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Tank cultivation of the red alga *Palmaria palmata*: Effects of intermittent light on growth rate, yield and growth kinetics

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Abstract

Tank cultivation of marine macroalgae involves air-agitation of the algal biomass and intermittent light conditions, i.e.periodic, short light exposure of the thalli in the range of 10 s at the water surface followed by plunging to low light or darkness at the tank bottom and recirculation back to the surface in the range of 1–2 min. Open questions relate to effects of surface irradiance on growth rate and yield in such tumble cultures and the possibility of chronic photoinhibition in full sunlight. A specially constructed shallow-depth tank combined with a dark tank allowed fast circulation times of approximately 5 s, at a density of 4.2 kg fresh weight (FW) $m^{-2}s^{-1}$. Growth rate and yield of the red alga *Palmaria palmata* increased over a wide range of irradiances, with no signs of chronic photoinhibition, up to a growth-saturating irradiance of approximately 1600 μmol m^{-2} s^{-1} in yellowish light supplied by a sodium high pressure lamp at 16 h light per day. Maximum growth rate ranged at 12% FW d^{-1}, and maximum yield at 609 g FW m^{-2} d^{-1}. This shows that high growth rates of individual thalli may be reached in a dense tumble culture, if high surface irradiances and short circulation times are supplied. Another aspect of intermittent light relates to possible changes of basic growth kinetics, as compared to continuous light. For this purpose on-line measurements of growth rate were performed with a daily light reduction by 50% in light-dark cycles of 1, 2 or 3 min duration during the daily light period. Growth rates at 10 °C and 50 μmol photon m^{-2} s^{-1} dropped in all three intermittent light regimes during both the main light and dark periods and reached with all three periodicities approximately 50% of the control, with no apparent changes in basic growth kinetics, as compared to continuous light.

Introduction

Palmaria palmata occurs on both sides of the North Atlantic (Lüning, 1990) and has been consumed as human food for hundreds of years in northern Europe (Guiry & Blunden, 1992). Several aspects have intensified research on use and cultivation of this red alga in recent years. *P. palmata* has been reported, for example, as the best suitable algal species to support fast growth rate of abalone (Evans & Langdon, 2000) or gonad development in sea urchins (Vadas et al., 2000). A related Northern Pacific species, *Palmaria mollis*, has been successfully co-cultured with red abalone with satisfactory growth rates of both the algae and the animal (Evan & Langdon, 2000). The relatively high protein content of *P. palmata* (20–25% dry weight) can be compared with other high protein foods such as beef (25%), canned salmon (20%) or soybean (30%), and therefore this species is considered as an appropriate dietary protein source for both humans and animals (Morgan et al., 1979; Galland-Irmouli et al., 1999). As an example for modern use in integrated mariculture, *P. palmata* takes up NH_4^+ three times faster than NO_3^- (Morgan & Simpson, 1981b) and is thus a valuable candidate for integration into fish farming for removing nutrients from the effluents. Under these conditions the algal protein content increases, and the alga constitutes, hence, an appropriate feed for animals (Rosen et al., 2000).

Tank cultivation of seaweeds using air agitation (tumble culture) was first developed in detail for the red alga *Chondrus crispus*, by Bidwell et al. (1985), with tank depths of 0.6–0.9 m and algal densities of 5–10 kg FW m^{-2} of tank surface. The individuals which are tumbled in a tank by the upward water current generated by aeration are exposed to the surface light only for a short time, in the order of 10 s, and then plunged to the dark or almost dark depths of the tank, usually at 0.6–0.8 m water depth, with water circulation times in the circulating cells of about 1.0 to 1.5 minutes. As an example, irradiance at 0.5 m depth in a 2000-L tank decreased to 0.05% of surface irradiance in a tank with a surface area of 2.6 m^2 and a depth of 0.8 m inoculated with *Palmaria palmata* at a density of 8 kg m^{-2} (Lüning & Pang, 2003).

