The Effects of Water Chemistry and Organism Source on Dysprosium Toxicity to *Hyalella Azteca*

by

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Author's Declaration

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

Rare earth elements (REEs) are a series of seventeen chemical elements, containing mostly lanthanides. Due to their multiple applications in industry and consumer electronics, global demand and production of REE has been increasing. Canada has the potential to be one of the major REE producers, with reserves mainly located in northern regions, yet there is limited knowledge of the potential environmental impact of mining and refining of REEs. Dysprosium (Dy) is one of the most widely used heavy REEs due to its magnetic strength. In this study, acute 96 h toxicity tests were conducted to determine the sensitivity to Dy for four different sources (Fort Hope, Hannah Lake, Daisy Lake, Low Water Lake, ON) of *Hyalella azteca* as well as *Daphnia pulex*. LC_{50}s and their confidence intervals derived from dissolved Dy concentrations indicated significant differences in Dy sensitivity as a function of the source of *H. azteca.* To determine the potential influence of toxicity modifying factors on Dy toxicity, acute (96 h) and chronic (28 d) toxicity tests using *H. azteca* were conducted with differing concentrations of Ca^{2+}, Mg^{2+} and Na^{+} (acute test only), differing qualities and/or concentrations of dissolved organic carbon (DOC) as well as differing pH. Based on LC_{50} data for 96 h tests, Ca^{2+} (2 mM), Na^{+} (2 mM), low pH (6.7) and DOC (Suwannee River, USA; 9.6 mg/L), but not Mg^{2+} (up to 2 mM) demonstrated protective effects against Dy toxicity. In addition, pH played an important role in Dy solubility. For 28 d exposures, protective effects against Dy toxicity were demonstrated for Ca^{2+} (2 mM), Mg^{2+} (0.5 mM) and DOC (White River and Luther Marsh, ON; 6.4 and 7.8 mg/L respectively) based on dissolved Dy LC_{50}s. Both types of DOC were found to partially ameliorate growth inhibition at low Dy concentrations. One of the sources of DOC (Luther Marsh) was very effective at reducing Dy bioaccumulation in *H. azteca*. The LC_{50} data for 96 h toxicity tests contributed to building a preliminary Dy biotic ligand model (BLM) of *H. azteca*, which will assist with estimations of Dy toxicity in aquatic systems that differ in water quality and provide knowledge to direct the establishment of water resource protection guidelines of REE in Canada.

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CHAPTER 1

General introduction

1.1 Rare earth elements

1.1.1 General background

Rare earth elements (REEs) are a series of 17 metallic elements, mostly discovered in the 19th and early 20th centuries, including scandium, yttrium and 15 lanthanides with atomic numbers from 57 to 71 (Table 1.1; Emsley, 2011). They exist in the earth's crust as oxides in mineral ores at moderate abundance; some are more abundant than Cu and Pb (USEPA, 2012; Humphries, 2013). They have very similar chemical properties because of their similar atomic configuration thus are difficult to separate efficiently (Caster and Hedrick, 2006). This class of elements is subdivided into two categories, light rare earth elements (LREEs) and heavy rare earth elements (HREEs) based on their electron configuration; LREEs include the first 8 lanthanides, from lanthanum to gadolinium, which have increasing unpaired electrons from 0 to 7; HREEs include the rest of the lanthanides, from terbium to lutetium, plus yttrium, which have paired electrons; scandium is neither LREE nor HREE (Generalic, 2014). Dysprosium (Dy), the REE of interest in this study has an atomic number of 66 and is classified as a HREE.

Table 1.1 A summary of the 17 rare earth elements.

Elements	Symbol	Atomic no.	Standard atomic weight	Sub-class[a]
Scandium	Sc	21	45.0	n/a
Yttrium	Y	39	88.9	HREE
Lanthanum	La	57	138.9	LREE
Cerium	Ce	58	140.1	LREE
Praseodymium	Pr	59	140.9	LREE
Neodymium	Nd	60	144.2	LREE
Promethium	Pm	61	145.0	LREE
Samarium	Sm	62	150.4	LREE
Europium	Eu	63	152.0	LREE
Gadolinium	Gd	64	157.3	LREE
Terbium	Tb	65	158.9	HREE
Dysprosium	Dy	66	162.5	HREE
Holmium	Ho	67	164.9	HREE
Erbium	Er	68	167.3	HREE
Thulium	Tm	69	168.9	HREE
Ytterbium	Yb	70	173.0	HREE
Lutetium	Lu	71	175.0	HREE

[a] HREE, heavy rare earth element; LREE, light rare earth element; n/a, not applicable

1.1.2 Global production and demand

Global REE production has been dominated, in succession, by South Africa, United States and China. China has been the leading producer since the 1980s and in 2009, REE production reached 120,000 metric tons per year, which accounts for 97% of the global production of approximately 124,000 metric tons per year (Humphries, 2010). Global REE production has been increasing and by 2020 it is predicted to be between 240,000 to 280,000 metric tons per year (CREEN, 2013). Due to multiple uses of REEs in clean energy and other high-technology applications, global REE demand has also been growing and it is forecast to reach approximately 375,000 metric tons per year in 2035 (Alonso et al., 2012). In general, elements such as cerium, lanthanum, neodymium, praseodymium, and yttrium comprise the majority (95%) of the REE demand (Alonso et al., 2012). As demand and production of REEs increases, there is a critical need to understand the environmental impacts of REEs in both aquatic and terrestrial ecosystems.

1.1.3 Dysprosium

Dysprosium (Dy) is the rare earth element with atomic number 66, belonging to HREE category. Dysprosium, as well as holmium, have the strongest known magnetic strength among all the elements (Emsley, 2001). Compared to the more widely used REEs such as lanthanum and cerium, Dy accounted for only about 1% of the global REE demand in 2010, but this percentage is predicted to reach 7% in the year of 2035 (Alonso et al., 2012). Due to its physio-chemical properties, Dy is primarily used as an additive to manufacture high strength permanent magnets and these magnets are installed in many products such as electronics, green energy vehicles, and wind energy turbines to improve heat resistance (Watanabe, 2012). Different Dy alloys can also be used to make laser lighting materials and nuclear reactor control rods (Emsley, 2001). Because of its ability to become luminescent when excited by ionizing radiation, Dy can also be utilized to make radiation indicators (Emsley, 2001). Due to the wide variety of applications of Dy including clean energy, nuclear technology and electronics manufacture, it is understandable that the global demand for Dy is increasing. In fact, a shortage of HREE has been predicted: in 2016, while the estimated global HREE production is 7,000 metric tons, the global demand for HREE (especially Tb, Dy, Er, Y) might reach 14,500 tonnes (CREEN, 2013).

In Canada, rare earth mineral deposits contain heavy REEs including Dy. Great Western Minerals Group (GWMG) of Canada and Avalon Rare Metals Inc. possess REE deposits with relatively high percentage of HREEs. In 2010, Avalon started mining the Thor Lake REE deposit in the Northwest Territories of Canada, which is considered to be one of the largest HREE-producing deposits worldwide (Humphries, 2013). Since Canada is positioned to become a major producer of HREE, the need to better understand the environmental issues and risk associated with the mining and refining of HREEs such as Dy is paramount.

1.1.4 Environmental concerns

One of the environmental concerns in the REE mining and refining industry is the presence of radioactive elements that often coexist with REEs. In general, industrial treatment systems can be developed to reduce the potential environmental impacts of radiation (Bourzac, 2010; Bradsher, 2010). However, there is uncertainty in terms of the potential for REEs to cause toxicity in aquatic ecosystems. Currently, there are only a few studies of REE aquatic toxicity and there are no water quality guidelines for REEs in Canada.

1.2 Dy toxicology

1.2.1 Dy toxicity

When dissolved in solution, a portion of Dy exists as free metal ion (Dy^{3+}, preferred oxidation state) depending on water chemistry (Emsley, 2011), which is highly bioavailable and commonly considered as the main cause of metal toxicity (Paquin et al., 2002). There are few studies on Dy toxicity with the majority focused on the effects of Dy to rodents (Bruce et al., 1963; Mogilevskaya and Roshchina, 1964; Haley et al., 1966; Hirano and Suzuki, 1996). For mice, the intraperitoneal administration LD_{50} was 585 mg $DyCl_3$ per kg body weight, and the oral administration LD_{50} was 7650 mg $DyCl_3$ per kg body weight (Hirano and Suzuki, 1996). A more recent study examined the effects of Dy on microbial communities (*Euglena gracilis* Z, *Tetrahymena thermophila* B and *Escherichia coli* DH5α) and found extinction of all species at 1000 µM Dy (Fuma et al., 2005). *Hyalella azteca* toxicity tests were conducted on 63 metals and metalloids, including Dy, and the 7-d nominal Dy LC_{50}s were 485 µg/L in soft water and 897 µg/L in tap (hard) water (Borgmann et al., 2005). The toxicity of Dy will be studied again on *H. azteca* in the project, and based on the mortality and water chemistry data, an attempt will be made to build a biotic ligand model (BLM) of the acute toxicity of Dy based on *H. azteca* data.

1.2.2 Biotic ligand model

Biotic ligand model (BLM, Figure 1.1) is a frequently used software tool in aquatic ecotoxicology which helps determine how the toxicity modifying factors (TMFs) influence the speciation and bioavailability of metals (Paquin et al., 2002). It is established as a prediction method for estimating metal toxicity and is an extension of two previous models: the free-ion activity model (FIAM) and the gill surface interaction model (GSIM), which also focused on the bioavailability of metal to aquatic organisms under changing water quality conditions (Paquin et al., 2002). Figure 1.1 illustrates that the BLM focuses on the interactions between free metal ion, competing cations, organic/inorganic ligands, and biotic ligand (BL). Each of the interactions has the potential to impact the bioavailability of the metal, and therefore the expression of toxicity. The strength of the BLM is that it is a mechanistically based model that applies geochemical equilibrium principles to estimate the binding of metal to receiving sites in organisms (BL). In order to develop a valuable BLM, the toxic effects of the metal in question must be tested under a complete set of different water chemistry conditions. The ultimate goal of building a BLM is to determine the binding affinity of the metal and various common inorganic cations in solution to the biotic ligand and the relationship between the biotic ligand occupancy and mortality so that toxicological endpoints such as LC_{50} can be calculated based on water chemistry only, therefore eliminating the need for costly toxicity tests. With a complete BLM the potential hazard of the metal in a specific water system can be expediently estimated despite the fact that different water systems have dissimilar water chemistry. Therefore, BLM will be valuable to assist the government in establishing water resources protection regulations in different regions. At this moment, efforts have been made to build BLMs of multiple common metal pollutants such as Cu and Ni (Santore et al., 2001; De Schamphelaere and Janssen, 2002; Schroeder, 2008), while BLM development of some other metals such as REEs are still in progress.

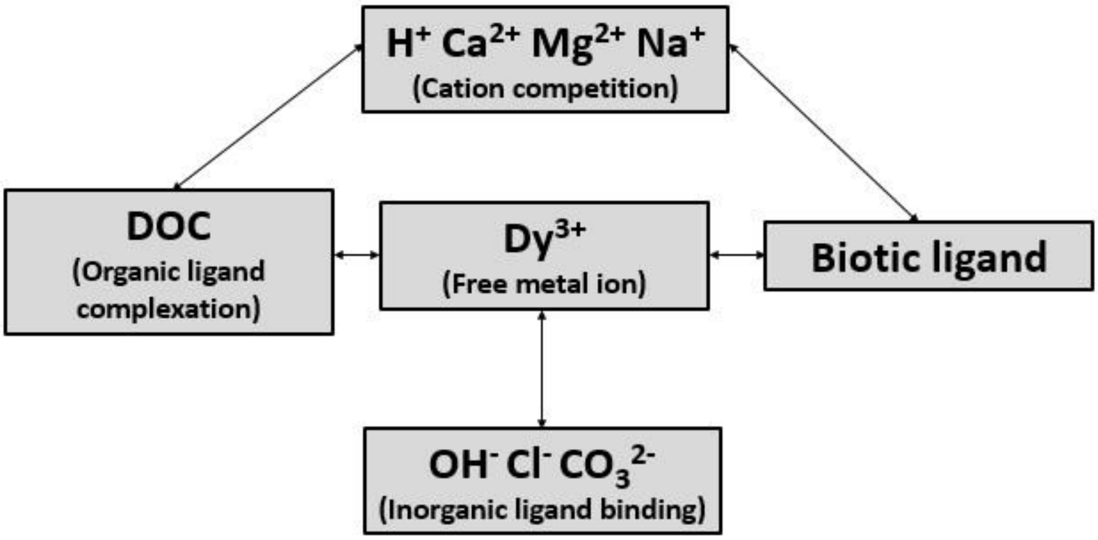

Figure 1.1 Schematic of the biotic ligand model showing toxic interaction of free metal ion with the biotic ligand and the TMFs (figure modified from Paquin et al., 2002).

1.2.3 Toxicity modifying factors

Generally, toxicity modifying factors (TMFs) are water chemistry parameters affecting metal bioavailability and toxicity. Based on Figure 1.1, the parameters can be inorganic cations (Ca^{2+}, Mg^{2+}, Na^+ etc.), inorganic ligands, DOC, and pH (H^+). As an important parameter of water chemistry, pH can substantially influence the speciation of many metals (Byrne et al., 1988). The general pattern is that the proportion of free metal ion increases (if free metal ion is toxic the toxicity increases) as the pH decreases. In addition, chemicals dissolved in solution also play an important role in metal speciation: organic/inorganic ligands and cations can affect free metal ion proportion/availability by complexation and competition (O'Shea and Mancy, 1978).

Organic/inorganic ligand complexation and cation competition are both important processes which affect the bioavailability/toxicity of free metal ion significantly. Organic and inorganic ligands can bind with free metal ion which results in a complex that does not bind as well with the BL. Dissolved organic carbon (DOC) is particularly notable as an aqueous ligand that can bind metal ions. Even though the composition of DOC from different water sources are not exactly the same, they are still generally considered protective against metal toxicity (Di Toro et al., 2001). At present DOC taken from different water systems have been qualitatively and quantitatively measured in order to study their protective effect. Inorganic anions (for example CO_3^{2-}, OH^-, SO_4^{2-} etc.) can also complex metals and similarly reduce the bioavailability/toxicity (Paquin et al., 2002). The relative abundance of some of these anions in solution is strongly influenced by pH.

For the cation competition, Zitko et al. (1976) reported that the hardness cations, Ca^{2+} and Mg^{2+}, were able to competitively interact with free metal ions and prevent their binding with BL. If the competition of a cation happens at the same site as the free metal ion, it will decrease the uptake/bioavailability of the metal. For example, in a previous study, increasing Ca^{2+}, Mg^{2+}, or Na^+ concentrations in the exposure medium increased the EC_{50} (i.e. decreases the toxicity) of Cu to *Daphnia magna* (De Schamphelaere and Janssen, 2002). Another study by Borgmann et al. (2005) also showed that in tap (hard) water, the LC_{50} of metals were generally higher compared to LC_{50} of metals in soft water (Borgmann et al., 2005). The strength of the BLM is that it is able to simultaneously account for both complexation reactions as well as competitive interactions to provide accurate estimates of metal toxicity.

1.3 *Hyalella azteca* and *Daphnia pulex*

Hyalella azteca is a common freshwater amphipod in North America, and can be found in many water systems in Canada. *H. azteca* is an important food source for fish and waterfowl (Krapu and Reinecke, 1992). They live near the sediment surface and feed on epibenthic algae, sediment microflora, or even decaying organic material (Hargrave, 1970; Canadian Museum of Nature, 2007). Unlike other species, *H. azteca* is tolerant to a wide range of environmental conditions. They are used for various toxicity bioassays because of their sensitivity (especially to metals), ease of culturing and good offspring production (Smith, 2001; Phipps et al., 1995). However, the generation time of *H. azteca* is very short, it is quite possible that diversification happens frequently (Witt and Hebert, 2000). Based on early study, 33 provisional species were discriminated within the complex of species represented by *H. azteca* (Witt et al., 2006). The relative sensitivity of the provisional species of *H. azteca* to contaminants is unknown because most of the toxicity testing has been done with provisional species from only a few sources.

The freshwater flea *Daphnia pulex* (*D. pulex*) is also a widely distributed species. It exists in almost all permanent and eutrophic water bodies (Miller, 2000). They are filter feeders, consuming bacteria, algae and detritus. They are also food for many fish species (Miller, 2000). They can switch their method of reproduction between sexual and asexual (parthenogenesis) modes depending on the seasons in order to survive under adverse environmental conditions (Miller, 2000). As a worldwide popular model organism, its mitochondrial genome has been completely sequenced (Crease, 1999).

1.4 Objectives

1.4.1 Acute Dy toxicity

The objectives of the acute study of this project are:

- To compare the sensitivity of *D. pulex* and *H. azteca* to Dy.
- To compare the sensitivity of *H. azteca* from different geographical locations to Dy.
- To assess whether TMFs that often have protective effects against other metals also influence the toxicity of Dy.
- To develop a Dy acute BLM based on FH *H. azteca* data.

The hypotheses for the acute study objectives are:

- *D. pulex* is more tolerant than *H. azteca* to Dy.
- *H. azteca* from different locations show various sensitivity to Dy.
- The TMFs provide *H. azteca* protective effects against Dy toxicity.

1.4.2 Chronic Dy toxicity

The objectives of the chronic study of this project are:

- To determine the chronic effects of Dy to *H. azteca*.
- To determine the long-term protection of TMFs to chronic Dy toxicity.

The hypotheses for the chronic study objectives are:

- Dy has adverse chronic effects on *H. azteca* in terms of survival and growth.
- TMFs have long-term protective effects on Dy toxicity.

CHAPTER 2

Acute toxicity of Dy to *Hyalella azteca* and the influence of toxicity modifying factors

2.1 Introduction

Rare earth elements (REEs) are a group of metallic elements including scandium, yttrium and 15 lanthanides, existing at moderate abundance in the earth's crust as oxides in rare minerals (Humphries, 2013). REEs are divided into two sub-categories based on small electronic differences (except scandium): light rare earth element (LREE) and heavy rare earth element (HREE; Generalic, 2014). REEs are used in a wide variety of applications as catalysts, alloys and permanent magnets. In 2009, Chinese REE production reached 120,000 metric tons per year, which accounted for 97% of the global production (approx. 124,000 metric tons per year; Humphries, 2010). As global REE demand continues to increase, production is predicted to increase to between 240,000 to 280,000 metric tons per year by 2020, with dominant production in China, followed by South Africa and United States (CREEN, 2013; Alonso et al., 2012; Humphries, 2010). Recently, in Canada, Avalon Rare Metals Inc. initiated mining (in 2010) on the Thor Lake REE deposit in the Northwest Territories which is considered to be one of the largest HREE-producing deposits worldwide (Humphries, 2013).

As a result of the increasing REE mining and refining activity in Canada, there are heightened concerns regarding the environmental protection of aquatic ecosystems. In the REE industry, it can often be the radioactive elements coexisting with REEs which are initially of concern. In general, treatment systems can be developed to minimize the potential environmental impacts of radiation (Bourzac, 2010; Bradsher, 2010). However, there is uncertainty regarding the potential for REEs to cause impacts in aquatic ecosystems, and currently there is little information on the aquatic toxicity of individual REEs and there are no Canadian water quality guidelines for any of these REEs.

Dysprosium (Dy), is a lanthanide and one of the HREEs of concern. Dy has the strongest known magnetic strength among all the elements (Emsley, 2001). Due to its physio-chemical properties, Dy is primarily used as an additive to manufacture high strength permanent magnets to increase heat resistance in electronics, green energy vehicles, and wind energy turbines (Watanabe, 2012). In addition, different Dy alloys can be used to make laser lighting materials and control rods for nuclear reactors (Emsley, 2001). Because Dy exhibits luminescence when excited by ionizing radiation, it can be utilized to make radiation indicators (Emsley, 2001). As a result of such wide applications, Dy is predicted to reach approximately 7% of global REE demand in the year of 2035, which was only 1% in 2010 (Alonso et al., 2012).

Currently there is limited information on aquatic toxicity of Dy and interactions between Dy and toxicity modifying factors (TMFs). When Dy is dissolved in solution, a certain portion of Dy exists as free metal ion (Emsley, 2011), which is considered to be the most toxic metal species (Paquin et al., 2002). Chemical speciation of metals may be substantially influenced by pH (Byrne et al., 1988); although no data is available for Dy specifically, in general, the proportion of free metal ion tends to increase as pH decreases, resulting in enhanced toxicity. Borgmann et al. (2005) studied the acute toxicity of Dy to *Hyalella azteca* and reported 7-day LC_{50} values that indicated reduced Dy toxicity in tap water (hardness 124 mg/L; LC_{50} 897 µg/L, nominal) compared to soft water (10% tap water; LC_{50} 485 µg/L, nominal). This is a good example of hardness cation competition, where Ca^{2+} and Mg^{2+} compete with free metal ions (Dy^{3+}) and prevent Dy^{3+} binding at the biotic ligand (BL) site; this reduces Dy^{3+} uptake/bioavailability and acute toxicity (Zitko et al., 1976). In addition to the effects of elevated Ca^{2+} and Mg^{2+}, increases in Na^{+} concentrations reduced the toxicity of Cu to *Daphnia magna* (De Schamphelaere and Janssen, 2002). Metal bioavailability and toxicity are also reduced by organic and inorganic ligands that bind with free metal ions to produce complexes that are unable to bind with biotic ligands. Dissolved organic carbon (DOC) is particularly notable as an aqueous organic ligand that can bind metal ions; the level of protection against metal toxicity is a function of DOC quality and quantity (Di Toro et al., 2001). Inorganic anions (e.g. CO_3^{2-}, OH^-, SO_4^{2-}) in solution, which are strongly influenced by pH, can also complex metals (even form precipitates) and similarly reduce metal toxicity (Paquin et al., 2002).

BLM was developed as a prediction method for estimating the toxicity of metals in water based on two earlier models; free-ion activity model (FIAM) and gill surface interaction model (GSIM). They both focused on the bioavailability of metals to aquatic organisms under different water quality conditions (Paquin et al., 2002). BLM is a frequently used software tool in aquatic ecotoxicology which aids in determining how TMFs influence chemical speciation and bioavailability of metals (Paquin et al., 2002). BLM considers the free metal ion to be the most bioavailable and toxic form of metals due to the ease of uptake by the organism. Inorganic cations and anions and dissolved organic carbon are TMFs which play an important role in metal toxicity in terms of competition for binding sites (cations) and complexation with metals (anions and DOC).The biotic ligand is the metal binding site of aquatic organisms, for example gills in fish and ion receptors in invertebrates. Mathematically, the model assumes the biotic ligand exists freely in solution similar to inorganic ligands (anions).

In order to develop a complete and valuable BLM, metal toxicity data (LC_{50}) generated for varying concentration of all the possible TMFs are required. Building a BLM will aid in determining the relative affinity of various cations (Dy^{3+},Ca^{2+}, Mg^{2+}, Na^+, H^+ in this study) in solution to bind to the biotic ligand and the relationship between the biotic ligand occupancy by Dy^{3+} and mortality. This type of model can then be applied in the future to predict Dy toxicity under specific environmental water quality conditions to assess environmental risk of Dy without the need to conduct expensive toxicity tests. With a usable BLM for Dy the potential hazard of Dy can be expediently estimated for a wide variety of sites despite potential differences in water chemistry. As a result, BLM becomes an extremely useful tool to assist the government in making water resource protection regulations in different regions. At this moment, BLMs of several common metal pollutants (e.g. Cu, Ni, Cd) have been developed (De Schamphelaere and Janssen, 2002; Schroeder, 2008), while some other metals such as REEs still need to be further studied. In the current study, one of the REEs, Dy, will be tested using *H. azteca* to generate toxicity data for various TMFs in order to attempt to build a BLM for Dy.

In the present study, the objectives were to: 1) assess the acute toxicity of Dy on two invertebrate species, freshwater amphipod (*H. azteca*) and freshwater flea (*Daphnia pulex*); 2) compare the acute toxicity of Dy on *H. azteca* from 4 different sources. While both test organisms are common in aquatic ecosystems and frequently used in toxicity tests based on their sensitivity to contaminants, ease of culturing and high offspring productivity, *H. azteca* has high genetic diversity with 33 provisional species discriminated within the complex of species represented by *H. azteca* (Witt et al., 2006). To ascertain the relative sensitivity of the *H. azteca* species complex to Dy, *H. azteca* is collected from 4 different locations in northern Ontario, Canada; 3) determine the effects of pH and different concentrations of individual cations (Ca^{2+}, Mg^{2+}, and Na^{+}) and dissolved organic carbon (DOC) on the toxicity of Dy; 4) use exposure (e.g. dissolved Dy concentrations in water) and effects (e.g. *H. azteca* mortality) data to build a BLM for Dy that will simultaneously account for chemical speciation, complexation reactions as well as competitive interactions to provide accurate estimates of metal toxicity.

2.2 Materials and Methods

2.2.1 *H. azteca* sources and *D. pulex*

Hyalella azteca were collected from 4 different locations in Ontario, Canada (Figure 2.2) and cultivated in the lab. Three of the sampling sites (Hannah Lake: 46.443741, -81.038141, Daisy Lake: 46.455342, -80.880002 and Low Water Lake: 47.152090, -81.694350) were located close to Sudbury, an area that has historically experienced severe sulfur dioxide and metal contamination (Spektor, 2003). The 4th site, located near Fort Hope (Eabamet Lake: 51.560457, -87.986864) in northwestern Ontario, is relatively pristine. A single culture of *D. pulex* was also maintained in McGeer's lab (McFarlane Lake, Sudbury, ON).

