Field and culture studies of the life history of Porphyra dioica (Bangiales, Rhodophyta) from Portugal

Article *in* Phycologia · November 2004

DOI: 10.2216/i0031-8884-43-6-756.1

CITATIONS
22

READS
389

3 authors:

Rui Pereira
A4F S.A.
62 PUBLICATIONS **1,737** CITATIONS
SEE PROFILE

Isabel Sousa Pinto
University of Porto
257 PUBLICATIONS **5,212** CITATIONS
SEE PROFILE

Charles Yarish
University of Connecticut
234 PUBLICATIONS **7,518** CITATIONS
SEE PROFILE

Status	Rapid Code	Branch Name	Start Date
New	UCW	Main Library	7/24/2006 6:49:10 AM
Pending	VTU	Main Library	7/24/2006 6:49:14 AM

CALL #: **Shelved by title**
LOCATION: **VTU :: Main Library :: Periodicals (B/H)**

TYPE: Article CC:CCG
JOURNAL TITLE: Phycologia
USER JOURNAL TITLE:
VTU CATALOG TITLE: Phycologia.
ARTICLE TITLE: Pereira, "Field and culture studies of the life history of Porphyra dioica (Bangiales, Rhodophyta) from Portugal"
ARTICLE AUTHOR:
VOLUME: 43
ISSUE: 6
YEAR: 2004
PAGES: 756 - 767
ISSN: 0031-8884
OCLC #: 1642231
CROSS REFERENCE ID: 21744156
VERIFIED:

BORROWER: **UCW :: Main Library**
PATRON: **Pereira, Rui**

PATRON ID: rpp02006
PATRON ADDRESS:
PATRON PHONE:
PATRON FAX:
PATRON E-MAIL: rui.pereira@uconn.edu
PATRON DEPT: EEB - Ecology & Evolutionary Biology
PATRON STATUS: member, employee, staff, faculty
PATRON NOTES:

Phycologia (2004) Volume 43 (6), 756–767 Published 30 December 2004

Field and culture studies of the life history of *Porphyra dioica* (Bangiales, Rhodophyta) from Portugal

RUI PEREIRA[1,2,3]*, ISABEL SOUSA-PINTO[1,2] AND CHARLES YARISH[3]

[1]*CIIMAR, Centre for Marine and Environmental Research, Rua dos Bragas 177, 4050-123 Porto, Portugal*
[2]*Botany Department of the University of Porto, Rua do Campo Alegre, 1191, 4150 Porto, Portugal*
[3]*Department of Ecology and Evolutionary Biology, University of Connecticut, One University Place, Stamford, CT 06901-2315, USA*

R. PEREIRA, I. SOUSA-PINTO AND C. YARISH. 2004. Field and culture studies of the life history of *Porphyra dioica* (Bangiales, Rhodophyta) from Portugal. *Phycologia* 43: 756–767.

Aspects of the life history of *Porphyra dioica* collected in Porto, Portugal, were investigated under laboratory and field conditions. This is one of the most common *Porphyra* species on the north coast of Portugal and can be found throughout the year. Field studies showed higher percentage cover, from 23% to 66%, from February to May in 2001 and 2002, compared to other times of the year. The effects of temperature, photon flux density (PFD) and photoperiod on growth and reproduction were tested. Zygotospores germinated fastest at 15°C and a PFD of 25 μmol photons m^{-2} s^{-1}. Growth rate of the conchocelis was more affected by temperature than by photoperiod. In the three photoperiods tested, growth rate was maximal at 15°C, under 25–75 μmol photons m^{-2} s^{-1}. Conchosporangium formation was greatest at 15°C, 25–75 μmol photons m^{-2} s^{-1} light and short-day, 8:16 h light–dark conditions. In all conditions tested, conchosporangium formation was almost nonexistent in free-floating conditions. Optimal conditions for conchosporangium maturation (15°C, 8:16 h light–dark and 5–25 μmol photons m^{-2} s^{-1}) also promoted spore release after 18 weeks. Aeration appeared to be crucial for normal blade development. No archeospores were observed. Preliminary findings of the conditions for growth of the gametophyte stage are also discussed.

INTRODUCTION

Porphyra C. Agardh is a well-known seaweed genus due to its value as an aquaculture crop (Hanisak 1998). According to data from the Food and Agriculture Organization of the United Nations, production of *Porphyra* in 2001 was valued at 1.2 billion US dollars (FAO 2003). Demand for *Porphyra* products, either as food or as commercial source of the red pigment r-phycoerythrin, appears to be growing (Levine 1998; Yarish *et al.* 1998), and a program is currently underway in New England (USA) to promote the domestication of indigenous *Porphyra* species for commercial cultivation (Yarish *et al.* 1998, 1999).

Since Drew's (1954) classical work, many studies have dealt with the life histories of different species of *Porphyra* (e.g. Bird *et al.* 1972; Lewmanomont & Chittpoolkusol 1993; Notoya & Magaura 1998; Notoya & Miyashita 1999). Different species have different requirements to complete their life cycles and to optimize growth and reproduction. The main triggers for life cycle events are temperature, photoperiod and photon flux density (PFD) (Krishnamurthy 1969; Kapraun & Lemus 1987; Waaland *et al.* 1987; Lewmanomont & Chittpoolkusol 1993; Nam-Gil 1999).

With our current understanding of this genus, we believe that there are at least five *Porphyra* species growing along the Portuguese coastline (Ardré 1970; South & Tittley 1986; Brodie & Irvine 1997). *Porphyra dioica* Brodie & Irvine is one of the most common and, because it can be found throughout the year, it may be a good candidate for aquaculture development. *Porphyra dioica* was described (Brodie & Irvine 1997) as differing from *P. purpurea* (Roth) C. Agardh in being dioecious and forming packets of 8 zygotospores and 64 spermatangia, whereas *P. purpurea* is monoecious (male and female regions separated by a distinct line) and typically has packets of 16 zygotospores and 64–128 spermatangia. Another distinctive characteristic is the distribution of the reproductive cells: along the margins in *P. dioica* and scattered in *P. purpurea*.

This paper describes some aspects of the population dynamics of the gametophyte and the effects of environmental conditions on zygotospore (*sensu* Nelson *et al.* 1999) germination and on growth, phenology and reproduction of the conchocelis phase of *P. dioica*. The objective of this study was to provide basic knowledge of the biology of a Portuguese *Porphyra* that may be useful for future aquaculture development.

MATERIAL AND METHODS

Field study

A population of *P. dioica* was monitored during 2 years, in an area 20 km north of Porto, Portugal (41°19′37″N, 8°45′40″W). The percentage cover and the presence of reproductive individuals were assessed monthly, between February 2000 and May 2002, in 13 quadrats of 0.25 m^2 each, fixed to the rocky substrate. Biomass was estimated based on the weight of the material collected in 10 quadrats of 0.04 m^2 each. The site was selected based on previous observations that a persistent bed of *Porphyra* existed in the area.

* Corresponding author (rpereira@cimar.org).

Zygotospore isolation

Female specimens of *P. dioica* were collected from the coast at Porto (41°10′N, 8°59′W) in September 2000. The surface of the blades was cleaned gently with a cotton ball using sterilized seawater. Small areas of reproductive tissue were excised and placed in Petri dishes with Von Stosch's modified (Ott 1965) enriched medium (VSE) plus 4 mg l^{-1} germanium dioxide (GeO_2) (within the range suggested by Lewin 1966), and left overnight at 15°C, 25 μmol photons m^{-2} s^{-1} and a 12:12 h light–dark cycle. Light sources used in all experiments were cool-white fluorescent tubes. Liberated spores were transferred to new Petri dishes (100 mm diameter) containing VSE.

Germination experiments

Zygotospores were collected with a modified Pasteur pipette (the tip was drawn thinner in a Bunsen burner flame) and distributed into Petri dishes (60 mm diameter) at 25 and 75 μmol photons m^{-2} s^{-1} and at 5°C, 10°C, 15°C and 20°C, all at 12:12 h light–dark. Three replicate plates per condition were observed periodically (daily until day 12 and then every 2–3 days), and the number of zygotospores germinating within the first 50 random spore observations was recorded. The percentage of germinating zygotospores through time was calculated and analysed statistically with ANOVA (Rohlf & Sokal 1981). A *post hoc* analysis based on the ANOVA results was done with Student–Newman–Keuls (SNK) test to assess differences between temperatures (Rohlf & Sokal 1981). An α equal to 0.05 was used for all the tests.

Conchocelis growth experiments

Conchocelis colonies derived from a single strain (PD2-1, Marine Biotechnology Laboratory, UCONN) were placed in 0.5 litre flasks with VSE and gently aerated, under 15°C, 30 ± 5 μmol photons m^{-2} s^{-1} and a 12:12 h light–dark cycle for 4–5 weeks to increase biomass. This strain originated from a single zygotospore from material collected in Porto, as described in the zygotospore isolation procedure. For these experiments, conchocelis were ground, using a common kitchen grinder, and passed through screen filters. The resulting filaments, between 50 and 70 μm in length, were suspended in seawater. One millilitre aliquots of this mixture were inoculated into cell wells (6 well with lid; Corning, Acton, MA, USA) containing 10 ml of VSE and placed under different combinations of light (25 or 75 μmol photons m^{-2} s^{-1}), temperature (5°C, 10°C, 15°C or 20°C) and photoperiod (16:8 h, 12:12 h or 8:16 h light–dark). The medium was changed weekly.

