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Toxicity of lead and zinc to developing mussel and sea urchin embryos: Critical tissue residues and effects of dissolved organic matter and salinity

Sunita R. Nadella [a,b,*], Margaret Tellis [a,b], Rachael Diamond [c], Scott Smith [c], Adalto Bianchini [b,d], Chris M. Wood [a,b]

[a] Dept. of Biology, McMaster University, Hamilton, ON L8S4K1, Canada
[b] Bamfield Marine Sciences Centre, 100 Pachena Road, Bamfield, BC V0R 1B0, Canada
[c] Dept. of Chemistry, Wilfrid Laurier University, University Avenue West, Waterloo, ON N2L3C5, Canada
[d] Universidade Federal do Rio Grande — FURG, Instituto de Ciências Biológicas, Campus Carreiros, Av. Itália km 8, 96.203-900 Rio Grande, RS, Brazil

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ABSTRACT

Lead (Pb) EC50 values in the very sensitive early development phases (48–72 h post-fertilization) of the mussels *Mytilus galloprovincialis* and *Mytilus trossolus* and sea urchin *Strongylocentrotus purpuratus* in 100% sea water were: *M. trossolus* — 45 (95% C.I. = 22–72) μg L^{-1}; *M. galloprovincialis* — 63 (36–94) μg L^{-1}; *S. purpuratus* — 74 (50–101) μg L^{-1}. Salinity thresholds for normal development varied: *M. trossolus* > 21 ppt; *M. galloprovincialis* > 28 ppt; *S. purpuratus* ≥ 30 ppt. Addition of two spectroscopically distinct dissolved organic matters (DOM) from fresh water (Nordic Reservoir) and sea water (Inshore) moderately decreased the toxicity of Pb to both mussels, but not in a concentration-dependent fashion, with only an approximate doubling of EC50 over the range of 1.4–11.2 mg C L^{-1}. Independent Pb binding capacity determinations for DOC explained the lack of a relationship between DOM concentration and toxicity. Salinity had no effect on Pb toxicity down to 21 ppt in *M. trossolus*, and low salinity (21 ppt) did not enhance the protective effect of DOC. Both DOMs increased the toxicity of Pb in developing sea urchin embryos, in contrast to mussels. Relative to Pb, the organisms were 6–9 fold less sensitive to Zn on a molar basis in 100% seawater with the following Zn EC50s: *M. trossolus* — 135 (103–170) μg L^{-1}; *M. galloprovincialis* — 172 (126–227) μg L^{-1}, *S. purpuratus* — 151 (129–177) μg L^{-1}. Nordic Reservoir and Inshore DOM (2–12 mg C L^{-1}) had no significant effect on Zn toxicity to mussels, in accord with voltammetry data showing an absence of any strong ligand binding for Zn by DOMs. As with Pb, DOMs increased Zn toxicity to urchin larvae. Critical Tissue Residues (CTR) based on whole body concentrations of Pb and Zn were determined for *M. galloprovincialis* at 48 h and *S. purpuratus* at 72 h. The median lethal CTR values (LA50s), useful parameters for development of saltwater Biotic Ligand Models (BLMs), were approximately 4-fold higher on a molar basis for Zn than for Pb. The latter were not altered by DOM exposure, despite increased EC50 values, in accord with the tenets of the BLM.

1. Introduction

The biotic ligand model (BLM) successfully predicts toxicity associated with metal accumulation on or in aquatic organisms, such that lethality occurs when a critical tissue concentration of the metal (LA50) is reached (DiToro et al., 2001; Paquin et al., 2002; Niyogi and Wood, 2004). In practice, LA50 values are often derived from iterative modeling of toxicity data, rather than direct measurements, but the latter are always more desirable for scientific ground-truthing of BLMs (Niyogi and Wood, 2004). The tissue residue approach (TRA) is a complementary tool for regulatory purposes (McCarty and Mackay, 1993; Adams et al., 2011; McCarty et al., 2011; Sappington et al., 2011). As the TRA associates toxicity with a particular threshold tissue metal burden (CTR = critical tissue residue) in the target organism, it is analogous to the BLM approach which relies on the LA50.

Freshwater BLMs for Cu (Santore et al., 2001) and Ag (McGeer et al., 2000) are well established, and the former has been adopted for regulatory purposes by the USEPA (2006). Acute and chronic BLMs for Zn toxicity to freshwater algae, daphnids and fish (Heijerick et al., 2002; Santore et al., 2002; De Schamphelaere et al., 2005) have been reported and used to evaluate current environmental quality standards in the US and Europe (Van Sprang et al., 2009; DeForest and Van Genderen, 2012). Similarly for Pb, a freshwater gill-binding model based on cation competition parameters and dissolved organic carbon (DOC) characteristics (McDonald et al., 2002) and a preliminary biotic ligand model founded on regression analysis for invertebrates (Esbaugh et al., 2012) have been described.

In the past few years, the focus has now shifted to the development of comparable regulatory tools for estuarine and marine environments. Work has centered on larval molluscs and echinoderms,

* Corresponding author at: Dept. of Biology, 1280 Main Street West, McMaster University, Hamilton, ON L8S4K1, Canada. Tel.: +1 905 525 9140x26389; fax: +1 905 522 6066.
E-mail address: nadellsr@mcmaster.ca (S.R. Nadella).

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which are good indicators of ecological damage because of their extreme sensitivity to contaminants (His et al., 1999). For example, in an earlier study, we showed that the embryo–larval life stage of the mussel *Mytilus trossolus* is 10–100 fold more sensitive to Cu than either sperm or eggs alone (Fitzpatrick et al., 2008).

Arnold (2005) and coworkers (Arnold et al., 2005, 2006, 2010; DePalma et al., 2011b) have pioneered BLM approaches for Cu in marine mussel larvae, but without direct measurements of LA50 or CTR. Dissolved organic matter (DOM), quantified as dissolved organic carbon (DOC), which can reduce the bioavailability of metals to target organisms, proved to be the most important component of the BLM, but analyses were performed only in 100% seawater, and not under estuarine conditions. Rosen et al. (2008) have explored the use of the CTR approach for Cu in embryo–larval stages of mussels and sea urchins, again in 100% seawater. They reported that whole body Cu residues were a better predictor of toxicity than exposure water Cu concentrations when DOM concentrations were varied. However, other metals such as Zn and Pb have not been studied in this context.

The BLM generally assumes that all DOMs, when expressed as DOC, have equivalent protective abilities. However, a recent review (Al-Reasi et al., 2011) of many freshwater studies has indicated that this is certainly not the case for Cu, and the same variability may apply to other metals. Optical properties of the various DOMs appeared to be a good predictor of their protective capabilities. DOMs also affect the physiology of organisms, so the influence of DOM source on metal toxicity is likely to depend on the metal, the organism, and the nature of the particular DOM (Wood et al., 2011). In marine systems, information on the possible DOM source-dependency of protective effects is sparse. Nadella et al. (2009) reported that different DOMs exerted differential protective effects against Cu toxicity to mussel larvae, but this was not seen in the results of DePalma et al. (2011b).

The purposes of the present study were to use acute larval toxicity bioassays (His et al., 1999) to measure LC50 values and whole body LA50 values (as CTRs), and to evaluate the effect of DOMs from several different sources and salinity on the toxicity of Zn and Pb in two species of blue mussels — *M. trossolus* and *Mytilus galloprovincialis* and the sea urchin *Strongylocentrotus purpuratus*. While the toxicity of Zn and to a lesser extent Pb have been explored in marine organisms (Hunt and Anderson, 1989; Phillips et al., 1998; Radenac et al., 2001; Novelli et al., 2003), there is little information on how physico-chemical factors such as DOM or salinity affect the toxicity of these metals. In the U.S.A., current chronic ambient water quality criteria (AWQC) for Zn and Pb in sea water are 81 μg L^{-1} (USEPA, 1996) and 8.1 μg L^{-1} (USEPA, 1985), respectively. These values have been derived from data available in the 1980s and have not been adjusted for salinity and DOM. There is a clear need to revisit these criteria with modern approaches incorporating the influence of DOM and salinity, the two factors likely to be the most important variables affecting the toxicity of these metals.

Often studies investigate metal toxicity independent of DOM chemistry, or vice versa, they investigate DOM chemistry with respect to metal speciation independent of associated toxicological responses. Here we bring the two approaches together, and attempt to rationalize observed toxicological responses based on speciation measurements, represented as metal binding capacity, for the same metals with the same DOM sources. Two spectroscopically distinct DOM sources are investigated in terms of invertebrate embryo LC50 tests as well as Zn and Pb binding capacities across DOM and salinity gradients. Indeed, the chemistry measurements provide a mechanistic explanation for a very unexpected toxicological result.

2. Materials and methods

2.1. Animals

Adult *M. trossolus* were collected from natural intertidal populations in the Broken Island Group, near Bamfield, B.C., Canada. *M. galloprovincialis* adults were obtained courtesy of Northwest Aquaculture Farm located on the Effingham Inlet in Barkley Sound on the west coast of Vancouver Island. *S. purpuratus* were supplied by Westwind Sealab Supplies, Victoria B.C., Canada. At all sites of origin, salinity was 28–35 ppt and conditions were pristine. In the laboratory, animals were cleaned and transferred to aerated flowing seawater baths maintained at 11–13 °C and allowed to settle for 24 h or longer. Representative seawater chemistry for the holding conditions and control exposures (33 ppt) is given in Table 1.

