

Relationship between Phycoerythrin and Nitrogen Content in *Gloiopeltis furcata* and *Porphyra yezoensis*

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Seasonal changes of pigment composition in two intertidal red algae, *Gloiopeltis furcata* (Postels et Ruprecht) J. Agardh, and *Porphyra yezoensis* Ueda, were investigated. Chlorophyll *a* and phycoerythrin levels were high during winter, but decreased in late spring or summer, with accompanying discoloration from deep red to green or yellow. This discoloration corresponded closely to the fluctuation of phycoerythrin content. Nevertheless, photosynthesis capacity was maintained by the increasing water temperature in the field, suggesting that large amounts of phycoerythrin are not necessary for photosynthesis. Phycoerythrin content correlated significantly with nitrogen content in both species when the nitrogen level was greater than the level of critical content (1.30% DW in *G. furcata*, and 2.26% DW in *P. yezoensis*), indicating that phycoerythrin plays a more important role in the nitrogen status as a nitrogen pool than that of nitrogen critical content. Furthermore, the dependence level of the algae on phycoerythrin as a nitrogen pool was greater in *P. yezoensis* than in *G. furcata* because of the remarkable increase of phycoerythrin content in *P. yezoensis* with increasing nitrogen content.

Key Words: discoloration, *Gloiopeltis furcata*, nitrogen, phycoerythrin, pigment composition, *Porphyra yezoensis*, red algae

INTRODUCTION

Red algae show a characteristic summer discoloration which is regarded as a loss of pigment related to environmental conditions (Neish *et al.* 1977). This discoloration often decreases the commercial value of seaweed. In Japan, fertilizer application has been attempted to prevent discoloration and to improve the quality of *Porphyra* (Nozawa 1959; Amano and Noda 1987). It is necessary to determine the discoloring mechanism for effective cultivation and management of commercial seaweeds. Algal pigment composition is influenced by light conditions. The changes in pigment composition are often represented by the phycoerythrin/chlorophyll *a* ratio (PE:Chl *a*). The phycobiliprotein/chlorophyll *a* ratio increases with water depth, and chlorophyll *a* and phycoerythrin contents are inversely proportional to light level (Ramus *et al.* 1976a, 1976b). Nitrogen amount also changes algal pigment composition, particularly the level of phycoerythrin (Lapointe 1981; Bird *et al.* 1982;

Lapointe 1985). Discoloration is tightly linked to the nutritional conditions of algae. It has been reported that nitrogen enrichment increases both chlorophyll *a* and phycobillin in *Gelidium latifolium* (Rico and Fernández 1996), *Chondrus crispus* (Neish *et al.* 1977) and *Porphyra yezoensis* (Amano and Noda 1987). Moreover, pigment content often changes with photosynthesis and respiration, influencing the growth and maturation of algae. However, no direct indicator has yet been identified to aid in the elucidation of the discoloration of seaweed.

This study aims first to clarify the seasonal changes of pigment composition in two intertidal red algae, *Gloiopeltis furcata* and *Porphyra yezoensis*, and second, to determine the relationship between pigment content and nitrogen content.

MATERIALS AND METHODS

Two red algae, *Gloiopeltis furcata* and *Porphyra yezoensis*, were collected once or twice a month at a fixed site in the intertidal zone at Usujiri, southern Hokkaido, Japan, in the period from December 1997 to August 1998. Seawater was collected from the same site in the period

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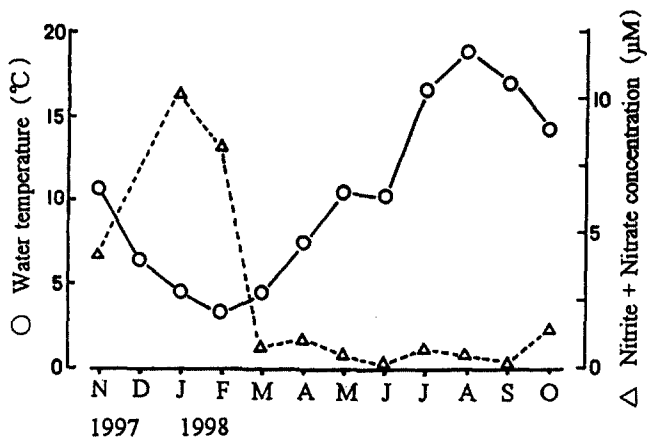


