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O.V. Kravtsova, V.I. Scherbak, M. I. Linchuk
Institute of Hidrobiology of NAS of Ukraine, Kyiv

THE REGULARITIES OF PHYTOPLANKTON FORMATION AT VARIOS BIOGENIC ELEMENTS AND ORGANIC MATTER CONCENTRATIONS

The seasonal dynamics of the concentration of nutrients in the form of inorganic nitrogen (NH_4^+ , NO_2 , NO_3^- , ΣN), dissolved phosphorus, organic matter and the connection with the development of phytoplankton in waters with high content of total inorganic nitrogen (from 23.31 to 102.65 mg N/dm³) and its compounds (ammonia - from 8.42 to 76.60, nitrate - from 4.94 to 15.93, nitrite - from 0.077 to 4.35 mg N/dm³) and organic matter (from 8.00 to 21.92 mg O/dm³ by permanganate oxidation values and from 58.46 to 265.2 mg O/dm³ by dichromate oxidation values) were analyzed in paper. The peculiarity of the hydrochemical regime of the reservoirs was phenomenally high relations $\Sigma\text{N}:\text{P}$ (133,54-12152,86) during the growing seasons. Found that response algal plankton communities such features hydrochemical regime is a simplification of the structure due to the predominance of representatives of departments Euglenophyta, Chlorophyta and Bacillariophyta, while Chrysophyta, Dinophyta, Charophyta and presented Cryptophyta 1-3 species. The response of phytoplankton to the high content of compounds of inorganic nitrogen is the increase in the number and biomass of green algae, and organic matter - eugenic algae.

Key words: biogenic elements, inorganic nitrogen, dissolved phosphorus, phytoplankton, species diversity, abundance, biomass, seasonal dynamics, anthropogenic load

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¹O.B. STOLIAR, L.L. ^{1,2}GNATYSHYNA, ¹V.V. KHOMA, ³G.H. SPRINĢE

¹Volodymyr Hnatyuk Ternopil National Pedagogical University
M. Kryvonosa Str., 2, Ternopil, 46027, Ukraine

²I.Ya. Horbachevsky Ternopil State Medical University
m.Voli, 1, Ternopil, 46001, Ukraine

³University of Latvia
Miera Str. 3, Salaspils, LV, 2169, Latvia
e-mail: oksana.stolyar@gmail.com

THE APPLICATION OF THE NOVEL INTEGRATIVE INDEX OF OXIDATIVE STRESS IN THE ASSESSMENT OF ENVIRONMENTAL IMPACT ON FRESHWATER MOLLUSKS

The adverse environmental impacts cause the oxidative stress response in the aquatic animals. However, depending on the severity and duration of impact, this response can be highly different. The aim of this study was the analysis of available results of the evaluation of antioxidant activities in the freshwater mollusks in the sense of the successfulness of the oxidative stress response. The recently proposed integrative index 'Preparation to the oxidative stress' (POS) was applied. Three populations of bivalve mollusks from the basin of the river Dniester were compared during three seasons, and in their ability to withstand heating (25° C and 30° C during 14 days) and exposure to ionizing radiation (14 days after the acute exposure to 2 mGy). The mussels were sampled in the low disturbed pristine

site, highly polluted agricultural region and the cooling pond of the nuclear power plant with the constantly elevated temperature. The parameters for the calculation of POS included superoxide dismutase activity, catalase activity, glutathione *S*-transferase activity, glutathione concentration, and metallothionein (from its thiol groups) concentration. The values were calculated as the magnitude of change (as % change) in comparison to the corresponding control (less disturbed field group or non-exposed group). Three criteria for POS were applied. The number of the positive and negative changes and their limits were indicated. The analysis have shown that the POS responses were in the limits of adaptive ability in all studied cases. However, the results of POS calculation allowed the distinguishing of the responses that are realized in the field and experimental exposures of mollusks. The most distinct responses were shown for the glutathione (mainly positive changes) whereas the metallothionein level was mainly oppressed, particularly under the heating. The depressive direction was estimated in the cases of extreme temperatures, irradiation and, mainly for the mollusks from the highly polluted sites. The key importance of POS as a survival strategy of the mussels exposed to adverse impact depending on the life history is evident.