The growth of conchocelis under the experimental conditions was recorded weekly, as an increase in the area (α). Area was estimated using the formula:

$$\alpha = \frac{\pi}{n} \times \left[\sum_{i=1}^{n} \left(\frac{D_i}{2} \right)^2 \right]$$

This formula is based on the area of a circle, where the ray ($D_i/2$) is obtained from the mean of two perpendicular diameter measurements for each conchocelis tuft (D_i).

The area values were used to calculate specific growth rates (μ), as the mean percent increase per day, using the formula:

$$\mu = (\mathrm{Ln}(\alpha_2/\alpha_1)) \times 100/t$$

where α_2 and α_1 are the conchocelis area at the end and at the beginning of the experiment, and t is the number of days. This formula (DeBoer *et al.* 1978), which assumes that growth is exponential, was also used by Chopin *et al.* (1999) and Stekoll *et al.* (1999) for conchocelis growth rate measurement.

The growth rates were analysed using a three-way ANOVA to assess differences between the three factors and possible interactions. For this analysis, the growth rate value from week 2 to 4 of each replicate was used because the first week was considered an adaptation period. The growth rates for 5°C were not used because growth under short-day conditions was not tested at this temperature, and this would cause an asymmetry in the analysis. A *post hoc* analysis was performed using the SNK test for differences between temperature and between photoperiod treatments (Rohlf & Sokal 1981). An α equal to 0.05 was used for all the tests.

Conchosporangia formation and conchospore release

The filaments grown under the conditions described above were also monitored for conchosporangium formation and conchospore release. The number of conchocelis with conchosporangia, within the first 30 randomly observed conchocelis in each well, was recorded weekly. The percentage of reproductive conchocelis was calculated. Simultaneously, vegetative conchocelis tufts from several conditions were transferred to different environmental conditions. Several combinations of changes in temperature, photoperiod and PFD were tested for conchosporangium formation and maturation. For these purposes, a PFD of 5 μmol photons m^{-2} s^{-1} was also tested. These conchocelis were also followed weekly for conchosporangium formation. Simultaneously, some gently aerated cultures, maintained as a source of inoculum for other experiments, were also followed for conchosporangium formation.

The ability of the conchospores to attach to nets typically used in *Porphyra* cultivation was also assessed. Pieces of synthetic string were added to the cultures with mature conchosporangia, and the attachment of the conchospores was confirmed by observation using a dissecting microscope.

RESULTS

Field observations

Porphyra dioica can be found on the rocky shores of the north coast of Portugal throughout the year. It is often attached to a rocky substrate in the eulittoral zone above the area with species of *Fucus* Linnaeus and below that of *P. umbilicalis* (Linnaeus) Kützing, but may be found dispersed among these other algae. During the period of study, *P. dioica* revealed an amazing capacity to tolerate periods of sand cover lasting several days. The severity of these episodes sometimes prevented the assessment of percent cover (Fig. 1), when the quadrats were completely buried. That was the case in March and May 2000 (the value for May 2000 is based on only one quadrat). Nonetheless, the algae subsequently reappeared in high densities. From February 2001 until May 2002 the percentage cover of *P. dioica* in Mindelo (20 km north of Porto) ranged

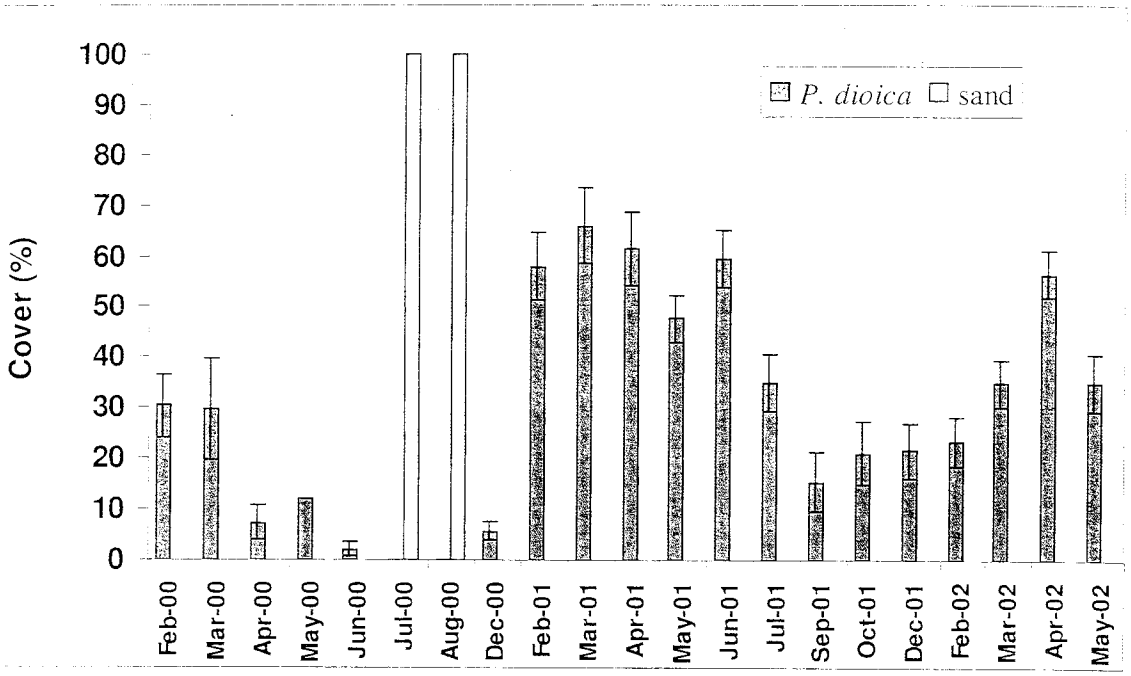


Fig. 1. Percentage cover of *Porphyra dioica* on Mindelo beach, 20 km north of Porto. Bars represent $\bar{x} \pm s_{\bar{x}}$ of 9–13 0.25 m^2 quadrats, affixed to the rock.

from 15% to 66%, corresponding to 80–714 g fresh weight m^{-2}, being higher during spring months (Fig. 1). Male and female individuals were found in the population all year long.

The morphological characters of the specimens from which strains were isolated for life history studies are presented in Table 1 and Figs 2–9. The specimens had morphological and cytological characters that, in general, matched the description of this species (Brodie & Irvine 1997). However, the diameter of vegetative cells of the Portuguese plants ranged from 9.9 to 21 μm compared to the 10–16 μm recorded by those authors. The zygotosporangium packets were larger in the specimens used in this work, 40–48 μm × 24–36 μm in surface view, vs the 19–22 μm × 17–20 μm of Brodie & Irvine (1997). Species identification was confirmed by Chris Neefus using restriction fragment length polymorphism assay comparison, as described by Teasdale *et al.* (2002).

