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Interspecies Peculiarities of Biomarkers Response of Marine Fish Embryos to Oil Pollution

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ABSTRACT

Shelf areas of the seas and oceans characterizing high productivity are the spawning sites of many aquatic organisms. However, they are strong impacted for anthropogenic pollution, including oil contamination, which negatively influence on marine organisms. The effects of mazut and diesel fuel in the concentrations of 0.05, 0.1 and 0.2 ml/l on the activity of antioxidant enzymes which are recognized as biomarkers of the oxidative stress namely superoxide dismutase (SOD), catalase (CAT), peroxidase (PER) and glutathione reductase (GR) in the developing embryos of two marine blennies *Parablennius sanguinolentus* and *Salaria pavo* (Perciformes: Blenniidae) on the V - VI developmental stages were studied. The results demonstrated higher mazut toxicity as compared with diesel fuel. In *P. sanguinolentus* embryos exposed to mazut the activity of key anti-oxidant enzymes SOD and CAT were significantly higher (+413% and +100% as compared with the control, $p<0.05$), while in the case of diesel fuel the enzymes level varied insignificantly. In the embryos of *S. pavo* SOD and CAT activities were also increased at mazut incubation (256% and 103% respectively, $p<0.05$), while the differences between enzymes level in embryos exposed to diesel fuel were lower. In contaminated *S. pavo* embryos enzyme activities varied less as compared with the embryos of *P. sanguinolentus*, therefore they are more resistant and adaptive to oil contamination. The possible mechanisms of fish embryos antioxidant system response to oil pollution are discussed.

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INTRODUCTION

The increasing risk of oil contamination is attributed with its production and oil spills in shelf zone, which can result the dramatic consequences for marine biota and for ecosystem generally (Adams et al., 2020; Beyer et al., 2016; Patin, 2015). Significant quantities of polycyclic aromatic hydrocarbons (PAHs), alkylated phenols (APs) and other thousands of compounds with varying physical-chemical properties and resulting toxicity for aquatic biota, are discharged to the marine environment by the offshore oil and gas industry. Constant small oil leakages in produced water are usual, they contaminate chronically water, accumulate in bottom sediments and biota. Despite many studies of oil effects on aquatic organisms, there is still a lack of understanding of how long-term exposure to low levels of oil and its products may affect marine organisms. For instance, in Gulf of Mexico which is recognized as a site of intensive oil production, approximately 10% of fish spawning areas and 12 % of the sites where the larvae develop are

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oil polluted. However, the investigators don't indicate, what is the reason of fish number and distribution decrease in this region and is this really connected with the oil pollution. Hence, for a reliable risk assessment individual petroleum product toxicity profiles are needed in natural and experimental conditions (Muhling et al.,2012; Rooker et al., 2013; Johann et al., 2019).

Oil contains many various compounds, which characterizing different toxicity and, therefore, they can influence on marine organisms for different pathways (Beiraoa et al., 2019; Rial et al.,2010). PAHs impact directly aquatic organisms, which accompanied with the absorption or accumulation of the toxicants, caused the damage of the living functions of the organism. The indirect effects are displayed as a result of the changes of environmental conditions namely the damage of gas exchanges in biotop, the formation of anoxic and hypoxic zones, the sedimentation of heavy oil components on the bottom and their accumulation in the sediments (Samuelsen et al., 2019). Additionally, both kinds of the effects are indicated in oil polluted areas. These factors are connected each with other, resulted the worsening the living conditions and marine animals health. Therefore, it is important not only identify and evaluate the toxic effects of the oil contamination, but also, to determine the possible mechanisms of aquatic organisms adaptation to unfavorable impacts. The investigators demonstrate the clear interspecies differences in sensitivity of fish to oil pollution both in adults (Mazmanidi, 1997) and in early developmental stages, in various levels of the biological organization (Perrichon et al., 2014). Exposure to oil products can lead to toxic effects in mollusks (Beyer et al., 2016; Jiang et al., 2017), fish (Samuelsen et al., 2019*)* and other marine organisms. At cellular and molecular levels, exposure to oil may also trigger the increase of the formation of reactive oxygen species (ROS) and induce detoxification responses with antioxidant system. Antioxidant enzymes are the biomarkers of oxidative stress because they protect the organisms against the damage of ROS (Geraudie et al., 2016; Jiang et al., 2017). However, responses of antioxidant system are not uniform, they depend on the level of toxicants, its chemical specificity and developmental stage of the organism, which is important to take into account at the embryotoxicity tests. Additionally, analysis of the oil and its compounds effects to the early developmental stages of fish provides to obtain the important information on resistance/sensitivity of the eggs and larvae to anthropogenic impact. Therefore, monitoring of adverse effects and modifications of cellular and molecular defense systems can both be used as biomarkers in the programs at the case of oil spills and for prediction of their consequences in marine areas at longtime periods, and for understanding the mechanisms of the adaptation of the aquatic organisms to the oil pollution (Martınez-Gomez et al., 2010).

