

Light-dark regimes in the intertidal zone and tetrasporangial periodicity in the red alga *Rhodochorton purpureum**

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ABSTRACT: The causal factors for observed differences in tetrasporangial periodicity in high- and midlittoral plants in an estuarine population of *Rhodochorton purpureum* (Lightf.) Rosenv. were analysed. In high littoral plants tetrasporangia were absent from early March to early November when tetrasporogenesis was blocked by long days (> c. 12 h) and, from mid-May on also by high temperatures (> c. 16°C). Midlittoral plants, in particular those growing at shaded sites, continued to form tetrasporangia until the end of May. Here, at high tide, high water turbidity and heavy shading by brown algae reduced light to such an extent that it was below the threshold for light perception in *R. purpureum* for several hours. Since, on successive days, high water marks regularly fell c. 50 min later, very irregular light-dark regimes were the result. Days with two light and two dark periods (high tide at midday) are followed by days with one light and one, long, dark period (one high tide at dawn, the next at dusk). Experiments showed that these regimes are effectively short days for *R. purpureum*. In summer and autumn tetrasporogenesis in the midlittoral plants is blocked by high temperatures. Minimal daily temperature-values are often below the critical temperature, but this did not induce tetrasporangia. Probably average or maximal values block tetrasporogenesis and this was supported by experimental results. In one of the field samples a diminutive male gametophyte was found which had grown from a tetraspore that had germinated in situ, and this is the first report of a gametophyte of *R. purpureum* from nature.

INTRODUCTION

Ultimately, an analysis of the causal factors for the reproductive phenology of benthic marine algae depends on experimental work. Only in the laboratory can environmental factors be varied independently and only in this way can their specific effects be ascertained.

In the red alga *Rhodochorton purpureum* (Lightf.) Rosenv. the formation of tetrasporangia is restricted to the winter halfyear. Long days and high temperatures are potentially blocking factors for tetrasporogenesis (West, 1972; Stegenga, 1978; Dring & West, 1983). Several regional daylength-temperature ecotypes have been found which

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differ in the critical limits for these key-factors (West, 1972; Stegenga, 1978; Dring & West, 1983).

In the Ems-Dollard estuary (Northern Netherlands) *R. purpureum* grows on the man-made dikes that constitute the only solid substrate for benthic algal growth. *R. purpureum* is found in the understory of a fucoïd canopy which extends from the dike-bases, at about the midtide level, to the upper littoral. The very regular construction of the substrate enabled sampling of the population along two clear-cut gradients: one is a tidal submergence gradient, the other a light exposure gradient. Such a sampling program seemed interesting since preliminary observations had drawn our attention to differences in tetrasporangial periodicity at different tide levels. Finally, we designed laboratory experiments to analyse the causal factors for the periodicity observed in the field.

MATERIAL AND METHODS

Location and sampling procedure

The studied *R. purpureum* population occurs on a man-made dike in the Ems-Dollard estuary (Northern Netherlands) (Fig. 1). The dike is covered with large granite blocks and is bordered, seaward, by a mudflat situated at c. 0.30 m below the NAP-level (NAP = Amsterdam Zero ≈ midtide) (Fig. 2). *R. purpureum* occupies its characteristic habitat in the understory of the large fucalean algae *Fucus spiralis*, *Ascophyllum nodosum* and *Fucus vesiculosus* (Fig. 2). In the *F. spiralis*-zone brown algal cover is c. 30 % and the plants are patchily distributed. In the *A. nodosum*- and the *F. vesiculosus*-zone brown algal cover is high (c. 90 % and c. 80 %, respectively) and the plants

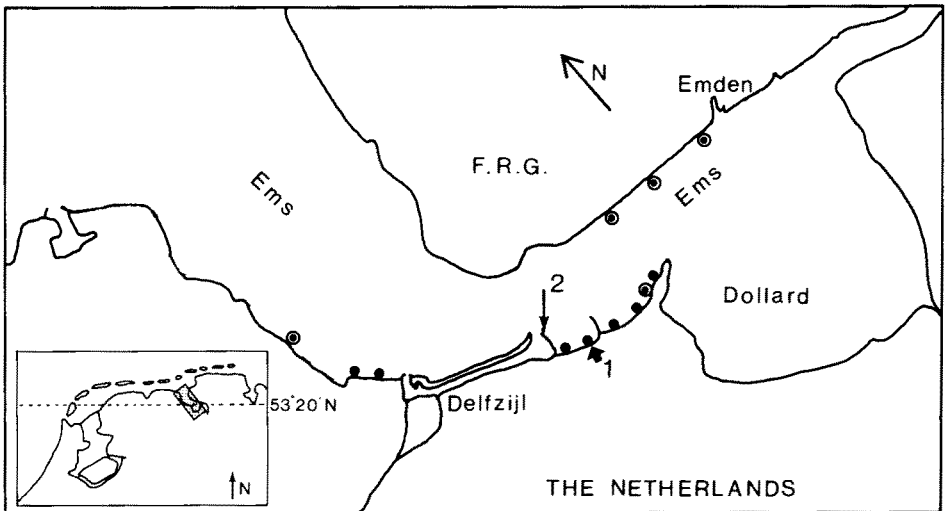


Fig. 1. *Rhodochorton purpureum*. Distribution in the Ems-Dollard estuary (northern Netherlands). *R. purpureum*-vegetation under fucoïd canopy (●), without canopy (○). Location of sample sites (arrow 1) and station where temperatures and tide-curves were recorded (arrow 2) (Havendienst Delfzijl, pers. comm.)

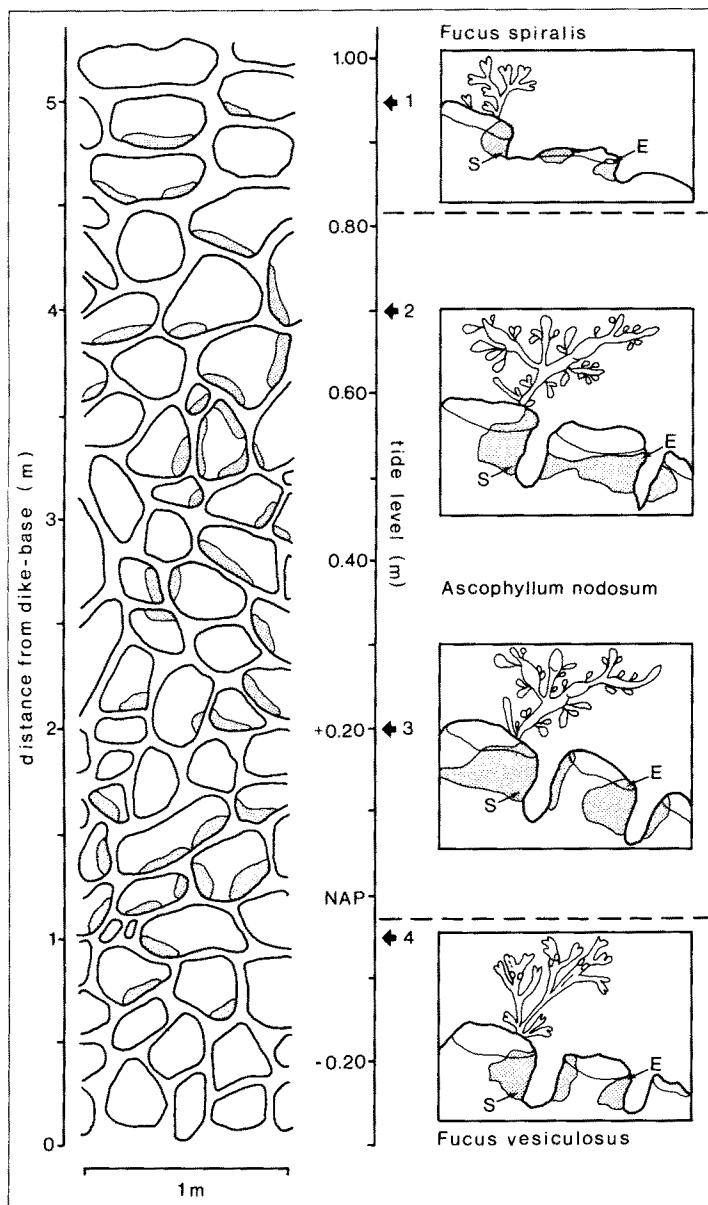


Fig. 2. *Rhodochorton purpureum*. Location of individual turf-patches (shaded) on the granite-blocks that face the dike (left) and diagram showing the location of sample-sites in exposed (E) and shaded (S) positions in the *F. spiralis*-zone (1), the *A. nodosum*-zone (2, 3) and the *F. vesiculosus*-zone (4) (right). Average tidal range: 2.80 m. Mean high water level at 1.24 m above NAP; MHWS at NAP + 1.50 m; MHWN at NAP + 0.94 m. NAP = Amsterdam Zero level \approx midtide

