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ORIGINAL ARTICLE

Liver antioxidant system of the Prussian carp and pumpkinseed as response to the environmental change

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The article presents the results of biochemical studies on the state of the antioxidant liver system of alien fish of the Samara Bay of the Zaporizke Reservoir (Dnipropetrovsk Region, Ukraine) on the example of the Prussian carp and pumpkinseed. The biochemical parameters, which indicate the essential sensitivity of the enzymatic activity of superoxide dismutase, catalase and glutathione peroxidase, as well as the level of malate dialdehyde in Prussian carp *Carassius gibelio* (Bloch, 1782), compared to the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), are determined. The obtained results indicate an increase in the catalase and superoxide dismutase activity in the liver of experimental species of fish, which plays an important role in the consistent elimination of superoxide anion and hydrogen peroxide during the intensive metabolic transformations occurring in the tissue. It is noted that the activity of enzymes and levels of malondialdehyde depend on the state of the antioxidant system of fish, anthropogenic loading on the aquatic environment, the influence of many environmental factors, seasonality and nutrition of each alien species.

Key words: antioxidant system; invasive fish; Prussian carp; pumpkinseed; enzymatic activity; superoxide dismutase; catalase; glutathione peroxidase

Introduction

In recent years, the works devoted to the study of oxidative stress in fish have discovered many areas of study of the physiology and biochemistry of fish. Adaptation to factors unnatural to the organism is molecular and biochemical mechanisms of adaptation to various, sometimes extreme, conditions of existence (Hochachka, Somero, 1988; Voronkova et al., 2016; Ananieva, 2017). Increasing the effectiveness of the antioxidant system in the fish organism in response to the intensification of peroxide lipid oxidation (LPO) is a normal mechanism for the elimination of LPO products and an important element of adaptation to environmental changes in the surrounding aquatic environment (Dudkin, 1990).

Studies of the antioxidant support system have shown that in the liver of fish, the activity of the antioxidant system is much higher than in the tissues of the skin and muscles, mucus, but lower than in red blood cells (Fitzgerald, 1992; Fedonenko et al., 2016b). The study of the state of the AS system of fish in ecological stress, with seasonal changes, with contamination of the reservoirs showed that the activity of such key enzymes of the antioxidant support system as superoxide dismutase (SOD) and catalase (CT) significantly increases (Keselman et al., 1997). Along with this, a considerable amount of work is devoted to studying the state of the AS system in fish tissues under the influence of heavy metals.

Despite the considerable amount of work on the study of separate parts of adaptation processes of fish for ecological stress, changes in habitats, pollution of reservoirs, etc., the mechanism of formation of adaptive processes under the influence of these factors remains incompletely studied. In addition, in recent years, the assessment of the potential of adaptation opportunities of invasive organisms in a new environment is of particular interest for researchers (Holoborodko et al., 2016; Fedonenko et al, 2016a; Marenkov et al. 2017).

In fishes, as well as in terrestrial vertebrates, the enzymes link of antioxidant support system plays an important role in disinfecting the products of the LPO (Tushnyts'ka et al., 2006). The content of LPO in the liver of fish is a biomarker, which characterizes not only the physiological state under the impact of toxic substances and pathogens, but also is one of the criteria for assessing the biochemical adaptation of invasive species to stressful environmental conditions (Tushnyts'ka et al., 2006). Previous studies have allowed determining the mechanism of adaptation of reproductive systems of invasive species to reproduction in new conditions of existence, which allows effective reproducing and increasing its size, creating competition to aboriginal species and reducing fish productivity of reservoirs (Marenkov, 2016, 2018).

