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Mechanisms of Nickel Toxicity in the Highly Sensitive Embryos of the Sea Urchin *Evechinus chloroticus*, and the Modifying Effects of Natural Organic Matter

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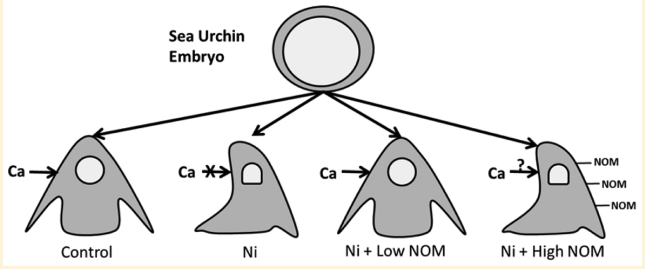
Supporting Information

ABSTRACT: A 96 h toxicity test showed that the embryos of the New Zealand sea urchin (*Evechinus chloroticus*) are the most sensitive of all studied marine species to waterborne nickel (Ni), with the EC_{50} for the development of fully formed pluteus larvae found to be 14 $\mu g\ L^{-1}$. Failure to develop a standard larval shape suggested skeletal impairment. Whole body ions (Na, Mg) increased with Ni exposure and calcium influx was depressed. The effects of natural organic matter (NOM) on Ni accumulation and toxicity were also examined in three different seawater sources (nearshore, offshore, and near the outlet of a "brown water" stream). At low dissolved organic carbon (DOC) concentrations the brown water NOM was protective against Ni toxicity, however at higher DOC concentrations it exacerbated developmental toxicity in the presence of Ni. These results show that sea urchin development is highly sensitive to Ni via a mechanism that involves ionoregulatory disturbance, and that Ni toxicity is influenced by environmental factors such as NOM. These data will be critical for the development of water quality guidelines for Ni in the marine environment.



INTRODUCTION

Metal contamination is of growing concern in aquatic settings owing to inputs from increasing industrial activity and fossil fuel production during the last century.[1] In a recent survey of coastal sediments of New Zealand's South Island, nickel (Ni) was the only metal that exceeded sediment quality guidelines,[2] with Ni also identified as a metal of concern in other studies of marine sediments worldwide.[3,4] In seawater itself, Ni concentrations range from 0.2 to 100 $\mu g\ L^{-1}$, with the highest values the result of anthropogenic inputs.[5] For example, in New Zealand, risk assessments have identified activities that disturb Ni-enriched sediments as contributing to local seawater Ni concentrations that may exceed regulatory criteria.[6]

Sea urchin embryos are considered to be excellent indicators of marine pollution, in part because they are the most sensitive of all tested organisms to metal toxicity.[7] For Ni, reported EC_{50}s (concentration required to generate a given toxic effect in 50% of the exposed population) vary from 15 to 341 $\mu g\ L^{-1}$ (Table 1). These lower concentrations are well within the range of environmental Ni, and are therefore of significant environmental relevance. For example, regulatory values in Canada, the U.S., and New Zealand fall close to, or are within, this range (8.2−75 $\mu g\ L^{-1}$).[8−10] The cause of the variation between toxicity studies remains poorly understood, largely because the mechanisms of Ni toxicity in sea urchins remain relatively unexplored.

There are three main mechanisms of Ni toxicity to aquatic biota: ionoregulatory disturbance,[11−16] respiratory impairment,[12,17,18] and oxidative damage.[11,12,19,20] Respiratory toxicity is generally considered the main mode of action in fish,[17,18] whereas ionoregulatory toxicity is the most reported mechanism of toxicity in invertebrates.[13,14] Water chemistry also influences Ni toxicity. For example, natural organic matter (NOM), a ubiquitous but variable component of natural waters, can bind metals, thereby reducing their bioavailability to the organism.[21] However, the composition and concentration of NOM will vary from source to source, as will its protective effects.[22,23] As many predictive modeling approaches, such as the biotic ligand model (BLM), utilize the relationship between free metal ion (the bioreactive form) and toxic effect,[24] then

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Table 1. Toxicity of Ni to Sea Urchin Embryo-Larval Development

species	common name	toxicity measure[a]	concentration (μg L^{-1})	exposure duration (h)	temperature (°C)	nominal (N) or measured (M) Ni	DOC (mg C L^{-1})	salinity (‰)	reference
Anthocidaris crassispina	murasaki uni	EC[b]	1200	12	28	N		34	65
Diadema antillarum	long-spined sea urchin	EC_{50}	15	40	20	M		33	31
Diadema savignyi	banded diadem urchin	EC_{50}	94	48	25	M	0.8	34	33
Diadema setosum	black longspine urchin	EC[b]	500	48	27	N			32
Evechinus chloroticus	kina	EC_{50}	14	96	15	M	0.5	32	this study
Hemicentrotus pulcherrimus	Chinese sea urchin	EC[b,c]	500	48	18	N			35
Hemicentrotus pulcherrimus	Chinese sea urchin	EC_{50}	34	64	16	N		32	58
Paracentrotus lividus	stony sea urchin	EC_{50}	320	72	18	N		35	36
Paracentrotus lividus	stony sea urchin	EC_{10}	217	72	16	M	1.0	38	7
Strongylocentrotus purpuratus	purple urchin	EC_{50}	341	96	15	N		34	66
Strongylocentrotus purpuratus	purple urchin	EC_{10}	335	48	16	M	1.2	30	7

