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ORIGINAL PAPERS
Ecology

A Comparative Study of Long-Term Pollution Effects of Marine Waters on the Blood Biomarkers of Two Benthic Fish Species: the Scorpion Fish *Scorpaena porcus* (Linnaeus, 1758) and the Round Goby *Neogobius melanostomus* (Pallas, 1814) from the Black Sea

E. N. Skuratovskaya*, T. B. Kovyrshina, and I. I. Rudneva*****

Kovalevsky Institute of the Marine Biological Research, Russian Academy of Sciences, Sevastopol, 299011 Russia
**e-mail: skuratovskaya2007@rambler.ru*
***e-mail: svg-41@mail.ru*
****e-mail: mtk.fam@mail.ru*

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Abstract—Blood antioxidant enzyme activities were studied in 2003 and 2012 in the scorpion fish *Scorpaena porcus* (Linnaeus, 1758) and the round goby *Neogobius melanostomus* (Pallas, 1814) caught in two Sevastopol bays with different pollution levels. The study revealed higher blood antioxidant enzyme activities in the fish caught in 2012 compared to 2003. Possible causes of these changes are discussed.

Keywords: scorpion fish, round goby, antioxidant enzymes activities, blood, pollution, Black Sea

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INTRODUCTION

Long-term pollution of coastal waters caused by effluent of waters containing industry wastes and municipal discharge results in accumulation of toxic substances in the bottom sediments and in living organisms, in changes of physical and chemical properties of waters and soils, this has a negative effect on biota. Inhabitants of such waters, adapting to the pollution stress, can change their habitat or adapt to adverse conditions of life, using phenotypic plasticity and evolutionary adaptations [19]. In the absence of physical barriers, the inhabitant organisms find new habitats; this strategy is not useful for sedentary fish. These fish are forced to adapt to life conditions either by increasing their energy expenditure in the defense reactions that are provided by the immune, antioxidant and biotransformation systems [16, 29] or through micro-evolutionary processes in populations that contribute to an improvement of the overall resistance at the genetic level [19, 22]. The remarkable changes in morphological and physiological parameters (growth rate, size, weight, and the nutritional index) and in the parameters that characterize metabolic processes, including oxidative stress are recorded in fish that inhabit waters that are subjected to constant chemical contamination [15, 35]. Chronic pollution of the environment is greatly exacerbated by changes in climate [24–26]. Any disturbances of the temperature regime in highly polluted waters significantly reorganizes the metabolism in animals living there and reduces the viability, reproduction, abundance, and biodiversity of these animals. In this connection, studies of the effects of long-term changes in aquatic organisms in waters with a high anthropogenic load are important and of great current interest.

Benthic organisms are most susceptible to the influence of chemical pollution, as they live directly in the bottom part where various toxicants settle and accumulate. In addition, benthic organisms feed on organisms and particles with increased contents of toxic substances taken from the bottom layers of water and soils. Thus, bottom fish species, especially predators that occupy the top of the trophic chain and accumulate toxicants, are at a particular risk [34].

Many bays located in the vicinity of the city of Sevastopol vary considerably in their ecological state depending on the characteristics of hydrological and hydrochemical conditions of the sea and also on economic activities in the adjoining coastal areas [5] (Fig. 1). Streletskaya Bay is more susceptible to anthropogenic impact than Karantinnaya Bay because of intensive navigation and recreation developed there; the fuel pier and the ship-repair yard located on the shores contaminate the marine environment with their sewage [10]. The content of petroleum hydrocarbons in bottom sediments of Strelets-

