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Influence of Ligand Complexation on Nickel Toxicity, Speciation and Bioavailability in Marine Waters

Samantha Sherman
sher2620@mylaurier.ca

Influence of Ligand Complexation on Nickel Toxicity, Speciation and Bioavailability in Marine Waters

By

SAMANTHA R. SHERMAN

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Currently there are no site-specific bioavailability-based prediction models for assessing the impacts of nickel (Ni) in marine environments although there are indications that these may be warranted. The aim of this research was to characterize the complexation of Ni in relation to toxicity and speciation. Various complexing ligands were used, and it was predicted that the binding affinity ($logK_f$) of ligands would be inversely correlated to toxicity based on dissolved Ni concentrations ($[Ni_D]$) but that on a free ion concentration ($[Ni^{2+}]$) basis, toxicity would not vary. A two-phased approach was used; the first was a proof of principle where synthetic ligands with known $logK_f$ values [EDTA, NTA, tryptophan (TRP), glutamic acid (GA), histidine (HD) and citric acid (CA)] were tested and the second, natural waters were characterized for binding capacity and Ni toxicity. Chronic Ni toxicity assays were performed using two marine species sensitive to Ni, the purple sea urchin (*Strongylocentrotus purpuratus*; where EC_{50} and EC_{20} values were determined) and the mysid (*Americamysis bahia*; LC_{50} and LC_{20}). Embryological development and mortality (respectively) were used as the toxicity endpoints. Ni was measured by graphite furnace atomic absorption spectroscopy (GFAAS). The $[Ni_D]$ E/LC_{50} values in unmodified artificial seawater (ASW) were 3.6 μM (95% CI 3.0-4.5 μM) for *S. purpuratus* and 2.6 μM (2.3-2.8 μM) for *A. bahia*. Tests with synthetic ligands provided significant protection based on $[Ni_D]$, particularly for those with strong complexation such as EDTA and NTA. However, when considered on the basis of $[Ni^{2+}]$, E/LC_{50} values were either similar or less than those in ASW. In natural seawater the E/LC_{50} values ranged from 2.0 to 7.0 μM based on $[Ni_D]$ and variability was reduced when expressed on a $[Ni^{2+}]$ basis. There were no significant

differences in Ni toxicity between natural waters and ASW. Overall, this study supports the theory that free Ni concentration is the best predictor of toxicity and confirms the applicability of a marine BLM for Ni. It also provides insight into the understanding of relationships between aquatic geochemistry, Ni speciation, complexation and toxicity from a biological perspective.

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I did it!

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I can't wait to see what's next.

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LIST OF ABBREVIATIONS AND SYMBOLS

˚C	Degrees Celsius
µM	Micromolar
µg/L	Micro grams per litre
95% CI	95% confidence intervals
ANOVA	Analysis of variance
ASW	Artificial seawater
BL	Biotic ligand
BLM	Biotic ligand model
Ca^{2+}	Calcium
CA	Citric acid
CETIS	Comprehensive environmental toxicity information system
Cd/Cd^{2+}	Cadmium
Cl^-	Chloride
Cu/Cu^{2+}	Copper
CRM	Certified reference material
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EC_{10}	10% effective concentration
EC_{20}	20% effective concentration
EC_{50}	Median (50%) effective concentration
EC	Environment Canada
EDTA	Ethylenediaminetetraacetic acid
FW	Freshwater
GA	Glutamic acid
GFAAS	Graphite furnace atomic absorption spectroscopy
h	Hour
HD	Histidine
HNO_3	Nitric acid
IC_{20}	20% inhibition concentration
IC_{50}	Median (50%) inhibition concentration
IET	Ion exchange technique
K'_{BL}	Binding affinity of the biotic ligand
K_f	Binding affinity
K^+	Potassium
KCl	Potassium chloride
L	Litre
LC_{20}	20% lethal concentration
LC_{50}	Median (50%) lethal concentration
Mg^{2+}	Magnesium
mg/kg	Milligrams per kilogram
mg C/L	Milligrams of carbon per litre
min	Minute
mL	Millilitre
mm	Millimetre

n	Sample size
Na^+	Sodium
NaOH	Sodium hydroxide
Ni	Nickel
Ni^{2+}	Nickel free ion
Ni_D	Dissolved nickel
Ni_T	Total nickel
nm	Nanometers
NOM	Natural organic matter
NTA	Nitrilotriacetic acid
pH	Negative log of the hydrogen ion concentration
ppt	Parts per thousand
R^2	Coefficient of determination
r	Pearson's product-moment correlation coefficient
RO	Reverse osmosis
RSD	Reproducibility standard deviation
SD	Standard deviation
SSD	Species sensitivity distribution
SW	Seawater/saltwater
TRP	Tryptophan
US EPA	United States environmental protection agency
WHAM	Windemere humic aqueous model
YSI	Yellow spring instrument
Zn/Zn^{2+}	Zinc

CHAPTER 1:

General Introduction

1.1 Ni in the environment

Nickel (Ni) is the 22nd most abundant metal and is one of the transition metals, similar to copper (Cu) and zinc (Zn; Pyle and Couture 2012; Meyer 1999). A transition metal means that chemically it can complex with different molecules in a solution to form coordinate compounds, which are where anions (complexing agents) bind to a central metal ion by coordinate covalent bonds. Ni is naturally occurring and environmental inputs can result from erosion and weathering of Ni-containing minerals, hydrothermal vent outputs, soil run-off, fire debris and vegetation (Eisler 1998). Ni is ubiquitous in the environment and is the 24th most abundant element in the earth's crust with a mean concentration of 75 mg Ni/kg (Chau and Kulikovsky-Cordeiro 1995). Natural concentrations range from 0.003 to 0.01 μM in the open ocean and can reach values of 0.15 μM in coastal environments (Wells et al. 2000). Low concentrations of Ni allow for use by plants as a micronutrient in regards to plant growth and by bacteria in the biosynthesis of different compounds; it is not clear if it is used by different aquatic invertebrates (Ragsdale 1998). These contributions are very low compared to inputs from anthropogenic sources.

Globally, the largest anthropogenic releases are from coastal mining, manufacturing and processing plants (Chau and Kulikovsky-Cordeiro 1995). Other anthropogenic sources include: municipal or industrial effluent, building material runoff, plumbing and anti-fouling paints which can lead to concentrations of Ni typically ranging from 0.02 to 1.70 μM in marine environments (Boyden 1975; Eisler 1998). A study conducted by Chester and Stoner (1974) summarized average dissolved Ni concentrations of estuarine and near shore sites globally. Some of which included: Tampamachoco Lagoon and Tuxpan River Estuary (Mexico) 0.39 to 0.65 μM, Jundiai River Estuarine (Brazil) 0.85

μM, Andoni River Estuary (Nigeria) 1.36 to 1.87 μM, Northeastern Atlantic 0.009 to 0.09 μM, South African Coast 0.01 to 0.09 μM, and China Sea 0.01 to 0.09 μM. Ni concentrations in plants, animals and abiotic materials are elevated in the vicinity of nickel smelters and refineries, nickel-cadmium battery plants, sewage outfalls, and coal ash disposal basins (Chau and Kulikovsky-Cordeiro 1995). Dissolved waterborne metal exists in solution partially as free metal ion. In general the free ion (i.e. Ni^{2+}) is the most bioavailable and therefore toxic geochemical form (species) although other geochemical species can also be associated with toxic effects (Landner and Reuther 2005; Niyogi and Wood 2004; Wood et al. 2011). Currently there are no site-specific bioavailability-based prediction models for assessing the impacts and risks of Ni in marine environments although there are indications that these may be warranted (Gissi et al. 2016). This is due to the fact that there is insufficient good quality chronic data on Ni toxicity to marine biota and read-across methods from freshwater (FW) databases are not permitted as the geochemical speciation within these models cannot be extrapolated to saltwater (SW) environments (Gissi et al. 2016). However, ongoing work is continuing towards the development of a robust SW model (Blewett and Leonard 2017).

1.2 BLM

Ni toxicity in marine environments is not as well understood as it is in FW where factors modulating toxic effects have been incorporated into site-specific bioavailability-based prediction models. Within FW systems, several modelling programs exist that account for geochemical speciation at equilibrium conditions, such as MINEQL+, Visual Minteq and the Windermere Humic Aqueous Model (WHAM VI). The binding affinity for inorganic complexes are well established in these programs, but reactions involving dissolved organic matter (DOM) are far more difficult to quantify (Stockdale et al. 2011,

2015). WHAM for example, has not been calibrated with marine specific ligands so the role of DOM in reducing toxicity in SW is less certain; however, at high salinities it predicts poor protection caused by the weak binding of DOM (Stockdale et al. 2011). Currently there are no models available that are able to predict Ni toxicity in marine systems, although frameworks are currently in development (Gissi et al. 2016).

The biotic ligand model (BLM) is a predictive modeling approach that utilizes the relationship between water chemistry and tissue-metal accumulation to estimate toxic effects. The BLM replaces the fish gill as this site of action with a more general 'biotic ligand' in order to allow the model to be applicable to other aquatic organisms (Fig. 1; Di Toro et al. 2001). The predictions of toxicity generated by the BLM are based on knowledge of the specific sites to which a metal binds including the total number of binding sites and the affinity of these sites for the metal (K_f) at the site of toxic action on an organism (the biotic ligand; Blewett and Wood 2015; Di Toro et al. 2001; Paquin et al. 2002). This toxicity is not only related to metal concentration but also metal-ligand complexation and metal interactions with competing cations as defined by the site-specific water chemistry conditions (Pagenkopf 1983; Paquin et al. 2002). The role of metal complexation is important to consider because formation of organic and inorganic metal complexes generally renders a significant portion of the total metal non-bioavailable (Di Toro et al. 2001). This complexation will reduce the bioavailability of the metal and its uptake into the biotic ligand, reducing toxicity (Playle et al. 1993). The BLM has been used to predict metal toxicity for many dissolved metals in FW (Arnold 2005; Crémazy et al. 2018; Meyer et al. 1999; Paquin et al. 2002) and for some in SW (Blewett et al. 2018; Nadella et al. 2013). Playle et al. (1993) determined metal-gill

stability constant values using modelling software, which allowed for predictions of metal accumulation on fish gills and toxicity. To do this they estimated free ion (Cu^{2+} and Cd^{2+}) concentrations on the gill of fathead minnows in FW, with and without added ligands. Their approach of inserting biological data into an aquatic chemistry program has been a useful tool for modelling and predicting metal accumulation and toxicity. Therefore, verifying the BLM's applicability to Ni will be useful in interpreting and predicting the toxicity of Ni and developing it as a regulatory tool related to marine waters.

1.3 Organic Matter

Recently there have been many studies starting to investigate the potential protective effects of changing water parameters [i.e. pH, salinity and marine natural organic matter (NOM)] with regards to Ni toxicity for marine organisms (Blewett et al. 2018; Blewett and Wood 2015; Ho et al. 1999; Lussier et al. 1999; Tellis et al. 2014). In SW, the components of natural waters formed by the physical breakdown or microbial processing of plant and animal materials is referred to as NOM (Thurman 1985). NOM input into the BLM is done as the measured dissolved organic carbon (DOC: any organic carbon that passes through a 0.45μm filter) concentrations as well as the % humic acid. In natural marine waters ranging from open ocean to coastal waters DOC concentrations vary from 0.5 to 10.0 mg DOC/L (Benner 2002). In current literature, there is agreement that DOC plays a key role in reducing the toxicity of many metals by its ability to bind to them, effectively reducing bioavailability to the biotic ligand (Blewett et al. 2018; Playle et al. 1993; Wood et al. 2011). Blewett et al. (2018) showed that DOC-metal binding is significant in marine waters using the species *Mytilus edulis* and *Strongylocentrotus purpuratus*; concentrations at 4.5 mg C/L provided a 5 fold reduction in toxicity

compared to artificial seawater (ASW). Therefore, studies examining the role of DOC in terms of concentration will make important contributions to understanding marine Ni toxicity.

Growing evidence suggests that NOM from different sources exhibit different metal binding capacities that depend on their composition (Al-Reasi et al. 2011; Arnold et al. 2005; Blewett et al. 2016, 2018). DOCs can be grouped into two forms: allochthonous and autochthonous. Allochthonous DOC originates from the breakdown of leaves and wood or other terrigenous sources where autochthonous is formed from algae within lakes and rivers by degradation of allochthonous DOC (Thurman 1985). These different sources have different aromatic characteristics and Ni complexing capacities, which would have an overall effect on protection to toxicity (De Schamphelaere and Janssen 2004). Measuring and determining properties such as the specific absorption coefficient at 340 nm (SAC_{340}), the specific UV absorbance at 254 nm (SUV_{254}) or the fluorescence index (FI) can provide estimates of the relative aromatic composition of DOC. These are important factors in predicting potential Ni toxicity as they have been shown to affect metal binding (Al-Reasi et al. 2011; Blewett et al. 2018; Wood et al. 2011). In SW, Blewett et al. (2018) found the inclusion of optical parameters (i.e. SAC_{340}) did improve correlations to toxicity response values for *M. edulis* and *S. purpuratus* compared to DOC concentration alone. Therefore, studies examining the role of DOC in terms of both concentration as well as composition will make important contributions to understanding marine Ni toxicity and the development of BLM approaches to predicting potential impacts of Ni.

1.4 Anions and Cations

Water chemistry parameters play important roles in influencing Ni toxicity by affecting the bioavailability of Ni to the organism (Di Toro et al. 2001; Playle et al. 1993). As the transition from FW to SW occurs, the key anionic and cationic species change. As previously mentioned, anions (i.e. Cl^- and NOM) bind to a central metal ion (cation) by coordinate covalent bonds. Toxicity to an organism could be viewed as the interaction of metals with anionic surface sites on the biotic ligand such as that of the gill or membrane of an embryo (Smith et al. 2015). The anionic species that are considered important in Ni complexation are SO_4^2 and Cl^-, which increase in concentration with an increase in salinity (Blewett et al. 2018). It has been reported that $NiCl_2$ makes up ~15% and $NiSO_4$ ~20% of the total inorganic Ni in SW (Sadiq 1989). When these species bind to Ni to form complexes they will decrease free ion concentrations, limit bioavailability and therefore reduce toxicity (Playle et al. 1993; Sadiq 1989). However, when $NiSO_4$ and NiCl are formed in SW there could be potential for these Ni-anionic complexes to be bioavailable and contributing to the toxicity to marine organisms (Landner and Reuther 2005).

In the BLM, the free metal ion competes with other cations for binding at the biotic ligand. The major cations in SW are calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) and are approximately 10-, 200-, 110-, and 724-fold greater respectively, than in FW (Blewett and Wood 2015). The presence of these cations in solution can mitigate toxicity, the degree of which is dependent on the concentrations and strength of their binding to the biotic ligand (Di Toro et al. 2001). This can occur as they compete for binding sites against Ni on the biotic ligand, effectively reducing bioavailability (Paquin et al. 2000). Recently it has been recognized that not only inorganic complexes but also

various common cations have the potential to contribute to the total toxicity of these metals to aquatic organisms (Landner and Reuther 2005). Therefore, investigating complexation with anions and competition for uptake to the biotic ligand among cations is important in predicting toxicity. Especially as these water quality parameters that influence toxicity are needed as inputs for the BLM, which allow for site-specific water criteria guidelines to be determined (Paquin et al. 2002).

1.5 Test Organisms

Using a species previously proven to be sensitive to Ni is the most appropriate for studies trying to parameterize a marine BLM. DeForest and Schlekat (2013) compiled chronic Ni toxicity data for a total of 17 marine species to create a species sensitivity distribution (Fig. 2; SSD). SSDs are the most common method used to derive water quality criteria, which describe the variability and range of sensitivities among individual taxa (Wang et al. 2015). DeForest and Schlekat (2013) showed that sea urchins were among the most sensitive species with variations of up to 2 orders of magnitude. Sea urchin embryo bioassays are commonly used as a method to determine marine water quality criteria and can also be used to examine the physiological effects of metal toxicity because these early developmental stages are extremely sensitive to a variety of contaminants (Blewett et al. 2016; Phillips et al. 2003; Tellis et al. 2014). As such, purple sea urchin embryos (*S. purpuratus*) were used as one of the model organism in this study because: they are sensitive to Ni, they have an important ecological roles and they have a wide distribution along the eastern edge of the Pacific Ocean (Uthicke et al. 2009). Therefore they are valuable indicators of potential ecological damage to aquatic communities (DeForest and Schlekat 2013; Silva et al. 2013). The same study deemed mysids (*Americamysis bahia*) as the second most sensitive marine species tested to date

(DeForest and Schlekat 2013). The mysid has a high sensitivity to Ni and toxicity tests are designed to measure effects on survival, growth, and maturation of juvenile mysids during a critical period of growth and sexual maturation (DeForest and Schlekat 2013; Lussier et al. 1985; Lussier et al. 1999). Both *S. purpuratus* and *A. bahia* are excellent model species for marine Ni toxicity studies and can add data to current SSDs and ongoing environmental regulatory tools such as the BLM.

1.6 Thesis Goals

The overall objective of this study is to understand toxicity and speciation of Ni in marine organisms and to estimate metal-biotic ligand stability constants. This is in coordination with the goal to generate new data in order to strengthen SSDs for Ni impacts to marine species, and to understand the factors that influence the bioavailability of Ni, particularly the role of organic matter complexation. This thesis aims to:

I. Determine the sensitivity of *Strongylocentrotus purpuratus* embryonic life stages to Ni toxicity

II. Investigate the protective effects of synthetic ligands with regards to Ni toxicity

III. Determine the dependence of toxicity on the speciation of Ni

IV. Investigate the Ni toxicity to *S. purpuratus* embryonic life stages in natural marine waters and determine the relationship between toxicity and speciation

V. Understand potential protective effects of DOM in marine waters

VI. Use the defined BLM to estimate the $\log K_f$ (binding affinity) of the biotic ligand

VII. Determine ligand responses with another species by:

    i. Determining the sensitivity of *Americamysis bahia* to Ni toxicity

ii, iii. Investigating the protective effects of both synthetic ligands and natural waters (DOM) on toxicity and mysid development

iv. Determining the dependence of toxicity on the speciation of Ni

v. Using the defined model to estimate the $\log K_f$ (binding affinity) of the biotic ligand

1.7 Hypotheses Tested

Overall there were five main hypotheses to be tested, as outlined below:

1. Ni toxicity (EC_{50} [Ni_D]) will correlate to the binding affinity of complexing agent
2. Metal free ion toxicity (EC_{50} [Ni^{2+}]) will be similar regardless of exposure
3. Ni toxicity (EC_{50} [Ni^{2+}]) will be similar regardless of source
4. DOC concentration and composition will be important in predicting toxicity
5. Ni-biotic ligand stability constants will be used to permit site specific estimates of toxicity
6. Ni toxicity (LC_{50} [Ni^{2+}]) will show consistent results regardless of species

1.8 Chapter Summaries

Chapter 2 looks to characterize the complexation of Ni in relation to toxicity using embryological development of the purple sea urchin (*S. purpuratus*). This was used as a proof of concept to test the assumptions of the BLM that metal free ion (Ni^{2+}) toxicity will be similar regardless of exposure to differing ligands. Ni toxicity was reduced by addition of synthetic ligand into solution relative to the binding affinity. EC_{50} of dissolved Ni varied between synthetic ligands, but the [Ni^{2+}] EC_{50} of all values were either similar [EDTA and citric acid (CA)] or less [NTA, glutamic acid (GA) and histidine (HD)] than the values for tests in ASW. The exact mechanism of toxicity seen within exposures GA, HD and NTA was not looked at directly.

Based on the findings of Chapter 2, Chapter 3 was designed to better understand chronic toxicity of Ni in natural waters by exploring the effects of NOM on Ni toxicity and speciation. Natural waters had no significant correlations with toxicity, and there were no conclusive trends showing site-dependent protection, indicating that toxicity is independent of DOC source and composition. However, the [Ni^{2+}] EC_{50} was similar regardless of exposure, showing this to be the best predictor of Ni toxicity and showing agreement with the BLM assumptions.

Chapter 4 explored the BLM assumptions using another species sensitive to Ni, the marine mysid, *A. bahia.* Synthetic ligands and natural waters did decrease Ni toxicity based on [Ni_D], however not significant compared to the ASW exposure. The LC_{50} values based on [Ni^{2+}] were less variable than those of [Ni_D], and all exposures were not significantly different from the ASW exposure. This data supports the theory that free Ni concentration is the best predictor of toxicity.

Chapter 5 summarizes the main findings of the experimental chapters of this thesis and discusses the potential relevance of this work for understanding Ni toxicity from a biological perspective. It also discusses the integrative nature of this research and finally, it provides future directions with regards to the study of Ni toxicity within marine environments.

1.9 Figures

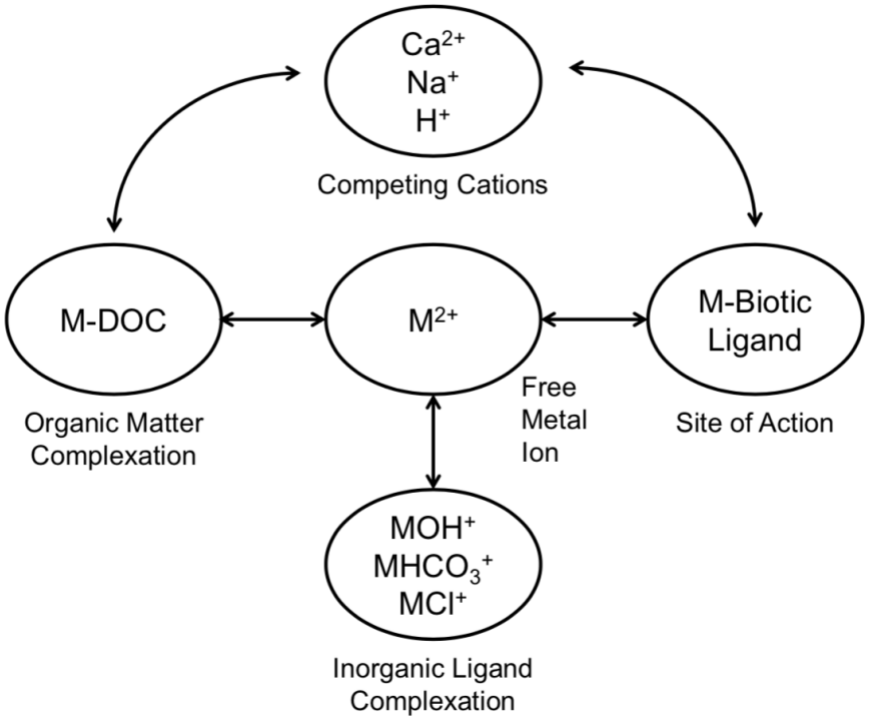

FIG. 1.1. A diagram displaying the biotic ligand model (Adapted from Playle et al. 1993; Di Toro et al. 2001).

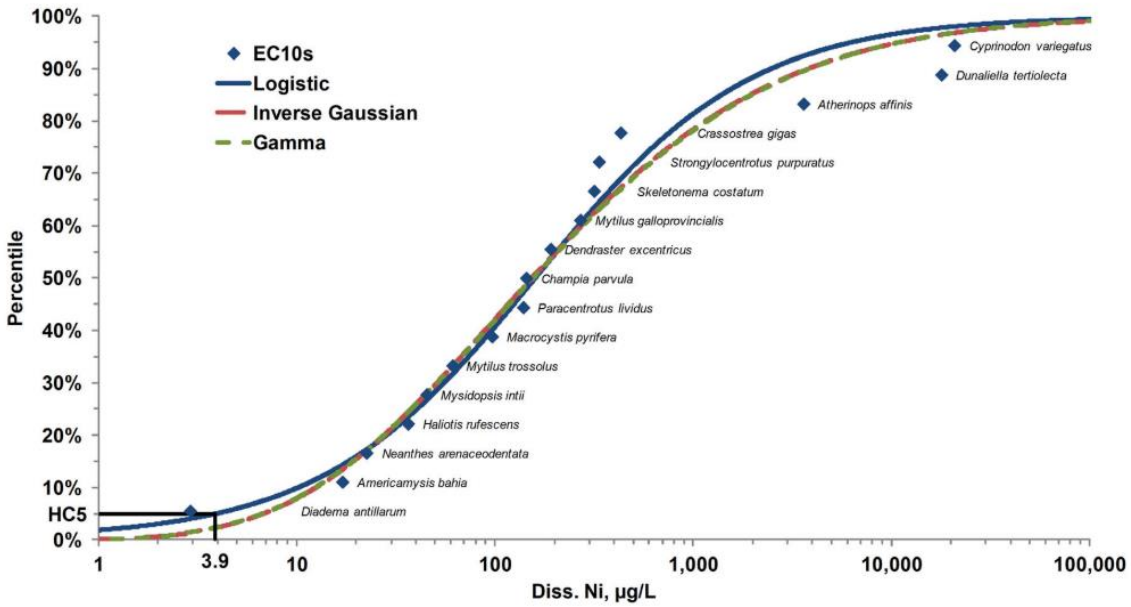

FIG. 1.2. A species sensitivity distribution (SSD) for 17 marine organisms for Ni in marine waters. Data and graph from DeForest and Schlekat (2013).

1.10 References

Al-Reasi HA, Wood CM, Smith DS. 2011. Physicochemical and spectroscopic properties of natural organic matter (NOM) from various sources and implications for ameliorative effects on metal toxicity to aquatic biota. *Aquat Toxicol*. 103:179-190.

Arnold WR. 2005. Effects of dissolved organic carbon on copper toxicity: Implications for saltwater copper criteria. *Integr Environ Assess Manag*. 1:34-39.

Benner R. 2002. Chemical composition and reactivity. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Elsevier, USA. 59–85.

Blewett TA, Dow EM, Wood CM, McGeer JC, Smith DS. 2018. The role of dissolved organic carbon concentration and composition on nickel toxicity to early life-stages of the blue mussel *Mytilus edulis* and purple sea urchin *Strongylocentrotus purpuratus*. *Ecotoxicol Environ Saf.* 160:162-170.

Blewett TA, Leonard EM. 2017. Mechanisms of nickel toxicity to fish and invertebrates in marine and estuarine waters. *Environ Pollut.*223:311-322.

Blewett TA, Smith DS, Wood CM, Glover CN. 2016. Mechanisms of nickel toxicity in the highly sensitive embryos of the sea urchin *Evechinus chloroticus*, and the modifying effects of natural organic matter. *Environ Sci Technol.* 50:1595-1603.

Blewett TA, Wood CM. 2015. Salinity-Dependent Nickel Accumulation and Oxidative Stress Responses in the Euryhaline Killifish (*Fundulus heteroclitus*). *Arch Environ Contam Toxicol*. 68:382-394.

Boyden CR. 1975. Distribution of some trace metals in Poole Harbour, Dorset. *Mar Pollut Bull.* 6:180-187.

Chau YK, Kulikovsky-Cordeiro OTR. 1995. Occurrence of nickel in the Canadian environment. *Environ Rev.* 3:95-120.

Chester R, Stoner JH. 1974. The distribution of zinc, nickel, manganese, cadmium, copper, and iron in some surface waters from the world ocean. *Mar Chem.* 2:17-32.

Crémazy A, Brix KV, Wood CM. 2018. Using the Biotic Ligand Model framework to investigate binary metal interactions on the uptake of Ag, Cd, Cu, Ni, Pb and Zn in the freshwater snail *Lymnaea stagnalis*. *Sci Total Environ.* 647:1611-1625.

DeForest DK, Schlekat CE. 2013. Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms. *Integr Environ Assess Manag*. 9:580-589.

De Schamphelaere KAC, Janssen CR. 2004. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environ Toxicol Chem.* 23:1365-1375.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem*. 20:2383-2396.

Eisler R. 1998. Nickel hazards to fish, wildlife, and invertebrates: A synoptic review. *Biol Sci Rep*. 34:95.

Gissi F, Stauber JL, Binet MT, Golding LA, Adams MS, Schlekat CE, Garman ER, Jolley DF. 2016. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. *Environ Pollut.* 218:1308-1323.

Ho KT, Kuhn A, Pelletier MC, Hendricks TL, Helmstetter A. 1999. pH dependent

toxicity of five metals to three marine organisms. *Environ Toxicol*. 14:235-240.

Landner L, Reuther R. 2005. Metals in society and in the environment. Environ Pollut. 1-9.

Lussier SM, Gentile JH, Walker J. 1985. Acute and chronic effects of heavy metals and cyanide on *Mysidopsis bahia* (Crustacea:Mysidacea). *Aquat Toxicol*. 7:25-35.

Lussier SM, Kuhn A, Comeleo R. 1999. An evaluation of the seven-day toxicity test with *Americamysis bahia* (formerly *Mysidopsis bahia*). *Environ Toxicol Chem*. 18:2888-2893.

Meyer JS, Santore RC, Bobbitt JP, Debrey LD, Boese CJ, Paquin PR, Allen HE, Bergmann HL, Ditoro DM. 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environ Sci Technol*. 33:913-916.

Nadella SR, Tellis M, Diamond R, Smith S, Bianchini A, Wood CM. 2013. Toxicity of lead and zinc to developing mussel and sea urchin embryos: Critical tissue residues and effects of dissolved organic matter and salinity. *Comp Biochem Physiol - C Toxicol Pharmacol*. 158:72-83.

Niyogi S, Wood CM. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ Sci Technol*. 38:6177-6192.

Pagenkopf GK. 1983. Gill surface interaction model for trace-metal toxicity to fishes: Role of complexation, pH, and water hardness. *Environ Sci Technol*. 17:342-347.

Paquin PR, Santore RC, Wu KB, Kavvadas CD, Di Toro DM. 2000. The biotic ligand model: A model of the acute toxicity of metals to aquatic life. *Environ Sci Policy*. 3:S175-S182.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F. 2002. The biotic ligand model: a historical overview. *Comp Biochem Physiol C Toxicol Pharmacol*. 133:3-35.

Phillips BM, Nicely PA, Hunt JW, Anderson BS, Tjeerdema RS, Palmer SE, Palmer FH, Puckett HM. 2003. Toxicity of cadmium-copper-nickel-zinc mixtures to larval purple sea urchins (*Strongylocentrotus purpuratus*). *Bull Environ Contam Toxicol*. 70:592-599.

Playle RC, Dixon DG, Burnissn K. 1993. Copper and cadmium binding to fish gills: Estimates of metal-gill stability constants and modelling of metal accumulation. *Can J Fish Aquat Sci*. 50:2678-2687.

Pyle G, Couture P. 2012. Nickel. In: Wood CM, Farrell AP, Brauner CJ (eds) Homeostasis and toxicology of essential metals. *Fish Physiol.* 31A: 254-281.

Ragsdale SW. 1998. Nickel biochemistry. *Curr Opin Chem Biol*. 2:208-215.

Sadiq M. 1989. Nickel sorption and speciation in a marine environment. *Hydrobiologia*. 176-177:225-232.

Silva C, Yáñez E, Martín-Díaz ML, Riba I, Delvalls TA. 2013. Integrated ecotoxicological assessment of marine sediments affected by land-based marine fish farm effluents: Physicochemical, acute toxicity and benthic community analyses. *Ecotoxicology*. 22:996-1011.

Smith KS, Balistrieri LS, Todd AS. 2015. Using biotic ligand models to predict metal toxicity in mineralized systems. *Appl Geochemistry*. 57:55-72.

Stockdale A, Tipping E, Hamilton-Taylor J, Lofts S. 2011. Trace metals in the open oceans: Speciation modelling based on humic-type ligands. *Environ Chem*. 8:304-

319.

Stockdale A, Tipping E, Lofts S. 2015. Dissolved trace metal speciation in estuarine and coastal waters: Comparison of WHAM/Model VII predictions with analytical results. *Environ Toxicol Chem*. 34:53-63.

Tellis MS, Lauer MM, Nadella S, Bianchini A, Wood CM. 2014. The effects of copper and nickel on the embryonic life stages of the purple sea urchin (*Strongylocentrotus purpuratus*). *Arch Environ Contam Toxicol*. 67:453-464.

Thurman EM. 1985. Organic geochemistry of natural waters. *Usgs*. 5-110.

Uthicke S, Schaffelke B, Byrne M. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecol Monogr*. 79:3-24.

Wang Y, Zhang L, Meng F, Zhou Y, Jin X, Giesy JP, Liu F. 2015. Improvement on species sensitivity distribution methods for deriving site-specific water quality criteria. *Environ Sci Pollut Res*. 22:5271-5282.

Wells ML, Smith GJ, Bruland KW. 2000. The distribution of colloidal and particulate bioactive metals in Narragansett Bay, RI. *Mar Chem*. 71:143-163.

Wood CM, Al-Reasi HA, Smith DS. 2011. The two faces of DOC. *Aquat Toxicol*. 105:3-8.

CHAPTER 2:
Complexation reduces nickel toxicity to purple sea urchin embryos (*Strongylocentrotus purpuratus*), a test of biotic ligand principles in seawater

2.1 Abstract

The potential for nickel (Ni) toxicity in seawater (SW) is of concern because of mining and processing activities in coastal regions. Determining speciation is vital to understanding and predicting Ni toxicity and for bioavailability-based Ni risk assessment. The goal of this study was to characterize the complexation of Ni in relation to toxicity using embryological development of purple sea urchin (*Strongylocentrotus purpuratus*). It was predicted that toxicity would vary based on total dissolved Ni concentrations ($[Ni_D]$) but that on a free ion concentration ($[Ni^{2+}]$) basis, toxicity would not vary. Synthetic ligands with known binding affinity ($logK_f$) values [EDTA, NTA, tryptophan (TRP), glutamic acid (GA), histidine (HD), and citric acid (CA)] were used to test the assumptions of the biotic ligand model (BLM) for Ni in seawater. $[Ni_D]$ was measured by graphite furnace atomic absorption spectroscopy (GFAAS) and Ni^{2+} was first quantified using the ion-exchange technique (IET) and then concentrations were measured by GFAAS; $[Ni^{2+}]$ was also estimated using aquatic geochemistry modelling software (Visual Minteq). The mean for EC_{50} values for $[Ni_D]$ in unmodified artificial seawater (ASW) was 3.6 µM (95% CI: 3.0-4.5) and the addition of ligands provided protection, up to 6.5-fold higher $[Ni_D]$ EC_{50} for EDTA. EC_{50} values based on $[Ni^{2+}]$ were less variable for some exposures; EDTA and CA showed 72% variability for $[Ni_D]$ and 17% for $[Ni^{2+}]$ and both were not significantly different from values in ASW. The results of this research provide a proof of principle for the application of biotic ligand-based prediction models for estimating Ni impacts in seawater.

2.2 Introduction

Currently there are no site-specific bioavailability-based prediction models for assessing the impacts and risks of nickel (Ni) in marine environments although there are indications that these may be warranted (Gissi et al. 2016). This is due to the fact that there is insufficient good quality chronic data on Ni toxicity to marine biota and read-across methods from freshwater (FW) databases are not permitted as the geochemical speciation within these models cannot be extrapolated to saltwater (SW) environments (Gissi et al. 2016). Therefore, Ni toxicity in SW is not as well understood as it is in FW where factors modulating toxicity have been incorporated into water quality assessments. Within FW systems, several modelling programs have been presented that account for geochemical speciation at equilibrium conditions, such as MINEQL+ and Visual Minteq. The binding affinity for inorganic complexes are well established in these programs, but reactions involving natural organic matter (NOM) are more difficult to quantify. The Windermere Humic Aqueous Model (WHAM VI) provides a robust model for the most difficult portion of the chemical speciation calculation, the complexation of metals to NOM (Stockdale et al. 2011; Stockdale et al. 2015). However, it predicts that for Ni in the marine environment there is poor protection by NOM, caused by its weak binding at high salinities (Stockdale et al. 2011). Given that WHAM has not been calibrated with marine specific ligands, the role of NOM in reducing toxicity in SW is less certain. Thus, research should be expanded to investigate toxicity caused by marine Ni and its speciation to generate data for the development of a robust SW model.

The biotic ligand model (BLM) is a predictive modeling approach that utilizes the relationship between water chemistry and tissue-metal accumulation to estimate toxic effects. The predictions of toxicity generated by the BLM are based on knowledge of the

specific sites to which a metal binds including the total number of binding sites and the affinity of these sites for the metal (K_f; Blewett and Wood 2015; Di Toro et al. 2001). The BLM makes the assumption that metal toxicity is primarily caused by free metal ions reacting with sites on the biotic ligand (organism), although other geochemical species can also be associated with toxic effects (Landner and Reuther 2005). A ligand may complex a metal that is otherwise bound to a binding site on the biotic ligand therefore reducing the bioavailability of the metal and its uptake into the biotic ligand, reducing toxicity (Playle et al. 1993). The BLM has been used to predict metal toxicity for many dissolved metals in FW (Arnold 2005; Crémazy et al. 2018; Meyer et al. 1999; Paquin et al. 2002) and for some in saltwater (Blewett et al. 2018; Nadella et al. 2013) and has proven a very valuable asset for marine risk assessments and to help establish site specific water quality criteria. Therefore, verifying the BLM's applicability to Ni will be useful in interpreting and predicting the toxicity of Ni and developing it as a regulatory tool related to marine waters.