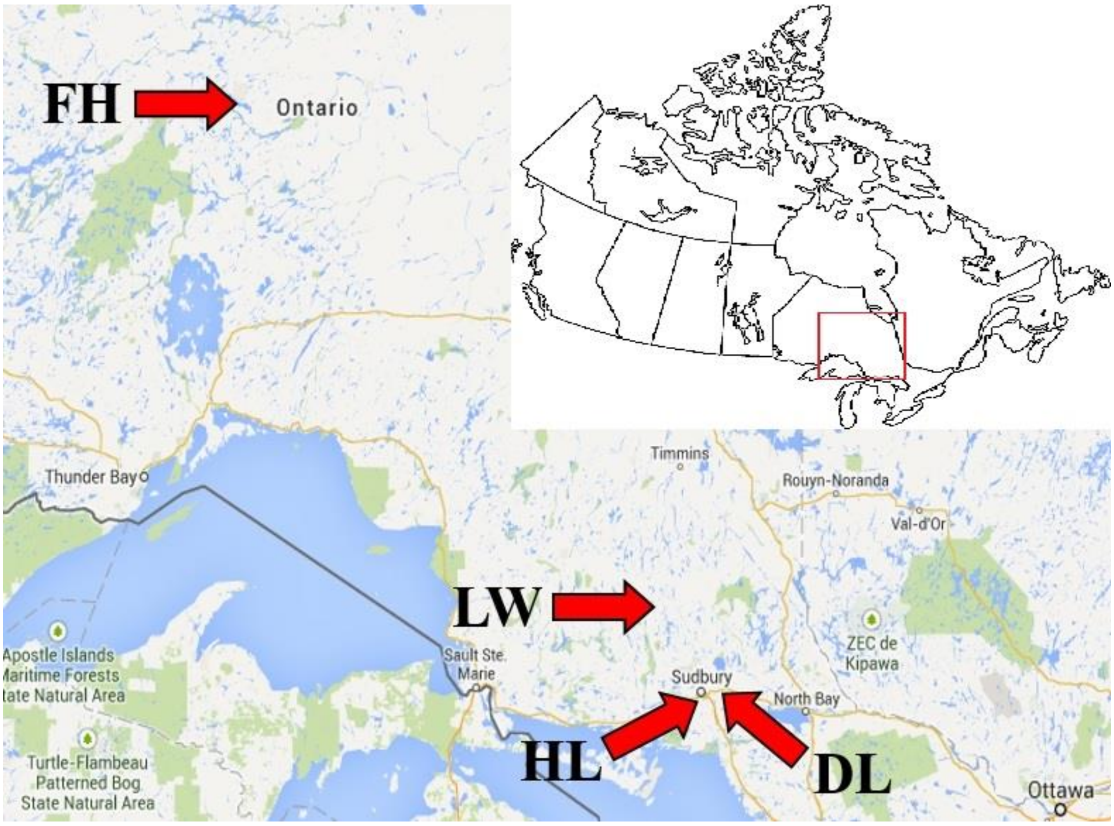

Figure 2.1 Four collection sites for *H. azteca* in northern Ontario, Canada (Fort Hope, FH; Hannah Lake, HL; Daisy Lake, DL; Low Water Lake, LW). Map of Canada insert on top right depicts collection area in northern Ontario.

2.2.2 *H. azteca* and *D. pulex* culture maintenance

For each *H. azteca* source about 20 adults were kept in 2L plastic beakers (Fisher Scientific, Nepean, ON) that contained 1600 mL of moderately soft medium (MSM; referred to Borgmann, 1996); pH was adjusted to 7.3±0.1 using HNO_3 or KOH solutions if needed (Table 2.1). A 5 cm by 10 cm piece of cotton gauze was added to each beaker as substrate. The beakers were kept at 23°C and a 16:8 hour light:dark cycle. Three times per week (Monday, Wednesday and Friday) the cultures were fed with 5 mg of TetraMin® tropical fish food. On each Wednesday, *H. azteca* adults and neonates were separated using 2 filter screens with different aperture diameters (650 μm and 275 μm) and placed into separate, clean beakers with new solution and gauze, and then fed as mentioned above. Neonates at age 2-9 days were used in experiments.

Daphnia pulex cultures were maintained under similar conditions as *H. azteca*: except that they were kept in 3 glass beakers (2L) containing 2L MSM and fed with 10 mL of algal mixture (*Pseudokirchneriella subcapitata* and *Chlorella vulgaris*) and 5 mL of yeast, Cerophyll™ and trout chow (YCT) 3 times a week. The neonates used for tests were less than 48 hours old.

Table 2.1 Nominal ion strength of the MSM used for culturing of *H. azteca*.

Cations		**Anions**	
Species	**Conc. (mM)**	**Species**	**Conc. (mM)**
Na^+	0.505	Cl^-	1.025
Ca^{2+}	0.5	HCO_3^{2-}	0.5
Mg^{2+}	0.125	SO_4^{2-}	0.125
K^+	0.025	Br^-	0.005

2.2.3 Test solution preparation

For each toxicity test, a 32000 μg/L nominal Dy stock solution (usually 250 mL) was made using the 1000±10 mg/L Dy atomic absorption standard (AAS, stabilized in 7% HNO_3; Inorganic Ventures, Mississauga, ON) and MSM (same medium used for cultures in Section 2.2.2). The Dy stock solution was then pH adjusted to 7.3 by adding known quantities of KOH. A volume of 4L of MSM was prepared and pH adjusted to 7.3 (see Section 2.2.2). Test solutions (500 mL each) at concentrations of 0 (control; MSM), 200, 800, 1600, 3200 and 6400 μg Dy/L were then prepared by mixing the Dy stock solution and MSM at appropriate ratios. Following mixing, 250 ml of each test solution was added to 2 polypropylene test beakers (400 mL, tri-cornered, Fisher Scientific, Nepean, ON). In addition, 2 pieces of cotton gauze (5 cm x 10 cm) were pre-soaked for 24 h in 20 ml of each test solution in 50 mL plastic beakers. All the containers were covered and held under culture conditions for 24 hours prior to testing.

For TMF tests, extra chemicals were added to MSM prior to use of this medium for the preparation of the Dy stock solution and test solutions. Table 2.2 lists the type and concentration of the TMFs tested as well as the specific chemical and source for each type of TMF used. For tests using 3-(*N*-morpholino)propanesulfonic acid (MOPS) as a pH buffer, the MOPS concentration was 1mM.

Table 2.2 Summary of tests for different types and concentrations of TMFs.

TMF	Conc. (mM)	Chemical	Source
Ca^{2+}	0.5(MSM) 1 2	$CaCl_2$	Fisher Scientific
Mg^{2+}	0.125(MSM) 0.25 0.5 2	$MgSO_4$	Sigma-Aldrich
Na^+	0.505(MSM) 1 2	NaCl	Sigma-Aldrich
DOC	4 (mg/L) 8 (mg/L)	Suwannee River DOC (powder)	International Humic Substances Society
pH	6.3	MOPS[a]	Sigma-Aldrich
	7.3(MSM)	HNO_3	Fisher Scientific
	8.3	KOH	Fisher Scientific

[a] MOPS, 3-(*N*-morpholino)propanesulfonic acid

2.2.4 *H. azteca* acute toxicity test

Hyalella azteca toxicity testing procedures followed modifications of U.S. Environmental Protection Agency's (USEPA) "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, Second Edition" (EPA/600/R-99/064; USEPA, 2000) and Environmental Canada's (EC) "Biological Test Method: Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca*" (EPS1/RM/33; EC, 2013). In the current study, a static 96 h water-only acute toxicity test was conducted. Test treatments consisted of 1-2 controls (for all the pH tests and the second trial of 8 mg/L

DOC test, there was an extra control without MOPS or DOC) and a series of exposure solutions (5-6 metal concentrations; mainly 200, 800, 1600, 3200, 6400 µg Dy/L) in duplicate (prepared according to section 2.2.3). At test initiation, one pre-soaked cotton gauze was added to each test beaker and test solutions were measured for pH and temperature. Ten neonates were then added to each beaker and the beaker was covered with a plastic petri dish to reduce evaporation. Test beakers were placed in the same incubator used for *H. azteca* cultures (23°C, 16h:8h light:dark cycle). Tests were 96 hours in duration and there was no feeding during the tests. At the end of the tests, each container was assessed for mortality of *H. azteca* and water quality (pH and temperature, MeterLab® PHM240 pH/ion meter, Radiometer Analytical, London, ON).

2.2.5 *D. pulex* acute toxicity test

Daphnia pulex testing procedures generally followed the EC standard aquatic biological test method for *Daphnia* spp. (EPS1/RM/11; EC, 1990). In this study, a static 48 h water-only acute toxicity test was conducted. Test solutions consisted of 100 ml of MSM control and 500, 1000, 2000, 4000 and 8000 µg Dy/L in 400 mL polypropylene beakers (2 replicates). Each test beaker contained 10 neonates that were less than 24 hours old. Test beakers were covered with plastic petri dishes and held in the same incubator used for *H. azteca* tests (23°C, 16h:8h light:dark cycle). Tests were 48 hours in duration and there was no feeding during the test. At the end of the tests, each container was assessed for water quality parameters (pH and temperature) and mortality of neonates.

2.2.6 Water chemistry of test solutions

Water samples were collected from test solutions for measurements of Dy and inorganic cation (Ca^{2+}, Mg^{2+}, Na^{+}) concentrations. For each acute toxicity test, solution samples were taken immediately following solution preparation (1 day prior to the start of the test; referred to as time 0 h) and at the end of the test (120 h for *H. azteca*; 60 h for *D. pulex*). A total volume of 10 ml was collected from all the test concentrations and controls (5 ml x 2 replicates). Solutions were stirred before sampling and a syringe was used to collect the sample. 2 types of 10 ml samples were collected to measure total concentration (whole water sample) and dissolved concentration (whole water filtered through a 0.45 µm filter (Tuffryn). Each sample was preserved with 200 µL of HNO_3 (Fisher Scientific, Nepean, ON; TraceMetal^{TM} Grade, 70%; approximately 2% acid). The samples were kept in 15 mL conical centrifuge tubes and stored at room temperature. Total and dissolved Dy concentrations were measured by Optima 8000 ICP-OES spectrometers (PerkinElmer, Woodbridge, ON). Inorganic cation concentrations were measured by SpectrAA 880 spectrophotometer (Varian, Mississauga, ON) or Optima 8000 ICP-OES spectrometers (PerkinElmer, Woodbridge, ON).

In addition to the toxicity tests, a test using the same Dy concentrations in MSM and the same test conditions but without any organisms was conducted. The purpose of the test was to study the change in total and dissolved Dy concentrations over time in the absence of test organisms. For this test, 4 replicates per concentration were used and solution samples were collected more frequently at 0 h (immediately following solution preparation), 12 h, 24 h (start of the toxicity test), 72 h and 120 h (end of the 96 h test). After the 120 h samples were taken, 3 mL of 70% nitric acid (Fisher Scientific, Nepean, ON; TraceMetal^{TM} Grade) was added to each test beaker to create a 2% acidity and a final set of total and dissolved samples were taken (120 h acid; Figure 2.4).

Water samples were also collected from test solutions for measurements of DOC concentrations (where applicable). For DOC analysis, 50 mL of test solution was filtered (0.45 µm, Tuffryn) and stored in 50 mL conical centrifuge tubes at 4°C in the dark. DOC content of the solution samples were measured by Shimadzu TOC-L Total Organic Carbon Analyzer (Mandel Scientific, Guelph, ON).

2.2.7 Data analysis

Visual MINTEQ 3.1 was used to predict the formation of Dy precipitation during the tests. LC_{50} values for the acute toxicity tests were calculated using measured total and dissolved Dy concentrations and produced by IBM SPSS Statistics 22 software. A Probit analysis was applied to calculate the LC_{50} data and confidence intervals. Dy concentrations were used as covariates and there was no transformations. The total number of neonates added to each test concentration was entered as "Total Observed" and the number of dead neonates was entered as "response frequency" (Jia, 2006). In options, the heterogeneity factor was not used since the use of this factor would sometimes make the model unable to calculate the confidence interval. Natural response rate was not filled in since the data used for LC_{50} calculation had already taken mortality of control into account. To compare LC_{50} values, no overlap of the confidence intervals would be considered as significantly different. If the confidence intervals overlapped, Litchfield-Wilcoxon method would be used to determine whether the LC_{50}s were significantly different (EC, 2005). Graphs in this thesis were made by SigmaPlot 12.

2.2.8 BLM development

The method used to build a BLM for Dy generally followed the Cu BLM built by De Schamphelaere and Janssen (2002). To build the BLM, Windermere Humic

Aquaous Model (version 7: WHAM7) was used to predict the speciation (mainly free ion concentration which was also used for LC_{50} calculation) of Dy based on the water chemistry. The temperature was set at 23°C and pCO_2 was fixed at $3.96*10^{-4}$ atm for all the tests (CO_2 data acquired from co2now.org). Cation concentrations and pH were filled in using the measured data, while the anion concentrations which had not been measured were calculated based on mass conservation. Matlab R2014b was used to calculate the important parameters that made up the BLM. In general, only simple linear regression and matrix operation were applied. The program is provided in Appendix A.

2.3 Results

2.3.1 General trends in Dy chemistry

Total and dissolved Dy concentrations in *H. azteca* toxicity tests

General trends for measured total and dissolved Dy concentrations in test solutions are presented in this section. Data from 7 typical *H. azteca* toxicity tests demonstrated that approximately 15 % to 35 % of total Dy concentration was present in the dissolved fraction at 0 h, and that precipitation of Dy increased at higher nominal Dy concentration (Figure 2.3). There was a decrease in total and dissolved Dy concentrations from 0 h and 120 h which may be a function of adsorption to the test container, as well as absorption and uptake by *H. azteca* (Figure 2.3).

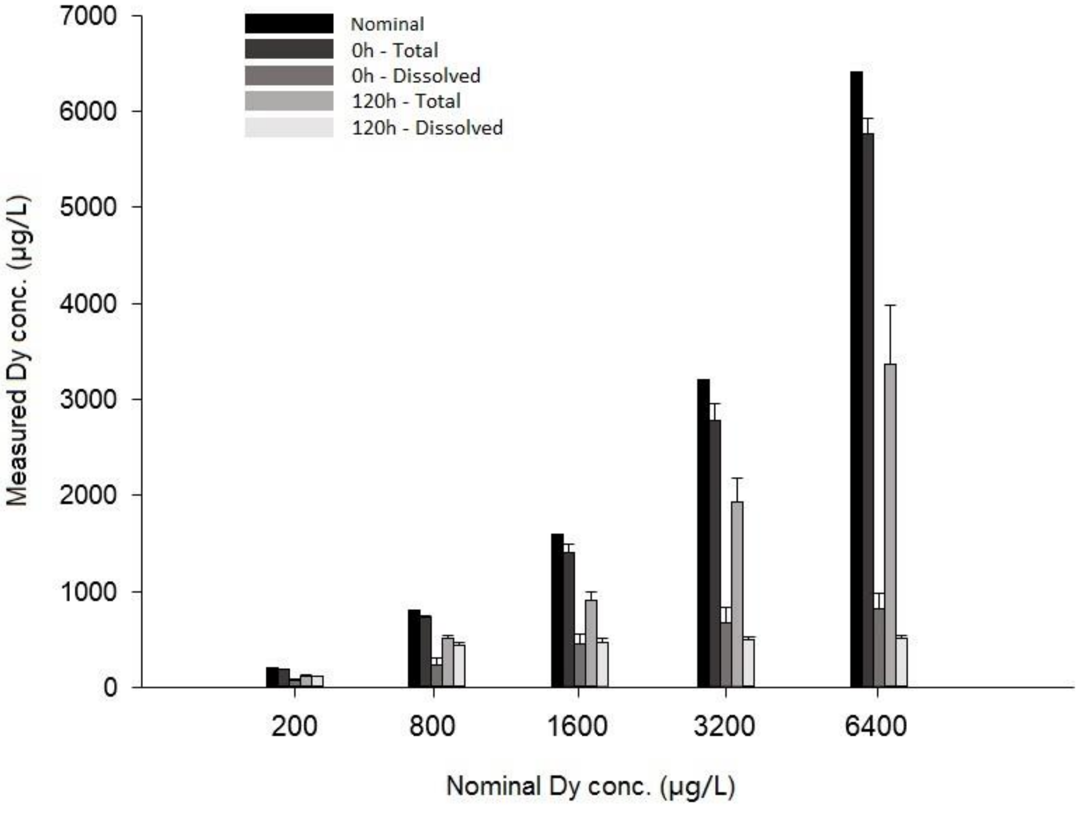

Figure 2.2 Nominal and measured (total and dissolved) Dy concentrations (mean ± standard error, n=7, µg/L) at 0 h and 120 h for various test exposures from typical *H. azteca* toxicity tests.

Total and dissolved Dy concentrations in test solutions without *H. azteca*

A test was conducted without *H. azteca* to examine changes in total and dissolved Dy concentrations as a function of adsorption to the test container and precipitation of dissolved Dy in the absence of absorption and uptake by *H. azteca.* Total Dy concentration at 120 h was moderately elevated in test solutions following the addition of acid (approximately 2% acid) (Figure 2.4a); this trend was more pronounced at higher nominal concentrations and indicated probable Dy desorption from the surfaces of the test container. In comparison, dissolved Dy concentration at 120 h was significantly elevated in test solutions with 2% acid relative to test solutions without acid (Figure 2.4b); the trend for dissolved Dy was also more pronounced at higher nominal concentrations. The increase in dissolved Dy under acidic conditions is a function of primarily increased solubility and to a lesser extent desorption.

Measurements of total and dissolved Dy concentrations over time (0, 12, 24, 72 and 120 h) were also determined for the test without *H. azteca* (Figure 2.5). There was clear evidence of the limited solubility of Dy over the course of the entire test period from 0 h (time of new solution preparation), 24 h (start of test) and 120 h (end of 96 h test). Raw data is provided in Appendix B.

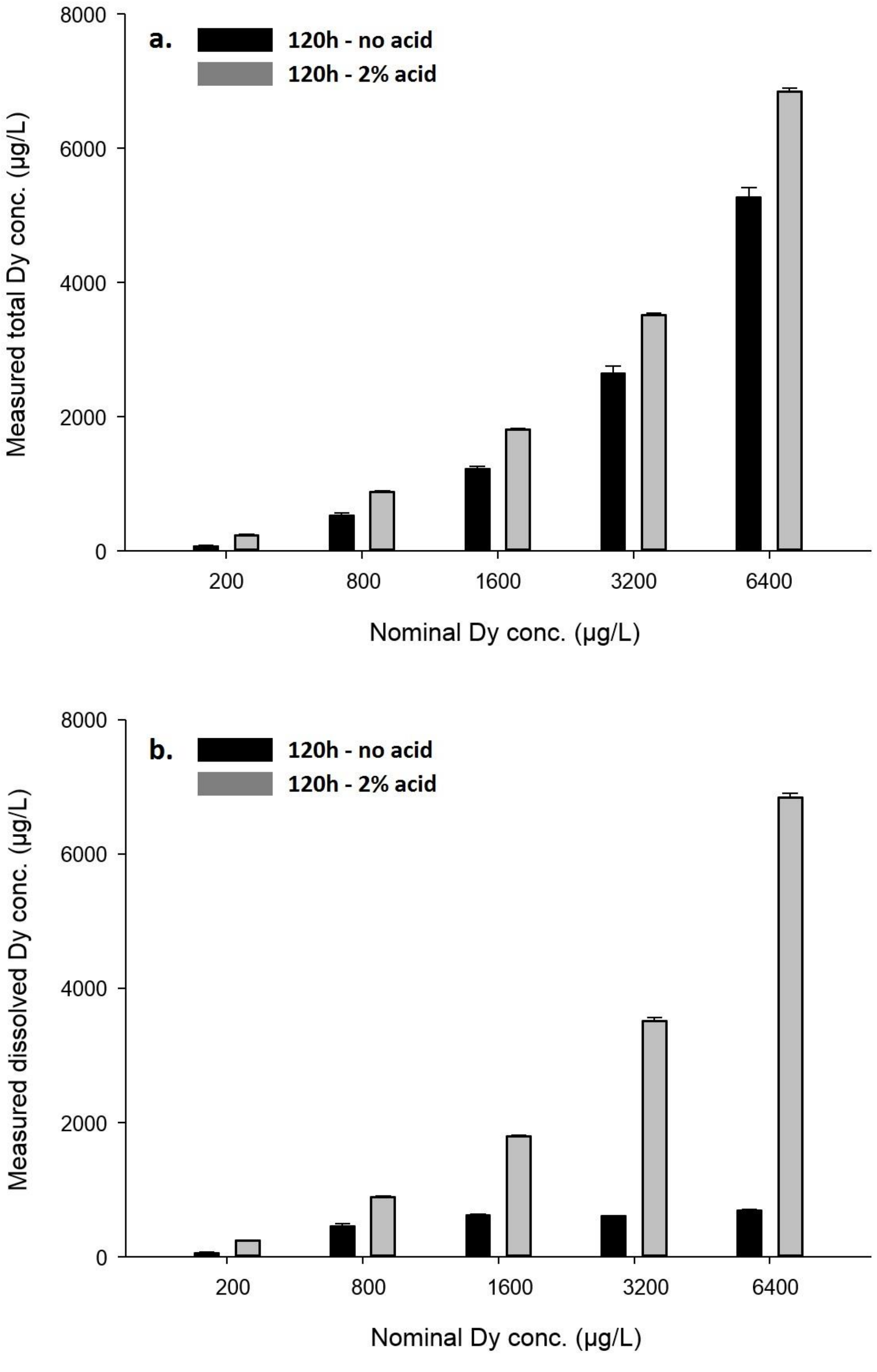

Figure 2.3 Measured total (a) and dissolved (b) Dy concentrations (including error bars, µg/L) before and after introducing acid to samples for different nominal Dy concentrations.

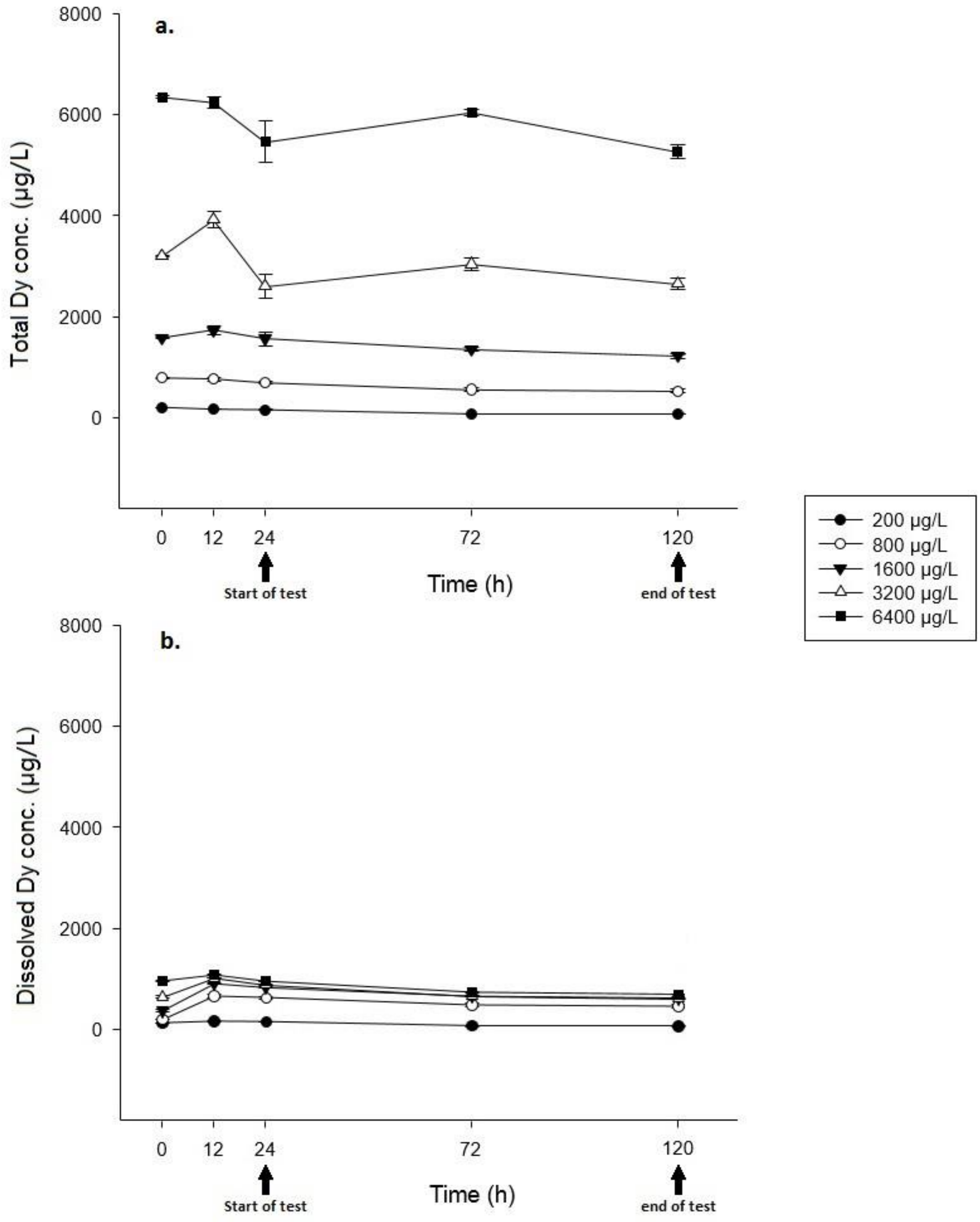

Figure 2.4 Measured total (a) and dissolved (b) Dy concentrations (including error bars, µg/L) over the duration of the test period for a test without *H. azteca*.