2.2. DOM sources

Two sources of DOM, quantified as DOC, were used in the study.

Nordic Reservoir DOC (a freshwater-derived DOM, collected by reverse osmosis and purchased as freeze-dried powder from the International Humic Substances Society, St. Paul, MN, USA) and Inshore DOM (a seawater-derived DOM collected by solid phase extraction by one of the authors, Dr. Adalto Bianchini, in inshore coastal waters of southern Brazil). The latter was extracted from seawater samples (2000 L) collected in March and April 2009 at the coast (inshore sample) near the Cassino Beach (Rio Grande, RS, Southern Brazil; 32°10′S, 52°20′W). Samples were filtered (10, 5, and 0.5-μm mesh filters; Cuno, Polyclean) and acidified (pH 2) with HCl. Dissolved DOM was extracted by solid phase using commercially pre-packed cartridges (Mega Bond Elut PPL, 5 GM 60 mL, 16/PK, Varian). Activation of the PPL cartridges, removal of salts, and rinsing, as well as DOM elution, drying and storage were performed as previously described (Rodrigues and Bianchini, 2007; Dittmar et al., 2008).

2.3. Mytilus 48 h embryo test

Embryo development was assessed using well-established ASTM protocols for mussel (ASTM, 2004a) and sea urchin larvae (ASTM, 2004b).

Briefly, adult *Mytilus* sp. were transferred to a 10-L filtered (0.20 μm) seawater bath (15–20 adults/bath) maintained at 22–25 °C for thermal shock, typically for a period of 0.5–2 h, which induced spawning. Spawning individuals were immediately moved to separate beakers containing 200 mL of filtered seawater, for isolation and collection of gametes. Egg quality and sperm motility were assessed using a microscope at 200× magnification. Eggs from several individual females were pooled and homogenized by gentle stirring, and an aliquot of sperm solution pooled from several male individuals was added to initiate fertilization. A subsample of this mixture was periodically observed until 80% or more of the eggs were fertilized. The test was initiated by adding 100 μL of fertilized embryos (approximately 600–1000

Table 1
Measured water chemistry parameters for representative test solutions (means ± SEM).

	33 ppt sea water (SW)	21 ppt SW	1000 μg Pb L^{-1} 33 ppt SW	12 mg C L^{-1} inshore DOC 33 ppt SW
pH	7.5 ± 0.09	7.4 ± 0.03	7.7 ± 0.13	7.6 ± 0.07
Na (mmol L^{-1})	443 ± 0.5	277 ± 0.7	430 ± 1.5	486 ± 0.9
K (mmol L^{-1})	9.3 ± 2.0	5.4 ± 1.0	9.2 ± 1.4	9.3 ± 1.0
Ca (mmol L^{-1})	7.8 ± 0.9	4.8 ± 0.4	8.6 ± 0.6	8.8 ± 0.7
Mg (mmol L^{-1})	67 ± 5.0	43 ± 1.6	63 ± 5.0	69 ± 7.0
Cl (mmol L^{-1})	535 ± 5.3	323 ± 1.3	304 ± 3.1	543 ± 0.9
DOC (mg L^{-1})	1.79 ± 0.02	0.61 ± 0.10	0.88 ± 0.03	10.38 ± 0.40

individuals) to each test vial containing 10 mL of test solution. Tests were conducted in a biological incubator maintained at a constant temperature of 20 °C ± 1 and a photoperiod of 16-h light:8-h dark. After 48 h, controls were examined to ensure more than 80% embryos developed into normal D-shaped prodissoconch larvae. Tests in which control development was less than 80% normal were discarded. Addition of 1 mL buffered formalin to each vial terminated the test. Microscopic observation using a Sedgewick–Rafter slide was employed to determine the percentage of embryos exhibiting normal development. At least 100 embryos in each replicate were assessed.

2.4. Sea urchin 72 h embryo test

Sea urchins were stimulated to spawn by injecting 1 M KCl into the gonads. Sperm and eggs were collected separately. After fertilization, 100 μL of fertilized embryos were added to 10 mL of test solution and the gametes were held in the same incubator at a constant temperature of 15 °C ± 1 and a photoperiod of 16-h light:8-h dark. The test was terminated at 72 h by addition of 1% buffered formalin, and microscopic observation was carried out as above. Again, a criterion of 80% normal development (to the pluteus stage) was employed, and at least 100 embryos in each replicate were assessed.

2.5. Whole-body residue determination

Exposures for this test were modified slightly from the above procedure according to methods from Rosen et al. (2008). In order to obtain sufficient biomass and determine weight and tissue burden, tests were conducted using larger water volumes (1 L) with embryo concentrations of ~60 embryos mL^{-1}. After the appropriate exposure period (48–72 h), each beaker was gently homogenized with a Pasteur pipette and a 5 mL aliquot was removed and preserved with 500 μL of formaldehyde for EC50 determination.

The remainder of the sample was filtered through a pre-weighed 8 μm polycarbonate filter (Whatman Nucleopore Track-Etch Membrane PC MB 47 MM 8.0 μm). The filter was dried at ~25 °C until a constant mass was achieved and the mass was recorded.

2.6. DOM characterization

Fluorescence spectra of the DOM samples were collected using a Varian Cary fluorescence spectrophotometer with 1 cm path-length quartz cuvettes. The fluorescence spectra were measured by using excitation wavelengths from 200 to 450 nm in 10 nm increments. The emission wavelengths were measured in the range of 250 to 650 nm for every 1 nm increment. The Fluorescence Index (FI) was calculated as the ratio of fluorescence at 450 nm divided by emission at 500 nm, both for excitation at 370 nm (McKnight et al., 2001).

In order to determine if metal binding capacity was dependent or independent of DOM source or concentration (at two different nominal concentrations of 2 and 12 mg C L^{-1}), Inshore and Nordic Reservoir DOC were dissolved separately in artificial seawater prepared from analytical grade salts according to a recipe of the Organization for Economic Cooperation and Development (OECD) given in the transformation/dissolution protocols (OECD, 2001).

Additionally, the 2 mg C L^{-1} DOM concentration was measured for each organic matter source at three different salinities (3, 15 and 30 ppt). The solutions (in triplicate in Teflon beakers) were held at a constant pH of 7.76 ± 0.02 and left to equilibrate for 24 h. Pb and Zn additions were made up to 900 μg L^{-1}. Anodic stripping voltammetry (ASV) was used for determination of binding capacity. A Metrohm Autolab instrument with a 663 VA stand was used with the NOVA 1.7 software to obtain scans and peak height. Electrochemically labile Pb was measured using square wave anodic stripping voltammetry (SWASV) with a deposition potential of −0.65 V, a deposition time of 30 s, equilibration time of 5 s, voltage scanning (−0.65 to −0.25 V), amplitude of 25 mV, frequency of 25 Hz, and a scan increment of 2 mV. This corresponds to the method of Sanchez-Marin et al. (2011).

2.7. Analytical chemistry — exposure water

As per US EPA (2001) recommendations, water chemistry parameters were measured in treatments critical to the toxicity tests. Dissolved Pb and Zn concentrations in sea water samples were measured after filtration through a 0.45 μm mesh filter, using a method modified from Toyota et al. (1982). Briefly, the representative metal was precipitated from 1 mL of sample by adding 1 μL of lanthanum oxide (10 mg La mL^{-1}) and 7.5 μL of 1 M Na_2CO_3, which brought the pH of the sample to approximately 9.8. The solution was gently stirred in a hot water bath maintained at 80 °C for 30 min to allow flocculation of precipitate. The solution was centrifuged at 1500 ×*g* for 15 min and the supernatant discarded. The remaining precipitate was dissolved in 1 mL of 1 N HNO_3. Pb concentrations were measured via graphite furnace atomic absorption spectroscopy (220, Varian, Palo Alto, CA. USA). Zn levels were measured via flame atomic absorption spectroscopy (220FS; Varian). Fisher Scientific calibration standards were used for every run of samples. Recovery was always ±10% as determined from similarly processed Analytical Reference material TM15 (Environment Canada, Natural Water Research Institute) for water samples. Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations in seawater samples were determined by atomic absorption spectroscopy (Varian SpectrAA-1275FS) and Cl^- by coulometric titration on a chloridometer (CMT 10 Chloride Titrator; Radiometer, Copenhagen, Denmark; Cl^-). Reference standards (Fisher Scientific and Radiometer) were used for the measurement of all ions studied.

Samples for DOC analysis were passed through a 0.45 μm filter. Total organic carbon (TOC) was measured using a Shimadzu TOC analyzer (5050A, Mandel Scientific). Organic Carbon Standards were prepared according to Shimadzu specifications.

2.8. Analytical chemistry — tissues

The dried filter on which exposed embryos were collected was digested overnight with 100 μL of trace metal grade concentrated HNO_3. The next day, 1 mL of de-ionized water was added and the sample stored for tissue Pb or Zn measurements. Pb concentrations were measured via graphite furnace atomic absorption spectroscopy (220, Varian). Zn levels were measured via flame atomic absorption spectroscopy (220FS; Varian). Recovery was ±10% as determined from similarly processed DORM-2 (dogfish muscle — National Research Council, Institute for National Measurement Standards, Ottawa, Canada).

