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ANTHROPOGENIC POLLUTION-INDUCED STRESS: CATECHOLAMINE DEPOSITION IN ERYTHROCYTES OF *Scorpaena porcus* LINNAEUS 1758

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ABSTRACT

The sympathoadrenal system plays an essential role in forming the body's response to the action of stimuli of various natures and intensities. The content of deposited catecholamines in peripheral blood erythrocytes is one of the most accessible parameters for an adequate description of the functional activity of the sympathoadrenal system in humans and rats. The aim of the work is to assess the accumulation of catecholamines in erythrocytes of sea ruffs *Scorpaena porcus* L. at various pollution levels in the bays of Sevastopol. The phenomenon of accumulation of catecholamines in erythrocytes of the peripheral blood of fish is revealed. The concentration of erythrocytes with deposited catecholamines was higher in fish from polluted bays. In 2006, fish from Alexander Bay exhibited a significantly higher erythrocyte content of deposited catecholamines (36.04%) compared to those from the background area (28.19%) and Quarantine Bay (30.74%) ($P < 0.01$). Similar levels were observed between Quarantine Bay and the coast of Balaklava. In 2007, fish from Alexander Bay maintained a high erythrocyte catecholamine content (35.2%), while those from Quarantine Bay exhibited a lower level (23.8%) ($P \leq 0.01$). In 2008, the erythrocyte catecholamine content in Quarantine Bay was six times higher than that observed in fish from Martynov Bay. These findings underscore the variability in catecholamine concentrations among fish populations inhabiting different coastal regions, suggesting potential environmental influences on stress responses..

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INTRODUCTION

The escalating issue of anthropogenic contamination in the World Ocean is a formidable concern in contemporary times. Primarily affecting coastal and shelf regions, this contamination profoundly impacts marine ecosystems, eliciting significant alterations in population dynamics and the functional structure of aquatic organisms (Häder et al., 2020; Ansari and Matondkar, 2014). Data analysis reveals a spectrum of ecological disturbances, including deviations in bioreproduction trends, abnormalities in specific structures, the depletion of vulnerable species, and disruptions in the life stages of aquatic organisms, among other ecological shifts. At the heart of these ecological transformations lies the sympathoadrenal system, a critical regulator shaping an organism's responses to diverse stimuli of varying magnitudes. Catecholamines, serving as chemical messengers of this system, intricately orchestrate physiological activities across a broad spectrum (Ilieva et al., 2020; Di Lorenzo et al., 2020). Understanding the physiological state of both endothermic and ectothermic organisms during their adaptation to nonspecific stimuli underscores the paramount importance of blood. As the primary conduit for responding to stimuli, blood plays a pivotal role in nonspecific and specialized immune responses, profoundly influencing an organism's resilience and capacity to confront threats (Seibel et al., 2021; Chujan et al., 2003). So, blood, and in particular hematological analysis, is also vital to understanding the health status of fish and the physiological response to environmental influences (Acar et al., 2021; Parrino et al., 2018).

The discovery of catecholamine linkage by erythrocytes led to the development of a rapid preliminary evaluation test for assessing stress levels in warm-blooded animals (Mardar and Kladienko, 1986; Morishima and Kasai, 2024). Subsequent research provided compelling evidence of changes in sympathoadrenal system activity in humans and rats under various influences (Chyan et al., 2003; Dziedzic et al., 2014). It became evident that the stress response in both fishes and higher vertebrates follows a similar pattern (Alfonso et al., 2021). Activation of stress hormone synthesis, including somatotrophic, adrenocorticotrophic, cortisol, and catecholamines, disrupts metabolic transformations, immune functions, and adaptive potential (Martemyanov, 2002; Mikryakov et al., 2007).

However, the role of catecholamines in maintaining protective and adaptive reactions in ectotherms and fishes remains poorly understood, with only previous and not current studies available (Gerwick et al., 1999; De Pedro et al., 2001). Consequently, there is a notable gap in the literature regarding the buildup of catecholamines in fish red blood cells.

This study aims to address this gap by investigating the buildup of catecholamines in the erythrocytes of *Scorpaena porcus* L. (sea ruffs) in Sevastopol bays under varying degrees of pollution. Through this research, we aim to elucidate the role of catecholamines in the stress response of fish and their potential implications for aquatic ecosystems subjected to anthropogenic contamination.

