Use of Non-linear Models Based on Saturation Kinetics to Determine Chronic Co, Se or Zn Toxicity for Either Exposure or Body Burden in *Hyalella azteca*

by

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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.

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ABSTRACT

Water chemistry can influence the bioavailability, and therefore the toxicity, of metals and other elements. Water chemistry measurements have been incorporated into many water guidelines for the concentration of metals in Canada. However, the application of these guidelines requires site-specific measurements of metal concentrations and can also require the measurement of water chemistry parameters. The amphipod *Hyalella azteca* has been used extensively in toxicity testing, and the whole-body concentration of an element in the organism can be related to toxic effects for some elements. The whole-body concentration is, therefore, assumed to be proportional to the concentration of the element at the site of toxic action. However, it is unknown if the water chemistry variables that influence element bioavailability and toxicity will also influence the whole-body concentration that is linked to a toxic effect. If the whole-body concentration of an element in *H. azteca* causing toxicity is not influenced by water chemistry conditions, then it could be a useful site assessment tool.

Several trace metals, including cobalt (Co) and zinc (Zn), as well as the element selenium (Se), are essential to many organisms for metabolic processes. However, above a certain threshold these elements will have toxic effects. Mortality is often the most sensitive toxic endpoint in *H. azteca*, so the acute and chronic of mortality of *H. azteca* were determined in organisms exposed to a range of concentrations of cobalt, selenium, or zinc in soft water exposure media that varied in pH, alkalinity, or dissolved organic carbon.

Non-linear regression models based on saturation kinetics were used to model mortality in both acute and chronic exposures, and to model bioaccumulation in chronic exposures in relation to exposure or body concentration of an element. From these models, lethal exposure concentrations and lethal body concentrations were determined for each element and each water chemistry scenario. Dissolved organic carbon (DOC) was protective against chronic exposure-based Co and Zn toxicity, but increased the toxicity of Se. The patterns of uptake of Se were also influenced by DOC, as well as pH and alkalinity. Concentrations of DOC greater than 5 mg L^{-1} influenced the uptake pattern of Co, but the lethal body concentrations of Co were not influenced by water chemistry. The lethal body concentrations of Zn in *H. azteca* were similarly not influenced by water chemistry, whereas concentrations of DOC greater than 5 mg L^{-1} decreased the Se body burden that caused mortality. The bioaccumulation models that were developed could predict observed body concentrations within two-fold of the actual value over 87% of the time for all elements.

The resulting non-linear regression models and lethal concentrations were compared to studies conducted in hard water that had similar data. Increased hardness was protective against exposure-based toxicity of all elements tested. Lethal body concentrations for each metal exposure were consistent regardless of the water hardness. The existing bioaccumulation models were not appropriate to model soft water data. However, the existing mortality models for Co and Zn were robust enough to estimate the lethal exposure and lethal body concentrations. Even though the existing Se model could predict lethal concentrations from the soft water data, there did not appear to be a consistent Se body concentration associated with mortality.

This research showed that non-linear models can be used to describe mortality and bioaccumulation of Co and Zn in many different water chemistry scenarios and predict both lethal water and lethal body concentrations. In addition, it was determined that whole-body concentration is a good predictor of mortality caused by Co or Zn exposure, regardless of water chemistry. The body concentration of Se causing mortality varies with water chemistry, so it is not advisable to use any Se model for toxicity prediction.

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LIST OF ABBREVIATIONS

°C	Degrees Celsius
µS/cm	Microsieverts per centimetre
µg g^{-1}	Microgram per gram
µg L^{-1}	Microgram per litre
µmol L^{-1}	Micromole per litre
ACS	American Chemical Society
Ag	Silver
Alk	Alkalinity
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
BCF	Bioconcentration factor
BLM	Biotic Ligand Model
C	Carbon
Ca	Calcium
$CaCO_3$	Calcium carbonate
Cb	Whole-body concentration
C_{bk}	Background concentration, control concentration
CCME	Canadian Council of Ministers of the Environment
Cd	Cadmium
CI	Confidence interval
Cl	Chloride
Co	Cobalt
CO_2	Carbon dioxide
CO_3	Carbonate
CRM	Certified reference material
Cu	Copper
C_b	Total body concentration
C_w	Concentration of metal in water
CWQG	Canadian Water Quality Guidelines
d.w.	Dry weight
D/W ratio	Dry weight to wet weight ratio

DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EDTA	Ethylenediaminetetraacetic acid
GFAAS	Graphite furnace atomic absorption spectroscopy
H	Hydrogen
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric acid
Hg	Mercury
HNO_3	Nitric Acid
IC10	Critical concentration associated with 10% growth inhibition
IC25	Critical concentration associated with 25% growth inhibition
IC50	Critical concentration associated with 50% growth inhibition
ISO	International Organization for Standardization
K	Potassium
K	Half saturation constant, concentration of metal in water where C_{TB} is halfway between C_{bk} and max
K_D	Partition coefficient
KOH	Potassium Hydroxide
K_b	Half-saturation constant, where the concentration of metal in organism's body associated with mortality is half the maximum
K_w	Half-saturation constant, where the concentration of metal in water associated with mortality is half the maximum
L	Litre
LBC10	The whole-body concentration of metal associated with 10% mortality
LBC25	The whole-body concentration of metal associated with 25% mortality
LBC50	The whole-body concentration of metal associated with 50% survival of unexposed controls
LC10	Concentration of metal in water associated with 10% mortality
LC25	Concentration of metal in water associated with 25% mortality
LC50	Concentration of metal in water associated with 50% survival of unexposed controls
m	Mortality rate

m'	Control mortality rate
max	Maximum above-background whole-body concentration
max/*K*	Ratio of maximum accumulated concentration to exposure concentration where accumulation is half maximum
MEAM	Metal effects addition model
Mg	Magnesium
mg kg^{-1}	Milligram per kilogram
mg L^{-1}	Milligram per litre
N	Number
Na	Sodium
$NaHCO_3$	Sodium bicarbonate
n.d.	Not determined
NICA	Non-ideal competitive adsorption
NLET	National Laboratory for Environmental Testing
nmol g^{-1}	Nanomoles per gram
nmol L^{-1}	Nanomole per litre
OH	Hydroxide
p	Probability
p.a.	Practical grade
Pb	Lead
Ph Eur.	European Pharmacopeia (also EP)
QA	Quality assurance
QC	Quality control
r^2	Coefficient of determination
reag.	Reagent
SAM	Standard Artificial Media
Se	Selenium
SeO_3^{2-}	Selenite
SeO_4^{2-}	Selenate
SO4	Sulfate
TKTD	Toxicokinetic Toxicodynamic
TMDW	Trace metals in drinking water

TOC	Total Organic Carbon
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
W	Wet weight
W'	Control wet weight
WHAM	Windermere Humic Aqueous Model
Zn	Zinc

CHAPTER 1
General Introduction

1.1 INTRODUCTION

There are several factors that complicate the development of site-specific water quality guidelines or tissue/body concentration guidelines for protection of aquatic life from toxic effect due to exposure to metals or other elements. The effects of physicochemical and biological variables and interactions are complex, and the models used to describe and predict toxicity are regularly being updated and improved. The elements of interest for this study are two transition metals, cobalt and zinc, as well as one non-metal, selenium. These elements were selected for study because when this study commenced, the guidelines for these elements were out of date (Se, Zn) or did not have a federal water quality guideline in Canada (Co). Water quality guidelines for elements indicate concentrations of elements in an aquatic system that are considered safe (Canadian Council of Ministers of the Environment, 2001). However, the fraction of the element that can be taken up by an organism (bioavailable) from the environment varies greatly with environmental conditions, including pH, organic matter, water hardness, and many other biotic and abiotic factors (Niyogi et al., 2008). Different species will have a range of toxic effects when exposed to an element (Mager et al., 2011), as elements will not only be taken up into organisms at different rates and through different routes, but they will also be metabolized, stored, and eliminated in diverse ways (Rainbow & Luoma, 2011). Organisms also have requirements and background concentrations for elements that are essential for life. There will often be a background concentration of non-essential elements present in an organism, as well. However, at a concentration above specific thresholds, there will be toxic effects. These toxic effects may change depending on exposure time and concentration and if there are other elements present, as well as the physicochemical and biological factors already stated (Niyogi & Wood, 2003).

This chapter will review the general chemistry, biology, toxicology, availability, and environment fates of Co, Se, and Zn. In addition, this chapter will discuss physicochemical factors that can alter the bioavailable fraction of these elements in the environment. It will also present exposure-based and whole-body concentration-based models that have been developed to model toxicity of trace elements.

1.2 ENVIRONMENTAL SOURCES, CONCENTRATIONS, AND FATE

1.2.1 Cobalt

Cobalt (Co) is the 33rd most abundant element on earth, composing 0.0025% of the Earth's crust (Smith et al., 1981; Watt, 2007), which is equivalent to about 20 – 25 mg kg^{-1} (Smith & Carson, 1981). Within the crust, Co is not found as a free metal, but combined with sulfides, arsenides, hydrates, and oxides to form over 70 different minerals. Cobalt can naturally be released into the environment through erosion, volcanic eruptions, and biogenic emissions (Kim et al., 2006; Lison, 2007). Cobalt is often associated with copper and nickel in the crust (Kim et al., 2006) and the majority of Co mining is a secondary product of other metal mining (Shedd et al., 2017). As of 2015, 50% of the world's Co was mined in the Democratic Republic of Congo, with twenty other countries producing less than 6% of the 126 kt mined globally (Shedd et al., 2017). Although Co is relatively scarce and, therefore, expensive, it commonly used as a cathode component in lithium-ion batteries. The demand for Li-ion batteries has increased due to its use in portable electronics and electric cars (Gulbinska, 2014; Shedd et al., 2017). Properties of Co that make it useful for technological applications include ferromagnetism, hardness, low electrical conductivity, and high melting point. Other anthropogenic sources of Co include the burning of fossil fuels, sewage sludge, and the processing of Co alloys (Lison, 2007).

In pristine environments, Co concentrations are less than 1 μg L^{-1} in freshwater, 1 ng m^{-3} in air as particulate matter, and 20 mg kg^{-1} in fresh water sediments (Smith and Carson, 1981). At sites close to historical silver mining operations, total Co concentrations in freshwater were reported greater than 2000 μg L^{-1} in Ontario (Environment Canada 2017) and greater than 600000 μg L^{-1} in Idaho (ATSDR, 1995; Kim et al., 2006). Soil concentrations range from 1 to 40 mg kg^{-1} in the United States (Kim et al., 2006). Cobalt will sorb to particles in the soil and sediment, and these two locations are major sinks for Co in the environment. Several variables affect the background concentration of Co in soil, including pH, organic matter, and clay mineral content (Hamilton et al., 1994). Cobalt will also bind strongly to organic matter (Kim et al., 2006).

1.2.2 Selenium

Selenium is widely, but unevenly, distributed in the environment as a naturally occurring component of organic and inorganic compounds (Sharma et al., 2014). Concentrations in the

earth's crust range from 0.05 – 0.09 μg g^{-1}; however, Se concentrations greater than 1000 μg g^{-1} can co-occur with sulfur and uranium, as well as in coal deposits (Högberg & Alexander, 2007). Selenium concentrations in soils also have a wide range, from 0.01 mg kg^{-1} to greater than 1000 mg kg^{-1} (Sharma et al., 2014). In freshwater systems, concentrations of Se can range from less than 0.1 μg L^{-1} to greater than 400 μg L^{-1} (Högberg & Alexander, 2007).

A major anthropogenic source of Se is coal mining, and its subsequent processing and combustion. Se waste produced during power generation from coal can have Se concentrations enriched over 1000 times compared to the surrounding soil (Lemly, 2004). This waste is stored in piles or tailing ponds, and Se can leach into ground or surface water (Lemly, 2004). Other anthropogenic sources include the refining of crude oil, mining, and irrigated agriculture (Young et al., 2010).

1.2.3 Zinc

Zinc is the 25th most abundant element in the earth's crust with an average content of 78 mg kg^{-1} (Sandstead & Au, 2007). In natural environments, Zn concentrations will vary greatly. Average background concentrations range from 87 to 277 ng Zn L^{-1} in the Great Lakes (Nriagu et al., 1995), but anthropogenic inputs of Zn have increased background concentrations in water over 1000 times at some mine sites (CCME, 2016). However, Zn will generally partition into the sediment in aquatic systems, and sediment concentrations can exceed 1000 mg kg^{-1} (WHO, 2001). Depending on the parent material, concentrations in soil can range from 10 mg kg^{-1} to over 100 mg kg^{-1}. After iron, aluminum, and copper, Zn is the fourth most used metal in the world. Its applications include galvanizing other metals, the production of brass and alloys, fertilizers, and batteries (WHO, 2001).

1.3 CHEMISTRY AND SPECIATION

1.3.1 Cobalt

Cobalt is a transition metal with atomic number 27, an atomic weight of 58.93, and three oxidation states Co(0), Co(II), and Co(III). Co has. At room temperature (20°C), Co(0) is a silver grey metallic solid with a density of 8.9 g cm^{-1} and a melting point of 1493°C (Kim et al., 2006). Co(0) is not soluble in water; however, several Co(II) compounds are soluble in water including

$Co(NO_3)_2$ and $CoCl_2$. $CoCl_2$ is the species of Co used in the current study and its solubility in water is 450 g L^{-1}. $CoCl_2$ is a blue salt at room temperature (Kim et al., 2006).

Partition coefficients (K_d) describe the ratio of sorbed metal (in mg kg^{-1}) to dissolved metal (in mg L^{-1}). These coefficients are often reported as log K_d. The soil/soil water K_d is 2.1 L kg^{-1}, the sediment/porewater Kd is 3.1 L kg^{-1}, the dissolved organic carbon/inorganic solution K_d is 3.8 L kg^{-1}, and the suspended particulate matter (SPM)/water K_d is 4.7 L kg^{-1} (USEPA, 2005). In a literature review by the USEPA (2005), Co was among the elements with the lowest K_d values, with only arsenic having less sorbed metal to sediment and nickel and arsenic sorbing less to SPM (USEPA, 2005). Co will bind to a biological ligand (a fish gill) more strongly than it will bind to CO_3^{2-} or OH^- (Richards & Playle. 1998). The logarithms of the equilibrium binding constants (log K) are 5.1, 3.2, and 4.8, respectively. The log K for Co to Luther Marsh dissolved organic matter (DOM) is also 5.1 (Richards & Playle, 1998). Copper (Cu) and cadmium (Cd) both bind more strongly to a biological ligand and to DOM (Playle, 1998). With stronger binding to a biological ligand, it is more likely the metal will be taken up by the organism and will be able to outcompete other ions that have lower a log K_d for uptake. Stronger binding to DOM could lead to a more protective effect from toxicity, as the metal will be less available in solution for uptake.

1.3.2 Selenium

Selenium is a non-metallic element with atomic number 34 and oxidation states including -2, 0, +4, and +6. There are three different Se allotropes (physical forms) that form under different conditions. The most common allotrope is grey Se, which is the only allotrope that exhibits some metalloid behaviour (Di Gregario, 2008; Young et al., 2010). Se is sometimes considered a metalloid; however, there is no universally accepted definition of metalloid. Selenium can act as an electron donor, like a metal, but also as an electron accepter (Hemat, 2004). Metals will form cations in aqueous solution, whereas Se will form oxyanions (Young et al., 2014). Partition coefficients (K_d) for Se are less than Co for soil/soil water at 1.3 L kg^{-1} and for DOC/inorganic solution at 2.0 L kg^{-1}. However, the sediment/porewater K_d is 3.6 L kg^{-1}, meaning that more Se will sorb to sediment than Co (USEPA, 2005).

In aquatic systems, inorganic species of Se are typically present as Se(VI), Se(IV), or Se(0). Se(VI) and Se(IV) form the oxyanions selenate (SeO_4^{2-}) or selenite (SeO_3^{2-} or $HSeO_3^-$), respectively (Cai, 2002). Selenate will form in strong oxidizing conditions, whereas selenite will be the dominant species in mildly oxidizing conditions. As pH increases in mildly oxidizing

conditions, the concentration of SeO_3^{2-} will also increase, while the concentration of $HSeO_3^-$ decreases (Sharma et al., 2014). Depending on the redox environment and the pH, there are over 10 different inorganic species that can form (Saji & Lee, 2013). Se(-II) can bind to amino acids and proteins, forming organoselenium compounds (Besser et al., 1993). This transformation occurs when inorganic Se is taken up by primary producers (Fan et al., 2002).

1.3.3 Zinc

Zinc is a transition metal with atomic number 30 and an atomic weight of 65.38. Zn has a melting point of 420°C and a boiling point of 908 °C (Sandstead & Au, 2007). At room temperature, Zn is a bluish-white solid with a density of 7.13 g cm^{-1}. The oxidation state of Zn is most commonly II and is present as the cation Zn^{2+} in aquatic systems. The Zn^{2+} ion can form bonds with chloride, sulfate, nitrate, or hydroxide anions; however, zinc hydroxides have low solubility in water (Bradl, 2005).

Compared with Co and Se, more Zn will bind to soil, sediment, DOC, and SPM, as its K_d are 2.7, 4.1, 5.1, and 5.1 L kg^{-1}, respectively. With higher K_d coefficients, a greater concentration of Zn will be taken out of solution by binding to organic and inorganic material (USEPA, 2005). At the site of uptake on *D. magna*, Zn will bind more strongly than Ca^{2+}, Mg^{2+}, and Na^+, with binding constants of 3.22, 2.69, and 1.90, respectively. However, H^+ binding strength is 5.77, which is similar to the Zn binding strength of 5.31 (Heijerick et al., 2005).

1.3.3.4 General complexation of metals

In addition to the binding constants and partition coefficients, variables like the concentration of complexing ligands can influence the speciation of Co and Zn. Complexes result from the formation of covalent bonds between a ligand and metal cation through a ligand-exchange reaction (Morel, 1983; Stumm and Morgan, 1996). Free metal ions are often associated with the surrounding water molecules through chemical bonding. Ligands that can replace water have a pair of free electrons and include Cl^-, NO_3^-, CO_3^{2-}, SO_4^2, PO_4^{3-} (Morel, 1983). The concentration of inorganic ligands that metals form complexes with is unaffected by complexation due to the large excess of such ions (Morel, 1983). When physicochemical parameters, such as pH or concentration of dissolved organic matter (DOM), are altered in a way that reduces complexation, there will be an increase in the concentration of the free metal ion (Rainbow & Dallinger, 1993). This species of metal can bind to biological ligands and cause toxicity (Campbell, 1995; Di Toro et al., 2001), although it has been suggested that it is not the

only bioavailable and toxic species of metal (De Schamphelaere et al., 2002; Hoang et al., 2004; Boullemant et al., 2011).

1.4 BIOLOGICAL ROLE AND TOXIC EFFECTS

1.4.1 Cobalt

Cobalt is an essential element for humans, animals, some algal species, and legumes. In humans, the required form is vitamin B_{12}, and both the metallic forms and Co salts can have toxicological effects (Lison, 2007). Such toxicological effects are mainly due to oxidative damage from the formation of reactive oxygen species. Cobalt can also interfere with hypoxia sensing in animal cells (Simonsen et al., 2012).

Major ions (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}) in aquatic systems compete with trace metals for uptake (Luoma & Rainbow, 2008), as channels in the cell membrane are often permeable to more than one type of ion. Ca^{2+} can compete with Co^{2+} for uptake at the gill epithelium and can reduce Co toxicity in fish (Richards & Playle, 1998; Kim et al., 2006). The dimensions of the channel and the interactions the ion has with the channel wall will determine whether an ion can pass through (Simkiss & Taylor, 1995). In the case of Ca^{2+} and Co^{2+}, both ions have the same charge and Co^{2+} has a slightly smaller atomic radius. The relative concentrations of the ions will affect the probability that one type of ion will displace another competing ion (Simkiss & Taylor, 1995). However, Co^{2+} can bind to the gill epithelium more strongly than Ca^{2+}. (Richards & Playle, 1998). Nutritional Co requirements for fish are 0.05 mg kg^{-1} in their diet (Watanabe et al, 1997).

The majority of Co taken up by humans is inorganic, with daily intake ranging from 5 to 50 μg (Lison, 2007), with a total body burden of 1.1 – 1.5 mg (Kim et al., 2016). Intestinal bacteria can then transform this inorganic Co into cobalamin, a component of vitamin B_{12} (Romero, 2017). Additional Co is in muscle and fat tissue, as well as the liver and heart. In animals, Co will be distributed to the liver, kidneys, and spleen after an oral dose (Domingo, 1989). Free Co will precipitate with phosphates and bind non-specifically to a variety of proteins (Lison, 2007). Generally, Co will not accumulate in humans and will be excreted in urine (Romero, 2017).

1.4.2 Selenium

Selenium, a component of antioxidant glutathione peroxidase, is essential in humans and animals (Wantanabe et al., 1997). Organic Se compounds can be absorbed from dietary sources or through inhalation (Högberg & Alexander, 2007). Dietary sources are the main route of

exposure to humans and an average of 14% inorganic and organic Se from food are absorbed in the small intestine (Peters et al, 2016). Some forms of Se can be taken up passively through a Na-mediated carrier mechanism or by competing with methionine (Peters et al., 2016). Selenium can be incorporated into methionine and cystine in the place of sulfur, and selenoproteins contain Se-Cys (Bierla et al., 2016). Selenoproteins are present in mammals and other eukaryotes including some algae, fish, and invertebrates (Gladyshev, 2016). Humans have 25 different selenoproteins, whereas an invertebrate like *C. elegans* only has one.

It is recommended that humans ingest from 40 to 75 μg per day, depending on their geographical location (Peters et al., 2016). Concentrations above and below what is required can lead to diseases and other effects in humans. Selenium deficiency can impair human development and immune function, while high concentrations of Se can lead to selenosis, which has symptoms that include gastroenteritis, fatigue, and nerve damage. Selenium toxicity rarely results in death in humans (Peters, 2016).

Whole-body or tissue Se concentrations as low as 3 $\mu g\ g^{-1}$ can be toxic to fish (Lemly, 1993). The difference between a beneficial and a toxic dietary intake of Se among fish is very small. Inorganic selenium in an aquatic system can be biotransformed by primary producers to form organic Se species, namely selenomethionine, which can bioaccumulate in organisms and biomagnify in the foodweb (Lenz & Lens, 2009). Among invertebrate species there are a wide range of sensitivities and these sensitivities vary with exposure route (DeBruyn and Chapman, 2007). In a flow-through system, the measured 96-h LC50 of sodium selenite to *H. azteca* was 340 $\mu g\ L^{-1}$ (4310 nmol L^{-1}), while the 14-day LC50 was 70 $\mu g\ L^{-1}$ (887 nmol L^{-1}) (Halter et al., 1980). The acute toxicity matches closely to the one-week static test LC50 of 371 $\mu g\ L^{-1}$ (4700 nmol L^{-1}) in moderately hard water (Borgmann et al., 2005). The uptake of Se is reduced at low pH, which could reduce its waterborne toxicity (Yu and Wang, 2002). There is also a wealth of literature that show reproductive toxicity is an important endpoint in many organisms, and that the egg/ovaries are significant locations for Se storage (Hamilton et al., 2004; Adams et al., 2010).

1.4.3 Zinc

Zinc is an element essential for life (Watanabe et al., 1997; Sandstead & Au, 2007), as it is a component of hundreds of enzymes (Sandstead & Au, 2007; Sosa-Torres & Kroneck, 2009). Zn has a role in all six classes of enzymes which include oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Sosa-Torres & Kroneck, 2009). Because of Zn

essentiality, organism have homeostatic regulation of the element. At high concentrations of Zn, the homeostatic pathway can be overwhelmed and metal-binding metallothionein proteins will mediate an Zn toxicity (McRae et al, 2016). Although metallothioneins sequester non-essential and potentially toxic metals like Cd, Hg, and Ag, they also sequester the essential metals Cu and Zn. Metallothioneins can act as an inducible metal buffer, extending the range of Zn that can be tolerated (Mason and Jenkins, 1995). These metals will be bound by the proteins when metal concentrations are high. When concentrations are lower, these metals will be released and used for cellular metabolism (Roesijadi, 1992). If both homeostasis and further regulatory mechanisms are overwhelmed, Zn can have toxic effects.

In 7-day acute toxicity tests, Borgmann et al. (2005) exposed *H. azteca* to Zn in both soft and moderately hard water. When the water hardness was increased, there was a four-fold increase in the LC50, showing that hardness ions (Ca^{2+} and Mg^{2+}) are protective against Zn toxicity. In a 6-week exposure of *H. azteca* to Zn in moderately hard water, the LC50 was 3100 nmol L^{-1} (Borgmann et al., 2004). This lethal concentration was not significantly different from the 7-day Zn LC50. Typically, there would be a difference between acute and chronic lethal concentrations, and these results indicate that Zn concentrations are tightly regulated.

The toxicity of Zn to rainbow trout (*Oncorhynchus mykiss*) can vary in exposures to different water chemistry. Increased Ca^{2+} concentrations caused a 12-fold reduction in both acute and chronic Zn toxicity. There was no significant difference in the acute and chronic lethal concentrations. Increased Mg^{2+}, Na^{+}, and H^{+} also resulted in at least a two-fold reduction in chronic toxicity (De Schamphelaere & Janssen, 2004).

1.5 WATER CHEMISTRY

1.5.1 Dissolved organic carbon (DOC)

Organic matter in aqueous systems is often reported as the concentration of DOC (Morel, 1983). DOC has phenolic and carboxylic groups with negative charges that can bind free metal ions, like Co^{2+} or Zn^{2+}. Humic acid can act as an electron accepter or donor in natural environments and Se can reversibly bind to N-, S-, or O- containing functional groups (Sharma et al., 2014). The two main components of DOC are humic and fulvic acids. Humic acids precipitate at a low pH, unlike fulvic acids (Stumm and Morgan, 1996). Humic acids generally make up 40-99% of the DOC (Morel, 1983), but the percentage and the overall concentration of

DOC depends on the net productivity, production of organic substances by phytoplankton, and the import/export of organic matter (Stumm and Morgan, 1996).

With increasing concentrations of DOC in an aquatic environment, the bioavailability of an element is expected to decrease (Richards et al., 1999). In freshwater, DOC concentrations range from 10^{-1} to 10^{+1} mM C (Morel, 1983; Stumm and Morgan, 1996), with higher concentrations in lakes and streams (Stumm and Morgan, 1996). Freshwater environments can receive organic matter from sources within the aquatic system (autochtonous), such as degraded phytoplankton and macrophyte material. They also have input from the terrestrial environment (allochthonous) via runoff and deposition (Wetzel & Likens, 2000). Natural organic matter (NOM) is organic material that contains many different functional groups that can be either dissolved, particulate, or colloidal. The darker the colour of NOM, the greater the concentration of aromatic groups, which contribute to its protective effect. This NOM typically has an allochthonous origin (Richards et al., 2001; Gheorghiu et al., 2010).

The addition of dissolved organic matter (DOM) reduces the uptake of Cd in *Danio rerio* eggs. In addition, the concentration of DOM is the most important factor in reducing Cd bioavailability (Burnison et al., 2006). Doig and Liber (2006) also noted that DOM reduced Ni toxicity, but the source and fraction of DOM had little to no effect on toxicity or accumulation. The binding of metals to DOM has been the basis for metal speciation models including the Windermere humic aqueous model (WHAM) and the NICA-Donnan model (non-ideal competitive adsorption). These models describe metal/proton binding and interactions with humic and fulvic acids (Tipping, 1998).

1.5.2 pH

Metal bioavailability can either increase or decrease, depending on the pH and the metal. At acidic pHs, a greater proportion of the dissolved metal concentration will be in the free ion form, which is often considered more bioavailable and therefore has the potential to be more toxic (Campbell, 1995). However, at an acidic pH, free metal ions can compete with the greater concentrations of hydrogen ions for binding sites and this competition could reduce the toxic effects (Simkiss and Taylor, 1995). At higher pH, with less H^+ competition, there can be more uptake and toxicity of the metal. However, there are many instances where H^+ competition is a factor in reducing the uptake and/or toxicity of some metals including Ni in *Lemna minor* (Gopalaapillai et al., 2012), Cd and Zn in *Chironomus riparius* (Bervoets & Blust, 2000), and Cu *in P. promelas* (Erickson et al., 1996). It was concluded that speciation, and not competition

between H^+ and Pb^{2+}, explained Pb toxicity in *C. dubia* chronic toxicity tests (Esbaugh et al., 2012). At higher pH, carbonate and other anionic compounds can form, which will reduce the concentration of free metal ions (Parametrix, 1995). Metals can also be displaced from organic matter by H^+, increasing the free metal ion concentration and bioavailability (Playle et al., 1993a).

1.5.3 Alkalinity

Alkalinity is a solution's acid neutralizing capacity in a titration where the endpoint is the CO_2 equivalence point (Morel, 1983). The most abundant weak acid in natural waters is carbon dioxide and with it, the entire carbonate system is responsible for the alkalinity of natural waters (Morel, 1983). Carbonate enters an aquatic system from the weathering of carbonate rock and through CO_2 dissolving in water. Aquatic plants also release carbon dioxide during respiration, which can change the pH of the water in their immediate environment (Morel 1983). The carbonate cycle is linked to the carbon cycle, as predators and decomposers can reoxidize organic material to CO_2 or to carbonate (Bowen, 1979).

With increased alkalinity, there is a greater concentration of anions that can complex metal ions, so as alkalinity increases, it is expected that bioavailable metal concentrations should decrease. However, some complexed metal species may contribute to toxicity (Richards & Playle, 1998; Santore et al., 2002; Clifford et al., 2009). Alkalinity did not prevent gill accumulation of Cd in rainbow trout; however, toxicity was significantly higher when alkalinity was increased three-fold from the control (Niyogi et al., 2008). They suggested that $CdHCO_3^+$ may contribute to toxicity (Niyogi et al., 2008). Mager et al. (2011) saw a protection effect from alkalinity in acute toxicity tests in *P. promelas*, as high alkalinity prevented mortality. The protective effect was not evident for *C. dubia* (Mager et al., 2011).

1.5.4 Water Hardness

Hardness is a measure of the concentrations of calcium and magnesium in an aquatic system. Depending on pH, these two elements can be cations with a charge of 2^+ or complexed with ions such as CO_3^{2-} or OH^-. Some metals currently have hardness-dependent water quality guidelines in Canada, including Cu and Pb, as their effect on organisms varies with water hardness (CCME, 1999). As cations, Ca^{2+} and Mg^{2+} are of a similar size and charge as other metal ions and can compete and block their uptake into an organism (Campbell et al., 1995).

Increased water hardness leads to greater competition of metals with Ca^{2+} and Mg^{2+} when interacting with membrane ions.

Hardness ions (typically Ca^{2+}) influence uptake and toxicity of several metals including Ni in *H. azteca, O. mykiss, and P. promelas* (Deleebeeck, et al., 2007; Schroeder et al., 2010), Cd in *D. magn*a (Tam & Wang, 2012), and Zn in O. mykiss (Alsop & Wood, 1999). Borgmann et al. (2005) determined that pH of the water affected the competition that Ca^{2+} and Na^{+} had with the free ion of Cu. Hydrogen ions can also compete with Cu for binding sites (Playle et al., 1993b; Borgmann et al., 2005). Both Ca^{2+} and Na^{+} are competing cations yet have varying degrees of protectiveness at different pH.

1.6 WATER QUALITY GUIDELINES

1.6.1 Canada

The Canadian Council of Ministers of the Environment is an inter-governmental group of provincial environment ministers. This council includes a water management committee that develops guidelines for concentrations of many potential toxicants in aquatic systems, including metals and other elements (CCME, 2014). Many Canadian water quality guidelines (CWQG) for the protection of aquatic life in freshwater were developed in 1987 from earlier research (Canadian Council of Ministers of the Environment, 2008), although some of the guidelines have been updated in recent years (CCME, 2016). Copper, lead, and nickel guideline concentrations are calculated from different equations when water hardness is between 82 and 180 mg L^{-1} $CaCO_3$ for Cu, greater or less than 60 mg L^{-1} as $CaCO_3$ for Pb, and when water hardness is between 60 and 180 mg L^{-1} as $CaCO_3$ (CCME 1987). In addition, there are several procedures to modify these guidelines to water quality objectives for specific sites when there is concern that the guideline value might be over- or under-protective given the unique physicochemical and/or biological conditions at a specific site (CCME, 2003). Federal Environmental Quality Guidelines (FEQG) have been created for elements, including cobalt, that do not have a CCME recommended benchmark (Environment and Climate Change Canada, 2017). The FEQG for Co was developed from a species sensitivity distribution that incorporated toxicity to different species, including fish, invertebrates, and plants. This guideline also includes water hardness as a variable and a guideline can be calculated for hardness ranging from 52 to 396 mg L^{-1} as $CaCO_3$ using the equation in Table 1.1. Over this hardness range, the guideline will range from 0.78 to 1.80 μg L^{-1} (ECCC, 2017). The CWQG for Se has not been updated since 1987 and only

considers inorganic species of Se in its derivation (CCME, 1987). An updated CWQG for Zn is in draft form (CCME, 2016). The guideline for chronic exposure to Zn can be calculated over a range of water hardness from 28.2 to 190 mg L^{-1} as $CaCO_3$ and from pH 6.5 to 8.13 using the formula in Table 1.1. At a hardness of 50 mg L^{-1} as $CaCO_3$ and pH 7.5 the guideline is 12 µg L^{-1}.

Table 1.1 Water quality guidelines for Co, Se, and Zn in North America

Metal	Canadian Water quality guideline µg L^{-1}	American Water quality criteria µg L^{-1}
Co	$e^{\{(0.414[\ln(\text{Hardness})] - 1.887\}}$ --a	0.7[d]
Se	1[b]	1.5[e]
Zn	$e^{0.995(\ln(\text{hardness}))+ 0.847(\ln(\text{pH})) + 4.932}$ -- c	$e^{0.8473(\ln \text{hardness}) + 0.884}$ -- f

[a] Federal Water Quality Guideline (ECCC, 2017)

[b] Water Quality Guideline for the Protection of Aquatic Life (CCME, 1987)

[c] Water Quality Guideline for the Protection of Aquatic Life (CCME, 2016)

[d] Arizona Drinking Water guideline (FSTRAC, 1996)

[e] Aquatic Life Ambient Water Quality Criterion (US EPA, 2016)

[f] Water Quality Criteria for the Protection of Aquatic Life (US EPA, 1996)

1.6.2 United States

The USEPA last published water quality criteria for the protection of aquatic life in 1986, but as with Canadian guidelines some of the criteria have been updated. These criteria are not law but provide guidance to States when setting water quality standards. Water quality standards are regulations to control pollution that incorporate these criteria along with designated uses, antidegradation requirements, and general policies (USEPA, 2014). The updated criterion for Se is based on dietary exposure and tissue concentrations (USEPA, 2016a). There is a wide variation in Se water quality criteria between Canadian federal, Canadian provincial, and US EPA guidelines (CCME, 1987; Beatty & Russo, 2014; USEPA, 2016a). As of 2016, the Cd criterion has been updated and is slightly higher than the criterion developed for Canada in 2014, but both are hardness-dependent (USEPA 2016b). A water-effects ratio procedure, similar to the Canadian procedures, can be used to modify the criteria for specific sites, (USEPA, 1994). The USEPA also adopted a biotic ligand model-based Cu criterion in 2007 (USEPA, 2007). Different

types of metal and element modelling for criteria development will be discussed in the next section.

1.7 Existing Toxicity Models

1.7.1 Cobalt

The Gill Surface Interaction Model (Pagenkopf, 1983) predicts the speciation and activity of metals associated with the fish gill surface using the concept of competitive equilibria. The model assumes that ions contributing to water hardness (Ca^{2+}, Mg^{2+}), would competitively inhibit metal ions from binding to the gill membrane. The model also relies on the assumption that the gill membrane had a finite number of binding sites and that death occurs due to the binding of a metal causing a change in gill function. Alkalinity and pH were two other water quality parameters considered in the development of this model, as these parameters can change the speciation of the metal. This model does not include complexation with organic matter. The author stated that in laboratory bioassays the water used had a low dissolved organic carbon content (Pagenkopf, 1983).

Richards and Playle (1998) developed a GSIM (Gill Surface Interaction Model) for Co and concluded that Ca^{2+} competition and complexation with dissolved organic matter (DOM) were the two main factors in reducing the binding of Co to rainbow trout gills. At concentrations above 15 mg/L DOC, there was no accumulation of Co in the gills.

The Biotic Ligand Model (BLM) draws from the GSIM and over 20 years of additional research (Paquin et al., 1999; Di Toro et al., 2001; Santore et al., 2002). The model incorporates the relationships and interactions (e.g.: competition, complexation) between the potentially toxic free metal ion, other cations (Na^{+}, H^{+}, Ca^{2+}, Mg^{2+}), abiotic ligands, and the biotic ligand (Niyogi & Wood, 2004). A terrestrial Biotic Ligand Model (BLM) was developed for the potworm *Enchytraeus albidus*, with increasing Ca^{2+}, Mg^{2+}, and H^{+} all decreasing the toxicity of Co (Lock et al., 2006).

The recently developed FEQG for Co uses a species sensitivity distribution to protect 95% of the species, followed by a multiple linear regression method to account for significant water chemistry effects. This two-part method considers both biological variation and water chemistry and does not need to directly account for the uptake or speciation of Co (ECCC, 2017).

1.7.2 Selenium

Measured whole-body residue-based approaches are not widely used, as the accumulation pattern of metals and other elements in many organisms do not allow for a relationship between whole-body metal concentration and toxicity to be modelled (Adams et al., 2010; Rainbow & Luoma, 2011). However, with certain organisms and with specific experimental conditions some whole-body models exist and have been field-validated (Adams et al., 2010). The USEPA has adopted a tissue-based draft criterion for selenium toxicity recommending that fish ovary concentrations are given precedence over water column concentrations (USEPA, 2016a).

1.7.3 Zinc

There have been several Zn BLMs created for fish, invertebrates, and algae at both the acute and chronic levels (Niyogi and Wood, 2004). The first models were acute models for rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas)* and *Daphnia magna* that were able to predict toxicity within a factor of two (Santore et al., 2002; Heijerick et al., 2002). More recent acute Zn BLMs include a *Daphnia pulex* model by Clifford and McGeer (2009). The protective effect of calcium (Ca^{2+}) was increased when compared to the other BLMs mentioned, which may be due to the difference in species sensitivities or the soft water test conditions (Clifford & McGeer, 2009). A chronic Zn BLM has also been developed (Heijerick et al., 2005). The major difference between the acute and chronic BLMs was that in the acute model, pH only influenced the speciation of Zn, but in the chronic model, H^+ appeared to be in competition with Zn. In both models, Na^+, Ca^{2+}, and Mg^{2+} also affected Zn toxicity (Heijerick et al., 2005).

1.7.4 Saturation kinetics-based models

1.7.4.1 Mortality Model

Michaelis-Menton kinetics-based mortality models, developed by Borgmann et al. (2004) to predict mortality in *H. azteca,* are the focus of this thesis. The mortality model uses similar saturation kinetics to the BLM, but links mortality to measured metal/element concentrations in water or whole-body concentrations (Norwood et al., 2007) instead of the level of saturation of the critical binding sites (Di Toro et al., 2001). This mechanistic model assumes that mortality rate reaches a maximum (Borgmann et al., 2004; Norwood et al., 2006). Borgmann et al. (2004) state that total body concentration is proportional to mortality in *H. azteca,* and therefore assumed to be proportional to the concentration at the site of toxic action. This assumption is necessary for

the mortality model to estimate lethal concentration based on whole-body concentrations. In many cases, the whole-body concentration is a better predictor of toxicity than the environmental concentrations since the whole-body concentration of an organism accounts for (i.e. eliminates the need to consider) environmental factors and chemical variables that influence the bioavailability and uptake of an element (Borgmann et al., 2004). Growth is considered a less sensitive endpoint in *H. azteca* than mortality (Borgmann et al., 2004) and therefore will not be discussed in this thesis. However, growth results are available in Appendices A, B, and C.

The mortality model from Borgmann et al. (2004) will be used in Chapters 2, 3, 4, and 5 to calculate critical concentrations as follows:

$$m = m' + (\ln(2)/t) \times [C_w(1/LC50+1/K_w)/(1+Cw/K_w)]^{nw} \quad \text{(Eq. 1.1)}$$

or

$$m = m' + (\ln(2)/t) \times [C_b(1/LBC50+1/K_b)/(1+C_b/K_b)]^{nb} \quad \text{(Eq. 1.2)}$$

where m is mortality rate, m' is the control mortality, t is time, C is the measured water (w) or body (b) concentration, LC50 (lethal concentration) is the concentration of an element in water resulting in 50% of control survival and LBC50 (lethal body concentration) is the whole-body element concentration resulting in 50% of control survival, K_w or K_b is the half-saturation constant (the concentration at which the mortality rate is halfway between the control and maximum mortality rates), n is an exponent. The LBC50 is used instead of an LD50, since the organisms were exposed to a concentration of an element in water, not a dose. Body concentrations are background corrected and gut-cleared.

Formulae 1.1 and 1.2 can be modified as follows to determine LC25 and LBC25 values as

$$LC25 = [(LC50^{-1} + K_w^{-1}) (\ln(4/3) \ln(2)^{-1})^{1/nw} - K_w^{-1}]^{-1} \quad \text{(Eq. 1.3a)}$$

and

$$LBC25 = [(LBC50^{-1} + K_b^{-1}) (\ln(4/3) \ln(2)^{-1})^{1/nb} - K_b^{-1}]^{-1} \quad \text{(Eq. 1.3b)}$$

where LC25 is the concentration of an element in water causing 25% mortality, LC50 is the concentration of an element in water causing 50% mortality, LBC25 is the whole-body (background corrected and gut-cleared) concentration of an element associated with 25% mortality, LBC50 is the whole-body concentration associated with 50% mortality, K_w" is the half-saturation constant (where the element concentration in water associated with mortality is

half the concentration causing maximum mortality), K_{TB}" is the half-saturation constant (where the element concentration in organism's body causing mortality is half the maximum), and nw and nb are exponents.

Formulae 1.1 and 1.2 can also be modified for LC10 and LBC10 determination as

$$LC10 = [(LC50^{-1} + K_w^{-1}) (\ln(10/9) \ln(2)^{-1})^{1/nw} - K_w^{-1}]^{-1} \quad \text{(Eq. 1.4a)}$$

and

$$LBC10 = [(LBC50^{-1} + K_b^{-1}) (\ln(10/9) \ln(2)^{-1})^{1/nb} - K_b^{-1}]^{-1} \quad \text{(Eq. 1.4b)}$$

where LC10 is the concentration of an element in water causing 10% mortality, LC50 is the concentration of an element in water causing 50% mortality, LBC10 is the whole-body (background corrected and gut-cleared) concentration of an element associated with 10% mortality, LBC50 is the whole-body concentration of an element associated with 50% mortality, K_W is the half-saturation constant (where the concentration of an element in water causing mortality is half the maximum), K_b is the half-saturation constant (where the concentration of an element in organism's body causing mortality is half the maximum), and nw and nb are exponents.

1.7.4.2 Bioaccumulation Model

The mechanistically-based, bioaccumulation saturation model of Borgmann et al (2004) is used to determine the relationship between bioaccumulation of cobalt and exposure as follows:

$$C_b = \max \times C_w \times (K + C_w)^{-1} + C_{Bk} \quad \text{(Eq. 1.5a)}$$

Where C_b is the whole-body concentration of the element, max is the maximum above-background body concentration, C_W is the concentration of an element in the exposure media, K is the half saturation constant, which is the concentration of an element in water at which the C_b is half way between the max and the C_{Bk} (background body concentration, or control concentration). The data used to fit this model was gut-cleared, whole-body concentration of an element on a dry weight basis. Gut-clearance is when organisms are transferred into clean water containing ethylenediaminetetraacetic acid (EDTA) for 24 hours to remove metal that has not been absorbed from their digestive tract or body surface (Neumann et al., 1999).