Variations in water-quality between FW and SW regarding BLM input parameters are not well characterized. The information currently available for FW models, show that metal bioavailability is affected by water quality parameters such as: pH, salinity, conductivity, and natural organic matter (NOM; Playle et al. 1993; Di Toro et al. 2001). Recently there have been many studies starting to investigate the potential protective effects of these water parameters with regards to Ni toxicity for marine organisms (Blewett et al. 2018; Blewett and Wood 2015; Ho et al. 1999; Lussier et al. 1999; Tellis et al. 2014). Blewett et al. (2018) showed that DOC binding is strong and can alter toxicity, contrary to the predictions of WHAM VI (Stockdale et al. 2011). It was

also suggested that the composition of the DOC rather than just the concentration plays a key role in altering Ni toxicity to marine organisms (Blewett et al. 2018). Hence evaluating whether Ni toxicity in SW is affected by changing water parameters including DOC is fundamental to testing the BLM assumptions and further developing a computational Ni BLM for marine waters.

Using a species previously proven to be sensitive to Ni is the most appropriate for studies trying to parameterize a marine BLM. DeForest and Schlekat (2013) showed that sea urchins were among the most sensitive species with variations of up to 2 orders of magnitude. Sea urchin embryo bioassays are commonly used as a method to determine marine water quality criteria and can also be used to examine the physiological effects of metal toxicity because these early developmental stages are extremely sensitive to a variety of contaminants (Blewett et al. 2016; Phillips et al. 2003; Tellis et al. 2014). As such, purple sea urchin embryos were used as the model organism in this study because: they are sensitive to Ni; they have an important ecological roles; they have a wide distribution along the eastern edge of the Pacific Ocean (Uthicke et al. 2009). Therefore they are valuable indicators of potential ecological damage to aquatic communities (DeForest and Schlekat 2013; Silva et al. 2013). This makes *Strongylocentrotus purpuratus* an excellent model species for marine Ni toxicity studies and can add to ongoing environmental regulatory tools such as the BLM. This data can also work towards extending the chronic Ni toxicity database for marine species to satisfy the criteria for creating SSDs (DeForest and Schlekat 2013).

This study aims to evaluate if the free Ni ion (Ni^{2+}) is the only species that contributes to toxicity, based on normal embryonic development to sea urchin embryos. The current

study had four goals. The first was to determine the sensitivity of *S. purpuratus* embryonic life stages to Ni toxicity. The second was to investigate the protective effects of synthetic ligands with regards to Ni toxicity. The third was to determine the dependence of toxicity on the speciation of Ni. As according to the assumptions of the BLM, if the Ni toxicity is dependent on $[Ni^{2+}]$, then protection (indicated by the median effective concentration; EC_{50}) of dissolved Ni ($[Ni_D]$) will vary between synthetic ligands, but the $[Ni^{2+}]$ EC_{50} will remain constant regardless of the addition of synthetic ligands in solution. Lastly, to be able to use the defined BLM model (assuming the application is valid, using IET measured EC_{50} values when 50% of BL is bound to $[Ni^{2+}]$, $[NiBL] = [BL]_{free}$) to estimate the $logK_f$ (binding affinity) of the biotic ligand. The results of this study will provide insight to understand Ni toxicity and speciation and establish whether development of a site-specific Ni BLM for marine waters is warranted.

2.3 Methodology

2.3.1 Animal care

Adult purple sea urchin (*S. purpuratus*) were obtained from WestWind SeaLabs in Victoria, British Columbia and held in artificial seawater (ASW) at a salinity of 30 ppt, pH of 8.1 and temperature of 15°C and following standard methods (EPS 1/RM/58 2nd edition 2014) from Environment Canada (EC 2014). The ASW was created by reconstituting sea salt (Kent Marine Reef Salt Mix, Big Als Canada Inc, Kitchener ON) with reverse osmosis (RO) water and this was held in a reservoir with continuous aeration. Salinity, pH and temperature were monitored daily using a hand-held conductivity meter (YSI 30, YSI Inc., Yellow Springs). The urchins were fed kelp three times weekly until satiety, and debris removed weekly.

2.3.2 Collection and fertilization of gametes

To collect gametes, spawning was induced by injecting 1 mL 0.5 M KCl into the hemocoel of the adult sea urchin. Sex was determined by visual inspection of the gametes. Sperm was collected from males (n = 4) using a transfer pipette and stored in a centrifuge tube on ice (4°C). Eggs were collected from spawning females (n = 4) by placing the aboral surface on top of a beaker filled with ASW, allowing eggs to fall to the bottom. Eggs were rinsed three times, added to 250 mL of ASW and examined to ensure egg quality using a compound microscope (EVOS, ThermoFisher Scientific, Nepean, ON) at 100x magnification with a Sedgewick-Rafter cell. Approximately 10 µL (2-3 drops) of sperm was diluted into 10 mL of ASW and added to the 250 mL beaker of eggs and mixed gently to facilitate fertilization. Fertilization success was confirmed under a microscope by the presence of a fertilization membrane around each egg. Once >80% success fertilization was achieved, approx. 15 min, 1 mL aliquots of eggs were added to 20 ml glass scintillation vials containing 9 mL of test solution to achieve a final density of 200 fertilized eggs per mL. Exposure duration was 96 h at 30 ppt, a pH value of 8.1, 15 ºC and in a 16:8 h light:dark cycle.

2.3.3 Preparation of test solutions and toxicity tests

Each exposure concentration was aliquoted into one of six 20 mL scintillation vials (9 mL for each). These included four biological replicates and two for measuring total and dissolved Ni concentrations. The remaining solution was used for [Ni^{2+}] determination by IET. Ni solutions were prepared using $NiCl_2$ soluble salt (1000 ppm; Sigma Aldrich). Tests were conducted in ASW only as well as with ASW with a synthetic ligand added. All methods were repeated for the four ASW treatments (Table 2.1). Six toxicity tests using 30 µM ethylenediaminetetraacetic acid (EDTA), 10 µM nitrilotriacetic acid (NTA),

250 µM tryptophan (TRP), 700 µM glutamic acid (GA), 10 µM histidine (HD) and 500 µM citric acid (CA; Sigma Aldrich, Oakville, ON) were conducted. For each test 2.5 L of ASW was equally split into eight (or nine) 250 mL Nalgene bottles which were then spiked with appropriate amounts of Ni and synthetic ligand (Table 2.1). The ligand concentrations were selected based on geochemical modelling (see section 2.3.6) so that each experiment would span a similar range of Ni free ion concentrations. Similarly, the range of Ni exposure concentrations were chosen based on the Ni-binding affinity of each ligand in order to compare Ni binding within a similar analytical window. The pH was adjusted to 8.1 ± 0.1 using 0.1 M NaOH.

2.3.4 Toxicity endpoint determination

At 96 h, development was terminated by the addition of 0.5 mL of 5% neutral buffered formalin (Sigma Aldrich, Oakville, ON) and vials were set aside for later observation. One hundred embryos were assessed for each replicate using a microscope (see section 2.3.2). Embryo development was quantified after 96 h of exposure by scoring as either normal or abnormal (Fig. 2.1). Abnormal development as defined as an embryo that did not display typical pluteus form, with reference to control embryos. Values were scored out of 100, so abnormal counts could be used as percent abnormal development (%).

2.3.5 Ni quantification by GFAAS

After 96 h one water chemistry vial was immediately acidified to 1% with nitric acid (trace metal grade, Fisher Scientific Mississauga ON) and this was used for measurement of total Ni concentration ($[Ni_T]$). The second chemistry vial was filtered (0.45 µm 25mm HT Tuffryn® Polysulfone Membrane Disc Syringe Filters, Pall Life Sciences, Houston, TX, USA) then acidified to 1% to measure $[Ni_D]$. Both $[Ni_T]$ and $[Ni_D]$ were measured by

GFAAS (Perkin Elmer PinAAcle 900T, TraceCERT, Sigma Aldrich, Oakville, ON) based on a daily calibration curve. A two-time dilution factor was applied by the instrument for automatically diluting solutions with MilliQ. Samples with Ni concentrations >50 µg/L were first manually diluted by appropriate factors with 2% HNO_3. The Zeeman background correction was applied to reduce the possible salt-induced interference. Samples were measured twice, and the results were finalized if the reproducibility standard deviation (RSD) was less than 10%, otherwise the samples were remeasured. The reliability and constancy of the GFAAS measurements were verified by running a certified reference material (CRM) containing 17.6 ± 2.4 µg/L Ni (TM 15.2, National Research Council Canada) every twelve samples. A paired *t* test was performed to compare the CRM value from the GFAAS measurement to determine any significant differences.

2.3.6 Determination of Ni speciation

IET measurements were done in parallel to the toxicity tests during this study to quantify the [Ni^{2+}] within solution and [Ni^{2+}] was also estimated using geochemical equilibrium-based software (Visual Minteq Ver. 3.1, KTH, Stockholm, Sweden) following the methods found in Chen et al. (unpublished). In order to validate the performance of the IET in SW, EC_x of measured [Ni^{2+}] was compared to modelled predictions. Visual Minteq software was also used to determine the concentration of Ni species bound to inorganic and organic ligands at the EC_{50} for each of the synthetic ligand treatments.

2.3.7 Calculation of Ni-Biotic ligand binding affinity

Assuming the BLM approach is valid, and that the Ni BLM complex is of 1:1 stoichiometry, the Ni-binding affinity of the biotic ligand (BL) conditional to SW matrix

at pH 8.1 (K'_{BL}) was estimated based on the IET measured EC_{50} values (reaction 1 and equation 1-2). The K'_{BL} was determined for every treatment and the average was calculated.

$Ni^{2+} + BL_{free} \leftrightarrow NiBL$ *Reaction 1*

where BL_{free} is the biotic ligand, and

NiBL is the Ni-biotic ligand complex

$$K'_{NiBL} = \frac{[NiBL]}{[Ni^{2+}][BL_{free}]}$$ *Equation 1*

where K'_{NiBL} is the stability constant for the metal-ligand complex,

when 50% of BL is bound to $[Ni^{2+}]$, $[NiBL] = [BL]_{free}$,

assuming that 50% bound Ni corresponds to 50% toxic response

$$K'_{NiBL} = \frac{1}{[Ni^{2+}]_{EC50}}$$ *Equation 2*

where $[Ni^{2+}]EC_{50}$ is the concentration of the free ion associated with a 50% toxic response

2.3.8 Statistical analysis

The 96 h median effective concentration (EC_{50}) and 20% effective concentration (EC_{20}) values 95% confidence intervals (95% CI) were determined for both $[Ni_D]$ and $[Ni^{2+}]$ using the Comprehensive Environmental Toxicity Information System (CETIS) software following the EPA ICPIN method based on linear interpolation with bootstrapping. Significant differences between exposures (and with ASW) were examined using the overlap of 95% CI; if they did not overlap, then the EC_{50} (or EC_{20}) values were considered significantly different (EC 2005). In order to determine the extent to which toxicity was related to $[Ni^{2+}]$ the relative variability of EC_{50} values across sites

for [Ni_D] and for [Ni^{2+}] was assessed by comparing coefficients of variation (CVs). The CVs were calculated by dividing the standard deviation of the EC_{50}s by the average EC_{50}s across sample sites. A one-way ANOVA was performed to determine if the individual inorganic species differed between exposures.

2.4 Results and Discussion

2.4.1 Water Chemistry

The measured [Ni_D] values were 93 ± 15% of [Ni_T] values (shown as mean ± SD; range of 39 to 144%), indicating negligible Ni precipitation over the 96-h toxicity test (Table 2.1). There was no significant difference between the certified reference material (CRM) value and the average value measured on the GFAAS (19% difference; $p>0.05$). Throughout the 96 h for all exposures, temperature in the water bath ranged from 14.7 to 15.4 °C (n=4), and within the test vials salinity ranged from 29.5 to 30.9 ppt (n=2) and pH ranged from 7.9 to 8.2 (n=2; Table 2.1).

2.4.2 Ni toxicity to fertilization success of S. purpuratus

All toxicity tests met the acceptable criteria where >80% normal development was reached within 96 h in unexposed controls (Table 2.2; ECCC 2014). From the 4 ASW exposures (i.e. ASW without ligands) the mean 96 h EC_{50} and EC_{20} for [Ni_D] with 95% CI for successful embryo development (development into pluteus stage) of *S. purpuratus* was 3.6 (95% CI: 3.0-4.6 µM; Fig. 2.2) and 1.5 (1.3-1.9 µM; Fig. 2.3) respectively (n=4). There were no significant differences in these EC values among the 4 ASW groups based on the confidence intervals. Trial 3 showed large confidence bands around the mean EC_{50}; the relatively high variability seen was likely associated with individual sensitivities within the test population. The sensitivity of *S. purpuratus* in their embryonic life stages determined by this study is in agreement with literature values which showed

EC_{50} values ranging from 4.0 to 5.8 µM indicating a similar sensitivity of *S. purpuratus* between studies (Blewett et al. 2018; Phillips et al. 2003). Previous research shows significant species sensitivity variations of up to 2 orders of magnitude, with EC_{10}s as low as 0.05 µM for the tropical long-spined sea urchin *(D. antillarum*) which may denote that *S. purpuratus* is more resilient to Ni toxicity (DeForest and Schlekat 2013).

2.4.3 Protection against Ni toxicity produced by ligands

Synthetic ligands (EDTA, NTA, TRP, GA, HD, CA) were used to represent organic ligands that bind to Ni with different complexation characteristics. This was used as a proof of concept to test the assumptions of the BLM that complexation of Ni and [Ni^{2+}] are important factors in determining toxicity. Metal-synthetic ligand complexation characteristics for these synthetic ligands are well known; it is possible to model Ni speciation using modeling programs such as Visual Minteq. To our knowledge this is the first study to examine the effects of complexing agents on Ni toxicity and speciation for this species. All synthetic ligands provided protection based on [Ni_D]; up to 6.5-fold higher [Ni_D] EC_{50} for EDTA compared to ASW exposures (Fig. 2.2). The same trends were found when looking at EC_{20} values, where EDTA had up to 11-fold higher [Ni_D] (Fig. 2.3). It was assumed that toxicity would be dependent on the magnitude of binding affinity for Ni of each synthetic ligand (Playle et al. 1992). For example, EDTA which has a high affinity for Ni and can form strong complexes, had nearly 99% Ni bound as Ni-EDTA. CA and weaker ligands had decreasing complexing ability with Ni and still showed increased EC_{50} values compared to ASW exposures.

2.4.4 Speciation

IET measurements were done in parallel to the toxicity tests during this study to quantify the [Ni^{2+}] within solution (Table 2.1). Measuring [Ni^{2+}] within SW provides a

method to test the complexation predictions provided by Visual Minteq and is important to assess the assumptions inherent to the BLM for validating its use for Ni in SW. This is based on the theory that the most bioavailable and toxic species of a metal in solution is the free ion (Landner and Reuther 2005). There were no significant differences among the 4 ASW groups where the average 96 h $[Ni^{2+}]$ EC_{50} was 2.6 (2.2-3.3 µM; Fig. 2.4) and EC_{20} was 1.1 (1.0-1.4 µM; Fig. 2.5).

The EC_{50}s for free metal ion in solution varied from 0.43 to 3.3 µM $[Ni^{2+}]$ between treatments with added synthetic ligand (Fig. 2.4). All values were either similar (EDTA and CA) or less (NTA, GA and HD) than the values for tests in ASW except for TRP (Fig. 2.4). EC_{50} values based on $[Ni^{2+}]$ did not show reduced variability when considering all exposures. However, based on the exposures that were considered to follow the BLM assumptions (EDTA and CA) there was 72% variability for $[Ni_D]$ and 17% for $[Ni^{2+}]$, showing reduced variability. EDTA in particular was very protective based on $[Ni_D]$, but when plotted on a $[Ni^{2+}]$ basis there were no significant differences from the ASW exposures; this was also seen for CA. NTA, GA, and HD treatments also showed Ni complexation and lower EC_{50} values between $[Ni_D]$ and $[Ni^{2+}]$ however compared to ASW exposures, the $[Ni^{2+}]$ values were significantly lower. This indicates that Ni bioavailability in these solutions was enhanced by the presence of ligand. The same trends were found when looking at the EC_{20} values (Fig. 2.5). The assumption that $[Ni^{2+}]$ values would be similar to ASW exposures was not met for TRP. For the TRP treatment, the EC_{50} and EC_{20} values for $[Ni^{2+}]$ were higher than other free ion ECx values and also, higher than the $[Ni_D]$ ECx values for that treatment. These results show that the IET measurements in the presence of TRP dramatically overestimated the actual $[Ni^{2+}]$ in

solution (Chen et al. unpublished). This is likely related to the adsorption of Ni-ligand complexes onto the IET resin as similar results were shown in two studies where [Zn^{2+}] and [Ni^{2+}] were overestimated by IET in the presence of amino acids and hydrophobic complexes (Fortin and Campbell 1998; Worms and Wilkinson 2008). A more recent study has tested synthetic ligands that contained similar functional groups to evaluate the IET performance. It was found that concentrations of amino acids less than 100 µM can give reliable IET-based measurements of [Ni^{2+}] in SW (Chen et al. unpublished). The IET results for measured [Ni^{2+}] in the ligand treatment with added TRP are not useful, nor relevant in further discussion.

There were no significant differences among the 4 ASW groups within their predicted EC_{50} values or within their EC_{20} values (Fig. 2.4; Fig. 2.5). As well, within these groups, predicted and measured values were similar (Fig. 2.4; Fig. 2.5). However, the agreement between measured and modelled ECx values varied within the different ligands (Fig. 2.4). For EDTA and CA, the model under-predicted [Ni^{2+}] in solution, resulting in an over-prediction of toxicity in comparison to the measured free ion. This may be due to inaccuracies within the model resulting in an overprediction of complexation and [Ni^{2+}] and this could result from ionic strength corrections around the stability constant. It may also be related to the fact that the predictive modelling only accounted for the ligand in SW and did not include the biotic ligand itself. For NTA, GA, and HD deviations were not significantly different between measured and modelled values, further displaying that NTA-, GA-, and HD-Ni complexes were somewhat toxic. The model over predicts toxicity for all ligands at the EC_{20} (Fig. 2.5).

Ni toxicity to embryo development may be caused from a number of factors. Speciation analysis using Visual Minteq predicted inorganic and organic species across all treatments. It is assumed the most bioavailable and toxic species of a divalent metal in aqueous solution is the free ion (e.g. Ni^{2+}; Landner and Reuther 2005). Recently, it has been recognized that some inorganic complexes as well as various common cations (e.g. Ca^{2+}, Mg^{2+}, and Na^{+}) have the potential to contribute to the total toxicity of these metals to aquatic organisms (Landner and Reuther 2005). The exact mechanism of Ni uptake into aquatic organisms is unknown but is theorized to occur either by being directly taken up by the organism or transported into cells via membrane transporters (Campbell et al. 2002). Sea urchin embryos reach their critical stage of development at some point after 48 h, where toxicity will begin to show noticeable developmental effects (Blewett et al. 2016; Hardin et al. 1992). Embryos utilize forms of calcium carbonate during this period of embryonic development to form their skeletons and spicules (Wilt 1999). As such, Ni is assumed to use ion mimicry to transport through both calcium (Ca) and magnesium (Mg) pathways to disturb homeostasis and inhibit transporters (Eisler 1998; McFarlane and Gilly 1998; Tellis et al. 2014). However, under the conditions employed in this study, Ca and Mg were not major species in any exposures and its contribution to Ni toxicity could be neglected (Table 2.3). Thus, the lower levels of $[Ni^{2+}]$ associated with ECx values for NTA, GA and HD could represent an increase in potential Ni-ligand uptake, representing a route of Ni exposure distinct from that of ion mimicry. Ni-organic and Ni-inorganic fractions relative to $[Ni_D]$ were plotted for all exposures (Fig. 2.6). TRP is shown with the Visual Minteq predicted $[Ni^{2+}]$ value only as it could not be measured by IET. All synthetic ligand treatments showed that Ni is mostly (>50%) bound to the

organic components, with a small portion bound to inorganic species (<15%; Fig. 2.6). The individual inorganic species did not differ significantly (p=0.34) between treatments and could therefore be disregarded as a possible cause for anomalous toxicity. It is possible the toxicity was caused by the NTA-, GA-, and HD-Ni complexes themselves. Previous studies have shown that through internalization of the metal complex by passive diffusion or ligand transporters metal complexes can contribute to bioavailability and toxicity (Phinney and Bruland 1994; Zhao et al. 2016). Exposures GA and HD showed high concentrations of neutral species of Ni (Ni-Glutamate and Ni-Histidine respectively; >45% of the total Ni bound; Table 2.4). These complexes are uncharged, lipophilic metal complexes that can passively diffuse through the lipid bilayer of biological membranes causing toxicity (Zhao et al. 2016). This passive diffusion has been seen for complexes involving synthetic organic ligands such as 8-hydroxyquinoline (Ox) and diethyl-dithiocarbamate (DDC; Phinney and Bruland 1994). For NTA it is hypothesized that a small fraction of the Ni-NTA anionic hydrophilic species that was calculated to be present in the medium may be transported across the membrane of the embryo, however this exact mechanism is unknown (Table 2.4). This has been seen for other metal complexes such as $Cu(Sox)_2^{2-}$ (copper-sulfoxine; Phinney and Bruland 1994).

2.4.5 Binding affinity

To date, conditional stability constants for sea urchin embryos have not been estimated for Ni in SW. IET-measured [Ni^{2+}] was used for the derivation of conditional stability constant (K') for each treatment and an average value was calculated (Table 2.5). $\log K'_{NiBL} = 6.3 \pm 0.4$ or $K'_{NiBL} = 10^{6.3 \pm 0.4}$, indicating that 50% of the Ni binding sites would be occupied at aqueous Ni concentrations of approximately$10^{-6.3}$ M. A similar

approach has been used in FW for many species and metals (Playle et al. 1993). This value can be used to develop a computational BLM for marine Ni and also aids in determining when ligands may be protective. For example, if a ligand has an equivalent K_f value to the biotic ligand ($K'_{NiBL} = 10^{6.3 \pm 0.4}$) at the same concentrations, then half of Ni will bind to each. However, if concentrations or binding affinities increase for one, it will out compete the other causing more Ni to bind to it. Using these K_f values to predict if Ni will bind more strongly to the biotic ligand or DOC will be important in predicting toxicity.

2.5 Conclusion

In the present study, we have better defined Ni-biotic ligand interactions in marine waters, as influenced by synthetic ligands. *Strongylocentrotus purpuratus* is a sensitive marine organism in regards to Ni toxicity. Ni toxicity was reduced by addition of synthetic ligand into solution relative to the binding affinity. EC_{50} of dissolved Ni varied between synthetic ligands, but the $[Ni^{2+}]$ EC_{50} of all values were either similar (EDTA and CA) or less (NTA, GA and HD) than the values for tests in ASW. GA, HD and NTA showed toxicity that likely occurred from the organic Ni complexes themselves through passive diffusion or active transport through the embryo membrane. This exact mechanism is unclear. The results of this study provide insights into the understanding of Ni toxicity from a biological perspective, which will be used to calibrate a computational marine BLM for Ni and help develop environmental regulations for the protection of marine species against Ni contamination (DeForest and Schlekat 2013). Further research that looks at the effects of other synthetic ligands as well as natural organic ligands (DOC) may be required.

2.6 Acknowledgements

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2.7 Tables and Figures

Table 2.1. Water chemistry parameters in ASW with (and without) added ligands. Ni exposure concentrations are given as nominal, $[Ni_D]$ and $[Ni^{2+}]$. $[Ni^{2+}]$ was both calculated using interpolation of an IET curve and also predicted using Visual Minteq. Means are given ± standard deviation (SD) for $[Ni_D]$ (µg/L; n=2), pH (n=2), salinity (ppt; n=2), and temperature (°C; n=4). Values with * were excluded from any calculations of $[Ni_D]$ as a % of [NiT].

Exposure		Nominal Ni (µg/L)	Dissolved Ni (µg/L) ± SD	Free Ni (µg/L)	Predicted Free Ni (µg/L)	pH ± SD	Temperature ± SD (°C)	Salinity ± SD (ppt)
ASW	1	0	0.4 ± 19.3*		0	8.0 ± 0.05	15.0 ± 0.1	30.80 ± 0.2
		25	26 ± 4.1	22	18			
		50	45 ± 1.5	30	32			
		100	86 ± 4.0	58	62			
		200	181 ± 7.2	128	130			
		400	317 ± 29.2	262	270			
		800	826 ± 14.3	737	594			
		1600	1905 ± 60.3	1033	1362			
	2	0	0.4 ± 14.0*		0	8.0 ± 0.05	15.0 ± 0.1	30.10 ± 0.2
		400	318 ± 20.4	224	228			
		800	721 ± 27.4	614	517			
		1200	1076 ± 16.4	832	771			
		2000	1560 ± 31.9	950	1119			
		2750	2163 ± 9.3	1098	1551			
		3750	3036 ± 66.6	1313	2177			
		5000	4478 ± 114.7	1734	3211			
	3	0	28 ± 0.4*		0	8.0 ± 0.05	15.1 ± 0.2	30.50 ± 0.1
		50	71 ± 0.06	48	51			
		100	83 ± 0.06	56	59			
		200	248 ± 7.5	180	178			
		400	660 ± 9.6	545	473			
		800	767 ± 17.4	666	550			
		1000	935 ±25.9	798	671			
		2000	1629 ± 31.4	966	1168			
		3000	3993 ± 12.6	1590	2863			
	4	0	2 ± 0.01*		2	8.1 ± 0.04	15.3 ± 0.1	30.10 ± 0.1
		25	27 ± 2.3	48	23			
		50	50 ± 0.5	59	42			
		100	99 ± 2.8	98	83			
		200	176 ± 1.1	150	149			

		300	276 ± 2.1	209	233			
		400	371 ± 0.4	221	313			
		800	821 ± 3.1	453	693			
		1600	1647 ± 21.3	803	1027			
EDTA		0	4 ± 16.2*		0.00002	8.0 ± 0.10	15.0 ± 0.1	30.30 ± 0.6
		400	295 ± 19.6	24	0.002			
		800	882 ± 13.8	88	0.01			
		1200	1152 ± 23.8	158	0.02			
		2000	1735 ± 45.7	263	1			
		2750	3000 ± 72.4	422	1046			
		3750	3636 ± 14.6	806	1583			
		5000	4232 ± 150.3	1041	2054			
NTA		0	0.3 ± 0.04*		0	8.1 ± 0.03	14.8 ± 0.1	29.70 ± 0.4
		250	149 ± 3.5	17	2			
		500	360 ± 12.5	29	8			
		600	404 ± 3.6	32	11			
		700	492 ± 4.2	49	21			
		800	596 ± 12.5	51	51			
		900	646 ± 6.4	54	76			
		1600	830 ± 3.1	132	197			
		2000	1313 ± 82.3	148	556			
TRP		0	8 ± 1.2*		3	8.0 ± 0.05	15.1 ± 0.2	30.30 ± 0.1
		250	161 ± 10.5	257	63			
		500	492 ± 11.4	991	194			
		1000	919 ± 11.8	1095	365			
		1500	1272 ± 30.3	1580	509			
		2000	2047 ± 34.2	2308	828			
		2500	2643 ± 38.5	2745	1079			
		3000	3181 ± 87.5	3112	1309			
GA		0	13 ± 16.3*		2	8.1 ± 0.10	15.1 ± 0.2	30.80 ± 0.2
		100	47 ± 2.4	6	7			
		200	240 ± 3.4	21	35			
		400	444 ± 3.7	32	65			
		800	952 ± 3.3	66	140			
		1200	1531 ± 16.4	109	230			

	1600	2286 ± 32.3	142	351			
	2000	2631 ± 27.4	151	408			
HD	0	4 ± 11.7*		0	8.1 ± 0.10	15.1 ± 0.2	29.90 ± 0.2
	125	79 ± 7.1	12	0.1			
	250	236 ± 6.9	30	2			
	500	488 ± 3.7	83	87			
	750	841 ± 0.2	191	325			
	1000	821 ± 99.0	195	311			
	1500	1467 ± 47.5	469	809			
	2000	1738 ± 28.7	648	1028			
CA	0	13 ± 7.6*		7	8.0 ± 0.10	15.0 ± 0.1	30.30 ± 0.2
	25	28 ± 1.1	15	16			
	50	62 ± 1.1	25	35			
	100	109 ± 1.0	41	62			
	200	242 ± 2.1	85	138			
	400	403 ± 0.01	148	230			
	800	647 ± 27.2	188	368			
	1600	1381 ± 37.6	398	790			

Table 2.2. The 96-hour chronic toxicity end-point of percent successful embryo development are shown as mean ± SD for all exposures (n=4).

Exposure		Nominal Ni (µg/L)	% successful embryo development ± SD (%)
ASW	1	0	96.75 ± 1.9
		25	88.50 ± 6.6
		50	89.00 ± 5.0
		100	84.25 ± 4.3
		200	60.25 ± 6.1
		400	7.25 ± 4.3
		800	0
		1600	0
	2	0	96.75 ± 2.9
		400	5.25 ± 3.3
		800	0
		1200	0
		2000	0
		2750	0
		3750	0
		5000	0
	3	0	95.50 ± 1.7
		50	86.00 ± 4.5
		100	74.75 ± 13.0
		200	42.75 ± 17.3
		400	6.50 ± 8.5
		800	0
		1000	0
		2000	0
		3000	0
	4	0	97.25 ± 0.9
		25	91.00 ± 3.4
		50	87.75 ± 5.3
		100	72.75 ± 2.6
		200	55.75 ± 3.6
		300	39.00 ± 2.2
		400	7.00 ± 4.8
		800	0

		1600	0
EDTA		0	96.25 ± 2.5
		400	87.00 ± 5.5
		800	85.25 ± 3.3
		1200	66.00 ± 3.9
		2000	11.75 ± 8.7
		2750	1.50 ± 2.4
		3750	0
		5000	0
NTA		0	99.25 ± 1.0
		250	94.75 ± 2.1
		500	83.75 ± 3.9
		600	83.00 ± 3.2
		700	66.00 ± 2.7
		800	51.75 ± 7.1
		900	41.75 ± 3.0
		1600	0
		2000	0
TRP		0	95.50 ± 2.4
		250	90.50 ± 2.1
		500	73.50 ± 6.6
		1000	0.75 ± 1.0
		1500	0
		2000	0
		2500	0
		3000	0
GA		0	97.50 ± 1.9
		100	91.75 ± 3.1
		200	77.25 ± 10.5
		400	42.00 ± 5.9
		800	1.25 ± 1.9
		1200	0
		1600	0
		2000	0
HD		0	97.50 ± 2.5

	125	92.25 ± 4.3
	250	69.50 ± 7.8
	500	42.00 ± 10.0
	750	0
	1000	0
	1500	0
	2000	0
CA	0	94.25 ± 3.1
	25	91.25 ± 3.0
	50	88.75 ± 4.8
	100	85.75 ± 4.6
	200	76.75 ± 3.3
	400	54.25 ± 4.0
	800	6.50 ± 3.3
	1600	1.50 ± 24

Table 2.3. Calculated Ca^{2+} and Mg^{2+} concentrations (mol/L) and their % bound to the synthetic ligand for all exposures; negligible <0.01.

Exposure	$[Ca^{2+}]$ (mol/L)	Ca bound to ligand (%)	$[Mg^{2+}]$ (mol/L)	Mg bound to ligand (%)
ASW	7.88E-03		3.21E-02	
EDTA	7.88E-03	0.075	3.21E-02	0.022
NTA	6.83E-03	negligible	3.16E-02	negligible
TRP	6.83E-03	0.044	3.16E-02	negligible
GA	7.87E-03	0.187	3.21E-02	0.047
HD	6.83E-03	negligible	3.16E-02	negligible
CA	7.81E-03	0.95	3.19E-02	0.72

Table 2.4. Calculated neutral Ni-organic species concentrations (mol/L) and their % Ni.

Exposure	Species	Charge	Concentration (mol/L)	% Ni
NTA	$NiNTA^{-1}$	-1	9.10E-06	89
GA	Ni-Glutamate	0	3.82E-06	56
HD	Ni-His	0	3.44E-06	46

Table 2.5. Calculated logK'$_{NiBL}$ values for all exposures.

Exposure		logK'$_{NiBL}$
ASW	**1**	6.4
	2	6.3
	3	6.4
	4	6.4
EDTA		6.5
NTA		5.9
TRP		7.3
GA		5.6
HD		6.1
CA		6.4

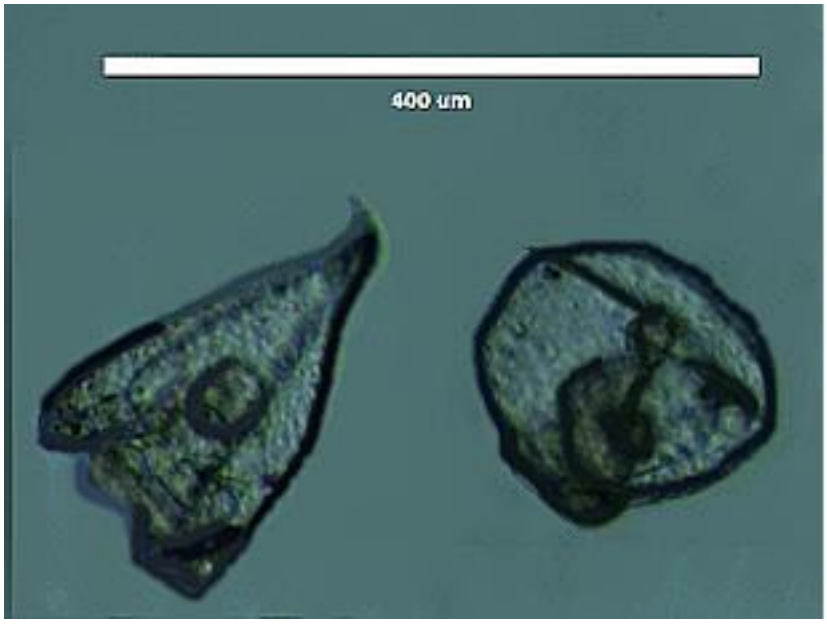

FIG. 2.1. Embryo development was quantified after 96 h of exposure to Ni by scoring as either normal or abnormal at 100x magnification using a Sedgewick-Rafter cell. Abnormal development was defined as an embryo that did not display typical pluteus form (right embryo), with reference to control embryos (left embryo). Values were scored out of 100, so abnormal counts could be used as percent abnormal development (%).

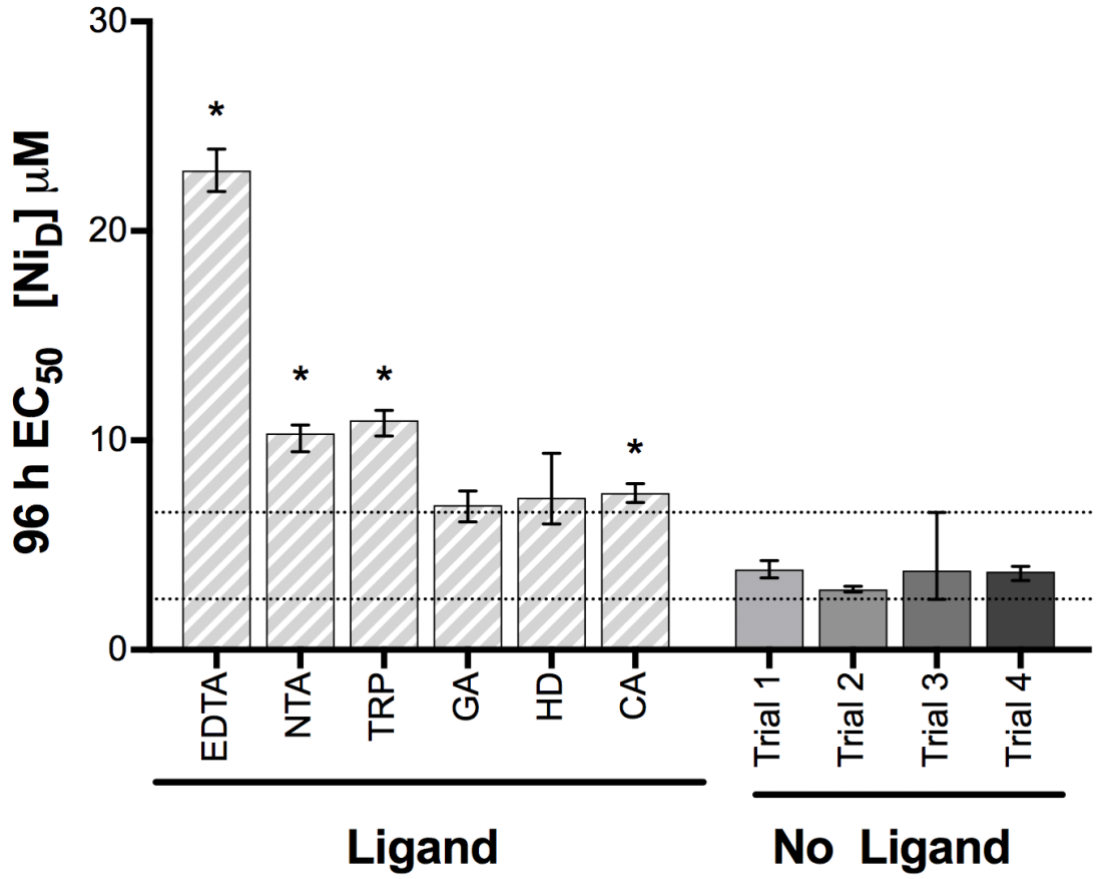

FIG. 2.2. The 96 h EC_{50} values for [Ni_D] for abnormal embryo development in purple sea urchin with (and without) added ligands. Error bars show 95% confidence interval and * indicates a significant difference in EC_{50} value compared to no ligand (ASW) exposures. Dotted horizontal lines represent the confidence limits of the ASW trials.