Fig. 1. Monthly changes in seawater temperature and ambient nitrite+nitrate concentration in the intertidal zone at Usujiri, Hokkaido, Japan.

from November 1997 to October 1998 and filtered with a Whatman GF/C glass fiber filter (Whatman International Ltd., Maidstone, England) to measure the nutrients present following the method described by Parsons *et al.* (1984). After removing the epiphytes, the plants were measured for fresh weight and surface area, and were then traced onto tracing paper to measure the surface area with a digital planimeter (KP-92N, Uchida Yoko Co., Ltd., Tokyo, Japan). The surface area of *G. furcata*, which has a cylindrical thallus, was estimated as a column by measuring the diameters and lengths of the axes and branches in each plant. The plants were also subjected to analysis of photosynthetic pigments. Chlorophyll *a* and carotenoid levels were determined following the method described by Evans (1988). Phycoerythrin and phycocyanin were extracted with 0.1M phosphate buffer and the absorbency of the extracts was measured using a spectrophotometer (type-220, Hitachi Ltd., Tokyo, Japan) following the method described by Beer and Eshel (1985). Variance of each pigment content was less than 4.4% of the mean value. The nitrogen content was analyzed using plants collected on three occasions in *G. furcata* (January, April and July 1998) and on four occasions in *P. yezoensis* (January, April, May, and June 1998). The nitrogen content was measured by the Kjeldhal method after the plants were dried under a vacuum desiccator and weighed. Accuracy was 3.2% of each mean value. Photosynthesis was measured by the dissolved oxygen method with an oxygen electrode (Model 57, Yellow Springs Instrument Co. Inc., Ohio, USA). After thirty minutes of acclimation, dissolved oxygen in a glass bottle (100 ml) agitated with a

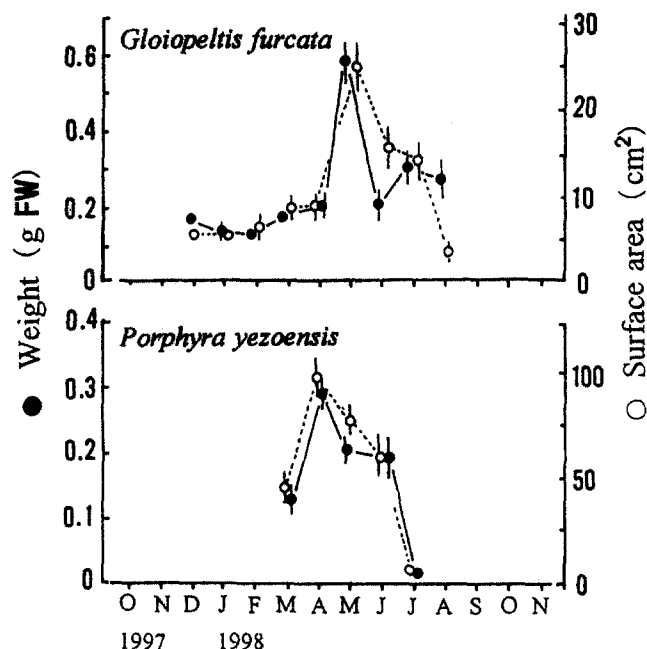


Fig. 2. Growth patterns of *Gloiopeltis furcata* and *Porphyra yezoensis* collected in the intertidal zone at Usujiri, Hokkaido, Japan. Data are mean \pm SE ($n = 4-6$).

stirrer was monitored for thirty minutes. Four plants of each type of algae were used for measuring photosynthesis, expressed as a mean value. Irradiance was adjusted to 0, 83, 298, 705, and 1195 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a photorelector lamp (PRF-500W, Matsushita Electric Industrial Co. Ltd., Osaka, Japan). Photosynthetic parameters were calculated by the theoretical equation, $P = bI(1 + aI)$, where P is the gross photosynthetic rate, I is irradiance, and a and b are constants. α is the initial slope ($= b$). I_k ($= 1/a$) is the saturation irradiance at which P reaches the maximum photosynthetic rate ($P_{\text{max}} = b/a$).

RESULTS

Monthly changes in water temperature and ambient nitrite+nitrate concentration are shown in Fig. 1. The water temperature reached its minimum (3.3°C) in February and its maximum (18.9°C) in August. The nitrite+nitrate concentration was 10.2 μM at its maximum in January, but decreased (1.0 μM) from March on. The ammonium concentration was always less than 1 μM in the period from May to September.