Momentary changes in irradiance with periods of seconds or minutes or a few hours also occur in the natural environment of seaweeds due to various factors such as canopy movement, waves, clouds and tides (Lobban & Harrison, 1994). Algal cells have developed mechanisms for integration of such short-term irradiance changes ‘so as to produce as constant and as rapid a growth rate as possible’ (Harris, 1980). As an example for irradiance integration in seaweeds, an *Ulva* sp. grown in outdoor, continuous flow culture tanks at low algal density and subjected to step changes between 9 and 100% of incident, natural light with periodicities from 15 min to 7 h exhibited an average value of the growth rates of control cultures in either 9 or 100% of incident light under steady state conditions, at all periodicities (Henley & Ramus, 1989). In tumble culture, the resulting, ‘averaging’ growth rates in fluctuating light will depend on various parameters such as algal species, surface irradiance, circulation time or tank depth. One objective of the present investigation was to measure the dependence of growth rate and yield upon irradiance in a tumble culture of *Palmaria palmata* with fixed circulation time and tank depth. First successful cultivation trials with *P. palmata* in small 8-L tanks with air-agitation had been reported by Morgan & Simpson (1981), with a maximum growth rate of 6% increase in fresh weight (FW) per day, at low algal density (1.0 kg FW m^{-2}) and in fluorescent light at an irradiance of approximately 300 μmol m^{-2} s^{-1} (reported as 86 langleys d^{-1} at 16 h light per day).

A second objective concerned the question, whether intermittent light with light-dark cycles of 1, 2 or 3 min duration during the day phase might affect the typical day-night growth kinetics in *Palmaria palmata* characterized by a growth maximum during the day and a minimum during the night (Lüning, 1992).

Materials and methods

Algal material

Palmaria palmata was originally collected near Roscoff, France and maintained by vegetative propagation in air-agitated tumble culture in greenhouse tanks, with 7–10 cm long thalli being used standard material for the study.

Cultivation system for assessing irradiance effects in tumble culture

The cultivation system consisted of a light tank for algal incubation (0.6 × 0.4 × 0.3 m; 0.24 m^2 in area) and a dark tank filled with additional, recirculating seawater for the light tank (Figure 1). This design with a rather shallow incubation tank allowed fast algal circulation times of 4–6 s, while a sufficiently large water volume was present in the additional dark reservoir. A biomass of 1 kg of *Palmaria* was tumbled at an algal density of 4.2 kg FW m^{-2} in approximately 60 L of plain seawater and irradiated for 16 h light per day. Light was provided by a high-pressure 400 W-sodium lamp (Philips SON-T Plus 400 W) mounted centrally above the culture tank. This lamp emits yellowish light with a spectrum containing 6%, 39% or 55% of total irradiance in the ranges 400–495, 595–590 or 590–700 nm, respectively (manufacturer’s data), and with no emission of UV-A (320–400 nm) or UV-B (290–320 nm). Different irradiance levels of photosynthetic active radiation (PAR; 400–700 nm) were obtained by adjusting the height of the lamp above the water surface. Irradiances were measured with a LI-190SA flat quantum sensor (LI-COR, Lincoln, Nebraska)

The somewhat larger dark tank received overflowing water from the light tank, and water was recirculated to the light tank via an underwater pump (Figure 1). Temperature was kept constant at 10 °C using a sea water cooler (type SK4, Aquamedic, Bissendorf, Germany). New water was pumped at hourly intervals

