

Patterns of tetraspore discharge in *Caloglossa* and *Murrayella* (Ceramiales, Rhodophyta)

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SUMMARY

Caloglossa released tetraspores only during dark periods in contrast to a marked light-period release exhibited by *Murrayella*. Both could be readily converted to a reversed light–dark cycle after a lag period of 1 day. Maximum tetraspore discharge by *Caloglossa* occurred approximately 4–5 h after the onset of darkness and by *Murrayella* 4–5 h after the onset of light. Neither 8- nor 16-h light periods affected spore release patterns in *Caloglossa*, while in *Murrayella* the spore release pattern was briefly disrupted by short day lengths, but recovered after several days. In both genera, the rhythmicity was disrupted in periods of continual darkness or continual light and appeared to be independent of light quality; however, minimum irradiance levels were critical to maintenance of release patterns. Possible mechanisms of tetraspore discharge periodicity are discussed but since both plants typically have a limited habitat as epiphytes on mangrove trunks and pneumatophores, any speculations as to the adaptive advantages of each pattern tend toward mutual contradiction.

Key words: *Caloglossa*, light quantity/quality, *Murrayella*, rhythmicity, tetraspore discharge.

INTRODUCTION

Caloglossa lepieurii (Montagne) J. Agardh, *Caloglossa apomeiotica* West et Zuccarello and *Murrayella pericladus* (C. Agardh) Falkenberg are typical epiphytic inhabitants on mangroves. *Caloglossa lepieurii* has been the subject of a number of taxonomic, morphological, life history and genetic studies (e.g. Post 1943; Papenfuss 1961; King and Puttock 1994; Kamiya *et al.* 1995, 1998), whereas *C. apomeiotica* is characterized by a lack of sexual reproduction (West *et al.* 1994). The complete life history of *M. pericladus* in culture has been outlined by Aponte and Ballantine (1987).

Aspects of red algal spore discharge have been investigated by a number of researchers (see reviews by Searles 1980; Guiry 1990; Hawkes 1990; Santelices 1990). Although a large number of reports have been published on the effects of light on algal growth and reproduction (reviews by Dring 1988; Rudiger and Lopez-Figueroa 1992), only relatively few studies

have carried out in-depth investigations on the photo-parameters surrounding the tetraspore discharge phenomenon. Perhaps one of the most thorough was performed by Sagromsky (1961) on *Nitophyllum punctatum* Stackhouse where the author included the effects of light quality on spore release. Since certain isolates of *Caloglossa* and *Murrayella* (Table 1) proved to be reliable tetraspore producers, the present investigation was launched in an effort to gather more information on tetraspore discharge patterns in the Rhodophyta under controlled culture conditions.

MATERIALS AND METHODS

The *Caloglossa* and *Murrayella* isolates used in this research are listed in Table 1. Culture methods are described in West and Calumpong (1988). Stock cultures of tetrasporangiate plants were maintained separately in the following conditions: 22–26°C, 12:12 light:dark photoperiods daily, 5–20 µmol photons m⁻² per s, in Pyrex (No. 3250, Corning Glass Works, Corning, NY, USA) 500 mL storage dishes containing 250 mL Modified Provasoli's Enriched Seawater (West and McBride 1999) adjusted to 30 p.p.t. salinity with MilliQ-filtered water. The medium and culture dishes were changed at 1–3-month intervals.

Portions of thalli (1–5 mm long) with one to three branches (stichidia) each bearing a mature sorus (group of tetrasporangia) were excised with microdissection scissors. On the basis of visual observations, sori of approximately the same size and maturity were chosen for each experiment. Each thallus portion was placed individually in polystyrene Cell Wells™ (Corning 25820, Corning Glass Works, Corning, NY, USA). Each of the 24 wells was 16 mm diameter and contained 1.5 mL medium. Alternatively, four 1 mL Nalgene™ cups housed in 35 mm polystyrene Petri dishes were used as experimental chambers. Plants were maintained in the above-described conditions with various light:dark regimes. Light quality experiments in the visible range were