Key words: Antioxidants; Reactive oxygen species; Oxidative stress; Integrative indexes; Bivalve mollusks

The response of the oxidative stress (OS) is the common biological phenomenon that is causing by the external or internal adverse effects in the organism [15]. Concerning the native freshwaters habitants, the most expected inducing factors are the disturbing of the hydrological regime, chemical pollution and climate changing [1, 4, 17]. However, depending on the duration and severity of the impact, the manifestations of OS can be different. Casual impacts can provoke the generation of reactive oxygen species (ROS) and, as a consequence of their signal activity, the elevated expression of the antioxidants, namely the ROS scavengers, both enzymes and non enzymatic substances, for example glutathione (GSH) [18]. The imbalance of the ROS generation and antioxidant activities provokes the oxidative injury, namely the lipid peroxidation, protein oxidative modifications, and DNA oxidative damage [15]. The particular response in the native populations can be manifested as the adaptation to the environmental impact or the exhausting of the stress responsibility and, as a consequence, the absence of the OS response [3, 14].

Bivalve mollusks, due to their suspension-feeding and sedentary lifestyle, are on the first line of impact from the aquatic effluents [5, 6]. Populations of freshwater bivalves are declining dramatically all over the world [11]. In Ukraine, this was partially confirmed by authors' own research experience on the evaluating the indices of stress responses and environmental toxicity in the basin of Dniester, the second largest river in Ukraine [7, 12].

Therefore, the aim of this study was the analysis of available results of the antioxidant activities in the freshwater mollusks in the sense of the successfulness of the oxidative stress response. The recently proposed integrative index 'Preparation to the oxidative stress' (POS), utilized to the evaluation of the responses of poikilothermic species exposed to anoxia/hypoxia [13], was applied. The analyzed here results were obtained previously within the scopes of the Ukrainian state funding grants to O. Stoliar [7, 8, 9, 10].

Materials and methods

The parameters for the calculation of POS included superoxide dismutase common activity (SOD), catalase activity (CAT), and glutathione total or reduced concentration (GSH). The metal buffering protein metallothionein (MT) was included in the set of antioxidants if it was detected from its thiol groups, responsible for the antioxidants properties [16]. Glutathione *S*-transferase (GST) is the II phase of biotransformation enzyme. Since the functionality of its plural forms is related to the reduction of organic hydroperoxides and to conjugation of LPO products with the participation of GSH [2], GST was also included in the antioxidants list. Moreover, the expression of MT and GST is regulated by the antioxidant response element [19]. We classified each group of mollusks as POS-positive, POS-negative or POS-neutral based on three different criteria. This classification was made for each tissue separately.

To distinguish the responses, three different criteria were applied. Initially, we calculated the magnitude of change (as % change) in comparison to the corresponding control (see the captions to tables). Only statistically significant differences, as stated by the authors, were considered.

Statistically insignificant changes were regarded as zero. When the values for each group were not available from the text or tables, they were estimated from the Figures by comparing the height of the column of the experimental group to that of the control group using an image editor software.

Based on the magnitude of change (as % change) compare to the corresponded control (season, less disturbed native group or untreated experimental group), we classified each group as POS-positive, POS-negative or POS-neutral, considering the three different criteria. Finally, the prognosis of the impact (health status) was made basing on the classification of the mature ecosystems according to [20].

The first criterion was the occurrence of at least one statistically significant up-regulation event of antioxidant defense regardless of what happened in another tissue. In this criterion, if no changes occurred at all, the tissue was flagged as neutral, but if any down-regulation occurred (and no up-regulation) it was flagged as negative. Correspondingly, the response was qualified 'in the limits of adaptive ability (+)' or as 'loss of the adaptive ability'.