[a]EC_x = Exposure concentration required to cause X% mortality at the given exposure duration. [b]"Effect concentration": lowest tested exposure concentration at which embryo development was abnormal. [c]Represents exposure where fertilization was also performed in the presence of Ni

understanding the role of NOM on Ni bioreactivity and toxicity is of significant regulatory value.

The current study aimed to determine the sensitivity of early life-stages of the New Zealand sea urchin *Evechinus chloroticus* to waterborne Ni. *Evechinus* is endemic to New Zealand, where it is of significant cultural and socioeconomic value.[25] However, nothing is known regarding the sensitivity of this species to metal toxicants. Following assessment of lethal Ni exposure concentrations, the potential role of ionoregulatory disturbance in generating embryo-larval Ni toxicity was investigated, and the effects of NOM on Ni toxicity were examined. This study identifies *E. chloroticus* as the most sensitive of all marine species to Ni, confirms an ionoregulatory mechanism of toxicity, and for the first time shows important direct and indirect roles for NOM in modifying sensitivity of sea urchin embryos to metal toxicants at environmentally relevant exposure concentrations.

MATERIALS AND METHODS

Animals. Adult sea urchins (*E. chloroticus*) were collected from Lyttleton Harbour, Canterbury, New Zealand and held at the University of Canterbury in a recirculating, constantly aerated, seawater system maintained at 15 °C. All subsequent experimental procedures were performed in a controlled temperature room set to 15 °C, in full-strength seawater (32 ppt, pH 8.1).

Fertilization. To induce spawning, 10 mL of 0.5 M KCl was injected into the hemocoel of adult sea urchins.[26] Sperm was collected from three males (pooled for each set of experiments) and stored on ice (4 °C) for up to an hour until eggs were collected. Eggs were collected from spawning females (N = 3) and added to 50 mL of seawater in plastic flasks to give a density of 100 eggs mL^{-1} (all densities based on counts made with a Sedgewick-Rafter cell). Approximately 200 μL of sperm was added to each egg-containing flask and stirred gently to facilitate fertilization. Once at least 80% fertilization was achieved, aliquots of eggs were added to 500 mL acid-washed glass beakers containing fresh seawater with an appropriate water chemistry (i.e., Ni and/or NOM; see below) to achieve a final density of 20 fertilized eggs per mL.

EC_{50} Determination. All EC_{50} exposures were conducted in natural seawater collected from Lyttelton Harbour (GPS coordinates: −43.604343, 172.714833; "UC NOM"). Concentrations of Ni (nominally: 0, 15, 30, 60, 120, 240, 480, and 960 μg L^{-1}; N = 3; from a stock solution of $NiCl_2 \cdot 6H_2O$; Sigma-Aldrich) were prepared in UC NOM 24 h in advance of the start of the experiment to allow equilibration. After 48 and 96 h, embryo development was scored. Abnormal development was defined as an embryo that did not display typical gastrula (48 h) or pluteus (96 h) form, relative to the time-matched control. After 96 h 15 mL of water containing 300 embryos was removed and development terminated by the addition of 1% neutral buffered formalin (NBF) for staging of development. Remaining embryos were taken for metal analysis by filtering solutions through a preweighed 8 μm polycarbonate filter, which was then taken for analysis of embryo element and Ni concentrations. Water samples were taken at the start and the end of the exposure (times 0 and 96 h). For Ni analyses, both unfiltered and filtered (0.45 μm syringe filter) samples were taken. Since there was less than a 5% difference between filtered and unfiltered samples, only filtered water Ni concentrations are reported.

