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Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossolus*) and the protective effect of dissolved organic carbon

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ABSTRACT

Marine water quality criteria for metals are largely driven by the extremely sensitive embryo–larval toxicity of *Mytilus* sp. Here we assess the toxicity of four dissolved metals (Cu, Zn, Ni, Cd) in the mussel *Mytilus trossolus,* at various salinity levels while also examining the modifying effects of dissolved organic carbon (DOC) on metal toxicity. In 48 h embryo development tests in natural seawater, measured EC50 values were 6.9–9.6 μg L^{-1} (95% C.I. = 5.5–10.8 μg L^{-1}) for Cu, 99 μg L^{-1} (86–101) for Zn, 150 μg L^{-1} (73–156) for Ni, and 502 μg L^{-1} (364–847) for Cd. A salinity threshold of >20 ppt (~60% full strength seawater) was required for normal control development. Salinity in the 60–100% range did not alter Cu toxicity. Experimental addition of dissolved organic carbon (DOC) from three sources reduced Cu toxicity; for example the EC50 of embryos developing in seawater with 20 mg C L^{-1} was 39 μg Cu L^{-1} (35.2–47.2) a 4-fold increase in Cu EC50. The protective effects of DOC were influenced by their distinct physicochemical properties. Protection appears to be related to higher fulvic acid and lower humic acid content as operationally defined by fluorescence spectroscopy. The fact that DOC from freshwater sources provides protection against Cu toxicity in seawater suggests that extrapolation from freshwater toxicity testing may be possible for saltwater criteria development, including development of a saltwater Biotic Ligand Model for prediction of Cu toxicity.

1. Introduction

Trace metals are natural components of the biosphere. Although some metals are essential for life (Engel and Sunda, 1979), all metals are toxic at sufficiently high concentrations; for some there is a narrow range of concentrations between what is essential and what is toxic. Elevated metal concentrations can cause a severe reduction or elimination of intolerant species, thereby having a significant effect on the diversity and trophic structure of the biological community (Peterson, 1986). However, the disruption of such systems is not determined merely by the concentration of the metal. A number of environmental and biological processes may influence the bioavailability of metals to organisms (Luoma, 1983). To date, most research has focused on the aspects of environmental water chemistry that modify the bioavailability of dissolved metals to freshwater species. This body of research has led to the development of the Biotic Ligand Model (BLM) which takes into account the protective effects of different components of water chemistry, either through competition with the metal or complexation of the metal. The BLM has proven to be effective in the prediction of toxicity for a variety of dissolved metals in freshwaters of diverse chemical composition making it a valuable resource for assessing environmental effects of metals in freshwater ecosystems (Di Toro et al., 2001; Paquin et al., 2002; Niyogi and Wood 2004).

Increased concerns regarding the use of oceans as a site for the disposal of anthropogenic wastes and the large scale use of copper and other metals in antifouling boat paint have prompted evaluation of metal toxicity to marine species (Apte and Day, 1998). Today, there is a considerable body of research outlining the toxicity of various metals to marine species. However, available data often exhibit wide variation in sensitivity. For instance mussel larvae of the genus *Mytilus* are widely accepted to represent one of the most sensitive marine organisms to Cu (e.g. Grosell et al., 2007) and therefore play a critical role in environmental regulations. Yet, the US EPA's (2003) ambient water quality criteria document reports mean acute values (EC50s in 48 h developmental tests) of 21.4 μg L^{-1} dissolved Cu (at 20 ppt salinity) for *Mytilus edulis* larvae but 6.1 μg L^{-1} dissolved Cu (at 28–30 ppt) salinity for *Mytilus* spp. respectively. This variation could reflect either the importance of water chemistry in influencing the

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Table 1
Measured water chemistry parameters for representative test solutions

	SW	60% SW	10 μg Cu L^{-1} 100% SW	100 μg Zn L^{-1} 100% SW	150 μg Ni L^{-1} 100% SW	500 μg Cd L^{-1} 100% SW	10 mg C L^{-1} NR 100% SW	10 mg C L^{-1} SW 100% SW	10 mg C L^{-1} LM 100% SW
pH	7.96	7.50	7.88	7.85	7.88	7.88	7.96	7.92	7.89
Na (mM)	492±13.1	282±7.5	497±12.5	503±8.7	511±11.4	507±12.7	499.5±20	502.5±22.3	497±20.5
K (mM)	9.1±1.5	5.3±0.5	9.0±1.1	9.8±0.1	9.5±0.5	9.5±1.2	9.1±0.7	9.0±1.3	9.1±0.8
Ca (mM)	11.6±0.3	6.6±2.5	11.2±0.1	11.5±0.1	11.5±1.2	11.3±0.9	11.5±1.1	11.8±2.1	11.8±1.0
Mg (mM)	50±0.8	32±9.1	48.8±1.7	56.3±1.5	53.7±3.4	56.4±2.5	50.1±4.1	52.0±3.1	49.4±1.1
Cl (mM)	539±10.1	343±22.5	539±18.5	545±10.5	544±32.5	544±22.2	534±17.1	533±15.7	539±20.1

(means±SEM).
Notes: 1. SW=seawater; NR=Nordic Reservoir NOM; SR=Suwanee River NOM; LM=Luther Marsh NO 2. All representative test solutions denoted by nominal values.

bioavailability of the metal, differences in physiological sensitivity of the organisms at different salinities, or true inter-species differences.