are very evenly spaced over the surface of the closely packed granite blocks. *R. purpureum* forms patches of dense velvety turf on the sides and upper edges of the granite blocks, with a preference for the lower, seaward facing sides of the blocks (Fig. 2). At their upper margins these patches are bordered by crusts of the red alga *Hildenbrandia prototypus* and the holdfasts of the fucalean algae. At their lower margins they are bordered by barnacles and other invertebrates and by silt. The *R. purpureum*-turf is very dense and homogeneous in height, which is attributed to heavy grazing by micrograzers (Hoeksema & Breeman, unpublished). Vertically, the *R. purpureum* population extends from the *F. vesiculosus*-zone, near the base of the dike, to the *F. spiralis*-zone at about 1 m above the NAP-level (Fig. 2).

We sampled this population at regular intervals during the autumn of 1980, throughout 1982 and during the spring of 1983. Samples were taken from eight different types of locations which represented two gradients: one is a tidal submergence gradient (sites 1 to 4, Fig. 2), the other a light exposure gradient. At each tide level (1–4, Fig. 2) separate samples were taken from relatively shaded and from relatively exposed sites. Shaded sites (marked S, Fig. 2) were situated at the lower margin of the *R. purpureum*-patches, deep in the crevices between the stones, and were, at the moment of sampling, completely covered by brown algal thalli. Exposed sites (marked E, Fig. 2) were situated at the upper margin of the *R. purpureum*-patches and were, at the moment of sampling, not covered by brown algal thalli.

Pieces of granite with the attached *R. purpureum*-turf were chipped off and a quantitative estimate of sporangial abundance was obtained by harvesting thirty subsamples of uniform size and recording the presence, in each subsample, of each of the following structures (Fig. 3): young sporangial branches (1), tetrasporangial initials (2), mature tetrasporangia (3), empty tetrasporangia (4), and regenerating remnants of old sporangial branch (5).

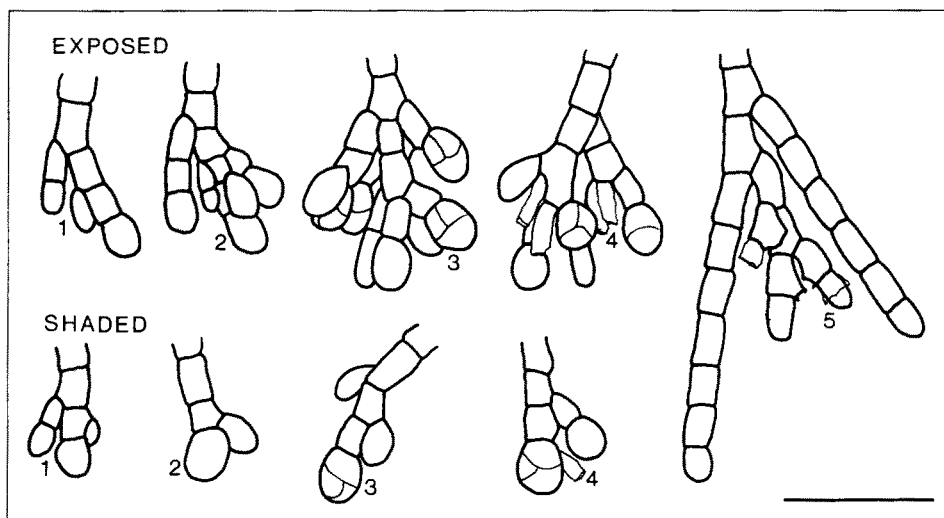


Fig. 3. *Rhodochorton purpureum*. Stages in the development of a tetrasporangial branch as observed in samples from exposed and shaded sites; initial of sporangial branch (1); tetrasporangial initial (2); mature tetrasporangium (3); empty tetrasporangium (4); regenerating remnant of old sporangial branch (5). Scale: 50 μ m

sporangial branches (5). This provided a general impression as to whether the process of spore-formation had only just started or was in a terminal phase. In the end the presence of tetrasporangial initials or mature tetrasporangia was chosen as an appropriate measure for sporangial abundance.

Climatological data

During the sampling period water temperatures, air temperatures and tide-curves were routinely recorded by the Havendienst Delfzijl (pers. comm.) at a station c. 0.5 km north of the sampling location (Fig. 1). Monthly averages for the daily irradiance curve were derived from long term solarimeter data recorded at 10 min intervals at Delfzijl (Fig. 1) during the years 1976–1980 (Colijn, 1982 and pers. comm.). Light attenuation in the waters of the Ems-Dollard estuary near Delfzijl was measured during 29 surveys between 1976 and 1980 by Colijn (1982).

Actual light conditions near the *R. purpureum*-plants were measured, both at low and at high tide, during 12 surveys carried out between 1982 and 1984. Light was measured with a LiCor 185 quanta meter, equipped, where necessary, with an underwater sensor. The degree of shading caused by the brown algal thalli at low tide was estimated in each of the fucallean zones. Comparative readings were taken with a quantum-sensor placed in full daylight and another sensor placed under the brown algal canopy. Shading by substrate topography was likewise estimated after the brown algal thalli had been removed. This shade-factor was equally valid at low and at high tide. At high tide two types of light measurements were carried out. In the first, light was measured at 5–10 min intervals throughout a tidal submergence cycle with the underwater-quantum sensor in a fixed position, either on the dike-surface between the *A. nodosum* thalli, or on the mudflat where there was no influence of the brown algal thalli. Simultaneously, the amount of light at the water-surface and the depth of the water-column flooding the sensor were recorded. These measurements were intended to get at first a rough impression whether the attenuating properties of the water varied much in the course of a high water period. They were carried out west of Delfzijl (Fig. 1) because these waters were clear enough to enable accurate readings ($> 1 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the underwater-sensor) throughout a high water period and this was not the case near the sampling station. Secondly, light versus depth-profiles were recorded near the sampling station, both in open water and in water with *A. nodosum*-thalli; this was done at various moments in the course of a high water period and in waterlayers of various depths. Unlike in clearer waters (Jerlov, 1977), in the very turbid waters of the Ems-Dollard estuary, attenuation of total PAR (Photosynthetic Active Radiation, $\lambda = 400\text{--}700 \text{ nm}$), which is measured with the quantum sensor, is approximately exponential with depth in the first few meters, in spite of the great spectral band width being measured (Colijn, 1982; Kirk, 1977). Therefore light attenuation in the whole watercolumn can be described with one quantum attenuation coefficient ($K_{400\text{--}700 \text{ nm}}$; m^{-1}).

Culture experiments

R. purpureum was collected on 24 May 1978 from the middle of the *A. nodosum*-zone. Unialgal cultures of the tetrasporophyte were kept at 12°C , 16:8 (L:D, h), c. $5 \mu\text{mol m}^{-2} \text{s}^{-1}$. Under these conditions no tetrasporangia were formed and growth was limited.

