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Adaptation of freshwater mussels to cyanobacterial toxins: Response of the biotransformation and antioxidant enzymes

Vanessa Burmester [a], Jorge Nimptsch [b], Claudia Wiegand [c,*]

[a] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Dept. Ecophysiology and Aquaculture, Müggelseedamm 301, 12587 Berlin, Germany
[b] Universidad Austral de Chile, Instituto de Ciencias Marinas and Limnologicas, Las Encinas 220, Valdivia, Chile
[c] University of Southern Denmark, Institute of Biology, Campusvej 55, 5230 Odense M, Denmark

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ABSTRACT

Freshwater mussels such as the invasive *Dreissena polymorpha* and the indigenous *Unio tumidus* nourish by high filtration rates and may accumulate cyanobacteria and their toxins during cyanobacterial blooms. Physiological adaptations to cyanotoxins enable organisms to endure cyanobacterial blooms but may differ between species. Biotransformation and excretion capacities for cyanobacteria and anthropogenic pollutants have been demonstrated for *Dreissena polymorpha* but less for unionid species. This study compares the activities of biotransformation (glutathione S-transferase, GST) and antioxidant enzymes (superoxide dismutase, SOD and catalase, CAT) in *Dreissena polymorpha* to *Unio tumidus* in response to cyanotoxin exposure (10 $\mu g\,L^{-1}$ and 50 $\mu g\,L^{-1}$ microcystin-LR, respectively, total microcystin from a cyanobacterial crude extract) for 24 h and 7 d exposure duration. Enzyme activities in *Dreissena polymorpha* were measured in the whole mussel tissue, digestive gland and in gills and in *Unio tumidus* in the digestive gland, gills, mantle, foot as well as in the remaining tissue. The sGST was elevated for the entire exposure period in the whole mussel tissue of *Dreissena polymorpha* but despite higher basal activities in digestive gland and gills of *Unio tumidus*, it was rather inhibited or unaltered in most of their tissues. Elevated SOD activity indicated oxidative stress response in *Dreissena polymorpha*, but not in *Unio tumidus*. The CAT activity was barely affected in both species, rather inhibited in *Unio tumidus*, despite again higher basal activities in digestive gland and remaining tissue. Compared to the indigenous *Unio tumidus*, the investigated biotransformation and oxidative stress combating enzymes respond stronger in the invasive *Dreissena polymorpha*.

1. Introduction

Decline of biodiversity or increase of migrating or invasive species is far more severe in freshwaters compared to terrestrial ecosystems (Sala et al., 2000; Dudgeon, 2010; Strayer and Dudgeon, 2010). In particular, stream ecosystems are increasingly impacted by multiple stressors causing a loss of sensitive species and an overall reduction in biodiversity (Palmer et al., 2010). Those stressors are mostly anthropogenic and include (i) a destruction or degradation of habitat structure, frequency and connectivity, (ii) a decrease of any other factor within the niche of the indigenous species, such as food availability, (iii) shelter from predation, non-excess parasitizing, and (iv) disturbance of any function for reproduction (Dudgeon et al., 2006; Ormerod et al., 2010). Also anthropogenic environmental pollutants contribute to biodiversity decline as has been demonstrated, e.g. for estuaries by Kennish (2002), for freshwater ecosystems by Dudgeon et al. (2006); or for Lake Ohrid, Macedonia by Kostoski et al. (2010). Consequently, there is a shift to more tolerant and general distributed species. Karatayev et al. (2009) evidenced invasive species amongst the more tolerant against organic pollution. In many of the bigger waterways worldwide invasive species successfully compete and reduce indigenous species (Malmqvist and Rundle, 2002). One of the highly endangered groups are the freshwater bivalve mollusks, in particular of the *Unionidea* superfamily, which face reduction worldwide, and several species are close to extinction (e.g. Bogan, 1993; Williams et al., 1993; Augspurger et al., 2007; Geist, 2010). In contrast, single species from other families, such as *Dreissenidae*, originating from the Caspian Sea exhibit a strong proliferating and invasive character in Europe and North America. Invasion of the *Dreissenidae* to North America causes high annual economical losses as the mussels settle in high densities on industrial structures, water pipes, navigational structures, ships and even fishing gear or cages. Decrease in the native unionid community following invasion of watercourses by *Dreissenidae* are well documented

* Corresponding author. Fax: +45 6550 2786.
E-mail address: wiegand@biology.sdu.dk (C. Wiegand).