If the element concentration does not reach a maximum in the organism, the following formula can be used to calculate the whole-body concentration as the addition of C_W will not affect K in this scenario,

$C_b = \max/K \times C_w + C_{Bk}$ (Eq. 1.5b)

A bioconcentration factor relating the concentration of the element in an organism to the concentration in water can be calculated as:

$BCF = C_b\, C_w^{-1} = \max\,(K + C_w)^{-1} + C_{Bk}\, C_w^{-1}$ (Eq. 1.6)

1.8 *HYALELLA AZTECA*

H. azteca are a freshwater amphipod species that lives at the water-sediment interface of slow moving streams or lakes (Environment Canada, 1997). It should be noted that *H. azteca* is a species complex (Major et al., 2013), and the effect of toxicants may vary between clades. The clade used in this thesis (clade 1) is more sensitive to Cu and Ni than clade 8 used in most North America laboratories (Leung et al., 2016). *H. azteca* are tolerant to a wide range of water chemistry parameters. A population of *H. azteca* can be maintained in water with pH as low as 5.8 (Grapentine & Rosenburg, 1992) and can withstand pulses of lower pH water (Pilgrim and Burt, 1993). This species is present in alkaline lakes and even estuaries, showing that they can adapt to high alkalinity, pH, hardness and salinity (Environment Canada, 1997).

1.9 PROJECT OBJECTIVES AND HYPOTHESES

This project was initiated in support of the Canadian Government's Chemicals Management Plan to investigate the toxicity of three elements that were identified as high risk to the environment and human health – Co, Se, and Zn. The amphipod *H. azteca* was selected to observe the toxicity and bioaccumulation of each element in chronic waterborne exposures. Endpoints based on both exposure and whole-body were determined using the mortality model (Section 1.7.4.1) and the bioaccumulation pattern of each element was determined using the bioaccumulation model (Section 1.7.4.2).

1.9.1 Objectives

1. Determine if pH or DOC influences the acute toxicity of an element tested singly (Co, Se, or Zn) to *H. azteca.*
2. Determine if pH, alkalinity or DOC influences the chronic toxicity of an element tested singly (Co, Se, or Zn) to *H. azteca*. The effect of water hardness will also be determined incorporating data from previous studies.

a) Determine how mortality rate and bioaccumulation of an element tested singly in *H. azteca* is related to exposure concentration and if water chemistry affects this relationship.

b) Determine how mortality rate and bioaccumulation of an element tested singly in *H. azteca* is related to whole-body concentration and if water chemistry affects this relationship.

3. Compare mortality and bioaccumulation relationships in water with different chemistry to models developed by Borgmann et al. (2004), Norwood et al. (2006, 2007), and Norwood et al. (unpublished) to determine if water chemistry is a variable necessary for the models to be good predictors of toxicity.

1.9.2 Hypotheses

1. Changes to water chemistry in chronic exposures to metals will have significant effects on rates of mortality and bioaccumulation of an element.

a) The toxicity and bioaccumulation of Co and Se will decrease with increasing pH and alkalinity.

b) The toxicity and bioaccumulation of Zn will have a u-shaped response to pH, with increased mortality at neutral pH. The toxicity of Zn will also have a u-shaped response to alkalinity.

c) The toxicity and bioaccumulation Co, Se, and Zn will decrease with increasing concentrations of DOC.

2. Whole-body concentration-based mortality will not be affected by changing water chemistry.

CHAPTER 2

Dissolved organic carbon and pH effects on the acute toxicity of Co, Se, and Zn to *Hyalella azteca*

OVERVIEW

This chapter presents 7-day toxicity data for Co, Se, and Zn to *Hyalella azteca* in lab-based experiments manipulating the pH or DOC concentration of the exposure environments. The toxicity endpoint examined was mortality. Two methods were applied to estimate the LC50, followed by a comparison of the results from each method. The two models used were the Trimmed Spearman-Karber method and the mortality model. The acute toxicity endpoints were used to define chronic toxicity testing ranges in Chapter 3, 4, and 5.

2.1 INTRODUCTION

The acute toxicity of environmental contaminants occurs at high concentrations of a compound over a short time. Typically, this toxicity will only occur in an aquatic environment when a contaminant is accidentally introduced or before benchmark environmental concentrations have been established (Nikinmaa, 2014). Chronic exposures occur when an organism is exposed for greater than 10% of its lifespan, at concentrations lower than what would cause an acute toxic effect (Nikinmaa, 2014). In terms of creating environmental criteria and for risk assessment, chronic endpoints are more useful; however, acute toxicity testing is still required to determine short term toxicity mechanisms, as chronic exposures introduce more complexity. During longer exposures, organisms will grow or potentially acclimatize, which can redistribute and/or remove toxic element from the organism (Niyogi & Wood, 2004). Grosell et al. (2006) noted that the water chemistry parameters affecting Pb toxicity were similar in both acute and chronic exposures. However, other studies have found that water chemistry can have different or no effects on acute and chronic endpoints (Heijerick et al., 2002; Heijerick et al., 2005; CCME, 2016).

The objectives of this chapter were to determine how water chemistry affects the acute toxicity of Co, Se, or Zn to *Hyalella azteca*. Seven-day exposures of *H. azteca* to single elements in different concentrations of dissolved organic carbon (DOC) and at different pH were completed with mortality as the endpoint. *H. azteca* have been previously exposed to Co, Se, and Zn in 7-day acute exposures in both soft water and hard water by Borgmann et al. (2005). Seven-day LC50s were greater in the hard water treatments, with the Co LC50 increasing four times, Se 7.5 times, and Zn four times. Schubauer et al. (1993) exposed *H. azteca* to Zn at three levels of pH and identified a four-fold decrease in toxicity between pH 8-8.5 and pH 6-6.5 in 96-hour exposures.

Two methods were used to estimate the concentration causing 50% mortality in *H. azteca* - the Trimmed-Spearman Karber method and the mortality model, as described in Section 1.7.4.1. The Trimmed-Spearman Karber method calculates an LC50 and its 95% confidence intervals using survival and concentration data. This method appears in literature; however, its potential weaknesses include pooling of replicates and using only monotonic data. This method has been used as tool to determine preliminary LC50s before additional modelling (Environment Canada, 2005).

2.2. METHOD

2.2.1 Experimental Set-up

The 7-day acute testing methods used were similar to those of Norwood et al. (2006; 2007). HDPE containers (500 mL) were acid-washed in 20% HNO_3 and rinsed 7-8 times with deionized water. Standard artificial media (SAM-5S) water (Borgmann, 1996) was diluted to 30% (SAM30: 0.33 mM $CaCl_2$, 0.01 mM NaBr, 0.075 mM $MgSO_4$, 0.33 mM $NaHCO_3$, 0.015 mM KCl), so the experiments took place in moderately-soft water. The concentration of the bromide ion remained at 0.01mM, as it is an essential ion for *H. azteca* (Borgmann, 1996). The water chemistry was further adjusted for each set of experiments (Table 2.1). The different treatment media were made in 25L carboys initially containing SAM30. Alkalinity, pH, and DOC adjustments were made to these carboys and then equilibrated for 48 hours prior to use in experiments. The animals were cultured in SAM30 and the young randomly transferred to the media set to the appropriate water chemistry for an acclimatization period of 4 hours prior to the start of the experiment. Fifteen 2-9 day old *H. azteca* were then transferred to 400 mL SAM water in the HDPE containers. Each container had a 2.5 x 2.5 cm piece of 100% cotton gauze as a substrate. The containers received 16-hour light/8-hour dark photoperiod in a 25°C walk-in incubator.

Table 2.1. Test parameters and methods to maintain the water chemistry

Parameter measurements		Method	Reference
pH	6.7, 7.7, 8.3	-1M HCl or KOH amendments	Taylor et al. (2002); Niyogi (2008)
Dissolved organic carbon	0.5, 2, 5, 10 mg C L^{-1}	Luther Marsh, natural organic matter	Gillis et al. (2010)

Test solutions containing a single element were equilibrated 4 hours in test containers before *H. azteca* were added. For the DOC experiments the test solutions were equilibrated 24 hours, so the element, DOC, and other ions would interact before the addition of organisms (Taylor et al., 2002; Niyogi et al., 2008).

Each test was repeated twice for a total of six control replicates (no element addition) and four replicates of exponentially increasing element concentrations (0, 32, 56, 100, 180, 320 μg L^{-1} (Table 2.2). The element additions were as follows: Cobalt as $CoCl_2 \cdot 6H_2O$ (Cobalt (II) chloride

hexahydrate, ACS reagent, 98%; Sigma Aldrich), Selenium as Na_2SeO_3 (Sodium selenite 99%, Sigma Aldrich), or Zinc as $ZnCl_2$ (Zinc Chloride puriss. p.a., ACS reagent, reag ISO, reag Ph. Eur., >98%, Sigma Aldrich). The nominal concentrations were selected based on the 7-day LC50s of these elements in soft water from Borgmann et al. (2005). The acute exposures from this chapter served as a range-finding tests for the chronic (28-day) element toxicity experiments in Chapters 3, 4, and 5.

Table 2.2 Measured concentration of elements in concentration series

	Measured Exposure Concentrations
Element	nmol L^{-1}
Co	1.43, 490, 851, 1690, 2790, 4690
Se	4.65, 428, 805, 1630, 2640, 4260
Zn	53.9, 451, 815, 1480, 2240, 4890

2.2.2 Sample collection and analysis

Water samples (1-mL unfiltered) were taken at the beginning and end of each one-week experiment from the first replicate of each treatment and preserved with 10µL ultrapure HNO_3 (70%, Fisher Scientific) for elemental analysis. Filtered (0.45 µm) 1-mL water samples were taken from control, 56 nmol L^{-1}, and 320 nmol L^{-1} test containers at the beginning and end of two turnover periods to determine dissolved element concentration. Total Co in water samples was determined with a Varian SpectraAA 400 graphite furnace atomic absorption spectrophotometer (GFAAS) with Zeeman background correction, while total Se or Zn in water was analyzed on a Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometer with SOLAAR Data Station V11.03 software. For the analyses of Co in water, the ash temperature was 1100°C and the atomization temperature was 2200°C, with Zeeman background correction. For Se, the ash temperature was 1100°C and the atomization temperature was 2500°C, with Zeeman background correction. Nickel was used as a matrix modifier. For Zn analyses, the ash temperature was 700°C and the atomization temperature was 1150°C with Zeeman background correction. Ammonium phosphate was used as a modifier. Method blanks, reference standards (CRM-TMDW Certified Reference Material - Trace Metals in Drinking Water, High-Purity Standards, Charleston, South Carolina), and element standards (High-Purity Standards: 10 µg/mL Co in 2% HNO_3, 10 µg/mL Se in 2% HNO_3, 10 µg/mL Zn in 2% HNO_3) were analyzed every five samples to ensure quality control (QA/QC) in the determination of background contamination, instrument

drift, detection limits and element recovery. Recovery of CRM-TMDW was 88.0 % ± 7.15 for the Co program, 99.3% ± 9.11 for the Se program, and 106% ± 6.56 for the Zn program. If any blank or reference test failed, the instrument was recalibrated.

Water samples (500 mL filtered) were collected from control, 56 μg L^{-1}, and 320 μg L^{-1} test containers for major ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}) and analyzed by Environment Canada's National Laboratory for Environmental Testing, Burlington, Ontario (NLET). Inductively coupled plasma-optical emission spectrometry was used to measure the cations, while ion chromatography was used to measure Cl^- and SO_4^{2-}. Water samples (100 mL unfiltered) were collected from control, 56 μg L^{-1}, and μg L^{-1} test containers to measure dissolved organic carbon (DOC) and inorganic carbon (DIC). These samples were analyzed on a Phoenix 8000™ UV-persulfate TOC Analyzer (Teledyne Tekmar). Water samples (500 mL filtered) were also collected at control, mid-level, and high element concentrations to determine alkalinity. Alkalinity was determined by NLET using a PC-Titrate automated system (Mandel Scientific) in a potentiometric titration with sulfuric acid (Environment Canada, 2008). Dissolved oxygen (Thermo Scientific Orion Model 080510), pH (Thermo Scientific Orion Model 8165BNWP), conductivity (VWR Scientific Model 1054), and ammonia concentrations (Aquarium Pharmaceuticals, Inc. NH_3/NH_4 test kit) were measured in subsamples of replicate one at the beginning of each experiment and in all containers at the end of the one-week exposure.

At the end of the seven-day test, the surviving organisms were counted to determine mortality. The surviving animals were then transferred into 50 μM ethylenediaminetetraacetic acid (EDTA) in SAM30 with a piece of gauze (2.5 X 2.5 cm) and 2.5 mg of TetraMin for 24-hour gut clearance and to remove external elements (Borgmann & Norwood, 1995b; Neumann et al., 1999). After 24h the animals were removed from the solution, placed on a Kimwipe to wick away excess water, and wet-weighed (Mettler Toledo micro-analytical balance) for growth analysis. The organisms were placed in acid-cleaned cryovials and dried at 60°C for 72 hours. Whole-body element concentrations were not measured due to the inability to characterize concentrations of elements in such small organisms after one week of growth.

2.2.3 Calculations and data analyses

2.2.3.1 Trimmed Spearman-Karber method

Concentrations of elements in water causing mortality were first determined using measured total element concentrations in water and the Trimmed Spearman-Karber method. The

Trimmed-Spearman Karber method estimates an LC50 and its 95% confidence intervals from the weighted averages of the midpoints between concentrations (Environment Canada, 2005). If the data does not have 0% and 100% effects, the ends of the distribution can be trimmed. This method appears in literature (Brinkman & Wooding, 2005; Ryan et al., 2009); however, its potential weaknesses include excessive trimming and using only monotonic data. If the distribution is trimmed without examining a plot of the data, it can shift the LC50 estimate. When the data are not monotonic it can be smoothed by taken the average of the non-monotonic data points, which can influence the confidence intervals. This method has been used as a tool to determine preliminary LC50s before modelling (Environment Canada, 2005).

2.2.3.2 Mortality Model

The mortality model was described in Section 1.7.4.1. Mortality data were fourth-root transformed to normalize the data before the above models were fitted in SYSTAT 10 (Norwood et al., 2007), after visual inspection of probability plots (Golding et al., 2013). In addition, Levene's test was performed on the absolute values of the residuals to ensure equality of variance (Environment Canada, 2005).

2.2.3.3 Confidence Intervals

To determine significant difference between the various treatment critical concentrations, the 95% confidence intervals for the two lethal concentrations must not overlap (Gillis et al., 2010). If the two confidence intervals do overlap, they were considered not significantly different.

2.3 RESULTS

2.3.1 Trimmed-Spearman Karber LC50 values

Table 2.3. Trimmed Spearman-Karber method LC50s and 95% confidence intervals in nmol L^{-1} of Co, Se, and Zn with differing water chemistry treatments.

Treatment	pH	DOC	Alkalinity	LC50		
		mg L^{-1}	mg $CaCO_3$ L^{-1}	nmol Co L^{-1}	nmol Se L^{-1}	nmol Zn L^{-1}
pH 6.5	6.53 (6.41 – 6.64)	0.3	15.9	1940 (1600 - 2340)*	1420 (1350 - 1490)*	2550 (2100 – 3100)
pH 7.6 †	7.61 (7.51 – 7.69)	0.3	15.9	1320 (1200 – 1460)	1850 (1630 - 2100)	3480 (3040 – 3980)
pH 8.3	8.29 (8.01 – 8.59)	0.3	15.9	1190 (1060 – 1340)	2870 (2490 - 3310) *	2230 (1960 – 2520)*
DOC 2	7.7	2.4	20.1	945 (788 – 1130)*	1950 (1710 - 2230)	3360 (2900 – 3900)
DOC 5	7.7	4.4	20.1	647 (524 – 800)*	nd	3550 (3220 – 4100)
DOC 10	7.7	9.1	20.1	2270 (1870 – 2760)*	2230 (1920 - 2600)	>4890

* Indicates a significant different from unmodified treatment (†)

2.3.1.1 Cobalt

The increased concentration of hydrogen ions had a protective effect, reducing cobalt toxicity by 30% between test pH values of 7.6 to 6.5, but not between 8.3 and 7.6 (Table 2.3). The addition both 2 and 5 mg C L^{-1} resulted in a 30% and 50% increase in Co toxicity compared to the test with no added DOC at a similar pH (pH 7.6). At 10 mg C L^{-1} there was a significant protective effect (Table 2.3).

2.3.1.2 Selenium

Using the Trimmed Spearman-Karber method, the results showed that selenium was 50% more toxic at pH 6.7 compared to pH 8.3. Increased DOC did not have a significant effect on LC50 values (Table 2.3).

2.3.1.3 Zinc

At pH 6.5 and pH 8.3 the toxicity of Zn was comparable; however, only the LC50 at pH 8.3 was significantly lower than at pH 7.6. (Table 2.3). The addition of dissolved organic carbon did not significantly affect the toxicity of Zn until the concentration of DOC was 10 mg C $L^{-1.}$ When 2 mg C L^{-1} and 5 mg C L^{-1} were present in the solution the Zn LC50s were not significantly different from each other nor from the test at a comparable pH (7.6) with no added DOC (Table 2.3). However, when 10 mg C L^{-1} was present, the LC50 was greater than 4890 nmol Zn L^{-1}, but it was not possible to establish statistical significance because the LC50 was estimated to be greater than the test concentration with the most Zn.

2.3.2 Mortality model LC50 determinations

Table 2.4 Mortality model LC50s and 95% confidence intervals in nmol L^{-1} of Co, Se, and Zn with differing water chemistry treatments.

Treatment	pH	DOC	Alkalinity	LC50		
		mg L^{-1}	mg $CaCO_3$ L^{-1}	nmol Co L^{-1}	nmol Se L^{-1}	nmol Zn L^{-1}
pH 6.5	6.53 (6.41 – 6.64)	0.3	15.9	1780 (1010 – 2560)	1390 (821 – 1970)	2690 (2040 – 3340)
pH 7.6 †	7.61 (7.51 – 7.69)	0.3	15.9	848 (285 – 1410)	1550 (1200 – 1900)	3600 (3290 – 3910)
pH 8.3	8.29 (8.01 – 8.59)	0.3	15.9	686 (339 – 1030)	2830 (2460 – 3230)*	2290 (1850 – 2720)*
DOC 2	7.7	2.4	20.1	777 (618 – 935)	1980 (1740 – 2460)	3640 (3000 – 4280)
DOC 5	7.7	4.4	20.1	436 (297 – 577)	nd	4290 (3130 – 5450)
DOC 10	7.7	9.1	20.1	1710 (748 – 3050)	2370 (1880 – 2860)	>4890

* Indicates a significant different from unmodified treatment (†)

2.3.2.1 Cobalt

The Co LC50s estimated by the mortality model did not change significantly as pH increased (Figure 2.1; Table 2.4). The mortality model estimates were not significantly different than those of the trimmed Spearman-Karber method except at pH 8.3 where the LC50 value was significantly lower than the trimmed Spearman-Karber value (Table 2.3 and Table 2.4). The DOC-modified Co LC50s were inconsistent in terms of trends. The addition of 5 mg L^{-1} DOC resulted in significantly reduced LC50 compared to addition of 2 mg L^{-1}. The addition of 10 mg L^{-1} resulted in a significant increase in LC50 compared to the two lower additions (Figure 2.1). However, there were no differences with any of the DOC additions compared to the test with no added DOC at a comparable pH (pH 7.6; Table 2.4). These values were not significantly different from the LC50s as determined by the trimmed Spearman-Karber method.

2.3.2.2 Selenium

Using the mortality model to estimate LC50s values, there was no change in toxicity between pH values of 6.5 and 7.6, but a significant reduction at pH 8.3 (Figure 2.2; Table 2.4).

The DOC treatments also had similar values to the trimmed Spearman-Karber method with a LC50 of 1980 (1740 – 2460) nmol Se L^{-1} at 2 mg C L^{-1} and 2370 (1880 – 2860) nmol Se L^{-1} at 10 mg C L^{-1}.

2.3.2.3 Zinc

The Zn LC50s determined by the mortality model were also not significantly different from the trimmed Spearman-Karber method, with values of 2690 (2040 – 3340) nmol Zn L^{-1} at

pH 6.5, 3600 (3290 -3910) nmol Zn L^{-1} at pH 7.5, and 2290 (1850 – 2720) nmol Zn L^{-1} at pH 8.3. This inverted u-shaped effect can be seen in Figure 2.3.

The DOC-modified Zn LC50s were not significantly different. However, increasing DOC concentrations in the exposure water provided a protective trend against Zn toxicity (Figure 2.3).

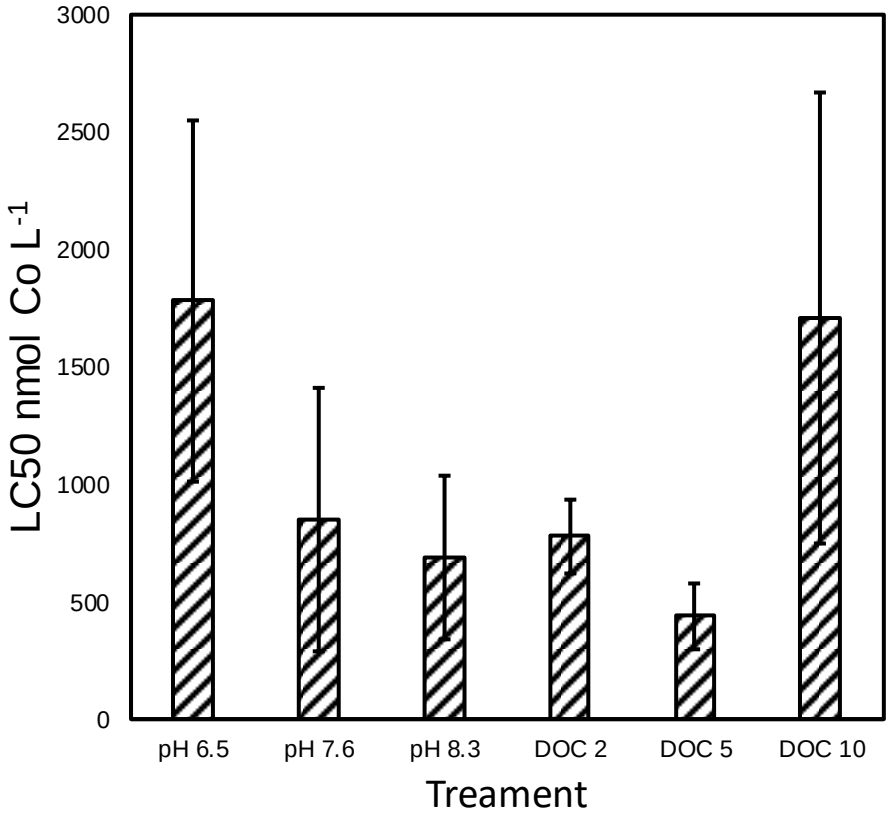

Figure 2.1 Acute toxicity of Co to *H. azteca* as LC50 values in nmol Co L^{-1} in varying pH and DOC exposures. LC50s were determined using the mortality model. Error bars are 95% confidence intervals.

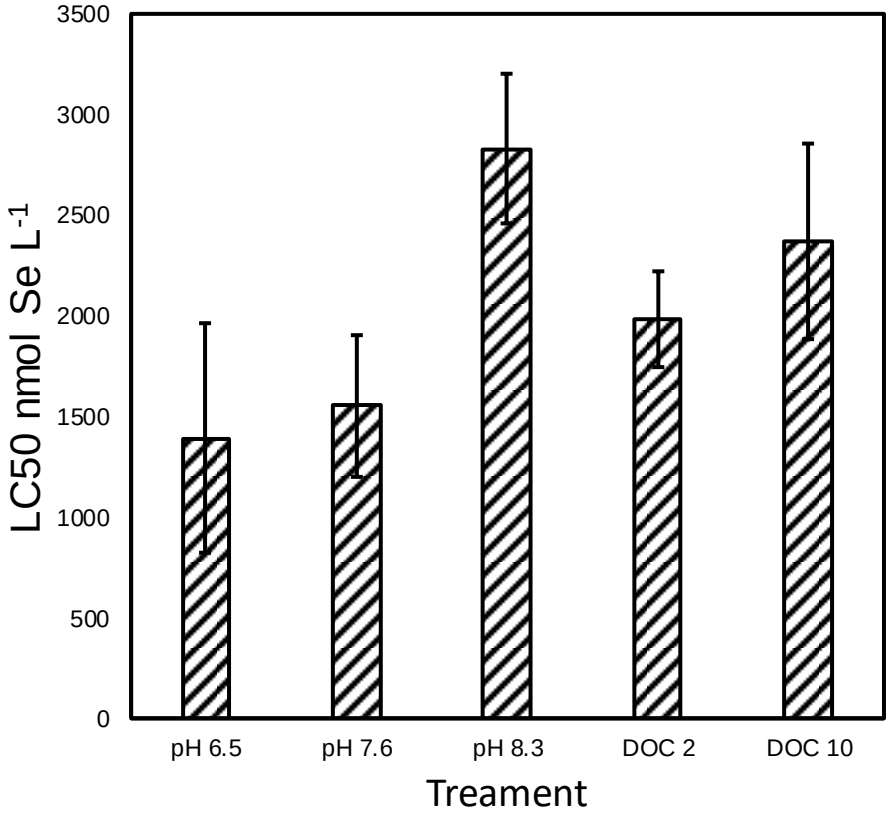

Figure 2.2 Acute toxicity of Se to *H. azteca* as LC50 values in nmol Se L^{-1} in varying pH and DOC exposures. LC50s were determined using the mortality model. Error bars are 95% confidence intervals.

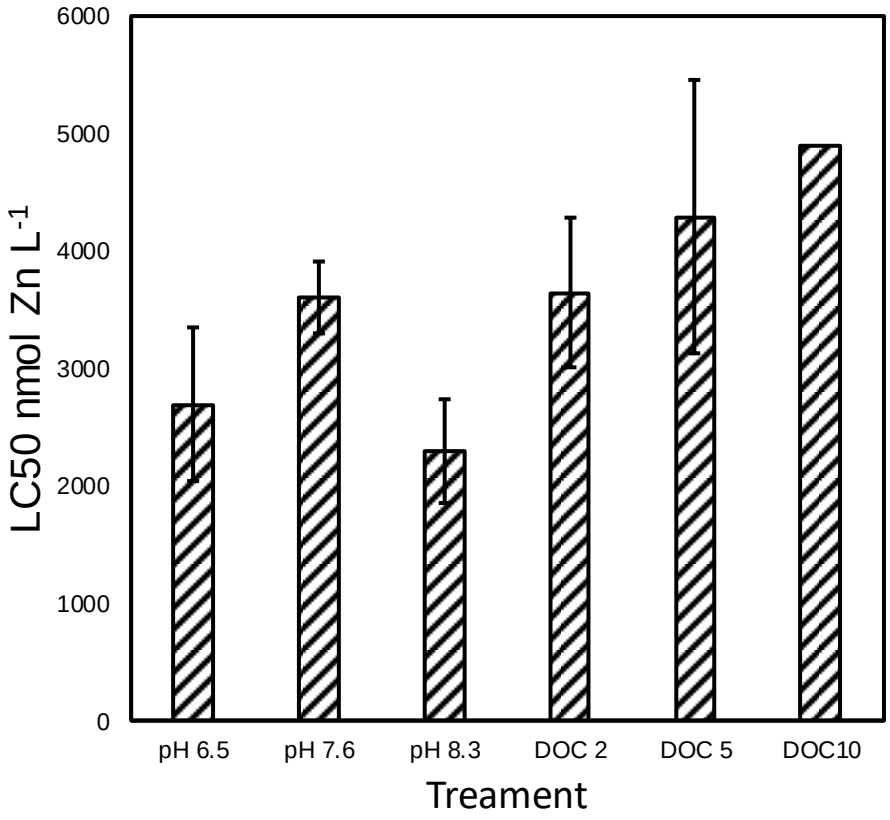

Figure 2.3 Acute toxicity of Zn to *H. azteca* as LC50 values in nmol Zn L^{-1} in varying pH and DOC exposures. LC50s were determined using the mortality model. Error bars are 95% confidence intervals.

2.4 DISCUSSION

2.4.1 Comparison of the two LC50 methods

Both the Trimmed Spearman-Karber method and the saturation mortality model predicted similar lethal concentrations, with the only significant difference being the LC50 of the Co treatment at pH 8.3. The confidence intervals from the mortality model were larger than those of the Trimmed Spearman-Karber method, as the monotonic requirement reduced the variability of the small data set. The sensitivity to variable data may be a weakness of the mortality model. Because the trimmed Spearman-Karber method is an accepted method to determine lethal concentration values and the two methods estimated similar LC50s, the mechanistic mortality model should be a reliable predictive tool.

2.4.2 Comparison of mortality model LC50s with literature

2.4.2.1 Cobalt

Environments with lower pH had a protective effect against Co toxicity, as the LC50 at pH 6.5 was significantly greater than at pH 8.3. Exposures with the highest DOC also had a protective trend against Co toxicity. However, the difference from the treatment with no added DOC and from the DOC-2 treatment was not significant. The trimmed Spearman-Karber method estimated that Co in the DOC-10 would be significantly less toxic than with no added DOC (pH 7.6 treatment); however, Co in the DOC-2 and DOC-5 treatments were significantly more toxic. Watanabe et al (2017) concluded that the molecular size of aquatic humic substances could affect the toxicity of Co to *Ceriodaphnia dubia* in a 7-day exposure. The affect the humic substance had on toxicity was also influenced by the Co concentration (Watanabe et al., 2017). The aquatic humic substances used in the study by Watanabe et al. (2017) had other metals bound, unlike the dissolved organic matter (DOM) used in the current study. Richards and Playle (1998) used DOM from the same source as the current study, observed that 25 mg L^{-1} DOC was able to complex 9 µmol Co L^{-1}. In the current study, the highest concentration tested was 4690 nmol Co L^{-1} and this concentration would not be completely complexed by the lower concentrations DOC added.

Borgmann et al. (2005) discovered the acute LC50 of Co for *H. azteca* was over three-fold less in soft water (18 mg L^{-1} as $CaCO_3$) compared in hard water (124 mg L^{-1} as $CaCO_3$) (Borgmann et al., 2005). This LC50 of 1040 (882 – 1220) nmol Co L^{-1} in hard water from Borgmann et al. (2005) was not significantly different from any of the pH treatments from the

current study; however, the soft water treatment with an LC50 of 274 (189 – 395) nmol Co L^{-1} from Borgmann et al. (2005) was significantly more toxic, which shows that hardness also has a protective effect.

2.4.2.2 Selenium

Se was significantly more toxic to *H. azteca* in the pH 6.5 treatment when compared to the pH 8.3 treatment. DOC did not significantly influence Se toxicity, although it was thought that it would be protective as humic acid can be an electron donor or accepter (Kunenkov et al., 2009; Sharma et al., 2014) the polarity of SeO_3^- also allows it to adsorb to DOM (Wiramanaden et al., 2010). Borgmann et al. (2005) completed 7-day experiments in soft and hard water for Se and the soft water LC50 for Se was 545 (456 – 659) nmol Se L^{-1} compared to the hard water LC50 of 4700 (3580 – 6170) nmol Se L^{-1}. The soft water Se LC50 was significantly lower than any of the treatments tested in the current study, while the hard water LC50 was significantly greater. Although no other specific water chemistry data were provided, it was likely that hardness was a factor affecting acute Se toxicity in *H. azteca* because Se can interfere with Ca metabolism in invertebrates (Short & Wilbur, 1980; Johnston, 1987; Ingersoll et al., 1990). Therefore, Ca-limited invertebrates in softer water would be more susceptible to toxicity.

2.4.2.3 Zinc

DOC has a protective trend against the toxicity of Zn to *H. azteca*. However, since the LC50 at the highest concentration of DOC was greater than the highest concentration tested, confidence intervals could not be determined, and the trend was not significant. Several studies have concluded that increased concentrations of DOC will decrease the toxicity of Zn (De Schamphelaere et al., 2005; Bringolf et al., 2006; Clifford & McGeer, 2009). At lower Zn concentrations, a greater proportion was complexed with DOC (De Schamphelaere et al., 2005). pH changes caused an inverted u-shaped effect with the pH 7.6 LC50 significantly greater than the LC50 at pH 8.3. The LC50s for the pH 6.5 and pH 7.6 treatments were not significantly different. It was possible that Zn^{2+} competed with H^+ for uptake and at pH 8.3 it complexed with anions (-OH, $-CO_3$), which reduced the bioavailability and toxicity (USEPA, 1980).

The soft water LC50 for Zn from (Borgmann et al., 2005) was 858 (704 – 1040) nmol Zn L^{-1} and 3400 (3080 – 3750) nmol Zn L^{-1} in hard water. The soft water LC50 was significantly lower than any of the treatments tested in the current study, indicating that hardness affects the toxicity of Zn. However, the hard water treatment was not significantly different from the DOC

treatments or the lower pH treatments. Without further pH data, it was difficult to discern the reason for these trends, but the higher hardness likely has a similar protective effect to the DOC treatments. In addition, the organisms used in the tests by Borgmann et al. were cultured in hard water, which could affect the number of binding sites for metal toxicity to occur compared to the organisms cultured in soft water of the current study (Khan et al., 2011).

2.4.3 Future work

Using the data from this study, water chemistry effects on both bioaccumulation and toxicity of these three elements will be determined in 28-day exposures. The concentration range selected for these chronic experiments will be based on the 7-day toxic endpoint.

2.5 SUMMARY

Acute toxicity of all three elements was influenced by varying pH. There was a trend of increased Co toxicity with increased pH, while Se became significantly less toxic as pH increased. Zn had an inverted u-shaped toxic response, with moderate pH contributing to lower toxicity. There were no significant effects with changing DOC concentrations.

CHAPTER 3

Using saturation kinetics-based non-linear regression models to predict the chronic toxicity and bioaccumulation of cobalt to *Hyalella azteca* under different water chemistry conditions

OVERVIEW

Water-based exposures of cobalt causing mortality in *H. azteca* can be influenced by water hardness and dissolved organic carbon concentrations. Using non-linear regression saturation-based mortality models, changes in water chemistry influenced the lethal concentrations of Co in water, but not lethal whole-body Co concentrations. In soft water the LC50 was 25.4 ± 5.4nmol $L^{-1,}$ which increased to 80.6 ± 38.3 nmol L^{-1} with 5 mg C L^{-1} added and to 183 ± 63 nmol L^{-1} in moderately hard water. The LBC50 was 144 ± 28 nmol g^{-1} in soft water, 192 ± 54 nmol g^{-1} in hard water, and 146 ± 31 nmol g^{-1} with added DOC. Varying alkalinity and pH did not affect mortality or bioaccumulation of Co. In soft water treatments, *H. azteca* had greater accumulation of Co than in hardwater at the same exposure concentrations, although the predicted maximum whole-body concentrations were not significantly different. The whole-body concentration of Co in *Hyalella azteca* was a more consistent predictor of toxicity over a range of water chemistry conditions when compared to exposure concentrations.

3.1. INTRODUCTION

Cobalt is a metallic element that is a micronutrient for humans and many other organisms including fish (Watanabe et al., 1997). Cobalt is not regulated in some invertebrates, including the marine amphipod *Echinogammarus pirloti* (Rainbow & White, 1990). However, other micronutrients, including Cu and Zn, are also not always regulated in invertebrates. Aquatic environments typically have background concentrations of Co less than 1 μg L^{-1} with concentration increasing to 10 μg L^{-1} in more populated areas (Smith & Carson, 1981; Environment Canada, 2017). Anthropogenic activity such as metal mining and smelting have led to Co concentrations in some aquatic systems greater than 2000 μg L^{-1} (Environment Canada, 2017). Canadian Water Quality Guidelines for the Protection of Aquatic Life developed by the Canadian Council of Ministers of the Environment currently do not have a recommended maximum acceptable concentration of Co; however, Environment Canada has developed a Federal Water Quality Guideline of 1.0 μg L^{-1} (17.0 nmol L^{-1}) which is the 5th percentile of a species sensitivity distribution indicating where there is a low risk of adverse effects to aquatic life (Environment Canada, 2017). The province of British Columbia has implemented a 30-d average concentration of 4 μg L^{-1} (67.9 nmol L^{-1}) and a maximum exposure concentration of 110 μg L^{-1} (1866 nmol L^{-1}). These guidelines were developed using long-term toxicity data for a variety of species and based on the most sensitive groups of species (Nagpal, 2004).

Water chemistry parameters have proven effects on the uptake of metals, which will affect bioaccumulation patterns (Richards & Playle, 1998; Verschoor et al, 2012) and toxicological endpoints (Heijerick et al., 2003; Niyogi & Wood, 2004). Guidelines for some metals, including zinc and lead, are dependent on hardness to determine a site-specific guideline (CCME, 2007); however, many guidelines do not account for water chemistry and may not be reflective of metal bioavailability and toxicity under different conditions. When there is a pH effect on metal toxicity, it is predicted that H^+ competes with metal ions for binding sites on the surface of the organism, so there is more competition for uptake at a lower pH. However, a higher proportion of metal is in a cationic form at low pH, which is more readily taken up by an organism (Di Toro et al., 2001). Cobalt carbonate forms at higher pH though covalent bonding, which will reduce the concentration of free metal ions and cause increase alkalinity (Parametrix, 1995). Dissolved organic carbon (DOC) has phenolic and carboxylic groups with negative charges that can complex free metal ions. With increasing concentration of DOC in an aquatic environment, the effect that the metal would have on an organism is expected to decrease, as it

would have reduced bioavailability through complexation (Richards et al., 1999). The effect of water chemistry on Co chemistry for this study is presented in Table 3.3. Richards and Playle (1998) studied cobalt binding to rainbow trout (*Oncorhynchus mykiss*) gills and the effect of water chemistry on accumulation. It was established that Ca competition and DOM complexation were the main factors in preventing Co accumulation on fish gills. DOC concentrations in south-central Ontario tertiary watersheds rarely exceed 10 mg L^{-1} (David et al., 1997), but can reach concentrations exceeding 14 mg L^{-1} in soft water lakes in the same region (Welsh et al., 1996).

The mortality model does not consider the previously mentioned water chemistry parameters, but instead links mortality to measured concentrations of Co in water or whole-body Co concentrations (Norwood et al., 2007) instead of the level of saturation of the critical binding sites (Di Toro et al., 2001). This mechanistic model assumes that mortality rate reaches a maximum (Borgmann et al., 2004; Norwood et al., 2006). Borgmann et al. (2004) state that total body concentration causing mortality in *H. azteca* is often proportional to the concentration at the site of action and is a better predictor of toxicity than environmental concentrations. In addition, the use of organism whole-body concentrations should eliminate the need to consider environmental factors and chemical variables that could affect the bioavailability of a metal. Using this model, a lethal concentration causing 50% mortality in *H. azteca* was 16 µg Co L^{-1} (271 nmol Co L^{-1}) in a one-week exposure (Borgmann et al., 2005). *H. azteca* were exposed to Co in moderately hard water for 28-days by Norwood et al., (2007). The concentration of Co in water causing 50% mortality was 183 nmol L^{-1} and the whole-body concentration causing 50% mortality was 192 nmol L^{-1}.

The research in this chapter investigated how manipulating pH, alkalinity, and DOC affects mortality of *H. azteca* based on whole-body or water concentrations of Co. Lethal concentrations were determined using the mortality model described in section 1.7.4.1 and compared to previous Co exposures in moderately hard water by Norwood et al. (2007). The influence of these water chemistry variables on the bioaccumulation of Co was also determined using the bioaccumulation model from section 1.7.4.2. It was hypothesized that the toxicity and bioaccumulation of Co will decrease with increasing pH, alkalinity, or DOC.

3.2. METHOD

3.2.1 Experimental Set-up

The 28-day chronic testing methods used were similar to those described in section 2.2.1, deviations were described in the subsequent text. In addition to testing different pH and DOC concentrations, alkalinity was also adjusted (Table 3.1), with final water chemistry recorded in Table 3.2.

Table 3.1. Test parameters and methods to maintain the water chemistry.

Parameter measurements		Method	Reference
pH	6.7, 7.7, 8.3	1M HCl or KOH amendments	Taylor et al. (2002); Niyogi (2008)
Alkalinity	16, 50, 100 mg L^{-1} as $CaCO_3$	Adjust $NaHCO_3$, maintain sodium ions using NaCl	Deleebeeck et al. (2007)
Dissolved organic carbon	0.5, 2, 5, 10 mg C L^{-1}	Luther Marsh, natural organic matter	Gillis et al. (2010)

Test solutions containing $CoCl_2 \cdot 6H_2O$ (Cobalt chloride, 99%, Sigma Aldrich) were equilibrated 24 hours in test containers before *H. azteca* were added, so the metal, DOC, and other ions would interact before the addition of organisms (Taylor et al., 2002; Niyogi et al., 2008). The water in each container was renewed and the organisms counted every 7 days. Each test consisted of three control replicates (no metal addition) and two replicates of exponentially increasing metal concentrations (e.g.: 10, 18, 32, 56, 100 nmol L^{-1}). The organisms were fed 2.5 mg finely ground TetraMin fish food (Tetra GMBH, Melle, Germany) twice during this period.

3.2.2 Sample collection and analysis

Water samples (1-mL unfiltered) were taken at the beginning and end of each one-week experiment from the first replicate of each treatment and preserved with 10µL ultrapure HNO_3 (70%, Fisher Scientific) for metal analysis. Filtered (0.45 µm) 1-mL water samples were taken in 0, 18, and 100 nmol L^{-1} Co concentrations at the beginning and end of two turnover periods to determine dissolved metal concentration. Total and dissolved Co concentrations in water samples were determined with a Varian SpectraAA 400 graphite furnace atomic absorption spectrophotometer (GFAAS), using the program described in Section 2.2.2.

Water analysis methods were the same as those described in section 2.2.2. Water samples (500-mL filtered) were collected from containers containing 0, 18, or 100 nmol L^{-1} Co for major ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}). Water samples (100 mL unfiltered) were collected from these treatments for dissolved organic carbon (DOC) and inorganic carbon (DIC) analysis. Water samples (500 mL filtered) were also collected from containers containing 0, 18, and 100 nmol L^{-1} Co to measure alkalinity. Dissolved oxygen, pH, conductivity, and ammonia concentrations (Aquarium Pharmaceuticals, Inc. NH_3/NH_4 test kit) were measured in subsamples of replicate one at the beginning of each experiment and in all containers at the end of the one-week.

After 28 days, the surviving organisms from each container were counted. This count and the survival from each previous week was used to determine mortality per week to be input into the mortality model. The surviving organisms from each container were treated with the other organisms from the same container for the rest of the procedure. Replicates were not pooled. The organisms were then transferred into 50 mL containers containing 50 μM ethylenediaminetetraacetic acid (EDTA) in SAM30 with a piece of gauze (2.5 X 2.5 cm) and 2.5 mg of TetraMin for gut clearance and to remove external Co. After 24 h the animals were removed from the solution, placed on a Kimwipe to wick away excess water, and wet-weighed (Mettler Toledo micro-analytical balance) for growth analyses. Wet weights per treated organism ranged from 0.113 to 1.063 mg, with an average weight of 0.623 mg for the control organisms. The organisms were placed in an acid-cleaned cryovial and dried at 60°C for 72 hours.

3.2.3 Whole-body Digests

The dried organisms were weighed and digested for 6 days using concentrated ultrapure nitric acid (70%, Fisher Scientific) followed by the addition of 30% analytical grade hydrogen peroxide (J.T. Baker) for 24 hours. Milli-Q water was added, so the final digest concentrations were 1.75% ultrapure HNO_3 and 0.60% H_2O_2 (Golding et al., 2013). The weight of the dried organisms ranged from 0.034 – 0.224 mg, with a control average weight of 0.145 mg. Digests of a certified reference material had a recovery value of 107 ± 14% (TORT2: lobster hepatopancreas; Co certified as 0.51 ± 0.09 mg kg^{-1} from the National Research Council of Canada).

Table 3.2. Water chemistry from treatments. Mean of all measurements at the beginning and end of turnover periods with 95% C.I and maximum/minimum pH.