MATERIALS AND METHODS

The research was conducted on *Scorpaena porcus* L., known as sea ruffs, captured between 2006 and 2008 in the bays of Sevastopol and along the Balaklava coast (Fig. 1). These areas were identified as subject to human-induced pollution's adverse effects (Soloveva et al., 2023; Kurinnaya and Orekhova, 2020).

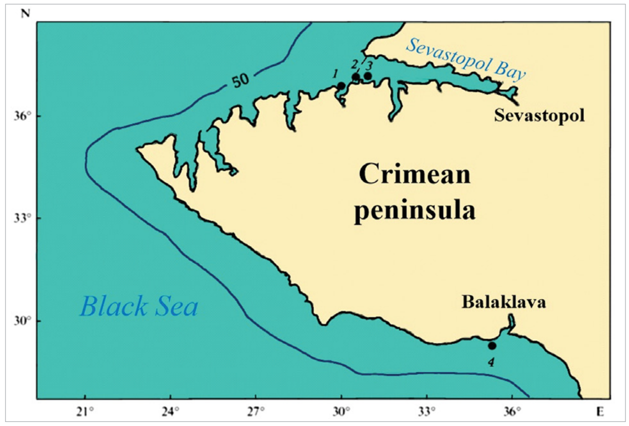

Fig 1. Schematic map of the study area and sampling locations (d) for 2006-2008: (1) Quarantine Bay, (2) Martynov Bay, (3) Aleksander Bay, (4) Balaklava coast

The fish were captured using a trap and then underwent biological investigation to assess the stages of development of their sexual glands. Every animal had a thorough exterior inspection to detect any indications of abnormalities or infestation, and all were found to be in good health. Blood samples were sampled by venipuncture from the caudal vein, followed by prompt preparation of blood smears (Ivanova, 1983). The blood samples were tested three times and no significant variations were observed between the measurements. The concentration of catecholamines in the erythrocytes of fish's peripheral blood was quantified using the methodology outlined by Mardar and Kladienko (1986) and Basova et al. (2017). The cytochemical technique quantifies catecholamine levels in non-nuclear erythrocytes found in the peripheral blood of humans and warm-blooded animals (Mardar and Kladienko, 1986). To evaluate catecholamines in fish erythrocytes, we adjusted the existing approach due to nuclei in these erythrocytes (Basova et al., 2017). 100 erythrocytes were examined in each blood smear to determine the number of cells harboring catecholamines. The catecholamine-containing cells were quantified using an OLYMPUS microscope equipped with a ×15 objective lens with immersion and a ×100 eyepiece.

The statistical data analysis was performed using Microsoft Excel 2007. Analytical data, represented as means ± standard error (SE), are the averages of three analyses carried out by the same operator. Generally accepted methods were used to compare the data, and the student's t-test was used to determine the differences.

RESULTS

The study displayed the concentrations of red blood cells containing catecholamines in fish inhabiting different regions of the Sevastopol and Balaklava coastlines between 2006 and 2008. In 2006, the erythrocyte content of deposited catecholamines in fish from Alexander Bay was 36.04%, which was substantially greater than the 28.19% discovered in ruffs from the background area and the 30.74% found in fish from Quarantine Bay ($P < 0.01$) (Fig. 2). The indicators at Quarantine Bay and the coast of Balaklava in 2006 were almost the same.

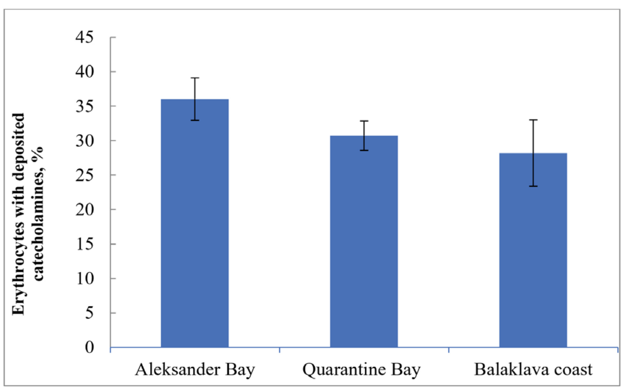

Fig 2. Erythrocytes with deposited catecholamines (%) in *Scorpaena porcus* from Alexander Bay, Quarantine Bay and coastal Balaklava ($P \leq 0.01$)

In 2007, the colossal content of erythrocytes with deposited catecholamines in the fish from Alexander Bay was 35.2% compared with 23.8% in ruffs from Quarantine Bay ($P \leq 0.01$), as shown in Fig. 3.

