Evaluation of the Reliability of Biotic Ligand Model Predictions for Copper Toxicity in Waters Characteristic of the Arid West

Final Report for Arid West Water Quality Research Project

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FOREWORD

The Arid West Water Quality Research Project (AWWQRP or "Project") was established in 1995 as a result of a federal appropriation (Public Law 103-327) and the establishment of an Assistance Agreement between the U.S. Environmental Protection Agency (USEPA) and Pima County Wastewater Management (PCWMD), Tucson, Arizona. The establishment of this Agreement provided a significant opportunity for western water resource stakeholders to (1) work cooperatively to conduct scientific research to recommend appropriate water quality criteria, standards and uses for effluent-dependent and ephemeral waters in the arid and semi-arid regions of the West ("arid West"), and (2) improve the scientific basis for regulating wastewater and stormwater discharges in the arid West. Effluent-dependent waters are created by the discharge of treated effluent into ephemeral streambeds or streams that in the absence of effluent discharge would have only minimal flow.

With the establishment of the AWWQRP, a management infrastructure was created to support the development of peer-reviewed research products. From within the Environmental Planning Division of PCWMD, the AWWQRP Project Director, Program Manager and support staff administer the Project. A Regulatory Working Group (RWG), comprised of 15 stakeholders representing both public and private interests, works to ensure that Project research has a sound regulatory basis and that research activities focus on important regulatory concerns. The Scientific Advisory Group (SAG), comprised of scientists with experience in water quality research, makes certain that project research has a sound scientific basis and that studies are properly designed and technically sound.

This report represents the fourth in a series of research reports produced by the AWWQRP, and builds upon already completed work. The first report in the series, *Pre-Research Survey of Municipal NPDES Dischargers in the Arid and Semi-Arid West*, resulted from an RWG recommendation that the Project survey arid West wastewater facilities to compile information about their effluent discharges and associated water quality concerns.

The second report, the *Habitat Characterization Study*, utilized the findings of the Discharger Survey. Recognizing that an understanding of the attributes of effluent-dependent waters was critical to the development of appropriate water quality criteria and standards for these waters, the RWG recommended that the AWWQRP commission a major study to describe the physical, chemical, and biological characteristics of effluent-created habitats.

The *Habitat Characterization Study* evaluated the physical, chemical and biological characteristics of effluent-dependent habitats at ten case study sites in the arid West: Santa Cruz River below Nogales and below Tucson, Arizona; Salt River below Phoenix, Arizona; Santa Ana River below San Bernardino, California; Fountain Creek below Colorado Springs, Colorado; South Platte River below Denver, Colorado; Las Vegas Wash below Las Vegas, Nevada; Santa Fe River below Santa Fe, New Mexico; Carrizo Creek below Carrizo Springs, Texas; and Crow Creek below Cheyenne, Wyoming (Figure F-1). The primary objectives of this effort were to (1) review existing physical, chemical and biological data; (2) conduct a site reconnaissance to characterize habitats using established protocols and protocols adapted for arid West conditions; (3) identify similarities and differences among sites; (4) discuss potential approaches to protect these habitats in the context of existing regulatory programs; and (5) recommend areas for additional study. The final report may be downloaded from the AWWQRP website, www.co.pima.az.us/wwm/wqrp, or obtained from the AWWQRP Office in a CD hyperlinked format.

The AWWQRP's third report, *Extant Criteria Evaluation*, evaluated the applicability of national water quality criteria, as well as the methods to modify those criteria, to effluent-dependent and ephemeral waters in the arid West. This work built upon the findings presented in the *Habitat Characterization Study* using the expertise of national water quality criteria researchers. The AWWQRP used the findings and recommendations contained in the *Extant Criteria Evaluation* as the primary driver for the selection and execution of three subsequent research projects, including evaluations of 1) the Biotic Ligand Model of copper toxicity in arid west streams, 2) use of the EPA recalculation procedure in effluent-dependent streams, and 3) potential hardness-modifications to ammonia toxicity and their implications for use of the water-effect ratio.

Figure F-1. *Habitat Characterization Study* Case Study Sites.

The purpose of this fourth report, *Evaluation of the Reliability of Biotic Ligand Model (BLM) Predictions for Copper Toxicity in Waters Characteristic of the Arid West*, ("BLM Study") is to evaluate the relevance of the BLM for deriving site-specific copper criteria in effluent-dependent and effluent-dominated streams in the arid West. The BLM was developed from studies conducted in relatively soft to moderately hard test waters, and so may not adequately represent chemical conditions often encountered in very hard waters. As a result, this study was initiated to help evaluate whether the BLM represents a viable alternative for derivation of site-specific copper criteria in effluent-dependent waters of the arid West.

The SAG provided a technical review of the findings from the BLM Study. After the SAG comments were addressed, the report was submitted to the RWG and USEPA for additional technical and regulatory review. Comments of a technical nature were covered in a response matrix, with major comments addressed in the report, as necessary. Most of the RWG and USEPA comments were more directly related to policy and implementation issues, rather than to the scientific content and recommendations in this report. As such, even though the findings of this study have received both technical and regulatory reviews, it is strongly recommended that local state and regional USEPA staff should be consulted prior to using these findings to support or propose regulatory change.

The AWWQRP has made a significant effort to share Project results and their implications in a variety of technical, regulatory, industry and public interest forums, including publication in the primary scientific literature. This outreach effort is designed to create a broader understanding of water quality issues unique to the arid West and provide scientific and regulatory data in support of a regional approach to the development of water quality criteria, standards and uses. Heightened interest in arid West water quality issues has been fueled by the recognition that treated effluent can have a valuable role in the support and enhancement of riparian ecosystems, particularly in light of increasingly limited water resources. The AWWQRP looks forward to continuing its support of research that not only provides critical data to address unique western water quality issues, but also supports the development of innovative solutions.

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ACRONYM LIST

µg/L	microgram(s) per liter
µm	micrometer
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
AWQC	ambient water quality criteria
AWWQRP	Arid West Water Quality Research Project
BLM	biotic ligand model
Ca	calcium
CCC	criterion continuous concentration (chronic criterion)
Cl	chloride
CMC	criterion maximum concentration (acute criterion)
CO_3	carbonate
Cu	copper
Cu^{2+}	hydrated copper ion
$CuOH^-$	copper hydroxide
DO	dissolved oxygen
DOC	dissolved organic carbon
FAV	final acute value
GMAV	genus mean acute value
h	hour
K	potassium
L	liter
LA50	median lethal accumulation concentration – point estimate for 50% mortality used in biotic ligand model
LC50	median lethal concentration – point estimate for 50% mortality
M	moles per liter (molar)
Mg	magnesium
mg/L	milligram(s) per liter
ml	milliliter
Na	sodium
NPDES	National Pollutant Discharge Elimination System
NH_3	ammonia
PCWMD	Pima County Wastewater Management Department
SMAV	species mean acute value

ACRONYM LIST (CONTINUED)

SO_4	sulfate
SS-AWQC	site-specific ambient water quality criterion
USEPA	U.S. Environmental Protection Agency
WER	Water-Effect Ratio

EXECUTIVE SUMMARY

Metal toxicity often varies as a function of water hardness, and so Ambient Water Quality Criteria (AWQC) for metals, including copper, are typically derived as a mathematical function of hardness. Since the inception of the hardness-based criteria for copper, the upper limit for application of this equation has been set at 400 mg/L as $CaCO_3$. However, copper toxicity in very hard surface waters (i.e., waters with hardness > 400 mg/L), such as effluent-dependent streams of the arid western U.S., may not be accurately represented by this equation. At the present time, there are only two formal recommendations from the USEPA for calculating a site-specific copper criterion in waters with hardness greater than 400 mg/L as $CaCO_3$; (1) calculate the criterion using a hardness of 400 mg/L in the hardness equation; or (2) calculate the criterion using a measured Water-Effect Ratio (WER; discussed below) and the actual ambient hardness of the surface water in the equation. However, both of these approaches have the potential of generating acute criteria that are under-protective of sensitive aquatic biota.

Because copper toxicity in arid western streams is often controlled by water quality variables other than hardness (e.g., alkalinity, pH, dissolved organic matter, and major ions), hardness equations or WERs may not be the most appropriate methods for determining site-specific criteria for copper. However, a computational model (called the Biotic Ligand Model; BLM) has been recently developed that considers how these water quality factors influence the chemical interactions between copper and the external binding sites on the organism that cause toxicity (e.g., fish gill). By understanding how water quality influences these chemical interactions, it is hoped that site-specific criteria can be appropriately applied to all surface waters. The BLM is also important because it is being proposed for use in derivation of acute freshwater criteria in USEPA's most current draft AWQC for copper. However, the BLM was developed from studies conducted in relatively soft to moderately hard test waters, and so may not adequately represent chemical conditions often encountered in very hard waters. As a result, it is as yet uncertain whether the BLM represents a viable alternative for derivation of site-specific copper criteria in effluent-dependent waters of the arid West.

The goal of this study was to evaluate the relevance of the BLM for deriving site-specific copper criteria in effluent-dependent and effluent-dominated streams in the arid West. The process of generating toxicity data for validation of the BLM in various surface waters with elevated hardness also provided the opportunity to determine the appropriateness of more traditional and currently recommended site-specific copper criteria derivation methodologies (hardness-based equation and Water-Effect Ratio methodology). To test these methods, we conducted acute toxicity tests with three different aquatic test species (*Ceriodaphnia dubia*, *Daphnia pulex*, and *Pimephales promelas*) in seven different effluent-dependent waters. The sites were representative of effluent-dependent or effluent-dominated streams common to the arid west and were chosen based on historical water quality data to ensure a wide range of water quality variables were tested. The sites selected for this study were:

EXECUTIVE SUMMARY (CONTINUED)

Location (City, State)	Drainage	Flow Condition Tested Base	Medium	High
Albany, OR	Drainage Swale	X	-	-
Denver, CO	South Platte River	X	X	X
Globe, AZ	Pinal Creek	X	-	-
Las Vegas, NV	Las Vegas Wash	X	-	-
Los Alamos, NM	Sandia Canyon	X	-	-
Phoenix, AZ	Salt River	X	X	X
Riverside, CA	Santa Ana River	X	X	X

CONCLUSIONS AND REGULATORY IMPLICATIONS

Conclusions from this study suggest that the BLM generates more appropriate and protective copper standards for waters with elevated hardness when compared to the hardness-based equation or Water-Effect Ratio (WER) approaches. Although the historical site-specific methods (hardness equation and WER) are useful for surface waters with low to moderate levels of hardness, the unique chemical conditions of arid West streams require site-specific methods that account for the influences of all water quality variables (i.e., pH, dissolved organic carbon, alkalinity, and major ions). Therefore, the BLM offers an improved alternative to the hardness-based and WER approach for modifying copper criteria, particularly for situations where the current methods would be under-protective of sensitive aquatic life.

Water Effect Ratios

WER values ranged from less than one to greater than 13 for the natural waters tested in this study, meaning that copper toxicity was up to 13-fold lower in site waters when compared to hardness-matched synthetic laboratory waters. WER values among species varied by up to 15-fold at individual sites and greater than tenfold among sites for individual species. Generally speaking, *C. dubia* produced the largest WER values and fathead minnows had the smallest (most conservative). However, results for Pinal Creek suggested that the invertebrates were equally sensitive to site and laboratory water (i.e., WER values approximating one) while the fathead minnows had a calculated WER of greater than ten. WER values also changed substantially during different flow conditions and were generally highest at base flow and decreased with elevated flows. However, WER values were relatively similar among flow levels for the Santa Ana River, most likely owing to significantly elevated flows being observed there throughout the study.

Several design strategies and implementation concerns should be considered prior to initiating a definitive WER study for waters of elevated hardness. First, because hardness was not correlated with alkalinity in site waters used in the present study, both parameters should be matched in concurrent reconstituted waters to account for confounding variables. Second, the current study clearly demonstrated the importance of matching ion ratios (primarily calcium and magnesium) of the concurrent reconstituted water to site conditions. Finally, calculating site-specific criteria from observed WER values coupled with use of the existing hardness equation is likely to be under-protective and, thus, not appropriate in waters with hardness greater than 200 mg/L as $CaCO_3$.

EXECUTIVE SUMMARY (CONTINUED)

Calcium vs. Magnesium Hardness

While both calcium and magnesium contribute to water hardness, they may not exert similar influences on copper toxicity to all freshwater organisms. In the present study, increasing total hardness from 200 to 1000 mg/L as $CaCO_3$ using either calcium or magnesium had considerably different effects on acute copper toxicity to *C. dubia* and fathead minnows. A tenfold addition of calcium doubled the LC50 for each species. However, while a similar protective effect from magnesium addition occurred for *C. dubia*, acute toxicity to fathead minnows remained constant. These results justified incorporation of a Mg-gill interaction into the BLM to account for competition between magnesium and copper on the biotic ligand of invertebrates. Additionally, an increase in copper toxicity to fathead minnows at the highest magnesium concentrations was observed which was also incorporated into the model to improve performance in high hardness waters.

Biotic Ligand Model Performance

The unmodified version of the BLM only predicted 61% of the copper toxicity values in the present study with reasonable accuracy (i.e., within two-fold of empirical toxicity values). While the majority of the unacceptable predictions were for the fathead minnow, the model performed remarkably well for the two invertebrates whose sensitivity to copper were closest to the acute criterion concentration. Further investigations revealed that carbonate precipitation was likely occurring in site and laboratory waters due to elevated concentrations of calcium and magnesium. Consideration of carbonate precipitation and interactions between magnesium and the biotic ligand of fish and invertebrates improved model predictions by 40%. Therefore, the present study demonstrated the utility of considering the influence of all water quality variables when deriving site-specific criteria for waters with elevated hardness.

