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SEASONAL DYNAMICS OF CATALASE ACTIVITY IN *Cystoseira crinita* (BLACK SEA) AND *Fucus vesiculosus* (BARENTS SEA)

Abstract: The seasonal dynamics of catalase activity of two related species of brown macroalgae, *Cystoseira crinita* (Desf.) Bory (1832) and *Fucus vesiculosus* L. (1753) was studied. In general, catalase activity (CA) in *C. crinita* was several times higher than in *F. vesiculosus*. The maximum values of CA in C. crinita were observed in November and the minimum ones in September. For *F. vesiculosus*, the maximum CA was found in January and the minimum in April. Abrupt changes in water temperature significantly affected the catalase activity in *C. crinita* and *F. vesiculosus*. In both species of algae, a similar seasonal trend in the change of CA was noted: two periods of adaptation adjustment associated with sharp changes in the temperature regime (spring and autumn) were distinguished. In spring, with a rapid increase in the temperature of the water masses, catalase inactivation occurred, whereas during summer to winter transition, accompanied by a sharp water cooling, catalase activity increases. Stabilization of the CA values of the studied macroalgae in the absence of sharp temperature variability was observed. However, this period of "stationary state" varies in time: in *Cystoseira crinita* it lasts from May to August, and in *Fucus vesiculosus* it lasts from May to December.

Keywords: catalase activity, *Cystoseira crinita*, *Fucus vesiculosus*, temperature, Black Sea, Barents Sea

Introduction

Brown algae *Cystoseira crinita* (Desf.) Bory and *Fucus vesiculosus* L., representatives of the order Fucales, of the family Sargassaceae and the family Fucaceae, respectively, are keystone species and primary producers in coastal ecosystems of the Black Sea and the Barents Sea. Occupying a vast area of the phytal zone, these species form the basis of food chains for many marine aquatic organisms.

Despite the fact that *C. crinita* is a low-boreal species, and *F. vesiculosus* is a boreal-arctic one, both species are characterized by similar environmental living conditions: they are mainly confined to the surf zone of an open seacoast, they prefer a solid substrate, grow at a depth of 0.2-5 m and have a high ecological tolerance amplitude [1, 2]. *F. vesiculosus* prefers a littoral zone. The stability of bottom phytocenosis depends on the condition of these species and it is determined, in particular, by their adaptation potential directly related to the antioxidant system (AOS).

The core of the antioxidant system (AOS) is the catalase enzyme (EC 1.11.1.6) (CAT) which, in comparison with the other AOS components, is characterized in marine

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hydrobionts by the largest range of response to changes in abiotic and biotic environmental factors [3, 4].

It is known that one of the primary abiotic factors provoking a high variation of AOS indices is temperature [5]. The data on the effects of temperature on catalase activity (CA) in the Black Sea *C. crinita* are fragmentary and unsystematized [3], and such studies are isolated for *F.vesiculosus* of the Barents Sea [6].

In accordance with that, the objective of this work consisted in examining the response of catalase activities in *C. crinita* and *F. vesiculosus* to seasonal changes in temperature in the coastal zones of the Black Sea and the Barents Sea. A comparative analysis of these data will make it possible not only to assess the adaptive potential of the studied species, but also to provide a prognostic assessment of the possible transformation of marine plant communities in changeable environmental and climatic conditions.

Methodology

The studies were conducted on two related species of macroalgae: *Cystoseira crinita* (Desf.) Bory and *Fucus vesiculosus* L. (1753) which grow in the water areas of the Sevastopol and Murmansk seacoast, respectively.

Description of the study areas

The water area of the Kruglaya Bay is characterized by the absence of household wastewater and domestic and industrial effluents [7]. The concentration of biogenic elements in the water is low (nitrites - 0.04-0.07 µmol/dm^3, nitrates - 0.02-0.6 µmol/ dm^3, ammonium ions - 0.11-0.33 µmol/dm^3) which corresponds to the MAC values approved for the water used for fisheries [7]. The abundance of *C. crinita* in the coastal zone and the high values of its biomass and density [8] are also indicative of the favorable environmental conditions in the study area.