In the Black Sea alone, oil and gas production has been increased, it was estimated that annually 80–130,000 tons of oil discharges the ecosystem, and 1% of this is associated with the accidents (Leonov and Fashuk 2006). In future, petroleum pollution in the shelf area of the Black Sea will rise intensively, and so the risk of oil spills and accidents in offshore zone will increase. It's well known that shelf zone is the spawning area and the growth and development of many marine organisms both fish and invertebrates, including commercial species. At present, the aquaculture intensively develops in the coastal waters, which requires the clean water for the optimal conditions of the growth of cultured organisms. However, oil contamination impacted negatively the early developmental stages of marine animals, which are highly sensitive to pollution and the changes of the living conditions (Johann et al., 2019; . Bender et al., 2021; Jin et al., 2020; Pasparakis et al., 2019; Phan et al., 2020). Therefore, the different ontogenetic stages of fish, including eggs, developing embryos and larvae are good tools in toxicological studies for experimental purposes and for the legal levels determinations for many toxicants (Martınez-Gomez et al., 2016; Rudneva, 2014). Few studies of oil toxicity have been conducted with freshwater and marine organisms in early developmental stages, and they indicate the response of the biomarkers to oil pollution (de Andrade Brito et al., 2018; Velisek et al., 2018).

The aim of present study is the comparative analysis of the biomarkers response namely antioxidants SOD, catalase, peroxidase and glutathione reductase in the embryos of two Black

Sea blennies *Parablennius sanguinolentus* and *Salaria pavo* in V -VI developmental stages to mazut and diesel fuel intoxication in the concentrations of 0.05, 0.1 и 0.2 ml/l.

MATERIALS AND METHODS

The eggs of both blennies *Parablennius sanguinolentus* (Pallas, 1814) and *Salaria pavo* (Risso,1810) were collected from Sevastopol coastal area at the spawning time in May–June. Both fish species are highly distributed in the shelf zone of the Black Sea. They are benthic settled euryphagous fish, their food is recognized as small invertebrates such as mollusks, crustacean, worms and polychaets.

After collection, the embryos at the V -VI stages of the development were immediately placed in the aerated tank and transfer to the laboratory, where they were incubated at 12.5° to 13° C at the constant aeration in different concentrations of mazut and disel fuel. The developmental stages V-VI are characterized the growth of the tail, the separation of the tail from the egg yolk, the formation of the hart, gut, liver and the fin, the cardiorhythm and embryos moving (Dechnic, 1973).

Mazut (black mineral oil) is a heavy, low-quality fuel oil used in generating plants and similar applications. It may be used as an energy fuel for heating houses and for ships. Diesel fuel is any liquid fuel specifically designed for use in a diesel engine. The most common type of diesel fuel is a specific fractional distillate of petroleum fuel oil.