Gloiopeltis furcata became visually perceptible in December and its weight and area increased to 0.57 ± 0.07 gFW and 25.2 ± 3.0 cm², respectively, in May (Fig. 2). Its color was yellow in July and August. *Porphyra yezoensis* was collected from March to July. In April, its

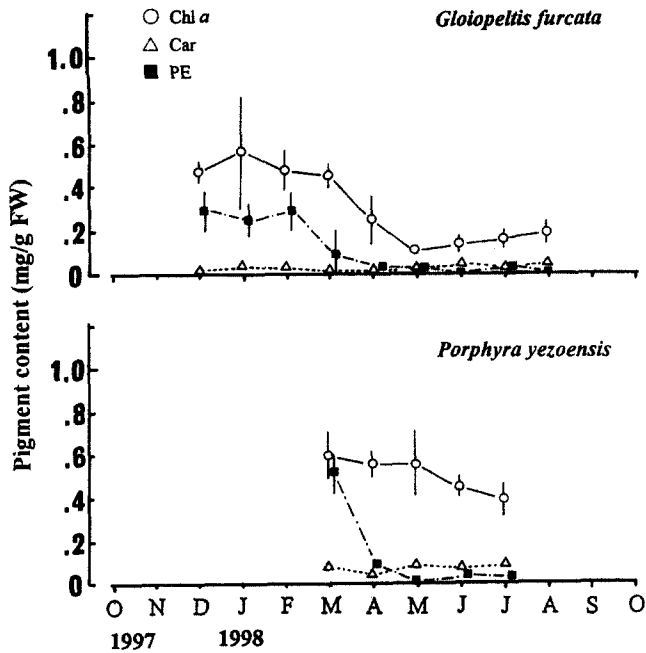


Fig. 3. Monthly changes of pigment contents (mean \pm SE, $n = 4$) in *Gloiopeltis furcata* and *Porphyra yezoensis* collected in the intertidal zone at Usujiri, Hokkaido, Japan.

weight and surface area reached 0.31 ± 0.04 gFW and 91.2 ± 9.8 cm², respectively, both of which were the maximum measurements for this species. The thallus color changed from red in April to green in May and June.

The monthly changes of the photosynthetic pigment contents of the two red algae are shown in Fig. 3. High chlorophyll *a* and phycoerythrin contents in both species were observed in the winter months. The chlorophyll *a* and phycoerythrin contents of *G. furcata* in February were more than 0.2 mg gFW⁻¹ and decreased as the plant shifted in color from red to yellow in the period from May to August. Its phycoerythrin content changed in a pattern similar to that of chlorophyll *a*. Carotenoid content ranged from 0.01 to 0.05 mg gFW⁻¹ without clear seasonality. In *P. yezoensis*, the maximum content of chlorophyll *a* and phycoerythrin reached 0.59 mg gFW⁻¹ and 0.51 mg gFW⁻¹ in February, respectively. During the period from April to August, the phycoerythrin content was less than 0.10 mg gFW⁻¹. However, carotenoid content did not show clear seasonality and was consistently less than 0.10 mg gFW⁻¹. In both species included in the present study, phycocyanin content was always less than 10% of the total photosynthetic pigment (< 0.05 mg gFW⁻¹).

The seasonal variations of accessory pigment/chlorophyll *a* ratio in the two red algae are shown in Fig. 4. The

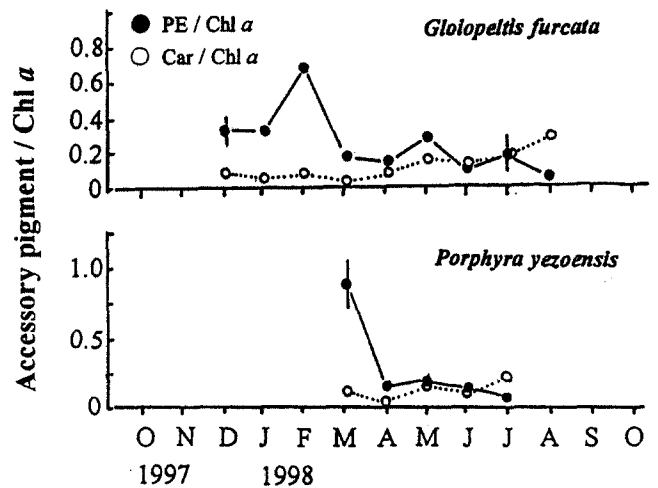


Fig. 4. Monthly changes of accessory pigment/chlorophyll *a* ratio (mean \pm SE, $n = 4$) in *Gloiopeltis furcata* and *Porphyra yezoensis* collected in the intertidal zone at Usujiri, Hokkaido, Japan.