It is known that during intense activity of an organism (migration, spawning, competition in the condition of existence) and other non-specific for fish effects (anthropogenic loading), there is an increase in oxidative processes, resulting in the accumulation of LPO products acting as the primary mediator of stress (Zenkov et al., 2001; Baraboy, 2006). Stress is a protective function of an organism to the negative effect of one or another factor. The extended effect of the stress state of an organism can lead to the exhaustion of the organism, and further death (Panin, 1983; Pustovhar et al., 2010). Scientific literature on the antioxidant system and the intensity of LPO in the organism of invasive fish is practically absent; separate works do not give a complete picture of the features of LPO and the activity of antioxidant support system in the organs and tissues of fish. Therefore, the purpose of the work was to determine the adaptive capacities of the species-invaders by assessing the processes of oxidative stress in the liver tissues.

Methods

The ichthyological material was selected at two monitoring stations located in the Samara Bay of the Zaporizke Reservoir (Odinkovka village 48°50'60"N, 35°18'87"E, Novoselovka village $48^0$57'35"N, $35^0$23'50"E) in summer of 2016. The Samara River, tributary of the Dnieper River is under significant influence of the coal mining industry of the Western Donbas. Constantly increasing anthropogenic pressure on the river (since 60th) has caused significant changes in the chemical composition of water. According multi-year research, it was found that the water mineralization of the bay has increased on average by 2.2 times (from 1490 mg/dm^3 to 2888 mg/dm^3) due to chlorides, sulfates, sodium and magnesium, which are the main components of mine water. Changed ecological conditions of the river led to significant hydrobiological changes: the deterioration of the species composition of hydrobionts, which freshwater forms are replaced by mesohalobiotic and salt-water ones (Fedonenko et al, 2018).

Samara Bay (the lower reach of the Samara River) is a part of the Zaporizke Reservoir, created in 1933–1935 after filling the estuarial part of the Samara River. After the destruction of the DneprHES dam (1941), the Samara Bay temporarily ceased to exist. It was restored in 1947. Specificity of hydroecological parameters caused the allocation of the bay into a separate structural unit of zoning, called the Edge Samara stretch. It is a large shallow water area, created in the flood plain of the Samara River. The total area of the bay is 5702 hectares. The average width of the bay is 2 km, the area of shallow water is 57.9% of the water area (Fedonenko et al, 2018).

The upper (overflow land) area of the Samara Bay is created on the partly flooded Samara flood plain. Shallow water areas here occupy up to 90% of the water area, and the thickets of the reeds alternate with submerged vegetation. The soils are represented by highly molten sands with detritus. In the summer, in the floating zone, there is often a decrease in oxygen dissolved in water to critical values (1–3 mg/dm^3), an increase in the values of carbon dioxide and nutrients (ammoniacal nitrogen – 0.65 mg/dm^3, phosphates – 0.62 mg/dm^3).

The upper part of the bay is almost completely cut off by the dam of the Kharkiv-Simferopol highway and is connected with the other part of the bay only by the riverbank of Samara. This has led to the degradation of spawning grounds on most of the floodplain areas above and below the dam, and they are unsuitable for reproduction for most representatives of aboriginal fish assemblages, especially from the phytophyllic group, including commercial species of fish.

The lower (stretch) area of the bay is formed on a completely flooded Samara flood plain. Shallow water areas here are 70% of the water area, occupied mainly by aero-aquatic plants. Soils are muddy sands. Water, due to the influence of highly mineralized mine waters of the Western Donbas and household wastewater, is highly mineralized (2.9 g/dm^3) and contains excessive organic matter. The high degree of organic substances contamination indicates increased values of permanganate oxidation – 9.1–23.1 mgO/dm^3 at a normal rate of 10 mgO/dm^3. Biological oxygen consumption in the summer period exceeds the limits for fish farming and reaches 15.1 mg/dm^3.