Formation of Dy precipitates in *H. azteca* toxicity tests

Due to the low dissolved Dy concentrations relative to total Dy concentrations, Visual MINTEQ 3.1 was applied to assess the formation of two possible Dy precipitates ($Dy(OH)_3$ and $Dy_2(CO_3)_3$). By analyzing the most representative Dy test concentrations (200, 800, 1600, 3200, 6400, 12800 µg/L) and water chemistry of MSM, the model predicted formation of the precipitate, $Dy(OH)_3$, at pH 7.6 and Dy concentrations greater than 800 µg/L (Table 2.3).

Table 2.3 Formation of Dy precipitates of various test exposures in MSM medium predicted by Visual MINTEQ 3.1.

				Precipitation formation			
Experimental environment	**Total Dy conc. (µg/L)**	**Dissolve Dy conc. (µg/L)**	**Dissolved Dy %**	**$Dy(OH)_3$ (s) (µg/L)**	**%**	**$Dy_2(CO_3)_3$ (s) (µg/L)**	**%**
23℃ pH=7.6	200	200	100	0	0	0	0
	800	800	100	0	0	0	0
	1600	1224	76.4	376	23.6	0	0
	3200	1224	38.2	1976	61.8	0	0
	6400	1224	19.1	5176	80.9	0	0
	12800	1224	9.6	11576	90.4	0	0

2.3.2 Acute toxicity and water chemistry data for *H. azteca* (4 sources) and *D. pulex*

Water chemistry and mortality data

Acute toxicity tests were conducted to assess the effects of Dy on *H. azteca* from 4 different sources and *D. pulex*. Water chemistry data for all the successfully conducted Dy toxicity tests with *H. azteca* from different sources and *D. pulex* are provided in Tables 2.4 and 2.5. Water chemistry parameters including pH and inorganic cation concentrations were relatively constant among the tests and different exposure concentrations of a single test. There was an unusual increase in Ca^{2+} and Na^{+} concentrations with an increase of Dy concentration for the *D. pulex* and FH *H. azteca* (trial 2) tests, respectively. Generally, there was a mild pH increase over the course of the tests. Concentration gradients for measured total and dissolved Dy concentrations were more pronounced at 0 h than 120 h. Percentage dissolved Dy concentrations (D/T %) were approximately 50% or lower at 0 h and decreased with increasing nominal concentration due to Dy precipitation (see section 2.3.1). Percentage mortality increased along the Dy concentration gradient and 100% mortality was observed at higher test concentrations for all tests except for HL trial 2 and *D. pulex* tests.

Table 2.4 A summary of the water chemistry data and mortality data of *H. azteca* (from 4 different sources: Fort Hope, FH; Daisy Lake, DL; Low Water Lake, LW; Hannah Lake, HL) (T, total Dy concentration; D, dissolved Dy concentration). Mean values are shown for water chemistry data (n = 2).

Test	Dy nominal conc.	Water chemistry[a]					Measured Dy (µg/L)						% Mortality
							newly made (0h)			end of test (120h)			
		pH (start)	pH (end)	$[Ca^{2+}]$ (µM)	$[Mg^{2+}]$ (µM)	$[Na^{+}]$ (µM)	T	D	D/T %	T	D	D/T %	
FH	0	7.4	7.6	557	139	562	0	2	n/a	0	2	n/a	0
Trial 1	200	7.4	7.6	567	156	579	189	37	19.6	99	93	93.8	5
	800	7.5	7.6	589	166	578	750	136	18.2	485	409	84.3	25
	1600	7.5	7.7	596	167	587	1501	337	22.4	999	468	46.8	45
	3200	7.5	7.6	574	165	590	3040	707	23.3	2133	563	26.4	90
	6400	7.5	7.7	514	163	580	6138	660	10.7	4548	485	10.7	100
FH	0	7.6	7.5	475	152	516	0	11	n/a	1	9	n/a	0
Trial 2	200	7.6	7.7	511	158	531	248	121	48.7	113	103	91.3	0
	800	7.5	7.6	480	149	559	886	415	46.8	547	490	89.5	35
	1600	7.6	7.7	510	157	667	1603	335	20.9	860	496	57.7	80
	3200	7.6	7.6	476	146	697	3452	571	16.5	4886	536	11.0	80
	6400	7.6	7.6	502	155	885	7275	757	10.4	4597	598	13.0	100
DL	0	7.6	7.8	568	157	543	1	0	n/a	6	1	n/a	5
Trial 1	200	7.6	7.8	568	176	563	198	103	52.2	82	77	93.7	0
	800	7.6	7.7	594	190	566	840	216	25.7	402	386	96.2	50
	1600	7.6	7.7	602	187	572	1083	320	29.6	471	491	104.3	100
	3200	7.6	7.7	566	182	580	1563	551	35.2	563	520	92.3	100
	6400	7.6	7.6	528	177	582	6927	1206	17.4	629	527	83.8	100
DL	0	-	7.8	639	167	587	0	10	n/a	3	7	n/a	10
Trial 2	200	-	7.7	637	185	600	208	69	33.2	170	168	99.2	10
	800	-	7.8	642	187	584	816	258	31.6	604	599	99.1	60
	1600	-	7.8	646	185	575	1708	522	30.6	1265	553	43.7	85
	3200	-	7.7	593	173	559	3328	677	20.4	608	533	87.7	95
	6400	-	7.7	546	170	555	6563	1005	15.3	5596	581	10.4	100

Table 2.4 continued

Test	Dy nominal conc.	Water chemistry[a]					Measured Dy (µg/L)						% Mortality
		pH (start)	pH (end)	$[Ca^{2+}]$ (µM)	$[Mg^{2+}]$ (µM)	$[Na^{+}]$ (µM)	newly made (0h)			end of test (120h)			
							T	D	D/T %	T	D	D/T %	
LW Trial 1	0	7.5	7.7	606	159	585	1	1	n/a[c]	9	2	n/a	5
	200	7.6	7.7	601	173	572	199	74	37.2	137	136	99.5	45
	800	7.5	7.7	637	184	596	803	266	33.2	563	569	101.1	70
	1600	7.6	7.7	644	186	603	1580	419	26.5	570	558	97.8	81
	3200	7.6	7.8	646	194	622	3108	724	23.3	2764	522	18.9	100
	6400	7.6	7.8	616	187	631	6029	994	16.5	3440	549	16.0	100
LW Trial 2	0	7.7	8.2	584	158	569	2	1	n/a	7	2	n/a	20
	200	7.7	8.1	581	177	571	327	93	28.4	282	278	98.5	35
	800	7.6	8.0	605	186	589	1416	273	19.3	925	533	57.6	75
	1600	7.6	8.0	607	182	574	2676	370	13.8	2174	522	24.0	95
	3200	7.6	7.9	577	181	583	5363	533	9.9	3303	605	18.3	100
HL[b] Trial 1	0	7.7	7.8	557	153	539	10	15	n/a	19	6	n/a	5
	200	7.8	7.7	585	190	671	96	36	37.8	52	43	82.7	20
	800	7.8	7.8	595	181	581	251	114	45.2	155	152	98.0	5
	1600	7.7	7.7	608	181	584	426	161	37.8	292	295	101.2	65
	3200	7.7	7.7	558	177	570	771	283	36.7	496	459	92.7	100
	6400	7.7	7.8	525	172	569	3588	508	14.2	519	462	88.9	100
HL[b] Trial 2	0	7.8	8.1	559	152	537	7	9	n/a	13	6	n/a	5
	200	7.5	7.9	569	178	559	309	158	51.1	234	230	98.2	15
	800	7.6	7.8	616	186	586	1282	241	18.8	544	506	93.2	48
	1600	7.6	7.8	624	187	587	2678	363	13.6	524	477	91.2	67
	3200	7.6	7.8	604	187	587	5215	510	9.8	693	484	69.9	95

[a] pH at the start and the end of each test; Ca^{2+}, Mg^{2+}, Na^{+} concentrations represent mostly the average of start and end of each test but a few are only for the start or end

[b] data from Oliver Vukov, McGeer Lab, Wilfrid Laurier University

[c] n/a, not applicable

Table 2.5 A summary of the water chemistry data and mortality data of *D. pulex* acute toxicity test (T, total Dy concentration; D, dissolved Dy concentration). Mean values are shown for water chemistry data (n = 2).

Test	Dy nominal conc.	Water chemistry[a]					Measured Dy (μg/L)						% Mortality
							newly made (0h)			end of test (72h)			
		pH (start)	pH (end)	$[Ca^{2+}]$ (μM)	$[Mg^{2+}]$ (μM)	$[Na^{+}]$ (μM)	T	D	D/T %	T	D	D/T %	
D. pulex	0	7.6	7.5	562	146	540	-4	-3	n/a[b]	-4	-3	n/a	0
	500	7.5	7.7	603	173	549	474	88	18.5	345	333	96.7	5
	1000	7.5	-	627	172	548	946	203	21.5	552	527	95.5	50
	2000	7.5	7.6	687	176	557	1892	279	14.8	639	546	85.4	50
	4000	7.6	7.6	738	172	553	3793	512	13.5	787	567	72.1	60
	8000	7.5	7.6	826	167	560	7704	692	9.0	1134	598	52.7	90

[a] pH at the start and the end of each test; Ca^{2+}, Mg^{2+}, Na^{+} concentrations represent mostly the average of start and end of each test but a few are only for the start or end

[b] n/a, not applicable

Toxicity data

Mortality data from Tables 2.4 and 2.5 were used to calculate the LC_{50}s and 95% confidence intervals of Dy for total, dissolved and calculated free ion Dy concentrations at 0 h only (Table 2.6 and Figure 2.6). Dy concentrations at 120 h (end of test) were not used in LC_{50} calculations due to a lack of concentration gradient for dissolved Dy concentrations with an increase in nominal Dy concentration. LC_{50} values calculated for each *H. azteca* source showed consistency between the 2 trials based on intersecting confidence intervals with the exception of HL. Similar sensitivities were observed among *H. azteca* from the 3 locations in the Sudbury area (DL, LW and HL) and *H. azteca* from these sources were more sensitive to Dy than *H. azteca* from FH. *H. azteca* from FH showed a similar tolerance to Dy as *D. pulex.*

Table 2.6 Dy LC_{50} and 95% confidence interval (CI) for *H. azteca* (different sources) and *D. pulex* acute toxicity tests.

Test	LC_{50} & 95% CI (0 h)[a]					
	Total Dy		**Dissolved Dy**		**Calculated free Dy ion**	
	µg/L	**nM**	**µg/L**	**nM**	**µg/L**	**nM**
FH (trial 1)	1665 (1352-2077)	10246 (8320-12782)	376 (301-477)	2314 (1852-2935)	14.8 (11.7-19.0)	91 (72-117)
FH (trial 2)	1160 (974-1374)	7138 (5994-8455)	372 (317-430)	2289 (1951-2646)	13.0 (10.7-15.3)	80 (66-94)
DL (trial 1)	723 (593-842)	4449 (3649-5182)	204 (174-237)	1255 (1071-1458)	6.5 (5.4-7.5)	40 (33-46)
DL (trial 2)	985 (691-1319)	6062 (4252-8117)	276 (206-347)	1698 (1268-2135)	8.0 (5.9-10.2)	49 (36-63)
LW (trial 1)	625 (353-895)	3846 (2172-5508)	186 (118-252)	1145 (726-1551)	5.9 (3.8-7.8)	36 (23-48)
LW (trial 2)	790 (414-1158)	4862 (2548-7126)	145 (90-195)	892 (554-1200)	2.9 (1.7-4.0)	18 (10-25)
HL (trial 1)[b]	370 (304-459)	2277 (1871-2825)	146 (120-178)	898 (738-1095)	3.7 (3.0-4.5)	23 (18-28)
HL (trial 2)[b]	1945 (1451-2533)	11969 (8929-15588)	277 (231-326)	1705 (1422-2006)	7.8 (6.5-9.1)	48 (40-56)
D. pulex	2984 (2215-3980)	18363 (13631-24492)	357 (289-435)	2197 (1778-2677)	14.7 (11.9-18.1)	90 (73-111)

[a] toxicity data calculated based on measured Dy concentrations at 0 h

[b] data from Oliver Vukov, McGeer Lab, Wilfrid Laurier University

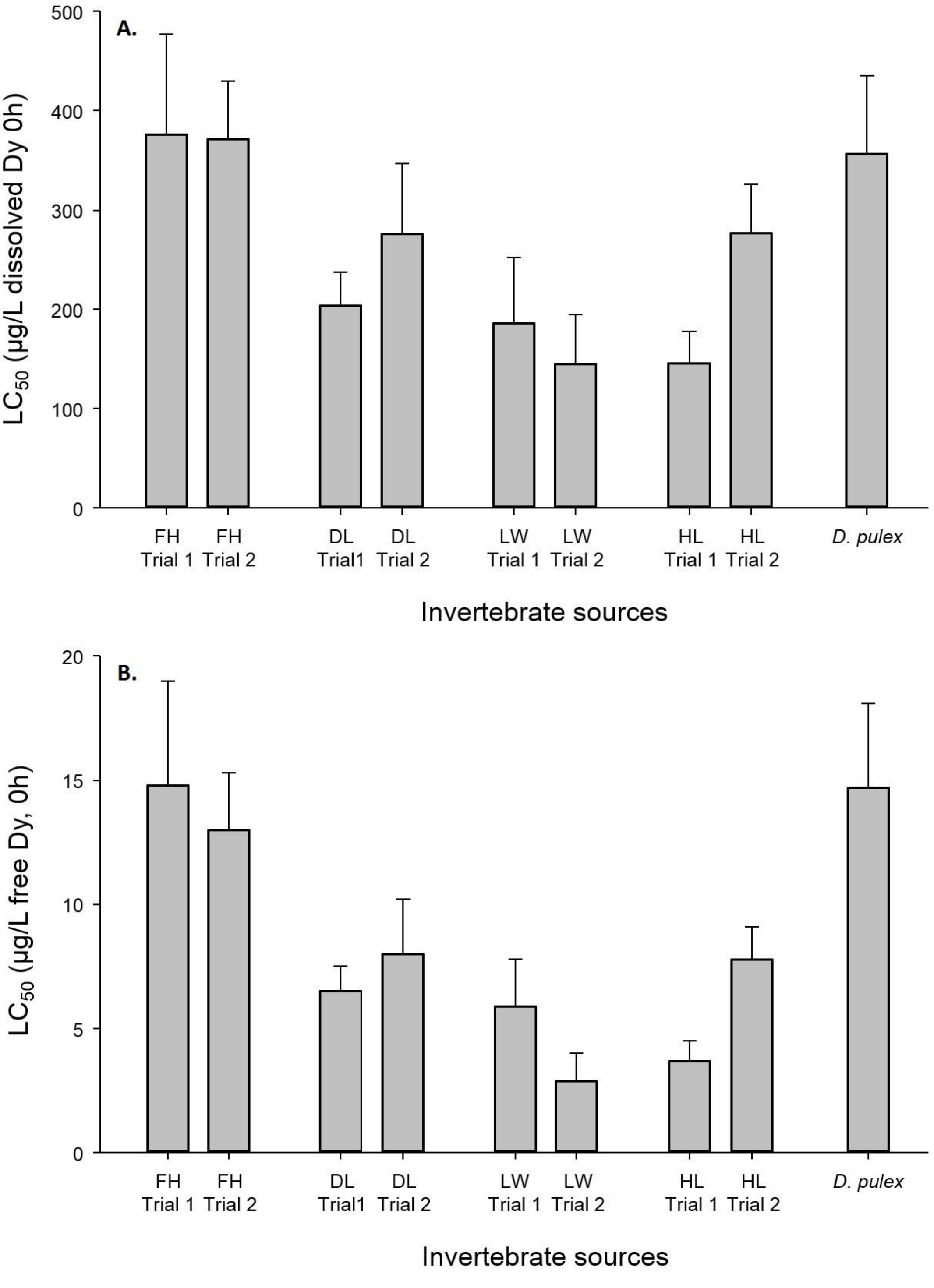

Figure 2.5 Comparison of the sensitivities of *H. azteca* from 4 different sources and *D. pulex* for dissolved Dy concentration (A) and free Dy ion concentration (B) at 0 h. Error bars show upper 95% confidence limit.

2.3.3 Effects of TMFs on Dy toxicity and water chemistry

Water chemistry and mortality data

Data for dissolved Dy concentrations indicated that some TMFs such as DOC and pH had a strong influence on dissolved Dy concentrations. The effects of inorganic cations were tested at cation concentrations that were 2 and 4 times greater than MSM for Ca^{2+}, Mg^{2+} (also 16 times) and Na^{+}. Dissolved Dy concentrations at 0 h increased slightly at elevated cation concentrations relative to MSM but there was no apparent effect on dissolved Dy concentrations at the end of tests (120 h). Additions of 4 and 8 mg/L (nominal) of SR DOC resulted in increased Dy solubility: at 6400 µg Dy/L (nominal), 0 h dissolved Dy concentration increased by 5-6 times while 120 h dissolved Dy concentration increased by approximately 4 times relative to MSM with no added DOC (exception, trial 2 at 8 mg/L DOC). Changes in pH had the strongest impact on Dy solubility: a pH drop of 0.9 (to pH 6.7) resulted in a 8 times increase of 0 h dissolved Dy concentration at 6400 µg Dy/L (nominal), while a pH increase of 0.5 (to pH 8.1) caused about a 50% reduction of 0 h and 120 h dissolved Dy concentrations relative to pH 7.6 (MSM).

Table 2.7 Summary of water chemistry data and mortality data of *H. azteca* (from Fort Hope) acute toxicity tests with different TMFs (T, total Dy concentration; D, dissolved Dy concentration). Shaded areas are used to distinguish between different types of TMF tests. Mean values are shown for water chemistry data (n = 2).

Test	Dy nominal conc.	Water chemistry					Measured Dy (µg/L)						% Mortality
		pH (start)	pH (end)	[Ca^{2+}] (µM)	[Mg^{2+}] (µM)	[Na^{+}] (µM)	newly made (0h)			end of test (120h)			
							T	D	D/T %	T	D	D/T %	
MSM Trial 1	0	7.4	7.6	557	139	562	0	2	n/a[a]	0	2	n/a	0
	200	7.4	7.6	567	156	579	189	37	19.6	99	93	93.8	5
	800	7.5	7.6	589	166	578	750	136	18.2	485	409	84.3	25
	1600	7.5	7.7	596	167	587	1501	337	22.4	999	468	46.8	45
	3200	7.5	7.6	574	165	590	3040	707	23.3	2133	563	26.4	90
	6400	7.5	7.7	514	163	580	6138	660	10.7	4548	485	10.7	100
MSM Trial 2	0	7.6	7.5	475	152	516	0	11	n/a	1	9	n/a	0
	200	7.6	7.7	511	158	531	248	121	48.7	113	103	91.3	0
	800	7.5	7.6	480	149	559	886	415	46.8	547	490	89.5	35
	1600	7.6	7.7	510	157	667	1603	335	20.9	860	496	57.7	80
	3200	7.6	7.6	476	146	697	3452	571	16.5	4886	536	11.0	80
	6400	7.6	7.6	502	155	885	7275	757	10.4	4597	598	13.0	100
1mM Ca^{2+} Trial 1	0	7.7	7.8	1084	147	553	2	13	n/a	4	2	n/a	5
	200	7.6	7.8	1077	162	562	170	167	98	117	112	96	10
	800	7.7	7.7	1105	180	577	704	606	86	455	505	111	30
	1600	7.6	7.7	1113	179	575	1480	975	66	832	531	64	60
	3200	7.6	7.7	1099	177	583	2855	1319	46	1213	510	42	60
	6400	7.6	7.7	1061	174	577	5778	1446	25	813	506	62	100
1mM Ca^{2+} Trial 2	0	7.5	7.6	941	139	492	0	1	n/a	0	0	n/a	5
	200	7.5	7.7	1025	155	559	165	52	32	80	78	97	0
	800	7.6	7.7	961	142	582	659	186	28	396	349	88	20
	1600	7.5	7.7	988	149	655	1418	433	31	899	462	51	40
	3200	7.5	7.6	965	142	705	2779	621	22	1625	476	29	85
	6400	7.5	7.6	1037	151	906	5508	969	18	2258	537	24	100

Table 2.7 continued

Test	Dy nominal conc.	Water chemistry					Measured Dy (µg/L)						% Mortality
							newly made (0h)			end of test (120h)			
		pH (start)	pH (end)	$[Ca^{2+}]$ (µM)	$[Mg^{2+}]$ (µM)	$[Na^{+}]$ (µM)	T	D	D/T %	T	D	D/T %	
1mM Ca^{2+} Trial 3	0	7.4	7.5	1044	126	568	0	2	n/a	0	2	n/a	0
	200	7.5	7.6	1070	143	589	194	70	36	142	134	94	20
	800	7.5	7.5	1068	158	599	752	278	37	551	526	95	80
	1600	7.5	7.5	1144	162	593	1471	612	42	1087	534	49	90
	3200	7.5	7.5	1148	154	584	2906	877	30	1505	545	36	95
	6400	7.4	7.5	1096	153	583	5900	1187	20	5021	600	12	100
2mM Ca^{2+} Trial 1	0	7.6	7.9	2010	156	554	0	12	n/a	2	7	n/a	0
	200	7.6	7.8	2020	163	556	181	82	45	131	130	99	0
	800	7.6	7.8	2048	176	564	771	319	41	556	516	93	0
	1600	7.5	7.7	2002	174	559	1526	528	35	982	558	57	15
	3200	7.5	7.7	1995	172	564	3062	692	23	1124	533	49	70
	6400	7.5	7.7	2008	176	578	6189	1058	17	3144	586	19	95
0.25mM Mg^{2+} Trial 1	0	7.6	-	655	334	589	0	1	n/a	0	1	n/a	0
	200	7.6	-	606	317	558	n/a	n/a	n/a	n/a	n/a	n/a	0
	800	7.6	-	638	352	571	802	171	21	537	465	87	15
	1600	7.6	-	638	349	566	1515	353	23	1155	532	46	70
	3200	7.6	-	611	346	576	3367	565	17	2773	554	20	75
	6400	7.5	-	556	338	563	6176	950	15	5790	603	10	100
0.5mM Mg^{2+} Trial 1	0	7.8	7.8	583	632	536	0	1	n/a	0	n/a	n/a	0
	200	7.9	7.7	589	663	565	194	44	22	143	138	97	5
	800	7.8	7.8	625	687	563	770	152	20	588	521	89	55
	1600	7.7	7.9	683	704	582	1540	290	19	995	549	55	85
	3200	7.7	7.8	726	667	563	3012	462	15	1412	508	36	90
	6400	7.6	7.7	854	601	557	6063	657	11	n/a	n/a	n/a	100

Table 2.7 continued

Test	Dy nominal conc.	Water chemistry						Measured Dy (µg/L)						% Mortality
								newly made (0h)			end of test (120h)			
		pH (start)	pH (end)	[Ca^{2+}] (µM)	[Mg^{2+}] (µM)	[Na^{+}] (µM)	DOC (mg/L)	T	D	D/T %	T	D	D/T %	
2mM Mg^{2+} Trial 1	0	7.6	7.7	533	2602	643	-	0	9	n/a	2	4	n/a	0
	200	7.6	7.7	514	2530	664	-	206	103	50	119	105	88	0
	800	7.6	7.7	484	2239	626	-	836	399	48	613	553	90	0
	1600	7.6	7.7	512	2502	753	-	1601	462	29	1340	758	57	65
	3200	7.6	7.7	458	2237	725	-	2690	617	23	2658	681	26	95
	6400	7.6	7.7	592	2586	1030	-	5866	1047	18	3265	769	24	100
1mM Na^{2+} Trial 1	0	7.7	7.6	578	152	936	-	0	3	n/a	0	1	n/a	0
	200	7.6	7.7	615	183	1258	-	183	70	38	127	126	99	10
	800	7.7	7.5	638	194	1299	-	741	209	28	624	443	71	35
	1600	7.6	7.5	632	197	1103	-	1465	474	32	1135	549	48	80
	3200	7.7	7.5	593	186	1056	-	2923	714	24	2491	592	24	80
	6400	7.7	7.5	565	184	1079	-	5696	843	15	2510	623	25	95
2mM Na^{+} Trial 1	0	7.5	7.7	442	142	1842	-	6	0	n/a	4	0	n/a	0
	200	7.6	7.8	446	143	1907	-	154	160	104	115	110	95	10
	800	7.4	7.7	443	143	1973	-	727	608	84	571	461	81	30
	1600	7.5	7.7	442	143	2125	-	2078	774	37	1944	500	26	75
	3200	7.5	7.7	437	142	1922	-	3075	880	29	3257	524	16	95
	6400	7.4	7.6	437	143	2122	-	5857	1174	20	6651	639	10	100
4mg/L SR[b] DOC Trial 1	0	7.6	7.6	564	138	592	9.6	0	4	n/a	5	6	n/a	5
	800	7.6	7.7	585	162	605	10.1	750	509	68	679	543	80	10
	1600	7.6	7.7	601	163	590	9.5	1545	n/a	n/a	1396	737	53	50
	3200	7.6	7.7	586	162	597	9.6	3013	1760	58	3325	1242	37	90
	6400	7.6	7.6	550	165	626	11.3	5994	3525	59	7757	2393	31	100
	12800	7.5	7.5	540	158	596	7.5	12326	6066	49	12002	4119	34	100

