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Vulnerability of biomarkers in the indigenous mollusk *Anodonta cygnea* to spontaneous pollution in a transition country

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ABSTRACT

The aim of this study was to estimate the sensitivity of biomarkers of stress and exposure in the bivalve mollusk *Anodonta cygnea* to spontaneous anthropogenic activities typical for the Western Ukraine. Three sites were examined during spring, summer and autumn: an agricultural site (A); the cooling pond of nuclear power plant (N) and a forestry close to the municipal water inlet (F). Common temporal changes of a battery of biochemical markers in the gills and hemolymph and morphological characteristics were shown by discriminant functional analysis. Classification trees built on the basis of the screened biomarkers demonstrated persistent peculiarities at each site: genotoxicity (nuclear abnormalities) at site A and endocrine disruption (high levels of vitellogenin-like proteins (Vtg-LP) in hemolymph) at site F. Interim local effects were best characterized by metallothionein (MT) concentrations, lipid peroxidation (LPO), activities of glutathione S-transferase and lactate dehydrogenase, and the conditional index of the gills. In autumn, the mollusks from the three sites revealed the highest differences in pollution status: the activation of antioxidant defense and cholinesterase were typical for site A, highest levels of MT related to high levels of Cu and Cd in the water at site B, and a steep increase in the level of Vtg-LP and the decrease of lysosomal membrane stability were recorded at the site selected as reference (F). The biomarker alterations recorded at site F were later related to an emergency event at the municipal dump located nearby. Thus, our case study demonstrated the reliability of using biomarkers of exposure to assess both long-term and accidental environmental pollution loads.

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1. Introduction

The Galicia–Volyn area (Western Ukraine) is recognized to be one of the most ecologically clean areas in Ukraine. It is explained by a collapsed large-scale economy due to the ongoing transfer of a socialist-type command economy to a market-based one, typical for transition countries. However a total destruction of the water purification systems accompanied by an increased usage of agrochemicals in local small farms caused both the communal services and agricultural wastes to be dangerous polluters of the surface waters in this area (Lebedynets et al., 2004; http://www.ua-reporter.com/eng/51314). In general, it appears that a substitution of point sources of pollution by diffuse and more complex pollution takes place here. Our knowledge about the changes of the health status of aquatic organisms caused by these spontaneous anthropogenic activities is limited. Our previous investigations based on biological responses to potentially dangerous inputs of anthropogenic loads in freshwater environments in the Western Ukraine confirmed local and season-dependent impact on the aquatic biota in this area (Stolyar et al., 2008; Falfushynska and Stolyar, 2009; Falfushynska et al., 2009). However, the extent of permanency, specificity and severity of the environmental effects on the health status of aquatic animals in this area is unclear.

Bivalve mollusks are considered reliable—sentinel species for the bioindication of aquatic pollution due to their sedentary nature, filter-feeding behaviour and ability to bioaccumulate pollutants. Their characteristics of oxidative stress, markers of genotoxicity and cytotoxicity are frequently utilized for the assessment of environmental impacts on their health status (Regoli, 1998; Baršienė et al., 2006; Bocchetti and Regoli, 2006; Hagger et al., 2006; Viarengo et al., 2007). However, the validity of biochemical characteristics in bivalve mollusks to reflect the chemical composition of pollution is rather questionable. Among common markers of exposure to specific pollutants, only metallothioneins (MTs), as markers of biologically available heavy metals in excess (Cd, Cu, Zn, Hg), are entirely approved for utilisation in mollusks. Other biochemical

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responses on specific kinds of pollution, such as carbamate and organophosphate pesticides, and environmental estrogens are considered to date as of low sensitivity and specificity (Riffeser and Hock, 2002; Hagger et al., 2006; Viarengo et al., 2007). However, some works with freshwater bivalves as bioindicators demonstrated cholinesterase activity (ChE) and vitellogenin-like protein (Vtg-LP) in hemolymph as adequate indicators of environmental pollution (de Lafontaine et al., 2000; Binelli et al., 2005). Additionally, in case of non-pointed and spontaneous sources of pollution, the influence of different environmental factors like high seasonal variability, and the complexity of pollutant mixtures might cause additive, synergistic or opposite effects on the measured molecular and/or cellular endpoints.