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(e.g. Nalepa et al., 1996 in Lake St. Claire; Schloesser and Kovalak, 1991 in Lake Erie; Martel et al., 2001 in the Rideau River).

In Germany, the Federal Nature Conservation Act protects all species of the *Unionidae*, as the population of Unionidae is endangered due to shoreline construction and water pollution (Jaedicke, 1997) as well as by competition for food with *Dreissena polymorpha* (Pallas, 1771). In contrast to the indigenous *Unio tumidus* (Philipsson, 1788), the invader *D. polymorpha* establishes sustainable populations in urban watercourses. The moderate sensitivity to anthropogenic pollutants, combined with hard substrates as habitats and a planktonic larvae ensured the success of *D. polymorpha* (Horgan and Mills, 1997).

The abundance of Unionaceae (besides other native species) decreases in Berlin's water bodies, partly as a result of the reduction of habitats due to shoreline reinforcements in the rivers Havel and Spree and all connecting channels. However, even in suitable habitats within the city the *Unionidae* are often lacking. Environmental pollution could be the cause as *U. tumidus* shows a higher abundance at less polluted parts of the river Spree (pers. comm. Krofta, IGB Berlin). The two species are not competing for the same habitat, as the *Unionidae* submerges in soft sediments, whereas *D. polymorpha* attaches to any kind of hard substrate. *D. polymorpha* is commonly found on stones used for shoreline reinforcements or on sheet pilings, hence it benefits from increasing habitat availability, but it also colonizes tree roots or dead wood and even shells of the *Unionidae* (Ricciardi et al., 1996). Larvae colonies of *D. polymorpha* were observed to completely enclose the siphonal region of *Unionidae*, preventing normal valve opening, hence decrease food availability for the larger species (Mackie, 1993). Furthermore, the burrowing of the carrying unionid is impaired and the additional weight – up to more than the weight of the "host" – causes it to sink to deeper layers in the sediment (Burlakova et al., 2000).

The different habitat requirements also contribute to higher possible exposure to environmental pollutants for the *Unionidae*, as juvenile and adult *Unionidae* live buried in the sediment, which is a sink for many environmental pollutants (Matisoff and Eaker, 1992). Besides filtrating surface waters above the sediments, *Unionidae* also take up from the upper deposit layers of the sediment or are exposed *via* their foot directly to the sediment pore water (Raikow and Hamilton, 2001; Vaughn and Hakenkamp, 2001). All of which may result in higher uptake of harmful substances compared to *D. polymorpha*, as this species is situated well above the sediment and filtrates from surface water solely.

Contrasting to the *Unionidae*, *D. polymorpha* has been regularly employed for bioaccumulation studies in the field (e.g. Minier et al., 2006; Binelli et al., 2008), and biomarker responses were used for assessing water quality (Contardo-Jara et al., 2009a; Binelli et al., 2010; Faria et al., 2010). Guerlet et al. (2010) demonstrated spatial and temporal variations, and recovery of cellular, tissue, and individual markers in a 90 day field study with transplanted mussels. DNA adducts proved the ability of *D. polymorpha* to biotransform PAHs to DNA binding metabolites and the applicability of this endpoint for biomonitoring purposes (Le Goff et al., 2006). Success of a renaturation program was evaluated by gene expression of biotransformation and antioxidant enzymes and general cellular stress markers (Contardo-Jara and Wiegand, 2008).