For experimental purposes, in our study, mazut and diesel fuel were mixed at the concentrations of 0.05, 0.1 and 0.2, ml/ l in the filtrated marine water (the Black Sea, salinity 18 g l^{-1}), and the mixtures were stripped using magnetic stirrer for 20–30 min, and after 30 min the mixture was used in experiments (Chesalina et al. 2000). Test emulsions of both toxicants with the corresponding concentrations were made using filtrated marine water. 50 embryos in stage V-VI were randomly transferred into test solutions in aerated tanks of the volume 1.5 l. Each treatment was replicated three times. At the end of embryogenesis the living embryos were homogenized in cold 0.85% NaCl using glass homogenizer, and the homogenates were centrifuged at 8000g 15 min at cool conditions. The supernatants were used for biochemical determinations.

Antioxidant enzyme activities in the supernatants were determined according to the methods described previously (Rudneva, 2019). The activity of superoxide dismutase (SOD) was assayed on the basis of inhibition of the reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm (Nishikimi et al., 1972). The enzyme activities were calculated as arbitrary units U per (min mg protein)$^{-1}$. Catalase (CAT) activity was measured by the method involving the reaction of hydroperoxide reduction. The enzyme activities were calculated in mg H_2O_2 per (min mg protein)-^{1}. Peroxidase (PER) activity was detected by a spectrophotometric method using benzidine reagent (Litvin, 1981). The enzyme activities were calculated in optical units. Glutathione reductase (GR) activity was assayed spectrophotometrically according the method Goldberg et al., (1987).

Biochemical measurements were detected in duplicate for each sample. Simple, descriptive statistics were performed using an ANOVA (Halafian 2008). P value of <0.05 was used for the determination of statistical significance between control values and the values of individual experimental group.

RESULTS AND DISCUSSION

The obtained results have shown that the enzyme activities were changed in the embryos of both fish species exposed to mazut and diesel fuel (Table 1). At tested mazut concentrations the uniform alterations of enzyme activities in the embryos of *P. sanguinolentus* were observed.

The significant increase of SOD activity (+413 %, $p<0.05$) and CAT activity (+50-100%) were shown, while the activity of PER and GR was dropped (-70-98% respectively) (Fig. 1).

Another trends were shown in response of the studying biomarkers in the embryos of *P. sanguinolentus* exposed to diesel fuel. At both concentrations of toxicant activity of SOD, was insignificantly decreased on 24-30% (Fig. 2), while CAT activity was elevated to 74-100%,

PER activity varied insignificantly at the concentration of 0.1 ml/l, while at high concentration it dropped on 50%. GR activity at low diesel fuel decreased on 63%, while at increasing level of toxicant it elevated on 104%

Antioxidant enzyme activities in the embryos of *S. pavo* exposed to both toxicants are present in Table 2. SOD and PER activities were progressively increased at both concentrations of mazut (+ 102-256% and 76-150% respectively), CAT activity was also higher as compared with the control (+ 51-103%), while GR activity decreased approximately in 2-fold (Fig. 3).

In the embryos of *S. pavo* exposed to diesel fuel the increasing trends of SOD activity (+ 19-98%) and CAT activity (+ 92-135%) were observed, while PER activity was insignificant varied

Table 1. Antioxidant enzyme activities in the embryos of *P. sanguinolentus* on V-VI developing stages (Mean ± SE), exposed to mazut and diesel fuel in the concentrations of 0.1 and 0.2 ml/l. SOD – superoxide dismutase, CAT – catalase, PER – peroxidase, GR – glutatgione reductase

Enzyme activities, per mg protein/min	**Mazut, ml/l**			**Diesel fuel, ml/l**	
	0	**0.1**	**0.2**	**0.1**	**0.2**
SOD, arbitrary units	119.9 ±15.8	615.2* ±36.8	161.8 ±25.3	84.6 ±15.6	91.1 ±6. 9
CAT, mg H_2O_2	0.05 ±0.01	0.08* ±0.01	0.06 ±0.01	0.07 ±0.01	0.08* ±0.01
PER, optical units	0.04 ±0.003	0.01* ±0.003	0.02* ±0.004	0.04 ±0.01	0.02* ±0.005
GR, nmol NADPH	1.74 ±0.38	0.50* ±0.30	0.054* ±0.015	0.63* ±0.05	3.56* ±0.35