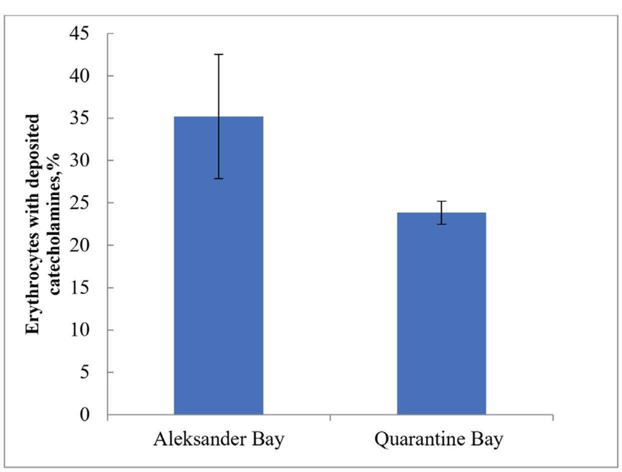

Fig 3. Erythrocytes with deposited catecholamines (%) in *Scorpaena porcus* from Alexander Bay and Quarantine Bay ($P \leq 0.01$)

In 2008, the content of red blood cells with deposited catecholamines in Quarantine Bay 1.6 times exceeded this indicator in fish from Martynov Bay (Fig. 4).

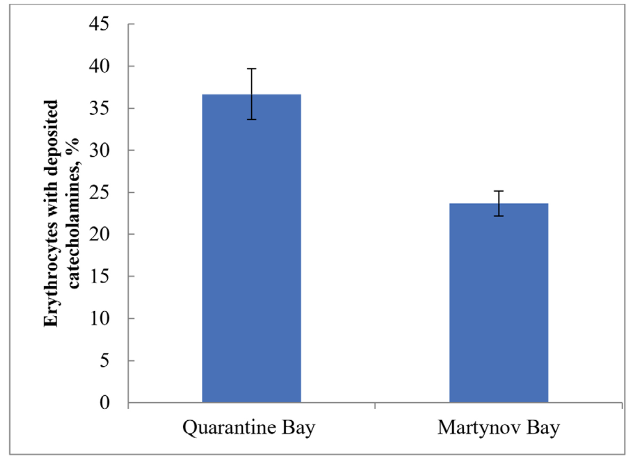

Fig 4. Erythrocytes with deposited catecholamines (%) in *Scorpaena porcus* from Quarantine Bay and Martynov Bay ($P \leq 0.01$)

DISCUSSION

Sevastopol Bay and the coastal waters of Sevastopol are heavily used and experience a significant influx of natural and man-made contaminants. The sources include raw sewage, stormwater drains, effluents from military installations, civilian fleets, and yacht clubs. The state components' distribution in the water region varies based on the topographical features and currents. Alexander Bay and Martynov Bay are components of the partially enclosed Sevastopol Bay, which is under ecological strain (Kurinnaya and Orekhova, 2020).

Alexander Bay is situated further away from the broad expanse of the sea. The seas of Sevastopol Bay have unrestricted communication with its interior waterways. The high amount of human effect on the Bay may be attributed to the increased flow of the Black River and stormwater runoff in the apical region (Soloveya et al., 2023). The levels of petroleum products and mercury in the bottom water layer of Alexander Bay exceed the Maximum Permissible Concentration (MPC) by a factor of 6-106 and 2.5-10.0 times, respectively. Additionally, the concentrations of copper, lead, and zinc in the bottom sediments are significantly high, posing a risk of secondary contamination (Mostafa et al., 2004). In addition, some areas of the Bay are more susceptible to midsummer die-off events caused by the decreased hydrodynamic activity, particularly in the lower layer (Subbotin et al., 2007). Martynov Bay is situated immediately next to the entrance to Sevastopol Bay, and there is a high level of water circulation in this energetically active region (Kuftarkova et al., 2011). The mercury content in the lower areas of this Bay is almost equivalent to the natural amount found in offshore bottom sediments. However, in other regions of Sevastopol Bay, mercury is 20 times higher (Kostova, 2005).