The water area of the southern knee of the Kola Bay is subject to significant quality impacts of the discharge of industrial, domestic wastewater and rainwater by the enterprises and organizations located along its shores. It is contaminated with biogenic elements, oil products and heavy metals. In 2014-2015 the water of the bay was classified as very dirty (the 5th degree of water quality) [9]. That is also confirmed by the observations of the state of coastal vegetation which was characterized by structural damage, a decrease in species diversity and a decrease in biomass of the dominant species [10].

Material

Samples of vegetatively adult plants of *C. crinita* were collected on a monthly basis at a depth of between 0.2 and 1 m in the coastal zone close to the Vostochny Cape, the Kruglaya Bay (Sevastopol Region) from 2014 to 2016. Catalase activity (CA) indices were determined for the apical vegetative parts of 2-3-year-old thalli. The age of the plants was calculated according to A.A. Kalugina-Gutnik [1]. CA was determined according to the method of Bach and Zubkova [7]. All analyses were performed 30 or 60 minutes after sampling. Between 2 and 4 g of thalli of the species under study were placed in vessels with 2.0 dm^3 of sea water. In the laboratory, a 1000 mg algal sample was ground with 10 cm^3 of normal saline in a homogenizer in the cold and then centrifuged for 15 minutes at 8000 rpm. The supernatant was used for analysis. A fixed volume of 1 % hydrogen peroxide (2 cm^3) was added to 1 cm^3 of extract and the sample was left for 30 min at a temperature of 18 °C so that the reaction could happen. The remaining amount of hydrogen peroxide was titrated with potassium permanganate solution, 0.1 N after 5 cm^3 of 10 % sulfuric acid had been added to the sample. The blanks were prepared by means of the enzyme deactivation by boiling for 10 minutes. The number of parallel measurements varied from 3 to 6 and the obtained results were statistically processed.

CA in *F. vesiculosus* cells was determined in plants which were collected on a monthly basis in the littoral area near the village of Abram-Mys of the Kola Bay in the first half of the day at low tide (first half of low tide) from 2015 to 2017.

The apical vegetative part of the thallus up to 0.5 cm long was used for analysis. The plants were treated within 2 hours after sampling. In the laboratory, 100 mg algal samples were ground with phosphate buffer in the cold and then centrifuged for 15 minutes at 8000 rpm. The modified spectrometric method, based on the ability of hydrogen peroxide to form a stable coloured complex with molybdenum salts, was used to determine CA [11]. The supernatant was used for analysis. Two cm^3 of hydrogen peroxide were added to 0.1 cm^3 of sample and the mixture was incubated for 10 min at a temperature of 18 °C. One cm^3 of 4 % ammonium molybdate was added to the mixture in order to stop the reaction. The measurements were carried out at 410 nm by SF-2000 spectrophotometer.

The number of parallel measurements of CA was 3 and the obtained results were statistically processed.

The STATISTICA 10 software package was used for statistical data analysis. Arithmetic mean value (M) and confidence intervals $CI = \pm\, t0.05 \cdot m$ for CA were calculated in the compared samples, where: $t0.05$ - Student's coefficient for a given number of degrees of freedom (df) and significance level $\alpha = 0.05$, m - standard error of the mean.

Both methods used are based on similar processes of enzymatic decomposition of hydrogen peroxide. In the course of the study, a comparative analysis of the results of measuring catalase activity obtained by different methods on the same thallus of *F. vesiculosus* was carried out, which showed minimal differences in absolute values (the difference was 0.01-0.03 units). This allows us to compare the results obtained. This paper compares the general trends in catalase activity of related species, rather than its absolute values.

The studied water areas vary in the degree of contamination and have different degree of quality, however, we found it possible to compare the obtained results, since it is known that the catalase in brown algae is more responsive to changes in such abiotic factors as temperature, salinity, etc. and it practically shows no response to anthropogenic pollution (oil products, surfactants, etc.), compared with green and red algal species [3].

The figures show data for one year of studies, although the work on the measurement of AC was carried out for several years and a comparison of this results showed a similar trend in their changes.

