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Assessment of Mazut Toxicity for Embryos of Two Sea Fish Species

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Abstract

Shelf areas of the seas and oceans characterizing high level of bioproductivity are significantly affected by anthropogenic pollution, including oil contamination. Early developmental stages of marine organisms are very sensitive to pollutants, which generate oxidative stress in them and provoke further pathological processes. We studied the influence of mazut in a concentration of 0.01 and 0.1 ml/L on the developing embryos of two benthic fish species: peacock blenny *Salaria pavo* and round goby *Neogobius melanosthomus* in Stage VI. We studied the following biomarkers: superoxide dismutase (SOD), catalase (CAT), peroxidase (PER) and glutathione reductase (GR) spectrophotometrically. The results showed high toxicity of mazut accompanied with the changes in the activity of key antioxidant enzymes in the embryos of both tested fish species, which generated oxidative stress in developing fish exposed to mazut. The general trends and peculiarities of the responses of embryo enzymes to the oil intoxication were shown, which depended on the morphological peculiarities of eggs of the tested fish species. The peacock blenny egg has thicker shell than the round goby egg, therefore, it is protected better from the environmental impact. The paper discusses possibilities of use of the demersal fish eggs for the assessment of ecological status of shelf areas in case of oil pollution.

Keywords*:* Black Sea, mazut, pollution, fish embryos, biomarkers, antioxidant enzymes

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Оценка токсичности мазута для икры двух видов морских рыб

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Аннотация

Шельфовые зоны морей и океанов, характеризующиеся высокой биопродуктивностью, в наибольшей степени подвержены антропогенному загрязнению, включая нефтяное. Ранние онтогенетические стадии гидробионтов очень чувствительны к действию загрязнителей, приводящих к возникновению окислительного стресса и развитию последующих патологических процессов. Исследовали влияние мазута в концентрации 0.01 и 0.1 мл/л на активность антиоксидантных ферментов, являющихся маркерами окислительного стресса, у эмбрионов собачки-павлина *Salaria pavo* и бычка-кругляка *Neogobius melanosthomus* на VI этапе развития. В качестве биомаркеров спектрофотометрическими методами исследовали активность ключевых антиоксидантных ферментов супероксиддисмутазы (СОД), каталазы (КАТ), пероксидазы (ПЕР) и глутатионредуктазы (ГР). Результаты позволили выявить токсичность мазута, что выразилось в изменении активности тестируемых ферментов в эмбрионах обоих видов рыб, свидетельствующем о развитии окислительного стресса у развивающихся зародышей, инкубированных в растворах токсиканта. Установлены характерные общие закономерности и особенности ответных реакций ферментов эмбрионов на интоксикацию мазутом, зависящие от морфологического строения икринок исследуемых видов. Икринка собачки-павлина имеет более толстую внешнюю оболочку, чем у бычка-кругляка и, следовательно, она лучше защищена от внешних воздействий. Обсуждается возможность использования демерсальной икры донных рыб в качестве тест-объектов для оценки экологического состояния прибрежных акваторий при нефтяном загрязнении.

Ключевые слова: Черное море, мазут, загрязнение, эмбрионы рыб, биомаркеры, антиоксидантные ферменты

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Introduction

Ecotoxicological methods of the assessment of the effects of various pollutants are recognized for the analysis of the state of aquatic ecosystems and their inhabitants, since they have a number of advantages, namely: they do not require any expensive equipment, they are quick, and owing to the responses of test organisms they allow to identify adverse changes in ecosystems within a fairly short period of time. From this perspective, the ecotoxicological approach has found wide application in ecology and aquatic toxicology. It is an integral part of some monitoring programs, which is widely used in environmental risk analysis [1]. The choice of test objects is of great importance here, as their reactions make

it possible to assess the effect of pollutants. One of the informative test systems is represented by fish embryos and larvae, which are sensitive to the effects of various pollutants and, therefore, are widely used on the practical side to establish legal levels of environmental pollution and assess the state of the aquatic environment. The fish embryos embryotoxicity test is an obligatory component of the standard technique [1)] for the threshold limit value (TLV) concerning fishery water bodies, including marine areas with high economic activities [2]. First of all, it is referred to the shelf zones of the seas and oceans, which are most susceptible to anthropogenic influence, which negatively affects the ecosystem and its inhabitants.