2.9. Statistical analysis

Water chemistry values and whole body tissue metal concentrations have been expressed as means ± 1 SEM (N). An Environmental Toxicity Data Analysis Software Tox Calc™ package v5.0 (Tidepool Scientific Software, CETIS, McKinleyville, CA, USA) was used to estimate EC50, EC20, and EC10 with 95% confidence intervals (CI), employing the responses and the measured dissolved metal concentration data from all exposures. No observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) have also been tabulated as they are used for regulatory purposes in some jurisdictions. The 48 h and 72 h LA50 values, together with 95% CI, for *Mytilus* sp. and *S. purpuratus* respectively, were calculated from linear regressions between log 48 h or 72 h whole body Pb or Zn accumulation and logit 48 h or 72 h abnormality respectively. Logit abnormality is $\log_{ln}$ (M / 1 − M) where M is proportion of abnormality. Whole-body Pb and Zn accumulation and abnormality were corrected for control levels prior to analysis.

3. Results

3.1. Water chemistry

Key salinity variables (Na, Cl, Mg, Ca, and pH), as well as actual concentrations of Pb, Zn and DOC were measured in all tests; representative values of salinity variables for the four exposure conditions are reported in Table 1. All EC values were calculated using measured metal concentrations. For details of Pb, Zn and DOC measured values please see Supplementary Tables 1–7 in Supplementary data.

3.2. Salinity tolerance

Embryo–larval development tests (48 h) were performed for *M. galloprovincialis*, *M. trossolus* and *S. purpuratus* over a range of salinities (15–32 ppt) (Fig. 1). *S. purpuratus* was the most sensitive to salinity changes (Fig. 1C), with normal embryo development declining significantly (<80% normal) at salinities lower than 30 ppt. Embryo development profiles in *M. galloprovincialis* (Fig. 1B) and *M. trossolus* (Fig. 1A) were less sensitive with salinity thresholds of 25 ppt and 21 ppt, respectively.

3.3. DOM characterization

The two DOM sources were found to be spectroscopically distinct (Fig. 2). The fluorescence "fingerprints" were very different with Nordic Reservoir DOC displaying typical humic and fulvic acid fluorescence, while the Inshore DOC exhibited additional fluorophores at wavelengths typical of amino acids (DePalma et al., 2011a). As a summary indicator of different source, Nordic Reservoir had a measured FI of 1.35 versus 1.52 for Inshore. For reference, a FI value of ~1.9 indicates a microbially derived (autochthonous) source and a FI of ~1.4 is typically terrestrial in origin (McKnight et al., 2001).

3.4. Pb series

3.4.1. Toxic effects

For *M. galloprovincialis* and *M. trossolus*, in 48 h embryo–larval toxicity tests in 100% sea water over a range (nominally 3.2–1000 μg L^{-1}) of Pb concentrations, we determined EC50s of 63 μg L^{-1} and 45 μg L^{-1}, respectively (Table 2). EC20 values representing possible chronic thresholds were 19 μg L^{-1} and 16 μg L^{-1} for the two species, respectively (Table 2). With the sea urchin *S. purpuratus* in 72 h embryo–larval toxicity tests over a nominal range (3.2–1000 μg L^{-1}) of Pb concentrations in 100% sea water, we determined an EC50 of 74 μg L^{-1} and EC20 of 31 μg L^{-1}, slightly, but not significantly, higher than for the two mussel species (Table 2).

3.4.2. DOM effects

The addition of DOM (Inshore or Nordic Reservoir) to test waters decreased the toxicity of Pb moderately. However, surprisingly, this protective effect did not demonstrate a concentration-dependent relationship with increasing DOC concentrations. For *M. galloprovincialis*, both types of DOM tested exhibited similar responses with dissolved EC50 values for Pb ranging between 134 μg L^{-1} at 2.5 mg L^{-1} of added Inshore DOC, and 157 μg L^{-1} at 10.5 mg L^{-1} of added Nordic Reservoir DOC. These EC50 values in the presence of elevated DOC were moderately but significantly increased from a control EC50 value of 63 μg L^{-1}. Similarly, for *M. trossolus* embryos, DOM provided marginal protection against Pb toxicity which was again not concentration-dependent, with EC50 values ranging from 97 μg L^{-1} at 1.4 mg L^{-1} of Inshore DOC to 108 μg L^{-1} at 11.2 mg L^{-1} of Nordic Reservoir DOC, compared to control values of 45 μg L^{-1} (Table 2). Therefore, this limited protection was independent of both the concentration and the type of DOM tested.

In the sea urchin *S. purpuratus*, Inshore DOC actually exacerbated Pb toxicity to the embryos, as indicated by a decrease in the Pb EC50 from 74 μg L^{-1} to between 46 and 57 μg L^{-1} when Pb treatments were spiked with 4.2 to 10.3 mg L^{-1} of DOC. Nordic Reservoir DOC proved toxic over the whole concentration range (Table 2).

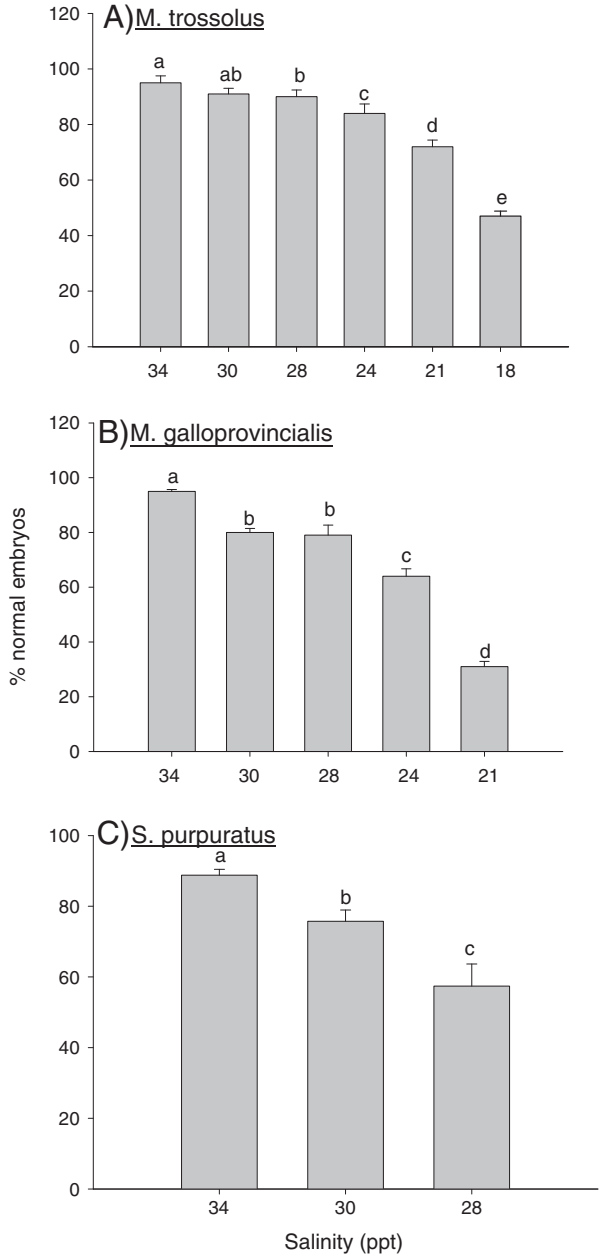

Fig. 1. The influence of salinity on percent normal development in embryos of (A). *Mytilus trossolus*, (B). *Mytilus galloprovincialis* and (C). *Strongylocentrotus purpuratus* in the absence of added metals. Values are means ± SEM of 5 replicates.

3.4.3. Salinity–DOM interactions

The effect of salinity on Pb toxicity was investigated for *M. trossolus*, the more low-salinity tolerant of the two mussel species. Salinity had no effect on Pb toxicity, with EC50 values of 67 μg L^{-1} in 100% sea water and 70 μg L^{-1} in 60% sea water (21 ppt; Table 2). As in 100% sea water, there was only modest protective effect at 21 ppt, for both Inshore DOC and Nordic Reservoir DOC. The EC50 increased from 70 μg L^{-1} to 156 μg L^{-1} in the presence of 5.5 mg C L^{-1} of Inshore DOC (Table 2) and 174 μg L^{-1} with 7.3 mg C L^{-1} of Nordic Reservoir DOC (Fig. 3C).