* - differences are significant $p < 0.05$ between the enzyme activities of the treated and control groups

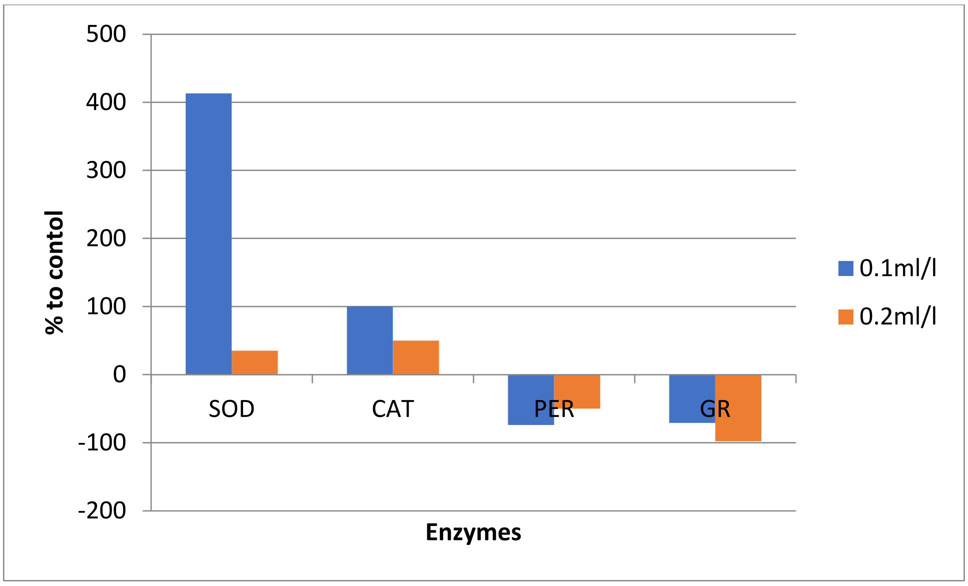

Fig. 1. Changes of antioxidant enzyme activities in embryos of *P. sanguinolentus* on V-VI developing stages, exposed to mazut in the concentration of 0.1 and 0.2 ml/l to the control as 100%. SOD – superoxide dismutase, CAT – catalase, PER – peroxidase, GR – glutathione reductase