Results and discussion

The dynamics of CA of *C. crinita* in the littoral zone of the water area of the Kruglaya Bay during 2014-2016 are shown in Figure 1. The values of the studied index varied during the year from the minimum values in September to the maximum ones in November. CA in *C. crinita* gradually increased from March to May at a water temperature of 10-15 °C. The stabilization of CA values was observed in the summer months (June-September). In early autumn, during the first phase of natural water cooling to 20 °C, a significant inactivation of the studied enzyme was noted compared with the summer period. The maximum CA for the whole series of observations was noted in November, as the water cooled down abruptly from 20 to 12 °C. In winter time, CA values in *C. crinita* were generally higher.

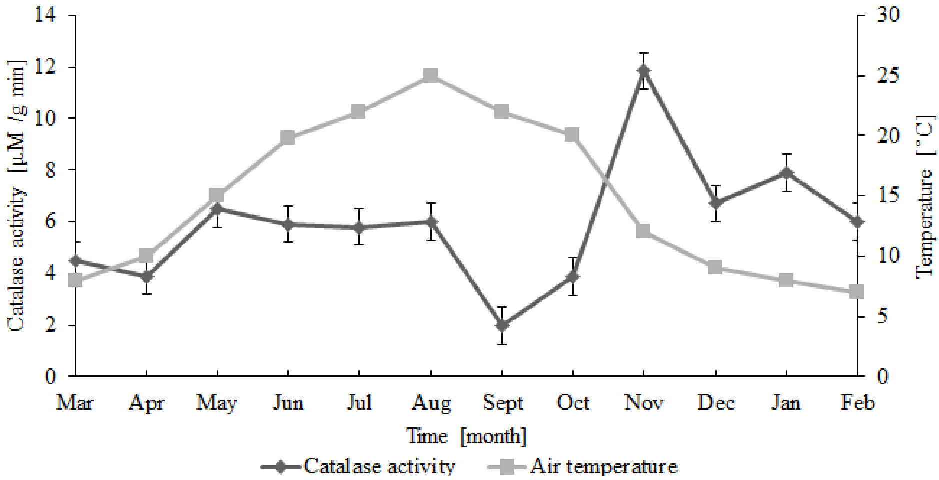

Fig. 1. Changes in catalase activity, CA in *Cystoseira crinita* and in water temperature in the littoral zone of the water area of the Kruglaya Bay, by month. The figures show data for the period from 2014-2016. The data is presented as an arithmetic mean and standard deviation

The dynamics of CA of *F. vesiculosus* growing in the littoral area of the village of Abram-Mys of the Kola Bay, during the year is shown in Figure 2. It is shown that the minimum CA values in *F. vesiculosus* were noted in April and the maximum ones in January.

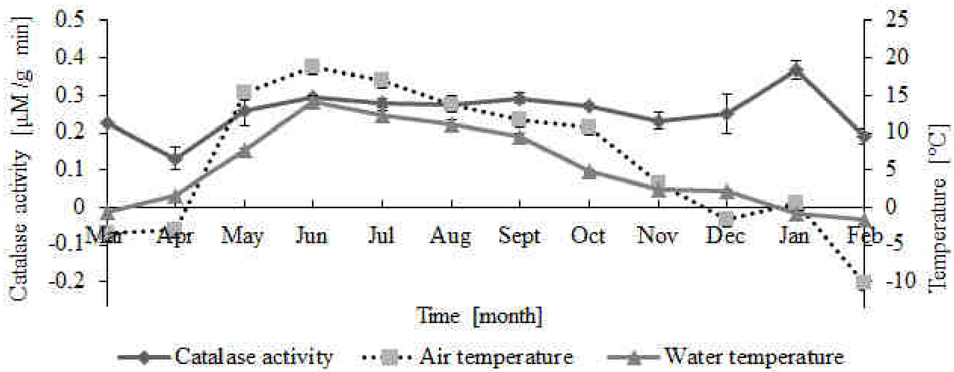

Fig. 2. Changes in catalase activity in *Fucus vesiculosus* in the littoral zone of the village of Abram-Mys of the Kola Bay, by month. The figures show data for the period from 2015-2017. The data is presented as an arithmetic mean and standard deviation

In spring, from March to April, a decrease in catalase level in *F. vesiculosus* was found, which was related to the increase in water temperature from negative to positive values. In May, a two-fold increase in CA was noted compared to one in April. The stabilization of CA values was observed during the summer and autumn seasons (till October). The decrease in CA in *F. vesiculosus* was observed in November but, in December, it began to increase, reaching its maximum value in January.