One of the most common and dangerous pollutants of the marine environment has still been oil and its products [3, 4]. At the same time, an increase in shelf area oil production, as well as the development of new areas, including in the Black Sea, inevitably cause an increase in pollution of the marine environment with petroleum hydrocarbons, drilling mud and other associated pollutants [5–7]. They include components characterizing different levels of toxicity [7], which affect marine organisms in different ways. Concurrently, the sea shelf is the most biologically productive area, where reproduction and development (at early developmental stages) of organisms occur, for which even the smallest concentration of oil pollution poses a significant danger [8–11]. Moreover, researchers note the manifestation of diverse disorders at different stages of biological organization. Such disorders are characterized by a delay in growth and development, deterioration of body functions [12, 13], appearance of anomalies, increase in mortality, and change in the time of hatching of larvae from fish eggs [14]. All these indicators can serve as convenient bioindicators of the toxic effect of oil on the early developmental stages of fish, so they are widely used in aquatic toxicology. However, primary toxic reactions unfold at the molecular level, and informative biomarkers in this case are represented by antioxidant enzymes that protect the body from oxidative stress [15–18]. The responses of the protective antioxidant system can depend on the fish species, stage of their development, and toxicant dose, which should be taken into account when planning ecotoxicological experiments and monitoring programs, including the determination of legal levels of pollutants in the marine environment.

The aim of this work is a comparative analysis of the responses of biomarkers, in terms of which the activity of four antioxidant enzymes in the fish eggs of peacock blenny *Salaria pavo* and round goby *Neogobius melanosthomus* were analyzed at developmental stage VI upon incubation exposed in the mazut solution in a concentration of 0.01 and 0.1 ml/L.

[1)] Russian Federal Fishery Agency, 2009. [*Guidelines for the Development of Water Quality Standards for Fishery Water Bodies, Including Standards for Threshold Limit Values of Harmful Substances in the Water of Fishery Water Bodies*]: Approved by Order no. 695 as of 4 August 2009 by the Russian Federal Fishery Agency (in Russian).

Materials and methods

The fish eggs of round goby *Neogobius malanostomus* and peacock blenny *Salaria pavo* at developmental stage VI were collected in the Sevastopol coastal waters. The fish eggs are benthic, and the embryos develop in the coastal area. Stage VI is the stage of the mobile embryo, which is characterized by the beginning of the moving activity of the embryo simultaneously with the heart pulsation [19].

Mazut in a concentration of 0.01 and 0.1 ml/L was added to filtered sea water, stirred for 20–30 minutes, settled for 30 minutes, and poured into aerated aquariums, in which the fish eggs in the amount of 50 pieces were placed at a water temperature corresponding to the sea water temperature [20]. Control fish eggs without any oil products were kept under similar conditions. At the end of stage VI, fish eggs were taken to determine the activity of enzymes, and the whole procedure was replicated three times.

The activity of such antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT), peroxidase (PER), and glutathione reductase (GR) was analyzed with the help of Specol-211 spectrophotometer (Carl Zeiss, Iena, Germany) in accordance with the methods that we had described previously [21]. The SOD activity was expressed in arbitrary units, the CAT activity – in milligrams H_2O_2. The PER activity was expressed in absorbance units, that of GR – in nanomoles NADPH. All units of enzyme activity were adjusted to milligram of protein per minute.

The research results were processed statistically by standard methods, and the arithmetic mean (*M*) and the error of the mean (*m*) were calculated. Significance of differences was determined by the Mann–Whitney test; differences were considered significant at $p < 0.05$.

Results

The obtained results made it possible to establish certain patterns and discover features of changes in the antioxidant enzyme activity in developing embryos of two fish species during incubation in a mazut solution in both concentrations (Table). In a concentration of 0.01 ml/L, the SOD activity decreased by 83.5 % significantly ($p < 0.05$) in peacock blenny embryos, but with an increase in the toxicant content, it increased almost 3 times as against the control (Table, Fig. 1). Concerning round goby embryos, a decrease in the enzyme activity was also stated in both mazut concentrations by 51–71 % (Fig. 2).

The CAT activity in peacock blenny embryos increased by almost 140 % in a low mazut concentration, and in a high concentration it remained 40 % higher as against the control. Concerning round goby, other relationship was established: in a concentration of 0.01 ml/L, the enzyme activity showed no changes, and in a concentration of 0.1 ml/L, it decreased by 50 % (Fig. 2).

The PER activity in peacock blenny embryos did not change in a low mazut concentration, but it increased by 150 % with a toxicant doze increase (Fig. 1). Round goby embryos showed a significant decrease in enzyme activity in both mazut concentrations by 79–81 % (Fig. 2). Under the influence of the toxicant, the GR activity decreased in peacock blenny embryos by 38–53 % as against the control, and in round goby embryos – by 69–54 %, respectively (Fig. 1).