For the experiments c. 1 cm long branched filaments were cut off and planted at regular distances in Whatmann GF/A 11 cm glass microfibre filter paper covering the bottom of a petridish (Ten Hoopen et al., 1983). Per dish 35–50 plants were inoculated and 2–3 dishes were placed in each of the experimental conditions. The response to a given treatment was assessed by counting the number of plants that had formed tetrasporangia every week. The experiment was continued until no further plants formed tetrasporangia, which in some experiments took more than 4 months. In the end the maximal response was taken as a measure for the effectiveness of a given treatment. General methods of cultivation and equipment have been described elsewhere (Breeman & Ten Hoopen, 1981). In this study Philips TL34 fluorescent tubes were used as a light source. Table 1 compares the relative spectral distribution of the fluorescent light with that of

Table 1. Relative spectral distribution (% of total quanta 400–700 nm) of experimental light source (Philips TL34; manufacturer's specification) and of underwater light at 1 m depth in Jerlov's watertype 9 (Jerlov, 1976; sun + sky; solar elevation 65°)

| Waveband (nm) | Philips TL34 (%) | Underwaterlight (%) |
|---------------|------------------|---------------------|
| 400–500 | 14 | 16 |
| 500–600 | 37 | 43 |
| 600–700 | 49 | 41 |

underwaterlight at 1 m depth in Jerlov's (1976) watertype 9, the most turbid type in the classification. At mean high water marks, c. 1 m water floods the midlittoral *R. purpureum* plants, for which light quantities in the field were compared with experimentally determined threshold light levels for daylength perception. The fluorescent light contains somewhat more red light (600–700 nm) and peak transmission is at 660 nm instead of at c. 600 nm. The Ems-Dollard waters are more turbid than Jerlov's watertype 9 but spectral transmittance is probably comparable though there is some evidence for a further shift in peak transmission towards the "orange" in estuarine waters (Champ et al., 1980).

RESULTS

Seasonal occurrence of tetrasporangia in the field

Tetrasporangia were abundant in winter from December to February at all sample-sites (Fig. 4). In March sporangial abundance started to decrease at the highest tide levels and by April sporangia were absent there, whereas at the lower tide levels, and particularly at the shaded sites, sporangia were abundant till the end of May. On one sampling date, in the last week of April, sporangial abundance had, in some samples, increased compared with that of the previous date, and the presence of many young sporangial branches (stages 1 and 2, Fig. 3) indicated that there tetrasporogenesis had started anew. In June sporangia were absent at all sites and the whole population was sterile throughout the summer. In November sporangia started to reappear at all tide levels, somewhat sooner at the exposed than at the shaded sites. The over-all annual

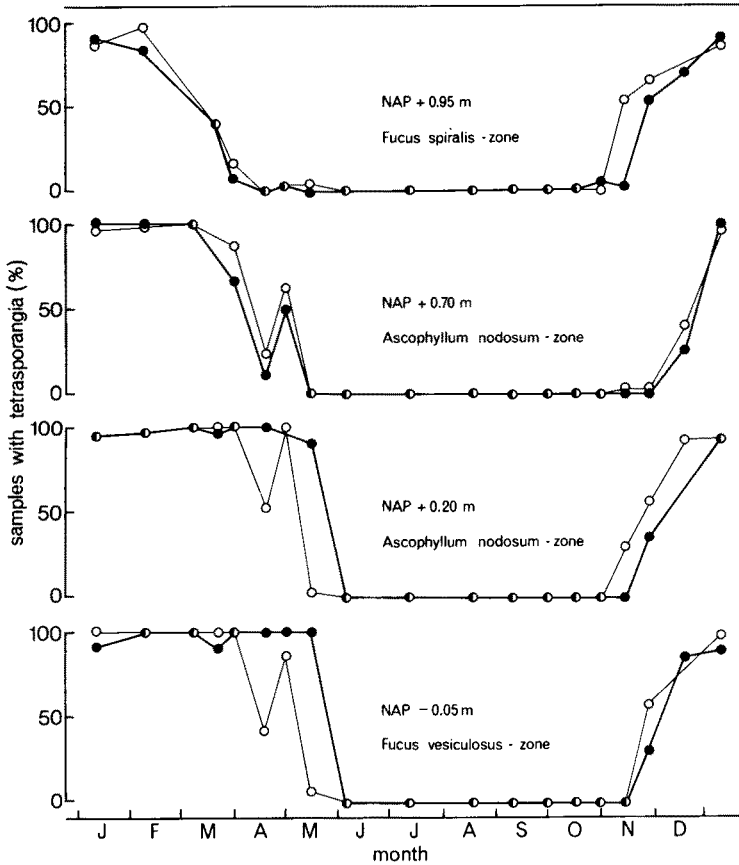


Fig. 4. *Rhodochorton purpureum*. Seasonal occurrence of tetrasporangia at four different tide levels at exposed (○) and shaded (●) sample sites (Fig. 2) as observed in 1982

pattern of tetrasporangial abundance as found on various sample-sites in 1982 (Fig. 4) was confirmed by data from the autumn of 1980 and the spring of 1983 (not shown).

Once only, during the entire sampling period did we find a gametophytic plant. A tetraspore had germinated in situ and had grown into a diminutive male gametophyte (Fig. 5). No other trace of male or female gametophytes was found, neither in the compact mat of rhizoidal filaments that constitutes the base of the *R. purpureum* turf, nor on the neighbouring bare granite substrate.

Causative factors for the observed tetrasporangial periodicity

Culture experiments

In order to analyse the causative factors for the observed periodicity we experimentally determined the influence of temperature, daylength and light quantity on tetrasporogenesis in a local *R. purpureum* isolate. Tetrasporangia were formed in short day and low temperature conditions only. Under optimal conditions tetrasporangia started to

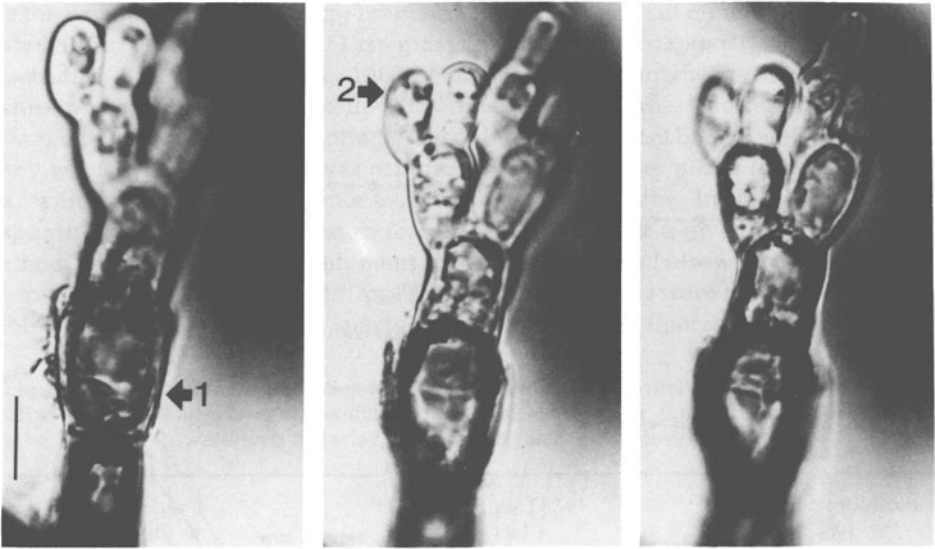


Fig. 5. *Rhodochorton purpureum*. Male gametophyte grown from a tetraspore that had germinated in situ (arrow 1); note spermatangia (arrow 2). Micrographs taken in 3 different plains of focus. Sample from shaded site 3 (Fig. 2) collected on 10 March 1982. Scale: 10 μm

appear after 3–4 weeks and it took c. 8 weeks for all plants to respond (Fig. 6). Under suboptimal conditions such as low temperatures or very low light levels, it took considerably longer (Fig. 6). The critical daylength was about 12 h both at 8° and at 12°C (Fig. 7), temperatures that are more or less typical for the periods in spring and autumn when the critical daylength of 12 h is reached (Figs 9 and 10). The 12 h critical daylength was valid for light levels above 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (up to 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$) but not at lower light levels where it was reduced to 11 h (Fig. 7). A light level as low as 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was still sufficient for tetrasporogenesis at daylengths of 8–11 h but not in very short days (Fig. 7).

The critical temperature lay between 16° and 18°C with 50 % response at 16.5°C (Fig. 8). This was found for plants pre-incubated at blocking high temperatures as well as for those pre-incubated at blocking long day conditions.

First estimation of key-factors

Comparing these culture results with field-data on temperature- and daylength-conditions during the sampling period (Figs 9, 10) we find periods when tetrasporogenesis would have been blocked by long days or high temperatures or both. If we assume (but see below!) that, for plants growing at very low light levels (< 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$), the day begins at sunrise and ends at sundown, but for those growing at higher light levels, the day includes civil twilight, we find that long days would block sporogenesis in all plants from early March to early October (Figs 10, 11).

