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By Göktuğ GÜL

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THE EFFECTS OF INCREASED SALINITY ON ORGANISMS IN FRESHWATER ECOSYSTEMS: A CASE STUDY OF FRESHWATER MUSSELS

***Göktuğ GÜL*[1,*], *Pınar ARSLAN*[2], *Aysel Çağlan GÜNAL*[3]**

[1] *Medical Laboratory Programme, Health Services Vocational School, Gazi University, Ankara, Türkiye*

[2] *Department of Biology, Faculty of Science, Çaknırı Karatekin University, Çankırı, Türkiye*

[3] *Department of Biology Education, Faculty of Education, Gazi University, Ankara, Türkiye*

Abstract

The melting of terrestrial glaciers, the expansion of the sea levels, and droughts due to global warming cause the increase of salinity levels in the freshwater. Notably, research on salinity in marine ecosystems is significantly higher than research in freshwater ecosystems. Freshwater mussels are an essential group of organisms that improve water quality by filtering the water body in which they are located. In addition, they are symbiotically related to other organisms in the feeding and reproduction cycle. These characteristics make freshwater mussels good model organisms. In this study, the effects of salinity increasing on total haemocyte levels (THCs) and lipid peroxidation by-product malondialdehyde (MDA) were investigated on freshwater mussels (*Unio delicatus*). After obtaining the freshwater mussels from local fishermen in Bursa (Türkiye), they were adapted to laboratory conditions for two weeks. Five freshwater mussels were placed in each aquarium and directly transferred to 12 ‰ salinity at a constant water temperature of 24 ºC for 1 hour and 24 hours in the experiment. There was also a positive control group. At the end of the exposure times, mussels were placed under ice anesthesia, and the hemolymph liquid, gill, and digestive gland tissues were taken. The THCs were investigated with the hemolymph liquid, and the levels of MDAs were calculated in gills and digestive tissues. The amount of THCs increased significantly at the end of 1 hour of salinity exposure but returned to the level of control group values at the end of 24 hours. According to the MDA results, levels in digestive and gill tissues did not change significantly within 1 hour after exposure but showed a tendency to increase during the rest of the exposure. It has been understood that mussels develop a rapid physiological and cellular response to salinity. The effects of salinity on freshwater mussels should be monitored using other parameters.

Keywords: *Salinity, Freshwater Mussel, Total Hemocyte Counts, MDA*

1. Introduction

The global warming initiated by the increased consumption of fossil fuels during the Industrial Revolution is currently perceived as one of the most critical environmental issues, posing a significant risk of ecological degradation. Global warming has given rise to climate change, with the world's average temperature increasing by 1°C during summer seasons and 2°C during winter seasons in each successive decade from the 1960s to the present. This escalation has led to the melting of glaciers, a rise in sea levels, and an expansion of marine areas, potentially resulting in the submersion of certain terrestrial regions beneath seawater. Additionally, it is anticipated that the mixing of freshwater resources with seawater may occur as a consequence of these temperature fluctuations. The intrusion of seawater or alluvium into river systems can be facilitated by various natural and anthropogenic mechanisms.

In addition to seawater contamination, increases in salinity levels in freshwater sources can occur due to various factors such as salting activities on asphalt roads during winter seasons, mining operations, industrial wastewater discharge, and the mixing of groundwater with contaminated and alluvial soils. The augmentation of salinity in freshwater sources can adversely impact agriculture, livestock farming, and aquaculture [1-4].

Countries with coastlines along the Mediterranean are known to be more significantly affected by climate change. Climate change influences the flow rates of rivers, known as the world's vascular by differentiating precipitation patterns and average temperatures. Aquatic ecosystems, which host a myriad of organisms, undergo continuous changes due to natural and human-induced impacts. In recent years, an increase in salinity has been added to these ecosystem differentiations. Changes in river flow observed in rivers lead to an increase in sediment load in the waters. [2-5]. The rise in sediment load and coastal erosion in freshwater systems, resulting from seawater intrusion due to increased mixing of seawaters with inland water systems, lead to significant increases in the mineral loads of freshwater sources. The increase in mineral quantities in the waters is expressed as salinity [6].

It is anticipated that decreases in the population numbers of freshwater mussels could lead to serious ecosystem damage [5]. Additionally, mussels are preferred organisms in ecological research due to their ability to inhabit

* Corresponding author. *e-mail address: goktuggul@gazi.edu.tr*

various habitats. Salinity, in addition to causing reproductive and feeding disorders in freshwater mussels, can lead to variations in shell formations [7]. Furthermore, it is known that mussels, especially non-adult and larval individuals, are sensitive to salinity [8]. Parasitic mussel larvae in the glochidia stage lose their affinities to attach to fish gills at high salinity levels [9]. This situation could cause significant damage to the reproduction and distribution of freshwater mussels.

