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Physiological measurements of the Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) from the Bulgarian Black Sea as biomarkers for multi-stressor environment

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. Wild and farmed mussels from the Bulgarian Black Sea were collected to study and compare the survival rates (stress on stress response) as a biomarker for multi-stressor environment. In sum, the survival time indicated that the farmed mussels were more tolerant to stress, even though they had a similar size and lived in similar conditions. Further research needs to be done to clarify why.

Key words: mussels, survival rate, stress ecology

Introduction

Pollution of aquatic ecosystems with various toxicants, including emerging contaminants has become a worldwide environmental problem in recent years. These contaminants have significant adverse effects on the health of aquatic organisms, such as mussels and may therefore also have potential consequences for human health through seafood consumption. The Black Sea is one of the largest inland seas in the world, which takes the discharge of Europe's largest rivers, such as the Danube, Dnieper and Don into its basin. The Black Sea communicates with the Mediterranean Sea to the south through the narrow Turkish Straits System, which includes the Bosporus and Dardanelles, and the Azov Sea to the north (Eryaşar *et al.*, 2022). Furthermore, the Black Sea, which drains a basin of approximately 2.200.000 km^2 in Europe and Asia, is under constant anthropogenic pressure from hundreds of cities and a population of more than 200 million people (Alkan *et al.*, 2008). *Mytilus galloprovincialis* (Lamarck, 1819) is included in the *M. edulis* complex. This complex has a great economic value, including in Bulgaria. In addition, *M. galloprovincialis* has been validated as a test species in ecotoxicological studies and risk assessment monitoring programs with both native and transplanted mussels. This mussel species has a broad geographic distribution, it is easily collected and handled, sessile and filter-feeding, characterized by a high fecundity and an effective population size (Ayari *et al.*, 2024). Ecological and toxicological risks likely to arise in the Black Sea may affect humans through the food chain (Makedonski *et al.*, 2017) because fishing and seafood farming are

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carried out intensively in the area (Balık, 2019). There are some studies on the human health risk assessment on mussel consumption and some on the ecological status of several mussel species from the Bulgarian Black Sea, however knowledge on a battery of selected physiological, biochemical, cellular or tissue-level biomarkers in this sentinel species is relatively scarce. This study was designed in order to follow multi-biomarker alterations in wild mussels compared to farmed mussels used as controls from the Bulgarian Black Sea. Here we present the results on stress on stress (SoS) response and the hypothesis being tested is that the wild mussels will have less ability to survive out of water.

Material and Methods

A total of 200 adult specimens (length 5.5 cm ± 2.5; weight 13.5 g ± 0.5) were purchased from a commercial farm located in Sozopol, Bulgaria in the end of January 2024 (Fig. 1).

Fig. 1. Map of Bulgaria, showing the location of the town of Sozopol.

Another 200 wild individuals (length 4.5 cm ± 3.5; weight 11 g ± 2.5) were hand-collected with the aid of local fishermen of the lower intertidal zone. The farm was considered as relatively non-impacted and the site where the wild mussels were collected from was considered as a potentially impacted site. From each site 150 specimens were selected for bioaccumulation, histopathological, and histochemical analyses, which will not be discussed here. The other 50 mussels were used for physiological analyses. Stress on stress response (SoS) was firstly investigated. This tolerance test is indicated by the survival time of 50% of sampled mussels (LT_{50}) when exposed to air (Thomas *et al.*, 1999). We compared 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 chi-square distance and the corresponding *P*-value (Harrington & Flemming, 1982). After collection, the mussels were transported to the laboratory in a clean glass tank with seawater and then subjected to anoxia by air. They were placed in two dishes in an incubator maintained at 15°C, with a regime of 12 h of light and dark. The mussels were checked on a daily basis for a response with a gentle tap on the shell or for open valves, mortality rates were recorded, and those mussels were removed from the incubator (Veldhuizen-Tsoerkan *et al.*, 1991; Viarengo *et al.*, 1995; Brooks *et al.*, 2018). Dissection was finally performed according to Benito *et al.* (2023), and the mussel's weight and length were recorded, too, using an analytical balance and calipers for the calculation of several condition factors, which will be reported in future separately.