Regulatory Implications

Neither of the formal recommendations from the USEPA for calculating a site-specific copper criterion in waters with hardness greater than 400 mg/L as $CaCO_3$ (i.e., capped hardness equation or the product of a measured WER and hardness equation) were protective of all sites used in this study. For example, the first approach would likely be under-protective of sensitive biota in the Albany Drainage Swale and Pinal Creek because the hardness equation produced a criterion equal to or greater than the acute lethal concentration of copper to *Ceriodaphnia dubia*. The consequence of regulating copper at an acutely lethal level could be decreased populations of sensitive biota. Similarly, the second approach (WER) would be under-protective of sensitive biota in six of the seven sites tested (Albany Drainage Swale, South Platte River, Pinal Creek, Las Vegas Wash, Salt River, and Santa Ana River).

In contrast, the BLM-derived acute criterion for copper was protective of sensitive biota for all seven of the sites used in this study. This demonstrates the utility of considering the influence of all water quality variables, particularly when deriving site-specific criteria for waters with elevated hardness. Our results suggest that the BLM offers an improved alternative to both of the current site-specific methods for modifying copper criteria, particularly for situations where the hardness equation and WER approach would under-protect sensitive aquatic life.

1. INTRODUCTION

Metal toxicity often varies as a function of water hardness (Wood et al. 1997), and so Ambient Water Quality Criteria (AWQC) for metals, including copper, are typically derived as a mathematical function of hardness (USEPA 1984c, 1985). Since the inception of the hardness-based criteria for copper, the upper limit for application of this equation has been set at 400 mg/L as $CaCO_3$ (USEPA 1984b, 2002b). However, copper toxicity in very hard surface waters, such as effluent-dependent streams of the arid western U.S., may not be accurately represented by this equation. Despite extensive research related to the chemical interactions between copper, water quality (e.g., pH, dissolved organic matter and major ions) and their effects on aquatic biota (de Schemphelaere and Janssen 2002, Erickson et al. 1996, Lauren and McDonald 1986, Welsh et al. 1993, Welsh et al. 2000a, Welsh et al. 2000b), effluent-dependent waters present unique combinations of water quality parameters that are not adequately represented by hardness-based equations. For example, hardness and alkalinity do not necessarily co-vary in surface waters of the arid western U.S. as they do in most natural systems (PCWMD 2002). As a result, hardness equations may not accurately represent the more realistic and complex factors which control copper toxicity in very hard waters.

Likewise, the relative ratios of calcium and magnesium (hardness cations) vary among water bodies and, thus, may also affect copper toxicity differently. For example, water hardness consisting primarily of calcium ions (i.e., Ca:Mg molar ratios of greater than two) is more protective of copper toxicity to freshwater fish (Naddy et al. 2002, Welsh et al. 2000b), while water hardness consisting of similar proportions of calcium and magnesium (i.e., Ca:Mg molar ratio of $\leq$ 1) is more protective of copper toxicity to aquatic invertebrates (de Schemphelaere and Janssen 2002, Gensemer et al. 2002, Naddy et al. 2002). The implications of these findings on water quality criteria development and site-specific modification are twofold. First, ephemeral waters and some effluent-dependent streams sometimes lack fish species and site-specific water quality criteria may best be based on available toxicological data for sensitive invertebrate species (e.g., Arizona's ephemeral waters; see also PCWMD 2005). Second, Ca:Mg molar ratios vary significantly by region (e.g., from two in effluent-dependent streams such as the Las Vegas Wash, NV, USA to 35 in gypsum-treated hardrock mine effluents), and absolute concentrations of magnesium can be relatively high compared to effluent-dependent waters in more mesic regions of the U.S.

1.1 THE BIOTIC LIGAND MODEL (BLM)

Recent efforts have been made towards developing computational models that incorporate chemical equilibrium and metal-gill binding to better represent the complex chemical factors that influence copper bioavailability (i.e., development of biotic ligand models; de Schemphelaere and Janssen 2002, Di Toro et al. 2001). Unlike the hardness equation, this type of model (termed the Biotic Ligand Model; BLM) explicitly accounts for individual water quality variables and is not linked to a particular correlation between toxicity and these variables. However, the mechanistic principles underlying the BLM follow general trends of copper toxicity as related to individual water quality variables and their combinations. The basic presumption is that any changes in water quality that decrease the concentrations of copper (primarily Cu^{2+} and $CuOH^-$ to a lesser degree) which can chemically bind to biological surfaces (i.e., the “biotic ligand”) are associated with decreasing copper toxicity (Di Toro et al. 2001, USEPA 2003). For example, increases in pH, alkalinity, or natural organic matter would all tend to decrease copper bioavailability and increase median-lethal concentrations (LC50) for copper (Erickson et al. 1996). Copper bioavailability may also be affected by competitive interactions at the biotic ligand (e.g., fish gill; Figure 1.1) with calcium and sodium, thereby increasing copper LC50 values (Erickson et al. 1996). The interactions between the biotic ligand and each of the dissolved chemical species with which it reacts are represented by characteristic binding site densities and conditional stability constants (Playle et al. 1993).

Predictions of copper toxicity are made by assuming that the dissolved copper LC50, which varies with water chemistry, is always associated with a fixed critical level of copper accumulation at the biotic ligand. While the median-lethal gill copper accumulation concentration (LA50) can vary based on species sensitivity (i.e., more or less copper-gill accumulation required to exert a similar toxic response), they are assumed to be constant for individual species, regardless of water quality (Meyer et al. 1999). The advantage of using a mechanistic model for predicting copper toxicity is that all thermodynamic constants used to simulate inorganic and organic equilibrium reactions are empirically derived. As such, the constants do not change for simulations involving different organisms. For example, although binding constants for copper and other cations were derived using fathead minnows, the values apply to invertebrates and other fish (Santore et al. 2001).

Analogous to criteria derivation based on normalizing available toxicity data to a similar hardness (USEPA 1984a), the BLM was used to generate water quality-normalized LA50 values in USEPA's latest draft AWQC for copper (USEPA 2003). Although not all historical studies reported concentrations of required water quality data, the dataset was supplemented by new data from current research (USEPA 2003). As a result, species sensitivity was ranked relative to species-specific LA50 values and the acute criterion was established that simulated the physiology of a hypothetical organism that was more sensitive than 95% of all freshwater fauna. As a tool for deriving site-specific criteria, the BLM predicts the acutely toxic concentration of copper for this hypothetical organism, based on the actual water quality conditions in the waterbody of concern. This approach represents a significant departure from the current hardness-based copper criteria, and so long as the BLM is adequately validated for a wide range of water quality conditions, it should provide more scientifically-defensible site-specific water quality criteria.

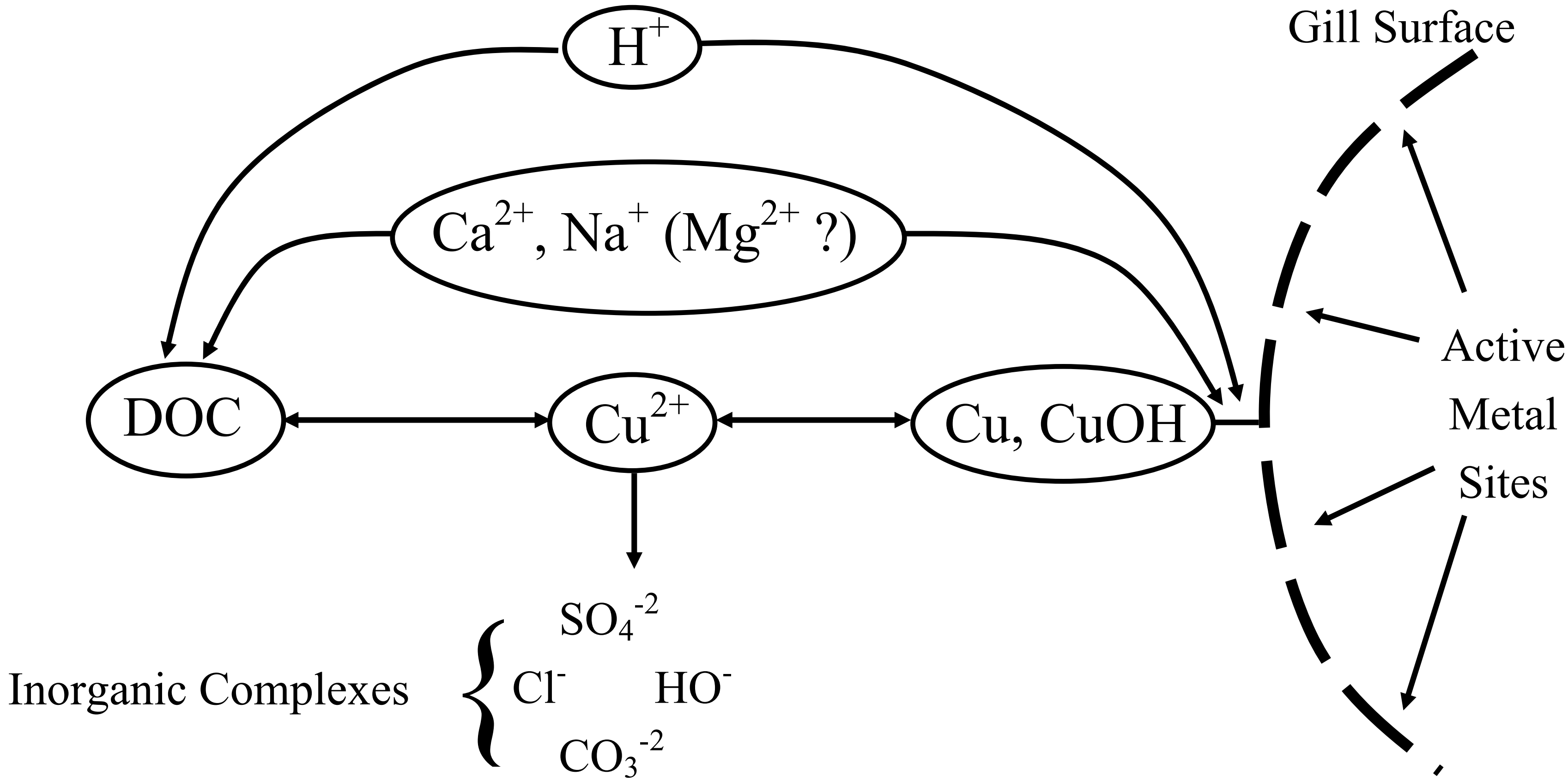

Figure 1-1. Conceptual diagram of the Biotic Ligand Model for copper which considers interactions with competing cations (calcium, sodium, and possibly magnesium) and complexing ligands such as inorganic moieties (e.g., carbonate, hydroxide, chloride, and sulfate) and organic compounds (gill surface and dissolved organic carbon; DOC). Diagram adapted from DiToro et al. (2001) and U.S. EPA (2003).

1.2 STUDY OBJECTIVES AND APPROACH

Even though the BLM was developed using data from relatively soft to moderately-hard waters, previous studies have suggested that model predictions may still be accurate even in very hard waters (Gensemer et al. 2002). Although these initial BLM results were promising, they were based on the outcome of a single study of copper toxicity in only three types of reconstituted water to the cladoceran *Ceriodaphnia dubia*. Evaluating the generality of this conclusion for copper requires testing with additional species and using natural waters with varying water quality. Similarly, Gensemer et al. (2002) proposed that the accuracy of BLM predictions could be improved by incorporating a Mg-gill interaction which is not presently included in the model (i.e., only Ca-gill interactions are used to represent the influence of hardness cations). This modification was based on the assumption that calcium and magnesium behaved similarly at the biotic ligand of *C. dubia*; this assumption has received limited empirical support for invertebrates (de Schemphelaere and Janssen 2002). However, a more rigorous experimental design is needed to independently control for the relative amounts of calcium and magnesium present in exposure waters in order to validate the inclusion of magnesium-biotic ligand interactions into models for both invertebrates and fish.

Therefore, the primary project objectives of this study were threefold:

1. Conduct acute copper toxicity tests with three different aquatic test species (*C. dubia*, *Daphnia pulex*, and *Pimephales promelas*) under a range of water quality conditions that were representative of waters in the arid West. This range of water quality conditions was generated by testing in natural waters from sites already studied in the Extant Criteria Evaluation (PCWMD 2003) and Habitat Characterization Study (PCWMD 2002) projects under both low and high flow conditions.

2. Conduct acute copper toxicity tests with two different aquatic test species (*C. dubia* and *P. promelas*) under a range of calcium and magnesium concentrations that are representative of waters in the arid West. By fluctuating the concentrations of calcium or magnesium within each toxicity test media, while maintaining constant levels of other water quality parameters (e.g., alkalinity, pH, sodium, etc.), the independent effects of calcium or magnesium on copper toxicity can be directly compared.

3. Conduct an analysis of both empirical relationships between water quality characteristics and copper toxicity, and an analysis of the accuracy and precision with which the BLM predicts copper toxicity in arid West waters. In addition, the incorporation of magnesium-gill interactions into the BLM for both *C. dubia* and *P. promelas* will be validated.

Our general approach was to conduct a series of Water-Effect Ratio (WER)-style studies to compare copper toxicity in natural waters to that of laboratory reconstituted waters which are matched to site hardness and alkalinity. This approach is important for several reasons. First, the Indicator Species, or WER, procedure is intended to take into account how water quality characteristics affect the toxicity of contaminants (most typically metals) in laboratory dilution water relative to that in site water. Second, comparative testing between natural and reconstituted waters provides the opportunity to design reconstituted water recipes specifically to evaluate the influence of ion composition on copper toxicity. Finally, conducting WER-style tests allows for a direct comparison between site-specific water quality standards generated by an empirical WER study, to standards generated by BLM predictions as is currently being proposed for the anticipated revision to the AWQC for copper (USEPA 2003).

