

Photoperiodism and life history of *Psilothallia dentata* (OKAMURA) KYLIN (Ceramiaceae, Rhodophyta) in culture

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The red alga *Psilothallia dentata* (Okamura) Kylin from Korea was studied in culture under different light : dark cycles (8 : 16, 8 : 7 : 1 : 8, 16 : 8, 24 : 0) and photon flux densities (180, 90, 60 $\mu\text{mol m}^{-2}\text{s}^{-1}$) at 17°C. A *Polysiphonia* type of life history was demonstrated. Tetrasporogenesis was under photoperiodic control and the tetraspores were only produced under short-day conditions. More than 10 short-day cycles were necessary for induction, and interruption of the dark period by a one hour night-break did not inhibit the formation of tetraspores. Gametophytes exhibited no photoperiodic responses. Growth was best under the longest photoperiods and the lowest photon flux densities tested. This is the first culture study of a species of *Psilothallia*.

Key Index Words: growth—life history—photoperiodism—*Psilothallia dentata*—Rhodophyta.

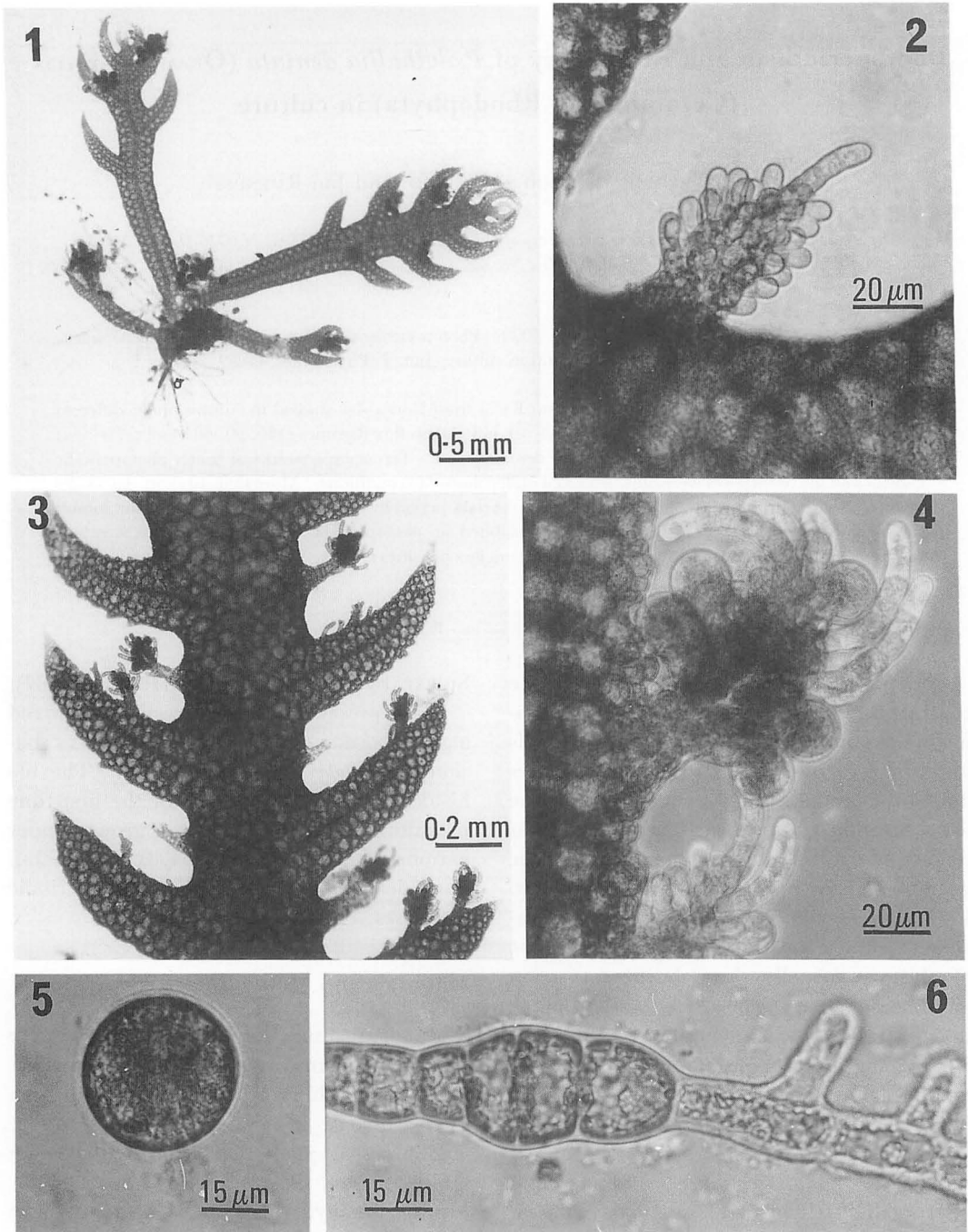
Photoperiodism could be defined as “the control of some aspect of a life cycle by the timing of light and darkness” (DRING 1984). It is important to distinguish between responses due to the amount of light and responses due to its duration. In the last 10 years numerous reports of algal species showing photoperiodic responses have been published (see DRING 1984 for review). Most of the responses involve a change of phase in a heteromorphic life history, usually through the formation and release either of spores by the sporophyte or gametes by the gametophyte generation. So far only two of the reported responses are to long-day regimes (TEN HOOPEN *et al.* 1983, HUTH 1979), all others are short-day responses. A genuine photoperiodic response should be all or nothing, and a night interrupted by a light-break should have the same effect as a long day. Although night-breaks have been shown to be effective in most of the short-day responses tested, this criterion should not be regarded as absolutely diagnostic of a genuine photoperiodic response (DRING 1988), as demonstrated in *Acrosymphyton purpuriferum* (J. AGARDH)

