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Seasonal Variations in the Metabolic Activity of Cells of *Fucus vesiculosus* Linnaeus, 1753 (Phaeophyta: Fucales) from the Barents Sea

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Abstract—The cellular metabolic activity was studied in the brown alga *Fucus vesiculosus* from the Barents Sea. Several phases of activity of physiological processes in cells of this alga during a 1-year period have been identified. This study shows that the onset and duration of the three main phases of seasonal development of alga, viz., the resting phase, growth, and the accumulation of reserve nutrients, can be determined based on the level and dynamics of the cellular metabolic activity. Each development phase in *F. vesiculosus* is characterized by a specific rhythm of the daily metabolic activity of cells.

Keywords: cell metabolic activity, *Fucus vesiculosus*, seasonal development, apical meristem, MTT assay, annual growth cycle, daily activity, Barents Sea

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INTRODUCTION

The climatic features of the Arctic region, viz., significant seasonal variations in illuminance, temperature, and the amount of nutrients, determine the annual periodicity in the development of macroalgae. The periods of their active growth, reproduction, and accumulation of nutrients are usually confined to a certain season [6, 20, etc.]. The time of the onset and duration of these periods in some algal species depend on the meteorological characteristics of the considered year, primarily on temperature, whereas other species manifest relationships with circannual rhythms to a greater extent [17, 22, etc.]. It is difficult to determine the beginning of a certain period in the development of algae using the standard research methods. However, each of these periods is accompanied by a change in the plant's physiological state, one of the indicators of which is the cellular metabolic activity (CMA).

The aim of this work was to study the seasonal and daily dynamics of metabolic activity of cells in the brown algae *Fucus vesiculosus* L. and to assess the possibility to determine the seasonal phases of algae development on the basis these dynamics.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Algology and the Biological Station of the Murmansk Marine Biological Institute, Kola Scientific Center, Russian Academy of Sciences, located in Zelenetskaya Bay, Barents Sea. The seasonal CMA dynamics in *F. vesiculosus* were studied from January 2010 to December 2011. Even-aged plants were collected in the intertidal zone of the Kola Bay (Cape Abram) during a low tide once a month. In addition, the daily CMA dynamics in thalli of *F. vesiculosus* were studied in various seasons of the year. A total of six round-the-clock measurements at 1-hour intervals were performed. The CMA was evaluated in the apical parts of the thallus as the most metabolically active zones of the plant.

For the analysis of the physiological state of the cells, we used the tetrazolium-based method (MTT assay) [21] modified for fucoid algae [10]. Samples of algae with a weight of 15 μg were placed in 200 μL of 0.8% MTT solution (a 5% MTT (Sigma) solution in 12 mM Tris-HCl buffer (pH 7.5) was diluted with seawater to the necessary concentration) and incubated with periodic agitation. After incubation, the sample was transferred into isopropanol (V = 1 mL) for 2 hours for extraction of the formed formazan. The extract was centrifuged for 5 minutes at 3000 rpm, and its absorbance was measured at a wavelength of 570 nm relative to the isopropanol extract of samples that had been in seawater without MTT. The metabolic activity of the cells was evaluated by the absorbance of the solutions, expressed per unit wet weight of algal sample, using Specord UV-VIS (daily dynamics)

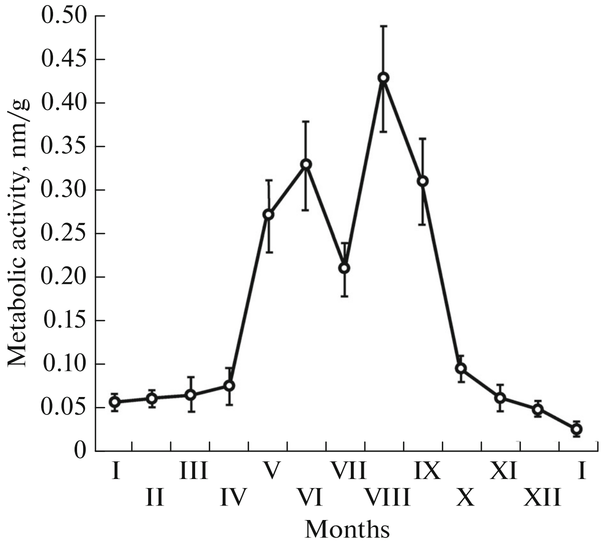

Fig. 1. Variations in the metabolic activity of cells of *Fucus vesiculosus* during the year.