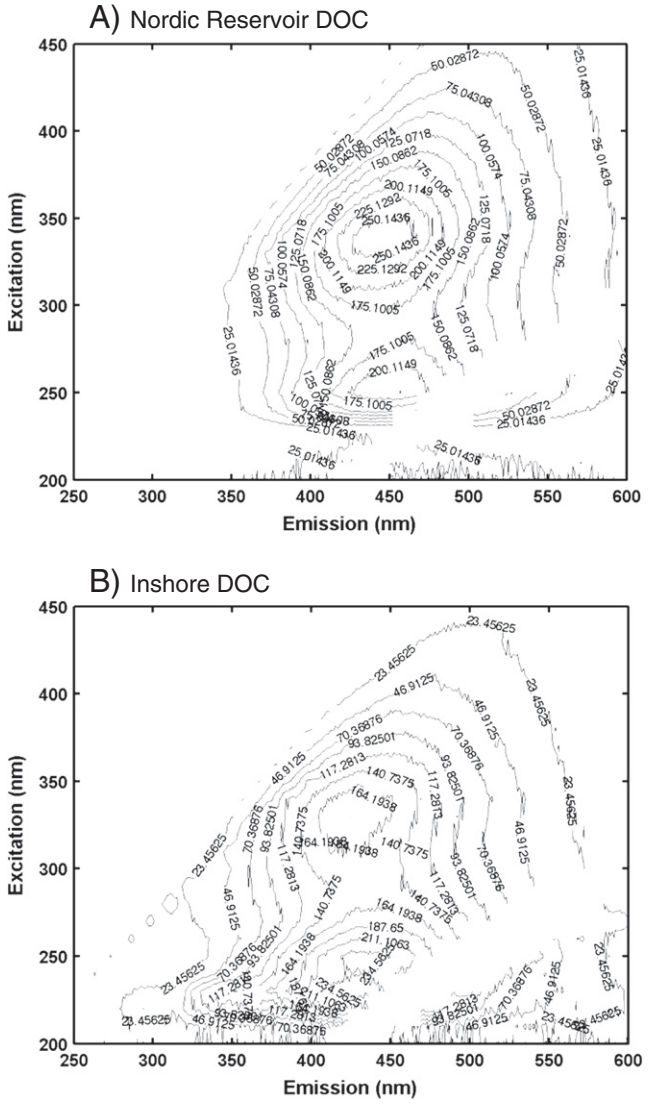

Fig. 2. Fluorescence of DOM samples. (A). Nordic Reservoir and (B). Inshore DOC. Scans were performed at 6 mg C L^{-1} and pH of approximately 8.0. Contour lines are indicated as the fluorescence emission intensity.

3.4.4. Tissue burdens

Critical Tissue Residues (CTR) of Pb, based on whole body concentrations at 48 h of Pb exposure (Fig. 3A) were determined for *M. galloprovincialis*. A good correlation was observed between tissue burden and percent abnormality ($r^2 = 0.88$) for *M. galloprovincialis* embryos. An LA50 for Pb of 575 μg g^{-1} dry mass (2.8 μmol g^{-1}) was determined from this relationship (Fig. 3B).

In embryos of *S. purpuratus*, whole-body Pb accumulation increased in a dose-dependent manner after a 72 h exposure to a range of Pb concentrations (Fig. 3C). Again, a good correlation was observed between tissue burden and percent abnormality ($r^2 = 0.88$). An LA50 of 316 μg g^{-1} dry mass (1.5 μmol g^{-1}) was determined from this relationship (Fig. 3D), which was not significantly different from the LA50 for Pb in *M. galloprovincialis* embryos.

In *M. trossolus*, elevations in DOC caused increases in EC50 but not in LA50 for Pb. Thus whole-body Pb accumulation increased significantly with increasing Pb levels (1.2–350 μg L^{-1}) in control embryos (embryos exposed to seawater without added DOC) and those exposed to Inshore and Nordic Reservoir DOC (~4 mg C L^{-1}; Fig. 5A–C). CTR correlated well with abnormality in control as well as DOC-exposed embryos (Fig. 5D–F). In confirmation of the results in Table 2, in these high volume tests, both forms of DOM were protective with EC50 values increasing significantly from 56 μg L^{-1} in control to 104 μg L^{-1} with Inshore DOC, and 96 μg L^{-1} with Nordic Reservoir DOC (Fig. 6A–C). However exposure to DOC did not have any significant effect on LA50 which measured 346 μg g^{-1}dry mass in controls, 295 μg g^{-1} dry mass with elevated Inshore DOC, and 426 μg g^{-1} dry mass with elevated Nordic Reservoir DOC respectively (Fig. 5D–F).

3.4.5. Larval weight

Larval tissue weight was evaluated as a possible additional endpoint of toxicity for both mussel and sea urchin larvae. Larval weight reduced significantly for *M. galloprovincialis* at a measured Pb concentration of 230 μg L^{-1} (Fig. 6A). For *S. purpuratus* larval weight was significantly lower at a measured Pb concentration of 2.7 μg L^{-1} but thereafter was stable through to 250 μg L^{-1}, with a significant decrease only at 970 μg L^{-1} (Fig. 6B).

3.5. Zn series

3.5.1. Toxic effects

For *M. galloprovincialis* and *M. trossolus*, in 48 h embryo–larval toxicity tests in 100% sea water over a range (nominally 3.2–1000 μg L^{-1}) of Zn concentrations, we determined EC50s of 172 μg L^{-1} and 135 μg L^{-1}, respectively. EC20 values representing possible chronic threshold were 101 μg L^{-1} and 69 μg L^{-1} for the two species, respectively. Thus Zn was somewhat less toxic (on a metal mass basis) than Pb (Table 3). When converted to a molar basis, this difference in toxicity becomes about 9-fold. With *S. purpuratus* in 72 h embryo–larval toxicity tests over a nominal range (3.2–1000 μg L^{-1}) of Zn concentrations in 100% seawater, sea urchins proved to have about the same sensitivity as mussel larvae, with EC50 values of 151 μg L^{-1} (Table 3). This was about 6-fold lower than Pb toxicity on a molar basis.

3.5.2. DOM effects

Surprisingly, the addition of DOM (Inshore and Nordic Reservoir) to test waters had no significant effect on Zn toxicity to mussel larvae. Both types of DOM tested showed similar (lack of) responses with dissolved EC50 values for Zn ranging between 164 μg L^{-1} and 239 μg L^{-1} with 2.1–9.6 mg L^{-1} DOC for *M. galloprovicialis* and EC50 values ranging from 135 to 184 μg L^{-1} over a DOC range up to 11.3 mg L^{-1} for *M. trossolus* (Table 3).

In the sea urchin *S. purpuratus*, Inshore DOC aggravated Zn toxicity to the embryos, as indicated by a decrease in the Zn EC50 to 101 μg $L^{-1}$when Zn treatments were spiked with 3.0 mg L^{-1} DOC. Adding a higher concentration (12.0 mg L^{-1}) of the same DOC proved to further exacerbate toxicity to the embryos with an EC50 of 77 μg L^{-1}. Nordic Reservoir DOC was toxic to sea urchin embryos even in the absence of added Zn (Table 3).

3.5.3. Tissue burdens

Critical Tissue Residues (CTR) values of Zn, based on whole-body concentrations at 48 h of Zn were determined for *M. galloprovincialis*. Whole-body burdens of Zn increased from controls initially at the lowest Zn exposure concentration, and then substantially at the two highest exposures (Fig. 4A). There was a good correlation between tissue burden and percent abnormality ($r^2 = 0.95$). An LA50 of 759 μg g^{-1} dry mass (11.7 μmol g^{-1}) was determined from this relationship (Fig. 4B). Thus despite the differences in toxicity, LA50 values were similar for Pb and Zn on a metal mass basis, but on a molar basis, Zn LA50 was 4-fold greater than Pb LA50.

In embryos of *S. purpuratus*, after a 72 h exposure to a range of Zn concentrations, whole-body accumulation showed significant dose-dependent increases (Fig. 4C). We measured an LA50 of 398 μg g^{-1}dry mass (6.1 μmol g^{-1}) ($r^2 = 0.82$) (Fig. 4D). The CTR for Zn was thus comparable to that for Pb in this species on a metal mass basis (but a 4-fold difference on a molar basis) and less than that in mussel embryos.

Table 2
48 h EC50, EC20, EC10, and NOEC values for abnormal development in embryos of *Mytilus galloprovincialis* and *Mytilus trossolus* and comparable 72 h values for *Strongylocentrotus purpuratus* for Pb in the presence of a potential modifying factor (DOC). All Pb and DOC concentrations are measured.