Zygotospore germination

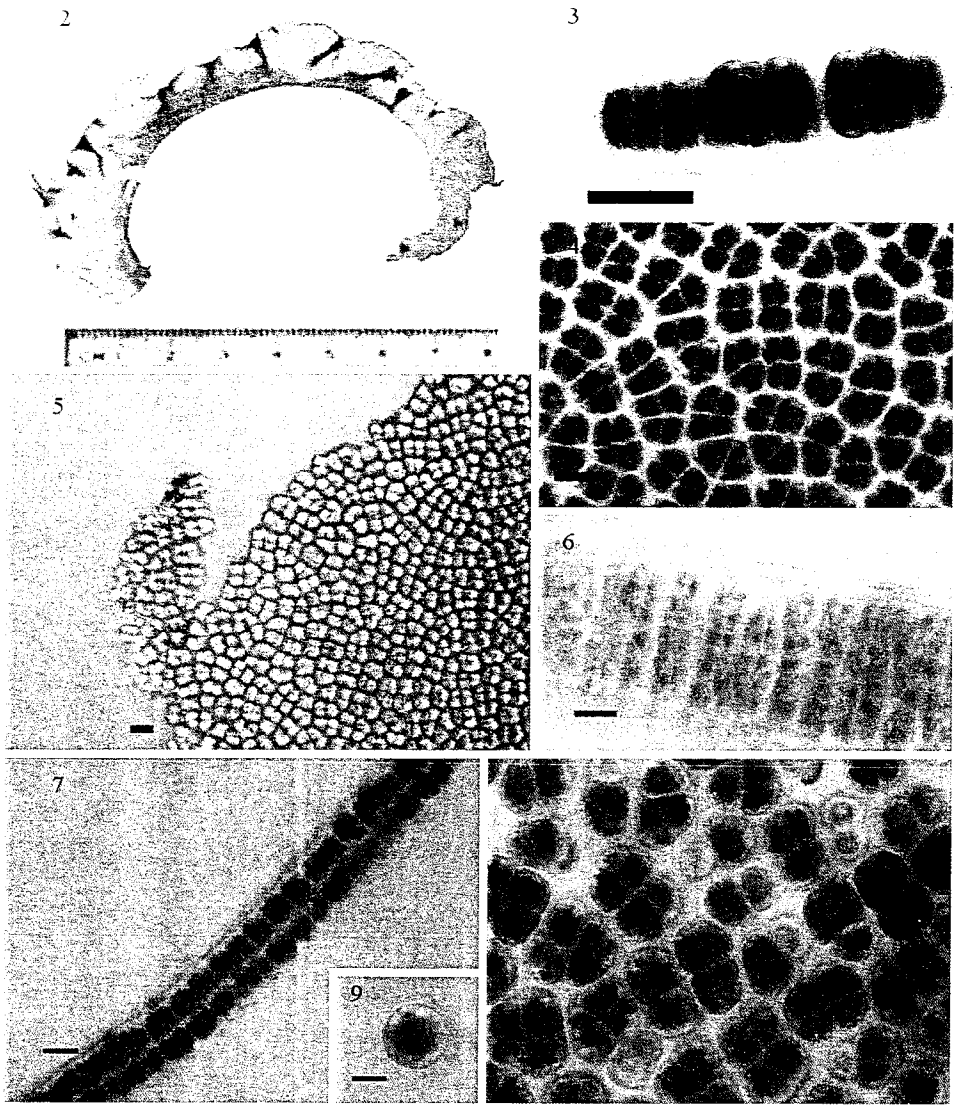
The germination rates of the zygotospores were higher at 15°C and 20°C than at other temperatures, regardless of the PFD (25 or 75 μmol photons m^{-2} s^{-1}; Fig. 10). According to the ANOVA using germination data from day 20, there was no significant difference between 25 and 75 μmol photons m^{-2} s^{-1} ($P > 0.05$) nor was there any interaction between light and temperature. On the other hand, there was a significant difference between temperatures ($P < 0.01$). The SNK test showed that there were differences in germination between 5–10°C and 15–20°C but not within these temperature pairs.

The change in germination rates through time (Fig. 11) combines data from 15°C and 20°C and compares only the two PFDs (because there was no difference in the germination rates between these two temperatures). At 25 μmol photons m^{-2} s^{-1}, 40% germination was recorded on the third day.

Table 1. Morphological characters of *P. dioica* specimens collected in Porto, Portugal.

	Vegetative thallus	Female thallus	Male thallus
Thallus thickness (μm)	1 cell layer: 52–65	65–76	70–74
Cell size in surface view (μm)	10–21[1]	12–18 × 18–24[2]	4–7[3]
Cell height in cross section (μm)	38–39	10–24	± 7.5
Cell length in cross section (μm)	12–15	8–22	7–9
Arrangement		2 × 2 × 2	2 × 4 × 8
Packet size in surface view (μm)		24–36 × 40–48[4]	13–17 × 20–25[5]

[1] Minimum and maximum cell lengths observed in cells in the centre of the thallus, away from the margins with reproductive or growing areas. Cells have irregular shapes.
[2] Minimum and maximum length and height of the cells in surface view. Cells are square to rectangular.
[3] Minimum and maximum diameters. Cells are round.
[4] Minimum and maximum packet length and height in surface view. Packets are slightly rectangular.
[5] Minimum and maximum packet length and height in surface view. Packets are rectangular.



Figs 2–9. Morphology of *Porphyra dioica* from Porto, Portugal. Scale bars = 20 μm (Figs 4–6, 8, 9) or 40 μm (Figs 3, 7).
Fig. 2. Mature female specimen.
Fig. 3. Cross section of vegetative area showing one cell layer.
Fig. 4. Surface view of vegetative cells.
Fig. 5. Surface view of spermatangia.
Fig. 6. Cross section of spermatangia.
Fig. 7. Cross section of zygotosporangia.
Fig. 8. Surface view of zygotosporangia.
Fig. 9. Zygotospore.

However, these high germination rates declined during the following days and, on the eighth day, were not significantly different ($P > 0.05$) from those at 75 μmol photons m^{-2} s^{-1}. At 75 μmol photons m^{-2} s^{-1}, only after 11–15 days was germination higher than 10% (Fig. 11). At 20°C and 25 μmol photons m^{-2} s^{-1}, germination levels of 90% were achieved after 22 days (data not shown), although this value is not significantly different from the one at 15°C and the same PFD ($P > 0.05$). At higher PFD, levels of 90% germination were recorded only on day 30.

The influence of temperature on germination was also confirmed through transferring zygotospores to different temperatures. At 25 μmol photons m^{-2} s^{-1}, zygotospores initially grown at 5°C and 10°C were transferred to 20°C on day 22. This caused an increase in the germination rate up to 60% and 89%, respectively, on day 30. This indicates that temperatures as low as 5°C, below those recorded in Portuguese coastal waters, are not lethal for zygotospores at least for up to 22 days of exposure.

Conchocelis growth

Well-developed conchocelis tufts were regenerated, in all conditions, starting from mostly single unbranched filaments 50–70 μm in length. No protothalli were produced. No occurrence of neutral conchospores or conchocelis archeospores (*sensu* Nelson *et al.* 1999) was noted. The cells of the filament were usually 7.5 ± 2.4 μm (actual range) in diameter and 76.6 ±

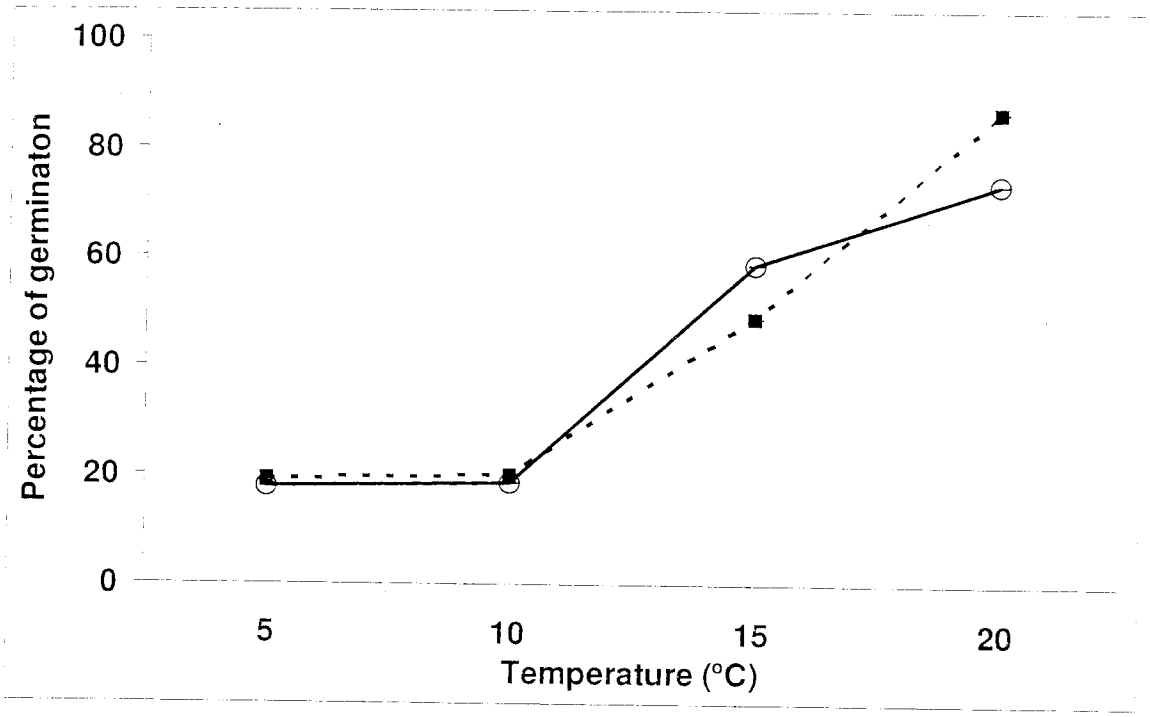


Fig. 10. *Porphyra dioica*. Percentage germination of zygotospores after 20 days in culture under different temperatures (5°C, 10°C, 15°C, 20°C) and light intensities (■, 25; ○, 75 μmol photons m^{-2} s^{-1}). Germination calculated from the first 50 random observations of spores. Values represent $\bar{x} = s_{\bar{x}}$, $n = 3$.

7.4 μm long, and no differences in these characters were noted under any of the growth conditions. On the other hand, the conchocelis produced many more ramifications when grown at 15°C and 20°C, regardless of the photoperiod and PFD. While the conchocelis at 5°C did not grow much until the fourth week, those at 10°C grew well but were less branched than the ones at 15°C and 20°C (data not shown). This difference gradually disappeared during the following 4–5 weeks. The conchocelis at 10°C then acquired a morphology similar to the conchocelis at the higher temperatures.