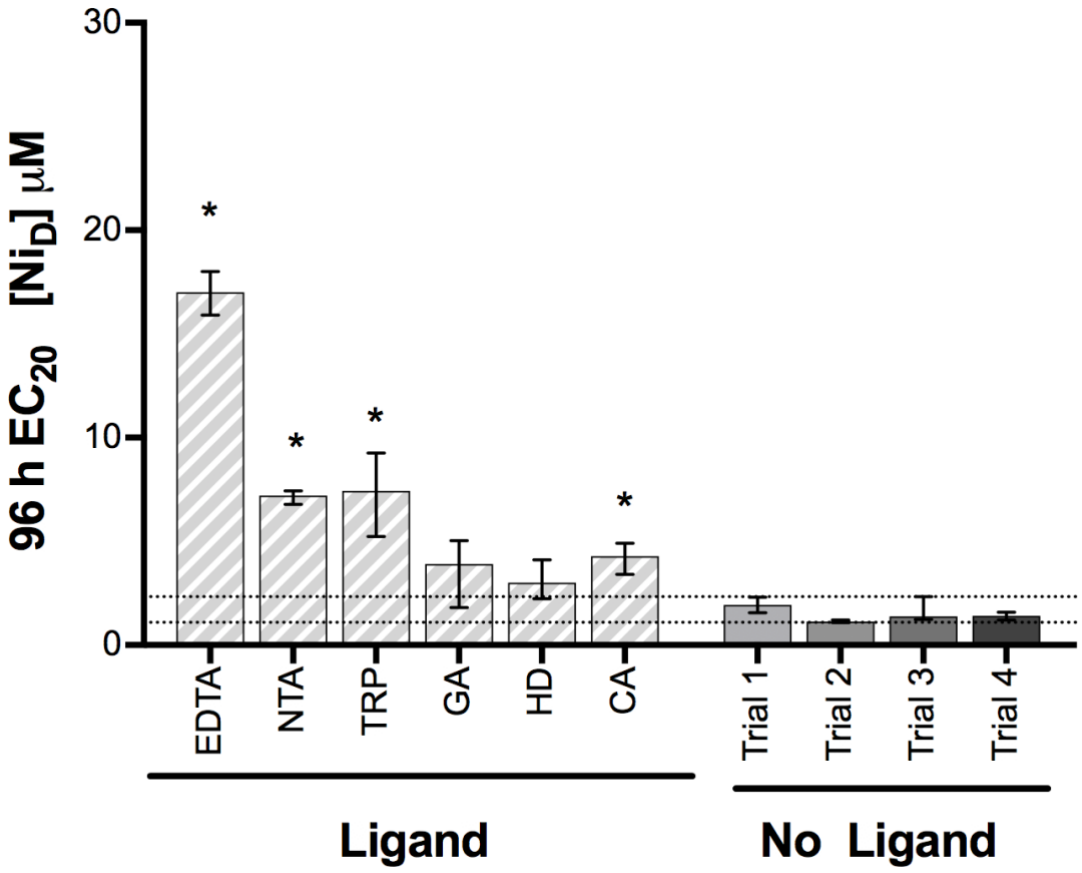

FIG. 2.3. The 96 h EC_{20} values for [Ni_D] for abnormal embryo development in purple sea urchin with (and without) added ligands. Error bars show 95% confidence interval and * indicates a significant difference in EC_{50} value compared to no ligand (ASW) exposures. Dotted horizontal lines represent the confidence limits of the ASW trials.

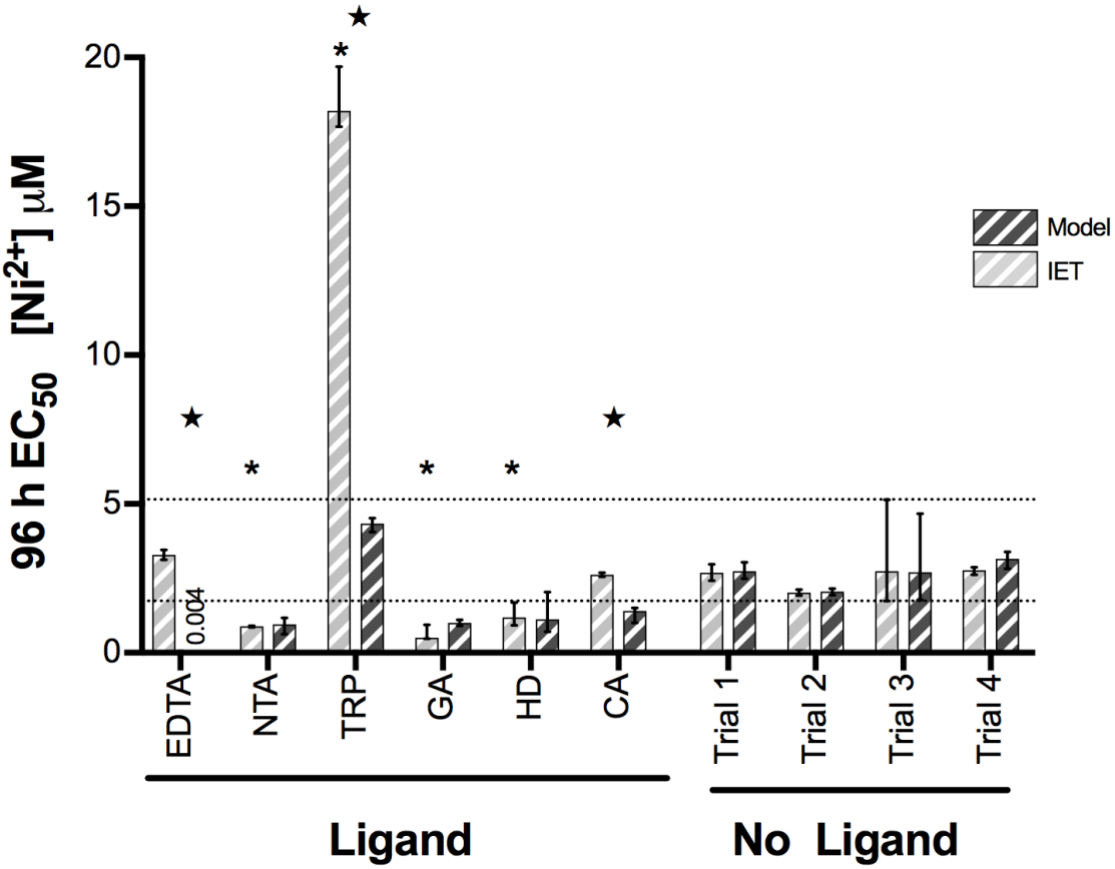

FIG. 2.4. The 96 h EC_{50} values for $[Ni^{2+}]$ for abnormal embryo development in purple sea urchin with (and without) added ligands. $[Ni^{2+}]$ endpoint determinations were calculated using either measured $[Ni^{2+}]$ by IET (dark gray stripes) or modelled $[Ni^{2+}]$ predicted by Visual Minteq (light gray stripes). Note: the predicted value for EDTA was very low, so the value is written in place of the bar. Error bars show 95% confidence interval and * indicates a significant difference in EC_{50} value compared to the no ligand (ASW) exposure and ★ indicates a significant difference in EC_{50} value between measured and predicted. Dotted horizontal lines represent the confidence limits of the ASW trials.

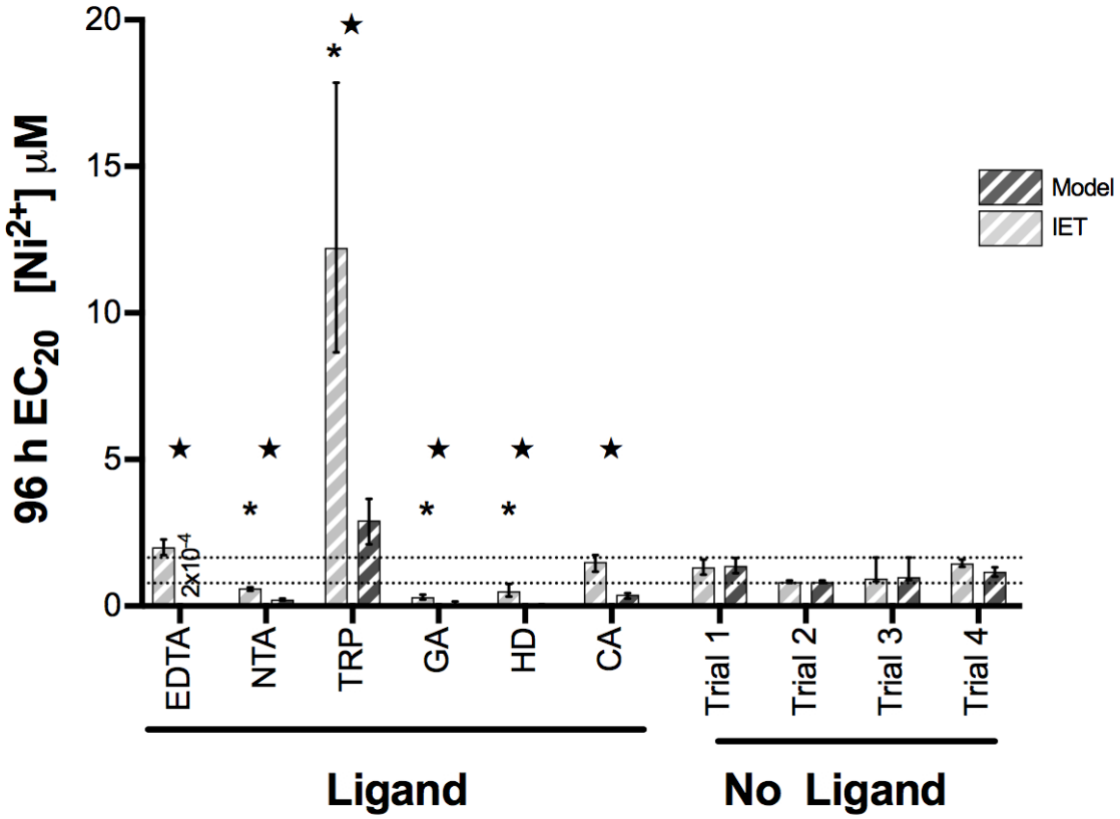

FIG. 2.5. The 96 h EC_{20} values for $[Ni^{2+}]$ for abnormal embryo development in purple sea urchin with (and without) added ligands. $[Ni^{2+}]$ endpoint determinations were calculated using either measured $[Ni^{2+}]$ by IET (dark gray stripes) or modelled $[Ni^{2+}]$ predicted by Visual Minteq (light gray stripes). Note: the predicted value for EDTA was very low, so the value is written in place of the bar. Error bars show 95% confidence interval and * indicates a significant difference in EC_{20} value compared to the no ligand (ASW) exposure and ★ indicates a significant difference in EC_{20} value between measured and predicted. Dotted horizontal lines represent the confidence limits of the ASW trials.

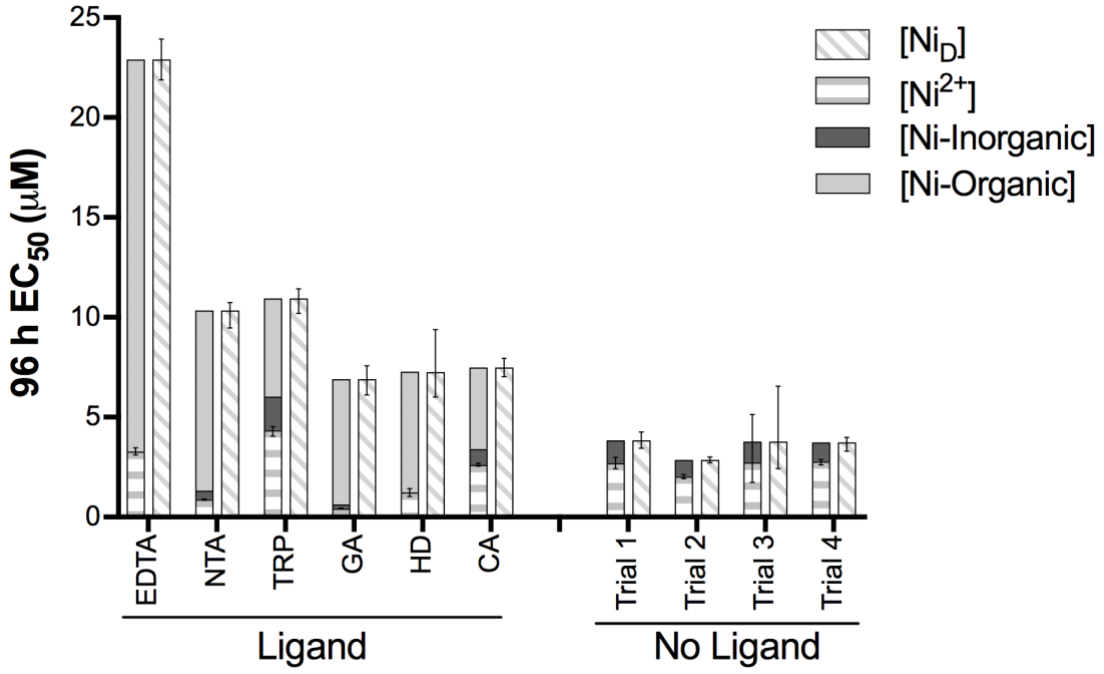

FIG. 2.6. The 96 h EC_{50} values for abnormal embryo development due to Ni with or without the addition of synthetic ligands. The full bar height indicates the [Ni_D] toxicity and fractions within the dissolved phase: Ni bound to inorganic complexes (dark gray), Ni bound to the added synthetic organic ligand (light gray), and [Ni^{2+}] as determined by IET (except for TRP which used predicted-free ion by Visual Minteq). Error bars show 95% confidence intervals for [Ni_D] and [Ni^{2+}]. See text for description of synthetic ligands.

2.8 References

Arnold WR. 2005. Effects of dissolved organic carbon on copper toxicity: Implications for saltwater copper criteria. *Integr Environ Assess Manag*. 1:34-39.

Blewett TA, Dow EM, Wood CM, McGeer JC, Smith DS. 2018. The role of dissolved organic carbon concentration and composition on nickel toxicity to early life-stages of the blue mussel *Mytilus edulis* and purple sea urchin *Strongylocentrotus purpuratus*. *Ecotoxicol Environ Saf.* 160:162-170.

Blewett TA, Leonard EM. 2017. Mechanisms of nickel toxicity to fish and invertebrates in marine and estuarine waters. *Environ Pollut.*223:311-322.

Blewett TA, Smith DS, Wood CM, Glover CN. 2016. Mechanisms of nickel toxicity in the highly sensitive embryos of the sea urchin *Evechinus chloroticus*, and the modifying effects of natural organic matter. *Environ Sci Technol.* 50:1595-1603.

Blewett TA, Wood CM. 2015. Salinity-Dependent Nickel Accumulation and Oxidative Stress Responses in the Euryhaline Killifish (*Fundulus heteroclitus*). *Arch Environ Contam Toxicol*. 68:382-394.

Chen W, Sherman SR, McGeer JC, Smith DS. Determining the concentration of free nickel ions in seawater by ion exchange technique: validation and application. *Manuscript in progress.*

DeForest DK, Schlekat CE. 2013. Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms. *Integr Environ Assess Manag*. 9:580-589.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem*. 20:2383-2396.

Dow E. 2017. Quantifying the interactions of nickel with marine dissolved organic matter

using fluorescence. Unpublished masters dissertation. Wilfrid Laurier University, Waterloo, Ontario. pp. 91.

Eisler R. 1998. Nickel hazards to fish, wildlife, and invertebrates: A synoptic review. *Biol Sci Rep*. 34:95.

Environment Canada (EC). 2005. Guidance document on statistical methods for environmental toxicity tests. Environment Canada, method development and application section. Ottawa, ON. Report EPS 1/RM/46. June 2007 amendments. pp 241.

Environment Canada (EC). 2014. Reference method for measuring the toxicity of contaminated sediment to embryos and larvae of echinoids (sea urchins or sand dollars). 1/RM/58. Science and Technology Branch Ottawa, Ontario. (July):1-91.

Fortin C, Campbell PGC. 1998. An ion-exchange technique for free-metal ion measurements (Cd^{2+} Zn^{2+}): Applications to complex qqueous media. *Int J Environ Anal Chem*. 72:173-194.

Gissi F, Stauber JL, Binet MT, Golding LA, Adams MS, Schlekat CE, Garman ER, Jolley DF. 2016. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. *Environ Pollut*. 218:1308-1323.

Hardin J, Coffman JA, Black SD, McClay DR. 1992. Commitment along the dorsoventral axis of the sea urchin embryo is altered in response to $NiCl_2$. *Development*. 116:671-685.

Ho KT, Kuhn A, Pelletier MC, Hendricks TL, Helmstetter A. 1999. pH dependent toxicity of five metals to three marine organisms. *Environ Toxicol*. 14:235-240.

Landner L, Reuther R. 2005. Metals in society and in the environment. Environ Pollut. 1-9.

Lussier SM, Kuhn A, Comeleo R. 1999. An evaluation of the seven-day toxicity test with *Americamysis bahia* (formerly *Mysidopsis bahia*). *Environ Toxicol Chem*. 18:2888-2893.

McFarlane MB, Gilly WF. 1998. State-dependent nickel block of a high-voltage-activated neuronal calcium channel. *J Neurophysiol*. 80:1678-1685.

Meyer JS, Santore RC, Bobbitt JP, Debrey LD, Boese CJ, Paquin PR, Allen HE, Bergmann HL, Ditoro DM. 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environ Sci Technol*. 33:913-916.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F, et al. 2002. The biotic ligand model: a historical overview. *Comp Biochem Physiol C Toxicol Pharmacol*. 133:3-35.

Phillips BM, Nicely PA, Hunt JW, Anderson BS, Tjeerdema RS, Palmer SE, Palmer FH, Puckett HM. 2003. Toxicity of cadmium-copper-nickel-zinc mixtures to larval purple sea urchins (*Strongylocentrotus purpuratus*). *Bull Environ Contam Toxicol*. 70:592-599.

Phinney JT, Bruland KW. 1994. Uptake of lipophilic organic Cu, Cd, and Pb complexes in the coastal diatom *Thalassiosira weissflogii*. *Environ Sci Technol*. 28:1781-1790.

Playle RC, Dixon DG, Burnissn K. 1993. Copper and cadmium binding to fish gills: Estimates of metal-gill stability constants and modelling of metal accumulation.

Can J Fish Aquat Sci. 50:2678-2687.

Silva C, Yáñez E, Martín-Díaz ML, Riba I, Delvalls TA. 2013. Integrated ecotoxicological assessment of marine sediments affected by land-based marine fish farm effluents: Physicochemical, acute toxicity and benthic community analyses. *Ecotoxicology*. 22:996-1011.

Stockdale A, Tipping E, Hamilton-Taylor J, Lofts S. 2011. Trace metals in the open oceans: Speciation modelling based on humic-type ligands. *Environ Chem*. 8:304-319.

Stockdale A, Tipping E, Lofts S. 2015. Dissolved trace metal speciation in estuarine and coastal waters: Comparison of WHAM/Model VII predictions with analytical results. *Environ Toxicol Chem*. 34:53-63.

Tellis MS, Lauer MM, Nadella S, Bianchini A, Wood CM. 2014. The effects of copper and nickel on the embryonic life stages of the purple sea urchin (*Strongylocentrotus purpuratus*). *Arch Environ Contam Toxicol*. 67:453-464.

Uthicke S, Schaffelke B, Byrne M. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecol Monogr*. 79:3-24.

Wilt FH. 1999. Matrix and mineral in the sea urchin larval skeleton. *J Struct Biol*. 126:216-226.

Worms IAM, Wilkinson KJ. 2008. Determination of Ni^{2+} using an equilibrium ion exchange technique: Important chemical factors and applicability to environmental samples. *Anal Chim Acta*. 616:95-102.

Zhao CM, Campbell PGC, Wilkinson KJ. 2016. When are metal complexes bioavailable?

Environ Chem. 13:425-433.

CHAPTER 3:
Speciation and toxicity of Ni to the purple sea urchin (*Strongylocentrotus purpuratus*) embryos in marine waters

3.1 Abstract

The biotic ligand model (BLM) is a predictive modeling approach that utilizes the relationship between water chemistry and tissue-metal accumulation to predict toxicity. Therefore, differing water chemistries, including varying dissolved organic carbon (DOC) concentrations and compositions, can alter these toxic effects. The goal of this study was to test the assumptions of the BLM by characterizing the complexation of Ni in natural marine water samples in relation to toxicity. Purple sea urchin (*S. purpuratus*) embryological development was used as the toxicity endpoint. It was predicted that DOC concentration would be inversely correlated to toxicity based on total dissolved Ni concentrations [Ni_D]; however, on a free ion concentration ([Ni^{2+}]) basis, effects concentrations should be constant. [Ni_D] was measured by graphite furnace atomic absorption spectroscopy (GFAAS) and Ni^{2+} was first quantified using Ion-Exchange Technique (IET) and then concentrations were measured by GFAAS. Natural waters were protective to varying degrees offering a 2.5-fold difference in protection across exposures (values ranging from 2.8 to 7.3 µM). The variability of the EC_{50} values for natural waters slightly decreases when expressed on a [Ni^{2+}] basis ranging from 1.5 to 4 µM. However, no significant correlation was found between toxicity and DOC or between toxicity and any of the DOC optical characteristics defining composition. Therefore, there were no conclusive trends showing site-dependent protection, indicating that protectivity is independent of DOC source and composition. The results of this research contribute to the development of biotic ligand-based prediction models for estimating Ni impacts in seawater.

3.2 Introduction

Nickel (Ni) toxicity in marine environments is not as well understood as it is in freshwater (FW) where factors modulating toxic effects have been incorporated into site-specific bioavailability-based prediction models. As such, there are currently no models available that are able to predict Ni toxicity in marine systems, although frameworks are currently in development. The biotic ligand model (BLM) has been used to predict metal toxicity for many dissolved metals in FW (Arnold 2005; Crémazy et al. 2018; Meyer et al. 1999; Paquin et al. 2002) and for some in saltwater (SW; Blewett et al. 2018; Nadella et al. 2013). The BLM predicts the degree of metal binding at the site of toxic action on an organism (the biotic ligand) and metal binding is a function of metal bioavailability as well as uptake, as defined by the site-specific water chemistry conditions (Paquin et al. 2002). In general the free ion (i.e. Ni^{2+}) is the most bioavailable and therefore toxic geochemical form (species) although other geochemical species can also be associated with toxic effects (Landner and Reuther 2005; Niyogi and Wood 2004; Wood et al. 2011).

The relationship between water chemistry and Ni accumulation is a key factor in determining Ni toxicity within aquatic systems. In order to assess the impacts and risks of Ni in marine systems studies have explored the potential protective effects of water chemistry (Blewett et al. 2018; Lussier et al. 1999; Tellis et al. 2014). Water chemistry parameters such as: H^+, Ca^{2+} and natural organic matter (NOM) play important roles in influencing Ni toxicity by affecting the bioavailability of Ni to the organism (Di Toro et al. 2001; Playle et al. 1993). Ni^{2+} may compete with cations (i.e. Ca^{2+}) for access to binding sites on the organisms. As well, complexation with NOM and anions (i.e. Cl^-) will decrease free ion concentrations, limit bioavailability and therefore reduce toxicity

(Playle et al. 1993). Investigating complexation with anions and competition for uptake to the biotic ligand among cations is important in predicting toxicity as water quality parameters that influence toxicity are needed as inputs for the BLM, thereby allowing site-specific water criteria guidelines to be determined (Paquin et al. 2002).

Incorporating the influence of important water quality parameters such as NOM is an essential feature of all BLMs (Di Toro et al. 2001; Santore et al. 2001; Paquin et al. 2002; Niyogi and Wood 2004). NOM input into the BLM is done as the measured dissolved organic carbon (DOC: any organic carbon that passes through a 0.45μm filter) concentrations as well as the % humic acid. DOC (in mg DOC/L) generally has greater protective effects than H^+, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+} (in mol/L), which are usually strong inorganic modifiers of metal toxicity (Wood et al. 2011). DOC may complex metal ions that would otherwise bind to sites on the biotic ligand therefore reducing the bioavailability, uptake into the biotic ligand and toxicity (Playle et al. 1993). In marine waters, which range from open ocean to coastal waters, DOC concentrations vary from 0.5 to 10 mg C/L (Benner 2002) and have the potential to provide significant protection. Blewett et al. (2018) showed that DOC-metal binding is significant in marine waters using the species *Mytilus edulis* and *S. purpuratus*; concentrations at 4.5 mg DOC/L provided a 5 fold reduction in toxicity compared to artificial seawater (ASW) and Ni controls. Therefore, studies examining the role of DOC in terms of concentration will make important contributions to understanding marine Ni toxicity.

Growing evidence suggests that NOM from different sources exhibit different metal binding capacities that depend on their composition (Al-Reasi et al. 2011; Arnold et al. 2005; Blewett et al. 2016, 2018). For example, Al-Reasi et al. (2011) found that for

copper (Cu) in FW there were significant positive relationships between the aromatic composition of different NOM sources and the measured toxic responses for three freshwater organisms (fathead minnows, rainbow trout, *Daphnia magna*). In SW, Blewett et al. (2018) found the inclusion of optical parameters (i.e. specific absorption coefficient at 340 nm; SAC_{340}) did improve correlations to toxicity response values for *M. edulis* and *S. purpuratus* compared to DOC concentration alone. To date very few studies have explored the role of DOC composition on toxicity related to Ni. DOCs can be grouped into two forms: allochthonous and autochthonous. Allochthonous DOC originates from the breakdown of leaves and wood or other terrigenous sources where autochthonous is formed from algae within lakes and rivers by degradation of allochthonous DOC (Thurman 1985). These different sources vary in Ni complexing capacities that could have an overall effect on protection to toxicity (De Schamphelaere and Janssen 2004). Allochthonous DOCs are optically darker compared to autochthonous DOCs as they tend to have more phenolic groups and have higher concentrations of humic and fulvic acids (Al-Reasi et al. 2011; Blewett et al. 2018, Wood et al. 2011). Allochthonous DOCs have shown to be more protective against metal toxicity in FW for metals such as copper, silver and lead (Wood et al. 2011). Measuring and determining properties such as the SAC_{340}, the specific UV absorbance at 254 nm (SUV_{254}) or the fluorescence index (FI) can provide estimates of the relative aromatic composition of DOC. These are important factors in predicting potential Ni toxicity as they have been shown to affect metal binding and it may important to update current available BLMs to account for it (Al-Reasi et al. 2011; Blewett et al. 2018; Wood et al. 2011). Therefore, studies examining the role of DOC in terms of both concentration as well as composition will make important

contributions to understanding marine Ni toxicity and the development of BLM approaches to predicting potential impacts of Ni.

In a recent study, we tested the assumptions of the BLM, using synthetic ligands with known complexation for Ni, using a sensitive marine species (*Strongylocentrotus purpuratus*; refer to chapter 2). We found that the addition of ligands provided protection compared to tests in ASW when we based endpoint determinations using dissolved Ni concentrations ([Ni_D]). However, when based on measured free ion concentration ([Ni^{2+}]) all endpoint values were either similar [EDTA and citric acid (CA)] or less [NTA, glutamic acid (GA) and histidine (HD)] than those from tests in ASW. The results of this research better define Ni-biotic ligand interactions in marine waters as influenced by synthetic ligands and provide a proof of principle for the application of biotic ligand-based prediction models for estimating Ni impacts in SW. The present study was designed to extend our research and better understand chronic toxicity of Ni by exploring Ni toxicity and speciation in natural waters. The current study had two goals. The first was to investigate the Ni toxicity to *S. purpuratus* embryonic life stages in natural marine waters and determine the relationship between toxicity and speciation. The second was the understand potential protective effects of NOM in marine waters.

3.3 Methodology

3.3.1 Animal care

Refer to chapter 2 section 2.3.1

3.3.2 Water collection, storage and DOC analysis

Samples of marine water were collected from coastal sites with the goal of having sources with varying NOM characteristics and DOC concentration. Sites in Rhode Island, Connecticut, Florida and along the Gaspé peninsula in Quebec provided 13

different sources (Table 3.1 and Fig 3.1). Additionally, an open ocean site in the Beaufort Sea was collected (Table 3.1 and Fig. 3.1). The sample from Florida was provided by K. Brix (EcoTox) and the one from the Arctic was donated by C. Guéguen (Trent University). A further sample was obtained from the foam fractionator (protein skimmer) of the marine system at a local aquarium supplies store (Living Aquarium, Cambridge, ON). This sample was diluted to yield 3 subsamples with a concentration gradient of high (9.0 mg C/L), medium (6.0 mg C/L) and low (3.0 mg C/L) DOC. Following Cooper et al. (unpublished), sites were chosen based on visual assessment of the local geography and on the drainage basins where they were located (using Google Maps and personal judgement upon arrival at the site). Ideal sample sites consisted of an area with a NOM rich fresh water tributary with no significant upstream anthropogenic inputs (agriculture and/or industry) that flowed into a water basin with a salinity above 10 ppt (Table 3.1).

Samples were collected using a submersible pump and filtered through a 0.45 µm filter (FHT-45 High Turbidity Inline Filter, Hoskin Scientific Ltd., Burlington, ON), placed in carboys and shipped in coolers to Wilfrid Laurier University where they were refrigerated until further analysis and use in the toxicity assay. At each site a sub sample was taken and measured for fluorescence using a field fluorescence biological oxygen demand (BOD) meter which measures proteinaceous fluorescence as a proxy to BOD (STS SMF 4 Fluorimeter, Pine Environmental, Toronto ON, Canada; Baker et al. 2015; Table 3.1). Salinity and pH were also measured on site when possible using a hand-held conductivity meter (YSI 30, YSI Inc., Yellow Springs; Table 3.1). Samples for DOC analysis (50 mL, also filtered) were acidified with 50 µL concentrated HCL and measured using a Shimadzu TOC-Vcph/CPN total organic carbon analyzer (Shimadzu

Corporation, Kyoto, Japan). The aromatic composition of the DOCs were estimated by examining SAC_{340}, SUV_{254}, FI, and charged cation concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}) by fellow author Chen et al. (unpublished) following the methods found in McKnight et al. (2001) and Schwartz et al. (2004). Briefly, SAC_{340} (equation 1), SUV_{254} (equation 2) and FI (equation 3) were calculated as:

$$SAC_{340} = \frac{(Abs340)}{[DOC]} \qquad \textit{Equation 1}$$

where Abs_{340} is the absorbance at 340 nm

$$SUV_{254} = \frac{(Abs254)}{[DOC]} \qquad \textit{Equation 2}$$

where Abs_{254} is the absorbance at 254 nm

$$ex370 = \frac{Em450}{Em500} \qquad \textit{Equation 3}$$

where ex370 is the FI index at 370 nm and Em450 and Em500 refer to emission intensities at 450 nm and 500 nm

3.3.3 Collection and fertilization of gametes

Refer to chapter 2 section 2.3.2

3.3.4 Preparation of test solutions and toxicity tests

Twenty-four hours before the toxicity test started, the salinity of the collected samples was increased to 30 ± 0.5 ppt using Kent Marine salt. Each exposure concentration was aliquoted into one of six 20 mL scintillation vials (9 mL for each). These included four biological replicates and two for measuring total and dissolved Ni concentrations which were measured on a graphite furnace atomic absorption spectroscopy (GFAAS). The remaining solution was used for [Ni^{2+}] determination by ion exchange technique (IET). Ni solutions were prepared using $NiCl_2$ soluble salt (1000 ppm; Sigma Aldrich). For each test 2.5 L of sample was equally split into polyethylene bottles which were then spiked

with appropriate amounts of Ni (Table 3.2). For each test at least 8 and up to 9 concentrations of Ni were used and all solutions were equilibrated for 24 h at 15°C before the addition of embryos. The pH was adjusted using 0.1 M NaOH to 8.1 ± 0.1.

3.3.5 Toxicity endpoint determination

Refer to chapter 2 section 2.3.4

3.3.6 Ni quantification by GFAAS

Refer to chapter 2 section 2.3.5

3.3.7 Determination of Ni speciation

Refer to chapter 2 section 2.3.6

3.3.8 Calculation of Ni-Biotic ligand binding affinity

Refer to chapter 2 section 2.3.7

3.3.9 Statistical analysis

The 96 h median effective concentration (EC_{50}) and 20% effective concentration (EC_{20}) values with upper and lower 95% confidence intervals (95% CI) were determined for both [Ni_D] and [Ni^{2+}] using the Comprehensive Environmental Toxicity Information System (CETIS) software following the EPA ICPIN method based on linear interpolation with bootstrapping. Significant differences between sites (and with ASW) were examined using the overlap of 95% CI; if they did not overlap, then the EC_{50} (or EC_{20}) values were considered significantly different (EC 2005). In order to determine the extent to which toxicity was related to [Ni^{2+}] the relative variability of EC_{50} values across sites for [Ni_D] and for [Ni^{2+}] was assessed by comparing coefficients of variation (CVs). The CVs were calculated by dividing the standard deviation of the EC_{50}s by the average EC_{50}s across sample sites. Pearson correlation analysis was carried out using GraphPad Prism software v. 7.0 to determine relationships between toxicity endpoints and either DOC

concentration or optical characteristics related to NOM composition (significant correlation if $p<0.05$).

3.4 Results and Discussion

3.4.1 Water Chemistry

The measured [Ni_D] values were 98 ± 19 % of [Ni_T] values (shown as mean ± SD; range of 41 to 165 %), indicating negligible Ni precipitation over the 96-h toxicity test (Table 3.2). There was no significant difference between the certified reference material (CRM) value and the average value measured on the GFAAS (19% difference; $p>0.05$). Throughout the 96 h for all exposures, temperature in the water bath ranged from 14.5 to 15.4 °C (n=4), and within the test vials salinity ranged from 29.6 to 31.4 ppt (n=2) and pH ranged from 8.0 to 8.1 (n=2; Table 3.2).

3.4.2 Protection against Ni toxicity caused by natural waters

All toxicity tests met the acceptable criteria where normal development of >80% of the embryos was reached within 96 h in unexposed controls (EC 2014; Table 3.3). Chronic toxicity of Ni to *S. purpuratus* embryos was assessed in the presence of each of the 14 natural water samples. Natural water samples show that the range of EC_{50} values for [Ni_D] varied by a factor of two ranging from 3.0 to 7.0 µM (Fig. 3.2). For [Ni_D] EC_{20} the values ranged from 0.5 to 2.0 µM (Fig. 3.3). Based on the EC_{50} value, site ML showed the greatest protection compared to other sites; all other sites from the Gaspé peninsula were similarly protective except for site GP, which showed to be least protective (Fig. 3.2). All Connecticut and Rhode Island sites grouped together and showed similar protection (Fig. 3.2). However, there were no definite trends in sampling location with toxicity. Considering the endpoint values and associated confidence intervals none of the samples were significantly protective compared to ASW exposures

(data shown in appendix A). However, this range of values within natural waters does indicate that some component is potentially offering some protectivity.

The effect of DOC concentration on Ni toxicity was investigated (Fig. 3.4). DOC concentration ranged from 1.3 to 5.7 mg C/L within the natural waters (Table 3.4). In ASW the DOC concentration was 0.5 mg C/L, which are similar to previous studies (0.88 mg C/L; Blewett et al. 2018). DOCs are thought to mitigate toxicity to organisms by forming metal-DOC complexes that cause the metal to be less bioavailable to an organism (Di Toro et al. 2001; Santore et al. 2001; Wood et al. 2011). This has been seen for many metals in SW such as copper and silver (Arnold 2005; Glover and Wood 2005; Nadella et al. 2009; Lorenzo et al. 2006). An analysis of the relationship between [Ni_D] EC_{50} and DOC concentration revealed that the relationship is not strong and no significant correlation was found (Fig 3.4; R^2= 0.005, r= 0.07, p= 0.78) in spite of a slight positive relationship (i.e. as DOC concentration increased the EC_{50} also increased). Therefore, any protection seen within the samples was not related to the concentration of DOC, which can be confirmed as the WHAM model predicts only a weak DOC-Ni binding relationship in full strength SW (Stockdale et al. 2015). Similar results were found by Nadella et al. (2013) who showed increased DOC concentrations did not provide protection against zinc toxicity for two marine organisms. However, this study did not consider the source and composition of DOC and these factors may influence the ability to protect against Ni toxicity. From this research it is apparent that DOC concentration is theoretically not the only factor influencing Ni toxicity, indicating that DOC concentration may not be a great indicator of toxicity and other markers should possibly also be used.