According to the Working Group on Biological Effects of Contaminants (WGBEC), there are four different methods for investigating the responses of mussels to various pollutant groups among aquatic organisms. These include detecting tissue residue levels, identifying cellular oxidative and antioxidant responses, and detecting tissue and growth [10]. The total hemocyte count is considered an important parameter in evaluating the health status of aquatic invertebrates [11]. Malondialdehyde levels are preferred in toxicology studies to indicate cellular membrane damage in oxidative stress and antioxidant mechanisms [12].

Mussels are considered model organisms in aquatic studies due to their filter-feeding behavior and symbiotic relationships with other organisms [13]. Numerous studies in the literature have investigated the population status of mussels at various salinity levels in ecosystems. However, the longevity of mussels is thought to contribute to a gradual decrease in population density. The Unionidae family, with 620 species, is the most extensive among freshwater mussels. *Unio delicatus*, belonging to this family, exhibits a wide distribution in Turkish inland water systems [13-14]. Because of this reason, this Unionid species was selected for research.

This study examines the physiological and antioxidant mechanism responses induced by acute increases in salinity in freshwater mussels (*U. delicatus*). Changes in the total hemocyte counts (THCs) from hemolymph and lipid peroxidation byproduct malondialdehyde (MDA) levels from gill and digestive tissues were investigated in freshwater mussels.

2. Materials and Methods

2.1. Test Materials and Organisms

Freshwater mussels (n=40) were procured from local fishermen in Bursa Province and brought live to the Biology Laboratories of Gazi University Gazi Faculty of Education in aerated tanks. Mussels were selected as medium and large-sized specimens with an average weight of 34.96±6.91 g. Water from the city network was allowed to rest in aerated aquariums for one week to ensure the removal of chlorine from the water. Mussels were placed in aquariums containing 50 L of rested water and subjected to a two-week acclimation process; during the process, they were fed with commercial spirulina once a day.

For the experiment, 4 aquariums containing 10 L of rested water each were used, and sea salt was added to achieve a salinity of ‰12. Aquarium water parameters were measured daily and maintained at a constant level (pH: 7.6±0.1; 24±1.3 ^{o}C; Salinity= ‰12±0.01). Five mussels were introduced into each aquarium. The experiment was conducted with a control group (Salinity=0.01 ppm) for 1 and 24 hours of exposure, with two replicates each.

2.2. Total Haemocyte Counts

Following exposure, mussels were anesthetized under ice, and hemolymph was taken, entering from the adductor muscle using 2.5 mL syringes. From the hemolymph, 1 mL was transferred to Eppendorf tubes and fixed with 4% formaldehyde at a ratio of (1:1). Hemocytes were counted using Thoma slides under a light microscope. The total hemocyte counts (THCs) were determined as cell/mL by analyzing according to the Yavuzcan and Benli method [15].

2.3. Lipid Peroxidation

Under ice anesthesia, gill and digestive tissues were dissected, quickly packaged in aluminum foil, and placed in liquid nitrogen. Lipid peroxidation analyses were conducted according to the method described by Mihara and Uchiyama. Gill and digestive tissues were homogenized in 1.15% KCl buffer in a cold environment, treated with thiobarbituric acid, and read at 535 nm using a spectrophotometer. The results were expressed in nM/mg tissue [16].

2.4. Statical Analysis

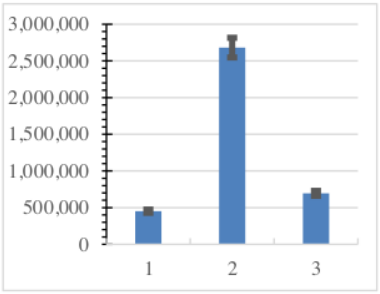
Total hemocyte counts (THCs) and malondialdehyde (MDA) results were calculated using Microsoft Excel, and the data were expressed as mean ± standard error. Graphs representing the data were also created using Microsoft Excel.

3. Results and Discussion

In recent years, the increase in salinity in freshwater has begun to raise concerns within the scientific community. Organisms exhibiting filter-feeding characteristics, such as mussels, tend to accumulate aquatic contaminants. Moreover, variations occurring in aquatic habitats lead to disruptions in various cellular, physiological, and antioxidant mechanisms [17-19]. Although there are predictions about the association of salinity impact mechanisms with hardness, information on this topic is very limited [1]. This study investigated responses induced acutely by marine salinity levels in freshwater mussels, focusing on total hemocyte counts (THCs) and the lipid peroxidation byproduct malondialdehyde (MDA) levels. No deaths occurred in the control and experimental groups during acute exposure.

3.1. Total Haemocyte Counts

Total hemocyte counts (THCs) were conducted on hemolymph tissues obtained from mussels at the end of the experiment. The results are presented in Figure 1. Following a 1-hour exposure in the experiment, THCs increased approximately 6-fold compared to the control groups. After a 24-hour exposure, despite a subsequent decrease, THCs were found to be 1.5 times higher than the levels in the control group ($p<0.05$).



1. Control Groups
2. Mussels exposed to a salinity level of ‰12 for 1 hour.
3. Mussels exposed to a salinity level of ‰12 for 24 hours.