NOM Collection, Preparation and Analysis. To determine the effect of NOM composition on toxicity, two additional NOM sources were obtained. Offshore NOM (OS NOM) was collected by boat approximately two kilometers from Banks Peninsula in the Pacific Ocean (−43.588257, 172.835396). West Coast NOM (WC NOM) was collected from Kumara on the West Coast, where a brown water stream, draining native beech forest, entered the Tasman Sea (−42.572311, 171.116101). This brackish sample (13 ppt at collection) was returned to the University of Canterbury, and concentrated by evaporation via gentle heating until the salinity reached 32 ppt. To make 10 and 50% WC NOM, the 100%

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WC NOM (that which had been concentrated to 32 ppt) was diluted with West Coast seawater, collected at the same time as the WC NOM, but 1 km south of the river inlet. Prior to exposures all NOM samples were passed through an 8 μm filter. A subsample of each NOM (including UC NOM) was shipped to Wilfrid Laurier University for measurement of dissolved organic carbon (DOC) concentration and molecular structural information via fluorescence−excitation-emission measurements (FEEM). For both analyses samples were filtered (0.45 μm GMF GD/C membrane). Measurement of DOC was performed using a Shimadzu TOC-Vcph/CPN total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan). For FEEM analysis fluorescence surface scans of emission wavelengths were recorded from 250 to 600 nm in a Varian Cary Eclipse Fluorescence spectrophotometer (Varian, Mississauga, ON) using a 1 cm quartz cuvette. During the scan fluorescence moved in 1 nm increments for every 10 nm excitation wavelength increment between 200 and 450 nm. Scan speed was set at 400 nm min^{-1} and the photomultiplier tube was set to high detection (800 V). The excitation and emission monochromator slit widths were both set to 5 nm for all measurements.

Fluorescence indices (FI) were determined, using the following calculation:[27,28]

$$FI_{ex370} = \frac{em450}{em500}$$

where FI_{ex370} is the fluorescence index at 370 nm and em500 and em450 are the emission intensities at 450 and 500 nm.

NOM Effects on Ni Toxicity. Fertilized eggs were separated into five different NOM treatments (UC NOM, OS NOM, 10% WC NOM, 50% WC NOM, 100% WC NOM). Within each treatment ($N = 5$) there was a control (0 $\mu g\ L^{-1}$ of Ni), and two concentrations of Ni (15 and 30 $\mu g\ L^{-1}$; that is, one concentration at the EC_{50}, one above). After 96 h the test was terminated by the addition of 1% NBF (see above). To assess development 100 embryos from each replicate were scored, and water samples for metals analysis were also taken, as described above.

Ion Influx. Fertilization and exposure was conducted as described above, and at each assay time-point (every 24 h for the first 96 h following fertilization) 1250 embryos were removed (following counting on Sedgewick-Rafter cell), and placed in 5 mL screwtop plastic vials. To each vial a matching volume (0.5−2.5 mL, varying depending on embryo density in exposure chambers) identical in water chemistry to the exposure was added containing 0.5 μC_i of either ^{22}Na or ^{45}Ca (PerkinElmer). The effect of Ni exposure (30 $\mu g\ L^{-1}$ in UC NOM) was tested relative to a Ni-free control (UC NOM alone). After 20 min incubation[29] embryos were collected on a 0.45 μm membrane filter and rinsed with 10 volumes of seawater. Filters containing embryos were counted directly for ^{22}Na analysis using a gamma counter (Wizard Wallac 1470; PerkinElmer), or for ^{45}Ca were digested in 2 mL of 2N HNO_3 before addition of 15 mL of scintillation fluor (Ultima-Gold) and counting via a liquid scintillation analyzer (TriCarb 2910TR: PerkinElmer). Triplicate 1 mL water samples (of spiking, rather than exposure, solutions to avoid harvesting of embryos) were taken for determination of specific activity. Quenching of ^{45}Ca samples was accounted for using the external standards ratio approach. Influx (J_{in}) was calculated as

$$J_{in}(\text{nmoles embryo}^{-1}\text{h}^{-1}) = \frac{\text{cpm/SA}}{n \times T}$$

where cpm is the counts in the embryos (quench corrected in the case of ^{45}Ca); SA is the specific activity (cpm/nmol); n is the number of embryos (1250) and T is time in hours. This exposure was repeated examining the effect of elevated NOM alone (10% WC NOM) and Ni (30 $\mu g\ L^{-1}$) in the presence of 10% WC NOM.

Embryo Element Analysis. Embryos and filter paper were digested in 2N HNO_3 at 65 °C for 48 h. Embryo digests were then diluted in nanopure water (>18 MΩ), and analyzed for metals and ions via inductively coupled plasma mass spectrometry (ICPMS; Agilent 7500cx). Quality assurance and quality control involved the addition of ^{103}Rhodium as an internal standard and the inclusion of a blank and spiked standards (2 and 20 $\mu g\ L^{-1}$) every 20 samples. Filter paper blanks (treated identically to embryo digests) were also run to determine the effect of the filter paper on the ionic composition of the embryos. These controls contributed negligible concentrations of Na, K, Ca, Mg, and Ni, however these blank values were subtracted from reported values for Ni and element concentrations. Water Ni concentrations for all exposures are provided in Tables S1 and S2, and other water chemistry parameters in Table S3.