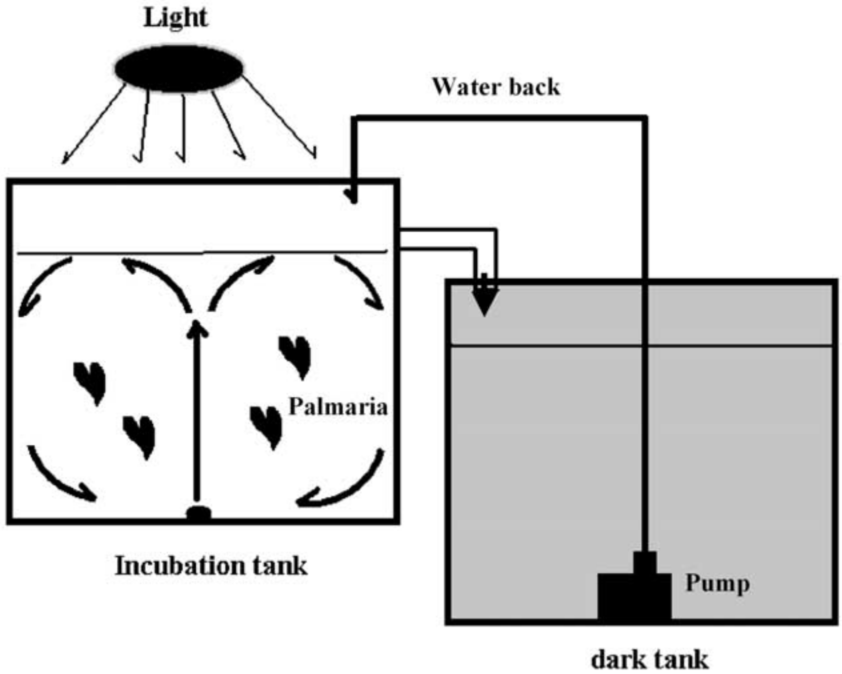

Figure 1. Schematic representation (not to scale) of the tank system used for testing the effects of irradiance in dense tumble culture of *Palmaria palmata*. A light incubation tank (0.24 m^2 in surface area) contained air-agitated algal thalli, at an algal density of 4.2 kg fresh weight m^{-2}. Outflowing water was entering a a 100-L dark tank and recirculated back to the light incubation tank. See text for further explanation.

from a sand-filter water system resulting in a two-fold water exchange per day. A daily morning-nutrient pulse using NH_4NO_3 and $(NH_4)_2HPO_4$ was applied to obtain approximately concentrations of 100 μm NH_4^+ and 10 μm PO_4^-.

Eight successive experiments, each of 3 days duration, were performed at different irradiances, ranging from 10 to 1600 μmol photon m^{-2} s^{-1} as measured at the water surface. The fresh weight of 5 tagged individuals and the total biomass in culture was measured at the end of the experiment after 3 days.

On-line measurements of growth rate and growth kinetics

On-line growth-rate measurements were performed by means of a computer-aided image analysis system (Lüning, 1992, 1994). Briefly, a 2 cm long top portion of *Palmaria* was placed in the middle of a Plexiglas chamber supplied constantly with flowing 10% PES (Provasoli's enriched seawater; Starr & Zeikus, 1987) in a constant temperature room at 10 °C. Every 2 min the infrared image (> 850 nm) of the growing alga were recorded by a CCD video camera and evaluated for thallus area by an on-line computer. Relative growth rate per h was calculated from the successive differences of the logarithms of thallus area.

The experimental *Palmaria* thallus was irradiated from above at an irradiance of 50 μmol photon m^{-2} s^{-1} by light from a projector via a semi-transparent mirror. The projector (Leitz Prado Universal) was fitted with a quartz-iodine lamp (24V, 250W) and combined with a 2 mm BG 38 Schott filter to mimic underwater light. The measurements were performed with a light regime of 12 h light per day. During the entire 12-h light phase the light source was automatically switched on and off by a timer-clock using 1, 2 or 3 min long light intervals followed by correspondingly long dark intervals. As a control, a non-interrupted light phase was employed.

Results

In a tank with fast circulation times of approximately 5 s, the growth rate of individual tagged thalli and the yield of the total *Palmaria* biomass increased with increasing irradiance up to a saturating irradiance near to 1600 μmol m^{-2} s^{-1} supplied during a light phase of 16 h per day (Figure 2). Maximum values at sat-