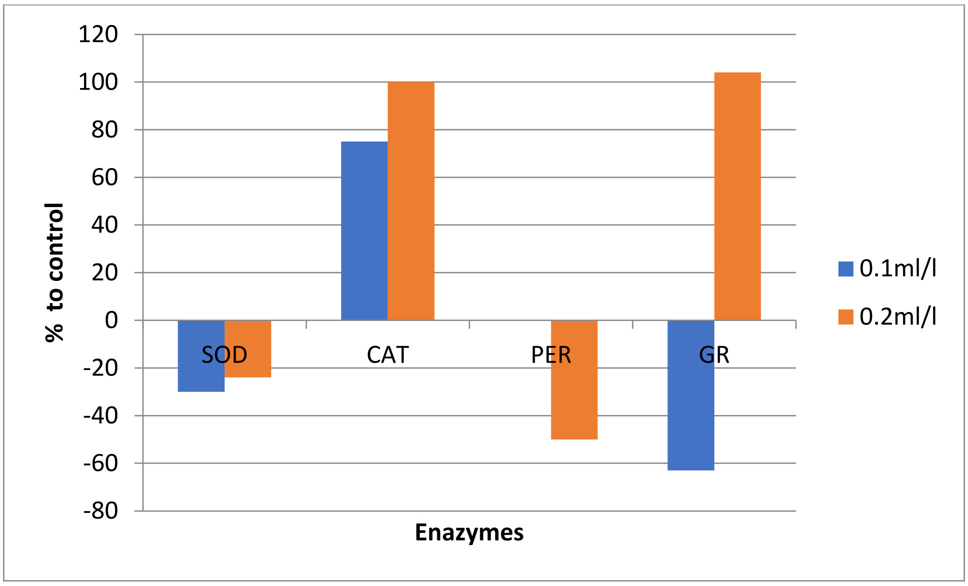

Fig. 2. Changes of antioxidant enzyme activities in embryos of *P. sanguinolentus* on V-VI developing stages, exposed to diesel fuel in the concentration of 0.1 and 0.2 ml/l to the control as 100%. The other abbreviations are the same as in the Fig. 1

Table 2. Antioxidant enzyme activity in the embryos *S. pavo* on V-VI developing stages (Mean ± SE), exposed to mazut and diesel fuel in the concentration of 0.05 and 0.1 ml/l. SOD – superoxide dismutase, CAT – catalase, PER – peroxidase, GR – glutathione reductase

Enzyme activities, per mg protein/min	**Mazut, ml/l**			**Diesel fuel, ml/l**	
	0	**0.05**	**0.1**	**0.05**	**0.1**
SOD, arbitrary units	137.4 ±18.6	277.2* ±26.5	489.5* ±28.5	160.2 ±17.4	272.4* ±18. 9
CAT, mg H_2O_2	0.05 ±0.01	0.10* ±0.02	0.07 ±0.01	0.09* ±0.015	0.12* ±0.04
PER, optical units	0.04 ±0.003	0.07* ±0.01	0.10* ±0.02	0.05 ±0.01	0.04 ±0.005
GR, nmol NADPH	4.35 ±1.38	1.96* ±0.63	2.04* ±0.15	0.32* ±0.09	7.82* ±2.35

as compared with the control. GR activity at low concentration of the toxicant progressively dropped on 93%, and at high concentration it was elevated approximately in 2-fold as compared with the control (Fig. 4).

Different antioxidant parameters, like the antioxidant enzymes namely superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, are used to assess exposure to toxicants in living organisms. Our results demonstrated the induction of oxidative stress in the developing embryos of both tested fish species following both oil compounds exposure which displayed the changes of antioxidant enzyme activities, their rations and balance of enzymatic antioxidant defense. Disturbance of prooxidant/antioxidant ratio following oil pollution was shown in many fish species and invertebrates in the majority of laboratory experiments and field collected samples (Martınez-Gomez et al., 2010; Mu et al., 2018). Generation high production of ROS at the case of exposure to various xenobiotics were observed in fish embryos (Incardona et al., 2011; Sehonova et al., 2017; Velisek et al., 2018). Increase of antioxidant enzymatic activities

