

COMMUNICATION

Biology

LEVELS OF DNA DAMAGE IN CRAYFISH HEMOCYTES

A. E. SIMONYAN¹, T. A. HARUTYUNYAN¹, A. L. GEVORGYAN¹, E. H. TADEVOSYAN¹,
B. K. GABRIELYAN², N. S. BADALYAN², R. M. AROUTIOUNIAN^{1*}

¹ Chair of Genetics and Cytology YSU, Armenia

² Institute of Zoology of NAS RA

The genotoxicity of water pollution in Lake Sevan, near the Sevan peninsula, village Shorja and its basin (rivers the Gavaraget and the Dzknaget) was investigated. An assessment of DNA integrity in hemocytes of crayfish (*Astacus leptodactylus*) was performed using the Comet assay. The obtained results of DNA damage level of crayfishes from the aquatic environment near village Shorja, the Gavaraget and the Dzknaget Rivers demonstrate significantly higher levels than from the peninsula of Lake Sevan. The results suggest that crayfishes are sensitive test objects for ecotesting of natural water bodies.

Keywords: Comet assay, genotoxicity, crayfish hemocytes, pollution of water bodies.

Introduction. Increased concern for environmental health problems resulted a considerable interest in monitoring the pollution status of aquatic ecosystems. It is known that a number of chemicals presented in freshwater can have genotoxic and even mutagenic properties. To analyze the genotoxic effect of pollution in different water bodies of Armenia, the hemocytes of crayfish *Astacus leptodactylus* have been chosen as a suitable test object [1–4]. In several studies crayfish has been used as indicators of aquatic pollution, since they tend to accumulate pollutants (e.g. metals) in their tissues [2]. Single-cell gel electrophoresis (comet) assay is a sensitive and effective method for determining DNA damage *in vitro* and *in vivo* [5].

The aim of our study was to evaluate the levels of DNA damage of crayfish caught from different water bodies of Armenia using the Comet assay and to estimate the sensitivity of these organisms as indicators of pollution.

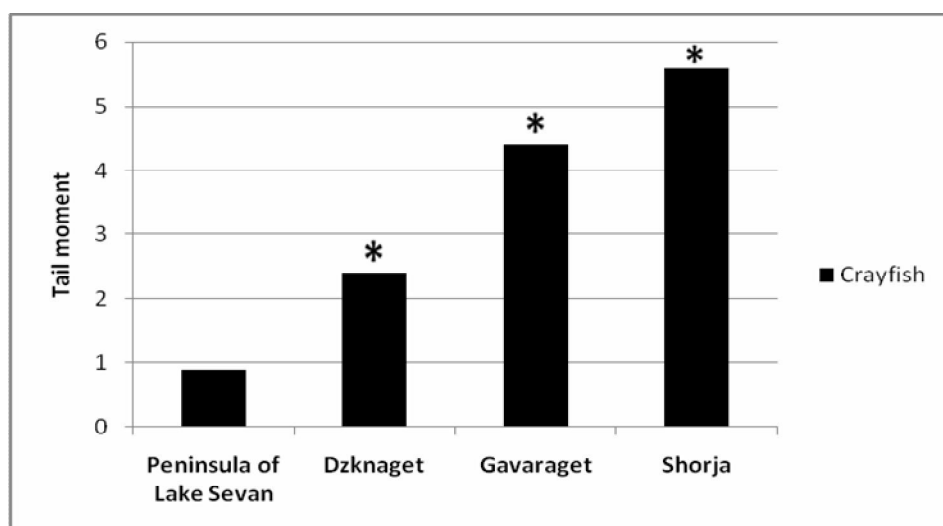
Experimental Part. Crayfish for experimental studies were provided by the Institute of Zoology of NAS RA.

Crayfish (*Astacus leptodactylus*) were caught from Lake Sevan: near the peninsula of Lake Sevan and village Shorja and its basin (rivers the Gavaraget and the Dzknaget). A group of crayfish from the peninsula of Lake Sevan was considered as a control. DNA damage was evaluated in hemocytes, obtained from

* E-mail: genetik@ysu.am

the pericardial sinus by syringe and needle. As an anticoagulant a homemade solution was used: 0.49 M NaCl, 30 mM trisodium citrate, 10 mM EDTA, pH 6 in ratio hemolymph : anticoagulant =1:1.5. The level of DNA damage was evaluated by standard alkaline single-cell gel electrophoresis (Comet assay) [5]. Slides were examined using fluorescent microscope (ZEISS, Germany) at 250×magnification. At least 150 cells were scored per animal (50 cells scored per each of three replicate slides). Images of comets were recorded with a video camera with high sensitivity (Variocam, PCO, Germany) and processed on a computer program Comet Assay IV (Version 4.3).

Statistical analysis of the results was performed using SPSS 19 with application of non-parametric Mann-Whitney test (U-test). The levels of DNA damage in hemocytes of crayfish from aquatorium of Lake Sevan nearby the peninsula and viillage Shorja and its basin (rivers the Gavaraget and the Dzknaget) were analyzed by parameter of Tail intensity and Tail moment (see Figure) of Comet assay.



The levels of Tail moment in crayfish hemocytes.

* – significant difference ($p < 0.01$) in comparison with the peninsula of Lake Sevan.

Significant differences in both parameters of DNA damage were detected ($p < 0.01$) in hemocytes of crayfish from rivers the Gavaraget and the Dzknaget and water near village Shorja in comparison with peninsula of Lake Sevan.

The levels of DNA damage by both parameters were significantly higher in crayfish from Shorja and the Gavaraget River, in comparison the levels of DNA damage of crayfish from the Dzknaget River ($p < 0.001$). So, according to obtained results of genotoxicity, we can classify the DNA damage in crayfish: Shorja \geq Gavaraget $>$ Dzknaget $>$ peninsula of Lake Sevan.

The correlation analysis on the base of comparison of the levels of DNA damage with several ions in studied water bodies was performed (see Table). The results reveal low correlation between parameters of DNA damage and analyzed ions in water.

Correlation coefficients between the levels of DNA damage (Tail intensity and Tail moment) and chemical content of investigated water reservoirs of Armenia

Parameters of comets	Nitrate ions	Si	Al	P	Fe	Mn	Cu	V
Tail intensity	0.44	0.33	0.2	0.5	0.26	0.23	0.17	0.49
Tail moment	0.35	0.22	0.09	0.4	0.14	0.1	0.05	0.42

Earlier we revealed such correlation between the levels of DNA damage with several ions in the same water bodies for *Carassius auratus gibelio* [6].

The possible reason of such discrepancy can be, since crayfish are inhabitants of benthos, the results have to be compared with ions concentrations in benthos. This research is now realized. However, our study confirmed that the application of crayfish for ecotoxicological research in water bodies can be informative.

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