3.4.3 Water composition and its role in Ni toxicity

Optical characteristics of the marine samples related to DOC composition were investigated as well. SAC_{340} (the specific absorbance coefficient at 340 nm), SUV_{254} (the specific UV absorbance at 254 nm), fluorescence index (FI), and charged cation concentrations were all measured (i.e. Na^+, K^+, Ca^{2+}, Mg^{2+}; Table 3.4). The data showed the SAC_{340} and SUV_{254} had no significant trends with $[Ni_D]$ EC_{50} values (R^2= 0.07, r= -0.27, p= 0.36 and R^2= 0.12, r= -0.34, p= 0.23 respectively). This is consistent with other studies that showed very weak correlation with toxicity (De Palma 2009; Tait 2013). A higher SAC_{340} indicates terrigenous origin and is suspected to decrease Ni availability and toxicity, increasing EC_{50} value, at least in FW (Schwartz et al. 2004). Similarly, allochthonous humic substances show higher average SUV_{254} values and is assumed to lower toxicity. However, the SAC_{340} and SUV_{254} values within this study were significantly lower (approximately 2 to 4x) than other recorded values (Schwartz et al. 2004; Wood et al. 2011), possibly indicating why no correlations were seen. Fluorescence Index (FI) had no correlation with $[Ni_D]$ EC_{50} and was not significant (R^2= 0.01, r= -0.12, p= 0.71). FI is a measure that distinguishes the source or origin of various DOMs (McKnight et al. 2001). A FI value of roughly 1.4 indicates terrestrially-derived and 1.9 of microbially-derived NOM (McKnight et al. 2001). Within this study, FI ranged from approximately 1.0 to 1.63, suggesting that samples encompassed both terrestrially and microbial sources. Most sources were found to be terrestrially-derived with FI indexes of 1.0 to 1.45, however CC had a FI of 1.63 suggesting the site has both terrestrial and microbial inputs. It has been proposed that terrestrially-derived organic matter is more protective than microbially-derived NOM and therefore it is assumed that as FI increases protectivity would decrease (Luider et al. 2004; Schwartz et al. 2004; Tait 2013). All

charged cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) showed no significant correlations with [Ni_D] EC_{50} (R^2= 0.22, r= 0.47, p= 0.11 for Ca^{2+}, R^2= 0.25, r= 0.50, p= 0.08 for Mg^{2+}, R^2= 0.30, r= 0.55, p= 0.05 for Na^{+} and R^2= 0.28, r= 0.53, p= 0.06 for K^{+}). Potential protective effects to an aquatic organism caused by Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} occur as they compete for binding sites against Ni on the biotic ligand, effectively reducing toxicity (Di Toro et al. 2001; Paquin et al. 2002; Niyogi and Wood 2004;). Therefore, increased concentrations of these cations cause more sites on the biotic ligand to be potentially bound, reducing the chance for Ni to be taken up by the organism, and reducing toxicity (Di Toro et al. 2001; Playle et al. 1993; Paquin et al. 2002).

The effect of initial fluorescence on Ni toxicity was also investigated. Fluorescence characterisation of organic matter in aquatic systems has advanced significantly in recent years with technological improvements to optical instrumentation (Baker et al. 2015). Portable instrumentation has been used to measure the intensity of fluorescence emitted at 350 nm through excitation of DOM at 280 nm and has been shown to relate to the water quality in aquatic environments and correlate with biological oxygen demand (BOD; Cumberland et al. 2012; Baker 2001; Baker et al. 2015). For this study, initial fluorescence values ranged from 107 to 1526 within natural waters (Table 3.1). Values of initial fluorescence were found to significantly correlate with EC_{50} values (Fig 3.5; R^2= 0.54, r= 0.73, p= 0.001). This means that a site with a higher initial fluorescence value would have more tryptophan-like fluorescence from protein molecules resulting in a higher EC_{50} value. This is because Ni can bind to proteinaceous sites, reducing the Ni available to bind to the biotic ligand and reducing toxicity. However, although toxicity between sites within this study did vary, they were not significantly different from ASW

exposures. Very few studies have used initial fluorescence as a marker for toxicity, though it may be one way that site-specific estimates of toxicity can be done in the field (Baker et al. 2015).

3.4.4 Speciation

IET measurements of free ion were performed in subsamples of the same solutions to measure the [Ni^{2+}] within natural waters and calculate ECx values (Table 3.2). For natural waters, EC_{50} values when expressed on a [Ni^{2+}] basis ranging from 1.5 to 4 µM (Fig 3.6). All samples had confidence bands that overlapped with the confidence bands of the ASW exposures and were therefore not significantly different (data shown in appendix A). For [Ni^{2+}] EC_{20} the values ranged from 0.25 to 2 µM (Fig. 3.7). Measuring [Ni^{2+}] within SW provides a method to test the complexation predictions important to assess the assumptions inherent to the BLM for validating its use for Ni in SW. [Ni^{2+}] EC_{50} was unchanged regardless of DOC concentration and no significant trends were found with DOC since [Ni^{2+}] was within a factor of two for all natural waters (R^2= 0.14, r= 0.37, p= 0.19). As well, there were no significant correlations with any of the DOC composition characteristics (SAC_{340}, SUV_{254}, FI, and Na^+, K^+, Ca^{2+} and Mg^{2+}; data shown in appendix A). These results show that [Ni^{2+}] is potentially the best predictor of Ni toxicity which agrees with the BLM assumptions and strengthen the data found in chapter 2 showing that free ion remains unchanged.

3.4.5 Gradient of DOC concentrations effect on Ni toxicity

Chronic toxicity was assessed for artificial aquarium water samples diluted to yield a DOC gradient. Artificial aquarium treatments were excluded from the natural water sample plots because they are not natural samples and instead they were examined separately to test the relationship of toxicity with DOC concentration. For [Ni_D] EC_{50}

values of the low and medium (SL and SM) DOC concentrations were not significantly different from one another based on confidence intervals but high (SH) showed significantly greater protection than both SL and SM (Fig. 3.2). For [Ni_D] EC_{20} all treatments similar to one another (Fig. 3.3). Aquarium waters showed strong positive trends with DOC but were not significant (Table 3.4; R^2= 0.93, r= 0.96, p= 0.17). However, more sample points are necessary to strengthen the relationship. Artificial aquarium waters showed decreased variability by 50% for the EC_{50} values when expressed on a [Ni^{2+}] basis compared to the value based on [Ni_D] (Fig 3.6). None of these samples were significantly different from one another or from ASW exposures (data shown in appendix A). The same trends were seen for [Ni^{2+}] EC_{20} (Fig. 3.7). This further supports the assumptions of the BLM and the data found previously that [Ni^{2+}] can predict toxicity.

3.4.6 Binding affinity

IET-measured [Ni^{2+}] was used for the derivation of conditional stability constant ($K^{'}$) for each site and an average value was calculated (Table 3.4). The $\log K^{'}_{NiBL}$= 6.4 ± 0.09, indicating that 50% of the Ni binding sites would be occupied at aqueous Ni concentrations of $10^{-6.4 \pm 0.09}$ M. Previous research has shown natural waters to have K_f values ranging from 3.8 to 7.1, which are comparable to the values calculated within this study (Chen et al. unpublished; Dow 2017). The $\log K^{'}_{NiBL}$ using model ligands in a previous study was found to be 6.3 ± 0.4= $10^{6.3}$ showing agreement between model ligands and natural samples (refer to chapter 2). This value can be used to develop a computational BLM for marine Ni and also aids in determining when ligands may be protective. For example, if a natural organic ligand (DOC) has an equivalent K_f value to

the biotic ligand ($K'_{NiBL}= 10^{6.3\pm 0.4}$) at the same concentrations, then half of Ni will bind to each. However, if concentrations or binding affinities increase for one, it will out compete the other causing more Ni to bind to it. Using these K_f values to predict if Ni will bind more strongly to the biotic ligand or DOC will be important in predicting toxicity in natural waters.

3.5 Conclusion

Natural waters decreased the toxicity of Ni to *S. purpuratus* embryos compared to ASW exposures. Natural waters showed varying EC_{50} values but were not significantly protective compared to ASW exposures. The variability seen in EC_{50} values showed no definite trends in sampling location with toxicity. There was no conclusive evidence of protection from Ni toxicity for any of the natural waters indicating that protectivity is independent of DOC source and composition. Initial fluorescence showed positive correlations with the EC_{50} value indicating it may be one way that site-specific estimates of toxicity can be done in the field. As well, $[Ni^{2+}]$ EC_{50} was similar between natural water sites regardless of DOC concentration or composition agreeing with the assumptions of the BLM. Overall, the relationship between marine Ni toxicity and DOC is complex. Further studies that reproduce results of the current study but expand the range of natural DOC concentrations are needed to better understand the relationship between natural waters and protection.

3.6 Acknowledgements

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3.7 Tables and Figures

Table 3.1. Location coordinates for DOM sampling sources, including water chemistry parameters at time of collection and DOC measurements (mg C/L). Note, that there are no initial fluorescence values for AR and JB treatments because upon collection there was no access to the BOD meter.

Location	Site Name	Site Code	GPS Coordinates	Salinity (ppt)	pH	Initial Fluorescence (RFU)
Rhode Island	Seaview Park	SVP	41°45'40.2"N 71°23'11.9"W	17.50	7.7	408
	Barbara Tufts Playground	BTP	41°39'29.6"N 71°26'48.4"W	18.00	7.4	278
	Perry Creek Access	PCA	41°21'50.2"N 71°37'36.5"W	28.00	8.3	364
Connecticut	Walnut Beach	WB	41°11'46.7"N 73°04'27.5"W	26.50	7.7	107
	Audubon Coastal Center	CCC	41°10'34.5"N 73°06'06.5"W	20.00	7.4	106
Gaspé	Gros Morne	GM	49°15'07.8"N 65°32'48.9"W	12.36	7.3	920
	Forillion National Park	FP	48°50'20.8"N 64°12'48.6"W	26.11	7.9	562
	Pit Caribou	PC	48°28'21.3"N 64°18'36.5"W	23.56	7.8	339
	Cap Chat	CC	49°05'51.6"N 66°40'47.3"W	18.90	7.6	1526
	Rimouski	RI	48°26'41.3"N 68°32'26.4"W	27.50	8.4	337
	Mont-Louis	ML	49°13'40.2"N 65°44'15.3"W	12.19	8.8	1008
	Grand Pabos	GP	48°20'38.5"N 64°42'16.9"W	9.62	7.5	143

Beaufort Sea	Arctic	AR	72°36'00.0"N 144°42'00.0"W	-	-	-
Florida	Jimbo	JB	25°46'28.9"N 80°08'43.4"W	-	-	-
Aquarium	Skimmer Low	SL	-	-	-	-
	Skimmer Medium	SM	-	-	-	-
	Skimmer High	SH	-	-	-	-

Table 3.2. Water chemistry parameters in ASW and collected natural waters. Ni exposure concentrations are given as nominal, [Ni_D] and [Ni^{2+}] measured by IET (except unexposed controls). Means are given ± standard deviation (SD) for [Ni_D] (µg/L; n=2), [Ni^{2+}] (µg/L; n=2), pH (n=2), salinity (ppt; n=2), and temperature (°C; n=4). Values with * were excluded from any calculations of [Ni_D] as a % of [NiT]. Some exposures only had enough sample for 1 measurement and therefore an SD could not be calculated.

Site	Nominal Ni (µg/L)	Dissolved Ni (µg/L) ± SD	Free Ni (µg/L) ± SD	pH ± SD	Temperature ± SD (°C)	Salinity ± SD (ppt)
SVP	0	3 ± 0.09*		8.1	15.0 ± 0.3	30.8 ± 0.04
	25	28 ± 0.05	18 ± 0.4			
	50	53 ± 0.9	31 ± 1.5			
	100	102 ± 8.3	60 ± 1.3			
	200	164 ± 4.5	98 ± 0.6			
	400	334 ± 3.5	231 ± 1.2			
	800	690 ± 20.2	358 ±2.7			
	1600	1899 ± 82.3	574 ±7.4			
BTP	0	2 ± 0.07*		8.1	15.0 ± 0.3	30.4 ± 0.06
	25	27 ± 0.2	16			
	50	52 ± 0.03	27			
	100	93 ± 3.3	70			
	200	158 ± 1.0	104			
	400	324 ± 0.9	199			
	800	739 ± 55.9	371			
	1600	1544 ± 368.5	664			
PCA	0	3 ± 0.9*		8.1	15.0 ± 0.3	31.1 ± 0.23
	25	28 ± 1.2	18 ± 1.5			
	50	52 ± 2.6	30 ± 0.8			
	100	98 ± 0.7	69 ± 1.2			
	200	177 ± 6.0	102 ± 5.2			
	400	347 ± 1.1	214 ± 0.5			
	800	997 ± 1.4	384 ± 29.1			
	1600	1693 ± 328.8	764 ± 46.0			

WB	0	2 ± 1.1*		8.1	15.0 ± 0.3	30.4 ± 0.25
	25	27 ± 1.5	16 ± 1.8			
	50	50 ± 0.4	34 ± 7.7			
	100	95 ± 0.7	53 ± 3.4			
	200	168 ± 0.03	119 ± 1.9			
	400	331 ± 1.6	268 ± 20.2			
	800	997 ± 20.5	446 ± 6.7			
	1600	1616 ± 355.3	854 ± 59.0			
CCC	0	3 ± 0.5*		8.1	15.0 ± 0.3	30.6 ± 0.23
	25	28 ± 0.04	27 ± 8.6			
	50	52 ± 1.4	38 ± 8.6			
	100	95 ± 1.4	66 ± 9.7			
	200	162 ± 3.7	121 ± 7.4			
	400	333 ± 3.5	259 ± 18.0			
	800	674 ± 17.9	391 ± 1.0			
	1600	1814 ± 76.1	844 ± 22.7			
GM	0	0 ± 0*		8.1	15.1 ± 0.1	30.1 ± 0.13
	25	37 ± 1.7	21			
	50	75 ± 1.2	40 ± 21.7			
	100	147 ± 0.7	27			
	200	227 ± 0.09	63			
	400	499 ± 4.5	142			
	800	963 ± 7.9	311			
	1600	1937 ± 23.6	634			
FP	0	0 ± 0*		8.1	15.1 ± 0.1	30.0 ± 0.33
	25	33 ± 0.8	24			
	50	38 ± 0.8	1			
	100	65 ± 2.7	49			
	200	225 ± 6.2	85			
	400	321 ± 12.3	189			

	800	624 ± 4.4	358			
	1600	2006 ± 22.0	727			
JB	0	0 ± 0*		8.1 ± 0.1	15.1 ± 0.1	30.6 ± 0.27
	25	28 ± 0.2	36			
	50	59 ± 21.0	34			
	100	121 ± 7.3	43			
	200	207 ± 0.2	73			
	400	456 ± 13.9	119			
	800	847 ± 10.8	179			
	1600	1755 ± 60.5	291			
PC	0	1 ± 0*		8.1	15.1 ± 0.1	30.4 ± 0.23
	25	22 ± 3.5	20 ± 2.3			
	50	52 ± 4.3	37 ± 3.2			
	100	98 ± 3.8	40 ± 19.3			
	200	227 ± 10.7	114 ± 12.8			
	400	495 ± 29.1	232 ± 9.9			
	800	930 ± 49.0	549 ± 117.9			
	1600	2181 ± 94.43	897 ± 126.1			
CC	0	0 ± 0*		8.1	15.1 ± 0.1	29.9 ± 0.37
	25	38 ± 3.1	27			
	50	67 ± 3.1	44			
	100	123 ± 4.8	62			
	200	255 ± 1.3	105			
	400	322 ± 7.1	206			
	800	920 ± 1.7	370			
	1600	1940 ± 47.8	721			
AR	0	1 ± 5.1*		8.0	14.9 ± 0.1	30.7 ± 0.18
	25	13 ± 1.5	32 ± 3.0			
	50	49 ± 3.2	33 ± 4.1			
	100	88 ± 1.6	71 ± 12.3			

	200	178 ± 7.1	102 ± 3.4			
	300	287 ± 11.5	157 ± 2.6			
	400	288 ± 3.9	155 ± 8.1			
	800	867 ± 8.9	317 ± 30.2			
	1600	1900 ± 12.2	518 ± 44.7			
RI	0	1 ± 7.2*		8.1	14.9 ± 0.1	30.8 ± 0.20
	25	13 ± 0.6	34 ± 10.3			
	50	65 ± 0.5	43 ± 9.1			
	100	73 ± 5.8	65 ± 8.9			
	200	146 ± 6.1	98 ± 10.7			
	300	348 ± 8.5	132 ± 27.4			
	400	337 ± 16.1	170 ± 1.4			
	800	629 ± 2.5	332 ± 99.3			
	1600	1379 ± 31.2	608 ± 36.8			
ML	0	1 ± 2.8*		8.1	14.9 ± 0.1	31.0 ± 0.17
	25	21 ± 0.9	48 ± 9.1			
	50	43 ± 1.1	54 ± 11.1			
	100	96 ± 11.8	84 ± 9.6			
	200	170 ± 1.3	130 ± 18.6			
	300	286 ± 9.1	204 ± 35.8			
	400	417 ± 44.1	228 ± 23.3			
	800	809 ± 41.8	387 ± 30.0			
	1600	1720 ± 0.9	702 ± 9.5			
GP	0	1 ± 0.02*		8.1	14.9 ± 0.1	31.2 ± 0.18
	25	19 ± 0.53	51 ± 35.8			
	50	47 ± 0.8	52 ± 28.4			
	100	87 ± 2.9	74 ± 34.6			
	200	93 ± 0.6	96 ± 39.8			
	300	258 ± 3.4	125 ± 19.7			

	400	347 ± 18.9	138 ± 50.8			
	800	686 ± 5.0	292 ± 57.1			
	1600	1520 ± 15.6	382 ± 88.9			
SL	0	1 ± 0*		8.0	15.2 ± 0.2	30.4 ± 0.46
	25	8 ± 1.1	22 ± 1.4			
	50	32 ± 0.2	32 ± 3.4			
	100	65 ± 2.1	55 ± 3.5			
	200	139 ± 1.0	84 ± 4.0			
	300	209 ± 0.7	107 ± 30.0			
	400	300 ± 4.7	132 ± 1.2			
	800	601 ± 9.6	218 ± 1.8			
	1600	1210 ± 29.5	292 ± 7.0			
SM	0	1 ± 11.3*		8.0	15.2 ± 0.2	30.2 ± 0.37
	25	9 ± 0.2	24 ± 3.3			
	50	31 ± 2.7	34 ± 0.7			
	100	60 ± 2.4	54 ± 4.0			
	200	130 ± 2.6	85 ± 1.8			
	300	211 ± 3.4	135 ± 13.3			
	400	306 ± 4.4	132 ± 9.1			
	800	562 ± 23.2	223 ± 62.5			
	1600	1118 ± 29.0	326 ± 28.7			
SH	0	1 ± 0*		8.0	15.2 ± 0.2	30.3 ± 0.17
	25	9 ± 0.2	29 ± 12.5			
	50	32 ± 0.7	40 ± 5.4			
	100	68 ± 2.1	45 ± 0.6			
	200	122 ± 2.0	65 ± 5.4			
	300	195 ± 0.09	88 ± 15.4			
	400	296 ± 1.9	112 ± 3.5			
	800	568 ± 10.8	259 ± 48.6			

	1600	1134 ± 0.4	333 ± 1.2			

Table 3.3. The 96-hour chronic toxicity end-point of percent successful embryo development are shown as mean ± SD for all exposures (n=4).

Site	Nominal Ni (µg/L)	% successful embryo development ± SD (%)
SVP	0	95.75 ± 3.3
	25	89.25 ± 9.0
	50	80.50 ± 3.4
	100	75.00 ± 3.6
	200	59.25 ± 2.2
	400	14.25 ± 4.4
	800	0
	1600	0.25 ± 0.5
BTP	0	95.50 ± 4.7
	25	89.75 ± 1.3
	50	84.75 ± 2.8
	100	77.75 ± 4.0
	200	57.00 ± 4.2
	400	12.50 ± 8.6
	800	0.50 ± 0.6
	1600	0
PCA	0	97.75 ± 2.6
	25	89.75 ± 6.4
	50	81.75 ± 8.5
	100	75.50 ± 2.5
	200	58.75 ± 2.6
	400	18.25 ± 2.5
	800	0
	1600	0
WB	0	97.50 ± 2.4
	25	88.25 ± 2.5
	50	78.00 ± 4.1
	100	71.00 ± 2.9
	200	55.00 ± 3.6
	400	4.25 ± 4.4
	800	0.25 ± 0.5
	1600	0
CCC	0	97.75 ± 1.3
	25	89.00 ± 6.2
	50	78.25 ± 2.5
	100	73.00 ± 2.9
	200	63.50 ± 3.7
	400	25.75 ± 4.6
	800	0.50 ± 1.0
	1600	0
GM	0	95.00 ± 4.7

	25	92.50 ± 5.3
	50	76.75 ± 3.9
	100	69.50 ± 3.9
	200	60.25 ± 8.1
	400	17.75 ± 10.1
	800	0.50 ± 1.0
	1600	0
FP	0	95.25 ± 2.8
	25	89.00 ± 2.2
	50	87.00 ± 1.8
	100	69.50 ± 3.3
	200	62.75 ± 2.9
	400	33.75 ± 5.1
	800	0.25 ± 0.5
	1600	0
JB	0	94.00 ± 2.4
	25	91.00 ± 6.4
	50	87.00 ± 5.6
	100	72.75 ± 2.6
	200	62.25 ± 4.6
	400	36.25 ± 4.6
	800	0
	1600	0
PC	0	95.25 ± 3.9
	25	91.75 ± 5.6
	50	80.50 ± 3.4
	100	72.50 ± 8.4
	200	57.00 ± 4.5
	400	9.75 ± 7.6
	800	0
	1600	0
CC	0	94.50 ± 5.2
	25	89.50 ± 1.3
	50	84.00 ± 1.6
	100	69.75 ± 2.8
	200	60.75 ± 1.5
	400	34.50 ± 7.0
	800	0
	1600	0
AR	0	95.75 ± 2.6
	25	87.00 ± 3.6
	50	80.50 ± 3.4
	100	75.25 ± 3.1
	200	58.00 ± 3.6
	300	39.00 ± 3.6

	400	11.75 ± 8.3
	800	0
	1600	0
RI	0	97.00 ± 2.9
	25	94.25 ± 4.3
	50	83.50 ± 4.4
	100	71.75 ± 2.2
	200	67.50 ± 3.5
	300	61.75 ± 3.5
	400	23.00 ± 3.9
	800	0.50 ± 1.0
	1600	0
ML	0	95.25 ± 2.4
	25	92.50 ± 5.1
	50	88.75 ± 4.3
	100	81.25 ± 3.0
	200	67.50 ± 2.4
	300	60.25 ± 1.0
	400	48.50 ± 7.0
	800	14.25 ± 4.0
	1600	0
GP	0	97.00 ± 2.9
	25	88.00 ± 3.6
	50	82.00 ± 2.9
	100	76.50 ± 3.9
	200	58.00 ± 2.2
	300	36.00 ± 2.6
	400	18.00 ± 1.6
	800	0
	1600	0
SL	0	96.50 ± 2.1
	25	89.75 ± 1.0
	50	83.50 ± 3.4
	100	77.75 ± 2.6
	200	66.50 ± 2.6
	300	53.25 ± 7.9
	400	39.75 ± 4.9
	800	0
	1600	0
SM	0	95.25 ± 2.5
	25	89.75 ± 1.9
	50	88.25 ± 1.7
	100	66.50 ± 2.6

	200	65.50 ± 4.8
	300	58.00 ± 2.2
	400	39.75 ± 2.6
	800	0.25 ± 0.5
	1600	0
SH	0	94.50 ± 2.1
	25	94.00 ± 4.5
	50	83.50 ± 2.1
	100	73.25 ± 4.0
	200	72.75 ± 2.2
	300	63.25 ± 7.3
	400	54.75 ± 3.5
	800	10.00 ± 5.6
	1600	0

Table 3.4. Measured DOC concentrations and optical characteristics for natural and artificial waters.

Site	DOC (mg C/L)	SAC_{340} (nm)	SUV_{254} (nm)	FI	$[Ca^{2+}]$ (mol/L)	$[Mg^{2+}]$ (mol/L)	$[K^{+}]$ (mol/L)	$[Na^{+}]$ (mol/L)
SVP	5.02	7.89	12.2	1.03	0.004	0.012	0.002	0.33
BTP	4.24	7.99	10.46	1.38	0.006	0.029	0.004	0.51
PCA	4.18	8.28	11.74	1.34	0.008	0.043	0.007	0.78
WB	3.34	9.53	11.7	1.2	0.008	0.043	0.007	0.80
CCC	4.13	8.62	11.93	1.32	0.006	0.028	0.004	0.50
GM	2.41	13.64	16.46	1	0.011	0.048	0.009	0.84
FP	2.25	14.11	17.45	1.17	0.010	0.046	0.007	0.86
PC	2.12	0.82	3.57	1.11	0.010	0.045	0.007	0.83
CC	3.06	11.2	14.48	1.63	0.012	0.048	0.008	0.85
RI	3.19	10.53	14.56	1.25	0.009	0.045	0.007	0.93
ML	3.77	8.78	11.53	1.1	0.011	0.046	0.008	0.86
GP	2.04	15.32	17.76	1.01	0.012	0.046	0.008	0.80
AR	1.31	0.13	0.14	-	-	-	-	-
JB	2.72	11.9	15.51	1.45	0.010	0.097	0.008	0.97
SL	3.68	0.09	0.10	-	-	-	-	-
SM	6.18	0.14	0.17	-	-	-	-	-
SH	9.04	0.15	0.20	-	-	-	-	-

Table 3.5. Calculated logK'$_{NiBL}$ values for all exposures.

Site	logK'$_{NiBL}$
SVP	6.4
BTP	6.3
PCA	6.3
WB	6.4
CCC	6.5
GM	6.2
FP	6.4
PC	6.4
CC	6.4
RI	6.4
ML	6.6
GP	6.3
AR	6.3
JB	6.2
SL	6.3
SM	6.4
SH	6.4

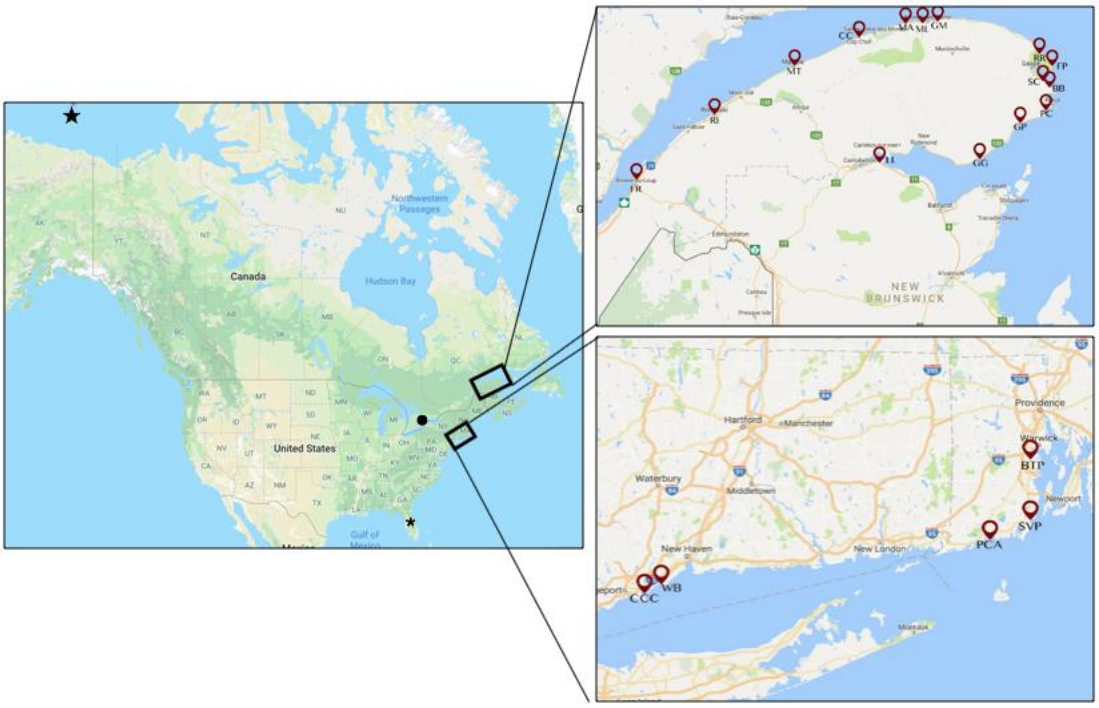

FIG. 3.1. Samples of marine water were acquired from Rhode Island, Connecticut and Florida (USA; asterisk), Gaspé peninsula (Quebec, Canada) and from the Arctic (star) as well as skimmer waste from a local aquarium store (circle).

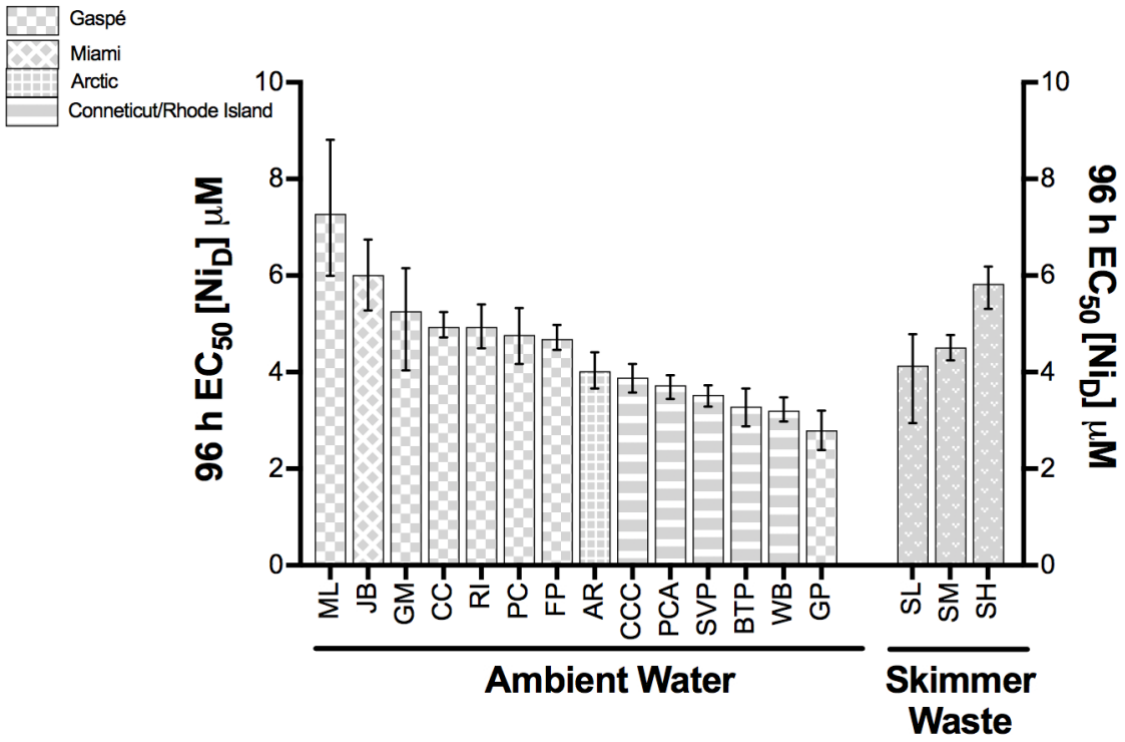

FIG. 3.2. The 96 h EC_{50} values for [Ni_D] for abnormal embryo development in purple sea urchin in ASW (with and without added synthetic ligand) and natural waters. Error bars show 95% confidence interval. Samples were collected from Gaspé, Quebec; Miami, Florida; Connecticut and Rhode Island, USA; Northern Canadian Arctic; and an aquarium store, Ontario (see table 3.1).

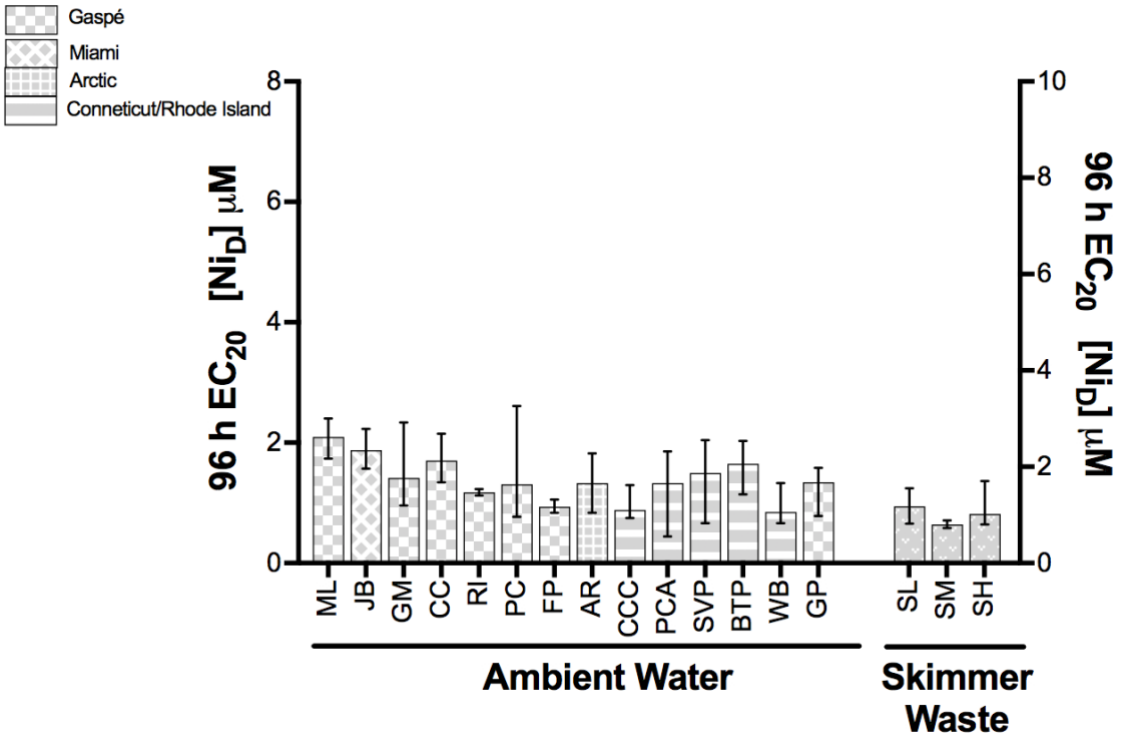

FIG. 3.3. The 96 h EC_{20} values for $[Ni_D]$ for abnormal embryo development in purple sea urchin in ASW (with and without added synthetic ligand) and natural waters. Error bars show 95% confidence interval. Samples were collected from Gaspé, Quebec; Miami, Florida; Connecticut and Rhode Island, USA; Northern Canadian Arctic; and an aquarium store, Ontario (see table 3.1).

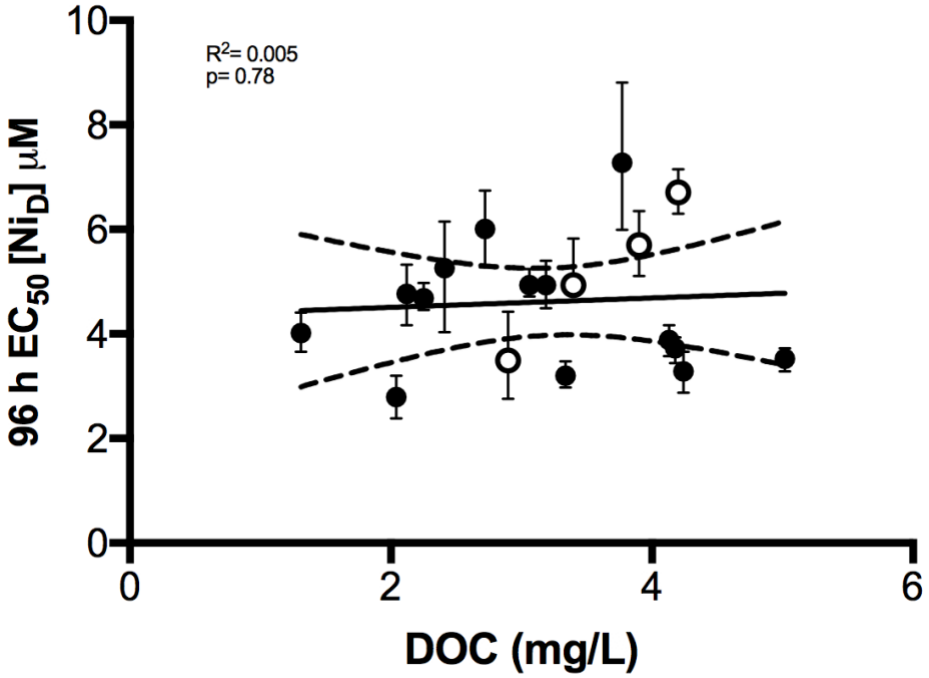

FIG. 3.4. The 96 h EC_{50} values for [Ni_D] for abnormal embryo development in purple sea urchin within natural waters shown as a function of measured DOC (mg C/L). Data from this study (filled circles) was pooled with data from Blewett et al. 2018 (open circles). Error bars show 95% confidence interval. The black line represents the linear regression and the dotted lines show the 95% confidence bands around the line.