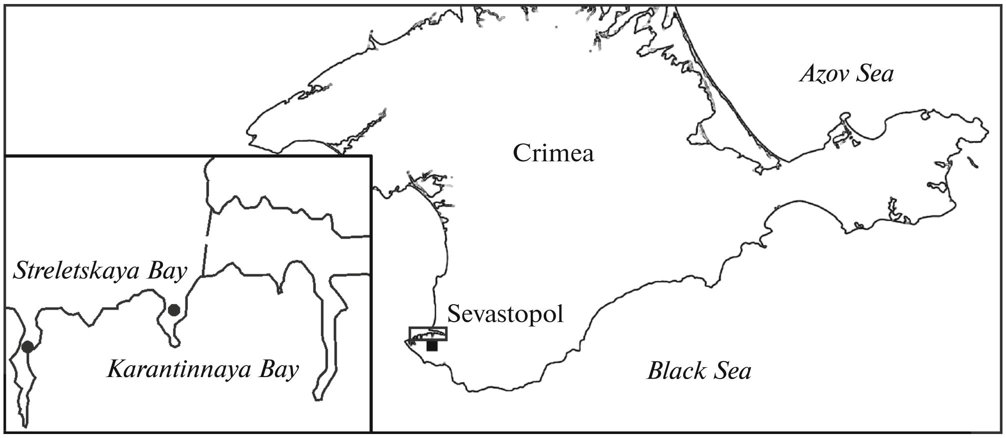

Fig. 1. The sites of fish catches in the Streletskaya and Karantinnaya bays in Sevastopol.

kaya Bay is 10 times higher than that of the Karantinnaya Bay [4], which is characterized by an intensive water exchange with the open part of the sea and, correspondingly, by quite active processes of self-purification [7]. In the last decade, the shores of the Streletskaya Bay have been involved in intensive construction of communal buildings, tourist and hotel complexes, and berths for recreation fleet vessels [5]. As a result, the concentration of petroleum hydrocarbons in this bay increased in 2003–2009 by 1.2–1.5 times compared to that in the 1990s [11]. At the same time, the level of contamination of the Karantinnaya Bay has not significantly changed from 2003 to 2008 [4, 6].

The aim of this work was to conduct a comparative study of the activity of antioxidant (AO) blood enzymes in two benthic species, typical representatives of bottom fish fauna of the Black Sea: the round goby *Neogobius melanostomus* (Pallas, 1814) (Perciformes: Gobiidae) and the scorpion fish *Scorpaena porcus* (Linnaeus, 1758) (Scorpaeniformes: Scorpaenidae).

MATERIALS AND METHODS

The round goby is the most abundant commercial species of gobies that occur along the entire coast of the Crimea Peninsula and is particularly abundant in coves and bays. The species inhabits the coastal zone to a depth of 20 m, preferring sandy and shell coasts. The species feeds on small mollusks, crustaceans, polychaete worms, and other invertebrates and fish fry. The scorpion fish is a typical ambush predator and is highly distributed in the coastal zone of the Black Sea. Most of the time this fish is still, hiding among rocks or in thickets of sea weeds and algae at depths of from 0.5–1.0 m to 30–40 m [2].

For the analysis, we selected male specimens of both species at the age of 3-4 years, which were caught in the spring in 2003 and 2012 in two bays located near the city of Sevastopol (see table; Fig. 1).

The research material consisted of hemolysates of red blood cells obtained by the method of Troitskaya (1977); these were used for determination of the activity of five AO enzymes. The activity of catalase (CAT) was analyzed by the reaction of hydrogen peroxide decomposition [1]; the activity of superoxide dismutase (SOD) was determined spectrophotometrically in the system of nitroblue tetrazolium phenazine methasulfate-nikotinamid dinucleotid (NBT-PMS-NADN) [31]. The activity of peroxidase (PER) was determined with the use of the benzidine reagent [9], while the activity of glutathione reductase (GR) was found by the reaction of the degradation of nikotinamid dinucleotid phosphatase (NADPH) and that of glutathione transferase (GT) was determined by accumulation of the conjugate in the presence of 2,4-dinitrochlorobenzol [12]. All determinations were carried out on a Spekol-211 spectrophotometer (Carl Zeiss, Jena, Germany). The activity of the enzymes was estimated as the concentration of hemoglobin (Hb). The Hb content was determined with the use of a standard kit of reagents.

