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ORIGINAL PAPERS

# The Response of Blood Biomarkers of the Round Goby *Neogobius melanostomus* (Pallas, 1814) (Perciformes: Gobiidae) to Chronic Coastal Pollution in the Sea of Azov

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**Abstract**—The long-term variations in blood biomarkers and weight–size characteristics of the round goby, *Neogobius melanostomus* (Pallas, 1814), have been studied in specimens collected from two sites of the southwestern Sea of Azov. The fish from catches of 2011–2012 showed a decrease in the size and weight parameters and an increase in the antioxidant enzyme activities and the level of oxidized serum proteins compared to the values in the fish caught in 2003. The ecological causes of the observed differences and the possibility of using the studied fish blood biomarkers in programs of monitoring the marine coastal waters are discussed.

**Keywords:** round goby, antioxidant enzymes, oxidized serum proteins, blood, oxidative stress, pollution, Sea of Azov

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## INTRODUCTION

To efficiently diagnose the ecological condition of marine waters, including those in the Black Sea and the Sea of Azov, the use of responsive reactions of benthic fish that lead a sedentary life can be an efficient approach [4, 14]. These fish species are members of the family Gobiidae, including the commercially valuable round goby, *Neogobius melanostomus* (Pallas, 1814) (Perciformes: Gobiidae), which is considered as a convenient object for ecotoxicological assessment of the state of marine and brackish bodies of water [25]. The deterioration of the ecological situation in the southwestern Sea of Azov [8, 9] necessitates biomonitoring research in this region of Crimea in accordance with the strategy developed and successfully applied in the world practice [18, 24, 26, 27]. In these cases, researchers use appropriate biomarkers to detect early biological effects triggered at the molecular and cellular levels [19, 22, 28]. The parameters of oxidative stress that occurs in an organism under the effect of negative factors such as chemical pollution remain of particular importance. Induction and/or inhibition of the activities of antioxidant (AO) enzymes is a nonspecific form of the response of an organism to the effects of various stressors, whereas the parameters of oxidative modification of proteins (OMP) serve as early indicators of pathological protein metabolism in oxidative stress [12, 19].

The goal of the present work was to study the long-term variations in the size–weight and morphophysiological characteristics, as well as in the activities of antioxidant enzymes and the level of oxidized forms of proteins in blood serum in round gobies from two areas of the southwestern Sea of Azov.

## MATERIALS AND METHODS

The object of the study was the round goby, *Neogobius melanostomus*, a Ponto-Caspian endemic and a typical member of the benthic ichthyofauna in the Sea of Azov. Fish were caught in the spring seasons of 2003 and 2011–2012 from the Bay of Arabat, Sea of Azov, near the villages of Semenovka and Mysovoye, located at capes Kiten' and Kazantip, respectively.

The areas of fish catches differed in ecological characteristics and sources of pollution. Thus, the coastal waters off Semenovka are exposed to nutrient elements from the nearby farmland. As a result, their concentration is higher here, while $BOD_5$ is lower than that recorded from the waters off Mysovoye [11], where the main sources of pollution of coastal waters are toxic wastes of petroleum production. In the latter area, the greatest hazard is posed by drilling mud containing organic and inorganic substances, surfactants, defoamers, lubricants, biocides, and heavy metals. In baryte, where heavy metals are present as an admixture, the Pb concentration reaches 0.22%; Cd,

0.124%; and Cu, 0.019% [13]. These metals can accumulate in the tissues of the round goby to substantial concentrations and affect the prooxidant–antioxidant status of the fish [3, 7].

In 2001, the pollution of bottom sediments in the western Sea of Azov was classified as class I according to the level of petroleum products; in 2009, it was class III [9]. A similar trend was observed for organochlorine compounds in water and bottom sediments, whose concentrations increased in 2005 compared to those in 2001 [8]. Thus, the ecological situation in the considered area of the Sea of Azov shows a tendency to deteriorate.

During the biological analysis of fish (101 fish in 2003 and 115 fish in 2011–2012), the total body length and body weight of an individual, gutted body weight (Wb), liver weight (Wl), and gonad weight (Wg) were measured, and the sex and maturity stage were identified. The age of the fish was assessed by otoliths. The liver index (LI, ‰) and gonadosomatic index (GSI, %) were calculated as reported previously [10]: LI = Wl × 1000/Wb; GSI = Wg × 1000/Wb.

