

Use of fish blood serum biomarkers for evaluating of black sea coastal waters health

Rudneva Irina I., Kovyrshina Tatyana B. and Skuratovskaya Ekaterina N.

Ichthyology Department, Institute of Biology of the Southern Seas, Nahimov AV.

ABSTRACT

The aim of the present study was to measure the biomarkers in blood serum of round goby *Neogobius melanostomus* and scorpion fish *Scorpaena porcus* (L.) caught in two bays of the Black Sea in Sevastopol region (Crimea) which were differed of pollution level. Concentration of oxidated proteins (OP) was studies in blood serum spectrophotometrically at 345, 370, 430 and 530 nm. The OP level in blood serum of fish from polluted area was higher than in less-polluted site. The interspecies differences of OP concentration in two tested fish species were shown and depended on specific features of biology and ecology. High anthropogenic impact leads oxidative stress in fish tissues and stimulates high production of ROS, which damage and modify cell membranes and biomolecules, including proteins. Examined biochemical parameters are important for the evaluating of fish abilities to protect against chemical pollution and keep their life in the polluted environments. They may potentially be used as indicators of chemical pollution of marine environment and fish health status.

Key words: *Scorpaena porcus*, *Neogobius melanostomus*, pollution, oxidated proteins, biomarkers, Black Sea

INTRODUCTION

Fish are very sensitive to anthropogenic pollution and some of them may be tested as biomonitors for the evaluating of the effects of contaminant-related stress on marine environment. The resistance of fish to unfavorable factors depends on their phylogenic position, ecological and biological characteristics, physiological status [1, 2, 3] and the presence of efficient detoxification mechanisms. Previously we described the polluted response of Black Sea teleosts which reflect their adaptive strategy and ability to cope with the environment [4, 5]. Ecotoxicological methods which use biomarkers of physiological status of fish are applied widely in assessing of water quality. Among them antioxidants level, lipid peroxidation parameters, enzyme activities and they are successfully used in many studies [3]. Molecular biomarkers of fish health are the most informative because they respond at very short time and reflect the changes of fish physiological status at the case of stress [3]. Previously we described the changes of antioxidant enzyme activities and lipid peroxidation level in fish from polluted and non-polluted sites [6, 7], the fluctuations of aminotransferase activities in the animals inhabited the locations characterized different anthropogenic impact [8]. We showed the correlation between heavy metals accumulation in fish tissues and biochemical parameters [9, 10]. Level of oxidative modification of proteins in tissues are good biomarkers for evaluation of oxidative stress in fish caused anthropogenic impact [11]. The further study of fish response on environmental stress led anthropogenic pollution is very important for the evaluation of water quality and fish population status.

Scorpion fish (*Scorpaena porcus*) and round goby (*Neogobius melanostomus*) are highly distributed fish species in the coastal waters of Black Sea. Both species are benthic forms, their biology and ecology are similar, but each species is characterized by several peculiarities.

Scorpion fish is among the most common fish species in Black Sea coastal waters and it was selected as biomonitor in our previous studies [4]. It is benthic form inhabiting bottom waters on the depth of 1-40 m, however it prefers the depth of 1-10 m. Spawning time covers the period from the end of May to the middle of September. Female

produces the eggs in twin sacks, which dissolve in marine water, and the embryos in the eggs develop in pelagic zone. Scorpion fish is a batch spawner, it produces many spawning portions and the eggs hatch every 1-2 day. Every portion contains 1100- 27200 eggs [12].

Round goby is highly distributed fish species in different kinds of aquatic ecosystems, including the coastal waters of Black Sea. It is benthic form. The feeding spectrum of fish includes mollusks, crustacean and worms. Spawning time covers the period from April to August . It is batch spawner, female spawns 6 portions of eggs. The egg is large, its size is approximately 4 mm [12].

The aim of the present study was to measure the level of oxidated proteins as biomarkers in blood serum of round goby *Neogobius melanostomus* and scorpion fish *Scorpaena porcus* (L.) caught in two locations of the Black Sea in Sevastopol region (Crimea) differed of pollution level.

