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Conchospore production and seasonal occurrence of some *Porphyra* species (Bangiales, Rhodophyta) in Washington State

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Abstract

The leafy thalli of species of the marine red algal genus *Porphyra* grow rapidly but persist for a relatively short time on rocky intertidal or subtidal substrata or as epiphytes on other marine plants. In most species, the large, short-lived leafy thalli alternate with small, presumably perennial, filamentous 'conchocelis' plants. Depending on the species of northeastern Pacific *Porphyra*, photoperiod and temperature are important regulators of conchospore formation and release. Data from laboratory studies of conchospore formation and release in five Washington species of *Porphyra* (*P. abottae, P. nereocystis, P. perforata, P. pseudolanceolata* and *P. torta*) indicate that conchospores are most likely to be released at a time that precedes the appearance of the leafy thalli in the field.

Introduction

The leafy thalli of species of the marine red algal genus *Porphyra* grow rapidly and may be conspicuous elements of marine communities in rocky intertidal or subtidal habitats. Some *Porphyra* species occur as epiphytes on other marine plants. Despite their rapid development and conspicuous appearance, most species persist for a relatively short time in the blade phase. In most species, the leafy thalli have been shown to alternate with a small, persistent and presumably perennial, filamentous phase called 'conchocelis'. We have undertaken studies of conchospore production in conchocelis of several species of *Porphyra* native to Washington State. Although the primary purpose of this research was to gain control of conchospore release in selected species for use in controlled net seeding for experimental nori farming (Merrill & Waaland, 1988), we have accumulated a significant amount of information regarding conchospore production in five *Porphyra* species (*P. abbottae, P. nereocystis, P. perforata*[1], *P. pseudolanceolata*, and *P. torta*). This paper summarizes information on the laboratory conditions that induce conchospore production and release (see also Mumford, 1980) and correlates these findings with the reported seasonal occurrences of the leafy thalli in the field (see also Conway & Cole, 1977).

[1] Note: Lindstrom & Cole (1990) are describing the entity called *P. perforata* in this paper as a new species (to be named *P. fallax*).

Material and methods

Cultures

Most of our conchocelis cultures were initiated from carpospores released by field collected specimens. Cultures of eleven strains of five species were used in these experiments: *P. abbottae*, #1626 collected 5/30/83, Rialto Beach, Clallam Co. (Hannach, 1989; Hannach & Waaland, 1989) and #1643 collected 6/23/85 La Push, Clallam Co.; *P. nereocystis*, #PN-1 collected 3/2/79, San Juan I., San Juan Co. (Mumford, 1980; Dickson & Waaland, 1985) and #1624 collected 5/30/83, Rialto Beach, Clallam Co.; *P. perforata*, #1620 collected 4/5/83, Golden Gardens, Seattle (Lindstrom & Cole, 1990), #1615 collected 3/2/83, Discovery Park, Seattle; *P. pseudolanceolata*, #1625 collected 5/30/83, Rialto Beach, Clallam Co. and #1645 collected 11/30/86, Cannon Beach, Oregon; *P. torta*, #1608 and #1610 both collected 3/24/82, San Juan I., San Juan Co. (Waaland *et al.*, 1987) and #GP7 collected 12/82, Green Point, Skagit Co. (Burzycki & Waaland, 1987). Field collected specimens were scrubbed to remove contaminants, then dried for several hours to days at approximately 10 °C and finally immersed in f/2 medium to induce spore release. Cultures were observed periodically for carpospore release, and carpospores were pipetted into petri dishes with fresh medum (usually with GeO_2 at approximately 1 mg/L to inhibit diatom growth). Cultures so obtained were incubated in reach-in or walk-in growth chambers in f/2 medium. The major differences in treatments have been temperature, photoperiod, and irradiance. Other factors, which were tested in some species, were salinity and growth or conchosporangium development in or out of oyster shell. Reference to typical culture procedures can be found in Dickson & Waaland (1985), Hannach & Waaland (1989), Waaland *et al.* (1987) and Waaland *et al.* (1983).