The studied water areas vary in the degree of contamination and have different degrees of quality. However, we found it possible to compare the obtained results, since it is known that catalase in brown algae is more responsive to changes in such abiotic factors as temperature, salinity, etc., and it shows practically no response to anthropogenic pollution (oil products, surfactants, etc.), compared with green and red ones [3].

It is likely that the adaptive defense mechanisms of brown algae under toxic stress do not use antioxidant enzymes to the full extent. They can also be associated with the activation of carotenoid and ascorbate defense systems which are more ancient in brown macroalgae [12]. Possibly, such features are also related to the morphological characteristics of plants, for example, the presence of a thick cuticular layer on the surface of the thallus consisting of polysaccharides which will either prevent any toxicant from penetrating into cells or slow down its penetration.

The comparison of two species of macroalgae in terms of the studied enzyme activity indicates that, in general, CA in *C. crinita* is greater than that in *F. vesiculosus*. Previously, Yakovleva and Belotsitsenko [13] showed that warm-water tropical-low-boreal macroalgae species at middle latitudes are characterized by a higher antioxidant capacity compared to boreal species. This is reflected in the greater activity of primary antioxidant enzymes (superoxide dismutase, glutathione reductase, ascorbate peroxidase, catalase) in comparison with cold-water ones.

The presence of two periods in the analyzed macroalgae (spring and autumn/winter) characterized by a change in CA was noted in the annual CA dynamics, however, their duration and level of change in the compared species is different and it is more pronounced in the southern species.

In April, a decrease in CA values compared with those in March was revealed in *C. crinita*, which can probably be explained by a rather sharp increase in temperature from winter to summer (from 4-6 °C in March to 10-12 °C in April) and by the increase in PAR (photosynthetically active radiation). It was demonstrated that as the water temperature rises, the adaptive changes in low-boreal and tropical macroalgae species consist of a decrease in the content of low molecular weight antioxidants, carotenoids, and in an inactivation of peroxide-detoxifying enzymes which also include catalase [14].

The similar adaptive processes, manifested in a decrease in CA, were also found in *F. vesiculosus* during this period. Hydrological winters and the lowest negative water temperatures at the high latitudes of the Barents Sea occur in March. However, the air temperature has positive values and the PAR level increases significantly. The temperature drop that occurs during high tide/low tide periods and a significant change in lightning can be of great importance for fucoids which are littoral plants. Such variations in temperature and in amount of light probably may lead as well to the adaptive changes described above in *F. vesiculosus*. In spring, a decrease in carotenoid concentration was also shown for the northern algae which take over the role of a free radicals neutralizer [15]. It is possible that during this period, polyphenolic compounds, for which the maximum accumulation was shown in March, also play a significant role in protecting brown macroalgae cells from intense lighting and, as a consequence, from reactive oxygen intermediates (ROI) [16].

In May, when the temperature quite smoothly changes during spring to summer transition, the metabolic adaptation of macroalgae to a sharp increase in water and air temperature ends and the process of intensive growth, anlage and/or development of the reproductive systems begins in *C. crinita* and *F. vesiculosus* [1]. This period is characterized by an almost 2-fold increase in CA in *C. crinita* compared with that in April and by a 2.2 increase in *F. vesiculosus*. A sharp increase in metabolic activity during this period is also shown for the latter species [6].

In summer, the CA values in *C. crinita* were quite stable, but in September-October, a sharp inactivation of catalase was again noted. Despite the fact that the photosynthetic apparatus of brown algae is the most resistant to high temperatures and insolation [1], nevertheless, the intensity of respiration and photosynthesis in *C. crinita* slows down at summer water temperatures above 25 °C and at a high PAR level [14]. Under such conditions, a decreases in the concentration of low molecular weight antioxidants and in the activity of antioxidant enzymes in macroalgae is noted [13]. Thus, it is likely that protracted summer photo-oxidative stress caused by the increased generation of ROI (reactive oxygen intermediates) in macroalgae cells manifests itself. Besides, in August, the mass elimination of the lateral branches takes place, and the growth of the thallus of *C. crinita* slows down. A decrease in CA to the minimum values may be indicative of a slowing down of metabolism or of a possible enabling of other mechanisms that neutralize ROI.