Quarantine Bay is highly contaminated, as shown by many previous studies conducted by Ryabinin (1996), Gruzinov et al. (2019), Kuftarkova et al. (2008), and Kravtsova et al. (2014). The concentration of chloroform-extractable compounds and petroleum hydrocarbons in the bottom sediments of the Bay is 161 and 46 mg/100 g, respectively, which is almost twice as high as that found in Martynov Bay (Rubtsova et al., 2013).

The Balaklava shore is marked by frequent wave processes and, as a result, a reduction in human impact and clearer seas. The eutrophication index, or E–TRIX, stands out as a particularly intriguing method for evaluating water quality among the other intricate ways available. This integral indicator is determined by crucial factors, including the thermohaline structure of fluids, the concentration of nutrients, and the level of chlorophyll "a." Based on the indicator values, the waters of Sevastopol Bay may be classified as transitional, ranging from the oligotrophic to the mesotrophic level (Gubanov et al., 2004). At various times, there was an observed increase in the E-TRIX value to a range of 4.5-4.9 in specific areas of Sevastopol and Balaklava bays. This increase was attributed to the release of domestic wastewater and oil pollution. However, the E-TRIX value decreased to 1.3-1.7 along the coasts of Sevastopol and Balaklava. These findings were reported by 2010, Kuftarkova et al. in 2006 and Kovrigina et al. in 2010.

Erythrocytes are crucial in warm-blooded animals and humans to store, move, and neutralize free catecholamines. Simultaneously, plasma catecholamines rapidly adapt to stress factors, while catecholamines stored in red blood cells play a crucial role in regulating long-term adaptation to both normal and abnormal stimuli (Manger et al., 1982; Mardar, 2001; Vizir and Berezin, 2001; Londe, 2006). It is well recognized that in most fish, hypoxia and metal pollution, such as manganese, lead to an elevation in the concentration of catecholamines in the plasma. This rise serves as an indicator of the development of stress (Barnhoorn et al., 1999; Gerwick et al., 1999). Therefore, the observed patterns of catecholamine metabolism demonstrate common characteristics in both warm-blooded animals and fish.

The findings indicate a propensity for an elevated presence of erythrocytes with accumulated catecholamines in the blood of fish inhabiting more polluted regions. The observed disparities show that the total pollution level in Alexander and Quarantine bays is much greater than in Martynov Bay and the surrounding background region. Consequently, fish inhabiting the highly contaminated Quarantinny and Alexander bays endure significant and protracted levels of stress, while the comparatively unpolluted coastal waters and energetically active Martynov Bay provide more ideal environments for sea ruff. Hence, the ruffs from the heavily polluted Alexander and Quarantine bays exhibit a more pronounced and enduring adaptation response compared to the fish from the comparatively uncontaminated reference region of the coastal waters of Balaklava and the dynamically active Martynov Bay. This finding is supported by concurrent investigations of the leukocyte concentration in the bloodstream of ruffs inhabiting these bays. The study demonstrated a positive correlation between pollution levels and the quantity of lymphocytes, as well as a negative correlation with the quantity of segmented neutrophils, in the white blood cells of scorpionfish ($P \leq 0.05$) (Basova, 2017).

Evidence obtained and made public indicates that the ruffs of Sevastopol bays are consistently subjected to anthropogenic contamination of different intensities. Therefore, the presence of catecholamines deposited in erythrocytes serves as a very comprehensive biomarker of stress.

Ruff, a kind of fish, inhabits lower layers of water where oxygen levels are very low. This environment is characterized by high levels of stress-inducing chemicals such as hydrogen sulfide, radionuclides, heavy metals, and other substances. Furthermore, a significant concentration of contaminants is found in the lower layers and sediments at the bottom. As a result, these animals consistently experience the aforementioned stressful situations. This assumes that ruff must possess extensive adaptive capacities and ecological flexibility. This is evident throughout its early stages of development. When ruff eggs are subjected to the concentrated wastewater from the city's sewage system, the embryos have an average lifespan 1.6-2.0 times longer compared to *Mullus barbatus*, carp *Diplodus annularis*, and hamsa *Engraulis engrasicholus*. Furthermore, during development in oil-contaminated waters, ruff species exhibits the lowest percentage of defective eggs (Bolgova, 1994).

