Aquatic Toxicology 155 (2014) 52–61

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

# Towards a better understanding of biomarker response in field survey: A case study in eight populations of zebra mussels

S. Pain-Devin[a,*], C. Cossu-Leguille[a], A. Geffard[b], L. Giambérini[a], T. Jouenne[c], L. Minguez[a], B. Naudin[c], M. Parant[a], F. Rodius[a], P. Rousselle[a], K. Tarnowska[d], C. Daguin-Thiébaut[d], F. Viard[d], S. Devin[a]

[a] Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC), Université de Lorraine, UMR 7360 CNRS, Metz, France
[b] Unité Interactions Animal-Environnement EA4689 – Université de Reims Champagne-Ardenne, Reims, France
[c] Laboratoire Polymères Biopolymères Surfaces, UMR CNRS 6270, Université de Rouen, France
[d] Laboratoire Adaptation & Diversité en Milieu Marin, Université Pierre et Marie Curie – CNRS, UMR 7144, Station Biologique de Roscoff, France

ARTICLE INFO

*Article history:*
Received 10 January 2014
Received in revised form 20 May 2014
Accepted 14 June 2014
Available online 20 June 2014

*Keywords:*
Populations
Zebra mussels
Biomarkers
Genetic structure
Proteomics

ABSTRACT

In order to provide reliable information about responsiveness of biomarkers during environmental monitoring, there is a need to improve the understanding of inter-population differences. The present study focused on eight populations of zebra mussels and aimed to describe how variable are biomarkers in different sampling locations. Biomarkers were investigated and summarised through the Integrated Biomarker Response (IBR index). Inter-site differences in IBR index were analysed through comparisons with morphological data, proteomic profiles and genetic background of the studied populations. We found that the IBR index was a good tool to inform about the status of sites. It revealed higher stress in more polluted sites than in cleaner ones. It was neither correlated to proteomic profiles nor to genetic background, suggesting a stronger influence of environment than genes. Meanwhile, morphological traits were related to both environment and genetic background influence. Together these results attest the benefit of using biological tools to better illustrate the status of a population and highlight the need of consider inter-population difference in their baselines.



## 1. Introduction

Field studies of aquatic ecotoxicology are mainly based on the use of sentinel organisms, such as mussels. In freshwater ecosystems, the zebra mussel *Dreissena polymorpha* is often used: numerous studies have confirmed its effectiveness to detect exposure to contamination or to describe toxic effects (Viarengo et al., 2007; Voets et al., 2010; Mantecca et al., 2010). Moreover, its abundance, wide distribution, functional role in ecosystems and easiness of collecting and handling are important features of sentinel organisms (Borcherding, 2010; Voets et al., 2010). Surveys are carried out by using either passive or active monitoring. Passive monitoring refers to the implementation of biological measures (contaminant accumulation in tissues, biomarkers, etc.) in organisms sampled in local populations (e.g. Mussel Watch in United States, Kimbrough et al., 2008; ROCCH-Réseau d'Observation de la Contamination Chimique du littoral-in France, Marchand et al., 2009). As mussels are sessile organisms, they integrate contamination of the site where they live, thus providing a "contamination life history" of the site. However, the complexity of this "contamination life history" (half-life of components, metabolites, synergistic or antagonist effects) added to the biological variability (age, sex and reproductive status, parasitism, etc.) and to the local environmental conditions (temperature, conductivity, etc.) makes it difficult to reliably link biomarkers and contaminant concentrations both in animal tissues (Viarengo et al., 2007; Brooks et al., 2009) and in water or sediments. Active monitoring refers to the use of caged organisms sampled in a wild population from a pristine area and deployed for a given duration (few weeks or months) in different locations. This methodology is recommended for monitoring studies because it enables avoiding biological variability inherent to the various origins of the organisms (Roméo et al., 2003; Bodin et al., 2004; Guerlet et al., 2007; Viarengo et al., 2007; Contardo-Jara and Wiegand, 2008). Whatever the strategy is, the results and their interpretation depend on the characteristics of the mussel population used for the experiment or survey. These population characteristics can be described by two interacting components: genes and environment.

* Corresponding author. Tel.: +33 3 87378452; fax: +33 3 87378512.
*E-mail address:* sandrine.devin@univ-lorraine.fr (S. Pain-Devin).