Treatment	Ca	Mg	Na	K	Cl	SO_4	DOC	DIC	Hardness	Alkalinity	pH	Conductivity
	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$mg\ L^{-1}$	$mg\ L^{-1}$	$mg\ L^{-1}$	$mg\ L^{-1}$		$\mu S\ cm^{-1}$
DOC 10	349 ± 9.36	97.4 ± 3.13	307 ± 6.65	15.0 ± 0.705	579 ± 21.9	86.8 ± 3.95	9.05 ± 0.44 (8.4 - 10)	4.22 ± 0.192	44.7	26.6 ± 3.39	7.44 ± 0.03 (7.17 - 7.71)	143 ± 0.896
DOC 5	350 ± 9.36	97.4 ± 3.14	308 ± 6.65	15.0 ± 0.706	580 ± 21.9	86.8 ± 3.96	4.37 ± 0.190 (4.2 -4.9)	4.17 ± 0.537	44.7	26.6 ± 3.40	7.45 ± 0.04 (7.18 - 7.59)	152 ± 3.60
DOC 2	351 ± 9.36	97.4 ± 3.15	309 ± 6.65	15.0 ± 0.707	581 ± 21.9	86.8 ± 3.97	2.42 ± 0.318 (2.1 - 2.9)	4.66 ± 0.662	44.7	26.6 ± 3.41	7.51 ± 0.03 (7.31 - 7.66)	157 ± 4.05
pH 6.5	297 ± 4.18	82.7 ± 1.69	389 ± 6.76	16.4 ± 0.990	979 ± 3.91	78.4 ± 0.77	0.6 ± 0.150	0.475 ± 0.090	38.2	).233 ± 0.27:	6.73 ± 0.06 (6.42 - 7.06)	144 ± 0.489
pH 8.5	318 ± 17.1	78.4 ± 2.82	340 ± 10.7	549 ± 12.5	652 ± 16.6	81.0 ± 0.71	0.5 ± 0.196	9.7 ± 0.196	39.7	43.3 ± 0.392	8.26 ± 0.07 (7.66 - 8.57)	173 ± 3.0
pH 7.5/Alk 16/DOC 0.5	310 ± 1.03	80.0 ± 0.180	348 ± 18.5	13.9 ± 0.375	632.3 ± 11.4	75.2 ± 0.228	0.28 ± 0.030	3.97 ± 0.109	39.2	16.1 ± 0.149	7.60 ± 0.01 (7.50 - 7.66)	150 ± 6.25
Alk 100	299 ± 1.26	75.6 ± 0.269	4000 ± 63.9	16.8 ± 0.380	2620 ± 16.9	75.4 ± 0.187	0.35 ± 0.080	23.4 ± 0.326	37.5	101 ± 0.165	8.35 ± 0.013 (8.09 - 8.49)	546 ± 2.14
Alk 50	314 ± 0.815	74.9 ± 0.208	3990 ± 47.5	16.8 ± 0.710	3620 ± 0.255	74.4 ± 0.254	0.22 ± 0.060	12.3 ± 0.166	38.9	51.7 ± 0.537	8.10 ± 0.03 (7.94 - 8.47)	562 ± 3.05
Hard water[a]	870 ± 0.410	351 ± 0.090	561 ± 0.310	40 ± 0.020	674 ± 0.530	314 ± 0.980	1.1 ± 0.360	20 ± 0.320	122	85 ± 1.06	8.2 ± 0.06	315 ± 6.50

a- Data from Norwood et al. (2006)

3.2.4 Total Cobalt Analyses

Total Cobalt in water and whole-body samples was determined with a Varian SpectraAA 400 graphite furnace, atomic absorption spectrophotometer with Zeeman background correction. The ash temperature was 1100°C and Co was atomized at 2200°C. Method blanks, reference standards, and metal standards were analyzed to ensure quality control (QA/QC) in the determination of background contamination, instrument drift, detection limits and metal recovery. All samples were corrected for background contamination and instrument drift.

There was no significant difference between filtered and unfiltered water samples (Two-way ANOVA, p= 0.469, N=12) or between day 0 and day 7 sample concentrations with an average % loss of 3.55 ± 13.6 (Two-way ANOVA, p=0.144, N= 90). Detection limits were calculated as three times the standard deviation of the method blanks (Norwood, 2008). The detection limit was 7.9 nmol L^{-1} for water samples. The detection limit for whole-body methods was 8.77 nmol L^{-1}. When measurements were below the detection limit, they were set at the limit for input into the models.

Exposure concentrations were mean measured dissolved Co concentrations of samples taken at the beginning and end of all turnover periods in nmol Co L^{-1}. Whole-body concentrations were mean measured Co concentrations of all surviving organisms in a replicate at each exposure concentration after 28 days in nmol Co L^{-1} d.w.

3.2.5 Data analyses

3.2.5.1 Mortality Model

The mortality model, as described in Section 1.7.4.1, was used to determine lethal water and lethal body concentrations. Mortality data were fourth-root transformed to normalize the data before the above models were fitted in SYSTAT 10 (Norwood et al., 2007), after visual inspection of probability plots (Golding et al., 2013) and the Shapiro Wilk test for normality (W=0.777, p=0.000 on untransformed data). In addition, Levene's test (F = 0.977, p = 0.512) was performed to ensure equality of variance of 4^{th} root transformed data (Environment Canada, 2005). Mortality rate was determined by converting percent survival (S) to mortality using the formula,

$$\text{mortality} = -\ln(S) \qquad \text{(Eq. 3.1)}$$

The slope of these mortality values was then calculated over four weeks to determine the mortality rate (Norwood et al., 2007).

3.2.5.2 Bioaccumulation

The bioaccumulation saturation model, as described in Section 1.7.4.2 was used to determine the relationship between bioaccumulation of cobalt and exposure.

3.2.5.3 Confidence Intervals

To determine significant effects, confidence intervals were used as described in section 2.2.3.3.

3.2.5.4 Comparison with Norwood et al. (2006, 2007)

Cobalt concentrations in water and whole-body concentration causing mortality in hard water have been previously determined by Norwood et al. (2007). Lethal concentrations and mortality model parameters for cobalt from Norwood et al. (2007) were used in this study to determine the effect of water hardness on Co toxicity. In addition, raw mortality data from Norwood et al. (2007) were used to calculate an LC10 and LBC10 in hard water.

The saturation model for the bioaccumulation of Co in hard water and its parameters from Norwood et al. (2006) were used to compare bioaccumulation in soft water to hard water.

3.2.5.5 Cobalt Speciation

The Windermere Humic Aqueous Model VI (WHAM VI) was used to estimate the free ion activity and Co-complex concentrations in the different water chemistry treatments (Table 3.3). WHAM VI is a program that simulates the chemical reactions of metals in water or soil, notably the reactions with humic substances. Luther Marsh DOM is 74% humic acid-like material (Gheorghiu et al., 2010) and humic and fulvic acids were assumed to be in particulate form.

Table 3.3. Cobalt speciation (% total Co) determined by WHAM VI.

Treatment	Free ion activity % of Co	Co^{2+}	$CoOH^{+}$	$Co(OH)_2$	$CoSO_4$	$CoCO_3$	$CoCl^{+}$	$CoHCO_3^{+}$	Co-HA	Co-FA
DOC10	34.5	40.9	0.2	0	0.5	1.9	0	0	48.1	4.61
DOC5	45.9	54.2	0.4	0	0.6	3.6	0.1	5.2	32.9	3.2
DOC2	53.6	63.7	0.1	0	0.8	4.3	0.1	6.2	22.03	1.96
pH 6.5	76.1	94	0.1	0	1.1	0.2	0.3	1.6	1.57	1.15
pH 7.5	54.7	65.1	0.8	0	0.8	15.8	0.1	13.4	2.81	1.21
pH 8.5	21.4	26	1.1	0.2	0.3	56.5	0	13	2.8	0.61
Alk 100	10.7	14.4	0.6	0.1	0.1	66.9	0.1	15.8	1.46	0.53
Alk 50	25.9	35	0.7	0.1	0.3	42.3	0.2	20	1.01	0.37
Hard water[a]	18.5	26.1	0.8	0.1	3.8	53.3	0	15.9	0.01	0.02

a- Calculated from data in Norwood et al. (2007)

3.3. RESULTS

3.3.1 Exposure-related mortality

3.3.1.1 DOC

The concentration of Co in water causing mortality decreased as the concentration of added DOC decreased (Table 3.4). The 28-day LC50s for DOC-10 (9.05 ± 0.44 mg C L^{-1}; DOC ± standard deviation), DOC-5 (4.37 ± 0.19 mg C L^{-1}), DOC-2 (2.42 ± 0.32 mg C L^{-1}), and DOC-0.5 (0.28 ± 0.03 mg C L^{-1}) were 90.2, 80.6, 68.3, and 24.7 nmol Co L^{-1}, respectively, with models that had r^2 values between 0.600 and 0.700 (Table 3.4). With increasing concentrations of DOC, the concentrations of Co causing mortality were not significantly different from each other when compared stepwise, but the differences between the DOC-0.5 and DOC-5 treatments were significant (Table 3.3). The DOC-10 treatment 28-day LC50 of 90.2 nmol Co L^{-1} was near the upper end of the Co exposure concentration range tested, so there was high variability in the estimate compared to the variability in the 28-day LC25 and LC10 estimates. Between the lowest and highest DOC concentration treatments there was over a three-fold increase in lethal Co concentrations (Table 3.4).

The mortality model parameters (Table 3.4) were used to generate mortality curves (Figure 3.1). The curve with the highest mortality rates on the graph represents the mortality rates of the DOC-0.5 treatment. The mortality rate in the DOC-2 treatment began to increase at Co concentrations of 15 nmol Co L^{-1}, while DOC-5 treatment did not deviate from control mortality until the Co concentration reached 30 nmol Co L^{-1}. Unlike the other DOC treatments, the DOC-0.5 treatment had a gradual increase in mortality rate. Except for the DOC-10 curve, all the other models approached a maximum (saturated) mortality rate within the concentration range tested. The mortality rate did not reach a maximum in the DOC-10 treatment, which indicates that the true LC50 was likely higher than 100 nmol Co L^{-1}. Control mortality was plotted at the detection limit of 7.9 nmol Co L^{-1} for all exposure-based mortality figures.

3.3.1.2 pH

There were no significant differences among the 28-day LC50s as pH was adjusted (Table 3.5). However, it should be noted that the control mortality rate in the pH-6.7 treatments (m′ in Table 3.5) was greater than 10% per week in several replicates, indicating that this pH was getting close to the tolerance for *H. azteca*. Acceptable control survival/mortality rates are 65% survival over 4 weeks or <10% mortality $week^{-1}$ (Borgmann, 2002). The control mortality rate of

the pH-8.3 treatment was also higher than the pH-7.7 treatment, but the survival was acceptable for a 28-d test. The parameters in Table 3.5 were modelled in Figure 3.2. The pH-6.7 curve was the highest curve on the graph; however, the data points for all treatments were overlapping.

3.3.1.3 Alkalinity

The mid-range alkalinity treatment Alk-50 (51.7 ± 0.54 mg L^{-1} as $CaCO_3$ equivalents) had a 28-day LC50 1.8 times greater than the high (Alk-100; 101 ± 0.17 mg L^{-1} as $CaCO_3$ equivalents) and low (Alk-16; 16.1 ± 0.15 mg L^{-1} as $CaCO_3$ equivalents) alkalinity treatments; however, there were no significant differences among these values (Table 3.6). The alkalinity treatment mortality model r^2 values were greater than 0.650. The models for Alk-100 and Alk-16 gradually increase in mortality rates as the Co exposure concentration (Figure 3.3). The Alk-50 treatment had sharp increase in mortality rate at about 20 nmol Co L^{-1} and reached a greater maximum mortality rate than the other treatments.

3.3.1.4 Hardness

The concentrations of Co causing mortality in soft water were significantly lower when compared to experiments by Norwood et al. (2007) conducted in hard water. (Table 3.7). The 28-day LC50 in Hardness-37.5 was 25.4 nmol Co L^{-1} and in Hardness 122 it was 183 nmol Co L^{-1}, while the 28-day LC25 were 16.6 and 68 nmol Co L^{-1}, respectively. Both models had r^2 values greater than 0.850. The two mortality curves (Figure 3.4) intersect at a low concentration of Co in water and deviate above this concentration. The curve of the mortality rate for hardness-37.5 has a greater increase and approaches a maximum mortality rate, whereas the hardness-122 curve has a lesser increase and does not approach a maximum over this concentration range.

Table 3.4. Mortality model parameters based on concentrations of Co in water of treatments with different dissolved organic carbon concentrations. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Treatment	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}			
DOC 10	0.010	-123	(-685 - 438)	90.2	(15.1 - 165)	60.5	(26.6 - 94.4)	27.9	(-70.8 - 127)	0.844	0.603
DOC 5	0.010	10.7	(0.208 - 21.1)	80.6	(42.3 - 119)	45.1	(28.2 – 62.0)	29	(14.3 - 43.7)	10.0	0.677
DOC 2	0.019	4.44	(-0.076 - 8.96)	68.3	(24.8 - 112)	27.3	(9.33 - 45.2)	15.5	(3.54 - 27.5)	10.0	0.673
DOC 0.5	0.033	1.94	(0.469 - 3.42)	24.7	(12.1 - 37.2)	10.9	(5.08 - 16.7)	6.42	(2.58 - 10.3)	10.0	0.655

Table 3.5. Mortality model parameters based on concentrations of Co in water of treatments with different pH. Parameters include control mortality rate (m′), half saturation constant (K_w''),28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Treatment	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}			
pH 6.7[a]	0.059	2.54	(0.807 - 4.27)	15.9	(9.20 - 22.5)	9.51	(4.85 - 14.2)	6.34	(2.95 - 9.73)	10.0	0.703
pH 7.7	0.033	1.94	(0.469 - 3.42)	24.7	(12.1 - 37.2)	10.9	(5.08 - 16.7)	6.42	(2.58 - 10.3)	10.0	0.655
pH 8.3	0.042	3.88	(0.748 - 7.02)	30.0	(15.7 - 44.3)	16.6	(7.72 - 25.6)	10.7	(4.28 - 17.1)	10.0	0.569

[a] higher than acceptable control mortality for 28-d assay

Table 3.6. Mortality model parameters based on concentrations of Co in water of treatments with different alkalinity. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Treatment	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}			
Alk 100	0.029	5.39	(3.04 - 7.74)	25.4	(19.9 - 30.8)	16.6	(12.4 - 20.9)	11.6	(8.23 - 15.0)	10.0	0.854
Alk 50	0.027	27.4	(-131 - 185)	44.9	(26.6 - 63.2)	18.3	(10.6 – 26.0)	8.78	(0.280 - 17.3)	2.00	0.763
Alk 16	0.033	1.94	(0.469 - 3.42)	24.7	(12.1 - 37.2)	10.9	(5.08 - 16.7)	6.42	(2.58 - 10.3)	10.0	0.655

Table 3.7. Mortality model parameters based on concentrations of Co in water of treatments with different hardness. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Treatment	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}			
Hardness 37.5	0.029	5.39	(3.04 - 7.74)	25.4	(19.9 - 30.8)	16.6	(12.4 - 20.9)	11.6	(8.23 - 15.0)	10.0	0.854
Hardness 122[b]	0.05	3900		183	(120 - 279)	68.0	(33 - 140)	22.3	(4.76 - 39.9)	0.913	0.860

[b] From Norwood et al. (2007)

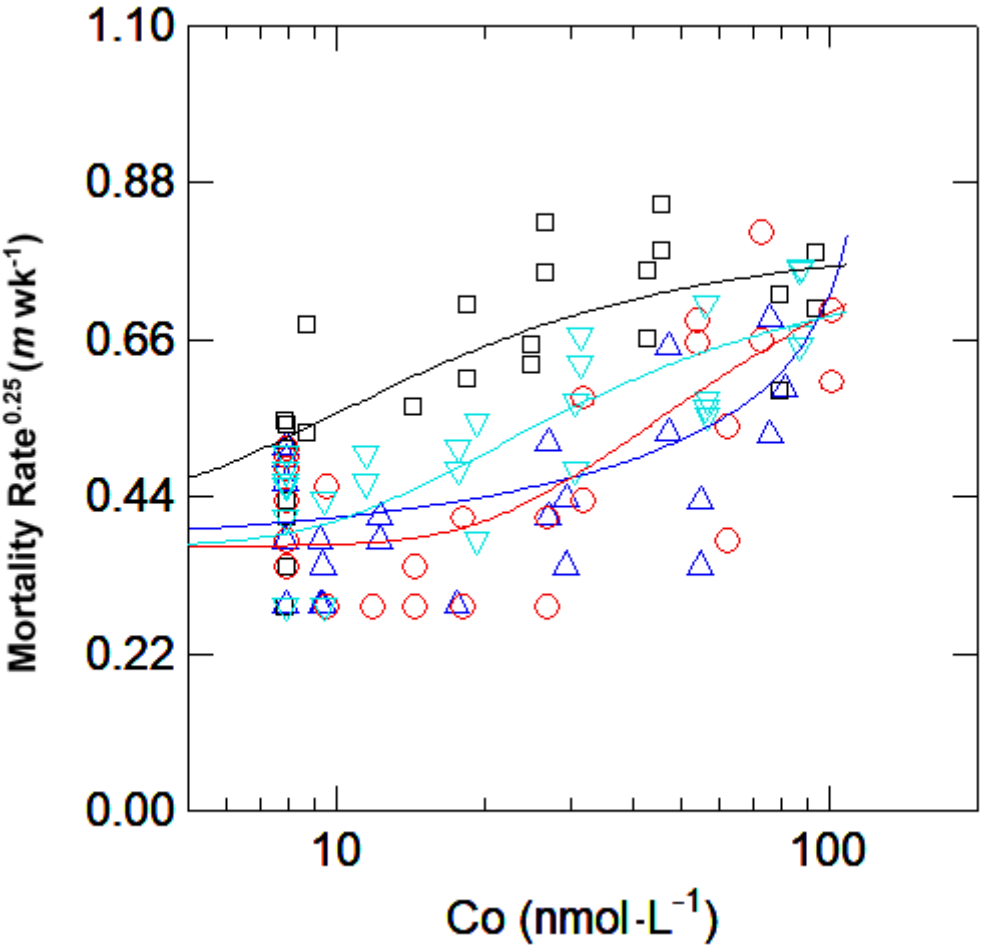

Figure 3.1. Waterborne Co mortality models with modified DOC based on parameters in Table 3.4. Data points are mortality rates at measured concentrations of Co in water. □ are data from DOC-0.5 experiments, ▽ are DOC-2, ○ are DOC-5, △ are DOC-10. The solid lines represent the corresponding Co mortality model.

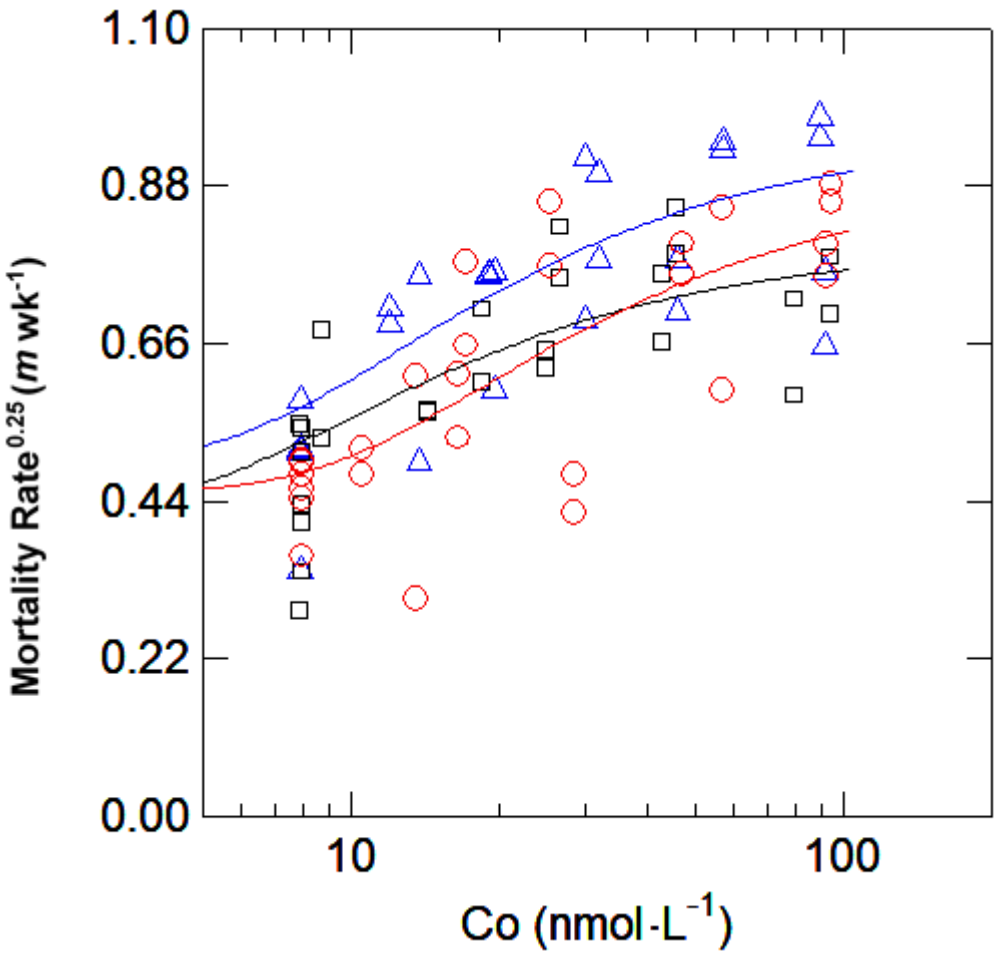

Figure 3.2. Waterborne Co mortality models with modified pH based on parameters in Table 3.5. Data points are mortality rates at measured concentrations of Co in water. △ are data from experiments pH-6.8, ○ are pH-7.7, and □ are pH-8.3. The solid lines represent the corresponding Co mortality model.

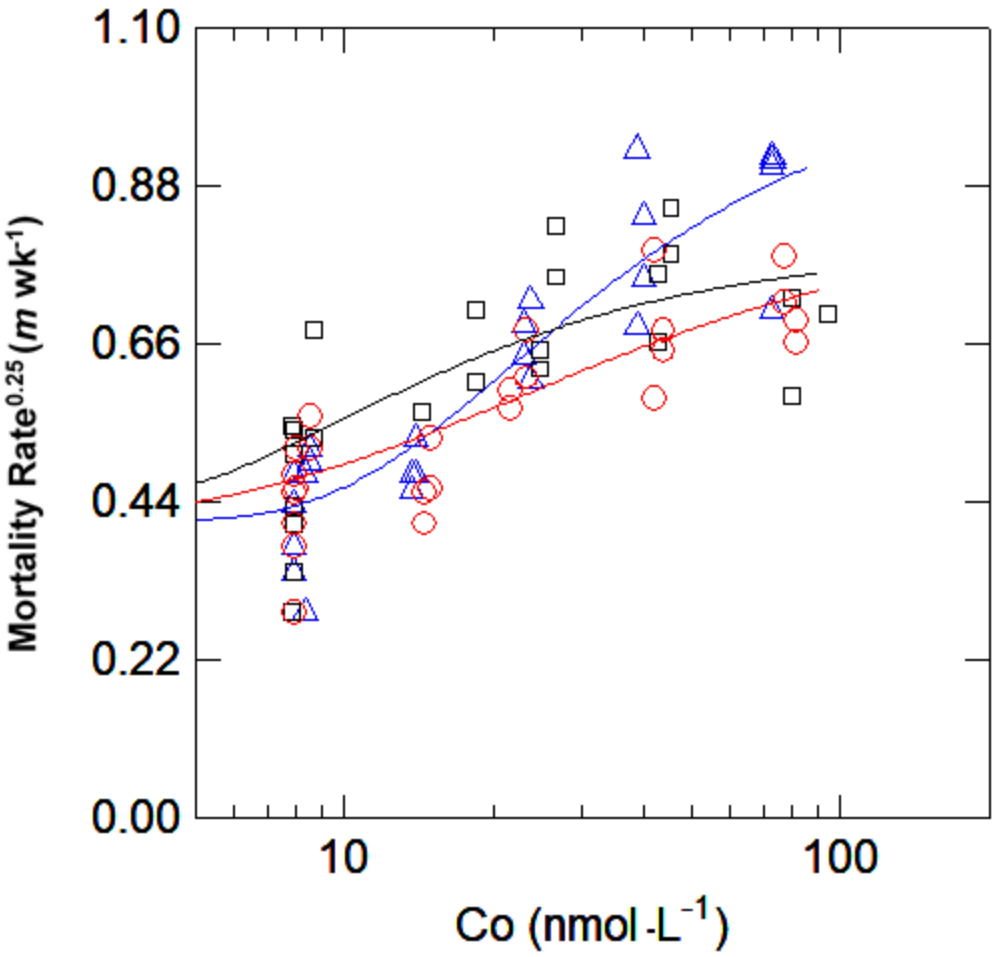

Figure 3.3. Waterborne Co mortality models with modified alkalinity based on parameters in Table 3.6. Data points are mortality rates at measured concentrations of Co in water. △ are data from Alk-100 experiments, ○ are Alk-50, and □ are Alk-16. The solid lines represent the corresponding Co mortality model.

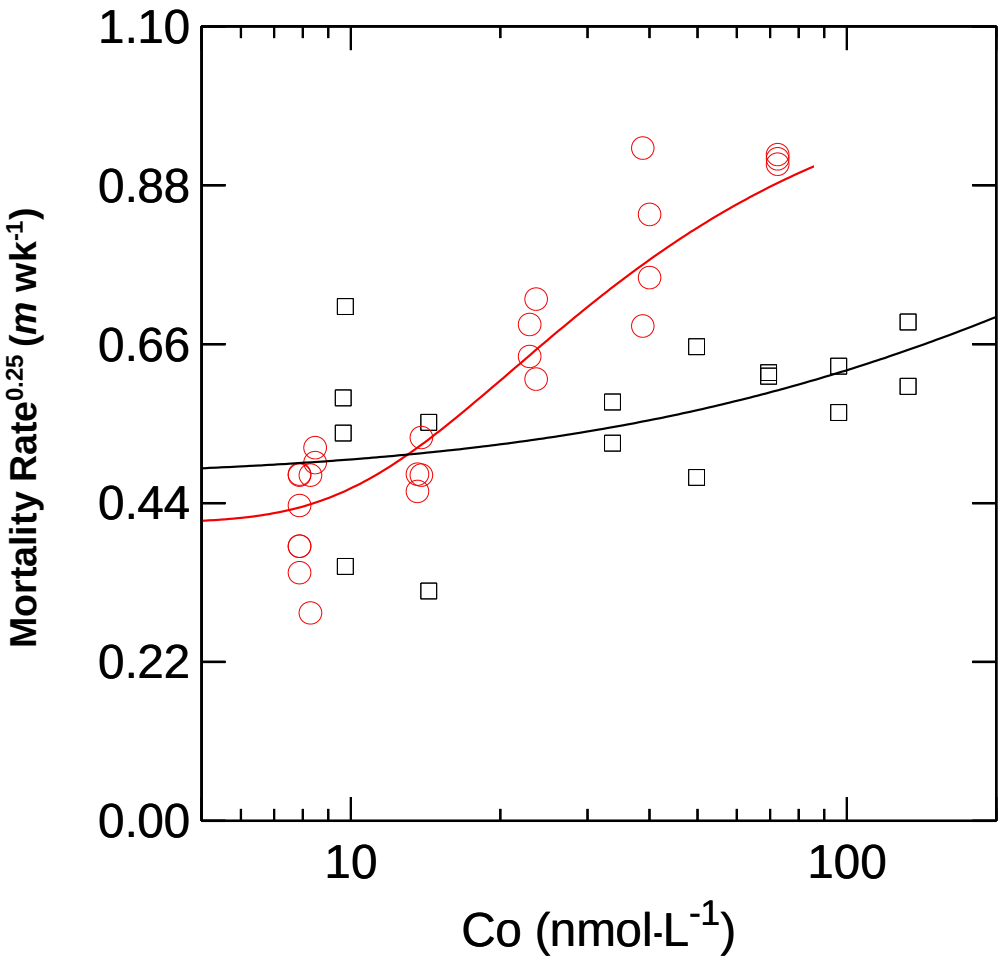

Figure 3.4. Waterborne Co mortality models with modified hardness based on parameters in Table 3.7. Data points are mortality rate at measured cobalt water concentration in SAM30 with modified hardness. ○ are Hardness-37.5 and □ are Hardness-122. The solid lines represent the corresponding Co mortality model.

3.3.2 Bioaccumulation

There was greater accumulation of Co in *H. azteca* in the soft water treatments of the current study when compared to hard water exposure of Norwood et al. (2006) over the exposure concentration ranged tested (Table 3.8). The pooled soft water model, based on pooled data except DOC-10 and DOC-5 treatments, predicted a maximum Co whole-body concentration of 720 nmol Co g^{-1} dry weight (d.w.), while the hard water model predicted a maximum of 674 nmol Co g^{-1} d.w. The DOC-10 and DOC-5 treatments had an accumulation pattern similar to the hard water model. The measured body concentrations in these DOC treatments were significantly different from other soft water treatments at several concentrations of Co in water (Figure 3.5), so were not included in the pooled soft water model (Tukey's HSD post hoc test, $p < 0.05$). At high DOC concentrations, the hard water model was a better predictor of bioaccumulation than the pooled soft water model.

Although the maxima were not significantly different for all models, the bioaccumulation factor for the soft water treatments was 2.5 times greater than the hard water treatment, which indicates a greater proportional rate of uptake at low concentrations of Co in water. The max/K value of the pooled soft water model was significantly higher than both other models (Table 3.8). The max/K ratio can be used to compare how bioaccumulation changes with the surrounding media as it is the ratio of maximum Co accumulation to the concentration of Co in water where half the maximum Co was accumulated. A higher max/K was indicative of greater Co accumulation.

The bioaccumulation models' predictions of Co body concentration were within two times the measured body concentrations 87.5% of the time and were used to predict body concentrations for exposures that did not have enough organisms alive at the end of 28-days to determine measured body concentrations (Figure 3.6). These predicted body concentrations were used in body concentration mortality models (Section 3.3.3).

Table 3.8. Co bioaccumulation model parameters with predicted maximum cobalt accumulation (max) and half saturation constant (K) with 95% confidence limits (CL), r^2, number of data points (N), mean dry to wet weight ratio (D/W), bioconcentration factor (BCF) calculated as max × K^{-1} × D/W × 1000, and background cobalt concentration in *H. azteca* (C_{bk}).

Treatment	N	r^2	max	CL	K	CL	MAX K^{-1}	CL	C_{bk}	D/W ratio	BCF
			nmol g^{-1}		nmol L^{-1}		L g^{-1}		nmol g^{-1}		
Pooled DOC 10, 5	38	0.792	679	(-405 - 1763)	322	(-249 - 894)	2.11	(1.65 - 2.56)	<8.77	0.234	494
Pooled soft water	87	0.454	720	(-63.8 - 1500)	130	(-39.9 - 300)	5.55	(4.09 – 7.00)	<8.77	0.259	1440
Hardness 122[a]	34	0.838	674	(395 – 1150)	378	(183 – 780)	1.79	(1.39 – 2.34)	<15.5	0.289	515

[a] From Norwood et al. (2006)

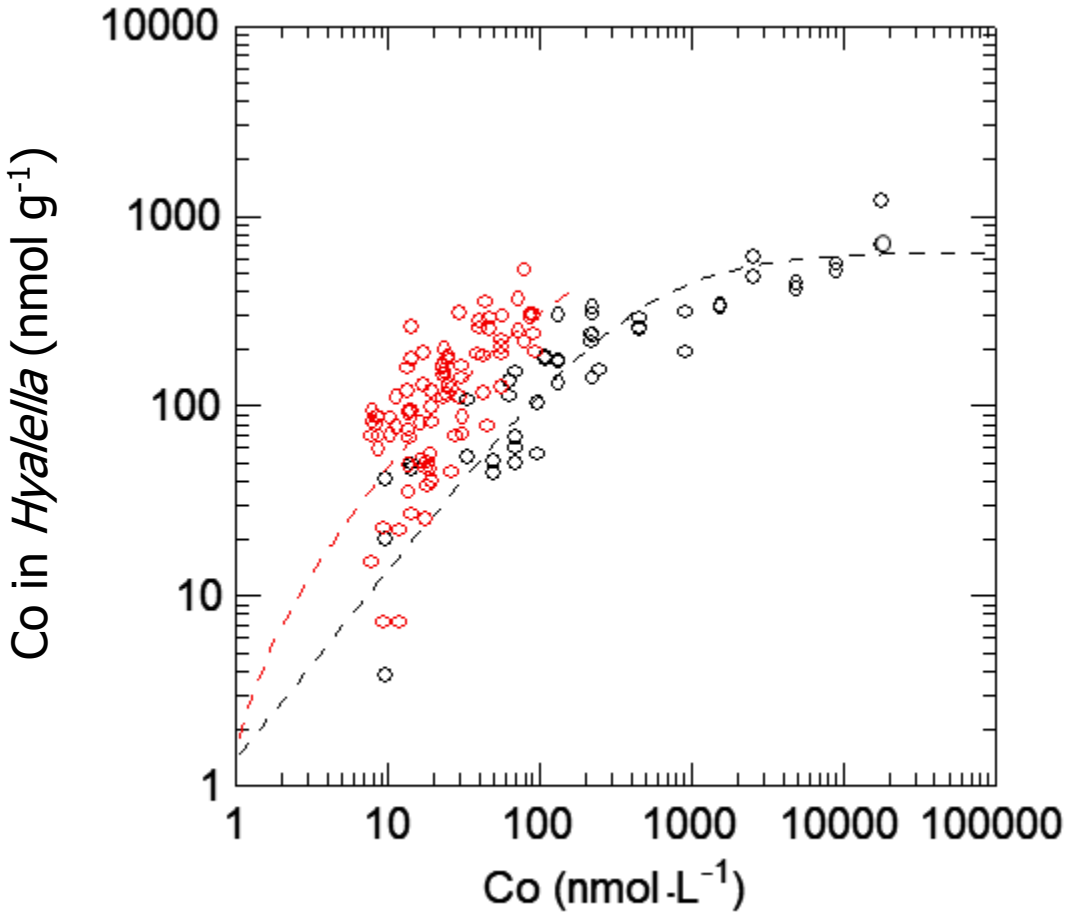

Figure 3.5. Influence of concentration of Co in water on Co concentration accumulated in *H. azteca* in a 4-week exposure in different soft water treatments (red) as compared to accumulation in hard water treatments by Norwood et al. (2007) (black). Dashed lines are bioaccumulation models calculated from parameters in Table 3.8.

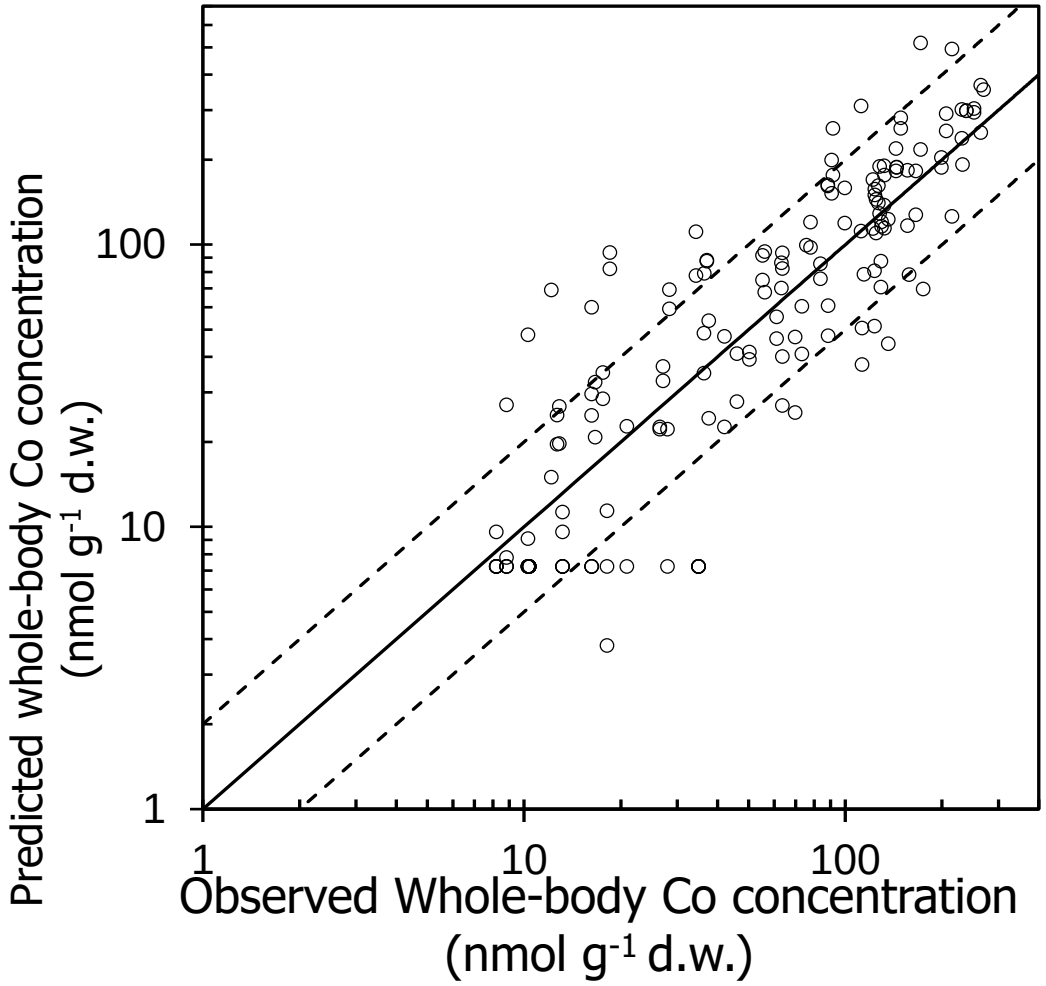

Figure 3.6. Predicted concentrations based on the bioaccumulation models from Figure 3.5 and calculated using the parameters in Table 3.8. Observed Co whole-body concentrations based on average dry weight for each replicate. The solid line indicates a 1:1 relationship and the dashed line is 2x overpredicted or 2x underpredicted

3.3.3 Body concentration-related mortality

3.3.3.1 DOC

DOC concentrations did not have a significant effect on the total body accumulation of cobalt causing mortality (Table 3.9). Although the lethal body concentration increased from 140 to 230 nmol Co g^{-1} d.w. between the DOC-0.5 and DOC-2 treatments, the DOC-10 28-day LBC50 was 174 nmol Co g^{-1} d.w. The 28-day LBC25 values were the most similar among the treatments ranging from 87.0 to 112 nmol Co g^{-1} d.w.

Three of the DOC models (DOC-0.5, DOC-5, DOC-10) had similar shapes (Figure 3.7), with a sharp increase in mortality rate when the whole-body Co concentrations reached 60 – 70 nmol Co g^{-1} d.w. The DOC-2 treatment had a more gradual increase in mortality; however, at body concentrations of about 100 nmol Co g^{-1} d.w., the treatments with added DOC (DOC-2, DOC-5, DOC-10) had the same mortality rate.

3.3.3.2 pH

The 28-day lethal body concentrations in the pH-6.7 treatment were lower than other treatment due to increased mortality from the effect of H^+, as previously described in Section 3.3.1.2. The lower pH resulted in whole-body lethal concentrations that were significantly lower than the pH-8.3 lethal body concentrations. The pH-8.3 lethal body concentrations were not significantly different from the pH-7.7 lethal body concentration or any of the DOC treatments' lethal body concentrations, despite the control mortality rate also being elevated (Table 3.10).

The mortality models for pH-8.3 and pH-7.7 were similar (Figure 3.8), with a slight difference due to the control mortality rate. The pH-6.7 model had a higher mortality rate over the body concentration range and deviated from the control mortality rate at 30 nmol g^{-1}, whereas the other two treatments had a sharp increase between 50 – 70 nmol Co g^{-1} d.w.

3.3.3.3 Alkalinity

The 28-day LBC50s for the three alkalinity treatments of Alk-16, Alk-50, and Alk-100 were 140, 166, and 144 nmol Co g^{-1} d.w., respectively (Table 3.11). There were no significant differences among the lethal concentrations. The mortality models (Figure 3.9) were virtually indistinguishable and all had inflection points between 60 and 80 nmol Co g^{-1} d.w.

3.3.3.4 Hardness

The 28-day lethal body concentrations for the two different hardness treatments were not significantly different from each other with the Hardness-37.5 28-day LBC50 at 144 nmol Co g^{-1} d.w. and the Hardness-122 LBC50 at 192 nmol Co g^{-1} d.w. (Table 3.12). The 28-day LBC25 values were 98.1 nmol Co g^{-1} d.w. for Hardness-37.5 and 90 nmol Co g^{-1} d.w. for Hardness-122.

The models (Figure 3.10) indicate that at 500 nmol Co g^{-1} d.w. mortality rates were similar, as the curves intersect; however, at lower Co concentrations the curves had different shapes due to lack of data in this range from Norwood et al (2007). The soft water curve had an increase in mortality at about 70 nmol Co g^{-1} d.w., but no such value can be accurately stated for moderately hard water.

Table 3.9. Mortality model parameters based on cobalt body concentrations in organisms exposed to treatments with different dissolved organic carbon concentrations. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	$weeks^{-1}$		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
DOC 10	0.019	34.6	(-18.1 - 142)	174	(48.1 - 300)	112	(82.2 - 142)	77.4	(44.4 - 110)	10.0	0.474
DOC 5	0.02	39.1	(1.61 – 76.6)	146	(115 - 177)	102	(75.1 - 128)	73.6	(45.7 - 101)	10.0	0.696
DOC 2	0.019	467	(-2860 – 3780)	230	(151 – 309)	103	(60.3 – 145)	46.5	(25.3 – 67.6)	1.46	0.684
DOC 0.5	0.026	25.1	(-2.90 - 53.1)	140	(69.0 - 210)	87	(29.1 - 145)	59.1	(13.6- 105)	10.0	0.572

Table 3.10. Mortality model parameters based on cobalt body concentrations in organisms exposed to treatments with different pH. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	$weeks^{-1}$		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
pH 6.7[a]	0.059	11.5	(4.43 - 18.5)	69.7	(46.5 - 92.9)	42.2	(25.5 - 59.0)	28.3	(15.7 - 40.9)	10.0	0.545
pH 7.7	0.026	25.1	(-2.90 - 53.1)	140	(69.0 - 210)	87	(29.1 - 145)	59.1	(13.6- 105)	10.0	0.572
pH 8.3	0.042	91.2	(10.3 - 172)	161	(122 - 201)	105	(71.0 - 140)	71.2	(42.2 - 100)	10.0	0.565

[a] higher control mortality than acceptable for a 28-d exposure

Table 3.11. Mortality model parameters based on cobalt body concentrations in organisms exposed to treatments with different alkalinity. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	$weeks^{-1}$		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
Alk 100	0.042	35.0	(18.9 – 51.0)	144	(116 – 172)	98.1	(74.4 – 122)	70.0	(50.3 – 89.6)	10.0	0.843
Alk 50	0.027	25.5	(17.9 - 33.2)	161	(136 – 186)	96.3	(80.6 - 112)	64.0	(51.9 - 76.2)	10.0	0.75
Alk 16	0.026	25.1	(-2.90 - 53.1)	140	(69.0 - 210)	87	(29.1 - 145)	59.1	(13.6- 105)	10.0	0.572

Table 3.12. Mortality model parameters based on cobalt body concentrations in organisms exposed to treatments with different hardness. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	$weeks^{-1}$		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
Hardness 37.5	0.042	35.0	(18.9 – 51.0)	144	(116 – 172)	98.1	(74.4 – 122)	70.0	(50.3 – 89.6)	10.0	0.843
Hardness 122[b]	0.050	-747		192	(138 – 264)	90.0	(42 – 177)	n/a		0.913	0.858

[b] From Norwood et al., 2007

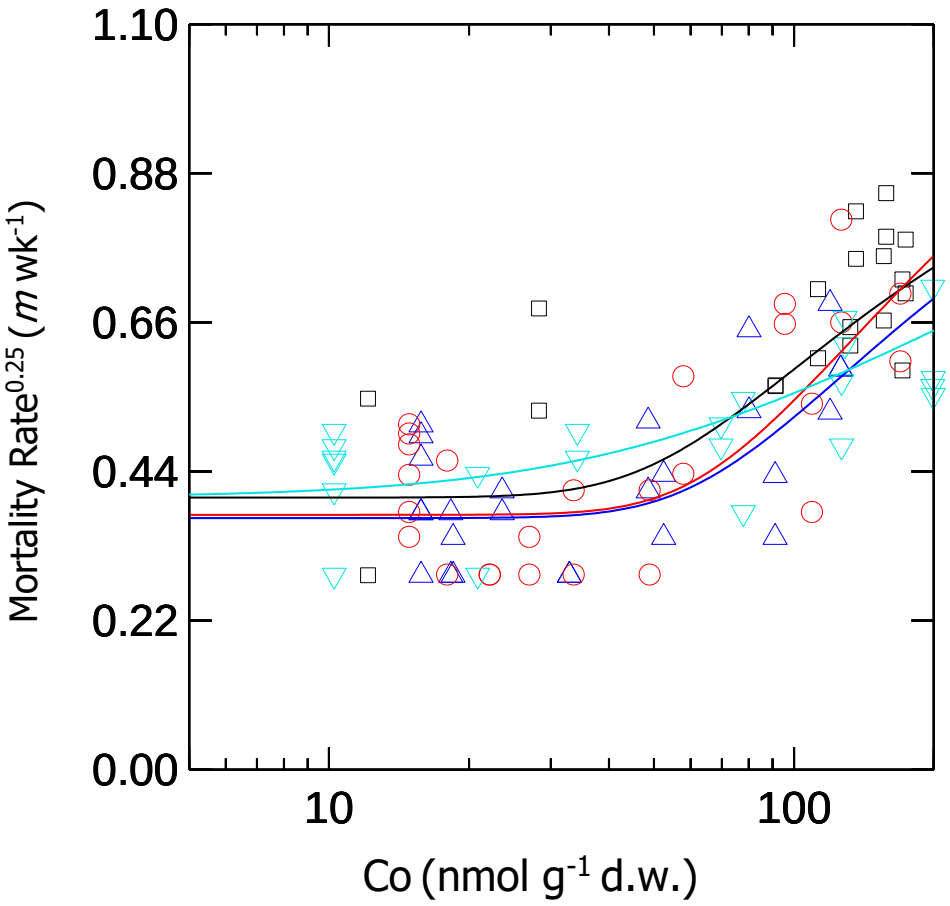

Figure 3.7. Co body-concentration mortality models based on parameters in Table 3.9 with modified DOC concentrations in the exposure water. Data points are mortality rate at the mean of measured whole-body concentrations of Co on a dry weight basis in organisms. □ are data from DOC-0.5 experiments, ▽ are DOC-2, ○ are DOC-5, and △ are DOC-10. The solid lines represent the corresponding Co mortality model.