Biochemical parameters were measured in 22 males caught in 2003 and 50 males in 2011–2012. The material for the biochemical studies was fish blood collected from the caudal artery with a syringe or Pasteur pipette. In hemolysates obtained by the method of Troitskaya [15], the activities of five antioxidant enzymes were assessed in accordance with the protocols described earlier [4, 14]. The activity of catalase (CAT) was determined by the reaction of hydrogen peroxide decomposition; superoxide dismutase (SOD) was determined by the spectrophotometric method in the nitro blue tetrazolium–phenazine methosulphate–nicotinamide dinucleotide (NBT–PMS–NADH) system; peroxidase activity (PER) was determined by the benzidine method; glutathione reductase (GR) was determined by the reaction of degradation of nicotinamide dinucleotide adenine phosphate (NADPH); and glutathione transferase (GT) was determined by the accumulation of conjugate in the presence of 2,4-dinitrochlorobenzene. Enzyme activity was expressed per 1 mg hemoglobin, which was measured using a standard set of reagents.

The levels of protein oxidation products in the blood serum were also analyzed. The method is based on the reaction of oxidized amino acid protein residues with 2,4-dinitrophenylhydrazine. The derivatives of 2,4-dinitrophenylhydrazone that form as a result of this reaction were detected at the following wavelengths (OD): neutral aldehyde and ketone products were detected at $\lambda$ = 346 and 370 nm, respectively; basic aldehyde and ketone products were detected at $\lambda$ = 430 and 530 nm, respectively [1]. All measurements were made on a Specol-11 spectrophotometer (Carl Zeiss, Jena, Germany). To exclude the influence of ecological, physiological, and seasonal factors on the prooxidant–antioxidant status of fish, the blood biomarkers were compared between spawning males from the same age group (age 2).

Statistical data analysis was carried out using the Student's *t*-test. The results were considered significant at $p < 0.05$ [5].

## RESULTS

A decrease in the body size and weight, as well as in the morphophysiological indices, was observed in fish from both studied water areas from 2003 to 2011–2012 (see Table 1). The fish caught from the coastal waters off Semenovka Village were characterized in most cases by higher mean values of body length and weight, as well as by higher gonad weight and GSI than those in the fish caught off Mysovoye. In 2003, the activities of AO enzymes in the blood (Fig. 1) and the level of protein oxidation products (Fig. 2) in spawning 2-year-old males did not differ between the areas. The activities of most AO enzymes and the level of oxidized serum proteins in the blood of fish caught in 2011–2012 were higher than those in fish caught in 2003. Along with the general tendency of SOD activity to increase, the round gobies from the two water areas in 2011–2012 showed some characteristics in their blood parameters that are specific for each of the considered areas. The activities of CAT ($p < 0.001$) and GR ($p < 0.01$) significantly increased in the fish from the water area off Semenovka, whereas in the blood of the fish from the coastal waters off Mysovoye Village, a decrease in CAT activity ($p < 0.001$) and an increase in PER activity ($p < 0.05$) were recorded. During this period, the GT activity also increased in the blood of the fish from both areas, but no significant differences were found (Fig. 1). The activities of CAT ($p < 0.01$) and GR ($p < 0.05$) were significantly higher and the PER activity was significantly lower in the erythrocytes of the fish from Semenovka compared to the activities of these enzymes in the fish from Mysovoye.

The level of oxidized serum proteins in the fish from both water areas of the Bay of Arabat in 2011–2012 significantly increased due to the neutral components (OD 346 and 370 nm). The concentration of basic products did not differ between the study periods (OD 430 and 530 nm) (Fig. 2). In the blood serum of fish from the coastal waters off Semenovka, the level of OMP, that is, a neutral aldehyde product (OD 346 nm), was lower ($p < 0.01$) than that in the fish from the area off Mysovoye.

## DISCUSSION

These data indicate a decrease in the size and weight characteristics of the round goby and an increase in the level of oxidized serum proteins and the activities of most AO enzymes in the blood of the fish caught in 2011–2012, compared to the values recorded in 2003. These variations can be explained by the development of oxidative stress in fish under the con-