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Table 1. Species, collection sites/dates, isolate numbers

Species	Collection site/date	Isolate no.
<i>Murrayella periclados</i>	Agana, Guam, 30 viii 1989	2999
<i>M. periclados</i>	Yacura Island, Cuvu Bay, Viti Levu, Fiji, 6 vi 1997	3731
<i>Caloglossa lepieurii</i>	St Lucia, Natal, South Africa, 22 xii 1991	3267
<i>C. lepieurii</i>	Towra Point, New South Wales, Australia, 1 vii 1992	3290
<i>Caloglossa apomeiotica</i>	Barra de Navidad, Jalisco, Mexico, 19 iii 1992	3244
<i>C. apomeiotica</i>	Isla Espiritu Santo, Baja Cal. S., Mexico, 19 iii 1992	3276
<i>C. apomeiotica</i>	Fort Pierce, Florida, USA, 19 vi 1994	3421

carried out by passing fluorescent white light (Thorne Cool White 18W, Thorne Lighting, Sydney, NSW, Australia) through Spectracoat Monopass™ Filters (Optics Technology Inc., Palo Alto, CA, USA) with resulting intensities of 2.5–5.5 $\mu\text{mol photons m}^{-2}$ per s as measured by a Li-Cor (Model LI-198, Lincoln, NB, USA) quantum/radiometer/photometer. Filters transmitting wavelengths of 423 nm, 486 nm, 520 nm, 579 nm, 656 nm, and 706 nm were used. The UVA light source was a 20 W NEC black light (H20–27, Nippon Electric Co., Osaka, Japan) fluorescent tube with peak emission at 365 nm and the UVB source was a 20 W Phillips (TL 20/12, Phillips Lighting, London, UK) fluorescent tube with peak emission at 310 nm. Where necessary, intensities were adjusted by varying the distance of the experimental chambers from the light source. Tabulations of released spores from preformed sporangia were carried out by direct observation for periods up to 12 days. Each experiment was replicated up to six times and tetrasporangial branches were transferred to fresh medium daily during test periods.

RESULTS

The relatively extended tetraspore release period (at least 10–12 days) of *Caloglossa* and *Murrayella* allowed detailed, long-term comparative observations. This contrasts with the situation in certain other members of the Ceramiales (e.g. 3–4 days of dark release in *Spyridia filamentosa* [Wulfen] Harvey) (D. L. McBride and J. A. West, unpubl. obs.). It should be emphasized that both *Caloglossa* and *Murrayella* discharged a variable number of spores during each release event. This appeared to be dependent on various factors including the size of sori and an asynchronous rate of maturity within each sorus, hence attempts were made to choose sori of approximately the same visual size and maturity for each experiment. Generally, spore release declined during observation periods, with the most marked decrease often occurring after approximately 8–10 days, when few mature tetrasporangia remained in sori.

In all *Caloglossa* isolates examined, spore discharge predominantly took place during the dark period of a 12:12 light:dark cycle (Fig. 1). This contrasts with the situation in *Murrayella* where spore discharge occurred during the light period of a 12:12 light:dark cycle

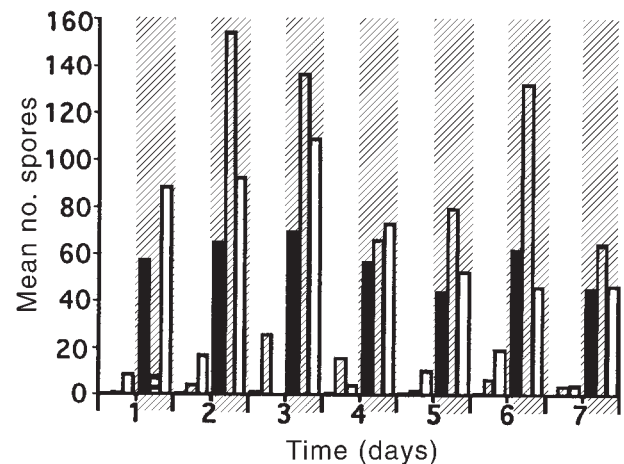


Fig. 1. *Caloglossa* tetraspore discharge pattern, 12:12 h light:dark: ■, Florida (*Caloglossa apomeiotica*) 3421; ▨, South Africa (*Caloglossa lepieurii*) 3267; □, Australia (*C. lepieurii*) 3290. Each shaded portion represents 12 h of darkness during each 24-h period.