The average growth rate of the conchocelis was higher at 15°C in all photoperiods, independent of irradiance tested

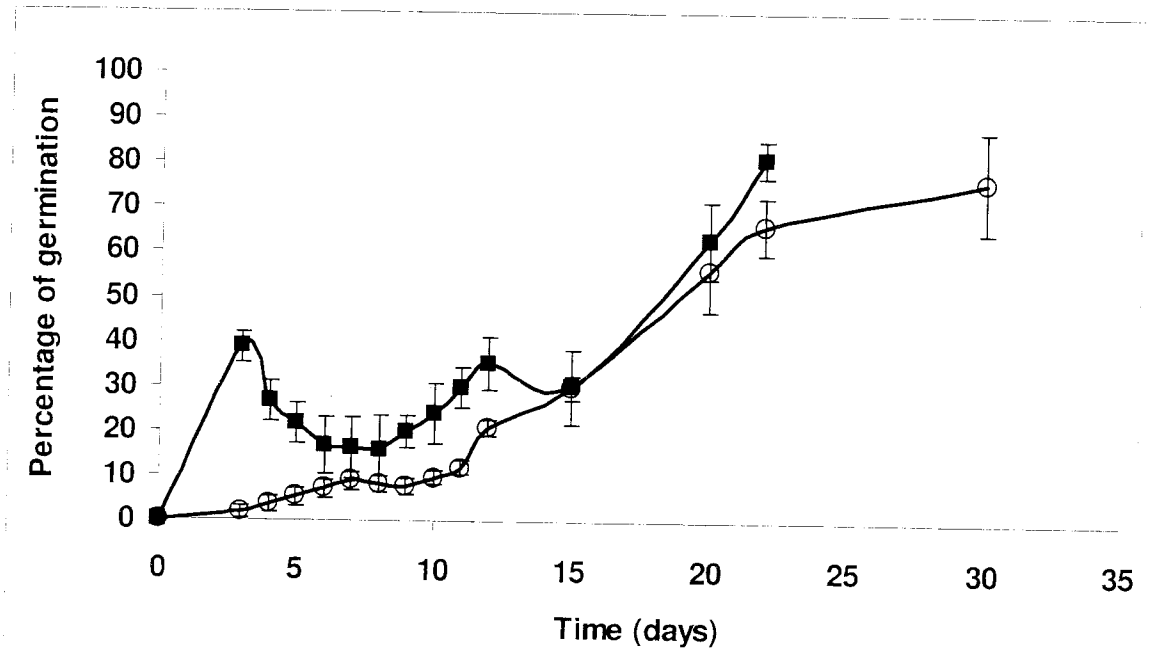


Fig. 11. *Porphyra dioica*. Change in mean percent germination of zygotospores at 15°C and 20°C under neutral-day conditions as a function of PFD (■, 25; ○, 75 μmol photons m^{-2} s^{-1}). Germination calculated from the first 50 random observations, 6 replicates per condition, with corresponding $s_{\bar{x}}$.

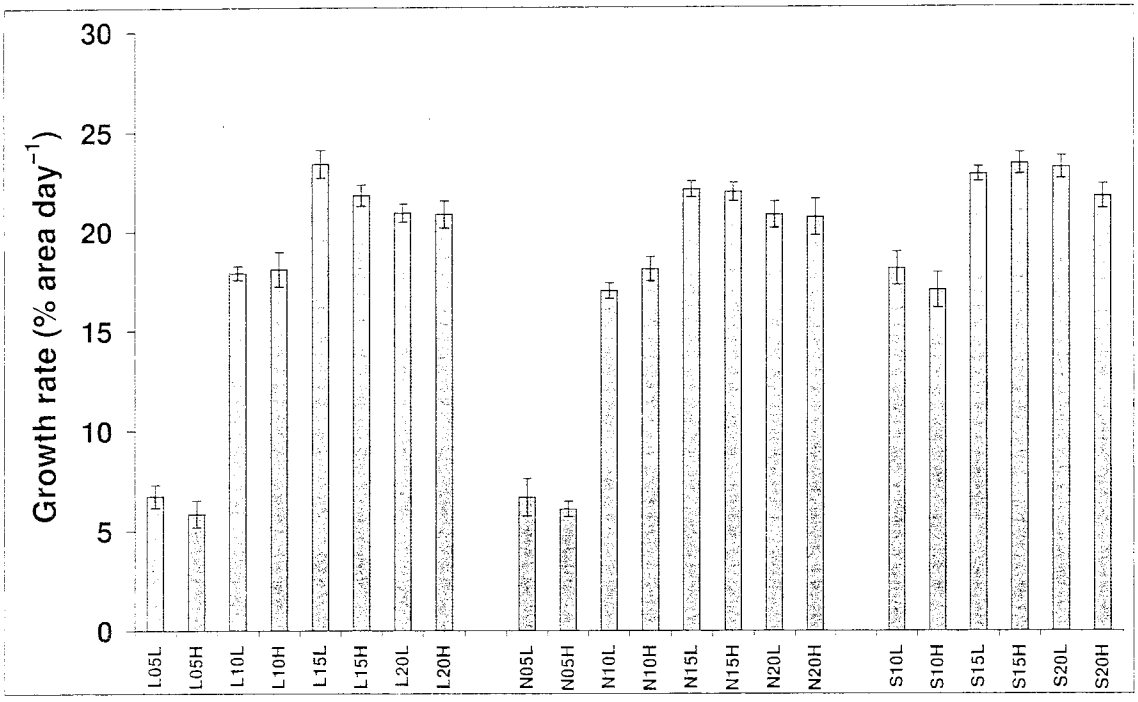


Fig. 12. *Porphyra dioica*. Growth rates of conchocelis under a combination of photoperiods, PFD and temperatures. L, N or S stands for 16 : 8 h, 12 : 12 h or 8 : 16 h light–dark cycles, respectively; 05, 10, 15 or 20 stands for the temperature in °C; L or H stands for low-light (25 μmol photons m^{-2} s^{-1}) or high-light (75 μmol photons m^{-2} s^{-1}), respectively. Bars represent $\bar{x} \pm s_{\bar{x}}$, $n = 6$, after 21 days in culture.

(Figs 12, 13). At 15°C and 20°C, the average area increase was always more than 20% day^{-1}, regardless of the photoperiod. During the same period, for 10°C and 5°C, the maximum mean growth rates recorded were 18.1% and 6.7%, respectively.

According to the three-way ANOVA, there were differences between temperatures ($P < 0.001$) and between photoperiods ($P < 0.05$) but not between the two PFDs tested ($P > 0.05$), and there were no interactions between any of the factors. The SNK test revealed that growth rates at 5°C were clearly different from those at all other temperatures. In respect to photoperiod, the SNK test detected a difference be-