Statistical and Speciation Analysis. Ion influx (with water chemistry and time as the two factors) was analyzed via two-way ANOVA. Following the identification of a lack of a time effect, data were expressed as a percentage of the time-matched control, then grouped across times. The effects of NOM on development (with NOM source and Ni concentration as the two factors) were analyzed via two-way ANOVA, with a Tukey's post hoc test. The EC_{50} values and 95% confidence intervals were determined from a sigmoidal logistic curve in SigmaPlot. Embryo element concentrations were assessed via a one-way ANOVA, followed by a Dunnett's post hoc test. Significance for all statistical tests was accepted at $\alpha = 0.050$. All data have been expressed as means ± SEM. Speciation of Ni was determined using Visual MINTEQ software (ver. 3.1 beta), and is provided in Table S4. This software uses a NICA- Donnan model to estimate the effect of NOM on metal speciation.[30]

RESULTS AND DISCUSSION

Ni Toxicity to Developing *E. chloroticus*. In a 96 h embryo-larval toxicity test the EC_{50} of Ni was determined to be 14 $\mu g\ L^{-1}$ (95% confidence intervals: 12−17 $\mu g\ L^{-1}$) (Figure 1A). This characterizes the early life stages of *E. chloroticus* as the most sensitive of any tested marine organism to Ni toxicity. This value is well below the Australian/New Zealand marine trigger value for protection of 95% of species (70 $\mu g\ L^{-1}$) with 50% confidence[8] and the U.S. Environmental Protection Agency (USEPA) acute ambient water quality guideline (75 $\mu g\ L^{-1}$) for Ni in marine waters,[10] but slightly above the USEPA chronic guideline marine value of 8.2 $\mu g\ L^{-1}$.[10] Previous research has shown that the sea urchin *Diadema antillarum* exhibits a similar sensitivity to that noted here (15 $\mu g\ L^{-1}$).[31] However, toxicity for other sea urchin species shows significant variation, with EC_{50} values as high as 341 $\mu g\ L^{-1}$ also being recorded (Table 1). Sensitivity does not appear to correspond to obvious phyletic groupings, with other *Diadema* species shown to be significantly less sensitive than *D. antillarum*.[32,33] Although embryo Ni burden increased with

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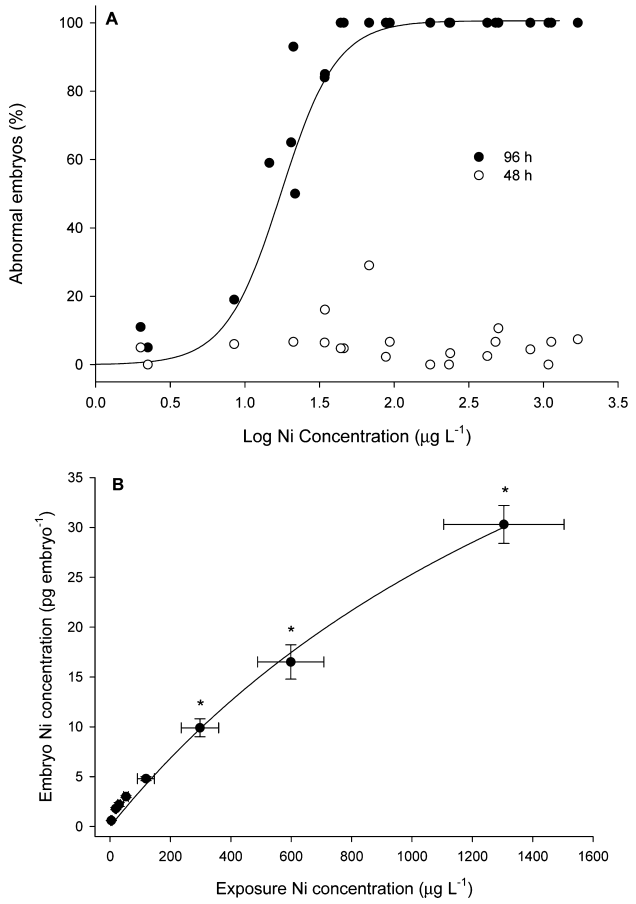

Figure 1. (A) Toxicity of Ni (determined using the end point of abnormal development) to sea urchin (*Evechinus chloroticus*) embryos after 48 h (open circles) and 96 h (closed circles), in seawater (UC NOM) at 15 °C. Plotted curve represents the best-fit sigmoidal logistic regression (Sigmaplot ver. 11.2). (B) Whole sea urchin (*Evechinus chloroticus*) embryo Ni concentration following Ni exposure in seawater (UC NOM) for 96 h at 15 °C. Plotted points represent means ± SEM (N = 3). Significant differences between control (4 µg L^{-1} Ni) and exposure concentrations (*) were determined by a one-way ANOVA followed by Dunnett's post hoc test (α = 0.050).