For cation competition studies, acute toxicity tests were conducted in reconstituted waters to elucidate the relative importance of calcium and magnesium concentrations on acute copper toxicity in very hard waters of the arid West. This approach is important for two reasons. First, although several researchers have alluded to the species-specific importance of calcium and magnesium at varying ratios, this phenomenon has not been characterized at hardness levels above 200 mg/L as $CaCO_3$. Similar to concerns related to the hardness-based criteria for copper, the influence of ionic composition at elevated levels of hardness needs to be properly characterized to validate proposed alternatives to criteria modification (i.e., BLM). Second, comparative testing between distinctly different aquatic taxa provides the opportunity to test hypotheses related to the mechanism of copper toxicity and bioavailability. Given that preliminary results of the BLM for acute copper toxicity to *C. dubia* have suggested possible need to include additional biotic ligand-metal interactions (e.g., Mg-gill), it would be valuable to further characterize this competition and calibrate the BLM for very hard waters often encountered in the arid West.

2. MATERIALS AND METHODS

2.1 SITE SELECTION

Water samples from seven effluent-dependent waters were used to evaluate acute copper toxicity across a range of water quality conditions. Sites were selected from an existing database of water quality for western U.S. rivers and streams (PCWMD 2002) based on two criteria. First, each site was representative of effluent-dependent (i.e., surface water consisting of treated wastewater that would otherwise be considered an ephemeral stream) or effluent-dominated (i.e., stream consists of more than 50% treated wastewater a majority of the time) streams common to the arid west. Second, sites were chosen based on historical water quality to ensure that copper toxicity was tested across a wide range of hardness, alkalinity and dissolved organic matter concentrations. Sites exhibiting elevated concentrations of copper, total ammonia, or total residual chlorine were avoided to prevent the potentially confounding effects of acute toxicity from other chemical constituents (USEPA 1994).

Seven different surface waters were selected from the western U.S. that satisfied the criteria given above and each was sampled during approximate base flow conditions (Table 3.1). Additionally, three of the sites were also sampled during two different elevated flow conditions (qualitative differentiation) to determine if there were any related effects on toxicity (i.e., dilution of water quality parameters). All sampling events were performed by personnel working at or near each wastewater discharge facility. To the best of the samplers' ability, water was collected from the receiving stream below the outfall and not directly from effluent outfalls. Samples were collected into pre-washed polypropylene containers, placed on ice in a cooler and shipped overnight to our testing facility in Albany, Oregon.

2.2 CULTURE METHODS

Larval fathead minnows and cladoceran neonates (*Ceriodaphnia dubia* and *Daphnia pulex*) used for toxicity tests were provided from in-house laboratory cultures maintained according to U.S. EPA methods (USEPA 2002a). Fathead minnow (*Pimephales promelas*) brood stocks were reared in a flow-through system using moderately-hard well water which was saturated with respect to dissolved oxygen, and maintained under constant conditions of pH 7.8, 25 ± 2 °C, and hardness and alkalinity of 100 and 100 mg/L as $CaCO_3$, respectively. Poly-vinyl chloride tiles within culture tanks were checked daily for the presence of eggs; tiles containing eyed eggs were removed, cleaned of debris using deionized water and placed in polypropylene pans containing moderately-hard reconstituted laboratory water that was continuously aerated and renewed every two days. Prior to hatch, tiles containing eyed eggs were placed in hard reconstituted laboratory water (HW; hardness and alkalinity of 220 and 140 mg/L as $CaCO_3$, respectively). Larval fish were fed *Artemia* nauplii three times daily until use in toxicity tests. Cultures were maintained in an environmental chamber having a 16:8 (light:dark) hour photoperiod at 25 ± 1 °C. All fathead minnows used for testing were between one and seven days old (USEPA 2002a).

Cladoceran mass cultures were established from cultures originally grown in moderately-hard water but the new mass cultures were maintained in HW for at least one month prior to conducting toxicity tests. Acclimation to conditions of elevated hardness has been reported to ameliorate the sensitivity of some organisms (e.g., rainbow trout and *C. dubia*) to copper (Erickson et al. 1997, Naddy et al. 2003, Welsh et al. 2000a, Welsh et al. 2000b). Even though this may influence copper toxicity to a minor degree for the cladocerans, we contend it would be best to conduct toxicity tests using organisms that are reared in waters of hardness and ion composition that are relatively representative of conditions encountered in many arid West streams.

New mass cultures (approximately 300-400 organisms) were initiated each week from HW-acclimated adults so that neonates used for testing were at least third generation acclimated-cladocerans. Mass cultures consisted of approximately 100 individual organisms in 2.0 L of HW that were fed 10 ml of a yeast : trout chow : cereal leaves/algae (*Selenastrum capricornutum*) suspension (USEPA 2002a) daily and transferred to new solution every two to three days. Cultures were maintained in a water bath having a 16:8 (light:dark) hour photoperiod at 23 ± 2 °C. Cladoceran neonates less than 24 h old were used in all studies.

2.3 SITE WATER TOXICITY TESTS

96-h static-renewal (80% volume replacement at 48 h) tests with larval fathead minnow and 48-h static tests with cladocerans were conducted according to U.S. EPA and American Society for Testing and Materials guidance (ASTM 2000, USEPA 1993, 2002a). The biological endpoint used for all toxicity tests was immobilization following gentle flushing with a transfer pipette. Organism mortality was determined at 24 h intervals and dead organisms were immediately removed. All fish in tests were fed 0.2 ml *Artemia* nauplii 2 hours prior to the 48 h renewal (USEPA 2002a). Cladocerans were not fed during testing. A 16:8 h light:dark photoperiod was maintained in an environmental chamber using cool white fluorescent tubes that provided 50–100 foot-candles at the beaker surface. Temperature was maintained at 20 ± 1°C and the dissolved oxygen concentration was > 80% of saturation.

Reconstituted laboratory waters were matched to site conditions using reagent grade salts ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$; Fisher Scientific, Pittsburg, PA, USA). Hardness was achieved using a Ca:Mg molar ratio of 1.82 to better approximate natural water composition (Welsh et al. 2000b). Exposure treatments in both site and laboratory waters were prepared by adding appropriate volumes of $CuCl_2$ stock (Fisher Scientific, Pittsburg, PA, USA) to dilution chambers and were allowed to equilibrate for at least one hour prior to distributing to exposure chambers for organism addition. Tests were conducted in 1-L polypropylene beakers (fathead minnows) containing 250 ml of test solution, or in 30-ml polypropylene cups (cladocerans) containing 25 ml of test solution. Four replicate exposure chambers were prepared for each of six toxicant concentrations (50% dilution series) and a negative control (unspiked dilution water). Exposures were initiated by randomly distributing organisms (ten fathead minnows or five cladocerans) directly into test solutions.

2.4 CATION COMPETITION TOXICITY TESTS

Larval fathead minnow tests were conducted using 96-h static-renewal (80% volume replacement at 48 h) procedures while *C. dubia* tests were conducted using static (48-h) procedures as described above. Exposure waters were prepared using reagent grade salts ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$; Fisher Scientific, Pittsburg, PA, USA) added to increase hardness primarily on the basis of calcium or magnesium. The hard water recipe used for culturing (nominal hardness of 220 mg/L as $CaCO_3$ with a Ca:Mg molar ratio of 0.67) served as the base recipe from which all exposure waters were prepared. To determine the influence of magnesium on copper toxicity, $MgSO_4$ was added to the base recipe to achieve nominal Ca:Mg molar ratios of 0.26, 0.16, 0.11 and 0.09 which corresponded to hardness levels of 400, 600, 800 and 1000 mg/L as $CaCO_3$, respectively. Similarly, to determine the influence of calcium on copper toxicity, $CaSO_4{\cdot}2H_2O$ was added to the base recipe to achieve nominal Ca:Mg molar ratios of 2.24, 3.86, 5.48 and 7.10 which also corresponded to hardness levels of 400, 600, 800 and 1000 mg/L as $CaCO_3$, respectively.

2.5 CHEMICAL ANALYSES

Water quality parameters measured in the toxicity tests included dissolved oxygen (DO), temperature, pH, hardness, alkalinity, total residual chlorine, ammonia, conductivity, dissolved organic carbon (DOC), total and dissolved copper and major ions required for BLM inputs (calcium, magnesium, sodium, potassium, chloride, and sulfate). All parameters were measured at test initiation. Measurements of DO, pH, and temperature were made daily. Samples for dissolved copper were also taken from freshly prepared test solutions just prior to renewal, and at test termination or complete mortality, whichever occurred first.

Measurements of total acid-extractable copper (referred to as total copper) and soluble copper (sample filtered through 0.45 µm nylon mesh filter [Pall-Gelman Laboratories, Ann Arbor, MI, USA] following a 5-ml rinse with water sample; referred to as dissolved copper) were made in the control water and each treatment. Samples were collected into polypropylene conical tubes and acidified to pH < 2 with concentrated nitric acid (ACS grade; Mallinckrodt, Phillipsburg, NJ, USA) before analysis by graphite or flame atomic absorption spectroscopy (AA800; Perkin-Elmer, Shelton, CT, USA).

Samples collected for DOC analysis were filtered through 0.45 µm nylon mesh following a 5-ml rinse with water sample, acidified with concentrated H_2SO_4, and stored in amber glass bottles. DOC concentrations were determined via combustion method (EPA method 415.1). Other analytes were analyzed by inductively coupled-plasma atomic emission spectroscopy (calcium, magnesium, sodium, and potassium; EPA method 200.7), ion chromatography (SO_4^{2-}; EPA method 300.1) or colorimetric titration (Cl^-, hardness and alkalinity). Water pH and ammonia were measured using ion-selective electrodes (Thermo Electron, Beverly, MA, USA) connected to a multi-channel pH/mV meter. Conductivity and total residual chlorine were measured with portable probes from Hach Co. (Loveland, CO, USA). DO was measured using a dissolved oxygen probe (Yellow Springs Instruments, Yellow Springs, OH, USA).

2.6 WATER-EFFECT RATIO CALCULATION

Water-effect ratio (WER) values were calculated from time-dependent median-lethal concentrations (LC50) calculated on the basis of dissolved copper in laboratory and site water toxicity tests. WER values were calculated by dividing the measured dissolved Cu LC50 in site water by the measured dissolved Cu LC50 in matched laboratory water (Equation 1; (USEPA 1994).

$$\mathrm{WER} = \frac{\mathrm{LC50}_{\text{Site Water}}}{\mathrm{LC50}_{\text{Lab Water}}} \tag{1}$$

2.7 BIOTIC LIGAND MODEL

The copper BLM (Ver 2.1.2; available at http://www.hydroqual.com/wr_blm.html) was used to predict copper LC50 values for *C. dubia*, *D. pulex*, and *P. promelas*. Measured values for pH, DOC, Ca, Mg, Na, K, Cl^-, SO_4^{2-}, and alkalinity (units of mg/L for DOC and ions, and in units of mg/L as $CaCO_3$ for alkalinity) were used as model input parameters for all site water and reconstituted water toxicity tests. In addition, default values for percent humic acid (10%) and sulfide (0.01 µM) were used.

2.8 DATA ANALYSIS

Median-lethal concentrations and 95% confidence limits were calculated from observed mortalities and measured copper concentrations using the trimmed Spearman-Karber method (Hamilton et al. 1977). The toxicant concentrations used in these calculations were averages of two or three measurements for each treatment within each toxicity test. Toxicological differences among species, sites, and flow levels were determined using an analysis of variance (ANOVA) following back-calculation of standard error values from 95% confidence limits. An alpha value of 0.05 was used to judge the significance of each statistical relationship.

3. RESULTS

3.1 COPPER TOXICITY IN NATURAL WATER

Acute copper toxicity (LC50 values) was significantly different among species ($P < 0.0001$) in each of the natural waters tested, where *C. dubia* was generally more sensitive to copper than *D. pulex* followed by *P. promelas* (fathead minnows). However, there was no statistical difference between *C. dubia* and *D. pulex* LC50 values in four of the seven site waters. Significant differences in toxicity among sites ($P < 0.0001$) also existed and LC50 values varied nearly 60-fold for *C. dubia* and *D. pulex*, and 10-fold for fathead minnows (Table 3.1). Copper toxicity to *C. dubia* was significantly different ($P < 0.0001$) between sites that were sampled during different flow conditions (Table 3.1). Toxicity varied nearly fourfold between different flow conditions in the South Platte River and Salt River, and twofold in the Santa Ana River. For the South Platte River and Salt River, protection from copper toxicity decreased as the flow increased while LC50 values were relatively similar among flow levels for the Santa Ana River. The influence on flow for the South Platte River was based on seasonal snowmelt (base flow in the early spring with higher flows in early summer; Table 3.2) while the Salt River and Santa Ana River were generally influenced by rainfall (higher flows in early spring and base flow typically during the summer; Table 3.2).