	EC50 (μg L^{-1})	EC20 (μg L^{-1})	EC10 (μg L^{-1})	NOEC (μg L^{-1})
M. galloprovincialis				
Pb	63 (36–94)	19 (7–33)	10 (3–20)	3.2
Inshore DOC (2.5 mg L^{-1})	134 (98–169)	68 (39–94)	48 (24–70)	4.4
Inshore DOC (10.5 mg L^{-1})	141 (99–182)	82 (43–113)	62 (27–91)	2.2
Nordic Reservoir DOC (2.1 mg L^{-1})	153 (141–165)	85 (75–95)	63 (54–72)	12.2
Nordic Reservoir DOC (8.8 mg L^{-1})	157 (141–172)	85 (71–99)	62 (49–75)	30
M. trossolus				
Pb	45 (22–72)	16 (5–30)	9 (2–19)	3.4
Inshore DOC (1.4 mg L^{-1})	97 (90–105)	65 (59–72)	53 (47–59)	4.3
Inshore DOC (10.1 mg L^{-1})	109 (100–118)	80 (71–88)	68 (60–76)	11.8
Nordic Reservoir DOC (3.1 mg L^{-1})	117 (77–157)	67 (31–96)	50 (19–76)	1.7
Nordic Reservoir DOC (11.2 mg L^{-1})	108 (83–133)	57 (37–75)	40 (24–57)	0.7
M. trossolus				
Pb (33 ppt SW)	67 (37–100)	27 (10–46)	17 (5–32)	2.7
Pb (21 ppt)	70 (34–109)	30 (8–53)	19 (3–38)	–
21 ppt SW + Nordic Reservoir DOC (7.3 mg L^{-1})	174 (111–248)	48 (19–81)	25 (7–47)	9.7
21 ppt SW + Inshore DOC (5.5 mg L^{-1})	156 (97–222)	52 (20–86)	30 (9–55)	<3.1
S. purpuratus				
Pb	74 (50–101)	31 (16–46)	19 (8–31)	2.7
Inshore DOC (4.2 mg L^{-1})	46(10–92)	17 (0.8–40)	10 (0.2–27)	2
Inshore DOC (10.3 mg L^{-1})	57 (29–89)	27 (7–45)	18 (3–34)	1.5
Nordic Reservoir DOC (2.7–10.3 mg L^{-1})	No survival			

3.5.4. Larval weight

Larval mass, as in the case for Pb, was not very sensitive to Zn exposures as a toxicity endpoint both for mussels and sea urchins. A significant drop in mass was observed at measured Zn concentrations of 342 μg L^{-1} for *M. galloprovincialis* (Fig. 6C) and for *S. purpuratus* there was no clear threshold up to a Zn concentration of 1374 μg L^{-1}(Fig. 6D).

3.6. Pb and Zn binding to DOM

Results of binding capacity analysis for Pb are shown in Table 4. Note that Pb binding capacity was independent of DOM concentration in a range of DOC concentrations up to 12.0 mg C L^{-1}, and at a range of salinities. This result correlates with the surprising finding that the modest ability of DOM to protect against Pb toxicity was independent

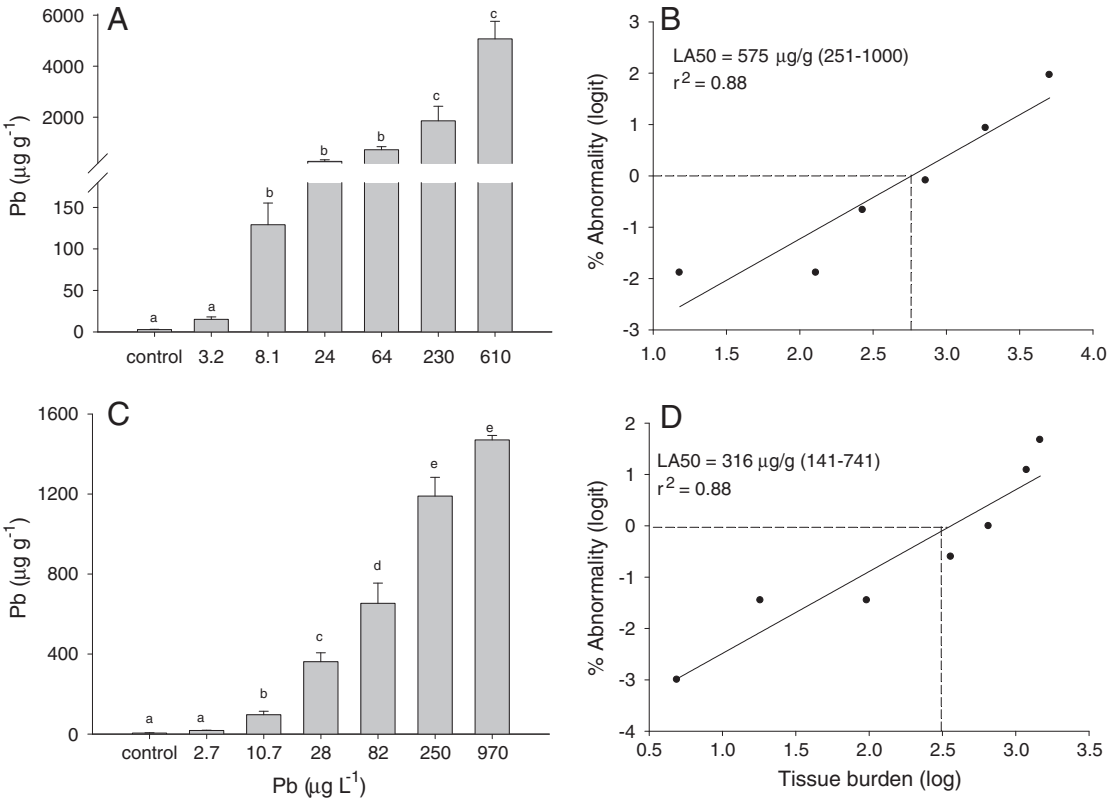

Fig. 3. (A). Whole body accumulation of Pb in *Mytilus galloprovincialis*. Values are means ± SEM of 5 replicates. (B). LA50 concentrations for *M. galloprovincialis* calculated from tissue accumulation relative to exposure Pb concentration and abnormality. (C). Whole body accumulation of Pb in *Strongylocentrotus purpuratus*. Values are means ± SEM of 5 replicates. (D). LA50 concentrations for *S. purpuratus* calculated from tissue accumulation relative to exposure Pb concentration and abnormality.

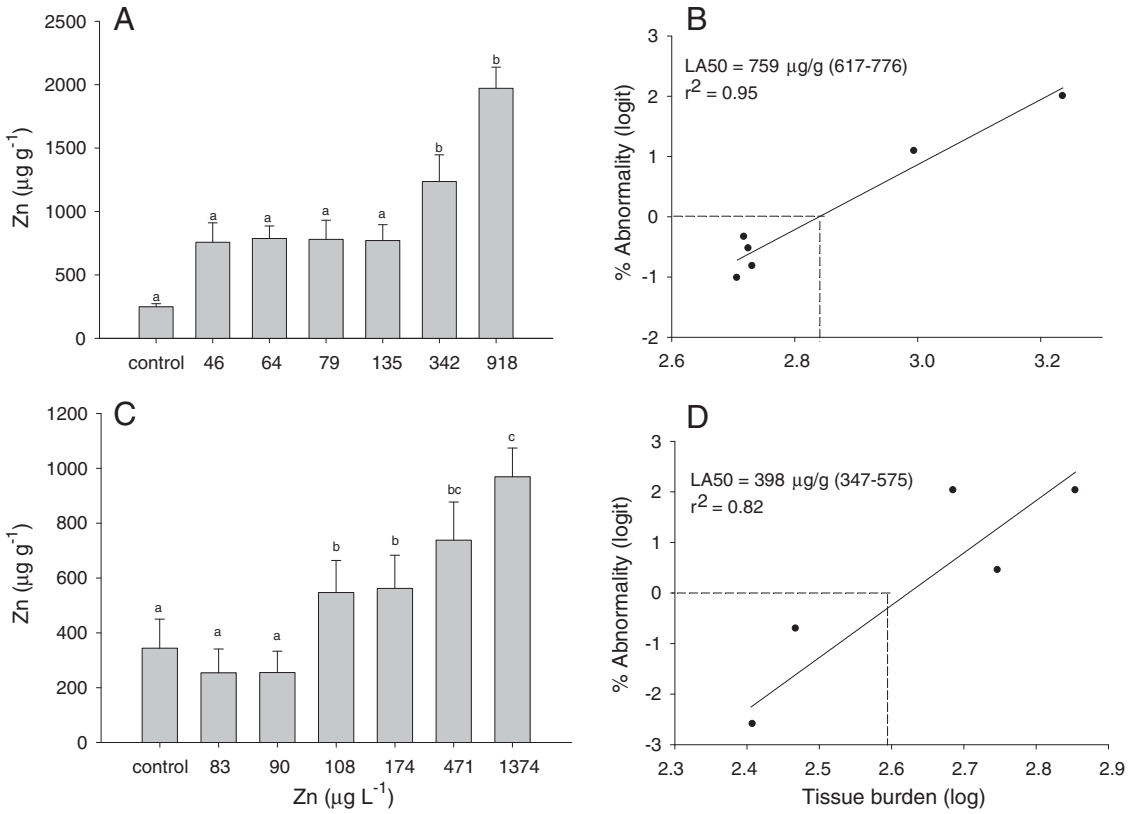

Fig. 4. (A). Whole body accumulation of Zn in *Mytilus galloprovincialis*. Values are means ± SEM of 5 replicates. (B). LA50 concentrations for *M. galloprovincialis* calculated from tissue accumulation relative to exposure Zn concentration and abnormality. (C). Whole body accumulation of Zn in *Strongylocentrotus purpuratus* Values are means ± SEM of 5 replicates. (D). LA50 concentrations for *S. purpuratus* calculated from tissue accumulation relative to exposure Zn concentration and abnormality.

of DOC concentration over this range and not affected by salinity (Table 2). The voltammetric method utilized in this work did not detect any strong ligand binding for zinc in the presence of DOM. This again correlates with the complete lack of protection seen in the toxicity studies (Table 3).