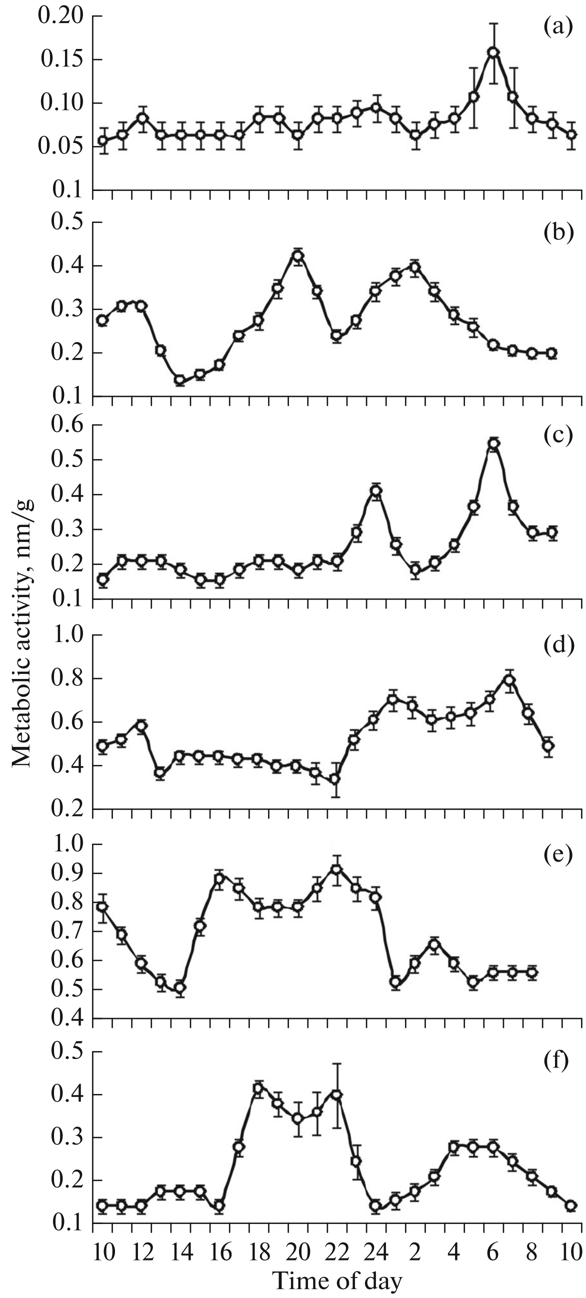

Fig. 2. Variations in the metabolic activity of cells of *Fucus vesiculosus* during a day in various seasons of the year: (a) December; (b) February; (c) April; (d) June; (e) August; (f) September.

and SF-46 (seasonal dynamics) spectrophotometers with 15-fold repetition. The data were processed using the algorithms of the MS Excel software package. The figures provide the arithmetic mean values and the standard deviation.

RESULTS AND DISCUSSION

During the year, the CMA in *F. vesiculosus* varied from 0.002 to 0.14 A570 nm/g (Fig. 1). The minimum CMA was recorded during the polar night. The daily CMA dynamics in this period were also characterized by the lowest values and a slight rise in the morning hours (Figs. 1 and 2a). In late January and early February, the physiological processes in algae intensified. When the level of illuminance increased, the intensity of photosynthesis in *F. vesiculosus* rose from 0.27 (January) to 0.6 mg O_2/g wet weight/h (April) [9], the same as in other algal species [12]. Nevertheless, the duration of the daylight period in this season is insignificant, approximately 6 hours. The previous studies showed that activation of the photosynthetic apparatus in fucoid algae requires a certain intensity, or dose, of solar radiation, which is achieved only by March or April [14]. The rate of algae growth also begins to increase in January [4, 5, 19]. The energy consumption for this process is provided through an increase in the rate of photosynthesis, which is accompanied by accumulation of photosynthetic pigments even with a significant increase of illuminance [6, 16]. The substantial increase in CMA in April also shows the activation of energy-consuming growth processes.

The dynamics of daily CMA variations during the season of low temperatures and short daylight periods are sinusoidal (Fig. 2b). The first peak of seasonal CMA dynamics in *F. vesiculosus* was observed in June (Fig. 1), when the algae growth rate is at a maximum [5]. This phase of the seasonal development of algae (growth) is characterized by the dynamics of daily CMA variations with an increase of this parameter in the night hours (Figs. 2c and 2d). This may be related to the active cell division processes at night [18]. By July, the growth processes had slowed down and the photosynthetic activity of *F. vesiculosus* decreased,