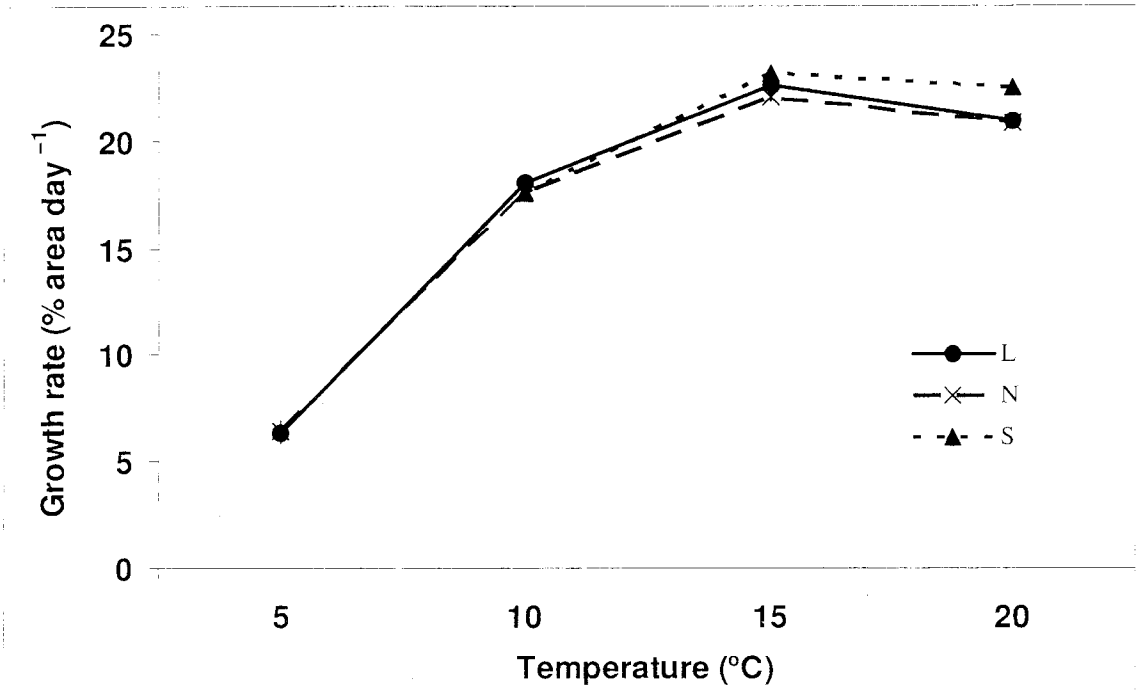
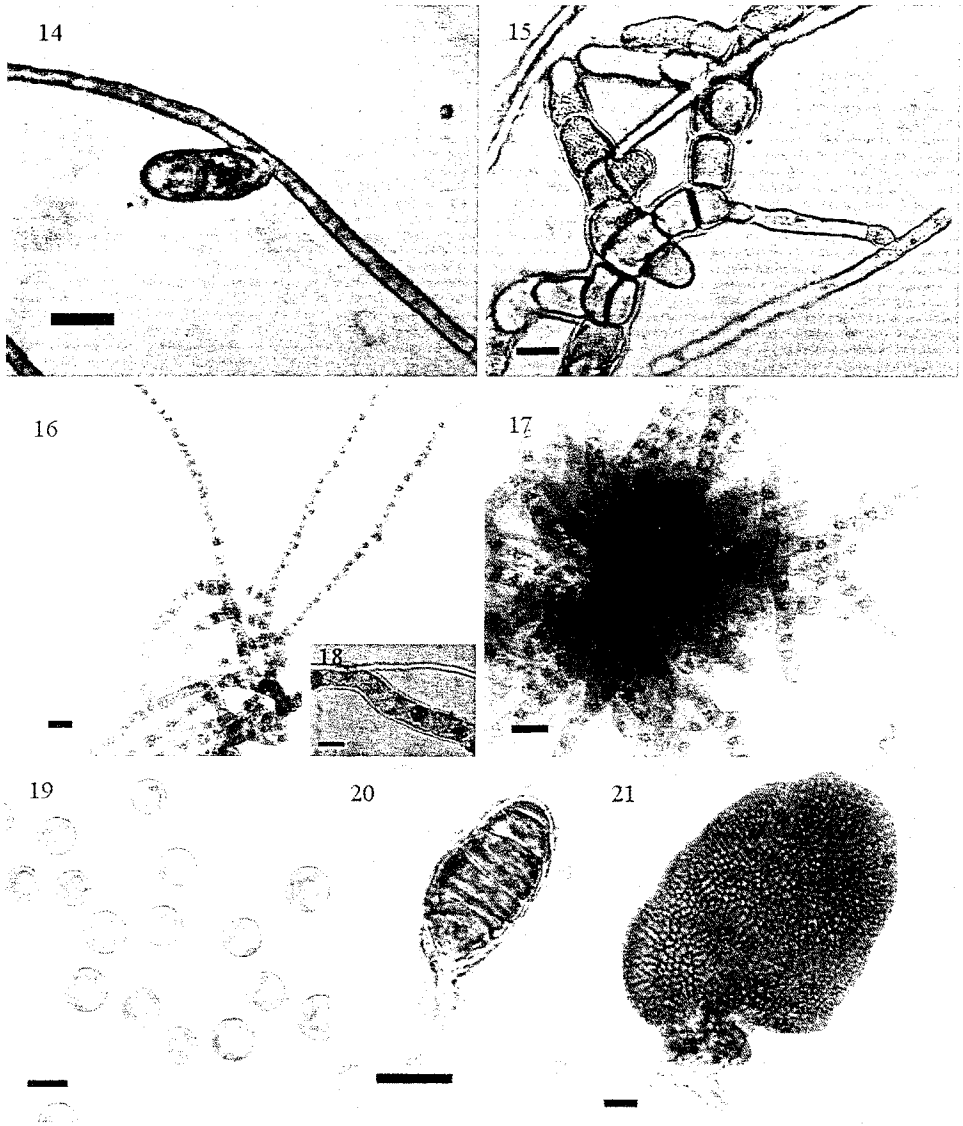


Fig. 13. *Porphyra dioica*. Average growth rates of conchocelis under a combination of photoperiods, PFD and temperatures. L, N or S stands for 16 : 8 h, 12 : 12 h or 8 : 16 h light–dark cycle, respectively; dots represent $\bar{x} \pm s_{\bar{x}}$, $n = 12$, after 21 days in culture.



Figs 14–21. Details of different stages in the life cycle of *Porphyra dioica* in culture. Scale bar = 20 μm (Figs 14, 15, 18–20), 40 μm (Figs 16, 17) or 50 μm (Fig. 21).

Fig. 14. Conchosporangial branch arising laterally from a vegetative filament, after 8 weeks at 15°C and 25 μmol photons m^{-2} s^{-1} under 8:16 h light–dark.

Fig. 15. Conchosporangial branch formed from the apical cell of a vegetative filament; same conditions as Fig. 14.

Fig. 16. Conchosporangial tuft formed by conchocelis growing on shell.

Fig. 17. Conchosporangial tuft formed by conchocelis growing on the surface of a Petri dish.

Fig. 18. Detail of conchosporangial cells.

Fig. 19. Released conchospores.

Fig. 20. Young thallus after 3 days at 15°C and 25 μmol photons m^{-2} s^{-1} under 16:8 h light–dark.

Fig. 21. Blade after 3.5 weeks.

tween growth rates at 8:16 h light–dark in relation to those at 12:12 h light–dark, but not between 12:12 h light–dark and 16:8 h light–dark, nor between 8:16 h light–dark and 16:8 h light–dark.

Conchosporangium formation and conchospore release

Conchosporangial branches can form either from the apical cell of a vegetative filament or laterally from cells in the middle of a vegetative filament (Figs 14, 15). The morphology of the conchosporangial tufts differed depending on the substrate. Those growing on shells were not as branched (Fig. 16) as those attached to the bottom of Petri dishes (Fig. 17). The conchosporangial branches in the *P. dioica* conchocelis were 20 ± 1 μm in diameter. In lateral view, the cells were square to rectangular (up to 37.8 μm long) when immature (Fig. 18). Released conchospores were 20 ± 2.5 μm in diameter (Fig. 19) and germinated to form new thalli. The young blades were approximately 53 μm long after 3 days and more than 500 μm long after 3.5 weeks (Figs 20, 21).

Conchosporangia were formed mainly in nonaerated cultures but also in aerated cultures with attached conchocelis. In aerated cultures with free-floating conchocelis, some larger cells were observed, but they never formed typical conchosporangia. In stationary cultures, the first conchosporangia

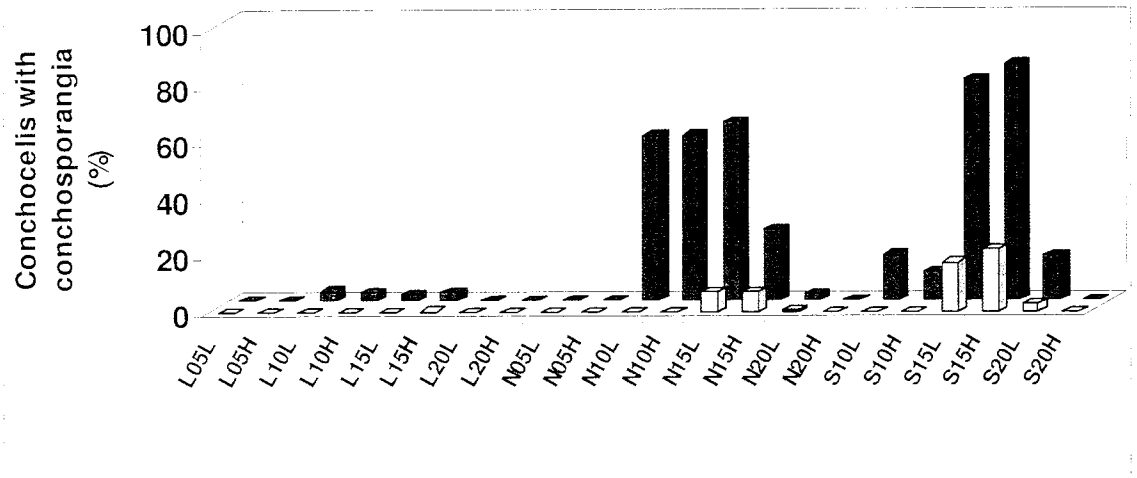


Fig. 22. *Porphyra dioica*. Percent conchocelis with conchosporangia after 7 (white columns) and 15 weeks (dark columns) in culture, under a combination of photoperiods, PFD and temperatures. L, N or S stands for 16 : 8 h, 12 : 12 h or 8 : 16 h light–dark cycles, respectively; 05, 10, 15 or 20 stands for the temperature in °C; L or H stands for low-light (25 μmol photons m^{-2} s^{-1}) or high-light (75 μmol photons m^{-2} s^{-1}), respectively. Columns represent the average of six replicates in each condition, using the first 30 random observations in each replicate.

were observed 6 weeks after germination of the zygotospores, at 15°C, 8 : 16 h and 12 : 12 h light–dark, but also at 20°C, 12 : 12 h and 16 : 8 h light–dark. However, after 15 weeks, it was clear that the higher frequencies of conchosporangium formation were achieved at 15°C and 8 : 16 h light–dark, without significant difference between the two PFDs (Fig. 22). Under these conditions, around 77% and 83% of the conchocelis formed conchosporangial branches at 25 and 75 μmol photons m^{-2} s^{-1}, respectively. In 12 : 12 h light–dark, at 10°C the frequencies were around 58% for both PFDs, whereas at 15°C the frequencies were 62% and 22% for 25 and 75 μmol photons m^{-2} s^{-1}, respectively.