Figure 1. Total Haemocyte Counts (THCs) (Mean±SEM) of freshwater mussels

The responses exhibited by mussels to environmental influences occur both at the cellular and extracellular levels. Any alteration in ecosystems will trigger mussels' immune systems and homeostatic mechanisms, resulting in abrupt fluctuations in the hemocyte counts within their circulatory systems. Therefore, total hemocyte counts (THCs) are a crucial parameter employed in immunological studies [20]. Responses developed against environmental stress factors have been examined in various studies. In the case of *Mytilus galloprovincialis* collected from an area where environmental conditions were disrupted, differences in THCs between stations have been reported [21]. Several studies have reported fluctuations, both increases and decreases, in THC counts in response to various environmental influences [22-24].

In a study investigating the combined effects of salinity and various contaminating pollutants, it was reported that salinity variations did not have a direct impact on THCs. Still, other humoral factors were directly influenced by salinity [25]. A study exploring the effect of water quality on THCs in ship oysters (*Barbatia decussate*) in the Iranian Basra Gulf indicated a relationship between salinity increase and THC [27]. A study examining the effects of hypoxic elevations in the diel cycle on the Hong Kong oyster (*Crassostrea hongkongensis*) reported an inverse relationship between salinity increases and THC levels [28]. Within the scope of this study, the 1-hour exposure appears to trigger the immune system, causing an acute increase in THC levels, followed by the intervention of homeostatic mechanisms to restore hemocyte counts to their original levels.

3.2. Lipid Peroxidation Byproduct Malondialdehyde Level

MDA levels were determined in gill and digestive system tissues at control, 1, and 24-hour intervals. While MDA levels showed no significant difference between control and 1-hour salinity exposures, they exhibited an

approximately 2-fold increase in gill tissue and an approximately 3-fold increase in digestive canal tissue after 24 hours (Figure 2).

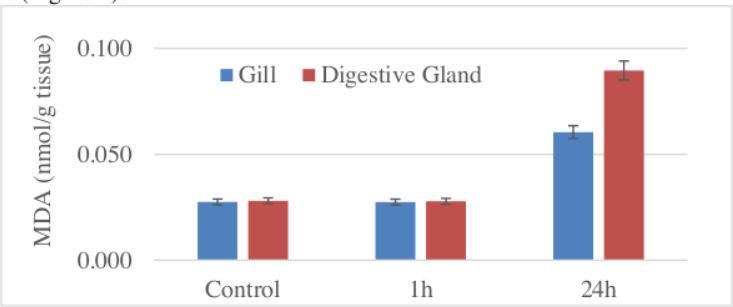


Figure 2. Changing of MDA levels in gill and digestive gland tissues in freshwater mussels

Organisms exposed to environmental stressors rapidly activate their immune systems, and the antioxidant defense system impedes cellular activities [29]. In ecotoxicology studies, lipid peroxidation is the most commonly utilized antioxidant mechanism [30]. When reviewing the literature, it has been established that malondialdehyde (MDA), a byproduct of lipid peroxidation, is used as a robust biomarker [31-32]. In this study, a significant difference was observed in both gill and digestive canal tissues of freshwater mussels exposed to 24 hours of salinity stress, particularly in comparison to the control and 1-hour groups. In *Unio ravoisieri* freshwater mussels exposed to different salinity levels for one week, it was reported that gill MDA levels increased from 0.55 to 5.35 μmol/mg, and digestive canal tissue levels increased from 1.7 to 3.6 μmol/mg [33].

A study involving Mediterranean mussels (*M. galloprovincialis*) found higher MDA levels at low salinity levels (‰14) due to cellular damage. Lower MDA levels were detected at high salinity levels (‰28-35) [34]. Another study on oysters (*Crassostrea gigas*) exposed them to salinity levels of 9-15-25-35 ppt for 10 and 17 days. In the 10-day exposure, MDA levels in oysters were similar at 9 and 35 ppt but significantly higher at 15 and 25 ppt. Additionally, in the 17-day exposure, MDA values were relatively close across all salinity levels [35].

4. Conclusion

Bu çalışma *U. delicatus*'ün tuzluluğa karşı duyarlı bir tür olduğunu göstermiştir. Sonuçlar *U. delicatus* türünün THCs ve MDA aktivitesi üzerine etkili olduğunu gösterdi. Şok etkisi olarak da adlandırılabilecek olan 1 saatlik maruziyette THCs önemli ölçüde artış göstermiş 24 saat sonunda homeostazinin devreye girerek bağışıklık sistemini dengelediği düşünülmektedir. İlk 1 saatlik maruziyette solungaç ve sindirim dokularına hücresel hasara bağlı MDA değerinde önemli bir değişiklik olmazken 24 saatlik maruziyet sonucunuda MDA seviyeleri 2-3 kat artış göstermiştir. Tuzluluğun ‰12 seviyelerine çıkmasının kısa sürede hücresel hasara sebep olabileceği düşünlümektedir. Bu çalışma ile tuzluluk ve tatlısularda yaşayan canlıların fizyolojik biyobelirteçleri arasındaki etkileşimin daha fazla araştırılması gerektiği anlaşılmaktadır.

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