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Effect of the exposure to suspended solids on the enzymatic activity in the bivalve *Sinonovacula constricta*

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A B S T R A C T

Aquatic animals are susceptible to sudden changes of their living environment but they adopt strategies to cope with adverse environmental challenges. Contamination by suspended solids, often associated with a dramatic change in the concentrations of important water-quality variables is a frequent occurrence in China's coastal waters and estuaries. Here we studied the impact of suspended solids on the activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), as well as adenosine triphosphates (including Na^{+} K^{+}-ATPase, $Mg^{+\ +}$-ATPase, $Ca^{+\ +}$-ATPase) and H^{+} K^{+}-ATPase in the gills and visceral mass tissues of the molluscan bivalve *Sinonovacula constricta* exposed (4, 8, 12, 16, 20, and 24 days) to various concentrations of suspended solids. Our results showed that the antioxidant enzymes cooperated closely to effectively scavenge superoxide anion free radicals and H_2O_2 (which can ultimately inhibit gill activity) through the modification of SOD and/or CAT enzymatic activities. ATPases activity (considered to be a sensitive indicator of toxicity) could play an effective role in the maintenance of functional integrity of the plasma membranes as well as some other intracellular functions. After the exposure, a decrease in the Na^{+} K^{+}-ATPase, $Mg^{+\ +}$-ATPase, and $Ca^{+\ +}$-ATPase activity of the gills was observed suggesting that they were inhibited by the treatments. These results also indicated that, from day 4 to day 16, exposure to high concentrations of suspended solids had an inhibitory effect on the activity of H^{+}-K^{+}-ATPase in the visceral mass of *S. constricta*. However, after a period of adaptation the H^{+}-K^{+}-ATPase activity was restored to original levels. Our results suggest that long-term exposure to high levels of suspended solids disturb osmoregulation, gastric acid secretion and digestion, cause oxidative damage, as a consequence of antioxidant enzymes inactivation which eventually damages the gills, affect the food intake and transformation, ultimately resulting in systems failure and eventually death.

1. Introduction

During the past few decades, intensive engineering projects in coastal areas, such as sea reclamation, harbour dredging and disturbance of oceanographic and atmospheric cycles (such as extreme weather events and wave action) have led to high variability in suspended solids levels, which poses a threat to health of marine organisms (Howlett, 2003; Orpin et al., 2004; Szostek, Davies, & Hinz, 2013). Recently, benthic shellfish aquaculture adjacent to marine construction areas in Liaoning Province in China are confronting high levels of mortality, which may be related to the high concentrations of suspended solids, amongst other factors (contaminants transported by the high levels of suspended solids, such as heavy metals, nutrients and organic compounds and buried suffocation effect). Analyses have shown that the concentration of suspended solids near the marine construction work zone can achieved 467.75 mg/L (Yang et al., 2009).

Anthropogenic disturbance due to towed bottom-fishing gear (Black & Parry, 1995), ocean engineering (Bilotta & Brazier, 2008) and dredged material deposition (Desprez, 2000; Robinson, Newell, Seiderer, & Simpson, 2005; Smith et al., 2008; Suedel, Lutz, Clarke, & Clarke, 2012) have resulted in an increase of suspended solid materials and turbidity in water. High concentrations of suspended solids have a negative impact on the physical (Bilotta & Brazier,

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2008), chemical (Dawson & Macklin, 1998; Haygarth et al., 2006; Kronvang, Laubel, Larsen, & Friberg, 2003; Miller, 1997; Russell, Walling, Webb, & Bearne, 1998) and biological properties (Birkett, Tollner, & Gattie, 2007; Galbraith, MacIsaac, Macdonald, & Farrell, 2006; Robertson, Scruton, & Clarke, 2007; Shaw & Richardson, 2001; Francoeur & Biggs, 2006; Suren, Martin, & Smith, 2005) of coastal waters.

Suspended solids are major stressors to bivalves, and cause abrasion of the gills, high variability in feeding rates, physiological stress, behavioural modifications and increase their susceptibility to diseases (Kiørboe et al., 1980; Leverone, 1995; Barillé, Prou, Héral, & Razet, 1997; MacDonald, Bacon, & Ward, 1998; Ellis, Cummings, Hewitt, Thrush, & Norkko, 2002; Zheng, Cui, Jia, & Bu, 2009). These factors can impair bivalve growth, survival and reproduction (Bricelj, Malouf, & De Quillfeldt, 1984; Gilmour, 1999; MacDonald et al., 1998; Meager & Batty, 2007). Behavioural modifications can include modifications in the filtering mechanism of bivalves, resulting in a reduction of feeding and filtration rates (Barillé et al., 1997; Ellis et al., 2002; Hewitt & Pilditch, 2004; Shin, Yau, Chow, Tai, & Cheung, 2002; Velasco & Navarro, 2003). Increasing suspended solids concentrations induce physiological responses in bivalves are often manifested as a decrease in the clearance rate (Bacon, MacDonald, & Ward, 1998; Ward & MacDonald, 1996) and oxygen consumption (Alexander, Thorp, & Fell, 1994; Grant & Thorpe, 1991). In bivalves, high levels of suspended solids impel a significantly higher rate of shell closures or “coughs” (to clear sediment from the mantle) and “claps” (an escape response) (Last, Hendrick, Beveridge, & Davies, 2011, p. P76), resulting in an accelerated heart rate, decrease of blood oxygen concentration and promote anaerobic metabolism (Guderley & Pörtner, 2010).