The aim of this study was the comprehensive estimation of the sensitivity of biomarkers of stress and exposure in the indigenous bivalve mollusk *Anodonta cygnea* to spontaneous anthropogenic activities and the selection of most important biomarkers. As the estimation of environmental effects via the comparison of specimens from at least three areas meets desirable demand of field studies (Da Ros et al., 2002; Lionetto et al., 2003), three mollusk populations from areas with different types of human activity of the Galicia–Volyn area were studied during spring, summer and autumn. The set of biomarkers included well recognized markers of oxidative stress (superoxide dismutase activity (SOD), concentration and redox state of the main nonprotein cellular thiol glutathione and lipid peroxidation (LPO)), cytotoxicity (lysosomal membrane stability) and genotoxicity (micronucleus test). The probable markers of exposure to specific kinds of pollution, glutathione S-transferase (GST) activity, although this marker in mollusks is mostly considered in relation to oxidative stress than to the ability of forming conjugates in the II phase of biotransformation (Viarengo et al., 2007), the ChE activity and Vtg-LP were evaluated. Biomarker measurements were performed in the respiratory organ, the gills as the first target tissue for contaminants (Salánki et al., 2003; Bebianno et al., 2004), and in the hemolymph.

2. Materials and methods

2.1. Experimental groups

The experiments were carried out during the middle of May, July and September. *A. cygnea* were collected manually from 0.5 to 1 m depth in three sites located in the Galicia–Volyn area, Western Ukraine (Fig. 1). The forestry (F) site is located in the upstream portion of river Seret (near the village Ivachiv, 49°49′N, 25°23′E) where no industrial contamination was expected, and close to the area of a municipal water inlet. The agricultural (A) site is situated in the lower portion of the Nichlava River near the boroughs of the city of Borshchiv (48°48′N, 26°00′E) that receives effluents from the region with intense agricultural activity and also from the city, in which a creamery is the most prominent source of pollution and the waste water plants are absent. The site with consistently higher water temperature (N) is located on the southern bank of the cooling pond of Khmelnytskyi Nuclear Power Plant (NPP) in Netishyn (in a forestry area on the tributary of river Goryn, 50°21′N, 26°38′E). The connection via water between these three sites is absent, and the distance between sites is about 100–200 km, so we may confirm that mollusks from the selected sites represent three different populations.

The samplings were carried out simultaneously in all sites. Individuals were transported to the laboratory in cages with native water and treated within a day after the sampling procedure. Water samples were also collected and transferred to the laboratory in iced packs for the determination of chemical parameters. For each biochemical parameter eight gills and hemolymph samples were prepared individually. Each procedure of tissue analysis was carried out at a temperature around 4 °C. Lysosomal membrane stability and micronuclei were determined in the hemocytes, Vtg-LP – in the hemolymph, all other biomarkers – in the gills.

For enzymatic measurements in the gills, tissue samples were homogenized (1/10 w/v) in 0.1 M pH 7.4 phosphate buffer contain-

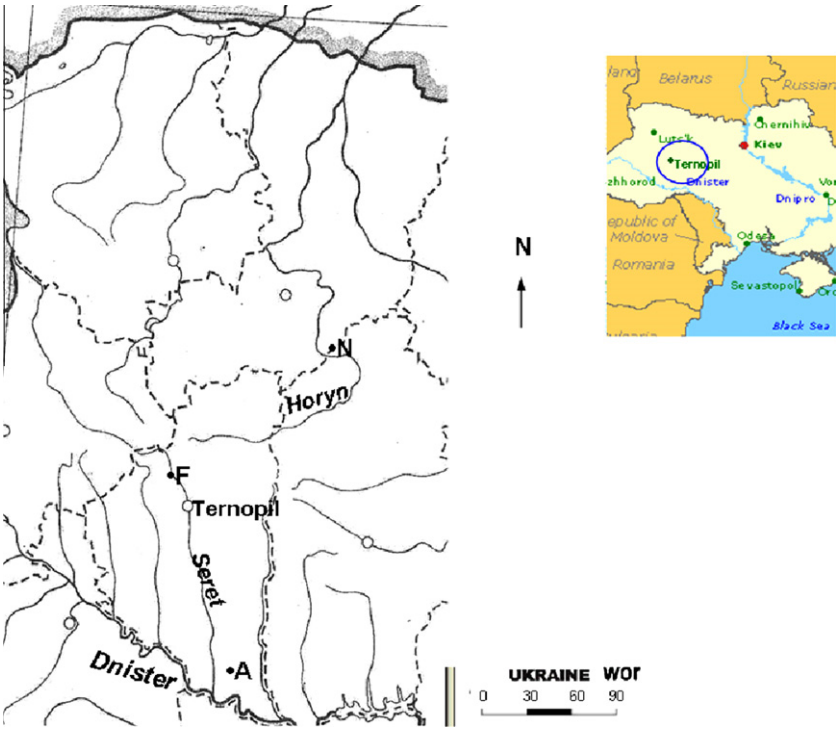

Fig. 1. Localization of the sampling sites in Galicia–Volyn area, Western Ukraine. Sites: 1, F; 2, A; 3, N. The map from http://worldmap.org.ua/Pages/Europe/Ukraine/Map_of_Ukraine_eng_0022.html was utilized.

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