High water temperatures would block sporogenesis for intermittent periods from mid May to mid September (Figs 9, 11).

Maximal daily air temperatures would be high enough to block sporogenesis

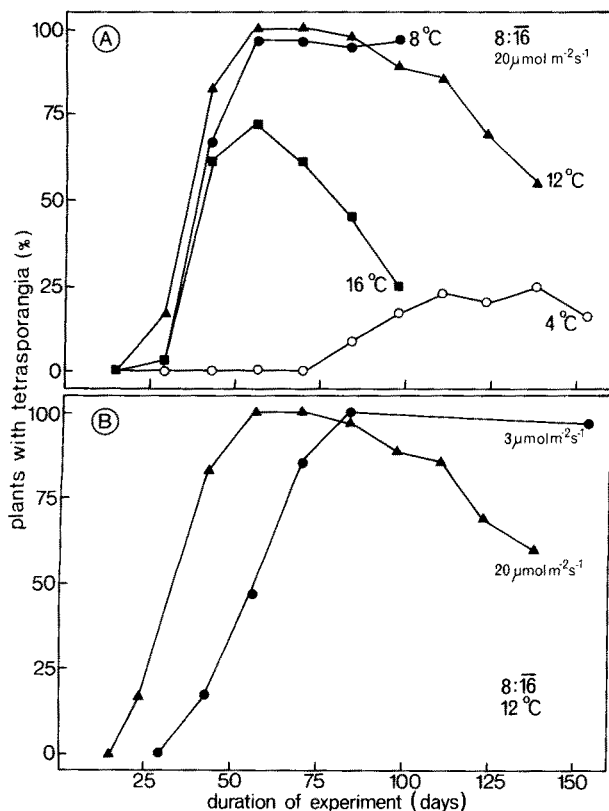


Fig. 6. *Rhodochorton purpureum*. Formation of tetrasporangia in cultures kept at various temperature- (A) and light (B) conditions in standard short days

throughout summer until early October, but minimal daily air temperatures would be rarely high enough to do so (Figs 9, 11).

Comparing the periods when one or more of these factors would have had high blocking values with the periods when sporangia were actually absent in the field (Fig. 11) we find that the occurrence of blocking long day conditions from early March does not agree with our observation that, at shaded sites at the lower tide levels, sporangia were being formed till the end of May. It is suggested that these plants experienced darkness for part of the day so that they were in fact subjected to short day instead of long day conditions.

The "effective daylength" for *R. purpureum*

Threshold value for light perception in the short day response

In order to test the above hypothesis we experimentally determined the threshold level for light perception in the short day response and compared this with the light conditions in the field at low and at high tide. The threshold level was determined in the following way: plants were subjected to a daylength-regime in which an 8 h main light

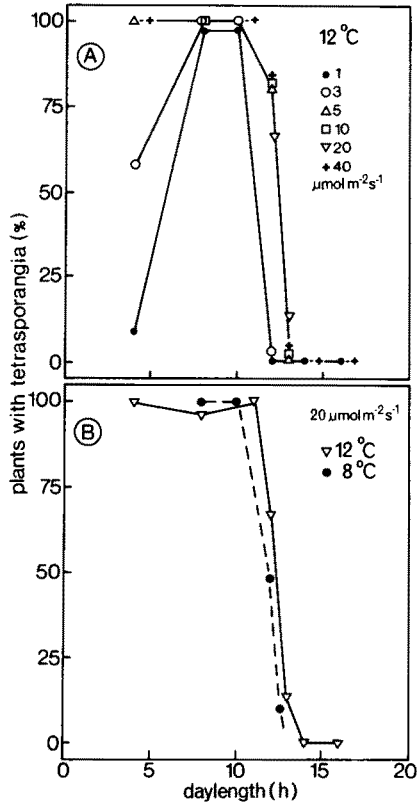


Fig. 7. *Rhodochorton purpureum*. Critical daylength for the formation of tetrasporangia at various light (A) and temperature (B) conditions

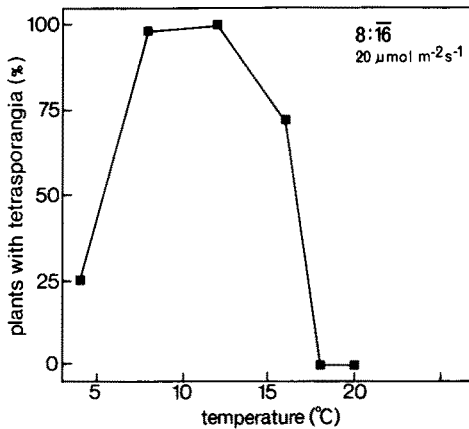


Fig. 8. *Rhodochorton purpureum*. Critical temperature for the formation of tetrasporangia in standard short days

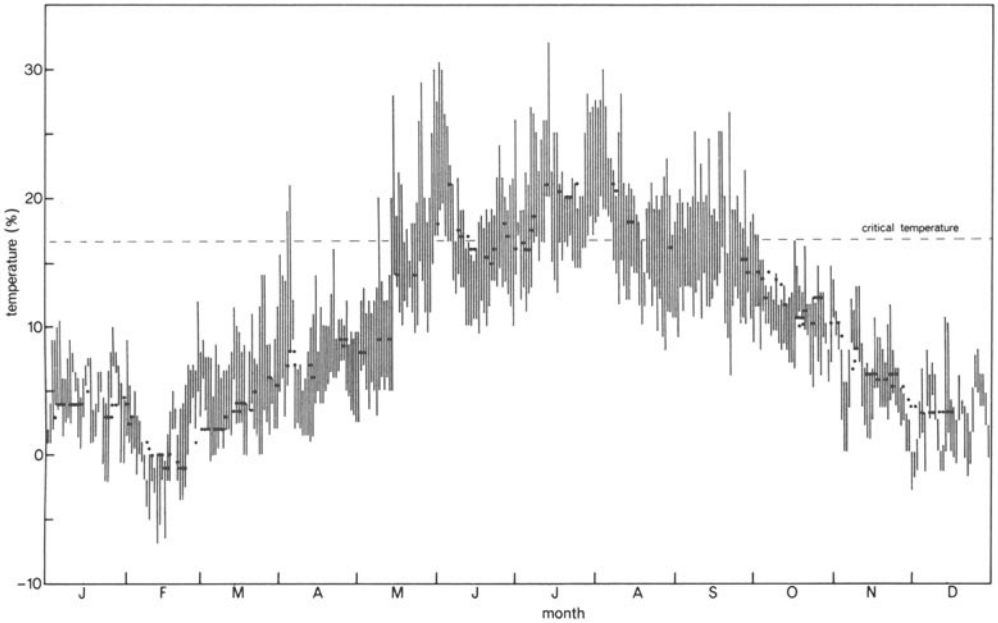


Fig. 9. Annual variations in water temperatures (●) and in daily ranges of air temperatures (vertical lines) during the sampling period as measured near Delfzijl (Fig. 1, arrow 2) (Havendienst Delfzijl, pers. comm.). Critical temperature for tetrasporogenesis in *Rhodochorton purpureum* indicated

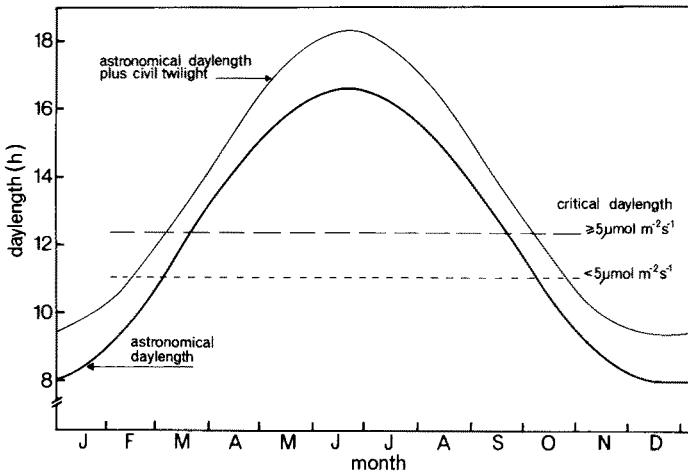


Fig. 10. Annual variations in the astronomical daylength and in the duration of the daylight period that begins and ends with civil twilight (from Smith, 1974 and daily irradiance-curves as measured at Delfzijl between 1976 and 1980 [Colijn, 1982 and pers. comm.]). Duration of civil twilight: 35 min at equinox, 52 min at midsummer. Critical daylength for tetrasporogenesis in *Rhodochorton purpureum* indicated for plants growing at very low ($< 5 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at higher light levels ($\geq 5 \mu\text{mol m}^{-2} \text{s}^{-1}$)