Table 2.7 continued

Test	Dy nominal conc.	Water chemistry						Measured Dy (µg/L)						% Mortality
		pH (start)	pH (end)	$[Ca^{2+}]$ (µM)	$[Mg^{2+}]$ (µM)	$[Na^{+}]$ (µM)	DOC (mg/L)	newly made (0h)			end of test (120h)			
								T	D	D/T %	T	D	D/T %	
8mg/L SR DOC Trial 1	0	7.6	7.6	531	145	552	13.8	0	4	n/a	3	5	n/a	10
	800	7.6	7.7	560	169	576	13.8	745	623	84	659	647	98	5
	1600	7.6	7.7	589	175	604	13.1	1501	1136	76	1233	1080	88	15
	3200	7.6	7.7	576	177	610	13.1	3154	2126	67	2404	1351	56	80
	6400	7.6	7.6	514	172	601	12.2	6052	4380	72	3878	1926	50	95
	12800	7.5	7.5	502	166	585	9.3	11971	6472	54	10680	6009	56	100
8mg/L SR DOC Trial 2	0	7.6	7.6	513	162	531	-	1	1	n/a	9	2	n/a	2.5
	800	7.6	7.6	461	139	478	-	198	68	34	145	137	94	5
	1600	7.6	7.7	544	166	649	-	579	222	38	540	496	92	0
	3200	7.6	7.7	467	139	600	-	768	240	31	704	646	92	10
	6400	7.6	7.7	548	168	813	-	1304	411	32	1275	898	70	65
	12800	7.5	7.7	457	139	785	-	7767	899	12	8232	1578	19	100
pH 6.3 Trial 1	0	6.7	6.8	534	149	559	-	0	10	n/a	4	0	n/a	7
	200	6.7	6.8	530	162	568	-	182	172	94	59	52	89	35
	800	6.7	6.8	574	179	574	-	710	751	106	495	445	90	70
	1600	6.7	6.7	660	181	579	-	1455	1431	98	1273	1286	101	85
	3200	6.7	6.7	665	180	578	-	2810	3026	108	2781	2740	99	90
	6400	6.7	6.7	640	176	580	-	5930	5667	96	5746	5771	100	100
pH 8.3 Trial 1	0	8.1	8.0	564	146	564	-	0	0	n/a	0	0	n/a	5
	200	8.1	8.0	588	162	576	-	180	15	9	101	90	89	5
	800	8.1	8.0	649	168	580	-	723	95	13	497	304	61	70
	1600	8.1	8.0	671	169	577	-	1431	212	15	1108	371	33	70
	3200	8.0	8.0	659	169	571	-	2897	243	8	2363	412	17	100
	6400	8.0	7.9	633	170	584	-	5982	313	5	5006	449	9	100

Table 2.7 continued

Test	Dy nominal conc.	Water chemistry					Measured Dy (µg/L)						% Mortality
							newly made (0h)			end of test (120h)			
		pH (start)	pH (end)	[Ca^{2+}] (µM)	[Mg^{2+}] (µM)	[Na^{+}] (µM)	T	D	D/T %	T	D	D/T %	
pH 8.3 Trial 2	0	8.1	8.0	537	153	572	0	2	n/a	1	1	n/a	7.5
	200	8.1	8.0	561	173	584	175	23	13	84	76	90	15
	800	8.1	8.0	614	180	587	651	74	11	406	255	63	50
	1600	8.1	8.0	631	177	579	899	111	12	572	274	48	75
	3200	8.1	8.0	611	177	580	1748	160	9	1024	304	30	100
	6400	8.1	8.0	591	175	580	4819	310	6	2256	379	17	100

[a] n/a, not applicable

[b] Suwanee River, USA

Toxicity data

Mortality generally increased along the Dy concentration gradient and 100% mortality was always observed at the highest test concentration for all tests except for 2mM Ca^{2+} and 1mM Na^{+} tests (95%, Table 2.7). Mortality data and measured Dy concentrations (at 0 h) in Table 2.7 were used to calculate LC_{50} and 95% confidence intervals for all the TMF tests (Table 2.8). Among the inorganic cations tested, protection against Dy toxicity was observed with increased quantities of Ca^{2+} and Na^{+}: at 2mM Ca^{2+} and Na^{+} LC_{50}s increased by 60% to 100%, approximately 4 times greater compared to lower concentrations of Ca^{2+} and Na^{+} in MSM. However, increases in Mg^{2+} concentration had no effect on LC_{50} values. DOC provided protection against Dy based on LC_{50}s for dissolved Dy concentrations except for trial 2 at 8 mg/L DOC which appeared to have no effect on Dy in solution (see dissolved Dy data in Table 2.7) and had a similar LC_{50s} compared to MSM. The effects of DOC on free Dy ion LC_{50} were unclear since the 4 mg/L DOC test showed a very low LC_{50}. Lower pH was very effective at increasing Dy solubility (Table 2.7) but the LC_{50} indicated lower toxicity than MSM. At higher pH, there was very low dissolved Dy concentration (Table 2.7) yet the calculated LC_{50} indicated greater toxicity than MSM which suggested that Dy precipitates may be toxic.

Table 2.8 Dy LC_{50} and 95% confidence interval (CI) for *H. azteca* (from FH) acute toxicity tests with various TMFs.

TMFs	Test	LC_{50} & 95% CI (0 h)[a]					
		Total Dy		**Dissolved Dy**		**Calculated free Dy ion**	
		µg/L	**nM**	**µg/L**	**nM**	**µg/L**	**nM**
Standard Artificial Medium (MSM)	MSM (trial 1)	1665 (1352-2077)	10246 (8320-12782)	376 (301-477)	2314 (1852-2935)	14.8 (11.7-19.0)	91 (72-117)
	MSM (trial 2)	1160 (974-1374)	7138 (5994-8455)	372 (317-430)	2289 (1951-2646)	13.0 (10.7-15.3)	80 (66-94)
Ca^{2+} addition	1mM Ca^{2+} (trial 1)	1859 (1421-2465)	11440 (8745-15169)	883 (735-1037)	5434 (4523-6382)	30.8 (25.4-36.5)	190 (156-225)
	1mM Ca^{2+} (trial 2)	1701 (1386-2123)	10468 (8529-13065)	428 (359-509)	2634 (2209-3132)	17.1 (14.1-20.8)	105 (87-128)
	1mM Ca^{2+} (trial 3)	709 (467-960)	4363 (2874-5908)	266 (182-351)	1637 (1120-2160)	13.4 (9.1-17.8)	82 (56-110)
	2mM Ca^{2+} (trial 1)	2953 (2478-3597)	18172 (15249-22135)	671 (605-751)	4129 (3723-4622)	31.2 (27.9-35.0)	192 (172-215)
Mg^{2+} addition	0.25mM Mg^{2+} (trial 1)	1881 (1432-2420)	11575 (8812-14892)	354 (286-428)	2178 (1760-2634)	11.8 (9.5-14.4)	73 (58-89)
	0.5mM Mg^{2+} (trial 1)	1114 (838-1430)	6855 (5157-8800)	198 (155-244)	1218 (954-1502)	4.9 (3.8-6.2)	30 (23-38)
	2mM Mg^{2+} (trial 1)	1596 (1374-1843)	9822 (8455-11342)	474 (446-508)	2917 (2745-3126)	15.8 (14.9-17.0)	97 (92-105)

Table 2.8 continued

TMFs	Test	LC$_{50}$ & 95% CI (0 h)[a]					
		Total Dy		Dissolved Dy		Calculated free Dy ion	
		µg/L	nM	µg/L	nM	µg/L	nM
Na$^+$ addition	1mM Na$^+$ (trial 1)	1562 (1130-2068)	9612 (6954-12726)	378 (301-458)	2326 (1852-2818)	14.5 (11.5-17.5)	89 (71-108)
	2mM Na$^+$ (trial 1)	1434 (1131-1784)	8825 (6960-10978)	609 (518-687)	3748 (3188-4228)	23.7 (19.9-27.1)	146 (122-167)
DOC addition	4mg/L SR DOC (trial 1)	1705 (1369-2117)	10492 (8425-13028)	1090 (835-1397)	6708 (5138-8597)	1.1 (0.7-1.5)	7 (4-9)
	8mg/L SR DOC (trial 1)	2617 (2096-3285)	16105 (12898-20215)	1868 (1500-2355)	11495 (9231-14492)	10.3 (5.6-18.0)	63 (34-111)
	8mg/L SR DOC (trial 2)	1236 (1042-1581)	7606 (6412-9729)	397 (337-501)	2443 (2074-3083)	10.9 (9.2-14.0)	67 (57-86)
pH adjustment	pH 6.3 (trial 1)	795 (535-1093)	4892 (3292-6726)	822 (546-1142)	5058 (3360-7028)	233.1 (151.8-327.7)	1434 (934-2017)
	pH 8.3 (trial 1)	866 (681-1112)	5329 (4191-6843)	113 (90-138)	695 (554-849)	1.2 (0.9-1.5)	7 (6-9)
	pH 8.3 (trial 2)	621 (496-781)	3822 (3052-4806)	72 (58-87)	443 (357-535)	0.7 (0.6-0.9)	4 (4-6)

[a] toxicity data calculated based on measured Dy concentrations at 0 h

To assess the effects of cation competition, LC_{50} values based on calculated Dy^{3+} concentrations and 95% confidence intervals (Table 2.8) were plotted with corresponding measured cations (Ca^{2+}, Mg^{2+}, Na^+, H^+) concentrations (Table 2.7) in Figure 2.7. Ca^{2+}, Na^+ and H^+ concentrations showed strong positive linear correlations with LC_{50s} whereas there was no linear correlation between Mg^{2+} concentration and LC_{50} values. This suggests that Ca^{2+}, Na^+ and H^+ may effectively compete with Dy for binding sites, thus decreasing Dy toxicity.

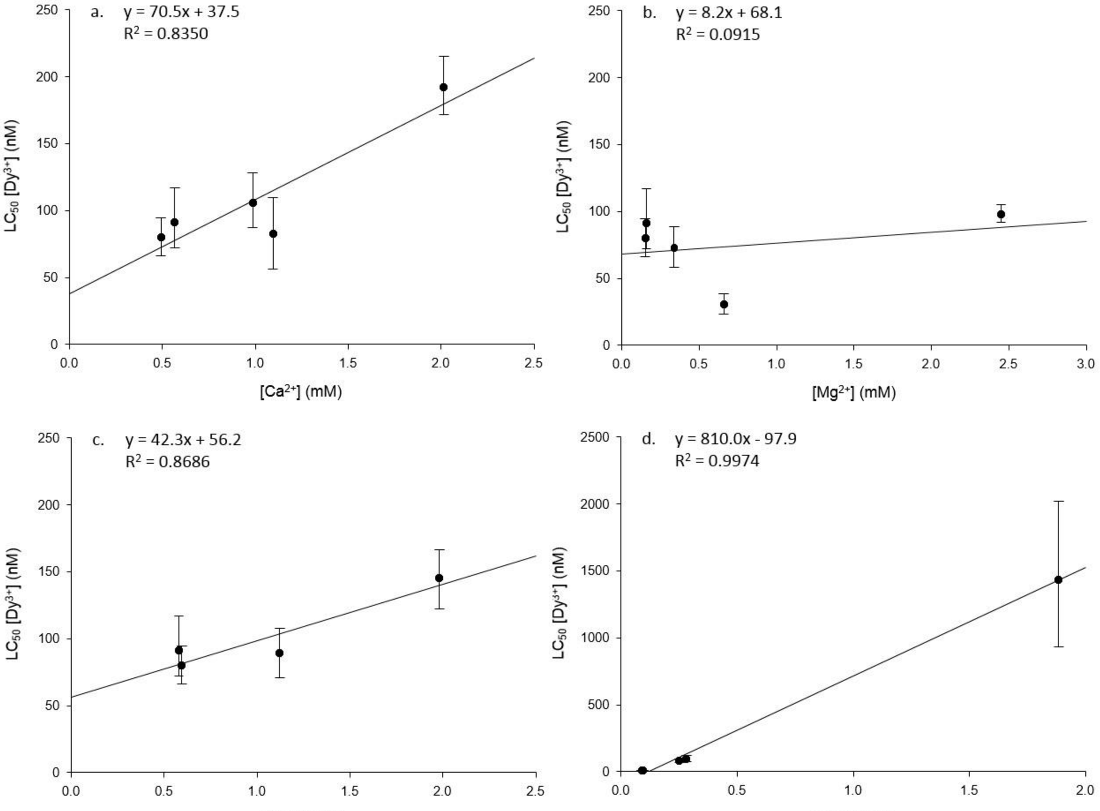

Figure 2.6 Linear regression of LC_{50}s (nM; LC_{50} and 95% confidence intervals) based on calculated Dy^{3+} (free metal ion) concentration and cation concentration for (a) Ca^{2+}, (b) Mg^{2+}, (c) Na^+ and (d) H^+. Regression line equation and R^2 values are showed.

2.3.4 BLM building for Dy

Dissolved Dy speciation in test solution

All the dissolved Dy species data modeled by WHAM7 for all the 17 TMF tests (including MSM tests) are demonstrated in appendix C.

According to WHAM7, 7 different Dy species will form in test solution: Dy^{3+}, $DyOH^{2+}$, $DyCO_3^+$, $Dy(CO_3)_2^-$, $DyHCO_3^{2+}$, $DySO_4^+$ and $Dy(SO_4)_2^-$. In all the 17 TMF tests, regardless of water chemistry, the concentrations of these complex ions followed a very similar pattern: $DyCO_3^+$ and $Dy(CO_3)_2^-$ always contributed to most of the Dy dissolved in solution (over 90% in most of the tests); concentrations of Dy^{3+} and $DyOH^{2+}$ were much less than the Dy-carbonate complexes but were dominant among the other Dy species identified by WHAM7; the other 3 Dy species were present in very limited quantities (especially $Dy(SO_4)_2^-$) and thus were disregarded. Based on the WHAM7 results, it is obvious that carbonate played an important role in Dy toxicity by binding almost all the Dy^{3+} in test solution.

Dy free metal ion was strongly affected by pH, according to WHAM7 for the pH tests (see Appendix C). A pH decrease by 0.9 resulted in an approximate 50-fold increase of Dy free metal ion, while a pH increase by 0.4 caused a 10-fold decrease of Dy free ion. Theoretically, the strong impact of pH on free metal ion concentration is due to its ability to affect inorganic carbon distribution (H_2CO_3, HCO_3^- and CO_3^{2-} in this case), and thus influence the binding between Dy^{3+} and carbonate. In addition to pH, another water chemistry factor that significantly influenced Dy speciation was DOC: its strong metal binding capacity can bring Dy back into solution, resulting in an approximately 6 times increase of dissolved Dy concentration (8 mg/L DOC).

First attempt at building BLM for Dy

The first attempt to build the Dy BLM was made following the method reported for a Cu BLM by De Schamphelaere and Janssen (2002). Unfortunately, negative stability constants were generated (Table 2.9), which do not fit their definition. However, these constants were effective at mathematically predicting Dy toxicity based on data observed in this study (Figure 2.8). The matlab program used to develop the BLM can be found in appendix A. With the "biotic ligand constants" in Table 2.9, LC$_{50}$s of Ca^{2+}, Na^{+} and pH TMF tests were again calculated using the BLM and compared to observed values in order to check the capacity of the BLM (Figure 2.8).

Table 2.9 Biotic ligand constants (K and log-K) of the first Dy BLM built.

Biotic ligand constants[a]	**K value**	**log(-K)**
K_{CaBL}	$-5.59*10^{-7}$	-6.25
K_{NaBL}	$-2.92*10^{-7}$	-6.53
K_{HBL}	$-4.21*10^{-2}$	-1.38
$\dfrac{f_{DyBL}^{50\%}}{\left(1-f_{DyBL}^{50\%}\right)*K_{DyBL}}$	-184.99	2.27

[a] Unit of Ca^{2+}, Na^{+}, H^{+} and Dy^{3+} concentrations in this BLM is nM. $f_{DyBL}^{50\%}$ is the percent of total biotic ligand that is occupied by Dy^{3+} when 50% mortality of *H. azteca* is observed.

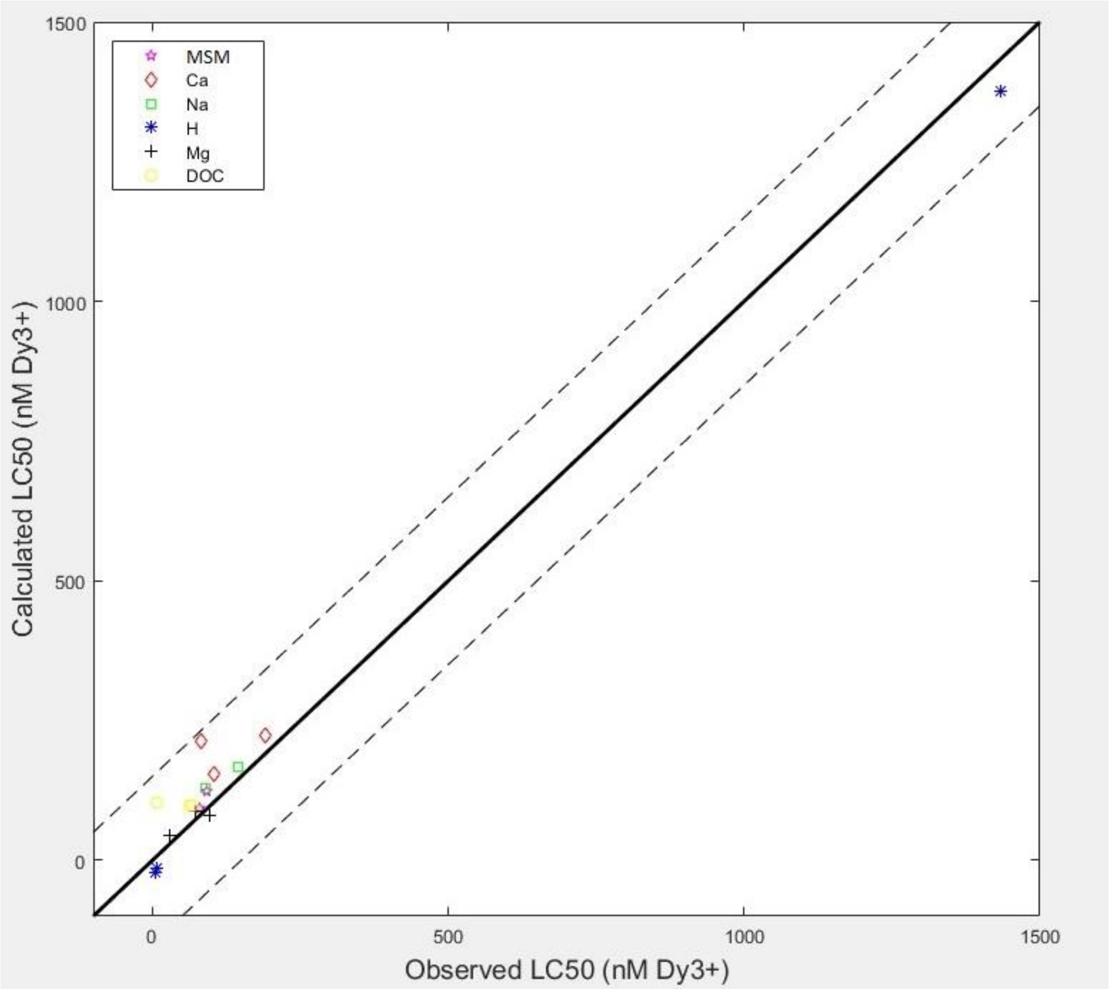

Figure 2.7 Relationship between BLM calculated and observed Dy LC_{50}s (nM Dy^{3+}) of all TMF and MSM tests. The thick solid line represents y=x and dashed lines represent ±150 µg/L.

Second attempt at building the BLM for Dy

Despite the fact that the first attempt to build the BLM for Dy could predict the LC_{50}s observed in this study, it was unacceptable since the negative biotic ligand constants do not have any meaning. Instead, a simpler 2-step model has been developed to replace the first BLM and predict the Dy toxicity. The first step was to estimate the LC_{50} only based on pH (assuming that pH was the only factor that affecting Dy toxicity and Dy^{3+} was the only toxic Dy species), since this TMF had a much stronger impact on LC_{50s} than other competing cations. The H^+-LC_{50} regression line in Figure 2.7 (d) is used:

$$y = 810x - 98$$

In this equation, y is the Dy free ion LC_{50} (nM), x is the proton concentration (10^{-7} M). This LC_{50} estimate was defined as LC_e.

Next, the LC_e was fixed with Ca^{2+} and Na^+ concentrations. It is already known that Ca^{2+} and Na^+ affect LC_{50} in a linear manner, so the equation can be:

$$LC_{50} = LC_e + C_{Ca} * ([Ca^{2+}] - 0.562) + C_{Na} * ([Na^+] - 0.591)$$

In this equation, 0.562 and 0.591 (mM) were averaged Ca^{2+} and Na^+ concentrations of the TMF tests, while C_{Ca} and C_{Na} were constants reflecting the strength of Ca^{2+} and Na^+ to influence LC_{50}. Based on the water chemistry and mortality data of 2 mM Ca^{2+} and 2 mM Na^+ tests, constants were estimated as $C_{Ca} = 77.22$ and $C_{Na} = 26.23$, so the final equation to calculate the Dy free metal ion LC_{50} will be:

$$LC_{50} = 810 * [H^+] + 77.22 * ([Ca^{2+}] - 0.562) + 26.23 * ([Na^+] - 0.591) - 98$$

Again, $[Ca^{2+}]$ and $[Na^{+}]$ were in mM, $[H^{+}]$ was in 10^{-7} M, and the resulting LC_{50} was in nM.

2.4 Discussion

2.4.1 Dy chemistry in aqueous phase

Nominal Dy concentrations compared to new solution (0h) total Dy concentrations indicated acceptable precision for solution preparation (Figure 2.3). There were reductions in total Dy concentrations over time from 0h to 120h, indicating potential adsorption of Dy to test vessels and cotton gauze, as well as absorption and uptake by *H. azteca*. There were also notably low dissolved Dy concentrations, inversely proportional to nominal concentration, relative to total Dy concentration at 0h (Table 2.4; Table 2.5; Table 2.7). Studies of other metals such as Cu have found similar low dissolved concentrations in polluted water sources, which is predictive of low toxic effects (Reash, 2004). Low dissolved Dy concentration relative to total Dy concentration is in part the result of precipitation. Also, in this study, consideration of pre-test acclimation of newly prepared test solutions (from 0h to 24h) is required since 0h Dy concentration was reported for these toxicity tests instead of 24h Dy concentration.

In general, total Dy concentrations were reduced by as much as 50% over time from 0h to 120h depending on nominal concentrations (Table 2.4; Table 2.5; Table 2.7). Factors such as adsorption to test vessels and cotton gauze, as well as absorption and uptake by *H. azteca* could explain the observed reductions in total Dy concentration (solutions were stirred before sampling). However, total Dy reduction in the test without *H. azteca* (Figure 2.5; Appendix B) didn’t show different pattern than invertebrate tests, which suggested that uptake and absorption by *H. azteca* was not a primary factor affecting total Dy concentration. The addition of 2% nitric acid at 120h resulted in an overall increase of total Dy concentration for all test vials compared to total Dy concentrations without acid addition (Figure 2.4) which implies the possibility of adsorptive potential of the test vessel and gauze. However, the polypropylene test vessel is not expected to have a strong binding capacity based on chemical structure (PipeSak, 2013) and adsorption to the cotton gauze is expected to be low since the gauze was pre-soaked in corresponding test concentrations for 24 hours to achieve saturation. Based on the fact that the stirring of solution before sampling could be incomplete, it is more likely that it was the precipitation that caused the reduction of total Dy concentration.

There were notably low dissolved Dy concentrations, ranging from 9% to 52% of the total Dy concentration at 0 h depending on nominal concentration (Table 2.4; Table 2.5). Visual MINTEQ 3.1 was applied to assess Dy salt precipitation by taking into account the relevant water quality conditions (Table 2.3). Based on the Visual MINTEQ stimulation, $Dy(OH)_3$ (s) is the only precipitate that will form and accounts for a significant percentage of total Dy loss (up to 80.9% at 6400 µg/L). Consequently, precipitation could be playing an important role in Dy distribution in solution.

2.4.2 Source-sensitivity relationship of *H. azteca* and comparison to *D. pulex*

Based on dissolved Dy and free Dy ion LC_{50} values for the *H. azteca* sources, FH *H. azteca* was overall more tolerant than the other 3 sources (except DL trial 2, which showed similar dissolved Dy LC_{50}). Although there was variability in LC_{50} values between trials, some trials of *H. azteca* from DL, HL and LW exhibited similar sensitivity to Dy. Considering the probability of *H. azteca* species diversification is high due to their short gene time and difficulty in making cross habitat divergent selection (Witt and Hebert, 2000), location is an important factor for estimating *H. azteca* sensitivity to Dy.

