ZooNotes 241: 1-4 (2024)
www.zoonotes.bio.uni-plovdiv.bg
ISSN 1313-9916
DOI: 10.69085/zn20240241

Stress on stress response in wild and farmed Mediterranean mussels (*Mytilus galloprovincialis* Lamarck, 1819) from Sozopol, Black Sea (Bulgaria)

VESELA YANCHEVA[1]*, ILIANA VELCHEVA[1], ELENKA GEORGIEVA[2], STELA STOYANOVA[2], BORISLAVA TODOROVA[1], KRISZTIAN NYESTE[3], LASZLO ANTAL[3]

[1]University of Plovdiv, Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tsar Assen Str., Plovdiv, 4000, Bulgaria
[2]University of Plovdiv, Faculty of Biology, Department of Developmental Biology, 24 Tsar Assen Str., Plovdiv, 4000, Bulgaria
[3]University of Debrecen, Department of Hydrobiology, Egyetem Sqr. 1, Debrecen, 4032, Hungary
**Corresponding author: vyancheva@uni-plovdiv.bg*

Abstract. As a continuation of our previous research, wild and farmed mussels from the town of Sozopol, located on the Southern Bulgarian Black Sea coast, were collected to assess and compare the survival rates (stress on stress response, SoS) as a biomarker for the multi-stressor environment. This time we aimed to investigate further if there could be any seasonal differences. Overall, the mussels collected in April had a longer survival time than those collected at the end of January 2024. However, the survival time of wild and farmed mussels had a shorter survival rate in the air than in other studies. The answer to the question "Why is that?" persists.

Key words: mussels, survival time, Black Sea

Introduction

According to Bozcaarmutlu *et al.* (2009) the Black Sea has been increasingly threatened by various chemical pollutants over the past decades as a result of accidental crude oil spills, dumping of toxic wastes, discharge of domestic wastes from coastal settlements, and industrial pollutants carried by rivers. Furthermore, the Black Sea is fed by a few large rivers flowing through Europe, including the Danube, Dnieper, Dniester, and Don.

The Black Sea is also a semi-closed sea, which deepens the environmental problems that it is already suffering (Eryaşar *et al.*, 2022; Onay *et al.*, 2023). In addition, in the Bulgarian part of the Black Sea coast, there are seventeen rivers and three lakes, which contribute to the pollution and eutrophication of the Black Sea (Mirinchev *et al.*, 1999). Martínez-Gómez et al. (2017) explain that mussels are an essential component of the integrated approach for assessing marine environmental quality. Different contaminants and contaminant-related biomarkers (molecular, cellular, tissue, physiological, and behavioral alterations) have been recommended to be monitored in a framework for risk assessment and monitoring programs of pollutants and their effects worldwide. In this regard, the SoS response can be used as a physiological index of mussel health and vitality. Its application is simple, and the index is calculated based on the survival time when animals are exposed to air. The SoS biomarker

Received: 13.05.2024, Accepted: 29.05.2024, Published: 11.06.2024
Университетско издателство "Паисий Хилендарски"
Plovdiv University Press "Paisii Hilendarski"

indicates whether environmental factors, including pollutants, have affected the capacity of molluscs to survive under stressful conditions, such as exposure to air.

This study was based on using mussels obtained from natural populations and farm mussels along the Southern Bulgarian Black Sea to expand our knowledge of multifactorial effects of the environment on SoS response as biomarker for pollution-induced effects.

Material and Methods

The fieldwork repeated our previous research (Yancheva et al., 2024). 200 adult specimens (length 6 cm ± 1.5; weight 12.5 g ± 2.5) were obtained from the same commercial farm located in Sozopol, Bulgaria at the end of April 2024. Approximately another 150 wild individuals (length 5.5 cm ± 2.5; weight 12 g ± 1.5) were hand-collected and provided by local fishermen. The SoS test was performed following a standard methodology, which indicates the survival time of 50% of sampled mussels (LT_{50}) when exposed to air (Veldhuizen-Tsoerkan *et al.*, 1991; Viarengo *et al.*, 1995; Brooks *et al.*, 2018). The survival of mussels was inspected every 24 hours after the sampling time until 100% mortality was reached. The mussels were considered alive when they resisted forcible valve separation and respectively dead when the valves gaped and external stimulus (squeezing of valves) did not respond. Similarly to Viarengo et al. (2007) and Vethaak et al. (2017), we further assessed the SoS response (expressed as days of air survival) against their corresponding background assessment criteria (BAC) and environmental assessment criteria (EAC). In addition, we followed the *post hoc* monitoring strategies proposed by assessing the SoS results using EACs jointly with the principle of the two-tier approach as follows: 1) If significant effects on SoS are not detected (LT_{50} values not exceeding the limit value set as EACs), the water environmental quality of the sampling site to be considered good enough and no other biological or chemical analysis to be required. The monitoring strategy to follow is categorized as type 0 (MS0). 2) If effects on SoS are moderate (LT_{50} value between its corresponding EAC and BAC), the water environmental quality of the sampling site is to be considered moderate, and analyses should be supplemented (second-tier) with a complete battery of biomarkers assessing the levels of pollutant-induced stress syndrome. The results of the complete battery of biomarkers will then guide the chemical analyses required. The monitoring strategy to follow is categorized as type 1 (MS1). Thus, the MS1 strategy would imply that investigative chemical monitoring is required to identify the cause of the less-than-good biological effect response. 3) If strong effects on SoS are observed (LT_{50} value lower than its corresponding EAC), the water environmental quality of the sampling site is considered poor, and then it should proceed directly to chemical analyses to identify the pollutants and their levels that induce biological effects. The monitoring strategy to follow is categorized as type 2 (MS2). We also compared the survival curves following pair-wise comparison with Bonferroni correction. "Survdiff" function ("survival" package) from R version 4.3.3. (R Core Team, 2015) was used to calculate the chi-square distance and the corresponding *P*-value (Harrington & Flemming, 1982).