4. Discussion

4.1. Salinity

The rank order of salinity tolerance for embryo development was *M. trossolus* > *M. galloprovincialis* > *S. purpuratus* (Fig. 1). Most species of echinoderms are intolerant to low salinities and sea urchins are no exception as they are stenohaline osmoconformers. Exposure of *S. purpuratus* to 70% seawater for as little as 3 h caused deleterious effects, both on adults and on developmental stages (Giese and Farmanfarmaian, 1963). On the other hand, the blue mussel (*M. galloprovincialis*) is a euryhaline osmoconformer and inhabits the intertidal zone which is subjected to frequent salinity shifts (Gilles, 1979). The greater sensitivity to salinity of *M. galloprovincialis* versus *M. trossolus* may reflect the fact that the former was procured from an aquaculture facility and raised in a stable water quality environment, whereas the latter was collected from the field. There is evidence from oysters (*Crassostrea virginica*) that optimum salinity for development of eggs was dependent on the salinity at which the parent oysters were acclimated immediately prior to spawning (Davis, 1958).

We hypothesized that low salinities would increase Pb toxicity to *M. trossolus* embryos, because metals competitively interact with cations for binding sites on ligands. Pb appears to act primarily as a Ca^{2+} antagonist, although evidence for inhibition of Na^{+} uptake in freshwater fish gills has been also reported (Rogers et al., 2003; Rogers and Wood, 2004). Indeed, Pb accumulation rates were found to be inversely proportional to salinity in the estuarine fish *Gillichthys mirabilis* (Somero et al., 1977). However, as in the case of Cu in our previous work (Nadella et al., 2009), we did not observe any effect of low salinity (60% sea water = 21 ppt) on Pb toxicity (Table 2). One reason could be that at the threshold of optimum salinity the toxic effects of Pb far exceeded any potential effect of salinity. For example, in *M. galloprovincialis* the effect of Pb on normal embryo development was reported to be greater than the effect of salinity (Hrs-Brenko et al., 1977).

4.2. Pb and Zn toxicity

The acute toxicity of Pb was similar between the two *Mytilus* sps. and *S. purpuratus* in terms of EC values as well as NOECs (Table 2). This was also true in the case of Zn toxicity to embryos of the three organisms tested (Table 3). Arnold et al. (2010) similarly reported *M. galloprovincialis* and *S. purpuratus* to be equally sensitive to Cu. However, as might be expected for a non-essential versus essential element, Pb toxicity was significantly higher than Zn toxicity, especially when compared on a molar basis, where the differences were 6 to 9-fold. Likewise in embryos of *Paracentrotus lividus*, Pb induced embryotoxicity was comparatively higher to Zn (Novelli et al., 2003). Pb is reportedly directly toxic in early life stages (Nacci et al., 2000) as it competes with Ca fixation and impairs skeletal differentiation (Warnau and Pagano, 1994). Zn is believed to cause indirect effects by inhibition of protein synthesis and interference in the biosynthesis of nucleic acids (Castagna et al., 1981).

Our EC50 values for Zn toxicity of 172 (126–227) µg L^{-1}, 135 (103–170) µg L^{-1}, and 151 (129–177) µg L^{-1} for *M. galloprovincialis*, *M. trossolus*, and *S. purpuratus* respectively (Table 3) compare reasonably

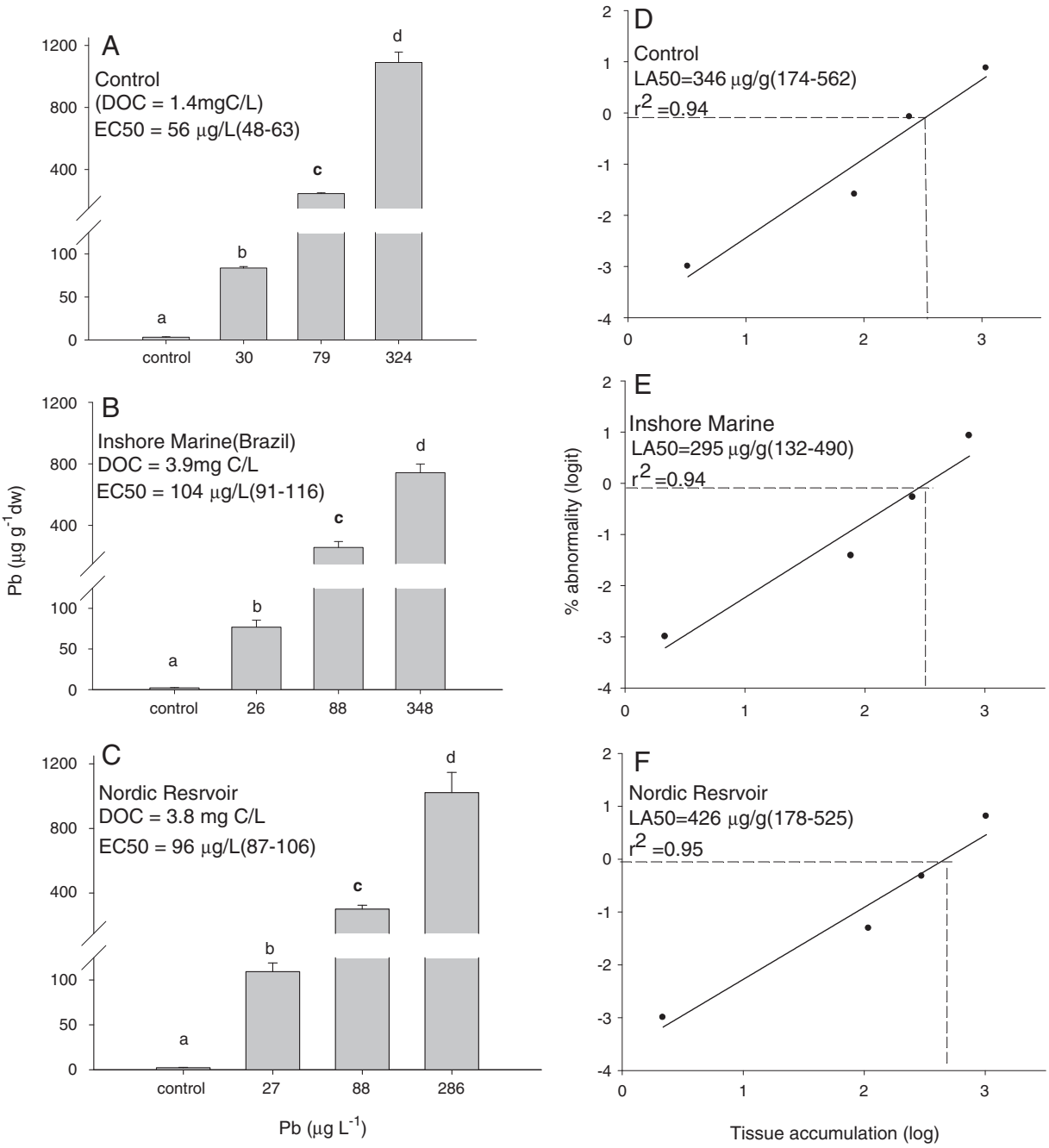

Fig. 5. Whole body accumulation of Pb in *Mytilus trossolus* (A) Control (B) Inshore DOC (C). Nordic Reservoir DOC. Values are means ± SEM of 5 replicates. LA50 concentrations of Pb in *M. trossolus* (D) Control (E) Inshore Marine DOC (F). Nordic Reservoir DOC calculated from tissue accumulation relative to exposure Pb concentration and abnormality.

well with literature values of 107 µg L^{-1} for *S. purpuratus* (Phillips et al., 1998) and 175 µg L^{-1} for *M. edulis* (Martin et al., 1981). EC50 values for Pb toxicity in sea urchin embryos (Table 2) compare closely with a few studies but are several fold lower in comparison to others (see comparison Table 5). The ultimate goal for all toxicity testing is to provide robust data that can be used in setting water quality criteria and such conflicting information leads to uncertainty. Perusal of the experimental conditions in Table 5 reveal differences in pH, temperature and chemical form of Pb used in each study. All of these factors are known to affect metal toxicity in general (e.g. Hrs-Brenko et al., 1977; Warnau and Pagano, 1994; Ho et al., 1999; Radenac et al., 2001). However, a closer examination of these studies also reveals variations in the indices used for quantification of larval abnormalities. For instance in the present study the developmental test for sea urchins was modeled on the ASTM guide, and criteria for normal development included formation of full length skeletal rods and differentiation of a three part gut, while Fernandez and Beiras (2001) used a less restrictive criterion for skeletal rods in their study. Most studies do not elaborate on the specifics used to quantify a subjective measure like abnormality. Toxicity testing will therefore benefit from developing more sensitive endpoints that can be objectively measured to restrict variability in data and allow development of accurate water quality criteria. Whole body tissue weight could provide one such measure. Rosen et al. (2008) found that whole-body tissue weight was a sensitive endpoint for Cu toxicity to *M. galloprovincialis*, though not to *S. purpuratus*.

