EVALUATION OF METALLOTHIONEINS LEVELS IN THE MARINE GASTROPOD *PHORCUS TURBINATUS* (BORN 1778) ALONG THE NORTHEASTERN AND EASTERN COASTS OF TUNISIA

Wafa Boulajfene 1*, Evangelia Strogyloudi 2, Ammar El Mlayah 3 and Sabiha Zouari-Tlig 4

¹ Université Tunis-El-Manar, Faculté des Sciences de Tunis- Unité de Biologie Intégrative et Ecologie Evolutive et Fonctionnelle des Milieux Aquatiques - wboulajfene@gmail.com

² Hellenic Centre for Marine Research - Institute of Oceanography – Ecotoxicology Laboratory

³ Technopôle de Bordj Cedria - Centre de Recherches et des Technologies des Eaux - Laboratoire de Géo-Ressources

⁴ Université Tunis-El-Manar, Faculté des Sciences de Tunis- Unité de Biologie Intégrative et Ecologie Evolutive et Fonctionnelle

des Milieux Aquatiques

Abstract

This work is an estimation of metallothioneins rates in the digestive gland of *Phorcus turbinatus*, collected from the northeastern and eastern coasts of Tunisia during different seasons. The results suggested a seasonal variation of the protein's levels (high concentration in winter), which could be explained by the strong correlations of these rates with the physicochemical environmental variables (temperature, salinity, pH and dissolved oxygen). Furthermore, the analyses of metallothioneins content suggest variations depending on intrinsic factors of organisms (size, weight, digestive gland weight).

Keywords: Gastropods, Rocky shores, Ecotoxicology, Tunisian Plateau

Introduction

Phorcus turbinatus (Born 1778) species is found in abundance at the rocky midlittoral of the Tunisian coasts. Researches on this bio indicator are still rather few. For this reason, we were interested in estimating the metallothioneins rates (low molecular weight protein) in the studied snail along the northeastern and eastern rocky coasts of Tunisia.

Material and methods

In order to estimate the variation of metallothioneins levels in *P. turbinatus*, a seasonal monitoring was conducted between April 2014 and January 2015 at six stations (Zarzouna, La Goulette, Korbous, Sidi Daoued, Kelibia and Monastir). Salinity values, water temperature, dissolved oxygen level and pH were recorded *in situ*. At each sampling, 30 adult individuals ([15-25mm]) were collected. The size, the total weight and the gland weight of each specimen were recorded. Digestive glands were extracted and homogenized. Proteins were isolated through a series of precipitations and resolubilizations and were allowed to react with DTNB ([1]). The colored solutions were analyzed using a spectrophotometer and the absorbences were correlated to MTs rates (µg MTs / g wet tissue) based on the values of standard solutions. The significance of the differences was evaluated using Kruskal-Wallis test.

Results

All studied stations, except La Goulette, presented a high rate of MTs in winter and less important levels in spring and autumn (Fig. 1). The maximum values of MTs were recorded at La Goulette in summer and at Zarzouna, Kelibia and Sidi Daoued stations in winter. The minimum value was observed at Korbous station in spring. The comparisons of MTs rates showed significant differences between seasons (p-value = 0.006) and not significant differences between stations (p-value = 0.721).



Fig. 1. Seasonal changes of metallothioneins (MTs) rates

At Zarzouna station, MTs content depends on dissolved oxygen level, pH, temperature, individuals' total weight and size. The levels of this protein in

La Goulette, vary according to the size and the weight of the individuals and to the weight of the digestive glands. At Korbous, the rate of MTs was closely related to temperature, dissolved oxygen and the total weight of individuals. It is also influenced by the size of the shell and the weight of the gland. As for Sidi Daoud station, it showed a MTs rate depending on the oxygen levels, salinity, temperature, pH and the size of the shells. At Kelibia station, the rate of MTs was strongly correlated to all the physicchemical variables. Except salinity and total weight of the individuals, the protein level in Monastir was influenced by the other studied variables.

Tab. 1. Estimated correlation between MTs rates and the various factors S = salinity; T $^\circ$ = water temperature; TL = total length; TW = total weight; GW = digestive gland weight; + Positive correlation; - Negative correlation

	S	10	pН	O2	TL.	IW	GW
Zarzouna	0.496	0.934 -	0.825 -	0.750 +	0.832 -	0.999 -	0.571 -
La Goulette	0.250	0.280	0.070	0.343	0.756 +	0.633 +	0.964 -
Korbous	0.428	0.971 -	0.330	0.761 +	0.519 -	0.794 -	0.540 -
Sidi Daoued	0.799 -	0.681 -	0.685 -	0.807 +	0.724 +	0.339	0.298
Kelibia	0.826 -	0.865 -	0.957 -	0.916 +	0.030	0.024	0.321
Monastir	0.353	0.566 -	0.756 -	0.572 +	0.679 +	0.458	0.679 -

Discussion and Conclusion

The absence of spatial variation in the rate of MTs could be related to the geographical proximity of the studied stations. The high levels of MTs, registered in winter, could be explained by the strong correlations of these concentrations with the physicochemical environmental variables (temperature, salinity, and pH decrease and dissolved oxygen level rise) ([2]). Furthermore, the analysis of MTs concentrations suggests variations depending on intrinsic factors of organisms (size, weight, digestive gland weight).

References

1 - 1- Ellman G.L. 1959, Tissue sulphydryl groups. Archives of Biochemistry and Biophysics, 82: 70-77.

2 - 2- Smaoui-Damak W., Berthet B. and Hamza-Chaffai A. 2009, *In situ* potential use of metallothionein as a biomarker of cadmium contamination in *Ruditapes decussates*. *Ecotoxicology and Environmental Safety*, 72: 1489-1498.