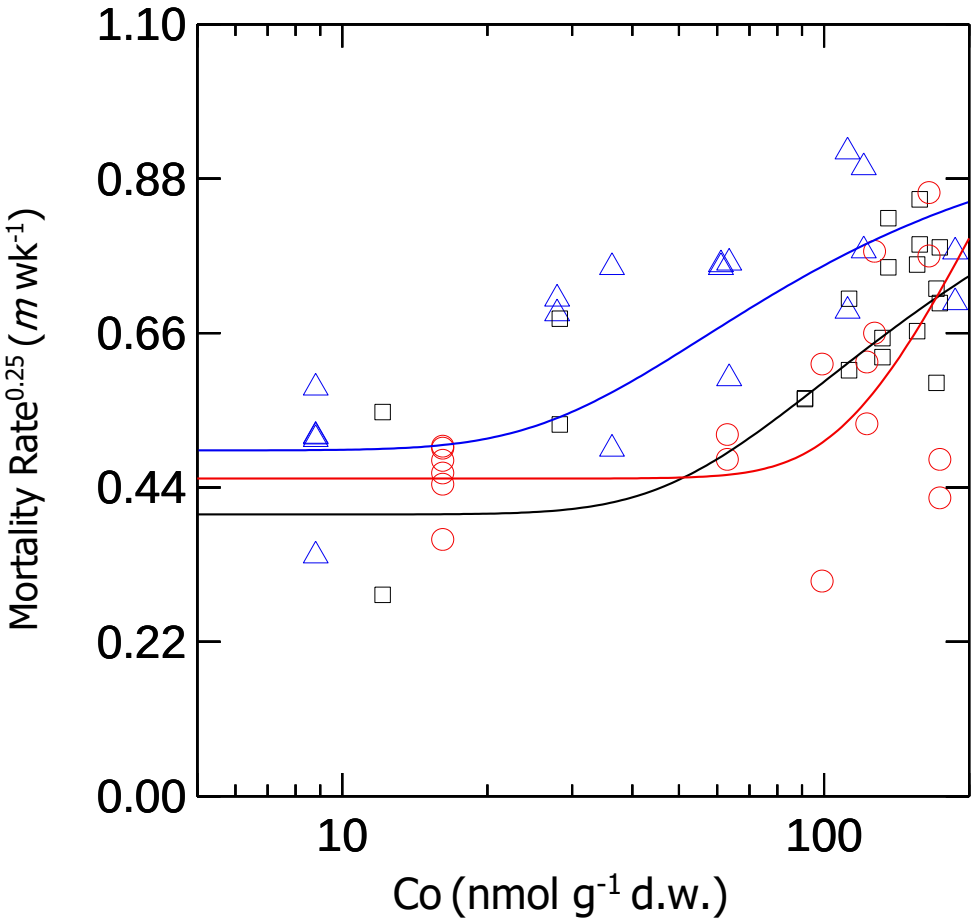

Figure 3.8. Co body-concentration mortality models based on parameters in Table 3.10 with modified pH of the exposure water. Data points are mortality rate at the mean of measured whole-body concentrations of Co on a dry weight basis. △ are data from experiments in pH 6.8, ○ are pH 7.7, and □ are pH 8.3. The solid lines represent the corresponding Co mortality model.

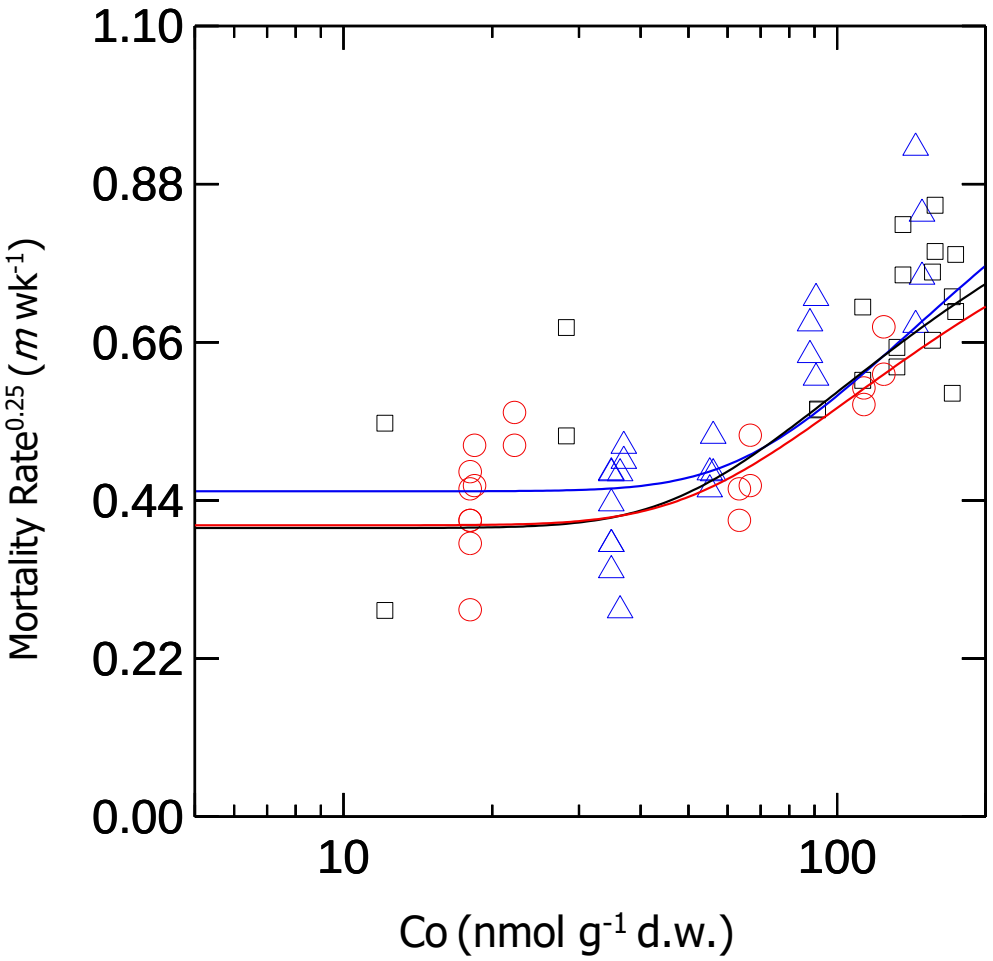

Figure 3.9. Co body-concentration mortality models based on parameters in Table 3.11 with modified alkalinity of the exposure water. Data points are mortality rate at the mean of measured whole-body concentrations of Co on a dry weight basis. △ are data from Alk-100 experiments, ○ are Alk-50, and □ are Alk-16. The solid lines represent the corresponding Co mortality model.

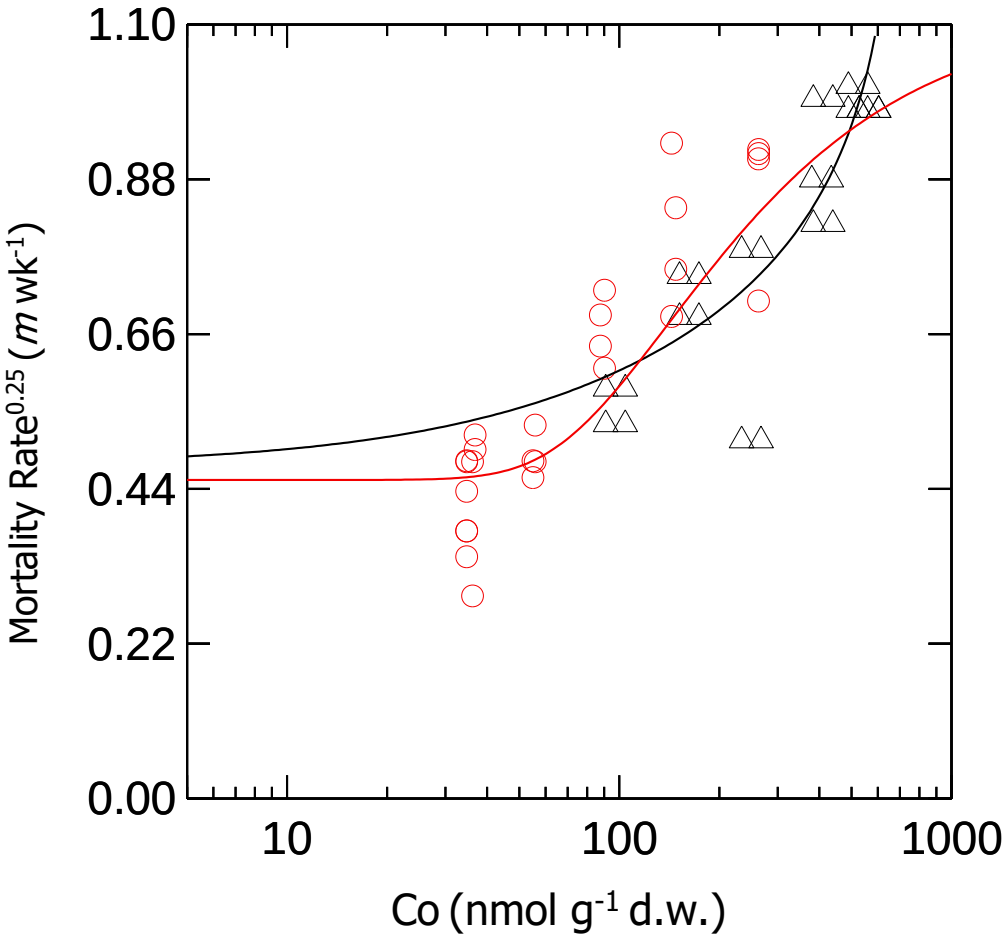

Figure 3.10. Co body-concentration mortality models based on parameters in Table 3.12 with modified exposure water hardness. Data points are mortality rates at the mean of measured whole-body concentrations of Co on a dry weight. △ are data from Hardness-37.5 experiments and ○ are Hardness-122. The solid lines represent the corresponding Co mortality model.

3.4. DISCUSSION

It was hypothesized in section 1.9.2 that treatments with increased DOC concentrations would decrease the toxicity of Co to *H. azteca*. It was also hypothesized that increased pH and alkalinity would also be less toxic. Higher hardness was protective against metal toxicity and it was expected that the concentration of Co in water causing mortality would be less than 183 nmol Co L^{-1} (Norwood et al. 2007) in moderately hard water, as Ca^{2+} will compete with Co^{2+} for uptake. In addition, it was hypothesized that the lethal Co body concentrations would not be affected by the concentration of Co in water.

3.4.1 DOC

The addition of 5 mg L^{-1} of DOC significantly reduced the waterborne toxicity of Co to *H. azteca*. The 28-day LC50 increased three-fold with this addition (Table 3.4). Wantanabe et al. (2017) discovered that 10 mg TOC L^{-1} of aquatic humic substances extracted from water in Brazil could not reduce the toxicity of 50 µg Co L^{-1} to *Ceriodaphnia dubia*; however, this concentration was much greater than the range tested in this study. The bioaccumulation patterns between low DOC treatments (DOC-0.5, DOC-2) and high DOC treatments (DOC-5, DOC-10) were different, as more Co was accumulated at lower Co exposure concentrations when there was less DOC present (Figure 3.5). The maximum Co body concentration predicted for all DOC models were predicted to be not significantly different, even with these different uptake patterns (Table 3.8). Richards and Playle (1998) observed that greater than 25 mg C L^{-1} of dissolved organic matter (DOM) was required to adequately complex 9000 nmol Co L^{-1} and prevent it from accumulating on the gills of rainbow trout. In the current study, 5 mg DOC L^{-1} prevented the accumulation of Co, which indicates that rainbow trout and *H. azteca* Co accumulation was not comparable.

The mortality caused by Co body concentrations was not affected by increasing DOC concentrations (Table 3.9). When comparing the 28-day LBC50 of DOC-0.5 to DOC-5, there was no significant different between the two values or between any of the other DOC additions. Ouelett et al. (2013) did not find any significant effect on mortality related to *Chironomus dilutus* body concentrations in media with 4.0 mg C L^{-1} compared to 6.4 mg C L^{-1}; however, this study was an effluent study with other metals present.

3.4.2 Alkalinity and pH

In this study, there was no significant difference in mortality influenced by the concentration of Co in water when pH was adjusted (Table 3.5). Decreasing pH can cause an

increase in the concentration of the free metal ion, which is more readily taken up in an organism (Di Toro et al., 2001). Conversely, metal toxicity and bioavailability can also be reduced with decreasing pH due to H^+ competition for binding to uptake sites (Playle, 1993; De Schamphelaere & Janssen, 2004). It is possible these two competing processes produced no net pH effect on the concentration of Co causing mortality or that the pH range tested did not significantly affect the concentration of toxic species.

It was expected that the whole-body concentration of Co causing mortality would not be affected by pH; however, whole-body concentrations causing mortality were significantly lower at pH 6.7. Low survival of *H. azteca* was observed by Borgmann et al (2001) in control containers after 4-weeks at pH 5.6 - 6.6 and was attributed to pH stress. However, in this study, the pH-8.3 treatment also had higher control mortality (but still within the acceptable range), and this treatment was significantly different from the pH 6.7 treatment.

The prediction of Richards & Playle (1998) of a decrease in Co binding to the site of uptake in alkaline water was not supported by accumulation data from this study (Table 3.11). There was no significant difference in the body concentrations causing mortality among the different alkalinity treatments and these treatments had the same accumulation patterns over the exposure range tested. However, after a 21-day exposure to mine process water effluent that had a five-fold increase in alkalinity compared to the reference water there was a two-fold increase in Co body concentration in *Chironomus dilutus* that did not cause mortality (Ouelett et al., 2013). It was unclear whether the organisms were gut-cleared in that study and it was also a multi-metal exposure in a mesocosm, so other interactions could have influenced the Co accumulation. It was also predicted by Richards and Playle (1998) that Co accumulation would decrease because concentrations of OH^- and CO_3^{2-} compounds would increase in more alkaline water. The authors theorized that $CoOH^+$ could also contribute to accumulation. The concentration of $CoOH^+$ was around 1% of the total aqueous Co (Table 3.3). However, in this study, there was no evidence that other Co species other than the free ion were taken up by *H. azteca*, so there was greater importance for this fraction. In systems with greater alkalinity and higher concentration of $CoOH^+$, this species could have a major influence on overall Co toxicity.

3.4.3 Hardness

It was expected that the Co lethal concentrations in the soft water of this study would be less than Co lethal concentrations in moderately hard water from Norwood et al. (2007) due to

the decrease in Ca^{2+} competition for uptake. With the decrease in hardness from 122 mg L^{-1} to 37.5 mg L^{-1} as $CaCO_3$ equivalents, the toxicity increased.

A similar increase in toxicity was seen in a 7-day toxicity test with *H. azteca*. In hard water (124 mg L^{-1} as $CaCO_3$ equivalents) and soft water (18 mg L^{-1} as $CaCO_3$ equivalents) the 7-day LC50s were 1035 nmol Co L^{-1} and 271 nmol Co L^{-1}, which was a decrease of 7 times to hardness and 3.5 times decrease to the lethal concentration (Borgmann et al., 2005). In 96h acute lethal bioassays using the fish *Capoeta fusca*, it was determined that decreasing the water hardness from 350 to 130 mg L^{-1} as $CaCO_3$ equivalents caused a similar decrease in toxicity of about 2.5 times, with the 96-h LC50 decreasing from 204.8 mg Co L^{-1} to 91.7 mg Co L^{-1} (Pourkhabbaz et al., 2011).

The accumulation of Co that caused 50% mortality in *H. azteca* in Norwood et al. (2007) was 192 ± 72 nmol Co g^{-1} d.w. and in this study, it was 106 ± 44.4 nmol Co g^{-1} d.w. Verschoor et al. (2012) compared 3-week bioaccumulation of Co and survival of *D. magna* and *Gammarus roeseli* at sites that varied in hardness from 113 to 303 mg L^{-1} as $CaCO_3$ equivalents. The most cobalt was accumulated in *D. magna* at the site that had the lowest hardness; however, it should be noted that the pH of some sites could also affect the uptake pattern (Verschoor et al., 2012). In water at a hardness of 122 mg L^{-1} as $CaCO_3$ equivalents, *G. roeseli* accumulated 390 nmol Co g^{-1} when exposed to 407 nmol Co L^{-1} over 3 weeks (Verschoor et al., 2012). In the current study, *H. azteca* were exposed to Co for four weeks and roughly the same concentration was accumulated; however, the exposure concentrations were lower by a factor of 4 and the hardness was three times lower than those stated in Verschoor et al. (2012). Despite the different species and experimental lengths, in all cases the increase in water hardness caused toxicity to decrease. Hardness is known to affect the toxicity of many metals including copper, cadmium, and zinc due to competition for uptake with calcium (Di Toro et al., 2001; Clifford & McGeer, 2010; Clifford & McGeer, 2009).

3.5 CONCLUSION

As expected, the different water chemistry treatments resulted in a wide range of toxic effects with a six-fold difference in 28-day LC50s. Hardness appears to be protective, as 28-day LC50 values in this study were lower when compared to those from a study done in harder water (Norwood et al 2007). However, decreasing pH was not protective and there were no significant differences in mortality. Changes in alkalinity treatments did not have a significant effect on toxicity but did show considerable variation. Therefore, because of the effect different water

chemistry treatments have on cobalt toxicity, it was apparent that concentrations of Co in water were not as reliable as predictors of toxicity than whole-body concentrations.

Lethal body concentrations ranged less than two-fold in all water chemistries tested. As expected, the LBC50s, LBC25, and LBC10s were not statistically different from each other, nor was there a statistical difference between the lethal body concentrations in soft water and the lethal body concentration in moderately hard water determined by Norwood et al. (2007), except for the lowest pH treatment which appeared to have a cumulative toxic effect from both the cobalt exposure and toxicity due to the low pH exposure.

Whole-body concentrations of Co causing mortality were less variable than concentrations of Co in water causing mortality over a wide range of water chemistries. These data support the hypothesis that the whole-body concentration of Co is a better predictor of toxic effects than the concentration of Co in water.

3.6 SUMMARY

1. Hardness was the most important water chemistry parameter influencing exposure-related Co toxicity in 28-day exposures based on the concentration of Co in water. Greater hardness reduces Co toxicity.
2. Dissolved organic carbon was protective against Co toxicity in 28-day exposures based on the concentration of Co in water.
3. The bioaccumulation of Co can be predicted by a saturation model in soft water.
4. The whole-body concentration of Co causing mortality in *H. azteca* was not significantly affected by different water chemistry parameters.
5. The whole-body concentration of cobalt was a better predictor of toxic effects than the concentration of Co in water

CHAPTER 4

Using saturation kinetics-based non-linear regression models to predict the chronic toxicity and bioaccumulation of selenium to *Hyalella azteca* under different water chemistry conditions

OVERVIEW

The effects of varying different water chemistry parameters on the toxicity of selenite to *Hyalella azteca* were investigated in 28-d exposures. Dissolved organic carbon, pH, alkalinity, and hardness all significantly affected the 28-day LC50s of Se, which ranged from 240 – 957 nmol Se L^{-1}. The highest DOC concentration of 10 mg L^{-1} was associated with the greatest Se toxicity. The bioaccumulation pattern of Se was also affected by these variables, with uptake increased at lower pH and increased DOC. The concentration of Se accumulated by *H. azteca* was not consistently associated with mortality, although this is the case for several metals. The 28-day lethal body concentrations (LBC50) ranged three-fold. The highest concentration of DOC was associated with the greatest Se toxicity on a body concentration basis as well.

4.1. INTRODUCTION

Selenium (Se) is an abundant element in the earth's crust associated with sulfur and in high concentrations in coal (Shamberger, 1981). The presence of Se in these environments means that it can be released into aquatic environments during the refining process of metal sulfide ores or into the atmosphere during the combustion of coal. Coal combustion is also a major source of Se aquatic contamination, as the coal combustion residues from coal plants are transported into settling ponds and can later be discharged (Lemly, 2004; Harkness, 2016). Human activity has also increased the leaching of Se from seleniferous soils in alkaline, oxidizing conditions due to agricultural irrigation (Lemly, 2004) and from waste rock from coal mining (Miller et al., 2013). Selenium can also be released into the environment from natural processes such as the weathering of rock and other anthropogenic sources like agricultural usage and oil refining (Pieterek and Pietrock, 2012). In aquatic systems, Se can have inorganic (-2, -1, 0, +4, +6) and organic forms; however, the most abundant species are the most oxidized forms selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (Lenz & Lens, 2009). The speciation is dependent on water chemistry variables such as pH, redox conditions, and organic matter, as well as physical and biological processes (Sharma et al., 2015). Concentrations of Se in fresh water average 1 µg L^{-1} (12.7 nmol L^{-1}) (Saiki and Lowe. 1987; Ingersoll et al., 1990) and are often less than 0.1 µg L^{-1} (1.27 nmol L^{-1}) when there is no anthropogenic input (Sharma et al., 2015).

Selenium is commonly an essential trace element to animals, including humans. It has an important role as an antioxidant in glutathione peroxidase and as part of thyroid hormone metabolism in thioredoxin reductase, and over 25 other selenoproteins in mammals (Choi et al., 2013). Such proteins are also present in algae, fish, and crustaceans (Pacitti et al., 2013; Qunitaneiro et al., 2015; Martínez-Ruiz et al., 2016). Although Se is an essential element, there is a small margin between essential and toxic concentrations of Se. At concentrations as low as 2 µg Se L^{-1} in the water, Se can cause adverse effects in fish and aquatic birds, notably their reproductive success (Lemly, 1993). To protect aquatic life, the Canadian water quality guideline for concentrations of Se in freshwater is 1 µg L^{-1} (12.7 nmol L^{-1}) (CCME, 2007) and the USEPA (2016) has reduced the selenium criterion from 5 µg L^{-1} (63.3 nmol L^{-1}) to 1.5 µg L^{-1} (19.0 nmol L^{-1}). However, algal and bacterial species can biotransform the abundant inorganic species of Se to organic forms, which are more toxic when ingested than waterborne inorganic Se (Hamilton et al., 2004). Food sources with concentrations greater than 3 µg/g Se dry weight can cause adverse effects, including lethality (Lemly, 1993). DeBruyn and Chapman (2007)

investigated how invertebrates have mainly been considered a food source, in that the bioaccumulation of Se and ingestion by fish is of greater significance than the mortality caused by Se. While organisms such as benthic invertebrates and zooplankton can accumulate Se up to 30 µg g^{-1} (380 nmol g^{-1}) in their tissue without major effects, dietary Se in wildlife and fish should not exceed a toxic dose of 3 µg g^{-1} (3.80 nmol g^{-1}) (Lemly et al., 1993). Selenite exposures caused 50% mortality in *Hyalella azteca* when whole-body Se concentrations reached 8.47 µg g^{-1} (107 nmol g^{-1}) (Norwood et al., unpublished manuscript). The USEPA has tissue concentrations for fish egg-ovary, whole-body, and muscle that range from 8.5 to 15.1 mg kg^{-1} dry weight (USEPA, 2016a), whereas the CCME has not introduced any dietary guidelines.

The objectives of this study were to determine the concentration of Se that can be accumulated from a waterborne SeO_3^{2-} source in different water chemistry conditions and how body concentration of Se relates to mortality. In addition, waterborne toxicity of Se was determined in the different water chemistry conditions. Lethal concentrations and bioaccumulation was modelled using saturation kinetics-based models described in Section 1.5.6.1 and 1.5.6.2.

4.2. METHODS

4.2.1 Experimental Set-up

The 28-day toxicity test methods were the same as Section 3.2.1, with the following deviations. The water chemistry was adjusted for each set of experiments as detailed in Table 4.1. pH adjustments were made using HCl for pH 6.8 treatments and KOH for pH 8.3, without alkalinity adjustments; however, control animal survival was not acceptable as it was less than 65% survival over 4 weeks (or <10% mortality $week^{-1}$) (Borgmann, 2002). Alkalinity was adjusted using $NaHCO_3$ and this adjustment also altered the pH.

Table 4.1. Test parameters and methods to maintain the water chemistry.

Parameter measurements		Method	Reference
pH[a]	6.8, 7.7, 8.3	1M HCl or KOH amendments	Taylor et al. (2002); Niyogi (2008)
Alkalinity/pH	5, 16, 100 mg L^{-1} as $CaCO_3$ equivalents pH 6.8, pH 7.7, pH 8.3	Adjust $NaHCO_3$ - maintained sodium ions using NaCl	Deleebeeck et al. (2007)
Dissolved organic carbon	0.5, 2, 5 mg C L^{-1}	Luther Marsh, natural organic matter	Gillis et al. (2010)

a- pH adjustments were made using HCl for pH 6.8 treatments and KOH for pH 8.3, without alkalinity adjustments; however, the survival of control animals was unacceptable.

Na_2SeO_3 (Sodium selenite 99%, Sigma Aldrich) additions to each container were equilibrated 24 hours before animals were added, so the Se and DOC would have time to interact (Taylor et al., 2002; Niyogi et al., 2008). Alkalinity, pH, and DOC adjustments were made to 25L carboys of 30% SAM water 48h in advance, so it was equilibrated at the start of each experiment.

The water in each container was renewed every 7 days and the organisms were counted at each turnover. The organisms were fed 2.5 mg finely ground TetraMin fish food (Tetra GMBH, Melle, Germany) twice during this period. TetraMin contains trace amounts of Se (10 nmol g^{-1}). Control animals did not accumulate more than 10 nmol g^{-1} over the 28-day exposure.

4.2.2 Sample collection and analysis

The methodology for collection and analysis of water and whole-body samples was the same as section 3.2.2. Water chemistry is summarized in Table 4.2. Total selenium in water and whole-body samples was determined with Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometer and SOLAAR Data Station V11.03 software using the method described in Section 2.2.2. Detection limits were calculated as three times the method blank standard deviations (Norwood, 2008) and were 9.78 nmol L^{-1} for water samples and 21.2 nmol g^{-1} for whole-body samples.

4.2.3 Whole-body Digests

Digest methodology was described in section 3.2.3. Digests of certified reference material had an average recovery value of 5.36 ± 0.36 mg kg^{-1} [TORT2: lobster hepatopancreas; Se certified as 5.63 ± 0.67 mg kg^{-1} from the National Research Council of Canada].

Table 4.2. Water chemistry from treatments. Mean with 95% C.I and maximum/minimum values.

Modifier	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	SO_4^{2-}	DOC	DIC	Hardness	Alkalinity	pH	Conductivity
	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}		µS cm^{-1}
DOC 5	376 ± 5.20	91.5 ± 2.03	323 ± 3.09	16.2 ± 0.97	nd	nd	6.05 ± 0.85	4.87 ± 1.36	46.7	26.6 ± 3.39	7.62 (7.55 - 7.80)	149
DOC 2	323 ± 14.8	93.0 ± 8.33	332 ± 11.9	16.9 ± 0.45	616 ± 15.5	74.1 ± 3.86	1.77 ± 0.35	4.87 ± 0.22	41.6	22.6 ± 1.57	7.79 (7.73 - 7.84)	143
DOC 0.5/pH 7.5/Alk 16	310 ± 1.03	80.0 ± 0.180	348 ± 18.5	13.9 ± 0.375	632 ± 11.4	75.2 ± 0.228	0.28 ± 0.030	3.97 ± 0.109	39.2	16.1 ± 0.149	7.52 (7.47 - 7.58)	134
Alk 100/pH 8.3	295 ± 24.2	80.8 ± 6.93	4350 ± 327	17.8 ± 1.80	3650 ± 55.5	74.3 ± 2.28	0.93 ± 0.69	24.9 ± 1.77	37.6	101 ± 0.165	8.40 (8.37 - 8.43)	546
Alk 5/ pH 6.8	297 ± 14.3	74.9 ± 1.8	4030 ± 96.4	16.4 ± 0.83	4390 ± 221	82.3 ± 3.96	0.88 ± 0.21	1.45 ± 0.54	37.2	7.54 ± 1.39	7.13 (7.03 - 7.23)	615
Hard water[a]	839 ± 17.0	351 ± 4.75	578 ± 11.9	114 ± 70.4	747 ± 3.19	406 ± 52.1	1.33 ±0.06	17.5 ± 0.79	131	85 ± 1.06	8.20 (8.14 - 8.26)	346

a- Norwood et al. (unpublished)

4.2.4 Data analyses

4.2.4.1 Mortality Model

The mortality model, as described in Section 1.7.4.1, was applied to determine lethal water and lethal body concentrations. Mortality data were log transformed before the above models were fit in SYSTAT 10, to ensure normality and equal variance. Normality was assessed by visual inspection of the probability plots and the Shapiro Wilk test on the mortality data ($W = 0.607$, $p = 0.000$ on untransformed data). Levene's test was performed for equal variance of log transformed data ($F=2.099$, $p=0.058$) (Golding et al., 2013).

4.2.4.2 Bioaccumulation

The bioaccumulation saturation model, as described in Section 1.7.4.2, was used to determine the relationship between bioaccumulation of Se and exposure.

4.2.4.3 Confidence Intervals

To determine significant difference between the various treatment critical concentrations, confidence intervals were used as described in section 2.2.3.3.

4.2.4.4 Comparison with Norwood et al. (unpublished data)

Selenium concentrations in water and whole-body concentrations causing mortality in hard water were previously determined by Norwood et al. (unpublished). Lethal concentrations and mortality model parameters for selenium from Norwood et al. (unpublished) were used in the current study to determine the effect of water hardness on Se toxicity. In addition, raw mortality data from Norwood et al. (unpublished) was used to calculate a 28-day LC10 and LBC10 in hard water.

4.3. RESULTS

4.3.1 Exposure-based mortality

4.3.1.1 DOC

Increased concentrations of DOC led to a trend of decreasing 28-day lethal concentrations when *H. azteca* were exposed to selenite from 240 (183 – 297) nmol Se L^{-1} with 6.05 mg added C L^{-1} (DOC-5) to 319 (134 – 504) nmol Se L^{-1} with 1.77 mg C L^{-1} (DOC-2) and 620 (334 – 906) nmol Se L^{-1} with 0.5 mg C L^{-1} (DOC-0.5) (Table 4.3). The DOC-0.5 LC50 was significantly higher than the DOC-5 treatment. The mortality models for DOC treatments had different shapes when the concentration of Se was greater than 100 nmol Se L^{-1}. However, the half saturation constants, K_w'', which is the concentration where mortality rate is half the maximum is extremely variable. In addition, the r^2 values for the DOC-2 and DOC-0.5 show a moderate correlation between the exposure concentrations and mortality. The curves for both DOC-5 and DOC-2 began to reach a maximum mortality rate near 1000 nmol Se L^{-1}, whereas the DOC 0.5 curve was still increasing. The mortality rate between 100 – 1000 nmol Se L^{-1} was highest for the DOC-5 treatment (Figure 4.1). However, the differences were not statistically significant. In low DOC water, the lower mortality rate at high Se concentrations is of interest for *H. azteca* as a potential food source, since Se can biomagnify in the food web.

4.3.1.2 pH/alkalinity

Se toxicity increased at low pH and low alkalinity. Reducing the alkalinity from 101 mg L^{-1} (pH 8.3/Alk-100) to 7.54 mg L^{-1} as $CaCO_3$ (pH 6.8/Alk-5) halved the 28-day LC50 from 600 (432 - 764) nmol Se L^{-1} to 274 (184 – 365) nmol Se $L^{-}1$. The 28-day LC25 for Alk-5 was also significantly lower than the 28-day LC25 at Alk-100 (Table 4.4). Until the concentration of Se reached 600 nmol Se L^{-1}, the resulting mortality model curves for the pH 7.7 /Alk-16 and pH 8.3 /Alk-100 treatments had similar shapes; however, at 600 nmol Se L^{-1}, the pH 8.3 mortality rate increased at a greater rate than the pH 7.7 curve. Both K_w'' and n_w, were markedly different for the pH 6.8/Alk 5 model, as the other two models did not reach a maximum mortality rate, so the concentration at maximum mortality could not be determined (Figure 4.2).

4.3.1.3 Hardness

Hardness had a significant effect on the lethal Se concentrations, with increased hardness being protective against Se toxicity. The Hardness-130 28-day LC50 was almost two-fold greater than the Hardness 37.5 28-day LC50 (Table 4.5). In both hardness treatments mortality does not reach a maximum over the concentration range tested (Figure 4.3).

Table 4.3. Mortality model parameters based on concentration of Se in water of treatments with different dissolved organic carbon concentrations. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$			
DOC 5	0.022	94.8	(55.5 - 134)	240	(183 - 297)	181	(135 - 228)	139	(100 - 177)	10	0.872
DOC 2	0.020	1040	(-12900 - 15000)	319	(134 - 504)	212	(94.4 - 329)	137	(13.8 - 260)	2.68	0.540
DOC 0.5	0.011	-2970	(-13000 - 7040)	620	(334 - 906)	375	(201 - 550)	201	(57.0 - 345)	1.46	0.663

Table 4.4. Mortality model parameters based on concentration of Se in water of treatments with different pH and alkalinity. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$			
pH 8.3/Alk 100	0.016	-2040	(-5620 - 1540)	600	(432 - 764)	434	(308 - 560)	290	(146 - 434)	2.06	0.763
pH 7.7/Alk 15	0.011	-2970	(-13000 - 7040)	620	(334 - 906)	375	(201 - 550)	201	(57.0 - 345)	1.46	0.663
pH 6.8/Alk 5	0.033	25.1	(-562 - 612)	274	(184 - 365)	195	(129 - 262)	146	(71.0 - 220)	26.4	0.762

Table 4.5. Mortality model parameters based on concentration of Se in water of treatments with different hardness. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$			
Hardness 37.5	0.016	-2040	(-5620 - 1540)	600	(432 - 764)	434	(308 - 560)	290	(146 - 434)	2.06	0.763
Hardness 130[a]	0.046	6330		957	(710 – 1210)	665	(375 - 954)	445	(100 - 788)	2.71	0.788

[a] Norwood et al. (unpublished)

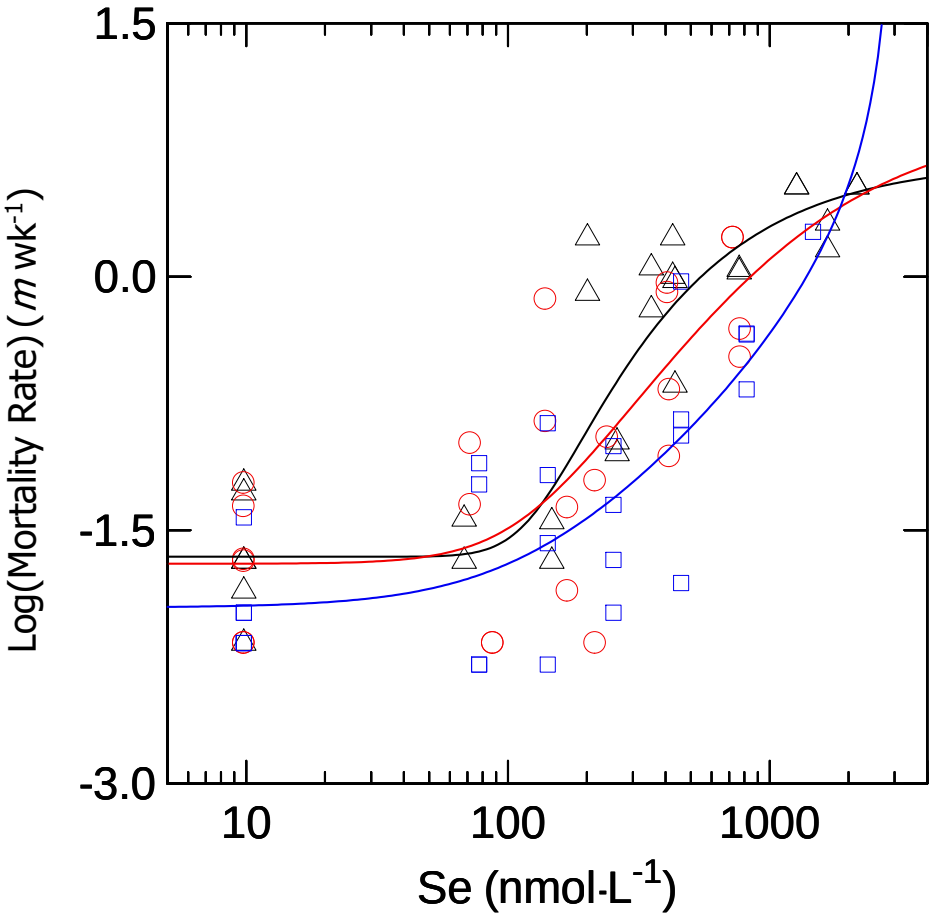

Figure 4.1. Waterborne Se mortality models with modified DOC based on parameters in Table 4.3. Data points are mortality rates (mortality per week) at measured Se water concentrations in SAM30 with modified DOC concentrations. □ are data from DOC 0.5 experiments, ○ are DOC-2, and △are DOC-5. Solid lines are corresponding mortality models.

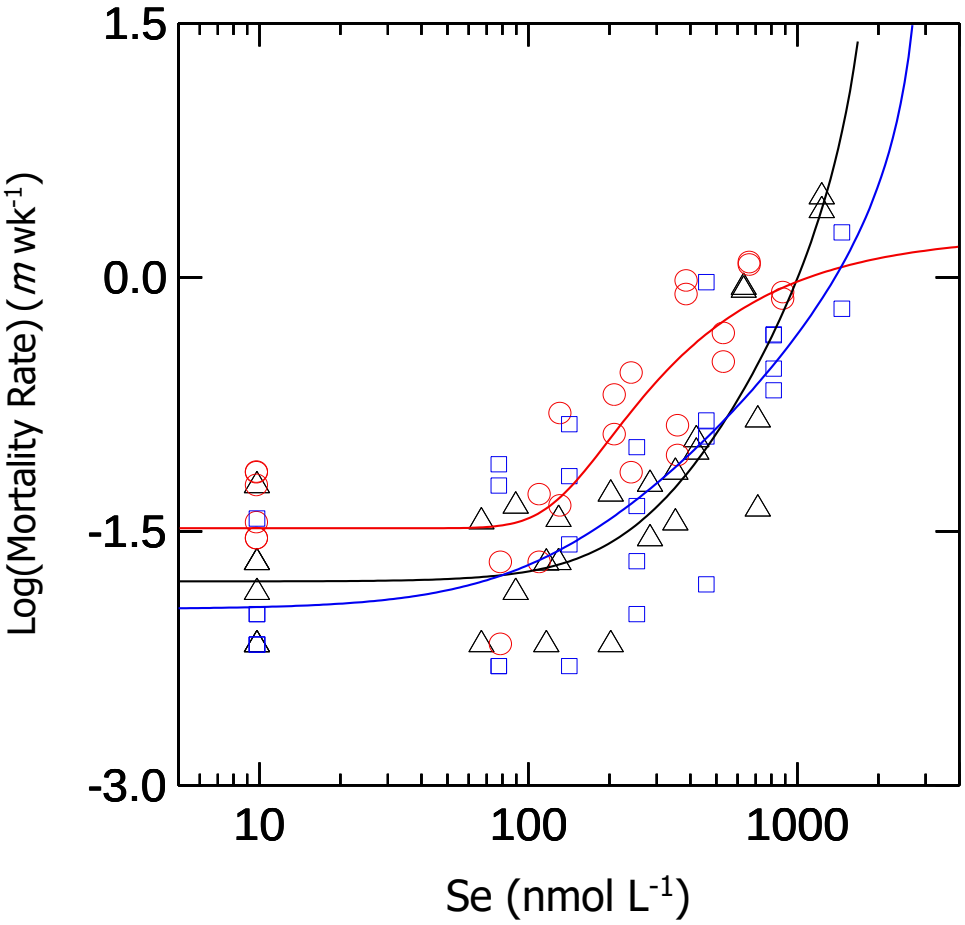

Figure 4.2. Waterborne Se mortality models with modified pH/alkalinity based on parameters in Table 4.4. Data points are mortality rates (mortality per week) at measured Se water concentrations in SAM30 with modified pH/alkalinity. ○ are data from pH 6.8/Alk 5 experiments, □ are pH 7.7/Alk 15, and △are pH 8.3/Alk 100. Solid lines are corresponding mortality models.

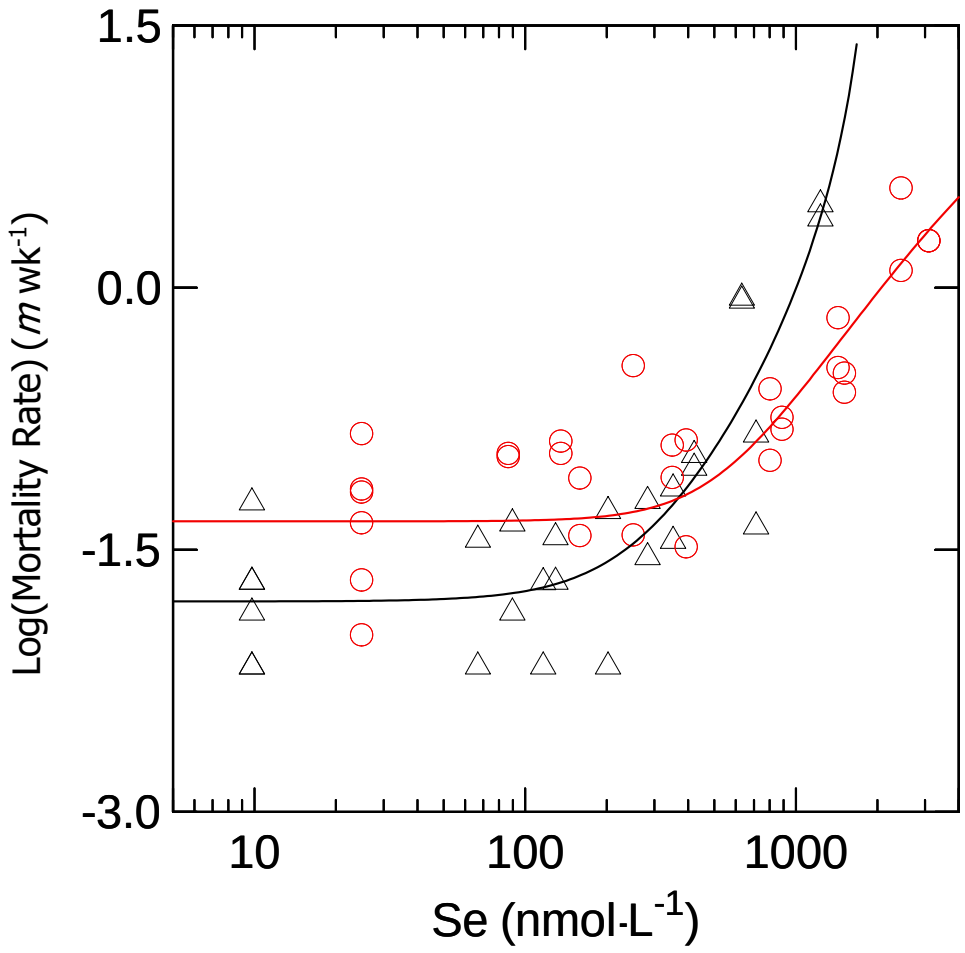

Figure 4.3. Waterborne Se mortality models with modified hardness based on parameters in Table 4.5. Data points are mortality rates (mortality per week) at measured Se water concentrations in SAM30 with modified hardness. △are data from Hardness-37.5 experiments and ○ are Hardness-130. The Hardness-130 data are from Norwood et al. (unpublished manuscript). Solid lines are corresponding mortality models.