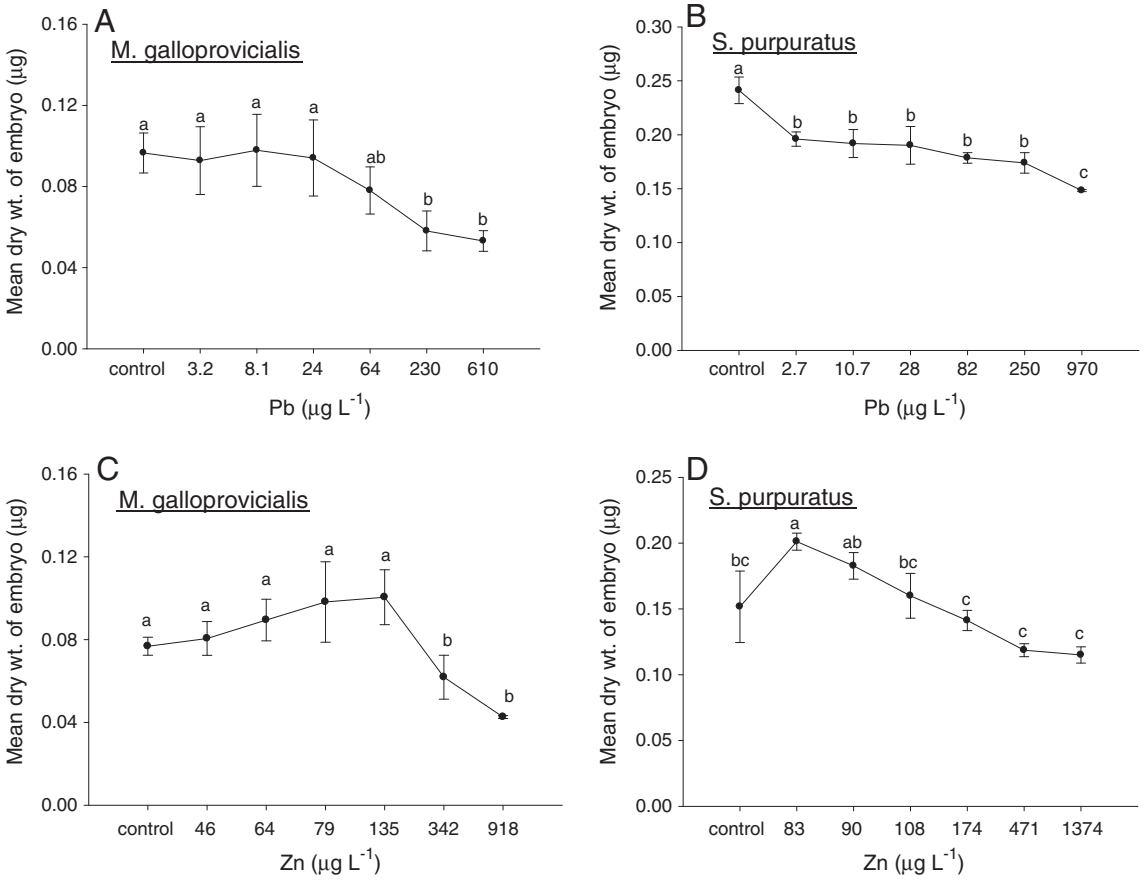

Fig. 6. Mean dry mass per embryo. Values are means ± SEM of 5 replicates relative to Pb exposures in (A) *Mytilus galloprovincialis* and (B) *Strongylocentrotus purpuratus*, and relative to Zn exposures in (C) *M. galloprovincialis* and (D) *S. purpuratus*.

However, in the present study larval tissue weight was not a sensitive endpoint for Pb or Zn toxicity in either of the tested species.

4.3. Effects of DOC

The presence of multiple binding sites for protons and cations on dissolved organic matter (DOM) characterizes its ability to function as a multi-site complexing ligand for metals, thereby ameliorating metal toxicity to organisms in aquatic environments (Al-Reasi et al., 2011). These characteristics of DOM in relation to metal toxicity have been well established for most metals in fresh water, but mostly only for Cu in seawater. Our results indicate DOM provides modest protection against Pb toxicity (Table 2) but not for Zn (Table 3) in *M. galloprovincialis* and *M. trossolus*.

Metal–ligand associations in NOM are complex and define the ability of DOM as protective agents, which can be assessed using conditional stability constants (Wood, 2001; Smith et al., 2002). Based on log K data (Smith et al., 2002), the strengths of Zn binding to carboxyl

Table 3
48 h EC50, EC20, EC10 and NOEC values for abnormal development in embryos of *Mytilus galloprovincialis* and *Mytilus trossolus* and comparable 72 h values in *Strongylocentrotus purpuratus* for Zn in the presence of a potential modifying factor (DOC). All Zn and DOC concentrations are measured.

	EC50 (µg L^{-1})	EC20 (µg L^{-1})	EC10 (µg L^{-1})	NOEC (µg L^{-1})
M. galloprovincialis				
Zn	172 (126–227)	101 (59–136)	76 (38–108)	46
Inshore DOC (3.0 mg L^{-1})	203 (138–290)	132 (59–180)	105 (36–150)	<98
Inshore DOC (9.6 mg L^{-1})	239 (177–317)	167 (100–216)	138 (72–185)	111
Nordic Reservoir DOC (2.1 mg L^{-1})	165 (80–279)	79 (19–137)	54 (8–102)	<51
Nordic Reservoir DOC (8.4 mg L^{-1})	164 (118–219)	98 (57–133)	75 (37–107)	<56
M. trossolus				
Zn	135 (103–170)	69 (44–92)	48 (28–68)	46
Inshore DOC (2.1 mg L^{-1})	168 (113–232)	99 (48–140)	76 (30–112)	89
Inshore DOC (10.4 mg L^{-1})	184 (137–236)	109 (65–144)	82 (42–115)	92
Nordic Reservoir DOC (3.1 mg L^{-1})	184 (169–198)	102 (89–113)	75 (64–85)	58
Nordic Reservoir DOC (11.3 mg L^{-1})	155 (119–192)	85 (56–112)	63 (37–87)	<49
S. purpuratus				
Zn	151 (129–177)	125 (95–144)	114 (80–132)	109
Inshore DOC (3.0 mg L^{-1})	101 (99–103)	88 (85–91)	82 (78–85)	<82
Inshore DOC (12.0 mg L^{-1})	77	41	29	78
Nordic Reservoir DOC (2.0–12.0 mg L^{-1})	No survival			

Table 4
Pb binding capacity for various DOC and salinity samples as determined using anodic stripping voltammetry. Bracketed values represent 95% confidence intervals about estimated concentrations. Measured DOC values for each characterized samples are indicated.

Sample	Pb binding capacity ($\mu g\ Pb\ L^{-1}$)
30 ppt SW + Nordic Reservoir DOC (1.0 mg C L^{-1})	320 (159–481)
15 ppt SW + Nordic Reservoir DOC (1.0 mg C L^{-1})	314 (301–327)
3 ppt SW + Nordic Reservoir DOC (1.0 mg C L^{-1})	307 (220–394)
30 ppt SW + Nordic Reservoir DOC (5.4 mg C L^{-1})	347 (251–443)
30 ppt SW + Inshore DOC (2.6 mg C L^{-1})	301 (274–326)
15 ppt SW + Inshore DOC (2.6 mg C L^{-1})	339 (276–402)
3 ppt SW + Inshore DOC (2.6 mg C L^{-1})	293 (270–316)
30 ppt SW + Inshore DOC (12.0 mg C L^{-1})	296 (264–328)

and amino components of DOM are comparable to those of Pb and Cu. However, Zn binding to the sulfidic-thiolate component (log $K = 6.8 \pm 1.8$) of DOM is much lower than for Pb (10.2 ± 2.2) and many fold lower than for Cu I (14.6 ± 0.8). Since thiolate–metal sulfide clusters are considered key ligands for Group B and some intermediate metals (Smith et al., 2002), this factor could account for the absence of DOM protection against Zn exposures.

Pb exposures supplemented with DOM from two sources (up to ~11 mg C L^{-1}) significantly increased Pb EC50 in *M. galloprovincialis* and *M. trossolus* by 2 to 3-fold (Table 2). The protective effect for Pb by DOM is less than reported for Cu (Arnold, 2005; Arnold et al., 2005, 2006, 2010; Nadella et al., 2009; DePalma et al., 2011b). However, Pb EC50 is about 2-fold higher than Cu EC50 on a molar basis. Pb speciation is affected by DOM, but to a lesser extent than Cu speciation as the higher EC50 for Pb would necessitate a corresponding increase in complexation for a similar protective effect (Sanchez-Marin et al., 2010).

Evidence from the literature for the DOM–Pb association is conflicting. Sanchez-Marin et al. (2007) reported a dose-dependent increase in Pb toxicity to *P. lividus* larvae and increase in Pb uptake by *M. edulis* gills with humic acid (HA). In another study using natural marine DOMs from several sources, some samples increased Pb toxicity to *P. lividus* embryos while others decreased it (Sanchez-Marin et al., 2010). Pb uptake by *M. edulis* gills was significantly lower when the exposure medium was supplemented with Fulvic Acid and Suwanee River DOM (Sanchez-Marin et al., 2011). In fresh water, DOM source-dependent effects were observed in relation to Pb toxicity to rainbow trout (Schwartz et al., 2004). These contrasting effects of DOM therefore appear to be related to physico-chemical properties of DOM, which emphasizes the need for including qualitative DOM characterization for site-specific water quality criteria.

