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Short communication

Temperature-dependent effects of cadmium on mitochondrial and whole-organism bioenergetics of oysters (*Crassostrea virginica*)

G. Lannig, A.S. Cherkasov, I.M. Sokolova *

Biology Department, University of North Carolina at Charlotte, 9201 University City Boulevard, Charlotte, NC 28223, USA

Abstract

Intertidal mollusks are exposed to multiple stressors in estuaries, including temperature and trace metals such as cadmium, which may interactively affect their physiology. We have studied the combined effects of temperature and cadmium stress on metabolism of oysters at the whole animal and mitochondrial levels. In vivo exposure to 50 μg L^{-1} Cd led to a significant increase in basal metabolic rate (BMR) in 20 °C-acclimated but not in 28 °C-acclimated oysters. Cadmium exposure resulted in a fast decrease in mitochondrial capacity to synthesize ATP in 28 °C-acclimated but not 20 °C-acclimated oysters indicating that mitochondria may be functioning closer to their capacity limits in the former group. This agrees with elevated mortality in Cd-exposed oysters at 28 °C but not 20 °C. In general, elevated temperature increased sensitivity of oysters to cadmium at mitochondrial and whole-organism levels suggesting that oyster populations may become more susceptible to trace metal pollution during seasonal warming and/or global climate change.

Keywords: Temperature; Cadmium; Basal metabolic rate; Mitochondria; ATP synthesis; Proton leak; *Crassostrea virginica*

Temperature and heavy metals are common stressors in estuaries, and their importance is increasing due to the global climate change and pollution of the coastal waters.

* Corresponding author. Tel.: +1 704 687 8532; fax: +1 704 687 2831.
E-mail address: insokolo@uncc.edu (I.M. Sokolova).

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Cadmium is an important environmental contaminant, which is bioaccumulated by marine mollusks including oysters (Roesijadi, 1996). As are most trace metals, cadmium is toxic at high concentrations, and increasing evidence points toward mitochondrial dysfunction as a mechanism of cadmium toxicity (Byczkowski and Sorenson, 1984; Sokolova, 2004; Wallace and Starkov, 2000). Temperature strongly affects mitochondrial function in poikilotherms including oxidation rates, coupling and ROS production (Abele et al., 2002; Keller et al., 2004; Poertner, 2002). Therefore, temperature and cadmium stress converge on a common intracellular target – mitochondria, and thus have a potential to synergistically affect energy metabolism. However, nothing is known about the impact of prolonged acclimation to elevated temperatures on cadmium effects upon mitochondrial function and bioenergetics.

The aim of this study was to determine the effects of cadmium exposure on mitochondrial and whole-organism bioenergetics of eastern oysters, *Crassostrea virginica*, acclimated to different temperatures within the environmentally relevant range (20° and 28 °C). Oysters were collected in pristine areas of Hewlett Creek and Stump Sound, NC. Water temperature at the time of collection 20–25 °C. Prior to the start of the experiment, oysters were acclimated for 6–7 days at 20°or 28 °C and 30‰ and then either kept in clean artificial seawater (ASW) (control) or ASW with addition of 50 μg L^{-1} Cd as $CdCl_2$ (Cd-exposed oysters) for 10–40 days at the same temperature. BMR was determined as oxygen consumption of resting oysters fasted for 12–18 h and measured in flow-through respiration chambers using fiber-optic oxygen microsensors (WPI, Sarasota FL, USA). Mitochondria were isolated from control and Cd-exposed oysters as described in Sokolova (2004). Oxygen consumption of isolated mitochondria was measured at 20 °C using Clarke-type oxygen electrodes (Qubit Systems, Kingston, ON, Canada). State 3 respiration indicative of the maximum rate of ATP synthesis was determined after addition of ADP and state 4+ respiration indicative of mitochondrial proton leak was measured in the presence of an ATPase inhibitor, oligomycin, as described in Sokolova (2004).

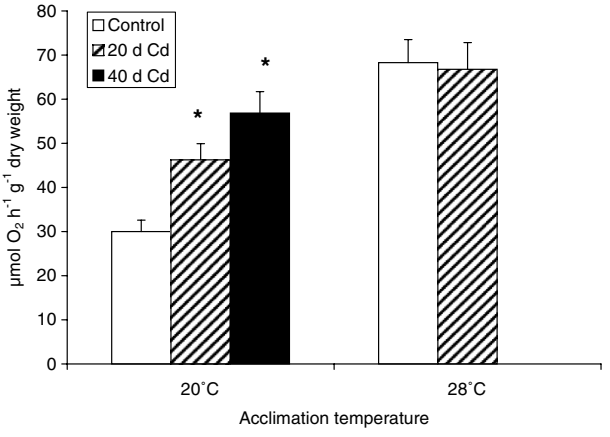

Fig. 1. Effects of cadmium exposure and acclimation temperature on basal metabolic rate of *C. virginica*. 20 d, 40 d – duration of cadmium exposure (days). Asterisks represent values significantly different from the respective controls ($P < 0.05$). $N = 4–6$.