**Table 1.** The size, weight, and morphophysiological characteristics of the round gobies, *Neogobius melanostomus*, caught from two areas of the Bay of Arabat, Sea of Azov, with different levels of pollution in 2003 and in 2011–2012

| Parameter | Semenovka | | Mysovoye | |
|---|---|---|---|---|
| | females | males | females | males |
| Number of specimens, fish | 57/31 | 7/20 | 29/17 | 8/47 |
| Total body length, cm | $14.92 \pm 0.13^{b}$ / $13.65 \pm 0.12$ | $19.35 \pm 0.79^{b}$ / $17.56 \pm 0.29$ | $15.23 \pm 0.2^{b}$ / $13.29 \pm 0.21$ | $15.15 \pm 1.41^{a}$ / $16.37 \pm 0.2^{a}$ |
| Body weight, g | $45.50 \pm 1.03^{b}$ / $35.24 \pm 0.94$ | $106.83 \pm 9.33^{b}$ / $73.54 \pm 4.96$ | $46.28 \pm 1.83^{b}$ / $32.60 \pm 1.62$ | $54.71 \pm 13.97^{a}$ / $61.05 \pm 2.26^{a}$ |
| Gonad weight, g | $4.69 \pm 0.24$ / $4.5 \pm 0.19$ | $1.12 \pm 0.18$ / $0.95 \pm 0.09$ | $4.18 \pm 0.29^{b}$ / $2.14 \pm 0.23^{a}$ | $1.12 \pm 0.13^{b}$ / $0.64 \pm 0.04^{a}$ |
| Liver weight, g | $1.48 \pm 0.084^{b}$ / $0.64 \pm 0.04$ | $4.15 \pm 0.38^{b}$ / $1.98 \pm 0.16$ | $1.57 \pm 0.12^{b}$ / $1.16 \pm 0.13^{a}$ | $1.85 \pm 0.45^{a}$ / $1.96 \pm 0.11$ |
| GSI, % | $12.82 \pm 0.67$ / $16.27 \pm 0.71^{b}$ | $1.11 \pm 0.13$ / $1.58 \pm 0.25$ | $10.89 \pm 0.71^{b}$ / $8.00 \pm 0.81^{a}$ | $2.92 \pm 0.51^{c}$ / $1.19 \pm 0.07$ |
| LI, ‰ | $39.36 \pm 2.03^{b}$ / $22.50 \pm 1.37$ | $42.73 \pm 2.2^{b}$ / $30.12 \pm 1.72$ | $40.59 \pm 2.81$ / $41.28 \pm 2.81^{a}$ | $52.66 \pm 6.46^{b}$ / $33.50 \pm 1.5$ |

[a] Significant differences between the study areas.
[b] Significant differences between the study periods.
[c] Significant differences between the study areas and periods.
Values (± error of the mean) in the numerator are data for 2003; in the denominator, for 2011–2012; age of fish is 2 years. GSI, gonadosomatic index; LI, liver index.

ditions of chronic pollution caused by human economic activities. Since 2000, the main sources of pollution in the studied area of the Sea of Azov have been the development and operation of offshore gas fields, bottom trawl fishing for the so-iuy mullet, and agriculture [8, 9]. As a result, a rise of the ground level was accompanied by an influx of pollutants that are not characteristic for bottom sediments in the natural ecosystem, as well as by a disruption of the structure of the bottom and biotopes. These processes could significantly alter the habitat conditions for the round goby in these waters and create a stressful situation, as is confirmed by the results of our study. Thus, in 2003, the activities of the analyzed blood biomarkers in the fish from two areas of the Bay of Arabat were not significantly different. In 2011–2012, the pattern changed: the activities of the key AO enzymes, SOD and CAT, in erythrocytes of the fish from the coastal waters off Semenovka significantly increased. The increase in the SOD and PER activities in the blood of the fish from the water area near Mysovoye was accompanied by a decrease in the CAT activity. Moreover, a higher level of oxidatively modified serum proteins was observed in the blood of the fish from both water areas. These data indicate that the increasing anthropogenic load on the study areas during the last decade has caused a more-pronounced manifestation of oxidative stress in fish, a change in the state of the AO enzyme defense system, as well as the accumulation of OMP products in their blood serum.

The non-uniform response of the AO system in the blood of the fish from the two considered water areas should also be noted. Induction of AO enzyme activities is an adaptive response of living organisms to the effects of various adverse factors, including environmental pollutants. Thus, a decrease in the activities of AO enzymes in tissues of fish from water areas with a high level of contamination is an evidence of toxic effect of the pollutants on the AO enzyme system and a shift in the prooxidant–antioxidant equilibrium towards the processes of oxidative damage to biomolecules that precede the development of pathological processes. The increase in the activities of AO enzymes (CAT, SOD, and GR) and the higher level of OMP in the blood of the round gobies from the coastal waters off Semenovka in 2011–2012 indicate the compensatory pattern of adaptation (compensation stage). The inhibition of enzymatic (CAT and GR) activities in the blood of the fish caught from the coastal waters off Mysovoye may be evidence of the development of a pathological state, as is confirmed by the high level of oxidized serum proteins (a direct indicator of oxidative stress) in fish blood [17, 20]. The revealed features can be considered to be a result of the deterioration of the ecological situation in the water area off Mysovoye, which is located in close proximity to the site of active development of oil and gas fields. Earlier, we noted similar trends when analyzing the long-term variations in the blood biomarkers of the round goby