Babin-Fenske et al. (2012) have already genetically analyzed *H. azteca* groups near Sudbury and divided LW *H. azteca* from HL and DL *H. azetca*. However, such genetic variance didn't result in different tolerance to Dy in this study. In the future, DNA barcoding of the FH *H. azteca* will be completed to determine if the more tolerant *H. azteca* is genetically different from the 3 other sources (LW, DL and HL). Other studies have found high genetic diversity between *H. azteca* sources and some of those *H. azteca* clades had differing sensitivity to metals: an approximate 2-fold difference in sensitivity to copper and nickel was observed for 2 *H. azteca* clades (Leung, 2014). Differences in metal toxicity within the *H. azteca* species complex as a function of *H. azteca* source/DNA divergence must be considered in future environmental monitoring and risk assessment of Dy. Routine DNA barcode identification of test cultures is recommended to verify that the culture is comprised and remains comprised of a single test species.

Dy toxicity tests were also conducted to compare the sensitivity of two common invertebrate test species, freshwater amphipod (*H. azteca*) and freshwater flea (*D. pulex*). Based on the LC_{50} values for dissolved Dy and free Dy ion concentrations, *H. azteca* from FH and *D. pulex* showed very similar sensitivity to Dy, and these tests were ranked as the most tolerant results among all the invertebrate tests in this study. Caution is used when comparing toxicity test results to compare species sensitivity for *H. azteca* and *D. pulex* since there are differences in test methods including test duration, test solution volume, need of gauze and neonate age. Previously, *H. azteca* was compared to *D. magna* in terms of sensitivity to several common toxic metals: differences in sensitivity were observed but the toxicity ranking of the metals for both organisms was the same (Borgmann et al., 2005; Nebeker et al., 1986). These comparisons were also made based on different test methods.

2.4.3 Effects of TMFs on Dy toxicity to *H. azteca*

Effects of Cations on Dy toxicity

Among the 3 inorganic cations tested, Ca^{2+} and Na^{+} exhibited a protective effect against Dy toxicity while Mg^{2+} did not show significant protection (Figure 2.7). Ca^{2+} and Na^{+} showed a protective effect at 1 mM and 2 mM; these concentrations were commonly observed in natural water sources of Canada (Government of Ontario, 2015). Similar protective effects of cations ($\log K_{\text{H-gill}} = 5.4$, $\log K_{\text{Na-gill}} = 3.0$, $\log K_{\text{Ca-gill}} = 3.6$) on Cu toxicity for a fathead minnow gill-binding model were reported (Santore et al., 2001). In another study, Jackson et al. (2000) found that as low as 0.75 mM of Ca^{2+} and 0.8 mM of Mg^{2+} were protective against Cd toxicity to *H. azteca* while Na^{+} was not. In previous short-term (7d) Ni exposures, only Ca^{2+} notably reduced the bioaccumulation (at 2 mM Ca^{2+}) and toxicity (at 4.25 mM Ca^{2+}) of Ni to *H. azteca* (Schroeder, 2008). In another study, the same Ca^{2+} protective effect (at 1 mM) was also observed for 28-d Cd toxicity on *H. azteca* (Borgmann et al., 2010).

In terms of cation competition, Ca^{2+} is recognized as a strong protector of several common test species against metal toxicity. Some well-studied metal ions such as Cd and Pb are recognized as analogues of Ca^{2+}, which can enter through Ca channels (Marchetti, 2013; Perfus-Barbeoch, 2002). This could explain the reduced Dy toxicity in the presence of extra Ca^{2+}. The cation competition theory could also apply to Na^{+} protection. In metal toxicity study, the inorganic cation profile is an important factor that should be taken into account.

Effects of pH on Dy toxicity

Acidity was an important water chemistry parameter that affected Dy toxicity by increasing Dy free ion proportion and allowing for potential proton competition at lower pH. On the other hand, increased pH could enhance Dy complexation thus resulting in decreased Dy free ion concentration. At lower pH (6.7), dissolved Dy concentration increased by about 10-fold at the highest nominal concentration tested (Table 2.7) yet surprisingly free Dy ion toxicity decreased 17-fold relative to the control medium (MSM) at pH 7.6 (Table 2.8). While lowering pH may cause the dissolution of Dy-carbonate complexes and subsequent increase in bioavailable Dy^{3+} concentration (Byrne et al., 1988), there was also an increase in H^+ concentration and the potential for H^+ competition with Dy^{3+} for binding sites (Paquin et al., 2002). Proton competition with toxic metal ions has been reported but the level of the effect of H^+ competition on metal toxicity was relatively low: 2-fold increase in 48 h EC_{50} of free Cu ion on *Daphnia magna* when pH decreased by 2 (from 7.92 to 5.98) (De Schamphelaere and Janssen, 2002). In the current study, the 17-fold decrease in the LC_{50} value with a shift in pH from 7.6 to 6.7 suggested the potential for H^+ competition with Dy^{3+} for binding sites, and also the possibility of toxicity of Dy-carbonate complexes.

At higher pH (8.1), while Dy solubility was generally less than 50% of the control medium (MSM; Table 2.7), a 14-fold stronger toxicity of free Dy ion was observed (Table 2.8). Speciation of dissolved Dy is driven by the strong capacity of carbonate to bind with Dy^{3+} ($\log K_f$ [$DyCO_3^+$] = 7.56; $\log K_f$ [$Dy(CO_3)_2^-$] = 12.91) (Luo and Byrne, 2004), producing Dy-carbonate complexes of $DyCO_3^+$ and $Dy(CO_3)_2^-$ that account for over 90% of the species present (see Dy speciation using WHAM7, Appendix C). The potential for a high proportion of $DyCO_3^+$ and $Dy(CO_3)_2^-$ relative to Dy^{3+} suggests that the observed increase in Dy toxicity to *H. azteca* at pH 8.1 may be a function of Dy-carbonate complexes. While significant carbonate binding with other toxic metals has been observed, metal toxicity to fish and/or Cladocera was reduced by binding of carbonate to Ni (Pyle et al., 2002) and Cu (Flemming and Trevors, 1989). In contrast, the formation of Cu complexes was found to be harmful to barnacles (Barnes and Stanbury, 1948) and algae cells (Gibson, 1972). Similarly, Wang et al. (2012) found that $CuCO_3$ was toxic to plants at pH>7. In addition, a previous study of Cu toxicity on *H. azteca* showed a similar increase (28-fold, LC_{50} from 717 to 26 nM) of 7 d free Cu ion toxicity as a function of a pH increase of 1.6 and a 100-fold increase of bicarbonate concentration (from 0.01 to 1 mM), which implied that Cu-carbonate complexes are toxic (Borgmann et al., 2005). For Dy, further study is required to confirm reduced toxicity to *H. azteca* via H^+ competition with Dy^{3+} at lower pH and increased toxicity due to the potential toxicity of Dy-carbonate complexes at higher pH.

Effects of DOC on Dy toxicity

Two concentrations (4 and 8 mg/L) of DOC from Suwanee River were evaluated. For trials with 8 mg/L DOC, there were significant differences in dissolved Dy concentration; trial 2 had uncharacteristically low dissolved Dy concentration and thus was not used to assess the effects of DOC on Dy toxicity.

According to Table 2.7, DOC prominently increased the amount of Dy in solution, resulting in a higher dissolved Dy percentages, due to its strong metal cation binding ability. As a result, 4 mg/L SR DOC test showed a 3-fold higher dissolved Dy LC_{50} than MSM tests. Furthermore, 8 mg/L SR DOC resulted in a further 2-fold decrease in dissolved Dy toxicity compared to tests with 4 mg/L SR DOC (Table 2.8). Such results imply the strong ability of DOC to bind and decrease the bioavailability of Dy. If free metal ion was the only bioavailable and toxic form of metal, in the presence of DOC, *H. azteca* should show a similar free metal ion LC_{50}. Considering the strong binding capacity of DOC, it could also reduce the inorganic cation competition by binding to competing cations, resulting in reduced free metal ion LC_{50}. As showed in Table 2.8, 8 mg/L (measured value, 12.55 mg/L) SR DOC test showed a free Dy LC_{50} of 10.3 µg/L while 4 mg/L (measured value, 9.6 mg/L) DOC test showed a very low free Dy ion LC_{50} of 1.1 µg/L. Obviously, more DOC tests should be conducted in order to give more persuasive data.

2.4.4 BLM development of acute data

De Schamphelaere and Janssen (2002) provided a very straight forward way to build a BLM based on linear regression and the assumption that free metal ion is the only species that causes metal toxicity (De Schamphelaere and Janssen, 2002). However, the same method did not work well on the data acquired from the TMF tests in this project. The strong impact of pH resulted in negative stability constants for all the cation-biotic ligand complexes, which had no practical significance. Mathematically, these negative stability constants can still be used to calculate LC_{50} values, but in terms of chemistry the constants have no meaning. Unfortunately, some useful BLM parameters like occupation percentage of total biotic ligand when 50% mortality is observed ($f_{DyBL}^{50\%}$) and LC_{50} when there is no cation competition ($[LC_{50(Dy3+)}]_0$) could not be derived from this BLM. For future work it would be advisable to include the pH results in BLM calculation.

The alternative method to build the model was even simpler and was based on multiple assumptions. The core of this model assumed that the estimation of the LC_{50} was only based on pH since pH was the dominant effect among the competing cations. The model then adjusted the LC_{50} estimate to take into consideration Ca^{2+} and Na^+ concentrations. It was understood that the competition of Ca^{2+} and Na^+ cannot be the same at different pHs, but since their effects were less significant compared to pH, the difference was assumed to be negligible. This model was built at a pH around 7.6, so it would give the best estimate at this pH. Similar to the first BLM built, the limitation of this model is that when pH is above a certain value (about 8), it will very likely give negative LC_{50} values.

2.5 Conclusion

Dy (MSM) tests on *H. azteca* from the 4 different sources and *D. pulex* have revealed the potential diversity among cryptic *H. azteca* species: FH *H. azteca* was the most tolerant species to Dy toxicity among the tested *H. azteca* sources, which was as sensitive as *D. pulex*; the other 3 *H. azteca* sources (HL, DL and LW) showed higher sensitivity than FH *H. azteca* and were similar to each other. In terms of TMFs, Ca^{2+}, Na^{+}, lower pH and SR DOC are protective against Dy toxicity by different mechanisms. The BLM building was not successful due to the negative K values generated. In the future, more TMF tests (i.e. more concentrations for TMFs) should be done in order to build a more accurate BLM: SR DOC should be looked into again because of unreliable data that was generated for trial 2, 8 mg/L; If possible, to better study the effect of pH, a test medium with less carbonate will be helpful. DNA barcoding can be done to genetically distinguish the *H. azteca* from various locations, and a more physiological approach can be made to look into the mechanism of Dy toxicity on *H. azteca*.

CHAPTER 3

Study of Dy chronic toxicity and bioaccumulation on *Hyalella azteca*

3.1 Introduction

As a rare earth element (REE), dysprosium (Dy) is a valuable material in various modern industrial fields such as green energy due to its physio-chemical properties (Emsley, 2001; Watanabe, 2012). Together with its adjacent element holmium (Ho), they are elements with the strongest magnetic strength (Emsley, 2011). At this moment, China is producing the majority of Dy and global demand of Dy is predicted to increase dramatically in the next 10 years (Emsley, 2011; Alonso et al., 2012). In Canada, Avalon Rare Metals Inc. and Great Western Minerals Group (GWMG) of Canada both possess ore deposits with a high proportion (up to 20%) of HREEs including Dy (Humphries, 2013). Thor Lake REE deposit, which is located in Northwest Territories (NWT) of Canada and operated by Avalon, is thought to be one of the largest REE deposits worldwide (Humphries, 2013). Under these circumstances, environmental toxicity of REEs in general and specifically Dy is a concern due to the current lack of toxicological information.

In previous studies, Dy toxicity on mammals as well as aquatic microbes and invertebrates has been evaluated. Mouse LD_{50}s were 585 mg (intraperitoneal) and 7650 mg (oral) $DyCl_3$ per kg body weight (Hirano and Suzuki, 1996). Hirano and Suzuki (1996) also observed adverse physiological effects of various Dy salts such as increased RNA polymerase II in liver, decreased kidney concentrating ability and conjunctivitis of eyes in rats or rabbits. Fuma et al. (2005) found microbial extinction of *Euglena gracilis* Z, *Tetrahymena thermophila* B and *Escherichia coli* DH5α at 1000 μM (162500 μg/L) Dy. Nominal Dy 7-day LC_{50}s of 485 μg/L (for dissolved Dy: 162 μg/L) and 897 μg/L for soft and moderately hard tap water, respectively, were observed for *H. azteca* (Borgmann et al., 2005). In this project, acute Dy toxicity was also observed on *H. azteca*: for the 4 *H. azteca* sources tested in MSM, dissolved Dy LC_{50}s ranged from 145 to 376 µg/L (Table 2.6, Chapter 2). Although there is acute toxicity data for Dy, currently there is no information on the sublethal or chronic effects of Dy on aquatic organisms. However, this type of information is needed to establish a water quality guideline for Dy in Canada. In the present study, a systematic approach that addresses both Dy chronic toxicity and chemistry was used to contribute knowledge of aquatic toxicity of Dy and aid in future water resources conservation.

The assessment of environmental risk of Dy in aquatic ecosystems is complicated by the presence of toxicity modifying factors (TMFs) such as inorganic cations, pH and DOC. As a result, water chemistry plays a determinative role with regard to metal toxicity and bioaccumulation by influencing metal speciation and bioavailability. For example, Ca^{2+} is the most frequently mentioned inorganic cation that can provide protection against metal toxicity by competitively binding to metal receptor sites of organisms (De Schamphelaere and Janssen, 2002; Schroeder, 2008). Similarly, Mg^{2+} and Na^{+} can provide the same kind of protection but were not usually as effective as Ca^{2+}, and sometimes no protection was demonstrated (Santore et al., 2001; Jackson et al., 2000; Schroeder, 2008). In the study of acute Dy toxicity, Ca^{2+} and Na^{+} reduced the acute toxicity of Dy to *H. azteca* however Mg^{2+} had no effect on Dy toxicity (Chapter 2). pH is also an important water chemistry parameter that can alter metal toxicity by proton competition for metal binding sites to protect against toxicity at lower pH (Paquin et al., 2002). In contrast, at higher pH, metal speciation can be affected significantly which may alter toxicity depending on the toxic potency and concentration of the metal species. The presence of metal species such as $DyOH^{2+}$ and $DyCO_3^{+}$ were predicted based on simulations by Windermere Humic Aquaous Model 7 (WHAM 7) using water chemistry data on various TMFs (Appendix C). In that case, acute toxicity of Dy was greater at higher pH (8.1) compared to lower pH (6.7 and 7.6) suggesting the potential toxicity of the metals species present at higher pH (mainly $DyCO_3^+$ and $Dy(CO_3)_2^-$) or a lack of proton competition. Dissolved organic carbon (DOC) is another factor that generally exists in water systems, its complexation capacity can reduce the bioavailability of metal, thus can protect aquatic organisms against metal toxicity (Paquin et al., 2002). In Chapter 2, DOC from SR showed great protective effect against Dy acute toxicity based on dissolved Dy concentration. In the present study, chronic toxicity of Dy and the effects of TMFs will be examined to improve our understanding of the potential risk of Dy in aquatic ecosystems.

Hyalella azteca is a broadly distributed freshwater crustacean (amphipod) in North America and commonly used model organism in toxicity experiments due to their ease of culture in the laboratory, considerable offspring productivity, and high sensitivity to toxicants (Phipps et al., 1995). A wide range of toxicants can be tested and various toxicity testing methods are available for *H. azteca* such as water-only tests, sediment tests and sometimes the toxicant can be added to their diet (EC, 2013; Golding, 2010). Compared to acute toxicity test, amphipod chronic toxicity tests were developed more recently and are less frequently applied in studies (Borgmann and Norwood, 1993). However, chronic toxicity tests provide more sensitive endpoints, such as growth and bioaccumulation, compared to mortality. The use of body concentration of amphipods could be a better approach to define exposure than environmental concentration (Norwood et al., 2007) although the reviews of McGeer et al. (2003) and Adams et al. (2011) clearly showed (theoretically and empirically) that bioaccumulation (particularly whole body concentrations) are not reliable predictors of effects. In this chapter, *H. azteca* will be used as the test organism to evaluate Dy chronic toxicity in water only exposures.

The objectives of this chapter were to study the long term effects of Dy, specifically lethality, growth and bioaccumulation of *H. azteca* and to determine the effects of TMFs on chronic Dy toxicity for comparison to Dy acute toxicity data (Chapter 2). To accomplish these objectives 4-week Dy chronic toxicity tests using water-only exposures were conducted on *H. azteca* from Fort Hope in northwestern Ontario to examine the effects of Dy and different TMFs. In this study two different sources of DOC were examined to determine the effect of DOC quality on Dy toxicity. In addition, to identify the quantity of Ca^{2+} required to reduce Dy toxicity, a test was conducted to examine various concentrations of Ca^{2+} in MSM at a constant concentration of 500 μg/L Dy (nominal).

3.2 Materials and Methods

3.2.1 *H. azteca* source and culture maintenance

Hyalella azteca from Fort Hope (FH) in northwestern Ontario (Figure 2.2) were used in the 4-week chronic toxicity tests. Culture conditions and maintenance were the same as the acute toxicity tests. Details of culture maintenance are found in Section 2.2.2 of Chapter 2.

3.2.2 Dy source and test solution preparation

Dy (atomic absorption standard, Inorganic Ventures) stabilized in 7% HNO_3 at a concentration of 1000±10 mg/L was used in the chronic toxicity test. One day prior to the start of the test, 10 L of test solution was prepared for each test concentration by mixing required volumes of MSM (Section 2.2.3, Chapter 2) and 1000±10 mg/L Dy. Test solutions included a minimum of 5 Dy concentrations (depended on TMF tested, from 10 to 2000 μg/L) and 1-2 controls (MSM and MSM with extra TMFs). The pH of the each solution was adjusted to 7.3±0.1 using KOH solutions. All stock solutions were then stored in sealed 10 L blue plastic carboys (Canadian Tire) for 24 h at room temperature (about 23°C). In addition, a sample volume of about 30 mL was used to pre-soak 3 pieces of 5 cm x 10 cm cotton gauze for 24 h for each corresponding test solution.

In addition to chronic tests using only MSM, tests were conducted with MSM with 2 mM Ca^{2+} (as $CaCl_2 \cdot 2H_2O$, Fisher Scientific, Nepean, ON) or 0.5 mM Mg^{2+} (as $MgSO_4 \cdot 7H_2O$, Sigma-Aldrich, Mississauga, ON). Tests using MSM were also conducted using two different sources of DOC at a concentration of 6 mg/L (nominal): White River, ON (WR) and Luther Marsh, ON (LM). LM DOC is a dark colored DOC with a high humic ratio (74%) and a high specific absorption coefficient (SAC) value of 37.8 (Gheorghiu et al., 2010), while WR DOC is lighter in color and has lower humic ratio than LM DOC (Livingstone, 2013). Additional chemicals for specific TMF tests were added to MSM prior to the preparation of Dy solutions. Due to the protective effects of Ca^{2+} observed for acute toxicity tests of Dy, the same chronic test was conducted to examine various concentrations of Ca^{2+} (0.5, 1, 2, 4, 8 mM) in MSM with a concentration of 500 µg/L Dy.

3.2.3 *H. azteca* chronic toxicity test procedures

Hyalella azteca chronic toxicity testing procedures followed modifications of Environment Canada's (EC) "Biological Test Method: Test for the survival and growth in sediment and water using freshwater amphipod *Hyalella azteca*" (EPS1/RM/33; EC, 2013) and procedures for *H. azteca* chronic sediment toxicity tests (Borgmann and Norwood, 1993). The water only *H. azteca* chronic test consisted of 3 replicates of 6-7 test solutions that were frequently renewed over the course of the 4-week exposure period. Neonates between 2-9 days old were used in testing. To start the test, 10 neonates were added to 250 mL of test solution in 400 mL tri-cornered polypropylene test beakers (Fisher Scientific, Nepean, ON) together with the pre-soaked cotton gauze. The test beakers were then covered with plastic petri dishes and kept at 23°C and 16 light: 8 dark photoperiod. Test solutions were completely renewed 3 times a week (Monday, Wednesday, Friday). At the time of renewal, neonates were counted (at least once a week). Neonates were fed 5 mg smashed TetraMin® tropical fish food immediately after solution renewal. The cotton gauze was not changed during the renewal.

3.2.4 Sample collection and water quality measurements

At the start of test (24 h after solution preparation), two 10 mL samples were collected for analysis of total and dissolved Dy following the same method used for the acute toxicity tests (see Section 2.2.6, Chapter 2). During the test, total and dissolved samples were also collected from both new and old test solutions at least once a week. Samples were placed in 15 mL conical centrifuge tubes, acidified immediately using 200 μL HNO_3 (Fisher Scientific, Nepean, ON, TraceMetal^{TM} Grade, 70%) to 2% acid and held at 4℃ prior to analysis. The samples were measured for Dy and inorganic cation (Ca^{2+}, Mg^{2+}, Na^{+}) concentrations using Optima 8000 ICP-OES spectrometers (PerkinElmer, Woodbridge, ON). To measure DOC concentration, a 50 mL filtered (0.45 μm, Tuffryn) sample was taken once a week from both new and old solutions for all treatment concentrations for selected tests only and later measured for DOC by Shimadzu TOC-L Total Organic Carbon Analyzer (Mandel Scientific, Guelph, ON). These DOC samples were placed in 50 mL conical centrifuge tubes and stored at 4°C in the dark.

3.2.5 *H. azteca* measurement endpoints

At the end of each chronic test, the number of surviving *H. azteca* was recorded. *H. azteca* from each test beaker was then placed into 40 mL E-pure water in 50 mL plastic beakers for 6 h for gut clearance (Neumann et al., 1999). After 6 h, the E-pure water was drained and *H. azteca* were dried at 80℃ for 48 h (Livingstone, 2013). Individual *H. azteca* from each test beaker were then weighed using Satorius (Mississauga, ON) SE2 ultramicrobalance and placed into 1.5 mL micro centrifuge tubes. For digestion, 25 μL of HNO_3 (Fisher Scientific, Nepean, ON; TraceMetal^{TM} Grade, 70%) was added to each tube, immersing the *H. azteca* for 6 days, and then 20 µL of 30% H_2O_2 (Sigma-Aldrich, Mississauga, ON) was added for another day (Neumann et al., 1999). The digested *H. azteca* were then diluted to 2 mL using 2% nitric acid and the samples were measured by Optima 8000 ICP-OES spectrometers (PerkinElmer, Woodbridge, ON) to determine body burdens of Dy.

3.2.6 Data analysis

LC_{50} data, as well as *H. azteca* body concentration at 50% mortality (LBC_{50}) data for the Dy chronic tests were calculated using Probit analysis provided by IBM SPSS Statistics 22 (for details see Section 2.2.7, Chapter 2). LC_{50} values were considered significantly different if their confidence intervals didn’t overlap. If the confidence intervals overlapped, Litchfield-Wilcoxon method would be used to determine whether the LC_{50}s were significantly different (EC, 2005). One-way ANOVA test with Dunnett’s post hoc comparison was run on IBM SPSS Statistics 22 to find significant difference between dry weights of exposures and control, and to compare water chemistry data. Regressions and graphs were made by SigmaPlot 12.

3.3 Results

3.3.1 Water chemistry and mortality data

Based on data in Table 3.1, average pH of old test solution was generally higher than new solution, the increased value usually ranged from 0.1 to 0.5, except the WR DOC test showed some abnormally high old solution pH. Mean Ca^{2+} and Mg^{2+} concentrations showed high consistency during each chronic test and between different Dy concentrations, while mean Na^{+} demonstrated slight increasing tendency along with the increase of nominal Dy concentration. Dissolved Dy concentrations were always similar to total Dy concentrations for new solutions. Both dissolved and total Dy concentrations were overall reduced in old solutions compared to new solutions. Also there was a decrease in dissolved Dy concentration compared to total Dy concentration (except for DOC tests) in old solutions. For measured DOC concentration, the WR DOC test had a mean concentration of 6.37 mg/L and 7.06 mg/L for new and old solutions, respectively, while the LM DOC test had a higher mean concentrations of 7.80 mg/L and 9.06 mg/L for new and old solutions, respectively. All the chronic tests showed generally increasing mortality as a function of Dy concentration; in most cases mortality was less than 100% at the highest Dy concentration. Control mortality was mostly below 10%, except for the MSM trial 2, which had 23.3% mortality.

Refer to Table 3.2, the water chemistry of the Ca tests with 500 μg/L Dy had average measured Ca^{2+} concentrations that were consistent with nominal concentrations. Total and dissolved Dy concentrations were overall similar for all test solutions except there was lower Dy concentrations for 2 mM Ca^{2+} + 500 μg/L Dy test solution. In general mortality decreased with increasing Ca^{2+} concentration up to 2 mM Ca^{2+} (which also corresponded to decreasing Dy exposure) but at higher Ca^{2+} concentrations above 2 mM (i.e. 4 and 8 mM) there was an increase in mortality and this was generally associated with higher Dy exposure (Table 3.2).

Table 3.1 Water chemistry (pH, inorganic cations, DOC, Dy) and mortality data of chronic toxicity tests including 2 MSM tests and 4 TMF (2mM Ca^{2+}, 0.5mM Mg^{2+}, 6mg/L WR DOC, 6mg/L LM DOC) tests. All the water chemistry and Dy data were average values (n varies from 4 to 27).