The number of specimens of fish caught in the Karantinnaya and Streletskaya bays

Species	Karantinnaya Bay		Streletskaya Bay	
	2003	2012	2003	2012
Scorpion fish *Scorpaena porcus* (Linnaeus, 1758)	27	15	12	15
Round goby *Neogobius melanostomus* (Pallas, 1814)	23	16	7	27

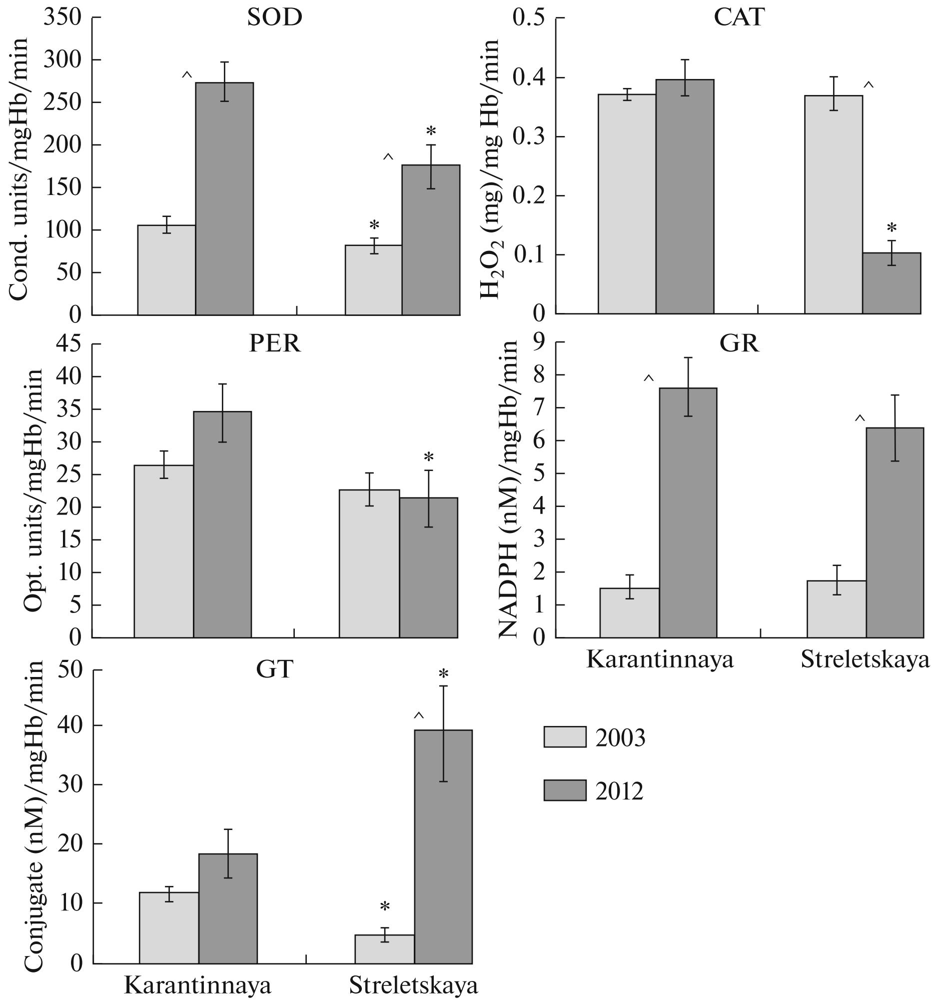

Fig. 2. The activity of antioxidant enzymes ($M \pm m$) in erythrocytes of the black scorpion fish from two Sevastopol bays in 2003 and 2012. Symbols: SOD, superoxide dismutase; CAT, catalase; PER, peroxidase; GR, glutathione reductase; GT, glutathione transferase. The reliable differences ($p < 0.05$) between the activity of AO blood enzymes of fish from two water areas, (*) from catches of 2003 and 2012 (^).

The statistical data analysis was performed using the Student *t*-test. The results were considered significant at $p < 0.05$ [8].

RESULTS AND DISCUSSION

In the blood of the scorpion fish caught in both bays that were tested in 2012, the activity of SOD, GR and GT was increased compared to that in 2003 (Fig. 2). The activity of CAT and PER in the blood of fish of the two bays in the studied period varied ambiguously. As an example, the CAT activity did not change in the blood of the scorpion fish inhabiting Karantinnaya Bay, but decreased more than 3 times in the blood of fish from Streletskaya Bay. The activity of PER in the blood of the scorpion fish from Streletskaya Bay in 2012, virtually did not change compared to that in 2003, while this value had a notable trend to an increase for fish from the Karantinnaya Bay.