MATERIALS AND METHODS

Sampling sites and animals

Scorpion fish *Scorpaena porcus* (L.) and round goby *Neogobius melanostomus* (Figure 1) were caught in autumn-winter period 2012 in two bays: Karantinnaya Bay and Streletskaia Bay in Sevastopol region (the Black Sea, Crimea) (Figure 2). The sampling sites are differed by level of anthropogenic impact: Streletskaia Bay is highly polluted region as compared with Karantinnaya Bay (Table 1). The animals were immediately placed in the aerated tank, transported to the laboratory and anesthesy. Blood samples were taken by caudal arteria puncture. The samples were stored at +4°C 2 hours and serum was separated. Oxidated proteins level was determined in blood serum immediately after preparation.



Figure 1. Scorpion fish *Scorpaena porcus* (1) and round goby *Neogobius melanostomus* (2)

Table 1. Anthropogenic impact on the sampling in Sevastopol region (Black Sea, Crimea)

Anthropogenic activity	Karantinnaya Bay	Streletskaia Bay
Recreation	–	+
Effluents of municipal and industrial sewage, m ³ day ⁻¹	50	350

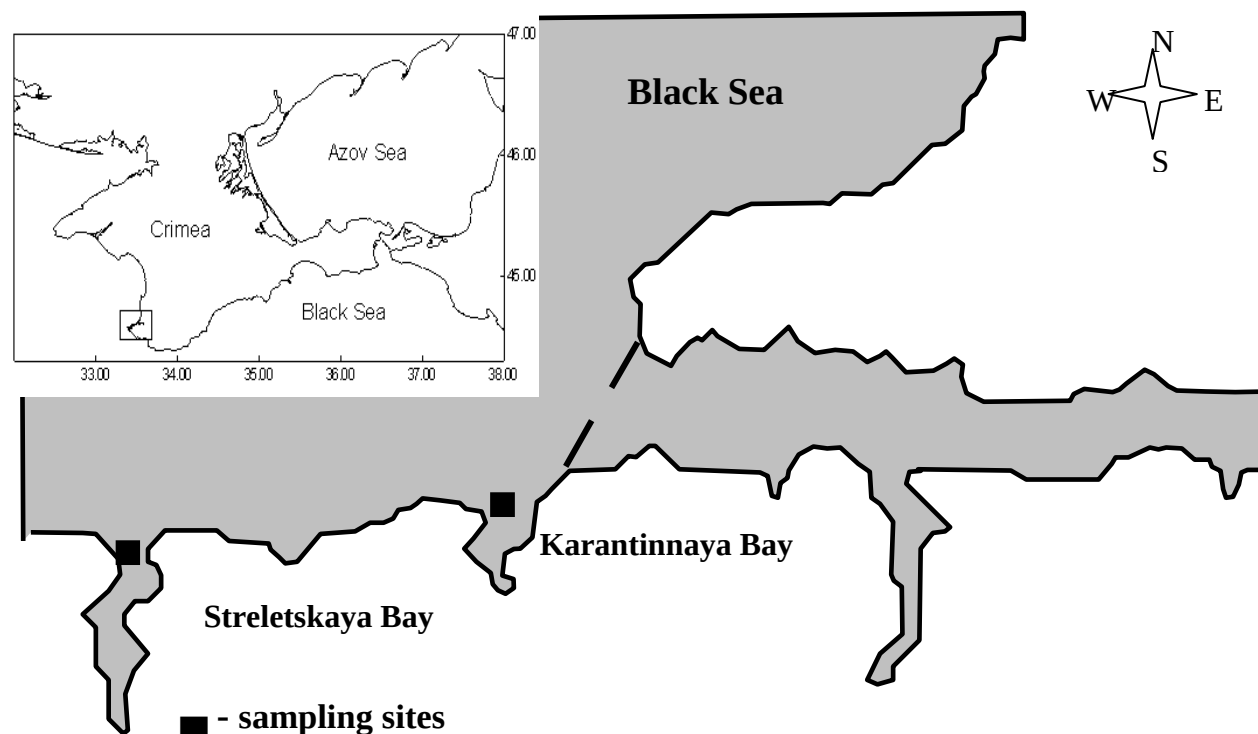
– - absence of impact, + - presence of impact