Table 3-1. Median-lethal concentrations (LC50 as µg/L with 95% Confidence Intervals) and Water-Effect Ratios (WER) for dissolved copper to *Ceriodaphnia dubia* (48-h), *Daphnia pulex* (48-h) and *Pimephales promelas* (96-h) in natural waters.[a]

Location			*C. dubia*		*D. pulex*		*P. promelas*	
(City, State)	**Drainage**	**Flow level** [b]	**LC50**	**WER**	**LC50**	**WER**	**LC50**	**WER**
Los Alamos, NM	Sandia Canyon	Base	130.66 (112.94 - 151.16)	12.53	149.26 (129.56 - 171.95)	11.05	1867.95 (1136.30 - 3070.71)	9.50
		Lab Water	10.43 (8.60 - 12.65)	-	13.50 (10.98 - 16.61)	-	196.57 (158.37 - 243.98)	-
Las Vegas, NV	Las Vegas Wash	Base	206.92 (194.54 - 220.08)	5.24	200.01 (178.09 - 224.62)	5.28	1389.27 (1236.64 - 1560.75)	4.42
		Lab Water	39.52 (31.80 - 49.13)	-	37.88 (29.75 - 48.23)	-	314.59 (276.11 - 358.43)	-
Globe, AZ	Pinal Creek	Base	5.38 (4.48 - 6.46)	1.07	6.68 (5.48 - 8.15)	0.70	180.02 (151.45 - 213.97)	10.32
		Lab Water	5.02 (4.42 - 5.69)	-	9.56 (5.25 - 17.38)	-	17.44 (13.90 - 21.89)	-
Albany, OR	Drainage Swale	Base	36.34 (29.87 - 44.21)	0.98	78.17 (61.32 - 99.66)	1.38	543.53 (468.70 - 630.32)	2.07
		Lab Water	36.98 (23.21 - 58.92)	-	56.59 (48.48 - 66.07)	-	262.04 (220.40 - 311.55)	-
Riverside, CA	Santa Ana R.	Base	44.06 (43.11 - 45.02)	2.17	56.27 (47.00 - 67.38)	3.77	683.57 (599.18 - 779.84)	2.74

Table 3.1. Continued

Location			*C. dubia*		*D. pulex*		*P. promelas*	
(City, State)	Drainage	Flow level [b]	LC50	WER	LC50	WER	LC50	WER
		Lab Water	20.27 (17.58 - 23.38)	-	14.91 (9.82 - 22.62)	-	249.88 (216.75 - 288.08)	-
		Medium	58.47 (51.14 - 66.86)	2.00	-	-	-	-
		Lab Water	29.25 (26.39 - 32.42)	-	-	-	-	-
		High	78.49 (70.24 - 87.70)	2.99	-	-	-	-
		Lab Water	26.24 (22.10 - 31.15)	-	-	-	-	-
Denver, CO	South Platte R.	Base	302.39 (266.61 - 342.97)	13.08	267.24 (214.24 - 333.36)	8.92	906.09 (790.30 - 1038.85)	3.02
		Lab Water	23.12 (20.20 - 26.45)	-	29.98 (22.58 - 39.79)	-	299.80 (265.19 - 338.92)	-
		Medium	147.56 (125.31 - 173.76)	8.34	-	-	-	-
		Lab Water	17.69 (11.88 - 26.33)	-	-	-	-	-
		High	77.43 (68.51 - 87.50)	5.33	-	-	-	-
		Lab Water	14.52 (12.64 - 16.68)	-	-	-	-	-

Table 3.1. Continued

Location			*C. dubia*		*D. pulex*		*P. promelas*	
(City, State)	Drainage	Flow level [b]	LC50	WER	LC50	WER	LC50	WER
Phoenix, AZ	Salt R.	Base	279.07 (249.36 - 312.33)	11.24	375.89 (318.70 - 443.34)	4.90	768.88 (656.30 - 900.76)	1.62
		Lab Water	24.82 (16.81 - 36.66)	-	76.73 (59.11 - 99.61)	-	473.21 (398.64 - 561.74)	-
		Medium	257.06 (236.33 - 279.60)	8.64	-	-	-	-
		Lab Water	29.76 (23.90 - 37.05)	-	-	-	-	-
		High	79.22 (70.51 - 89.00)	7.59	-	-	-	-
		Lab Water	10.44 (8.49 - 12.83)	-	-	-	-	-

[a] Water-Effect Ratios were calculated by dividing the LC50 value in the site water by the LC50 value measured in laboratory water that is matched to hardness and alkalinity of the site (Equation 1).

[b] Base flow levels corresponded to approximate design flow conditions at sampling locations. Medium and high flow levels are relative to base flow conditions.

Table 3-2. Site information and water quality measurements for natural waters used in toxicity tests and Water-Effect Ratio calculation.[a]

Location			Sampling	Major ions (mg/L)							pH	DOC[e]
(City, State)	Drainage	Flow level [b]	date	Ca	Mg	Na	K	SO_4	Cl	HCO_3[c]	SU[d]	mg/L
Los Alamos, NM	Sandia Canyon	Base	02-May-05	18.3	4.9	88.8	8.5	50.3	64.7	151.2	8.1	4.4
		Lab Water	-	18.1	6.0	67.6	4.0	50.2	3.9	165.9	8.4	< 0.5
Las Vegas, NV	Las Vegas Wash	Base	14-Feb-05	174.0	87.4	274.0	29.4	718.0	325.0	156.1	8.2	5.4
		Lab Water	-	202.0	64.0	64.1	10.7	704.0	6.5	151.2	8.3	< 0.5
Globe, AZ	Pinal Creek	Base	27-Apr-05	403.0	50.4	80.0	4.6	1090.0	70.0	19.5	7.2	0.7
		Lab Water	-	316.0	111.0	9.1	5.8	1040.0	9.7	24.4	7.6	< 0.5
Albany, OR	Drainage Swale	Base	12-May-05	66.7	30.9	98.0	2.9	1.2	172.0	278.0	8.2	1.2
		Lab Water	-	72.0	25.4	105.0	14.7	296.0	12.5	273.2	8.3	< 0.5
Riverside, CA	Santa Ana R.	Base	24-May-05	56.1	11.4	45.6	6.6	48.2	46.5	200.0	8.1	2.5
		Lab Water	-	37.0	15.4	76.5	6.8	167.0	7.3	190.2	8.3	< 0.5
		Medium	26-Apr-05	73.7	15.8	60.6	5.9	50.7	54.5	234.1	8.4	2.5
		Lab Water	-	67.0	22.6	99.9	7.1	184.0	9.4	239.0	8.3	< 0.5
		High	28-Feb-05	61.8	16.6	60.6	7.9	57.7	58.6	224.4	8.2	3.5
		Lab Water	-	50.8	22.6	104.0	7.4	233.0	8.3	224.4	8.2	< 0.5

Table 3.2. Continued

Location (City, State)	Drainage	Flow level [b]	Sampling date	Major ions (mg/L) Ca	Mg	Na	K	SO_4	Cl	HCO_3 [c]	pH SU [d]	DOC [e] mg/L
Denver, CO	South Platte R.	Base	02-Feb-05	71.9	26.3	150.0	14.1	161.0	200.0	248.8	8.2	9.8
		Lab Water	-	72.0	26.1	98.9	7.1	256.0	6.5	234.1	8.2	< 0.5
		Medium	12-May-05	77.4	18.3	92.1	7.9	115.0	49.9	190.2	8.0	6.4
		Lab Water	-	79.5	25.9	84.0	6.2	214.0	8.6	195.1	8.1	< 0.5
		High	16-Apr-05	44.6	11.6	46.1	6.3	76.9	49.9	131.7	8.0	5.8
		Lab Water	-	43.3	13.7	52.2	10.3	142.0	10.6	136.6	8.3	< 0.5
Phoenix, AZ	Salt R.	Base	19-Apr-05	104.0	43.3	370.0	16.6	185.0	530.0	219.5	8.0	6.9
		Lab Water	-	115.0	38.3	110.0	5.6	318.0	8.4	268.3	8.5	< 0.5
		Medium	24-May-05	84.1	33.7	292.0	18.6	321.0	436.0	224.4	8.0	7.7
		Lab Water	-	90.4	29.9	81.9	7.4	327.0	8.4	204.9	8.4	< 0.5
		High	07-Mar-05	35.4	14.8	68.7	5.5	19.8	124.0	146.3	8.1	6.3
		Lab Water	-	41.1	13.5	60.0	8.7	142.0	10.2	151.2	8.0	< 0.5

[a] Reported values are measurements taken from control waters at test initiation. Values for pH are averages from each treatment at test initiation.
[b] Base flow levels corresponded to approximate design flow conditions at sampling locations. Medium and high flow levels are relative to base flow conditions.
[c] Bicarbonate estimated by dividing alkalinity by 0.82.
[d] SU = standard units.
[e] DOC = Dissolved organic carbon.

Although water quality varied significantly among the various natural waters, acute toxicity was generally influenced by different parameters for fish than were invertebrates (Figure 3.1). Toxicity did not change significantly as a function of hardness or alkalinity for any species even though the parameter values ranged from 65 to 1200 mg/L as $CaCO_3$, and 16 to 230 mg/L as $CaCO_3$, respectively. Because one site (Pinal Creek) exhibited the upper and lower extremes of these ranges (i.e., hardness and alkalinity of 1200 and 16 mg/L as $CaCO_3$, respectively), there was no overall correlation between hardness and alkalinity values in the natural water samples (Table 3.3). In fact, the lowest copper LC50 values recorded for all three organisms was in water collected from or matched to Pinal Creek (Table 3.1). One noticeable hardness-based correlation was between hardness cations (Ca^{2+} and Mg^{2+}), suggesting that the concentration of each cation increased as hardness values increased in natural waters (Table 3.3).

Table 3-3. Pearson correlation coefficients for relationships between log-transformed water quality parameters

	DOC	Ca	Mg	Na	Alkalinity	Hardness
DOC	1.000					
Ca	-0.406	1.000				
Mg	-0.109	**0.857**	1.000			
Na	0.410	0.351	**0.669**	1.000		
Alkalinity	0.544	**-0.559**	-0.212	0.178	1.000	
Hardness	-0.330	**0.986**	**0.932**	0.465	-0.470	1.000

Numbers in bold denote coefficients that are significant at $P < 0.05$.

Copper toxicity was also compared against the concentrations of other water quality parameters that have known competitive (calcium, magnesium, or sodium) or complexation (DOC) interactions with copper (de Schemphelaere and Janssen 2002, Di Toro et al. 2001). Similar to correlations with total hardness and alkalinity, no significant relationship between copper LC50 values and calcium or magnesium was observed for any of the organisms tested (data not shown). However, both DOC and sodium were good linear predictors of copper toxicity in natural waters for invertebrates, yet no single parameter significantly influenced toxicity to fathead minnows (Figure 3.1).

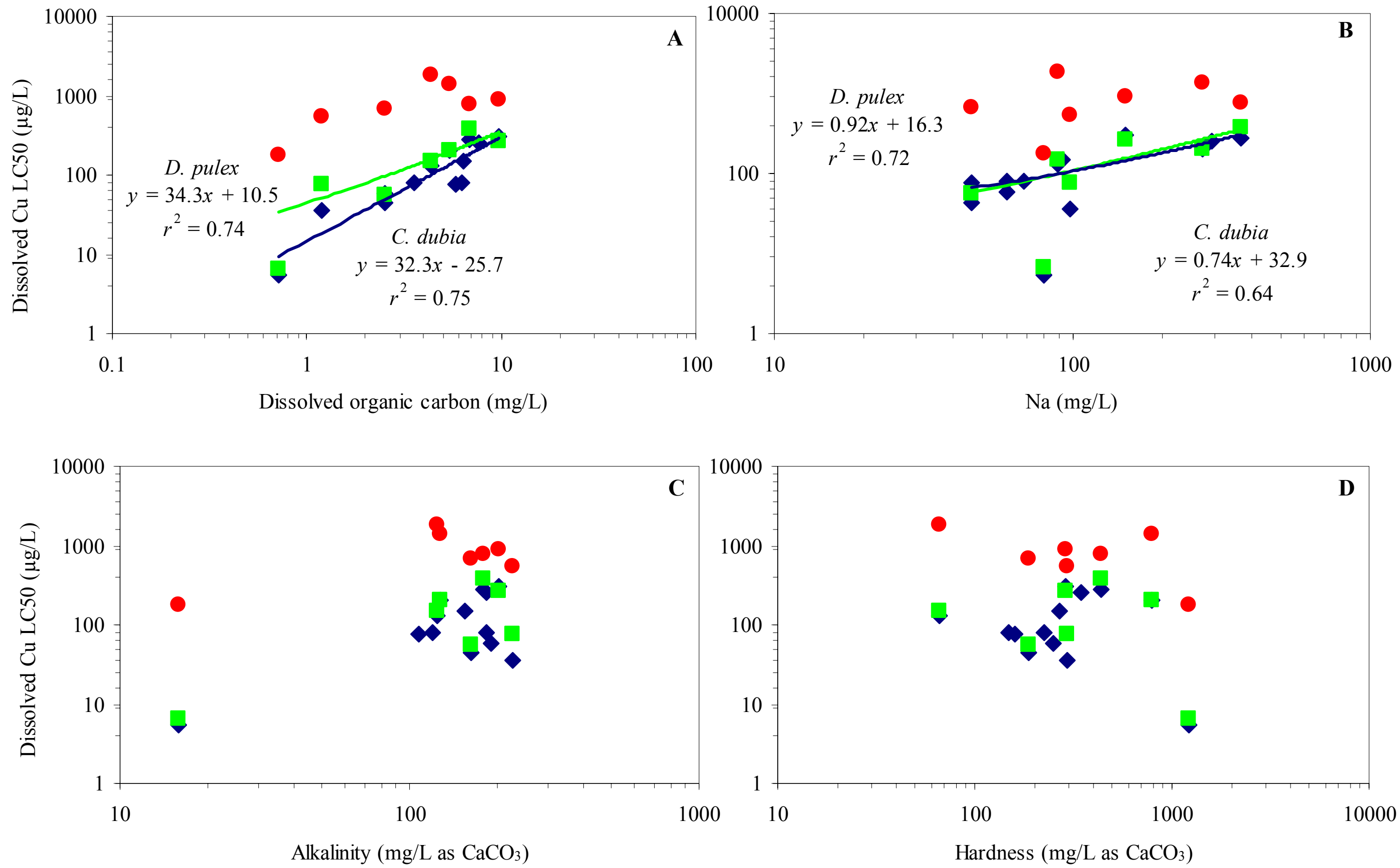

Figure 3-1. Relationship between time-dependent median-lethal concentrations (LC50) for *Ceriodaphnia dubia* (48-h; diamonds), *Daphnia pulex* (48-h; squares), and *Pimephales promelas* (96-h; circles) and concentrations of dissolved organic carbon (A), sodium (B), alkalinity (C), and total hardness (D). Linear regressions with slopes significantly different than zero are denoted by solid lines (see Table 3.3 for respective Pearson correlation coefficients).