The usual interpretation for DOM protective effects against metal toxicity involve decreased bioavailability of the metal cation due to complexation with DOM. Although DOM provides protection to *Mytilus* embryos against Pb toxicity, this effect is not concentration-dependent (Table 2). This could be due to formation of toxic metal–DOM complexes (Sanchez-Marin et al., 2007) or it could be due to a decrease in availability of organic matter binding sites. DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory can actually be used to predict colloid stability (Christenson, 1984). Basically a colloid (such as DOM) has a tendency to interact with other colloids by intermolecular forces such as van der Waals forces. At the same time, there are electrostatic repulsive forces because of surface charge on the colloids. Increased salt can balance the attractive and repulsive forces and promote colloid aggregation. If such aggregation were to occur, the functional groups involved in the aggregation would no longer be available for Pb binding. Only the external functional groups would remain available. Aggregation is a concentration-dependent phenomenon; at low DOM concentrations, the DOM molecules would not be close enough on average to interact but higher concentrations would induce interactions provided that the salt concentration is sufficient to overcome the repulsive effects. Thus, the surprising lack of dose-dependence of DOM protection for Pb is hypothesized to be a result of salt-induced colloid aggregation of DOM particles, and is in perfect accord with the anodic stripping voltammetry data shown in Table 4 which indicate a constant Pb binding capacity at a variety of DOC concentrations.

It was also surprising that the two sources of DOM had no protective effect against either Pb or Zn toxicity in developing *S. purpuratus* embryos (Tables 2 and 3). Exposure to Nordic Reservoir DOM resulted in total loss of embryo survival even in the absence of added Pb or Zn. Physico-chemical characteristics of DOM and functional organization of the epithelial interface (which could be species-specific) both play a role in defining the DOM–ligand relationship (Sanchez-Marin et al., 2011). There is abundant evidence for a direct interaction of DOM molecules with biological surfaces (Myers et al., 1975; Campbell et al., 1997; Galvez et al., 2009; Wood et al., 2011) associated with their lipophilic nature. Clearly, this appears to be a species-specific phenomenon as adverse effects were not observed in *Mytilus* sp. embryos when exposed to either Inshore or Nordic Reservoir DOM.

4.4. Salinity–DOC interactions

Pb toxicity to developing *M. trossolus* embryos was not significantly different in the presence of Inshore or Nordic Reservoir DOC at 21 ppt compared to embryos exposed at the same DOC concentrations at 33 ppt (Table 2). Thus, Pb binding to biotic ligand sites is stronger than Na and Ca cation binding. Indeed, binding models for Pb in freshwater fish also show DOM–Pb binding constants of 8.4 in comparison to binding constants of 5.0 for DOM–Ca (Niyogi and Wood, 2004). The lack of a toxicity change with salinity in the presence of DOM implies that Pb out-competes Na and Ca for binding at DOM molecules as well, and is in agreement with the anodic stripping voltammetry data shown in Table 4.

4.5. Critical tissue residues (CTR)

Our data from whole-body metal concentrations illustrate the different mechanisms organisms utilize to regulate non-essential and

Table 5
Median effect concentration (EC50) values for embryo toxicity test compared to literature values. Exposure Pb range concentrations are nominal; EC50 values are measured or nominal, as specified.

Species	Pb range ($\mu g\ L^{-1}$)	Exposure duration	Medium	pH	Temperature	EC50 ($\mu g\ L^{-1}$)	Reference
Strongylocentrotus purpuratus	3.2–1000 Pb $(NO_3)_2$	72 h	33 ppt filtered sea water	7.6	15 °C	74 (51–101) (measured)	Present study
Paracentrotus lividus	0–500 Pb $(NO_3)_2$	72 h	35 ppt artificial sea water	8.0	18 °C	68 (57–80) (nominal)	Novelli et al. (2003)
P. lividus	10–250 Pb $(NO_3)_2$	48 h	34 ppt artificial sea water	8.3	22 °C	40 (nominal)	Radenac et al. (2001)
P. lividus	10–1200 Pb $(OCOCH_3)_2$	48 h	33 ppt filtered sea water		21 °C	482 (101) (nominal)	His et al. (1999)
P. lividus	250–4000 Pb $(NO_3)_2$	48 h	Artificial sea water		20 °C	509 (measured)	Fernandez and Beiras (2001)

essential metals. Pb whole-body concentrations in *M. galloprovincialis* and *S. purpuratus* increased significantly from controls with each increasing exposure level, while Zn whole body concentrations in the two species were maintained close to control levels except in the higher exposure concentrations (Figs. 3 and 4) Clearly, for Zn, which is essential, the organisms were able to maintain a balance between uptake and excretion with minimal sequestration till they reached a threshold at 342 μg L^{-1} for *M. galloprovincialis* and 108 μg L^{-1} for *S. purpuratus*. Rosen et al. (2008) reported a similar pattern of regulation for whole-body Cu burden, though over a lower absolute range of concentrations, in these same two species. Like Zn, Cu is an essential element. To the best of our knowledge, no other study has reported whole-body tissue levels in *Mytilus* larvae after Pb or Zn exposures. However, reported control Pb and Zn concentrations were 7 μg g^{-1} wet mass and 90 μg g−1 wet mass in adult *M. edulis* (Chou and Uthe, 1991) are comparable to our values in control embryos of *M. galloprovincialis*. In embryos of *P. lividus* Radenac et al. (2001) reported whole-body tissue levels ranging from 3 to 3105 μg g^{-1} dry mass for Pb (at 0–250 μg Pb L^{-1}) and 185–534 μg g^{-1} dry mass for Zn (at 0–500 μg Zn L^{-1}) after 48 h exposure which compare well to values measured in this study. We measured 5–1470 μg g^{-1} dry mass for Pb (at 0–1000 μg L^{-1}) and 344–969 μg g^{-1} dry mass for Zn (at 0–1000 μg L^{-1}) in another species of sea urchin *S. purpuratus* after 72 h exposure. Therefore values appear to be generally similar between the two species.

The CTR approach is based on the idea that a tissue residue concentration is a measured value of metal actually taken up by the organism, regardless of bioavailability conditions in the exposure medium (Adams et al., 2011). Our data show that the toxicity of Pb to *M. galloprovincialis* and *S. purpuratus* is about 6 to 9-fold higher on a molar basis than the toxicity of Zn using measured EC50s from the waterborne exposures, and about 4-fold higher using CTRs. Thus, whole-body LA50 values (on a molar basis) were 4-fold higher for Zn than for Pb in both species. This can be related to the fact that although both these metals are Ca^{2+} antagonists and share a common mechanism of uptake via Ca^{2+} pathways, Pb is more effective in disturbing Ca^{2+} uptake than Zn. In support of this argument, Rogers and Wood (2004) reported Log K_i = 6.3 for Pb inhibition of Ca^{2+} uptake in freshwater rainbow trout, in close agreement the gill-binding model for Pb (McDonald et al., 2002) predicts a log K_i of 6.0 for Ca^{2+} uptake in the presence of Pb. In comparison, a log K_i of 5.5 was reported for Ca^{2+} uptake in the presence of Zn (Santore et al., 2002). Interestingly, Rosen et al. (2008) reported LA50s for Cu of 49 μg g^{-1} in *M. galloprovincialis* and 142 μg g^{-1} in *S. purpuratus*. When translated to a molar basis, these are in the same range as the Pb LA50 and far below the Zn LA50 values of the present study, though Cu, which is essential, is generally not thought to be a Ca^{2+} antagonist (Wood, 2001).

EC50 and LA50 values for Pb toxicity were measured in *M. trossolus* in the presence of ~4 mg of DOC (Inshore Marine and Nordic Resrvoir) to illustrate how bioavailability of the metal defines these two parameters. Toxicity of Pb decreased in the presence of DOC with EC50 increasing from 56 μg L^{-1} in control embryos to 104 μg L^{-1} in the presence of Inshore DOC and 96 μg L^{-1} (Fig. 5A–C) in exposures with Nordic Reservoir DOC. The data clearly indicate that bioavailability of Pb was compromised by the presence of DOM in the water. However, there were no changes in LA50 values derived from measurements of tissue Pb burden and abnormality between controls (346 μg g^{-1} dry mass) and DOC exposed embryos (295 μg g^{-1} and 426 μg g^{-1} dry mass; Fig. 5D–F). Our data are in agreement with the basic tenets of both the BLM approach and the CTR approach that toxicity is directly proportional to metal burden on the toxic site, regardless of bioavailability (Niyogi and Wood, 2004). We are not aware of any other study that shows similar data for Pb in marine organisms to provide a comparative validation. LA50 data such as these may eventually lead to development of BLM constants based on direct tissue residue measurements on the organisms of interest, rather than extrapolation from other sources as outlined in the Introduction.

5. Conclusions

In summary, the study demonstrates that Pb is more toxic to embryos of *Mytilus* and *S. purpuratus* compared to Zn and DOC offers moderate protection against Pb toxicity but has no effect on Zn toxicity. Salinity in the 21–33 ppt seawater range does not alter Pb toxicity and measured whole body metal burdens co-related well with abnormality in the case of both Pb and Zn. The Biotic Ligand Model (BLM) approach was validated by our demonstration that increased DOC concentration shifted the Pb EC50 to a higher level, without changing the LA50.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpc.2013.04.004.

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