Results and Discussion

Figure 2 presents the average LT_{50} for both farmed and wild mussels. Typical stress on stress (SoS) reactions occurred in both groups at different times. However, the lowest 50% lethal threshold (LT_{50}) values were recorded in the wild mussels - 4 days, followed by the farmed mussels (LT_{50} = 10 days). The survival analysis has shown that there was a significant difference between the two groups (x^2=24.5, DF=10, P<0.01). Mussels can survive

for a long time in air and this ability has been shown to provide reliable data in biological effects studies. Furthermore, few reports showed general survival of blue mussels with a size of 4-5 cm even as long as 30 days (Hammen, 1976; Smaal *et al.*, 1991; Hellou & Law, 2003; Brooks *et al.*, 2015; Martínez-Gómez *et al.*, 2017), but in general, the survival time of mussels from polluted sites is much shorter, between 3 and 7 days (Wepener *et al.*, 2008). The survival time in our study indicated that the farmed mussels were more tolerant to stress, and the wild ones were less tolerant, i.e., dying more quickly. Overall, the survival time of both groups was less than in previous reports, even for the mussels from the reference site (aquaculture). The stress on stress (SoS) response can also be significantly influenced by the seasonal variations of environmental factors, such as temperature, food availability, spawning, etc. (Petrović *et al.*, 2004). In addition, according to Thomas *et al.* (1999) smaller mussels survive longer than larger ones. Since one size range and one holding temperature were tested in our study, we consider that the effects on this general biomarker for physiological fitness could be pollution-induced. Our results confirmed that individuals stressed by pre-exposure to pollutants show greater mortality than controls or individuals collected from a reference location.

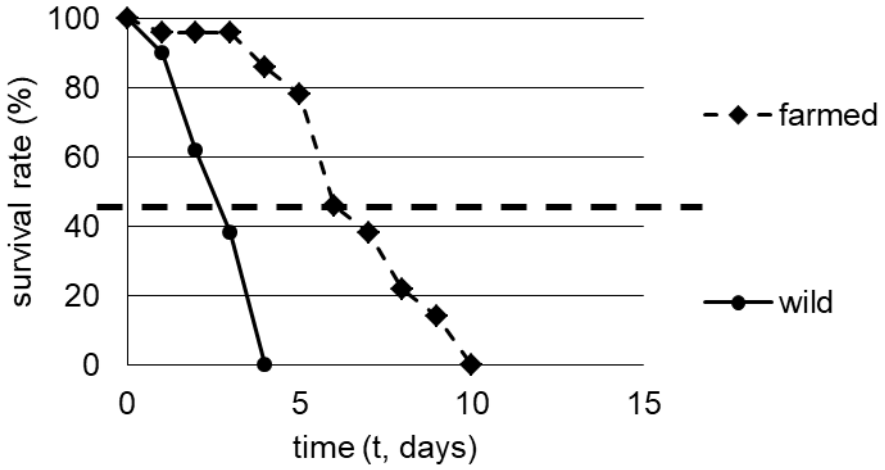

Fig. 2. Survival rate of farmed and wild mussels in stress on stress (SoS) response test (n = 100). The dashed line represents the 50% survival rate (LT_{50}).

Conclusion

To our knowledge, this is the first study on stress on stress (SoS) response on mussels from the Bulgarian Black Sea, which has many ecological problems. We confirmed that the tested methodology is simple, rapid, and low in cost and does not require sophisticated equipment. However, since it is not that sensitive we suggest that the stress on stress (SoS) response should be combined with other cellular or tissue biomarkers along with bioaccumulation analyses as a monitoring tool for the assessment of contaminated coastal areas in order to further study the reasons for the shorter survival time.

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