The SOD activity in the blood of the scorpion fish of the Karantinnaya Bay was higher than that of fish from Streletskaya Bay in both years of study. The activities of CAT and PER did not differ in the blood of fish from both bays in 2003, while in 2012 the activity of these enzymes was significantly lower in fish from the Streletskaya Bay than that of fish from Karantinnaya Bay. The GR activity in the blood of fish from both bays did not differ significantly in 2003, but increased by more than 4 times in 2012. The GT activity in the blood of the scorpion fish from Streletskaya Bay was lower in 2003 compared to that of the fish from the Karantinnaya Bay, whereas in 2012 this value in the blood of fish in Streletskaya Bay was more than 2 times higher than that of fish in the Karantinnaya Bay. Thus, in 2012 compared to 2003, the scorpion fish showed a significant change in the activity of the studied antioxidant enzymes in the blood.

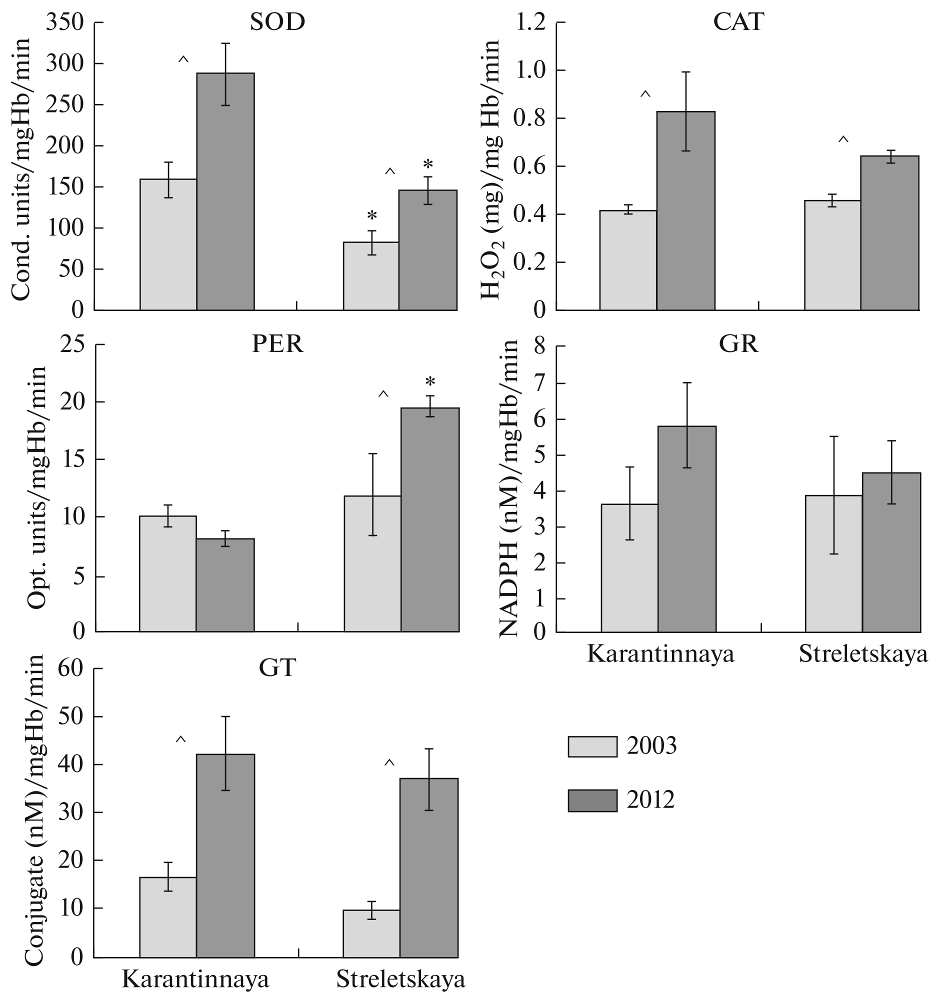

Fig. 3. The activity of antioxidant enzymes ($M \pm m$) in the red blood cells of the round goby from two Sevastopol bays in 2003 and 2012. Symbols as in Fig. 2.