Moreover, the Samara stretch is under a considerable anthropogenic impact due to pollution by the emergency discharges of under-treated sewage by the water treatment facilities of the water service company from the left-bank part of the Dnipro city. This contaminated wastewater contains a large amount of nutrients: phosphates up to 1.3 mg/dm^3, nitrates up to 9.84 mg/dm^3, with corresponding levels in the water in the zone of discharge: phosphates up to 0.625 mg/dm^3, nitrates up to 1.16 mg/dm^3, nitrites – up to 0.06 mg/dm^3, ammonium nitrogen – up to 0.33 mg/ dm^3, permanganate oxidation – up to 16.5 mgO/dm^3. The pH values at different points varied, on average, in the range from 6.85 to 8.4, with a strong predominance in the alkaline range. In general, this is typical for the Zaporizke Reservoir, where, for many years of observation, the average pH is 7.0–8.0. Only in the summer, in conditions of oxygen deficit in the bottom layers, the pH values there reduce up to 6.0. Also, Samara Bay is under constant toxicological stress caused by the increased content of heavy metals in water and bottom sediments of the reservoir.

Thus, the hydroecological conditions of the Samara Bay are tense, which is reflected in the physiological and biochemical state of hydrobionts (Marenkov, 2016; Fedonenko et al, 2018).

The object of the study was two alien species of fish: Prussian carp *Carassius gibelio* (Bloch, 1782) and pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758). Age of experimental individuals was 4 years. Age of fish was determined according to standard ichthyological methods of V. L. Bruzgin (1969) and I. I. Chugunova (1959).

In the liver of Prussian carp and pumpkinseed, the content of LPO products was determined, the concentration of TBA-active products (Korobeynikova, 1989), as well as the activity of antioxidant enzymes, such as superoxide dismutase (KF1.15.1.1) (Dubinina, 1983), glutathione peroxidase (KF1.11.1.9) (Moin, 1986) and catalase (KF 1.11.1.6) (Korolyuk, 1986).

To determine the activity of antioxidant enzymes and LPO products, samples of liver tissue were used, which were homogenized (in a ratio of 1:3, weight/volume) in phosphate buffer (pH 7.4). After homogenization, the homogenate was centrifuged for 15 minutes at 7000 g (4°C) (Yilmaz, Borchert, 2006). For further research, a supernatant was used.

The concentration of TBA-active products in the tissue homogenate was measured by the color reaction of malonic dialdehyde (MDA) with thiobarbituric acid (TBC) under high temperature and acidic environments, resulting in the formation of a trimethine complex containing one MDA molecule and two TBC molecules (Korobeynikova, 1989; Oleksyuk, Yanovich, 2010).

The activity of SOD was determined by the method, the principle of which is the reduction of nitrotetrazolium by superoxide radicals, which are formed in the reaction between phenazine methosulphate and the reduced form of Nicotinamide Adenine Dinucleotide (NADH) (Dubinina, 1989; Oleksyuk, Yanovich, 2010).

The activity of glutathione peroxidase (GP) was determined by the rate of oxidation of reduced glutathione (RG) before and after incubation with tertiary butyric hydroperoxide by color reaction with 5,5-dithiobis-2-nitrobenzoic acid, resulting in a colored product, thionitrofenyl anion (Moin, 1986; Oleksyuk, Yanovich, 2010). The activity of catalase was determined by the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts (Korolyuk, 1986; Oleksyuk, Yanovich, 2010).

All received results were statistically processed. Statistical data processing was carried out by conventional methods using software packages for personal computers Statistica 8.0 (StatSoft Inc., USA). All results are given as the mean ± standard deviation (SD). There liability of the difference between data samples was determined using one-factor ANOVA dispersion analysis at a significance level $p<0.05$.