The research has shown that the organism exhibits slow movement, decreased metabolic activity, a low blood volume (equivalent to 1.4% of body weight), and a notably delayed response to stress and hormonal changes, as evidenced by the distinctive pattern seen in the White Blood Cell Count (Basova, 2017). Significant disparities have been seen in the buildup of catecholamines in erythrocytes among persons residing in places with different pollution levels. Previous studies also support this physiological result. For example, the current and very popular microplastic pollution has been reported to cause differences in catecholamine levels in sea bream (Hoyo-Alvarez, 2022). This outcome is the consequence of the adaption of ruff to survive in the coastal area of Sevastopol, where there is a persistent high level of human activity and prolonged exposure to toxins. The accumulation of catecholamines in the red blood system, specifically in erythrocytes, after a long-term adaption, demonstrates this cold-blooded animal's ecological adaptability.

It is important to highlight that many plant and animal species inhabit the coastal area while facing intense human-induced stress. These aquatic animals possess a unique set of adaptive characteristics that enable them to survive under harsh environmental circumstances such as hypoxia, anoxia, temperature and salinity variations, and high concentrations of contaminants. These capabilities are a result of their biological potential and ecological flexibility. The physiological and metabolic processes shown during adaptation to environmental circumstances in these creatures may serve as biomarkers, and the organisms themselves can be used as indicator species for evaluating the status of the aquatic environment. Sea ruff is a characteristic example of aquatic invertebrates found in the Black Sea.

CONCLUSIONS

In conclusion, our study on erythrocytes in the peripheral blood of sea ruff *Scorpaena porcus* L. has demonstrated that these cells perform a catecholamine depositing function. We found that sea ruffs inhabiting more polluted bays exhibit a higher concentration of erythrocytes with deposited catecholamines, highlighting a potential relationship between environmental pollution and catecholamine accumulation in these fish. Furthermore, our results indicate that the described method for assessing catecholamine deposition in fish erythrocytes is a practical and applicable approach for monitoring studies, providing insights into the physiological responses of poikilothermic organisms to various environmental stressors.

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STRES IZAZVAN ANTROPOGENIM ONEČIŠĆENJEM: TALOŽENJEM KATEKOLAMINA U ERITROCITIMA *Scorpaena porcus* (LINNAEUS, 1758)

SAŽETAK

Simpatoadrenalni sustav igra bitnu ulogu u formiranju odgovora tijela na djelovanje podražaja različite prirode i intenziteta. Sadržaj deponiranih katekolamina u eritrocitima periferne krvi jedan je od najdostupnijih parametara za adekvatan opis funkcionalne aktivnosti simpatoadrenalnog sustava ljudi i štakora. Cilj rada je procijeniti akumulaciju kateholamina u eritrocitima škarpuna *Scorpaena porcus* L. pri različitim razinama onečišćenja u zaljevima Sevastopolja. Otkriven je fenomen nakupljanja kateholamina u eritrocitima periferne krvi riba. Koncentracija eritrocita s taloženim kateholaminima bila je veća u riba iz onečišćenih uvala. U 2006. godini, ribe iz Aleksandrijskog zaljeva pokazale su značajno veći sadržaj deponiranih kateholamina u eritrocitima (36,04%) u odnosu na one iz udaljenijeg područja (28,19%) i Karantinskog zaljeva (30,74%) (P < 0,01). Slične razine uočene su između Karantinskog zaljeva i obale Balaklave. Godine 2007. ribe iz Aleksandrijskog zaljeva zadržale su visok sadržaj kateholamina u eritrocitima (35,2%), dok su one iz Karantinskog zaljeva pokazale nižu razinu (23,8%) (P ≤ 0,01). Godine 2008., sadržaj kateholamina u eritrocitima u Karantinskom zaljevu bio je šest puta veći od onoga zabilježenog u ribama iz zaljeva Martinov. Ovi nalazi naglašavaju varijabilnost koncentracija kateholamina među ribljim populacijama koje obitavaju u različitim obalnim regijama, što ukazuje na potencijalne utjecaje okoliša na reakcije na stres.

Ključne riječi: antropogeno zagađenje, simpatoadrenalni sustav, kateholamina, eritrociti, fiziološki pokazatelji

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