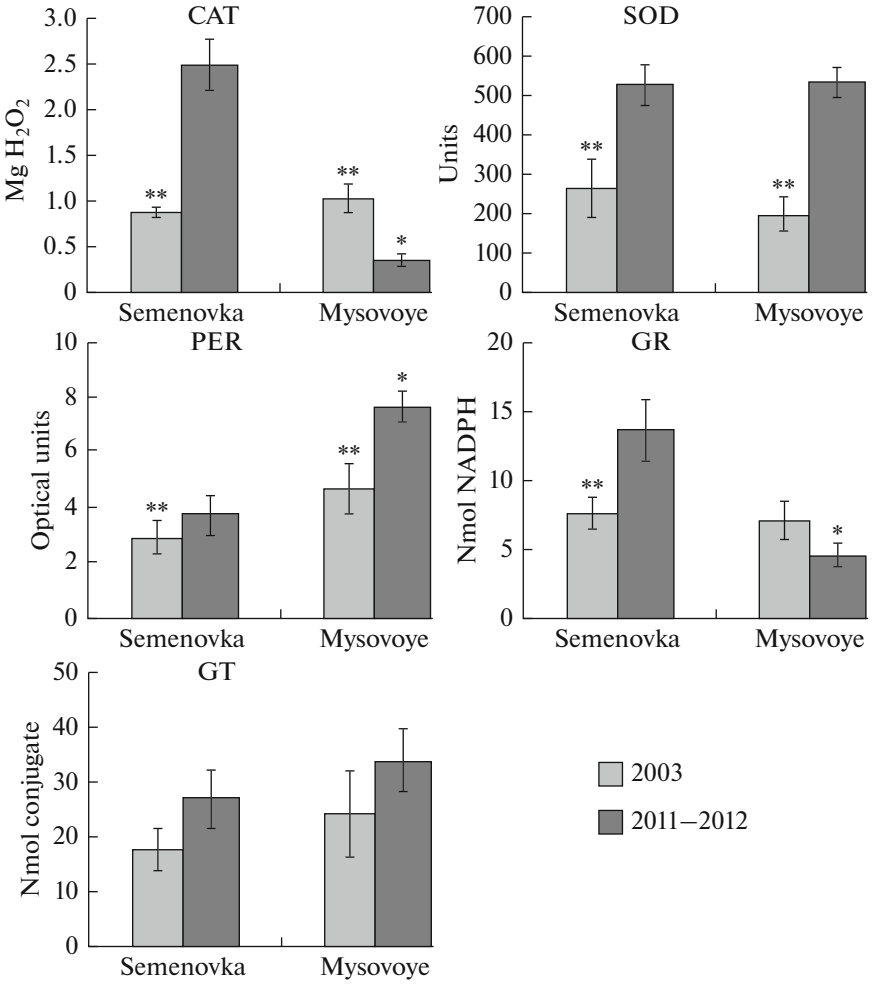


**Fig. 1.** The enzyme activity (mg Hgb/min; $M \pm m$) in the blood of male *Neogobius melanostomus* from the Bay of Arabat, Sea of Azov. *Significant differences for fish from different areas of the bay; **significant differences for fish examined in 2003 ($n = 22$ fish) and in 2011–2012 ($n = 50$ fish) ($p < 0.05$).