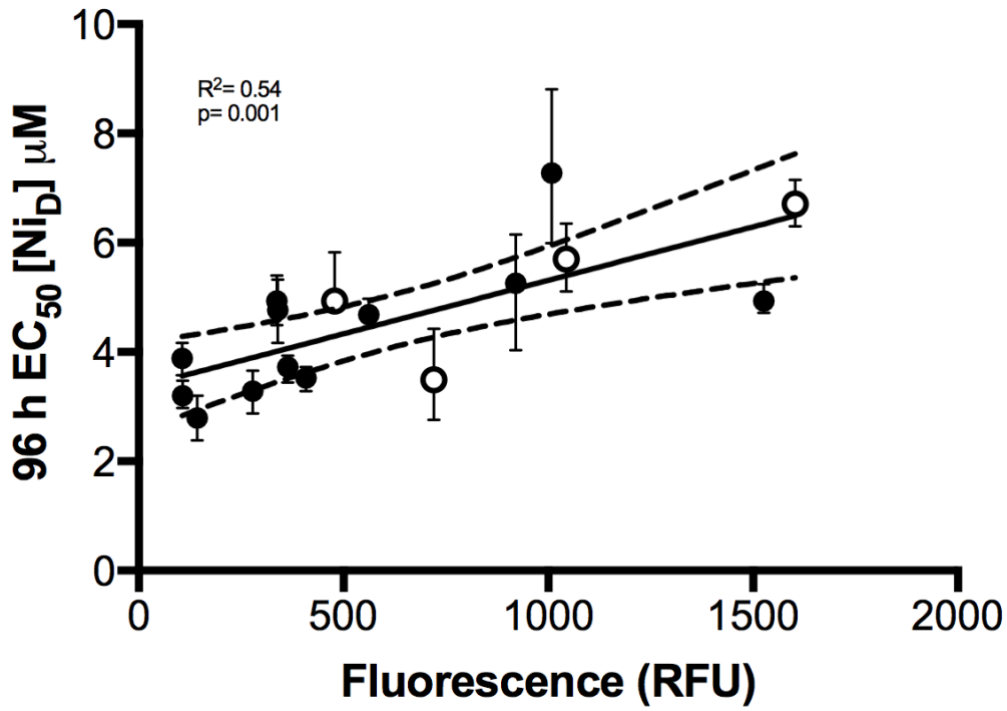

FIG. 3.5. The 96 h EC_{50} values for [Ni_D] for abnormal embryo development in purple sea urchin within natural waters shown as a function of initial fluorescence (RFU). Data from this study (filled circles) was pooled with data from Blewett et al. 2018 (open circles). Error bars show 95% confidence interval. Note: initial fluorescence for sites AR and JB were not measured. The black line represents the linear regression and the dotted lines show the 95% confidence bands around the line.

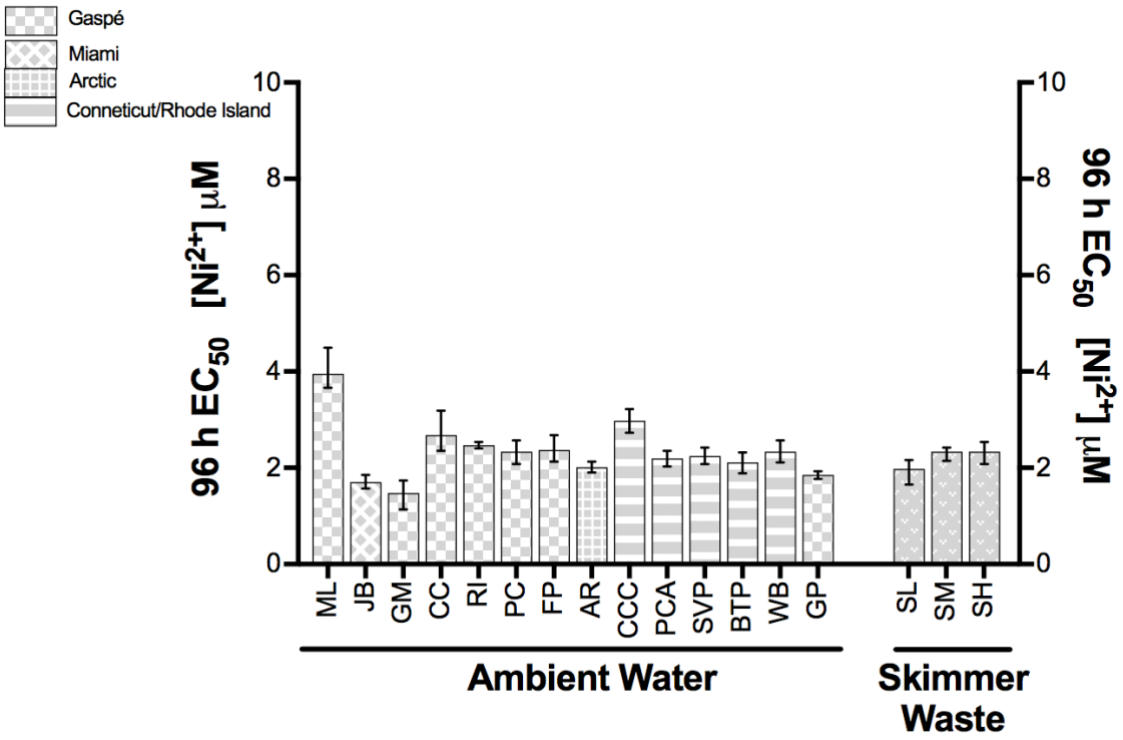

FIG. 3.6. The 96 h EC_{50} values for $[Ni^{2+}]$ for abnormal embryo development in purple sea urchin in ASW (with and without added synthetic ligand) and natural waters. The $[Ni^{2+}]$ endpoint determinations were measured by IET. Error bars show 95% confidence interval. Samples were collected from Gaspé, Quebec; Miami, Florida; Connecticut and Rhode Island, USA; Northern Canadian Arctic; and an aquarium store, Ontario (see table 3.1).

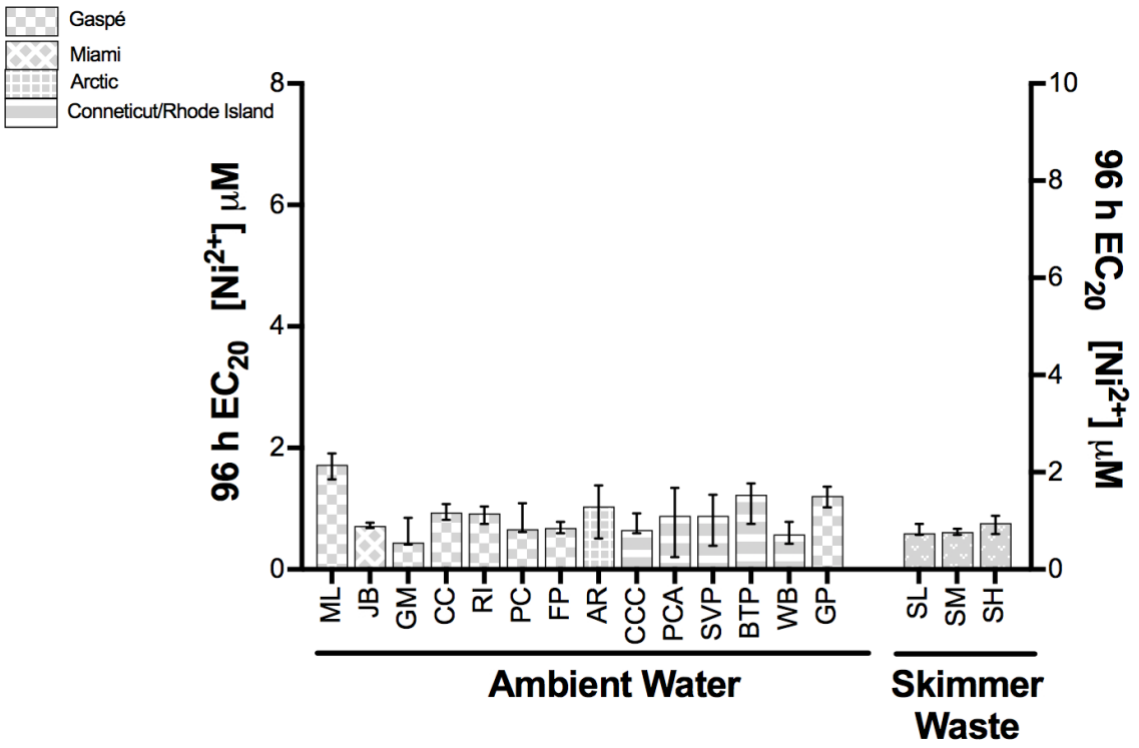

FIG. 3.7. The 96 h EC_{20} values for $[Ni^{2+}]$ for abnormal embryo development in purple sea urchin in ASW (with and without added synthetic ligand) and natural waters. $[Ni^{2+}]$ endpoint determinations were measured by IET. Error bars show 95% confidence interval. Samples were collected from Gaspé, Quebec; Miami, Florida; Connecticut and Rhode Island, USA; Northern Canadian Arctic; and an aquarium store, Ontario (see table 3.1).

3.8 References

Al-Reasi HA, Wood CM, Smith DS. 2011. Physicochemical and spectroscopic properties of natural organic matter (NOM) from various sources and implications for ameliorative effects on metal toxicity to aquatic biota. *Aquat Toxicol*. 103:179-190.

Arnold WR. 2005. Effects of dissolved organic carbon on copper toxicity: Implications for saltwater copper criteria. *Integr Environ Assess Manag*. 1:34-39.

Baker A. 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers.*Environ Sci Technol.* 35:948-953.

Baker A, Cumberland SA, Bradley C, Buckley C, Bridgeman J. 2015. To what extent can portable fluorescence spectroscopy be used in the real-time assessment of microbial water quality? *Sci Total Environ*. 532:14-19.

Benner R. 2002. Chemical composition and reactivity. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Elsevier, USA. 59–85.

Blewett TA, Dow EM, Wood CM, McGeer JC, Smith DS. 2018. The role of dissolved organic carbon concentration and composition on nickel toxicity to early life-stages of the blue mussel *Mytilus edulis* and purple sea urchin *Strongylocentrotus purpuratus*. *Ecotoxicol Environ Saf.* 160:162-170.

Chen W, Sherman SR, McGeer JC, Smith DS. Determining the concentration of free nickel ions in seawater by ion exchange technique: validation and application. *Manuscript in progress.*

Cooper CA, Nasir R, Mori J, McGeer JM, Smith DS. Influence of dissolved organic

carbon concentration and source on chronic 7-day Ni toxicity to the mysid, *Americamysis bahia*. *Manuscript in progress.*

Crémazy A, Brix KV, Wood CM. 2018. Using the biotic ligand model framework to investigate binary metal interactions on the uptake of Ag, Cd, Cu, Ni, Pb and Zn in the freshwater snail *Lymnaea stagnalis*. *Sci Total Environ.* 647:1611-1625.

Cumberland S, Bridgeman J, Baker A, Sterling M, Ward D. 2012. Fluorescence spectroscopy as a tool for determining microbial quality in potable water applications. *Environ Technol.* 33:687-693.

DePalma SGS. 2009. Characterization of dissolved organic matter and reduced sulfur in coastal marine and estuarine environments: Implications for protective effects on acute copper toxicity. Unpublished masters dissertation. University of Waterloo, Waterloo, Ontario. pp. 175.

De Schamphelaere KAC, Janssen CR. 2004. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environ Toxicol Chem.* 23:1365-1375.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem*. 20:2383-2396.

Dow E. 2017. Quantifying the interactions of nickel with marine dissolved organic matter using fluorescence. Unpublished masters dissertation. Wilfrid Laurier University, Waterloo, Ontario. pp. 91.

Environment Canada (EC). 2005. Guidance document on statistical methods for environmental toxicity tests. Environment Canada, method development and

application section. Ottawa, ON. Report EPS 1/RM/46. June 2007 amendments. pp 241.

Environment Canada (EC). 2014. Reference method for measuring the toxicity of contaminated sediment to embryos and larvae of echinoids (sea urchins or sand dollars). Report EPS 1/RM/58. Science and Technology Branch Ottawa, Ontario. (July):1-91.

Glover CN, Wood CM. 2005. Accumulation and elimination of silver in *Daphnia magna* and the effect of natural organic matter. *Aqua Toxicol.* 73:406-417.

Landner L, Reuther R. 2005. Metals in society and in the environment. Environ Pollut. 1-9.

Lorenzo JI, Nieto O, Beiras R. 2006. Anodic stripping voltammetry measures copper bioavailability for sea urchin larvae in the presence of fulvic acids. *Environ Toxicol Chem*. 25:36-44.

Luider CD, Crusius J, Playle RC, Curtis PJ. 2004. Influence of natural organic matter source on copper speciation as demonstrated by Cu binding to fish gills, by ion selective electrode, and by DGT gel sampler. *Environ Sci Technol*. 38:2865-2872.

Lussier SM, Kuhn A, Comeleo R. 1999. An evaluation of the seven-day toxicity test with *Americamysis bahia* (formerly *Mysidopsis bahia*). *Environ Toxicol Chem*. 18:2888-2893.

McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Anderson DT. 2001. Spectroflourometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol Oceanogr*. 46:38-48.

Meyer JS, Santore RC, Bobbitt JP, Debrey LD, Boese CJ, Paquin PR, Allen HE, Bergmann HL, Ditoro DM. 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environ Sci Technol*. 33:913-916.

Nadella SR, Fitzpatrick JL, Franklin N, Bucking C, Smith S, Wood CM. 2009. 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. *Comp Biochem Physiol - C Toxicol Pharmacol*. 149:340-348.

Nadella SR, Tellis M, Diamond R, Smith S, Bianchini A, Wood CM. 2013. Toxicity of lead and zinc to developing mussel and sea urchin embryos: Critical tissue residues and effects of dissolved organic matter and salinity. *Comp Biochem Physiol - C Toxicol Pharmacol*. 158:72-83.

Niyogi S, Wood CM. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ Sci Technol*. 38:6177-6192.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F. 2002. The biotic ligand model: a historical overview. *Comp Biochem Physiol C Toxicol Pharmacol*. 133:3-35.

Playle RC, Dixon DG, Burnissn K. 1993. Copper and cadmium binding to fish gills: Estimates of metal-gill stability constants and modelling of metal accumulation. *Can J Fish Aquat Sci*. 50:2678-2687.

Santore RC, Di Toro DM, Paquin PR, Allen HE, Meyer JS. 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to accute copper toxicity in freshwater fish and *Daphnia*. *Environ Toxicol Chem*. 20:2397-2402.

Schwartz ML, Curtis PJ, Playle RC. 2004. Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem*. 23:2889-2899.

Stockdale A, Tipping E, Lofts S. 2015. Dissolved trace metal speciation in estuarine and coastal waters: Comparison of WHAM/Model VII predictions with analytical results. *Environ Toxicol Chem*. 34:53-63.

Tait TN. 2013. Determination of copper speciaion, bioavailability and toxicity in saltwater environments. Unpublished masters dissertation. Wilfrid Laurier University, Waterloo, Ontario. pp. 208.

Tellis MS, Lauer MM, Nadella S, Bianchini A, Wood CM. 2014. The effects of copper and nickel on the embryonic life stages of the purple sea urchin (*Strongylocentrotus purpuratus*). *Arch Environ Contam Toxicol*. 67:453-464.

Thurman EM. 1985. Organic geochemistry of natural waters. *Usgs*. 5-110.

Wood CM, Al-Reasi HA, Smith DS. 2011. The two faces of DOC. *Aquat Toxicol*. 105:3-8.

CHAPTER 4:
Towards development of a multi-species BLM: Determining toxicity and speciation of Ni and the modifying effects of dissolved organic carbon to a marine invertebrate, *Americamysis bahia*

4.1 Abstract

The biotic ligand model (BLM) has been widely adopted for predicting metal toxicity (both acute and chronic) in freshwater (FW), however, saltwater (SW) models for nickel (Ni) are still in development. Due to the absence of research supporting the underlying BLM assumptions for a more diverse range of model conditions (i.e. Ni in SW) there is a lack of a more widespread application of the BLM approach. The aim of this study was to assess the assumptions of the BLM by characterizing the complexation of Ni by complexing agents in relation to toxicity and speciation. This toxicity was evaluated in the marine mysid (*Americamysis bahia*) using artificial seawater (ASW) with synthetic ligands [EDTA and citric acid (CA)] and also within natural marine water samples. It was predicted that toxicity, measured as LC_{50}, would vary based on total dissolved Ni concentrations [Ni_D] but that on a free ion concentration [Ni^{2+}] basis, toxicity would be constant. [Ni_D] was measured by graphite furnace atomic absorption spectroscopy (GFAAS) and Ni^{2+} was first quantified using Ion-Exchange Technique (IET) and then concentrations were measured by GFAAS; Ni^{2+} was also estimated using aquatic geochemistry modelling software (Visual Minteq). The LC_{50} for [Ni_D] in unmodified artificial seawater was 2.6 (95% CI: 2.3-2.8) µM and the addition of ligands provided protection, up to 10-fold higher [Ni_D] LC_{50} for EDTA. Natural waters were protective to varying degrees with LC_{50} values ranging from 2.2 to 4.5 µM. When expressed on a [Ni^{2+}] basis values ranged from 1.2 to 2.0 µM for synthetic ligand solutions and 1.2 to 2.5 µM in natural waters. Overall, LC_{50} values based on [Ni^{2+}] were less variable (145% variability for [Ni_D] to 32% for [Ni^{2+}]) and not significantly different from the ASW exposure. The results of this research provide novel insights into the relationships

between water chemistry, Ni accumulation and Ni toxicity which will aid in the adoption of a BLM approach for marine Ni.

4.2 Introduction

The biotic ligand model (BLM) has been widely adopted for predicting toxicity in freshwater (FW) and saltwater (SW) for many metals. The Environmental Protection Agency (EPA) has applied the BLM to update the water quality criterion for selected metals including copper (Arnold 2005; Meyer et al. 1999; Paquin et al. 2002) and silver (Paquin et al. 1999) and is considering its use for cadmium and lead (Federal Register 1999). SW computational models for nickel (Ni) have yet to be implemented. The lack of a more extensive implementation of a marine BLM approach for Ni is due to the absence of research supporting the underlying BLM assumptions for a more diverse range of model conditions (Blewett et al. 2018). For Ni specifically, there is a lack of knowledge regarding the relationship between accumulation and toxicity in marine settings.

Recent studies have investigated Ni toxicity in SW and the development of a marine BLM for Ni has been suggested (Gissi et al. 2016). The toxicity of Ni and its impacts has been studied regarding the potential protective effects of water chemistry (i.e. H^+, Ca^{2+} and natural organic matter (NOM); Blewett et al. 2018; Blewett and Wood 2015; Ho et al. 1999; Lussier et al. 1999; Tellis et al. 2014). These parameters all play important roles in influencing Ni toxicity and incorporating their influence is an essential feature of all BLMs (Di Toro et al. 2001; Santore et al. 2001; Paquin et al. 2002; Playle et al. 1993; Niyogi and Wood 2004). NOM input into the BLM is done as the measured dissolved organic carbon (DOC: any organic carbon that passes through a 0.45μm filter) concentrations as well as the % humic acid. DOC in particular has strong binding to most metals and can alter toxicity through complexation and decreased metal bioavailability. It has more recently been suggested that the composition of the DOC as well as the concentration may play a key role in altering Ni toxicity to marine organisms (Blewett et

al. 2018). Hence evaluating Ni toxicity in SW as a factor of both DOC concentration and composition is essential to further developing a computational marine BLM for Ni.

In two recent studies using the sea urchin (*Strongylocentrotus purpuratus*) the assumptions of the BLM were first tested by using synthetic ligands with known complexation characteristics for Ni (refer to chapter 2) and then toxicity was examined in natural waters (refer to chapter 3). We found that the addition of ligands provided protection based on [Ni_D] and that based on [Ni^{2+}] all endpoint values were either similar [EDTA and citric acid (CA)] or less [NTA, glutamic acid (GA) and histidine (HD)] than the endpoint values from tests in artificial seawater (ASW). Natural waters were not significantly different from ASW based on [Ni_D], and free Ni at the EC_{50} was also not significantly different from ASW for any site. Overall, there were no conclusive trends showing site-dependent protection, indicating that any variability of the EC_{50} values were independent of DOC concentration and composition. With these studies we have better outlined Ni-biotic ligand interactions in marine waters, as influenced by complexation and speciation.

The current study continues the research efforts by examining the effects of complexing agents and DOC on Ni toxicity and speciation for the marine mysid *Americamysis bahia*. Using a species known to be sensitive to Ni is the most relevant in trying to parameterize a marine BLM. DeForest and Schlekat (2013) compiled chronic Ni toxicity data for a total of 17 marine species to create a species sensitivity distribution (SSD). This study showed that *A. bahia* was the second most sensitive species with an EC_{10} of 17 mg Ni/L (DeForest and Schlekat 2013). This makes *A. bahia* an excellent

model species for marine Ni toxicity studies and can add data to current SSDs and ongoing environmental regulatory tools such as the BLM.

This study had five goals. The first was to confirm the sensitivity of *Americamysis bahia* to Ni. The second and third were to investigate the protective effects of synthetic ligands and then natural waters on toxicity. The fourth was to determine the dependence of toxicity on the speciation of Ni. Lastly, to be able to use the defined model to estimate the $\log K_f$ (binding affinity) of the biotic ligand. The results of this study will provide insight to understand Ni toxicity and help to establish development of a site-specific Ni BLM for marine waters.

4.3 Methodology

4.3.1 Animal care

Six-day old mysids (*Americamysis bahia*) were obtained from Aquatic Research Organisms (ARO, Hampton, New Hampshire, USA) and held in ASW at a salinity of 30 ppt, pH of 8.1 and temperature of 26°C following standard methods (EPA-821-R-02-014 3rd edition; US EPA 2002). The ASW was created by reconstituting sea salt (Kent Marine Reef Salt Mix, Big Als Canada Inc, Kitchener ON) with reverse osmosis (RO) water and this was held in a reservoir with continuous aeration. The mysids were fed brine shrimp (*Artemia nauplii*, Brine Shrimp Direct, Ogden, UT, USA) twice daily at a rate of 75 *Artemia*/neonate and a 90% water change was done daily to remove debris and dead mysids. The mysids were held in lab until they reached 7 days old.

4.3.2 Water collection, storage and DOC analysis

Samples of marine water were collected at 2 sites along the coast of Connecticut, USA (Table 4.1; Fig. 4.1) following the methods described in chapter 3. Samples were shipped in coolers to Wilfrid Laurier University where they were refrigerated until further

analysis and use in the toxicity assay. Samples for DOC analysis (50 mL, also filtered) were acidified with 50 µL concentrated HCL and measured using a Shimadzu TOC-Vcph/CPN total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan).

4.3.3 Preparation of test solutions and toxicity tests

Toxicity tests were static renewal 7-day chronic tests. Twenty-four hours before the toxicity test started, the salinity of the collected sample was increased to 30 ± 0.5 ppt using Kent Marine salt and held in a separate water bath at 26°C. The pH was adjusted to 8.1 ± 0.1 using 0.1 M NaOH. Toxicity tests using 30 µM EDTA and 500 µM CA (Sigma Aldrich, Oakville, ON) and two using natural natural waters were conducted. All methods were repeated for the ASW treatment (Table 4.2). For each exposure 120 L of sample (either ASW or natural water) was equally split into six 25 L carboys which were then spiked with appropriate amounts of Ni (Table 4.2). Ni solutions were prepared using $NiCl_2$ soluble salt (1000 ppm; Sigma Aldrich). Each exposure concentration was aliquoted into one of six 1 L glass beakers (500 mL for each). These included four biological replicates and the other two for measuring total and dissolved Ni concentrations. The biological replicates had 10 7-day old neonates per beaker. The remaining solution was used for $[Ni^{2+}]$ determination by IET. Salinity, pH and temperature were monitored daily for one replicate per concentration using a hand-held conductivity meter (YSI 30, YSI Inc., Yellow Springs).

4.3.4 Toxicity endpoint determination

After 7 days of exposure mortalities were counted (Table 4.3) and alive mysids were saved for subsequent assessment of length and maturation.

4.3.5 Ni quantification by GFAAS

Refer to chapter 2 section 2.3.5

4.3.6 Determination of Ni speciation

Refer to chapter 2 section 2.3.6

4.3.7 Calculation of Ni-Biotic ligand binding affinity

Refer to chapter 2 section 2.3.7

4.3.8 Statistical analysis

The 7-day median lethal concentration (LC_{50}) and the 20% lethal concentration (LC_{20}) values with 95% confidence intervals (95% CI) were determined for both [Ni_D] and [Ni^{2+}] using Comprehensive Environmental Toxicity Information System (CETIS) software following the EPA ICPIN method based on linear interpolation with bootstrapping. Significant differences between exposures and ASW were assessed using the overlap of 95% CI; if they did not overlap, then the LC_{50} (or LC_{20}) values were considered significantly different (EC 2005). In order to determine the extent to which toxicity was related to [Ni^{2+}] the relative variability of LC_{50} values across sites for [Ni_D] and for [Ni^{2+}] was assessed by comparing coefficients of variation (CVs). The CVs were calculated by dividing the standard deviation of the LC_{50}s by the average LC_{50}s across sample sites.

4.4 Results and Discussion

4.4.1 Water Chemistry

The measured [Ni_D] values were 99 ± 12% of [Ni_T] values (shown as mean ± SD; range of 64 to 118%), indicating negligible Ni precipitation over the 96-h toxicity test (Table 4.2). There was no significant difference between the certified reference material (CRM) value and the average value measured on the graphite furnace atomic absorption spectroscopy (GFAAS; 19% difference; $p>0.05$). Throughout the 7 days for all

exposures, temperature in the water bath ranged from 25.8 to 27.1°C (n=7), salinity ranged from 29.1 to 30.9 ppt (n=7), and pH ranged from 7.8 to 8.1 (n=7; Table 4.2).

4.4.2 Ni toxicity to A. bahia

All toxicity tests met the acceptable criteria where survival of >80% of the mysids was reached within 7 days in unexposed controls (Table 4.3; US EPA, 2002). The mean survival of mysids in the unexposed (control) solution of ASW was 82.5 ± 5% (n=4; Table 4.3). From the ASW exposure the mean 7 day LC_{50} and LC_{20} for [Ni_D] was 2.6 (95% CI: 2.3-2.8 µM; Fig. 4.2) and 1.8 (1.2-1.9 µM; Fig. 4.3) respectively. *A. bahia* was found to be very sensitive to Ni, which is in concurrence with previous studies where LC_{50} values range from 1.5 to 3.9 µM (Cooper et al., unpublished; Lussier et al., 1985).

4.4.3 Protection against Ni toxicity caused by ligands

Chronic toxicity of Ni to *A. bahia* was assessed in the presence of two synthetic ligands, EDTA and CA. The results from Ni toxicity tests with EDTA and CA added into test solutions showed that EC_{50} values based on [Ni^{2+}] were not significantly different from the ASW exposure, using the purple sea urchin *S. purpuratus* (refer to chapter 2). There was a concentration dependent effect of Ni in all exposures, where higher Ni concentrations resulted in decreased survival. EDTA provided protection based on [Ni_D]; up to 10-fold higher [Ni_D] LC_{50} at 25.6 (24.1-26.2 µM; Fig. 4.2). The same trends were found for [Ni_D] LC_{20} values, where EDTA had up to 4.5-fold higher LC_{20} value compared to the ASW exposure (Fig. 4.3). Results agree with predictions where EDTA, which has a strong binding affinity to Ni, would have the greatest LC_{50} value offering the most protection to the mysids. However, CA which has a weak affinity for Ni showed effect concentrations lower than the ASW exposure (Fig. 4.2). The toxicity seen in the CA exposure was not expected, however CA, due to its relatively weak binding, has been

shown to not reduce accumulation or alter toxicity for other metals such as copper or cadmium on fathead minnow gills (Playle et al. 1993).

4.4.4 Protection against Ni toxicity caused by natural waters

Chronic toxicity of Ni to *A. bahia* was assessed in the presence of 2 natural waters. DOC concentration was measured within natural waters at 3.9 mg C/L for site IN and 3.2 mg C/L for site CCC. Site IN showed a higher LC_{50} value compared to the ASW exposure although it was not significant based on overlapping confidence intervals (Fig. 4.2). Compared to ASW, site CCC was not significantly different offering no protection to Ni toxicity (Fig. 4.2). Similarly, based on LC_{20} values no protection was observed (Fig 4.3).

4.4.5 Speciation

IET measurements were done in parallel to the toxicity tests during this study to quantify the $[Ni^{2+}]$ within solution (Table 4.1). Measuring $[Ni^{2+}]$ within SW provides a method to test the complexation predictions provided by Visual Minteq. Estimating free ion is important to assess the assumptions inherent to the BLM for validating its use for Ni in SW. In the aquatic environment the free ion (Ni^{2+}) is considered to be the most toxic species of a metal as it is thought to be the most bioavailable form meaning it can move into the organism causing toxicity (Pyle and Couture 2012). Speciation is affected by complexation, which can decrease Ni bioavailability and possibly reduce toxicity (Niyogi and Wood 2004). Therefore, the speciation of a metal has a significant role in predicting toxicity, which makes it valuable to measure and use alongside toxicity values. The 7-day $[Ni^{2+}]$ LC_{50} of the ASW exposure was 1.8 (1.6-2.0 µM; Fig. 4.4) and LC_{20} was 1.2 (0.9-1.3 µM; Fig. 4.5). $[Ni^{2+}]$ LC_{50} values for synthetic ligands were either similar (EDTA) or less (CA) than the values for tests in ASW (Fig. 4.4). The same trends were

found for the LC_{20} values for EDTA (Fig. 4.5). The CA exposure showed $[Ni^{2+}]$ values that were significantly lower than the ASW exposure. However, the LC_{50} $[Ni^{2+}]$ was higher than the $[Ni_D]$ LC_{50} value for that treatment. These results indicate that potentially the mysid toxicity tests did not work for the CA exposure or that IET measurements in the presence of CA dramatically overestimated the actual $[Ni^{2+}]$ in solution; although, CA has been shown to recover speciation in agreement with Visual Minteq calculations for the marine IET method with Ni (Chen et al. unpublished). The same trends were found for the LC_{20} values (Fig. 4.5). When expressed on a measured $[Ni^{2+}]$ basis, values ranged from 1.2 to 2.5 µM (Fig 4.4). The variability of the $[Ni^{2+}]$ LC_{50} values for natural waters was similar to the variability seen for the $[Ni_D]$ values. The 95% CI for the LC_{50}s of the two natural water sites overlapped with the confidence bands of the ASW exposure and were therefore not significantly different (Fig 4.4). When looking at the LC_{20}, values ranged from 0.4 to 1.3 µM; site CCC LC_{50} $[Ni^{2+}]$ was significantly lower than the ASW exposure but site IN was not significantly different (Fig. 4.5). Overall, there was a decreased variability when expressed on a $[Ni^{2+}]$ basis compared to values based on $[Ni_D]$ (145% variability for $[Ni_D]$ to 32% for $[Ni^{2+}]$). Measuring $[Ni^{2+}]$ within SW provides a method to test the complexation predictions important to assess the assumptions inherent to the BLM for validating its use for Ni in SW.

The LC_x of measured $[Ni^{2+}]$ was compared to modelled predictions using the methods found in Chen et al. (unpublished) in order to validate the performance of the IET in SW (Fig. 4.4; Fig. 4.5). There were no significant differences within the ASW exposure for the predicted and measured LC_{50} or LC_{20} values (Fig 4.4; Fig 4.5). The agreement between measured and modelled LCx values varied within the different synthetic ligand

exposures (Fig. 4.4; Fig. 4.5). This comparison has been done previously for model ligands (refer to chapter 2). For EDTA and CA, the model under-predicted [Ni^{2+}] in solution, resulting in an over-prediction of toxicity, which is what has been seen previously (refer to chapter 2). This may be due to inaccuracies within the model that are not fully understood. The results from experiments using *S. purpuratus* (refer to chapter 2 and 3) and *A. bahia*, provide general conclusions that strengthen the assumptions of the BLM across multiple species. Suggesting that [Ni^{2+}] is likely the most bioavailable form and causes toxicity, and that complexation produces some amount of protection for Ni in SW.

4.4.6 Binding affinity

IET-measured [Ni^{2+}] was used for the derivation of conditional stability constant ($K^{'}$) for each treatment and an average value was calculated (Table 4.4). The $\log K^{'}_{NiBL} = 6.2 \pm 0.18 = 10^{6.2}$ using model ligands and in natural waters $\log K^{'}_{NiBL} = 6.2 \pm 0.15 = 10^{6.2}$, indicating that 50% of the Ni binding sites would be occupied at aqueous Ni concentrations of $10^{-6.2}$ M. In a previous study using *S. purpuratus* the $\log K^{'}_{NiBL}$ was found to be $6.3 \pm 0.4 = 10^{6.3}$ in solutions with synthetic ligands (refer to chapter 2) and $6.4 \pm 0.09 = 10^{6.4}$ in natural waters (refer to chapter 3). This data shows *A. bahia* to have a similar binding affinity for Ni as *S. purpuratus*. Determining $\log K^{'}_{NiBL}$ values can be helpful in development of a computational BLM for marine Ni and also aids in determining when ligands may be protective. For example, if two ligands have equivalent K_f values at the same concentrations, then half of Ni will bind to each. However, if concentrations or binding affinities increase for one, one will out compete the other

resulting in more Ni being bound to it. These values will be used to implement a computational BLM and aid in predicting toxicity for marine Ni.

4.5 Conclusion

When chronic toxicity data of *A. bahia* is compared to those of *S. purpuratus* and other marine organisms, the mysid are found to be highly sensitive to Ni. This is in agreement with previous literature which show the mysid to be the second most sensitive organism to Ni out of 17 species studied (DeForest and Schlekat 2013). The addition of ligands provided protection for EDTA based on [Ni_D] LC_{50} but natural waters showed no protection compared to the ASW exposure. Based on natural waters tested so far, Ni complexation and decreased Ni bioavailability is not a large factor. However, a marine BLM for Ni may be useful for other waters with higher complexing ligands, such as sediment porewaters and wastewater impacted sites. The trends for [Ni^{2+}] LC_x also varied within synthetic ligands and natural waters. Overall, LC_{50} values based on [Ni^{2+}] were less variable (145% variability for [Ni_D] to 32% for [Ni^{2+}]) and all exposures (except CA) were within the confidence bands of the ASW exposure. This study provides novel insights into the relationships between water chemistry and Ni toxicity for the marine organism *A. bahia* and will aid in the potential development and adoption of a BLM approach for marine Ni. Further studies that continue the work by expanding the scope of exposures to encompass a wider range of complexation are needed to better understand the relationship between toxicity, speciation and complexation.

4.6 Acknowledgements

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4.7 Tables and Figures

Table 4.1. Location coordinates for natural waters and their measured DOC (mg C/L). Note, that there are no initial fluorescence or pH values because upon collection there was no access to the BOD meter or YSI.

Location	Site Name	Site Code	GPS Coordinates	DOC (mg C/L)
Connecticut	Gulf Pond	IN	41°13'26.6" N 73°02'06.0" W	3.9
	Audubon Coastal Center	CCC	41°10'34.5"N 73°06'06.5"W	3.2

Table 4.2. Water chemistry tests in ASW and collected natural waters. Ni exposure concentrations are given as nominal, [Ni_D] and [Ni^{2+}] with the latter both as measured by IET (except unexposed controls) and also predicted using Visual Minteq. Means are given ± standard deviation (SD) for [Ni_D] (µg/L; n=2), [Ni^{2+}] (µg/L; n=2), pH (n=7), salinity (ppt; n=7), and temperature (°C; n=7). Values with * were excluded from any calculations of [Ni_D] as a % of [NiT].

