal, meristematic zone (Schmitz & Lobban, 1976). A third possibility of higher photoinhibition for *L. digitata* in the tanks than in natural populations may be ruled out, because growth rates in the tank were similar to those in the sea during June of the first year, and because the maximum PAR doses reaching the surface of *L. digitata* blades in the tank under ambient daylength conditions in June amounted to approximately 12 mol m^{-2} day^{-1}, which corresponds to a mean daily irradiance of approximately 200 μmol m^{-2} s^{-1} (daylength = 17 h). This is below the critical irradiance for photoinhibition of photosynthesis in laminarian blades (300–600 μmol m^{-2} s^{-1} in Arctic Laminariales; Hanelt, 1998; Hanelt *et al.*, 1997).

During the season of low light from October to February, the decline in growth rate of the experimental sporophytes in the outdoor tanks in constant SD was clearly due to light limitation, since the critical, mean daily irradiance of approximately 80 μmol m^{-2} s^{-1} in October lies within the linear part of the *P* vs *I* curve for laminarian species, with saturation occurring above about 150 μmol m^{-2} s^{-1} (Lüning, 1979). Mean daily irradiance in the sea at the lower limit of *Laminaria digitata* near Helgoland (2 m water depth) decreases from 100 μmol m^{-2} s^{-1} in August to 58 μmol m^{-2} s^{-1} in September (Lüning & Dring, 1979), and this suggests that the critical light level for growth in *L. digitata* is similar in the sea and in the tank experiment. It might have been possible to maintain a high growth rate in constant SD throughout the year by increasing the irradiance (e.g. by removing the screen and mounting floodlights above the tanks), as shown previously for laminarian species under constant conditions in the laboratory (Lüning, 1991, 1993; tom Dieck, 1991; Schaffelke & Lüning, 1995), but this was not attempted in the outdoor experiment.

It remains an open question whether the seasonal growth activity of perennial, marine macroalgae, other than laminarians – in particular, commercially valuable red algae – also possess endogenous, circannual oscillators, and whether continuous SD treatment would also result in constant growth activity in these species. The general occurrence of circadian and circannual clocks throughout the plant and animal kingdoms would support such a notion and suggests that further experiments along these lines would be worthwhile.

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