SJOEST. by BREEMAN and TEN HOOPEN (1987).

In the present study we investigated the red alga *Psilothallia dentata* (OKAMURA) KYLIN (basionym: *Ptilota dentata* OKAMURA). The life history has been completed for the first time in culture, and the alga was grown under various regimes of photoperiods and photon flux densities to test for photoperiodic responses.

Materials and Methods

Unialgal cultures of *Psilothallia dentata* were established from carposporophytic plants collected from fishing nets near Kangreung, on the east coast of Korea, in August 1986. Reproductive plants were brought to the University of Oslo, Norway, Section for Marine Botany, where the experiments were carried out. Carposporelings were grown in polystyrene Petri dishes containing 25 ml enriched seawater medium (IMR/2, EPPLEY *et al.* 1967) at 17°C and under a 16 : 8 light : dark photoperiod. When the carposporelings reached a length of about 1–2 mm, they were transferred to the following

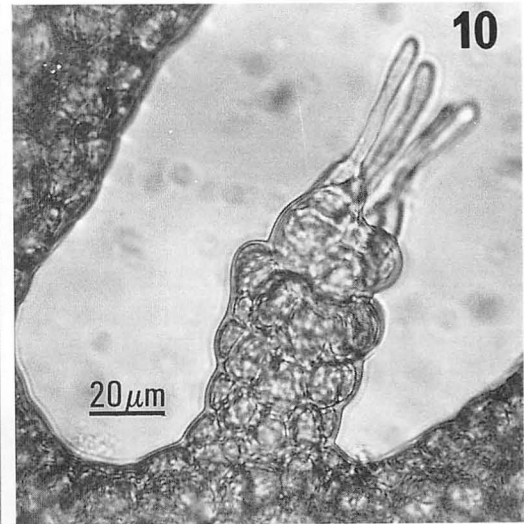
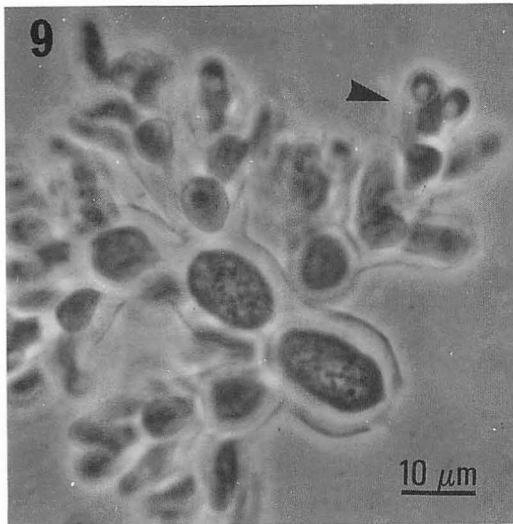
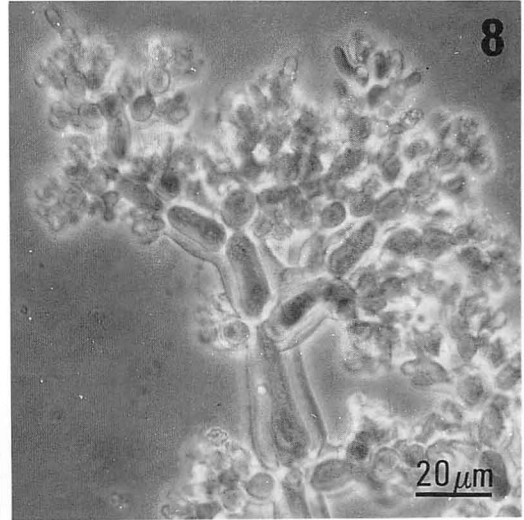
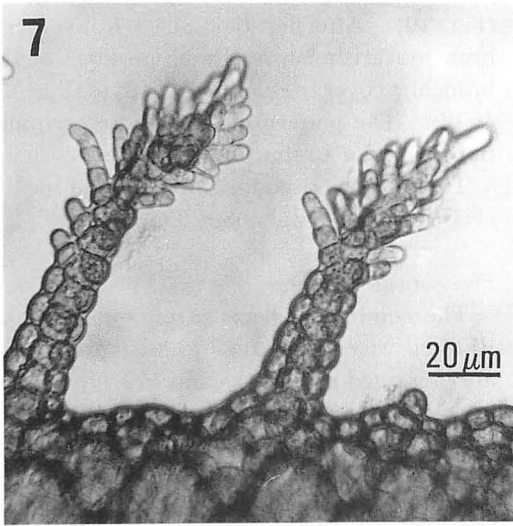


Figs. 1-6. *Psilothallia dentata* (OKAMURA) KYLIN.

Fig. 1. Habit of fertile tetrasporophyte after two months in culture under short-day conditions (8 : 16). Fig. 2. Early stage in the formation of tetrasporangial branchlet. Fig. 3. Mature tetrasporophyte showing the position of fertile branchlets. Fig. 4. Detail of mature tetrasporangia surrounded by colourless, sterile filaments. Fig. 5. Newly settled tetraspore. Fig. 6. Bipolar germination of tetrasporeling.

regimes of photoperiod and photon flux density (PFD): short day (SD) 8:16, short day with night break (NB) (8:7:1:8), long day (LD) 16:8, and continuous light (CL) 24:0. For each daylength, three different photon flux densities (PFD) were tested: 180, 90 and 60 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Light was provided by Philips fluorescent tubes (TL E 32W/33 and TL E 40W/33) and was measured with a QLS-100 Laboratory Quantum Scalar irradiance meter with a spherical quantum sensor measuring photosynthetically active radiation (PAR).

Three parallel dishes, each containing 5 carposporelings, were kept under each culture regime. During the experiment, which lasted for two months, medium was changed every fourth day and the plants were examined in a dissecting microscope to check the development of reproductive structures. The



Figs. 7–10. *Psilothallia dentata* (OKAMURA) KYLIN.

Fig. 7. Early stage in the development of spermatangial branchlets. Fig. 8. Mature spermatangial branchlet showing one row of axial cells with budding side branches. Fig. 9. Detail of spermatangial branch system showing that one spermatangial mother cell gives rise to two spermatangia (arrow). Fig. 10. Female branchlet prior to fertilization, bearing a group of four carpopogonial branches surmounting the apex.

length of the main axis of each of the 180 experimental plants was measured after two months in culture.