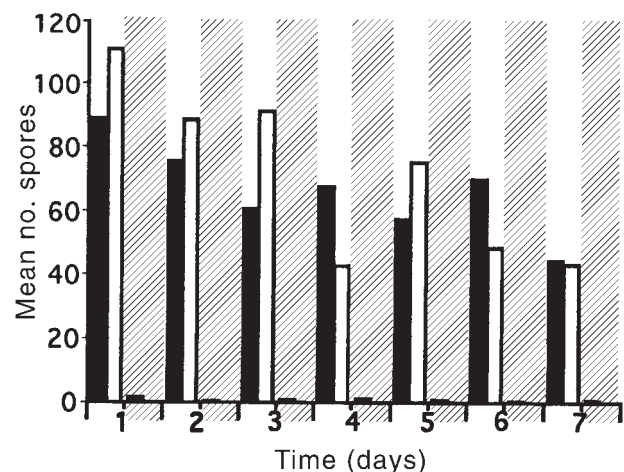


Fig. 2. *Murrayella periclados* tetraspore discharge patterns, 12:12 h light:dark: ■, Guam (*Murrayella periclados*) 2999; □, Fiji (*Murrayella periclados*) 3731. Each shaded portion represents 12 h of darkness during each 24-h period.

(Fig. 2). In both genera, a reversal of the 12:12 light:dark cycle at day 5 of observation resulted in the spore release pattern also reversing (Figs 3, 4). The reversal

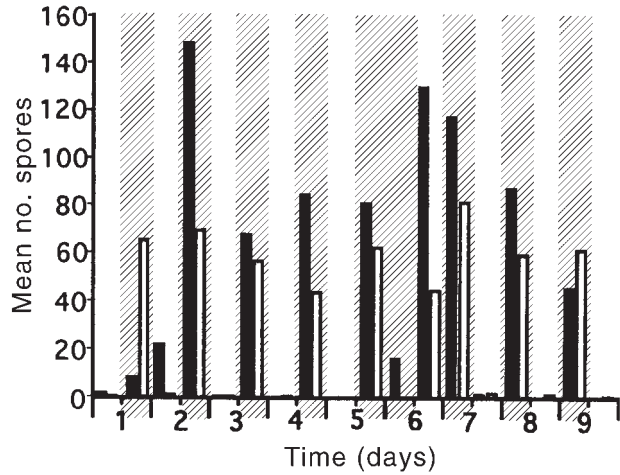


Fig. 3. *Caloglossa* tetraspore discharge pattern, 12:12 h light:dark, reversed: ■, Mexico (*Caloglossa apomeiotica*) 3244; □, Florida (*Caloglossa apomeiotica*) 3421. Each shaded portion represents 12 h of darkness during each 24-h period. Note light-dark reversal after day 5 and the subsequent 24-h lag period until the tetraspore release pattern also reversed.

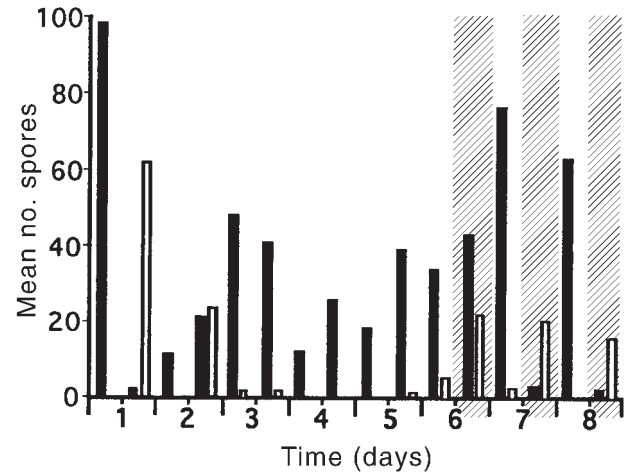


Fig. 5. *Caloglossa* and *Murrayella* tetraspore discharge pattern in continual light. Note resumption of 12:12 h light:dark after day 5 and the subsequent 24-h lag period until a normal release pattern was re-established: ■, Florida (*Caloglossa apomeiotica*) 3421; □, Guam (*Murrayella pericladus*) 2999.

