Conference Paper

Seasonal Changes in the Concentration of Photosynthetic Pigments *Palmaria palmata* (Linnaeus) F. Weber & D. Mohr

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Abstract

The article presented the overview of the main seasonal dynamics of photosynthetic pigments (chlorophyll a, carotenoids and phycobiliproteins) as well as their ratios for the mass species of the Murmansk coast of the Barents Sea red algae *Palmaria palmata*. The concentration of pigments depends on the level of illumination and temperature which is statistically confirmed. The results of the research showed that the chlorophyll content of *Palmaria palmata* is characterized by a significant decrease in summer and an increase in winter which is in contrast to the change in the level of illumination. It was proved that in winter with a decrease in light, the efficiency of photosynthesis is provided by the accumulation of pigments including phycobilins. It is suggested that the relationship of reproduction, namely the release of tetrascospores with the reduction of phycobiliproteins, since the presented process requires significant energy costs. It is shown that the concentration of phycobilins decreases with increasing light intensity. Prolonged presence of algae under high light intensities reduces the amount of photosynthetic pigments. The results of the research showed that the number of pigments decreases with low temperatures and vice versa their number increases with increasing temperature. A significant negative correlation between the water temperature and the main pigments of the photosynthetic apparatus -- chlorophyll a and carotenoids was revealed. The concentration of carotenoids and phycobilins depends on the function they perform: in summer - photoprotectors, in winter-additional light collectors. Palmaria has a maximum content during the polar night. Significant changes in the ratios of Car/Chl a, PhE/ Chl a, PhE / PhE depending on environmental factors are shown.

Keywords: *Palmaria palmata*, illumination, pigments, temperature, Barents sea

1. Introduction

*Palmaria palmata* (Linnaeus) F. Weber & D. Mohr is phytocenosis-forming species which is often used by other plants as a basiphyte. It is characterized by the availability of endophytes [1, 2].
The intensity and direction of assimilation processes in marine red algae are mostly determined by the number and ratio of pigments of their light-absorbing complex (LAC). Adaptive adjustments of the latter in response to seasonal changes in environmental factors (lighting, temperature and concentration of nutrients in water, etc.) contribute to maintaining a high level of algae primary production during the year. Among the pigments characteristic of red algae, it is possible to distinguish phycobiliproteins (phycoerythrin and phycocyanin), different ratios of which cause their color from bright crimson to blue. These pigments are actively involved in photosynthesis processes in algae and are viewed as reserve proteins. Seasonal changes in the content and ratio of pigments can be regarded as one of the ways of mechanisms regulating the productivity of algae communities [3].

In addition to a significant ecological role this species is also interesting as a source of a number of valuable biological substances, such as vitamins, mycosporin-like amino acids. Algae are characterized by a high content of protein, macro-and microelements. *Palmaria* is used in the medical industry as a source of antioxidant components [4], in the cosmetic industry for correction, hyperpigmentation of the human body, as a modeling and sunscreen cosmetics. Photosynthetic pigments are harmless natural dyes, they are used for the food and cosmetic industry, phycobilins can be also used in medicine as immune stimulators and while creating new diagnostic drugs [5, 6].

The aim of the work is to analyze seasonal changes in the accumulation of photosynthetic pigments (chlorophylls, carotenoids and phycobilin pigments) of *Palmaria palmata* on the Murmansk coast of the Barents Sea and to identify the factors determining their changes.

### 2. Methods and Equipment

#### 2.1. Methods

The researches upon *Palmaria palmata* were carried out from November 2018 to September 2019. Samples were taken once a month during syzygy tides from the littoral of the Abram-Cape of the Kola Bay (South knee) of the Barents Sea. The samples were processed within 2 hours after sampling; the concentration of photosynthetic pigments (chlorophyll A, carotenoids, phycobilins (phycocyanin, phycoerythrin)) was determined by the use of spectrophotometry methods. During plants gathering salinity, water temperature and air were measured every month.
The extraction of chlorophyll a and carotenoids was carried out by the method of D. I. Sapozhnikov (1964) [7]. The concentration of chlorophyll a in acetone solution was calculated according to the formula proposed by Jeffrey and Humphrey (1975) [8]. The concentration of carotenoids in acetone solution was calculated according to the formula proposed by Seeley and co-authors (1972) [9].

The isolation of phycobiliproteins. Algae weighing 200-500 mg were ground with glass sand, seawater (pH 6.8) was added for complete homogenization of the sample (5-7 ml), the homogenate was centrifuged for 15 minutes at 5000 rpm after worked with a supernatant. The concentration of PBP was calculated by Rosenberg formulas [10].

The composition of pigments was expressed in µg/g wet weight of algae. The determination of the algae pigments amount was carried out in three-fold repetition. The arithmetic rate values of the indicator were calculated on the basis of three measurements.

A nonparametric Spearman Rank test was used to identify the connection between pigment content and environmental factors. The relationship was considered reliable at \( P < 0.05 \). Statistical data processing was performed using Microsoft Excel 2010.