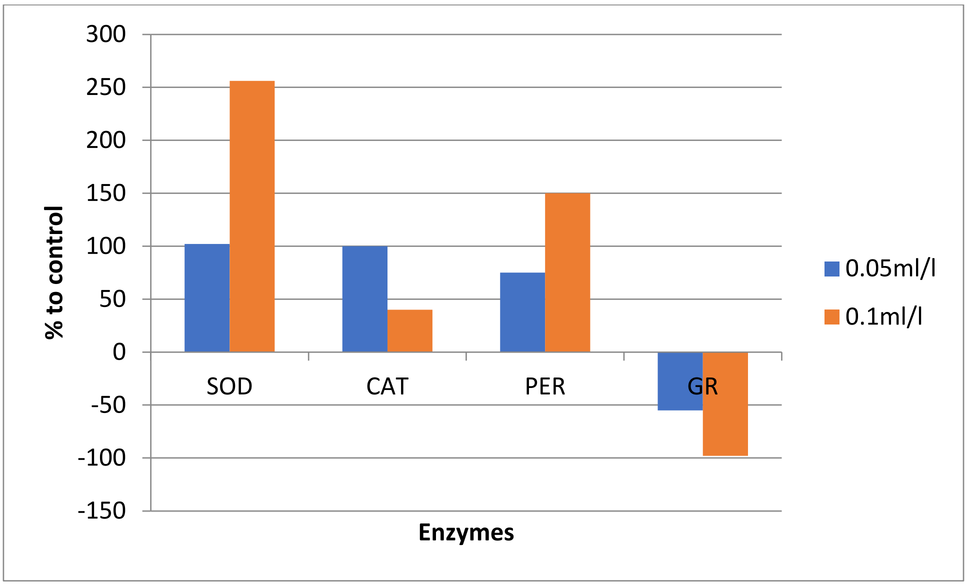

Fig. 3. Changes of the antioxidant enzyme activities in the embryos of *S. pavo* on V-VI developing stages, exposed to mazut in the concentration of 0.05 and 0.1 ml/l. The other abbreviations are the same as in the Fig. 1

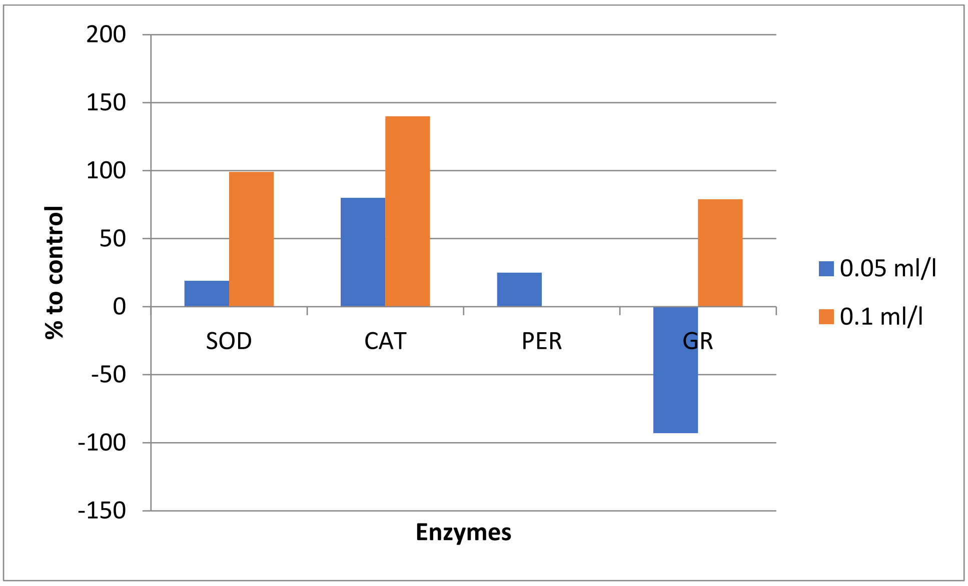

Fig. 4. Changes of the antioxidant enzyme activities in the embryos of *S. pavo* on V-VI developing stages (Mean ± SE), exposed to diesel fuel in the concentration of 0.05 and 0.1 ml/l. The other abbreviations are the same as in the Fig. 1

is accompanied with the defense against the harmful effects of ROS, which level elevates due intoxication and induction of oxidative stress. Simultaneously the induction of cytochrome P4501A (CYP1A) has been shown, which is the indicator of the oil hydrocarbons accumulation in the organism (Incardona et al., 2011).