After the formation of tetrasporangia and release of tetraspores, tetrasporelings were isolated into separate dishes and incubated under short days and long days at 17°C and a PFD of 90 $\mu\text{mol m}^{-2}\text{s}^{-1}$. These plants produced dioecious gametophytes, and carposporophyte development occurred following fertilization. Carpospores were again isolated and grown into mature tetrasporophytes. Thus the complete life cycle was accomplished in culture.

Results

Reproduction and life history in culture

The first sign of the maturation of tetrasporophytes is the formation of special fertile branchlets in an adaxial position on the lateral branches of limited growth, or on the side of the main axis between laterals (Figs. 1–4). The branchlets consist of colourless sterile filaments surrounding the tetrahedrally divided sporangia (Fig. 4). Tetraspores have a diameter of 35–40 μm (Fig. 5) and germinate in a bipolar manner typical of the

Ceramiales (Fig. 6).

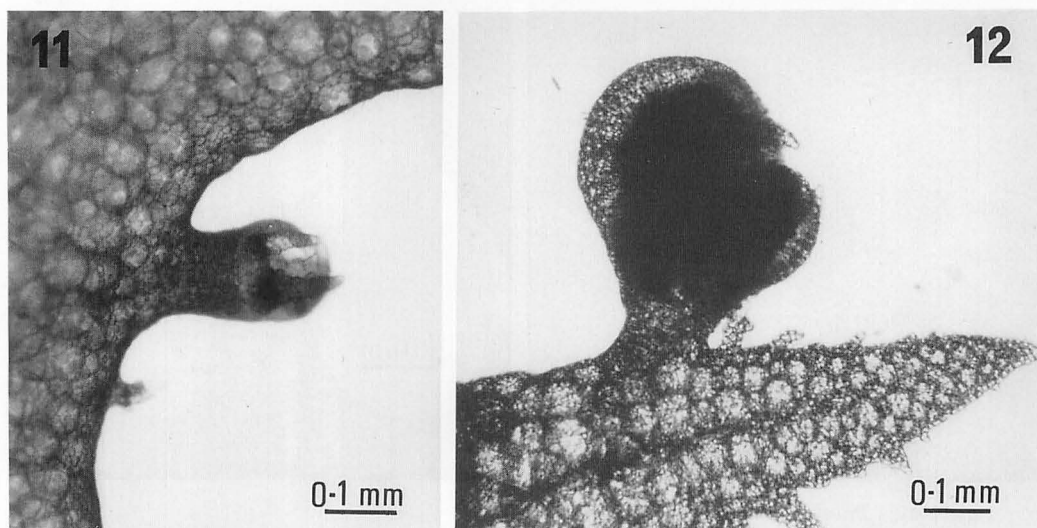
Spermatangial structures are formed terminally on special branchlets (Fig. 7) and consist of densely branched, monosiphonous shoots (Fig. 8), on which the spermatangia are formed in an apical position (Fig. 9).

The female reproductive structures are borne on special branchlets in a similar position to the tetrasporangial and male branchlets. Carpogonial branches occur in groups surmounting apices of the branchlets and with two to four protruding trichogynes (Fig. 10). After fertilization, 4–6 involucrel branches arising from lower portions of the branchlet cover the carposporophyte (Figs. 11 & 12). The pattern of carpospore germination is similar to that of tetraspores.

The time required for completion of the life cycle in culture was about 5 months.

Photoperiodic responses

The combined effects of photoperiod and PFD on vegetative growth and reproduction are presented in Figs. 13 and 14. Vegetative growth was determined as the mean length of ca. 15 tetrasporophytes (started from carpospores) after two months of growth under each regime. As seen in Fig. 13 the longest



Figs. 11 and 12. *Psilothallia dentata* (OKAMURA) KYLIN.