Biochemical assays

Oxidated proteins concentration in fish blood serum was determined spectrophotometrically according the methods [13] with some modifications. 0.05 ml serum was added 1 ml 20% TCA solution and 1 ml 2,4-dinitrophenylhydrazine (DNP) in 2 M HCl. The mixture was incubated 1hr at room temperature and then it was centrifuged at 3000 g due 15-20 min. Sediment was washed by ethanol-ethylacetat (1:1) solution and it was centrifuged again. 2.5 ml 8 M urea was added to dry sediment and it was solved in boiling water. Optical density (OD) of carbonyl groups was measured at 346 (neutral aldehyde), 370 (neutral ketone), 430 (basic aldehyde) and 530 nm (basic ketone) used spectrophotometer Specol-211 (Carl Zeiss, Iena, Germany). The results were shown in arbitrary units (OD per ml serum).

Statistical analysis

The results were processed to statistical evaluation with ANOVA one-way test. All numerical data are given as means ± SEM [14]. The significance level was 0.05.



RESULTS

Concentration of oxidated proteins in blood serum of scorpion fish and round goby, caught in two Sevastopol bays is present in Fig. 3 and 4. In both tested fish species the level of neutral compounds measured at 346 and 370 nm was higher as compared with the value of basic substances concentration assayed at the wave length 430 and 530 nm. The level of neutral ketones was higher than neutral aldehydes, while basic ketones concentration was greater as compared with basic aldehydes level. In both tested bays the level of blood serum OP in round goby was higher than in scorpion fish.

Figure 2. Sampling sites of fish specimens in Sevastopol bays, Black Sea, Crimea

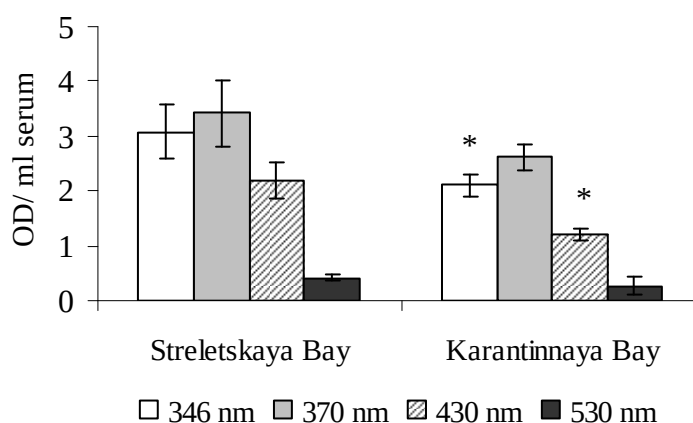


Figure 3. Concentration of oxidated proteins in blood serum of scorpion fish caught in Streletskaia Bay (n=15) and Karantinnaya Bay (n=15), mean $\pm$ SEM. * - significant differences between the values of fish from two tested bays.

In scorpion fish from Streletskaia Bay, the values of oxidated protein concentration were higher than in fish from Karantinnaya Bay. However, the differences between ketones concentration were insignificant while the differences between aldehydes levels were significant ($p < 0.05$).

In round goby from both examined locations the differences were not significant (Figure 4). At the other hand, we could note the decrease of basic ketones level in fish serum from Karantinnaya Bay as compared with the animals from Streletskaia Bay.

The ratio of OD₃₄₆:OD₃₇₅:OD₄₃₀:OD₅₃₀ is present in Table 2. The data obtained show that in scorpion fish from Streletskaia Bay the relative concentration of basic aldehydes was higher than in fish from Karantinnaya Bay. Opposite, in round goby serum the relative content of basic ketones was greater in fish from Streletskaia Bay than in Karantinnaya.