The second criterion was based on the definition of thresholds for up- and down-regulation events. This criterion was the occurrence of at least one up-regulation above a 50% threshold. On the other hand, the threshold for down-regulation was set at 25%. Species were classified as in criterion 1, but only considering changes that reached the thresholds. The response was classified as the state of elastic stability (1 event), the state of resistant stability (2 and more events), or inert state (0 events);

The third criterion was the occurrence of more cases of up-regulation in comparison to down-regulation within a tissue. Thus, only studies that measured at least two of the antioxidant parameters listed above were considered in criterion 3. For all three criteria, the occurrence of one positive-flagged tissue was enough to classify the response as POS-positive. Correspondingly, POS-positive response means the recovering direction, POS-negative response – depressive direction, and POS-neutral response – the state of equilibrium. Prevalence of specific antioxidants in POS-positive species was further analyzed, namely which antioxidant was most commonly up-regulated in these situations of environmentally realistic conditions.

Results and discussion

The results of the calculations for the mussels studied in their native populations (Fig. 1) have shown that the responses of the specimens from the pristine (control), agricultural (B) and artificial (N) sites were different in three seasons. The highest deviations were shown in autumn. The worsted POS response in the disturbed (B, N) sites versus control were detected in the digestive gland and in spring. From this calculation, it is also evident that the thiols MT and GSH (in the digestive gland) represent almost constantly oppressed biomarker, whereas KAT and GST were up-regulated in several exposures.

According to these results, the generalized POS responses of mussels and correspondent health state of the populations were classified (Table 1). In each case, the general state was within the limits of adaptive response. However, in the mussels from the highly polluted agricultural site B, the depressive direction was predominant, whereas in the artificial reservoir, namely cooling reservoir of the nuclear power plant that was not polluted and had the constantly elevated temperature of the water, the recovering direction of the POS response was predominant. The season depending differences were evident. They can be explained by the differences in the physiological state of mussels (exhausting of the energy storages in spring and their accumulation in the autumn).