At 20°C and 8 : 16 h light–dark, the frequencies recorded were not reliable. In these conditions we experienced problems with contaminants that prevented normal development of the conchocelis. The 16% value for 20°C and 8 : 16 h light–dark at 25 μmol photons m^{-2} s^{-1} is based on only one of the six replicates.

There is a clear difference in the frequency of conchosporangium formation when the conchocelis were transferred to an 8 : 16 h light–dark cycle, in relation to those transferred to a 12 : 12 h or a 16 : 8 h light–dark cycle (Table 2). In the transfers to or within an 8 : 16 h light–dark cycle, only the ones moved from 15°C to 10°C did not produce any conchosporangia. At 20°C, conchocelis transferred from a 12 : 12 h to an 8 : 16 h light–dark cycle formed conchosporangia with a frequency of 36.6% after 12 weeks. A very low percentage (15% or less) of conchocelis formed conchosporangia when transferred to 12 : 12 h light–dark. This frequency was even smaller when the conchocelis were transferred to a 16 : 8 h

Table 2. Frequency of conchocelis with conchosporangia 12 weeks after transfer of 4.5 week old cultures to different photoperiods, temperatures or both. Values are the means of two replicates for each new condition, and frequency is based on the first 30 random observations. N, S and L, 12 : 12 h, 8 : 16 h or 16 : 8 h light–dark cycles, respectively; 10, 15 and 20, temperature in °C; L and H, 25 and 75 μmol photons m^{-2} s^{-1}, respectively.

From N to (or within) short-day			From N to (or within) long-day			From L or S to (or within) neutral-day		
Origin	New	%	Origin	New	%	Origin	New	%
N15L	S15L	11.6	N15L	L15L	0.0	S15L	N15L	0.0
N20H	S15H	35.5	L15H	L15L	0.0	L15H	N15L	0.0
S20H	S15H	20.0	N15L	L15H	0.0	S15L	N15H	6.6
S15L	S10L	0.0	N15H	L15H	0.0	S15H	N15H	1.6
N20H	S20H	36.6	N15H	L20H	0.0	L15H	N15H	6.6
			L15H	L20H	0.0	N20H	N15H	15.0
			N20H	L20H	1.6	N10H	N15H	5.0
						N15L	N10L	0.0
						N15H	N20H	0.0
	Average	20.7		Average	0.2		Average	3.8

Table 3. List of other conditions tested but which did not produce conchospore release. All transferred conchosporangia were 10 weeks old and were formed at 15°C, 8 : 16 h light–dark and 25 μmol photons m^{-2} s^{-1}.

Temperature (°C)	Photoperiod (h light:dark)	PFD (μmol photons m^{-2} s^{-1})	Aeration	Time (weeks)
10	8 : 16	25	no	6.6
10	8 : 16	5	no	4.6
10	12 : 12	25	no	6.6
10	12 : 12	25	yes	6.6
10	12 : 12	5	yes	4.6
10	12 : 12	10	no	4.6
15	8 : 16	25	no	6.6
15	12 : 12	25	no	6.6
15	12 : 12	25	yes	6.6
15	12 : 12	5	no	4.6
15	12 : 12	5	yes	4.6
20	8 : 16	25	no	6.6
20	8 : 16	10	no	4.6
20	8 : 16	25	yes	6.6
20	12 : 12	25	no	6.6
20	12 : 12	25	yes	4.6

light–dark cycle. It is interesting to note, however, that the only changes in cultures transferred to 15°C and a 12 : 12 h light–dark cycle were observed at 75 μmol photons m^{-2} s^{-1}. These cultures were originally at the same temperature but different photoperiods or under the same photoperiod but at different temperatures. The same transfers but to 25 μmol photons m^{-2} s^{-1} did not produce any conchosporangia. These results are different from those observed in cultures not subjected to any change in conditions, where there was no difference between the two light intensities. In any case, in the cultures transferred to or within neutral-day, the highest frequency of conchocelis with conchosporangia was only 15%.

Conchospores of *P. dioica* (Fig. 19) were first released, after 18.1 weeks, only at 15°C and an 8 : 16 h light–dark cycle. These conchocelis were maintained at 5 μmol photons m^{-2} s^{-1} for 9 weeks, followed by an increase in light and the introduction of aeration. Although the conchosporangia were formed under 25 and 75 μmol photons m^{-2} s^{-1}, a period of 9–10 weeks under 5 μmol photons m^{-2} s^{-1} seems to promote faster maturation of the conchospores and release even without aeration. Conchosporangia transferred back to 25 μmol photons m^{-2} s^{-1}, after 9 weeks at 5 μmol photons m^{-2} s^{-1}, also released spores at the same time or slightly sooner if aerated.

When the conchosporangia were transferred from 15°C, 8 : 16 h light–dark and 25 μmol photons m^{-2} s^{-1} to other conditions, only the ones at 10°C, same photoperiod and light, released spores and generated blades. No released spores were observed in any of the other conditions tested (Table 3).

Conchosporangia maintained at 15°C, 8 : 16 h light–dark, 25 μmol photons m^{-2} s^{-1} and aerated released spores that attached to the bottom and walls of the flask and formed young blades (Figs 20, 21), first visible 21.6 weeks after the beginning of the experiment and 4.6 weeks after aeration was started. Young blades were also visible in cultures transferred from 15°C to 10°C, still in 8 : 16 h light–dark and 25 μmol photons m^{-2} s^{-1}, with aeration, 6.6 weeks after the transfer and the start of aeration. Under these conditions the exact timing of the spore release was not determined. The conchocelis were free-floating in 1 litre flasks, and released conchospores were never observed. We assume that the release occurred between the 18th and 20th weeks in culture and around 3 weeks after the beginning of aeration.

Another option for spore release is to use the conchosporangia produced in 15°C and 12 : 12 h light–dark. These cultures had mature conchosporangia after 19 weeks and released conchospores, but only 1–2 weeks after transfer to 15°C or 10°C and 8 : 16 h light–dark. Optimal conditions for the growth and reproduction of the conchocelis stage are summarized in Table 4.

Young blades were not obtained from the spores released at 15°C, 8 : 16 h light–dark, 5 and 25 μmol photons m^{-2} s^{-1} and not aerated. Only two spores were observed germinating under each of these conditions, but none of them developed into a macroscopic blade. Conchospores transferred from 25 to 75 μmol photons m^{-2} s^{-1}, within that same photoperiod, as well as conchospores transferred from 8 : 16 h to 12 : 12 h light–dark, 25 and 75 μmol photons m^{-2} s^{-1}, always at 15°C and kept without aeration, did not germinate.

Conchospores attached to synthetic string commonly used for nori cultivation, as well as to PVC tubing and glass. Germination and subsequent formation of gametophytes occurred on all three substrates. No archeospores or any other kind of asexual spore (*sensu* Nelson *et al.* 1999) were produced by the young blades in culture.

DISCUSSION

The germination rate of the zygotospores of *P. dioica*, under neutral-day, is higher at 15°C and 20°C. Despite the clear advantage of the use of temperatures between 15°C and 20°C, the growth rate of the conchocelis at 10°C cannot be neglected. Conchocelis cultivated at 10°C grow and acquire normal morphology, although slowly, and the conchocelis initially have fewer ramifications than the ones grown at higher temperatures. The results also show that 5°C delays, but does not stop, growth of the conchocelis after a period of acclimation. The conchocelis can even continue to grow during the first week after being transferred. This indicates an acclimation capacity to temperatures below those that are usual in Portuguese coastal waters.

Germination rates of zygotospores were independent of the PFD, after 20 days, within the range tested. Although the first impression is that the lower light promotes faster germination, the high germination values after only three days rapidly decline. After 1 week, the difference between the two light levels is not significant. This variation is probably explained by the death of some of the first spores to germinate. Despite this, 25 μmol photons m^{-2} s^{-1} should be preferred over the higher irradiance for zygotospore germination and isolation of conchocelis. Besides the energy saving, which may not be critical at the laboratory level but is important at larger scales, conchocelis at 25 μmol photons m^{-2} s^{-1} were generally more pigmented.

This result shows that the conchocelis of *P. dioica* does not require high PFD, as was observed in other *Porphyra* species. Waaland *et al.* (1990) found that the conchocelis of *P. abbottae* V. Krishnamurthy, *P. nereocystis* C.L. Anderson, *P.*

Table 4. Summary of the best conditions for growth and reproduction of the conchocelis of *Porphyra dioica*.

