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Assessing environmental stress through lysosomal membrane stability in wild and farmed mussels (*Mytilus galloprovincialis* Lamarck, 1819) across seasons

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Abstract. This study assessed lysosomal membrane stability (LMS) in hemocytes of *Mytilus galloprovincialis* from wild and farmed populations, using the Neutral Red Retention Time (NRRT) assay as a biomarker of environmental stress. Mussels were sampled in summer and autumn to evaluate seasonal variability, and the results were compared with our previous winter and spring data. NRRT values showed a significant reduction in both populations across seasons, indicating compromised lysosomal integrity and exposure to environmental stressors. Notably, wild mussels consistently exhibited lower NRRT values compared to farmed individuals, suggesting a higher stress burden. The underlying causes of this difference remain to be clarified.

Keywords: Black Sea, mussels, LMS.

Introduction

The Black Sea, a partially enclosed body of water surrounded by densely industrialised and populated regions, is a dynamic region affected by various environmental pressures (Da Ros *et al.* 2011). Mussels, filter-feeding and sessile bivalve molluscs, are useful sentinel organisms to assess the ecological stress because of their ability to accumulate different classes of pollutants, thusfor assessing ecological stress due to their ability to accumulate different classes of pollutants, thereby providing a time-integrated image of their bioavailability. Biomarkers that reflect a rapid contaminant-induced response at the molecular, biochemical, and cellular levels have often been considered sensitive 'early warning' indicators of organisms' responses to toxic chemicals (Moore, 1990). Therefore, we

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aimed to ass the lysosomal membrane stability (LMS), which is considered as a general stress biomarker of chemical pollution has been widely recommended by different organizations such as the Barcelona Convention, International Council for Exploration of the Sea (ICES), and Oslo and Paris Conventions (OSPAR) (Martínez-Gómez *et al.* 2015) in *M. galloprovincialis* from the Bulgarian Black Sea.

Material and Methods

As this was a continuation of our previous research, the field and laboratory work, as well as the applied statistical analyses, were the same (Yancheva *et al.* 2025). LMS was assessed using the NRRT (min) assay in hemocytes, and they were also scored for lysosomal alterations, following Viarengo *et al.* (2007) and Martínez-Gómez *et al.* (2015).

Results and Discussion

Table 1 shows the average NRRT in mussels from Sozopol, Black Sea, and the severity scores in the lysosomes of both wild and farmed mussels. The NRRT varied significantly between seasons and between wild and aquaculture groups (Kruskal–Wallis test: $H = 36.27$, $p = 6.45 \times 10^{-6}$). Post hoc Mann–Whitney pairwise comparisons revealed clear seasonal patterns (Fig. 1). In winter and spring, no significant differences were detected between wild and aquaculture mussels, and both groups showed relatively high and stable NRRT values (median values around 60–90 min.). In contrast, during summer and autumn, wild mussels displayed significantly lower NRRT values compared to aquaculture mussels (Mann–Whitney, $p < 0.01$), with medians between 15–30 min. Aquaculture mussels maintained higher retention times in these seasons (median 60–90 min.), significantly exceeding those of wild individuals. The compact letter displayed on the boxplot confirmed these patterns: a: winter-wild, winter-aquaculture, spring-wild, spring-aquaculture, autumn-aquaculture; b: summer-wild, autumn-wild; ab: summer-aquaculture. This indicates that wild mussels in summer and autumn formed a distinct group with significantly reduced NRRT. In contrast, aquaculture mussels were more stable across seasons, showing an intermediate position in summer.

Table 1. Neutral red retention time and severity scores in mussels from Sozopol, Bulgaria.

Season	NRRT (min.)		Severity score	
	wild	aquaculture	wild	aquaculture
Winter	41.25	69	2.75	2
Spring	45.75	57	2.8	1.9
Summer	30	41.25	3.35	2.25
Autumn	31.5	56.25	2.7	2.05

The severity scores of mussels showed clear variation between seasons and groups (Fig. 2). The Kruskal–Wallis test revealed a highly significant overall difference among groups ($H = 42.15$, $p = 4.87 \times 10^{-7}$). Post hoc Mann–Whitney U pairwise comparisons indicated that: Winter: Wild and aquaculture mussels did not differ significantly ($p > 0.05$), both displaying low to moderate severity scores (medians around 2–3). Spring: No significant difference was observed between wild and aquaculture mussels ($p > 0.05$). Summer: wild mussels had significantly higher severity scores (median = 3–4) compared to aquaculture mussels (median = 2; $p < 0.01$). Autumn: wild and aquaculture mussels did not differ significantly from each other, but both exhibited higher severity scores (median = 3–4) compared to winter and spring groups ($p < 0.01$). The compact letter display assigned the following groups: a: winter-wild, winter-aquaculture, spring-wild, spring-aquaculture b: summer-wild, autumn-wild, autumn-aquaculture ab: summer-aquaculture (overlaps with both clusters). This pattern demonstrates that mussels in wild populations, particularly in

summer and autumn, exhibited elevated severity scores indicative of higher stress, while aquaculture mussels maintained relatively stable scores across most seasons.