Growth rates

For most species conchocelis growth rates have been measured by following changes in diameter of free conchocelis tufts (Waaland *et al.*, 1987) or patches in shells. Field growth data on blades of some species have been obtained from length and/or area measurements on cultivated specimens growing on net or twine (Mumford *et al.*, 1985; Waaland *et al.*, 1986). Area measurements have been used to measure growth in laboratory cultures (Hannach & Waaland, 1989).

Induction of sporangia and sporulation

Photoperiod, temperature and irradiance are the major environmental factors we have manipulated in testing for induction of conchosporangia in vegetative conchocelis and for triggering conchospore release from conchosporangial conchocelis. Typical details of these treatments can be found in Dickson & Waaland (1985) and Waaland *et al.* (1987).

Field observations and data

Most of the data on seasonal occurrence of the blade phases of these species have been obtained from Conway & Cole (1977), Harlin (1969) and Thom *et al.* (1976). Reports on particular species (e.g., Mumford, 1975, 1980; Hawkes, 1977, 1978; Woessner, 1981), herbarium records (Conway *et al.*, 1975; Garbary *et al.*, 1980) and our own field observations also have been used.

Results and discussion

Conchocelis growth

We examined conchocelis growth in five species of *Porphyra* and found that all grew well at very low photon fluence rates (Table 1). In fact, growth was light saturated as low as 5 μE m^{-2} s^{-1}. Thus conchocelis for most of our experi-

Table 1. Growth conditions for conchocelis of five species of *Porphyra* from Washington State.

Species	Maximum growth rate	Optimal conditions		Good growth range		Upper lethal temp
	% day^{-1} volume increase	Light μE m^{-2} s^{-1}	Temp °C	Light μE m^{-2} s^{-1}	Temp °C	Temp °C
P. abbottae v	7.8	100	12	5–100	10–15	18
P. nereocystis v	7.5	5–100	12	5–100	8–12	18
P. nereocystis c	7.5	25	13	5–300	8–16	18
P. perforata v	9.5	25	15	5–100	10–15	>18
P. pseudolanceolata v	7.1	100	15	5–100	10–15	>18
P. torta c	9.2	100	15	5–100	12–15	18

v = vegetative c = conchosporangial
medium = f/2

ments has been grown at photon fluence rates between 5–40 μE m^{-2} s^{-1}.

Conchosporangium induction

Of the species tested, only one, *P. torta*, changed from vegetative conchocelis morphology (long, narrow, highly vacuolate cells with parietal, ribbon shaped chloroplasts) to conchosporangial morphology (short, wide, densely cytoplasmic cells with a stellate chloroplast) under all conditions tested (Table 2); the only way to observe vegetative morphology was in freshly germinated conchocelis less than six weeks old. After six weeks, *P. torta* will grow indefinitely (since 1982 in our lab) in the conchosporangial morphology (Waaland *et al.*, 1987).

All other species we tested require a particular combination of photoperiod and temperature to trigger the development of conchosporangia (Table 2). *Porphyra perforata* is noteworthy in that despite exposure to a wide range of culture conditions, it only formed conchosporangia when growing in shells. In these species, once conchosporangia are formed, they can be isolated and then will grow indefinitely in the conchosporangial morphology until they are exposed to

Table 2. Optimal conditions for conchosporangial initiation in five species of *Porphyra* from Washington state[1].

Species	Light μE m^{-2} s^{-1}	Temp °C	Photo-period[2]	Time weeks
P. abbottae	5–300	12–15	LD	4–8
P. nereocystis	5–100	15–18[6]	LD	3–4
P. perforata[3]	10–25	10–15	SD	8–10
P. pseudolanceolata[4]	10	10–15	SD	8–12
P. torta[5]	5–100	8–12	LD & SD	4–8

[1] All experiments in f/2 medium; conditions summarized here have routinely produced conchosporangia in our laboratory; however, extensive tests have not been done to rule out other possible combinations of conditions under which conchosporangia will form.
[2] LD = 16L : 8D, SD = 8L : 16D
[3] in shells, not in vitro
[4] in shells and in vitro
[5] remains vegetative only in shells
[6] long exposure to 18 °C may be lethal

environmental conditions that trigger conchospore release or to conditions that favor reversion to the vegetative morphology (with the exception of *P. torta*).