4.3.2 Bioaccumulation

At low concentrations of Se in water, the whole-body concentrations in the different treatments were not significantly different between treatments (at 70.9 nmol Se L^{-1} p=0.123 and at 127 nmol Se L^{-1} p=0.293, two-way ANOVA using Tukey post hoc analysis). However, at 228 nmol L^{-1}, the *H. azteca* in the Alk-5 treatment had significantly different bioaccumulated Se from both the DOC-5 (p=0.045) and the Alk-100 treatment (p=0.009). At the highest nominal concentration of Se, the two alkalinity treatments had significantly different bioaccumulation from each other and from the DOC treatments.

Body concentration of Se increased with increasing Se exposure concentrations in all water chemistry treatments (Figure 4.4). The concentration at which body concentration started to increase and the Se uptake pattern varied with the water chemistry. In addition, a maximum Se value could not be accurately predicted for any of the treatments from the observed results, as the concentration of Se in the organisms did not reach a maximum/point of saturation. This was likely due to mortality at higher exposure concentrations and thus there were no living organisms available for Se body concentration analysis. Therefore, the max/K ratio was used to compare the bioaccumulation patterns as it is the ratio of Se accumulation to the exposure concentration at half saturation. The max/K of the hard water and the Alk-100 treatment were both significantly lower than the pH-7.7 and Alk-5 treatments (Table 4.6). In addition to the hard water treatment having greater Ca and Mg, it also had an alkalinity of 85 mg L^{-1} and a pH of 8.3, which were similar pH and alkalinity values to the Alk-100 treatment.

If it was assumed that the treatments all have the same theoretical maximum Se body concentration, therefore the greater max/K ratio indicates a greater Se accumulation at lower concentrations of Se in water. The highest max/K value occurred in the treatment with the lowest alkalinity and pH, indicating this treatment had increased uptake of Se at lower concentrations of Se in water.

Using the bioaccumulation model parameters (Table 4.6), the body concentrations that could not be determined due to mortality can be predicted. Figure 4.5 shows all the observed Se body concentrations and how well the above models can predict the body concentrations. The model could predict the observed body concentration within 2x the observed concentration for 87% of the data points, with the remainder of the points close to the 2x overpredicted or 2x underpredicted lines. It should be noted that there were no experimental data at high Se exposure concentrations, so the model cannot predict where bioaccumulation would saturate. The

predicted body concentrations from the model could therefore be greater than actual body concentration if organisms had survived for body Se analysis. Nevertheless, the predicted data were used to estimate the lethal body concentrations in the next section.

Table 4.6. Saturation model parameters for selenium bioaccumulation with the max/K ratio of the predicted maximum Se accumulation (max) and half saturation constant (K) with 95% confidence limits (CL), coefficient of determination (r^2), number of data points (N), mean dry to wet weight ratio (D/W), bioconcentration factor (BCF) calculated as max × K-1 × D/W × 1000, and background Se concentration in *H. azteca* (C_{bk}).

Modifier	N	r^2	MAX/K	CL	D/W ratio	BCF	C_{bk}
				L g^{-1}			nmol g^{-1}
DOC 5	14	0.851	0.382	(0.125 - 0.507)	0.283	108	21.2
DOC 2	22	0.697	0.959	(-0.111 - 2.03)	0.236	226	21.2
pH 8.3/Alk 100	24	0.873	0.162	(0.133 - 0.192)	0.276	44.7	21.2
pH 7.7/Alk 15	25	0.831	0.309	(0.215 - 0.404)	0.266	82.2	21.2
pH 6.8/Alk 5	20	0.740	0.754	(0.466 - 1.04)	0.240	181	21.2
Hard water[a]	60	0.589	0.131	(0.045 – 0.176)	0.258	33.8	6.67

[a] Norwood et al. (unpublished)

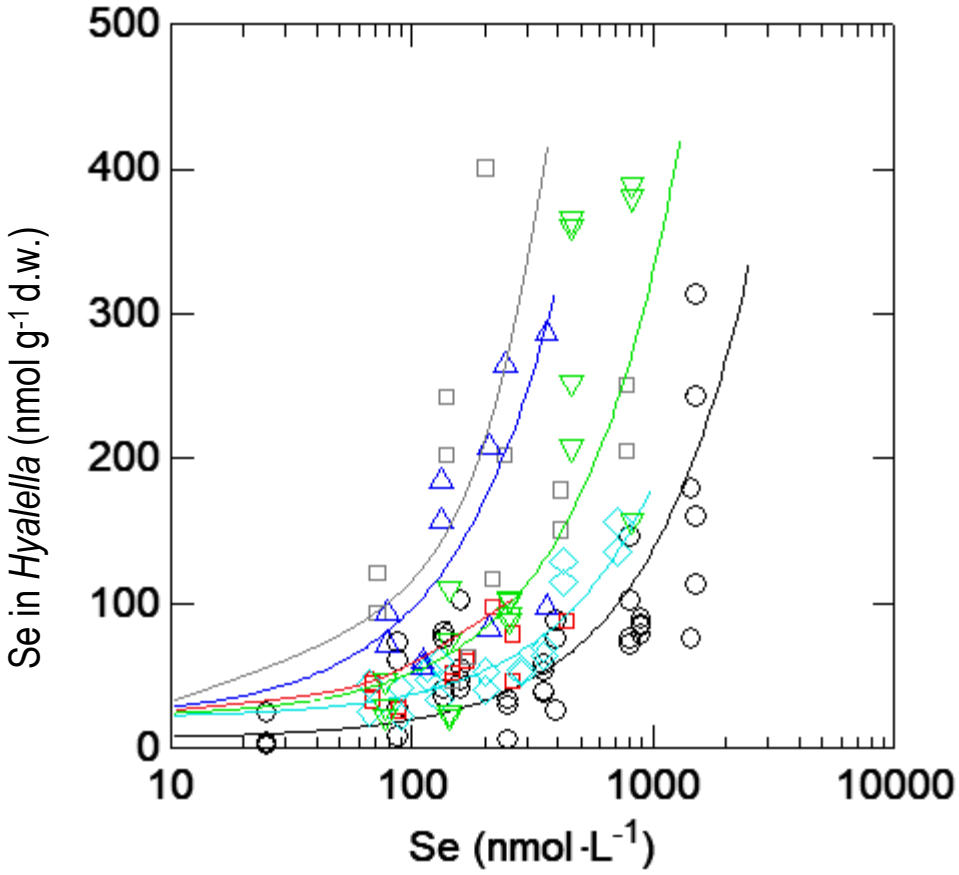

Figure 4.4. Selenium concentrations accumulated in *H. azteca* in a 4-week exposure of pH DOC2 (□), 6.8/Alk-5 (△), DOC10 (□), pH 7.7/Alk-16 (▽), and pH 8.3/Alk-100 (◊) compared to increased hardness treatments (○) by Norwood et al. (unpublished manuscript). Body Se concentration are mean dry weight of surviving *H. azteca* in each replicate at each concentration after 4-weeks. Water Se concentrations are mean measured concentrations.

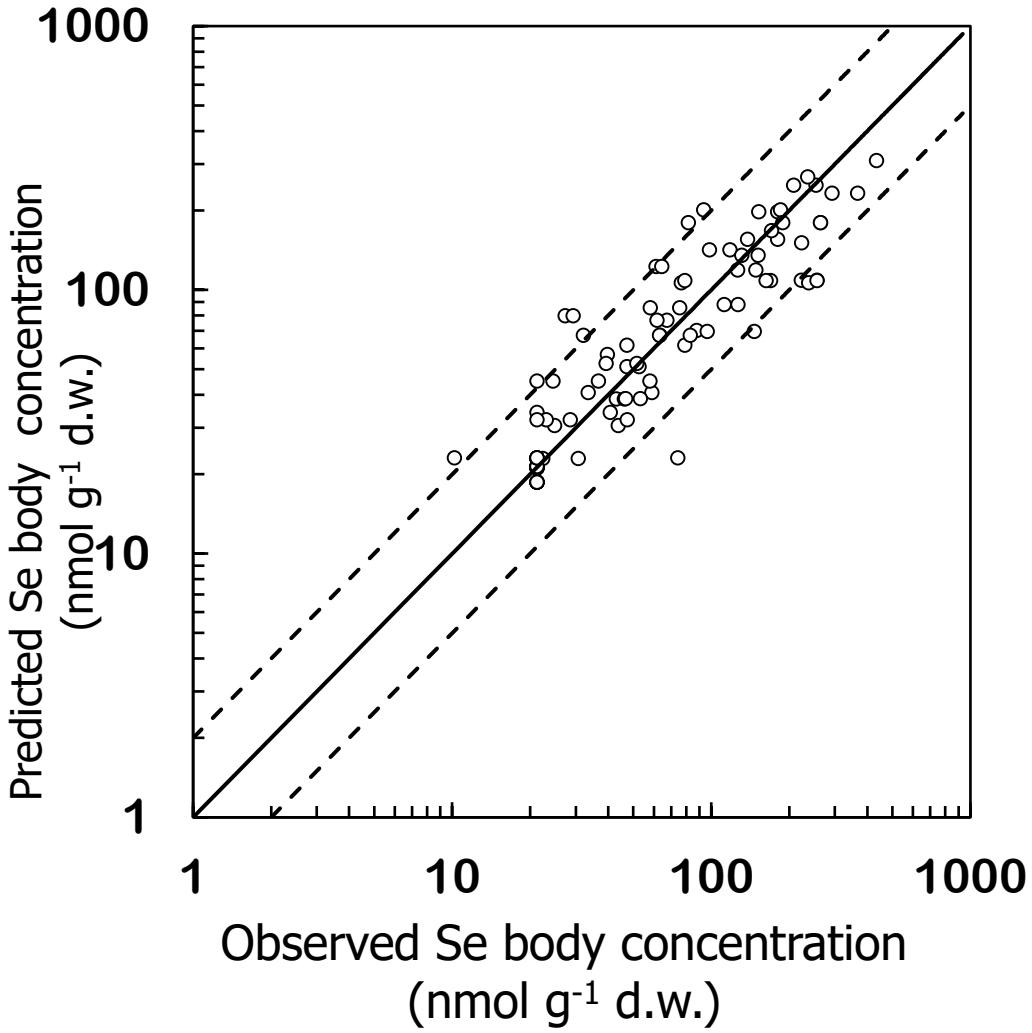

Figure 4.5. The predicted concentrations are based on the bioaccumulation models from Figure 4.4 and calculated using the parameters in Table 4.6. The observed Se whole-body concentrations was based on average dry weight for each replicate. The solid line indicates a 1:1 relationship and the dashed line is 2x overpredicted or 2x underpredicted.

4.3.3 Body concentration-based mortality

4.3.1 DOC

The 28-day LBC50 for the highest DOC treatment, DOC-5, was significantly lower than treatments where there was less DOC present (Table 4.7). The 28-day LBC50 for DOC-5 was 54.9 (48.1 – 61.6) nmol Se g^{-1} d.w., while it was 162 (117 – 207) nmol Se g^{-1} d.w. with 2 mg C L^{-1} and 128 (88.9 – 168) nmol Se g^{-1} d.w. with 0.5 mg C L^{-1}. There was also a significant different between the 28-day LBC25 values for the DOC-5 and DOC-2 treatment. The lethal body concentrations for DOC-2 and DOC-0.5 were not significantly different. The DOC-5 treatment curve in Figure 4.1 had a sharp inflection at a lower Se concentration than the DOC-2 treatment, although the curves had similar shapes. The mortality rate in DOC-5 treatments increased more quickly at lower body concentrations. The DOC-0.5 treatment mortality rate gradually increased rate, but once whole-body Se exceeded 40 nmol Se g^{-1} d.w. the mortality rates were similar to the DOC-2 treatment (Figure 4.6).

4.3.2 pH/alkalinity

As alkalinity in treatments Alk-5, Alk-16, and Alk-100 increased, the 28-day LBC50s decreased from 178 (153 – 203), 128 (88.9 – 168), to 113 (92.1 – 134) nmol Se g^{-1} d.w (Table 4.8). There was a significant difference between the 28-day LBC50s and 28-day LBC25s for Alk-100 and Alk-5 treatments. There were similar lethal body concentrations for the Alk-16 and Alk-100 treatments. The mortality model for both pH-8.3/Alk-100 and pH-7.7/Alk-16 treatments had similar mortality rates at Se body concentrations up to 100 nmol Se g^{-1} d.w. (Figure 4.7). At higher concentrations, the pH-8.3/Alk-100 treatment mortality rate had a greater increase over a smaller concentration range. The pH-6.8/alk-5 treatment mortality rate had a sharp increase at 100 nmol Se g^{-1} d.w., whereas the other treatments had gradual increases in mortality rates.

4.3.3 Hardness

Hardness did not have a significant effect on the 28-day lethal body concentrations in Table 4.9. The LBC50 in soft water was 113 (92.1 – 134) nmol Se g^{-1} d.w. and 107 (74.0 – 141) nmol Se g^{-1} d.w. in hard water. At body concentrations below 100 nmol Se g^{-1} d.w., the two models predicted similar mortality rates and both model predicted the LBC50 for the data sets to be around 110 nmol Se g^{-1} d.w. (Figure 4.8). When the body concentrations were greater than 200 nmol g^{-1}, the treatment in soft water was predicted to have a greater mortality rate. However,

there were few surviving animals in either treatment that have body concentrations that exceeded 200 nmol Se g^{-1} d.w.

Table 4.7. Mortality model parameters based on selenium body concentrations in organisms exposed to treatments with different dissolved organic carbon concentrations. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	weeks^{-1}		nmol g^{-1}d.w.		nmol g^{-1}d.w.		nmol g^{-1}d.w.		nmol g^{-1} d.w.		
DOC 5	0.023	-194	(-304 - -84.1)	54.9	(48.1 - 61.6)	46.7	(39.1 - 54.2)	38.3	(30.7 - 46.0)	4.00	0.811
DOC 2	0.022	-1080	(-22700 - 20600)	162	(117 - 207)	126	(74.5 - 178)	93.8	(9.39 - 178)	3.04	0.565
DOC 0.5	0.007	-1420	(-17000 - 14100)	128	(88.9 - 168)	81.9	(41.5 - 122)	48.3	(-1.43 - 122)	1.82	0.718

Table 4.8. Mortality model parameters based on selenium body concentrations in organisms exposed to treatments with different pH/alkalinity. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	weeks^{-1}		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1}d.w.		
pH 8.3/Alk 100	0.014	-396	(-1200 - 410)	113	(92.1 - 134)	86	(61.5 - 111)	61.2	(25.2 - 97.2)	2.41	0.829
pH 7.7/Alk 15	0.007	-1420	(-17000 - 14100)	128	(88.9 - 168)	81.9	(41.5 - 122)	48.3	(-1.43 - 122)	1.82	0.718
pH 6.8/Alk 5	0.036	47.0	(-1060 - 1160)	178	(153 - 203)	149	(117 - 182)	125	(73.7 - 178)	22.7	0.78

Table 4.9. Mortality model parameters based on selenium body concentrations in organisms exposed to treatments with different hardness. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	weeks^{-1}		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
Hardness 37.5	0.014	-396	(-1200 - 410)	113	(92.1 - 134)	86	(61.5 - 111)	61.2	(25.2 - 97.2)	2.41	0.829
Hardness 130[a]	0.046	6330		107	(74.0 - 141)	62.3	(41.0 - 83.7)	33.7	(5.07 - 62.3)	1.64	0.555

[a] Norwood et al. (unpublished)

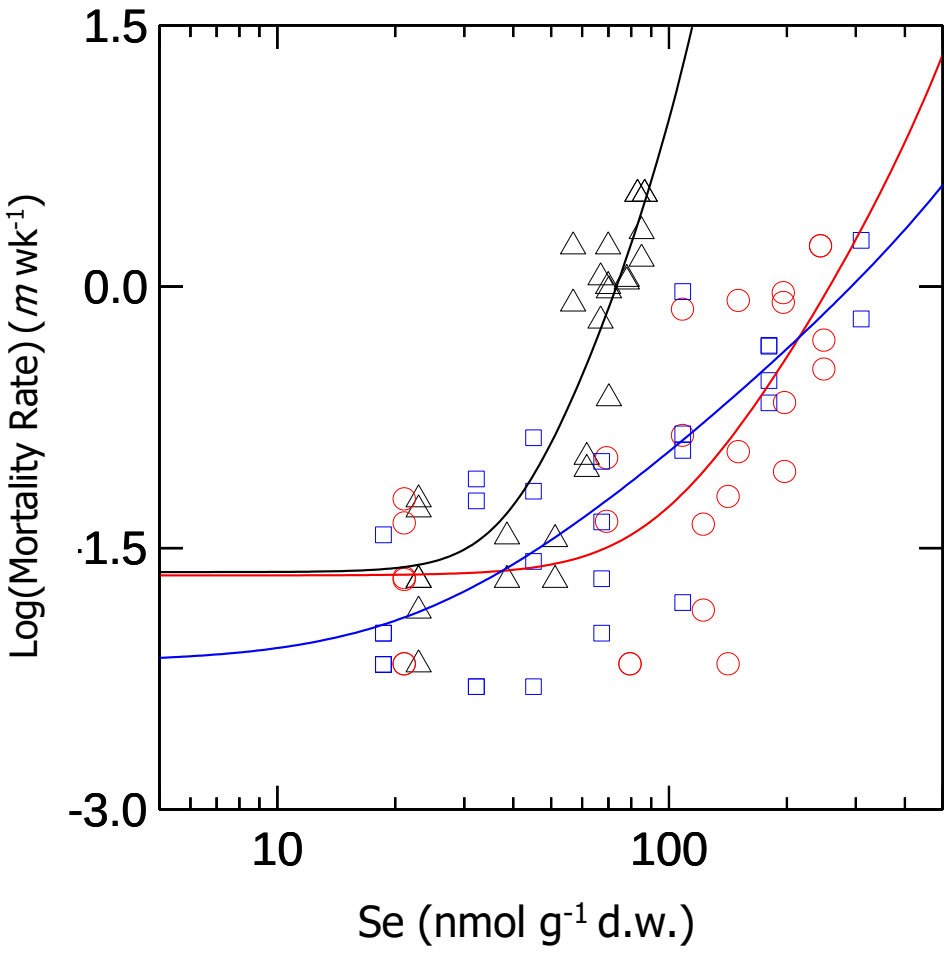

Figure 4.6. Se body-concentration mortality models based on parameters in Table 4.7 with modified DOC concentrations in the exposure water. Data points are mortality rate (mortality per week) at mean measured selenium body concentrations on a dry weight basis in organisms exposed to Se in SAM30 with modified DOC concentrations. □ are data from DOC-0.5 experiments, ○ are DOC-2 , and △ are DOC-5. Solid lines are corresponding mortality models.

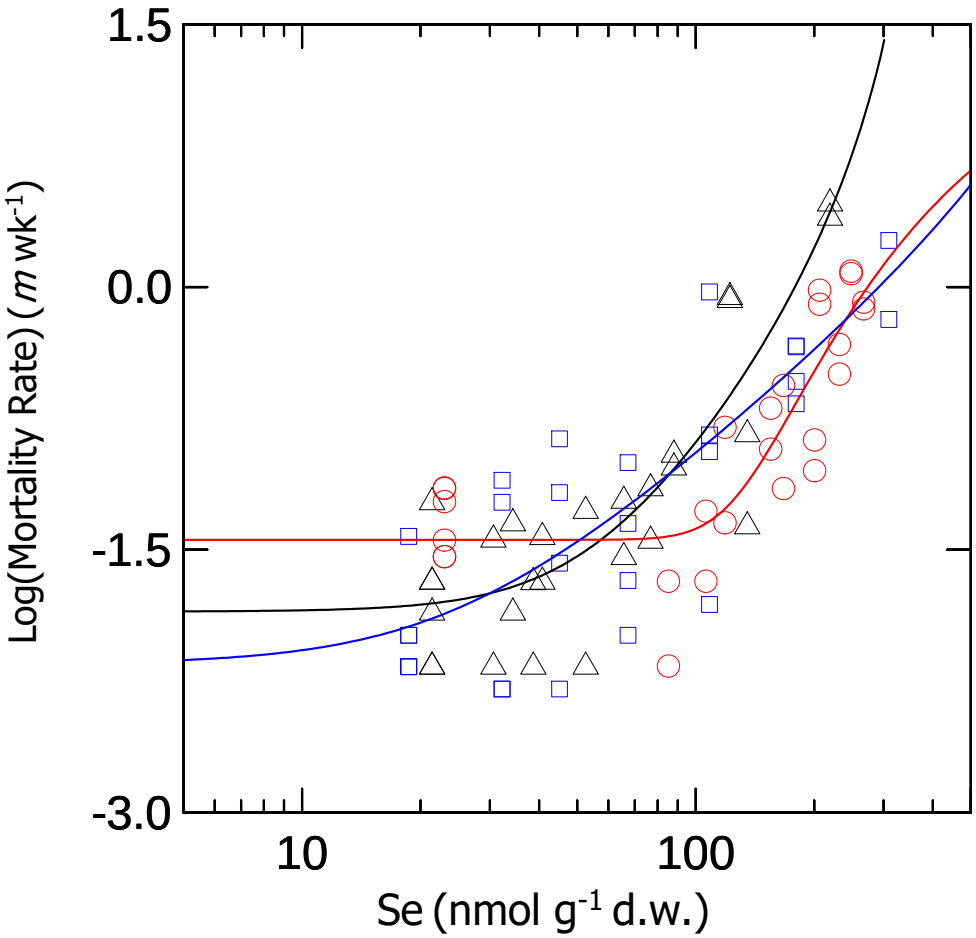

Figure 4.7. Se body-concentration mortality models based on parameters in Table 4.8 with modified pH/alkalinity exposure water. Data points are mortality rate (mortality per week) at mean measured selenium body concentrations on a dry weight basis in organisms exposed to Se in SAM30 with modified pH/alkalinity. ○ are data from pH 6.8/Alk 5 experiments, □ are pH 7.7/Alk 15, and △ are pH 8.3/Alk 100.

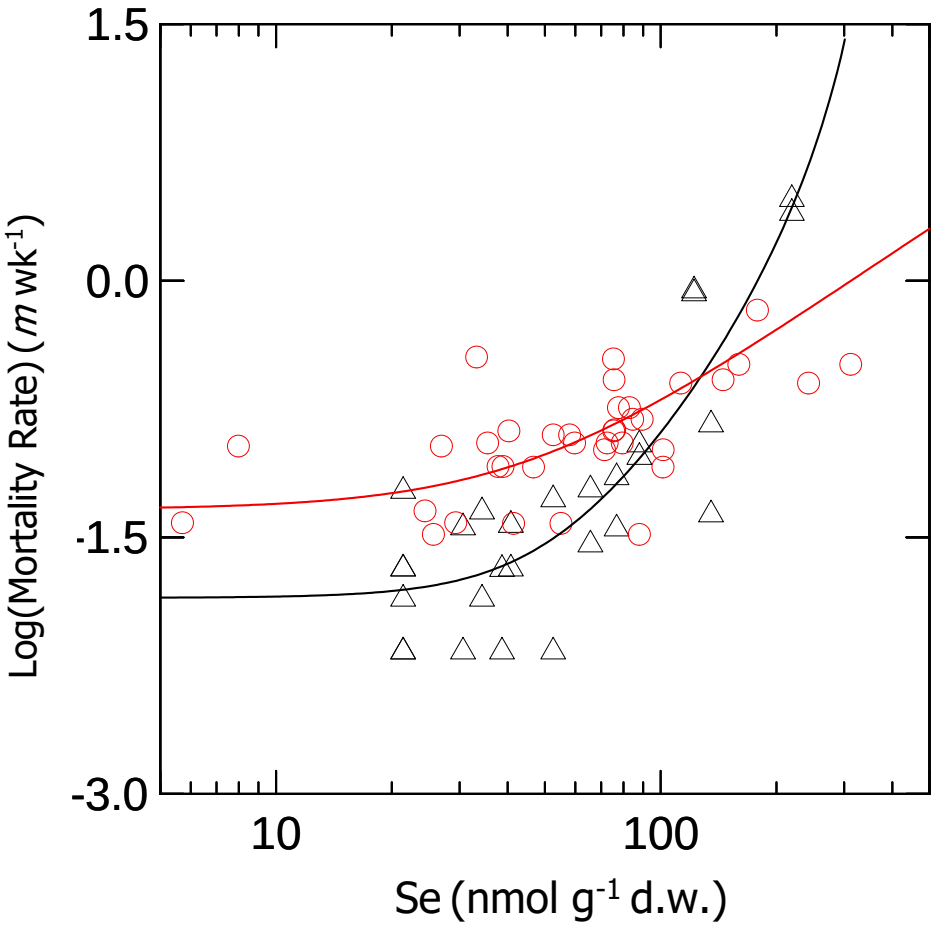

Figure 4.8. Se body-concentration mortality models based on parameters in Table 4.9 with modified exposure water hardness. Data points are mortality rate (mortality per week) at mean measured selenium body concentrations on a dry weight basis in organisms exposed to Se in SAM30 with modified hardness. △ are data from Hardness-37.5 experiments and ○ are Hardness-130. The Hardness-130 data are from Norwood et al. (unpublished manuscript).

4.4 DISCUSSION

4.4.1 Dissolved organic carbon

Increased DOC was not protective against Se toxicity based on both the concentration of Se in water (Table 4.3) and the concentration of Se in the organism (Table 4.6) even though it was hypothesized that the polarity of the oxyanion SeO_3^{2-} would bind to DOC and reduce its toxicity (Wiramanaden et al., 2010). In many organisms, Se is more toxic when taken up in an organic form and from a dietary source (Lemly, 1993; DeBruyn & Chapman, 2007). In the present study, *H. azteca* had visible organic material from the added DOC in their digestive tract. The DOC could also promote the growth of bacteria and algae that can convert the selenite into an organic form that could be taken up through their diet or in a dissolved form (Schlekat et al., 2002; Phibbs et al., 2011).

Different species (inorganic versus organic) of Se may be bioaccumulated differently, as there could be different uptake rates or mechanisms of action. In the invertebrate *Lumbriculus varuegatus,* it has been shown that exposure to both dissolved selenite or selenomethionine significantly reduces Na/K ATPase activity and increases lipid peroxidation; however, uptake of selenomethionine was greater, with over 30 times greater accumulation (Xie et al., 2016).

4.4.2. Alkalinity and pH

Se was more toxic at lower pH and lower alkalinity (Table 4.4). It was hypothesized that an increase in pH would decrease toxicity, as uptake of SeO_3^{2-} is less rapid when compared to the $HSeO_3^-$ (Riedel & Sanders, 1996) and the ratio of SeO_3^{2-} to $HSeO_3^-$ increases as pH increases. Halter et al. (1980) determined that the two-week selenite LC50 for fathead minnow was 7 times lower than the lethal concentration observed by Cardwell et al. (1976). This difference was attributed to fish age and study duration as it was assumed all Se would be of the form $HSeO_3^-$. However, there were marked differences in the water chemistry including a two-fold difference in hardness and pH 7.3 versus pH 7.8 water. This change in pH would cause a change in speciation, which could affect Se uptake (Riedel & Sanders, 1996).

Wang et al. (2016) determined that acute HC10s from the species sensitivity distribution for freshwater organisms from the USEPA's ECOTOX database were significantly higher at pH above 7.8 versus at pH 7.3 and pH 7.4. The HC10 at pH 7.3 was determined to be 63 (5.6 – 261) μg L^{-1} and increased to 573 (301 – 972) μg Se L^{-1} at pH 8.1. Both HC10s were greater than the

lethal concentrations from the current study. It should be noted that the other water chemistry parameters in Wang et al. (2016) were not significantly different; however, there were not enough DOC data to perform statistics. The pH 7.3 factor had one DOC concentration of 33 mg C L^{-1} and pH 8.1 had one DOC concentration of 4.0 mg C L^{-1}. Both pH and DOC had significant effects on the toxicity of Se to *H. azteca* in the current study.

The current study also found a significantly greater 28-day LBC50 at lower pH (Table 4.7). This result does not follow the concept of a single lethal body concentration in *H. azteca* for Se (Norwood et al., 2013). It is suspected the form and route of Se uptake influenced the lethal body concentration, as inorganic Se can be taken up more rapidly at lower pH. Franz et al. (2011) established that the speciation of Se within *C. dilutus* is not dependent on its uptake speciation. The internal Se speciation is predominantly organic selenides or diselenides when exposed to either inorganic or organic Se (Franz et al., 2011). However, since there were different lethal Se body concentrations in *H. azteca*, the internal mixture of Se forms is still potentially important and may cause variation in the concentration that causes mortality.

In higher alkalinity water Se can adsorb to iron-oxyhydroxides or co-precipitate with calcite, reducing its bioavailability; however, there was no significant difference between the 28-day LBC50s of the treatment at 100 mg L^{-1} as $CaCO_3$ equivalents (at pH 8.3) and 16 mg $L^{-1\,1}$ as $CaCO_3$ equivalents (at pH 7.7). Ouelett et al. (2013) determined that alkalinity increase from 23.6 ± 2.5 to 103.8 ± 4.3 mg L^{-1}, with a pH increase from 7.2 to 8.1 caused the Se body burden to decrease in *C. dilutus*, but it was not significant.

4.4.3 Hardness/calcium interference

The 28-day LC50 in soft water was significantly lower than in hard water (Table 4.5). There was also no significant difference between the lethal body concentrations between the two levels of hardness for soft and moderately hard water (Table 4.8). This indicates that the hardness level influenced the uptake of selenium so that at higher hardness, increased selenium exposure concentrations were required for selenium to accumulate in the body to have a toxic effect. This is consistent with the findings of Ingersoll et al. (1990) who noted that daphnids were more sensitive to Se in soft water in a 48-h exposure. Se has been shown to interfere with Ca metabolism in invertebrates (Short & Wilbur, 1980; Johnston, 1987; Ingersoll et al., 1990). When there is less Ca^{2+} available for uptake, the same concentration of Se will have a greater influence than if there was more Ca^{2+} present in the surrounding media.

4.4.4 Water quality guidelines and toxicity predictions

The water quality guideline for Se is set at 1 µg L^{-1} in Canada (CCME, 1987), which is equal to 12.7 nmol L^{-1}. This water quality guideline for Se is protective for *H. azteca*. However, the USEPA fish whole-body concentration guideline is set at 8.5 mg kg^{-1} (107 nmol g^{-1}) (USEPA, 2016a) and whole-body concentrations of Se in *H. azteca* that contribute to 50% mortality were around this concentration. Therefore, despite the concentration of Se in water being acceptable, the concentration of Se in a dietary source may contribute to toxic effects higher in the food chain.

4.5 CONCLUSION

The process to determine a site-specific guideline for Se is complicated, as both concentrations of Se in water causing mortality and whole-body concentrations of Se causing mortality in *H. azteca* can be affected by the water chemistry of the surrounding media. DOC, pH/alkalinity, and hardness can all affect the LC values of Se, with a 3.5-fold range in 28-day LC50s. DOC and pH can also affect the lethal body concentrations of Se, with a similar range of 3.3-fold LC50s. To improve estimates of both LC and LBC values, the speciation and source of Se should specifically be considered. The effect on water chemistry guidelines will be discussed in Chapter 6.

4.6 SUMMARY

1. Dissolved organic carbon can increase the toxicity of Se on both a body and water concentration basis.
2. Lower pH/alkalinity has a somewhat protective effective against Se toxicity on a body concentration basis. Lower pH/alkalinity increase Se toxicity based on concentrations of Se in water.
3. Increased hardness affects Se toxicity based on concentrations of Se in water, but not in terms of body concentration.
4. Se speciation alters uptake of Se into an organism, with $HSeO_3^-$, the form with the highest concentration at lower pH, having the greatest uptake at low concentrations of Se in water.

CHAPTER 5

Using saturation kinetics-based non-linear regression models to predict the chronic toxicity and bioaccumulation of zinc to *Hyalella azteca* under different water chemistry conditions

OVERVIEW

Zinc is an essential metal to *Hyalella azteca* and it is partially regulated within the organism when it is accumulated from waterborne exposures. *Hyalella azteca* were exposed to Zn over 28-days in conditions with varying water chemistry. Using a bioaccumulation model, the maximum body concentration was not dependent on water chemistry; however, this accumulation pattern was dependent on water hardness. Twenty-eight-day lethal concentrations were determined using a saturation kinetics-based mortality model. Both water hardness and dissolved organic carbon can protect *H. azteca* from Zn toxicity in 28-day exposures and LC10s ranged from 103 to 1290 nmol L^{-1}. Twenty-eight-day lethal body concentrations were also predicted using this non-linear model, as *H. azteca* body metal concentration is a good indicator of toxicity. Water chemistry parameters did not significantly affect the critical body concentrations causing toxicity, which was approximately 2000 nmol g^{-1}.

5.1 INTRODUCTION

Zinc (Zn) is a metallic element that is essential to living organisms. It is required for the proper function of enzymes and other proteins. However, if internal Zn concentrations are not effectively regulated, there can be toxic effects including changes to growth, reproduction, and survival. Background concentrations are less than 0.3 µg L^{-1} (4.59 nmol L^{-1}) in the Great Lakes (Nriagu et al., 1995), although other systems in Canada have higher background concentrations that range from 0.434 µg L^{-1} (6.64 nmol L^{-1}) dissolved Zn in the St. Lawrence River to 20 µg L^{-1} (306 nmol L^{-1}) in British Columbia (CCME, 2016). Zn can enter the environment from both natural and anthropogenic sources, including metal mining and industrial effluent (CCME, 2016). Sites affected by mining activity can have concentrations of Zn in water over 6000 µg L^{-1} (91800 nmol L^{-1}) (Bonnail et al., 2016).

The Canadian water quality guideline for the protection of aquatic life is 30 µg L^{-1} (459 nmol L^{-1}) (CCME, 1987), although there are draft guidelines for both short-term and long-term exposure that sets the recommended Zn concentration in the environment based on a species sensitivity distribution, and includes water hardness, dissolved organic carbon (DOC) concentration, and pH (CCME, 2016). The ions that contribute to water hardness (Ca^{2+} and Mg^{2+}) can both interact with Zn to affect its bioavailability to organisms. The concentration of Ca^{2+} is the most important factor in decreasing the toxicity of Zn^{2+} in both rainbow trout (*Oncorhynchus mykiss*) (De Schamphelaere & Janssen, 2004), *Daphnia manga* (Heijerick et al., 2005), and *Daphnia pulex* (Clifford & McGeer, 2009). It has been suggested that Zn^{2+} and Ca^{2+} compete for the same uptake channel in the cell membrane (Santore et al., 2002), as Zn^{2+} can also inhibit the uptake of Ca^{2+} (Muyseen et al., 2006). In experiments with *D. magna*, the 21-day EC50 of Zn increased from 1.94 to 5.25 µmol L^{-1} as the concentration of Ca^{2+} increased from 0.25 to 3.00 mmol L^{-1} (Heijerick et al., 2005). In addition to water hardness, pH (Heijerick et al, 2005) and DOC concentrations (Bringolf et al., 2006; Clifford & McGeer, 2009) also affect the waterborne effective and lethal concentrations of Zn to different organisms.

Previous studies of Zn toxicity to *Hyalella azteca* have been conducted by many different groups (Schubauer-Berigan et al., 1993; Borgmann et al., 1995a, Eisenhauer et al., 1999). The average background concentration of Zn in *H. azteca* is 1000 nmol g^{-1} ranging from 740 to 1200 nmol g^{-1} (Borgmann et al., 1995b). Borgmann et al. (1993) determined that Zn is significantly toxic to *H. azteca* at double the background body concentration of 1140 nmol g^{-1} in 10-week toxicity tests. The lethal body concentration causing 50% mortality was 2020 (1880 – 2180)

nmol g^{-1} using the data from Borgmann et al. (1993) in a saturation-kinetics based mortality model (Borgmann, 2004). A saturation-based bioaccumulation model, described in Chapter 1, has also been used to predict maximum Zn accumulation in *H. azteca* using data from Borgmann et al. (1995a). This model predicted a maximum Zn body concentration of 3550 (2980 – 4110) nmol g^{-1} (Borgmann, 2004). The objectives of this chapter were to show if and how water chemistry effects 1) concentrations of Zn in water causing mortality, 2) bioaccumulation of Zn, and 3) whole-body concentrations of Zn in *H. azteca* causing mortality. A final objective of this chapter was 4) to observe if water quality guidelines for Zn should consider water chemistry.

5.2 METHODS

5.2.1 Experimental Set-up

The 28-day toxicity test methods used were the same as Section 2.2.1 and 3.2.1, with the following deviations. The water chemistry was adjusted as follows in Table 5.1 for each set of experiments:

Table 5.1. Test parameters and methods to maintain the water chemistry

Parameter measurements		Method	Reference
pH	6.8, 7.7[a], 8.3	M HCl or KOH amendments	Taylor et al. (2002); Niyogi (2008)
Alkalinity	5, 16[a], 100 mg L^{-1} $CaCO_3$	Adjust $NaHCO_3$ - maintained sodium ions using NaCl	Deleebeeck et al. (2007)
Dissolved organic carbon	0.5[a], 2, 10 mg C L^{-1}	Luther Marsh, natural organic matter	Gillis et al. (2010)

[a] unmodifed SAM30 exposures

Zinc as $ZnCl_2$ (Zinc Chloride puriss. p.a., ACS reagent, reag ISO, reag Ph. Eur., >98%, Sigma Aldrich) additions to each container were equilibrated with the appropriate water chemistry 24 hours before animals were added (Taylor et al., 2002; Niyogi et al., 2008). Alkalinity, pH, and DOC adjustments were made to 25L carboys of 30% SAM water 48h in advance.

The organisms were fed 2.5 mg finely ground TetraMin fish food (Tetra GMBH, Melle, Germany) twice during this period. TetraMin contains 1150 nmol Zn g^{-1} dry weight, which is the approximate background concentration of Zn required by *H. azteca* as determined by Borgmann et al. (1995b).

5.2.2 Sample collection and analysis

Water and whole-body samples were collected and analyzed as stated in Section 2.2.2. Measurements of water chemistry were summarized in Table 5.2. Total zinc in water and whole-body samples were determined with a Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometer and SOLAAR Data Station V11.03 software. The ash temperature was 700°C and the atomization temperature was 1150°C with Zeeman background correction. Ammonium phosphate was used as a modifier. Method blanks, reference standards, and metal standards were analyzed to ensure quality control (QA/QC) in the determination of background contamination, instrument drift, detection limits and metal recovery. Detection limits were calculated as three times the method blank standard deviations (Norwood, 2008) and were 71.1 nmol L^{-1} for water samples and 76.2 nmol g^{-1} for whole-body samples. The recovery value of Zn from certified reference material CRM-TMDW Trace Metals in Drinking Water (High Purity Standards) was 106% ± 6.56. All samples were corrected for background contamination and instrument drift. Filtered Zn samples were contaminated, so all further references to Zn were total Zn measurements.

Table 5.2. Water chemistry from Zn treatments. Mean of all measurements at the beginning and end of turnover periods with 95% C.I and some (maximum and minimum) values.

Treatment	Ca	Mg	Na	K	Cl	SO_4	DOC	DIC	Hardness	Alkalinity	pH	Conductivity
	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}		µS cm^{-1}
DOC 5	348 ± 10.0	98.4 ± 2.40	303 ± 8.06	5.1 ± 0.7(	569 ± 13.4	88.4 ± 2.22	5.74 ± 0.649	5.48 ± 0.422	44.7	28.3 ± 2.54	7.90 ± 0.019 (7.69 - 8.30)	184
DOC 2	311 ± 9.33	91.4 ± 1.86	310 ± 7.06	4.8 ± 0.5:	575 ± 11.1	87.2 ± 1.57	1.85 ± 0.778	4.53 ± 0.314	40.3	24.2 ± 1.59	7.81 ± 0.016 (7.59 - 8.11)	172
pH 6.5	311 ± 11.3	87.7 ± 2.05	332 ± 4.96	7.4 ± 1.1	766 ± 16.8	80.6 ± 1.37	1.05 ± 0.271	1.68 ± 0.352	39.9	5.55 ± 1.48	6.98 ± 0.097 (6.70 - 7.29)	152
pH 8.5	316 ± 7.60	94.6 ± 4.15	335 ± 7.26	458 ± 18:	609 ± 4.15	82.1 ± 3.46	1.30 ± 0.069	8.7 ± 1.52	41.1	40.1 ± 15.3	8.29± 0.06 (7.81 - 8.48)	166
pH 7.5	310 ± 1.03	80.0 ± 0.180	348 ± 18.5	3.9 ± 0.3:	632.3 ± 11.4	75.2 ± 0.228	0.28 ± 0.030	3.97 ± 0.109	39.2	16.1 ± 0.149	7.60 ± 0.01 (7.50 - 7.66)	150
Alk 100	311 ± 11.0	89.2 ± 3.25	4120 ± 133	7.0 ± 0.4:	2810 ± 122	84.2 ± 3.71	1.13 ± 0.765	26.7 ± 1.58	40.0	115 ± 5.57	8.47 ± 0.044 (8.37 - 8.59)	619
Alk 5	291 ± 8.56	79.7 ± 7.05	4050 ± 277	6.4 ± 1.8	4940 ± 663	76.5 ± 3.16	0.55 ± 0.098	3.40 ± 1.68	37.1	6.70 ± 3.39	7.36 ± 0.083 (7.14 - 7.54)	729
Hard water	870±0.41[a]	351±0.09[a]	561±0.31[a]	40±0.02[a]	674±0.53[a]	314±0.98[a]	1.1±0.36[a]	20±0.32[a]	130[b]	90[b]	7.9 - 8.6[b]	315±6.5[a]

a- Data from Norwood et al. (2006)

b- Data from Borgmann et al. (1995a)

5.2.3 Whole-body Digests

Organisms were digested following the method of section 3.2.3. Digests of a certified reference material had an average recovery value 185 ± 18 µg g^{-1} d.w. [TORT2: lobster hepatopancreas; Zn certified as 180 ± 6 µg g^{-1} dry weight from the National Research Council of Canada].

5.2.4 Data analyses

5.2.4.1 Mortality Model

The mortality model as described in Section 1.7.4.1 was used to determine lethal water and lethal body concentrations. Mortality data were log transformed before the above models were fit in SYSTAT 10, to ensure normality and equal variance. Normality was assessed by visual inspection of the probability plots and the Shapiro Wilk test on the mortality data (W = 0.421, p = 0.000 4 on untransformed data; W=0.979, p =0.074 on log transformed). Levene's test was performed for equal variance of log transformed data (F=0.967, p=0.122) (Golding et al., 2013).

5.2.4.2 Bioaccumulation

The bioaccumulation saturation model, as described in Section 1.7.4.2 was used to determine the relationship between bioaccumulation of zinc and exposure. Measured concentrations were log transformed before the above models were fit in SYSTAT 10, to ensure normality and equal variance. Normality was assessed by visual inspection of the probability plots and the Shapiro Wilk test on the mortality data (W = 0.716, p = 0.0004 on untransformed data; W=0.982, p = 0.055 on transformed data). Levene's test was performed for equal variance of log transformed data (F=0.427, p=0.252).