PE:Chl *a* ratio changed seasonally in both species, and ranged from 0.03 to 0.67 in *G. furcata*, and from 0.00 to 0.90 in *P. yezoensis*. The PE:Chl *a* ratio was higher in high ambient nitrate concentrations and low water temperatures than in low nitrate conditions and high water temperatures. In contrast, the carotenoid/chlorophyll *a* (Car:Chl *a*) ratio showed no seasonal change in either species.

The fluctuation of nitrogen content and photosynthetic parameters during the discoloration of the two red algae are summarized in Table 1. The mean nitrogen content of *G. furcata* was at its maximum (3.65% DW) in January, but was less than 2.0% DW after April. The mean nitrogen content of *P. yezoensis* was highest in March, consistently showing more than 2.15% DW. The maximum photosynthetic rate and respiratory rate in *G. furcata* showed no significant change during the collection period, while the maximum photosynthetic rate (P_{max}) and respiratory rate (R) in *P. yezoensis* were highest in January. Compensation irradiance (I_c) in *G. furcata* was consistently higher than that in *P. yezoensis*. On the contrary, saturation irradiance (I_k) in *G. furcata* was consistently lower than that in *P. yezoensis*. Initial slope (α) in both species increased from the winter months to the late spring months.

DISCUSSION

Pigment content changes according to environmental

Table 1. Nitrogen content and photosynthetic parameters of *Gloiopeltis furcata* and *Porphyra yezoensis* during the discoloration period at Usujiri, Hokkaido, Japan

Month	Water temp. (°C)	Nitrogen	Pmax	Respirations	I _c	I _k	α
		%DW (s.e.)	μmolO ₂ ·cm ⁻² ·hr ⁻¹	μmolO ₂ ·cm ⁻² ·hr ⁻¹	μE·m ⁻² ·s ⁻¹	μE·m ⁻² ·s ⁻¹	(μmolO ₂ ·cm ⁻² ·hr ⁻¹)·(μE·m ⁻² ·s ⁻¹) ⁻¹
<i>Gloiopeltis furcata</i>							
Jan.	3.3	3.65(0.19)	105.6	1.76	32.93	197.6	0.08
Apr.	7.4	1.64(0.39)	102.6	1.71	27.58	239.1	0.11
Jul.	18.5	1.57(0.52)	115.8	1.93	34.57	122.7	0.29
<i>Porphyra yezoensis</i>							
Jan.	3.3	4.40(0.20)	75.0	2.50	17.57	408.8	0.19
Apr.	7.4	3.24(0.53)	37.2	1.24	25.15	532.3	0.12
May	10.4	2.15(0.32)	37.8	1.26	28.57	358.7	0.19
Jun.	10.2	2.36(0.06)	42.0	1.40	10.93	209.8	0.41

factors. As red algae in deep waters are rich in phycoerythrin (Ramus *et al.* 1976a, 1976b), light is considered to be a major factor influencing pigment composition. However, light did not seem to induce discoloration of the two red algae in this study because the plants were collected from the intertidal zone. Lapointe (1981, 1985) reports that a high nitrogen environment results in an increase of phycoerythrin and chlorophyll *a* in *Gracilaria foliifera*. In *Porphyra yezoensis*, phycoerythrin content was increased five times by nitrogen enrichment (Amano and Noda 1988). Rico and Fernández (1996) also report that nitrogen and phycoerythrin in *Gelidium latifolium* decreases in low ambient nitrate conditions. As noted above, these observations indicate that phycoerythrin plays an important role as a nitrogen pool for red algae. Accordingly, it is important to determine the relationship between phycoerythrin and nitrogen content in order to understand the discoloration process.

