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Short note

Seasonal differences on stress on stress responsiveness in the Mediterranean mussel from the Southern Bulgarian Black Sea – an indicator for an ecosystem health decline?

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Abstract. In this short note, we present new results on stress on stress response measurements in both farmed and wild Mediterranean mussels from the town of Sozopol, Bulgaria, collected in the summer of 2024 (August) and compare them with our previous results for the winter and spring season.

Key words: mussels, biomarkers, stress on stress, pollution, Black Sea.

Introduction

The Black Sea is known as one of the most degraded marine ecosystems in the world, with restrained exchanges with the Marmara and Azov Seas. In addition, the Black Sea, which is almost completely isolated from the Atlantic Ocean, is only connected to the Mediterranean by the narrow Bosporus Strait. It moves to the Sea of Marmara and then to the Aegean Sea via the Dardanelles (Bat et al., 2024).

The Black Sea is suffering from various types of pollution. Its estuaries - the Danube, which accounts for 75% of freshwater inputs per year, the Dnieper, Dniester, Don, and the Kuban, are further polluting the sea, which is semi-enclosed (Chevalier et al., 2024; Toschkova et al., 2024).

Biomarkers are extensively applied in marine pollution monitoring to assess the biological impacts on the investigated species. Overall, biomarkers can be classified into three groups based on their prognostic purposes, namely biomarkers of exposure (e.g., defense mechanisms against xenobiotics), biomarkers of effect (e.g., biological damage caused by xenobiotics), and biomarkers of growth potential (e.g., biological indices that forecast growth efficiency) (Leung et al., 2024).

According to Dagsuyu et al. (2024), the increasing interest in understanding the biological effects of hazardous substances has led to the development of the "biomarker approach" across various aquatic organisms.

In this regard, mussels have gained widespread recognition as suitable bioindicators in environmental studies and programs, including initiatives like the famous Mussel Watch Program (Goldberg, 1975). Moreover, mussels are highly useful for assessing pollution in aquatic environments because they are sessile organisms with a wide geographic distribution, which are also readily collectible, abundant in coastal regions prone to anthropogenic pollution, and used as food sources by many nations.

In this short note, we aim to present new results on stress on stress response measurements in both farmed and wild Mediterranean mussels from the town of Sozopol, Bulgaria, collected in the summer of 2024 (August). In addition, we further aim to see if there are seasonal fluctuations, as we already presented results for this rapid, simple, and low-cost biomarker for mussels collected in the winter (January) and spring (April) of 2024 (Yancheva et al. 2024a,b).

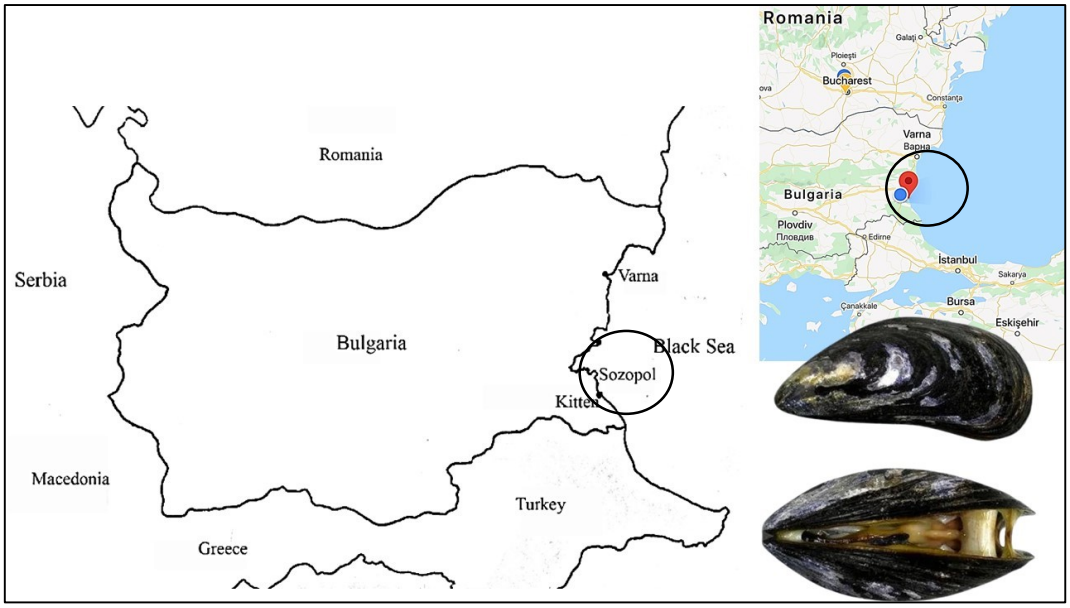

Fig. 1. Geographical location of the town of Sozopol in Bulgaria.

Materials and Methods

Survival data for farmed and wild individuals were collected over three seasons: winter, spring, and summer. For each group, the number of surviving individuals was recorded daily until all had died or reached the study endpoint. A total of 50 individuals were monitored in each group.

In August 2024, about 200 adult specimens (length 6.5 cm ± 1.5; weight 13.5 g ± 1.5) were purchased from the commercial farm we used earlier in the winter and spring, located in the town of Sozopol, Southern Bulgaria (Fig, 1). Another 150 wild specimens (length 6 cm ± 2.5; weight 12.5 g ± 0.5) were hand-collected with the help of local fishermen. Stress on stress responsiveness (SoS) was studied according to a standard methodology (Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995) before any dissection for other biomarkers investigation was performed. As explained before by Thomas et al. (1999), this tolerance test is based on the survival time of 50% of individuals (LT_{50}) when exposed to air. The survival of mussels was inspected every 24 hours until 100% lethality was reached.