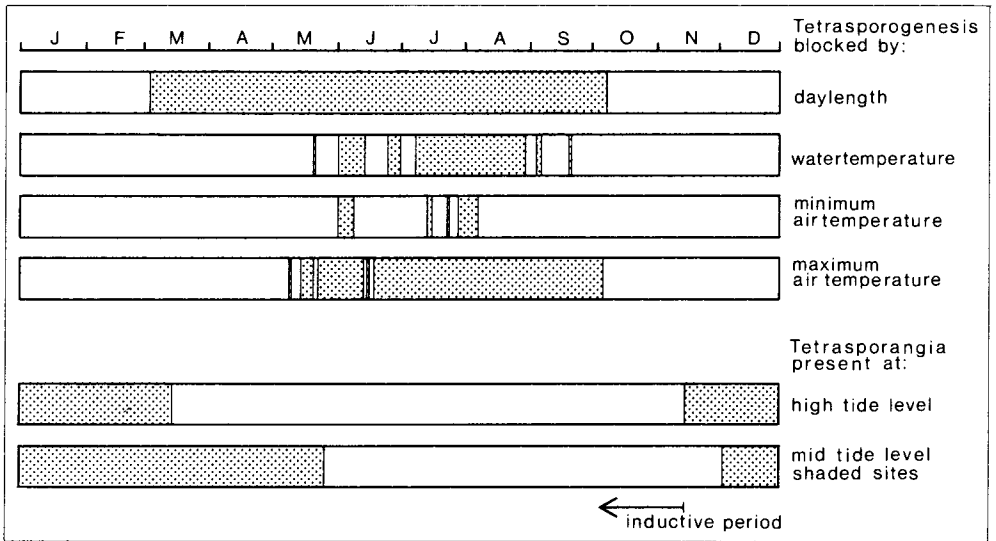


Fig. 11. *Rhodochorton purpureum*. Seasonal occurrence of possibly limiting factors and tetrasporangial presence at different sample sites

period was followed or preceded by an additional light period of 6 h at various low light levels (Table 2). If these were below the threshold for light perception the plants would form tetrasporangia. The threshold-value is not very sharply defined, because when light levels in the additional light period were raised from 0.2 to 0.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the number of plants that formed tetrasporangia decreased only gradually (Table 2). Moreover, the formation of sporangia was delayed in all treatments with additional light period: they only appeared after 7–8 weeks (compare Fig. 6). The sensitivity to light was slightly higher at the end than at the beginning of the main light period (Table 2). In

Table 2. *Rhodochorton purpureum*. Threshold for light perception in the short day response in tetrasporogenesis. Light/dark regimes: L = main light period (h), 16 $\mu\text{mol m}^{-2} \text{s}^{-1}$; \bar{L} = additional light period (h) at various low light levels; \bar{D} = dark period (h). Temperature: 12°C

| Regime (L : \bar{L} : \bar{D}) | Light level in additional light period ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | Plants with tetrasporangia (%) |
|-------------------------------------|---|--------------------------------|
| 14 : $\bar{10}$ | – | 0 |
| 8 : $\bar{16}$ | – | 100 |
| 8 : $\bar{6}$: $\bar{10}$ | 0.9 | 14 |
| | 0.2 | 21 |
| | 0.05 | 90 |
| $\bar{6}$: 8 : $\bar{10}$ | 0.9 | 25 |
| | 0.4 | 46 |
| | 0.2 | 50 |
| | 0.1 | 92 |

treatments where plants had received a light level of $0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ or less, during the additional light period more than 90 % eventually formed tetrasporangia. Therefore, we assume that, in the field, light levels below $0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ are no longer perceived by the plants.

Light conditions in the field at low tide

At low tide, shading is caused by the brown algal canopy and substrate topography. Shading is heaviest in the *A. nodosum*-zone and at the shaded sample sites (Fig. 2 sites 2S, 3S; Table 3). Here, light transmission through the algal canopy was, on the average, only 5 %. Only 9 % of the remaining light penetrates far enough into the crevices between the granite blocks to reach the *R. purpureum* plants at the shaded sample-sites. On a bright spring day, with an incident light quantity of, say, $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ at midday, only $4.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ would reach these plants, but this is still far above the threshold for light perception ($0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$, Table 2) which will, in fact, be exceeded within 30 min after sunrise. Moreover, individual plants are not as heavily shaded at every single low tide. Individually marked patches of *R. purpureum*-turf, which had been selected because they were completely covered by the *A. nodosum*-canopy, were, on subsequent low tides, all occasionally left uncovered. Then, the threshold for light perception will have been exceeded before sunrise, for plants in exposed positions directly after the beginning of civil twilight. However, accurate predictions on the precise moment when the threshold will be surpassed cannot be made on the basis of the culture experiments because these did not simulate the gradual in- and decrease of light at dawn and at dusk. Moreover, natural daylight contains more red and blue light than the artificial light source.

Nevertheless, we may conclude that heavy shading at low tide does not cause any substantial shortening of the light period for *R. purpureum* in spring.

Light conditions in the field at high tide

At high tide, light is reduced by attenuation in the watercolumn, brown algal shading and shading through substrate-topography. The latter factor will have the same value as it has at low tide (Table 3). The amount of light that penetrates a watercolumn

Table 3. Shade factors for *Rhodochorton purpureum* at low tide as measured during 6 surveys on 4, 19 and 25 Feb. and 10, 19 and 31 March 1983

| | Remaining light (%) | | Number of observations (n) |
|----------------------------|---------------------|--------------------|-------------------------------|
| | mean | standard deviation | |
| Algal canopy | | | |
| <i>Fucus spiralis</i> | 18 | 9 | 55 |
| <i>Ascophyllum nodosum</i> | 5 | 3 | 55 |
| <i>Fucus vesiculosus</i> | 11 | 6 | 48 |
| Substrate topography | | | |
| vertical/overhanging | 46 | 24 | 59 |
| entrance of crevices | 32 | 20 | 63 |
| deep in crevices | 9 | 5 | 45 |

depends on its depth and on the turbidity of the water. In the course of a high water-period, waterdepth varies for any sample-site, and the tidal submergence curve is quite different for sample sites situated at different tide levels. For instance site 4, situated near the mid-tide level, is flooded for c. 7 h with at most 1.3 m water; whereas site 1, situated 1 m higher, is flooded for only 3 h with at most 0.3 m water (Figs 2; 12A). In the very turbid waters of the Ems-Dollard estuary (mean attenuation-coefficient $K_{400-700 \text{ nm}}$ in this region is 5.88; Colijn, 1982) this difference would result in a 500-fold difference in light quantity at these sites during high water. However, the situation is much more