Ni exposure concentration (Figure 1B), this metric was not significantly correlated with toxicity (see below). A hyperbolic curve fit of the relationship between embryo Ni concentration and toxicity yielded a 96 h LA_{50} (accumulation leading to 50% mortality) of 1.5 ± 0.5 pg $embryo^{-1}$ (data not shown).

After 48 h of exposure to Ni, embryos were developing normally (Figure 1A), and it was only by 96 h where notable abnormal development occurred. Similar findings have been reported previously, with Ni exposure at early stages of development having no noticeable effect relative to the severe effects observed at later developmental stages.[34−36] After 48 h sea urchin embryos are entering gastrulation. This phase includes the formation of primary mesenchyme cells, which will eventually form the skeleton/spicule of the pluteus larvae.[37] During this period the skeleton, which is composed largely of Ca carbonate, undergoes biomineralization, which is dependent on Ca uptake from the environment.[38]

Exposure to Ni has been previously shown to impair skeletal development.[34] It has been speculated that Ni prevents proper cell migration and commitment during development, potentially via an inhibition of intercellular communication.[39,40] Interestingly manganese, which like Ni is a Ca channel blocker, disrupts extracellular signal-regulated kinase (ERK)-mediated signaling pathways,[41] which perform critical roles in primary mesenchyme cell processes in sea urchins.[42] This effect was likely mediated by an effect on Ca uptake and resulted in impaired skeletogenesis in sea urchins.[41] These morphological and functional changes are similar to those observed in the current study, indicating that this is likely the pathway by which Ni impairs development. It is of note that homologous developmental pathways are differentially regulated between different species of sea urchins,[43,44] which may help explain the variation in sensitivity from species to species (Table 1).

Further evidence for an effect on Ca metabolism was provided by ion influx experiments (Figure 2). In contrast to a

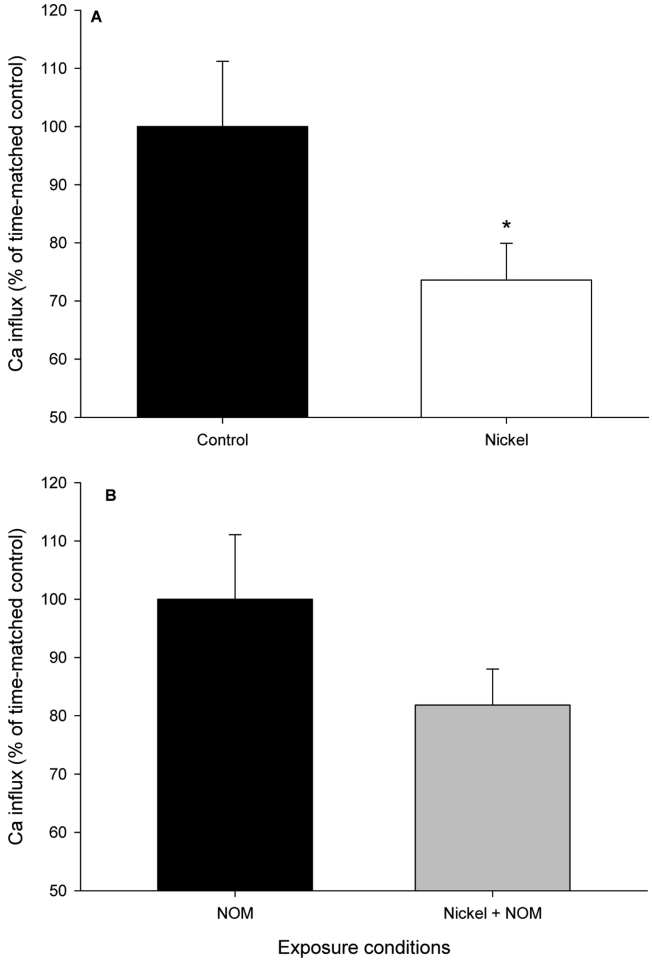

Figure 2. Unidirectional Ca influx (nmol $embryo\ h^{-1}$) in sea urchin embryos (*Evechinus chloroticus*) exposed to 30 µg L^{-1} of Ni in the absence (A) or presence (B) of NOM (10% WC NOM). Initial analysis of two-way ANOVA outcomes indicated a lack of a time effect, and thus data were collated by expressing as a percentage of the time matched control, and grouped between times. Plotted points represent means ± SEM (N = 5), where differences between control and Ni exposure groups are indicated by an asterisk.

lack of effect on Na (data not shown), and a lack of the effect of Ni in the presence of elevated NOM (P = 0.612; Figure 2B), Ni was observed to inhibit Ca influx (P = 0.045; Figure 2A). Effects of Ni on Ca homeostasis have been previously described in a range of organisms, including developing sea urchin embryos.[29,45−49] For example, Ni has been shown to impair the