More knowledge about water chemistry effects could lead to the development of saltwater criteria adjusted for water chemistry, similar to the Biotic Ligand Model approach that has been used in freshwater. Collecting toxicity data from diverse sources, Arnold et al. (2005, 2006) have recently presented evidence that the toxicity of dissolved copper to *Mytilus* spp. is a function of the dissolved organic carbon (DOC) concentration of the test sample. However this analysis was based on correlations using natural variations in DOC concentration rather than experimental manipulation of DOC concentrations (Arnold, 2005; Arnold et al., 2006). Previous experimental studies in freshwater (Schwartz et al., 2004; De Schamphelaere et al., 2004; Glover et al., 2005) have shown that DOC from different sources can offer differential protection against toxicity of dissolved metals based on the heterogeneity of physicochemical properties of the DOC sample.

With this background in mind, we chose *Mytilus trossolus* to assess the toxicity of dissolved metals as it is a member of the most sensitive genus in the US EPA's database for saltwater copper quality criteria (WQC), but is a species for which copper toxicity has not been characterized previously. Current criteria are based on toxicity tests with *M. galloprovincialis* and *M. edulis* (e.g. US. EPA, 2003).We employed the standard 48 h embryo–larval development test (NIWA, 2005) to measure embryo survivorship after exposure to a toxicant. Very recently, Fitzpatrick et al. (2008) used *M. trossolus*, in this test and showed that the embryo–larval life-stage is 10 to 100-fold more sensitive than either sperm or eggs alone.

The main objectives of the study were (i) to quantify the toxicities of four dissolved metals, Cu, Zn, Ni and Cd to *M. trossolus* larvae; (ii) based on results of objective (i), to experimentally evaluate the effect of two potential modifying factors of water chemistry (salinity and DOC) on the toxicity of the most toxic one, which turned out to be Cu; and (iii) to determine whether DOC collected from natural sources will provide heterogeneous protection against the toxicity of dissolved Cu. The metals tested were selected because of their common occurrence in municipal and industrial effluents as well as their known toxicity at elevated concentrations to marine organisms.

2. Materials and methods

2.1. Collection and maintenance of adult mussels

Adult *M. trossolus* were collected from natural intertidal populations in the Broken Island Group, near Bamfield, B.C. (Canada) in June–July of 2006 and 2007. Heath et al. (1996) used PCR markers to investigate interaction between sympatric *Mytilus* species and reported that *M. trossolus* is the only bay mussel species found on the west coast of Vancouver Island. More recently, Arkester and Martel (2000) confirmed the bay mussel species in the Barkley Sound Inlet (the location of our study site on Vancouver Island) was indeed *M. trossolus*, based on shell morphology.

In the laboratory, animals were cleaned and transferred to aerated flowing seawater baths maintained at 11–13 °C and allowed to acclimate for 24 h. Representative seawater chemistry is given in Table 1.

2.2. Test solutions

Metals were tested for toxicity using the following inorganic salts (analytical grade): $CuCl_2\ 2H_2O$, $NiCl_2\ 6H_2O$, $CdCl_2\ 5H_2O$ (all Sigma) and $ZnSO_4$ (Anachemia). Stock solutions of these metals were made in deionized water and stored in airtight polyethylene containers. Metal stock solutions were serially diluted with filtered seawater (0.20 μm) to achieve required concentrations in the test vials prior to the day of testing and allowed to equilibrate overnight.

Similarly stock solutions of DOC from three sources—Luther Marsh (LM), Ontario, Canada, obtained by reverse osmosis (for details see Schwartz et al., 2004), Nordic Reservoir (NR), and Suwannee River (SR), the latter two purchased as freeze-dried powders from the International Humic Substances Society (St. Paul, MN. USA) were reconstituted in filtered seawater. DOC derived from marine waters is

Table 2
Nominal versus measured concentrations (means±SEM) for Cu, Zn, Ni and Cd in test solutions used to determine metal toxicity in developing embryos of *M. trossolus*

Copper		Zinc		Nickel		Cadmium	
Nominal μg L^{-1}	Measured μg L^{-1}	Nominal μg L^{-1}	Measured μg L^{-1}	Nominal μg L^{-1}	Measured μg L^{-1}	Nominal μg L^{-1}	Measured μg L^{-1}
0	1.5±0.1	0	5±1	0	BDL*	0	BDL*
0.32	1.1±0.1	50	65±3	50	25±4	50	60±7
1	2.3±0.3	100	108±10	100	40±6	100	115±4
3.2	4.6±0.6	125	153±7	150	75±19	150	250±12
10	11.6±0.2	150	204±5	200	120±19	200	300±15
32	27.4±0.1	200	300±12	500	415±25	500	800±9
100	71.0±6.9	300	576±10	1000	760±16	1000	1200±19

*BDL=Below Detection Limit.