Superoxide dismutase (SOD) and catalase (CAT) play a crucial antioxidant role and constitute the primary defence line against xenobiotics through a neutralizing ROS (Stegeman et al., 1992; Yuan, Chen, Zhou, Liu, & Yang, 2010). It has been shown that high levels of suspended solids interfere with the antioxidant enzymatic defence mechanism in mollusc tissues, such as in *Ostrea plicatula* and in *Haliotis diversicolo* (Shen, Ma, & Chen, 2007; Wang, Xie, Yu, & Lan, 2007).

Adenosine triphosphatases (ATPases) are membrane-bound enzymes that play a role in intracellular functions such as osmotic pressure, membrane permeability, and cellular volume. They are considered to be sensitive indicators of toxicity (Sancho, Fernandez-Vega, Ferrando, & Andreu-Moliner, 2003; Yadwad, Kallapur, & Basalingappa, 1990) and thus can be used for assessing membrane fragility of the gills in aquatic animals (Stagg, Rusin, & Brown, 1992). The H^+-K^+-ATPase enzyme is responsible for the acid secretion mediated by the parietal cell of the gastric mucosa (Caplan, 1998) and catalyses the electro-neutral exchange of intracellular H^+ and extracellular K^+ coupled with the hydrolysis of cytoplasmic ATP (Shin, Munson, Vagin, & Sachs, 2009; Weidemüller & Hauser, 2009), which is an important gastric acid regulator in the gastric membrane vesicles (Zhang et al., 2013a,b).

The present study aims to quantify the long-term impact of high levels of suspended solids on the physiology of the adult bivalve mollusc *Sinonovacula constricta*. In this study, two laboratory based experiments were performed: (1) to test the adaptability and survival rate of *S. constricta* exposed to suspended solids, and (2) to investigate the enzyme activity in the gills and visceral mass of adult *S. constricta* exposed to high levels of suspended solids.

2. Materials and methods

2.1. Bivalves preparation

Sinonovacula constricta bivalve molluscs were collected at low tide from the Yingkou Coast, Bohai Sea, Yingkou City, Liaoning Province, China, and obtained from an aquaculture farmer in October 2011. Prior to the beginning of the experiments, molluscs were maintained in the laboratory for a month in 80 L glass aquarium (60 cm × 50 cm × 40 cm) filled with filtered seawater without suspended solids. During the acclimation period, aeration was provided continuously, with the bivalve density being approximately 123 ind/m^2 (i.e. 40 per aquarium). Seawater was completely changed daily and the water temperature was maintained at (15 ± 1) °C. Specimens collected from tanks were kept under natural-photoperiod conditions and fed with a mixed diet of *Chaetoceros* sp. and *Chlorella* sp. twice a day (8:00 a.m. and 17:00 p.m.). Shell length and height and the wet weight of *S. constricta* was (7.08 ± 0.22) cm, (2.38 ± 0.06) cm and (19.9 ± 0.89) g, respectively.

2.2. Mud sample preparation

Mud samples were collected from the surface sediment along the Yingkou coast, ventilated to dryness and then baked to a constant weight at 60 °C. Dried mud samples were ground to a fine powder and filtered using a standard sieve mesh of 400 meshes, which retrained particles of less than 38 μm. The portion that passed through the sieve was sealed and stored for use in experiments. The concentration of heavy metals, sulphides, oils, and organic matter (TOC) in the sediment were determined using standards for marine monitoring (GB17378-2007). The content of Cu, Pb, Zn, Cd, As, Hg, S, oils and TOC were: Cu: 15.2–18.6 mg/kg; Pb: 15.4–23.8 mg/kg; Zn: 35.5–54.5 mg/kg; Cd: 0.06–0.22 mg/kg; As: 2.06–4.17 mg/kg; Hg: 0.005–0.035 mg/kg; S: 93.6–116.5 mg/kg; oils: 23.2–45.3 mg/kg: and COD: 0.13–0.27 mg/kg. These complied with the standard concentrations required for a first class marine sediment quality.

2.3. Experimental procedure

A 24-day experiment was set-up to analyse the impact of suspended solids on the enzyme activity of *S. constricta*. Animals were exposed to nominal concentrations of suspended solids of 0 mg L^{-1} (Control: Group A), 100 mg/L (Level 1: Group B), 1000 mg/L (Level 2: Group C), and 10,000 mg/L (Level 3: Group D) based on (Fahey & Coker, 1992; Liu, He, Shao, Zhang, & Song, 1998; Shin et al., 2002; Ma, Gong, Liu, Chen, & Song, 2004; Song et al., 2006; Zheng et al., 2009). Glass aquariums (60 cm × 50 cm × 40 cm) were filled with 90 L filtered seawater from the Heishijiao seashore in Dalian (Liaoning Province, China) and maintained at a constant temperature (12–14 °C) and water circulation achieved with a submersible stream pump (Voyager 2, Sicce, Italy) and four 10 cm-long rectangular air stones connected to an air compressor. Each treatment consisted of three replicates and each replicate contained 30 individuals. The water quality during the experiments was maintained as follows: dissolved oxygen >(7.2 ± 1.0) mg/L; salinity: 31‰–34‰; and pH at 8.2 ± 0.05 and the animals were maintained under a photoperiod 10:14 (Light: Dark) hour cycle. Seawater was renewed daily and the sediment was replenished to maintain approximately constant concentration levels and to avoid sedimentation. The concentration of suspended solids in the seawater was calibrated using turbidity and measuring it with a HACH RA710XR Turbidmeter. *S. constricta* were fed with a mixture of *Chaetoceros* sp. and *Chlorella* sp. twice daily (at 8:00 a.m. and 17:00 p.m.). The control and exposed (three replicates in which two individuals were tested per treatment) groups were collected randomly at days 4, 8, 12, 16, 20 and 24 and frozen at −80 °C until further analysis. Experimental groups were checked daily and dead individuals removed.