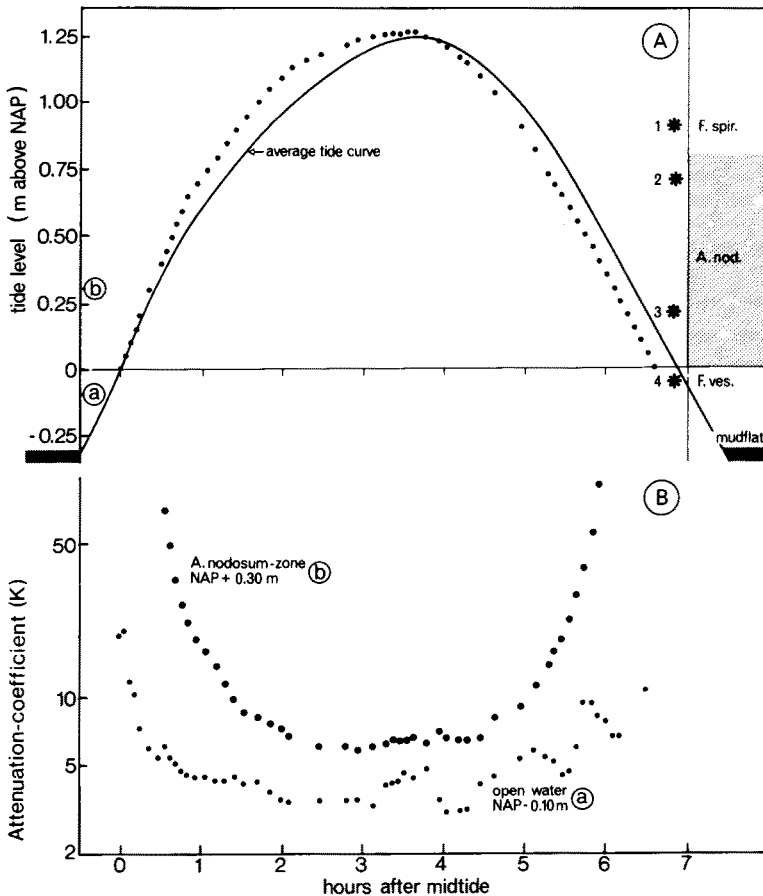


Fig. 12. A: Average tide curve for Delfzijl (upper part) (Getijtafels voor Nederland) and tide curve during high water period when light measurements were taken (●) sample sites 1–4 (Fig. 2) indicated. B: variations in the "attenuation coefficient" ($K_{400-700 \text{ nm}}$; m^{-1}) in the course of a high water period as measured in open water (a) and in water with *A. nodosum*-thalli (b). Underwater-quantum sensors were placed on the mudflat (a, upper graph) or on the substrate of the dike amidst the *A. nodosum*-thalli (b, upper graph). "Attenuation coefficients" calculated from: $K (\text{m}^{-1}) = (\ln I_0 - \ln I_d) / d$ where: I_0 = amount of light at watersurface; I_d = amount of light at underwater-sensor; d = depth of waterlayer flooding the underwater-sensor. Example typical for 4 similar series of measurements based on data from 25 Oct. 1983

complicated, because the light attenuating properties of the flooding water are not constant in the course of a high water period. In open water, attenuation-coefficients are highest directly after midtide, when the flooding water has just passed over the muddy tideflat (Fig. 12B); and they are lowest at slack high water when clearer water from the seaward side of the estuary has come in (Fig. 12B; Colijn, 1982). Nevertheless, these variations are of minor importance compared to those caused by brown algal shading. In water with brown algal thalli the total "attenuation-coefficient", as it results from the combined effect of water turbidity and brown algal shading, is much higher in shallow than in deep water (Figs. 12B; 13; 14). This must be attributed to the fact that in water

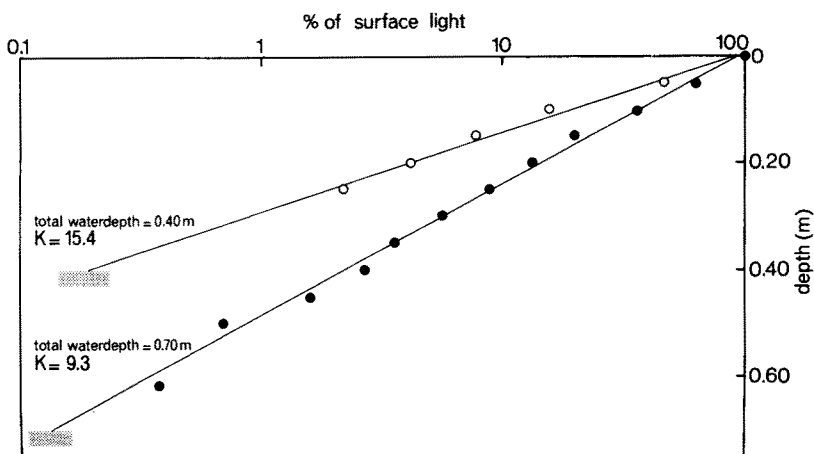


Fig. 13. Percentage of surface quanta (400–700 nm) in relation to waterdepth as measured in water with *A. nodosum*-thalli differing in total waterdepth. Two examples based on measurements carried out on 18 Feb. 1984

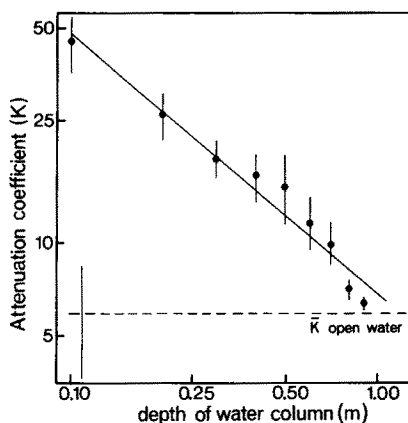


Fig. 14. Light "attenuation coefficients" ($K_{400-700 \text{ nm}}$; m^{-1}) in water with *A. nodosum*-thalli in relation to total waterdepth. Values based on light vs. depth profiles (i.e. Fig. 13) as measured on 11 July 1982; 28 March, 27 April and 25 Oct. 1983 and 18 Feb. 1984, all in normal weather-conditions. Mean values for individual depths based on 4–17 profiles (total $n = 92$). Average value for open water based on Colijn (1982 and pers. comm.) ($n = 29$). Vertical bars = st. dev.

shallower than the total length of the brown algal thalli these will assume a more horizontal position and thereby increase light attenuation in the water column considerably. At rising and falling tide water will be more shallow for some time, therefore the total "attenuation-coefficient" will vary strongly in the course of a tidal submergence period (Fig. 12). These variations within one tidal cycle caused by brown algal shading are much larger than seasonal variations caused by changing water-turbidity (Figs 12, 14; Colijn, 1982 and pers. comm.). However, after stormy periods, especially when winds have been blowing from the north-west, along the axis of the estuary, attenuation-coefficients in open water may also increase to over 20 (Colijn, 1982 and pers. comm.).

The fact that in waterlayers with brown algal thalli a lesser waterdepth goes with a higher "attenuation-coefficient" neutralizes to a certain extent the difference in the amount of light that reaches the bottom as the tide rises. Light reduction is very high directly on tidal submergence, and remains so for the entire duration of the high water period. In the following section we will find that this causes a substantial shortening of the light period for the *R. purpureum* plants in spring, in particular for those growing at shaded sites at the lower tide levels.

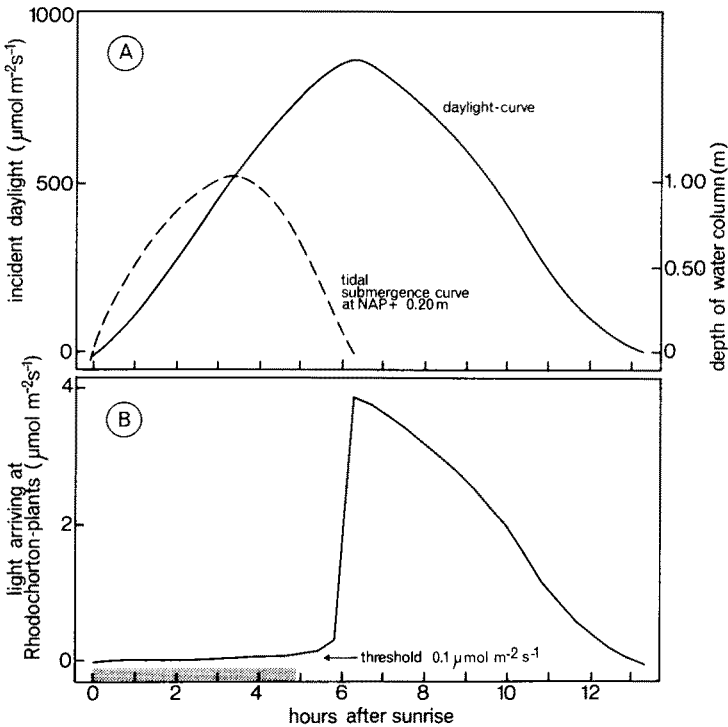


Fig. 15. A: Average daily light curve (smoothed) for the first two weeks of April as based on measurements taken in Delfzijl between 1976 and 1980 (Colijn, 1982 and pers. comm.) and average tidal submergence curve for sample site 3 (NAP + 0.20 m, Fig. 2). B: The amount of light arriving at *Rhodochorton purpureum*-plants at sample site 3 S (shaded) (Fig. 2) during high and during low tide in 25 min intervals calculated from the upper graph (a), Fig. 14 and Table 3. Threshold for light perception in *R. purpureum* indicated (from Table 2); black bar shows extra dark period caused by tidal submergence

Light/dark regimes for *R. purpureum* caused by tidal flooding

The average amount of light arriving at the *R. purpureum* plants in the course of a high water period may now be calculated using the following data: (1) the average daily irradiance-curve for the period concerned (Colijn, 1982 and pers. comm.; Fig. 15A), (2) the average tidal submergence-curve at the tide level concerned which gives the variations in waterdepth in the course of the high water period (Figs 12A; 15A), (3) the average total "attenuation-coefficient" valid for each waterdepth in waterlayers with brown algal thalli (Fig. 14), and (4) the average amount of shading by substrate-topography (Table 3).