5.2.4.3 Confidence Intervals

To determine significant effects, confidence intervals will be used as described in section 2.2.3.3.

5.2.4.4 Comparison with Borgmann et al. (2004)

Zinc concentrations in water and whole-body concentrations causing mortality in hard water have been previously determined by Borgmann et al. (2004). Lethal concentrations and

mortality model parameters for zinc from Borgmann et al. (2004) were used in the current study to determine the effect of water hardness on Zn toxicity.

5.2.4.5 Zinc speciation

The Windermere Humic Aqueous Model VI (WHAM VI) was used to estimate the free ion activity and Zn-complex concentrations in the different water chemistry treatments, as described in section 3.2.5.5 (Table 3.3).

Table 5.3. Zinc speciation (% total Zn) determined by WHAM VI

Treatment	Free ion activity % of Zn	Zn^{2+}	$ZnOH^{+}$	$Zn(OH)_2$	$ZnSO_4$	$ZnCO_3$	$ZnCl^{+}$	$ZnHCO_3^{+}$	Zn-HA	Zn-FA
DOC5	5.67	6.75	0.47	0.44	0.08	0.39	0.01	1.17	79.9	10.7
DOC2	10.8	12.9	0.79	0.55	0.02	0.09	0.002	2.46	79.8	2.56
pH 6.5	71.4	88.3	0.41	0.02	1.05	0.03	0.38	1.21	6.01	2.64
pH 7.5/DOC 0.5/Alk 16	52.0	61.8	3.55	2.32	0.80	2.55	0.09	10.1	14.7	3.98
pH 8.5	26.7	32.6	6.83	16.5	0.40	12.0	0.04	12.9	15.6	3.13
Alk 100	20.3	27.3	5.32	12.5	0.26	21.4	0.13	23.7	7.76	1.74
Alk 5	49.6	68.9	6.58	7.66	6.15	1.36	0.62	3.0	3.84	1.89
Hard water[a]	24.0	32.6	5.63	11.7	5.07	16.9	0.04	21.1	5.69	1.26

a - Calculated from water chemistry measurements from Norwood et al. (2007) and Zn concentrations from Borgmann et al., (1993)

5.3. RESULTS

5.3.1 Exposure-based mortality

5.3.1.1 DOC

The toxicity of Zn to *H. azteca* decreased as the concentration of DOC increased (Table 5.4). The 28-day LC10 of the DOC5 treatment was significantly higher than that of the DOC2 treatment, with a 28-day LC10 of 1290 (364 – 2220) nmol L^{-1} compared to 103 (11.0 – 196) nmol L^{-1}. Since the predicted 28-day LC50 and 28-day LC25 of the DOC5 treatment were greater than the exposure concentration range, the model was extrapolated, which caused wide confidence intervals. However, the lethal concentrations were ten times greater than those of the DOC2 and the unmodified DOC0.5 treatment. The DOC2 treatment and the DOC0.5 treatments lethal Zn concentrations were not significantly different.

The mortality rate of the DOC5 treatment did not increase from control until the concentration of Zn in water was almost 1000 nmol L^{-1}, whereas the DOC2 and DOC0.5

mortality rates increased at a lower concentration of Zn in water (Figure 5.1). Both treatments with added DOC (DOC2, DOC5) approached a mortality rate maximum as the curves began to plateau, while the unmodified treatment (DOC0.5) did not approach a maximum rate. Control exposure concentrations were plotted at 71.1 nmol L^{-1} when they were less than the detection limit; however, the detection limit was not used for modelling.

5.3.1.2 pH

There were no significant pH effects on Zn toxicity (Table 5.5). There was also no significant difference between the 28-day LC25s or 28-day LC10s over all the pH treatments. As well, the adjusted pH Zn lethal concentrations were not significantly different from the DOC2 28-day lethal concentrations. The pH models plotted in Figure 5.2 were overlapped.

5.3.1.3 Alkalinity

Alkalinity had little effect on the toxicity of Zn to *H. azteca*. The only significant effect was low alkalinity treatment 28-day LC10 which was greater than the high alkalinity treatment (Table 5.6). The Alk-16 treatment was not significantly different from either adjusted alkalinity treatments at any endpoint. The Alk-100 treatment (pH 8.33) when compared to the pH-8.3 treatment (alkalinity of 43 mg L^{-1} as $CaCO_3$ equivalents) did not cause significantly different lethal concentrations (Table 5.5 and Table 5.6). The lethal concentration for the Alk-5 treatment (pH 7.05), was also not significantly different from the pH-6.8 treatment. The alkalinity models also have a moderately strong fit to the data with an r^2 value of 0.718 for the Alk-100 treatment and 0.683 for the Alk-5 treatment (Table 5.5). Overall the model parameters and trends indicate that alkalinity does not affect mortality due to Zn exposure (Figure 5.3).

5.3.1.4 Hardness

The 28-day lethal concentration of Zn in soft water was significantly lower than in hard water experiments completed by Borgmann et al (2004) (Table 5.7). No control mortality rates were provided in Borgmann et al (2004), so the mortality rate for their controls was set to the soft water control mortality rate of the current study to plot the curve in Figure 5.4. The two curves have different shapes and the data points from the soft water experiments do not fit the hard water model.

Table 5.4. Mortality model parameters based on concentration of Zn in water of treatments with different dissolved organic carbon concentrations. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$			
DOC5	0.019	597	(-16100 - 17300)	10000[a]	(-172000 - 192000)	2580[a]	(-920 - 6080)	1290	(364 - 2220)	5.84	0.402
DOC2	0.02	1280	(-5280 - 7840)	1440	(236 - 2640)	345	(103 - 586)	103	(11.0 - 196)	0.962	0.598
DOC0.5	0.035	-2160	(-2560 - -1760)	1060	(832 - 1300)	421	(52.0 - 850)	122	(-122 - 366)	0.676	0.945

[a] Greater than Zn exposure range.

Table 5.5. Mortality model parameters based on concentration of Zn in water of treatments with different pH. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$			
pH-8.3	0.036	242	(50.7 - 434)	669	(421 - 918)	497	(291 - 704)	376	(202 - 704)	10.0	0.831
pH-7.7	0.035	-2160	(-2560 - -1760)	1060	(832 - 1300)	421	(52.0 - 850)	122	(-122 - 366)	0.676	0.945
pH-6.8	0.020	-5770	(-38100 - 26600)	1000	(586 - 1430)	528	(292 - 763)	238	(91.5 - 384)	1.18	0.687

Table 5.6. Mortality model parameters based on concentration of Zn in water of treatments with different alkalinity. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	weeks^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Alk-100	0.015	763	(-2520 - 4050)	816	(359 - 1270)	390	(188 - 562)	201	(102 - 301)	2.08	0.718
Alk-16	0.035	-2160	(-2560 - -1760)	1060	(832 - 1300)	421	(52.0 - 850)	122	(-122 - 366)	0.676	0.945
Alk-5	0.033	478	(27.4 - 929)	897	(615 - 1180)	710	(443 - 976)	562	(318 - 806)	10.0	0.683

Table 5.7. Mortality model parameters based on concentration of Zn in water of treatments with different hardness. Parameters include control mortality rate (m′), half saturation constant (K_w''), 28-day lethal concentrations causing mortality (LC50, LC25, LC10), an exponent to modify the curve shape (n_w), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_w''	CL	LC50	CL	LC25	CL	LC10	CL	n_w	r^2
	weeks^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Hardness 37.5	0.015	763	(-2520 - 4050)	816	(359 - 1270)	390	(188 - 562)	201	(102 - 301)	2.08	0.718
Hardness 130[b]	nd	2000		3100	(2600 – 3690)	2520	(2050 – 3100)	nd		10.3	nd

[b] Borgmann et al. (2004)

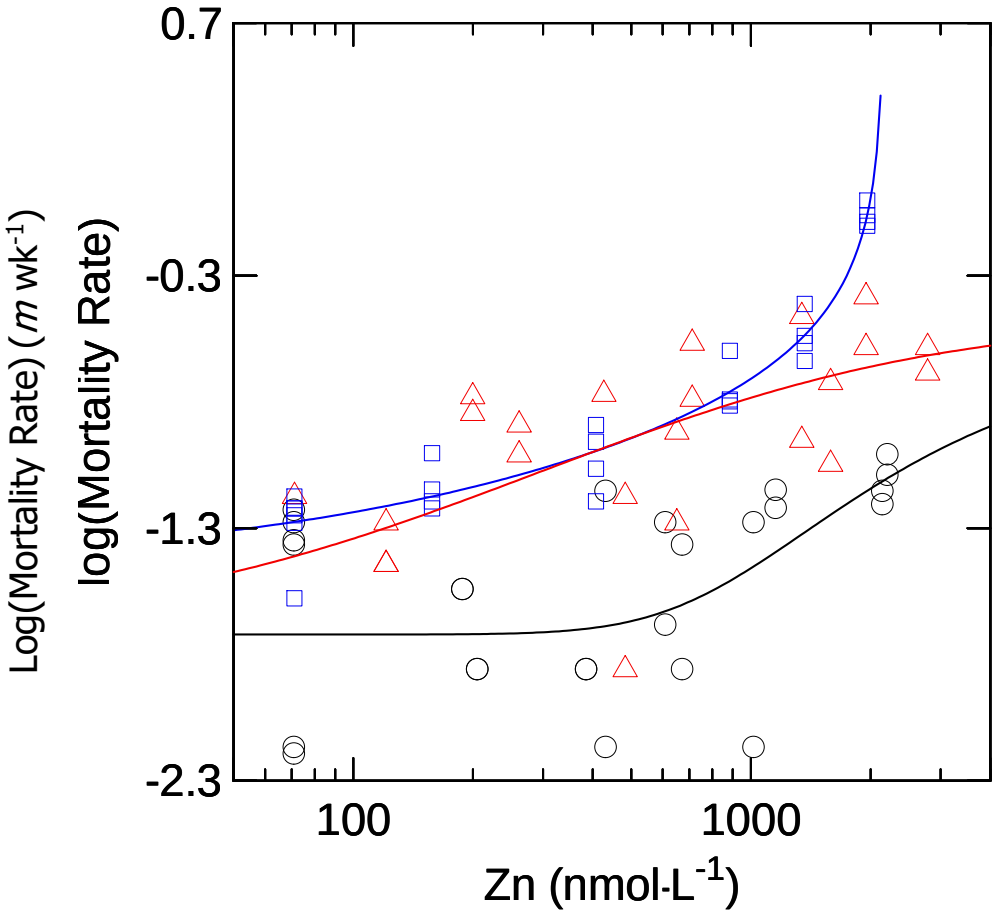

Figure 5.1. Waterborne Zn mortality models with modified DOC based on parameters in Table 5.4. Data points are mortality rate (mortality per week) at mean measured Zn exposure concentrations in SAM30 with modified DOC concentrations. □ are data from DOC-0.5 experiments, △ are DOC-2 , and ○ are DOC-5. The solid lines represent the corresponding Zn mortality model.

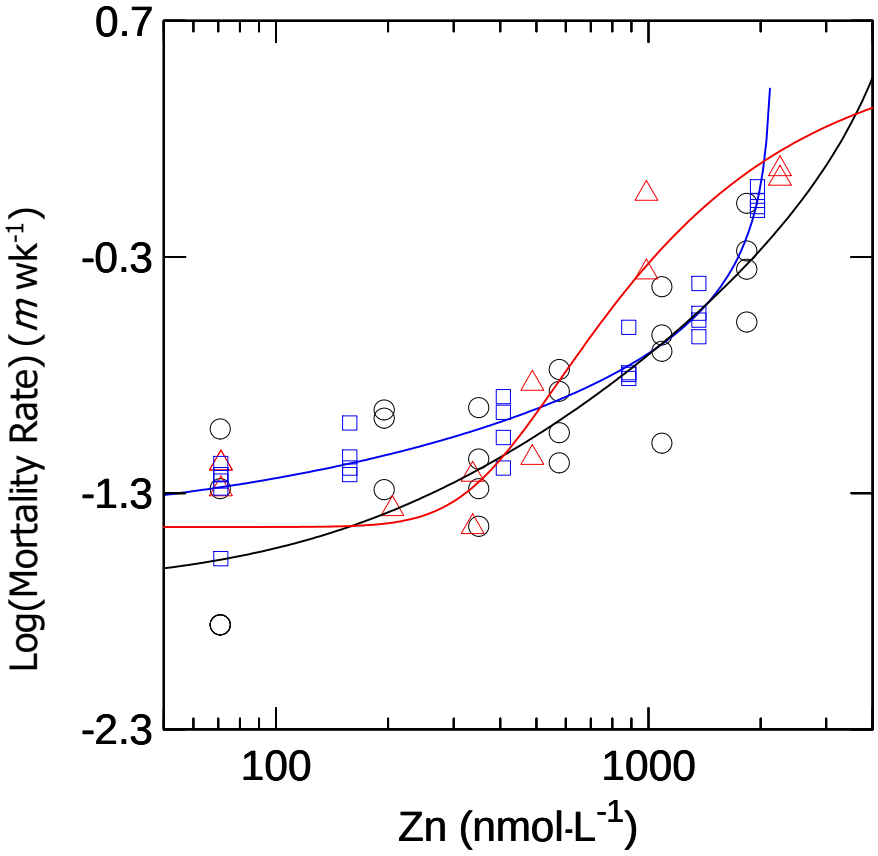

Figure 5.2. Waterborne Zn mortality models with modified pH based on parameters in Table 5.5. Data points are mortality rate (mortality per week) at mean measured Zn exposure concentrations in SAM30 with modified pH. ○ are data from the pH 6.8 treatment, □ at pH 7.7, and △ at pH 8.3. The solid lines represent the corresponding Zn mortality model.

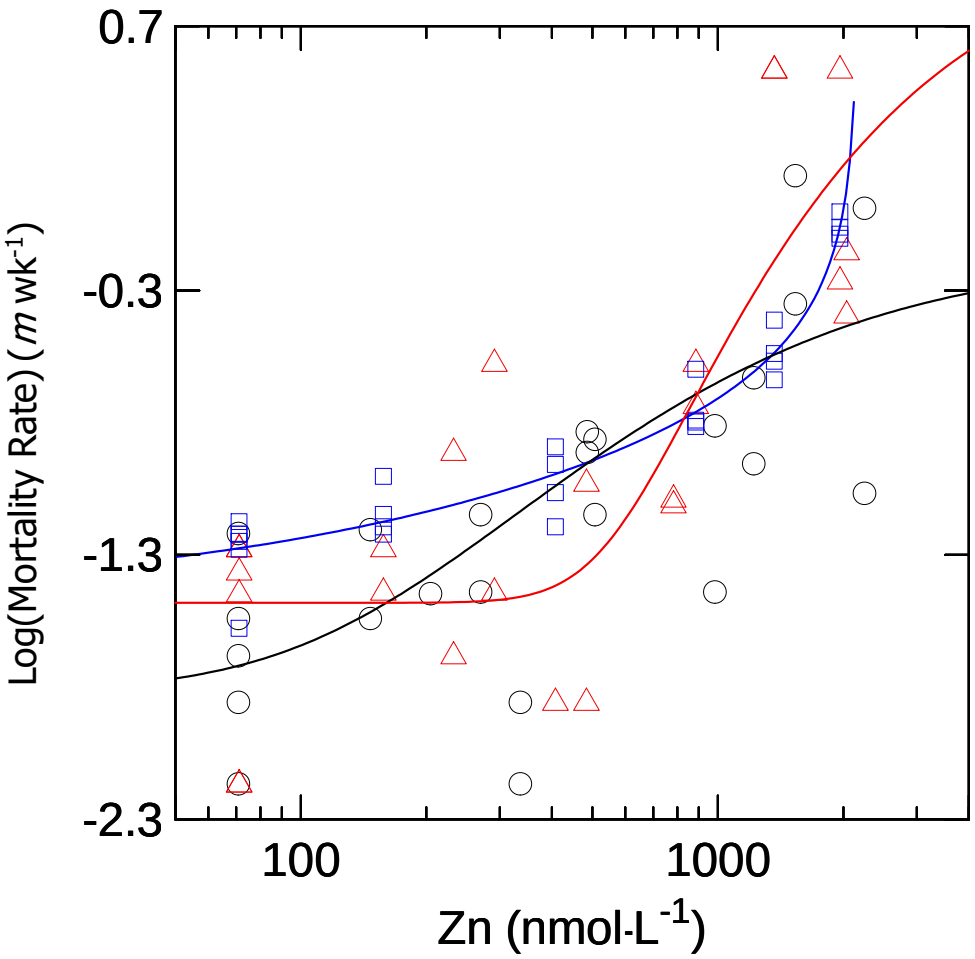

Figure 5.3. Waterborne Zn mortality models with modified alkalinity based on parameters in Table 5.6. Data points are mortality rate (mortality per week) at mean measured Zn exposure concentrations in SAM30 with modified alkalinity. ○ are Alk-100 treatments, □ are Alk-16 treatments, and △ are Alk-5 treatments. The solid lines represent the corresponding Zn mortality model.

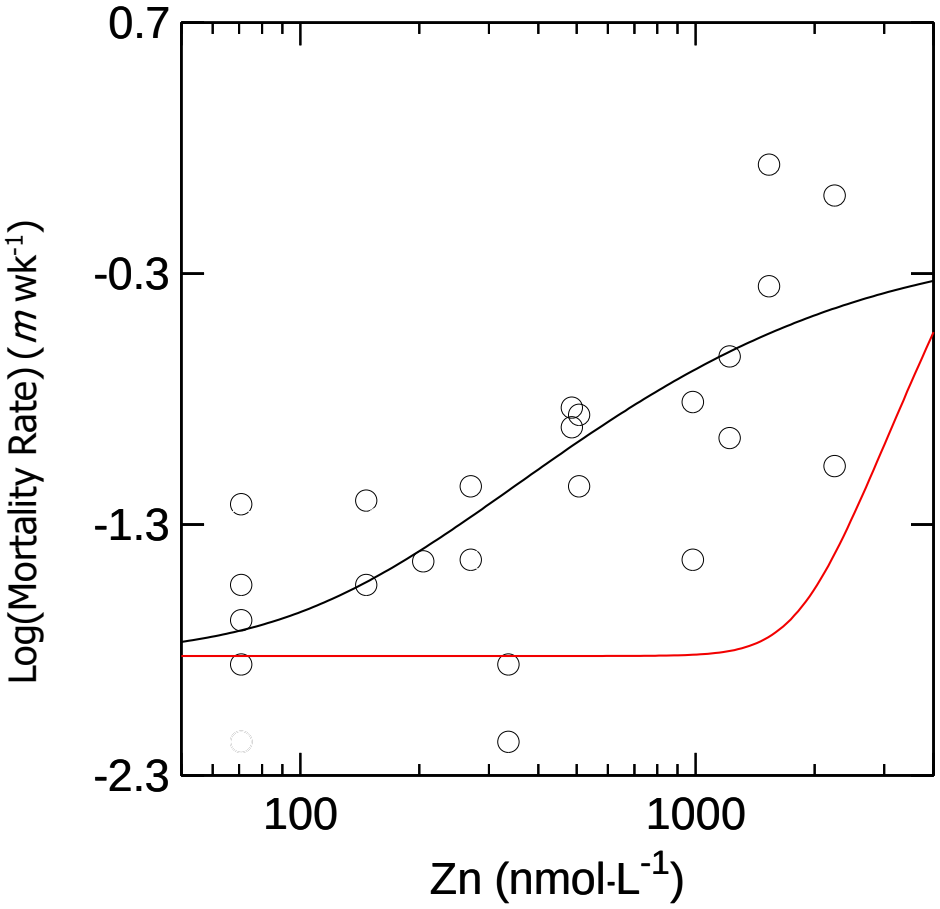

Figure 5.4. Waterborne Zn mortality models with modified hardness based on parameters in Table 5.7. Data points are mortality rate (mortality per week) at mean measured Zn exposure concentrations in SAM30 with modified hardness. ○ are the Hardness-37.5 treatment, the black line is the mortality model for Zn Hardness 37.5 and the red line is the mortality model for Zn Hardness-130 from Borgmann et al. (2004).

5.3.2 Bioaccumulation

The bioaccumulation of Zn in soft water increased with increasing Zn exposure concentrations (Figure 5.5). Whole-body concentration of Zn did not significantly increase above control levels until Zn exposure concentration reached 490 nmol L-1 ($p = 0.322$). Data for all experiments (DOC, pH, and alkalinity) from this study were pooled, as bioaccumulation at each nominal Zn exposure concentration were not significantly different between all water chemistry conditions (Two-way ANOVA at 153 nmol L^{-1} $p = 0.558$, at 276 nmol L^{-1} $p = 0.086$, at 490 nmol L^{-1} $p=0.217$, 858 nmol L^{-1} $p=0.561$, 1530 nmol L^{-1} $p = 0.149$). When the pooled soft water bioaccumulation data were compared to hard water accumulation data from Borgmann et al., 2004, water hardness did not affect the maximum predicted concentration of Zn accumulated in *H. azteca* after 28-days (Table 5.8).

The geometric mean control body concentration for *H. azteca,* determined in the current study, was 591 (501- 680, 95% CI) nmol g^{-1} (Table 5.8). This was significantly different from 1000 (980 – 1030) nmol g^{-1} determined by Borgmann et al. (1995b); however, it was similar to the background concentration of 705 (595–835) nmol g-1 determined by Neumann et al. (1999), using the same *H. azteca* culture as Borgmann et al. (1995b). These values indicate that there wass variation in the background concentrations of Zn in *H. azteca*. This variation may be due to slightly different analytical techniques, feeding regimes, or the exposure duration (Borgmann et al 1993, Borgmann et al 1995a & 1995b, Neumann et al 1999).

The max/K value is the uptake ratio of bioaccumulation to exposure, and there was a two-fold greater max/K in soft water compared to hard water (Table 5.8). This indicates greater accumulation of Zn at lower Zn exposure concentrations in soft water compared to the accumulation in hard water. Therefore, the bioaccumulation model from Borgmann et al. (2004), did not fit the data for the current study well, but indicated a similar trend (Figure 5.5).

Calculated whole-body concentrations obtained with the bioaccumulation model were used to determine body concentration-based lethal concentrations. This was done since some high exposure concentrations did not have surviving organisms for whole-body Zn analysis. Predicted values were within the 2:1 line of predicted versus observed Zn whole-body concentrations for 88.5% of data points (Figure 5.6).

Table 5.8. Bioaccumulation model parameters for zinc bioaccumulation with predicted maximum zinc accumulation (max) and half saturation constant (K) with 95% confidence limits (CL), r^2, number of data points (N), mean dry to wet weight ratio (D/W), bioconcentration factor (BCF) calculated as max × K^{-1} × D/W × 1000, and background Zn concentration in *H. azteca* (C_{bk}).

Treatment	N	r^2	max	CL	K	CL	MAX K^{-1}	CL	Cbk	CL	D/W ratio	BCF
			nmol g^{-1}		nmol L^{-1}		L g^{-1}		nmol g^{-1}			
Soft Water	121	0.643	4290	(2070 - 6520)	1420	(86.2 - 2750)	3.02	(1.59 - 4.46)	591	(501 - 680)	0.242	731
Hard water[a]	nd	nd	3550	(2980 - 4110)	2345		1.51		1000		nd	nd

[a] Borgmann et al. (2004) [data from Borgmann et al. (1995a)]

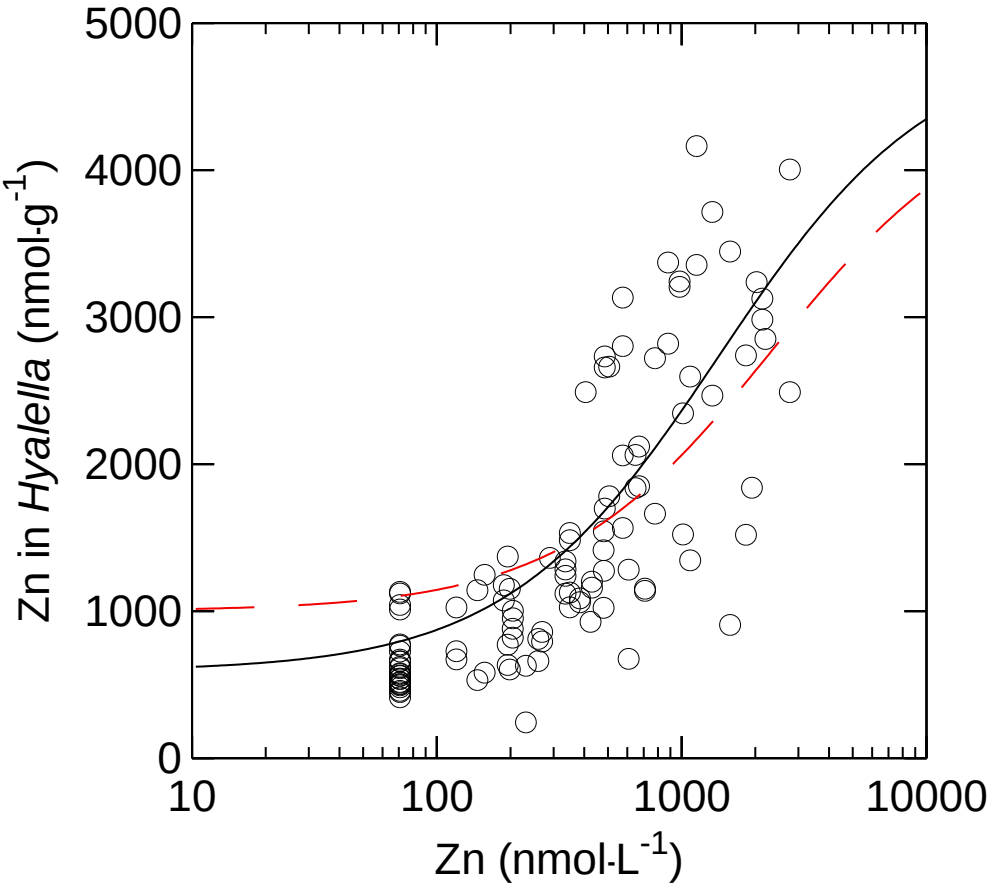

Figure 5.5. Zinc concentration accumulated in *H. azteca* in a 4-week exposure in soft water treatments (○). Solid black line is the soft water bioaccumulation model from this study calculated from parameters in Table 5.8. Dashed red line is hard water Zn bioaccumulation model from Borgmann et al. (2004).

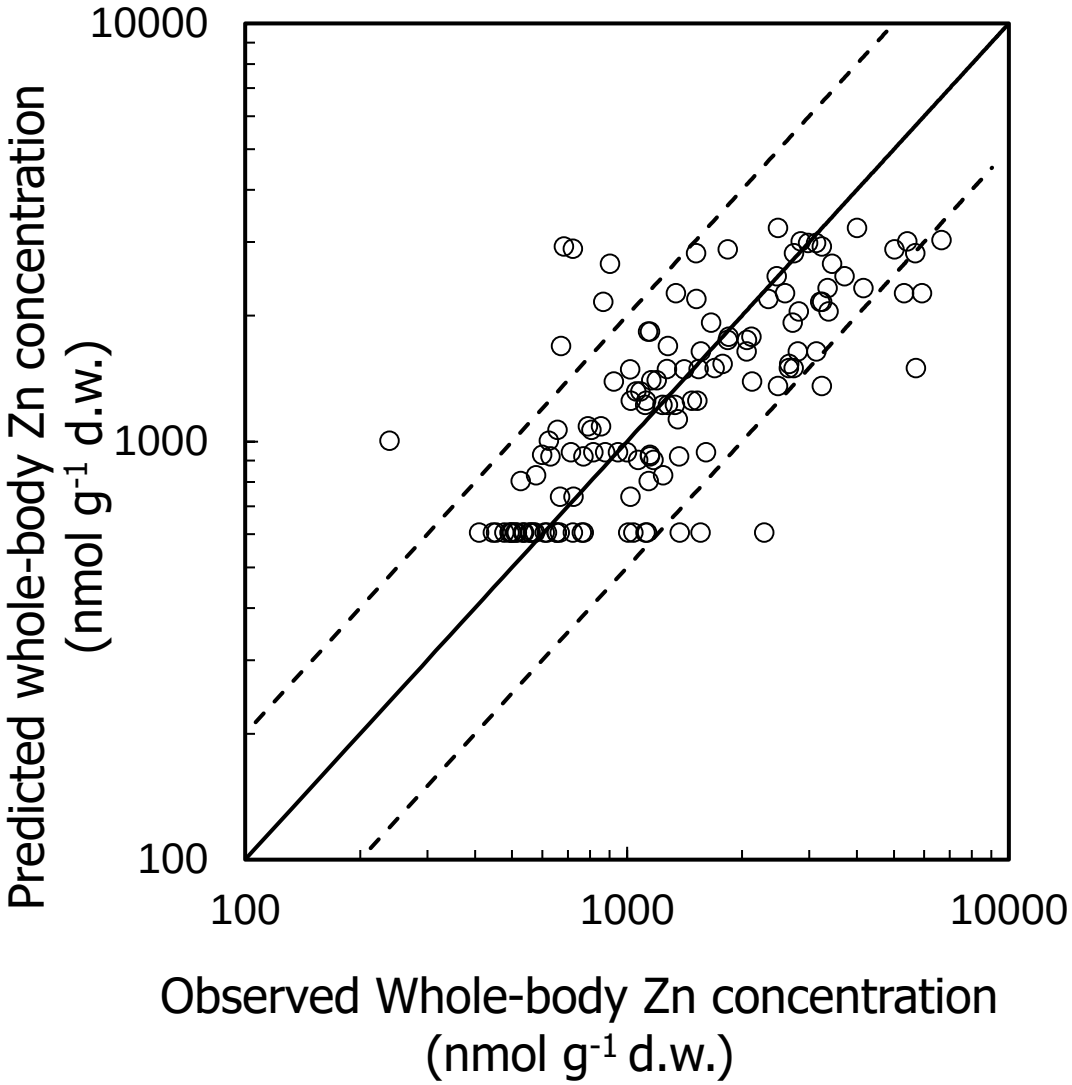

Figure 5.6. The predicted concentrations are based on the bioaccumulation models from Figure 5.5 and calculated using the parameters in Table 5.8. The observed Zn whole-body concentrations are based on average dry weight for each replicate. The solid line indicates a 1:1 relationship and the dashed lines are 2x overpredicted or 2x underpredicted.

5.3.3 Body concentration-based mortality

5.3.3.1 DOC

The lethal body concentration of Zn at which 50% of the organisms survived (LBC50) increased as the DOC concentration increased (Table 5.9), which is indicative of a somewhat protective effect. However, this increase was not significant, due to the large variation in the data. There were significant differences between LBC25s and LBC10s. There was over a two-fold increase in the LBC25s between the DOC0.5 and DOC5 treatments and an almost three-fold increase of the LBC10s of the same treatments.

There was only a slight increase in mortality rate at the highest body concentration of Zn for the DOC5 treatment (Figure 5.7). Both added DOC treatments had a weak fit to the mortality model, which can also be attributed to greater data variability than the other treatments.

5.3.3.2 pH, Alkalinity, and Hardness

Changes in pH, alkalinity, and water hardness did not significantly affect the 28-day lethal body concentrations of Zn (Table 5.10, Table 5.11, Table 5.12). Hard water and soft water 28-day lethal body concentration were almost identical at 2020 (1880 – 2180) and 2260 (1720 – 2800) nmol Zn g^{-1}, respectively. The model curves have slightly different shapes, but the data points have overlapping distributions (Figure 5.8, Figure 5.9, Figure 5.10). The models have moderate to strong fits with r^2 values ranging from 0.584 to 0.937 for these remaining treatments.

Table 5.9. Mortality model parameters based on zinc body concentrations in organisms exposed to treatments with different DOC concentrations (Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	$weeks^{-1}$		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
DOC5	0.023	2180	(-3610 - 7970)	4020	(1470 - 6570)	3190	(2390 - 3990)	2530	(1880 - 3180)	10.0	0.324
DOC2	0.032	-5730	(-65800 - 54400)	2760	(-1310 - 6830)	1480	(-7360 - 10300)	581	(-8960 - 10100)	0.9	0.395
DOC0.5	0.028	-5820	(-16300 - 4630)	1960	(1720 - 2210)	1370	(806 - 1940)	862	(23.7 - 1700)	1.75	0.937

Table 5.10. Mortality model parameters based on zinc body concentrations in organisms exposed to treatments with different pH. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	$weeks^{-1}$		nmol g^{-1} d.w.		nmol g^{-1}		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
pH-8.3	0.035	1350	(303 - 2390)	1760	(1430 - 2090)	1460	(1110 - 1800)	1190	(855 - 1530)	10.0	0.887
pH-7.7	0.028	-5820	(-16300 - 4630)	1960	(1720 - 2210)	1370	(806 - 1940)	862	(23.7 - 1700)	1.75	0.937
pH-6.8	0.015	-3460	(-6940 - 10.9)	2130	(1600 - 2650)	1380	(-270 - 3020)	672	(-1640 - 2990)	0.998	0.749

Table 5.11. Mortality model parameters based on zinc body concentrations in organisms exposed to treatments with different alkalinity. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	weeks^{-1}		nmol g^{-1} d.w.		nmol g^{-1} d.w.		nmol g^{-1}d.w.		nmol g^{-1}d.w.		
Hardness 37.5	0.025	3270	(-74700 - 81200)	2260	(1720 - 2800)	1800	(1190 - 2410)	1410	(507 - 2320)	6.20	0.584
Hardness 130[a]	nd	10000		2020	(1880 - 2180)	1840	(1660 - 2030)	nd		10.3	nd

[a] Borgmann et al. (2004)

Table 5.12. Mortality model parameters based on zinc body concentrations in organisms exposed to treatments with different hardness. Parameters include control mortality rate (m′), half saturation constant (K_b''), 28-day lethal whole-body concentrations causing mortality (LBC50, LBC25, LBC10), an exponent to modify the curve shape (n_b), the coefficient of determination (r^2), and confidence limits (CL).

Modifier	m′	K_b''	CL	LBC50	CL	LBC25	CL	LBC10	CL	n_b	r^2
	weeks^{-1}		nmol g^{-1} d.w.		nmol g^{-1}d.w.		nmol g^{-1} d.w.		nmol g^{-1} d.w.		
Alk-100	0.025	3270	(-74700 - 81200)	2260	(1720 - 2800)	1800	(1190 - 2410)	1410	(507 - 2320)	6.20	0.584
Alk-16	0.028	-5820	(-16300 - 4630)	1960	(1720 - 2210)	1370	(806 - 1940)	862	(23.7 - 1700)	1.75	0.937
Alk-5	0.032	2150	(-961 - 5250)	1880	(1420 - 2340)	1610	(1090 - 2120)	1360	(830 - 1880)	10.0	0.649

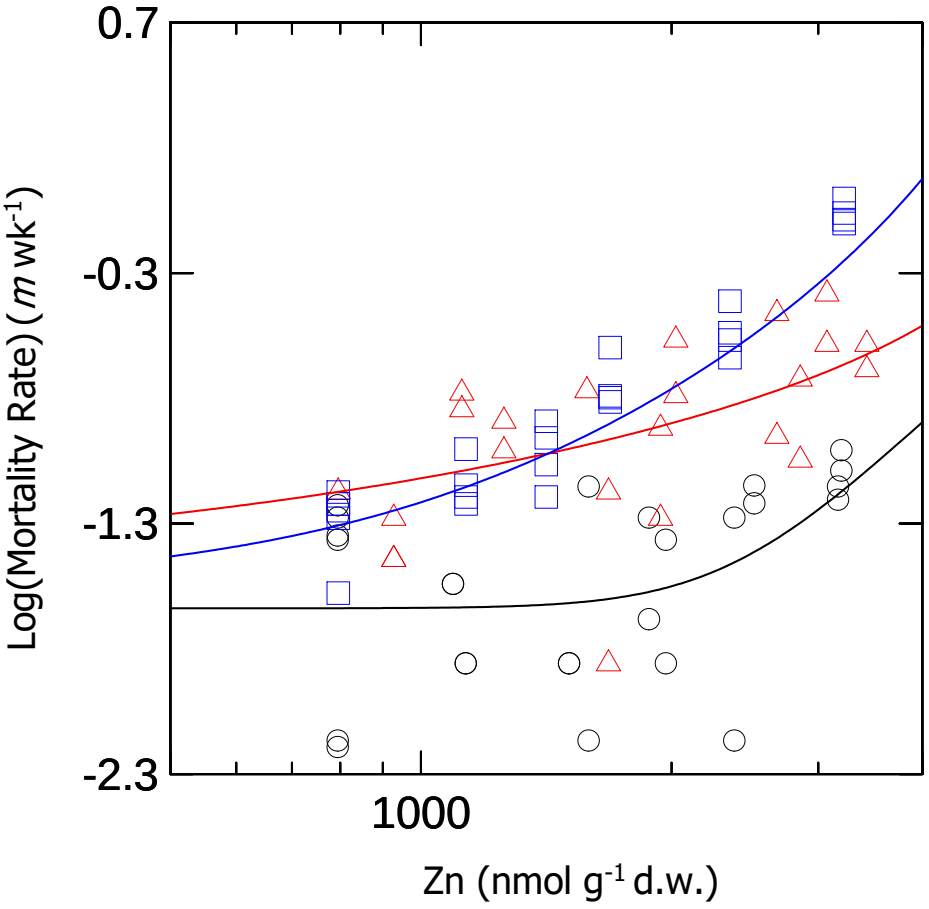

Figure 5.7. Zn body-concentration mortality models based on parameters in Table 5.9 with modified DOC concentrations in the exposure water. Data points are mortality rate (mortality per week) at mean measured Zn body concentrations on a dry weight basis in organisms exposed to Zn in SAM30 with modified DOC concentrations. Mortality rate was determined as the slope of mortality over 4-weeks. □ are from the DOC0.5 treatment, △ are DOC2, and ○ are DOC5. The solid lines represent the corresponding Zn mortality model.

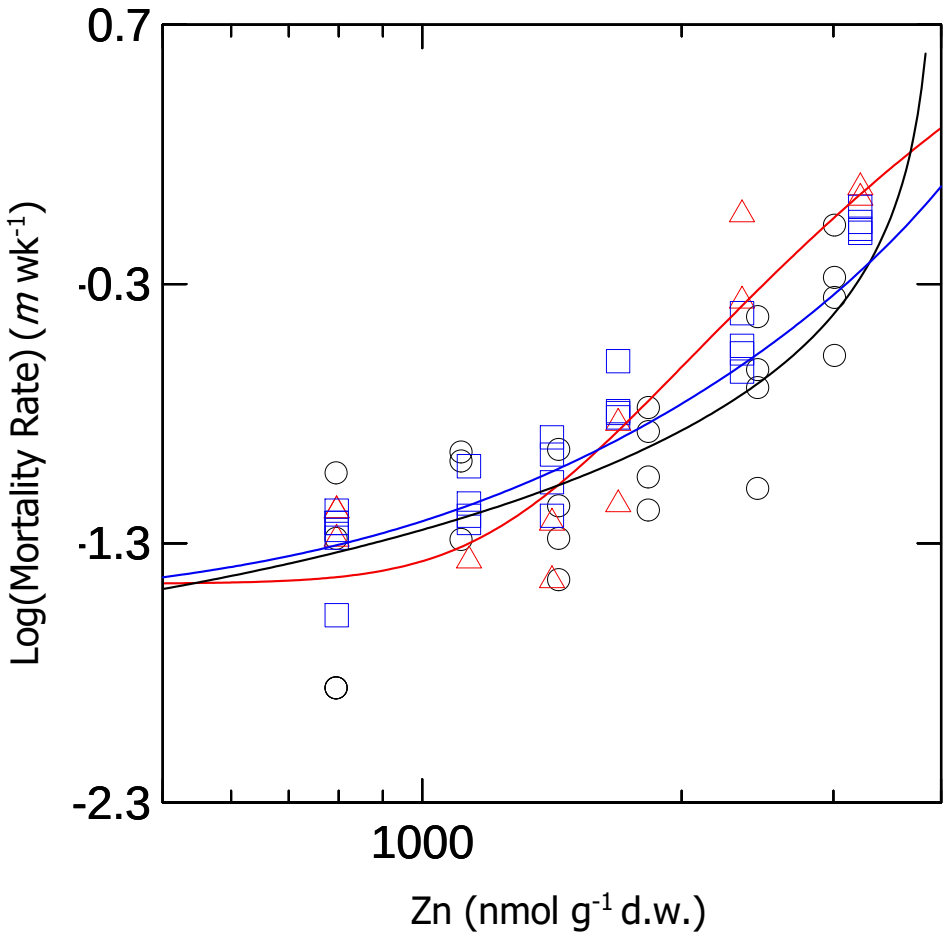

Figure 5.8. Zn body-concentration mortality models based on parameters in Table 5.10 with modified pH exposure water. Data points are mortality rate (mortality per week) at mean measured Zn body concentrations on a dry weight basis in organisms exposed to Zn in SAM30 with modified pH. ○ are data from experiments in pH 6.8, □ at pH 7.7, and △ at pH 8.3. The solid lines represent the corresponding Zn mortality model.

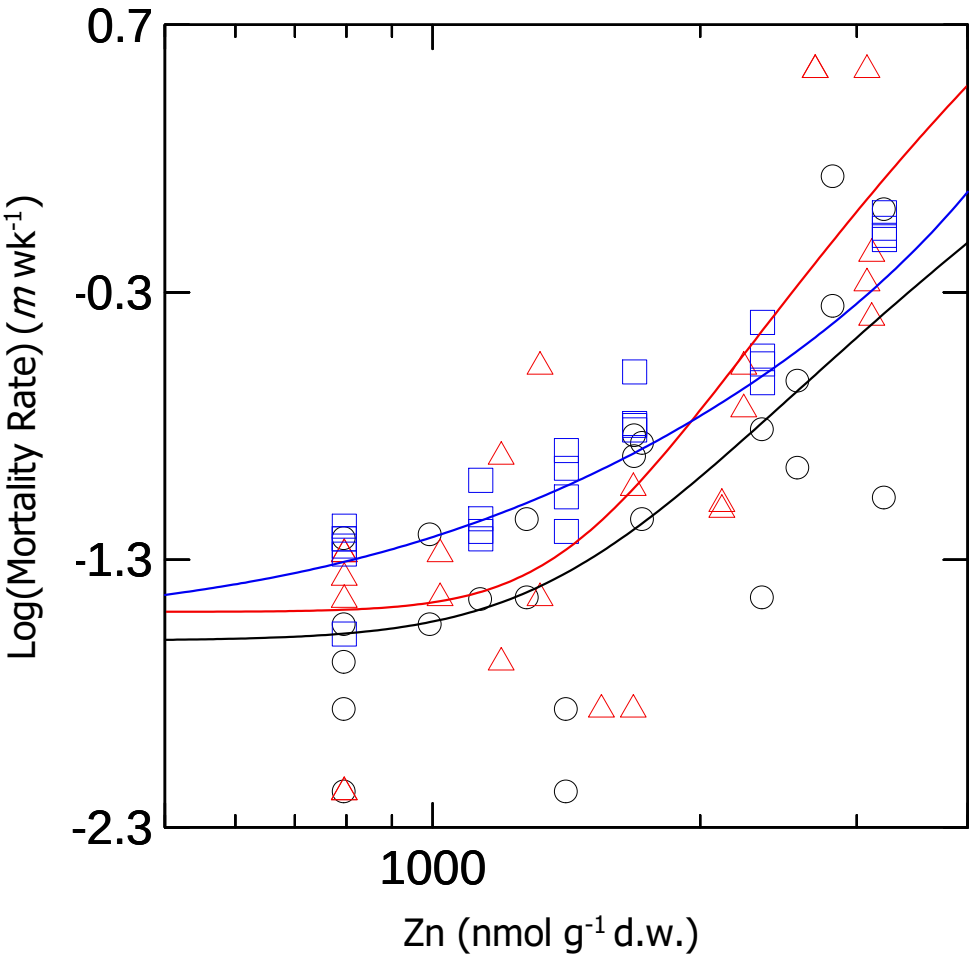

Figure 5.9. Zn body-concentration mortality models based on parameters in Table 5.11 with modified alkalinity exposure water. Data points are mortality rate (mortality per week) at mean measured Zn body concentrations on a dry weight basis in organisms exposed to Zn in SAM30 with modified alkalinity. ○ are data from Alk-100 experiments, □ are Alk-16, and △ are Alk-5. The solid lines represent the corresponding Zn mortality model.