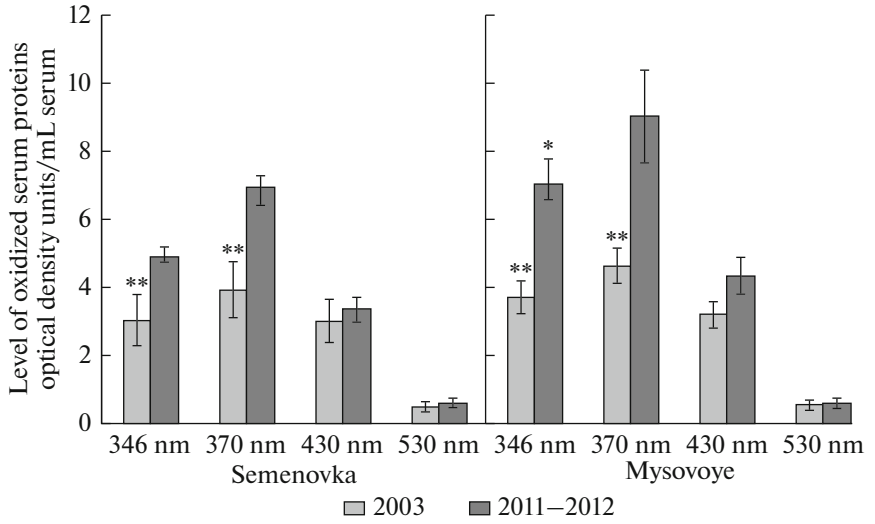


**Fig. 2.** The level of oxidized serum proteins ($M \pm m$) in the blood of male *Neogobius melanostomus* from the Bay of Arabat, Sea of Azov. *Significant differences for fish from different areas of the bay; **significant differences for fish examined in 2003 ($n = 16$ fish) and in 2011–2012 ($n = 14$ fish) ($p < 0.05$).

and black scorpionfish from bays off Sevastopol in the Black Sea [4, 14].

The consequences of chronic stress and the associated energy expenditure for defense reactions had an influence on the size and weight parameters of the round gobies caught in 2011–2012. Fish living in unfavorable conditions are characterized by a decrease in their growth rate, since the energy cost of generative metabolism prevails over the energy cost of development processes [6, 16]. We found that in the study periods the body size and weight of the female and male round gobies, as well as the size of gonads and GSI in the females caught in the area off Semenovka were higher than the values of these parameters in the fish from the coastal waters off Mysovoye. This can be explained by the more favorable conditions for feeding and spawning round gobies in the Semenovka area due to the abundant forage resources, whose growth was facilitated by the increased concentrations of nitrogen, phosphorus, and silicon in the water [11]. The high concentrations of nutrients create favorable conditions for the proliferation of phytoplankton consumed by mollusks, which are the main food item in the diet of the round goby. The sufficient forage resource probably contributed to more successful adaptation of the fish to the conditions of chronic pollution due to the uptake of low-molecular-weight antioxidants and trace elements that are available in food that are necessary for the synthesis of enzymes [21].

Thus, the results of our work allowed us to identify the effects of complex pollution of the habitat on the studied blood biomarkers in fish that inhabit the southwestern Sea of Azov. These biomarkers are indicators of the health of the fish and the conditions of its habitat, which can be unfavorable and sometimes toxic. The components of the AO system respond to a variety of exogenous and endogenous factors; therefore, they allow evaluation of the integrative response to variations in these factors [18, 23, 24, 27]. The variations in the activities of most of the AO enzymes in the blood of fish from the Bay of Arabat, Sea of Azov, over the past 10 years indicates a deterioration of the ecological condition of this water body, which is associated with the high level of economic activities in the coastal waters of this region. The fish had greater energy expenditures for the increase in the adaptive potential (higher activities of AO enzymes due to activation of their synthesis) and further redistribution of energy and constructive resources due to the rearrangement of metabolic processes. This resulted in a decrease in the size, weight, and morphophysiological parameters of the round gobies in 2011–2012 compared to those caught in 2003, because a substantial portion of their energy resources are used for the processes of adaptation of fish to unfavorable habitat conditions. It should be noted that similar results were obtained for round gobies from other waters of the Sea of Azov with increased levels of pollution [2]. It was found that the toxic effects of the key pollutants in some coastal areas of the Sea of Azov exceeded the adaptive capabilities of the round goby, which led to morphological pathologies and a decrease in the reproductive potential and reproductive success at the population–species level. However, a comprehensive analysis of the prooxidant–antioxidant status and the size, weight, and morphophysiological characteristics of round gobies from two areas of the Bay of Arabat, Sea of Azov, has shown the more ecologically favorable conditions for life and reproduction of this species near Semenovka. The conclusion can be made that applying the biomarkers of the round goby to assess the ecological condition of coastal waters is informative and can be used in monitoring programs. However, for a more detailed analysis of the adaptive and pathological processes that occur in the round goby populations in different waters of the Sea of Azov that are exposed to the impacts of economic activities, the use of molecular-genetic methods of analysis and the identification of adequate genetic markers seem more promising.

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