Conchospore release

In all species investigated, we have found that release of conchospores, a necessary prerequisite to blade appearance in nature, requires an environmental trigger (Table 3). Whereas all species are responsive to photoperiod (*P. abbottae* and *P. perforata* are long-day plants, *P. nereocystis* is a dual-daylength plant, *P. pseudolanceolata* is an intermediate-day plant, and *P. torta* is a short-day plant), we have found that some species have other environmental limits or windows (e.g., temperature) within which the photoperiodic response is operable. *Porphyra abbottae*, a long-day plant, also requires a decrease in temperature (from 10 to 6–8 °C), and *P. pseudolanceolata* releases more spores when the salinity is changed from the usual 30.0‰ to 32.5‰. Moreover, each species tested requires a different treatment to trigger spore release. These treatments all differ from the treatment used for cultivars of *P. yezoensis* grown in Washington (Melvin *et al.*, 1986). For example, whereas our native species, *P. torta*, releases spores in response to a short-day photoperiod as does *P. yezoensis*, the temperature that works for *P. yezoensis* (a drop from 25 to 16 °C) would be lethal to *P. torta* conchocelis, which dies above 18 °C (Table 1).

We have observed that conchocelis of some species have very tight control of spore formation and/or release and only will produce or release conchospores in response to a very precise set of conditions (e.g., *P. abbottae*, *P. nereocystis*, and *P. torta*), whereas others are less stringent in their conchospore release requirements (e.g., *P. perforata* and *P. pseudolanceolata*). Mumford (1980) has suggested that species exhibiting stringent control should be called 'specialists' and those with a wider latitude response should be called 'generalists'. We have observed that some species will release nearly all their spores in a large, synchronous pulse in one or two days (e.g., *P. torta*); a specialist such as this only produces conchospores in autumn or winter when conditions are

Table 3. Conditions for conchospore maturation and release in five species of *Porphyra* from Washington State and seasonal occurrence of *Porphyra* blades.[1]

Species	Light (μE m^{-2} s^{-1})	Temperature (°C)	Photoperiod	Time (days)	Fertile blades observed season
P. abbottae	25–40	6–8	16L : 8D	30–40	spring-summer[7]
P. nereocystis	25–40	first 12–15	@ 8L : 16D	for 30–40	fall-winter[8]
		then 8–10	@ 16L : 8D	for 24–30	
P. perforata[2]	10–350	6–12	16L : 8D	3–5[3]	
	35–350	6–10	12L : 12D	3–6[4]	spring-summer[7]
P. pseudolanceolata	35–40	6–10	12L : 12D[5]	14–21	
			14L : 10D	30–40	
		8[6]	12L : 12D	30	mid-fall-spring[9]
P. torta	25–40	8–12	8L : 16D	7–10	winter-early spring[7]

[1] All experiments in f/2 medium
[2] In shells & in shell-free fragments
[3] Recommended for maximum spore release
[4] Slower with fewer spores released
[5] 12L : 12D causes greater spore release than longer photoperiods
[6] 8 °C always gives better spore release than 6° or 10 °C (Mumford, unpublished)
[7] Data: Conway & Cole (1977)
[8] Data: Dickson & Waaland (1985) & Herbert (pers. comm.)
[9] Observations by Waaland, Dickson & Duffield

likely to be appropriate for development and growth of the blade phase. On the other hand, generalists also may require a very precise 'window' of conditions to trigger spore release, but they will continue to release conchospores over a much longer time span of one to several weeks (e.g., *P. pseudolanceolata*). The spores of such generalist species are thus exposed to a greater range of conditions than those of the specialists, but development still occurs within the season that typically has appropriate conditions for development of the blades. *P. perforata* combines traits of both the specialist and the generalist. In response to long days and bright light, this species releases a large, synchronous pulse of spores just a few days after experiencing the appropriate conditions (see Table 3), but it also releases a few spores almost all the time under a wide range of conditions. This 'combination' strategy permits *P. perforata* conchospores to 'sample' the suitability of the environment over a very wide range of conditions. *P. nereocystis* with its dual-daylength requirement for triggering spore release is a definite specialist with respect to the seasonal window that triggers conchospore release. However, once conchospore release is initiated, it may continue for several weeks, a generalist trait which is a useful adaptation considering its epiphytic habitat on the annual kelp *Nereocystis*. Thus our observations agree with the concept of 'specialists' and 'generalists' in spore release patterns. We would, however, add a third 'bet-hedger' or 'combination' type to the two proposed by Mumford. Our findings also would place some species in a different group than that assigned by Mumford.