Results and Discussion

It is known that one of the indicators, which can characterize both the physiological and biochemical state of the organism, is the activity of the LPO (Kozlov, 1985; Kucherenko, Vasilev, 1985; Pustovhar et al., 2010; Matviyenko, Drahan, 2013). The intensity of peroxide lipid oxidation in the cell membranes of fish tissues directly depends on the degree of unsaturation of fatty acids in the membrane phospholipids (Oleksyuk, Yanovich, 2010; Martines-Alvarez et al., 2005; Olsen et al., 2005). The intensity of the LPO in the tissues can be determined by the accumulation of malonic dialdehyde (MDA), one of the end products of peroxide oxidation. With this indicator, both the activity of the metabolism in the body of fish, and the degree of its imbalance in adverse environmental conditions can be estimated (Bogach et al., 1981; Pustovhar et al., 2010; Matviyenko, Drahan, 2013). The data in Fig. 1 shows that the content of TBA-active compounds in fish of the two species is significantly different. Thus, the decrease of TBA-active products in liver tissues of the pumpkinseed is 33% compared to the value of this indicator in the Prussian carp. Possibly higher content of MDA in liver tissue of Prussian carp is associated with peculiarities of nutrition, range and seasonality.

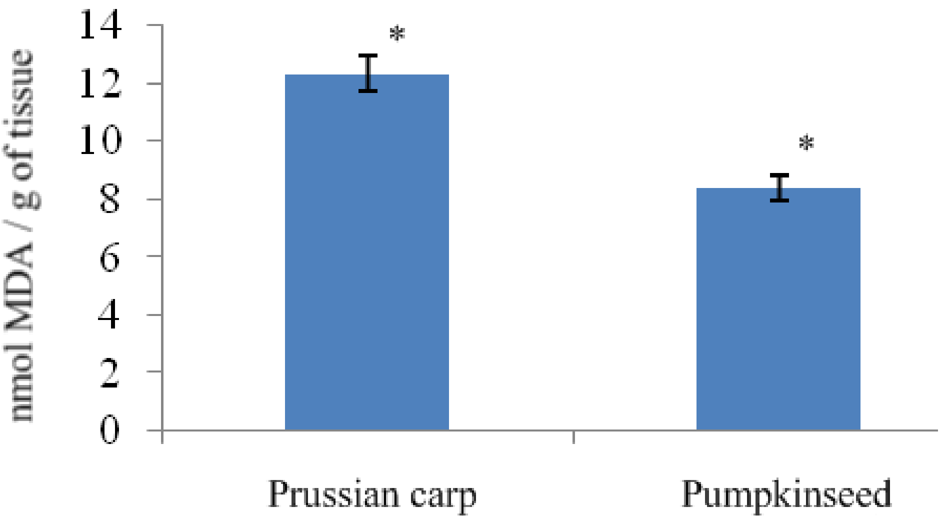

Figure 1. The content of TBA-active products in the liver tissue of fish, (x ± SD, n=6, $p < 0.05$).

Decrease in the content of MDA in liver tissue of the pumpkinseed can be explained by more intense neutralization of free radicals that are formed in the organism of fish because of aerobic metabolism, which leads to a decrease in the formation of TBA-active products (Baraboy, 2006). LPO is one of the important indicators of lipid metabolism, an active metabolic and regulatory factor that reflects the accumulation of intermediate and terminal metabolites, the results can indicate an appropriate protective reaction of the organism at the physiological and biochemical level to the effect of various stressors. Similar results were shown in studies on the analysis of LPO in carp, trout, silver carp and a monkey goby (Oleksyuk, Yanovich, 2010; Pustovhar et al., 2010).

The imbalance between the oxidizing processes and the antioxidant support system of the body causes oxidative stress, resulting in tissue damage (Trenzado et al., 2006).

In the antioxidant support system, an enzyme and non-enzymatic components are involved against oxidative stress (Antonyak et al., 2000; Osoba, 2013). The most important ones are the SOD, which converts the superoxide anion (O_2^-) to H_2O_2, the catalase that catalyzes the reaction of the decomposition of hydrogen peroxide to water, glutathione peroxidase-selenium-containing enzyme, which catalyzes the non-radical decomposition of lipid hydroperoxides using reduced glutathione ((Yilmaz, Borchert, 2006; Osoba, 2013).

