Antioxidant enzymes in the liver of *Chelidonichthys obscurus* from the Montenegrin coastline


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Abstract: The activities of antioxidant defence enzymes - total, manganese and copper zinc containing superoxide dismutase (Tot SOD, Mn SOD, CuZn SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and biotransformation phase II enzyme glutathione-S-transferase (GST) - in the liver of longfin gurnard (*Chelidonichthys obscurus*) from the Montenegrin coastline (Adriatic sea) were investigated. The specimens were collected in winter (February) and late spring (May) at two localities: Platamuni (PL, potentially unpolluted) and the Estuary of the River Bojana (EB, potentially polluted). The obtained results show that the activities of Mn SOD, CAT, GSH-Px and GST in winter were significantly lower at EB than at PL. In spring, the activities of CAT and GST were decreased, while GR activity was increased at EB in comparison to PL. The activities of Mn SOD and GST at PL were decreased and GSH-Px, GR and GST activities at EB were increased in spring compared to winter. Our work represents the first study of liver antioxidant enzymes of longfin gurnard from the Montenegrin coastline and reveals that locality, as a variable, has a greater influence on antioxidant enzymes and biotransformation phase II enzyme GST activities compared to season.

Keywords: Antioxidant enzymes • Reactive oxygen species • Pollution • Season • Longfin gurnard

1. Introduction

Some reactive oxygen species (ROS) (superoxide anion radicals, hydrogen peroxide and hydroxyl radicals) are produced as side products of an aerobic metabolism. They can also be formed intracellularly under the influence of various xenobiotics. ROS can arise as by-products in some metabolic processes or in some signal pathways [1]. They are very reactive molecules and thus very dangerous for normal cellular function [2]. During the evolution of the aerobic metabolism, cells developed various mechanisms in order to defend themselves from ROS. One of these mechanisms includes antioxidant defence enzymes, such as: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR). Phase II biotransformation components glutathione-S-transferase (GST) and reduced/oxidized glutathione system (GSH/GSSG) are also included in the defence against ROS [3]. ROS generation and antioxidant defences may be influenced by many environmental factors. Consequently, many abiotic and biotic influences should be taken into account when interpreting antioxidant defence biomarkers [4]. Marine ecosystems possess many specificities and many marine organisms have fine cellular control between production of ROS and antioxidant defence mechanisms [5]. The activity of antioxidant defence enzymes can be used as potential biomarkers for various environmental influences and aquatic contamination because these factors can directly or indirectly change the balance between the pro-oxidants and antioxidants [6]. Antioxidant defence enzymes are also related to changes in environmental factors such as temperature, salinity, food availability and dissolved oxygen levels, as well as to intrinsic biological factors such as gonadal development or the reproductive cycle [7]. Since changes at the organism level lead to changes at the population and community

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levels, antioxidant defence enzymes can be used as early warning signals of environmental disturbance [8].

Fishes play a number of roles in the food chain, bioaccumulate toxic substances and respond to low concentration of xenobiotics, and thus are often use as bioindicators [9]. Fishes are thermo- and oxygen-conformer organisms, meaning that ROS levels are dependent on these physical variables. Studies on oxidative stress biomarkers in poikilotherms show strong correlation between environmental influences and their metabolic needs [3,10]. Lower metabolic rate implies lower capacity of antioxidant defence. The main problem in fishes is to determine the role of individual components of antioxidant defence system in the achieving and in maintaining of homeostasis. Fishes are suitable model organisms, because they are on the top of the food chain and also very sensitive in response to stress conditions [11]. Fish liver plays a major role in various processes, such as the uptake, biotransformation and excretion of pollutants. The teleost liver has been shown to be very sensitive to pollutant exposure [9]. As many toxic compounds tend to accumulate in this organ, the magnitude of liver exposure to contaminants is greater than that of the environment or of other organs, resulting in various biochemical and histocytopathological changes [12].

Longfin gurnard *Chelidonichthys obscurus* (Walbaum 1792) was chosen for investigation because it is a territorial fish of commercial interest in fisheries and aquaculture. Longfin gurnard is a perciform fish which occurs in the benthic zone and inhabits sand, muddy sand or gravel bottoms. Longfin gurnard feeds mainly with fish and various invertebrates, such as crustaceans and mollusks. It has three rays on the pectoral fin which mainly help them in locating food on the soft bottom [13]. During their life-span, longfin gurnard is always close to the sediments when they find food and protection from predators. Therefore, it was expected to be exposed to contaminants associated with the sediment. Maturation of gonads in female gurnards in the Mediterranean takes place from October to May. In January and February more than 80% of females were sexually mature, while from June to September no mature females were observed [14].

The general aim of our study was to establish differences in antioxidant enzyme activities in the liver of longfin gurnard *C. obscurus* between two investigated localities in the Adriatic Sea: Platamuni (PL, northwestern part of the Montenegrin coastline) and the Estuary of the River Bojana (EB, southeastern part of the Montenegrin coastline), as well as between periods of low metabolic activity in winter and basal metabolic activity in spring [17]. Investigated areas have similar climate conditions, with the lowest mean water temperature in February and the highest in August. The bottoms of the biotopes are covered with a thick layer of fine terrigenous sludge containing particles of detritus. From each investigated location, 10 specimens of male longfin gurnard were collected in winter and 10 in spring (in total, 40 individuals).

2. Experimental Procedures

2.1 Study area and sampling

Fish samples were caught by trawling in winter (February) and late spring (May) at two localities: Platamuni (PL) and the Estuary of the River Bojana (EB) in the Adriatic Sea (Figure 1). The localities were chosen based on our earlier investigations [15,16] in order to compare the activity of antioxidant defence enzymes in the liver of longfin gurnard between the northwestern part of the Montenegrin coastline (open sea, 5 km from the coast) potentially with lower levels of pollution (PL) and the southeastern part of the Montenegrin coastline (1 km from the coast) with higher levels of urban and agricultural pollution (EB), as well as between periods of low metabolic activity in winter and basal metabolic activity in spring [17]. Investigated areas have similar climate conditions, with the lowest mean water temperature in February and the highest in August. Environmental parameters (depth, salinity, temperature, oxygen concentration and oxygen saturation) were measured at each locality. Figure 1. The geographical position of investigated localities: Platamuni (PL) and Estuary of the River Bojana (EB) in the Adriatic Sea.