The activities of SOD, CAT, and GT in erythrocytes of the round goby from both bays were in 2012 significantly higher than in 2003 (Fig. 3). The GR activity also tended to increase, but the differences between the values for 2003 and 2012 were found to be unreliable. In 2012, the PER activity significantly increased in the blood of the round goby from Streletskaya Bay, but did not change in fish from the Karantinnaya Bay. Thus, the scorpion fish and the round goby of the two Sevastopol bays with varying degrees of exposure to anthropogenic pollution showed differed antioxidant enzyme activities in the blood in the studied periods. In most cases, we revealed a trend to an activity increase compared to the values for 2003; the trend was most pronounced for SOD and GT. The GR activity in the blood of both the scorpion fish and the round goby of both tested bays increased significantly, while differences between the values for 2003 and 2012 were not significant. The induction of key enzymes in the blood of the two studied species may be due to an increasing role of the antioxidant protective system of fish that live under conditions of chronic pollution to provide their resistance to the effects of oxidative stress.

The increasing activity of enzymes in 2012 was more pronounced in the blood of the scorpion fish than in the round goby (Fig. 4). These recorded changes may indicate an active defense reaction of the organism to toxic effects of pollutants that are contained in waters and sediments of the Streletskaya Bay and stimulated the development of oxidative stress in the tested species. However, the fish that lived in the ecologically unfavorable waters that are characterized by high levels of pollution, as well as in experimental conditions at extreme concentrations of toxicants, showed a decreased activity of some antioxidant enzymes [13, 23, 30].

In 2003, the PER activity was nearly the same in the blood of fish from the two tested bays, while in 2012 it increased by almost 1.5 times in the blood of the round goby from Streletskaya Bay, which may be due to a compensatory effect or a response to high lev-

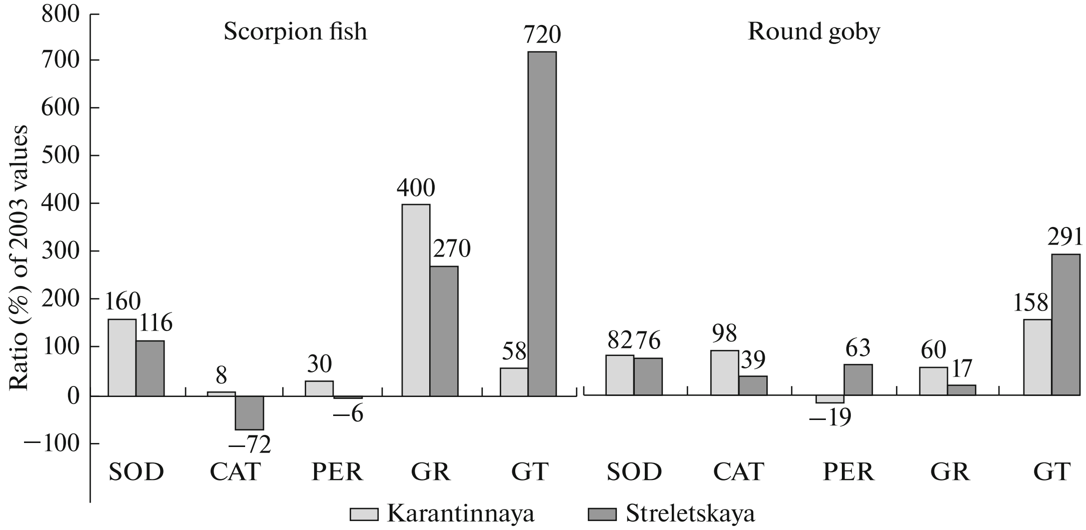

Fig. 4. The comparative characteristics of the activity of antioxidant enzymes in the red blood cells of the black scorpion fish and the round goby caught in Karantinnaya and Streletskaya bays in 2012, compared to the indices for 2003.