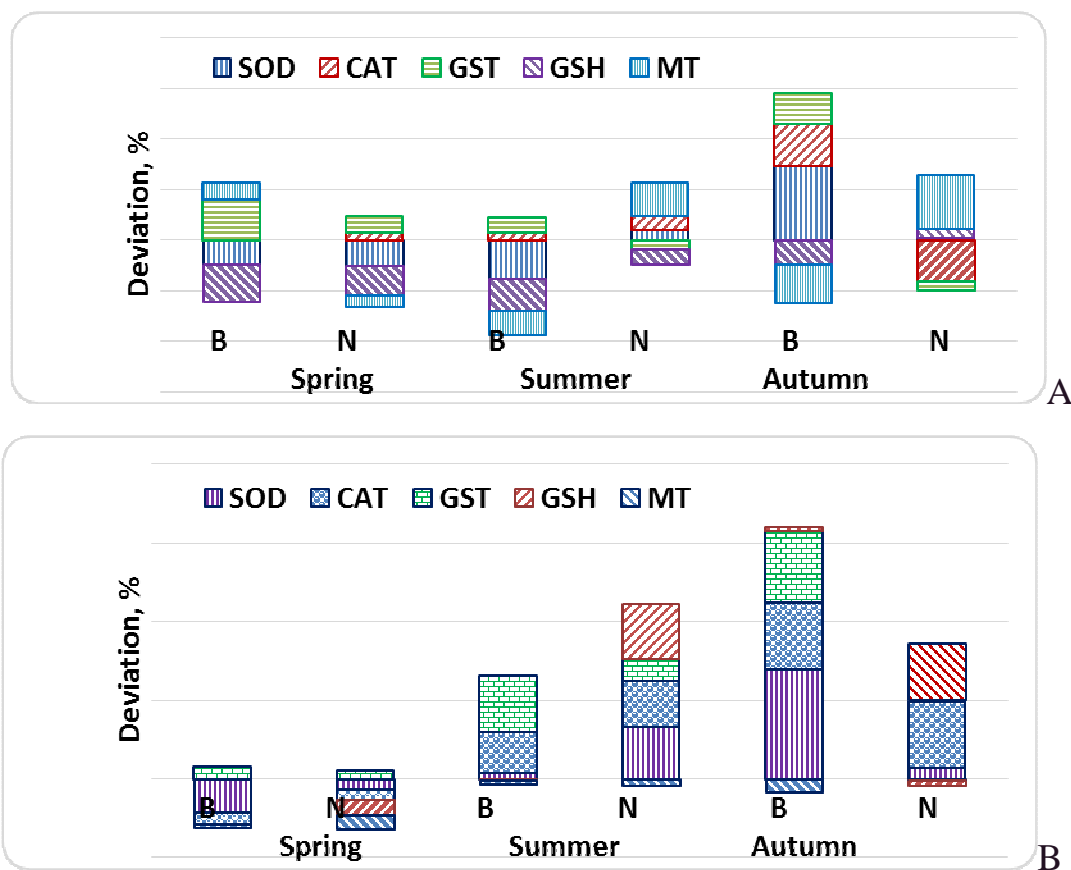


Fig. 1. Relative changes (expressed as the percentage of change) of the values of POS biomarkers in the digestive gland (A) and gills (B) of bivalve mollusk from the agricultural (B) and artificial (N) sites in comparison with the data for the pristine site (control) in spring, summer and autumn, calculated from the data represented in [7].

Table 1

The classification of the responses of the bivalve mollusk from the two impacted sites (B and N) in the comparison with the control (group from pristine site) in three seasons. The POS responses classified as '+, -, 0'.

Season	Tissue	Criterion POS			The health state of population
		1	2	3	
Group B					
Spring	Digestive gland	+	1+/-	-	In the adaptive limits, resistant stability, depressive direction
	Gills	+	0+/-	-	
Summer	Digestive gland	+	0+/-	-	In the adaptive limits, resistant stability, depressive direction
	Gills	+	2+0-	-	
Autumn	Digestive gland	+	3+/-	+	In the adaptive limits, resistant stability, recovering direction
	Gills	+	+/-	+	
Group N					
Spring	Digestive gland	+	0+/-	-	In the adaptive limits, resistant stability, depressive direction
	Gills	+	0+/-	-	
Summer	Digestive gland	+	1+/-	+	In the adaptive limits, resistant stability, recovering direction
	Gills	+	4+0-	+	
Autumn	Digestive gland	+	1+/-	+	In the adaptive limits, elastic stability, recovering direction
	Gills	+	1+0-	+	

To evaluate the ability of each studied population to withstand the heating, the mussels were subjected to the experimental exposure to the temperatures 25° C and 30° C during 14 days [8]. Basing on the obtained results, it was difficult to indicate the most heating-tolerate group due to the variability of differences between the groups. The results of the POS analysis for this experiment are represented in Fig. 2, Tabl. 2.

The comparison of the groups demonstrated the greatest vulnerability of the responses in the digestive gland in comparison with the gills. The highest sensitivity was shown for the SOD, whereas GSH demonstrated the best response of POS, particularly in the B- and N-groups. It was also indicated similar responses to two heating regimes within the group, whereas the responses of different populations were distinct, particularly in gills.