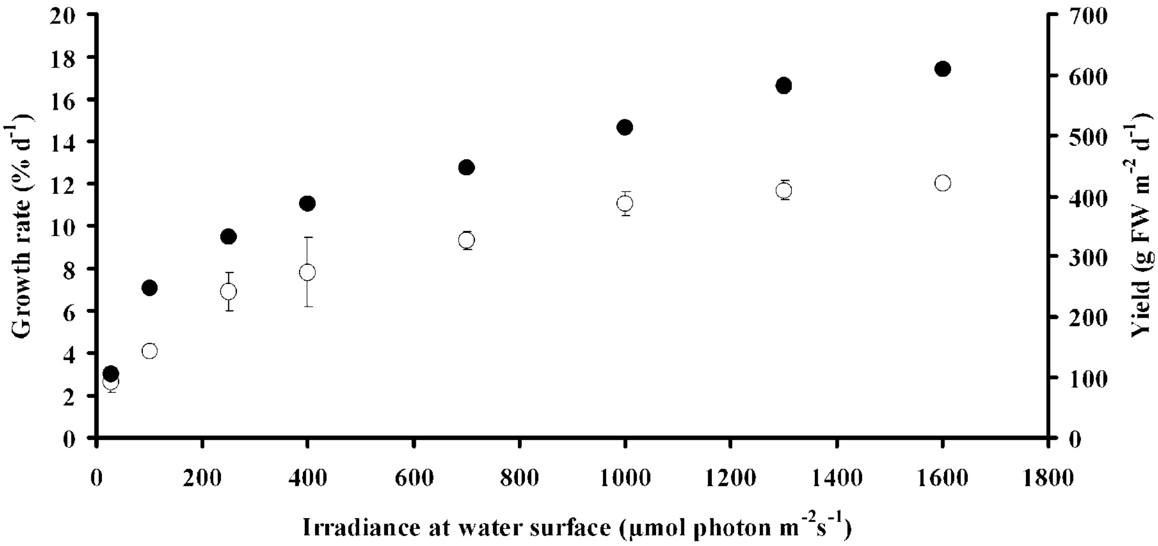

Figure 2. Growth rate of tagged individuals (open circles) and yield of total *Palmaria* biomass (closed circles) at different irradiances in 3-day experiments at 8 different irrdiances and in darkness (10 °C, 16 h light per day; daily nutrient pulse; 2 water renewals per day). Fresh weight of tagged individuals (n=5) and the total biomass were evaluated after 3 and 6 days, and the represented values indicate averages from the two measurements. Biomass density was maintained at 4.2 kg m^{-2} at the start of each measurement.

urating irradiance were 12% increase per day in fresh weight for growth rate and 609 g FW m^{-2} d^{-1} for yield. No photo-inhibition was observed even near to 1600 μmol m^{-2} s^{-1} supplied for three successive days.

The growth kinetics of isolated *Palmaria* thallus tips measured by means of the on-line system exhibited a day peak and a night trough both with continuous as with intermittent light (Figure 3A-C). A drop of growth rate occurred immediately upon the start of the intermittent light regimes on day 3 in all three regimes reducing growth rate during the day as well as during the night phases (Figure 3A-C).

The extent of growth reduction in intermittent light was calculated as a percentage of-the control in non-intermittent light and ranged at approximately 50%, with no apparent difference between light-dark cycles of 1, 2 or 3 min duration, both during the main light and main dark phases (data not shown).

Discussion

Palmaria palmata exhibited an increase in growth rate and no signs of chronic photoinhibition up to an irradiance of 1600 μmol m^{-2} s^{-1} for 16 h light per day, if cultivated at a density of 4.2 kg FW m^{-2} in tumble tank culture. This irradiance (PAR; 400–700 nm) resembles full sunlight (Lüning,1990), except for absence of the harmful UV-B and UV-A present in sunlight. In contrast, individual, fully light-exposed thalli of *P. palmata* became photo-inhibited for their growth rate in white fluorescent light in the irradiance range 290–570 μmol m^{-2} s^{-1}, with light-saturation of growth occurring above an irradiance of 140 μmol m^{-2} s^{-1} (Sagert & Schubert, 2000). The finding that growth saturation was hardly reached in the present investigation even at 1600 μmol m^{-2} s^{-1} may be seen as a special advantage of tumble culture with an increase in growth rate over a wide range of irradiances.

