MUSSEL DNASE ACTIVITY AS A NEW BIOMARKER FOR ENVIRONMENTAL CONTAMINATION: RESPONSE TO MODEL POLLUTANTS

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Abstract
In this study the determination of pollutant effect on acid DNase activity was performed on mussel *Mytilus galloprovincialis* hemocytes and hepatocytes. The specific enzyme activity in unexposed mussels from mariculture area was higher in hemocytes than in digestive gland. Acid DNase activity response to detergent, gasoline and copper sulphate exposure was both pollutant- and tissue-specific.
Keywords: Mollusca, Ecotoxicology, Enzymes, Toxins

Introduction
Mussels adjust their function to environmental changes by reacting to various contaminants accumulated from the surrounding water and serve as bioindicators of coastal water quality. The aim of this study was to investigate the changes in the specific acid DNase activity in the mussel *Mytilus galloprovincialis* exposed to three types of model pollutants, a detergent as a surfactant, gasoline as a polycyclic aromatic hydrocarbon mixture and copper as a heavy metal. Digestive gland as a detoxifying organ and hemocytes as a major internal defense tissue were selected as target organs.

Materials and methods
The mussel *M. galloprovincialis*, average mass 10± 2 g and length 4± 1 cm were obtained from mariculture and served as a control group and reference sample. Mussels (30 specimens) were exposed in 201 tanks to ariel detergent (0.05, 0.5 or 5 mg/l), gasoline (0.05, 0.5 or 5 mg/l) or copper sulphate, $CuSO_4$x $7H_2O$ (2.5, 25, or 250 mg/l) for 3 days. DNase activity was measured fluorometrically by a method originally developed for neutral DNases [1] and adapted to acid DNases [2].

Results and discussion
Comparison of specific acid DNase activity in hemocytes and digestive gland of control mussels revealed hemocytes as a tissue with the higher enzyme activity. This result indicates that the requirement for enzyme activity is headed to restriction of DNA in the process of phagocytosis in hemocytes. Acid DNase activity response in hemocytes and digestive gland of mussels exposed to detergent, gasoline and copper sulphate is presented in Fig. 1A. In specimens exposed to detergent or gasoline the significant increase in enzyme activity was observed in both tissues. The highest enzyme activity was detected in mussels exposed to gasoline. A dramatic change in acid DNase activity was observed in the digestive gland of mussels exposed to gasoline and copper sulphate. Exposure to copper sulphate increased enzyme activity in the digestive gland but significantly suppressed it in hemocytes. Detergent and copper sulphate induced a fast response (24 h) of the enzyme activity in both tissues (Fig. 1B1, 1B3) while metabolic biotransformation of lipophylic constituents was probably the cause of the delayed enzyme response (48 h in hemocytes and 72 h in digestive gland) to gasoline (Fig. 1B2). Suppression of enzyme activity as a specific response of hemocytes acid DNase to copper sulphate is consistent with its effect on the cells of the immune system in bivalves [3]. Acid DNase activity response in hemocytes and digestive gland of mussels exposed to model pollutants was both tissue- and pollutant specific but the potential of acid DNase response in digestive gland of mussel to serve as an "all or nothing response" biomarker of contaminant exposure is promising.

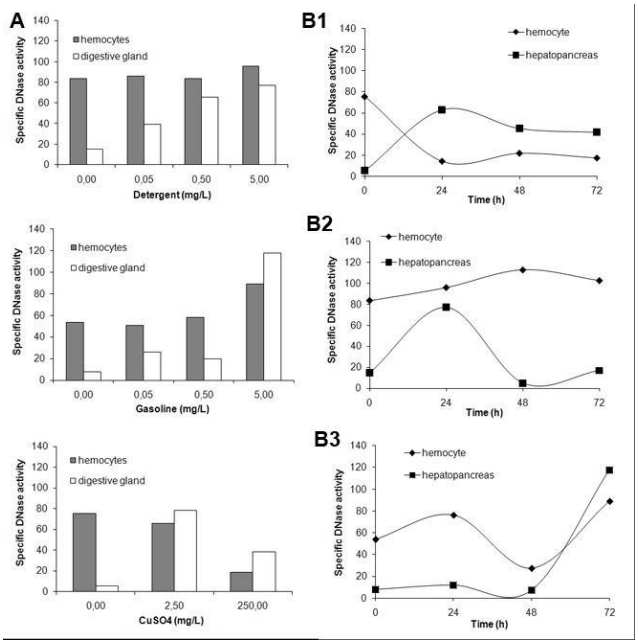

Fig. 1. Dose (A) and time (B1, B2, B3) response of acid DNase activity of hemocytes and the digestive gland of the mussel Mytilus galloprovincialis to different pollutants. B1- detergent (5 mg/l), B2- gasoline (5 mg/l), B3- copper sulphate (250 mg/l).

References
1 - Choi, S.-J.,Szoka, F.C., 2000. Fluorometric determination of deoxyribonuclease I activity with Picogreen. *Anal.Biochem*. 281, 95-97.
2 - Fafandel, M., Bihari, N., Peric, L., Cenov, A., 2008. Effect of marine pollutants on the acid DNase activity in the hemocytes and digestive gland of the mussel *Mytilus galloprovinciallis. Aquat. Toxicol*. 86, 508-513.
3 - Anderson, R., Mora, L., Thompson, S., 1994. Modulation of oyster (Crassostrea virginica) hemocyte function by copper as measured by luminal-enhanced chemiluminiscence. *Comp. Biochem. Physiol*. C 108, 218- 220.