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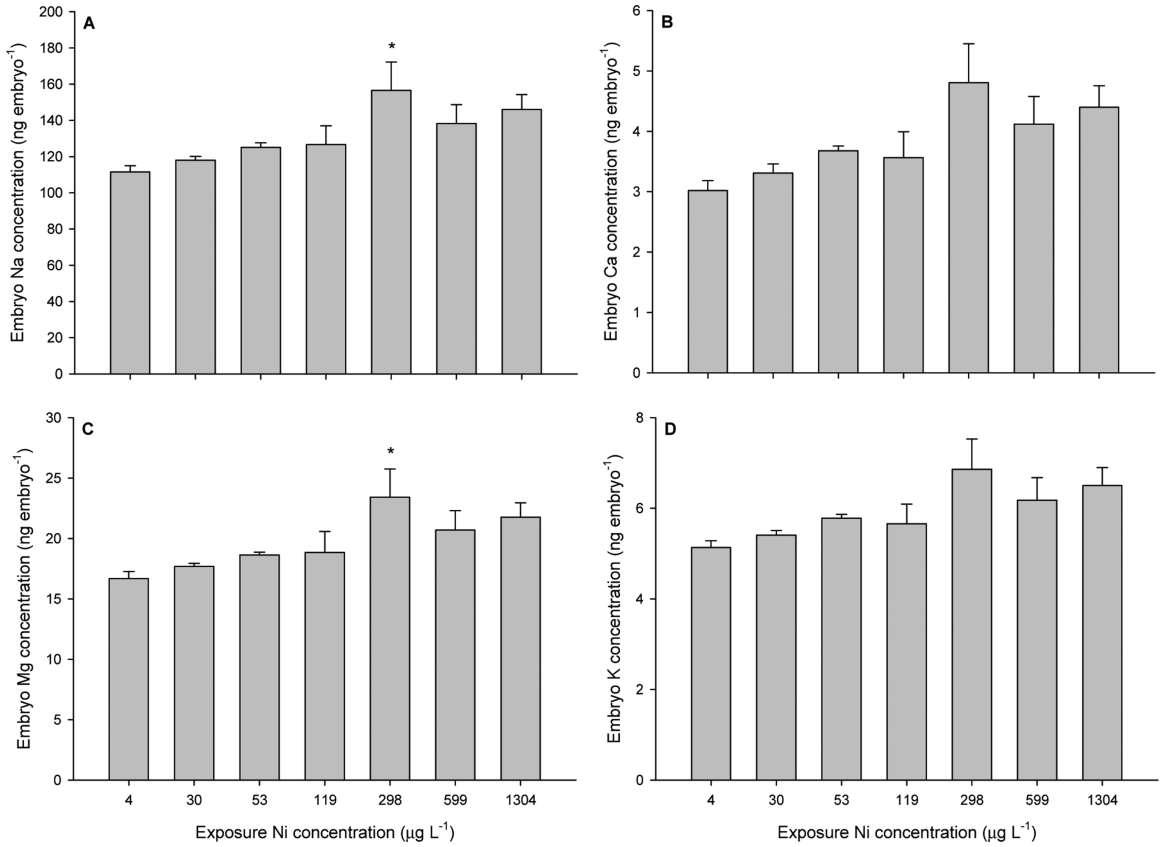

Figure 3. Whole sea urchin (*Evechinus chloroticus*) embryo Na (A); Ca (B); Mg (C) and K (D) concentration following Ni exposure in seawater (UC NOM) for 96 h at 15 °C. Plotted points represent means ± SEM (N = 3). Significant differences between control (4 μg L^{-1} Ni) and exposure concentrations (*) were determined by a one-way ANOVA followed by Dunnett's post hoc test (α = 0.050).

activity of the basolateral Ca-ATPase in the early life-stages of another sea urchin species (*S. purpuratus*).[29] Such inhibition may restrict the movement of Ca from ectodermal tissue to the primary mesenchyme cells, which are primarily responsible for spicule formation.[50] Furthermore, an impaired efflux is likely to have led to increased intracellular Ca, a phenomenon supported in the current study by the trend of increasing embryo Ca burden (Figure 3). This in turn may have dissipated the concentration gradient facilitating apical Ca transport (likely via an epithelial Ca channel[47]), thus corresponding to a decrease in Ca influx. The lack of effect in the presence of NOM indicates that the effect was mediated by the free Ni ion, consistent with the hypothesis that Ni^{2+} is a Ca^{2+} mimic.[29]