The mussels were transported to the laboratory in a clean glass tank with seawater and then treated for anoxia by air exposure. They were placed in glass dishes in an incubator at 15°C, with a regime of 12 h of light and dark, and observed regularly for a response with a gentle tap on the shell or for open valves. The lethality rates were recorded, and those mussels were disposed of (Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995; Brooks et al., 2018).

Kaplan-Meier survival curves were generated to compare survival rates between the farmed and wild groups within each season, and pairwise comparisons were made using the log-rank test.

The survival analysis was performed using the survival and survminer packages in R version 4.3.3. (R Core Team, 2015). The significance of differences between the farmed and wild groups in each season (winter, spring, and summer) was assessed using the log-rank test, and P-values were reported.

Results and Discussion

The statistical analysis revealed significant differences between the farmed and wild groups in all three seasons: winter (χ^2 = 218, DF = 1, P < 0.001), spring (χ^2 = 97.9, DF = 1, P < 0.001), and summer (χ^2 = 57.9, DF = 1, P < 0.001). Across all seasons, both groups exhibited shorter survival times compared to values typically reported in the literature. Among the seasons, the mussels collected in summer exhibited the most extended survival, followed by those gathered in spring, with the shortest survival observed in winter (χ^2 = 678, DF = 5, P < 0.001) (Fig. 2).

According to Anestis et al. (2010), the duration of air exposure depends on the positioning of the bivalves between low and high water lines and on the form of the tidal cycle. Most of the bivalves that inhabit the intertidal zone have developed mechanisms for surviving thermal stress and oxygen deprivation at low tide (Anestis et al., 2010).

However, "survival in the air" showed to be a sensitive response parameter for indicating pollution-induced environmental stress in bivalve species, such as *Mytilus*, decades ago (Eertman et al., 1993). Yet, little has been studied about this biomarker in any mussel species from the Bulgarian Black Sea.

From an ecological point of view on this biomarker, our previous work showed that it is probably a matter of chronic pollution of the waters of the Bulgarian Black Sea. This undoubtedly leads to disturbances in a number of biological indicators in aquatic organisms, such as the studied physiological indicator in the Mediterranean mussel. During the summer season, we found a slight increase in the resistance of mussels to atmospheric oxygen exposure. This was valid for both wild and aquaculture mussels. However, the time to withstand atmospheric oxygen was lower than in other reported field studies. In the present field study, we once again used mussels from one age-size group. From our perspective, the presented results could possibly be explained by individual resistance or better abiotic factors in summer, which would lead to better individual resistance, such as food availability, water temperature, etc. Still, the stress on stress time was shorter than two weeks. Therefore, we suggest that this easy-to-perform and relatively inexpensive biomarker should be combined with other biomarkers, such as histopathological alterations, oxidative stress enzymes, or lysosomal membrane stability, to further explore the physiological state of *M. galloprovincialis* along with simultaneous sediment and surface water chemical analyses.

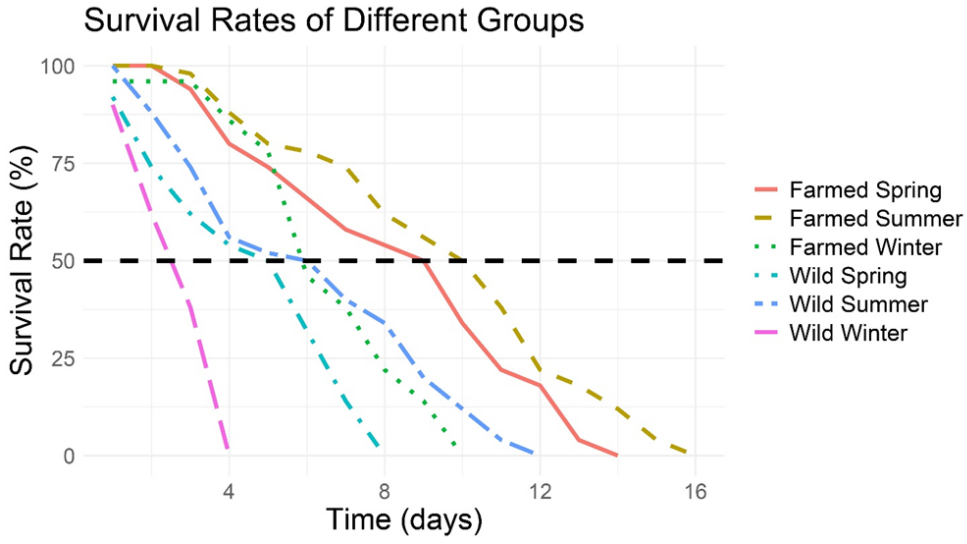

Fig. 1. Survival rates of farmed and wild mussels across winter, spring, and summer in stress-on-stress (SoS) response tests (n = 50 per group). The dashed line indicates the 50% survival threshold.

Conclusions

In summary, we can conclude that both mussel populations are experiencing stress based on the results obtained for the studied biomarker in all three seasons. We believe that the low survival time is related to the pollution of the Black Sea waters, and further research is needed regarding this ecological concern.

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