Different petroleum derivates demonstrate different toxicity. High toxicity are those which contain cyclic carbohydrates, and they in 3-5-fold more toxic than the non-cyclic compounds. They display the damage of cardiorythm in fish embryos (Brette et al., 2014; Crower et al., 2014; Córdova de la Cruz et al., 2022), caused decrease of their moving activity which requires high energetic costs, and, therefore, oxygen consumption. However, at the case of oil pollution it is a problem due the disturbance of gas exchanges between water and air. Decrease of oxygen concentration in the water results the risk for hatching process of the larvae and their further survival (Klinger et al., 2015). Additionally, at the petroleum polluted areas the investigators demonstrated damage of gene expression, which code the sex differentiation, growth and development of fish. As compared with the control, in embryos exposed to different oil concentrations the delay of development and hatching, decrease their survival (Dubansky et al., 2014) and generation of high ROS production (Crower et al., 2014; Pereira et al., 2018) were observed. At the end stages of embryogenesis antioxidant enzyme activities increase, which is connected with the hatching and environmental oxidative stress because larvae leaves the egg shell and enters to the environment. The presence of toxicants in the environment requires additional efforts of antioxidant system for detoxification of ROS.

Hence, the oxidative stress caused the oil pollution in fish embryos can develops in several mechanisms, such as (Mazmanidi, 1997; Jiang et al., 2017; Crower et al., 2014):

1. Due to their lipophilic nature, PAHs oil and its compounds are present in the entire marine waters, sediments and food web. They can penetrate through the egg shell which contains lipids, disturb physical and chemical properties of cells, generate ROS production and lipid peroxidation, and provoke the imbalance of prooxidant/antioxidant ratio;
2. The compounds forming due oil hydrocarbons hydroxylation by Cytochrome P450, involve in oxidative-reductive reactions in the organism. These compounds can conjugate with glutathione-S-transferase, which decreases the level of glutathione (GSH) and the total antioxidant activity and provokes further alterations of antioxidant enzymatic activity;
3. Simultaneously, several compounds are involved in redox-cycle and change it, because they are donors or acceptors of the electrons. The other substances conjugate with glutathione and decrease its level, and, therefore, total antioxidant activity (AOA), the third ones can inhibit antioxidant enzymes and decrease total antioxidant defense also.
4. Due all these processes it needs the outflow of the energy to antioxidant defense, resulted decrease of the embryos survival, provision of the normal processes of the growth, development and hatching. Changes of the energy pathways for detoxification modify genetic processes, including gene expression, connected with the antioxidant enzyme synthesis, transcription and translation, increase ROS level.

Taking into account all these scenarios, the developing embryo should re-organize its metabolism due the optimization of antioxidant enzymatic defense for the purpose of hatching success and detoxification of pollutants in the environment. We have shown the changes in antioxidant response in the embryos of both tested fish species exposed to oil products. The effects were more clear at the case of mazut intoxication as compared with diesel fuel. For instance, SOD activity in the embryos of *P. sanguinolentus* exposed to mazut solutions increased in 413%, and in *S. pavo* in 256% as compared with the control. There were no any differences in embryos *P. sanguinolentus* exposed to diesel fuel as compared with intact group, while it increased in the embryos *S. pavo* at high concentration of the toxicant. SOD is the key enzyme of the antioxidant defense against oxidative stress, because it converts the toxic superoxide radical O_2^- to lower toxic compound such as hydrogen peroxide (H_2O_2) (Van der Oost et al., 2003). We could propose that mazut provokes oxidative stress and the formation of ROS more intensively than diesel fuel, and this induces SOD activity.

CAT is an enzyme promoting the conversion of hydrogen peroxide to water and molecular oxygen and can be used as a biomarker of oxidative stress in fish embryos. CAT activity following both toxicants exposure showed the increase in 50-135% in contaminated organisms as compared with the control group. SOD and CAT responses were therefore indicative in this study of a stress exposure, because SOD and CAT work in association and they showing the same trend.

The response of PER and GR was not uniform and the enzymatic activities varied less, than SOD and CAT. However, the obtained results suggest that peroxidase and glutathione reductase were used to detoxify cells in presence of tested oil products, because GR increased in embryos of *P. sanguinolentus* and *S. pavo* in high concentration of diesel fuel, and PER level was increased in *S. pavo* in both mazut concentrations.