3.2 WATER-EFFECT RATIOS

WER values calculated from each site and matched (with respect to hardness and alkalinity) laboratory reconstituted water LC50 values ranged from less than one to greater than 13 for the natural waters tested in this study. A comparison of WER values had similar results among species and sites as observed among LC50 values. WER values among species varied by up to 15-fold at individual sites and greater than tenfold among sites for individual species (Table 3.1). Generally speaking, *C. dubia* produced the largest WER values and fathead minnows had the smallest (most conservative). However, results for Pinal Creek suggested that the invertebrates were equally sensitive to site and laboratory water (i.e., WER values approximating one) while the fathead minnows had a calculated WER of greater than ten. WER values also changed substantially during different qualitative flow conditions (Table 3.1). Similar to comparisons among LC50 values, WER values were generally highest at base flow and decreased with elevated flows. However, WER values were relatively similar among flow levels for the Santa Ana River.

3.3 CATION COMPETITION

Measured water quality parameters were within acceptable deviations (± 15%) from nominal values. Among treatments, the concentrations of sodium, potassium, chloride and alkalinity were similar in order to keep copper speciation relatively consistent among tests (Table 3.4). Increasing total hardness from 200 to 1000 mg/L as $CaCO_3$ using either calcium or magnesium had considerably different effects on acute copper toxicity to *C. dubia* and fathead minnows (Figure 3.2). A tenfold addition of calcium to the base water recipe (Ca:Mg = 0.63; Table 3.4) doubled the LC50 for each species. However, while a similar protective effect from magnesium addition to the base water recipe occurred for *C. dubia*, acute toxicity to fathead minnows remained constant although a non-significant increase in copper toxicity at the highest magnesium concentrations was observed.

Table 3-4. Water quality and median-lethal concentrations (LC50) for dissolved copper to *Ceriodaphnia dubia* (48-h) and *Pimephales promelas* (96-h) in very hard waters with varying ratios of calcium and magnesium

	Major ions (mg/L)							pH	LC50 (µg/L with 95% CI) [d]	
Treatment [a]	Ca	Mg	Na	K	SO_4^{2-}	Cl^-	HCO_3 [b]	SU [c]	C. dubia	P. promelas
0.18 (0.11)	43.0	239.0	73.0	0.6	1160	9.1	185.4	8.4	43.27 (36.51 - 51.29)	65.12 (52.94 - 72.90)
0.19 (0.12)	36.6	189.0	74.7	0.6	923	8.6	175.6	8.4	37.61 (31.81 - 44.48)	129.87 (114.83 - 146.89)
0.25 (0.15)	42.1	166.0	86.4	6.2	549	8.9	185.4	8.4	23.42 (19.22 - 28.54)	86.12 (72.52 - 102.27)
0.43 (0.26)	43.5	101.0	84.1	5.9	378	8.7	180.5	8.2	20.44 (13.48 - 30.99)	104.46 (85.02 - 128.36)
1.04 (0.63)	32.9	31.5	72.1	7.2	197	24.0	175.6	8.0	21.86 (18.23 - 26.21)	116.79 (96.77 - 140.96)
3.62 (2.19)	105.0	29.0	66.1	12.0	346	19.5	170.7	8.1	15.48 (11.91 - 20.12)	287.62 (249.88 - 331.05)
5.20 (3.15)	172.0	33.1	69.0	10.1	503	11.5	170.7	8.1	16.54 (12.92 - 21.19)	285.24 (248.76 - 327.08)
9.25 (5.60)	271.0	29.3	69.5	6.9	305	7.4	170.7	8.1	23.21 (17.90 - 30.10)	320.17 (280.12 - 365.95)
9.92 (6.01)	383.0	38.6	82.1	21.0	918	9.1	146.3	8.2	28.67 (22.29 - 36.88)	322.31 (278.13 - 373.52)

[a] Measured Ca:Mg ratios are presented on a bass basis and molar basis parenthetically.
[b] Bicarbonate estimated by dividing alkalinity by 0.82.
[c] SU = standard units.
[d] CI = confidence interval.

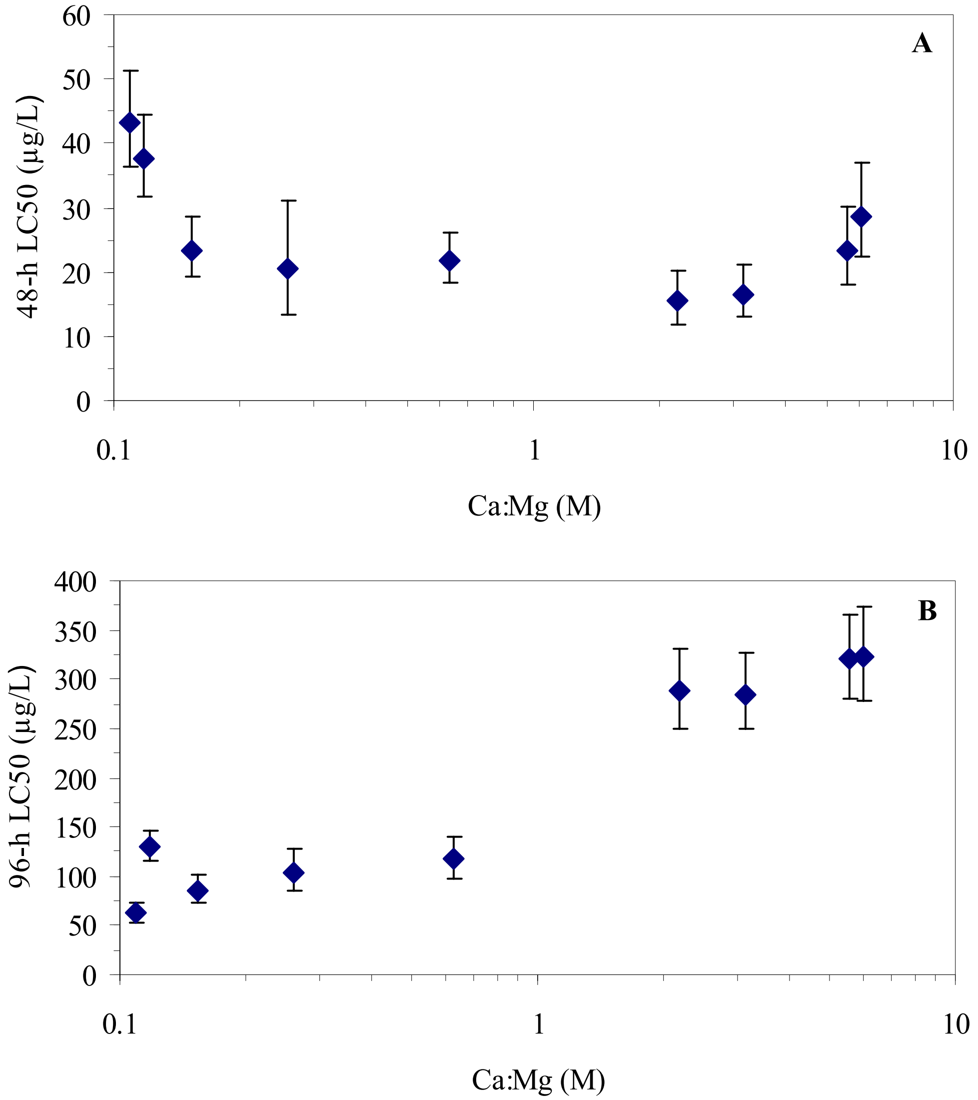

Figure 3-2. Influence of increasing calcium or magnesium concentration on dissolved copper median-lethal concentrations (LC50) for *Ceriodaphnia dubia* (A) and *Pimephales promelas* (B). Cation concentration expressed as the molar ratio of calcium to magnesium. Calcium concentrations are increasing to the right of the base water recipe (Ca:Mg = 0.63; hardness = 200 mg/L as $CaCO_3$) up to hardness of 1000 mg/L as $CaCO_3$ (Ca:Mg = 6.01). Magnesium concentrations are increasing to the left of the base water recipe up to hardness of 1000 mg/L as $CaCO_3$ (Ca:Mg = 0.11).

3.4 BIOTIC LIGAND MODEL

Dissolved copper LC50 values were predicted from measured water quality parameters from all toxicity tests and compared against the observed LC50 values (Figure 3.3A; $n = 72$). BLM predictions are typically considered to be of acceptable accuracy if they are within ± two of the line of perfect agreement with the empirical toxicity data (Di Toro et al. 2001, Di Toro et al. 2000). Using the base (i.e., unmodified) model, 61% of the empirical data were accurately predicted based on this ± factor of two criterion. Due to the static nature of the BLM (i.e., no variance associated with estimated metal-gill stability constants), results have been compared non-statistically, according to current practice (Di Toro et al. 2001). The BLM under-predicted toxicity (i.e., over-predicted LC50s) for most of the fathead minnow data (only 13% of the data were within ± factor of two from observed values), but demonstrated substantially better performance for *C. dubia* and *D. pulex* data (83% and 86% within ± factor of two from observed values, respectively).

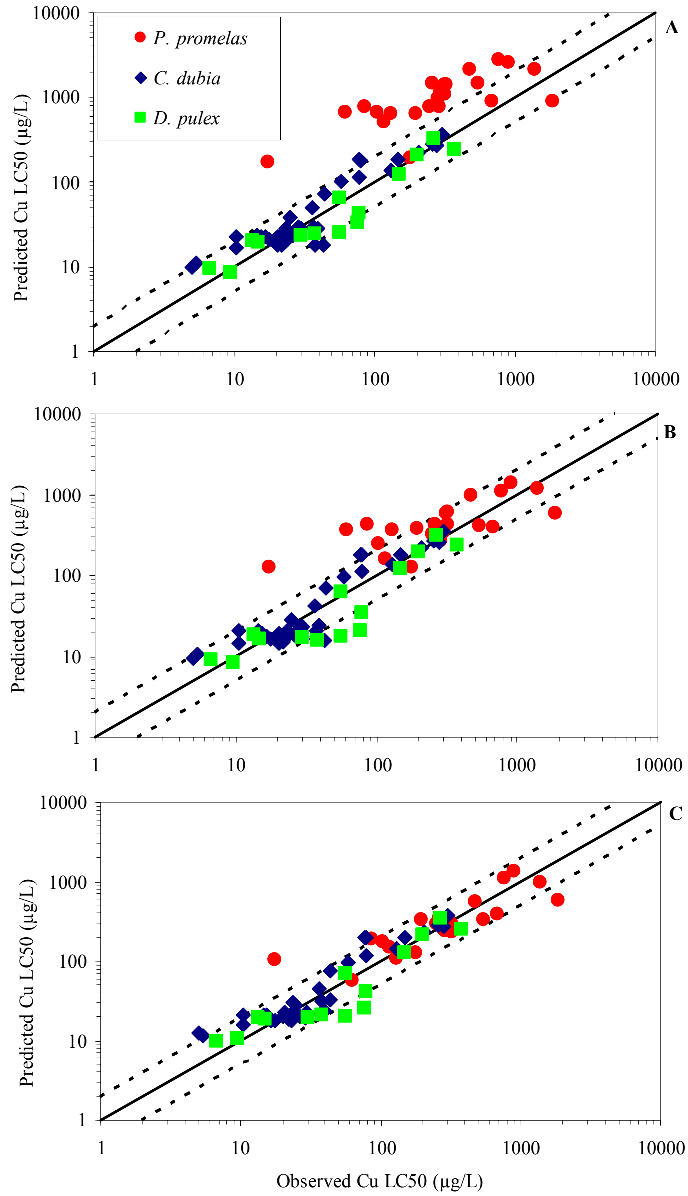

Figure 3-3. Comparison of observed and Biotic Ligand Model (BLM) predicted dissolved copper median-lethal concentrations (LC50). Plots represent BLM prediction made using measured water quality data and no model optimization (A; 61% in bounds); BLM predictions after taking into account carbonate complexation/ precipitation with calcium and magnesium and carbonate losses to the atmosphere (B; 79% in bounds); and, BLM predictions after carbonate considerations in (B) and considering Mg-gill interaction for invertebrates and magnesium sensitivity to fathead minnows (C; 86% in bounds). Solid line represents ideal fit between observed and predicted data (*x* = *y*). Dashed lines represent plus or minus a factor of two from line of ideal fit.