Exposure	Nominal Ni (µg/L)	Dissolved Ni (µg/L) ± SD	Free Ni (ug/L) ± SD	Predicted Free Ni (ug/L)	pH ± SD	Temperature ± SD (°C)	Salinity ± SD (ppt)
ASW	0	0 ± 4.6*		0	7.9 ± 0.06	26.4 ± 0.2	29.81 ± 0.5
	30	6 ± 4.0*	8	5	7.9 ± 0.04	26.3 ± 0.2	29.91 ± 0.5
	60	22 ± 3.0*	21	18	7.8 ± 0.08	26.4 ± 0.2	30.06 ± 0.7
	120	78 ± 0.4	53	65	7.9 ± 0.10	26.5 ± 0.4	30.25 ± 0.4
	240	161 ± 0.3	112	136	7.9 ± 0.09	26.7 ± 0.3	29.78 ± 0.4
	480	327 ± 4.1	229	276	8.0 ± 0.07	26.7 ± 0.3	29.68 ± 0.4
CA	0	0 ± 0.02*		0	7.9 ± 0.05	26.6 ± 0.05	30.32 ± 0.2
	30	15 ± 0.04*	51 ± 18.0	3	8.0 ± 0.05	26.7 ± 0.1	30.24 ± 0.3
	60	35 ± 0.04	70 ± 11.8	24	8.0 ± 0.05	26.6 ± 0.05	30.35 ± 0.5
	120	99 ± 0.003	69 ± 7.1	55	8.0 ± 0.05	26.6 ± 0.1	30.29 ± 0.4
	240	227 ± 0.06	131 ± 8.4	133	8.0 ± 0.04	26.7 ± 0.1	30.18 ± 0.4
	480	422 ± 0.06	168 ± 5.5	246	8.0 ± 0.04	26.7 ± 0.1	29.98 ± 0.4
EDTA	0	0 ± 0.3*		0	7.9 ± 0.05	26.4 ± 0.3	30.32 ± 0.2
	400	309 ± 2.4	24 ± 0.56	0.002	7.9 ± 0.05	26.6 ± 0.3	30.24 ± 0.2
	800	609 ± 17.0	34 ± 3.1	0.005	8.0 ± 0.05	26.6 ± 0.2	30.49 ± 0.3
	1200	876 ± 5.8	62 ± 7.5	0.01	8.0 ± 0.06	26.6 ± 0.3	30.21 ± 0.4
	1600	1643 ± 143.0	100 ± 0.3	0.03	8.0 ± 0.06	26.6 ± 0.4	30.03 ± 0.3
	2000	1426 ± 14.1	150 ± 2.6	0.05	8.0 ± 0.08	26.4 ± 0.3	30.00 ± 0.4
IN	0	0 ± 2.0*			8.0 ± 0.04	26.7 ± 0.2	30.49 ± 0.2
	30	23 ± 3.8	32 ± 0.1		8.0 ± 0.04	26.6 ± 0.2	30.44 ± 0.1
	60	50 ± 1.5	56 ± 1.9		8.0 ± 0.05	26.7 ± 0.2	30.52 ± 0.2
	120	74 ± 4.3	118 ± 5.5		8.0 ± 0.05	26.7 ± 0.2	30.48 ± 0.3
	240	207 ± 2.9	90 ± 0.7		8.0 ± 0.07	26.7 ± 0.2	30.16 ± 0.4
	480	442 ± 0.08	227 ± 4.5		8.1 ± 0.05	26.7 ± 0.1	30.10 ± 0.2
CCC	0	0 ± 1.3*			8.0 ± 0.04	26.3 ± 0.4	30.36 ± 0.2
	30	34 ± 6.3	29 ± 3.5		8.0 ± 0.00	26.3 ± 0.4	30.51 ± 0.1
	60	75 ± 2.5	36 ± 21.9		8.0 ± 0.05	26.3 ± 0.4	30.47 ± 0.2
	120	141 ± 4.5	78 ± 2.1		8.1 ± 0.05	26.3 ± 0.4	30.40 ± 0.3
	240	271 ± 32.2	100 ± 5.1		8.1 ± 0.05	26.3 ± 0.3	30.38 ± 0.4

	480	578 ± 0.7	189 ± 16.1		8.1 ± 0.05	26.3 ± 0.3	30.40 ± 0.3

Table 4.3. The 7-day chronic toxicity end-point of percent survival shown as mean ± SD for all exposures (n=4).

Exposure	Nominal Ni (µg/L)	% Survival ± SD
ASW	0	82.5 ± 5.0
	30	67.5 ± 38.6
	60	90.0 ± 8.2
	120	85.0 ± 5.8
	240	37.5 ± 5.0
	480	7.5 ± 9.6
CA	0	80.0 ±
	30	60.0 ±
	60	42.5 ± 9.6
	120	27.5 ± 9.6
	240	35.0 ± 5.8
	480	10.0 ±
EDTA	0	90.0 ±
	400	77.5 ± 9.6
	800	57.5 ± 39.5
	1200	77.5 ± 15.0
	1600	67.5 ± 20.6
	2000	5.0 ± 5.8
IN	0	80.0 ±
	30	75.0 ± 5.8
	60	77.5 ± 12.6
	120	57.5 ± 5.0
	240	50.0 ± 27.1
	480	7.5 ± 5.0
CCC	0	85.0 ± 5.8
	30	55.0 ± 12.9
	60	62.5 ± 9.6
	120	40.0 ± 21.6
	240	15.0 ± 12.9
	480	10.0 ± 8.2

Table 4.4. Calculated $logK'_{NiBL}$ values for all exposures.

Site	$logK'_{NiBL}$
ASW	6.3
EDTA	6.1
CA	6.3
IN	6.4
CCC	6.1

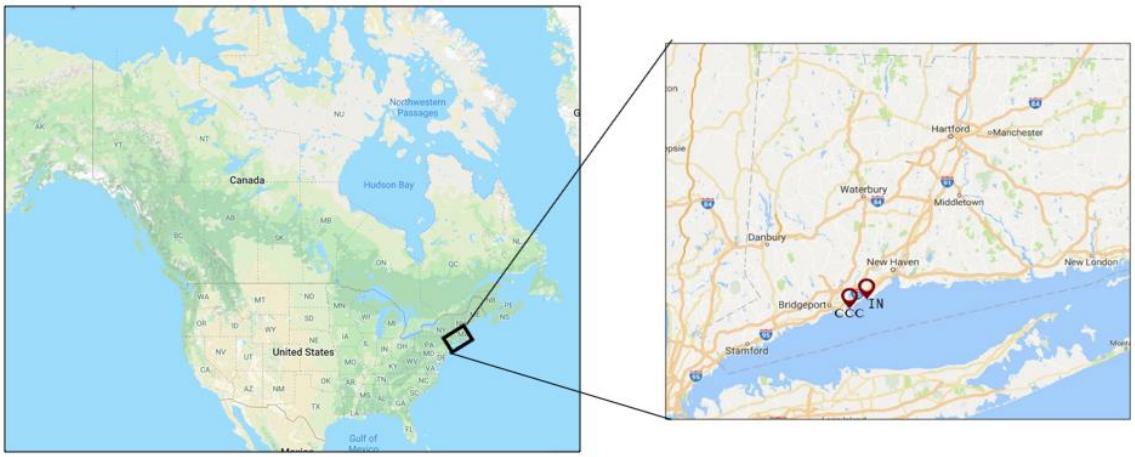

FIG. 4.1. Samples of marine water were acquired from Connecticut (USA).

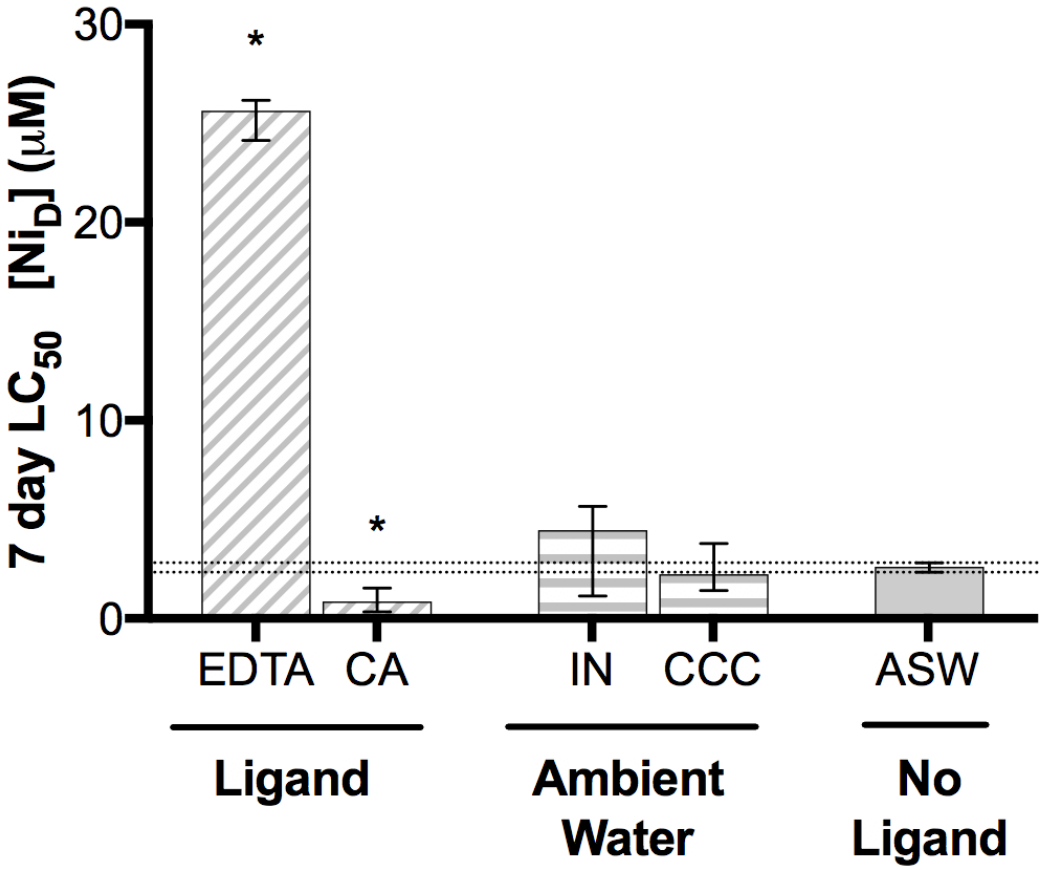

FIG. 4.2. The 7 day LC_{50} values for [Ni_D] for *A. bahia* survival in ASW (with and without added synthetic ligand) and natural waters. Error bars show 95% confidence interval and * indicates a significant difference in LC_{50} value compared to the ASW exposure. Dotted horizontal lines represent the confidence intervals of the ASW exposure. Samples were collected from Connecticut, USA (see table 4.1).

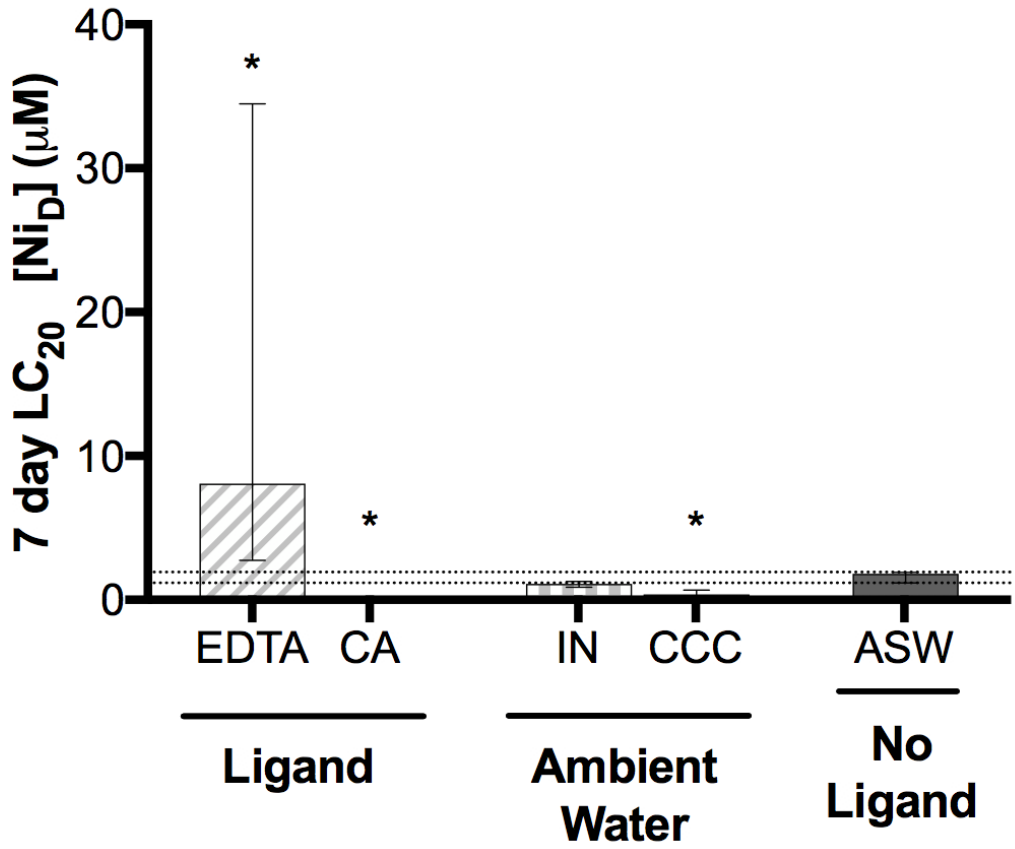

FIG. 4.3. The 7 day LC_{20} values for [Ni_D] for *A. bahia* survival in ASW (with and without added synthetic ligand) and natural waters. Error bars show 95% confidence interval and * indicates a significant difference in LC_{20} value compared to the ASW exposure. Dotted horizontal lines represent the confidence intervals of the ASW exposure. Samples were collected from Connecticut, USA (see table 4.1).

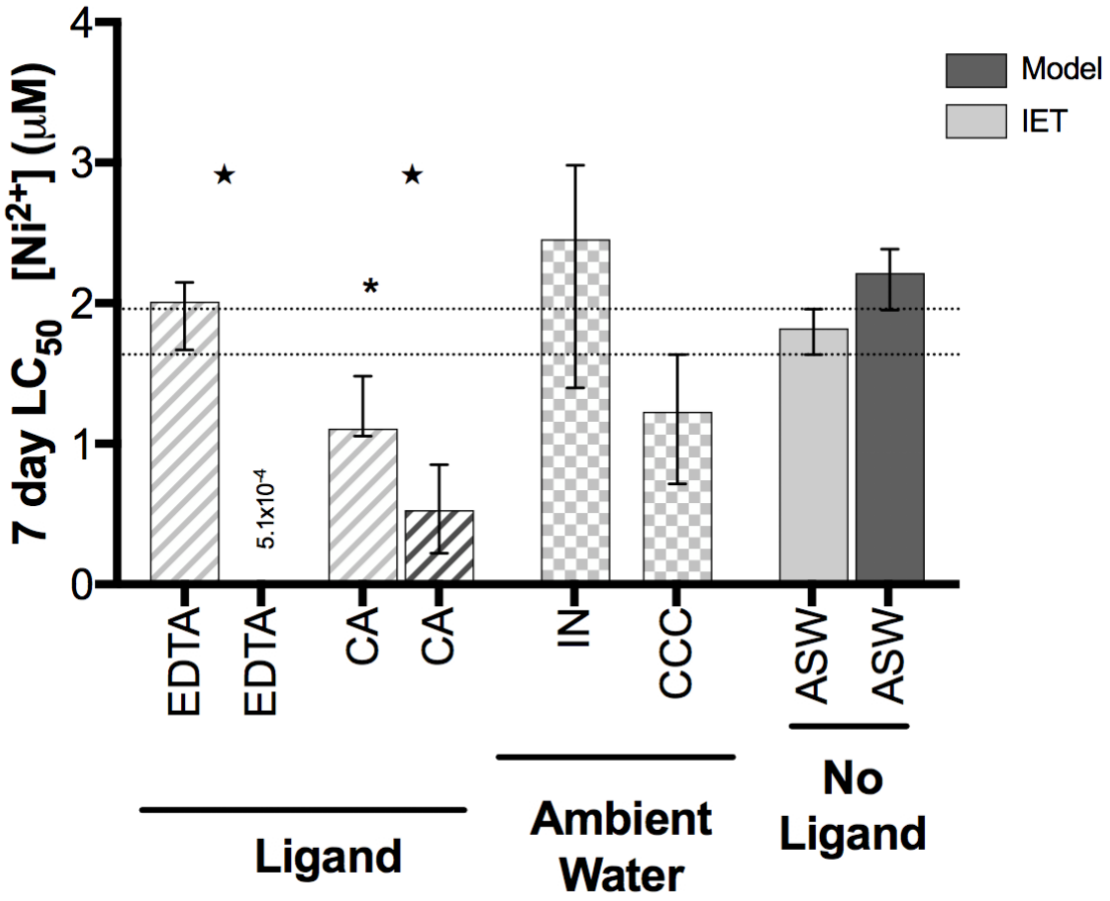

FIG. 4.4. The 7 day LC_{50} values for $[Ni^{2+}]$ for *A. bahia* survival in ASW (with and without added synthetic ligand) and natural waters. $[Ni^{2+}]$ endpoint determinations were calculated using either measured $[Ni^{2+}]$ by IET (light gray stripes) or modelled $[Ni^{2+}]$ predicted by Visual Minteq (dark gray stripes). Note: the predicted value for EDTA was very low, so the value is written in place of the bar. Error bars show 95% confidence interval and * indicates a significant difference in LC_{50} value compared to the no ligand (ASW) exposure and ★ indicates a significant difference in LC_{50} value between measured and predicted. Dotted horizontal lines represent the confidence limits of the ASW exposure. Samples were collected from Connecticut, USA (see table 4.1).

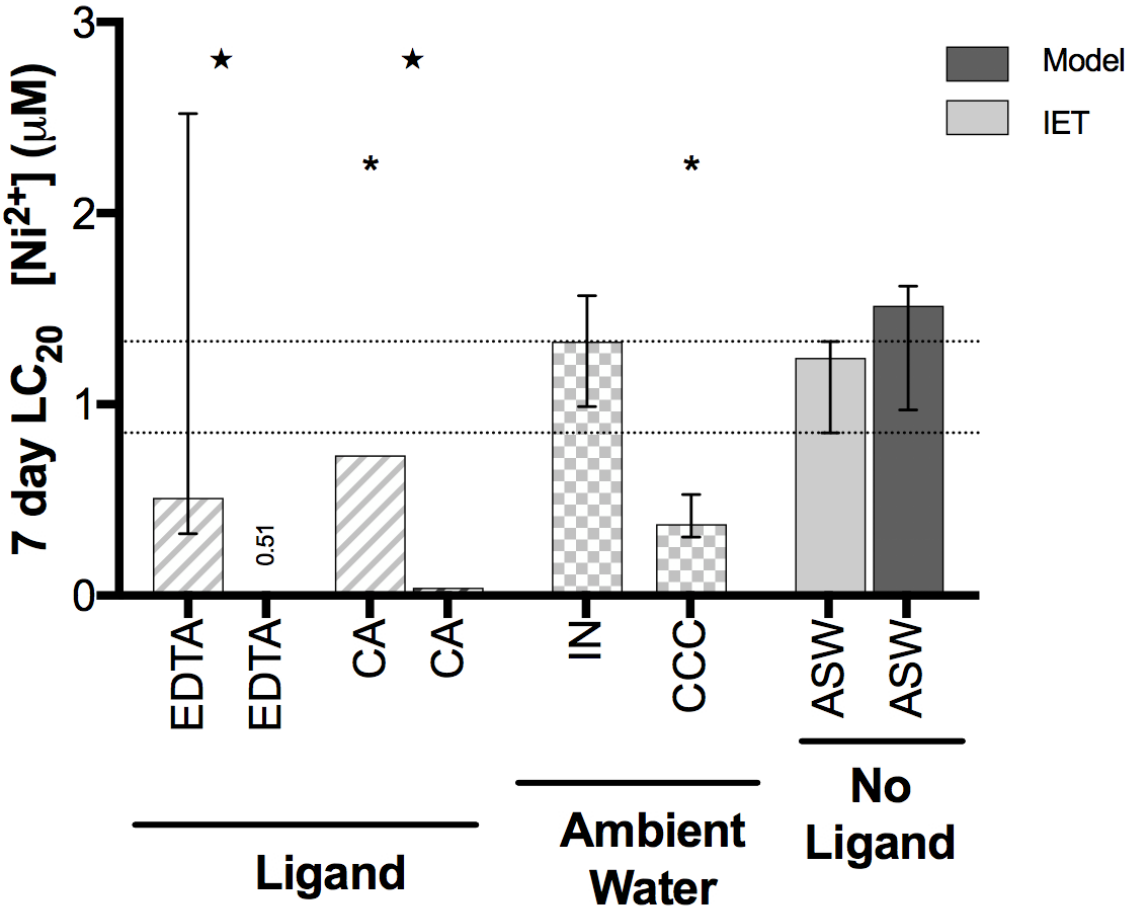

FIG. 4.5. The 7 day LC_{20} values for $[Ni^{2+}]$ for *A. bahia* survival in ASW (with and without added synthetic ligand) and natural waters. $[Ni^{2+}]$ endpoint determinations were calculated using either measured $[Ni^{2+}]$ by IET (light gray stripes) or modelled $[Ni^{2+}]$ predicted by Visual Minteq (dark gray stripes). Note: the predicted value for EDTA was very low, so the value is written in place of the bar. Error bars show 95% confidence interval and * indicates a significant difference in LC_{20} value compared to the no ligand (ASW) exposure and ★ indicates a significant difference in LC_{20} value between measured and predicted. Dotted horizontal lines represent the confidence limits of the ASW exposure. Samples were collected from Connecticut, USA (see table 4.1).

4.8 References

Arnold WR. 2005. Effects of dissolved organic carbon on copper toxicity: Implications for saltwater copper criteria. *Integr Environ Assess Manag*. 1:34-39.

Blewett TA, Dow EM, Wood CM, McGeer JC, Smith DS. 2018. The role of dissolved organic carbon concentration and composition on nickel toxicity to early life-stages of the blue mussel *Mytilus edulis* and purple sea urchin *Strongylocentrotus purpuratus*. *Ecotoxicol Environ Saf.* 160:162-170.

Blewett TA, Wood CM. 2015. Salinity-Dependent Nickel Accumulation and Oxidative Stress Responses in the Euryhaline Killifish (*Fundulus heteroclitus*). *Arch Environ Contam Toxicol*. 68:382-394.

Chen W, Sherman SR, McGeer JC, Smith DS. Determining the concentration of free nickel ions in seawater by ion exchange technique: validation and application. *Manuscript in progress.*

Cooper CA, Nasir R, Mori J, McGeer JM, Smith DS. Influence of dissolved organic carbon concentration and source on chronic 7-day Ni toxicity to the mysid, *Americamysis bahia*. *Manuscript in progress.*

DeForest DK, Schlekat CE. 2013. Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms. *Integr Environ Assess Manag*. 9:580-589.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem*. 20:2383-2396.

Environment Canada (EC). 2005. Guidance document on statistical methods for environmental toxicity tests. Environment Canada, method development and application section. Ottawa, ON. Report EPS 1/RM/46. June 2007 amendments.

pp 241.

Federal Register. 1999. Notice of Intent to Revise Aquatic Life Criteria for Copper, Silver, Lead, Cadmium, Iron and Selenium. USEPA 64 (October): 58409-58410.

Gissi F, Stauber JL, Binet MT, Golding LA, Adams MS, Schlekat CE, Garman ER, Jolley DF. 2016. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. *Environ Pollut*. 218:1308-1323.

Ho KT, Kuhn A, Pelletier MC, Hendricks TL, Helmstetter A. 1999. pH dependent toxicity of five metals to three marine organisms. *Environ Toxicol*. 14:235-240.

Lussier SM, Gentile JH, Walker J. 1985. Acute and chronic effects of heavy metals and cyanide on *Mysidopsis bahia* (Crustacea:Mysidacea). *Aquat Toxicol*. 7:25-35.

Lussier SM, Kuhn A, Comeleo R. 1999. An evaluation of the seven-day toxicity test with *Americamysis bahia* (formerly *Mysidopsis bahia*). *Environ Toxicol Chem*. 18:2888-2893.

Meyer JS, Santore RC, Bobbitt JP, Debrey LD, Boese CJ, Paquin PR, Allen HE, Bergmann HL, Ditoro DM. 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environ Sci Technol*. 33:913-916.

Niyogi S, Wood CM. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ Sci Technol*. 38:6177-6192.

Paquin PR, Di Toro DM, Santore RC, Trivedi D, Wu KB. 1999. A Biotic ligand model of the acute toxicity of metals. 3. Application to fish and daphnia exposure to silver. In: Integrated approach to assessing the bioavailability and toxicity of

metals in surface waters and sediments. EPA-822-E-99-001. US EPA, Washington, DC. pp. 3-59-3-102.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F. 2002. The biotic ligand model: a historical overview. *Comp Biochem Physiol C Toxicol Pharmacol*. 133:3-35.

Playle RC, Gensemer RW, Dixon DG. 1992. Copper accumulation on gills of fathead minnows: Influence of water hardness, complexation and pH of the gill micro-environment. *Environ Toxicol Chem*. 11:381–391.

Playle RC, Dixon DG, Burnissn K. 1993. Copper and cadmium binding to fish gills: Estimates of metal-gill stability constants and modelling of metal accumulation. *Can J Fish Aquat Sci*. 50:2678-2687.

Pyle G, Couture P. 2012. Nickel. In: Wood CM, Farrell AP, Brauner CJ (eds) Homeostasis and toxicology of essential metals. *Fish Physiol.* 31A: 254-281.

Tellis MS, Lauer MM, Nadella S, Bianchini A, Wood CM. 2014. The effects of copper and nickel on the embryonic life stages of the purple sea urchin (*Strongylocentrotus purpuratus*). *Arch Environ Contam Toxicol*. 67:453-464.

United States Environmental Protection Agency. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms fourth edition october 2002. EPA-821-R-02-014 Office of Water, Washington, DC. (October):1-350.

CHAPTER 5:
General Conclusions

5.1 Main findings and conclusions

The overall objective of this research was to understand toxicity and speciation of nickel (Ni) in marine environments and estimate metal-biotic ligand stability constants for Ni in order to determine potential impact. This is in coordination with the goal to generate new data in order to strengthen species sensitivity distributions (SSD) for Ni impacts to marine species, and to understand the factors that influence the bioavailability of Ni, particularly the role of organic matter complexation. To do this, chronic Ni toxicity tests were done on samples of artificial and natural origin encompassing a wide range of complexation characteristics with Ni using two marine organisms (*Strongylocentrotus purpuratus* and *Americamysis bahia*). The central findings of this research were as follows:

5.1.1 The sensitivity of marine organisms to Ni toxicity

Using a species that has been shown to be sensitive to Ni in literature is the most effective for use in toxicity tests in order to parameterize a marine BLM. Purple sea urchin embryos (*S. purpuratus*) are sensitive to Ni, they have important ecological roles and they are commonly used in toxicity bioassays as a method to determine marine water quality criteria (Uthicke et al. 2009). The mysid (e.g. *A. bahia*) has a high sensitivity to Ni as well and toxicity tests measure effects on survival, growth, and maturation of juvenile mysids that are exposed during a critical period of growth and sexual maturation (DeForest and Schlekat 2013; Lussier et al. 1985; Lussier et al. 1999). Both *S. purpuratus* in their embryonic life stages and *A. bahia* were found to be very sensitive to Ni, which is in agreement with literature values (Blewett et al. 2018; DeForest and Schlekat 2013; Lussier et al. 1999). Previous research deemed mysids (*A. bahia*) as the second most sensitive marine species tested to date following behind a tropical sea urchin species

(DeForest and Schlekat 2013).

5.1.2 The protective effects of synthetic ligands with regards to Ni toxicity

Chronic Ni exposures to *S. purpuratus* (96 h) and *A. bahia* (7 day) revealed that synthetic ligands do provide protection against Ni toxicity. For *S. purpuratus* synthetic ligands provided protection based on dissolved Ni concentration ($[Ni_D]$). It was assumed that toxicity would be dependent on the magnitude of binding affinity for Ni of each synthetic ligand. EDTA has a high affinity for Ni and can form strong complexes and also demonstrated the strongest protection based on toxicity (EC_{50}) values. Citric acid (CA) and weaker ligands had decreasing complexing ability with Ni but still showed increased EC_{50} values for fertilization success of *S. purpuratus* compared to exposures in artificial seawater (ASW). For *A. bahia* treatments, EDTA also showed increased LC_{50} values, indicating a high protection against Ni toxicity. However, the CA exposure showed values lower than the ASW exposure based on $[Ni^{2+}]$ LC_{50}, however, there were inconsistencies showing Ni^{2+} concentrations higher than $[Ni_D]$.

5.1.3 The protective effects of natural marine waters with regards to Ni toxicity

Natural organic matter (NOM) is a well-established toxicity modifying factor in both fresh and marine environments (Blewett et al. 2018; DePalma 2009; Tait 2013). However, its protective capacity for marine Ni is not well understood. Chronic Ni exposures to *S. purpuratus* (96 h) and *A. bahia* (7 day) revealed that natural waters show varied protection against Ni toxicity. Natural waters show that the range of EC_{50} values for $[Ni_D]$ for *S. purpuratus* varied by a factor of two. This variation of toxicity was not significantly different from ASW exposures and had no definite trends with sampling location. The sampling locations for *A. bahia* were chosen to go back to previous locations that were used with *S. purpuratus*. The LC_{50} values for $[Ni_D]$ for *A. bahia*

showed no significant differences from the ASW exposure.

The DOC concentration in all collected natural waters ranged from 1.3 to 5.7 mg DOC/L. An analysis of the relationship between [Ni_D] EC_{50} and DOC concentration for *S. purpuratus* exposures showed no significant correlation. Nadella et al. (2013) also showed that increased DOC concentrations did not provide protection against zinc toxicity for two marine organisms. Correlations between DOC concentration and *A. bahia* [Ni_D] LC_{50} values could not be considered as there were only two data points. From this research it is apparent that DOC concentration is theoretically not the only factor influencing Ni toxicity, indicating that DOC concentration may not be a great indicator of toxicity and other markers should possibly also be used.

5.1.4 The dependence of toxicity on the composition of DOC

The composition of DOC may influence the ability to protect against Ni toxicity. Optical characteristics of the marine samples related to DOC composition such as SAC_{340} (the specific absorbance coefficient at 340 nm), SUV_{254} (the specific UV absorbance at 254 nm), fluorescence index (FI), and charged cation concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were all measured. The data showed the SAC_{340} and SUV_{254} had no significant trends with *S. purpuratus* [Ni_D] EC_{50} values which is consistent with other studies that showed very weak correlation with toxicity (DePalma 2009; Tait 2013). It was assumed that as FI increases, protectivity would decrease (Schwartz et al. 2004), however FI also showed no significant correlation with [Ni_D] EC_{50}. This has been observed in other studies as well (Blewett et al. 2018; Luider et al. 2004; Tait 2013). It is predicted that protective effects are seen by increased concentrations of cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) because they can compete for binding sites against Ni on the biotic ligand, effectively reducing bioavailability (Di Toro et al. 2001; Paquin et al. 2002; Niyogi and Wood

2004). However, all charged cations also had no significant correlation with [Ni_D] EC_{50}. Initial fluorescence was also measured for all natural waters at time of collection, and values ranged from 107 to 1526. Initial fluorescence was found to correlate positively with [Ni_D] EC_{50} values, showing that a site with a higher initial fluorescence value had a higher EC_{50} value. This is likely because Ni can bind to proteinaceous sites, reducing the Ni available to bind to the biotic ligand and reducing toxicity. Very few studies have used initial fluorescence as a marker for toxicity, though it may be one way that site-specific estimates of toxicity can be done in the field (Baker et al. 2015). However, although toxicity (based on [Ni_D] EC_{50}) between sites did vary, they were not significantly different from ASW exposures. Correlations between optical characteristics of DOC and *A. bahia* [Ni_D] LC_{50} values could not be determined as there were only two data points. Overall, the results from this study in conjunction with previous literature suggest that protection is not source dependent and markers such as DOC concentration and composition may not be good predictive measures of Ni toxicity.

5.1.5 The dependence of toxicity on the speciation of Ni

Ion-exchange technique (IET) measurements were done in parallel to the toxicity tests during this study to quantify the free ion Ni concentration ([Ni^{2+}]) within solution. Measuring [Ni^{2+}] within solution is important to assess the assumptions of the BLM that considers Ni^{2+} to be the most toxic and bioavailable form. If this assumption is met then [Ni^{2+}] toxicity will be similar for different samples, regardless of exposure. This has been done previously for copper in saltwater (SW) where free copper at the LC_{50} for each site remained constant while the LC_{50} values ranged from 333 to 980 nM (Tait 2013). This data is inherent to validating the use of the BLM in SW and also provides a method to test the complexation predictions provided by Visual Minteq. The EC_{50}s for fertilization

success to *S. purpuratus* for [Ni^{2+}] in solution varied between treatments with added synthetic ligand, however, all values were either similar (EDTA and CA) or less [NTA, glutamic acid (GA) and histidine (HD)] than the values for tests in ASW except for tryptophan (TRP). For TRP, the IET measurements dramatically overestimated the actual [Ni^{2+}] in solution. The EC_{50} values based on [Ni^{2+}] did not show reduced variability when considering all exposures but when considering only the ligands that did follow the BLM assumptions (EDTA and CA) there was reduced variability. EDTA in particular was very protective based on [Ni_D], but when plotted on a [Ni^{2+}] basis there were no significant differences from the ASW exposures. Similarly, [Ni^{2+}] LC_{50} values for *A. bahia* for synthetic ligands (EDTA) was similar to the values for tests with ASW. For CA, there were inconsistencies between Ni^{2+} and Ni_D concentrations.

Visual Minteq was used to predict [Ni^{2+}] speciation within all exposures. For EDTA and CA, the model under-predicted [Ni^{2+}] in solution, resulting in an over-prediction of toxicity in comparison to the measured [Ni^{2+}] for both *S. purpuratus* and *A. bahia* exposures. This may be due to inaccuracies within the model. For NTA, GA, and HD deviations were not significantly different between measured and modelled values, further displaying that NTA-, GA-, and HD-Ni complexes were potentially contributing to the toxicity seen, however, this exact mechanism of toxicity was not looked at directly.

The variability of the EC_{50} values for fertilization success to *S. purpuratus* and *A. bahia* in natural waters decreases slightly when expressed on a [Ni^{2+}] basis compared to values based on [Ni_D]. All samples within exposures using *S. purpuratus* tests had overlapping confidence bands with those of the ASW exposures and were therefore considered not significantly different. For *A. bahia* exposures, the confidence intervals of

both sites were within the confidence bands of the ASW exposure and was therefore not significantly different. The results from experiments using *S. purpuratus* and *A. bahia* provide general conclusions that strengthen the assumptions of the BLM indicating that $[Ni^{2+}]$ is the best predictor of toxicity, and that complexation produces some amount of protection for Ni in SW.

5.1.6 The binding affinity of the biotic ligand

IET-measured $[Ni^{2+}]$ was used for the derivation of conditional stability constant ($K^{'}$) for each treatment and an average value was calculated. This value can be used to develop a computational BLM for marine Ni and also aids in determining when ligands may be protective. The $\log K^{'}_{NiBL}$ for *S. purpuratus* using model ligands was found to be $6.3 \pm 0.4 = 10^{6.3}$ and in natural waters the $\log K^{'}_{NiBL} = 6.4 \pm 0.09 = 10^{6.4}$. Previous research has shown natural waters to have K_f values ranging from 3.8 to 7.1, which are comparable to the values calculated within this study (Chen et al. unpublished; Dow 2017). These values show agreement between model ligands and natural samples. Using another organism, *A. bahia* the $\log K^{'}_{NiBL} = 6.2 \pm 0.18 = 10^{6.2}$ using model ligands and in natural waters $\log K^{'}_{NiBL} = 6.2 \pm 0.15 = 10^{6.2}$. This data shows that *A. bahia* has a similar binding affinity for Ni as *S. purpuratus*. Thus, if these two biotic ligands have equivalent K_f values at the same concentrations, then half of Ni will bind to each. However, if concentrations or binding affinities increase for one, it will start to out compete the other and Ni will bind more strongly to it. These values will be helpful in determining site specific estimates of toxicity and in implementing a computational BLM for marine Ni.

5.2 Integrative Science and Significance

This research is inherently integrative through its methodology and practice. The hypotheses tested throughout this thesis were addressed through a variety of biological, physiological, and geochemical means. In order to understand the full scope of Ni toxicity in marine systems, bioassays using two marine organisms were conducted, chemical analyses were utilized to measure dissolved and free concentrations of Ni as well as characterize the DOC within different natural waters. Inherent to the BLM principles, toxicity can be altered by cationic competition, DOM complexation and inorganic (anionic) complexation; all of which require a basic understanding of water chemistry. Not only are these components important to biological toxicity tests, but they are also important to input into geochemical modelling programs such as Visual Minteq, that was used to predict free ion concentrations within exposures. Furthermore, as this knowledge about how water chemistry parameters affects Ni toxicity is built upon, it can be applied to potential site-specific estimates of toxicity in natural waters. Metal toxicity was studied from a biological perspective and while the mechanisms at the site of action on the biotic ligand were not studied directly, a basic understanding of principles on how Ni may use transporter sites at the biotic ligand that may cause toxicity was gained. For example, how ions (i.e. Ca^{2+}) are required for developing sea urchin embryos for calcification of exoskeleton and other cellular functions and can be disrupted in the presence of Ni. Lastly, statistical methods were used throughout this thesis. Statistical tests were required for the analysis of all results including the calculation of effective and lethal concentrations values, means and standard deviations, significant differences and any correlations between two variables.

Looking at the broader scope, this work is relevant in order to understand Ni toxicity from both a biological and geochemical perspective. By using a BLM approach site-specific estimates can be made, making it useful in industry and policy development. Currently, there are few studies examining Ni toxicity in marine environments, and a marine BLM has yet to be developed, though it may be warranted. Therefore, continuing research efforts is important to development of a marine BLM for Ni. The results herein will be used to calibrate a computational marine BLM for Ni and help develop site-specific estimates of toxicity, as well as hopefully aid in creating environmental regulations for the protection of marine species against Ni contamination. Although aquatic toxicology is the specific field of this research, it is inherently integrative in that it draws on the knowledge and concepts from many fields of science.

5.3 Future work

The current study highlights important areas of research that need to be further studied in order to fully understand Ni toxicity in marine systems. The use of Visual Minteq was limiting since Ni toxicity was overestimated in the presence of most ligands, this may be due to ionic strength corrections around the stability constant or that the model does not account for the biotic ligand itself. Future use of modeling software should manually input these factors in order to predict free ion Ni concentrations with greater certainty. Based on natural waters tested so far, Ni complexation, protection and decreased Ni bioavailability was largely not seen. However, a marine BLM for Ni may be useful for other waters with higher complexing ligands. Future work should continue efforts in more extreme environments (i.e. with high proteinaceous DOC concentrations) such as sediment porewaters and wastewater impacted sites to see if general conclusions of the BLM can be applied. The initial fluorescence suggested that very protein rich sites might

be protective, so such sites should be tested in future work. As well, further studies that continue the work by expanding the scope of exposures to encompass a wider range of complexation with multiple marine organisms are needed to better understand the influence of ligand complexation on Ni toxicity, speciation and bioavailability.

