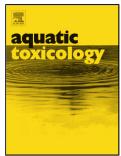
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## ACCEPTED MANUSCRIPT

## EFFECTS OF ELEVATED CO<sub>2</sub> levels on subcellular distribution of trace metals (CD and CU) in marine bivalves

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Highlights

- Effects of  $CO_2$  on Cu and Cd accumulation were studied in bivalves.
- Cd strongly accumulated in organelles and enzymes of clams and oysters.
- Cu accumulated in mitochondria of oysters but not in those of clams.
- Elevated CO<sub>2</sub> increased Cd accumulation in organelles and potentially Cd toxicity.
- Elevated CO<sub>2</sub> enhanced Cd detoxification in clams and suppressed it in in oysters.

**Abstract.** Hypercapnia (elevated CO<sub>2</sub> levels) and pollution with trace metals such as Cu and Cd are common stressors in estuarine habitats that can negatively affect physiology and health of marine organisms. Hypercapnia can modulate toxicity of trace metals including Cu and Cd; however, the physiological and cellular mechanisms of the metal-CO<sub>2</sub> interactions are not well understood. We investigated the effects of elevated  $P_{CO2}$  (~800 and 2000 µatm) and metal exposure (50 µg l<sup>-1</sup> of Cu or Cd) on subcellular distribution of metals in two common species of marine bivalves, Eastern oysters *Crassostrea virginica* and hard shell clams *Mercenaria mercenaria*. Oysters accumulated higher burdens of Cu and Cd in the gill tissues compared to clams. In both studied species, Cu was predominantly associated with the metabolically active cell compartments (mitochondria, lysosomes, microsomes and cytosolic enzymes), with a modest fraction sequestered by metallothioneins ( $\sim$ 30%) and the insoluble metal-containing granules (MCG) (~15-20%). Unlike Cu, Cd was largely sequestered by metallothioneins ( $\sim$ 60-70%), with a relatively small fraction associated with the organelles and the cytosolic enzymes. Mitochondria were the main intracellular target for trace metals accumulating higher concentrations of Cd (and in the case of ovsters – of Cu) than other organelles or cytosolic enzymes. Cu accumulation in the metabolically active cellular compartments was independent of the CO<sub>2</sub> levels, while Cd content of the organelles

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