Test	Dy nominal conc.	Medium water chemistry						Measured Dy (μg/L)						% Mortality
								new			old			
		pH (new)	pH (old)	$[Ca^{2+}]$ (μM)	$[Mg^{2+}]$ (μM)	$[Na^{+}]$ (μM)	DOC (mg/L)	T	D	D/T %	T	D	D/T %	
MSM Trial 1	0	7.4	7.5	515	147	539		0	1	n/a[b]	0	0	n/a	6.7
	10	7.4	7.8	511	146	508		4	5	108	2	1	78	3.3
	20	7.5	7.7	515	147	543	N: 1.01	12	11	97	5	3	51	6.7
	50	7.5	7.8	509	146	525	O: 1.83	37	35	94	14	7	50	20
	100	7.5	7.6	517	147	532		81	75	93	44	20	47	20
	200	7.4	7.7	526	147	547		178	163	91	115	53	46	16.7
	500	7.5	7.6	505	144	555		348	331	95	176	115	65	63.3
MSM Trial 2	0	7.3	7.8	455	129	459	-	-3	-3	n/a	-3	-3	n/a	23.3[a]
	50	7.3	8.2	444	127	460	-	39	38	97	18	7	40	33.3
	100	7.2	8.3	426	126	447	-	85	85	100	43	21	49	16.7
	250	7.4	8.3	444	126	477	-	148	147	99	97	56	58	30
	500	7.5	7.9	459	130	508	-	463	467	101	283	168	60	73.3
	750	7.4	7.7	460	131	532	-	712	719	101	470	290	62	96.7
	1000	7.4	7.7	461	131	546	-	951	940	99	612	413	67	96.7
2mM Ca^{2+}	0	7.3	7.3	502	135	486	-	3	9	n/a	-1	-2	n/a	13.3
	0+2mM Ca^{2+}	7.3	7.4	1919	133	542	-	-2	2	n/a	-3	-3	n/a	3.3
	250	7.3	7.2	1891	129	534	-	244	236	97	142	61	43	16.7
	500	7.1	7.2	1896	130	556	-	507	482	95	266	164	62	16.7
	750	7.3	7.4	1882	128	572	-	756	733	97	476	337	71	40
	1000	7.3	7.5	1913	130	605	-	1015	1005	99	666	481	72	86.7
	1250	7.3	7.4	1869	126	601	-	1266	1215	96	873	606	69	96.7
0.5mM Mg^{2+}	0	7.4	7.8	493	142	486	-	-5	-5	n/a	-6	-7	n/a	6.7
	0+0.5mM Mg^{2+}	7.4	7.9	487	502	497	-	-6	-7	n/a	-7	-7	n/a	13.3
	250	7.3	7.9	498	508	515	-	229	223	97	126	53	42	23.3
	500	7.3	7.7	494	506	533	-	466	460	99	279	135	48	20
	750	7.3	7.8	501	510	568	-	704	702	100	492	266	54	63.3
	1000	7.3	7.7	495	507	581	-	983	960	98	671	423	63	93.3
	1500	7.4	7.7	486	502	615	-	1467	1333	91	944	673	71	100

Table 3.1 continued

Test	Dy nominal conc.	Medium water chemistry						Measured Dy (µg/L)						% Mortality
								new			old			
		pH (start)	pH (end)	$[Ca^{2+}]$ (µM)	$[Mg^{2+}]$ (µM)	$[Na^{+}]$ (µM)	DOC (mg/L)	T	D	D/T %	T	D	D/T %	
6mg/L	0	7.4	7.8	489	139	482		1	4	n/a	-3	0	n/a	3.3
DOC	0+6mg/L DOC	7.5	8.5	499	142	502		-4	4	n/a	-4	-4	n/a	13.3
WR	250	7.4	9.0	488	139	500	N: 6.37	240	242	101	220	200	91	10
	500	7.4	8.7	492	141	536	O: 7.06	490	481	98	447	407	91	10
	750	7.5	8.3	494	141	556		665	656	99	610	560	92	20
	1000	7.4	7.8	503	143	590		1023	994	97	845	790	93	83.3
	1500	7.5	7.6	492	139	605		1504	1494	99	1289	1210	94	80
6mg/L	0	7.3	7.6	462	118	472		-8	-9	n/a	-9	-9	n/a	6.7
DOC	0+6mg/L DOC	7.3	7.6	454	118	458		-10	-10	n/a	-10	-10	n/a	16.7
LM	500	7.2	7.5	452	118	501	N: 7.80	472	472	100	426	420	99	30
	750	7.4	7.8	456	117	537	O: 9.06	731	728	100	653	637	97	26.7
	1000	7.2	7.7	462	119	567		978	976	100	896	863	96	56.7
	1500	7.4	7.7	460	119	594		1460	1462	100	1302	1264	97	46.7
	2000	7.5	7.7	460	119	635		1940	1933	100	1764	1681	95	76.7

[a] EC (2013) required a control survival rate of over 80 % in 14-day test for the test to be valid

[b] n/a, not applicable

Table 3.2 Water chemistry (pH, inorganic cations, DOC, Dy) and mortality data of the Ca test with 500 µg/L Dy. All the water chemistry and Dy data were average values (n varies from 4 to 13).

Test	Test conc.	Medium water chemistry						Measured Dy (µg/L)						% Mortality
								new			old			
		pH (start)	pH (end)	[Ca^{2+}] (µM)	[Mg^{2+}] (µM)	[Na^{+}] (µM)	DOC (mg/L)	T	D	D/T %	T	D	D/T %	
different Ca^{2+}	0	7.2	7.4	470	141	491		0	0	n/a[a]	0	0	n/a	13.3
	8mM Ca^{2+}	7.2	7.5	7835	150	499		-1	-1	n/a	-1	-2	n/a	26.7
	Dy+0.5mM Ca^{2+}	7.1	7.4	537	143	537	N: 1.15	443	441	99	209	112	53	46.7
	Dy+1mM Ca^{2+}	7.4	7.4	952	143	548	O: 1.55	468	461	99	227	167	74	23.3
	Dy+2mM Ca^{2+}	7.4	7.5	1952	144	578		375	357	95	198	157	79	16.7
	Dy+4mM Ca^{2+}	7.4	7.5	3924	147	516		467	442	95	275	199	72	30
	Dy+8mM Ca^{2+}	7.4	7.5	7921	151	499		466	477	102	281	224	80	53.3

[a] n/a, not applicable

3.3.2 LC_{50} data

On the basis of Table 3.3, the 2 chronic tests with MSM showed good consistency in Dy toxicity with LC_{50} values of 281 and 268 μg Dy/L. All the TMF tests (2 mM Ca^{2+}, 0.5 mM Mg^{2+}, 6 mg/L WR DOC, 6 mg/L LM DOC) had significantly higher LC_{50} values than MSM tests, indicating protective effects against Dy toxicity. Based on the LC_{50} data, the strength of protection in order from highest to lowest is: 6 mg/L LM DOC > 6 mg/L WR DOC > 2 mM Ca^{2+} > 0.5 mM Mg^{2+}.

Table 3.3 LC_{50}s (dissolved Dy of new test solution, μg/L) and 95% confidence intervals of chronic toxicity tests with MSM and TMFs.

Tests	LC_{50} & 95% CI (new, dissolved Dy) (μg/L)
MSM Test 1	281 (226-376)
MSM Test 2	268 (204-337)
2mM Ca^{2+}	681 (596-776)
0.5mM Mg^{2+}	548 (469-636)
6mg/L DOC WR	898 (781-1045)
6mg/L DOC LM	1215 (1006-1501)

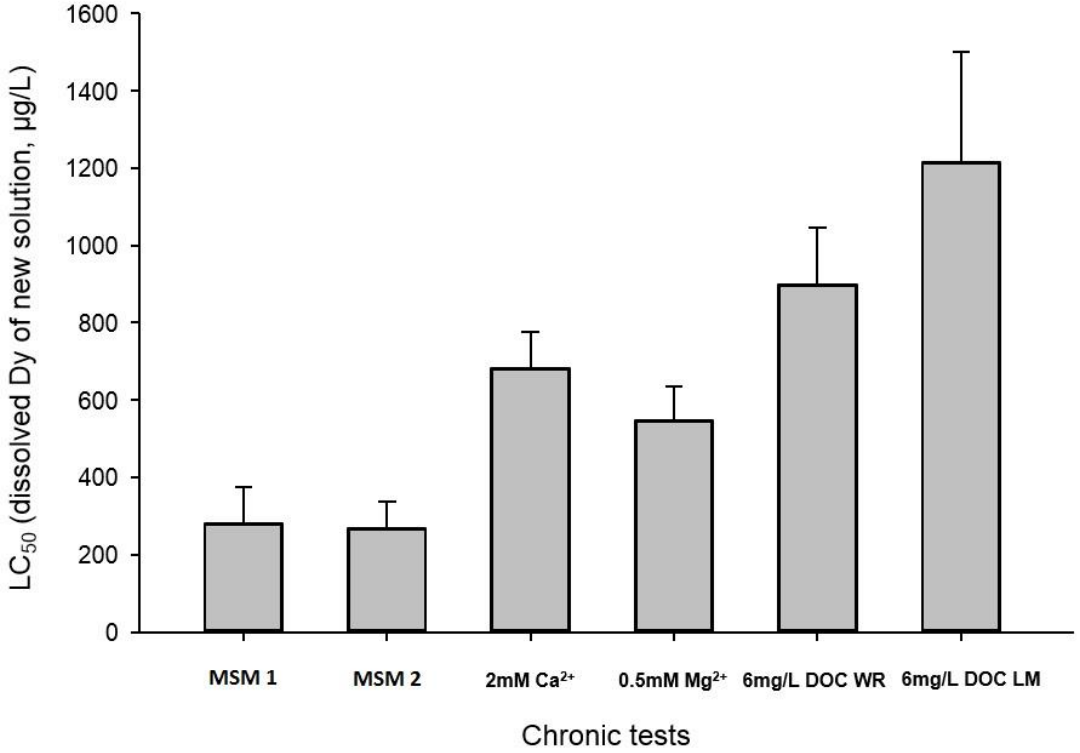

Figure 3.1 LC$_{50}$s (dissolved Dy of new test solution, µg/L) and upper 95% confidence limit of Dy chronic toxicity tests with MSM and different additional TMFs.

3.3.3 Dy and TMFs effects on *H. azteca* dry weight

Dry weight data was used only when more than 1 *H. azteca* survived. For the MSM trial 1, dry weight of the control was not used in the linear regression due to low values. For tests with extra TMFs, only the dry weight data of controls with additional TMFs were used. Based on the general growth model $W = W'(1 + aC^n)^{-1}$ (W' was control weight, C was exposure concentration, a and n were constants; Norwood et al., 2013), Figure 3.2, showed the adverse effect Dy had on *H. azteca* growth. Data in Table 3.4 showed that after 28 days, *H. azteca* in the controls were heavier than those exposed to Dy solutions. However, when different TMFs existed in solution, the harmful influence of Dy was inhibited to varying degrees (Table 3.4). Compared to MSM test trial 2, 2 mM Ca^{2+}, 6 mg/L WR DOC and 6 mg/L LM DOC all had protective effects against the growth inhibition of Dy.

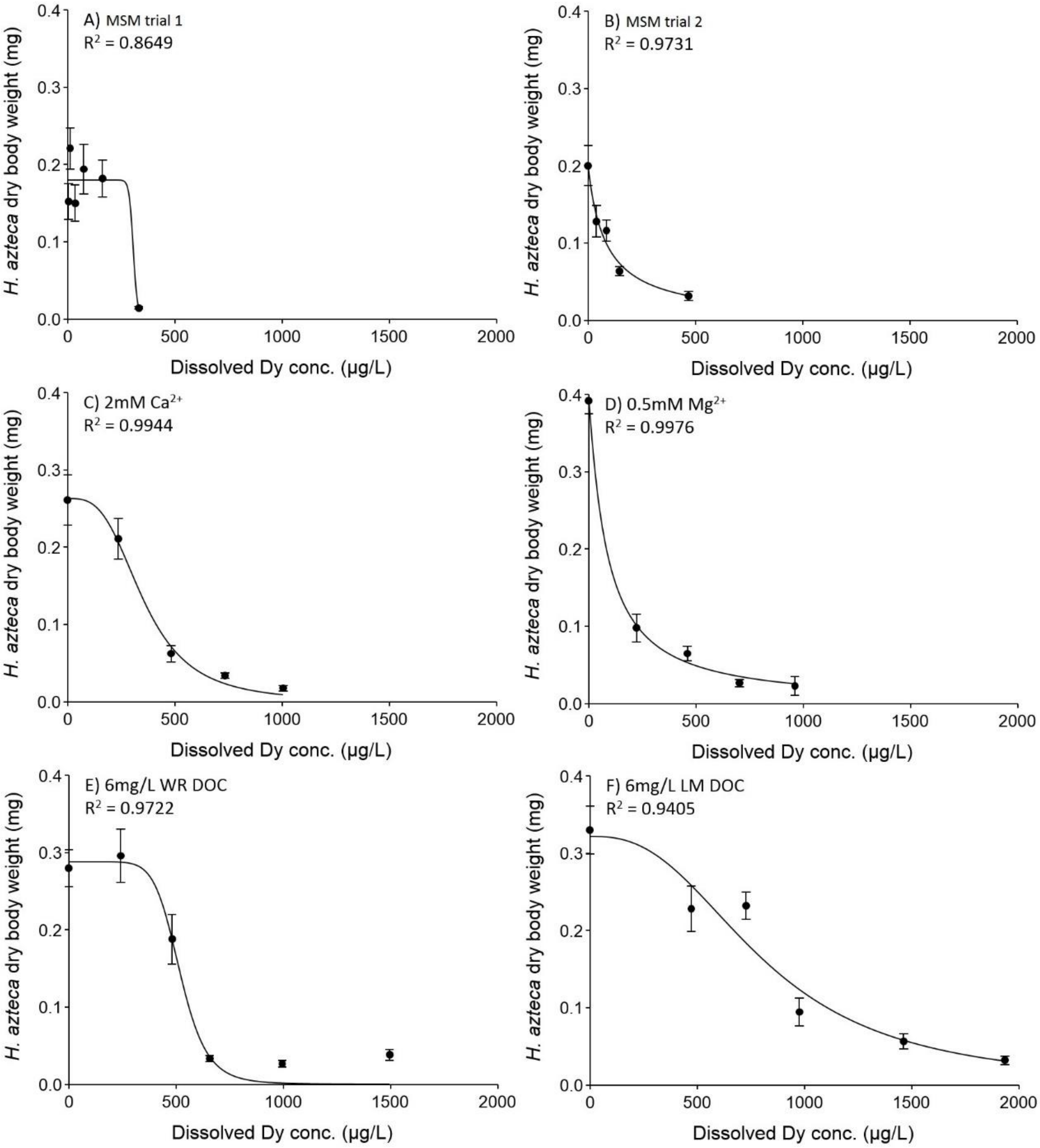

Figure 3.2 Dose response and nonlinear regression of *H. azteca* dry weight (mean ± SE, mg) as a function of dissolved Dy concentration (new solution, µg/L) for A) MSM trial 1, B) MSM trial 2, C) 2 mM Ca^{2+}, D) 0.5 mM Mg^{2+}, E) 6 mg/L WR DOC and F) 6 mg/L LM DOC. All the regression curves have R^2 values greater than 0.9 except MSM trial 1 (0.8649).

Table 3.4 EC_{25}, EC_{50} and LOEC of Dy (new, dissolved, µg/L) on *H. azteca* growth for all the chronic tests calculated by *H. azteca* growth model (EC_{25}, EC_{50}) and one-way ANOVA: Dunnett's post hoc test (LOEC).

Tests	**EC_{25} (new, dissolved Dy) (µg/L)**	**EC_{50} (new, dissolved Dy) (µg/L)**	**LOEC (new, dissolved Dy) (µg/L)**
MSM Trial 1	296	307	n/a
MSM Trial 2	27	85	147
2mM Ca^{2+}	251	354	482
0.5mM Mg^{2+}	31	85	223
6mg/L DOC WR	454	518	656
6mg/L DOC LM	531	813	976

In the presence of 500 μg/L Dy, growth of *H. azteca* was significantly inhibited relative to the controls. The 2 control groups showed similar mean dry weight. Among the Ca^{2+} concentrations with additional Dy, the only significant difference was observed between 0.5 mM Ca^{2+} and 4 mM Ca^{2+} (Figure 3.3).

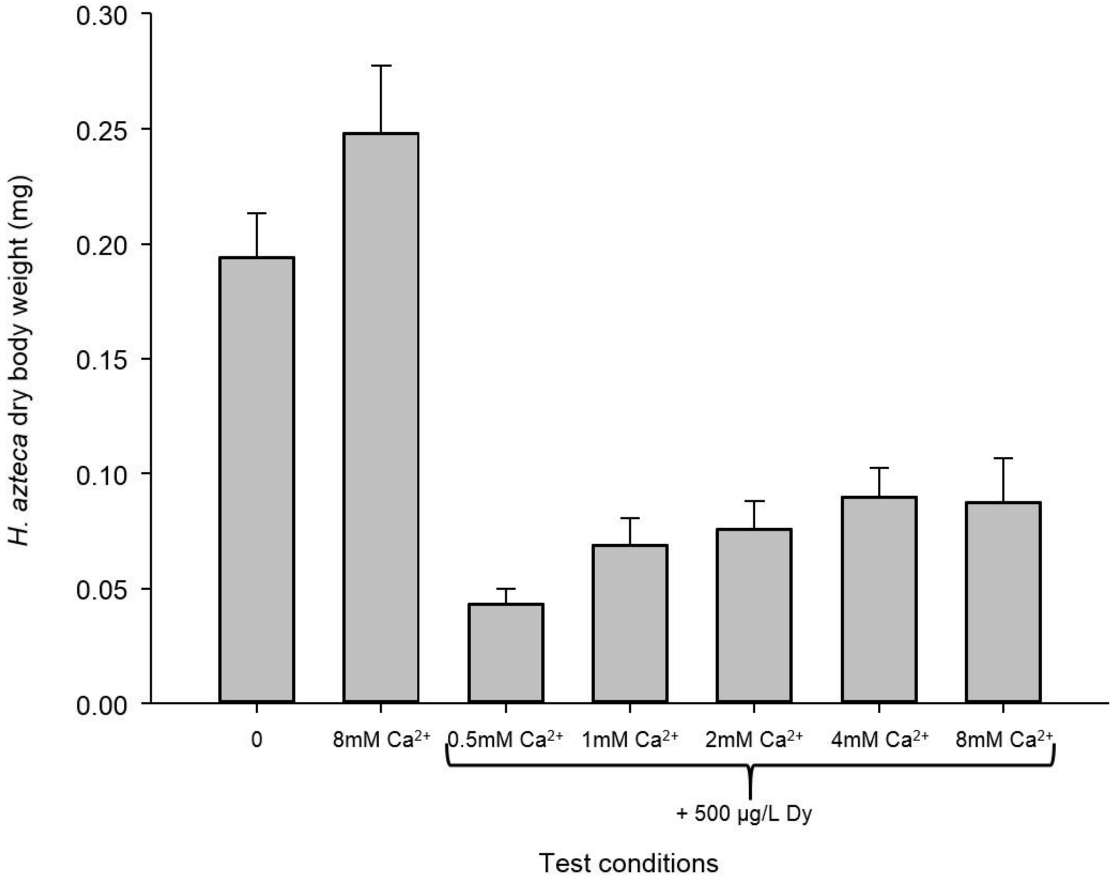

Figure 3.3 Dry weight of *H. azteca* (mean ± SE, mg) for varying Ca^{2+} concentrations. Ca^{2+} test concentrations that contain 500 μg/L Dy (nominal) are indicated.

3.3.4 Dy chronic bioaccumulation

In some cases, there was insufficient sample number to determine Dy bioaccumulation at higher dissolved Dy concentrations, as a result they were not showed in Figure 3.4. Dy bioaccumulation was not determined for controls. All the chronic tests showed increasing Dy bioaccumulation in *H. azteca* (μg Dy/mg dry weight) as dissolved Dy concentration (μg/L) increased (high R^2 values). The two MSM tests demonstrated different Dy bioaccumulation (slopes: trial 1, 0.0031 and trial 2, 0.0011). The addition of Ca^{2+} (2 mM) and Mg^{2+} (0.5 mM) resulted in similar Dy bioaccumulation in *H. azteca* based on slopes of 0.0045 and 0.0034, respectively. The presence of WR DOC (slope: 0.0013) resulted in higher bioaccumulation in *H. azteca* than LM DOC (slope: 0.0008).

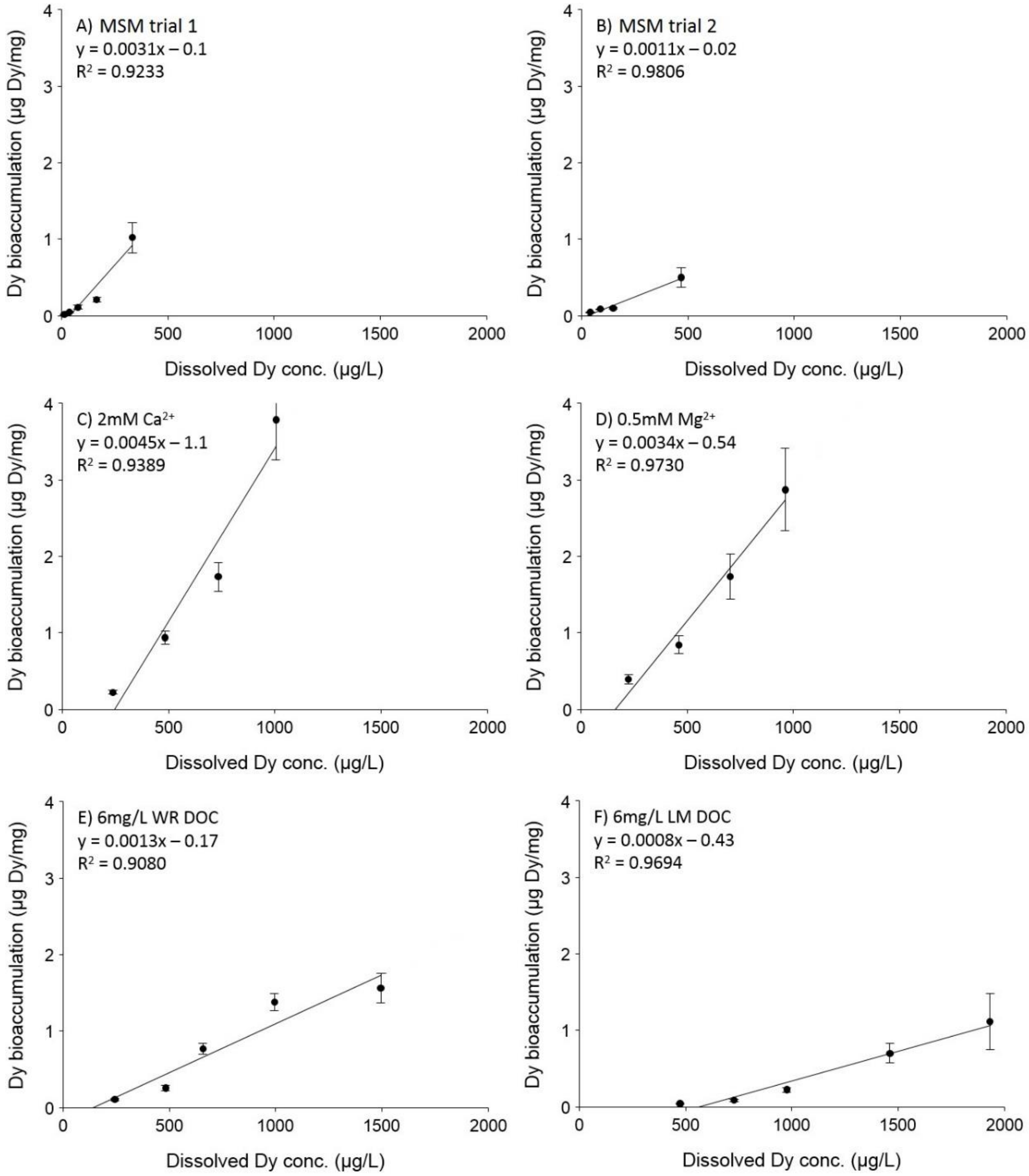

Figure 3.4 Linear regression of Dy bioaccumulation (mean ± SE, µg Dy/mg dry weight) in *H. azteca* and dissolved Dy concentration for new solutions (µg/L) for A) MSM trial 1, B) MSM trial 2, C) 2 mM Ca^{2+}, D) 0.5 mM Mg^{2+}, E) 6 mg/L WR DOC and F) 6 mg/L LM DOC. Linear equations and R^2 values are exhibited.

For the Ca test with 500 µg/L Dy, when compared to 0.5 mM Ca^{2+}, only Ca^{2+} concentration of 4 mM significantly inhibited Dy bioaccumulation in *H. azteca*.