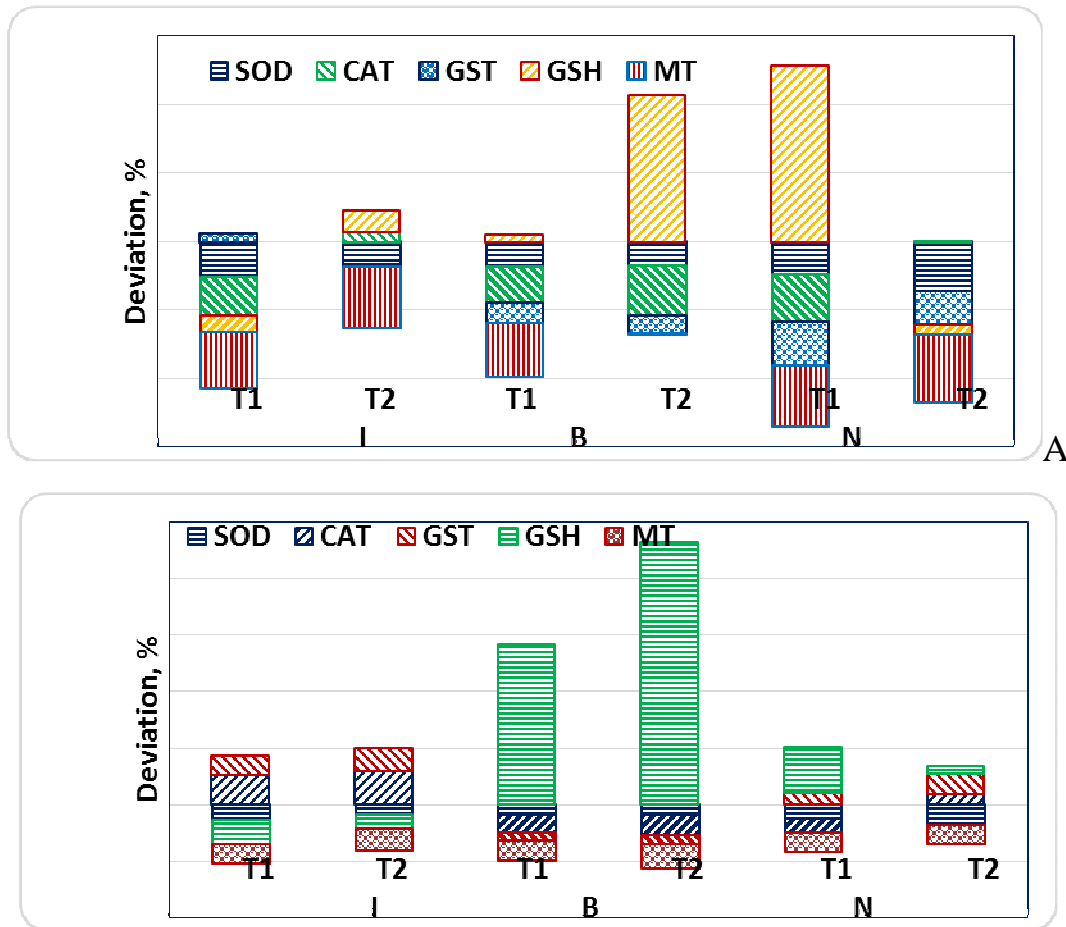


Fig. 2. Relative changes (percentage of change) of the mean values of POS biomarkers in the digestive gland (A) and gills (B) of bivalve mollusk from the pristine (I), agricultural (B) and artificial (N) sites exposed to 25° C (T1) and 30° C (T2) during 14 days in comparison with control (18° C). The data represented in [8] were utilized.

However, the generalization of the indexes has shown that all responses were within the adaptive limits. Importantly, B-group from the highly polluted biotope, despite the great up-regulation of GSH, demonstrated the depressive direction in both exposures. On the other hand, I group from the pristine site was at the equilibrium state. Moreover, the group adapted to the heating in its field environment (N group from the cooling pond) has shown even recovering direction indicating the greatest ability to withstand heating. Probably, this particular ability to withstand heating was explained by the adaptation of this population in its environment. Importantly, each was not able to make these crucial conclusions concerning the health status of populations basing only on the set of the biochemical responses even when we studied plural biomarkers of stress, injury and exposure [8].

Table 2

The classification of the responses of the bivalve mollusk from the pristine (I), agricultural (B) and artificial (N) sites exposed to 25° C (T1) and 30° C (T2) during 14 days in comparison with control (18°C).