### 3. Results

Seasonal changes in the main environmental factors on the Murmansk coast of the Barents sea, recorded during the sampling of algae, are presented in Table 1.

| Month | T of water °C | T of air °C | Salinity ‰ | Illumination W/m² |
|-------|---------------|-------------|------------|-------------------|
| X     | 4,9           | 0,8         | -          | 40                |
| XI    | 2             | -0,27       | 29         | 10                |
| XII   | 0,6           | -11,5       | 27         | 0                 |
| I     | 0,1           | -7          | 26         | 2                 |
| II    | 3,8           | 4,4         | 30         | 55                |
| III   | 2,9           | 6,3         | 24         | 184               |
| IV    | 7,2           | 13,3        | 16         | 240               |
| V     | 10            | 10,2        | 7          | 245               |
| VI    | 9,1           | 9,6         | 15         | 237               |
| VII   | 11,1          | 12,2        | 16         | 170               |
| VIII  | 8,8           | 8,8         | 18         | 115               |
| IX    | 7,9           | 5,6         | 18         | 60                |

Table 1: Indicators of abiotic factors at the time of sampling affecting the physiological processes of *Palmaria palmata*. 
The maximum water temperature during the study period was recorded in July, the minimum in January. The maximum air temperatures were recorded in April, the minimum in December. Salinity during the research varied significantly with a minimum value in May and a maximum value in February. The maximum average monthly intensity of illumination according to literary data [11] on the Murmansk coast was during the period from May to June, the minimum was in winter period.

Chlorophyll a and carotenoids. In November, the maximum concentration of chlorophyll a (1.09 mcg/g of crude mass) was found, in winter time the changes are insignificant, in summer there is a decrease in the concentration of chlorophyll, with a minimum in September (0.25 mcg/g of crude mass).

The composition of carotenoids increases in winter and decreases in summer periods. The maximum is observed in November (0.32 µg / g of crude mass) and May (0.27 µg/g of crude mass), the minimum concentration is recorded in September (0.069 µg/g of crude mass) (Figure 1).

Phycobiliproteins (FBP). The analysis of phycobilins revealed the dominant pigment-phycoerythrin, the content of which ranged from 0.09 to 0.32 µg / g of crude mass (Figure 2), with a minimum in January and a maximum in November.

4. Discussion

During the polar night the high content of chlorophyll a in the cells allows *P. palmata* using low light intensity. A decrease in the pigment was observed which is associated with an increase in the amount of light with spring coming. The amount of chlorophyll decreases while intense solar activity. The amount of chlorophyll increases with reduced solar activity (Figure 1).
In winter, according to the research of M. V. Makarov [11], *P. palmata* has the maximum development of the light-absorbing complex. Due to the multifunctional orientation of carotenoids their quantitative change occurs.

Carotenoids protect chlorophyll from photodestruction at high light intensity in the spring and summer months when the intensity of illumination on the Murmansk coast reaches maximum rates.

They perform the role of additional "light collectors" at a lower level increasing the efficiency of light energy use in winter period [12, 13].

In general, the chlorophyll composition of *Palmaria palmata* is characterized by a significant decrease in summer and an increase in winter which is in contrast to the change in the level of illumination. In the researches done on the far Eastern algae there is also an increase in the winter period. The adaptation to low light can be seen as a result. [3].

In winter with a decrease in light, the efficiency of photosynthesis is provided by the accumulation of pigments including phycobilins. The formation of reproduction organs *Palmaria palmata* requires significant energy costs which is timed to the polar winter. The decrease in phycobilin pigments in January may be due to the consumption of spare substances during the winter period and the release of tetraspores. The further increase is due to higher light intensity for more efficient operation of the photosynthetic apparatus and its protection from excess lights. It is shown that with increasing dose of UV radiation the concentration of phycoerythrin can decrease [14].

While high visible light intensities on *Chondrus pinnulatus* are exposed, a decrease in the content of PBP was noticed in laboratory conditions [15]. The decrease in phycobilins in *P. palmata* in the summer is also due to exposed high visible light intensities.
The quantitative composition of pigments in thalli depends on the illumination level and temperature of water. Phycobiliproteins and carotenoids play an important role in the adaptation of plants to low or high light levels which contribute to the preservation of viability, prevent inhibition of photosynthesis and destruction of the photosynthetic apparatus [16].

The number of carotenoids increases with increased photosynthetically active radiation (PhAR) as they are photoprotectors of the plant photosystem. Prolonged presence of algae under high light intensities reduces the number of photosynthetic pigments, which was demonstrated in studies [12].