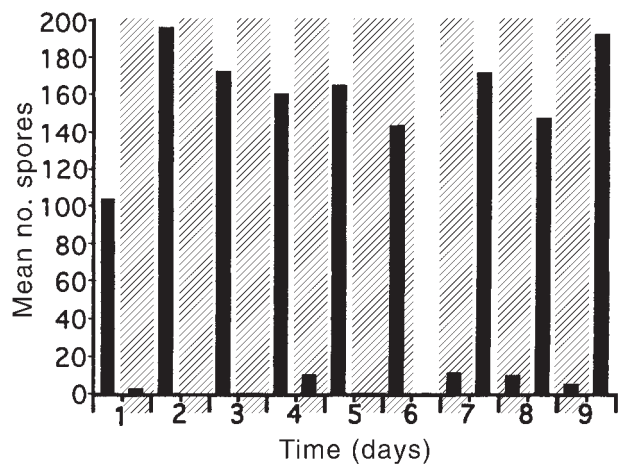


Fig. 4. *Murrayella pericladus* 2999 tetraspore discharge pattern, 12:12 h light:dark, reversed. Each shaded portion represents 12 h of darkness during each 24-h period. Note light-dark reversal after day 5 and the subsequent 24-h lag period until tetraspore release pattern also reversed.

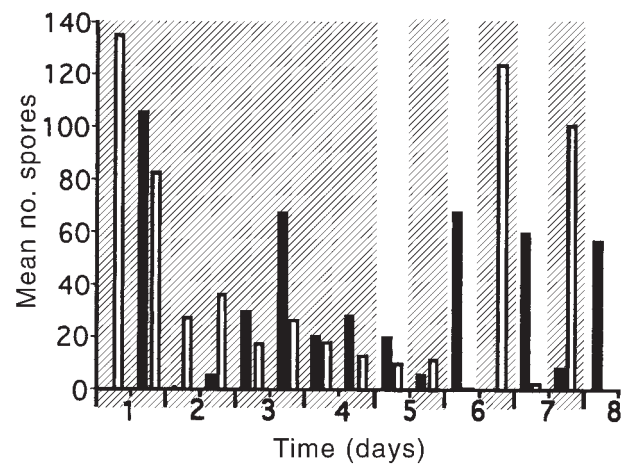


Fig. 6. *Caloglossa* and *Murrayella* tetraspore discharge pattern in continual darkness. Note resumption of 12:12 h light:dark after day 4 and the subsequent 24-h lag period until a normal release pattern was re-established: ■, Florida (*Caloglossa apomeiotica*) 3421; □, Guam (*Murrayella pericladus*) 2999.

took an unvarying single day to complete, with only one discharge event being out of phase.

Both genera also reacted in a similar manner when exposed to continual light. After 1–2 days, the normal rhythmic discharge pattern was lost and a variable number of spores was continuously released independent of the previously established light:dark cycle (Fig. 5). Resumption of the 12:12 light:dark cycle resulted in the re-establishment of each discharge pattern, once again after a single day lag period (Fig. 5). A like pattern was noted when either *Caloglossa* or *Murrayella* was exposed to continual darkness and similarly, after a single lag day, the

periodicity showed a characteristic re-emergence upon resumption of the 12:12 light:dark cycle (Fig. 6).