	Temperature (°C)	Photoperiod (h light–dark)	PFD (μmol photons m^{-2} s^{-1})	Time (weeks)
Growth of conchocelis	15–20	8:16, 12:12, 16:8	25–75	4–6
Conchosporangium formation[1]	more at 15 also at 10	more at 8:16 also at 12:12	25–75	6–7
Conchosporangium maturation	15	8:16, 12:12	5–25[2]	± 10
Conchospore release	10[3] and 15	8:16	25–75	1–2 weeks when transferred from 12:12 to 8:16

[1] Formation occurred only in nonaerated cultures or in cultures aerated but with the conchocelis attached to glass or to shell.
[2] Maturation seemed to be promoted under 5 μmol photons m^{-2} s^{-1}.
[3] Release at 10°C and 8:16 h light–dark occurred in cultures transferred from 15°C, both 8:16 h and 12:12 h light–dark cycles and aerated.

fallax S.C. Lindstrom & K.M. Cole (as *P. perforata* J. Agardh; Lindstrom & Cole 1990), *P. pseudolanceolata* V. Krishnamurthy and *P. torta* V. Krishnamurthy, from Washington State, USA, all grew well from 5 to 100 μmol photons m^{-2} s^{-1}, with growth light-saturated at 5 μmol photons m^{-2} s^{-1} in some cases. In species like *P. amplissima* (Kjellman) Setchell & Hus [as *P. miniata* (C. Agardh) C. Agardh; Lindstrom & Cole (1993)], the conchocelis also displays a wide tolerance to light intensities, growing from 200 to 5000 lux – equivalent to *c.* 3.6–90 μmol photons m^{-2} s^{-1} (Chen *et al.* 1970). An interesting case is that of the conchocelis of *P. abbottae*. Stekoll *et al.* (1999), using a culture from Alaska, reported growth inhibition in light over 40 μmol photons m^{-2} s^{-1} at 15°C or higher, whereas the conchocelis from the same species collected in Washington was found by Waaland *et al.* (1990) to show good growth in the range 10–15°C and 5–100 μmol photons m^{-2} s^{-1}. The only difference between the two cultures was their origin. This example emphasizes the need for independent investigations of species collected from distant locations and probably adapted to local conditions.

There are not many studies that compare conchocelis growth under different photoperiods. The gametophytes of most *Porphyra* species are seasonal, and the conchocelis phase typically occurs during the opposite season or during the season that precedes the gametophyte season. Therefore, previous research has usually focused more on temperature and other factors, using photoperiod only to trigger spore formation and release (e.g. Chiang & Wang 1980; Waaland *et al.* 1990; Lewmanomont & Chittpoolkusol 1993; Nam-Gil 1999; Stekoll *et al.* 1999). Due to the year-round presence of the gametophytes of *P. dioica*, we were interested in finding out what happens to the sporophyte under different photoperiods. The conchocelis of *P. dioica* showed growth rates higher than 20% day^{-1} in all photoperiods between 15°C and 20°C and grew also between 5°C and 10°C. This result suggests that the conchocelis of *P. dioica* can be present throughout the year on the north Portuguese coast. Notoya & Miyashita (1999) found a similar result for *P. moriensis* H. Ohmi, which grew equally well at 10:14 h and 14:10 h light–dark cycles at 15–25°C, and survived from 5°C to 25°C.

In regard to conchosporangium formation and release, it is possible to find species with very specific requirements; on the other hand, there are others that form conchosporangia in a wide range of conditions and others that need a sequence of changes in conditions. The main factor is photoperiod, although a more or less specific combination of temperature and irradiance is required, depending on the species. Species like *P. abbottae*, *P. fallax* (as *P. perforata*), *P. pseudolanceolata* and *P. nereocystis* (Waaland *et al.* 1990) all respond to photoperiod but have different temperature and light requirements. *Porphyra abbottae* produces conchosporangia from 5 to 300 μmol photons m^{-2} s^{-1} but needs temperatures between 12°C and 15°C. *Porphyra nereocystis*, on the other hand, is less temperature-specific, needing 10–15°C, but requires exactly 10 μmol photons m^{-2} s^{-1}. Other species, like *P. torta* (Waaland *et al.* 1990), *P. columbina* Montagne (Avila *et al.* 1986) and *P. leucosticta* Thuret (Sidirelli-Wolff 1992) respond to two different photoperiods. An interesting species, which shows a total absence of response to photoperiod for conchosporangium formation, is *P. angusta* Okamura & Ueda (Chiang & Wang 1980). This species has a frequency of con-

chosporangium formation of 100%, or very close to it, as long as cultures are at 27–29°C, but no information is given on the requirements for conchospore release.

Porphyra dioica is able to form conchosporangia in all photoperiods tested, from 10°C to 20°C and from 25 to 75 μmol photons m^{-2} s^{-1}, always without aeration. No conchosporangia were observed in aerated cultures with free-floating conchocelis. This result is hard to explain because, obviously, there are no still waters in the natural habitat. On the other hand, conchocelis aerated while attached to the flask or to pieces of shell, and therefore not free-floating, did produce conchosporangia. There are other reports of species that rarely, if ever, form reproductive structures if the plants are not attached (Lobban & Harrison 1994). Norton & Mathieson (1983) reject the lack of a fixed orientation as the cause for this infertility. Instead they suggest, among other possibilities, that unattached seaweeds become locked into a juvenile stage in which they are unresponsive to the environmental factors that typically would trigger reproduction.

The frequencies of conchosporangium formation obtained under neutral-day, 10°C and 15°C should not be neglected. Although conchosporangium formation is not very condition-specific, maturation and release occur preferentially under a combination of short days and 15°C. This is also the condition that produces more conchosporangia, thus making photoperiod and temperature important factors. The observations during this study suggest that the kind of culture (free-floating or fixed) and substrate influence the frequency of conchosporangium formation and also maturation and release. Typical conchosporangial branches and clearly mature conchosporangia were never observed in free-floating cultures. Moreover, conchocelis grown on shells seem to produce more conchosporangia than those grown on glass. This is based on simple observation, and no quantitative data are available at present. Further studies are needed, specifically designed to understand the possible interactions between the kind of substrate, water motion and the frequency of conchosporangium formation.

The different branching patterns of the conchosporangial tufts are probably related to the direction from which the filaments receive light. Filaments growing attached to shells were clearly less branched than those attached to glass, as mentioned earlier. The filaments growing on glass receive light from all directions except from below, whereas the ones on the shells seem to grow towards the light, received mainly from above. However, we have no explanation as to how light direction affects branching.

Besides temperature and photoperiod, light intensity plays an important role in maturation of the conchospores. Our results show that a period of 9 weeks at 5 μmol photons m^{-2} s^{-1} promotes maturation and release of conchospores. This happens without water movement, apart from that unavoidably caused by handling the cultures. Conchospores also matured and were released in the cultures at 25 μmol photons m^{-2} s^{-1} when aeration was provided.

Blades were only obtained at 10–15°C with aeration. The temperature range for blade formation agrees with that found on the north coast of Portugal during the months of higher abundance of gametophytes in the field, i.e. February to April. The first attempts to grow blades showed that they grew under all three photoperiods, at least at 15°C.

The wide tolerance of the zygotospores and conchocelis to the range of temperatures tested allows for the occurrence of conchocelis throughout the year, just as happens with the gametophytes. However, a continuous production of spores and constant renewal of the gametophyte population is unlikely to occur. Besides the fact that the presence of the conchocelis phase in nature has not been confirmed by us, conchospore release in the laboratory required a particular combination of temperature, photoperiod and PFD. These observations preclude the possibility of a constant renewal of gametophytes in nature. Our fieldwork also shows that there is a clear increase in the population during winter and early spring months. With a constant renewal of the population, we would expect percentage cover to be more stable throughout the year. On the other hand, the fact that we were able to grow blades under all three photoperiods supports the idea of a possible continual renewal of the population. The possibility of a constant renewal of the population, maybe with different intensities through the year, cannot be excluded. Holmes & Brodie (2004) also reported the occurrence of *P. dioica* throughout the year at Sidmouth Bay (Devon, UK), with young blades present during all seasons, suggesting a constant renewal of the population. In their study, the highest proportion of individuals that were < 1.00 g wet mass was recorded in February. This agrees with the period of increase in percentage cover (late winter to spring months) in our study, preceded by the period in which natural conditions matched those that resulted in conchospore release in the lab.

One explanation for the presence of gametophytes in nature all year long could be the formation of archeospores or other kind of asexual spore, although such spores were never observed in the laboratory. Nor is there, to our knowledge, information on how long gametophytes can last in nature. From our experience, blades produced in the lab grow for several weeks (or up to 2 months), and then reproduction is followed by disintegration.