Results and Discussion

The lethal threshold expresses the results of SoS for 50% mortality (LT_{50}), a median of the survival time, or the day on which 50% of mussels from a site were dead. The results on the average LT_{50} for both farmed and wild mussels collected and assessed in April are presented in Fig. 1. Similar to our previous research, we found that typical SoS reactions occurred in both groups at different times. The lowest 50% lethal threshold (LT_{50}) values were recorded for the wild mussels - 5 days, followed by the farmed mussels (LT_{50} = 9 days). The statistical analysis showed that there was a significant difference between the two tested groups collected in winter (x^2=7, DF=1, P<0.01) and in spring ((x^2=7, DF=1, P<0.01). Overall, both groups of mussels had shorter survival times than usually reported in scientific literature. Still, those collected in spring compared to winter survived longer when exposed to air (x^2=27.9, DF=3, P<0.001). The SoS response can be seriously influenced by the seasonal variations of environmental factors, such as temperature, food availability, spawning, etc. (Petrović *et al.*, 2004), which we believe was partially the case in our study, comparing the results obtained in January and April. According to De Zwaan & Mathieu (1992), mussels can survive aerial exposure for several days, and their ability to keep their valves closed and to resist aerial exposure is thought to be related to the amount of adenosine triphosphate (ATP), which is available to maintain the tonus in the adductor muscle. However, exposure to pollutants can impact the enzymes involved in the anaerobic pathways that mussels utilize during emersion to maintain basal metabolism, which might have happened with the mussels in our study. Our results once again confirmed that individuals stressed by pre-exposure to pollutants would show greater mortality than individuals collected from a reference location.

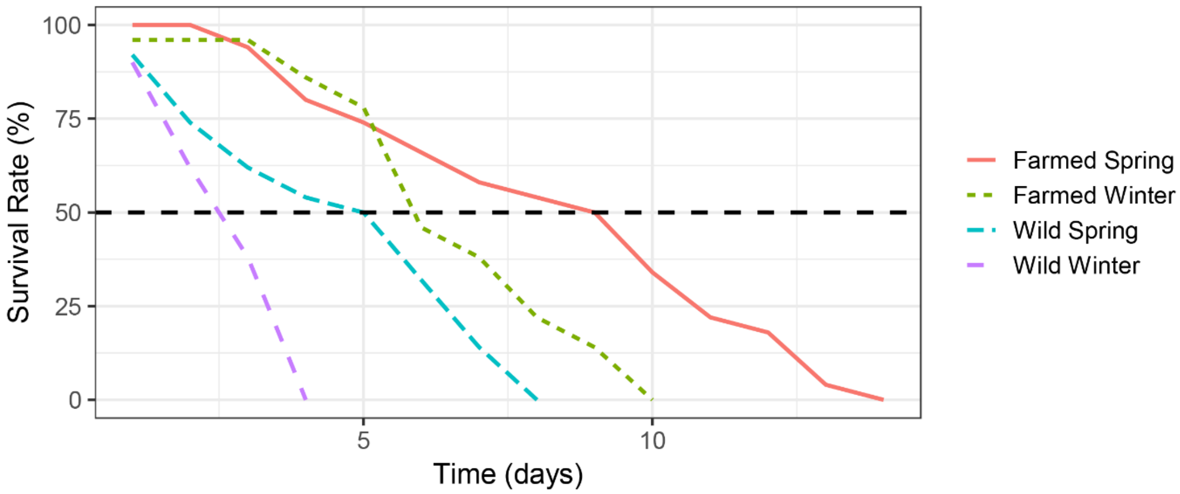

Fig. 1. Survival rate of farmed and wild mussels collected in winter and spring in stress on stress (SoS) response test (n = ohn P. Bignell, Ketil Hylland, 50 per group). The dashed line represents the 50% survival rate.