Antioxidant enzyme activity (mg protein/min, $M \pm m$) in the peacock blenny and round goby embryos at developmental stage VI exposed to mazut at a concentration of 0.01 and 0.1 ml/L

Enzymes	Peacock blenny			Round goby		
	Mazut concentration, %					
	0	0.01	0.1	0	0.01	0.1
SOD	137.4 ± 18.6	22.2 ± 8.6	489.5 ± 28.3	150.8 ± 29.0	43.7 ± 8.6	74.2 ± 12.3
CAT	0.05 ± 0.01	0.14* ± 0.08	0.07 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.05 ± 0.01
PER	0.04 ± 0.003	0.040 ± 0.003	0.10 ± 0.02	0.44 ± 0.01	0.080 ± 0.005	0.08 ± 0.01
GR	4.35 ± 1.38	2.7* ± 0.8	2.04 ± 0.15	27.53* ± 1.50	7.6 ± 0.9	12.7 ± 1.5

Note: SOD – superoxide dismutase, CAT – catalase, PER – peroxidase, GR – glutathione reductase.

* The differences are significant at $p < 0.05$ between the enzyme activity to the control.

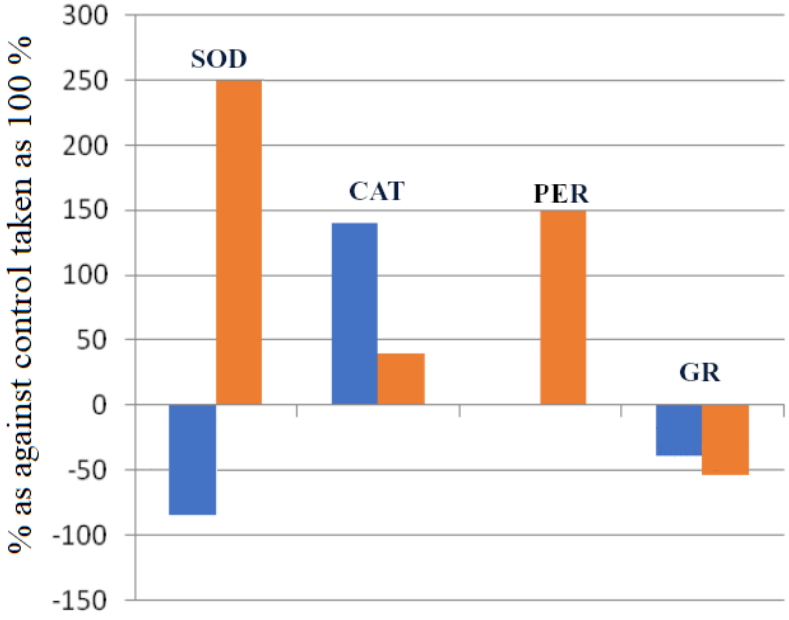

Fig. 1. The changes of the antioxidant enzyme activity in the developing peacock blenny embryos exposed to mazut as against the control taken as 100 % (mazut concentration: blue – 0.01 ml/L; orange – 0.1 ml\L)

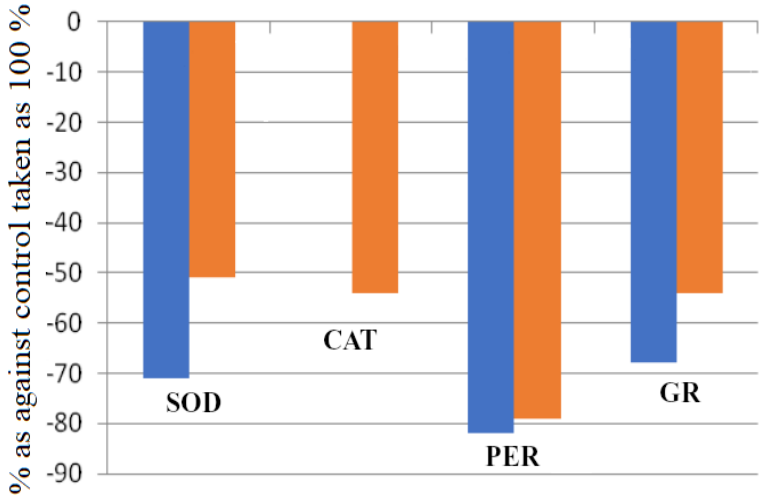

Fig. 2. The changes of the antioxidant enzyme activity in the developing round goby embryos exposed to mazut as against the control taken as 100 %. The other nomenclatures are the same as for Fig. 1