4. DISCUSSION

4.1 COPPER TOXICITY IN VERY HARD WATER

Results suggest that copper toxicity to all three aquatic organisms in very hard natural waters (i.e., average hardness of 360 mg/L as $CaCO_3$) is not accurately predicted as a function of hardness. Although significant differences were observed in copper toxicity among the different sites, these differences could not be explained by hardness alone. In fact, due to atypical water quality at one site (i.e., hardness and alkalinity highly uncorrelated in Pinal Creek water sample), negative relationships between hardness and LC50 values were calculated for each species, although the trends were not statistically significant (Figure 3.1D). While these conclusions are counter to studies performed with invertebrates and fish in relatively soft to moderately-hard waters (de Schemphelaere and Janssen 2002, Erickson et al. 1996, USEPA 1984a, Van Genderen et al. 2005), they support previous results with *C. dubia* conducted in very hard reconstituted waters (Gensemer et al. 2002). Hardness may, in fact, ameliorate copper toxicity at all hardness levels when tested in reconstituted waters using controlled manipulations of water quality variables, but studies conducted with natural water or additions of natural organic matter often conclude that hardness alone can not adequately predict copper toxicity (Sciera et al. 2004, Villavicencio et al. 2005). Moreover, while it is critical for the advancement of water quality criteria development that the influence of major ions on copper toxicity be characterized, the numerous confounding factors that exist in natural waters likely preclude the use of simple predictive relationships between acute copper toxicity and any one water quality variable.

One water quality factor that is intimately related to hardness is the relative ratios of cations (i.e., primarily calcium and magnesium) that make up the composite measurement of total hardness. Although calcium has been considered to be the more important hardness cation relative to competition with copper for binding sites on DOC and biotic ligands (e.g., fish gill), some studies have suggested that the ratio between calcium and magnesium may be more important than their absolute concentrations. For example, several studies have reported that hardness consisting primarily of calcium (Ca:Mg molar ratios of ≥ 1) is protective of both fish (Erickson et al. 1996, Naddy et al. 2002, Welsh et al. 2000b) and invertebrates (de Schemphelaere and Janssen 2002, Naddy et al. 2002). However, hardness consisting primarily of magnesium (Ca:Mg molar ratios of ≤ 1) has only been shown to be important for invertebrates (de Schemphelaere and Janssen 2002, Naddy et al. 2002). As a result, while magnesium is essential to survival, it has been not been considered to be an important cation that can chemically compete for copper-biotic ligand binding sites at the fish gill (Erickson et al. 1996, Naddy et al. 2002, Welsh et al. 2000b).

The calcium to magnesium molar ratios measured in the natural waters used in the present study ranged between one and five which is similar to the majority of natural waters throughout the U.S. (Welsh et al. 2000b). The extremes in the distribution of calcium to magnesium ratios found in natural waters (Ca:Mg molar ratios of 0.1 – 6.0) were tested in the present study using reconstituted waters to independently determine the impact of elevated calcium or magnesium concentrations on copper toxicity to *C. dubia* and fathead minnows. Results suggested that increasing hardness from 200 to 1000 mg/L as calcium or magnesium had a similar protective effect from copper toxicity to *C. dubia* (Figure 3.2A). Likewise, increasing hardness from 200 to 1000 mg/L as calcium demonstrated a protective effect on fathead minnows while hardness as magnesium exerted no impact on toxicity (Figure 3.2B). Although the present study is not the first to determine the influence of cation ratios on copper toxicity, the total hardness levels used were significantly higher than any other available study. Even so, our results are consistent with conclusions made by other researchers related to the toxicological importance of calcium and magnesium on copper toxicity to fish and invertebrates. However, these elevated calcium and magnesium concentrations may suggest that modifications to the BLM could improve model predictions; this is discussed further below.

Because hardness alone can not describe the variation we observed in acute copper toxicity to three aquatic species in natural waters, other water quality parameters, or combinations of water quality parameters, could be considered to improve predictive power of the BLM in very hard waters. The characteristics with the most potential for ameliorating copper toxicity by decreasing copper bioavailability are competing cations (Ca^{2+}, Mg^{2+}, Na^{+}, and H^{+}) and complexing ligands (DOC, OH^{-}, Cl^{-}, and CO_3^{2-}) (Di Toro et al. 2001). Given that one of the primary objectives of this study was to evaluate the accuracy of copper BLM predictions across a wide range of water quality in very hard waters, we purposefully selected natural waters that were known to encompass wide ranges of hardness, alkalinity, etc. As such, the concentrations of competing ions and complexing ligands could not be experimentally controlled, with the exception of the cation competition studies. However, because there was no correlation between concentrations of hardness and DOC, sodium, or alkalinity, this dataset can still be used to draw statistically meaningful (i.e., relatively few water quality variables were auto-correlated) comparisons between water quality and copper toxicity in effluent-dependent waters with elevated hardness (Table 3.3).

Unlike the other competing cations, sodium concentrations were significantly correlated with decreasing copper toxicity for the invertebrates tested in this study. Although this has also been observed with fathead minnows (Erickson et al. 1996), the present study did not detect a significant interaction in the exposures with natural water. However, analysis of the concurrent laboratory water data for fathead minnows demonstrated a significant positive correlation between sodium concentrations and LC50 ($r^2 = 0.73$, $P = 0.0148$). The influence of sodium on acute copper toxicity has been linked to hindering the disruption of ionoregulation by copper at the gill epithelium (i.e., decreased Na^{+}/K^{+}-Adenosine Triphosphatase activity; (Playle et al. 1993). Although the mode of toxicity in invertebrates is still unclear, the ameliorative effects of increased sodium concentrations on copper toxicity (de Schemphelaere and Janssen 2002) support the presumption that sodium acts in a similar fashion as in fish.

Aside from competing cations, copper complexation to organic and inorganic ligands in exposure waters can provide significant reductions in copper bioavailability. Alkalinity is considered an indicator of copper complexation and bioavailability because it is a measure of the amount of dissolved inorganic carbon present in solution. In typical natural and reconstituted waters, hardness and alkalinity are auto-correlated, thereby suggesting that both variables fluctuate relative to one another. In the natural waters used in this study, however, hardness and alkalinity were not auto-correlated (Table 3.3). This is likely due to the effluent-dominated nature of these waterbodies which are impacted by very different waste streams depending on the location and wide range of activities of the dischargers selected for this study (e.g., hard-rock mining, well-water discharges, municipal waste discharges, etc.). The best example of this is Pinal Creek which exhibited the highest hardness and lowest alkalinity in the dataset (Table 3.2). In fact, this site generated the lowest LC50 values measured in the entire study which suggested that toxicity was likely a function of carbonate complexation capacity, and hardness played a negligible role (Figure 3.1). Although significantly positive relationships existed between alkalinity and LC50 values for *C. dubia* and *D. pulex*, the relationships were driven by the results from Pinal Creek (Figure 3.1C). If these data points are removed from the analysis, the significance of the alkalinity relationships for *C. dubia* and *D. pulex* are eliminated (P = 0.693 and 0.984, respectively). Similar to results with sodium, although the relationship between alkalinity and LC50 values for fathead minnows was not significant (Figure 3.1A), analysis of the concurrent laboratory water data demonstrated a significant positive correlation (r^2 = 0.73, P = 0.0148). Again, this demonstrates the complexity of natural systems and the inability of any one water quality parameter to be the single best predictor of copper toxicity in all cases.

Although different sources of organic matter have been shown to influence copper toxicity to varying degrees (de Schemphelaere and Janssen 2004, Ryan et al. 2004, Sciera et al. 2004), the concentration of organic matter (as DOC) has been considered one of the most important factors influencing copper bioavailability (de Schemphelaere and Janssen 2002, Di Toro et al. 2001, Van Genderen et al. 2005, Villavicencio et al. 2005). The variation in DOC concentrations observed among the natural waters tested in this study (0.7 to 9.8 mg/L) was an unforeseen benefit considering that each of the sites were chosen based on major ion characteristics and the effluent dependence of each location. Dissolved organic carbon concentration significantly reduced the toxicity of copper to both invertebrates and accounted for approximately 75% of the variation observed in the datasets (Figure 3.1A). However, the fathead minnows did not respond as predictably to increasing DOC concentrations. While the relationship between DOC and acute toxicity to fathead minnows was not considered statistically significant (r^2 = 0.16, P = 0.37), increasing DOC concentrations up to 4 mg/L were associated with increasing LC50 values (Figure 3.1A).

4.2 WATER-EFFECT RATIOS

Water-Effect Ratio guidance was designed as an alternative to the hardness-based regulatory approach for metals (USEPA 1994). Continuous point sources of copper (e.g., municipal effluents which contain significant levels of organic matter) that have demonstrated low risks for toxicity are common situations where both the standard and "streamlined" WER approach has been applied (USEPA 2001). The WER procedure compares the measured toxicity of copper under conditions mimicking in-stream effluent concentrations (i.e., conducting tests with effluent or dilutions of effluent to design flow concentrations) to the toxicity of copper in laboratory water of similar composition to the original toxicity studies that were used to derive the current hardness-based criteria. This is facilitated by conducting a concurrent toxicity test in upstream dilution water or in reconstituted water that is matched to the hardness of the receiving stream. However, the majority of hardness values from the original calibration dataset for copper criteria derivation ranged from 40 to 400 mg/L as $CaCO_3$, so the hardness of the reconstituted water is restricted to this range, similar to the upper and lower bounds of the hardness equation (USEPA 1984a).

Because hardness and alkalinity co-vary in standard reconstituted waters, alkalinity is usually closely matched in the process of matching hardness. With this in mind, the current study was designed to match the actual hardness and alkalinity of the natural waters tested in order to remove confounding variables from our characterization of toxicity in very hard surface water. Although cation ratios are a significant consideration as well, it was not possible to measure calcium and magnesium concentrations prior to setting up toxicity tests and remain within the 36 hour holding time suggested by WER guidance (USEPA 2001). Instead, we used a calcium to magnesium molar ratio of 1.82 (3.0 by weight) as the recipe for all reconstituted waters that were matched to site hardness for WER determination. This value corresponds to the most common value (mode) from a distribution of surface waters throughout the U.S. (Welsh et al. 2000b).

The range of WER values observed in this study (0.70 to 13.08) is within the range of values that have been calculated by other researchers for copper (Van Genderen et al. 2005, Welsh et al. 2000a). The range of WER values among sites was similar for each species and the magnitude of values was typically similar for each organism at a given site. However, three sites in particular (Pinal Creek, the South Platte River, and the Salt River) exhibited large differences between WER values for the three species. In the case of Pinal Creek, both invertebrate species experienced similar toxicity in reconstituted and site water (i.e., WER approximated one). On the contrary, toxicity to fathead minnows in the reconstituted water was ten times lower than what was measured in the site water (i.e., WER = 10). A high WER value can be attributed to either an extremely high LC50 in the site water or an extremely low LC50 in the reconstituted water. The toxicity of copper to fathead minnows in the reconstituted water was relatively similar to that for *C. dubia* and *D. pulex* (LC50 values of 17.44, 5.03, and 9.54, respectively), so this initially suggested that some characteristic of the site water protected fish more so than invertebrates. However, based on evidence from speciation calculations provided by the BLM (Figure 4.2; discussed below), we determined that excessive amounts of magnesium present in the reconstituted water may have increased toxicity to fathead minnows. Because we could not match the calcium and magnesium concentrations in the concurrent test water to the site, the water recipe to achieve a hardness level of 1200 mg/L as $CaCO_3$ required two times the magnesium measured in the corresponding site water (i.e., measured magnesium concentration in the reconstituted water was 111 mg/L, or 4.5 mM, which is 2.5 times the threshold of acute response; Figure 4.2B). It is unknown, therefore, whether the observed toxic effects are based on additivity between copper and magnesium concentrations.

In the case of the South Platte River and the Salt River, large differences in WER magnitudes among species may be related to differences in copper sensitivity. Ideally, the selection of toxicity test (species, life stage, test duration, etc.) is based upon using an available organism whose sensitivity to copper is as close to the acute criterion value as possible (USEPA 1994). In the case of copper, *Ceriodaphnia* has a genus mean acute value similar to the Final Acute Value, and has been considered a model test system for determining site-specific copper criteria using the WER approach (USEPA 2001). In general, more sensitive organisms (*C. dubia*) typically produce larger WER values (e.g., South Platte River and the Salt River; Table 3.1). However, WER values obtained from insensitive organisms may also be equal to (e.g., Sandia Canyon, Las Vegas Wash, and Santa Ana River) or greater (e.g., Pinal Creek and Albany Drainage Swale) then values obtained for sensitive biota (Table 3.1).

Another consideration that can influence WER magnitude is stream flow. Because National Pollutant Discharge Elimination System (NPDES) permit limits are calculated based on the design-flow condition of a stream (i.e., the flow used for steady-state wasteload allocation modeling), site-specific criteria must be developed to be protective of aquatic life under design flow conditions. However, site-specific criteria must also offer adequate protection during higher flows. These generalizations made in WER guidance concerning the influences of stream flow are related to dilution (hardness, alkalinity, and conductivity), significant contributions (DOC, total suspended solids, and non-point source pollutants), and re-suspended sediment (USEPA 1994). Because the WER approach was developed for use at municipal effluent discharges, it is assumed that the highest concentrations of organic matter (a metal-complexing ligand that will diminish copper toxicity) will be present during design flow. In the case of the present study, two of the three sites that were sampled during various flow conditions demonstrated this phenomenon (Table 3.2; South Platte River and Salt River). In fact, both of these streams primarily (> 95%) consist of municipal effluent and elevated flows likely occurred due to surface runoff from precipitation or snowmelt (i.e., reduced WER values are likely a product of effluent dilution). The third site (Santa Ana River) is also a municipal discharger into an effluent-dominated stream channel, but flows recorded during the months of sampling (February to May 2005) were uncharacteristically high due to rain events through the entire study period, and water samples contained significant amounts of suspended solids. Likely due to these unusual flow conditions, WER values were very similar among sampling events because there was negligible change in flows or among water quality characteristics for the different samples (Table 3.2).