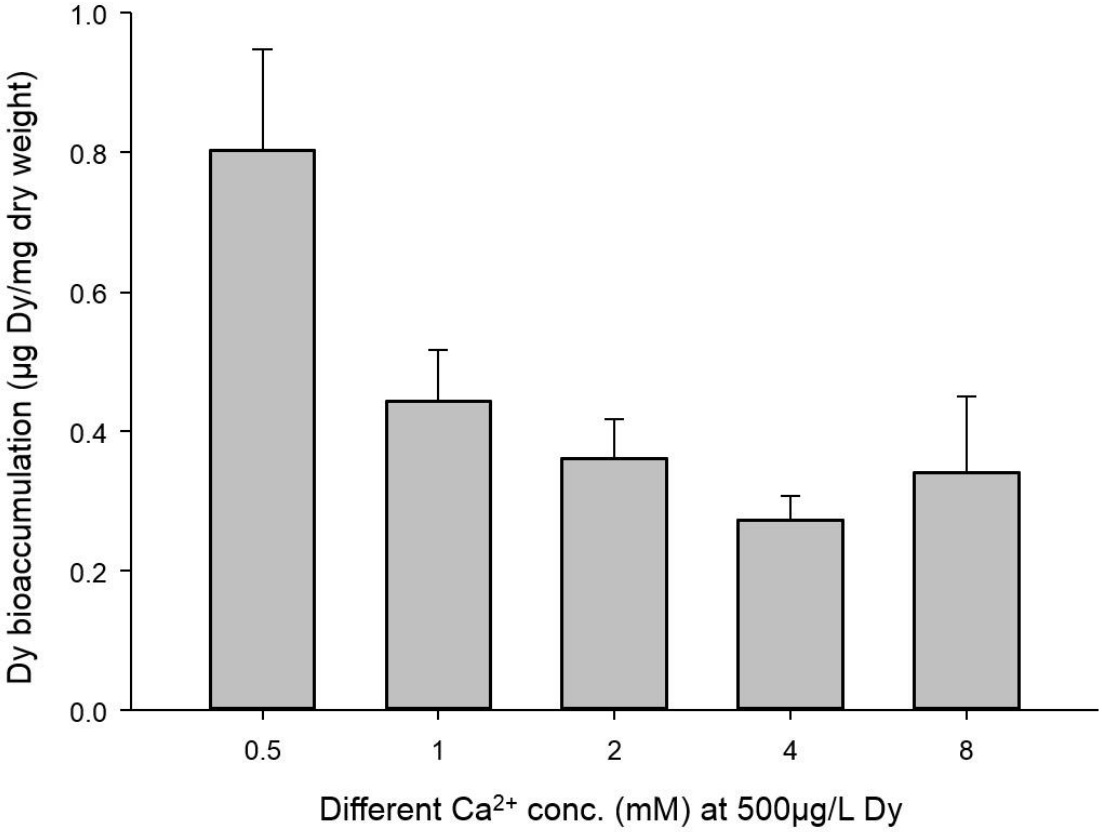

Figure 3.5 Dy bioaccumulation (mean ± SE, µg Dy/mg dry weight) in *H. azteca* at 500 µg/L Dy (nominal) as a function of increasing Ca^{2+} concentration.

LBC_{50} data for all the chronic tests were compared by Litchfield-Wilcoxon method (Table 3.5). There was low consistency for LBC_{50}s for the 2 MSM trials. The two MSM trials showed significantly different LBC_{50}s (0.789 and 0.287 µg Dy/mg dry body weight). The LBC_{50s} for cations (Ca^{2+} and Mg^{2+}) were significantly higher than both the MSM trials. In contrast, both types of DOC (WR and LM) had similar LBC_{50}s as MSM trial 1.

Table 3.5 Dy LBC_{50}s with 95% CI for all the *H. azteca* chronic tests calculated using Dy bioaccumulation data.

Tests	**LBC_{50} & 95% CI (µg Dy/mg body weight)**
MSM Trial 1	0.789 (0.607-1.115)
MSM Trial 2	0.287 (0.205-0.433)
2mM Ca^{2+}	2.120 (1.744-2.635)
0.5mM Mg^{2+}	1.390 (1.151-1.685)
6mg/L DOC WR	0.995 (0.857-1.161)
6mg/L DOC LM	0.576 (0.432-0.791)

3.4 Discussion

3.4.1 Dy chemistry in chronic tests

The new solutions for the chronic tests had good consistency in terms of pH (7.1-7.5), however there were pH increases observed in old test solutions (up to 1.6; Table 3.1, Table 3.2); this trend was not observed in the 96 h acute toxicity tests (Chapter 2). There was visible evidence of increasing algal growth in test vessels for all chronic tests over time; likely due to nutrients associated with *H. azetca* food. The presence of algae and the ability of photosynthesis of algae to increase the medium pH (Axelsson, 1988) would explain the higher pH observed in the chronic tests. In Chapter 2, pH had a strong effect on Dy chemistry - increased pH caused less dissolved Dy concentration. To control for algal growth and subsequent pH shifts in these chronic tests, clean test vessels could be used or complete vessel cleaning could be done at the time of water renewal.

The dissolved/total Dy ratios for new solutions were close to 100% for all the chronic tests while the ratios for the old solutions were reduced (except for DOC tests) but never lower than 40%. The test concentrations used for chronic tests were lower than the acute tests, thus less Dy precipitation would be expected in the chronic tests. However, the difference between total and dissolved Dy concentrations for old solutions was generally higher than predicted by Visual MINTEQ 3.1 (Table. 2.4) which may be due to: 1) higher pH observed in chronic tests for old solutions, thus potentially higher precipitation would form than acute tests and 2) feed in chronic tests may have provided more potential sources of complexation.

3.4.2 Dy chronic toxicity on *H. azteca*: Effects on survival

Dy LC_{50}s calculated for chronic 28-d MSM tests (281 and 286 µg/L) were lower than those for acute 96-h MSM tests (376 and 372 µg/L; Chapter 2) however based on

Litchfield-Wilcoxon method, the only significant difference was observed between acute MSM trial 2 and chronic MSM trial 2 (Table 3.6). Greater toxicity of Dy for chronic toxicity tests is expected since *H. azteca* were exposed to Dy for a longer period of time; however this trend was not generally observed in this project. In previous studies similar trends were observed: for *Eurytemora affinis*, Cu 48-h and 96-h LC_{50} values varied significantly with an increase in exposure duration: respectively 83 (75.21-91.68) and 69.4 (60.7-78.45) μg Cu/L (Hall et al., 1997). When comparing LC_{50}s of different test duration, consideration must be given to the test conditions that may influence organism tolerance to toxicants, such like feeding during chronic tests. That could explain why the fed *H. azteca* had similar or higher chronic LC_{50} when compared to acute LC_{50} data.

Table 3.6 Dissolved Dy (new solution) acute and chronic LC_{50}s and 95% CI for FH *H. azteca*.

Tests	**Acute LC_{50} & 95% CI (μg/L)[a]**	**Chronic LC_{50} & 95% CI (μg/L)**
MSM	376 (301-477)	281 (226-376)
	372 (317-430)	268 (204-337)
2mM Ca^{2+}	671 (605-751)	681 (596-776)
0.5 mM Mg^{2+}	198 (155-244)	548 (469-636)

[a] Acute data from Chapter 2

In chronic tests, all the TMFs tested showed protective effects against Dy toxicity based on estimated LC_{50}s calculated using dissolved Dy concentrations of new solutions (verified by clearly separate confidence intervals; Table 3.3). LC_{50}s were ranked from weak to strong protection: 0.5 mM Mg^{2+}, 2 mM Ca^{2+}, 6 mg/L WR DOC, 6 mg/L LM DOC. Ca^{2+} concentrations of 4 and 8 mM caused higher mortality for the Ca^{2+} test with 500 μg/L Dy. The mechanisms of protection of inorganic cations as competitors for metal binding sites and DOC in the formation of non-bioavailable Dy-DOC complexes were well documented in Chapter 2 for acute toxicity tests. However, Mg^{2+}, which did not display significant protection against dissolved Dy toxicity in acute toxicity tests when compared to acute MSM tests, showed protective effect in the chronic toxicity test compared to chronic MSM tests. Previous studies by Peters et al. (2011) confirmed the protective effects of Ca^{2+} and Mg^{2+} against chronic manganese toxicity for the invertebrate *Ceriodaphnia dubia*, however a similar Mg^{2+} effect was not observed for fish (*Pimephales promelas*) and freshwater unicellular green alga (*Pseudokirchneriella subcapitata*).

In the current study, 2 different sources of DOC were examined. LM DOC is a well-characterized allochthonous highly colored DOC with relatively high protein-to-carbohydrate ratio and degree of aromaticity (Richards et al., 2001). It also has a high SAC value (37.8) and a high humic acid-like material ratio (74%, Gheorghiu et al., 2010). These measurements reflect the dark color of LM DOC and correspond to its strong protection against metal toxicity (Al-Reasi et al., 2011). In a previous study, LM DOC showed the strongest protection against metal mixture (Pb, Hg, Cd, Cu, Ag and Co) toxicity and Pb/Cu bioaccumulation in rainbow trout among the 3 tested DOC sources (Luther Marsh, Beverly Swamp and Sanctuary Pond, ON) (Richards et al., 2001). Compared to LM DOC, the lighter-colored WR DOC, with less humic ratio, might not be able to provide the same strong protection as LM DOC (Livingstone, 2011; Al-Reasi et al., 2011). In the current study, the protection of LM DOC was significantly stronger than WR DOC based on LC_{50} values for dissolved Dy concentration, despite their confidence interval intersection. However, the mean measured LM DOC concentration was 20-30% higher than WR DOC, so the difference in protection could be due to the concentration gap, not their characteristics.

3.4.3 Dy chronic toxicity on *H. azteca*: Effects on growth

Long term (28-day) exposure to Dy caused reductions in average *H. azteca* dry weight. For the MSM trial 2, average dry weight was significantly reduced at dissolved new solution Dy concentrations of 147 µg/L (Table 3.4; such data was not applicable for MSM trial 1 due to low control dry weight). Similar growth inhibition effect of *H. azteca* was also found for other metals such as As, Co and Mn after 4-week exposures (Norwood et al., 2007).

On the basis of Table 3.4, all the 4 tested TMFs except 0.5 mM Mg^{2+} had the ability to reduce the adverse effect of Dy on *H. azteca* dry weight and the results were ranked as more to less effective: LM DOC > WR DOC > 2 mM Ca^{2+}). In a previous 4-week Ni chronic study on *H. azteca*, protective growth effect was observed with 1 mM Ca^{2+} and 9 mg/L DOC from two sources (Plastic Lake, Muskoka, ON and Daisy Lake, Sudbury, ON; Chan, 2010); although the effect of Mg^{2+} on growth was not evaluated in that study, Mg^{2+} was usually found to be less protective than Ca^{2+} against metal toxicity (96-h Cu study on rainbow trout and chinook salmon; Welsh et al., 2000).

In the absence of Dy, controls with TMFs had higher growth compared to controls without TMFs for all the TMFs tested except 2 mM Ca^{2+}. The increased mean dry weight was generally around 1.3-1.6 mg, which was 50% to 100% of the dry weight of controls without TMFs. In addition, in the Ca^{2+} test with constant 500 μg/L Dy, no significant higher *H. azteca* dry weight was observed in 8 mM Ca^{2+} control than MSM control (Figure 3.3). Cations such as Ca^{2+} and Mg^{2+} as well as DOC may have nutritional value. For example, Ca^{2+} (in the presence of Br^-) was found to be beneficial in long term survival (but not sure in terms of growth) of *H. azteca*, while Mg^{2+} was not necessary for survival if sufficient Br^- was present, but was beneficial in *H. azteca* growth (Borgmann, 1996). DOC was a nutritional supplement to zebra mussels (Roditi et al., 2000) and had potential to be nutritional to *H. azteca* (Chan, 2000). However, overdose of Ca^{2+} and Mg^{2+} could also be toxic to invertebrates. In *H. azteca* medium preparation, Ca^{2+} showed certain toxicity in the absence of Br^- but data was not provided, 0.35 mM Mg^{2+} caused 100% mortality when there was no Ca^{2+} in medium (Borgmann, 1996). For *D. magna* without feeding, Ca^{2+} and Mg^{2+} had 48-h LC_{50}s of 1.3 mM and 5.8 mM respectively in Lake Superior water (Biesinger and Christensen, 1972).

3.4.4 Dy bioaccumulation in *H. azteca*

In terms of inorganic cations, based on the regression lines in Figure 3.4, 2 mM Ca^{2+} and 0.5 mM Mg^{2+} both could prevent Dy bioaccumulation at low levels of dissolved Dy concentrations. However, refer to their regression line slopes (2 mM Ca^{2+}: 0.0045, 0.5 mM Mg^{2+}: 0.0034, MSM: 0.0031 and 0.0011), when dissolved Dy concentration increased in 2 mM Ca^{2+} or 0.5 mM Mg^{2+} solution, Dy bioaccumulation rise in *H. azteca* would be no less than *H. azteca* in MSM. In previous study excess Ca^{2+} (at approx. 2 mM Ca^{2+}) reduced 7-d Ni bioaccumulation in *H. azteca* (Schroeder, 2008). However, there were other studies found no change in Cu bioaccumulation in the presence of excess Ca^{2+} for freshwater macrophyte (*Ceratophyllum demersum*) (at up to 3.35 mM Ca^{2+}; Markich et al., 2006) or juvenile yellow catfish (*Pelteobagrus fulvidraco*) (at 1.25 mM Ca^{2+}; Chen et al., 2012).

By comparing Dy bioaccumulation at dissolved Dy concentration greater than 500 µg Dy/L, DOC tests showed less pronounced Dy bioaccumulation than cation tests, indicating that DOC effectively limited Dy bioaccumulation. For the 2 DOC tests, LM DOC test was associated with a greater reduction in Dy bioaccumulation (regression slope 0.0008) compared to WR DOC (regression line slope 0.0013). According to Section 3.4.2, such difference was likely due to difference in measured DOC concentration. DOC is widely studied and is generally recognized for the ability to reduce metal bioavailability (Paquin et al., 2002). Previous studies found reduced Ni bioaccumulation in *H. azteca* (Doig and Liber, 2006) and reduced Cu bioaccumulation in marine mussel larvae (Deruytter et al., 2014) in the presence of DOC.

A study of Ni toxicity found that *H. azteca* body metal concentration was a more reliable indicator of toxicity than environment metal concentration (Borgmann, et al., 2001). In the present study, both DOC tests showed similar LBC_{50}s as MSM trial 1, but Ca^{2+} and Mg^{2+} tests demonstrated significantly higher LBC_{50}s. Such results corresponded to previous study of McGeer et al. (2003) that whole body concentration was not a reliable indicator of toxicity. These results also suggest that in addition to the competition effects on Dy bioaccumulation of the cations, Ca^{2+} and Mg^{2+} might also have physiological effects on *H. azteca* which could increase their tolerance to metal toxicity. The beneficial effects of Ca^{2+} and Mg^{2+} in terms of survival and growth of *H. azteca* have been observed in a *H. azteca* culture medium study (Borgmann, 1996). Further study of the physiological response of *H. azteca* in the presence of cations and metals is required to better understand the metal tolerance of *H. azteca*.

3.5 Conclusion

Dy chronic toxicity tests of *H. azteca* all had high dissolved/total Dy ratio of their new solutions (close to 100%). Generally, Dy chronic toxicity tests of *H. azteca* did not show significantly lower LC_{50}s from acute toxicity tests. All the TMFs tested (2 mM Ca^{2+}, 0.5 mM Mg^{2+}, 6 mg/L WR DOC and 6 mg/L LM DOC) provided protection against Dy chronic toxicity, while Mg^{2+} did not show any protection again Dy acute toxicity. In terms of *H. azteca* growth, all the TMFs except 0.5 mM Mg^{2+} revealed beneficial effects to *H. azteca* growth or protection against growth inhibition of Dy. Dy bioaccumulation was similar for cation TMF tests whereas LM DOC exhibited reduced bioaccumulation of Dy compared to WR DOC. Uniformity of LBC_{50}s was not observed among the TMF tests: both DOC sources showed similar LBC_{50}s as MSM trial 1 but Ca^{2+} and Mg^{2+} showed significantly higher LBC_{50}s than MSM tests, suggesting unreliability of the use of whole body concentration to predict metal toxicity. In the future, additional Dy chronic toxicity tests of both MSM and TMFs should be conducted to provide more toxicity data and build a Dy chronic BLM of *H. azteca*.

CHAPTER 4

General summary and implications

4.1 Summary of findings

4.1.1 Acute 96-h toxicity of Dy

Differences in Dy tolerance for *H. azteca* sources based on Dy acute toxicity tests

- Among the 4 *H. azteca* sources tested (FH, HL, DL and LW), FH *H. azteca* had the highest tolerance (dissolved Dy LC_{50}: 374 µg/L, Dy free metal ion LC_{50}: 13.9 µg/L, about 2-fold higher than the others) to acute Dy toxicity than the other 3 sources.
- For the more sensitive *H. azteca* sources (HL, DL and LW), no significant difference in sensitivity to Dy was observed.
- *D. pulex* has similar sensitivity to Dy as FH *H. azteca*, thus more tolerant that HL, DL and LW *H. azteca*.

Effects of TMFs on Dy toxicity to FH *H. azteca* based on Dy acute toxicity tests

- Among the inorganic cations tested (Ca^{2+}, Mg^{2+}, Na^{+}), Ca^{2+} (2.01 mM) and Na^{+} (1.98 mM) showed protective effects (about 2-fold higher dissolved Dy and Dy free metal ion LC_{50}s than MSM) against acute Dy toxicity, while Mg^{2+} (as high as 2.45 mM) had no effect.
- Dy showed lower toxicity at lower pH than higher pH (pH range: 6.7-8.1): when compared to pH 7.6, pH 6.7 showed a 2-fold higher dissolved Dy LC_{50} and a 17-fold higher Dy free metal ion LC_{50}, while pH 8.1 showed a 4-fold lower dissolved Dy LC_{50} and a 15-fold lower Dy free metal ion LC_{50}.
- Additional DOC was also protective according to dissolved Dy LC_{50} data: at 12.6 mg/L SR DOC, a 5-fold higher dissolved Dy LC_{50} was observed compared to MSM with no extra DOC added, while a 3-fold higher dissolved Dy LC_{50} was demonstrated at 9.6 mg/L SR DOC.

Dy acute BLM development

- A valid Dy BLM of *H. azteca* was not successfully generated based on the toxicity data in hand. The over-strong effect of pH had resulted in negative values of all the stability constants of biotic ligand.
- According to the negative stability constants, binding capacity ranking of the protective inorganic cations (including H^+) was (from stronger to weaker): H^+, Ca^{2+}, Na^+.

4.1.2 Chronic 4-week toxicity of Dy

Dy chronic toxicity: Effects on survival of *H. azteca*

- Compared to 96-h acute tests, MSM 4-week chronic tests didn't show overall reduced dissolved Dy LC_{50}.
- All the TMFs tested (Ca^{2+}, Mg^{2+}, WR DOC, LM DOC) had protective effects against Dy chronic toxicity: 4.5-fold and 3-fold increase in dissolved Dy LC_{50}s were observed for LM and WR DOC tests, respectively; 2.5-fold and 2-fold higher dissolved Dy LC_{50}s were found in Ca^{2+} and Mg^{2+} additions, respectively.

Dy chronic toxicity: Effects on *H. azteca* growth

- *H. azteca* growth was inhibited as Dy concentration increased: in MSM tests almost no growth was observed at approx. 500 μg/L dissolved Dy concentration.
- All the TMFs tested except Ca^{2+} had beneficial effects on *H. azteca* growth for controls: average *H. azteca* dry body weights were about 50% to 100% higher in TMF controls than in MSM controls.
- All the TMFs except Mg^{2+} reduced the inhibition effect of Dy on *H. azteca* growth: EC_{25} values for Ca^{2+}, WR DOC, LM DOC were 251, 454, 531 μg/L

respectively; EC_{50} values were 354, 518, 813 μg/L respectively.

Dy chronic toxicity: Dy bioaccumulation in *H. azteca*

- Dy bioaccumulation in *H. azteca* increased as a function of dissolved Dy concentration, however no consistency was observed between the Dy bioaccumulation patterns of the two MSM tests.
- Among the TMFs tested, only LM DOC resulted in lower Dy bioaccumulation in *H. azteca*: LM DOC almost prevented Dy from accumulating when dissolved Dy concentration was below 500 μg/L, compared to MSM tests.
- LBC_{50}s calculated based on bioaccumulation data didn't show good consistency among the chronic tests: only the 2 DOC tests (0.995 and 0.576 μg Dy/mg body weight, for WR and LM respectively) showed intersected confidence intervals as the first trial of MSM tests (0.789 μg Dy/mg body weight); cation tests showed higher LBC_{50}s (2.120 and 1.390 μg Dy/mg body weight, for Ca^{2+} and Mg^{2+} respectively) than MSM tests.

4.2 Implications of study

In the study, *H. azteca* from different sources had varying sensitivities to Dy exposure. Some morphologic divergences including size and color were also observed during culture maintenance. As a cryptic species, highly diverse *H. azteca* causes difficulties when comparing data from different *H. azteca* sources/studies. Further cross contamination of cultures with multiple *H. azteca* clades could also influence test results. DNA barcoding to genetically differentiate *H. azteca* clades will improve the reliability and comparability of *H. azteca* toxicological research. Due to high diversity, it will be more appropriate to use local *H. azteca* clades for environmental risk assessment in order to get the most representative responses for that specific location. Efforts should be made to ensure the *H. azteca* gene database is frequently updated because of the great possibility for species diversification to happen (Witt and Hebert, 2000). It will be a good idea to use a relatively stable and well-studied invertebrate species as a reference in *H. azteca* studies, such as *D. pulex* in this project.

In this study Dy demonstrated a less predictable pattern when dissolved in solution; ratios of dissolved:total Dy concentration was not very constant, thus making the measurement of test solution samples more necessary. An abundant precipitation formation was observed in high Dy concentrations, making the bioavailability consideration of Dy more complicated. For dissolved fraction of Dy, its speciation was also complicated: besides Dy^{3+}, there were Dy complexes such as $DyOH^{2+}$, $DyCO_3^+$, $DySO_4^+$ etc based on WHAM 7. These Dy complexes may have different bioavailability to aquatic organisms and thus can contribute to the Dy toxicity. Again, water chemistry, especially carbonate, pH and DOC, are able to affect Dy speciation significantly. In conclusion, for metal toxicity tests and environmental assessments, water chemistry is a very important factor that can strongly influence the final results and comparison with other results for toxicity tests as well as estimation of Dy risk for environmental samples. In natural water systems, these water chemistry parameters could provide protection, or harm to local organisms.

Dy acute lethality and chronic lethality, inhibition of growth and changes in bioaccumulation were observed for *H. azteca* in this project. Compared to a previous toxicity study of multiple metals on *H. azteca* (Borgmann et al., 2005; 7-day tests in softer water), Dy (dissolved 96-h LC_{50}: 374 μg/L) was found to be less toxic than the other common toxic metals such as Ni (dissolved LC_{50}: 75 μg/L), Cu (dissolved LC_{50}: 36 μg/L), Ag (dissolved LC_{50}: 0.25 μg/L) and Cd (dissolved LC_{50}: 0.15 μg/L). In addition, based on data compiled for dissolved REE concentrations measured from over a thousand water samples from a wide range of natural water systems (average Dy: 16 ng/kg for groundwater; 163 ng/kg for lake water; 16 ng/kg for river water; 2 ng/kg for sea water) (Noack et al., 2014), the LC_{50} data (acute dissolved Dy LC_{50} range for all the *H. azteca* sources in MSM: 145-376 µg/L = 145000-376000 ng/L) generated in this study was much higher than the measured values of environmental samples, indicating low environmental risk of Dy based on *H. azteca* acute (96-h) toxicity tests. Similarly, chronic toxicity data (28-day dissolved Dy LC_{50} for FH *H. azteca* in MSM: 275 µg/L = 275000 ng/L) from the present study were higher than dissolved Dy in environmental samples indicating low environmental risk of Dy based on *H. azteca* chronic (28-day) toxicity tests. Assessments of Dy and REEs in general should include both acute and chronic toxicity tests.

4.3 Future studies

DNA barcoding of the *H. azteca* used in this project should be done in the future in order to determine if the *H. azteca* sources tested in this study were genetically different.

A deficiency of this project is that the Dy acute BLM of *H. azteca* has not been successfully built. Acute tests with more concentrations of TMFs should be done on *H. azteca* in order to develop a better BLM. In addition, different levels of carbonate should be tested to evaluate potential toxicity of Dy-carbonate species. Short-term bioaccumulation of Dy in *H. azteca* can also be measured, which was not included in this study.

For chronic study, more tests with TMFs should be done, so a chronic BLM can be made and compared to the acute BLM. There is still uncertainty about Dy bioaccumulation pattern and its relationship to *H. azteca* mortality, it should be revealed in the future.

Mechanism of Dy (REEs) toxicity, including uptake, absorption and site of toxic action should be examined in the future, so the toxicity of REE complexes can be modelled.