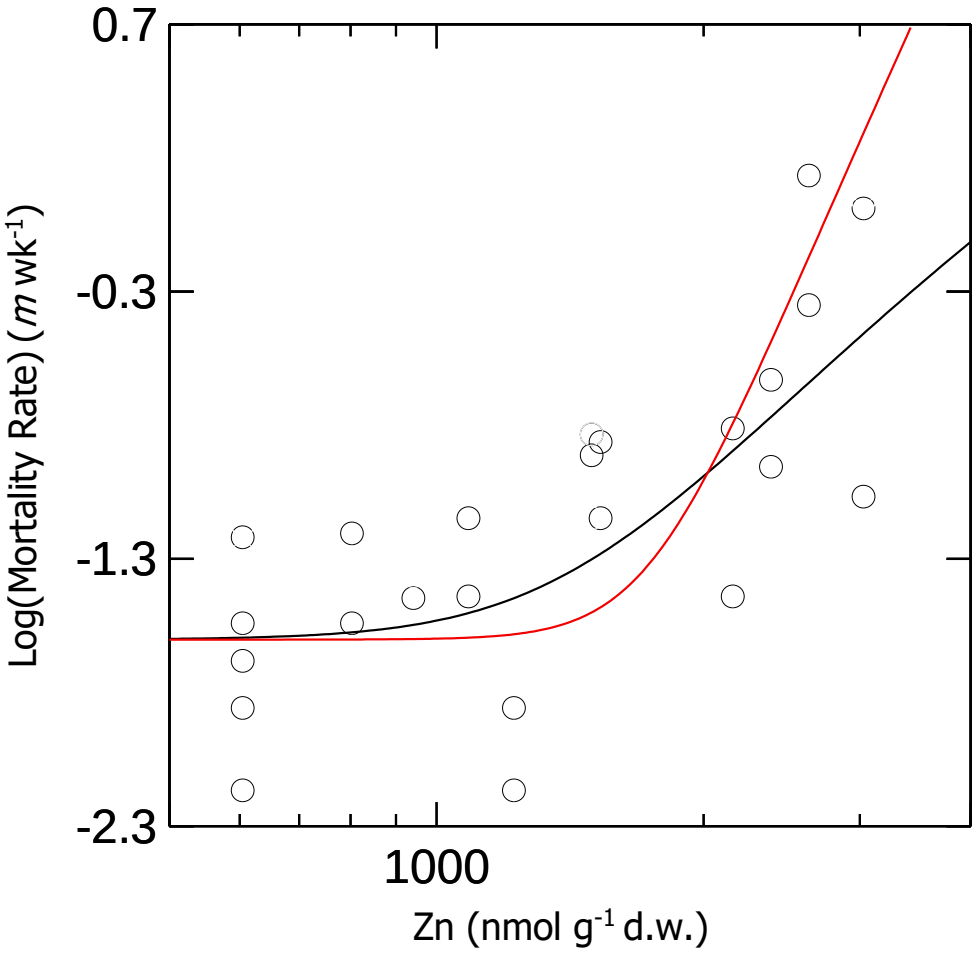

Figure 5.10. Zn body-concentration mortality models based on parameters in Table 5.12 with modified exposure water hardness. Data points are mortality rate (mortality per week) at mean measured Zn body concentrations on a dry weight basis in organisms exposed to Zn in SAM30 with modified hardness. ○ are from hardness-37.5 experiments. The solid lines represent the corresponding Zn mortality model, with the red line representing the model for Zn at hardness 130 mg L^{-1} as $CaCO_3$ equivalents from Borgmann et al., (2004).

5.4 DISCUSSION

5.4.1 Exposure-based concentrations

5.4.1.1 DOC

The addition of 5.74 mg DOC L^{-1} (DOC5 treatment) led to a marked decrease in the toxicity of Zn when compared to other treatments. This is consistent with Bringolf et al. (2006) who determined 11 mg L of dissolved organic carbon would provide a protective effect against Zn toxicity in larval fathead minnows (*Pimephales promelas*). DOC also had a protective effect in *D. pulex* at 6.1 mg DOC L^{-1} (Clifford and McGeer, 2009). Lower concentrations were not tested, so it is uncertain what the threshold is for a protective effect in this species.

5.4.1.2 pH and Alkalinity

In *H. azteca* it was determined that decreasing the pH of the exposure media from pH 8.3 to pH 6.8 did not significantly affect the Zn lethality. Change in alkalinity also did not alter mortality due to Zn exposure, except for the 28-day LC10s. The biotic ligand model for Zn by Santore et al. (2002) predicted a u-shaped toxic response in rainbow trout, with lower and higher pH being somewhat protective against Zn toxicity. At lower pH (< pH 6) there would be competition with H^+ and at higher pH (> pH 8) there is less free Zn available for uptake as $Zn(OH)_2$ forms at higher concentrations. At values between pH 6 and pH 8 the predicted LC50 concentrations do not differ greatly (Santore et al., 2002), as observed in the current study. There was also no significant effect of pH on Zn toxicity in *D. pulex* over a pH range of 6.0 to 8.3 (Clifford and McGeer, 2009). In addition, the difference in the lethal concentrations of Zn to rainbow trout at pH 6.5 and 7.5 was not significant, but when the pH was lowered to pH 5.5 Zn was significantly less toxic (De Schamphelaere & Janssen, 2004). However, in 96-h experiments by Schubauer-Berigan et al. (1993) increasing pH from 6.3 to 8.3 made Zn significantly more toxic in very hard water. This trend was also seen in *Ceriodaphnia dubia*, but the u-shaped toxic response was seen in the fish species, *P. promelas* (Schubauer-Berigan et al., 1993).

It should be noted that the 28-day LC10s of the alkalinity treatments were significantly different, such that increased alkalinity resulted in increased mortality. Increased alkalinity results in greater concentrations of Zn complexes like $ZnOH^+$ and $ZnCO_3^{\cdot}$ This may have contributed to the increased toxicity of Zn at these lower exposure concentrations in the high alkalinity treatment. However, these complexes did not contribute to Zn toxicity in the development of an acute Zn BLM for *D. pulex* in soft water (Heijerick et al., 2002) or for a

chronic BLM for *D. magna* (Heijerick et al., 2005). However, $ZnOH^+$ was included as a parameter in the BLM for *D. magna* by Santore et al. (2002). Clifford et al. (2009) discovered only 4% of total Zn was of the form $ZnOH^+$ at pH 8.0, but did not discount that it could contribute to toxicity. In the further development of Zn multi-species, multi-metal BLMs $ZnOH^+$ is to be included as a toxicologically active species (Santore & Ryan, 2015).

5.4.1.3 Hardness

The 28-day lethal concentrations of Zn in soft water to *H. azteca* were significantly lower than the lethal concentrations determined by the mortality model in Borgmann et al., (2004). The ions that contribute to water hardness (Ca^{2+} and Mg^{2+}) can both interact with Zn to affect its bioavailability to organisms. The concentration of Ca^{2+} wass the greater factor in decreasing the toxicity of Zn^{2+} in both rainbow trout (*Oncorhynchus mykiss*) (De Schamphelaere & Janssen, 2004), *D. manga* (Heijerick et al., 2005), and *D. pulex* (Clifford & McGeer, 2009). It has been suggested that Zn^{2+} and Ca^{2+} compete for the same uptake channel in the cell membrane (Santore et al., 2002), as Zn^{2+}can also inhibit the uptake of Ca^{2+} (Muyseen et al., 2006). Hardness, and more specifically the Ca^{2+} ion, has been shown to affect the uptake and toxicity of other metals including Cd^{2+} (Bourgeault et al., 2010) and Ni^{2+} (Leonard & Wood, 2013).

5.4.2 Bioaccumulation and lethal body concentrations

Total bioaccumulation was not affected by water hardness; however, the max/K value in soft water was two-fold greater than in hard water, which indicates greater uptake of Zn in soft water at lower Zn exposure concentrations. This difference in the uptake pattern did not significantly alter the lethal body concentrations. These 28-day lethal concentrations were two to three times greater than the background concentrations, discussed in section 5.3.2. The threshold body concentration for Zn toxicity was 2080 nmol g^{-1} in ten-week exposures, which was double the background Zn concentration (Borgmann et al., 1993). The predicted LC10 values for DOC 2 and pH 6.8 treatments in the current study were less than the physiologically required background concentration of Zn.

Increased DOC concentrations had a protective effect against Zn toxicity on a body concentration basis compared to all other treatments. Nguyen et al. (2012) determined that *H. azteca* had increased body burdens of Zn when the organisms were fed Zn-spiked food in sediment tests. It was noted in the current study that *H. azteca* ingested organic matter in the

DOC treatments that may have adsorbed Zn. This exposure route was not considered to be an important route of toxic exposure (Nguyen et al., 2012).

Rainbow and Luoma (2011) have argued against the use of critical accumulated body concentration to predict toxicity in aquatic invertebrates, as total internal metal concentrations were often stored in detoxified form. However, in some rare cases where no detoxification method is present, a critical body concentration could be possible. For *H. azteca*, internal Zn concentration were only partially regulated (Borgmann et al., 1993). In the current study, some organisms that were exposed to the highest concentration of Zn had body concentrations more than five times the control concentration, despite a 24-hour depuration period in EDTA. This excess Zn could be due to an excretion process failure due to the toxic concentrations, as seen by Rainbow & White (1989) in the shrimp *Palaemon elegans* that can only regulate Zn up to exposure concentrations of 316 µg L^{-1}. However, this would require extended time measures of Zn body concentrations to determine if there was a Zn loss over time (i.e. a slow depuration rate).

5.4.3 Water quality guidelines and toxicity predictions

The existing Zn Canadian Water quality guideline (CCREM, 1987) of 30 µg L^{-1} (459 nmol L^{-1}) exceeded or was not significantly different from the 28-day LC25s and 28-day LC10s for many of the soft water treatments, in contrast to the protective hard water and high DOC scenarios.

The draft Canadian guideline includes water hardness and DOC in its derivation. For the non-DOC treatments from this study, the Zn short-term benchmark would be about 400 nmol L^{-1}. The long-term Zn benchmark is calculated using water hardness and pH. Although the studies used in creating the model for the draft Canadian guideline included a significant DOC term, the toxicity predictions were more accurate using the two-factor model. In the current study, there were no significant differences in the lethal concentrations at different pH values, but at pH-8.3 the 28-day LC50 was 1.5 times lower than at pH 6.7. The long-term Zn benchmark is 3.5 times lower at pH 8.0 compared to pH 6.5, with benchmarks of 72.0 compared to 260 nmol Zn L^{-1}, which would be protect at least 90% of *H. azteca* in all water chemistry tested (CCME, 2016).

Body concentrations were not typically used for metal toxicity predictions, as not all organisms accumulate metals so that their whole-body concentrations would not be proportional to the concentration of metal at the site of action and therefore would not be related to toxicity (Rainbow & Luoma, 2011). However, except for a higher DOC environment, there is a consistent

lethal body concentration for *H. azteca* in all other exposure conditions. The use of metal body burden in *H. azteca* to predict toxicity will be discussed in detail in Chapter 6.

5.5 SUMMARY

1. Soft water can lead to greater uptake of Zn in *H. azteca* at low Zn exposure concentrations compared to hard water.
2. Increased water hardness and dissolved organic carbon have protective effects against waterborne Zn toxicity.
3. High concentrations of DOC have a protective effect against the body burden of Zn that causes a toxic effect.

CHAPTER 6

General Discussion and Project Summary

The overall objectives of this study were to determine if and how water chemistry affected the individual toxicity of three elements to *H. azteca* in different water chemistry. It was of interest to observe how water chemistry influenced the whole-body concentration of a single element associated with mortality, as it was previously assumed to be specific and constant in *H. azteca*. If whole-body concentration associated with mortality in *H. azteca* remained constant regardless of water chemistry, it would potentially be a useful site assessment tool. The following chapter details the findings and conclusions for the main objectives.

6.1. Objectives and findings

1. Determine if pH or DOC influences the acute toxicity of an element tested singly (Co, Se, or Zn) to *H. azteca* [Chapter 2].

Table 6.1. LC50 trends for Co, Se, or Zn when altering one water chemistry in a 7-day toxicity test.

Element	Increase pH	Increase DOC
Co	No significant effect, trend of increased toxicity	No significant effect
Se	Decrease toxicity	No significant effect
Zn	U-shaped response, with decreased toxicity at moderate pH	No significant effect

The first set of experiments presented in Chapter 2 were 7-day toxicity tests that served as range finding experiments for the further chronic toxicity testing. The LC50s were determined using the Trimmed Spearman-Karber method, which is a commonly used method for preliminary LC50 calculations. The mortality model was also used to compute the LC50s at different water chemistries. Both LC50 determination methods output similar results and trends. Using these morality models, pH significantly influenced the toxicity of Se and Zn. DOC did not have any effects on the acute toxicity of Co, Se, or Zn (Table 6.1). However, the LC50 for both Co and Zn exceed the concentration range of the experiments.

2. Determine if pH, alkalinity or DOC influences the chronic toxicity of an element tested singly (Co, Se, or Zn) to *H. azteca.* The effect of water hardness will also be determined incorporating data from previous studies [Chapter 3, 4, 5].

a) Determine how mortality rate and bioaccumulation of an element tested singly in *H. azteca* is related to exposure concentrations and if water chemistry affects this relationship.

Table 6.2. Effects of water chemistry on the chronic toxicity (LC50, LC25, and LC10) of Co, Se, or Zn to *H. azteca.*

Element	Increase pH	Increase Alkalinity	Increase DOC	Increase Hardness
Co	No significant effect	No significant effect	Decrease toxicity	Decrease toxicity
Se	Decrease toxicity	Decrease toxicity	Increase toxicity	Decrease toxicity
Zn	Increase toxicity	No significant effect. 1.8-fold decrease in LC25, 4-fold decrease in LC10	Decrease toxicity	Decrease toxicity

Both DOC and hardness had significant effects on the waterborne toxicity of all elements considered in this study (Table 6.2). The toxicity of the two metals, Co and Zn, were both reduced as these water chemistry parameters were increased. The toxicity of Se was also reduced by increased hardness, as well as by increasing pH and alkalinity. However, increased concentrations of DOC led to the greater toxicity of Se and was thought to be due to the potential contribution of dietary Se. Chapters 3, 4, and 5 concluded that water chemistry can affect the toxicity of these elements in chronic exposures to *H. azteca* and that water chemistry needs to be considered for these elements when developing and updating water quality guidelines.

Table 6.3. Select lethal concentrations of Co, Se, and Zn from soft water experiments compared to Canadian water quality guidelines.

Metal	Lowest LC50	Lowest LC25	Lowest LC10	Water quality guideline
		$\mu g\ L^{-1}$		
Co	1.44 (0.705 - 2.17)	0.635 (0.293 - 0.974)	0.374 (0.150 - 0.600)	0.78[a]
Se	18.9 (14.4 - 24.3)	14.3 (10.7 - 18.0)	11.0 (7.89 - 14.0)	1[b]
Zn	43.7 (27.5 - 59.9)	22.5 (6.73 - 38.3)	6.73 (0.718 - 12.8)	6.2 - 22[c]

[a] Federal Water Quality Guideline at hardness 52 mg L^{-1} (Environment Canada, 2013)

[b] Water Quality Guideline for the Protection of Aquatic Life (CCME, 1987)

[c] Water Quality Guideline for the Protection of Aquatic Life hardness 40 mg L^{-1} (CCME, 2016)

The water quality guideline for Co was developed using a species sensitivity distribution and the most sensitive species in that distribution was *H. azteca* in a hard water experiment. The guideline can be modified for water hardness between 52 and 396 mg L^{-1}; however, there is no modifying factor for DOC as the results from the current study would suggest is necessary (Table 6.2). Despite this guideline not being protective for *H. azteca* in some water chemistry conditions (Table 6.3), it was protective of 95% of species 95% of the time (Environment Canada, 2017).

The water quality guideline for Se was set very low to prevent known bioaccumulation in organisms and biomagnification in the food web (CCME, 1987). Thus, this guideline would be protective against all waterborne Se toxicity in the current study, as guideline is lower than the lowest Se concentration tested in the current study (Table 6.3). However, the current study also indicated that Se toxicity is more complicated than a waterborne route of uptake, so the inclusion of water chemistry variables would not lead to an improvement of the guideline.

The CCME guideline for Zn has been 30 µg L^{-1} since 1987, which would not be protective of *H. azteca* in soft water conditions. Updated draft Zn criteria include hardness and pH modification in a multiple linear regression to calculate site specific water quality guidelines. The study found DOC to significant affect Zn toxicity as well; however, its inclusion did not improve the accuracy the regression model (CCME, 2016). With these additional factors, this guideline would be protective against Zn toxicity in *H. azteca*, as no significant mortality occurred at or below the concentrations of 6.2 – 22 µg L^{-1} (Table 6.3).

Table 6.4. Effect of water chemistry on the bioaccumulation of Co, Se, or Zn in *H. azteca* in chronic single metal exposures.

Element	Increase pH	Increase Alkalinity	Increase DOC	Increase Hardness
Co	No significant effect	No significant effect	Reduces uptake at low concentrations, does not impact maximum biaccumulation	Reduces uptake at low concentrations, does not impact maximum biaccumulation
Se	Decrease uptake	Decrease uptake	No effect	Reduces uptake at low concentrations, unable to determine impact on maximum concentration
Zn	No significant effect	No significant effect	No significant effect	Reduces uptake at low concentrations, does not impact maximum biaccumulation

Water chemistry did not change the predicted bioaccumulated concentrations of Co and Zn (Table 6.4). The bioaccumulation model was unable to predict the maximum Se concentration, as the body concentration of Se in *H. azteca* did not approach a maximum concentration. An increased in the exposure hardness, caused the bioaccumulation pattern for all metals to change, as there was less uptake at low exposure concentration due to a greater ratio of competing ions. Bioaccumulated Se concentrations were not pooled, as the body concentrations accumulated at the exposure Se concentrations were statistically different among the water chemistry conditions. Both Co and Zn bioaccumulated concentrations were not statistically different among the soft water treatments of this study, except for Co in high DOC scenarios, which had a pattern more similar to Co hard water accumulation.

The bioaccumulation model was useful when there were no or few surviving organisms at high exposure concentrations. An expected body concentration was calculated using the bioaccumulation model, and this value was used for body concentration to mortality rate modelling and calculation of lethal body concentrations. However, maximum bioaccumulation should be verified in short-term exposures, in which greater survival is expected, resulting in available tissue for whole-body metal analysis.

b) Determine how mortality rate and bioaccumulation of an element tested singly in *H. azteca* is related to whole-body concentrations and if water chemistry affects this relationship.

Table 6.5. Effects of water chemistry on the chronic body concentration-based toxicity (LBC50, LBC25, and LBC10) of Co, Se, or Zn to *H. azteca*.

Element	Increase pH	Increase Alkalinity	Increase DOC	Increase Hardness
Co	No significant effect	No significant effect	No significant effect	No significant effect
Se	Increase toxicity	Increase toxicity	Increase toxicity	No significant effect
Zn	No significant effect	No significant effect	No significant effect, 1.5 fold increase in LBC50	No significant effect, 1.5 fold decrease in LC50

The body concentration of Co or Zn where *H. azteca* have reduced survival was not significantly affected by changes in exposure water chemistry (Table 6.5). However, Zn toxicity changed by 1.5-fold when the hardness or DOC conditions of the exposure environment were altered. Zinc is partially regulated in *H. azteca*, so while not statistically significant, this change could be important.

Water chemistry significantly affects Se body concentrations causing toxicity. An increase in exposure pH, alkalinity, and DOC contributed to greater mortality with less total Se in an organism. It was suspected the different exposure environments altered the Se species and exposure routes, which are known to alter Se toxicity.

3. Compare mortality and bioaccumulation relationships in different water chemistry to models developed by Borgmann et al. (2004), Norwood et al. (2006, 2007), and Norwood et al. (unpublished) to determine if water chemistry is a variable necessary for the models to be good predictors of toxicity.

Maximum concentrations of Co and Zn accumulated by *H. azteca* were not significantly affected by different water chemistry. However, at low exposure concentrations the "hard water" bioaccumulation model did not accurately describe the soft water bioaccumulation data. Selenium did not have a bioaccumulation model that fit all soft water data, so water chemistry must be considered in order to predict Se accumulation.

Mortality models can be used to predict lethal endpoints in different water chemistry scenarios in soft water. Mortality models have been developed previously for Co, Se, and Zn in hard water. Although hard water had significant effects on the waterborne toxicity of these elements throughout this study, the lethal body concentrations were not significantly altered by changes in hardness.

Using the whole-body based mortality models, a metal effects addition model (MEAM) was created by Norwood et al. (2013) to predict the toxicity of metal mixtures. This model uses the critical body concentrations causing toxicity in *H. azteca* to predict toxicity in up to a 10-element mixture. Mortality models for Se and V have since been developed. However, these models were developed in hard water and it was unknown if water chemistry would affect the critical body concentration of *H. azteca* and if it would change the toxicity prediction of the MEAM.

The remainder of this section will examine whether the current "hard water" mortality models used in the MEAM were robust enough to accurately predict the same lethal water and lethal body concentrations as the models that were developed in different water chemistries for Co, Se, and Zn in the previous chapters of this study. The model will be considered robust if there were no significant differences among the predicted LBC50s and LBC25s. Mortality model parameters and lethal concentrations predicted using the "hard water" models are in Appendix D.

Table 6.6. Robustness of “hard water” Co model when predicting lethal body concentrations from experiments conducted in soft water.

Treatment	Change in lethal body concentrations when determined using a "hard water" model	r^2
DOC 10	No significant differences, but overpredicts LBC50 two-fold.	0.429
DOC 5	No significant differences, but overpredicts LBC50 by 1.59.	0.496
DOC 2	No significant differences.	0.684
DOC 0.5	No significant differences, but underpredicts LBC10 over two-fold.	0.475
pH 6.7	No significant differences, but overpredicts LBC50 by 1.5.	0.479
pH 7.7	No significant differences, but underpredicts LBC10 over two-fold.	0.475
pH 8.3	LC10 significantly lower.	0.550
Alk 100	LC10 significantly lower.	0.822
Alk 50	LC10 significantly lower.	0.693
Alk 16	No significant differences, but underpredicts LBC50 over two-fold.	0.475

The “hard water” model for lethal body concentrations had few significant differences in endpoint predictions (Table 6.6); however, this “hard water” model overpredicted soft water LC50s and under-predicted soft water LC10s.

Table 6.7. Robustness of “hard water” Se model when predicting lethal body concentrations from experiments conducted in soft water.

Treatment	Change in lethal body concentrations when determined using a "hard water" model	r^2
DOC 5	No significant differences.	0.760
DOC 2	No significant differences.	0.498
DOC 0.5	No significant differences.	0.684
pH 8.3/Alk 100	No significant differences, but underpredicts LBC10 two-fold.	0.737
pH 7.7/Alk 15	No significant differences.	0.684
pH 6.8/Alk 5	No significant differences, but underpredicts LBC10 over two-fold.	0.584

The lethal body concentrations predicted by the Se “hard water’ model were not significantly different from the models used in the current study to predict the endpoints (Table 6.7). Some LC10s were under-predicted and can be attributed to the lack of data at low concentrations to fully develop the “hard water” model. Even though the “hard water” model can

predict the lethal body concentrations adequately, Se body concentrations were not a consistent predictor of toxicity under different water chemistry conditions, as stated previously (Table 6.4).

Table 6.8. Robustness of "hard water" Zn model when predicting lethal body concentrations from experiments conducted in soft water.

Modifier	Change in lethal body concentrations when determined using a "hard water" model	r^2
DOC 5	No significant differences.	0.305
DOC 2	No significant differences, but LBC25 is two-fold greater and LBC10 is over four-fold greater.	0.268
DOC 0.5	LC25 and LC10 significantly greater.	0.960
pH 8.3	All endpoints significantly greater.	0.777
pH 7.7	LC25 and LC10 significantly greater.	0.960
pH 6.8	No significant differences, but LC25 is 1.5 times greater and LC10 is almost three-fold greater.	0.681
Alk 100	No significant differences.	0.496
Alk 16	LC25 and LC10 significantly greater.	0.960
Alk 5	No significant differences.	0.574

The lethal body concentrations predicted by the "hard water" model were not significantly different from the soft water models' predictions in all cases except for the pH 8.3 treatment, where the endpoints were over-predicted (Table 6.8). The over-predictions by the model at this pH were all by less than 1.6 times the prediction by the other model. The "hard water" model does not have a good fit to the DOC data; however, the fit was comparable to the soft water models' fit, as the DOC data were variable.

6.2 Implications for metal mixture toxicity predictions

The MEAM uses mortality models based on gut cleared, background correct body concentrations for 10 different metals to calculate the predicted toxicity at a given site (Norwood et al., 2013). It set parameters for LBC50, K_{TB}, and n_b. The present study has shown that water chemistry does not affect the lethal body concentrations of Co or Zn. This chapter also showed that the "hard water" whole-body models can be used to predict the soft water LBC50s. The "hard water" model did not perform as well when predicting LC10s, but these values were not required for the MEAM.

The lethal body concentrations of Se were variable in different water chemistry scenarios and this is an issue since the MEAM uses a constant LBC50 value. Although the "hard water"

mortality model can by used to adequately predict the lethal body concentrations of Se in soft water, the lethal body concentration would need to be modified for use in the MEAM in other water chemistry scenarios, like environments with different DOC concentrations.

6.3 Additional research considerations

1. Additional DOC experiments with expanded concentration ranges of both added DOC and of each element.

DOC can have protective effects against metal toxicity but can enhance Se toxicity. Future work should include an expanded range of DOC to assess what concentrations have a significant effect compared to a control without added DOC. The concentration of DOC in wetlands can range upwards of 30 mg L^{-1} (Mann & Wetzel, 1995). In addition, the exposure concentrations of Co and Zn should be increased, as some lethal concentrations could only be determined by extrapolation as low mortality was observed. The expanded concentration ranges should follow the ranges tested in previous hardwater studies, for better comparison between soft and hard water.

2. Short term exposure to confirm bioaccumulation.

Since there was low or no survival in some treatments, one-week bioaccumulation experiment should be conducted to determine actual body concentrations at high exposure concentrations. This work will confirm the predicted bioaccumulation models developed in this study.

3. Se speciation and targeted uptake route experiments.

An area of further research is what form of Se is taken up by *H. azteca* and speciation inside the organism. Other research questions include how the forms of Se can influence both the bioaccumulation and toxicity to *H. azteca*.

4. Water chemistry effects of other metal toxicity to *H. azteca*.

It has been shown that body concentrations of Co and Zn were related to the mortality rate of *H. azteca* and it is of interest to determine if this holds true for other elements that were

included in the MEAM. Critical body concentrations of *H. azteca* could have potential to be used for site-specific evaluations if water chemistry conditions do not affect other lethal body concentrations.

5. The effects of water chemistry on two-metal or metal mixture toxicity.

Since metals rarely occur in isolated environments, combining water chemistry effects with metal mixtures is important to see if and how different scenarios change the above conclusions. Single element effects would first need to be established for additional elements, followed by binary-element or multi-element exposures in a range of water chemistry.

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APPENDIX A

SUPPLEMENTARY CO DATA ANALYSES

Growth model outline

Growth inhibitory concentrations will be determined using the growth model (Norwood et al., 2007) as follows:

$$W = W'(1 + aCn)^{-1} \quad \text{(Eq. A.1)}$$

where W is the wet weight, W' is the control wet weight, C is the concentration of metal in water, and a and n are constants. Using the constants from the above equation, critical growth inhibition concentrations IC10, 25 and 50 can be calculated as follows:

$$IC50 = (a)^{-(1/n)} \quad \text{(Eq. A.2)}$$

$$IC25 = (3a)^{-(1/n)} \quad \text{(Eq. A.3)}$$

$$IC10 = (9a)^{-(1/n)} \quad \text{(Eq. A.4)}$$

Where IC50, IC25, and IC10 are the concentrations where growth is inhibited 50%, 25%, or 10%, and a and n are constants.

1. COBALT EXPOSURE CONCENTRATION-BASED GROWTH MODELS

Table A.1.1. Growth model parameters based on Co water concentrations in treatments with different dissolved organic carbon concentrations. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
DOC 10	0.015	0.868	0.797	125	(-13.9 - 265)	35.3	(12.6 - 58.1)	9.96	(-4.77 - 24.7)	0.260
DOC 5	0.036	0.757	0.648	81.9	(28.8 - 135)	19.2	(6.51 - 31.9)	**4.5**	(-1.88 - 10.89)	0.395
DOC 2	0.083	0.669	0.623	41.4	(18.4 - 64.4)	8.01	(-0.346 - 16.4)	1.55	(-1.58 - 4.68)	0.362
DOC 0.5	0.002	1.48	0.400	65.4	(18.3 - 113)	31.1	(6.17 - 56.1)	14.8	(-6.35 - 36.0)	0.258

Table A.1.2. Growth model parameters based on Co water concentrations in treatments with different pH. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
pH 6.5	0.000	2.28	0.283	82.6	(28.7 - 136)	51.0	(13.8 - 88.1)	31.5	(-10.2 - 73.2)	0.211
pH 7.5	0.002	1.48	0.400	65.4	(18.3 - 113)	31.1	(6.17 - 56.1)	14.8	(-6.35 - 36.0)	0.258
pH 8.5	0.029	0.938	0.524	43.8	(16.0 - 71.5)	13.6	(0.679 - 26.4)	4.20	(-3.14 - 11.6)	0.318

[a] higher control mortality than acceptable for a 28-d exposure

Table A.1.3. Growth model parameters based on Co water concentrations in treatments with different alkalinity. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Alk 100	0.025	1.14	0.736	24.9	(14.7 - 35.1)	9.53	(3.35 - 15.7)	**3.65**	(-0.453 - 7.75)	0.463
Alk 50	0.076	0.685	0.629	42.7	(4.52 - 80.8)	8.59	(-3.26 - 20.4)	1.73	(-3.06 - 6.52)	0.368
Alk 16	0.002	1.48	0.400	65.4	(18.3 - 113)	31.1	(6.17 - 56.1)	14.8	(-6.35 - 36.0)	0.258

Table A.1.4. Growth model parameters based on Co water concentrations in treatments with different water hardness. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Hardness 37.5	0.025	1.14	0.736	24.9	(14.7 - 35.1)	9.53	(3.35 - 15.7)	**3.65**	(-0.453 - 7.75)	0.463
Hardness 122[b]	0.013	0.829	0.944			48.7	(10.7 - 221)			0.490

[b] Norwood et al., 2007

2. COBALT WHOLE-BODY CONCENTRATION-BASED GROWTH MODELS

Table A.2.1. Growth model parameters based on Co body concentrations in organisms exposed to treatments with different dissolved organic carbon concentrations. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}	
DOC 10	0.006	0.979	0.797	205	(-0.740 - 411)	65.5	(27.3 - 104)	**20.9**	(-7.30 - 49.1)	0.262
DOC 5	0.016	0.839	0.648	139	(57.9 - 220)	**37.6**	(15.0 - 60.1)	**10.1**	(-2.95 - 23.2)	0.397
DOC 2	0.013	0.849	0.623	164	(94.1 - 235)	45.1	(8.38 - 81.7)	12.4	(-6.95 - 31.7)	0.384
DOC 0.5	0.001	1.22	0.400	283	(-7.57 - 574)	115	(21.6 - 209)	47.0	(-32.7 - 127)	0.269

Table A.2.2. Growth model parameters based on Co body concentrations in organisms exposed to treatments with different pH. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}	
pH 6.5[a]	0.001	0.993	0.260	1050	(-6940 - 9050)	348	(-850 - 1546)	115	(-198 - 428)	0.199
pH 7.5	0.001	1.22	0.400	283	(-7.57 - 574)	115	(21.6 - 209)	47.0	(-32.7 - 127)	0.269
pH 8.5	0.02	1.20	0.524	172	(88.6 - 255)	68.6	(17.0 - 120)	27.4	(-10.4 - 65.3)	0.335

[a] higher control mortality than acceptable for a 28-d exposure

Table A.2.3. Growth model parameters based on Co body concentrations in organisms exposed to treatments with different alkalinity. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}	
Alk 100	0.001	1.39	0.736	111	(74.3 - 148)	50.5	(23.4 - 77.6)	22.9	(1.74 - 148)	0.478
Alk 50	0.013	0.838	0.629	171	(47.4 - 294)	46.1	(-6.16 - 98.3)	**12.4**	(-15.8 - 40.7)	0.375
Alk 16	0.001	1.22	0.400	283	(-7.57 - 574)	115	(21.6 - 209)	47.0	(-32.7 - 127)	0.269

Table A.2.4. Growth model parameters based on Co body concentrations in organisms exposed to treatments with different water hardness. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg		nmol L^{-1}		nmol L^{-1}		nmol L^{-1}	
Hardness 37.5	0.001	1.39	0.736	111	(74.3 - 148)	50.5	(23.4 - 77.6)	22.9	(1.74 - 148)	0.478
Hardness 122[b]	0.000 09	1.71	0.890			117	(52.1 - 258)			0.498

[b] Norwood et al., 2007

APPENDIX B

SUPPLEMENTARY SE DATA ANALYSES

1. SELENIUM EXPOSURE CONCENTRATION-BASED GROWTH MODELS

Table B.1.1. Growth model parameters based on Se water concentrations in treatments with different water chemistry. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
DOC 10	0.001	1.01	0.967	913	(-2510 - 4340)	309	(-141 - 759)	104	(-193 - 402)	0.126
DOC 2	0.001	1.08	0.955	607	(169 - 1040)	219	(58.7 - 379)	79.0	(-33.4 - 191)	0.438
pH 7.5/DOC 0.5/Alk 15	0.158	0.12	0.734	>5000		483	(-2170 - 3140)	**0.053**	(-1.22 - 1.33)	0.014
Alk 100/pH 8.3	0.120	0.234	1.12	>5000		79.7	(-150 - 309)	**0.724**	(-7.45 - 8.90)	0.044
Alk 5/pH 7.0	0.001	0.991	0.674	1070	(-343 - 2480)	352	(95.3 - 609)	116	(-73.7 - 306)	0.397

Table B.2.1. Growth model parameters based on Se body concentrations in organisms exposed to treatments with different water chemistry. Bolded values are significantly lower than corresponding lethality endpoint.

2. SELENIUM BODY CONCENTRATION-BASED GROWTH MODELS

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
DOC 10	0.001	1.10	0.967	535	(-1890 - 2960)	197	(-144 - 539)	72.5	(-104 - 249)	0.102
DOC 2	0.001	1.19	0.955	337	(-37.2 - 712)	134	(48.8 - 219)	53.0	(35.8 - 142)	0.365
pH 7.5/DOC 0.5/Alk 15	0.011	0.809	0.734	272	(-104 - 648)	70.0	(27.6 - 112)	18.0	(-8.62 - 44.6)	0.136
Alk 100/pH 8.3	0.024	0.706	1.12	202	(-191 - 595)	42.7	(3.34 - 82.0)	9.00	(-15.7 - 33.7)	0.081
Alk 5/pH 7.0	0.001	1.29	0.674	212	(11.6 - 412)	90.3	(40.9 - 140)	38.5	(-4.25 - 81.3)	0.427

APPENDIX C

SUPPLEMENTARY ZN DATA ANALYSES

1. ZINC EXPOSURE CONCENTRATION-BASED GROWTH MODELS

Table C.1.1. Growth model parameters based on Zn water concentrations in treatments with different dissolved organic carbon concentrations. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
DOC 10	0.001	0.791	0.707	6220	(-3980 - 16400)	1550	(494 - 2610)	**387**	(-104 - 877)	0.458
DOC 2	0.001	0.708	0.589	>10000		3660	(-5700 - 13000)	776	(-841 - 2390)	0.242
DOC 0.5				nd						

Table C.1.2. Growth model parameters based on Zn water concentrations in treatments with different pH. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
pH 6.5	0.006	0.705	0.668	1320	(127 - 2520)	278	(20.9 - 536)	**58.6**	(-61.1 - 178)	0.371
pH 7.5				nd						
pH 8.5	0.001	1.21	0.779	**304**	(179 - 430)	**123**	(16.8 - 228)	49.4	(-25.8 - 125)	0.923

Table C.1.3. Growth model parameters based on Zn water concentrations in treatments with different alkalinity. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Alk 100	0.001	0.832	0.846	2840	(-1300 - 6980)	759	(220 - 1300)	203	(-131 - 537)	0.243
Alk 16				nd						
Alk 5	0.016	0.444	0.634	>10000		990	(-2300 - 4270)	83.3	(-481 - 648)	0.037

Table C.1.4. Growth model parameters based on Zn water concentrations in treatments with different water hardness. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Hardness 37.5	0.001	0.832	0.846	2840	(-1300 - 6980)	759	(220 - 1300)	203	(-131 - 537)	0.243
Hardness 122[a]				nd						

[a] Borgmann et al., 2004

2. ZINC BODY CONCENTRATION-BASED GROWTH MODELS

Table C.2.1. Growth model parameters based on Zn body concentrations in organisms exposed to treatments with different dissolved organic carbon concentrations. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
DOC 10	0.001	0.676	0.707	>10000		5390	(-7700 - 18500)	1060	(-753 - 2880)	0.451
DOC 2	0.001	0.587	0.589	>10000		>10000		3080	-7730 - 13900	0.181
DOC 0.5				nd						

Table C.2.2. Growth model parameters based on Zn body concentrations in organisms exposed to treatments with different pH. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
pH 6.5	0.001	0.833	0.668	3990	(-1250 - 9240)	1070	(193 - 1940)	286	(-414 - 986)	0.387
pH 7.5				nd						
pH 8.5	0.001	0.92	0.779	1780	(-931 - 4500)	543	(-1010 - 2090)	165	(-926 - 1260)	0.922

Table C.2.3. Growth model parameters based on Zn body concentrations in organisms exposed to treatments with different alkalinity. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Alk 100	0.001	0.749	0.846	>10000		2330	(127 - 4540)	538	(-723 - 1800)	0.236
Alk 16				nd						
Alk 5	0.001	0.739	0.634	>10000		2600	(-3460 - 8650)	587.0	-2090 - 3260	0.040

Table C.2.4. Growth model parameters based on Zn body concentrations in organisms exposed to treatments with different water hardness. Bolded values are significantly lower than corresponding lethality endpoint.

Treatment	a	N	CON W	IC50	CI	IC25	CI	IC10	CI	r^2
			mg	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Hardness 37.5	0.001	0.749	0.846	>10000		2330	(127 - 4540)	538	(-723 - 1800)	0.236
Hardness 122[a]				nd						

[a] Borgmann et al., 2004

APPENDIX D

SUPPLEMENTARY COMPARISONS WITH HARD WATER MODELS

1. CO EXPOSURE CONCENTRATION-BASED LETHAL CONCENTRATIONS PREDICTED USING A "HARD WATER MODEL"

Table D.1.1. Mortality model LC output using Co water model parameters from Norwood et al. (2007) and data from soft water modified DOC experiments.

Treatment	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
DOC 10	0.010	201	(95.9 - 307)	74.5	(36.7 - 112)	24.5	(12.2 - 36.7)	0.417
DOC 5	0.010	134	(60.8 - 207)	50.1	(23.3 - 76.9)	16.5	(7.76 - 25.3)	0.483
DOC 2	0.019	79.4	(54.1 - 105)	29.9	(20.5 - 39.4)	9.92	(6.81 - 13.0)	0.684
DOC 0.5	0.033	39.3	(22.7 - 56.0)	14.9	(8.34 - 21.2)	4.95	(2.87 - 7.04)	0.468

Table D.1.2. Mortality model LC output using Co water model parameters from Norwood et al. (2007) and data from soft water modified pH experiments.

Treatment	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
pH 6.7[a]	0.059	22.2	(13.8 - 30.7)	8.45	(5.24 - 11.7)	2.81	(1.74 - 3.87)	0.479
pH 7.7	0.033	39.3	(22.7 - 56.0)	14.9	(8.34 - 21.2)	4.95	(2.87 - 7.04)	0.468
pH 8.3	0.042	40.0	(21.5 - 58.6)	15.2	(8.20 - 22.2)	5.04	(2.73 - 7.36)	0.550

[a] higher than acceptable control mortality for 28-d assay

Table D.1.3. Mortality model LC output using Co water model parameters from Norwood et al. (2007) and data from soft water modified alkalinity experiments.

Treatment	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
Alk 100	0.029	31.3	(18.6 - 44.1)	11.9	(7.08 - 16.7)	3.95	(2.36 - 5.55)	0.826
Alk 50	0.027	57.4	(38.3 - 76.4)	21.7	(14.6 - 28.8)	7.19	(4.83 - 9.55)	0.693
Alk 16	0.033	39.3	(22.7 - 56.0)	14.9	(8.34 - 21.2)	4.95	(2.87 - 7.04)	0.468

Table D.1.4. Mortality model LC output using Co water model parameters from Norwood et al. (2007) and data from modified hardness experiments.

Treatment	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
Hardness 37.5	0.029	31.3	(18.6 - 44.1)	11.9	(7.08 - 16.7)	3.95	(2.36 - 5.55)	0.826
Hardness 122[b]	0.05	183	(120 - 279)	68.0	(33 - 140)	22.3	(4.76 - 39.9)	0.860

[b] From Norwood et al. (2007)

2. CO BODY CONCENTRATION-BASED LETHAL CONCENTRATIONS PREDICTED USING A "HARD WATER MODEL"

Table D.2.1. Mortality model LBC output using Co body model parameters from Norwood et al. (2007) and data from soft water modified DOC experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
DOC 10	0.019	318	(192 - 444)	165	(76.2 - 253)	64.3	(23.8 - 105)	0.429
DOC 5	0.020	233	(125 - 342)	110	(46.6 - 174)	40.7	(14.6 - 66.8)	0.496
DOC 2	0.019	273	(219 - 327)	135	(100 - 169)	51.0	(36.1 - 65.8)	0.684
DOC 0.5	0.026	159	(110 - 209)	70	(45.0 - 95.0)	24.9	(15.4 - 34.3)	0.475

Table D.2.2. Mortality model LBC output using model Co body parameters from Norwood et al. (2007) and data from soft water modified pH experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
pH 6.7[a]	0.059	105	(70.9 - 139)	43.9	(28.3 - 59.6)	15.2	(9.57 - 20.9)	0.479
pH 7.7	0.026	159	(110 - 209)	70	(45.0 - 95.0)	24.9	(15.4 - 34.3)	0.475
pH 8.3	0.042	170	(109 - 230)	75.3	(44.2 - 106)	26.9	(15.0 - 38.7)	0.550

[a] higher control mortality than acceptable for a 28-d exposure

Table D.2.3. Mortality model LBC output using model Co body parameters from Norwood et al. (2007) and data from soft water modified alkalinity experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
Alk 100	0.042	149	(93.5 - 205)	65.1	(37.2 - 92.9)	23.0	(12.6 - 33.4)	0.822
Alk 50	0.027	220	(170 - 271)	103	(73.9 - 132)	37.7	(26.0 - 49.5)	0.693
Alk 16	0.026	159	(110 - 209)	70	(45.0 - 95.0)	24.9	(15.4 - 34.3)	0.475

Table D.2.4. Mortality model LBC output using model Co body parameters from Norwood et al. (2007) and data from modified hardness experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	weeks^{-1}	nmol g^{-1}		nmol g^{-1}		nmol g^{-1}		
Hardness 37.5	0.042	149	(93.5 - 205)	65.1	(37.2 - 92.9)	23.0	(12.6 - 33.4)	0.822
Hardness 122[b]	0.050	192	(138 – 264)	90.0	(42 – 177)	n/a		0.858

[b] From Norwood et al., 2007

3. SE EXPOSURE CONCENTRATION-BASED LETHAL CONCENTRATIONS PREDICTED USING A "HARD WATER MODEL"

Table D.3.1. Mortality model LC output using Se water model parameters from Norwood et al. (unpublished) and data from soft water modified DOC experiments.