Conclusion

Since 2008, the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) has emphasized on the importance of assessing critical biological responses to evaluate the health of organisms and to link any observed responses to pollutant exposure and their levels. We observed strong effects on SoS (LT_{50} values were lower than its corresponding EAC) thus the water environmental quality of the sampling sites was considered poor. That is why we strongly suggest that the SoS test is applied along with direct chemical analyses of waters and sediments to better study the physiology of *Mytilus galloprovincialis* from a multi-stressor environment and to fully assess the ecological status of the Bulgarian Black Sea in different seasons.

Acknowledgments. This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01.

References

Bozcaarmutlu, A., Sapmaz, C., Aygun, Z. & Arinç, E. (2009) Assessment of pollution in the West Black Sea Coast of Turkey using biomarker responses in fish. *Marine Environmental Research*, 67: 167-176.

Brooks, S.J., Escudero-Oñate, C., Gomes, T. & Ferrando-Climent, L. (2018) An integrative biological effects assessment of a mine discharge into a Norwegian fjord using field transplanted mussels. *Science of The Total Environment*, 644: 1056-1069.

De Zwaan, A. & Mathieu, M., (1992) Cellular biochemistry and endocrinology. *In*: Gosling, E. (Ed.), The Mussel *Mytilus*. Ecology, Physiology, Genetics and Culture. *Elsevier Science Publishers BV, Amsterdam,* pp. 223-307.

Eryaşar, A.R., Gedik, K. & Mutlu, T. (2022) Ingestion of microplastics by commercial fish species from the southern Black Sea coast. *Marine Pollution Bulletin*, 177: 113535.

Harrington, D.P. & Fleming, T.R. (1982) A class of rank test procedures for censored survival data. *Biometrika*, 69: 553-566.

Martínez-Gómez, C., Robinson, C. D., Burgeot, T., Gubbins, M., Halldorsson, H. P., Albentosa, M., Bignell, J.P., Hylland, K., & Vethaak, A. D. (2017) Biomarkers of general stress in mussels as common indicators for marine biomonitoring programmes in Europe: The ICON experience. *Marine Environmental Research*, 124: 70-80.

Mirinchev, A., Tzankov, Tz., Kostova, I. & Mirincheva, M. (1999) Impact of Bulgarian rivers on heavy metal pollution of the black sea. *Water Science and Technology*, 39(8): 9-12.

Onay, H., Karslı, B., Minaz, M. & Dalgıç, G. (2023) Seasonal monitoring of microplastic pollution in the Southeast Black Sea: An example of red mullet (Mullus barbatus) gastrointestinal tracts. *Marine Pollution Bulletin,* 191: 114886.

Petrović, S., Semenčić, L., Ozretić, B. & Ozretić, M. (2004) Seasonal variations of physiological and cellular biomarkers and their use in the biomonitoring of north Adriatic coastal waters (Croatia). *Marine Pollution Bulletin*, 49: 713-720.

Veldhuizen-Tsoerkan, M., Holwerda, D. & Zandee, D. (1991) Anoxic survival time and metabolic parameters as stress indices in sea mussels exposed to cadmium or polychlorinated biphenyls. Archives of Environmental Contamination and Toxicology, 20: 259-265.

Vethaak, A., Davies, I., Thain, J., Gubbins, M., Martínez-Gómez, C., Robinson, C., Moffat, C., Burgeot, T., Maes, T., Wosniok, W., Giltrap, M., Lang, T. & Hylland, K., (2017) Integrated indicator framework and methodology for monitoring and assessment of hazardous substances and their effects in the marine environment. *Marine Environmental Research*, 124: 11-20.

Viarengo, A., Canesi, L., Pertica, M., Mancinelli, G., Accomando, R., Smaal, A.C. & Orunesu, M. (1995) Stress on stress response: a simple monitoring tool in assessment of a general stress syndrome in mussels. *Marine Environmental Research*, 39: 245-248.

Viarengo, A., Lowe, D., Bolognesi, F., Fabbri, E. & Koehler, A. (2007) The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. *Comparative Biochemistry and Physiology, Part C*, 146: 281-300.

Yancheva, V., Velcheva, I., Georgieva, E., Stoyanova S., Todorova, B., Nyeste, K. & Antal, L. (2024). Physiological measurements of the Mediterannean mussel (*Mytilus galloprovincialis* Lamarck, 1819) from the Bulgarian Black Sea as biomarkers for multi-stressor environment. *ZooNotes*, 238: 1-4.