Figure 5 shows the relationship between phycoerythrin and nitrogen contents in the two red algae. Both species showed a significant positive correlation ($p < 0.05$) between phycoerythrin and nitrogen contents. These relations can be expressed by the following equations: $y = 0.108x - 0.140$ ($r = 1.00$) for *G. furcata*, and $y = 0.191x - 0.431$ ($r = 0.92$) for *P. yezoensis*. Phycoerythrin was depleted when the nitrogen level was 1.30%DW in *G. furcata* and 2.26%DW in *P. yezoensis*. These values indicate the critical nitrogen content necessary for storing phycoerythrin. The critical nutrient content is generally calculated from the relationship between nutrient contents in algae and their maximal growth rates, and has often been used for estimating the nutrient condition of several species of green and brown algae (Wong and Clark 1976; Chapman *et al.* 1978; Gordon *et al.* 1981; Probyn and Chapman 1983; Rosenberg *et al.* 1984;

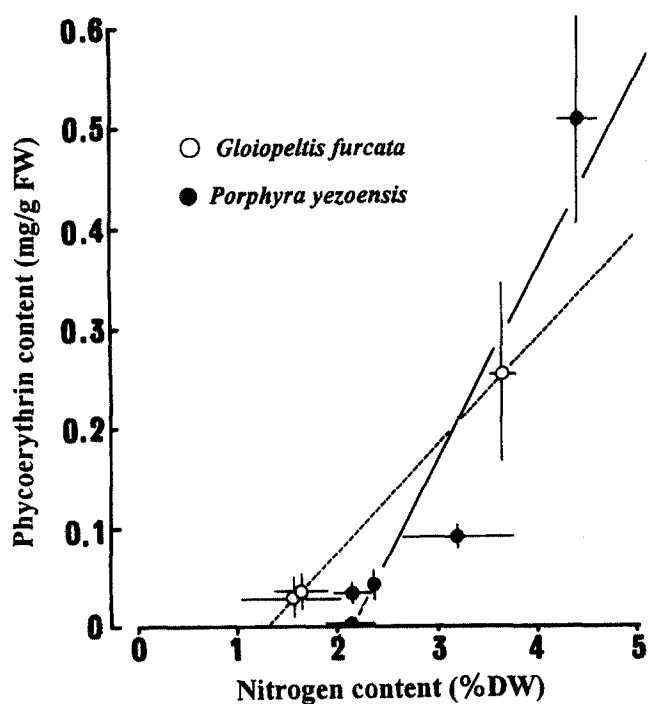


Fig. 5. Relationships between phycoerythrin accumulation and nitrogen content in *Gloiopeltis furcata* and *Porphyra yezoensis* collected in the intertidal zone at Usujiri, Hokkaido, Japan. Symbols show the mean values (\pm SE), which were shown in Fig. 3 and Table 1.

Mizuta *et al.* 1992). The critical nitrogen content necessary for growth has been determined to be about 2% in several red algae species: *Gelidium sesquipedale* (Vergara *et al.* 1993), *Gracilaria tikvahiae* (Hanisak 1990), *Gelidium latifolium* (Rico and Fernandez 1996), *Gracilaria foliifera*, and *Agardhiella subulata* (DeBoer 1981). In the current study, the critical nitrogen content for the accumulation of phycoerythrin appears to be consistent with the critical nitrogen contents found for other species of algae.

Thus, critical nitrogen content is useful in judging the initiation of discoloration in red algae. In addition, the slope of the function in Fig. 5 decreased in the order of *P. yezoensis* (0.191), and *G. furcata* (0.108). This order may be indicative of a dependence level on phycoerythrin as a nitrogen pool.

The relationship between pigment content and environmental factors, particularly light, has been reported for many macrophytes, but the effects of pigment content on the growth of seaweeds are not well understood. Ramus *et al.* (1976a) hypothesize that photosynthesis and growth are proportional to the increase of pigment content only in algae with thin thalli. However, in this study photosynthesis in both species was maintained even as phycoerythrin content decreased. It is likely that increasing water temperature and residual chlorophyll *a* support the maintenance of photosynthetic capacity. The phycobiliprotein/chlorophyll *a* ratio increases with water depth (Ramus *et al.* 1976a, 1976b) and chlorophyll *a* and phycoerythrin contents are inversely proportional to light level (Lapointe, 1981). These observations suggest that the contribution of phycoerythrin to photosynthesis increases with water depth. Accordingly, the function of phycoerythrin as a nitrogen pool in red algae may be greater in shallow populations than in deep ones.

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