In conclusion, our results show that the main factor controlling the growth of conchocelis is temperature, assuming that nutrients are not limiting. Conchosporangia production by *P. dioica* requires a combination of short days at 15°C, without aeration, and the release of conchospores is promoted by a period of time under very low light. Aeration is also crucial for the germination of the conchospores and production of blades.

One of the key factors for the success of *Porphyra* cultivation is the establishment of a constant and readily available supply of 'seedstock' of juvenile organisms (Yarish *et al.* 1998, 1999). The simplicity of requirements for conchocelis growth and conchospore production makes *P. dioica* a good candidate for cultivation. Some species require more specific conditions or a specific chain of events, in terms of temperature and photoperiod, in order to complete their life cycles; this increases the complexity and cost of operation. Working at a commercial scale, the simplicity of the processes and low costs are important factors. The possibility of a constant natural renewal of the gametophytes can allow several harvests without the intervention of the conchocelis phase. This characteristic, if confirmed by our ongoing work, is also important for aquaculture purposes. The best conditions for the growth of the gametophytes and their possible applications (pigments, amino acids and assimilation of nutrients) are now under investigation.

ACKNOWLEDGEMENTS

R. Pereira was sponsored by the Portuguese Foundation for Science and Technology (FCT), through a PhD grant (BD 21792/99). Work at the University of Connecticut was also sponsored by the Perkin Elmer Analytical Division of EG & G, Wellesley, MA, USA, by the Connecticut Sea Grant College Program, by the National Oceanic and Atmospheric Administration's National Marine Aquaculture Initiative and by the State of Connecticut Critical Technology Grant Program. The first author thanks Dr Raquel Carmona for help in culture maintenance at the UCONN Marine Biotechnology Laboratory. The authors also thank Prof. C. Neefus for confirming the identification of the species used in this work.

REFERENCES

ARDRÉ F. 1970. Contribution à l'étude des algues marines du Portugal. I – La flore. *Portugaliae Acta Biologica* (B) 10: 1–423.

AVILA M., SANTELICES B. & MCLACHLAN J. 1986. Photoperiod and temperature regulation of the life history of *Porphyra columbina* (Rhodophyta, Bangiales) from central Chile. *Canadian Journal of Botany* 64: 1867–1872.

BIRD C.J., CHEN L.C.-M. & MCLACHAN J. 1972. The culture of *Porphyra linearis* (Bangiales, Rhodophyta). *Canadian Journal of Botany* 50: 1859–1863.

BRODIE J. & IRVINE L.M. 1997. A comparison of *Porphyra dioica sp. nov.* and *P. purpurea* (Roth) C. Ag. (Rhodophyta: Bangiophycidae) in Europe. *Cryptogamie, Algologie* 18: 283–297.

CHEN L.C.-M., EDELSTEIN M., OGATA E. & MCLACHLAN J. 1970. The life history of *Porphyra miniata*. *Canadian Journal of Botany* 48: 385–389.

CHIANG Y.-M. & WANG J.-C. 1980. A study on the production of conchosporangia in the conchocelis phase of *Porphyra angusta* Okamura *et* Ueda. *Phycologia* 19: 20–24.

CHOPIN T., YARISH C., WILKES R., BELYEA E., LU S. & MATHIESON A. 1999. Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. *Journal of Applied Phycology* 11: 463–472.

DEBOER J.A., GUIGLI H.J., ISRAEL T.L. & D'ELIA C.F. 1978. Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. *Journal of Phycology* 14: 261–265.

DREW K.M. 1954. Studies in the Bangioidea. III. The life-history of *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Light.) J. Ag. *Annals of Botany, N.S.* 18(70): 183–209.

FAO 2003. *Yearbook. Fishery statistics, aquaculture production 2001*, vol. 92/2. (Formerly published as FAO Fisheries Circular N.815 'Aquaculture Production Statistics' at http://www.fao.org/fi/statist/statist.asp. Accessed 18 September 2003.)

HANISAK M.D. 1998. Seaweed cultivation: global trends. *World Aquaculture* 29: 18–21.

HOLMES M.J. & BRODIE J. 2004. Morphology, seasonal phenology and observations on some aspects of the life history in culture of *Porphyra dioica* (Bangiales, Rhodophyta) from Devon, UK. *Phycologia* 43: 176–188.

KAPRAUN D.F. & LEMUS A.J. 1987. Field and culture studies of *Porphyra spiralis* var. *amplifolia* Oliveira Filho *et* Coll (Bangiales, Rhodophyta) from Isla de Margarita, Venezuela. *Botanica Marina* 30: 483–490.

KRISHNAMURTHY V. 1969. The conchocelis phase of three species of *Porphyra* in culture. *Journal of Phycology* 5: 42–47.

LEVINE I.A. 1998. Commercial cultivation of *Porphyra* (nori) in the United States. *World Aquaculture* 29: 37–38, 45–47.

LEWIN J. 1966. Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia* 6: 1–12.

LEWMANOMONT K. & CHITTPOOLKUSOL O. 1993. Life cycle of *Porphyra vietnamensis* Tanaka & Pham-Hoang Ho from Thailand. *Hydrobiologia* 260/261: 397–400.

LINDSTROM S.C. & COLE K.M. 1990. *Porphyra fallax*: a new species of Rhodophyta from British Columbia and northern Washington. *Japanese Journal of Phycology* 38: 371–376.

LINDSTROM S.C. & COLE K.M. 1993. The systematics of *Porphyra*: character evolution in closely related species. *Hydrobiologia* 260/261: 151–157.

LOBBAN C.S. & HARRISON P.J. 1994. *Seaweed ecology and physiology*. Cambridge University Press, New York. 366 pp.

NAM-GIL K. 1999. Culture studies of *Porphyra dentata* and *P. pseudolinearis* (Bangiales, Rhodophyta), two dioecious species from Korea. *Hydrobiologia* 398/399: 127–135.

NELSON W.A., BRODIE J. & GUIRY M. 1999. Terminology used to describe reproduction and life history stages in the genus *Porphyra* (Bangiales, Rhodophyta). *Journal of Applied Phycology* 11: 407–410.

NORTON T.A. & MATHIESON A.C. 1983. The biology of unattached seaweeds. *Progress in Phycological Research* 2: 333–386.

NOTOYA M. & MAGAURA K. 1998. Life history and growth of the epiphytic thallus of *Porphyra lacerata* (Bangiales, Rhodophyta) in culture. *Algae* 13: 207–211.

NOTOYA M. & MIYASHITA A. 1999. Life history, in culture, of the obligate epiphyte *Porphyra moriensis* (Bangiales, Rhodophyta). *Hydrobiologia* 398/399: 121–125.

OTT F.D. 1965. Synthetic media and techniques for the xenic cultivation of marine algae and flagellate. *Virginia Journal of Science* 16: 205–218.

ROHLF F.J. & SOKAL R.R. 1981. *Biometry*, ed. 2. W.H. Freeman, San Francisco, CA, USA. 887 pp.

SIDIRELLI-WOLFF M. 1992. The influence of temperature, irradiance and photoperiod on the reproductive life history of *Porphyra leucostica* (Bangiales, Rhodophyta) in laboratory culture. *Botanica Marina* 35: 251–257.

SOUTH G.R. & TITTLEY I. 1986. *A checklist and distributional index of the benthic marine alga of the North Atlantic Ocean*. Huntsman Marine Laboratory, St Andrews, UK, and British Museum (Natural History), London. 76 pp.

STEKOLL M.S., LIN R. & LINDSTROM S.C. 1999. *Porphyra* cultivation in Alaska: conchocelis growth of three indigenous species. *Hydrobiologia* 398/399: 291–297.

TEASDALE B.W., WEST A.L., TAYLOR H. & KLEIN A.S. 2002. A simple restriction fragment length polymorphism (RFLP) assay to discriminate common *Porphyra* (Bangiales, Rhodophyta) taxa from the northwest Atlantic. *Journal of Applied Phycology* 14: 293–298.

WAALAND J.R., DICKSON L.G., DUFFIELD E.C.S. & CARRIER J.E. 1987. Conchocelis growth and photoperiodic control of conchospore release in *Porphyra torta* (Rhodophyta). *Journal of Phycology* 23: 399–406.

WAALAND J.R., DICKSON L.G. & DUFFIELD E. 1990. Conchospore production and seasonal occurrence of some *Porphyra* species (Bangiales, Rhodophyta) in Washington State. *Hydrobiologia* 204/205: 453–459.

YARISH C., WILKES R., CHOPIN T., FEI X.G., MATHIESON A.C., KLEIN A.S., NEEFUS C.D., MITMAN G.G. & LEVINE I. 1998. Domestication of indigenous *Porphyra* (nori) species for commercial cultivation in Northeast America. *World Aquaculture* 29: 26–29, 55.

YARISH C., CHOPIN T., WILKES R., MATHIESON A.C., FEI X.G. & LU S. 1999. Domestication of nori for northeast America: the Asian experience. *Bulletin of the Aquaculture Association of Canada* 99: 11–17.

Received 16 October 2003; accepted 19 July 2004
Communicating editor: S. Lindstrom