DNASE ACTIVITY AS A NEW BIOMARKER FOR MARINE ENVIRONMENTAL CONTAMINATION: FIELD STUDY

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Abstract
In mussels *Mytilus galloprovincialis* collected at different sampling sites and in those injected with organic seawater extracts the DNase activity in the hepatopancreas was higher than in mussels from a maricultured area or control mussels respectively. The enzyme activity in hemocytes decrease, increase or remain constant depending on the specific contamination of the sampling site. The DNase activity, as a novel biomarker, can be useful for the monitoring of marine environmental contamination.
Keywords: Pollution, Adriatic Sea, Bivalves, Enzymes

Introduction
The wide range of mollusks response to pollutants provides grounds for the development of various methods for water toxicity evaluation and development of new biomarkers. The activity of lytic enzymes, such as acid DNase, can provide a new promising model for such studies. The response of acid DNase activity to toxic industrial pollutants in the freshwater snail [1] and in mussels from contaminated areas [2] or in mussels exposed to marine pollutants [3] was recently reported. The aim of this study was to validate the acid DNase activity as a new biomarker for environmental contamination in mussels collected at sampling sites with different pollution histories and in mussels injected with organic extracts of different seawater samples.

Materials and methods
Mussels *Mytilus galloprovincialis* were collected at 6 different locations: shipyard (S2, S6), harbor (S3), river mouth (S4) and urban/industrial waste area (S7). Mussels from a maricultured area served as a reference sample. Another group of mussels was injected with organic extracts of seawater samples collected from different locations along the Croatian Adriatic Coast. The water samples have been collected in a protected area (S1), shipyard (S5), harbor (S9) and urban/industrial waste areas (S7, S8). Mussels injected with DMSO were used as experimental control. After 1h the acid DNase activity was measured in hemocytes and hepatopancreas according to Fafandel et al. [3].

Results and discussion
The specific DNase activity in hemocytes and hepatopancreas of native and injected mussels is presented in Table 1. In mussels collected at sampling sites S2, S3, S4, S6 and S7 the DNase activity in the hepatopancreas was significantly higher than in mussels from a maricultured area. The enzyme activity in hemocytes was significantly higher only at sampling sites S3, S4 and S7. The highest DNase activity in both tissues has been detected in mussels collected at sampling site S7, an area under high influence of urban/industrial wastes. In mussels injected with organic extracts of seawater from various sampling sites (S1, S5, S7 and S9), the DNase activity in the hepatopancreas significantly increased, when compared to the control. The highest enzyme activities (cca. twice as higher as in the control) have been detected in mussels injected with organic extracts of seawater collected in a shipyard ad harbor. The enzyme activity in hemocytes significantly increased only in those mussels injected with organic extracts from the sampling site S9 (harbor) and significantly decreased for those injected with extract from the sampling site S8 (urban/industrial waste area). The suppression of enzyme activity as a specific response of hemocyte acid DNase to organic extract S8 (urban/industrial waste area) could be consistent with its effect on cells of the bivalve immune system [4], including fluctuations in hemocyte numbers [5]. The DNase activity in the hepatopancreas of both native and injected mussels revealed sampling site S7 as the polluted one.

Tab. 1. Specific DNase activity in hemocytes and hepatopancreas of native and injected mussels

Sampling site	HEMOCYTES Specific DNase activity			HEPATOPANCREAS Specific DNase activity		
	ΔF/mg/min*10^{-1}	% of cont.	p < 0.05	ΔF/mg/min*10^{-1}	% of cont.	p < 0.05
Native						
Mariculture	70 ± 22	100	*vs* mari.	11 ± 13	100	*vs* mari.
S2	79 ± 18	112		104 ± 5	914	*
S3	103 ± 12	146	*	91 ± 4	806	*
S4	107 ± 7	152	*	105 ± 2	926	*
S6	86 ± 18	122		105 ± 4	926	*
S7	103 ± 10	146	*	110 ± 6	968	*
Injected						
Control/DMSO	53 ± 14	100	*vs* cont.	30 ± 9	100	*vs* cont.
S1	52 ± 3	97		47 ± 7	155	*
S5	70 ± 8	132		58 ± 3	190	*
S7	52 ± 3	98		48 ± 12	159	*
S8	39 ± 4	74	*	34 ± 8	113	
S9	71 ± 11	134	*	57 ± 15	187	*

Conclusion
The potential use of the acid DNase response in hepatopancreas and hemocytes of mussels *Mytilus galloprovincialis* for the determination of mixed marine environmental contamination is confirmed. The DNase activity as a biomarker could be applied for both native and injected mussels. The latter might be used when the native ones are not available. At the same time, the DNase activity could be included during the determination of various enzymes activities in protein microarrays in the monitoring of integrative biological effects of contaminants.

References
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