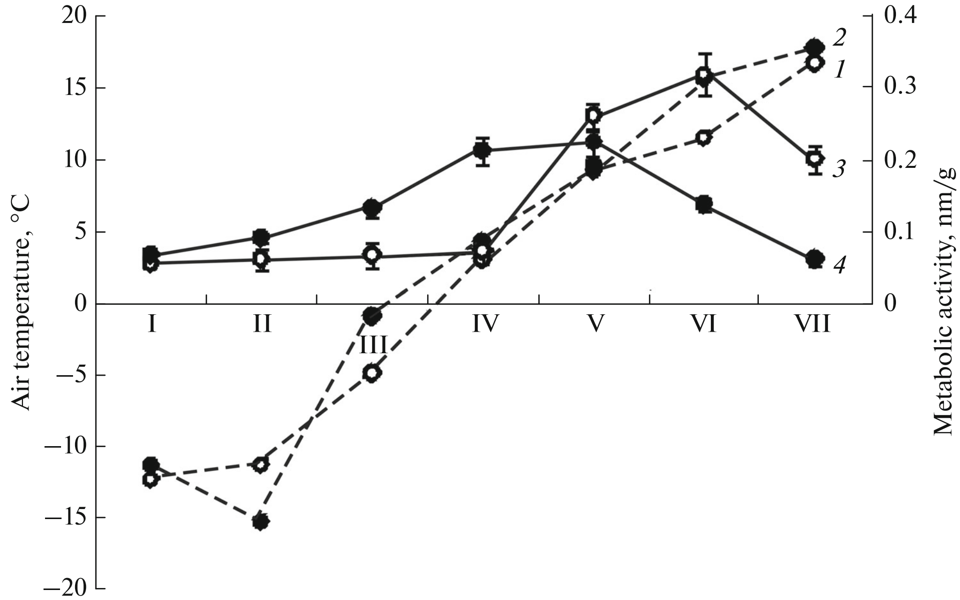

Fig. 3. The metabolic activity of cells of *Fucus vesiculosus* (solid lines) and the mean air temperature in the spring–summer periods (dashed lines) of 2010 (*1, 3*) and 2011 (*2, 4*).

which was accompanied by a decline in CMA. The hypothesis was made that the slowing of the algae growth rate at the maximum illuminance and a quite high water temperature in the middle of summer is related to the decrease in the water salinity or in the occurrence of biogenic substances [1, 4, 13]. In our opinion, the reorganization of algal metabolism from growth to preparation for the winter season occurs in this period.

A second peak of seasonal CMA dynamics in *F. vesiculosus* was observed in August (Fig. 1). At this time, the dry-matter content in algae increased and alginic acid and fucoidan accumulated [8]. As previously shown, the algae growth rate is the lowest in August–January [7, 18]. This period of their seasonal development can be considered as a phase of accumulation of reserve nutrients. The daily CMA dynamics in *F. vesiculosus* in the late summer and early autumn were characterized by a higher activity in the evening hours (Figs. 2e and 2f). The decline in CMA in July and the variations in daily dynamics of this parameter may indicate a reorganization of the energy system and a change of the direction of physiological processes from growth to accumulation of reserve nutrients. In the autumn season, the CMA in *F. vesiculosus* gradually decreased, while in the winter the activity of physiological processes was the lowest (growth was absent and photosynthesis and respiration significantly reduced) (Fig. 1). It has long been believed that the state of "hibernation" or "mesabiosis" (metabolism of cells at a lower level) is characteristic for fucoids in the period of the polar night. However, recent studies have shown the presence of photosynthetic activity in macrophytic algae at high latitudes during the polar night [2, 6, 16, 22], while the daily photosynthetic balance is negative. During this period, formation of reproductive organs starts in fucoids [2] and soluble polyphenols are synthesized in their cells [11].

As a result of the analysis of data on CMA variations during a 2-year period and their comparison with the temperature conditions, it has been established that low air temperatures in the winter affect the activation of growth processes in the intertidal alga *F. vesiculosus*. Thus, due to the low temperatures in February 2010, the CMA in algae increased from April; in 2011, from March. In this regard, the first peak in the seasonal cell activity in 2010 also shifted to June (Fig. 3). A probability of the effect of temperature on the duration of cycles has been shown for the infradian rhythms of reproduction in the green algae *Ulvaria fenestrata* [3]: a decrease in the water temperature to a level below the optimum (to 5°C) prevented thalli of this alga from transiting to the generative phase, whereas an increase in the temperature to values above the optimum facilitated frequent and regular gametogenesis. A number of macrophytes manifest a slowdown of physiological processes and an inhibition of gametogenesis and sporulation under temperatures below the optimum [15].

Thus, three development phases can be differentiated in the annual cycle of growth and reproduction of the brown alga *F. vesiculosus*: the resting phase, growth, and the accumulation of reserve nutrients, which are characterized by their specific dynamics of daily and seasonal changes in the metabolic activity of cells. The onset of the phase of growth activation is determined to a greater extent by the temperature conditions in the winter.

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