Variations in daylength also produced some interesting comparisons between the two genera. Both showed only normal response to a 16:8 light:dark cycle, continuing to discharge spores in their particular rhythm (Fig. 7). In addition, *Caloglossa* exhibited no change in the dark release pattern when subjected to a 16:8 light:dark cycle (Fig. 8). In *Murrayella*, however, the characteristic light release pattern was disrupted for a period of 1–3 days when the plants were subjected to either a 10:14 light:dark cycle or a

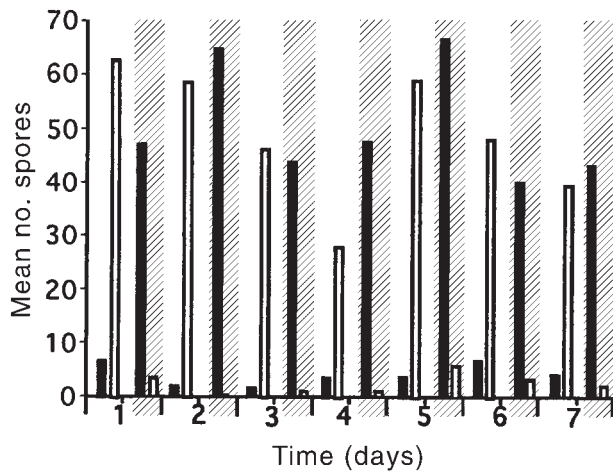


Fig. 7. *Caloglossa* and *Murrayella* tetraspore discharge pattern: 16:8 h light:dark: ■, Florida (*Caloglossa apomeiotica*) 3421; □, Guam (*Murrayella pericladus*) 2999. Each shaded area represents an 8-h dark period.

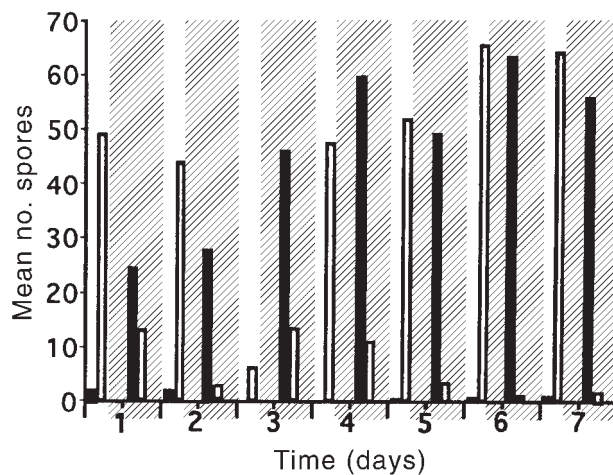


Fig. 8. *Caloglossa* and *Murrayella* tetraspore discharge pattern: 16:8 h light:dark: ■, Florida (*Caloglossa apomeiotica*) 3421; □, Guam (*Murrayella pericladus*) 2999. Each shaded area represents a 16-h dark period.

16:8 light:dark cycle (Fig. 8), but regardless of the length of this delay, in all cases the normal rhythm was re-established. Alterations of light:dark regimes where any period was less than 8 h were not feasible due to the elapsed time required for an individual release event (up to 8 h, see Figs 9,10).

Results of hourly spore counts from each genus proved to be similar. In *Caloglossa*, spore discharge peaked approximately 4 h after the onset of darkness (Fig. 9), while in *Murrayella* the maximum number of spores were discharged approximately 4 h after the onset of the light period (Fig. 10).

Light quantity proved to be much less of a determining factor in tetraspore spore discharge than one would have hypothesized. Intensities as low as 2.5 $\mu\text{mol photons m}^{-2}$ per s had no apparent effect on the periodicity