Water temperature affects the daily rhythm of algae and their seasonal development. This factor affects metabolic processes. The number of pigments decreases at low temperatures and vice versa their number increases when temperature is becoming higher [13]. A significant negative correlation between the water temperature and the main pigments of the photosynthetic apparatus -- chlorophyll a and carotenoids was revealed (Table 2).

| Pigment      | Water t, °C | Air °C t, °C | Salinity, ‰ | Illumination W/m |
|--------------|-------------|-------------|-------------|------------------|
| Chlorophyll a (Chl a) | -0.818***   | -0.664***   | 0.800***    | -0.573*          |
| Carotenoids (Car)   | 0.510**     | 0.420*****  | 0.483****** | -0.224           |
| Phycoerythrin (PhE) | 0.145       | -0.018      | -0.195      | 0.1              |
| Phyccyanin (PhC)    | -0.082      | -0.155      | -0.195      | -0.064           |
| Car / Chl a        | 0.077       | 0.336*****  | -0.207      | 0.559*           |
| PhE / Chl a        | 0.773**     | 0.6*        | -0.798***   | 0.664*           |
| PhE / PhT          | 0.818**     | 0.7***      | -0.740***   | 0.618*           |

**Note:** reliable values of the influence of environmental factors on the pigment content at P < 0.05 (*), P < 0.1 (* *), P < 0.01 (* * *), P < 0.3 (* * * * ) P < 0.2 (* * * * * ) are marked in bold.

There was a positive correlation between illumination and the ratio of Car/Chl a, PhE/Ch a, PhE/PhT (Table 2). A decrease in the Car/Chl ratio a (Figure 3, A) is a potential bioindicator of photo-oxidative damage in plant cells under the action of strong light. During the research period, the indicator remained at a constant level except in May when the ratio increased sharply and amounted to 0.63 mcg / g of crude mass and also marked the minimum value in July (0.22 µg/g) of crude mass.

The change in ratio may be due to the destruction of chlorophylls or additional synthesis of carotenoids. The increase in this ratio seems to be the result of the strengthening of the protective function of yellow pigments that inhibit lipid peroxidation.
processes in the algal thalloma [17]. In spring when the lighting is maximal, the role of carotenoids increases, they protect chlorophylls from photodestruction.

![Graphs showing seasonal ratios of carotenoids to chlorophyll (Car/Phl a) (a), phycoerythrin to chlorophyll (PhE/Chl a) (b), phycoerythrin to phycocyanin (PhE/PhC) (c).](image)

**Figure 3:** Seasonal ratios of carotenoids to chlorophyll (Car/Phl a) (a), phycoerythrin to chlorophyll (PhE/Chl a) (b), phycoerythrin to phycocyanin (PhE/PhC) (c).

The ratio of phycoerythrin to chlorophyll a varies significantly (from 0.12 to 1.03) (Figure 3, B). All above factors in table 2 have a statistically significant impact on this indicator. The seasonal PhE/PhCh ratio (Figure 3, B) was generally assessed as stable, with the exception of July when there was a sharp rise in the index and the winter months (October and January) with minimal values.

During the spring-summer period, the value of PhE/Chl a (Figure 3, B) increases in comparison with the winter period as the intensity of lighting, water and air temperatures increase. In these months, phycoerethrin acts as a photoprotector the increase in the ratio of PhE/Chl a in *Palmaria palmata* in September is due to a general decrease in chlorophyll a, as demonstrated in Figure 3, phycoerythrin appears to act as an additional light collector (photosensitizer). In the work of Makarov M. V. [11] the maximum size of the light-absorbing complex is seen in April and December. According to our data, the maximum size is in May and September-November. These differences may be associated with different frequency of measurements and the region of research (central and eastern Murmansk), these areas may have a shift in the onset of hydrological phases and seasonal differences in temperature.
The maximum ratio of the PhE/PhC indicator is fixed simultaneously with the minimum value of the Car/Ch ratio a (Figure 3, A). Perhaps the increase in phycobilin pigments was an adaptive reaction of the photosynthetic apparatus to photo-oxidative damage.

5. Conclusion

The data showed that changes in the light-absorbing complex *Palmaria palmata* on the Murmansk coast of the Barents Sea during the year, in addition to internal factors are largely regulated by the seasonal dynamics of abiotic factors photosynthetically active radiation (PAR) and temperature.

The shown changes in the concentration and ratio of photosynthetic pigments reflect adaptive rearrangements of the light-absorbing complex in response to seasonal changes in light and temperature regimes. With increasing solar activity the concentration of Chlorophyll a decreases in order to avoid the destruction of the photosynthetic apparatus. The amount of carotenoids and phycobilins depends on the function they perform: in summer - photoprotectors, in winter will be additional light collectors. *Palmaria palmata* has a maximum number of them during the polar night.

Thus, the illumination and temperature regime influence the quantitative distribution of photosynthetic pigments. The results of the research can be applied in the future in industry. It is necessary to have an idea about the seasonal dynamics of extracted substances in order to identify the appropriate time for the procurement of raw materials for the rational use of biological resources. It is also required to prepare the pigment complex *Palmaria palmata* in conditions of reduced light intensity.

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Conflict of Interest

The authors have no conflict of interest to declare.

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