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Integrated multi-biomarker responses in two dreissenid species following metal and thermal cross-stress☆

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ABSTRACT

With current global changes, the combination of several stressors such as temperature and contaminants may impact species distribution and ecosystem functioning. In this study, we evaluated the combined impact of two metals (Ni and Cr) with a thermal stress (from 12 to 17 °C) on biomarker responses in two bivalves, *Dreissena rostriformis bugensis* and *Dreissena polymorpha*. Biomarkers are informative tools to evaluate exposure and effects of stressors on organisms. The set of 14 biomarkers measured here was representative of both physiologic (filtration activity) and cellular antioxidant and detoxification mechanisms. Our aim was to study the response pattern of both species, and its meaning in terms of invasive potential. The implications for the use of these mussels in environmental monitoring are also discussed. Results evidenced that the two species do not respond to multiple stressors in the same way. Indeed, the effects of contamination on biomarker responses were more marked for *D. polymorpha*, especially under nickel exposure. While we cannot conclude as to the effect of temperature, invasiveness could be influenced by species sensitivity to contaminants. The physiological and cellular differences between *D. polymorpha* and *D. r. bugensis* might also be of concern for environmental risk assessment. The two species present differential bioaccumulation patterns, filtration activity and cellular biomarker responses. If *D. polymorpha* populations decline, their substitution by *D. r. bugensis* for biomonitoring or laboratory studies will not be possible without a deeper understanding of biomarker responses of the new invasive.

1. Introduction

Depending on scenarios, a 0.3–0.7 °C increase is predicted in the mean surface temperature by 2016–2035. The Earth's global warming could even exceed 1.5 °C by the period 2081–2100 (IPCC, 2014). Under the current climate change, further stressors such as contaminants might impact species distribution and abundance and could modify ecosystem structure and functioning. Recent studies point out that heat stress enhances metal and polycyclic hydrocarbon toxicity (Sokolova and Lannig, 2008; Kamel et al., 2012; Attig et al., 2014). The ability of ectothermic organisms to handle such changes will depend on their acclimation or adaptation capacities (Falfushynska et al., 2014; Sulmon et al., 2015). Many tools are available to evaluate the impact of such factors on ecosystems and organisms, from physico-chemical measurements to biodiversity and community indices (Vasseur and Cossu-Leguille, 2003; Allan et al., 2006). However, while the first explore exposure and the latter describe effects at the ecosystem level, intermediate levels of biological organization need to be considered to understand mechanisms of action and develop Adverse Outcome Pathway models (Groh et al., 2015).

Biomarkers are biochemical or physiological parameters that can be measured in individuals to indicate exposure to environmental chemicals and, for some of them, to detect toxic effects (Lagadic, 2002). Their use enables the linkage of exposure with effects on individuals and seeks to predict long-term effects at higher levels of biological organization, before populations are affected. They are considered as sensitive early-warning tools to detect the impact of stressors on organisms and can be used in biomonitoring, since a modification in biomarker response may evidence that a modification in environmental conditions occurred

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(Adams et al., 2001; Depledge and Fossi, 1994; Moore et al., 2004).

The zebra mussel, *Dreissena polymorpha*, is currently used in biomonitoring as a sentinel organism to evaluate freshwater contamination. It is a widespread and abundant species with a high filtration rate, thus it is often used to evaluate the accumulation of contaminants in its tissues and to study their subsequent effects on biological processes (de Lafontaine et al., 2000; Binelli et al., 2010, 2015; Guerlet et al., 2007). It presents a moderate sensitivity against pollutants and many studies focus on the use of physiological reactions as biomarkers (Contardo-Jara et al., 2009; Pain-Devin et al., 2014). Even in bivalve species quite tolerant to environmental changes, their biomarker responses have shown a great precocity and sensitivity to metal exposure and temperature variations (Tsangaris et al., 2008; Attig et al., 2014; Kamel et al., 2014). However in the last few decades, *D. polymorpha* populations have been declining all over the world, and are being replaced by a sister-species, the quagga mussel *Dreissena rostriformis bugensis* (Nalepa et al., 2010; Matthews et al., 2014).

In order to understand the dynamic of invasion and the switch between the two species, their biology and ecology need to be compared (Ram et al., 2012). Most of the studies dealing with both species focused on their morphological (Rosenberg and Ludyanskiy, 1994; Beggel et al., 2015) or functional differences (Diggins, 2001; Peyer et al., 2009; Grutters et al., 2012), fitness-relative traits (MacIsaac, 1994; Baldwin et al., 2002; Stoeckmann, 2003; Karatayev et al., 2011) or on their bioaccumulation capacities (Rutzke et al., 2000; Richman and Somers, 2005). To our knowledge, the only study dealing with several cellular biomarkers was conducted by Schäfer et al. (2012) and focused on DNA damage, heat shock proteins, lipid content, body mass and bioaccumulation.