T°	Tissue	Criteria POS			Health status
		1	2	3	
Group I					
T1	Digestive gland	+	0+/3-	-	In the adaptive limits, resistant stability, depressive direction
	Gills	+	2+/2-		
T2	Digestive gland	+	2+/2-	0	In the adaptive limits, resistant stability, equilibrium state
	Gills	+	2+/2-		
Group B					
T1	Digestive gland	+	0+/4-	-	In the adaptive limits, resistant stability, depressive direction
	Gills	+	2+/2-		
T2	Digestive gland	+	1+/3-	-	In the adaptive limits, resistant stability, depressive direction
	Gills	+	1+/4-		
GroupN					
T1	Digestive gland	+	1+/4-	-	In the adaptive limits, resistant stability, depressive direction
	Gills	+	2+/2-		
T2	Digestive gland	+	0+/3-	-	In the adaptive limits, resistant stability, recovering direction
	Gills	+	2+/1-		

Whereas the heating is the typical confounding factor for the bivalves, the effect of the ionizing radiation was utilized as an unusual stress factor for each populations studied. The analysis of the represented results [9] (Fig. 3, Tabl. 3) indicated the highly variable responses depending on the population. The most variable index was the GSH level. The common feature for three populations was the preference of the positive changes of the indices. However, according to the number of the events of activation or oppression of the indices, the direction of the health status changes was estimated for B- and N-groups as depressive and only for the I-group had the features of recovery.

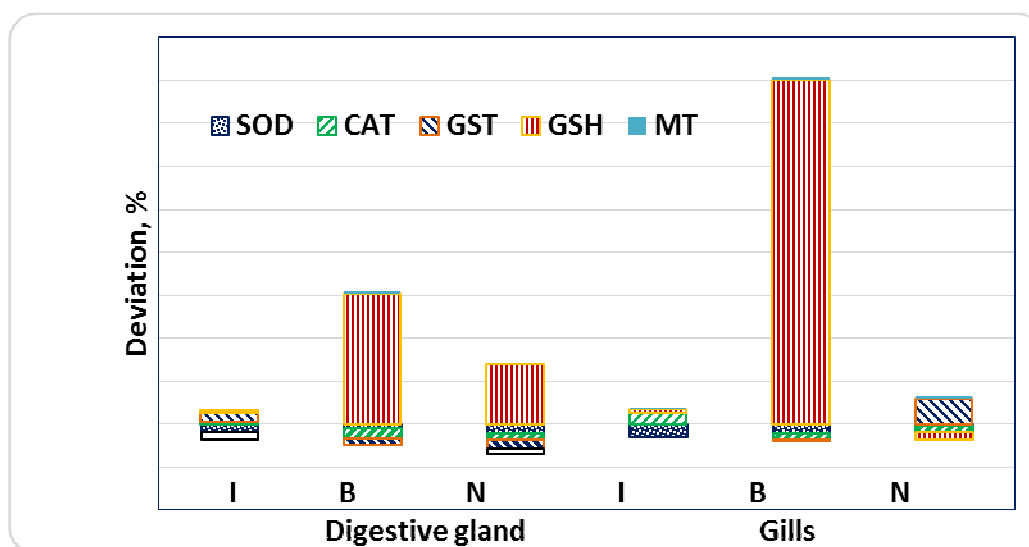


Fig. 3. Relative changes of the mean values of POS biomarkers in the digestive gland and gills of bivalve mollusk from the pristine (I), agricultural (B) and artificial (N) sites 14 days after the acute exposure to ionizing radiation in comparison with control. The data represented in [9] were utilised.