Different adaptation capacities to environmental stress between both mussel species could add to the spreading of *D. polymorpha*, but these are less investigated in *U. tumidus*. Exposure of *U. tumidus* to contaminated field sites revealed decreased antioxidant enzymes and glutathione pool resulting in increased lipid peroxidation (Cossu et al., 1997; Doyotte et al., 1997). Similar, a decrease in antioxidant levels was paralleled by an increase in lipid peroxidation and mortality after laboratorial exposure of *U. tumidus* to methyl methacrylate, an intermediate in polymers production (Coffinet et al., 2008). *U. tumidus*, transplanted for 21 day to the Mosel River, strongly increased the transcription of antioxidant and biotransformation enzymes, as well as that of metallothioneins (Bigot et al., 2010). Also DNA damage of haemocytes of the related *Unio pictorum* proved to be a reliable biomarker for genotoxic effects at polluted sites (Stambuk et al., 2009).

Whether or not *D. polymorpha* is less sensitive to environmental pollutants, and which physiological mechanism would facilitate a better adaption compared to the endangered *Unionidae* species, is a recently started research field. For *D. polymorpha* larvae Faria et al. (2010) evidenced a higher susceptibility to metal and polychlorbiphenyl exposures compared to *U. elongates* glochidia. Investigations comparing adult mussels of both species are still scarce.

Besides anthropogenic pollutants, cyanobacterial mass developments recurrently threaten the urban waterbodies of Berlin, as they flow very slowly and carry a high nutrient load. Cyanobacteria can be hazardous due to the production of toxic metabolites (cyanotoxins, Carmichael, 2001). The group of microcystins are the most common cyanotoxins in limnic ecosystems, with microcystin-LR (MC-LR) as most toxic structure variant (Sivonen and Jones, 1999). Fastner et al. (2001) showed that the total cell-bound microcystin concentration in the pelagic zone of several German lakes ranged from <0.01 up to 454 $\mu g\,L^{-1}$ MC and the extracellular microcystin concentration from <0.1 up to 16 $\mu g\,L^{-1}$ MC. Microcystins generally remain inside healthy cells, hence the extracellular concentrations increases during bloom lysis events. Compared to the pelagial zone, extracellular MC concentrations are often much higher in the littoral zone where *U. tumidus* live in the soft sediment receiving the sedimenting cyanobacteria. *D. polymorpha,* attached to hard substrate, encounters the wind driven biomasses at the water surface. Microcystin bind and inactivate the cellular steering enzymes protein phosphatase type 1 and 2A (MacKintosh et al., 1990). By this mechanism, as well as causing oxidative stress, microcystins and other cyanobacterial compounds affected limnic organisms including phytoplankton, zooplankton, invertebrates and fish (reviewed in Wiegand and Pflugmacher, 2005; Zurawell et al., 2005), and possibly the two mussel species to a different extent.

Physiological adaptations enable organisms to live in cyanobacterial-contaminated water bodies up to specific densities. Biotransformation *via* glutathione S-transferase (GST) detoxifies microcystin. Conjugation to glutathione enhances the water solubility of the toxin for better excretion (Pflugmacher et al., 1998), which is further aided by activity of the P-glycoprotein, a transporter protein belonging to the multi-xenobiotic resistance facilitating proteins (Contardo-Jara et al., 2008). Antioxidant enzymes protect cells from oxidative stress caused by cyanobacterial toxins (Prieto et al., 2007; Amado and Monserrat, 2010).

Freshwater mussels such as *D. polymorpha* and *Unionids* are filter feeders, thus accumulate the cyanotoxins, however, to different extents: *D. polymorpha* accumulated up to 11 μg microcystin g DW^{-1} within one week, whereas *Unio douglasiae* took up 130–250 μg MC g DW^{-1} after only five days exposure to the cyanobacteria *M. aeruginosa* (Pires et al., 2004; Yokoyama and Park, 2003). Contardo-Jara et al. (2008) observed an immediate and continuous uptake from dissolved microcystin in *D. polymorpha* (21 $\mu g\,g\,WW^{-1}$ after 1 h exposure to 100 $\mu g\,L^{-1}$ MC-LR). To our knowledge dissolved microcystin uptake in *U. tumidus* has not been researched.

The aim of this study was to analyze if *D. polymorpha* has better capacities for biotransformation (*via* the enzyme GST) of the cyanobacterial toxin microcystin and is better equipped to cope with oxidative stress (*via* antioxidant enzymes SOD and CAT) compared to *U. tumidus*. The freshwater mussels in this study were