4.3 BLM VALIDATION AND MODIFICATION

Considering that the BLM takes into account all of the influences on copper bioavailability that have been discussed thus far (i.e., competitive interactions including cation ratios and complexing ligands), the base (i.e., unmodified) model still only predicted 61% of the copper toxicity values in the present study with reasonable accuracy. However, the model performed remarkably well for the two invertebrates whose sensitivity to copper are very close to the criterion value. This suggests that the performance of the BLM as a tool for deriving site-specific copper criteria is acceptable for very hard surface waters, at least for acutely sensitive invertebrates.

To better evaluate the poor model performance with fathead minnows, an examination of BLM input data (i.e., residual analysis; regression between ratio of predicted vs. observed values and water quality parameters) was implemented. Results demonstrated that the influence of pH or alkalinity (which are chemically related) on deviations between predicted and observed LC50 values were not consistent. Instead, toxicity test waters appeared to be oversaturated with carbonate complexes (Figure 4.1) which could exert a significant impact on the bioavailability of free copper in the very hard waters tested here. Based on thermodynamic equilibrium speciation of measured ion concentrations (Table 3.2 and 4), it was determined that increased copper bioavailability may have occurred due to carbonate complexation with calcium and magnesium (e.g., $CaCO_3$ and $MgCO_3$; Morel and Herring 1993). The consequence of this condition on BLM predictions would be an over-prediction of LC50 values because actual dissolved inorganic carbon concentrations would be lower than reported. Due to complexation of calcium and magnesium with carbonate, the available concentrations of these ions would also have decreased. As a result, the average equilibrium concentrations of alkalinity, calcium, and magnesium were 31%, 70%, and 99% of measured values, respectively (data not shown). Additionally, the degree of complexation was similar between site and reconstituted waters.

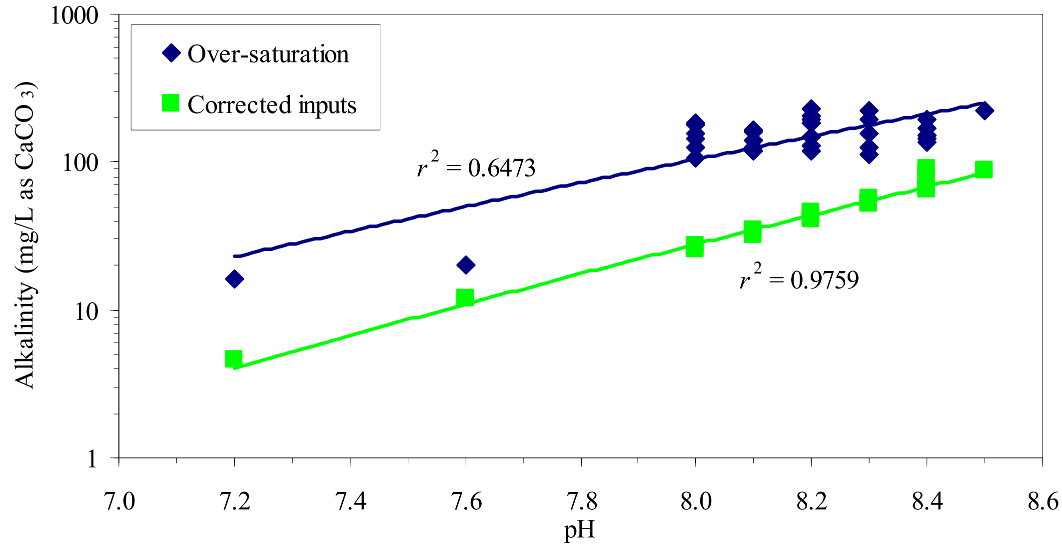

Figure 4-1. Comparison between measured alkalinity concentrations (over-saturation) and equilibrium-corrected alkalinity concentrations (corrected inputs). Alkalinity adjustment needed to reflect actual dissolved inorganic carbon concentrations in test waters.

To validate this finding, we prepared reconstituted waters similar to those used in the cation competition studies (hardness of 1000 mg/L as $CaCO_3$ with Ca:Mg molar ratio of 6.0 and 0.1 and alkalinity of 200 mg/L as $CaCO_3$), allowed the waters to equilibrate for four days with an open atmosphere, and then measured alkalinity in filtered (0.10 and 0.45 µm porosity nylon mesh) and unfiltered samples. Results were identical among all measurements for both waters. However, this only suggests that the solid complexes may have been smaller than the filter pore sizes that we used. The use of ultra-centrifugation could potentially remove carbonate precipitates for definitive analysis of this phenomenon. Despite the inability to validate the presence of carbonate complexes empirically, the input data for the BLM was adjusted to reflect the equilibrium conditions of dissolved inorganic carbon, calcium, and magnesium following loss of CO_2 to the atmosphere (less than five percent of total loss) and/or the precipitation of $CaCO_3$ and $MgCO_3$. Use of this adjusted dataset increased the number of acceptable predictions (i.e., within factor of two from ideal fit) from 61 to 79% (Figure 3.3B). Although a few of the predictions for *D. pulex* fell outside the acceptable bounds (i.e., more than factor of two from observed values), significant improvements to the fathead minnow predictions were observed (from 13 to 70% within bounds).

It is important to note that we considered two other adjustments to the input data that were justified empirically from measured water quality data. Because our initial assertion that carbonate was over-saturated in test waters came from an inconsistent residual analyses of pH and carbonate, we performed a model sensitivity analysis with respect to pH. First, pH values were increased to levels that corresponded with measured alkalinity concentrations. As expected, predicted LC50 values increased (representing decreased bioavailability) and so BLM-predicted toxicity deviated farther from observed values. Second, the initial exposure condition (pH measurements taken at test initiation) was also considered as input values to predict LC50 values. This was done to represent initial bioavailability conditions that would best correspond to sub-cellular mechanistic toxicity of metals that have been shown to occur within hours of exposure (Morgan et al. 2004, Rogers et al. 2005). This strategy also accounted for more bioavailable copper to be present in test waters (lower pH), but does not remedy the inconsistencies between pH and carbonate concentrations. Although predictions using initial pH values were roughly 20% lower than original predictions, only one additional prediction was brought within acceptable bounds of observed results. Overall, accounting for carbonate speciation from equilibrium computations provides the most accurate representation of copper bioavailability and is the preferable method for determining BLM input parameters when carbonate precipitation is suspected due to elevated concentrations of calcium and magnesium.

An additional modification to the BLM was consideration of Mg-gill interactions for both invertebrates and fathead minnows. Results from the cation competition studies with *C. dubia* suggested that calcium and magnesium equally protected against copper toxicity at cation concentrations up to 10 mM (Figure 3.2). As such, a Mg-gill interaction was added to the BLM program to account for this competitive interaction. The chemical affinity of this interaction was considered to be similar to calcium (log K = 3.6) as suggested by other researchers (de Schemphelaere and Janssen 2002). As a result, increased competition at the biotic ligand decreased copper binding and, hence, increased LC50 values. Because BLM predictions for *C. dubia* and *D. pulex* using the unmodified model were already considered to be of acceptable accuracy, the inclusion of magnesium competition at the gill improves model performance for invertebrates by only two percent (one additional prediction within bounds; Figure 3.3C).

Although BLM predictions for fathead minnows improved substantially following consideration of carbonate complexation, we recognized that data points remaining outside the bounds of acceptability had relatively high concentrations of magnesium. Additionally, residual analysis for fathead minnow LC50 values (regression between ratio of predicted vs. observed values and magnesium) suggested that the ability of the BLM to predict the toxicity of copper significantly decreased (increased deviation from perfect fit) as the magnesium concentration increased (Figure 4.2A). To correct for this, the BLM was modified so that it allowed the median-lethal accumulation of gill-copper (LA50) to change as a function of magnesium (Figure 4.2B). This type of adjustment required the BLM include a function to specify the LA50 at various magnesium concentrations. The function describing the relationship between LA50 and magnesium was calibrated to the present dataset since no other fathead minnow studies were available that contained exposures at Mg > 2 mM.

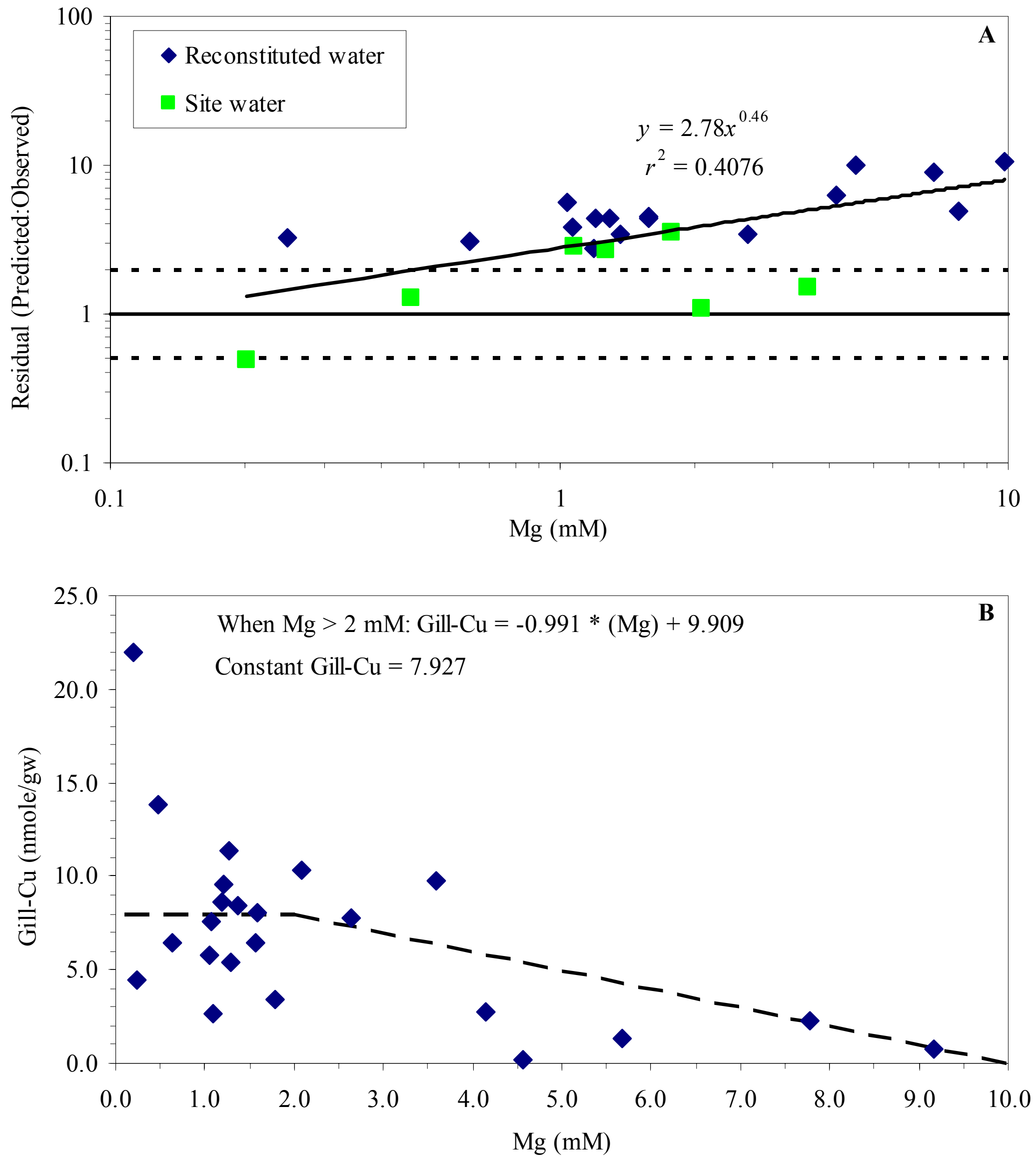

Figure 4-2. (A) Justification for adding a magnesium-copper interaction to the Biotic Ligand Model for fathead minnows based on a residual analysis of predicted vs. observed 96-h median-lethal concentrations for fathead minnows and measured magnesium concentrations. Solid horizontal line represents ideal fit between observed and predicted data ($x = y$). Dashed horizontal lines represent plus or minus a factor of two from line of ideal fit. Slope of regression for all data was significantly different than zero ($P < 0.0001$). (B) Derivation of a function to describe the influence of magnesium on copper toxicity. A least-squares minimization procedure determined that Mg $\geq$ 2 mM significantly ($P = 0.0403$) decreased amount of gill-copper needed to produce 50% mortality in fathead minnows. Copper toxicity at magnesium concentrations below 2 mM were described by the default gill-copper constant (7.927 nmol Cu/g gill wet weight; (Di Toro et al. 2001).

First, LA50 values were calculated for each fathead minnow toxicity test by utilizing the BLM in speciation mode. Then the LA50 values were plotted as a function of magnesium, and a sum of squares minimization procedure was used to fit a numerical function to the data (Figure 4.2B). Of the eight waters that contained magnesium concentrations above 2 mM, two were site waters (Las Vegas Wash and Pinal Creek). As a result, less copper accumulation is required to cause toxic effects at elevated magnesium concentrations which decreased predicted LC50 values for several data points and, hence, improved model performance for fathead minnows by an additional 17 %. Based on all manipulations that were performed (accounting for both carbonate and magnesium effects), model predictions for all three species improved from 61 to 86% within acceptable bounds across the entire dataset. It should be noted, however, that this modification is not part of the publicly available version of the model because this finding has not been empirically validated for larval fathead minnows or other species of fish.