High toxicity of mazut than diesel fuel was demonstrated at the case of PER response. The enzyme activity in the embryos of *S. pavo* exposed to solutions of diesel fuel was the similar as in control, while in the embryos of *P. sanguinolentus* at the concentration of 0.1 ml/l it was the same as in intact group and then decreased at the concentration of 0.2 ml/l. However, at tested mazut concentrations enzyme activity decreased in the embryos of *P. sanguinolentus* and increased in the embryos of *S. pavo.* GR activity in both species exposed to mazut, decreased. The similar response was shown in lower concentration of diesel fuel. If the concentration of diesel fuel elevated, GR activity increased also, which characterized the defense response of the organism to toxicant intoxication.

Therefore, the obtained results demonstrated the oxidative stress in the developing embryos of both marine blenny species as a response on the both oil toxicants, which are agreed with the data of the other researchers. They have shown that oil and its derivatives generate multiple stress in aquatic organisms, which attributes with the high level of ROS production, disturbance of the reproduction, development, cardiorhythm, and behavior, damage of DNA, decrease of immunity (Martınez-Gomez et al., 2010; Mu et al 2014; Córdova de la Cruz et al., 2022; de Andrade Brito et al., 2018). However, the investigators noted that the responses of antioxidant enzymatic system of aquatic organisms to oil pollution demonstrated both general effects and peculiarities, connected with the interspecies differences, and they can be not uniform. For instance, the induction of antioxidants production and decrease of immunity were observed in the tissues of gold fish *Carassius auratus,* exposed to water soluble diesel fuel at concentration of 0.05 и 0.1 mg/l at the period of 40 days (Zhang et al., 2004). The neurotoxic responses were shown in oil contaminated invertebrates (Geraudie et al., 2016). The increase of antioxidant enzymes levels SOD, CAT and GST was indicated in mollusks exposed to water soluble diesel fuel at the concentration 4 mg/l (Jiang et al., 2017). The researchers have shown the retard development and decrease of the number of hatching larvae, high mortality of embryos, exposed to high oil concentrations (20-40 mg/l). Similar responses attributed with the oxidative stress were identified in fish embryos, exposed to various oil products (Sehonova et al., 2017; Stancova et al., 2017; Velisek et al., 2018).

The increase of enzyme activities demonstrated the adaptive response of antioxidant system in the embryos, against the toxic effects of ROS, while the decrease of enzymatic activity can be connected with the disturbance of living processes and changes in metabolism. Hence, we could propose, that the embryos of S. *pavo* are more resistant to oil pollution, and they have effective adaptive mechanisms, than the embryos of *P. sanguinolentus*, because their enzymatic activity increased to oil pollution or no changes with the control generally, opposite of contaminated *P. sanguinolentus,* which enzymes tended to inhibit.

Additionally, one of the reasons of the differences could be attributed with the interspecies peculiarities of the eggs morphology of both tested blenny. The thickness of egg shell of both fish is the identical (5 μm), while surface square of the egg of S. *pavo* is 2-fold less (2.07 μm^2) as compared with the egg of *P. sanguinolentus* (5.72 μm^2) (Dechnik, 1973; Chesalina et al., 2000). Therefore, much more toxicants could penetrate via the egg shell and accumulate in the embryo of *P. sanguinolentus* as compared with S. *pavo* egg. The obtained results reflect that the interspecies peculiarities of the defense antioxidant system display in early stages of fish life and they are very important for the further development and fish adaptation to the changing environment.

CONCLUSIONS

Hence, the results demonstrated that early fish development can be affected by environmental pollutants such as various oil products (mazut and diesel fuel), causing alterations especially in antioxidant defense system. The activity of the antioxidant system represented by SOD, CAT, PER and GR was changed under stress induced by the mazut exposure stronger, than in the case of diesel fuel, and depends on interspecies differences of the fish developing embryos.

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CONFLICT OF INTEREST

The author declares that there is not any conflict of interests regarding the publication of this manuscript

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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