CA values in *F. vesiculosus* varied slightly from May to October. The stably high catalase activity in *F. vesiculosus* during that period is indicative of the intensive efficacy of the components of the antioxidant system, which is also confirmed by the increased metabolic activity of *F. vesiculosus* cells, especially in July-August [6]. These facts are in line with the data [16] that show the high resistance of *F. vesiculosus* to high temperatures, PAR (photosynthetically active radiation) intensity and UV (ultraviolet radiation) compared with other dominant boreal macroalgae species (*Saccharina latissima = Laminaria saccharina* (Linnaeus) J.V. Lamouroux, *Palmaria palmata* (Linnaeus) F.Weber& D.Mohr).

An abrupt decrease in temperature from summer to winter (from 20 to 10 °C) (Black Sea) also induced metabolic rearrangements resulting in activation of the catalase in *C. crinita* to the maximum values during the whole series of observations in November.

Metabolism of *F. vesiculosus* slows down in November when the light level decreases significantly and the temperature gets cooler, which also results in a decrease in catalase activity. In the winter period between December and February, during low tide periods, the catalase values will be determined by low air temperatures. A peak in catalase activity was observed in January when the water temperature changes from high values to stably negative values. CA increases when the temperatures are relatively low and it decreases at temperatures below 10 °C in winter.

A 1.5 to 3-fold increase in CA during the preparation of macroalgae for winter, compared with the summer period, was described by other researchers [3, 4]. These data are also consistent with the studies by Yakovleva and Belotsitsenko [13]. They show the accumulation of carotenoids and increased activity of AO enzymes in the dominant macroalgae of the Sea of Japan when the temperature gets cooler according to season.

Thus, CA in the studied macroalgae is mainly influenced by the temperature. Specifically, in sublittoral plants it is influenced by the water temperature, whereas in littoral ones it is influenced by the water temperature or by the air/water temperature in combination, as well as by the photosynthetically active radiation which enhances or weakens the temperature effects on phototrophic microorganisms. The activity of AO enzymes increases in the winter period, which ensures acclimation to low temperatures and prevents the development of photooxidative stress [4, 14]. A similar phenomenon has been observed in fucoids from the coast of the Atlantic Ocean. They showed higher levels of activity in winter (February) than in August and October [15]. At the same time, such changes look more contrasting in southern plants in comparison with northern ones. For example, a decrease in CA in *F. vesiculosus* in April can be caused by rather intense lighting and low temperatures. This is associated with decrease in the activity of processes occurring in Photosystem II [4]. CA inactivation during summer is shown for *F. vesiculosus* living at middle latitudes [4]. The data on significant species-specificity in catalase activity are presented in works on Arctic algae: it increases by the end of summer in *Monostroma sp.* and *Palmaria*, and increased activity is maintained in *Devaleria sp.* during all summer season [12], similar changes are observed in another more southern algae *Hypnea musciformis* [18]. An increased CA during the summer period was also observed for the Barents Sea species *F. vesiculosus*, which provides protection against photooxidative stress for the plant.

In general, the relationship between the activity level of AO enzymes and resistance to photooxidative stress was noted, apart from brown macroalgae, in red macroalgae of the North Atlantic [18-21].

Conclusion

Similar trends in the seasonal dynamics of catalase activity of two related species of brown macroalgae, *Cystoseira crinita* and *Fucus vesiculosus*, were observed in this study. Two periods of adaptive adjustments related to sharp temperature fluctuations (spring and autumn) were found. Sudden changes in water temperature had a significant effect on catalase activity in *C. crinita* and *F. vesiculosus*.

In spring, when the temperature of water masses increases rapidly, inactivation of the catalase of the studied species occurs. During summer to winter transition, accompanied by rapid water cooling, the catalase activity increases.

Stabilization of CA values of the studied macroalgae in the absence of significant variation in temperature was observed. However, this period of “stationary state” varies in time: in *C. crinite*, it lasts from May to August, and in *F. vesiculosus*, it lasts from May to December.

Thus, a change in catalase activity in brown algae, *Cystoseira crinita* and *Fucus vesiculosus,* is one of the essential adaptive mechanisms that enable maintenance of a stable state of these macrophytes in the presence of significant temperature fluctuations.

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