els of the content of petroleum hydrocarbons in this water area [10, 11]. In the scorpion fish from the bay, the PER activity in the blood was reduced in 2012 compared to that in the fish from Karantinnaya Bay; this may reflect the species peculiarities of the adaptation of the fish to adverse environmental conditions. The SOD activity in both fish species from Streletskaya Bay was lower than that of the fish from Karantinnaya Bay in both periods. The GR activity did not significantly differ in the blood of both species of the two bays in 2003 and in 2012. While in 2003, the GT activity in fish from the two tested water areas differed slightly, in 2012 this trend continued in the round goby, while in the scorpion fish from Streletskaya Bay the GT activity appeared to be more than 2 times higher than that of the specimens from the Karantinnaya Bay. This again indicates the presence of species-specific responses of protective system, namely GT, which is a phase II biotransformation enzyme that carries out the conjugation reaction, as we noted earlier [32].

Other authors have also shown that the reactions that characterize the state of the oxidative stress in fish under the influence of negative factors are complex. As an example, different fish species (*Diplodus vulgaris, D. sargus, Dicentrarchus labrax, Gobius niger,* and *Liza ramada*) that were caught in an estuary showed different enzyme reactions of the antioxidant system of muscles under a temperature increase [24]. Species-specific features of the enzyme responses of the antioxidant system have been revealed in red blood cells of the fish species *Astyanax fascialus* and *Pimelodus maculates*, which inhabit waters with different levels of pollution [33]. Researchers have noted that fish of smaller size are more susceptible to negative effects of toxicants than larger species [20]. We should also take the peculiarities of feeding behavior and their food composition into account, which somewhat differ between the black scorpion fish and the round goby [2].

The modifying effects of toxicants on the activities of the enzymes of the antioxidant system depend on the level of contamination of water areas [17, 18, 36]. The increase in the enzyme activity in the red blood cells of the scorpion fish and the round goby in 2012 indicates an adaptive response of these fish species to oxidative stress caused by the influence of adverse factors, including chemical pollution of the environment. This effect is clearly expressed in fish from Karantinnaya Bay. At the same time, the high level of pollution in the Streletskaya Bay led to the depletion of the protective functions of the body, which is confirmed by a partial reduction in the activities of antioxidant enzymes.

Thus, our study showed that benthic fish, which include the round goby and the scorpion fish living in ecologically unfavorable environments, exhibit a pronounced response to pollution. Chronic exposure to toxicants exerts an oxidative stress on fish and leads to enhancement of the processes of reactive oxygen species (ROS), which damage biomolecules, enzymes and membranes, and cause various pathologies. Induction of the main antioxidant enzymes in the fish blood indicates the manifestation of protective adaptive responses; however, at a high level of pollution we observe a reverse effect caused by reduced resistance to enhanced toxic effects.

Based on these studies, we can conclude that the decline in the level of pollution in the Sevastopol bays in the current period compared to the 1990s–beginning 2000s has not led to restoration of the blood antioxidant system in the two bottom fish species. Existence under conditions of long-term pollution caused corresponding posteffects in the round goby and in the scorpion fish, as shown by an increased activity of the antioxidant system of the blood in fish. This is confirmed by the data on the accumulation of oxidative stress products, as we noted in our previous studies [5]. Obviously, the system of regulation of vital functions in fish, including protective functions, moved to a new level in order to defend the fish in an environment altered by anthropogenic influence [3]. Therefore, the biomarkers used for these fish, namely the indices of the activities of the enzymes of the antioxidant system of red blood cells, can be applied in long-term monitoring programs. This becomes especially important in the case of tanker accidents, when large amounts of oil pour into the sea, settle to the bottom, and remain there for a long period of time [21, 27, 28]. The danger of oil pollution is also relevant to the coastal waters of Sevastopol. In this connection, further study of the effects of oil pollution on fish and the search for appropriate markers are important and timely. Moreover, under climatic changes, these processes can be subjected to substantial modifications. It is important to consider the peculiarities of the biology and ecology of the studied species.

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