BMR significantly increased in Cd-exposed oysters by nearly 100% above the respective control levels after 40 days at 20 °C (Fig. 1) indicating an elevated cost of living induced by cadmium stress in these oysters ($P < 0.01$, $N = 4$). Acclimation at 28 °C also resulted in strongly elevated BMR of oysters ($P < 0.01$, $N = 6$). However, there was no further increase in BMR due to Cd-exposure in the warm-acclimated group ($P > 0.5$). Combined exposure to cadmium and elevated temperature resulted in significantly higher mortality of oysters than temperature stress or cadmium stress alone. After 30 days of exposure, no mortality was observed at 20 °C, whereas at 28 °C, 25% and 45% of oysters died in control and Cd-exposed groups, respectively.

Cadmium effects on mitochondrial function were also strongly temperature-dependent (Fig. 2). At 20 °C, we found no effects of cadmium exposure on mitochondrial ATP synthesis or proton leak (ANOVA: $F_{1,33} = 1.65$, $P = 0.21$ and $F_{1,33} = 0.51$, P=0.458, respectively). In contrast, acclimation to 28 °C resulted in a gradual decrease in mitochondrial rates of ATP synthesis and proton leak, which was significantly faster and more pronounced in Cd-exposed oysters (ANOVA: $F_{1,30} = 4.66$, $P = 0.04$ and $F_{1,30} = 4.65$, $P = 0.04$, respectively).

Thus, combination of elevated temperature and cadmium stress are significantly more damaging to oysters than exposure to a single stressor alone. Elevated BMR in Cd-exposed oysters at 20 °C suggests an increased cost of basal maintenance possibly due to the activation of detoxification mechanisms such as synthesis of metallothioneins, replacement of damaged proteins and/or maintenance of intracellular ion homeostasis (Cattani et al., 1996; Leung and Furness, 2001; Roesijadi, 1996). Acclimation to 28 °C goes hand in hand with a dramatic increase in BMR reflecting elevated costs of maintenance of ion gradients across cellular and mitochondrial membranes at high temperatures (Somero, 1998; Poertner, 2002). At 28 °C there was no further increase in BMR due to cadmium exposure suggesting that detoxification mechanisms may be energy-limited at high temperatures. This agrees with the observation that Cd-exposed oysters experienced elevated mortality at 28 °C indicating failure of detoxification mechanisms and increased physiological stress. This elevated stress cannot be explained by higher cadmium uptake

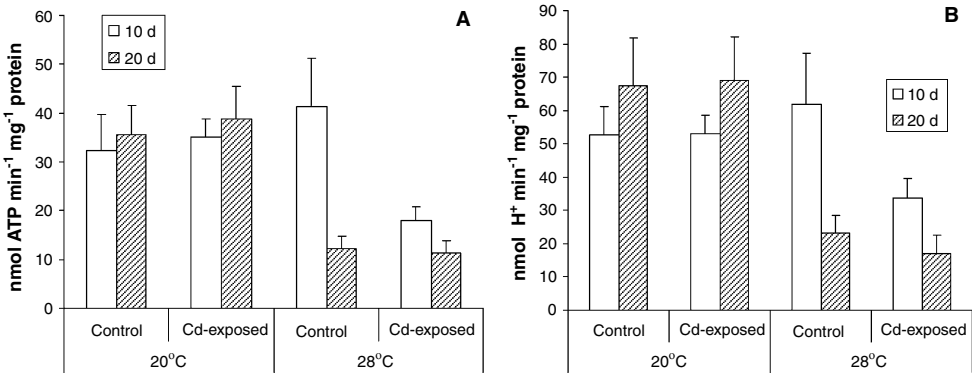

Fig. 2. Effects of cadmium exposure on rates of mitochondrial ATP synthesis (A) and proton leak (B) in *C. virginica* acclimated at 20 and 28 °C for 10 or 20 days. ATP synthesis rates were calculated from state 3 respiration rate assuming P/O ratio of 1.36 determined in our preliminary experiments. Rate of proton leak was calculated from state 4+ respiration assuming translocation of 6 mol protons per mol consumed O. $N = 5$–12.

rates, which were similar in oysters at 20°C and 28 °C (130.7 ± 27.6 and 98.4 ± 27.8 μg Cd g^{-1} dry gill weight after 20 days, $P > 0.1$, $N = 4$–5). Mitochondrial capacity for ATP synthesis did not keep up with elevated BMR in Cd-exposed or warm-acclimated oysters suggesting that mitochondria may be working closer to their capacity limits under conditions of elevated temperature and cadmium stress. Overall, enhancement of cadmium toxicity by elevated temperatures suggests that oyster populations subjected to temperature rise due to seasonal warming or global climate change may become more susceptible to trace metal pollution and vice versa. This emphasizes importance of considering environmental temperature when studying cadmium toxicity in ectotherms and has important implications for understanding the environmental effects of cadmium in the face of the global climate change in estuaries.

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