5.4 References

Baker A, Cumberland SA, Bradley C, Buckley C, Bridgeman J. 2015. To what extent can portable fluorescence spectroscopy be used in the real-time assessment of microbial water quality? *Sci Total Environ*. 532:14-19.

Blewett TA, Dow EM, Wood CM, McGeer JC, Smith DS. 2018. The role of dissolved organic carbon concentration and composition on nickel toxicity to early life-stages of the blue mussel *Mytilus edulis* and purple sea urchin *Strongylocentrotus purpuratus*. *Ecotoxicol Environ Saf.* 160:162-170.

Chen W, Sherman SR, McGeer JC, Smith DS. Determining the concentration of free nickel ions in seawater by ion exchange technique: validation and application. *Manuscript in progress.*

DeForest DK, Schlekat CE. 2013. Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms. *Integr Environ Assess Manag*. 9:580-589.

DePalma SGS. 2009. Characterization of dissolved organic matter and reduced sulfur in coastal marine and estuarine environments: Implications for protective effects on acute copper toxicity. Unpublished masters dissertation. University of Waterloo, Waterloo, Ontario. pp. 175.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem*. 20:2383-2396.

Dow E. 2017. Quantifying the interactions of nickel with marine dissolved organic matter using fluorescence. Unpublished masters dissertation. Wilfrid Laurier University, Waterloo, Ontario. pp. 91.

Luider CD, Crusius J, Playle RC, Curtis PJ. 2004. Influence of natural organic matter

source on copper speciation as demonstrated by Cu binding to fish gills, by ion selective electrode, and by DGT gel sampler. *Environ Sci Technol*. 38:2865-2872.

Lussier SM, Gentile JH, Walker J. 1985. Acute and chronic effects of heavy metals and cyanide on *Mysidopsis bahia* (Crustacea:Mysidacea). *Aquat Toxicol*. 7:25-35.

Lussier SM, Kuhn A, Comeleo R. 1999. An evaluation of the seven-day toxicity test with *Americamysis bahia* (formerly *Mysidopsis bahia*). *Environ Toxicol Chem*. 18:2888-2893.

Nadella SR, Tellis M, Diamond R, Smith S, Bianchini A, Wood CM. 2013. Toxicity of lead and zinc to developing mussel and sea urchin embryos: Critical tissue residues and effects of dissolved organic matter and salinity. *Comp Biochem Physiol - C Toxicol Pharmacol*. 158:72-83.

Niyogi S, Wood CM. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ Sci Technol*. 38:6177-6192.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F. 2002. The biotic ligand model: a historical overview. *Comp Biochem Physiol C Toxicol Pharmacol*. 133:3-35.

Phinney JT, Bruland KW. 1994. Uptake of lipophilic organic Cu, Cd, and Pb complexes in the coastal diatom *Thalassiosira weissflogii*. *Environ Sci Technol*. 28:1781-1790.

Playle RC, Gensemer RW, Dixon DG. 1992. Copper accumulation on gills of fathead minnows: Influence of water hardness, complexation and pH of the gill micro-environment. *Environ Toxicol Chem*. 11:381–391.

Playle RC, Dixon DG, Burnissn K. 1993. Copper and cadmium binding to fish gills:

Estimates of metal-gill stability constants and modelling of metal accumulation. *Can J Fish Aquat Sci*. 50:2678-2687.

Schwartz ML, Curtis PJ, Playle RC. 2004. Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem*. 23:2889-2899.

Tait TN. 2013. Determination of copper speciaion, bioavailability and toxicity in saltwater environments. Unpublished masters dissertation. Wilfrid Laurier University, Waterloo, Ontario. pp. 208.

Uthicke S, Schaffelke B, Byrne M. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecol Monogr*. 79:3-24.

Zhao CM, Campbell PGC, Wilkinson KJ. 2016. When are metal complexes bioavailable? *Environ Chem*. 13:425-433.

APPENDICES

Appendix A

Tables and Figures

Table 1. Results of Pearson correlations done between [Ni^{2+}] EC_{50} values of fertilization success of *S. purpuratus* and characteristics of the natural waters showing the coefficient of determination (R^2), Pearson's product-moment correlation coefficient (r) and p-value. Significance was determined at $p<0.05$.

Characteristic	R^2	r	p-value
DOC concentration	0.14	0.37	0.19
SUV_{254}	0.01	-0.10	0.73
SAC_{340}	0.02	-0.15	0.62
Fluorescence Index	0.007	0.08	0.79
[Ca^{2+}]	0.004	-0.06	0.84
[Mg^{2+}]	0.01	-0.11	0.73
[Na^{+}]	0.003	-0.06	0.86
[K^{+}]	0.003	-0.06	0.85
Initial Fluorescence	0.07	0.27	0.40
DOC concentration (Skimmer waste)	0.72	0.85	0.36

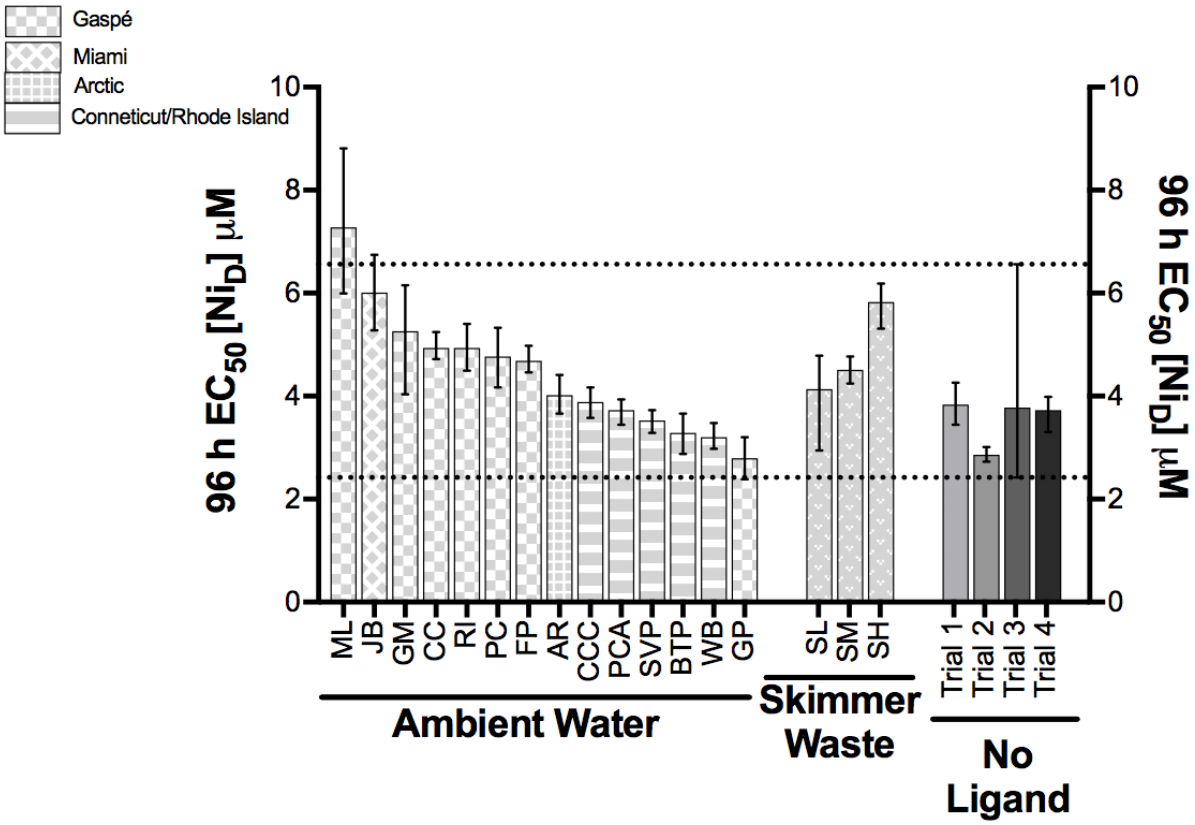

FIG. 1. The 96 h EC_{50} values for total dissolved Ni [Ni_D] for abnormal embryo development in purple sea urchin within natural and artificial waters. Total dissolved Ni [Ni_D] endpoint determinations were measured by GFAAS. Samples were collected from Gaspé, Quebec; Miami, Florida; Connecticut and Rhode Island, USA; Northern Canadian Arctic; and an aquarium store, Ontario. Error bars show 95% confidence interval and * indicates a significant difference in EC_{50} value compared to no ligand (ASW) exposures. Dotted horizontal lines represent the upper most and lowest most confidence limit of the ASW trials.

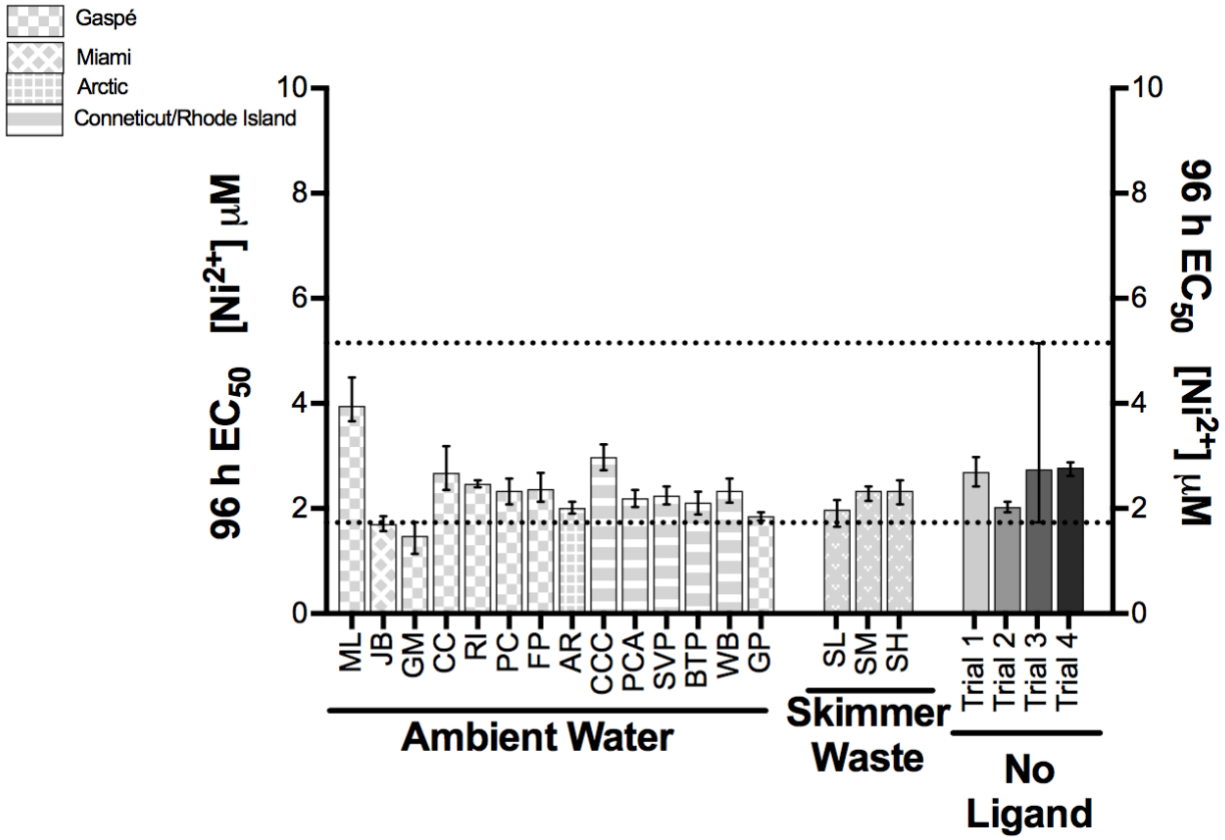

FIG. 2. The 96 h EC_{50} values for free Ni ion [Ni^{2+}] for abnormal embryo development in purple sea urchin within natural and artificial waters. Free Ni [Ni^{2+}] endpoint determinations were measured by IET. Samples were collected from Gaspé, Quebec; Miami, Florida; Connecticut and Rhode Island, USA; Northern Canadian Arctic; and an aquarium store, Ontario (see table 3.1). Error bars show 95% confidence interval and * indicates a significant difference in EC_{50} value compared to no ligand (ASW) exposures. Dotted horizontal lines represent the upper most and lowest most confidence limit of the ASW trials.

Appendix B
Methodology

Toxicity endpoint determination

At the end of 7 days surviving mysids were examined under an AMG EVOS compound microscope (Fisher Scientific, Toronto, ON) at 100x magnification and images were taken to determine the sex of the organism as well as the brood sac development score following the methods of Cooper et al. (unpublished; Fig. 1). Sexual maturation was determined using the brood sac development score by taking the score of each individual mysid at 7 days and subtracting that value from the average score of a 0 h starter population. Mysids were placed onto a petri dish and wet weight was measured as an average per replicate on a Sartorius CP224S balance (Sartorius Mechanatronics Corp., Bohemia, NY), then mysids were separated into individual bullet tubes and dried in an oven at 60°C for 24 h. Mysids were then individually weighed on a Sartorius SE2 Ultra Microbalance (Sartorius Mechanatronics Corp., Bohemia, NY). Growth was measured by taking the dry weight of each individual mysid at 7 days and subtracting that value from the average dry weight value of a 0-h starter population.

Bioaccumulation

Approximately thirty 7-day old mysids were placed in each of the 6 beakers that paralleled the concentration range of the current exposure. At 24 h 10 mysids were taken out and dipped into an EDTA 10^{-4} M, lightly dried on a KimWipe and placed into one bullet tube. The bullet tube (n=10) was weighed on a Sartorius CP224S balance to determine wet weight. Mysids were digested in 50 µL 1N HNO_3 and 150 µL trace metal grade HNO_3 at 60°C for 24 h, with samples vortexed at 24 h to aid in digestion. Embryo digests were then diluted appropriately in Milli-Q and analyzed for Ni on the GFAAS. Ni

accumulation was divided by the total weight of mysids per concentration to get Ni accumulation per gram (in µM Ni/g wet weight). At 48 h, 10 more mysids were taken out and the same procedures were followed.

Statistical analysis

The 7-day median inhibitory concentration (IC_{50}) and 20% inhibitory concentration (IC_{20}) values with 95% upper and lower confidence intervals (95% U&L CI) were determined. The ICx values were expressed by both [Ni_D] and [Ni^{2+}]. The ICx determination was done using linear regression analysis and extrapolation therefore confidence limits could not be determined.

Results and Discussion

All toxicity tests met the acceptable criteria where ≥0.20 mg/mysid and ≥2.5 maturation score (females scored 0 to 5 based on how sexually mature they were; Fig. 1) was reached in all unexposed controls (Table 1; US EPA 2002; Cooper et al. unpublished). The mean dry weight of the ASW exposure mysids was 0.26 ± 0.09 mg and the mean sexual maturation score was 3.26 ± 0.5 (n=33; Table 1).

Growth

Growth was measured by using dry weight values. Increasing Ni concentrations showed varying effects on growth (Table 1). However, all exposures were found to be higher than ASW exposure. All IC_x values had to be extrapolated from the data. EDTA had higher IC_{50} values higher than ASW and CA could not be calculated due to increasing weight values with increasing Ni concentrations (Fig. 2). The same trends were seen with IC_{20} values (Fig. 3). IN showed no change in growth with increasing Ni concentrations and CCC decreased over the 7-day toxicity test. However, both IN and CCC had higher IC_{50}

values compared to the ASW exposure (Fig. 2). The same trends were seen with IC_{20} values (Fig. 3). This has been seen in literature values where a significant decrease in Ni toxicity to these biological endpoints would be observed only when high DOC concentrations (> 20 mgC/L) were present in natural waters (Cooper et al. unpublished). Measurement of growth in higher concentration exposures raises concerns as the sample size is decreased and as such, can have skewed results. Few mysids surviving at higher concentrations are relatively large, either because larger mysids are more tolerant or due to cannibalism (Hunt et al. 2002). Therefore, growth endpoints have previously been found to be less sensitive than survival in mysid exposures with trace-metal toxicants (Hunt et al. 1997).

Maturation

For mysids, growth and maturation are often linked and this positive relationship between body length and brood size is well established (Lussier et al. 1999). Delayed reproduction often results from delayed maturation caused by reduced growth rate (Lussier et al. 1999). Maturation and reproduction are seen as valuable indicators of toxicity as they are very sensitive to abiotic and biotic factors. As such, maturation was measured by a brood sac development score. Sexual maturation decreased with increasing Ni concentrations for all exposures (Table 1). All exposures were found to be higher or the same as the ASW exposure. All IC_x values had to be extrapolated from the data. EDTA had a higher IC_{50} value than the ASW exposure and CA was slightly lower (Fig. 4). The same trends were seen with IC_{20} values (Fig. 5). For natural waters, both IC_{50} values for IN and CCC were similar, however both were lower than the ASW exposure (Fig. 4). The same trends

were seen with IC_{20} values (Fig. 5). Similar to growth, maturity can be skewed due to hardy individuals surviving in the higher concentrations.

Bioaccumulation

Whole body Ni bioaccumulation was examined after acute exposure to Ni (24 h and 48 h; Fig. 6). Whole-body Ni content of mysids increased at all lower concentrations of Ni until plateauing at an appeared equilibrium at the highest concentration. Accumulation between 24 h and 48 h Ni was similar within all exposures. Previous studies have shown that maximal deposition of a metal toxicant can occur 1 h up to 6 h after exposure and can have predictive effects of toxicity (Blust et al. 1995; Playle et al. 1992). As such, 24 h Ni accumulation was plotted against 7-day mortality values to determine if any correlation could be defined (Fig. 7). Exposures EDTA, IN and CCC with LA_{50}s of 2.3, 2.4 and 2.3 µM Ni/g wet weight respectively accumulated more Ni than the ASW exposure (LA_{50} = 1.8 µM Ni/g wet weight) while CA accumulated less (LA_{50} = 0.6 µM Ni/g wet weight). To our knowledge this is the first study to examine the effects of complexing agents on Ni accumulation and its relation to mortality for this species, so direct comparisons could not be made. Previous work investigating Cu accumulation in fish species have shown an inverse correlation between whole-body accumulation and toxicity (Dixon and Sprague 1981; Winner and Gauss 1986). What is seen for EDTA, IN and CCC is likely due to the majority of accumulated metal being in its non-toxic form (Dixon and Sprague 1981). For CA, although Ni accumulation was reduced compared to control the LC_{50} value showed the solution to be more toxic. This could be due to toxic action occurring at the organism-water interface during the first 24 h that is irreversible over the 7 days. A similar relationship was seen in the blue mussels (*Mytilus edulis*)

where cadmium (Cd) complexed with EDTA to form Cd-EDTA which was transferred across the membrane via carrier ligands, immobilizing interactions with essential enzymes within the cell eventually causing toxic effects (George and Coombs 1977). From this data it seems that bioaccumulation cannot be directly related to bioavailability nor to toxicity and further analysis is required to make any definite conclusions.

Conclusions

For growth and maturation all exposures had higher or similar IC_xs to control, but the magnitude of protection varied. The toxicity of Ni to the three biological endpoints do not overlap, and as such, more than one biological response should continue to be measured in order to identify the true toxic effects of a metal contaminant. From the data presented, 24 h bioaccumulation cannot be directly related to 7-day toxicity; because some Ni accumulated seems to be non-toxic. Ni is less bioavailable in some exposures but more toxic whereas in others more bioaccumulation occurred and there was more protection seen. Overall, this study provides novel insights into the relationships between water chemistry, Ni accumulation and Ni toxicity for the marine organism *A. bahia* and will aid in the adoption of a BLM approach for marine Ni.

Tables and Figures

Table 1. The 7-day chronic toxicity end-points of brood-sac development score and dry weight are shown as mean ± SD for all exposures.

Exposure	Nominal Ni (µg/L)	Brood-sac Development Score ± SD	Dry Weight (mg) ± SD	n
ASW	0	3.26 ± 0.5	0.26 ± 0.09	33
	30	2.40 ± 0.7	0.24 ± 0.05	27
	60	2.04 ± 0.5	0.27 ± 0.04	36
	120	1.75 ± 0.7	0.23 ± 0.01	34
	240	2.50 ± 1.1	0.17 ± 0.03	15
	480	2.00 ± 1.4	0.15 ± 0.004	3
CA	0	2.93 ± 0.4	0.31 ± 0.07	32
	30	2.48 ± 0.6	0.31 ± 0.05	24
	60	1.85 ± 0.8	0.32 ± 0.03	17
	120	2.04 ± 0.9	0.32 ± 0.04	11
	240	1.67 ± 0.7	0.29 ± 0.05	14
	480	1.50 ± 0.7	0.37 ± 0.04	4
EDTA	0	2.62 ± 0.7	0.33 ± 0.02	36
	400	2.62 ± 0.8	0.28 ± 0.03	31
	800	2.33 ± 0.2	0.28 ± 0.006	23
	1200	2.55 ± 0.5	0.27 ± 0.02	31
	1600	1.63 ± 0.3	0.26 ± 0.01	27
	2000	3.00 ±	0.25 ± 0.07	2
IN	0	3.43 ± 0.2	0.35 ± 0.02	32
	30	3.00 ± 0.7	0.32 ± 0.04	30
	60	2.03 ± 0.5	0.29 ± 0.02	31
	120	2.02 ± 0.5	0.32 ± 0.03	23
	240	1.73 ± 0.6	0.30 ± 0.06	20
	480	1.67 ± 1.2	0.36 ± 0.04	3
CCC	0	3.37 ± 0.8	0.36 ± 0.01	34
	30	2.98 ± 0.5	0.35 ± 0.03	22
	60	2.48 ± 0.7	0.30 ± 0.04	25
	120	2.73 ± 0.7	0.36 ± 0.01	16
	240	2.75 ± 0.4	0.33 ± 0.05	6
	480	2.50 ± 0.7	0.32 ± 0.09	4

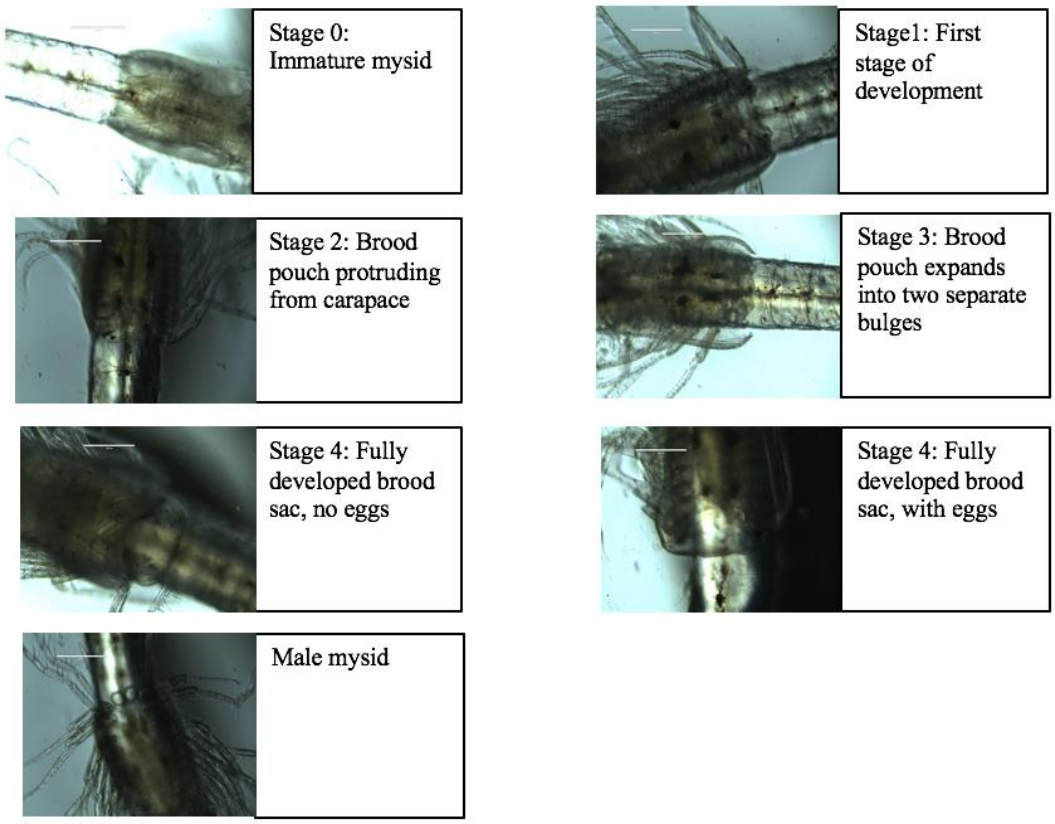

FIG. 1. Brood-sac development score (as a measurement of sexual maturation score). Brood sacs are rated on a scale from 0 (immature) to 5 (fully mature female with eggs in the brood sac). A male mysid is also depicted.

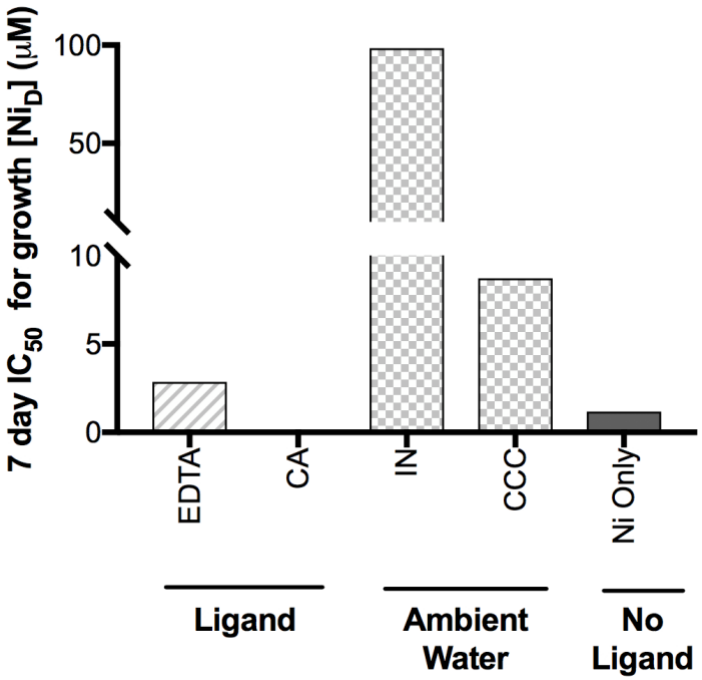

FIG. 2. 7-day IC_{50} values for total dissolved Ni [Ni_D] for mysid growth with (and without) added ligands.

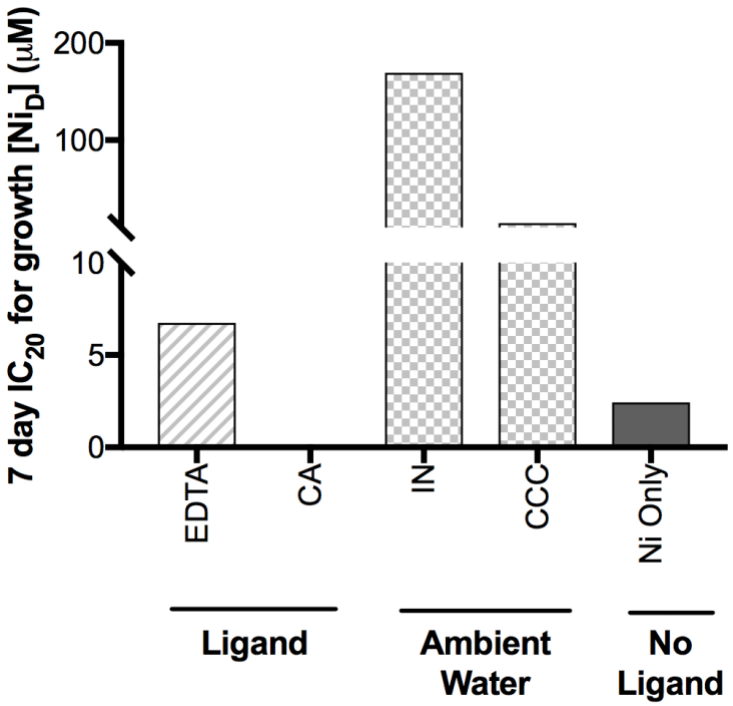

FIG. 3. 7-day IC_{20} values for total dissolved Ni [Ni_D] for mysid growth with (and without) added ligands.

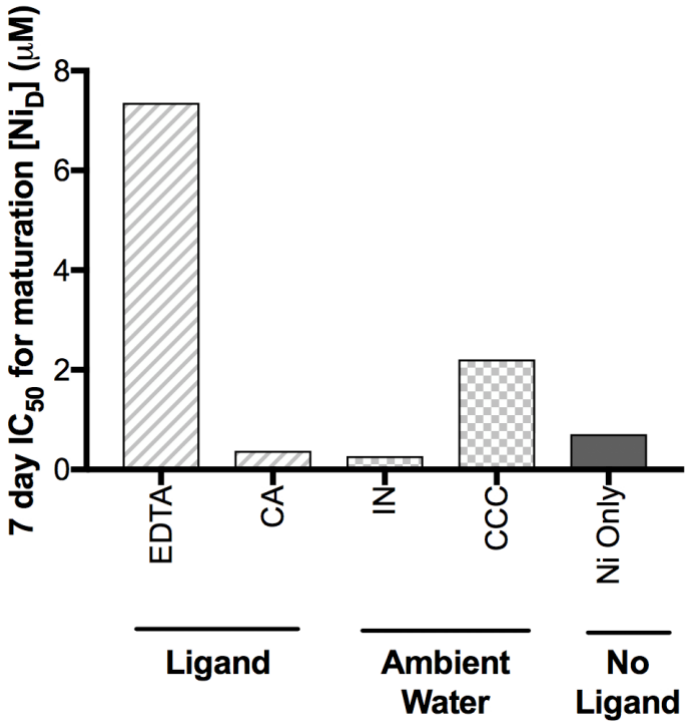

FIG. 4. 7-day IC_{50} values for total dissolved Ni [Ni_D] for mysid maturation with (and without) added ligands.

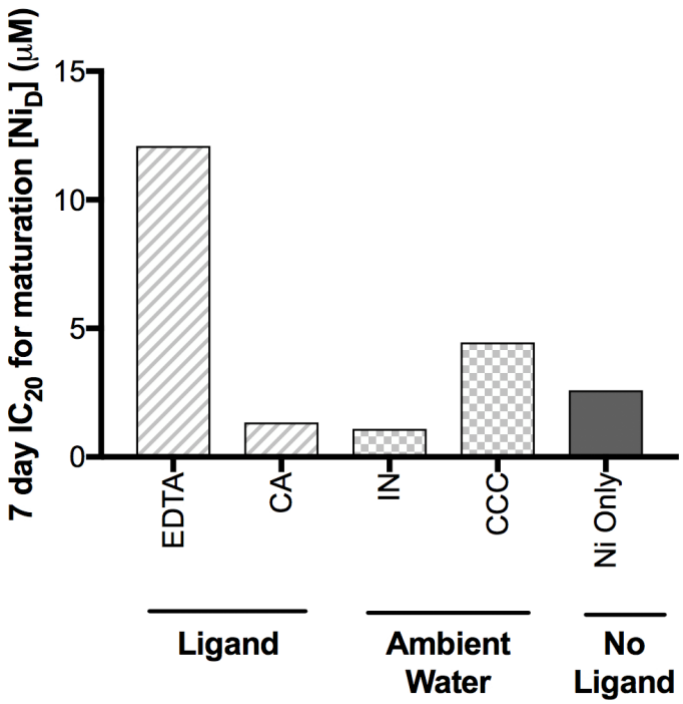

FIG. 5. 7-day IC_{20} values for total dissolved Ni [Ni_D] for mysid maturation with (and without) added ligands.

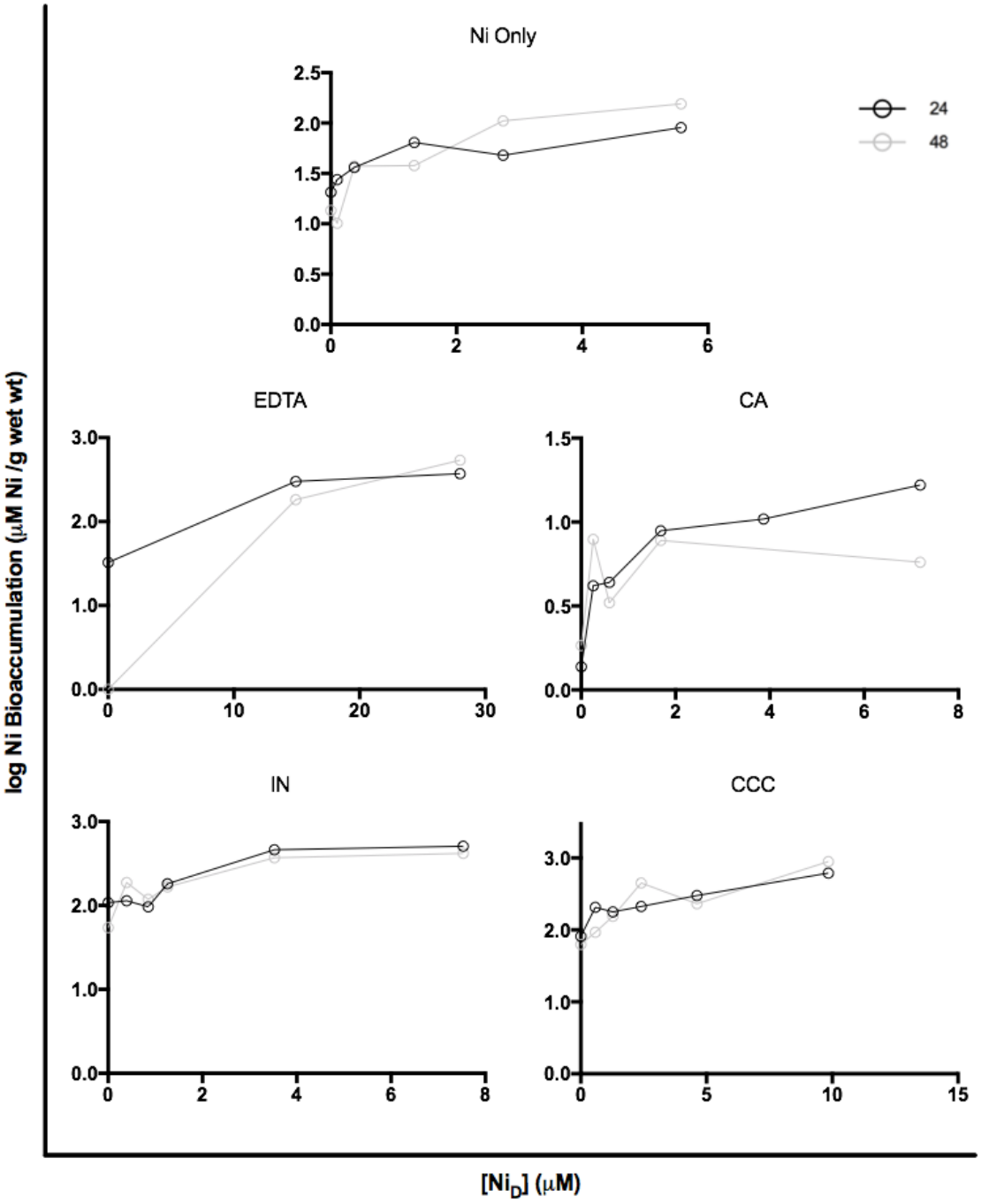

FIG. 6. 7-day Ni bioaccumulation values plotted against total dissolved Ni [Ni$_D$] for mysids with (and without) added ligands.

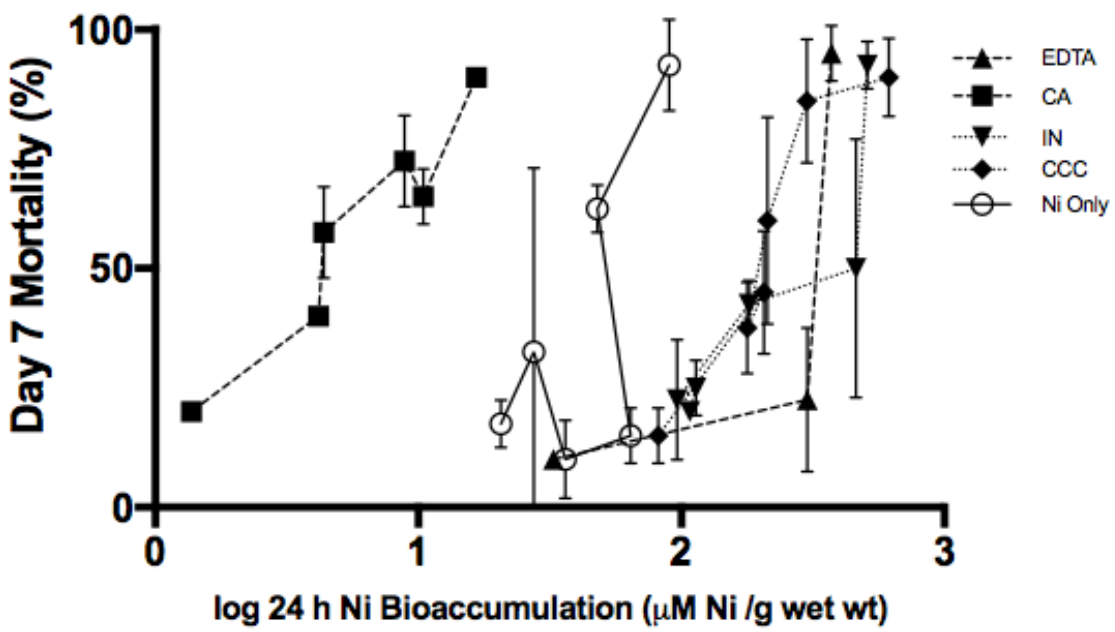

FIG. 7. 7-day Ni bioaccumulation values plotted against day 7 mortality (%) for mysids with (and without) added ligands.