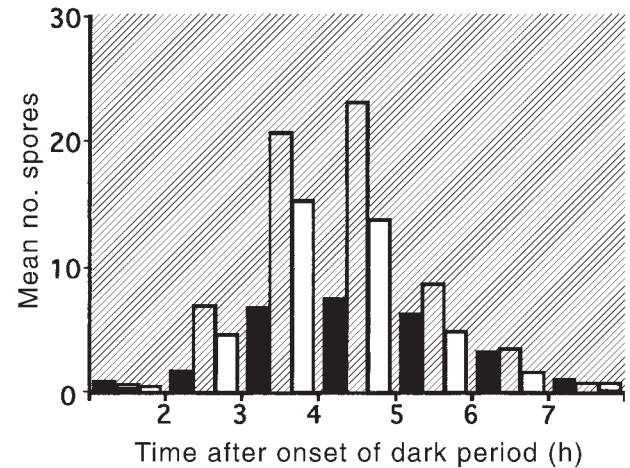


Fig. 9. *Caloglossa apomeiotica* tetraspore hourly discharge pattern: ■, Mexico (*C. apomeiotica*) 3244; ▨, Florida (*C. apomeiotica*) 3421; □, Mexico (*C. apomeiotica*) 3276.

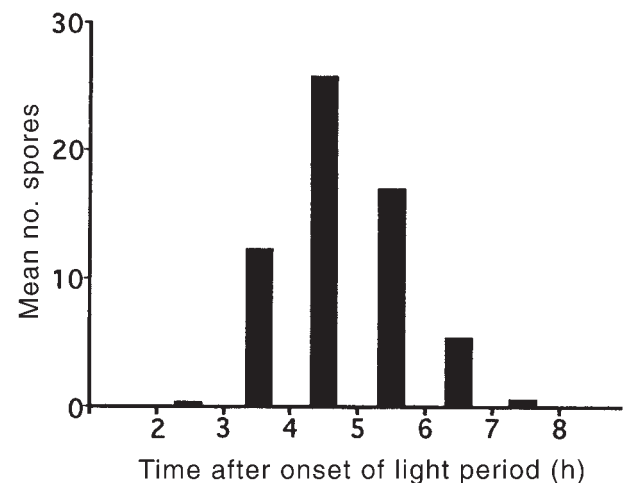


Fig. 10. *Murrayella pericladus* 2999 tetraspore hourly discharge pattern.

of tetraspore release in *Caloglossa*. *Murrayella* retained its rhythmicity at only slightly higher intensities (4.5–5.5 $\mu\text{mol photons m}^{-2}$ per s).

To determine the effect of light quality, a number of experiments were carried out. *Caloglossa* showed no evidence of loss of discharge pattern at any wavelength (365–706 nm, see Materials and Methods) at the particular light intensity tested (2.5–3.5 $\mu\text{mol photons m}^{-2}$ per s), while *Murrayella* showed no loss of periodicity at only slightly higher light intensities (4.5–5.5 $\mu\text{mol photons m}^{-2}$ per s). An additional preliminary experiment to test the effect of UVB light at higher intensities (> 5.5 $\mu\text{mol photons m}^{-2}$ per s) on spore discharge in both genera resulted in the somewhat predictable events of cessation of spore release, chlorosis and death in 2–3 days.

DISCUSSION

Of immediate interest is the obvious difference between the tetraspore release patterns of *Caloglossa* and *Murrayella*. Typically, *Murrayella* released spores only during periods of illumination. Indeed, a number of researchers have reported release of a variety of red algal spores during daylight (e.g. Suto 1950; Kurogi and Hirano 1955; Umamaheswara Rao 1974; Ngan 1979; Ngan and Price 1983). The pattern reported here is not dissimilar to that reported by Sagromsky (1961) in *Nitophyllum*. Sagromsky showed not only that a reversal of the spore-release pattern can be precipitated by an appropriate light reversal, but also that maximum spore release occurred several hours after the onset of light. However, in contrast to our results, it was also indicated that discharge periodicity was maintained in green and blue light but was disrupted in red light.

Caloglossa discharges tetraspores only during periods of darkness. This phenomenon was reported by Ngan (1979) and Ngan and Price (1983) in *Caloglossa lepreurii* var. *hookeri* as an evening spore release which the authors correlated primarily with tidal rhythms. They did not attempt to reverse the light:dark cycle, nor to test the effect of light quality. Other red algae such as *Spyridia* (West and Calumpong 1989; West and McBride 1999); *Bostrychia binderi* and *Catenella nipae* (Ngan and Price 1983) as well as various Gigartinales and Gracilariales (Umamaheswara Rao and Subbarangaiyah 1981) have been shown to have spore release events during periods of darkness. The latter authors suggest that light plays no role in controlling diurnal periodicity of spore discharge in *Gracilaria corticata* J. Agardh, *Gracilaria textorii* (Suringar) J. Agardh, *Gracilariopsis sjoestedtii* (Kylin) Dawson and *Hypnea valentiae* (Turner) Montagne; however, their experiments appear to have been carried out for only 24 h, which could represent the lag period reported here.