http://dx.doi.org/10.1016/j.aquatox.2014.06.008
0166-445X/

Genetic variability and acquired tolerance/resistance have been extensively studied in several contexts: laboratory acute and chronic tests using model organisms as *Daphnia magna* (see studies by Barata and colleagues, for example, Barata et al., 2002; Breitholtz et al., 2006), resistance of insects to insecticides (Feyereisen, 1995; Amichot et al., 2000; ffrench-Constant et al., 2004), impact of pollution on genetic diversity (Bickham et al., 2000; Belfiore and Anderson, 2001; Van Straalen and Timmermans, 2002; Costa et al., 2012) and search for markers of pollution-induced responses (Laroche et al., 2002; David et al., 2012). Together these studies demonstrated that populations from different locations are often genetically different. This is not an unexpected result: the genetic background of the populations inhabiting different locations may have been selected for adaptations to the local environmental conditions. Such evolutionary adaptations are facilitated – or even promoted – when populations are little connected by gene flow, an evolutionary force balancing divergent selection in different environments. Genetic variability among populations is, however, little included in biomarker interpretation, even in the case of passive monitoring where different wild populations are used. Besides the classical links drawn between the variations of biomarker and the effects of seasonal and other environmental (water quality and levels of contaminants) variations on them, the genetic background needs to be considered as a confounding or explanatory factor of the observed response (Evenden and Depledge, 1997; Theodorakis, 2001; Coutellec and Barata, 2011). At high taxonomic level (i.e. between species), this was investigated by recognizing the existence of sensitive or resistant species. For instance, Bignell et al. (2008) or Brooks et al. (2009) recently highlighted the need to identify with accuracy the species when using mussels (*Mytilus edulis*, *Mytilus galloprovincialis* and their hybrids) because of possible misinterpretation when comparing two different species with different genetic background and physiological properties. Focusing on one defence system used as a biomarker, the multixenobiotic defence MXR, Smital et al. (2000) showed that species originating from polluted environment expressed higher basal activity of MXR than species originating from less polluted environment. This work concluded that the basal level of the defence system could be a result of evolutionary process. What about the different populations of the same species? In order to optimise understanding the responses of organisms when used as sentinel for the assessment of biomarkers in the field experiment/survey, we believe that it is important to focus not only on the delineation of species, but also at the population level.

In this context we conducted a multidisciplinary study coupling ecotoxicology, proteomics and population genetics. In order to compare the inter-population difference of basal biomarker levels, we collected zebra mussels from eight localities, in rivers or canals within four watersheds, contaminated or not, to provide a representative sampling of various environmental situations. It was postulated that basal biomarker levels would vary across populations because these basal levels are an adaptive or acclimation response to the local environment influenced by both genetic background and environmental conditions of the sampling sites.

We focused mainly on biomarkers which are early warning systems involved in (1) protection of organisms against the entry of contaminants, (2) their sequestration, (3) their inactivation (metabolism) and (4) their elimination. The following endpoints (methodology) were studied: multixenobiotic defence MXR (transport assay), glutathione-S-transferases π (gene expression; in this article the phrase gene expression is used synonymous to gene transcriptioa, although it is acknowledged that gene expression is also regulated, e.g., by translation and protein stability), lysosomal defence (histochemical determination), anti-oxidant defence (selenium-dependent glutathione peroxidase gene expression) and metallothioneins (level determination and gene expression). malondiadlehyde (MDA) was also assessed in order to provide information about toxic effects of pollutants in collected organisms. In parallel, biometric measurements were also performed. A proteomic approach was implemented to complete the information. And finally, results of this work were compared to data obtained from a population genetics study previously done (Tarnowska et al., 2013). Tarnowska et al. (2013) investigated the same localities at the same time, which allowed determining to which extent the study populations of this study were genetically differentiated.

## 2. Materials and methods

### 2.1. Site description

Zebra mussels were collected from eight sites located in four basins from the north of France (Fig. 1). In the east, we focused on the River Moselle, a tributary of the Rhine River used for commercial shipping. Three sites were chosen, covering approximately 100 kilometres of this river. Mo1 (E06°10′39″; N49°08′03″) is located in the Metz agglomeration whereas Mo2 (E06°12′91″; N49°10′46″) is located a few km downstream, after the discharge of the main waste water treatment plant; Mo3 (E06°22′05″; N49°29′01″) is located 90 km downstream in the Luxembourg part of the river. All the Mo sites are under strong industrial and urban influence. For the Meuse basin, we sampled two stations located in two canals: Me1 (E05°31′46″; N48°48′16″) is a little canal (Canal de l'Est Branche Nord) dedicated to recreational activities (boating, shipping, and fishing) and used in spring and summer. Me2 (E05°41′07″; N48°42′39″) is a larger canal (Canal de la Marne au Rhin) used for commercial shipping (e.g. goods transport). In the Seine basin, we focused on two sites, Se1 (E01°06′14″; N49°20′23″) and Se2 (E00°57′16″; N49°21′01″) located in the Rouen agglomeration, 100 km upstream the estuary. This area is under the influence of both tides and strong industrialisation and urbanisation. In the west, the last site Vi (W02°06′13″; N47°35′41″) is located in the River Vilaine used for touristic purposes (boating, shipping, etc.).

For each site, water and sediment samples were collected for physicochemical and contamination analyses.

### 2.2. Zebra mussel collection

At each site, 150–300 adult zebra mussels were retrieved by section of the byssal threads and carried to the laboratory in the water of origin (mean length in mm of the mussel shell for each river basin: Me 24.5 ± 2.5; Vi 25.6 ± 2.6; Se 21 ± 2.7; Mo 17.9 ± 2.0). Then mussels were analysed or stored, depending on the targeted endpoints (see biological endpoints for details).

Sampling was conducted in late April (Me1, 2), May (Vi) and mid June 2009 (Se1, 2, Mo1, 2, 3).

### 2.3. Biological endpoints

#### 2.3.1. Biometric data

For each site, biometric data were obtained from 20 zebra mussels: length, width and height of shell were measured (mm) and the shell volume was then calculated.

#### 2.3.2. Biomarkers – detailed protocols are available in supplementary materials

*2.3.2.1. Metallothionein quantification.* MTs were quantified in extracts prepared from individual digestive gland ($n = 8$) by differential pulse polarography (DPP-Metrohm 797 VA Computrace) with the procedure described in Thompson and Cosson (1984).