Discussion

Thus, the obtained results showed significant differences in the responses of antioxidant enzymes in the embryos of two fish species exposed to mazut. At the same time, the antioxidant system of the round goby embryos turned out to be more sensitive to the effect of mazut if we compare it with the peacock blenny defense system. The activity of all the studied enzymes in the round goby embryos under the influence of the tested mazut concentrations was reduced by 50–89 % as against the control, while the peacock blenny indicators changed ambiguously and depended on the toxicant dose. Thus, the peacock blenny embryos SOD activity during the incubation in the mazut solution with a lower concentration decreased, but with its increase, it increased by more than 2 times as against the control. This indicates that intoxication with mazut develops a protective reaction on the part of the antioxidant system key enzyme, which converts the superoxide radical to less toxic products, in particular, to hydrogen peroxide. The activity of CAT decomposing hydrogen peroxide increased by 40–145 % under the action of the toxicant as against the control in peacock blenny embryos, which also indicated the processes of H_2O_2 detoxification as a protective reaction of embryos to the effect of oil products [22]. The same response was stated for PER, which degrades organic peroxides. In the studied mazut concentrations, GR activity in peacock blenny embryos was slightly inhibited.

The obtained data demonstrate the development of oxidative stress in the embryos of two fish species in response to mazut exposure, which is consistent with the results of other authors. These data showed that oil caused multiple stress in marine organisms, accompanied by an increase in the production of free radical products, impaired reproduction, DNA damage, and developmental and behavioral abnormalities, as well as a decrease in immunity [23, 24].

An increase in enzyme activity indicates the formation of an adaptive response of the antioxidant system of embryos to neutralize the products of reactive oxygen species, while a decrease in the activity of enzymes is due to intoxication, which causes their inhibition and leads to their inability to perform protective functions. In all cases, there is an outflow of energy for antioxidant protection, which reduces the metabolic rate of the developing embryo, impairs the provision of normal processes of growth, development, and hatching [17, 25]. The presented data are indicative of the informative value of the antioxidant system parameters for the assessment of the toxic effects in marine organisms under the action of oil pollution. In this connection, they were chosen within this research as biomarkers for the analysis of the mazut embryotoxicity.

Our research showed the reorganization of the enzyme antioxidant system in developing embryos of peacock blenny and round goby depending on different concentrations of the toxicant. As previously noted [16], before the larvae emerge from the shell of the fish egg, which protects it from the adverse effects of the external environment, there is an increase in the antioxidant enzyme activity,

which is associated with the protection of the body from the upcoming oxidative stress. However, under the influence of the tested mazut concentrations in the developing fish eggs, the response reactions of antioxidant protection were modified. Subject to the existence of a toxicant, additional efforts are required for the processes of its detoxification, which results in corresponding metabolism reorganization. It is necessary to note the species-specific reaction of fish embryos to the mazut action, which can be stipulated by the peculiarities of the morphological structure of the fish eggs. The fish egg shell thickness of round goby makes 4 μm, and of peacock blenny – 5 μm [19]. This means that a peacock blenny fish egg is better protected against the environmental impact than a round goby fish egg, which corresponds to the biochemical studies data. In addition, a round goby fish egg is almost twice as large (1.9 mm in diameter) as a peacock blenny fish egg (0.75–0.80 mm in diameter). The larger surface of a round goby fish egg makes it possible to adsorb more of the toxicant, which penetrates and accumulates in the developing embryo up to the concentrations that cause intoxication of the body and decrease in its protective reactions expressed in the enzyme inhibition.

Conclusion

The results of this research demonstrate the effectiveness of the use of molecular biomarkers represented by the protective antioxidant system enzymes in order to assess the state of the early ontogenetic stages of fish that respond to a stressor (oil). At the same time, the species specificity of the responses of the peacock blenny and round goby embryos to the action of mazut due to the specific structure of the fish eggs of the two tested species was established. A peacock blenny fish egg is better protected from external influences than a round goby fish egg, since the thickness of its shell is greater than that of the round goby (5 μm and 4 μm, respectively). Aside from that, a round goby fish egg is almost 2 times larger than a peacock blenny fish egg and, thus, it is able to adsorb more toxicant that penetrates inside and accumulates in the developing embryo, which causes intoxication and decrease in protective reactions. This should be taken into account when choosing test objects and their biomarkers in order to develop biotesting methods, systems of rapid response and elucidation of the mechanisms of adaptation of marine fish early developmental stages to environmental impact.

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