The various physiological responses of algal species to long/short day conditions are well documented, including information on photoperiodic control of reproduction and 'circadian' or endogenous rhythms (reviews by Dring 1984, 1988; Kain and Norton 1990; Murray and Dixon 1992), although, as stated by Santelices (1990), 'many experiments have confounded patterns of spore production with patterns of spore release and most have restricted themselves to testing single factor effects...'. Thus, the reaction of *Caloglossa* and *Murrayella* to such modified light regimes also bears discussion. Both algae respond in a similar fashion to periods of continual light, continual darkness and variation in light quality. Also, their responses to a variation in daylength and light quantity are only slightly different. This leads one to hypothesize that the underlying controlling photoperiodic mechanism is fundamentally similar in spite of the different timing of tetraspore discharge.

There was no indication of any particular wavelength of light influencing *Caloglossa* and *Murrayella* tetraspore discharge periodicity. In fact, the discharge periodicity is maintained at very low light intensities, perhaps associated with the photosynthetic compensation points of the algae. Karsten and West (1993) have reported growth at extremely low light levels in *Caloglossa* and low photosynthetic compensation points have been reported in other mangrove rhodophytes such as *Bostrychia simpliciuscula* (Karsten *et al.* 1994). These authors suggest that the phenomenon could be correlated with the relatively shaded habitat of mangrove-dwelling red algae. Our results indicate that the rhythmic response may not be mediated by a specific photoreceptor, but perhaps by the ability of the alga to accumulate sufficient photosynthetic product to allow the rhythmic discharge phenomenon to occur. Transmission electron microscope studies have shown that extensive mucilage synthesis and production is associated with spore discharge in various Rhodophyta (review by Pueschel 1990). Results from high resolution time-lapse video microscopy corroborate this (J. Pickett-Heaps, pers. comm.). Due to the volume of product released in conjunction with spore discharge, this process is probably sequential to photosynthesis rather than a storage product conversion. Indeed, there appears no marked reduction in numbers of floridean starch grains in released spores of *Smithora naiadum* (D.L. McBride unpubl. obs.). Since it appears critical to spore release, it could offer a possible explanation for the association between discharge phenomena and light regimes (i.e. discharge periodicity is dependent on light duration and intensity, not light quality). It should be noted that spore release during periods of constant light and dark was sporadic and unpredictable. Alternatively, if a photoreceptive pigment is involved as suggested by Sagromsky (1961), it would be biochemically unusual for such a substance to be equally photoreactive throughout the spectrum, although authors have proposed the presence of red algal photoreceptors for several spectral ranges to be involved in physiological responses (Dring 1988; Rudiger and Lopez-Figueroa 1992). Or, as Sagromsky (1961) has suggested, perhaps the periodicity is actually controlled by cell division and a 'meiotic control' rather than 'release control' may be operative with spore discharge following the period of cell division as a consequential phenomenon. Further experimentation is needed to gain insight into this control mechanism at a cellular/molecular level.

Regardless of the innate mechanism, remaining is the question of the difference in timing of spore release between the two genera. A hypothesis which immediately comes forward would be one involving a specific biochemical time lag needed to initiate the response necessary for spore release. The different durations of such proposed delay periods could account for the dark versus light release between the two genera examined here.

An extensive array of possible reasons for spore release rhythms has been proposed. These range from reducing competition for space available for settlement, through correlation with tidal rhythms to avoid desiccation, to avoidance of diurnal grazers, to having sufficient light available for early critical periods of germination and growth (review by Santelices 1990). Speculations as to the adaptive advantages of each pattern in the genera examined here tend to be mutually contradictory.

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