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# How stressed are two populations of the Mediterranean mussels (*Mytilus galloprovincialis* Lamarck, 1819) from the Black Sea (Bulgaria)? - Stress on stress biomarker responses

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**Abstract**. This article aims to present the latest results on the stress on stress responses in farmed and wild mussels from Sozopol, Bulgaria, focusing on the autumn season of 2024. Additionally, we compare these findings with the results from the other three seasons.

**Key words**: mussels, biomarkers, Black Sea.

## Introduction

The catchment area of the Black Sea is 1,9 million $km^2$ and encompasses 23 countries (Vespremeanu & Golumbeanu 2018). As Snigirova *et al.* (2024) explain, the inland seas that harbor a dense coastal population, significant river discharge, and well-developed shipping are especially vulnerable to pollution. In Europe, the Black Sea is an example of a nearly enclosed sea that suffers from land-based pollution and is considered one of the most polluted European seas, according to Bat & Öztekin (2022). Furthermore, Ciucă *et al.* (2025) explain that the semi-enclosed permanently anoxic Black Sea basin of ca. 423,000 $km^2$ and a total coastline length of approximately 4000 km is recognized as being one of the most polluted seas in the world with various pollutant substances, including plastics. Recent research showed that the Black Sea has almost two times more floating plastic concentrations than the Mediterranean Sea, which is considered the worst polluted sea by floating plastics (Pogojeva *et al.* 2020). In particular, the Northwestern Black Sea is highly affected by river discharge, with the Danube River strongly affecting the hydrological regime (Strokal *et al.* 2022). In addition, the Danube is the major river delivering 58% of the total freshwater (203 $km^3$ $yr^{-1}$) and sediment inputs to the Black Sea (Müftüoglu 2013). The Danube River, with its 2850 km and a drainage area of 817,000 $km^2$, together with its significant tributaries Dnieper, Dniester, Don, and Kuban, drains the central and eastern European industrial towns and the agricultural regions and transports substantial amounts of pollutants which end up eventually in the Black Sea. According to FAO (2024), global mussel production has reached 2,1 million tons in 2022, valued at approximately 4,5 billion USD. Moreover, aquaculture is by far the primary source of mussels and is responsible for over 90 % of total landings (FAO 2024). On the other hand, mussels play a crucial role in

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environmental biomonitoring studies as sedentary organisms with a broad geographic range and a high tolerance for ecological fluctuations (Singh & Gupta 2021). Similarly, to other European countries, the Mediterranean mussels in Bulgaria are cultivated, but the wild collection is also present. Environmental research highlighted *Mytilus galloprovincialis* as an excellent model organism for ecotoxicological studies because of its wide distribution, filter-feeding habits, and sensitivity to environmental pollutants (Impellitteri *et al.* 2024).

The present study assesses the autumn values of the biomarker stress on stress response and compares the results for the two mussel populations throughout the entire calendar year of 2024.

## Material and Methods

The experiment is a continuation of our previous research (Yancheva *et al.* 2024a,b,c). Similarly, mussels were purchased from a local farm and also hand-collected with the help of fishermen and boats in October 2024 (Fig. 1). They were transported in clean glass tanks with seawater to the Plovdiv University where the stress on stress (SoS) test was performed following a standard methodology, which indicates the survival time of 50% of sampled mussels ($LT_{50}$) when exposed to air (Viarengo *et al.* 1995). The survival of mussels (n=50 per population) was registered 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. The survival data were analyzed using the R software (version 4.3.3; R Core Team, 2024), with the packages survival and survminer. The Kaplan-Meier method was employed to estimate survival probabilities for each group, stratified by season (winter, spring, summer, and autumn) and type (farmed and wild). The survival curves were compared using the log-rank test, implemented via the survdiff function in the survival package (Harrington & Flemming, 1982). For pairwise comparisons between farmed and wild groups within each season, the log-rank test was applied to each pair of survival curves, and Bonferroni correction was used to adjust for multiple testing. Additionally, pairwise comparisons between seasons were performed for farmed and wild groups separately to assess seasonal variations in survival. The chi-squared statistics ($x^2$), degrees of freedom (DF), and adjusted p-values were reported for each comparison. Statistical significance was set at $\alpha = 0.05$ after correction. The survival dataset included the number of live individuals recorded daily for each group and season. Survival time (days) and event status (1 for death, 0 for censored) were used to create a survival object for analysis. Pairwise comparisons were performed for the following combinations: (1) farmed vs wild within each season and (2) between seasons for each type (farmed or wild). The results were summarized in terms of chi-squared values and adjusted p-values.