References

Blust R, Baillieul M, Decleir W. 1995. Effect of total cadmium and organic complexing on the uptake of cadmium by the brine shrimp, *Artemia franciscana*. *Mar Biol.* 123:65-73.

Cooper CA, Nasir R, Mori J, McGeer JM, Smith DS. Influence of dissolved organic carbon concentration and source on chronic 7-day Ni toxicity to the mysid, *Americamysis bahia*. *Manuscript in progress.*

Dixon DG, Sprague JB. 1981. Copper bioaccumulation and hepatoprotein synthesis during acclimation to copper by juvenile rainbow trout. *Aquat Toxicol.* 1:69-81.

George SG, Coombs TL. 1977. The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. *Mar Biol.* 268:261-268.

Hunt JW, Anderson BS, Turpen SL, Englund MA, Piekarski W. 1997. Precision and sensitivity of a seven-day growth and survival toxicity test using the west coast marine mysid crustacean *Holmesimysis costata*. *Environ Toxicol Chem*. 16:824-834.

Hunt JW, Anderson BS, Phillips BM, Tjeerdema RS, Puckett HM, Stephenson M, Tucker DW, Watson D. 2002. Acute and chronic toxicity of nickel to marine organisms: implications for water quality criteria. *Environ Toxicol Chem*. 21:2423-2430.

Lussier SM, Kuhn A, Comeleo R. 1999. An evaluation of the seven-day toxicity test with *Americamysis bahia* (formerly *Mysidopsis bahia*). *Environ Toxicol Chem*. 18:2888-2893.

Playle RC, Gensemer RW, Dixon DG. 1992. Copper accumulation on gills of fathead minnows: Influence of water hardness, complexation and pH of the gill micro-

environment. *Environ Toxicol Chem*. 11:381–391.

United States Environmental Protection Agency. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms fourth edition october 2002. EPA-821-R-02-014 Office of Water, Washington, DC. (October):1-350.

Winner RW, Gauss JD. 1986. Relationship between chronic toxicity and bioaccumulation of copper, cadmium and zinc as affected by water hardness and humic acid. *Aquat Toxicol*. 8:149-161.

Appendix C

Tables and Figures

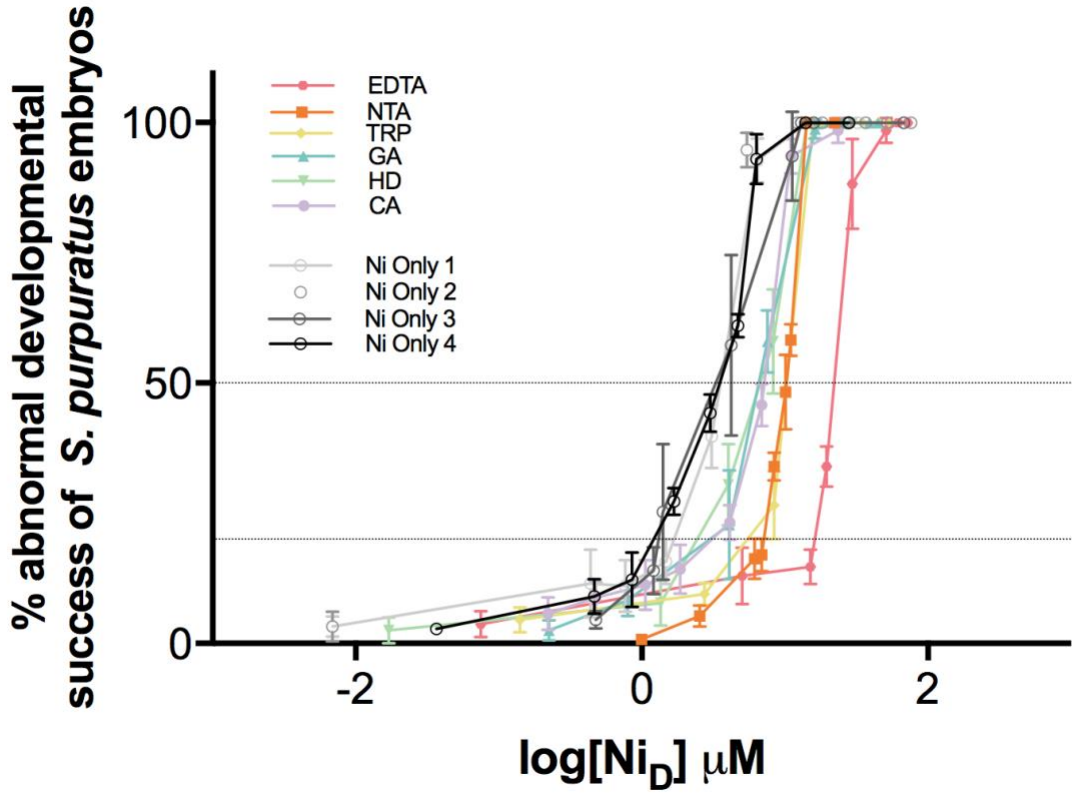

FIG. 1. Response of purple sea urchin embryos exposed to Ni with (and without) added ligands over 96 hr. Mean for % abnormal embryos (± std dev, n=4) are shown as a function of total dissolved Ni concentration (log_{10}).

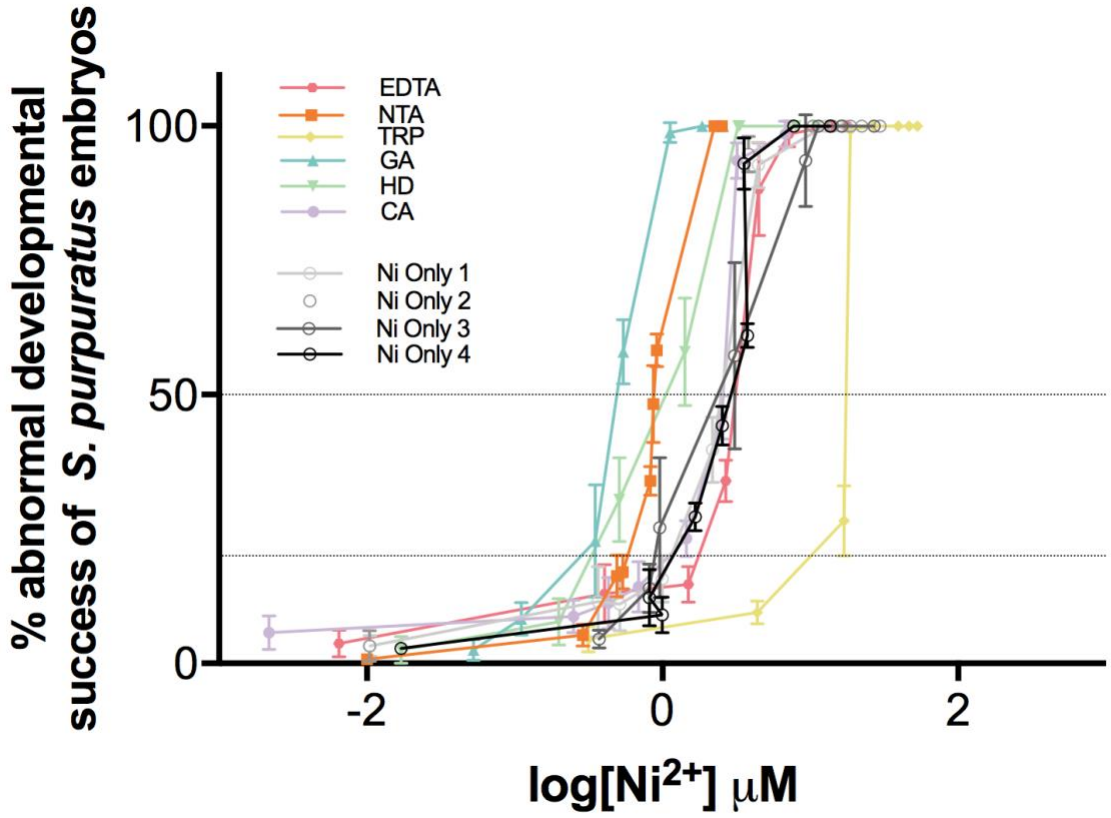

FIG. 2. Response of purple sea urchin embryos exposed to Ni with (and without) added ligands over 96 hr. Mean for % abnormal embryos (± std dev, n=4) are shown as a function of free Ni ion concentration (log_{10}).

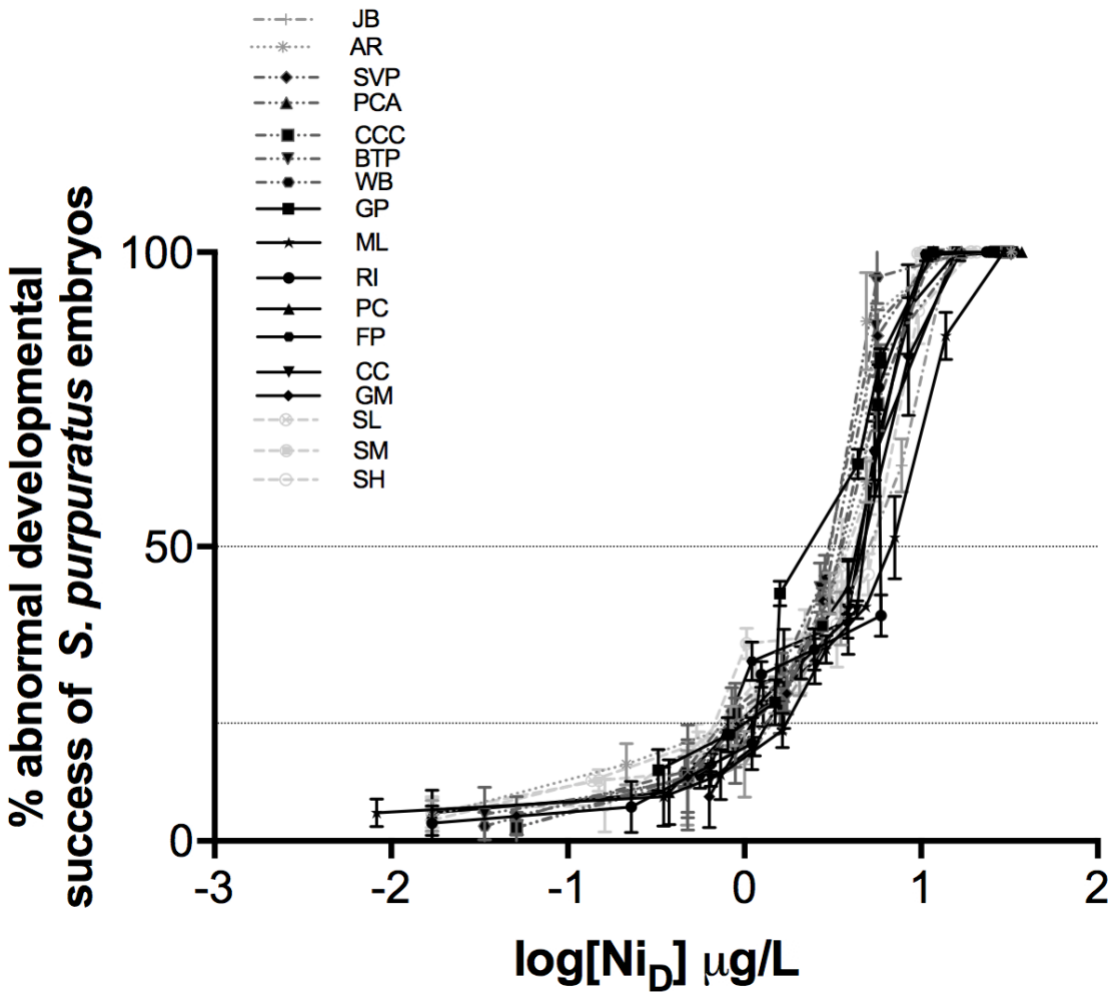

FIG. 3. Response of purple sea urchin embryos exposed to Ni with (and without) natural waters over 96 hr. Mean for % abnormal embryos (± std dev, n=4) are shown as a function of total dissolved Ni concentration ($\log_{10}$).

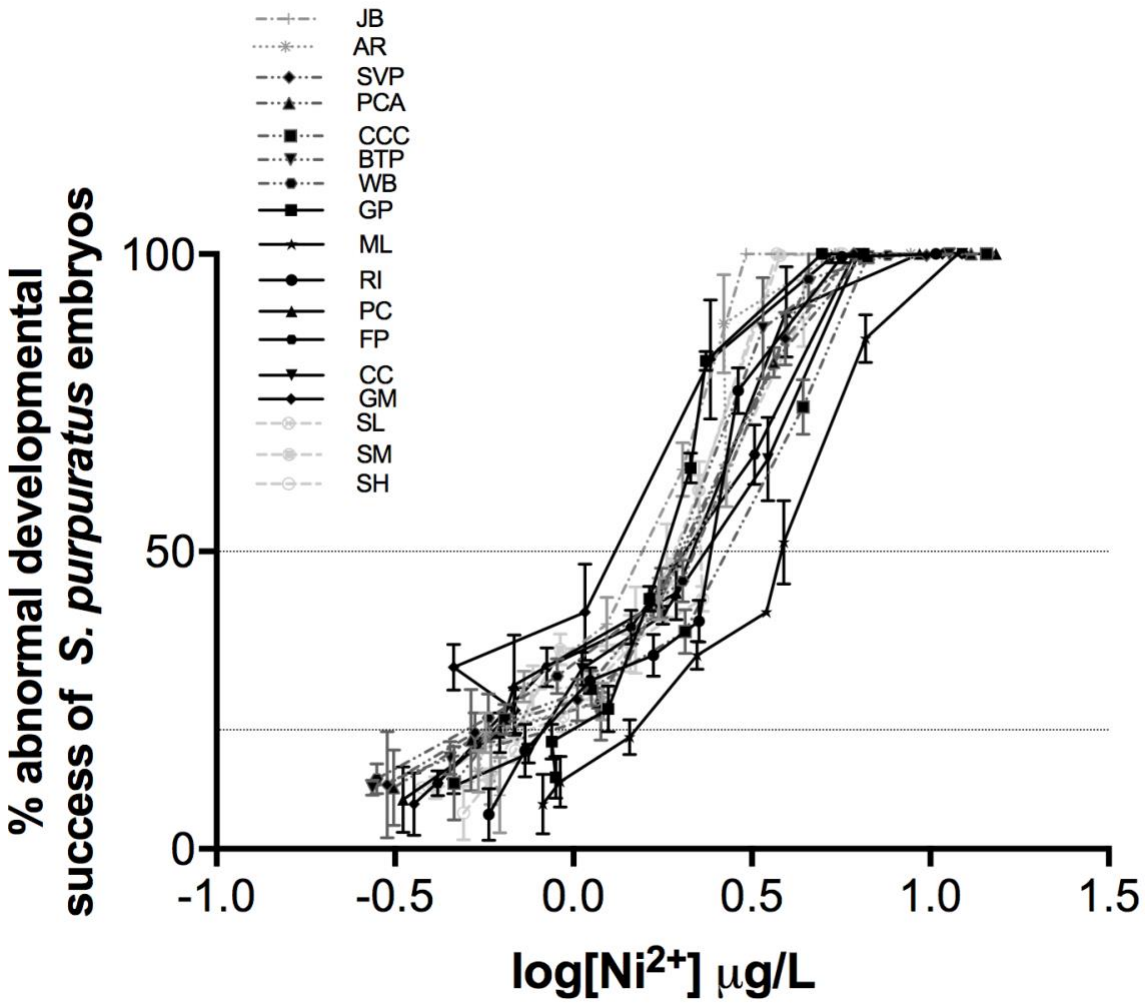

FIG. 4. Response of purple sea urchin embryos exposed to Ni with (and without) natural waters over 96 hr. Mean for % abnormal embryos (± std dev, n=4) are shown as a function of free Ni ion concentration (log_{10}).

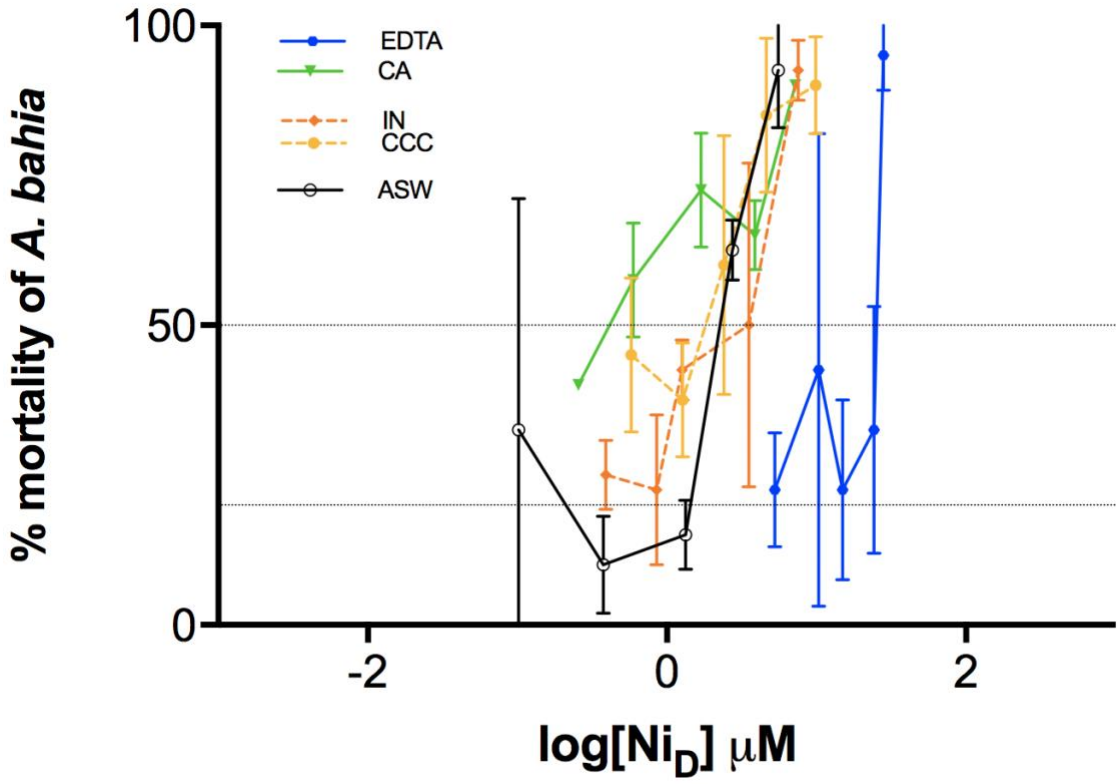

FIG. 5. Response of mysids exposed to Ni with (and without) added ligands and natural waters over 7 days. Mean for % survival (± std dev, n=4) are shown as a function of total dissolved Ni concentration (log_{10}).

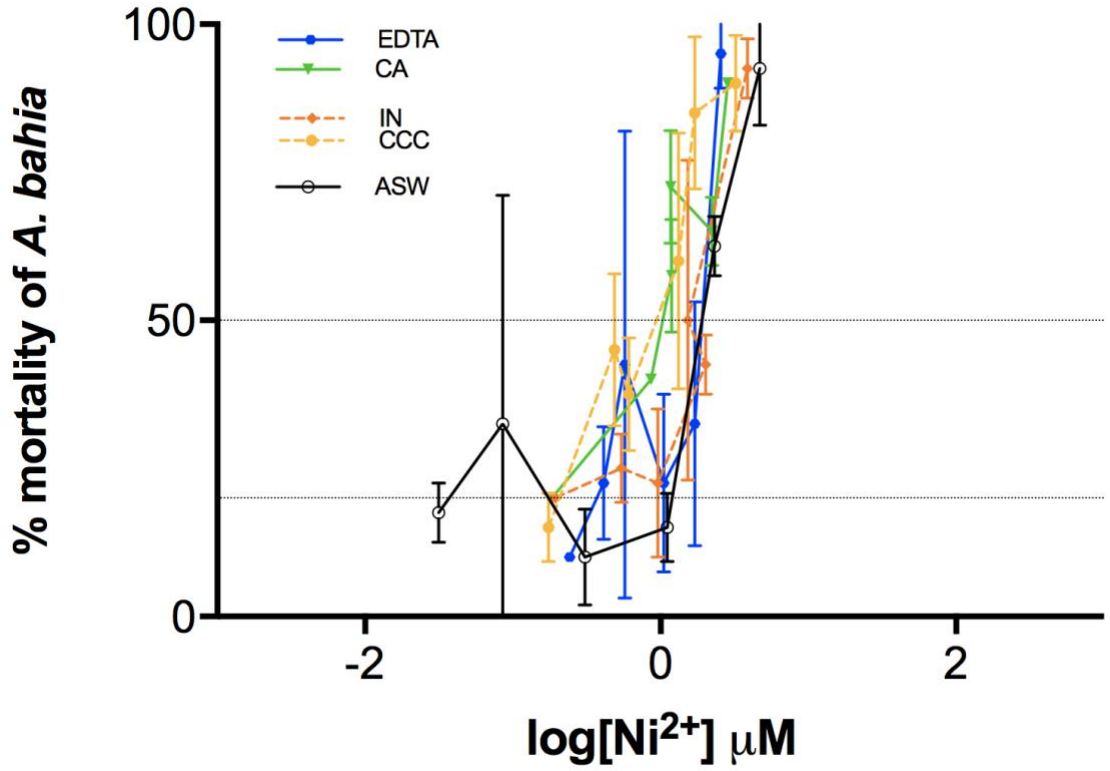

FIG. 6. Response of mysids exposed to Ni with (and without) added ligands and natural waters over 7 days. Mean for % survival (± std dev, n=4) are shown as a function of free Ni ion concentration (log_{10}).

COMPILED REFERENCE LIST

Al-Reasi HA, Wood CM, Smith DS, 2011. Physicochemical and spectroscopic properties of natural organic matter (NOM) from various sources and implications for ameliorative effects on metal toxicity to aquatic biota. *Aquat Toxicol*. 103:179-190.

Arnold WR. 2005. Effects of dissolved organic carbon on copper toxicity: Implications for saltwater copper criteria. *Integr Environ Assess Manag*. 1:34-39.

Baker A. 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers.*Environ Sci Technol.* 35:948-953.

Baker A, Cumberland SA, Bradley C, Buckley C, Bridgeman J. 2015. To what extent can portable fluorescence spectroscopy be used in the real-time assessment of microbial water quality? *Sci Total Environ*. 532:14-19.

Benner R. 2002. Chemical composition and reactivity. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Elsevier, USA. 59–85.

Blewett TA, Dow EM, Wood CM, McGeer JC, Smith DS. 2018. The role of dissolved organic carbon concentration and composition on nickel toxicity to early life-stages of the blue mussel *Mytilus edulis* and purple sea urchin *Strongylocentrotus purpuratus*. *Ecotoxicol Environ Saf.* 160:162-170.

Blewett TA, Leonard EM. 2017. Mechanisms of nickel toxicity to fish and invertebrates in marine and estuarine waters. *Environ Pollut.*223:311-322.

Blewett TA, Smith DS, Wood CM, Glover CN. 2016. Mechanisms of nickel toxicity in the highly sensitive embryos of the sea urchin *Evechinus chloroticus*, and the modifying effects of natural organic matter. *Environ Sci Technol.* 50:1595-1603.

Blewett TA, Wood CM. 2015. Salinity-Dependent Nickel Accumulation and Oxidative Stress Responses in the Euryhaline Killifish (*Fundulus heteroclitus*). *Arch Environ Contam Toxicol.* 68:382-394.

Blust R, Baillieul M, Decleir W. 1995. Effect of total cadmium and organic complexing on the uptake of cadmium by the brine shrimp, *Artemia franciscana*. *Mar Biol.* 123:65-73.

Boyden CR. 1975. Distribution of some trace metals in Poole Harbour, Dorset. *Mar Pollut Bull.* 6:180-187.

Chau YK, Kulikovsky-Cordeiro OTR. 1995. Occurrence of nickel in the Canadian environment. *Environ Rev.* 3:95-120.

Chen W, Sherman SR, McGeer JC, Smith DS. Determining the concentration of free nickel ions in seawater by ion exchange technique: validation and application. *Manuscript in progress.*

Chester R, Stoner JH. 1974. The distribution of zinc, nickel, manganese, cadmium, copper, and iron in some surface waters from the world ocean. *Mar Chem.* 2:17-32.

Cooper CA, Nasir R, Mori J, McGeer JM, Smith DS. Influence of dissolved organic carbon concentration and source on chronic 7-day Ni toxicity to the mysid, *Americamysis bahia*. *Manuscript in progress.*

Crémazy A, Brix KV, Wood CM. 2018. Using the Biotic Ligand Model framework to investigate binary metal interactions on the uptake of Ag, Cd, Cu, Ni, Pb and Zn in the freshwater snail *Lymnaea stagnalis*. *Sci Total Environ.* 647:1611-1625.

Cumberland S, Bridgeman J, Baker A, Sterling M, Ward D. 2012. Fluorescence

spectroscopy as a tool for determining microbial quality in potable water applications. *Environ Technol.* 33:687-693.

DeForest DK, Schlekat CE. 2013. Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms. *Integr Environ Assess Manag*. 9:580-589.

DePalma SGS. 2009. Characterization of dissolved organic matter and reduced sulfur in coastal marine and estuarine environments: Implications for protective effects on acute copper toxicity. Unpublished masters dissertation. University of Waterloo, Waterloo, Ontario. pp. 175.

De Schamphelaere KAC, Janssen CR. 2004. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environ Toxicol Chem.* 23:1365-1375.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem*. 20:2383-2396.

Dixon DG, Sprague JB. 1981. Copper bioaccumulation and hepatoprotein synthesis during acclimation to copper by juvenile rainbow trout. *Aquat Toxicol*. 1:69-81.

Dow E. 2017. Quantifying the interactions of nickel with marine dissolved organic matter using fluorescence. Unpublished masters dissertation. Wilfrid Laurier University, Waterloo, Ontario. pp. 91.

Eisler R. 1998. Nickel hazards to fish, wildlife, and invertebrates: A synoptic review. *Biol Sci Rep*. 34:95.

Environment Canada (EC). 2005. Guidance document on statistical methods for environmental toxicity tests. Environment Canada, method development and

application section. Ottawa, ON. Report EPS 1/RM/46. June 2007 amendments. pp 241.

Environment Canada (EC). 2014. Reference method for measuring the toxicity of contaminated sediment to embryos and larvae of echinoids (sea urchins or sand dollars). Report EPS 1/RM/58. Science and Technology Branch Ottawa, Ontario. (July):1-91.

Federal Register. 1999. Notice of Intent to Revise Aquatic Life Criteria for Copper, Silver, Lead, Cadmium, Iron and Selenium. USEPA 64 (October): 58409-58410.

Fortin C, Campbell PGC. 1998. An ion-exchange technique for free-metal ion measurements (Cd^{2+} Zn^{2+}): Applications to complex qqueous media. *Int J Environ Anal Chem*. 72:173-194.

George SG, Coombs TL. 1977. The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. *Mar Biol.* 268:261-268.

Gissi F, Stauber JL, Binet MT, Golding LA, Adams MS, Schlekat CE, Garman ER, Jolley DF. 2016. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. *Environ Pollut*. 218:1308-1323.

Glover CN, Wood CM. 2005. Physiological characterisation of a pH- and calcium-dependent sodium uptake mechanism in the freshwater crustacean, *Daphnia magna*. *J Exp Biol*. 208:951.

Hardin J, Coffman JA, Black SD, McClay DR. 1992. Commitment along the dorsoventral axis of the sea urchin embryo is altered in response to $NiCl_2$. *Development*. 116:671-685.

Ho KT, Kuhn A, Pelletier MC, Hendricks TL, Helmstetter A. 1999. pH dependent toxicity of five metals to three marine organisms. *Environ Toxicol*. 14:235-240.

Hunt JW, Anderson BS, Turpen SL, Englund MA, Piekarski W. 1997. Precision and sensitivity of a seven-day growth and survival toxicity test using the west coast marine mysid crustacean *Holmesimysis costata*. *Environ Toxicol Chem*. 16:824-834.

Hunt JW, Anderson BS, Phillips BM, Tjeerdema RS, Puckett HM, Stephenson M, Tucker DW, Watson D. 2002. Acute and chronic toxicity of nickel to marine organisms: implications for water quality criteria. *Environ Toxicol Chem*. 21:2423-2430.

Landner L, Reuther R. 2005. Metals in society and in the environment. Environ Pollut. 1-9.

Lorenzo JI, Nieto O, Beiras R. 2006. Anodic stripping voltammetry measures copper bioavailability for sea urchin larvae in the presence of fulvic acids. *Environ Toxicol Chem*. 25:36-44.

Luider CD, Crusius J, Playle RC, Curtis PJ. 2004. Influence of natural organic matter source on copper speciation as demonstrated by Cu binding to fish gills, by ion selective electrode, and by DGT gel sampler. *Environ Sci Technol*. 38:2865-2872.

Lussier SM, Gentile JH, Walker J. 1985. Acute and chronic effects of heavy metals and cyanide on *Mysidopsis bahia* (Crustacea:Mysidacea). *Aquat Toxicol*. 7:25-35.

Lussier SM, Kuhn A, Comeleo R. 1999. An evaluation of the seven-day toxicity test with *Americamysis bahia* (formerly *Mysidopsis bahia*). *Environ Toxicol Chem*. 18:2888-2893.

McFarlane MB, Gilly WF. 1998. State-dependent nickel block of a high-voltage-

activated neuronal calcium channel. *J Neurophysiol*. 80:1678-1685.

McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Anderson DT. 2001. Spectroflourometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol Oceanogr*. 46:38-48.

Meyer JS, Santore RC, Bobbitt JP, Debrey LD, Boese CJ, Paquin PR, Allen HE, Bergmann HL, Ditoro DM. 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environ Sci Technol*. 33:913-916.

Nadella SR, Fitzpatrick JL, Franklin N, Bucking C, Smith S, Wood CM. 2009. 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. *Comp Biochem Physiol - C Toxicol Pharmacol*. 149:340-348.

Nadella SR, Tellis M, Diamond R, Smith S, Bianchini A, Wood CM. 2013. Toxicity of lead and zinc to developing mussel and sea urchin embryos: Critical tissue residues and effects of dissolved organic matter and salinity. *Comp Biochem Physiol - C Toxicol Pharmacol*. 158:72-83.

Niyogi S, Wood CM. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ Sci Technol*. 38:6177-6192.

Pagenkopf GK. 1983. Gill surface interaction model for trace-metal toxicity to fishes: Role of complexation, pH, and water hardness. *Environ Sci Technol*. 17:342-347.

Paquin PR, Di Toro DM, Santore RC, Trivedi D, Wu KB. 1999. A Biotic ligand model of the acute toxicity of metals. 3. Application to fish and daphnia exposure to

silver. In: Integrated approach to assessing the bioavailability and toxicity of metals in surface waters and sediments. EPA-822-E-99-001. US EPA, Washington, DC. pp. 3-59-3-102.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F. 2002. The biotic ligand model: a historical overview. *Comp Biochem Physiol C Toxicol Pharmacol*. 133:3-35.

Paquin PR, Santore RC, Wu KB, Kavvadas CD, Di Toro DM. 2000. The biotic ligand model: A model of the acute toxicity of metals to aquatic life. *Environ Sci Policy*. 3:S175-S182.

Phillips BM, Nicely PA, Hunt JW, Anderson BS, Tjeerdema RS, Palmer SE, Palmer FH, Puckett HM. 2003. Toxicity of cadmium-copper-nickel-zinc mixtures to larval purple sea urchins (*Strongylocentrotus purpuratus*). *Bull Environ Contam Toxicol*. 70:592-599.

Phinney JT, Bruland KW. 1994. Uptake of lipophilic organic Cu, Cd, and Pb complexes in the coastal diatom *Thalassiosira weissflogii*. *Environ Sci Technol*. 28:1781-1790.

Playle RC, Gensemer RW, Dixon DG. 1992. Copper accumulation on gills of fathead minnows: Influence of water hardness, complexation and pH of the gill micro-environment. *Environ Toxicol Chem*. 11:381–391.

Playle RC, Dixon DG, Burnissn K. 1993. Copper and cadmium binding to fish gills: Estimates of metal-gill stability constants and modelling of metal accumulation. *Can J Fish Aquat Sci*. 50:2678-2687.

Pyle G, Couture P. 2012. Nickel. In: Wood CM, Farrell AP, Brauner CJ (eds)

Homeostasis and toxicology of essential metals. *Fish Physiol.* 31A: 254-281.

Ragsdale SW. 1998. Nickel biochemistry. *Curr Opin Chem Biol*. 2:208-215.

Reck BK, Müller DB, Rostkowski K, Graedel TE. 2008. Anthropogenic nickel cycle: Insights into use, trade, and recycling. *Environ Sci Technol*. 42:3394-3400.

Sadiq M. 1989. Nickel sorption and speciation in a marine environment. *Hydrobiologia*. 176-177:225-232.

Santore RC, Di Toro DM, Paquin PR, Allen HE, Meyer JS. 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to accute copper toxicity in freshwater fish and *Daphnia*. *Environ Toxicol Chem*. 20:2397-2402.

Santore RC, Ryan AC, Kroglund F, Rodriguez PH, Stubblefield WA, Cardwell AS, Adams WJ, Nordheim E. 2018. Development and application of a biotic ligand model for predicting the chronic toxicity of dissolved and precipitated aluminum to aquatic organisms. *Environ Toxicol Chem*. 37:70-79.

Schwartz ML, Curtis PJ, Playle RC. 2004. Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem*. 23:2889-2899.

Silva C, Yáñez E, Martín-Díaz ML, Riba I, Delvalls TA. 2013. Integrated ecotoxicological assessment of marine sediments affected by land-based marine fish farm effluents: Physicochemical, acute toxicity and benthic community analyses. *Ecotoxicology*. 22:996-1011.

Smith KS, Balistrieri LS, Todd AS. 2015. Using biotic ligand models to predict metal toxicity in mineralized systems. *Appl Geochemistry*. 57:55-72.

Stockdale A, Tipping E, Hamilton-Taylor J, Lofts S. 2011. Trace metals in the open

oceans: Speciation modelling based on humic-type ligands. *Environ Chem*. 8:304-319.

Stockdale A, Tipping E, Lofts S. 2015. Dissolved trace metal speciation in estuarine and coastal waters: Comparison of WHAM/Model VII predictions with analytical results. *Environ Toxicol Chem*. 34:53-63.

Tait TN. 2013. Determination of copper speciaion, bioavailability and toxicity in saltwater environments. Unpublished masters dissertation. Wilfrid Laurier University, Waterloo, Ontario. pp. 208.

Tellis MS, Lauer MM, Nadella S, Bianchini A, Wood CM. 2014. The effects of copper and nickel on the embryonic life stages of the purple sea urchin (*Strongylocentrotus purpuratus*). *Arch Environ Contam Toxicol*. 67:453-464.

Thurman EM. 1985. Organic geochemistry of natural waters. *Usgs*. 5-110.

United States Environmental Protection Agency. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms fourth edition october 2002. EPA-821-R-02-014 Office of Water, Washington, DC. (October):1-350.

Uthicke S, Schaffelke B, Byrne M. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecol Monogr*. 79:3-24.

Wang Y, Zhang L, Meng F, Zhou Y, Jin X, Giesy JP, Liu F. 2015. Improvement on species sensitivity distribution methods for deriving site-specific water quality criteria. *Environ Sci Pollut Res*. 22:5271-5282.

Wells ML, Smith GJ, Bruland KW. 2000. The distribution of colloidal and particulate

bioactive metals in Narragansett Bay, RI. *Mar Chem*. 71:143-163.

Wilt FH. 1999. Matrix and mineral in the sea urchin larval skeleton. *J Struct Biol*. 126:216-226.

Winner RW, Gauss JD. 1986. Relationship between chronic toxicity and bioaccumulation of copper, cadmium and zinc as affected by water hardness and humic acid. *Aquat Toxicol*. 8:149-161.

Wood CM, Al-Reasi HA, Smith DS. 2011. The two faces of DOC. *Aquat Toxicol*. 105:3-8.

Worms IAM, Wilkinson KJ. 2008. Determination of Ni^{2+} using an equilibrium ion exchange technique: Important chemical factors and applicability to environmental samples. *Anal Chim Acta*. 616:95-102.

Zhao CM, Campbell PGC, Wilkinson KJ. 2016. When are metal complexes bioavailable? *Environ Chem*. 13:425-433.