Season of blade occurrence

From both laboratory and field experiments we know that *Porphyra* blades become visible to the naked eye three to four weeks after conchospore release (Dickson & Waaland, 1985; Mumford *et al.*, 1985; Hannach & Waaland, 1989; Waaland *et al.*, 1986, 1987). They usually can be assigned to a particular species six to eight weeks after spore release when they become reproductive. In general our predictions agree well with the observations of seasonal occurrence summarized in Conway & Cole (1977) and others (*op. cit.*) for these same species (Table 3).

A significant problem with attempting to correlate seasonal occurrence of *Porphyra* blades with predicted time of conchospore release is the spotty nature of seasonal occurrence records of the blade phase. With few exceptions (Harlin, 1969; Thom *et al.*, 1976) checklists and similar reports usually record only the positive occurrence of a species rather than its non-occurrence. The ephemeral nature of the blades of many species complicates implementation of intensive seasonal investigations at many sites. Thus *Porphyra* blades might be observable during as few as 3–4 low tides. In Puget Sound and the Strait of Juan de Fuca, fall and winter observations are limited and complicated by the occurrence of low spring tides well after dark. During spring and summer, low spring tides occur during midday and winter *Porphyra* species may readily succumb to unfavorable conditions on a hot, sunny, spring day. It is important to note that differing climate and tidal conditions can result in shifted seasonal occurrences of species in different localities (e.g., *P. nereocystis* on the outer coast of Washington or in California; it also can be difficult to find in mid-summer in the San Juan Islands). Sometimes a few individuals of a species may be found 'out of season' if local weather or habitat features support the species (e.g., persistence of *P. torta* on rocks in shade caused by cliffs, docks or bridges).

Some ecophysiological data relevant to blade occurrence are available for some of these *Porphyra* species from the work of Herbert & Waaland (1988) and Herbert (1984, 1988) who have shown that *Porphyra* plants from winter (*P. torta*) or shaded habitats (*P. nereocystis*) differ significantly in their response to high photon fluence rates and are readily damaged by high light as compared to a summer species (*P. perforata*). Furthermore, we have observed that low intertidal and subtidal species (*P. gardneri, P. miniata, P. nereocystis, P. thuretii*)

are intolerant of desiccation, whereas summer and higher intertidal species (*P. abbottae, P. fucicola, P. perforata, P. torta*) do survive prolonged desiccation. We might predict that some seasons are more hazardous to some *Porphyra* species than others. For example, a 'summer' species such as *P. perforata* might persist 'out of season' into the fall, but a 'winter' species such as *P. torta* would quickly succumb to the heat, bright light, and dry conditions of a fine spring day.

The conchocelis phase of *Porphyra* is cryptic and difficult to find in nature as Mumford (1980), Conway & Cole (1977) and others have pointed out. In the Washington-British Columbia area the occurrence of 17 species of *Porphyra* complicates the task of relating a particular field-collected conchocelis with its *Porphyra* blade. Furthermore, there are only three reports of occurrence of conchocelis in the field in this region (Jao, 1937; Mumford, 1975; E. Martinez, 1988, pers. comm.). More information about a particular field-collected conchocelis might be gleaned by examining the conditions that induce conchosporangia and conchospore release under laboratory conditions.

Our observations on the ecophysiology of conchospore formation and release correlate well with the time when these species are observed in nature. The conchocelis phases of these *Porphyra* species respond to major and dependable environmental stimuli (especially photoperiod and temperature cues) that ensure that the blade phase will be initiated at a season of the year when it is most likely to encounter environmental conditions to which it is adapted. Much remains to be learned about the biology of conchocelis and clearly, as Mumford (1980) pointed out, the biology of conchocelis in nature remains an enigma.

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