Science of the Total Environment 543 (2016) 449–459

Contents lists available at ScienceDirect

Science of the Total Environment

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

Differences in tolerance to anthropogenic stress between invasive and native bivalves

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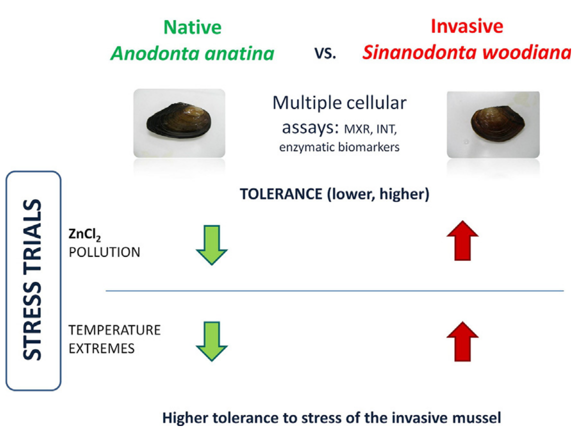
HIGHLIGHTS

- We compared tolerance to anthropogenic stress in invasive *vs.* native mussels.
- Animals were exposed to thermal stress and trace metal zinc pollution.
- RB accumulation, INT reduction capacity and enzymatic biomarkers were measured.
- Invasive bivalve showed higher tolerance to unfavourable conditions.
- Thermal stress tolerance could facilitate invasion in the context of climate change.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 May 2015
Received in revised form 31 October 2015
Accepted 9 November 2015
Available online xxxx

Editor: M. Hanson

Keywords:
Enzymatic biomarkers
Freshwater mussels
INT reduction capacity
Invasion success

ABSTRACT

Tolerance towards environmental stress has been frequently considered as one of the key determinants of invasion success. However, empirical evidence supporting the assumption that invasive species can better endure unfavorable conditions compared with native species is limited and has yielded opposing results. In this study, we examined the tolerance to different stress conditions (thermal stress and trace metal zinc pollution stress) in two phylogenetically related and functionally similar freshwater bivalve species, the native *Anodonta anatina* and the invasive *Sinanodonta woodiana*. We assessed potential differences in response to stress conditions using several cellular response assays: efficiency of the multixenobiotic resistance mechanism, respiration estimate (INT reduction capacity), and enzymatic biomarkers. Our results demonstrated that the invasive species overall coped much better with unfavorable conditions. The higher tolerance of *S. woodiana* was evident from (i) significantly decreased Rhodamine B accumulation indicating more efficient multixenobiotic resistance mechanism; (ii) significantly higher INT reduction capacity and (iii) less pronounced alterations in the activity of

Abbreviations: ABC, ATP-binding cassette; BR, Bradford reagent; BSA, bovine serum albumine; CAT, catalase; ChE, cholinesterase; ETS, electron transport system; GST, glutathione S-transferase; HB, homogenization buffer; INT, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride; MXR, multixenobiotic resistance; PB, potassium buffer; PBS, phosphate buffer saline; RB, rhodamine B; SS, substrate solution; StS, stopping solution; TL, total length.

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http://dx.doi.org/10.1016/j.scitotenv.2015.11.049

MXR mechanism activity
Tolerance to stress

stress-related enzymes (glutathione-S-transferase, catalase) and of a neurotoxicity biomarker (cholinesterase) in the majority of treatment conditions in both stress trials. Higher tolerance to thermal extremes may provide physiological benefit for further invasion success of *S. woodiana* in European freshwaters, especially in the context of climate change.

1. Introduction

A major component of human-induced rapid environmental changes involves the invasion of non-indigenous species (Sih et al., 2011; Simberloff et al., 2013). Invasive species are among the most important drivers of global biodiversity loss and ecosystem degradation (Pyšek and Richardson, 2010; Sala et al., 2000), albeit only a small fraction of all introduced species worldwide successfully passes through all stages of invasion process to become invasive (Lockwood et al., 2013; Williamson and Fitter, 1996). Due to such high variability in invasion success between different non-indigenous species, the prediction of successful invaders represents a major challenge for invasive species management (Kolar and Lodge, 2001). Tolerance to pollution and environmental stress has been frequently considered among the key determinants of invasion success (Braby and Somero, 2006; Cassey, 2002; Jeschke and Strayer, 2006; Kolar and Lodge, 2001; Marchetti et al., 2004; Olden et al., 2006; Williamson and Fitter, 1996). However, empirical evidence that invasive species generally cope better with unfavorable conditions is limited and has yielded opposing results (Faria et al., 2010b; Lenz et al., 2011; Prenter et al., 2004).

In order to examine whether invasive species are more tolerant to stress compared with their native counterparts, we performed a series of trials to determine the enzymatic and physiological responses to thermal stress and trace metal (zinc) pollution stress of two freshwater mussel species. Freshwater ecosystems are highly susceptible to biological invasions, exhibit a high degree of endemism and extinction rates (Strayer, 2010), and face increasing human pressures (Ricciardi, 2001; Strayer, 2010). Mussels are important components of these ecosystems (Vaughn and Hakenkamp, 2001) in terms of density and biomass and due to their key role in ecosystem structure and functioning through filtration, bioturbation and availability of shells as a feature of habitat heterogeneity (Lopes-Lima et al., 2014). In recent decades freshwater bivalves are experiencing a pronounced global decline due to a suite of anthropogenic pressures (Lopes-Lima et al., 2014). Due to their sedentary lifestyle, mussels are particularly exposed to environmental stress. The inability to regulate body temperature makes bivalves sensitive to acute changes in water temperature, and filtration feeding leads to high exposure to pollutants (Ambasht and Ambasht, 2012; Geist, 2010; Sousa et al., 2015). Some of their characteristics, such as broad geographical distribution, high reproductive potential, ease of collection and the ability to survive in polluted environments and bioaccumulate toxic pollutants, have prompted the wide use of mussels in ecotoxicological studies during the last decades (Della Torre et al., 2014; Farris and Van Hassel, 2006; Kelly et al., 2010; Schloesser and Schmuckal, 2012).