The classification of the responses of the bivalve mollusk from the pristine (I), agricultural (B) and artificial (N) sites after the acute exposure to ionizing radiation

Tissue	Criteria POS			Health status
	1	2	3	
Group I				
Digestive gland	+	2+/1-	+	In the adaptive limits, resistant stability, recovering direction
Gills	+	1+/1-		
Group B				
Digestive gland	+	1+/2-	-	In the adaptive limits, resistant stability, depressive direction
Gills	+	1+/2-		
Group N				
Digestive gland	+	1+/3-	-	In the adaptive limits, resistant stability, depressive direction
Gills	+	1+/3-		

Overall, the analysis of the results, obtained on the mollusks from different populations in the season-depending field exposures and experimental exposures to heating and ionizing radiation have given the clear forecast of their health status. Importantly, the most sensitive constituents of each response were different. Nevertheless, the specimens from all three populations give the responses in the adaptive limits and support the resistant stability. However, some exposures can possess critical changes in their health, particularly in the spring and under the additional loading by heating and irradiation. The group from the polluted area (B group) is the most vulnerable group. It can be explained by the continuous induction of OS in the mussels from this area [15].

Conclusion

The key importance of POS as a survival strategy of the mussels exposed to adverse impact depending on the life history is evident. Therefore, the developed method of the POS calculation can be useful tool for the prognosis of the environmental health. As far as we know, there is no analysis available of the prevalence of POS among mollusks depending on their history of population.

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О. Б. Столяр, Л. Л. Гнатишина, В. В. Хома, Г. Х. Спринге

Тернопільський національний педагогічний університет імені Володимира Гнатюка
ДВНЗ "Тернопільський державний медичний університет імені І.Я. Горбачевського МОЗ України"
Латвійський університет

ЗАСТОСУВАННЯ НОВІТНЬОГО ІНТЕГРАЛЬНОГО ІНДЕКСУ ОКИСНОГО СТРЕСУ В ОЦІНЦІ ВПЛИВУ ДОВКІЛЛЯ НА ПРІСНОВОДНИХ МОЛЮСКІВ

Несприятливі чинники довкілля викликають у водних тварин реакцію окисного стресу. Проте, залежно від сили та тривалості дії чинника, ця реакція може сильно відрізнятись. Метою роботи був аналіз отриманих результатів оцінки антиоксидантних активностей у прісноводних молюсків з точки зору успішності реакції окисного стресу. Було застосовано нещодавно запропонований інтегральний індекс «Приготування до окисного стресу» (ПОС). Порівнювали три популяції двостулкових молюсків з басейну ріки Дністер упродовж трьох сезонів за їх здатністю витримувати вплив нагрівання (25°C та 30°C упродовж 14 діб) та іонізуюче

випромінювання (14 діб після одноразової експозиції до 2 мГр). Для дослідження молюски відбирали в умовно чистій місцевості, сільськогосподарській місцевості із високим рівнем забруднення та у ставі-охолоджувачі атомної електростанції з постійно підвищеною температурою води. Параметри для розрахунку ПОС включали загальну супероксиддисмутазну активність, каталазну та глутатіонS-трансферазну активність, концентрацію глутатіону та металотіонеїнів (визначену за вмістом тіолів). Значення були розраховані як величина відхилення (% відхилення) у порівнянні з відповідним контролем (референтна місцевість у польовому дослідженні або група, що не піддавалась впливу чинника у лабораторних умовах). Розрахунок ПОС включав три критерії оцінки вірогідних відхилень від контролю: фіксували кількість позитивних та негативних змін та їх діапазон. Аналіз показав, що реакції ПОС були в межах адаптивної здатності у всіх досліджених ситуаціях. Результати обчислення ПОС дозволили розрізнити чотири стадії реакції окисного стресу, що реалізуються в молюсків у польових та експериментальних умовах. Найпомітніші зміни були відзначені для глутатіону (здебільшого позитивні), тоді як рівень металотіонеїну був переважно пригніченим, особливо за впливу нагрівання. Депресивне спрямування реакції ПОС було встановлено у випадках впливу екстремальної температури, іонізуючої радіації, особливо для молюсків із сильно забруднених територій. Очевидною є вирішальна роль ПОС як стратегії виживання молюсків за умови впливу несприятливих чинників залежно від їх життєвої історії.

Ключові слова: антиоксиданти, активні форми кисню, окисний стрес, інтегральні індекси, двостулкові молюски

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