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Appendix A Matlab program for BLM building

```
function L=BLM

% water chemistry and mortality data
data=[7.55 0.566 0.159 0.579 91
    7.6 0.492 0.153 0.594 80
    7.58 0.986 0.146 0.599 105
    7.49 1.095 0.149 0.586 82.5
    7.66 2.014 0.17 0.563 192
    7.55 0.566 0.159 0.579 91
    7.6 0.492 0.153 0.594 80
    7.64 0.617 0.339 0.571 72.6
    7.77 0.641 0.659 0.561 30
    7.65 0.516 2.449 0.682 97
    7.55 0.566 0.159 0.579 91
    7.6 0.492 0.153 0.594 80
    7.61 0.604 0.183 1.122 89
    7.59 0.441 0.143 1.982 146
    7.55 0.566 0.159 0.579 91
    7.6 0.492 0.153 0.594 80
    6.725 0.601 0.171 0.573 1434.5
    8.025 0.627 0.164 0.575 7.4
    8.05 0.591 0.173 0.58 4.3];

% data organization
dataEC50=data(:,5);
dataCa=data(:,2);
dataMg=data(:,3);
dataNa=data(:,4);
datapH=data(:,1);
dataH=10.^-datapH;
dataOH=10^-14./dataH;

% linear regression of TMFs
% Ca2+
figure (1);
plot(dataCa(1:5),dataEC50(1:5),'b.','markersize',15)
hold on;
[paramCa,SCa]=polyfit(dataCa(1:5),dataEC50(1:5),1);
yfitCa=polyval(paramCa,dataCa(1:5));
R2Ca=norm(yfitCa-mean(dataEC50(1:5)))^2/norm(dataEC50(1:5)-
mean(dataEC50(1:5)))^2;
plot([0,2.5],[paramCa(2),polyval(paramCa,2.5)],'k','linewidth',2)
title('Ca2+ to Dy toxicity','fontsize',15)
xlabel('Ca2+ (mM)','fontsize',14); ylabel('Dy3+ (nM)','fontsize',14)
R2Castr=num2str(R2Ca);text(0.2,220,['r2=',R2Castr])
slopeCa=num2str(paramCa(1));interceptCa=num2str(paramCa(2));
text(0.2,230,['y=',slopeCa,'x+',interceptCa])
axis([0,2.5,0,250])

% Ca2+
figure (1);
plot(dataCa(1:5),dataEC50(1:5),'b.','markersize',15)
hold on;
[paramCa,SCa]=polyfit(dataCa(1:5),dataEC50(1:5),1);
yfitCa=polyval(paramCa,dataCa(1:5));
R2Ca=norm(yfitCa-mean(dataEC50(1:5)))^2/norm(dataEC50(1:5)-
mean(dataEC50(1:5)))^2;
plot([0,2.5],[paramCa(2),polyval(paramCa,2.5)],'k','linewidth',2)
```

```
title('Ca2+ to Dy toxicity','fontsize',15)
xlabel('Ca2+ (mM)','fontsize',14); ylabel('Dy3+ (nM)','fontsize',14)
R2Castr=num2str(R2Ca);text(0.2,220,['r2=',R2Castr])
slopeCa=num2str(paramCa(1));interceptCa=num2str(paramCa(2));
text(0.2,230,['y=',slopeCa,'x+',interceptCa])
axis([0,2.5,0,250])

% Mg2+
figure (2);
plot(dataMg(6:10),dataEC50(6:10),'b.','markersize',15)
hold on;
[paramMg,SMg]=polyfit(dataMg(6:10),dataEC50(6:10),1);
yfitMg=polyval(paramMg,dataMg(6:10));
R2Mg=norm(yfitMg-mean(dataEC50(6:10)))^2/norm(dataEC50(6:10)-
mean(dataEC50(6:10)))^2;
plot([0,2.5],[paramMg(2),polyval(paramMg,2.5)],'k','linewidth',2)
title('Mg2+ to Dy toxicity','fontsize',15)
xlabel('Mg2+ (mM)','fontsize',14); ylabel('Dy3+ (nM)','fontsize',14)
R2Mgstr=num2str(R2Mg);text(0.2,220,['r2=',R2Mgstr])
slopeMg=num2str(paramMg(1));interceptMg=num2str(paramMg(2));
text(0.2,230,['y=',slopeMg,'x+',interceptMg])
axis([0,2.5,0,250])

% Na+
figure (3);
plot(dataNa(11:14),dataEC50(11:14),'b.','markersize',15)
hold on;
[paramNa,SNa]=polyfit(dataNa(11:14),dataEC50(11:14),1);
yfitNa=polyval(paramNa,dataNa(11:14));
R2Na=norm(yfitNa-mean(dataEC50(11:14)))^2/norm(dataEC50(11:14)-
mean(dataEC50(11:14)))^2;
plot([0,2.5],[paramNa(2),polyval(paramNa,2.5)],'k','linewidth',2)
title('Na+ to Dy toxicity','fontsize',15)
xlabel('Na+ (mM)','fontsize',14); ylabel('Dy3+ (nM)','fontsize',14)
R2Nastr=num2str(R2Na);text(0.2,220,['r2=',R2Nastr])
slopeNa=num2str(paramNa(1));interceptNa=num2str(paramNa(2));
text(0.2,230,['y=',slopeNa,'x+',interceptNa])
axis([0,2.5,0,250])

% H+
figure (4);
dataHx=dataH*10000000;
plot(dataHx(15:19),dataEC50(15:19),'b.','markersize',15)
hold on;
[paramH,SH]=polyfit(dataHx(15:19),dataEC50(15:19),1);
yfitH=polyval(paramH,dataHx(15:19));
R2H=norm(yfitH-mean(dataEC50(15:19)))^2/norm(dataEC50(15:19)-
mean(dataEC50(15:19)))^2;
plot([0,2],[paramH(2),polyval(paramH,2)],'k','linewidth',2)
title('H+ to Dy toxicity','fontsize',15)
xlabel('H+ (10^-7 M)','fontsize',14); ylabel('Dy3+
(nM)','fontsize',14)
R2Hstr=num2str(R2H);text(0.2,2200,['r2=',R2Hstr])
slopeH=num2str(paramH(1));interceptH=num2str(paramH(2));
text(0.2,2300,['y=',slopeH,'x+',interceptH])
axis([0,2,0,2500])

% calculation of the stability constants
RCa=paramCa(1)/paramCa(2);
RNa=paramNa(1)/paramNa(2);
```

```
RH=paramH(1)/paramH(2);
avgNa4Ca=mean(dataNa(1:5));
avgH4Ca=mean(dataHx(1:5));
avgCa4Na=mean(dataCa(11:14));
avgH4Na=mean(dataHx(11:14));
avgCa4H=mean(dataCa(15:19));
avgNa4H=mean(dataNa(15:19));
matrixA=[1 -RCa*avgNa4Ca -RCa*avgH4Ca
    -RNa*avgCa4Na 1 -RNa*avgH4Na
    -RH*avgCa4H -RH*avgNa4H 1];
matrixC=[RCa
    RNa
    RH];
% matrixB=[KCaBL;KNaBL;KHBL]
matrixB=matrixA\matrixC

% determination of f50/[(1-f50)*KCuBL]
K1=paramCa(1)/matrixB(1);
K2=paramNa(1)/matrixB(2);
K3=paramH(1)/matrixB(3);
avgK=(K1*paramCa(1)+K2*paramNa(1)+K3*paramH(1))/(paramCa(1)+paramNa(1
)+paramH(1))

% capacity of the BLM
% Ca
for i=1:5;

CaBLM(i)=avgK*(1+matrixB(1)*dataCa(i)+matrixB(2)*dataNa(i)+matrixB(3)
*dataHx(i));
end
% Na
for i=1:4;

NaBLM(i)=avgK*(1+matrixB(1)*dataCa(i+10)+matrixB(2)*dataNa(i+10)+matr
ixB(3)*dataHx(i+10));
end
% H
for i=1:5;

HBLM(i)=avgK*(1+matrixB(1)*dataCa(i+14)+matrixB(2)*dataNa(i+14)+matri
xB(3)*dataHx(i+14));
end

figure (5);
plot(dataEC50(1:5),CaBLM,'rd',dataEC50(11:14),NaBLM,'gs',dataEC50(15:
19),HBLM,'b*');
hold on;
plot([0,1500],[0,1500],'k','linewidth',2);
hold on;
plot([150,1500],[0,1350],'k--');
hold on;
plot([0,1350],[150,1500],'k--');
title('BLM capacity','fontsize',15)
xlabel('Observed LC50 (nM Dy3+)','fontsize',14); ylabel('Calculated
LC50 (nM Dy3+)','fontsize',14)
legend('Ca','Na','H','Location','NorthWest');
axis([0,1500,0,1500])

end
```

Appendix B Dy measurements of test without invertebrates

Table B.1 A summary table that includes all the Dy readings of the test with no invertebrates. T represents total conc. and D represents dissolved conc. Both the stabilization (0-24h) and test (24-120h) periods were measured.

Nominal conc. (µg/L)	Rep.	0h Dy (µg/L)			12h Dy (µg/L)			24h Dy (µg/L)		
		T	D	D/T %	T	D	D/T %	T	D	D/T %
200	1	206	123	60	153	141	92	137	133	97
	2	206	126	61	172	164	95	166	158	95
	3	192	115	60	174	166	96	167	160	96
	4	189	118	62	149	142	96	138	136	98
800	1	778	170	22	749	668	89	727	630	87
	2	792	192	24	833	683	82	712	662	93
	3	777	193	25	803	688	86	713	667	94
	4	775	218	28	662	574	87	599	553	92
1600	1	1563	325	21	1919	886	46	1409	822	58
	2	1588	351	22	1572	908	58	1357	830	61
	3	1581	387	24	1608	909	57	1926	811	42
	4	1575	420	27	1815	917	51	1462	801	55
3200	1	3171	606	19	4038	1017	25	2534	830	33
	2	3217	623	19	4258	1040	24	3090	855	28
	3	3204	651	20	3485	1003	29	2543	884	35
	4	3185	688	22	3871	1002	26	1968	854	43
6400	1	6270	916	15	6014	1089	18	5755	936	16
	2	6380	962	15	8190	1068	13	4232	913	22
	3	6387	957	15	6330	1074	17	5258	928	18
	4	6327	980	15	6365	1044	16	6064	909	15

Table B.1 continued

Nominal conc. (µg/L)	Rep.	76h Dy (µg/L)			120h Dy (µg/L)				120h Dy 2% acid (µg/L)	
		T	D	D/T %	T	D	D/T %	% reduced from 0h	T	D
200	1	53	42	78	61	40	65	70	237	240
	2	88	65	74	80	62	78	61	233	233
	3	83	73	89	75	70	93	61	224	227
	4	67	60	90	61	58	96	68	232	233
800	1	587	511	87	566	486	86	27	846	854
	2	554	478	86	535	451	84	32	859	867
	3	588	503	86	538	481	89	31	860	865
	4	427	386	90	409	364	89	47	885	890
1600	1	1261	630	50	1209	593	49	23	1745	1742
	2	1415	638	45	1076	644	60	32	1798	1789
	3	1278	638	50	1218	596	49	23	1751	1735
	4	1294	654	51	1255	580	46	20	1757	1757
3200	1	3309	635	19	2658	565	21	16	3402	3328
	2	2858	647	23	2569	599	23	20	3403	3384
	3	2794	639	23	2810	604	21	12	3434	3457
	4	2898	637	22	2292	588	26	28	3491	3550
6400	1	6058	713	12	4740	688	15	24	6656	6612
	2	5933	724	12	5320	684	13	17	6590	6738
	3	5834	711	12	5367	664	12	16	6698	6805
	4	5758	701	12	5125	653	13	19	6809	6582

Appendix C WHAM7 modelled Dy speciation

Table C.1 Concentrations of all the possible Dy species existing in solution for all the 17 TMF tests modeled by WHAM7 based on measured Dy 0h dissolved concentration and water chemistry which are also provided in the table.

Test	Test Condition (mM)	Dy nominal conc. (µg/L)	Dy 0h dissolved Conc. (nM)	pH	Dy species						
					$[Dy^{3+}]$ (nM)	$[DyOH^{2+}]$ (nM)	$[DyCO_3^+]$ (nM)	$[Dy(CO_3)_2^-]$ (nM)	$[DyHCO_3^{2+}]$ (nM)	$[DySO_4^+]$ (nM)	$[Dy(SO_4)_2^-]$ (nM)
	$[Na^+] = 0.579$										
	$[Mg^{2+}] = 0.159$	200	228	7.50	11	6	179	28	1	3	0.01
MSM	$[K^+] = 0.025$	800	837	7.55	34	21	651	117	4	10	0.02
Trial 1	$[Ca^{2+}] = 0.566$	1600	2074	7.60	74	51	1595	323	9	22	0.04
	$[Cl^-] = 1.157$	3200	4351	7.55	178	109	3384	606	21	53	0.09
	$[SO_4^{2-}] = 0.159$	6400	4062	7.60	146	100	3124	631	17	43	0.07
	$[CO_3^{2-}] = 0.574$										
	$[Na^+] = 0.594$										
	$[Mg^{2+}] = 0.153$	200	745	7.65	22	17	563	133	3	6	0.01
MSM	$[K^+] = 0.025$	800	2554	7.55	100	61	1984	367	12	29	0.05
Trial 2	$[Ca^{2+}] = 0.492$	1600	2062	7.65	62	48	1559	367	8	18	0.03
	$[Cl^-] = 1.009$	3200	3514	7.60	121	83	2698	562	15	35	0.06
	$[SO_4^{2-}] = 0.153$	6400	4658	7.60	160	110	3578	743	20	47	0.08
	$[CO_3^{2-}] = 0.589$										
	$[Na^+] = 0.571$										
	$[Mg^{2+}] = 0.17$	200	1028	7.70	32	26	767	192	3	8	0.01
1mM	$[K^+] = 0.025$	800	3729	7.70	115	94	2784	692	13	30	0.05
Ca^{2+}	$[Ca^{2+}] = 1.09$	1600	6000	7.65	213	155	4552	1000	23	56	0.09
Trial 1	$[Cl^-] = 2.205$	3200	8117	7.65	289	211	6161	1348	31	76	0.13
	$[SO_4^{2-}] = 0.17$	6400	8898	7.65	318	232	6755	1476	34	83	0.14
	$[CO_3^{2-}] = 0.566$										

Table C.1 continued

Test	Test Condition (mM)	Dy nominal conc. (µg/L)	Dy 0h dissolved Conc. (nM)	pH	Dy species						
					$[Dy^{3+}]$ (nM)	$[DyOH^{2+}]$ (nM)	$[DyCO_3^{+}]$ (nM)	$[Dy(CO_3)_2^{-}]$ (nM)	$[DyHCO_3^{2+}]$ (nM)	$[DySO_4^{+}]$ (nM)	$[Dy(SO_4)_2^{-}]$ (nM)
1mM Ca^{2+} Trial 2	$[Na^+]$ = 0.599										
	$[Mg^{2+}]$ = 0.146	200	320	7.60	12	8	245	51	1	3	0.00
	$[K^+]$ = 0.025	800	1145	7.65	37	28	864	202	4	9	0.01
	$[Ca^{2+}]$ = 0.986	1600	2665	7.60	100	65	2042	422	12	23	0.03
	$[Cl^-]$ = 1.997	3200	3822	7.55	164	96	2963	542	19	38	0.06
	$[SO_4^{2-}]$ = 0.146	6400	5963	7.55	256	150	4625	842	29	60	0.09
	$[CO_3^{2-}]$ = 0.594										
1mM Ca^{2+} Trial 3	$[Na^+]$ = 0.586										
	$[Mg^{2+}]$ = 0.149	200	431	7.55	19	11	334	60	2	4	0.01
	$[K^+]$ = 0.025	800	1711	7.50	87	45	1338	212	10	20	0.03
	$[Ca^{2+}]$ = 1.095	1600	3766	7.50	191	99	2945	465	21	44	0.06
	$[Cl^-]$ = 2.215	3200	5397	7.50	275	142	4221	664	30	64	0.09
	$[SO_4^{2-}]$ = 0.149	6400	7305	7.45	424	195	5743	797	46	98	0.14
	$[CO_3^{2-}]$ = 0.581										
2mM Ca^{2+} Trial 1	$[Na^+]$ = 0.563										
	$[Mg^{2+}]$ = 0.17	200	505	7.70	18	14	377	91	2	4	0.01
	$[K^+]$ = 0.025	800	1963	7.70	70	54	1466	351	7	15	0.02
	$[Ca^{2+}]$ = 2.014	1600	3249	7.60	151	92	2490	469	15	31	0.05
	$[Cl^-]$ = 4.053	3200	4258	7.60	199	121	3264	613	20	41	0.06
	$[SO_4^{2-}]$ = 0.17	6400	6511	7.60	306	186	4991	934	30	61	0.09
	$[CO_3^{2-}]$ = 0.558										

Table C.1 continued

Test	Test Condition (mM)	Dy nominal conc. (µg/L)	Dy 0h dissolved Conc. (nM)	pH	Dy species						
					$[Dy^{3+}]$ (nM)	$[DyOH^{2+}]$ (nM)	$[DyCO_3^+]$ (nM)	$[Dy(CO_3)_2^-]$ (nM)	$[DyHCO_3^{2+}]$ (nM)	$[DySO_4^+]$ (nM)	$[Dy(SO_4)_2^-]$ (nM)
	$[Na^+] = 0.571$										
	$[Mg^{2+}] = 0.339$	800	1052	7.65	35	26	790	177	4	20	0.07
0.25mM	$[K^+] = 0.025$	1600	2172	7.65	72	54	1632	365	8	41	0.14
Mg^{2+}	$[Ca^{2+}] = 0.617$	3200	3477	7.65	116	86	2613	582	13	66	0.23
Trial 1	$[Cl^-] = 1.259$	6400	5846	7.60	223	148	4446	875	25	128	0.44
	$[SO_4^{2-}] = 0.339$										
	$[CO_3^{2-}] = 0.566$										
	$[Na^+] = 0.561$										
	$[Mg^{2+}] = 0.659$	200	271	7.80	6	7	191	60	1	6	0.04
0.5mM	$[K^+] = 0.025$	800	935	7.80	22	23	661	205	2	22	0.14
Mg^{2+}	$[Ca^{2+}] = 0.641$	1600	1785	7.80	42	43	1261	391	5	42	0.27
Trial 1	$[Cl^-] = 1.307$	3200	2843	7.75	78	71	2046	563	8	77	0.49
	$[SO_4^{2-}] = 0.659$	6400	4043	7.65	144	104	2989	647	15	143	0.91
	$[CO_3^{2-}] = 0.556$										
	$[Na^+] = 0.682$										
	$[Mg^{2+}] = 2.449$	200	634	7.65	21	14	432	110	2	54	1.05
2mM	$[K^+] = 0.025$	800	2455	7.65	82	53	1672	425	9	209	4.07
Mg^{2+}	$[Ca^{2+}] = 0.516$	1600	2843	7.65	95	62	1936	492	11	242	4.72
Trial 1	$[Cl^-] = 1.057$	3200	3797	7.65	127	83	2586	657	14	323	6.31
	$[SO_4^{2-}] = 2.449$	6400	6443	7.65	217	141	4389	1110	24	551	10.75
	$[CO_3^{2-}] = 0.677$										

Table C.1 continued

Test	Test Condition (mM)	Dy nominal conc. (µg/L)	Dy 0h dissolved Conc. (nM)	pH	Dy species						
					$[Dy^{3+}]$ (nM)	$[DyOH^{2+}]$ (nM)	$[DyCO_3^+]$ (nM)	$[Dy(CO_3)_2^-]$ (nM)	$[DyHCO_3^{2+}]$ (nM)	$[DySO_4^+]$ (nM)	$[Dy(SO_4)_2^-]$ (nM)
	$[Na^+]$ = 1.122										
	$[Mg^{2+}]$ = 0.183	200	431	7.65	14	10	325	76	2	4	0.01
1mM	$[K^+]$ = 0.025	800	1286	7.60	47	31	984	203	6	15	0.03
Na^+	$[Ca^{2+}]$ = 0.604	1600	2917	7.55	122	73	2258	411	14	38	0.07
Trial 1	$[Cl^-]$ = 1.85	3200	4394	7.60	162	108	3364	690	19	51	0.10
	$[SO_4^{2-}]$ = 0.183	6400	5188	7.60	192	127	3972	813	22	60	0.12
	$[CO_3^{2-}]$ = 0.587										
	$[Na^+]$ = 1.982										
	$[Mg^{2+}]$ = 0.143	200	985	7.70	28	23	731	192	3	7	0.01
2mM	$[K^+]$ = 0.025	800	3742	7.55	160	94	2902	528	18	39	0.06
Na^+	$[Ca^{2+}]$ = 0.441	1600	4763	7.60	178	118	3653	749	21	44	0.07
Trial 1	$[Cl^-]$ = 2.384	3200	5415	7.60	203	134	4154	850	24	50	0.08
	$[SO_4^{2-}]$ = 0.143	6400	7225	7.50	353	185	5653	905	40	87	0.13
	$[CO_3^{2-}]$ = 0.587										
	$[Na^+]$ = 0.601										
	$[Mg^{2+}]$ = 0.158										
4mg/L	$[K^+]$ = 0.025	800	3132 (3)[a]	7.65	0	0	2	1	0	0	0.00
SR DOC	$[Ca^{2+}]$ = 0.571	3200	10831 (414)	7.65	12	9	313	75	2	4	0.01
Trial 1	$[Cl^-]$ = 1.167	6400	21692 (6387)	7.60	222	151	4899	1024	27	65	0.11
	$[SO_4^{2-}]$ = 0.158	12800	37329 (20190)	7.50	935	507	15831	2533	110	272	0.46
	$[CO_3^{2-}]$ = 0.596										
	DOC = 9.6 mg/L										

Table C.1 continued

Test	Test Condition (mM)	Dy nominal conc. (µg/L)	Dy 0h dissolved Conc. (nM)	pH	Dy species						
					$[Dy^{3+}]$ (nM)	$[DyOH^{2+}]$ (nM)	$[DyCO_3^+]$ (nM)	$[Dy(CO_3)_2^-]$ (nM)	$[DyHCO_3^{2+}]$ (nM)	$[DySO_4^+]$ (nM)	$[Dy(SO_4)_2^-]$ (nM)
8mg/L SR DOC Trial 1	$[Na^+] = 0.588$ $[Mg^{2+}] = 0.167$ $[K^+] = 0.025$ $[Ca^{2+}] = 0.545$ $[Cl^-] = 1.115$ $[SO_4^{2-}] = 0.167$ $[CO_3^{2-}] = 0.583$ DOC = 12.55 mg/L	800	3834 (3)	7.65	0	0	2	0	0	0	0.00
		1600	6991 (14)	7.65	0	0	10	2	0	0	0.00
		3200	13083 (272)	7.65	8	6	205	48	1	3	0.00
		6400	26954 (6772)	7.60	240	164	5202	1064	29	74	0.13
		12800	39828 (17665)	7.50	829	451	13852	2179	96	257	0.46
8mg/L SR DOC Trial 2	$[Na^+] = 0.643$ $[Mg^{2+}] = 0.152$ $[K^+] = 0.025$ $[Ca^{2+}] = 0.498$ $[Cl^-] = 1.021$ $[SO_4^{2-}] = 0.152$ $[CO_3^{2-}] = 0.638$ DOC = 0 mg/L[b]	800	418	7.60	13	9	319	72	2	4	0.01
		1600	1366	7.65	37	29	1023	261	5	11	0.02
		3200	1477	7.65	40	31	1106	282	5	12	0.02
		6400	2529	7.65	69	54	1894	482	9	20	0.03
		12800	5532	7.60	175	120	4216	947	23	50	0.08
pH 6.3 Trial 1	$[Na^+] = 0.573$ $[Mg^{2+}] = 0.171$ $[K^+] = 0.025$ $[Ca^{2+}] = 0.601$ $[Cl^-] = 1.227$ $[SO_4^{2-}] = 0.171$ $[CO_3^{2-}] = 0.568$	200	1058	6.75	276	27	631	14	25	86	0.16
		800	4622	6.75	1209	116	2751	59	108	377	0.69
		1600	8806	6.70	2537	217	4952	92	218	789	1.43
		3200	18622	6.70	5405	463	10430	191	459	1669	3.01
		6400	34874	6.70	10249	877	19404	349	854	3131	5.60

Table C.1 continued

Test	Test Condition (mM)	Dy nominal conc. (µg/L)	Dy 0h dissolved Conc. (nM)	pH	Dy species						
					$[Dy^{3+}]$ (nM)	$[DyOH^{2+}]$ (nM)	$[DyCO_3^+]$ (nM)	$[Dy(CO_3)_2^-]$ (nM)	$[DyHCO_3^{2+}]$ (nM)	$[DySO_4^+]$ (nM)	$[Dy(SO_4)_2^-]$ (nM)
pH 8.3 Trial 1	$[Na^+]$ = 0.575										
	$[Mg^{2+}]$ = 0.164	200	74	8.05	1	1	45	26	0	0	0.00
	$[K^+]$ = 0.025	800	585	8.05	6	11	357	208	1	2	0.00
	$[Ca^{2+}]$ = 0.627	1600	1305	8.05	13	25	797	464	2	4	0.01
	$[Cl^-]$ = 1.279	3200	1495	8.00	17	30	949	492	2	5	0.01
	$[SO_4^{2-}]$ = 0.164	6400	1926	7.95	26	40	1266	583	3	8	0.01
	$[CO_3^{2-}]$ = 0.570										
pH 8.3 Trial 2	$[Na^+]$ = 0.580										
	$[Mg^{2+}]$ = 0.173	200	142	8.05	1	3	86	51	0	0	0.00
	$[K^+]$ = 0.025	800	455	8.05	4	9	277	163	1	1	0.00
	$[Ca^{2+}]$ = 0.591	1600	683	8.05	7	13	416	245	1	2	0.00
	$[Cl^-]$ = 1.207	3200	985	8.05	10	19	599	353	1	3	0.01
	$[SO_4^{2-}]$ = 0.173	6400	1908	8.05	19	36	1162	682	2	6	0.01
	$[CO_3^{2-}]$ = 0.575										

[a] number in brackets is total aqueous concentration after eliminating the Dy bound to DOC

[b] based on the measured Dy concentration the DOC is not working, so use 0 here