4.4 REGULATORY IMPLICATIONS

At the present time, there are only two formal recommendations from the USEPA for calculating a site-specific copper criterion in waters with hardness greater than 400 mg/L as $CaCO_3$; (1) calculate the criterion using a default WER of 1.0 and using a hardness of 400 mg/L in the hardness equation (USEPA 2002b); or (2) calculate the criterion using a WER and the actual ambient hardness of the surface water in the equation (USEPA 2002b). The first alternative simply suggests that all wastewater discharges into streams with hardness greater than 400 mg/L as $CaCO_3$ should be permitted at 400 mg/L regardless of actual site water hardness (Table 4.1). While this approach would be protective for the Las Vegas Wash (i.e., *C. dubia* LC50 value was greater than the hardness-based criterion), a criterion for Pinal Creek would be extremely under-protective of acutely sensitive species (i.e., *C. dubia* LC50 value was ten times lower than the recommended hardness-based criterion; Table 4.1). Results from the Albany Drainage Swale (hardness = 294 mg/L as $CaCO_3$) draw a similar conclusion of under-protection where the hardness equation produced a criterion equal to the LC50 for *C. dubia*. Because even one-half the LC50 concentration is considered the threshold of acute toxicity (USEPA 1985), the consequence of regulating copper at an acutely lethal level would be decreased populations of sensitive biota. As acknowledged by the USEPA (2002b) and as we concluded from the present study, hardness and related inorganic water quality characteristics do not have the same magnitude of effect on toxicity of copper at higher hardness levels as they do at lower hardness levels. Additionally, related water quality characteristics do not correlate as well in high hardness water as they do in waters of relatively low hardness.

Table 4-1. Comparison of different methodologies for deriving site-specific copper water quality criteria for the seven sites used in this study. Biotic Ligand Model (BLM) predictions simulate toxicity to the hypothetical criterion organism (fifth percentile of sensitivity), and these BLM-based criteria were calculated as one-half of the predicted LC50 value, which is equivalent to the Criterion Maximum Concentration. Criterion concentrations in bold text are greater than the corresponding 48-h LC50 value for Ceriodaphnia dubia, which suggests a concentration that would not be protective of this acutely sensitive species. [a]

Location		Hardness	C. dubia 48-h	Dissolved Cu	Copper Acute Water Quality Criterion (µg/L)				
(City, State)	Drainage	(mg/L as $CaCO_3$)	LC50 (µg/L)	WER	HB [b]	SS [c]	BLM (1) [d]	BLM (2) [e]	BLM (3) [f]
Los Alamos, NM	Sandia Canyon	65.8	130.7	12.53	9.4	118.2	51.3	51.2	53.3
Las Vegas, NV	Las Vegas Wash	794.4	206.9	5.24	51.7	**516.5**	85.1	79.7	91.9
Globe, AZ	Pinal Creek	1213.8	5.4	1.07	**51.7**	**157.8**	3.5	3.4	3.7
Albany, OR	Drainage Swale	293.8	36.3	0.98	**38.6**	**38.0**	17.1	14.0	17.1
Riverside, CA	Santa Ana R.	218.1	58.7	2.35	29.2	**68.6**	34.4	31.5	35.8
Denver, CO	South Platte R.	230.9	151.2	8.35	30.8	**257.1**	84.7	83.4	91.6
Phoenix, AZ	Salt R.	283.6	178.5	9.03	37.4	**337.7**	88.2	86.5	94.1

[a] Hardness values, dissolved Cu median-lethal concentrations (LC50), Water-Effect Ratio (WER) values, and BLM predictions for sites with multiple sampling events (i.e., Santa Ana River, South Platte River, and Salt River) are presented as the geometric mean for all events.
[b] Hardness-based (HB) criterion = exp(0.9422*ln(Hardness)-1.700); hardness greater than 400 mg/L as $CaCO_3$ calculated as 400 mg/L (USEPA 1984a).
[c] Site-specific (SS) criterion = Hardness-based criterion (ambient hardness) * dissolved Cu WER.
[d] BLM (1) predictions based on measured quality data (i.e., unmodified input data).
[e] BLM (2) predictions based on adjustments to alkalinity, calcium, and magnesium concentrations following considerations of carbonate complexation/precipitation.
[f] BLM (3) predictions based on adjustments to alkalinity, calcium, and magnesium concentrations following consideration of carbonate complexation/precipitation and incorporation of magnesium-gill interaction (i.e., Mg-gill included in model; affinity characterized by long K = 3.6).

Whereas the first option is thought to result in a more protective aquatic life criterion, the second hardness-based option (multiply actual site hardness by the WER) is expected to result in the level of protection that is intended from the original guidelines (USEPA 1984c, 1985). Because hardness was not correlated with toxicity in site waters, specifying a criterion above 400 mg/L as $CaCO_3$ using the hardness equation can generate site-specific criteria that are equal to or greater than observed LC50 values for *C. dubia* from the actual site water. Such a situation could potentially result in site-specific criteria that are under-protective of the most acutely sensitive species. For example, site-specific criteria generated from the hardness equation (based on ambient hardness) and observed WER values for *C. dubia* in water from Las Vegas Wash and the Salt River were approximately two times greater than their corresponding LC50 values in each of the site waters (Table 4.1). Even the Sandia Canyon site (relatively low hardness) would result in a site-specific criterion equal to the observed LC50 for *C. dubia*. As a result, U.S. EPA's currently available options concerning the influence of hardness and other water quality parameters on site-specific copper criteria may not be adequately protective of aquatic life in waters with elevated hardness.

The magnitude of WER results generated in this study were very similar to previous studies with soft to moderately-hard waters (Van Genderen et al. 2005, Welsh et al. 2000a). However, several design strategies and implementation concerns should be considered prior to initiating a definitive WER study for water of elevated hardness. First, because hardness was not correlated with alkalinity in site waters used in the present study, both parameters should be matched in concurrent reconstituted waters to account for confounding variables. Second, the current study clearly demonstrated the importance of matching ion ratios (primarily calcium and magnesium) of the concurrent reconstituted water to site conditions. This consideration could significantly influence WER results for any hardness (Van Genderen et al. 2005, Welsh et al. 2000a) or species (de Schemphelaere and Janssen 2002, Erickson et al. 1996, Naddy et al. 2002, Welsh et al. 2000a). Finally, calculating site-specific criteria from observed WER values coupled with use of the existing hardness equation is likely to be under-protective and, thus, not appropriate for high hardness waters.

Our results suggest that the BLM offers an improved alternative to both of these current site-specific methods for modifying copper criteria, particularly for situations where the hardness equation and WER approach would continue to under-protect sensitive aquatic life. To illustrate this, the BLM was run in criteria mode (i.e., simulating an organism which represents the fifth percentile of sensitivity) to predict LC50 values and calculate site-specific Criterion Maximum Concentrations (i.e., acute criterion) for copper at each of the sites used in this study (Table 4.1; USEPA 2003). In contrast to either of the standard USEPA methods discussed above, the BLM-derived acute criterion for copper was protective of *C. dubia* for all of the sites, regardless of the manipulations that were made to the input data (Table 4.1). This demonstrates the utility of considering the influence of all water quality variables when deriving site-specific criteria for waters with elevated hardness.

Furthermore, even without justifiable manipulations to the input data (i.e., carbonate complexation/precipitation with calcium and magnesium) and gill interactions, the BLM accurately predicted 86% of the observed LC50 values for the copper-sensitive invertebrates (i.e., *C. dubia* and *D. pulex*). With both of these modifications to the BLM, overall performance increased substantially from 61% to 86% accuracy. Therefore, if these modifications can be supported by additional empirical evidence, we suggest that the BLM represents a greatly improved method for site-specific derivation of copper criteria in effluent-dependent and effluent-dominated waters of the arid West. Additionally, without the BLM, we would not have noted inconsistencies between pH and dissolved inorganic carbon, or been able to interpret the biological effects of elevated magnesium concentrations on fathead minnows. This study, therefore, provides an example of how the BLM can help interpret data to extend our understanding of copper speciation and bioavailability in the aquatic environment, regardless of whether the BLM will eventually include recalculation of alkalinity values or Mg-gill interactions for invertebrates.

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6. ACKNOWLEDGMENTS

6.1 ARID WEST WATER QUALITY RESEARCH PROJECT

This study was conducted as part of the Arid West Water Quality Research Project (AWWQRP) under the direction of the Pima County Wastewater Management Department in Tucson, Arizona. Funding for the AWWQRP was provided by USEPA Region IX under Assistance Agreement #XP-99926701. The AWWQRP was administered by the following individuals:

- Michael Gritzuk, P.E., Director, Pima County Wastewater Management Department
- Paul M. Bennett, P.E., Deputy Director, Pima County Wastewater Management Department
- Edward F. Curley, Project Director, Pima County Wastewater Management Department
- Karen Ramage, Program Manager, Pima County Wastewater Management Department
- Richard D. Meyerhoff, Ph.D., Research Manager, Camp Dresser & McKee
- Robyn Stuber, EPA Project Officer, EPA Region IX, San Francisco, California

6.2 REGULATORY WORKING GROUP (RWG)

The Regulatory Working Group (RWG) was established by the AWWQRP to assist in the identification of regulatory issues needing to be addressed by scientific research. The RWG includes representatives from State and regulatory agencies, municipalities, Indian Tribes, industry, environmental organizations, consulting firms, and universities. The RWG also provided critical review of draft reports and project presentations. Currently, the RWG consists of the following individuals:

- Michael Gritzuk, P.E., Pima County Wastewater Management, Tucson, Arizona
- Edward C. Anton, California State Water Resources Control Board, Sacramento, California
- Rodney W. Cruze, Riverside Regional Water Quality Control, Riverside, California
- Steve Davis, P.E., Malcolm Pirnie, Inc., Tucson, Arizona
- Paul D. Frohardt, Colorado Water Quality Control Commission, Denver, Colorado
- Robyn Stuber, USEPA, Region IX, San Francisco, California
- Andy Laurenzi, The Nature Conservancy, Marana, Arizona
- Patrick J. Maley, Mining Industry Representative, Boise, Idaho
- Lynn Wellman, U.S. Fish & Wildlife Service, Albuquerque, New Mexico
- James F. Pendergast, USEPA, Office Science and Technology, Washington, D.C.
- Sam Rector, Arizona Department of Environmental Quality, Phoenix, Arizona
- Eric Rich, EPA - Navajo Nation, Tuba City, Arizona

- Daniel Santantonio, Ph.D., City of Las Cruces, Utility/Water Division, Las Cruces, New Mexico
- Gary Ullinskey, City of Phoenix Water Services, Phoenix, Arizona

6.3 SCIENTIFIC ADVISORY GROUP (SAG)

The Scientific Advisory Group (SAG) was established to provide technical oversight and peer review of ongoing and planned research for the AWWQRP. The SAG provided critical review for all sections of this report. SAG members include:

- Paul Adamus, Ph.D., Corvallis, Oregon (Oregon State University)
- Gary Chapman, Ph.D., Paladin Water Quality Consulting, Corvallis, Oregon
- Karmen King, Colorado Mountain College, Leadville, Colorado
- Robert McFarlane, Ph.D., McFarlane & Associates Environmental Consultants, Houston, Texas
- Benjamin Parkhurst, Ph.D., HAF, Inc., Laramie, Wyoming

ALTERNATES:

- Robert Gray, Ph.D., Richland, Washington
- Carlton Sims White, Ph.D. University of New Mexico, Albuquerque, New Mexico

6.4 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

All AWWQRP research products were also reviewed to ensure compliance with the project QA/QC plan. This review was provided by:

- Frederick A. Amalfi, Ph.D., Aquatic Consulting and Testing, Tempe, Arizona

6.5 RESEARCH TEAM

This project was conducted by a multi-disciplinary research team consisting of aquatic toxicologists, ecologists, and ecological modelers. Key participants included the following:

Parametrix, Inc.

- Robert W. Gensemer, Ph.D., Principal Investigator, Albany, Oregon
- Eric Van Genderen, Ph.D., Laboratory Manager, Parametrix Environmental Research Laboratory (PERL), Albany, Oregon
- Carrie Smith, Study Director, PERL, Albany, Oregon
- Jeffrey R. Wirtz, QA/QC, PERL, Albany, Oregon
- William A. Stubblefield, Ph.D., Technical Director, PERL, Albany, Oregon
- Allison Cardwell, Aaron Edgington, Rene Jones, Dinah Nasvik, Laura Power, and Amber Young, Laboratory Technicians, PERL, Albany, Oregon

HydroQual, Inc.

- Robert C. Santore, Camillus, New York
- Adam C. Ryan, Ph.D., Camillus, New York
- Paul R. Paquin, Mahwah, New Jersey

6.6 DISCHARGERS

We are particularly grateful to the dischargers who participated in the WER portion of the study by collecting and shipping site water samples to PERL.

Albany Drainage Swale

- PERL staff, Albany, Oregon

South Platte River

- Todd Harris, Metro Wastewater Reclamation District, Denver, Colorado

Pinal Creek

- Jay Spehar, Pinal Creek Group / Phelps Dodge Miami Inc., Claypool, Arizona

Las Vegas Wash

- Dan Fischer and Randy Hadland, City of Las Vegas, Las Vegas, Nevada

Sandia Canyon

- Bruce Gallaher, Los Alamos National Laboratory, Los Alamos, New Mexico

Salt River

- Bob Hollander and Peggy Parma, City of Phoenix Pollution Control, Phoenix, Arizona

Santa Ana River

- Rod Cruze and Annie Yambot, Riverside Public Works Department, Riverside, California

6.7 OTHER PARTICIPANTS

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- Sharon Grimm, Parametrix, Albany, Oregon
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- Joseph Volosin, Parametrix, Buffalo, New York