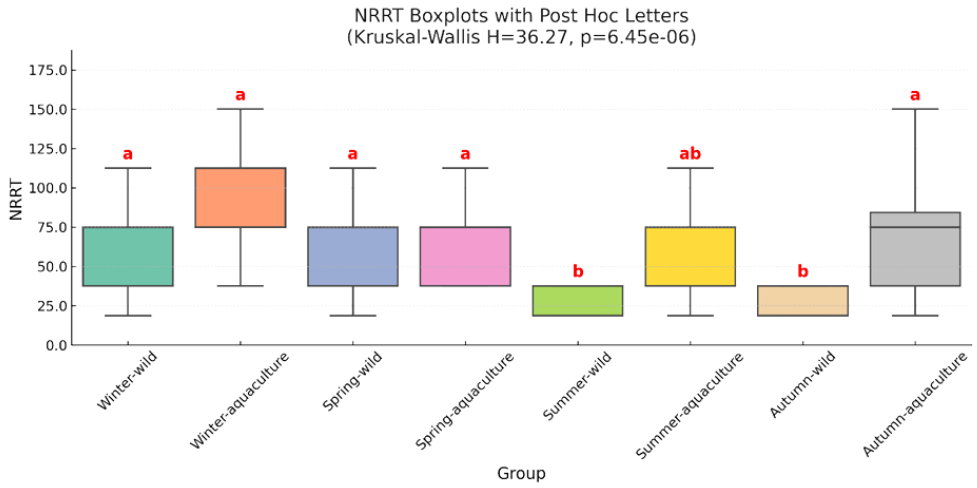

Fig. 1. Boxplots of neutral red retention time (NRRT) in wild and aquaculture mussels across seasons. Red letters above the boxes denote statistical groupings based on Mann–Whitney U post hoc tests. Groups sharing the same letter are not significantly different ($p > 0.05$).

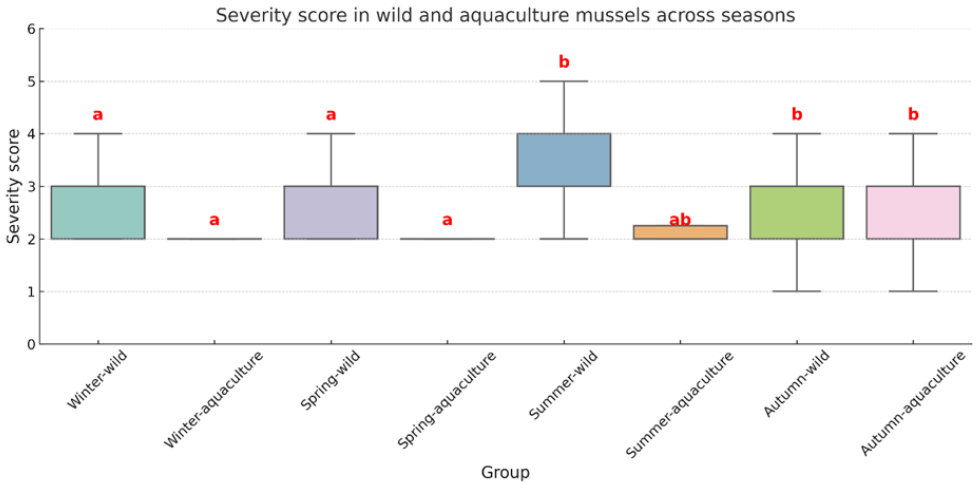

Fig. 2. Severity scores of wild and aquaculture mussels across seasons. Red letters denote statistical groupings based on Mann–Whitney U post hoc tests. Groups sharing the same letter are not significantly different ($p > 0.05$).

The results of our study on LMS are consistent with the idea that increased cellular stress, caused by various stressors including aquatic pollution, compromises the integrity of the lysosomal membrane (Lowe *et al.* 1992). This likely reduces cell viability and ultimately leads to cell death (Hauton *et al.* 1998). Our findings also correspond with those of other authors regarding this biomarker under field conditions (Shaw *et al.* 2011), i.e., that lysosomal membrane stability is weakened, especially as a result of a polluted aquatic environment. A recent study by Pantea *et al.* (2023) on the Romanian Black Sea, focusing on lysosomal membrane stability in *M. galloprovin*cialis, reported that lysosomes exhibited compromised integrity due to marine pollutants such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, heavy metals, and others. Neutral red retention times ranged from 0 to 60 min. in all samples, indicating low lysosomal membrane stability in mussels from the Romanian Black Sea. In comparison, our results showed higher retention values,

with no recordings below 30 min., suggesting that mussels from the Bulgarian Black Sea display greater resilience regarding this biomarker. Nevertheless, retention times in our study did not reach 90–120 min. considered indicative of healthy lysosomal membranes.

Lysosomal membrane stability (LMS) is a well-established biomarker of cellular stress in bivalves, reflecting organismal responses to environmental pressures. Comparative analysis revealed that wild mussels consistently displayed lower NRRT values than their farmed counterparts, suggesting a higher stress burden in natural populations. These findings highlight the sensitivity of NRRT as a diagnostic tool for assessing environmental quality and mussel health. The consistently higher stress levels in wild mussels raise important questions about the role of habitat conditions, pollutant exposure, and other ecological factors in shaping physiological resilience. Further investigation is required to clarify the drivers of these differences and to strengthen the application of LMS as a biomarker in environmental monitoring programs in Black Sea.

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