The lack of photoinhibition for growth rate in intermittent light in a dense, air-agitated algal biomass can only be due to the beneficial dark intervals preventing chronic photoinhibition of photosynthesis and, hence, of growth rate. The momentary exposure to strong light at intervals of the order of 10 s might cause dynamic (reversible) photoinhibition, but not chronic (non-reversible) photoinhibition associated with damage of mainly photosystem II occurring only at longer exposure times (Hanelt et al., 1992; Franklin & Forster, 1997; Russell et al., 1995). The

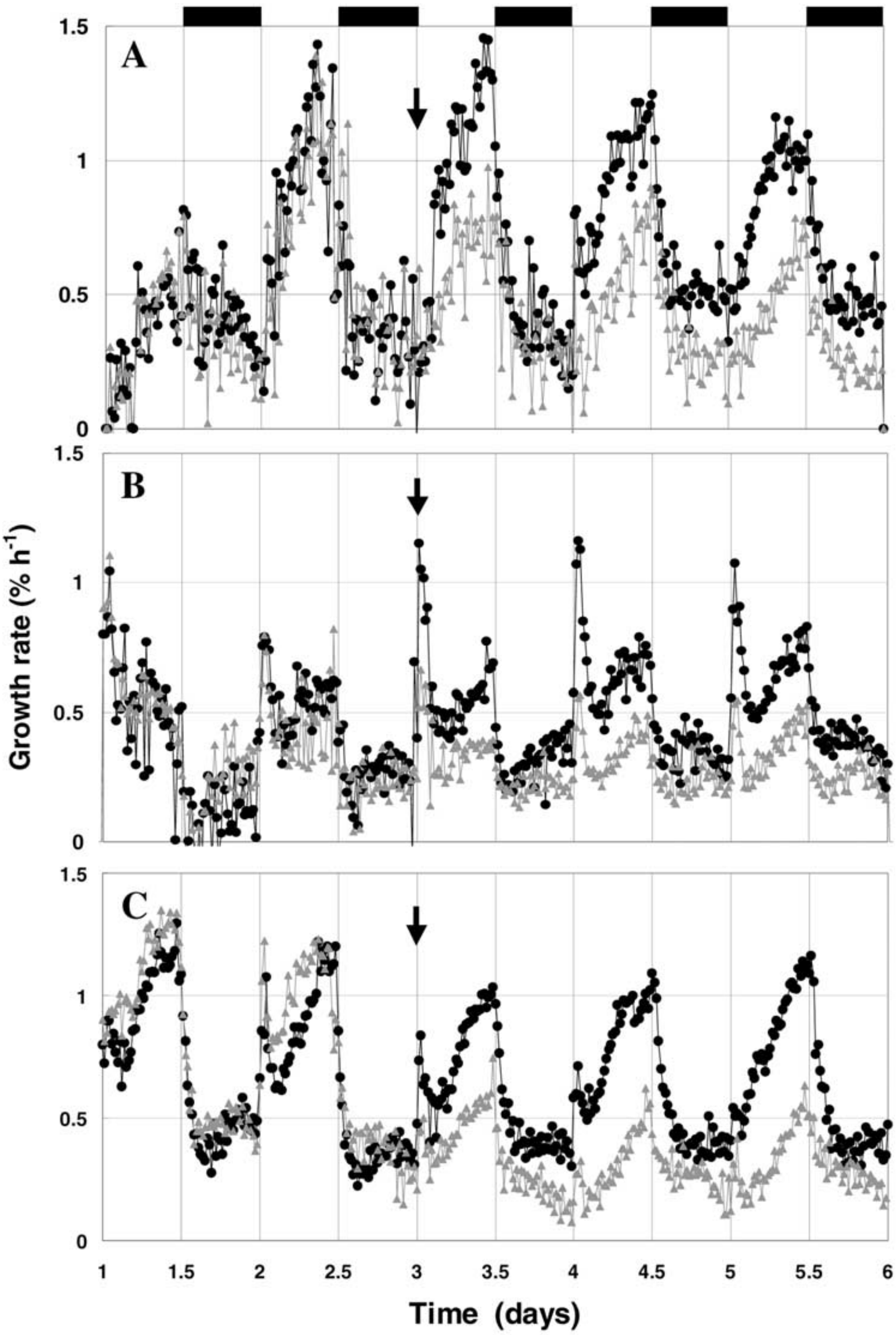

Figure 3. Growth kinetics of top portions of *Palmaria palmata* at 12 h light per day (50 μmol m^{-2} s^{-1}), with intermittent light (triangles; grey curves) or continuous light (circles; black curves). Intermittent light: 1 min (A) , 2 min (B) or 3 min (C) light followed by equally long dark intervals (10 °C; 50 μmol m^{-2} s^{-1} imitated underwater light). Filled horizontal bars indicate dark periods. Days 1–2: normal, non-intermittent light, days 3–5: intermittent light. Arrows indicate the start of intermittent light.

beneficial effect of short dark intervals in strong light had been observed already by Bidwell et al. (1985) in individual, fully light-exposed thalli of *Chondrus crispus*. These exhibited over 50% lower photosynthetic rates in continuous strong light at 700 μmol m^{-2} s^{-1}, as compared to algae exposed to light-dark cycles of 1.0 to 1.5 min duration.

Seaweed species originating from the upper sublittoral zone, such as *Palmaria palmata* or *Chondrus crispus* (Lüning, 1990) may thus be grown with no photodamage in tumble culture in full sunlight, due to high algal density and, probably, shielding off UV-A and UV-B. In the present investigation, the circulation time of the algae was only 4–6 s, much shorter than the normal 1–1.5 min circulation time in tanks with 0.6–0.8 m water height (Bidwell, 1985), and thus photoinhibition would become even more unlikely under such conditions. This may be not be valid, however, for seaweed species from the lower sublittoral zone, such as the light-sensitive red alga *Delesseria sanguinea* (Pang et al., 2001).