Unlike other tissues, liver tissues of fish are unique indicator and biomarker, which are the first to respond to the development of oxidative stress by changes in the main indicators of the AS system (Yilmaz, Borchert, 2006).

The regulation of this process in the cell includes several elements that inhibit the formation of free radicals or inactivating peroxide oxidation products.

The decrease in the content of TBC-active products may be due to an increase in the activity of antioxidant enzymes, as shown in the work (Oleksyuk, Yanovich, 2010). It is shown that there is a slight difference in the activity of the key enzymes of the antioxidant support system for the two species under study (Table 1).

Table 1. The activity of key enzymes of the antioxidant system in the liver tissues of the Prussian carp and the pumpkinseed (x ± SD, n=6)

Parameters	Prussian carp	Pumpkinseed
SOD activity, conventional units/mg of protein	5.65±0.21	5.12±0.30
CT activity, nmol H_2O_2/min.·1 mg of protein	7.68±0.56*	3.76±0.32*
GP activity, µmol GSH/min·1 mg of protein	3.82±0.21*	2.09±0.09*

* – significant at $p < 0.05$.

In the experimental studies on animals, there is a steady correlation between the activity of SOD and the toxic effect of oxygen (Perez-Campo et al., 1993); it also shows the highest activity of SOD in liver and erythrocytes. We found no significant difference in the results of superoxide oxidase activity in the liver tissues of Prussian carp and pumpkinseed. Our results agree with literary data, which also shows the greatest activity of SOD in the liver of other species of fish (Cassini et al., 1993; Wilhelm-Filho et al., 1993; Trenzado et al., 2006).

The leading role in protecting cells from oxidative stress belongs to catalase, which utilizes hydrogen peroxide (Wilhelm-Filho et al., 1993; Trenzado et al., 2006). The obtained results showed that the catalase activity in the Prussian carp significantly exceeds (by 2 times) this index in the pumpkinseed. One of the assumptions for such changes in the activity of catalase can be the inactivation of the enzyme, which may be due to the excess of active oxygen metabolites, the increased generation of which in the development of oxidative stress has been proved in several papers (Halliwell, 1994). However, it is known from literary sources (Fridovich, 1995), that the superoxide radical is an inhibitor of catalase. In addition, this may be due to the oxidative destruction of DNA under oxidative stress (Halliwell, 1994) or the fact that catalase is involved in other parallel reactions associated with the ionic processes of H_2O_2 metabolism (Levadnaya et al., 1998; Trenzado et al., 2006).

Reduced glutathione is a donor of hydrogen, which is used in the H_2O_2 cleavage reaction by glutathione peroxidase (Wefers, Sies, 1983; Videla, 1984). Our studies have shown that the activity of glutathione peroxidase in the liver of Prussian carp and the pumpkinseed is slightly different in the fish of both species: for Prussian carp, this indicator was 3.8, and for the pumpkinseed it was 2.09 µMGSH min^{-1} * mg $protein^{-1}$. The activity of this enzyme is complementary to the activity of catalase, and especially when detoxifying hydroperoxides at low concentrations of the substrate (Perez-Campo et al., 1993; Halliwell, Gutteridge, 2004; Trenzado et al., 2006).

Conclusions

The combination of the results obtained in the study of MDA, SOD, catalase, glutathione peroxidase indicates the significant sensitivity of the enzymatic layer of antioxidant protection of the Prussian carp and pumpkinseed. The higher indexes of superoxide dismutase, catalase and glutathione peroxidase activity, as well as the level of MDA in Prussian carp as compared to the pumpkinseed, are shown. Increase of catalase and superoxide dismutase activity in liver tissues plays an important role in the consistent elimination of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) during intense metabolic transformations occurring in the liver.

Differences in the activity of enzymes and levels of MDA depend on the state of the antioxidant system, anthropogenic load, the impact of many environmental factors, seasonality and nutrition of each invasive species.

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