Table 2. The ratio of oxidated compounds concentration in fish serum from two Sevastopol Bays

Bay	OD ₃₄₆ :OD ₃₇₅ :OD ₄₃₀ :OD ₅₃₀	
	Scorpion fish	Round goby
Karantinnaya	1:1.21:0.57:0.13	1:1.11:0.66:0.10
Streletskaia	1:1.11:0.71:0.13	1:1.11:0.66:0.16

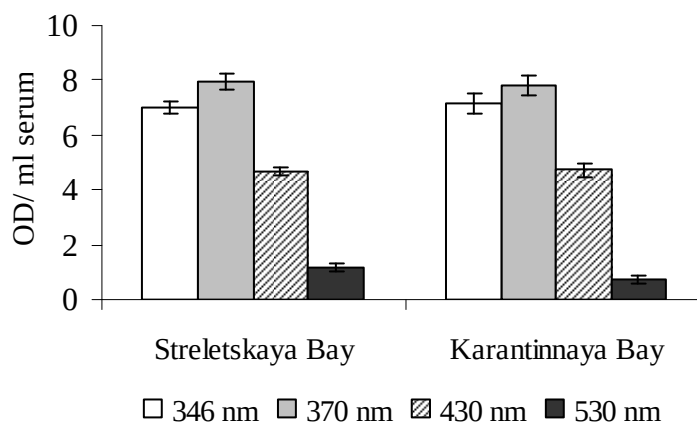


Figure 4. Concentration of oxidated proteins in blood serum of round goby caught in Streletskaia Bay (n= 7) and Karantinnaya Bay (n= 10), mean $\pm$ SEM

DISCUSSION

Our findings demonstrated the similar trends of increasing of oxidated proteins in blood serum of round goby and scorpion fish from high polluted site as compared with the animals from less polluted area. There are several factors including abiotic (physical and chemical parameters of the water and sediments in marine location, water temperature, pH, salinity, and etc.), biotic (specificity of fish biology, feeding behavior, age, sex, swimming activity and metabolic rate) and anthropogenic (level of pollution caused human activity) that may change fish physiology and biochemistry [15, 16, 17, 18]. Different level of pollution in biotopes causes the different accumulation of xenobiotics in fish tissues. Taking into account the specificity of the biology and ecology of tested fish species the aim of the present study was to compare their biochemical characteristics and to indicate their differences in blood serum because blood biochemistry is very important in evaluation of fish physiological and ecological status.

Oxidative stress caused unfavorable living conditions is an imbalance toward the pro-oxidant side of the pro-oxidant / antioxidant homeostasis. The content of protein carbonyl groups are good biomarkers of oxidative stress because they have some advantages in comparison with the measurement of other oxidation products: they are the relative early formation and the relative stability of carbonylated (oxidated) proteins [19, 20]. Carbonyl (CO) groups (aldehydes and ketones) are produced on protein side chains when they are oxidized. These moieties are chemically stable, therefore protein carbonyl content is the most general indicator of protein oxidation. The accumulation and increase of carbonyls in proteins has been observed in several human diseases [19, 20]. Oxidative damage on proteins may lead to negative consequences because among the proteins there are enzymes, hormones, mediators and other biomolecules which play an important role in metabolism and homeostasis of the organism.

Our results show that oxidatively modified proteins concentration increased in the blood serum of both fish species caught in marine polluted area. We could propose that pollutants stimulate oxidative stress in fish. High level of ROS causes inducible prooxidants overproduction and protein carbonylation. However, our findings demonstrated the interspecies differences in serum OP level: in scorpion fish the concentration was lower than in round goby in both tested locations. In addition we note that in Streletskaia Bay the level of serum OP in scorpion fish was significantly higher than in Karantinnaya Bay, while in round goby we could show the insignificant increase of OP concentration in polluted site. Thus we could propose that scorpion fish is more sensitive to pollution as compared with round goby. Previously we also demonstrated the interspecies differences of OP concentration between several Black Sea teleosts and their relations to heavy metals concentration in fish tissues [9]. We indicated that high OP

level correlated with the increase of concentrations of trace elements in fish tissues, several of which are well known as prooxidants, caused overproduction of ROS. We also demonstrated the seasonal dynamics of serum OP concentration and its relation to antioxidant enzymes activity in the blood of round goby in Black Sea and the Sea of Azov and we showed that the tested parameters depended on fish physiological status [11].

Therefore, blood serum OP concentration is informative biomarker of fish health and the ecological status of their habitats and could be successfully used in monitoring programs of the coastal areas of water bodies.

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