Studies on zebra mussels have shown that cellular endpoints responded early to environmental contamination and preceded effects at the individual level (Contardo-Jara et al., 2009; Guerlet et al., 2010). Multi-biomarker approaches are really useful in environmental quality assessment, to evaluate the impact of environmental conditions on organism's health and physiological status (Viarengo et al., 2007; Binelli et al., 2010). In our experiment, we measured a set of biomarker responses, representative of both antitoxic (antioxidant system, detoxification mechanisms) and physiological functions (cellular energetic, filtration rates) from the cellular to the individual scale. The large set of data obtained from multi-biomarker measurements can be analysed through multivariate analysis in order to integrate all the individual data and obtain a more synthetic overview of stress affecting both bivalve species.

To evaluate biomarker modulation, we exposed mussels to nickel (Ni) or chromium (Cr) and to a thermal stress (from 12 to 17 °C). The first temperature (12 °C) corresponds to the field temperature during the sampling, in March, and the second (17 °C) is considered as a thermal stress for this period. Ni is one of the priority metals under the European Water Framework Directive (2000/60/EC, 2000). It is mainly used for stainless steel production, plating and in many alloys to manufacture coins, tools, kitchen tools and mineral pigments. Ni represents approximately 0.9% of the Earth's crust, but its release into the environment is mainly due to anthropogenic sources (coal and fuel combustion, waste incineration, mining, steel manufacturing and plating) (INERIS, 2006). It is thus a metal of high environmental relevance, and many studies have shown its toxicity to aquatic organisms (Tsangaris et al., 2008; Attig et al., 2010), including *D. polymorpha* (Stuijfzand et al., 1995; Minguez et al., 2012), and its role in free radical formation (Stohs and Bagchi, 1995). Hexavalent Cr is also a pro-oxidant agent, toxic for aquatic organisms, that can cross cellular membranes and lead to the formation of reactive oxygen species during its intracellular reduction in trivalent Cr, which is able to interact with DNA (Vignati et al., 2010; Barmo et al., 2011). Cr is also used in stainless steel production and in several alloys, because it enhances metal's hardness and resistance to corrosion. It is emitted in the atmosphere through industry and gas combustion, and in watercourses via chroming, tanning, the textile industry and the manufacture of dyes and pigments (INERIS, 2005).

The aim of our study was to assess the response pattern of both species in the case of increasing water temperature and metal stress, in order to answer the following questions (1) is the quagga mussel more tolerant to environmental stressors compared to the zebra mussel, and thus may it enhance its invasiveness? (2) could the quagga mussel be used as a sentinel species for biomonitoring in case of zebra mussel disappearance?

2. Material and methods

2.1. Organism collection and biometric data

Organisms were hand-collected in March 2015 by section of their byssus and quickly transferred to the laboratory. *D. polymorpha* from Madine reservoir (Meuse, France, E05°44′52.2″ N48°55′41″) were 16.5 ± 2 mm in length and *D. r. bugensis* from the Moselle river (Jouy-aux-arches, France, E06°04′43″ N49°03′58″) were 27 ± 3 mm. The Madine population was the only population of *D. polymorpha* identified in the Moselle River basin at that time. The two species were differentiated according to morphometric parameters (Pavlova and Izyumov, 2014).

Just after sampling, 18 mussels of each species were measured (length, width and height of shell, in mm), soft tissues were weighed (without byssus, in mg) and a condition index was calculated for each individual, according to the following formula (Guerlet et al., 2010):

$$\text{Condition Index (mg mm}^{-3}) = \text{wet weight}/(\text{length} \times \text{width} \times \text{height})$$

2.2. Acclimation

The remaining mussels were acclimated for 96 h at 12 °C, which corresponds to the field temperature, without feeding before the experiment. During the first 3 days, 240 individuals of each species were placed in two separated 15 L aquaria continuously aerated and under a 10:14 light:dark period. Their water of origin was progressively replaced by spring water (Cristaline®, 1/3 per day; Ca^{2+}: 106; Mg^{2+}: 4.2; Na^{+}: 3.5; K^{+}: 1.5; HCO_3^{-}: 272; SO_4^{2-}: 50; Cl; F^{-}: 0.9 mg L^{-1}). After 72 h, 160 organisms of each species were placed in 250 mL experimental beakers (two individuals of the same species per beaker) to allow byssal fixation (80 beakers containing 2 zebra mussels each, and 80 beakers containing 2 quagga mussels each). Beakers were filled with 200 mL of Cristaline® and placed in a water bath to maintain a 12 °C temperature, under natural photoperiod.

2.3. Exposure treatments

After acclimation, mussels were treated for 96 h, under natural light and without any food source. Beakers containing pairs of mussel were emptied and Cristaline® was replaced by 200 mL of exposure media. There were five different treatments (16 replicates per treatment, *i.e.* 32 organisms): control (only water); Ni chloride (20 and 500 μg L^{-1} total ion Ni); potassium dichromate (5 and 15 μg.L^{-1} total ion Cr). Ni chloride ($Cl_2Ni \cdot 6H_2O$, ref 25851.236) and potassium dichromate ($K_2Cr_2O_7$, ref 26784.231) were purchased from VWR (Radnor, Pennsylvania). Stock solutions of Ni