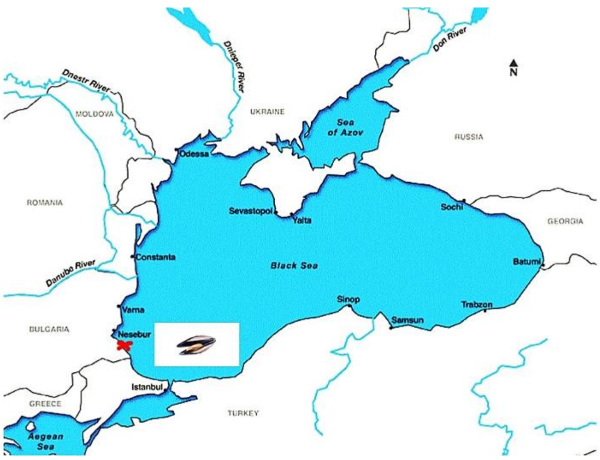


**Fig. 1.** Red Cross (x) shows the location of the town of Sozopol in Bulgaria.

## Results and Discussion

The statistical analysis revealed significant differences in survival between the farmed and wild groups across all seasons. In winter, the farmed mussels exhibited significantly better survival compared to wild mussels ($x^2 = 217.5$, DF = 1, $P < 0.001$). Similarly, in spring ($x^2 = 97.9$, DF = 1, $P < 0.001$), and summer ($x^2 = 57.9$, DF = 1, $P < 0.001$), the farmed groups showed higher survival rates than the wild groups. However, in autumn, survival differences were less pronounced, with the farmed group only marginally outperforming the wild group ($x^2 = 12.5$, DF = 1, $P = 0.012$ after Bonferroni correction) (Fig 2.). Among the seasons, the mussels collected in summer exhibited the longest survival, followed by those collected in spring, while the shortest survival was observed in winter. Pairwise comparisons among seasons revealed significant differences in survival between winter and spring ($x^2 = 46.4$, DF = 1, $P < 0.001$), winter and summer ($x^2 = 85.4$, DF = 1, $P < 0.001$), and winter and autumn ($x^2 = 42.1$, DF = 1, $P < 0.001$). These seasonal trends align with environmental conditions, as warmer temperatures during summer likely provide favorable conditions for physiological processes, whereas harsher winter conditions contribute to reduced survival (Fig 2.). Yet, the survival times in air were much shorter compared to other studies, linking these results with the poor ecological status of the Black Sea. The overall comparison across all groups and seasons indicated highly significant differences in survival ($x^2 = 678$, DF = 7, $P < 0.001$) (Fig 2.).

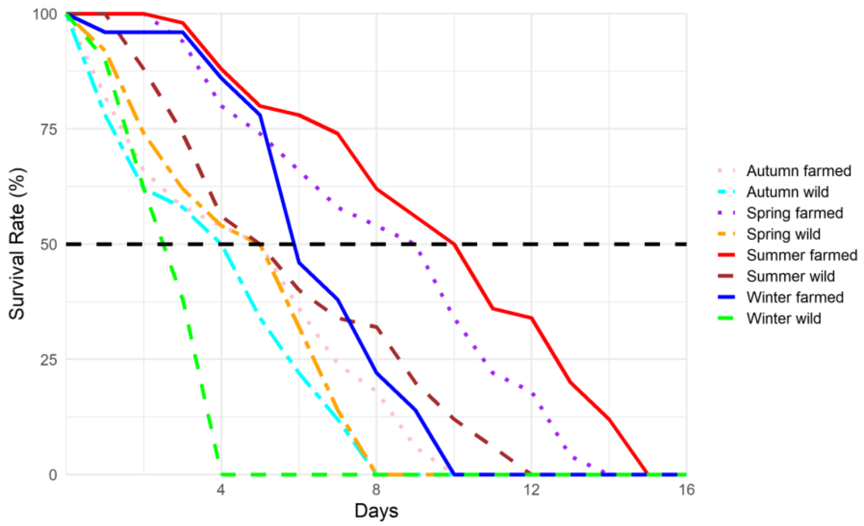


**Fig. 2.** Survival rates of farmed and wild mussels across winter, spring, summer, and autumn in stress on stress (SoS) response tests (n = 50 per group). The dashed line represents the 50% survival threshold.

## Conclusion

The Black Sea's farmed and wild mussel populations exhibit stress across all seasons, with $LT_{50}$ values below the Environmental Assessment Criteria (EAC), indicating compromised environmental quality. The shorter survival times point to poor water quality and pollution as driving factors, necessitating expanded monitoring tools to better understand the impacts of multi-stressor environments on *Mytilus galloprovincialis*. Our research is the first to investigate the seasonal stress on stress (SoS) responses in mussels from the Bulgarian Black Sea. This pioneering approach highlights the methodology's potential innational ecological monitoring.

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