Modifier	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	weeks^{-1}	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
DOC 10	0.022	430	(340 - 519)	305	(253 - 367)	207	(166 - 249)	0.659
DOC 2	0.020	361	(279 - 444)	257	(199 - 315)	175	(167 - 214)	0.589
DOC 0.5	0.011	544	(435 - 654)	384	(309 - 460)	260	(210 - 310)	0.693

Table D.3.2. Mortality model LC output using Se water model parameters from Norwood et al. (unpublished) and data from soft water modified pH/alkalinity experiments.

Modifier	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	weeks^{-1}							
pH 8.3/Alk 100	0.016	486	(407 - 564)	343	(289 - 398)	233	(197 - 270)	0.801
pH 7.7/Alk 15	0.011	544	(435 - 654)	384	(309 - 460)	260	(210 - 310)	0.693
pH 6.8/Alk 5	0.033	361	(305 - 418)	257	(217 - 297)	175	(149 - 202)	0.727

Table D.3.3. Mortality model LC output using Se water model parameters from Norwood et al. (unpublished) and data from modified hardness experiments.

Modifier	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	weeks^{-1}	nmol L^{-1}		nmol L^{-1}		nmol L^{-1}		
Hardness 37.5	0.016	486	(407 - 564)	343	(289 - 398)	233	(197 - 270)	0.801
Hardness 130[a]	0.046	957	(710 – 1210)	665	(375 - 954)	445	(100 - 788)	0.788

[a] Norwood et al. (unpublished)

4. SE BODY CONCENTRATION-BASED LETHAL CONCENTRATIONS PREDICTED USING A "HARD WATER MODEL"

Table D.4.1. Mortality model LBC output using Se body model parameters from Norwood et al. (unpublished) and data from soft water modified DOC experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
DOC 10	0.023	75.3	(57.7 - 92.9)	43.8	(33.6 - 54.0)	23.7	(18.2 - 29.2)	0.760
DOC 2	0.022	162	(97.6 - 227)	94	(56.9 - 131)	50.6	(30.8 - 70.5)	0.498
DOC 0.5	0.007	107	(75.5 - 139)	62.4	(44.0 - 80.8)	33.7	(23.8 - 43.5)	0.684

Table D.4.2. Mortality model LBC output using Se body model parameters from Norwood et al. (unpublished) and data from soft water modified pH/alkalinity experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
pH 8.3/Alk 100	0.014	99.3	(65.4 - 133)	57.7	(38.1 - 77.3)	31.1	(20.6 - 41.7)	0.737
pH 7.7/Alk 15	0.007	107	(75.5 - 139)	62.4	(44.0 - 80.8)	33.7	(23.8 - 43.5)	0.684
pH 6.8/Alk 5	0.036	183	(117 - 248)	106	(68.3 - 143)	56.9	(36.9 - 76.8)	0.584

Table D.4.3. Mortality model LBC output using Se body model parameters from Norwood et al. (unpublished) and data from modified hardness experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
Hardness 37.5	0.014	99.3	(65.4 - 133)	57.7	(38.1 - 77.3)	31.1	(20.6 - 41.7)	0.737
Hardness 130[a]	0.046	107	(74.0 - 141)	62.3	(41.0 - 83.7)	33.7	(5.07 - 62.3)	0.555

[a] Norwood et al. (unpublished)

5. ZN EXPOSURE CONCENTRATION-BASED LETHAL CONCENTRATIONS PREDICTED USING A "HARD WATER MODEL"

Table D.5.1. Mortality model LC output using Zn water model parameters from Borgmann et al. (2004) and data from soft water modified DOC experiments.

Modifier	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
DOC5	0.019	2730	(2220 - 3240)	2250	(1870 - 2630)	1850	(1570 - 2130)	0.277
DOC2	0.020	2680	(1910 - 3460)	2220	(1640 - 2800)	1820	(1390 - 2250)	0.161
DOC0.5	0.035	1420	(1320 - 1520)	1230	(1150 - 1310)	1060	(989 - 1120)	0.872

[a] Greater than Zn exposure range.

Table D.5.2. Mortality model LC output using Zn water model parameters from Borgmann et al. (2004) and data from soft water modified pH experiments.

Modifier	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
pH-8.3	0.036	1570	(1260 - 1870)	1350	(1110 - 1600)	1150	(960 - 1350)	0.649
pH-7.7	0.035	1420	(1320 - 1520)	1230	(1150 - 1310)	1060	(989 - 1120)	0.872
pH-6.8	0.020	1460	(1290 - 1640)	1270	(1120 - 1410)	1090	(968 - 1200)	0.590

Table D.5.3. Mortality model LC output using Zn water model parameters from Borgmann et al. (2004) and data from soft water modified alkalinity experiments.

Modifier	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
Alk-100	0.015	1590	(1330 - 1860)	1370	(1160 - 1590)	1170	(1000 - 1340)	0.386
Alk-16	0.035	1420	(1320 - 1520)	1230	(1150 - 1310)	1060	(989 - 1120)	0.872
Alk-5	0.033	1260	(1060 - 1470)	1100	(935 - 1270)	952	(814 - 1090)	0.393

Table D.5.4. Mortality model LC output using Zn water model parameters from Borgmann et al. (2004) and data from modified hardness experiments.

Modifier	m′	LC50	CL	LC25	CL	LC10	CL	r^2
	$weeks^{-1}$	$nmol\ L^{-1}$		$nmol\ L^{-1}$		$nmol\ L^{-1}$		
Hardness 37.5	0.015	1590	(1330 - 1860)	1370	(1160 - 1590)	1170	(1000 - 1340)	0.386
Hardness 130[b]	nd	3100	(2600 – 3690)	2520	(2050 – 3100)	nd		nd

[b] Borgmann et al. (2004)

6. ZN BODY CONCENTRATION-BASED LETHAL CONCENTRATIONS PREDICTED USING A "HARD WATER MODEL"

Table D.6.1. Mortality model LBC output using Zn body model parameters from Borgmann et al. (2004) and data from soft water modified DOC experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
DOC5	0.023	3430	(3090 - 3780)	3070	(2770 - 3370)	2710	(2450 - 2960)	0.305
DOC2	0.032	3140	(2770 - 3510)	2810	(2490 - 3130)	2480	(2210 - 2760)	0.268
DOC0.5	0.035	2510	(2430 - 2590)	2260	(2190 - 2330)	2010	(1950 - 2070)	0.960

Table D.6.2. Mortality model LBC output using Zn body model parameters from Borgmann et al. (2004) and data from soft water modified pH experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
pH 8.3	0.035	2330	(2100 - 2550)	2100	(1900 - 2300)	1870	(1690 - 2040)	0.777
pH 7.7	0.035	2510	(2430 - 2590)	2260	(2190 - 2330)	2010	(1950 - 2070)	0.960
pH 6.8	0.015	2390	(2220 - 2570)	2160	(2000 - 2310)	1920	(1790 - 2050)	0.681

Table D.6.3. Mortality model LBC output using Zn body model parameters from Borgmann et al. (2004) and data from soft water modified alkalinity experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
Alk100	0.025	2510	(2300 - 2710)	2250	(2070 - 2440)	2000	(1840 - 2160)	0.496
Alk 16	0.035	2510	(2430 - 2590)	2260	(2190 - 2330)	2010	(1950 - 2070)	0.960
Alk5	0.032	2140	(1960 - 2320)	1930	(1770 - 2090)	1720	(1580 - 1860)	0.574

Table D.6.4. Mortality model LBC output using Zn body model parameters from Borgmann et al. (2004) and data from modified hardness experiments.

Modifier	m′	LBC50	CL	LBC25	CL	LBC10	CL	r^2
	$weeks^{-1}$	$nmol\ g^{-1}$		$nmol\ g^{-1}$		$nmol\ g^{-1}$		
Hardness 37.5	0.025	2510	(2300 - 2710)	2250	(2070 - 2440)	2000	(1840 - 2160)	0.496
Hardness 130[a]	nd	2020	(1880 - 2180)	1840	(1660 - 2030)	nd		nd

[a] Borgmann et al. (2004)

APPENDIX E

RAW MORTALITY DATA

1. RAW MORTALITY DATA FOR COBALT EXPERIMENTS.

Table E.1.1. DOC-10 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
DOC-10	0	0.000	0.143	0.143	0.223	0.059
DOC-10	0	0.000	0.000	0.223	0.223	0.067
DOC-10	0	0.069	0.069	0.069	0.223	0.045
DOC-10	0	0.000	0.000	0.069	0.069	0.021
DOC-10	0	0.000	0.000	0.000	0.034	0.007
DOC-10	0	0.000	0.069	0.069	0.069	0.021
DOC-10	10	0.000	0.000	0.069	0.069	0.021
DOC-10	10	0.000	0.000	0.000	0.034	0.007
DOC-10	10	0.069	0.069	0.069	0.069	0.014
DOC-10	10	0.000	0.000	0.000	0.034	0.007
DOC-10	18	0.000	0.069	0.069	0.069	0.021
DOC-10	18	0.143	0.143	0.143	0.143	0.029
DOC-10	18	0.000	0.000	0.000	0.034	0.007
DOC-10	18	0.000	0.000	0.000	0.034	0.007
DOC-10	32	0.143	0.223	0.223	0.310	0.070
DOC-10	32	0.143	0.143	0.143	0.143	0.029
DOC-10	32	0.069	0.143	0.143	0.143	0.036
DOC-10	32	0.069	0.069	0.069	0.069	0.014
DOC-10	56	0.143	0.143	0.310	0.310	0.079
DOC-10	56	0.511	0.629	0.762	0.762	0.178
DOC-10	56	0.000	0.000	0.000	0.069	0.014
DOC-10	56	0.000	0.000	0.069	0.143	0.036
DOC-10	100	0.310	0.405	0.511	0.511	0.122
DOC-10	100	0.310	0.405	0.511	0.511	0.122
DOC-10	100	0.069	0.223	0.223	0.310	0.077
DOC-10	100	0.511	0.762	0.916	0.916	0.224

Table E.1.2. DOC-5 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
DOC-5	0	0.069	0.143	0.223	0.223	0.060
DOC-5	0	0.000	0.069	0.069	0.143	0.036
DOC-5	0	0.000	0.000	0.069	0.069	0.021
DOC-5	0	0.000	0.000	0.223	0.223	0.067
DOC-5	0	0.000	0.000	0.000	0.069	0.014
DOC-5	0	0.143	0.223	0.223	0.223	0.053
DOC-5	10	0.000	0.000	0.000	0.034	0.007
DOC-5	10	0.000	0.069	0.143	0.143	0.043
DOC-5	10	0.000	0.000	0.000	0.034	0.007
DOC-5	10	0.000	0.000	0.000	0.034	0.007
DOC-5	18	0.000	0.000	0.000	0.069	0.014
DOC-5	18	0.000	0.000	0.000	0.000	0.000
DOC-5	18	0.000	0.000	0.000	0.143	0.029
DOC-5	18	0.000	0.000	0.000	0.034	0.007
DOC-5	32	0.000	0.000	0.000	0.000	0.000
DOC-5	32	0.000	0.000	0.000	0.143	0.029
DOC-5	32	0.069	0.069	0.143	0.143	0.036
DOC-5	32	0.405	0.511	0.511	0.511	0.113
DOC-5	56	0.069	0.143	0.762	0.762	0.222
DOC-5	56	0.069	0.069	0.405	0.762	0.186
DOC-5	56	0.000	0.143	0.223	0.310	0.084
DOC-5	56	0.000	0.000	0.069	0.069	0.021
DOC-5	100	0.000	0.405	0.629	0.629	0.189
DOC-5	100	0.000	0.511	1.099	1.609	0.432
DOC-5	100	0.000	0.069	0.223	1.099	0.242
DOC-5	100	0.223	0.223	0.511	0.511	0.131

Table E.1.3. DOC-2 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
DOC-2	0	0.000	0.000	0.000	0.034	0.007
DOC-2	0	0.143	0.143	0.143	0.143	0.029
DOC-2	0	0.000	0.069	0.143	0.143	0.043
DOC-2	0	0.000	0.069	0.069	0.223	0.052
DOC-2	0	0.223	0.223	0.223	0.223	0.045
DOC-2	0	0.143	0.143	0.143	0.310	0.062
DOC-2	10	0.000	0.000	0.000	0.034	0.007
DOC-2	10	0.069	0.069	0.143	0.143	0.036
DOC-2	10	0.223	0.223	0.223	0.223	0.045
DOC-2	10	0.310	0.310	0.310	0.310	0.062
DOC-2	18	0.069	0.069	0.143	0.223	0.052
DOC-2	18	0.000	0.069	0.223	0.223	0.067
DOC-2	18	0.000	0.000	0.069	0.069	0.021
DOC-2	18	0.143	0.143	0.223	0.405	0.089
DOC-2	32	0.069	0.143	0.511	0.762	0.197
DOC-2	32	0.223	0.310	0.511	0.629	0.154
DOC-2	32	0.143	0.143	0.405	0.405	0.107
DOC-2	32	0.069	0.143	0.143	0.223	0.052
DOC-2	56	0.000	0.310	0.310	0.310	0.093
DOC-2	56	0.223	0.069	0.310	0.511	0.111
DOC-2	56	0.143	0.223	0.511	1.099	0.256
DOC-2	56	0.000	0.000	0.000	0.511	0.102
DOC-2	100	0.069	0.310	0.629	0.629	0.182
DOC-2	100	0.000	0.405	1.099	1.099	0.330
DOC-2	100	0.143	0.511	0.629	0.762	0.201
DOC-2	100	0.143	0.916	1.322	1.099	0.338

Table E.1.4. pH 6.7 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
pH-6.7	0	0.069	0.069	0.143	0.310	0.069
pH-6.7	0	0.000	0.000	0.000	0.069	0.014
pH-6.7	0	0.000	0.069	0.069	0.310	0.069
pH-6.7	0	0.000	0.143	0.223	0.223	0.067
pH-6.7	0	0.069	0.223	0.405	0.405	0.115
pH-6.7	10	0.223	0.310	0.916	0.916	0.253
pH-6.7	10	0.223	0.310	0.629	0.916	0.224
pH-6.7	10	0.069	0.223	0.223	0.223	0.060
pH-6.7	10	0.069	0.310	1.099	1.099	0.323
pH-6.7	18	0.000	0.000	0.000	0.629	0.126
pH-6.7	18	0.069	0.310	0.762	1.322	0.334
pH-6.7	18	0.069	0.405	1.099	1.099	0.323
pH-6.7	18	0.000	1.322	1.099	1.099	0.330
pH-6.7	32	0.000	0.511	0.762	0.762	0.229
pH-6.7	32	0.310	1.099	2.015	2.708	0.712
pH-6.7	32	0.310	1.099	1.322	1.322	0.366
pH-6.7	32	0.069	1.099	1.099	2.708	0.645
pH-6.7	56	0.223	1.099	2.708	2.708	0.790
pH-6.7	56	0.629	2.015	2.708	2.708	0.750
pH-6.7	56	0.511	0.762	0.916	1.609	0.362
pH-6.7	56	0.143	0.511	0.762	0.916	0.245
pH-6.7	100	0.405	0.916	2.708	3.401	0.910
pH-6.7	100	0.310	0.762	1.609	3.401	0.810
pH-6.7	100	0.310	0.629	0.629	0.762	0.184
pH-6.7	100	0.069	0.310	0.762	1.322	0.334

Table E.1.5. pH 7.7/Alk-16/DOC-0.5 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
pH-7.7/Alk-16/DOC-0.5	0	0.069	0.069	0.069	0.069	0.014
pH-7.7/Alk-16/DOC-0.5	0	0.069	0.069	0.143	0.143	0.036
pH-7.7/Alk-16/DOC-0.5	0	0.069	0.223	0.310	0.310	0.086
pH-7.7/Alk-16/DOC-0.5	0	0.069	0.069	0.069	0.069	0.014
pH-7.7/Alk-16/DOC-0.5	0	0.000	0.223	0.223	0.223	0.067
pH-7.7/Alk-16/DOC-0.5	0	0.000	0.000	0.000	0.143	0.029
pH-7.7/Alk-16/DOC-0.5	10	0.143	0.310	0.310	0.310	0.079
pH-7.7/Alk-16/DOC-0.5	10	0.143	0.223	0.762	0.762	0.214
pH-7.7/Alk-16/DOC-0.5	10	0.000	0.000	0.000	0.034	0.007
pH-7.7/Alk-16/DOC-0.5	10	0.223	0.310	0.310	0.405	0.090
pH-7.7/Alk-16/DOC-0.5	18	0.000	0.223	0.223	0.405	0.103
pH-7.7/Alk-16/DOC-0.5	18	0.000	0.405	0.405	0.310	0.103
pH-7.7/Alk-16/DOC-0.5	18	0.069	0.310	0.405	0.511	0.136
pH-7.7/Alk-16/DOC-0.5	18	0.223	0.916	0.916	0.916	0.253
pH-7.7/Alk-16/DOC-0.5	32	0.000	0.405	0.511	0.511	0.153
pH-7.7/Alk-16/DOC-0.5	32	0.069	0.511	0.629	0.629	0.182
pH-7.7/Alk-16/DOC-0.5	32	0.069	0.916	1.099	1.099	0.323
pH-7.7/Alk-16/DOC-0.5	32	0.223	1.322	1.609	1.609	0.461
pH-7.7/Alk-16/DOC-0.5	56	0.223	0.916	1.322	1.099	0.330
pH-7.7/Alk-16/DOC-0.5	56	0.223	0.511	0.629	0.762	0.193
pH-7.7/Alk-16/DOC-0.5	56	0.405	1.609	1.609	2.015	0.523
pH-7.7/Alk-16/DOC-0.5	56	0.143	1.099	1.322	1.322	0.382
pH-7.7/Alk-16/DOC-0.5	100	0.223	0.310	0.405	0.511	0.120
pH-7.7/Alk-16/DOC-0.5	100	0.223	0.762	0.762	1.099	0.274
pH-7.7/Alk-16/DOC-0.5	100	0.223	1.099	1.322	1.322	0.374
pH-7.7/Alk-16/DOC-0.5	100	0.310	0.916	0.916	0.916	0.244

Table E.1.6. pH 8.3 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
pH-8.3	0	0.069	0.223	0.223	0.223	0.060
pH-8.3	0	0.069	0.069	0.143	0.223	0.052
pH-8.3	0	0.223	0.223	0.223	0.223	0.045
pH-8.3	0	0.000	0.000	nd	0.069	0.018
pH-8.3	0	0.000	0.069	nd	0.143	0.039
pH-8.3	0	0.000	0.143	nd	0.223	0.061
pH-8.3	10	0.143	0.223	0.223	0.310	0.070
pH-8.3	10	0.143	0.223	0.223	0.223	0.053
pH-8.3	10	0.000	0.000	nd	0.034	0.009
pH-8.3	10	0.000	0.405	nd	0.511	0.143
pH-8.3	18	0.310	0.310	0.511	0.629	0.146
pH-8.3	18	0.143	0.310	0.310	0.310	0.079
pH-8.3	18	0.223	0.405	nd	0.762	0.188
pH-8.3	18	0.000	0.762	nd	1.322	0.362
pH-8.3	32	0.143	0.223	0.223	0.223	0.053
pH-8.3	32	0.105	0.143	0.143	0.143	0.032
pH-8.3	32	0.223	1.609	nd	2.015	0.545
pH-8.3	32	1.003	0.762	nd	1.609	0.350
pH-8.3	56	0.405	1.609	1.609	2.015	0.523
pH-8.3	56	0.629	0.629	0.629	0.629	0.126
pH-8.3	56	0.223	0.629	nd	1.609	0.413
pH-8.3	56	0.310	0.629	nd	1.322	0.331
pH-8.3	100	1.322	2.015	2.015	2.708	0.611
pH-8.3	100	0.629	2.015	2.015	2.015	0.542
pH-8.3	100	0.405	1.099	nd	1.609	0.410
pH-8.3	100	0.405	0.762	nd	1.322	0.327

Table E.1.7. Alk-100 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
Alk-100	0	0.000	0.069	0.069	0.069	0.021
Alk-100	0	0.069	0.143	0.143	0.143	0.036
Alk-100	0	0.000	0.069	0.069	0.069	0.021
Alk-100	0	0.069	0.069	0.069	0.069	0.014
Alk-100	0	0.143	0.143	0.223	0.223	0.053
Alk-100	0	0.069	0.143	0.143	0.223	0.052
Alk-100	10	0.000	0.000	0.000	0.034	0.007
Alk-100	10	0.069	0.143	0.143	0.223	0.052
Alk-100	10	0.223	0.310	0.310	0.310	0.071
Alk-100	10	0.069	0.223	0.223	0.223	0.060
Alk-100	18	0.143	0.310	0.310	0.310	0.079
Alk-100	18	0.069	0.223	0.143	0.223	0.052
Alk-100	18	0.143	0.143	0.223	0.223	0.053
Alk-100	18	0.000	0.143	0.143	0.143	0.043
Alk-100	32	0.069	0.511	0.511	0.629	0.170
Alk-100	32	0.069	0.629	0.762	0.762	0.222
Alk-100	32	0.143	0.405	0.511	0.511	0.139
Alk-100	32	0.405	0.916	0.916	1.099	0.271
Alk-100	56	0.405	0.762	0.762	0.916	0.219
Alk-100	56	0.629	2.708	2.708	2.708	0.750
Alk-100	56	1.099	2.015	2.015	2.015	0.495
Alk-100	56	0.223	0.310	0.762	1.322	0.318
Alk-100	100	0.916	2.708	2.708	2.708	0.721
Alk-100	100	1.099	2.708	2.708	2.708	0.703
Alk-100	100	0.629	1.099	2.015	2.708	0.680
Alk-100	100	0.629	0.762	0.916	1.099	0.248

Table E.1.8. Alk-50 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
Alk-50	0	0.000	0.223	0.143	0.143	0.043
Alk-50	0	0.000	0.000	0.069	0.069	0.021
Alk-50	0	0.143	0.143	0.223	0.223	0.053
Alk-50	0	0.143	0.143	0.143	0.143	0.029
Alk-50	0	0.000	0.000	0.000	0.034	0.007
Alk-50	0	0.143	0.143	0.143	0.143	0.029
Alk-50	10	0.223	0.143	0.310	0.310	0.071
Alk-50	10	0.223	0.310	0.223	0.223	0.045
Alk-50	10	0.223	0.223	0.310	0.310	0.071
Alk-50	10	0.223	0.223	0.405	0.405	0.099
Alk-50	18	0.143	0.143	0.143	0.143	0.029
Alk-50	18	0.000	0.143	0.143	0.143	0.043
Alk-50	18	0.069	0.069	0.069	0.223	0.045
Alk-50	18	0.143	0.310	0.310	0.310	0.079
Alk-50	32	0.143	0.405	0.762	0.762	0.214
Alk-50	32	0.223	0.310	0.629	0.511	0.143
Alk-50	32	0.143	0.310	0.405	0.405	0.107
Alk-50	32	0.511	0.511	0.511	0.629	0.126
Alk-50	56	0.143	0.629	0.762	0.762	0.214
Alk-50	56	0.069	0.511	0.629	0.629	0.182
Alk-50	56	0.000	0.069	0.143	0.511	0.116
Alk-50	56	0.223	0.762	0.916	1.609	0.391
Alk-50	100	0.069	0.916	0.916	0.916	0.268
Alk-50	100	0.223	1.322	1.322	1.322	0.374
Alk-50	100	0.223	0.629	0.629	0.762	0.193
Alk-50	100	0.143	0.223	0.629	0.916	0.232

2. RAW MORTALITY DATA FOR SELENIUM EXPERIMENTS.

Table E.2.1. DOC-5 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
DOC-5	0	0.000	0.000	0.000	0.034	0.007
DOC-5	0	0.000	0.069	0.069	0.069	0.021
DOC-5	0	0.069	0.000	0.069	0.069	0.014
DOC-5	5.6	0.000	0.000	0.069	0.069	0.021
DOC-5	5.6	0.000	0.000		0.143	0.037
DOC-5	10	0.000	0.000	0.069	0.069	0.021
DOC-5	10	0.000	0.000	0.069	0.143	0.036
DOC-5	18	0.223	0.223	0.310	0.405	0.090
DOC-5	18	0.069	0.069	0.310	0.405	0.105
DOC-5	32	0.000	0.000	0.762	0.762	0.229
DOC-5	32	0.000	0.000	2.708	3.401	0.951
DOC-5	56	0.069	0.916	3.401		1.105
DOC-5	56	0.143	0.629	3.401		1.069
DOC-5	100	0.511	1.609	3.401		1.445
DOC-5	100	1.322	3.401			2.079
DOC-5	0	0.000	0.000	0.069	0.069	0.021
DOC-5	0	0.143	0.143	0.223	0.223	0.053
DOC-5	0	0.069	0.143	0.223	0.223	0.060
DOC-5	5.6	0.143	2.708	2.708	2.708	0.798
DOC-5	5.6	0.310	3.401			1.701
DOC-5	10	0.223	1.322	3.401		1.130
DOC-5	10	0.069	0.405	2.015		0.638
DOC-5	18	0.629	2.015			1.007
DOC-5	18	1.099	3.401			1.701
DOC-5	32	3.401				3.401
DOC-5	32	3.401				3.401
DOC-5	56	3.401				3.401
DOC-5	56	3.401				3.401

Table E.2.2 DOC-2 treatment data by week and with slope of four weeks (mortality rate).

Treatment	Nominal Concentration nmol L^{-1}	Mortality during week 1	2	3	4	m
DOC-2	0	0.051	0.051	0.163	0.163	0.044
DOC-2	0	0.000	0.000	0.000	0.034	0.007
DOC-2	0	0.069	0.143	0.223	0.223	0.060
DOC-2	5.6	0.069	0.069	0.069	0.223	0.045
DOC-2	5.6	0.000	0.069	0.223	0.405	0.103
DOC-2	10	0.143	0.223	0.511	0.511	0.139
DOC-2	10	0.069	2.015	2.015	2.708	0.736
DOC-2	18	0.000	0.000	0.310	0.405	0.112
DOC-2	18	0.143	0.223	1.609	3.401	0.827
DOC-2	32	0.069	2.015	2.708	2.708	0.806
DOC-2	32	0.069	1.099	2.708		0.915
DOC-2	56	0.143	3.401			1.701
DOC-2	56	0.069	3.401			1.701
DOC-2	0	0.000	0.069	0.069	0.069	0.021
DOC-2	0	0.069	0.069	0.143	0.069	0.021
DOC-2	0	0.000	0.000	0.000	0.034	0.007
DOC-2	5.6	0.000	0.000	0.000	0.034	0.007
DOC-2	5.6	0.000	0.000	0.000	0.034	0.007
DOC-2	10	0.000	0.000	0.000	0.069	0.014
DOC-2	10	0.000	0.069	0.143	0.143	0.043
DOC-2	18	0.000	0.069	0.000	0.034	0.007
DOC-2	18	0.310	0.310	0.310	0.310	0.062
DOC-2	32	0.000	0.223	0.310	0.916	0.214
DOC-2	32	0.069	0.143	0.310	0.310	0.086
DOC-2	56	-0.065	0.223	1.609	1.609	0.489
DOC-2	56	0.069	0.223	0.762	1.322	0.334

Table E.2.3. pH 6.8/Alk-5 treatment data by week and with slope of four weeks (mortality rate).

Treatment	Nominal Concentration nmol L^{-1}	Mortality during week 1	2	3	4	m
pH-6.8/Alk-5	0	0.000	0.143	0.143	0.223	0.059
pH-6.8/Alk-5	0	0.000	0.000	0.069	0.143	0.036
pH-6.8/Alk-5	0	0.069	0.069	0.069	0.143	0.029
pH-6.8/Alk-5	5.6	0.000	0.000	0.000	0.034	0.007
pH-6.8/Alk-5	5.6	0.000	0.000	0.069	0.069	0.021
pH-6.8/Alk-5	10	0.143	0.143	0.143	0.223	0.045
pH-6.8/Alk-5	10	0.000	0.143	0.310	0.629	0.157
pH-6.8/Alk-5	18	0.069	0.069	0.143	1.322	0.272
pH-6.8/Alk-5	18	0.143	0.143	0.223	0.310	0.070
pH-6.8/Alk-5	32	0.000	0.223	1.099	3.401	0.790
pH-6.8/Alk-5	32	0.000	0.762	2.708	3.401	0.951
pH-6.8/Alk-5	56	0.000	1.609	3.401		1.181
pH-6.8/Alk-5	56	0.000	2.015	3.401		1.222
pH-6.8/Alk-5	0	0.223	0.310	0.310	0.310	0.071
pH-6.8/Alk-5	0	0.069	0.069	0.069	0.143	0.029
pH-6.8/Alk-5	0	0.143	0.143	0.223	0.310	0.070
pH-6.8/Alk-5	5.6	0.069	0.143	0.143	0.223	0.052
pH-6.8/Alk-5	5.6	0.000	0.069	0.069	0.069	0.021
pH-6.8/Alk-5	10	0.223	0.405	0.405	0.916	0.201
pH-6.8/Alk-5	10	0.069	0.223	0.223	0.511	0.118
pH-6.8/Alk-5	18	0.069	0.143	0.143	0.405	0.089
pH-6.8/Alk-5	18	0.000	0.143	0.069	0.629	0.133
pH-6.8/Alk-5	32	0.000	0.629	1.322	0.916	0.315
pH-6.8/Alk-5	32	0.000	0.069	0.629	2.015	0.466
pH-6.8/Alk-5	56	0.000	0.310	2.015	2.708	0.743
pH-6.8/Alk-5	56	0.000	0.069	1.322	3.401	0.812

Table E.2.4. pH 7.7/Alk-16/DOC-0.5 treatment data by week and with slope of four weeks (mortality rate).

Treatment	Nominal Concentration nmol L^{-1}	Mortality during week 1	2	3	4	m
pH-7.7/Alk-16/DOC-0.5	0	0.000	0.051	0.051	0.163	0.038
pH-7.7/Alk-16/DOC-0.5	0	0.000	0.000	0.000	0.051	0.010
pH-7.7/Alk-16/DOC-0.5	0	0.051	0.051	0.051	0.051	0.010
pH-7.7/Alk-16/DOC-0.5	5.6	0.000	0.000	0.000	0.025	0.005
pH-7.7/Alk-16/DOC-0.5	5.6	0.000	0.000	0.000	0.025	0.005
pH-7.7/Alk-16/DOC-0.5	10	0.000	0.000	0.000	0.025	0.005
pH-7.7/Alk-16/DOC-0.5	10	0.051	0.105	0.105	0.105	0.026
pH-7.7/Alk-16/DOC-0.5	18	0.105	0.105	0.105	0.105	0.021
pH-7.7/Alk-16/DOC-0.5	18	0.000	0.000	0.000	0.051	0.010
pH-7.7/Alk-16/DOC-0.5	32	0.000	0.223	0.288	0.431	0.115
pH-7.7/Alk-16/DOC-0.5	32	0.000	0.000	0.051	0.051	0.015
pH-7.7/Alk-16/DOC-0.5	56	0.051	0.916	1.386	1.609	0.455
pH-7.7/Alk-16/DOC-0.5	56	0.000	1.050	1.386	1.609	0.461
pH-7.7/Alk-16/DOC-0.5	100	0.223	1.609	1.897	2.303	0.653
pH-7.7/Alk-16/DOC-0.5	100	0.000	2.303	3.689		1.844
pH-7.7/Alk-16/DOC-0.5	0	0.000	0.000	0.000	0.034	0.007
pH-7.7/Alk-16/DOC-0.5	0	0.000	0.000	0.000	0.034	0.007
pH-7.7/Alk-16/DOC-0.5	0	0.000	0.000	0.000	0.034	0.007
pH-7.7/Alk-16/DOC-0.5	5.6	0.000	0.069	0.143	0.310	0.079
pH-7.7/Alk-16/DOC-0.5	5.6	0.000	0.143	0.143	0.223	0.059
pH-7.7/Alk-16/DOC-0.5	10	0.000	0.143	0.310	0.511	0.136
pH-7.7/Alk-16/DOC-0.5	10	0.000	0.223	0.223	0.223	0.067
pH-7.7/Alk-16/DOC-0.5	18	0.069	0.223	0.223	0.405	0.099
pH-7.7/Alk-16/DOC-0.5	18	0.143	0.143	0.143	0.223	0.045
pH-7.7/Alk-16/DOC-0.5	32	0.000	0.223	0.405	0.511	0.143
pH-7.7/Alk-16/DOC-0.5	32	0.000	0.405	2.015	2.708	0.937
pH-7.7/Alk-16/DOC-0.5	56	0.000	0.069	0.629	0.762	0.215
pH-7.7/Alk-16/DOC-0.5	56	0.000	0.223	0.629	1.099	0.289

Table E.2.5. pH 8.3/Alk-100 treatment data by week and with slope of four weeks (mortality rate).

Treatment	Nominal Concentration nmol L^{-1}	Mortality during week 1	2	3	4	m
pH-8.3/Alk-100	0	0.000	0.000	0.069	0.069	0.021
pH-8.3/Alk-100	0	0.000	0.000	0.143	0.223	0.059
pH-8.3/Alk-100	0	0.000	0.069	0.000	0.069	0.014
pH-8.3/Alk-100	5.6	0.000	0.069	0.000	0.223	0.045
pH-8.3/Alk-100	5.6	0.000	0.000	0.000	0.069	0.014
pH-8.3/Alk-100	10	0.000	0.000	0.069	0.069	0.021
pH-8.3/Alk-100	10	0.143	0.069	0.069	0.223	0.037
pH-8.3/Alk-100	18	0.069	0.223	0.223	0.223	0.060
pH-8.3/Alk-100	18	0.000	0.000	0.000	0.143	0.029
pH-8.3/Alk-100	32	0.000	0.310	0.310	0.310	0.093
pH-8.3/Alk-100	32	0.143	0.223	0.223	0.511	0.110
pH-8.3/Alk-100	56	0.000	1.609	1.609	3.401	0.841
pH-8.3/Alk-100	56	0.069	2.015	2.015	3.401	0.875
pH-8.3/Alk-100	100	0.405	3.401			2.996
pH-8.3/Alk-100	100	0.916	3.401			2.485
pH-8.3/Alk-100	0	0.000	0.069	0.069	0.069	0.021
pH-8.3/Alk-100	0	0.000	0.000	0.000	0.034	0.007
pH-8.3/Alk-100	0	0.000	0.000	0.000	0.034	0.007
pH-8.3/Alk-100	5.6	0.000	0.000	0.000	0.034	0.007
pH-8.3/Alk-100	5.6	0.069	0.143	0.143	0.143	0.036
pH-8.3/Alk-100	10	0.223	0.143	0.143	0.143	0.021
pH-8.3/Alk-100	10	0.000	0.000	0.000	0.034	0.007
pH-8.3/Alk-100	18	0.000	0.000	0.000	0.034	0.007
pH-8.3/Alk-100	18	0.143	0.143	0.223	0.223	0.053
pH-8.3/Alk-100	32	0.000	0.000	0.069	0.143	0.036
pH-8.3/Alk-100	32	0.223	0.310	0.310	0.310	0.071
pH-8.3/Alk-100	56	0.000	0.223	0.405		0.144
pH-8.3/Alk-100	56	0.000	0.000	0.143		0.043

3. RAW MORTALITY DATA FOR ZINC EXPERIMENTS.

Table E.3.1. DOC-5 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
DOC-5	0	0.000	0.143	0.143	0.143	0.043
DOC-5	0	0.143	0.000	0.069	0.069	0.006
DOC-5	0	0.000	0.069	0.143	0.223	0.059
DOC-5	0	0.069	0.069	0.069	0.223	0.045
DOC-5	0	0.143	0.223	0.223	0.223	0.053
DOC-5	0	0.000	0.000	0.000	0.034	0.007
DOC-5	5.6	0.069	0.069	0.069	0.069	0.014
DOC-5	5.6	0.069	0.069	0.069	0.069	0.014
DOC-5	5.6	0.000	0.000	0.000	0.143	0.029
DOC-5	5.6	0.143	0.143	0.143	0.143	0.029
DOC-5	10	0.000	0.000	0.000	0.034	0.007
DOC-5	10	0.143	0.143	0.223	0.310	0.070
DOC-5	10	0.069	0.143	0.069	0.069	0.014
DOC-5	10	0.000	0.000	0.000	0.069	0.014
DOC-5	18	0.000	0.143	0.143	0.143	0.043
DOC-5	18	0.069	0.069	0.069	0.069	0.014
DOC-5	18	0.143	0.223	0.223	0.223	0.053
DOC-5	18	0.000	0.069	0.069	0.069	0.021
DOC-5	32	0.069	0.143	0.223	0.223	0.060
DOC-5	32	0.223	0.310	0.310	0.310	0.071
DOC-5	32	0.143	0.143	0.223	0.223	0.053
DOC-5	32	0.000	0.000	0.000	0.034	0.007
DOC-5	56	0.310	0.223	0.310	0.405	0.081
DOC-5	56	0.143	0.310	0.310	0.405	0.098
DOC-5	56	0.223	0.223	0.223	0.310	0.062
DOC-5	56	0.143	0.143	0.223	0.310	0.070

Table E.3.2 DOC-2 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
DOC-2	0	0.143	0.223	0.223	0.223	0.053
DOC-2	0	0.069	0.143	0.143	0.143	0.036
DOC-2	0	0.069	0.143	0.143	0.143	0.036
DOC-2	0	0.000	0.143	0.223	0.223	0.067
DOC-2	0	0.000	0.223	0.223	0.223	0.067
DOC-2	0	0.069	0.069	0.069	0.069	0.014
DOC-2	5.6	0.143	0.223	0.310	0.405	0.098
DOC-2	5.6	0.143	0.405	0.405	0.511	0.128
DOC-2	5.6	0.223	0.629	0.629	0.629	0.166
DOC-2	5.6	0.000	0.310	0.405	0.511	0.143
DOC-2	10	0.000	0.143	0.223	0.223	0.067
DOC-2	10	0.069	0.069	0.069	0.069	0.014
DOC-2	10	0.069	0.223	0.511	0.629	0.170
DOC-2	10	0.143	0.405	0.405	0.405	0.107
DOC-2	18	0.405	0.629	0.916	1.099	0.271
DOC-2	18	0.143	0.405	0.511	0.629	0.162
DOC-2	18	0.143	0.223	0.223	0.223	0.053
DOC-2	18	0.223	0.405	0.405	0.511	0.120
DOC-2	32	0.223	0.223	0.310	0.405	0.090
DOC-2	32	0.143	0.405	0.511	0.762	0.189
DOC-2	32	0.310	0.405	0.405	0.511	0.112
DOC-2	32	0.511	0.916	1.322	1.322	0.345
DOC-2	56	0.223	0.310	0.629	1.099	0.260
DOC-2	56	0.223	0.629	0.762	0.762	0.206
DOC-2	56	0.511	0.762	0.916	1.099	0.260
DOC-2	56	0.405	0.762	1.322	1.609	0.414

Table E.3.3. pH 6.8/Alk-5 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
pH-6.8	0	0.000	0.000	0.000	0.069	0.014
pH-6.8	0	0.069	0.069	0.069	0.069	0.014
pH-6.8	0	0.000	0.000	0.000	0.069	0.014
pH-6.8	0	0.000	0.000	0.000	0.069	0.014
pH-6.8	0	0.069	0.143	0.143	0.223	0.052
pH-6.8	0	0.000	0.223	0.310	0.310	0.093
pH-6.8	5.6	0.000	0.069	0.069	0.223	0.052
pH-6.8	5.6	0.000	0.000	0.000	0.034	0.007
pH-6.8	5.6	0.000	0.069	0.310	0.405	0.112
pH-6.8	5.6	0.000	0.069	0.223	0.405	0.103
pH-6.8	10	0.069	0.069	0.143	0.223	0.052
pH-6.8	10	0.069	0.143	0.143	0.310	0.069
pH-6.8	10	0.069	0.069	0.143	0.143	0.036
pH-6.8	10	0.069	0.143	0.405	0.405	0.115
pH-6.8	18	0.223	0.310	0.310	0.405	0.090
pH-6.8	18	0.000	0.223	0.405	0.629	0.166
pH-6.8	18	0.223	0.310	0.310	0.629	0.134
pH-6.8	18	0.000	0.223	0.223	0.223	0.067
pH-6.8	32	0.405	0.405	0.405	0.405	0.081
pH-6.8	32	0.762	0.762	0.916	0.916	0.199
pH-6.8	32	0.405	0.511	0.916	1.609	0.373
pH-6.8	32	0.629	0.762	0.762	1.099	0.233
pH-6.8	56	1.322	1.322	1.322	1.322	0.264
pH-6.8	56	1.099	1.609	2.708	3.401	0.841
pH-6.8	56	0.405	0.916	1.609	1.609	0.442
pH-6.8	56	0.762	0.916	2.015	2.015	0.528

Table E.3.4. Alk-5 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
Alk-5	0	0.000	0.000	0.000	0.034	0.007
Alk-5	0	0.000	0.143	0.143	0.143	0.043
Alk-5	0	0.143	0.143	0.223	0.223	0.053
Alk-5	0	0.000	0.000	0.069	0.143	0.036
Alk-5	0	0.000	0.000	0.000	0.034	0.007
Alk-5	0	0.143	0.223	0.223	0.223	0.053
Alk-5	5.6	0.143	0.143	0.223	0.223	0.053
Alk-5	5.6	0.069	0.069	0.143	0.143	0.036
Alk-5	5.6	0.000	0.069	0.069	0.069	0.021
Alk-5	5.6	0.000	0.310	0.405	0.405	0.122
Alk-5	10	0.143	0.143	0.143	0.143	0.029
Alk-5	10	0.069	0.069	0.069	0.069	0.014
Alk-5	10	0.069	0.069	0.143	0.143	0.036
Alk-5	10	1.322	1.322	1.322	1.322	0.264
Alk-5	18	0.916	0.916	0.916	0.916	0.183
Alk-5	18	1.322	1.322	1.322	1.322	0.264
Alk-5	18	0.069	0.069	0.069	0.069	0.014
Alk-5	18	0.000	0.223	0.310	0.310	0.093
Alk-5	32	3.401				3.401
Alk-5	32	3.401				3.401
Alk-5	32	0.405	0.223	0.405	0.405	0.081
Alk-5	32	0.762	0.762	0.511	0.511	0.077
Alk-5	56	2.708	2.708	2.708	2.708	0.542
Alk-5	56	3.401				3.401
Alk-5	56	0.916	0.916	1.099	3.401	0.698
Alk-5	56	0.762	0.762	0.762	2.015	0.403

Table E.3.5. Alk-100 treatment data by week and with slope of four weeks (mortality rate).

	Nominal Concentration	Mortality during week				
Treatment	nmol L^{-1}	1	2	3	4	m
Alk-100	0	0.000	0.000	0.000	0.034	0.007
Alk-100	0	0.000	0.000	0.000	0.034	0.007
Alk-100	0	0.000	0.069	0.069	0.069	0.021
Alk-100	0	0.069	0.223	0.223	0.223	0.060
Alk-100	0	0.000	0.000	0.000	0.069	0.014
Alk-100	0	0.069	0.069	0.069	0.143	0.029
Alk-100	5.6	0.069	0.069	0.069	0.143	0.029
Alk-100	5.6	0.143	0.143	0.143	0.310	0.062
Alk-100	5.6	0.000	0.069	0.143	0.223	0.059
Alk-100	5.6	0.000	0.069	0.069	0.143	0.036
Alk-100	10	0.223	0.310	0.310	0.310	0.071
Alk-100	10	0.069	0.143	0.143	0.143	0.036
Alk-100	10	0.000	0.000	0.000	0.034	0.007
Alk-100	10	0.069	0.000	0.069	0.069	0.014
Alk-100	18	0.405	0.511	0.511	0.629	0.136
Alk-100	18	0.223	0.223	0.310	0.310	0.071
Alk-100	18	0.000	0.223	0.405	0.405	0.122
Alk-100	18	0.069	0.000	0.000	0.762	0.146
Alk-100	32	0.143	0.223	0.223	0.511	0.110
Alk-100	32	0.629	0.629	0.762	1.099	0.233
Alk-100	32	0.069	0.143	0.143	0.143	0.036
Alk-100	32	0.000	0.511	0.511	0.511	0.153
Alk-100	56	2.708	2.708			1.354
Alk-100	56	1.609	2.015	2.015	2.015	0.444
Alk-100	56	0.916	0.511	0.511	0.629	0.085
Alk-100	56	0.629	2.708	2.708		1.020