Fig. 11. Young cystocarp showing developing involucrel branches. Fig. 12. Mature cystocarp covered by involucrel branches.

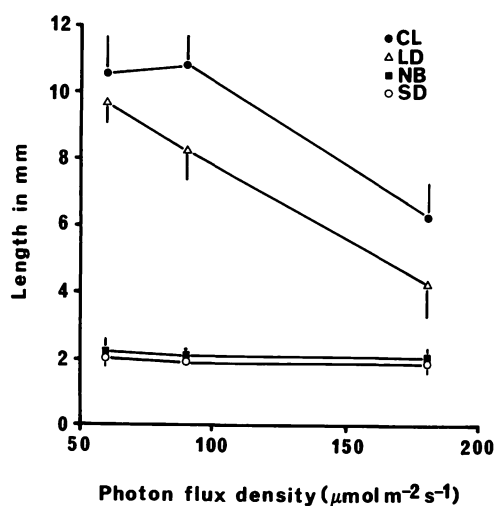


Fig. 13. *Psilothallia dentata* (OKAMURA) KYLIN. Mean length of tetrasporophytes (vertical bars indicate SD, $n=15$) grown for two months under various combinations of photon flux density and light: dark cycles. CL, continuous light; LD, long day (16 : 8); NB, night break (8 : 7 : 1 : 8); SD, short day (8 : 16).

plants were found at the lowest PFD under the longest daylight regimes (16 : 8 and continuous light). The plants grown at high irradiances (180 $\mu\text{mol m}^{-2} \text{s}^{-1}$) under a short-day regime (8 : 16) receive the same daily quantum dose as the plants grown under continuous light at the lowest irradiance (60 $\mu\text{mol m}^{-2} \text{s}^{-1}$). However, the vegetative growth under the two conditions differs significantly, being much less under a short-day regime.

Tetrasporangia were formed only under short-day conditions and were never observed under a long-day regime or in continuous light (Fig. 14). Interruption of the long night by a light break of one hour did not prevent the formation of tetrasporangia. A minimum of 10–14 short-day cycles was required for the induction of tetrasporogenesis. Plants transferred to non-inductive conditions after a period of about 2 weeks under short days formed tetrasporangia within a month. Plants not more than 2 mm long produced tetrasporangia under a short-day regime, while plants more than 12 mm remained vegetative under a long-day regime. Tetraspores incubated under various day-

			Photoperiod (light:dark)
14 T $n=14$	14 T $n=14$	13 T $n=13$	8 : 16 (SD)
13 T $n=13$	15 T $n=15$	15 T $n=15$	8 : 7 : 1 : 8 (NB)
0 T $n=15$	0 T $n=15$	0 T $n=15$	16 : 8 (LD)
0 T $n=14$	0 T $n=14$	0 T $n=13$	24 : 0 (CL)
180	90	60	Photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$)

Fig. 14. *Psilothallia dentata* (OKAMURA) KYLIN. The number of fertile tetrasporophytes (T) produced under each combination of photon flux density and photoperiod. Initial number of plants was 15 under each condition, and n is number of plants surviving after two months in culture.

length regimes produced mature gametophytes both under a short-day regime and in continuous light.

Field data

In Korea *Psilothallia dentata* is apparently a rare species. It has been collected on the eastern coast near Kangreung as an epiphyte on *Pachyarthron cretaceum* (POSTELS et RUPRECHT) MANZA at a depth of 18 m (Kim *et al.* 1983). The material used in the present study was collected in the same area in August and cultures were started from carpospores. Five specimens were recorded at the same site in September, four of them being tetrasporophytes and one a female plant bearing carposporophytes. The specimen bearing carposporophytes was 5.2 cm high, and the tetrasporophytes were from 1.7 to 4.7 cm high. In April and May the next year males, females and tetrasporophytes were recorded. The dimensions of 25 tetrasporangia were measured and the average was $60.5 \pm 4.8 \times 57.7 \pm 4.8 \mu\text{m}$.