Effects of Ni were not restricted to Ca. Nickel exposure also led to embryo Na and Mg concentrations that were significantly increased (P = 0.034 (Figure 3A) and 0.038 (Figure 3C), respectively). This effect was only statistically significant at a Ni exposure concentration of 298 μg L^{-1} (Figure 3AC). Similar trends were observed for Ca and K, but these eluded statistical significance (P = 0.052 (Figure 3B) and 0.079 (Figure 3D), respectively). Nickel is considered an ionoregulatory toxicant in invertebrates and has been previously reported to disturb Mg, Na, Ca, and K homeostasis via ionic mimicry and/or inhibition of transporters.[12,13,15,29] This may indicate an inhibition of Na^+/K^+ ATPase, as this basolateral transporter generates gradients for cellular transport of all ions. Previous evidence has shown that Na^+/K^+ ATPase activity can be disrupted at 8.2 μg L^{-1} of Ni in adult crabs, resulting in perturbed hemolymph Na.[19]

Effects of NOM on Embryo Ni Concentration and Toxicity. A two way ANOVA highlighted overall significant effects of NOM (P < 0.001), Ni concentration (P < 0.001) and the interaction between these two factors (P = 0.04) with respect to normal development of sea urchin embryos. There were no significant differences across control treatments (Figure 4A). Post hoc tests revealed that the 10% WC NOM exposure resulted in a higher percentage of normal development at 52% (15 μg L^{-1}) and 41% (30 μg L^{-1}) relative to the reference NOM (UC NOM) at the same Ni concentrations (42% in 15 μg L^{-1}and 13% in 30 μg L^{-1}). This finding is in agreement with the protective role of NOM against Ni toxicity.[21] NOM displays multiple binding sites for cations and therefore functions as a multisite complexing ligand for metals. This decreases the availability of the metal to the organism in the aquatic environment. Speciation analysis (Table S4) showed that in 100% WC NOM, only 46% of the total Ni was present as the free ion, generally considered the bioavailable form.[51] The controlling effect of NOM complexation of Ni is illustrated clearly in Figure 4B, which exhibits a strong linear relationship between free Ni ion, and embryo Ni concentration (r^2 = 0.78; P < 0.001). Confirming the protective effects of NOM, the overall inhibitory effect of Ni on Ca influx rate (Figure 2A) was not observed in the presence of 10% WC NOM (Figure 2B), lending support to the evidence for ionoregulatory impairment as an important underlying mechanism of Ni toxicity to *E. chloroticus* embryos.

The protective effects of 10% WC NOM are likely attributable to its higher concentration (1.5 mg C L^{-1}) relative to UC NOM (0.5 mg C L^{-1}) and OS NOM (0.2 mg C L^{-1}; see

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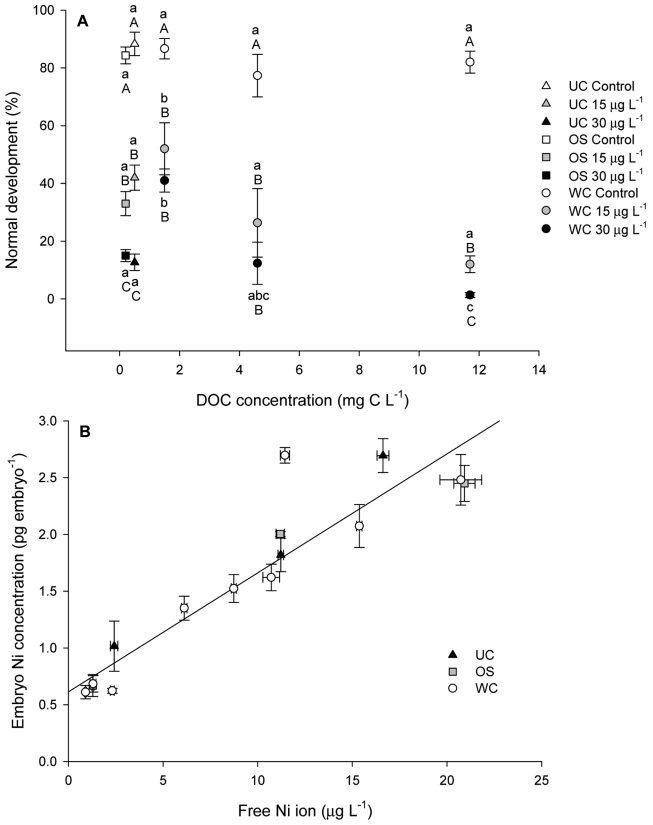

Figure 4. Ni toxicity (A) and embryo Ni concentration (B; pg embryo^{-1}) in sea urchin (*Evechinus chloroticus*) embryos exposed to one of five different NOM treatments (UC NOM, OS NOM, 10% WC NOM, 50% WC NOM, and 100% WC NOM) and three different concentrations of Ni (0, 15, and 30 μg L^{-1}) for 96 h at 15 °C. Plotted points represent means ± SEM (N = 5). In panel A statistical significance was determined by a two-way ANOVA and Tukey post hoc test (α = 0.050). Plotted points sharing lower case letters are not significantly different across NOM treatments within a Ni concentration. Bars sharing upper case letters are not significantly different between Ni concentrations within an NOM treatment. Free Ni ion in panel B was based on speciation analysis in Table S4.