The maximum growth rate of 12% FW d^{-1} in tumble culture at a density of 4.2 kg FW m^{-2} and a temperature of 10 °C corresponds to a similar value reported for individual thalli of *P. palmata* fully exposed to fluorescent white light at 290 μmol m^{-2} s^{-1} (Sagert & Schubert, 1995) for 16 h light per day (12°C). The yellowish light of a sodium high pressure lamp, with the majority of quanta emitted in the green and red part of the spectrum is obviously optimally absorbed by the phycobilins of a red alga (Sagert & Schubert, 1995). High growth rates of individual thalli as high may thus be reached in a dense tumble culture at a density of 4.2 kg FW m^{-2}s, provided that one supplies (1) high surface irradiances, allowing sufficiently high photosynthetic rates at greater depths within the dense algal biomass, and (2) short circulation times by means of a shallow-depth design like in the presently used dual tank system.

The maximum yield for *Palmaria palmata* of 609 g FW m^{-2} d^{-1} at a density of 4.2 kg FW m^{-2} was of similar order as the yield reported for tumble culture with *Palmaria mollis* (400–500 g FW m^{-2} d^{-1}; Evans & Langdon, 2000), at a density of 6.5 FW kg m^{-2}. A yield of 280 g FW m^{-2} d^{-1} (corresponding to 28 g dry weight) had been obtained by Morgan et al. (1980) in tumble culture of *P. palmata* in small tanks of 8 L.

The fast drop of growth rate upon onset of intermittent light, as compared to a control thallus in continuous light, points to close coupling of growth rate to photosynthesis, i.e. the rapid use of newly produced photosynthate for the growth process. Similarly, the reduction of growth rate during night after a day phase with intermittent light shows the close dependence of growth rate on photosynthates produced during the preceding day.

As expected, the extent of growth decrease, compared to continuous light, was near to 50% in light-dark cycles of 1, 2 or 3 min duration, which are all equivalent to a reduced light input by 50% during the daily light phase. This example demonstrates again that growth integration of short-term irradiance changes does not depend on period in the range of minutes, as noted before for phytoplankton (Harris, 1980) or for *Ulva* sp. (Henley & Ramus, 1989). Similarly, periodicities in the range of minutes used for intermittent light during the day phase were obviously too short for changing the basic day-night growth kinetics as observed in continuous light during the day phase.

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