Discussion

Growth in *Psilothallia dentata* appears to be light-saturated at low light intensities ($\text{PFD} < 100 \mu\text{mol m}^{-2} \text{s}^{-1}$), which fits with its occurrence in relatively deep water. Vegetative growth was much less in plants growing under short-day conditions than under long-day conditions and in continuous light. Even though the daily quantum dose was the same, vegetative growth was less under short-day conditions and regimes with a night break than under long-day conditions at the lowest PFD. There is a negative correlation between the formation of tetrasporangia and vegetative growth. Apparently the induction of tetrasporangia leads to a shift in allocation of photosynthetic surplus to reproductive, rather than vegetative processes.

Photoperiodic responses have been reported for a number of algal species during the last ten years (DRING 1984, 1988). Most of the responses involve a change of phase in a heteromorphic life history, in red algae usually through the induction of tetrasporogenesis by a short-day regime. There are only a few examples in which the photoperiodic response occurs in algae with isomorphic generations (GUIRY and CUNNINGHAM 1984, MAGGS and GUIRY 1982, RIETEMA and BREEMAN 1982, KAIN 1987). KAIN (1987) demonstrated in field and laboratory experiments with *Delesseria sanguinea* (HUDS.) LAMOUR. that both gametogenesis and tetraspore production are under photoperiodic control. This is the only species in the Ceramiales for which photoperiodic responses have been demonstrated before the present study of *Psilothallia*. In *Delesseria sanguinea* there is a pronounced seasonality, and the environmental triggers and timing of the changes between vegetative and reproductive processes seem to be well adapted to the ecological conditions. In *Psilothallia dentata* we have too few field observations to suggest a possible ecological adaptation behind the short day response observed in culture.

The interruption of the long dark period by

a short night-break of light may inhibit the short-day response. This response has been used as a test for genuine photoperiodic responses (VINCE-PRUE 1975). In *Psilothallia dentata* a night break was not effective, and in this respect its response was similar to that reported for *Audouinella purpurea* (LIGHTF.) WOELKERL. (DRING and WEST 1983) and *Acrosymphyton purpuriferum* (J. AGARDH) SJOEST. (BREEMAN and TEN HOOPEN 1987). However, as pointed out by DRING (1988) this criterion should not be regarded as an absolute diagnostic for photoperiodic responses.

Another characteristic of genuine photoperiodic responses is induction (RIETEMA 1982). Plants transferred from inductive to non-inductive daylengths will remain in the induced state for some time after return to non-inductive conditions. In *Psilothallia dentata* we demonstrated that 10–14 short-day cycles were required to induce tetrasporangium formation and the plants remained induced when placed in continuous light. We therefore conclude that tetrasporogenesis in *P. dentata* is caused by light period and not by light quantity, and hence that this is an example of a photoperiodic response.

Acknowledgements

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S. FREDRIKSEN¹ · S.-M. BOO² · J. RUENESS¹ : 培養によるベニヒバ
(紅藻イギス科) の光周性と生活史の研究

韓国で採集した紅藻ベニヒバ *Psilothallia dentata* (OKAMURA) KYLIN を種々の光周期 (8:16, 8:7:1:8, 16:8, 24:0) および光強度 (180, 90, 60 $\mu\text{mol m}^{-2}\text{s}^{-1}$) の下で 17°C で培養した結果, *Polysiphonia* 型の生活史をもつことが示された。四分孢子形成は光周期によって制御されており, 四分孢子は短日条件下のみで形成された。四分孢子形成の誘導には10回以上の短日サイクルを必要とし, 暗期中に1時間光中断を行っても四分孢子的形成は阻害されなかった。配偶体は光周期には反応しなかった。成長は, 実験を行った最も長い明期と最も低い光強度の下で最も良好であった。本研究はベニヒバ属の1種で行われた最初の培養による研究である。(¹Department of Biology, Marine Botany, University of Oslo, P.O. Box 1069 Blindern, N-0316 Oslo, Norway; ²Department of Biology, Chungnam National University, Daejeon 301-764, Korea)

