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THE BIOCHEMICAL BASIS OF THE PREFERENCES OF BIVALVE MOLLUSK *DREISSENA POLYMORPHA* IN A NEW ENVIRONMENT. UNIQUE OPPORTUNITY TO COMPARE NATIVE AND INVASIVE POPULATIONS IN THE FIELD AND EXPERIMENTAL EXPOSURES

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Since the discovery of invasive zebra mussels *Dreissena polymorpha* (Pallas, 1771), biological invasions are described as the second leading cause of extinction behind habitat destruction. Zebra mussel is one of most active filter-feeders and sedimentators in the water column [1]. Consequently, the spread of zebra mussel is crucial for the preservation of global biodiversity and ecosystem function. On the other hand, zebra mussel is valuable bioindicator species [1;3]. Therefore, the question is arisen concerning the reasons for the preferences of *D. polymorpha* in the new surrounding that provides its wide distribution. Two points of view exist to explain this phenomenon. First one proves that the reasons for these preferences can be the metabolic plasticity. Second concept explains their

performances by the reproduction benefits that allows rapid population recovery after environmental extremes. Therefore the goal of this study was to compare the plasticity of the biochemical responses in the native and invasive populations of mussels in their habitats and under the additional confounding pressure. We utilized the opportunity to compare the native population of *D. polymorpha* in the Ponto-Caspian region and invasive populations from West Ukraine and North France. The responses of apoptotic activities and cytotoxicity were selected for this study. The specimens of mussels were sampled from the river Dnipro near the city of Kherson (Kh, native population), in the Ternopil municipal pond, Ukraine (Tn), and in the Champagne Ardennes region, France (Der and Orne ponds) in June of 2021. The molluscs from the Kh and Tn populations were additionally exposed in the laboratory to the microplastics (MP-groups, 1 mg L^{-1}); caffeine (Caf-groups, 20.0 μg L^{-1}); their combination (Mix-groups), elevated temperature (T-groups, 25 °C) or combination of MP, Caf and T (MixT-groups) for 14 days. MP is one of the most persistent pollutants in the surface waters. Besides its own toxicity, it can be a vector for other substances in water. Caf represents the contaminant of concern due to its widespread occurrence in the aquatic environment and potential to be biologically active. Bivalves as filters have highly developed processes of intracellular internalization of MP that distort the responses to xenobiotics [4].

The comparison of two populations had shown the prominent differences between Kh and invasive populations. The caspase-3 activity was higher in all invasive populations, particularly in the Orne (~by three times). On the contrary, Cathepsin D (CtD) total activity and its efflux were lesser in these groups when in Kh-group by two-three times with the increase of the efflux rate by 37-50%. Despite low difference in the Acid phosphatase (AcP) total activity, its free activity was higher and membrane-bound activity was lesser even by ten times in the invasive populations. The assessment of lysosome membrane integrity (NRR test) confirmed the loss of this stability in the Der and Orne groups comparing native population. These changes were accompanied by the elevated concentrations of protein carbonyls in the Der and Orne-groups. Finally, the ratio Zn/Cu in the soft tissues was by 2-10 times lower in the invasive populations than in the native

population, mostly due to the higher level of Cu. Consequently, all these particularities of invasive populations can be assessed as the lysosomes injury and depression of their biogenesis, utilizing of apoptosis and autophagy as a protective measure against damaged cells, and imbalance of metals bioavailability that can indicate the shortness of the life cycle.

In the experimental exposures of Kh- and Tn-groups, the responses of lysosomal integrity, and CtD (both with one exception) to each exposure were similar in both populations, attesting the common strategy to withstand the experimental challenges. Caspase-3 activity was also increased in the exposures, particularly by MP and Caf, in both populations. CtD total and extralysosomal activities were reduced in the exposures to MP and caffeine in both populations, whereas heating and mixtures provoked significant increase of cathepsin D total in the TnT- and both Mix-group. In the KhMix-group it was accompanied by the extralysosomal enzyme activity. Among these manifestations, most common one was the increase of lysosomal integrity detected in all exposures, including heating, and for both populations (despite low level of lysosomal stability in the Kh-groups). Generally, in the bivalve molluscs, the immune response to bacterial infection is associated with the decrease of the lysosomal membrane stability, the same as the responses to plural xenobiotics, including Caf and MP. However, the invasive species, zebra mussel, had the preferences in the ability to support lysosomal integrity comparing to the native molluscs. The detected in our study increase of the lysosomal integrity can be explained by the induction of the lysosome biogenesis, that is one of the most important mechanisms for lysosomal adaptation. It increases lysosome numbers to meet different cellular demands such as starvation-induced autophagy and the dispensation of lysosomes to daughter cells during cell division.

Consequently, in the experimental exposures to single substances the responses of mussels were similar to their manifestations in the invasive species comparing to native population. Nonetheless, the heating alone and in the combine exposure distorted these responses.

To summarize, we can conclude that the ability to mobilize the apoptotic activities is the important component of *D. polymorpha* populations' resistance in the new confounding circumstances, both

field and experimental. The distorting effect of heating is common manifestations with other studied bivalves, like *Unio tumidus* [2]. Consequently, all these particularities of invasive populations can be indicated as the lysosomes injury but compensatory changes in their biogenesis, utilizing of apoptosis and autophagy as a protective measure against damaged cells, and imbalance of metals bioavailability that can indicate the shortness of the life cycle. The finding coordinates two mentioned hypotheses concerning the invasive success of this species. To continue our experiment, the populations of zebra mussels should be tested by the proteomic and, particularly, immune specificity for the understanding of the resistance reasons in the new surroundings.

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