We calculated these data for the period in spring (early April) when the critical daylength had been passed and the plants growing at the highest tide level had stopped forming tetrasporangia, whereas those growing at shaded sites at the lower tide levels continued having tetrasporangia (Figs 4; 11). We compared sample sites located in the *A. nodosum*-zone at 0.20 m above the NAP-level in exposed and shaded positions. At mean high water mark these sites were flooded with 1.04 m water. At the shaded site tetrasporangia were still abundant on a subsequent sampling-date (Fig. 4; 20 Apr.); whereas at the exposed site tetrasporangial abundance had started to decrease (Fig. 4).

At the shaded site (site 3S, Fig. 2), in the course of the day, light conditions at the *R. purpureum* plants vary in the following way (Fig. 15): If tidal flooding starts at daybreak, the high water period will last till midday (Fig. 15A). In the first part of the day very little light will reach the plants and light-levels will be below the threshold for light perception ($0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$, Table 2) for c. 5 h (Fig. 15B). Consequently, the night will be extended with 5 extra hours of darkness to a total duration of 16 h (Fig. 16A, first day of the fortnight). However, on the following day, high water mark is not reached before 50 min later and then, a light break is inserted between the night and the dark period caused by tidal flooding (Fig. 16A). During a fortnight light/dark regimes will be very variable. There are some days with two light and two dark periods, when the high tide

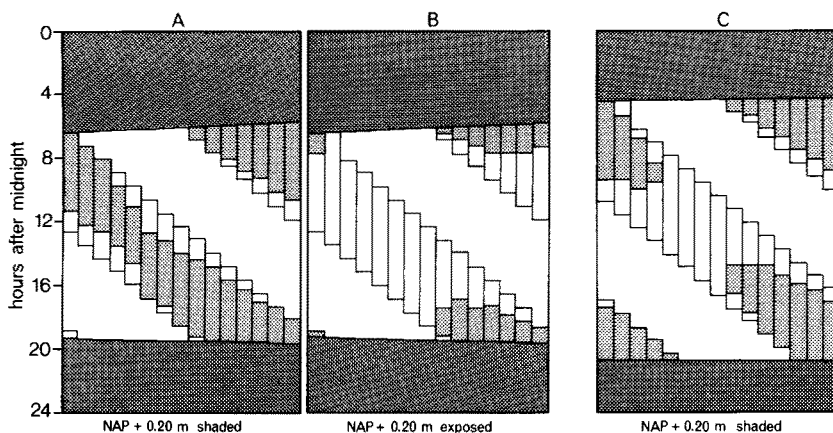


Fig. 16. *Rhodochorton purpureum*. Light/dark regimes as caused by tidal submergence for *R. purpureum*-plants growing at shaded and exposed sample-sites in the *A. nodosum*-zone at NAP + 0.20 m calculated (as in Fig. 15) for two 2-week periods (early April [A, B] and June [C]) preceding sampling-dates when tetrasporangial abundance had decreased at B and C, but not at A (Fig. 4)

falls in the middle of the day; and others with one light and one, long, dark period, when one high tide falls at the beginning and the next at the end of the day. Then, the night is extended both at dawn and at dusk (Fig. 16A).

At the exposed site (site 3E; Fig. 2) high tides falling in the middle of the day did not cause an extra dark period (Fig. 16B). Here shading through substrate topography was less than at the shaded site (Table 3). At midsummer, tidal flooding at midday no longer caused an extra dark period on the shaded site (Fig. 16C) as the incidental light quantity was too high.

At both sites (Fig. 16A, B) light-dark regimes varied in the course of a fortnight (Table 4). The number of days with uninterrupted long nights was no higher at the

Table 4. *Rhodochorton purpureum*. The presence of tetrasporangia at various sample-sites and characteristics of the light/dark regimes at these sites as caused by the interaction of the normal light/dark cycle and the occurrence of extra dark periods during tidal flooding

| Period | Early April | | June |
|---|-------------|---------|--------|
| Sample site (Fig. 16): | A | B | C |
| - tide level (m above NAP) | 0.20 | 0.20 | 0.20 |
| - exposure | shaded | exposed | shaded |
| Tetrasporangia on next sampling date (Fig. 4) | + | - | - |
| Hours of daylight/24 h (mean) | 8.2 | 12.2 | 12.1 |
| Number of days per fortnight with: | | | |
| - uninterrupted long nights | 6 | 6 | 3 |
| - uninterrupted long days | 0 | 7 | 4 |
| - light/dark ratio (/24 h) > 1 | 0 | 8 | 8 |

shaded site, where tetrasporangia were still being formed, than it was at the exposed sites where tetrasporangial abundance had started to decrease. However, there days with light:dark ratios over 1 (critical daylength = 12 h: light:dark ratio = 1) did not occur and the average duration of daylight per 24 h was markedly below that in the critical daylength (Table 4; Fig. 7). These comparisons suggest that the formation of tetrasporangia may rather depend on the average light:dark ratio than on the presence of uninterrupted long nights. To confirm this, we did a number of culture experiments.

Light/dark regimes that are effectively "short days" for *R. purpureum*

We tested the effect of night-break regimes such as occurred in the field as a result of tidal flooding. Plants were given a 10 h main dark period and, also, an extra dark period of 6 h, either in the morning or in the afternoon (Fig. 17). None of these treatments caused any inhibition of tetrasporogenesis. Thereafter, we compared the effect of regimes with one light and one dark period daily with those of equal light:dark-ratios but two light and two dark periods daily. The response depended on light:dark-ratios rather than on the duration of single light or dark periods (Fig. 17). These results were in close accordance with field-data. At the shaded site, where light:dark ratios were below 1 every day, tetrasporangia were still being formed, although several days with night-

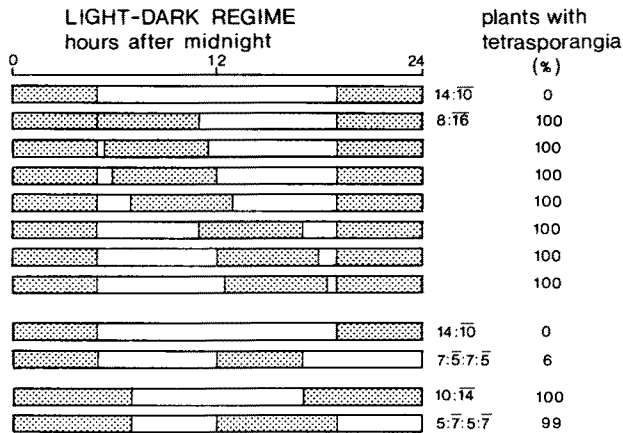


Fig. 17. *Rhodochorton purpureum*. The effect of light/dark regimes with "night-breaks" on the formation of tetrasporangia. Light level: $20 \mu\text{mol m}^{-2} \text{s}^{-1}$; temperature: 12°C

breaks occurred (Table 4). At the exposed site days with light:dark ratios over 1 were of frequent occurrence.