In this study, we analyzed two phylogenetically related and functionally similar bivalves, the native *Anodonta anatina* (Linnaeus, 1758) and the invasive *Sinanodonta woodiana* (Lea, 1834). The native *A. anatina* is widespread throughout Europe (Froufe et al., 2014; Lopes-Lima et al., 2015), and in Croatia it inhabits watercourses belonging to both Adriatic and Black Sea drainage (Lajtner, pers. data). *S. woodiana* is a successful invader of European freshwaters. It has been introduced to Europe unintentionally through aquaculture of the East Asian cyprinid species from the Amur River, which bore glochidia (the parasite larval stage of the bivalve) in their gills (Benkő-Kiss et al., 2013) and spread rapidly across Europe (Benkő-Kiss et al., 2013; Guarneri et al., 2014). In Croatia, its invasive range covers the whole continental part of the country. It was first recorded in Croatia in 2001 in the Danube River, but is assumed to have been present in Croatian water bodies for at least two decades (Lajtner and Crnčan, 2011). It negatively affects native European Unionids, such as *A. anatina*, through direct competition for food, habitat and fish hosts for its larvae (Douda et al., 2012; Sousa et al., 2014). Also, it exhibits higher growth rates and higher reproductive potential compared to native Unionid species (Dudgeon and Morton, 1983; Pou-Rovira et al., 2009). Severe declines of *A. anatina* or even its complete replacements by the invasive *S. woodiana* have already been reported in the literature (Cappelletti et al., 2009). Lastly, the two species co-occur extensively in the large rivers in Croatia (Lajtner, pers. data).

Interspecific differences in physiological/cellular responses to stress were studied using several biomarkers that were expected to respond to both stress conditions, rather than using specific biomarkers of metallic or thermal stress. The following biomarkers were used: efficiency of the multixenobiotic resistance (MXR) mechanism, respiration estimate (INT reduction capacity), and enzymatic biomarkers of stress response (glutathione-S-transferase, GST; catalase, CAT) or neurotoxicity (cholinesterase, ChE). MXR mechanism activity in gill tissue of many aquatic organisms represents a first line of cellular defense against diverse xenobiotics (Luckenbach and Epel, 2005; Smital et al., 2004). High MXR efflux activity in gills was previously reported for many aquatic species, including bivalves, protecting them from various contaminants, e.g. trace metals (Bielen et al., 2014; Bošnjak et al., 2014; Luckenbach and Epel, 2005; Smital et al., 2004; Stevenson et al., 2006). In particular, zinc exposure induced the MXR mechanism in brown mussel *Perna perna* (Franco et al., 2006) and in the freshwater clam *Corbicula fluminea* (Achard et al., 2004). Moreover, physical stressors, e.g. temperature, can also induce MXR activity in gill tissue of bivalves (Eufemia and Epel, 2000; Farcy et al., 2009; Minier et al., 2000). INT reduction capacity assay has been frequently employed as a proxy for cellular respiratory potential and is often referred to in literature as electron transport system (ETS) activity assay (García-Martín et al., 2014; Martínez-García et al., 2009; Musko et al., 1995; Schmidlin et al., 2015; Simčič, 2005; Simčič and Brancelj, 2004; Simčič et al., 2014; Žagar et al., 2015). INT reduction capacity is known to be affected by temperature and trace metals in aquatic invertebrates, including bivalves (Gagné et al., 2007; Schmidlin et al., 2015). Finally, enzymatic biomarkers used in this study are the most frequently analyzed biomarkers in ecotoxicological tests (Badiou-Bénéteau et al., 2012; Canesi et al., 2012; Jemec et al., 2010; Klaper et al., 2009). ChE hydrolyses substrates such as acetylcholine in the synaptic cleft of cholinergic nerves (Sussman et al., 1991), and the level of its inhibition is an established biomarker of neurotoxicity (Fulton and Key, 2001). On the other hand, enhanced ChE activity may indicate an inflammation process due to tissue injury (De Oliveira et al., 2012; Falugi et al., 2012; Falugi and Aluigi, 2012). GST belongs to the class of detoxifying enzymes, and its activity is induced during the detoxification processes (Jemec et al., 2007). Similarly, the induction of CAT activity, an antioxidative enzyme that decomposes the hydrogen peroxide to water and oxygen, is an indication of oxidative stress. The activity of both ChE and antioxidant enzymes such as CAT and GST in bivalves have been found very susceptible to thermal stress (Pfeifer et al., 2005; Park et al., 2008).

2. Materials and methods

2.1. Reagents

Reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), Kemika (Zagreb, Croatia), Merck Milipore (Germany) and Pierce (Rockford, IL, USA). All chemicals used in RB accumulation, INT reduction