ASSESSMENT OF THE OXIDATIVE STRESS AND INDICATOR POTENTIAL OF THE BIOLOGICAL RESPONSE OF THE BLACK SEA MUSSEL *Mytilus galloprovincialis* Lam. AS A GENERAL INDICATOR OF THE COASTAL ECOSYSTEMS FUNCTIONAL STATUS

Lachezar Petrov Yakimov

Supervisor: Prof. Nesho Chipev, PhD

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria

Summary of PhD Thesis

The aim of this study was to evaluate changes in the oxidative status in tissues of the mussel *Mytilus galloprovincialis* Lam. and its spatial and temporal dynamics, as well as the potential of the biological response of this species as a biomarker for the state of the marine environment and ecosystems. To achieve this, representative sampling points were selected from areas in the northern (north of cape Emine) and southern (south of cape Emine) Black Sea coast regions with different use patterns and with different characteristics of the abiotic environmental conditions. Sampling was conducted in two conditional seasons - spring (June-July) and autumn (September-October) in two consecutive years - 2017 and 2018.

The results of the ICP-OES analysis of mussel and seawater samples showed that *M. galloprovincialis* species were a macro-concentrator of the metals copper, lead, zinc, cadmium and nickel in the coastal Black Sea habitats. The content of accumulated metals in the mussel tissues varied significantly between study stations (regions), reflecting the content of metals in the aquatic environment of the habitats. The calculated coefficient of bioconcentration indicated that the soft (edible) tissues of the mussels can accumulate significantly more metal contaminants than the biomineral shells. Thus, the results for the accumulation of metals in mussel tissues obtained in this study fully supported the suitability of *M. galloprovincialis* for bioindication of metal pollution of marine coastal ecosystems.

Marker reactions of lipid peroxidation and total glutathione, as well as the activity of the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, phosphate dehydrogenase and glutathione-S-transferase were used To determine and evaluate the oxidative status of the gill, leg and digestive gland of the test mussels. The results of the biochemical analyzes strongly suggested that the mussels studied were sensitive to oxidative stress.

Different mussel organs showed different responses to the environmental pressures, as manifested by different response of the oxidative stress biomarkers. The most sensitive organ were the gills, probably due to their direct contact with the environment and intense oxygen exchange. The individual reactions of the oxidative stress biomarkers were correlated significantly (excluding catalase activity) with

copper, lead and cadmium content in the mussel tissues, demonstrating a high indicator potential for ecotoxicological monitoring purposes.

The redox balance in the studied *M. galloprovincialis* appeared to be sensitive to seasonal changes, with increased levels of oxidative stress reported in the mussels collected in fall compared to those collected in spring. A well-pronounced difference between the redox status and its dynamics in mussels from the northern and from the southern coastal regions of the Bulgarian Black Sea was found. As a whole, high levels of pro-oxidant pressures have been reported to be present in the southern regions and in places where significant levels of pollution were present (i.e. port complexes).

A tissue-specific pattern of oxidative status marker dynamics, associated with the bioaccumulation of metal pollutants in the mussel tissues was present. Further, this was related with the general conditions of the habitats environment. These findings may provide opportunities for novel applications, with different bioanalytical resolution, of the bioindication of ecosystem health, using *M. galloprovincialis*.

In order to achieve an integral assessment of the health of the coastal shallow ecosystems three indexes were constructed: PrO – pro-oxidant index measuring the effect of pro-oxidants in mussels, AOE – antioxidant enzyme index, indicating the level of the antioxidant system in mussels and SOS – specific oxidative stress index, indicating the level (risk) of oxidative stress. The biotic response of *M. galloprovincialis,* as measured by SOS, indicated the presence of oxidative stress or stressful conditions of the marine environment of the ecosystems inhabited by the mussels. The indexes are still under evaluation and verification, and in the present thesis we present the first comparative results.

An initial study of DNA damages in the studied mussels caused by pollution and other environmental pressures was carried out using the alkaline comet assay (CA). The measurement of the Tail Mean Intensity, as CA parameter, showed that the intensity of genotoxic effects in *M. galloprovincialis* was directly related to the state of their habitat environment and the accumulated metal contaminants in tissues. In this case, the genome of mussels from conditionally contaminated sites (e.g. ports) tended to be relatively more resistant to the pollution pressure damage than the genome of the mussels from conditionally clean sites, probably as a result of adaptive activation of repair mechanisms of DNA.

In conclusion, the results of the assessment of the state of the marine environment and health of coastal ecosystems using the biotic responses of the mussel *M. galloprovincialis*, measured through its redox balance and oxidative stress, can refine the evaluation of the state and stressfulness of the marine environment.