We experimentally tested how many "long-days" could be inserted in a short day regime before any inhibition of tetrasporogenesis occurred (Table 5). If one third of the

Table 5. *Rhodochorton purpureum*. The influence of repeated long-day interruptions of a short-day regime on the formation of tetrasporangia. Long days: 16:8; short days: 8:16; Light level: $20 \mu\text{mol m}^{-2} \text{s}^{-1}$; Temperature: 12°C

| Repeated treatment with short days + long days | Plants with tetrasporangia (%) |
|--|--------------------------------|
| 8 + 4 | 94 |
| 6 + 6 | 40 |

days were long ones tetrasporogenesis was not inhibited, but if half the days were long ones tetrasporogenesis was inhibited by 60 % (Table 5). Again, these results correspond closely with field-data. At the exposed sample-site, where tetrasporangial abundance had started to decrease (Table 4), 7 days in the fortnight were long ones and 8 had light:dark-ratios over 1.

Summarizing, we may conclude that the differences in tetrasporangial abundance at various sample-sites as they occurred in the spring were caused by differences in the effective daylength prevalent at the sites. These must be attributed to differences in the duration and frequency of the occurrence of extra dark periods during tidal flooding. For plants growing at shaded sample-sites at the lower tide levels shading was so heavy that effectively short-day regimes persisted far into the spring, though not all summer (Fig. 16C). Weather-conditions may influence the effective daylength markedly. For instance, on one occasion we found that tetrasporogenesis had started anew at sample-sites where it had already ended (Fig. 4, 30 April). Prior to this, severe storms had been blowing for

2–3 weeks causing water-turbidity to be above the average and mean high water marks to have risen by 0.6 m. These conditions enhanced light extinction at high tide to such an extent that, once more, short day conditions, as perceived by the alga, were prevalent.

The absence of tetrasporangia at shaded sites at the lower tide levels throughout the summer and autumn until November cannot be attributed now to the occurrence of blocking long day conditions there (Figs 4, 11). Therefore, the influence of temperature as a potentially blocking factor for tetrasporogenesis has been further examined.

Blocking temperatures for tetrasporogenesis in Rhodochorton purpureum

During late summer and autumn, when effective daylengths no longer have a blocking high value for plants growing at shaded sites at the lower tide levels, temperatures fluctuate around the critical temperature. Daily minimal air temperatures, to which all plants are regularly subjected, never exceed the critical temperature, but water temperatures do so regularly and maximal air temperatures do so continuously (Figs 9, 11).

We experimentally tested whether inductive low temperatures, when applied during part of the day, might induce tetrasporogenesis when, meanwhile, mean temperatures were of blocking high values (Table 6). However, no tetrasporangia were formed,

Table 6. *Rhodochorton purpureum*. The influence of daily temperature fluctuations on the formation of tetrasporangia. Daylength regime: 8:16; Light level: 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The period with a high temperature started at the beginning of the light period

| Temperature (°C) | | | High temperature for (h) | Plants with tetrasporangia (%) |
|----------------------|---------|------|-----------------------------|-----------------------------------|
| maximum | minimum | mean | | |
| 18 | 11 | 13 | 8 | 84 |
| 21 | 11 | 18 | 16 | 0 |
| critical temperature | | | | 50 |

and this is in accordance with their absence in the field. We also tested whether blocking high temperatures when applied for part of the day, might inhibit tetrasporogenesis and found that, in this case, fewer plants formed tetrasporangia (Table 6). These results suggest that in *R. purpureum* tetrasporogenesis may be more influenced by average or maximal daily temperatures than by daily minima. This is in agreement with our observation that, in the field, tetrasporangia had disappeared from the entire population about 3 weeks after maximal air temperatures had reached blocking high values; and that they reappeared in November, about 4 weeks after maximal air temperatures had dropped below these values. However, plants growing on shaded places are probably never subjected to maximal air temperatures and there only the occasional high water temperatures, prevalent till mid-September (Figs 9, 11) may have been the blocking factor for tetrasporogenesis. Further experiments, extensive sampling and more accurate in situ temperature-measurements will be required before the exact nature of the temperature-block existing in autumn is fully understood.

DISCUSSION

This study has shown that for benthic marine algae, growing in heavily shaded habitats, the effective daylength may differ considerably from the astronomical daylength. For midlittoral *R. purpureum*-plants, flooding with turbid estuarine water and heavy shading by the fucoïd canopy reduced light to such an extent that it was "night" for these plants during part of the high water period. In spring, this caused an extension of the period with "effective" short days and the plants responded with a continued production of tetrasporangia.

Dring (1984) calculated that light reduction during tidal flooding in turbid coastal waters with a large tidal range may be so high that the mean light level over a 14 days spring-neap tidal cycle is below the compensation point for fucoïd photosynthesis at shore levels above ELWS.

The studied *R. purpureum* population grows at a location which is particularly heavily shaded. The estuarine turbidity maximum is situated nearby (near Emden, Fig. 1; Colijn, 1982) and this causes maximal light attenuation in the watercolumn. Moreover, shading through the brown algal canopy is very heavy because the fucoïd thalli are evenly and densely packed on the gradually sloping substratum. Finally, substrate topography causes extra shading because the *R. purpureum*-plants grow deep in the crevices between the closely packed granite blocks. *R. purpureum* is a notoriously shade-tolerant species (Børgesen, 1908; Dellow & Cassie, 1955) and may elsewhere also occur in locations where it is so heavily shaded that the "effective" daylength is reduced.

In photoperiodic responses the duration of daylight is probably perceived by the same photomorphogenetic pigments that can be demonstrated in night break experiments. In *R. purpureum* there is some evidence about the spectral sensitivity of this pigment. Night breaks with blue (448 nm) and red (667 nm) light were most effective; those with green (547 nm) and farred (730 nm) light were relatively ineffective (Dring & West, 1983). This implies that our estimate of the threshold for light perception may be somewhat too low when applied to the amount of light arriving at the midlittoral sites at mean high water marks, because our light source contained more red light than will penetrate the c. 1 m water column flooding these sites. Consequently, we may conclude that at mean high water mark these plants will definitely be in darkness, and possibly that the duration of darkness is even longer than has been estimated. However, the spectral distribution of the light arriving at any shore level will change in the course of a high water period as waterdepth changes. In shallower water, a larger proportion will be in the blue and red spectral ranges to which the plants are more sensitive. The tide curve of Delfzijl is flat-topped and very steep at midtide, so that waterdepth will approach its maximum soon after tidal submergence. Nevertheless, our estimates of the duration of darkness during tidal flooding cannot be more than an approximation. Variations in the level of high water mark through the spring-neap tide cycle and variations due to weather conditions will cause further irregularities in the light-dark regimes caused by tidal flooding. In fact there was some evidence for the marked influences of weather conditions when *R. purpureum*-plants started tetrasporogenesis anew after a period with extra high water turbidity and extra high water levels.

The semidiurnal tides, with 12.4 h between consecutive high water marks, cause the dark period during high water to "shift" over the day. On some days this causes "night break" regimes, with both the duration of the "night-break" and the main light period

changing from one day to the next. Dring & West (1983) found that 1 h night breaks inhibited tetrasporogenesis in *R. purpureum* when combined with daylengths approaching the critical daylength, but not in shorter days. They described the "over-all" effect of these 1 h night breaks as to cause an increase in the "effective" daylength with 3.5–4 h (which means an increase of 2.5–3 h in the total duration of light per 24 h). If such an effect were also valid for longer night breaks this would still cause an extension of the period with effective short days for the midlittoral *R. purpureum* plants in the Ems-Dollard estuary far into the spring. However our experimental results suggest that in regimes with longer night breaks the response may depend on light:dark ratios. In regimes with a light:dark ratio approaching the critical daylength (e.g. 10:14; critical daylength = 12:12) the same response was induced when there were two light and two dark periods per 24 h (5:7:5:7) as when there was only one light and one dark period per 24 h (10:14).

In summer and autumn high temperatures prevent the formation of tetrasporangia on all shore levels. Since, in autumn, tetrasporogenesis in the midlittoral plants is not blocked by long day conditions, it was possible to analyse the nature of this temperature-effect more precisely. Daily temperature minima were below the critical temperature almost continually, but no tetrasporangia were being formed and this was also found in culture experiments. Therefore the temperature-effect is rather a blocking effect of high temperatures than an inductive effect of low ones.

This study has shown that, through the influence of microclimatic factors, plants of the same population growing nearby may differ in their reproductive phenology and are, in this, regulated by different environmental factors.

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