Table S3). However, NOM composition may also be important. All three NOM sources were found to have similar optical components but with varying contributions. The WC NOM samples had the highest fluorescence intensity reflecting their relatively high DOC concentrations (see Figure S1). Structurally the WC fluorophores contain more humic and fulvic acid-like fluorescence with emissions in the range 400–450 nm, characteristic of allochthonous NOM. The OS NOM and UC NOM also exhibited fluorescence in the humic/fulvic acid range but also displayed fluorescence in the range normally attributed to proteinaceous substances (wavelengths between 280 and 350 nm. Our FI indices are also in accordance, with the low values calculated for WC NOM (1.2), indicating a more fulvic/humic source.[27] Sources of NOM that are rich in humic and fulvic acids have been shown to have a greater protective effect against metal toxicity,[52−55] consistent with the data shown here. It should be noted that in order to achieve a salinity suitable for embryo development the WC NOM was gently heated to achieve evaporation. It is known, at least for soil NOMs and copper, that heating can increase metal complexation capacity.[56] As such the data for WC may overestimate the 'natural' protective effects of this NOM against Ni burden and toxicity.

The relationships between water chemistry, tissue metal burden and toxicity are key tenets in regulatory tools such as the BLM. In the current study, there was a strong linear relationship between free Ni ion and embryo Ni concentration (Figure 4B), supporting this concept. As previously mentioned, as NOM concentration increased, more complexing ligands were available to bind Ni, reducing the free ion form that is bioavailable to the embryo. However, the relationship between body Ni burden and toxic effect was less clear. For example, although the EC_{50} was 14 μg L^{-1} (with corresponding LA_{50} of 1.5 pg embryo^{-1}), a statistically significant effect of exposure concentration on embryo Ni burden was only seen at concentrations 20 times this (see Figure 1B). Furthermore, there were no significant correlations between Ni body burden and effects on embryo element concentrations (linear regression P value range 0.64 to 0.92, all r^2 < 0.01; data not shown), although it was notable that significant effects of Ni exposure on both Ni burden and embryo Na and Mg were apparent at the same exposure concentration (298 μg L^{-1}). A lack of correlation between body metal burden and toxic effect is commonly observed in invertebrates, owing to their ability to sequester metal in detoxified forms, that contribute toward metal concentration but not bioreactivity.[57] For example, Ni is known to induce expression of the metal-chelating enzyme metallothionein in sea urchin larvae,[58] and Ni may also be directly incorporated into the calcium carbonate endoskeleton of developing sea urchins.[50]

In the 100% WC NOM exposures there was an unexpected effect on development. In this water chemistry normal development was reduced from 82% in the control treatment to 12% in 15 μg L^{-1}and just 1% in 30 μg L^{-1} exposed embryos (Figure 4A). It is known that NOM has a direct effect on membrane function, thought to be manifested by alterations in membrane fluidity.[54,59−61] Such an effect would be most prevalent at high NOM concentrations and may explain the observed decrease in normal embryo development in the high WC NOM exposures. However, this is an effect that was only observed in the presence of Ni, suggesting that the mechanism of effect relies on the presence of the metal. It is also important to note that this effect is independent of embryo Ni burden (see Figure 4B), and was not associated with changes in embryo element concentrations (Table S5). Previous research has shown that changes in Ca metabolism may disrupt membrane fluidity.[62,63] Given the effect of Ni on cellular Ca handling, the presence of NOM may have an additive or synergistic effect (possibly via NOM chelation of Ca), leading to impaired cellular transport functions that depend on membrane fluidity.[64]

The use of naturally collected SW sources is advantageous in that the NOM component will be representative of marine NOMs, and thus environmentally relevant. However, variations in ion concentrations between the different SW treatments may be a complicating factor, and could have influenced the results observed. For example, the 10% WC treatment has the highest water Ca concentration (Table S3), and in the presence of Ni also has the highest proportion of normally developing embryos (Figure 4A). It is therefore possible that under conditions where Ca transport is compromised (i.e., in the presence of Ni), that the additional water Ca is sufficient to maintain embryo Ca requirements. Although there are similar patterns in terms of other major ions (Na, K, Mg; Table S3),

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these correlate less well with protection (i.e., highest concentrations do not correspond with greatest toxicity amelioration; Na, Mg) and/or are ions for which there is little evidence of impairment in the current study (K). Future studies that specifically manipulate the concentrations of ions, while maintaining NOM constant, may be required to delineate between the Ni toxicity ameliorating effects of water chemistry in general, and specific effects mediated by the composition and concentration of the NOMs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b05626.

Tables of exposure water chemistry, Ni speciation, and whole embryo ions following NOM exposures. FEEM's of NOM sources are also included (PDF)

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Notes

The authors declare no competing financial interest.

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