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Hazard/Risk Assessment

Copper Toxicity in Bristol Bay Headwaters: Part 1–Acute Mortality and Ambient Water Quality Criteria in Low-Hardness Water

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Abstract: The world-class Alaskan Bristol Bay salmon fishery and vast deposits of copper (Cu) and other metals in the watershed warrant further investigation into the potential toxicity of Cu to salmonids under the low water-hardness conditions that occur in the watershed. Therefore we investigated the acute toxicity of Cu to rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*) in low-hardness water (~ 30 mg/L as $CaCO_3$) formulated in the laboratory and collected from the Bristol Bay watershed. The median lethal concentration (LC50) for rainbow trout exposed to Cu in low-hardness laboratory water was 16 μg Cu/L (95% confidence intervals [CIs]: 12, 21; dissolved Cu, filtered to 0.45 μm). The LC50 values for fathead minnows exposed to Cu in low-hardness laboratory water or site water were 29 and 79 μg Cu/L (95% CIs: 23, 35 and 58, 125; dissolved Cu), respectively. The biotic ligand model (BLM) LC50 estimates for these bioassays were 1.3 to 2.3 times higher than the actual LC50 values. We also calculated and analyzed acute Cu water quality criteria, also known as criterion maximum concentration (CMC), using hardness-based methods and the BLM for water samples collected throughout the Bristol Bay watershed in 2007. Biotic ligand model CMCs ranged from 0.05 to 17.5 μg Cu/L and hardness-based CMCs ranged from 2.3 to 6.1 μg Cu/L for the 65 samples analyzed. Our results show the need for site-specific research and subsequent water quality guidelines in low-hardness aquatic habitats. *Environ Toxicol Chem* 2018;9999:1–8. © 2018 SETAC

Keywords: Copper; Hardness; Biotic ligand model; Bristol Bay

INTRODUCTION

Alaska's Bristol Bay supports the largest commercial sockeye salmon fishery in the world, accounting for 44% of the average annual global harvest (148 543 metric tons, including the United States, Canada, Japan, and Russia) for the 32-yr span from 1980 to 2012. During this same period, statewide sockeye landings in Alaska accounted for an average of 74% of the average annual global harvest (109 630 metric tons), including the Bristol Bay fishery (Alaska Department of Fish and Game 2016). Knapp et al. (2013) estimated that the Bristol Bay salmon industry contributes more than $1 billion USD annually in total commercial economic value across multiple industries and states. The Bristol Bay sockeye fishery is also an important subsistence fishery for native communities in Alaska (Westing et al. 2006). By whatever metrics it is assessed, it is clear that the Bristol Bay salmon fishery is a world-class wild salmon fishery of major economic, cultural, and ecological importance.

The spawning and rearing grounds for the Bristol Bay salmon fishery are located throughout the vast and pristine Bristol Bay watershed. Woody and O'Neal (2010a, 2010b) have documented the presence of numerous species of anadromous salmonids, including sockeye salmon, and other fish in headwater streams in the watershed. They reported the presence of anadromous salmon in 74% of the 92 sites they surveyed in the watershed from 2008 to 2010. Many of these sites were located in and around the Pebble Deposit—an area that has received much attention as a possible location for a very large copper (Cu) and gold mine (US Environmental Protection Agency 2014). The deposit is located at or near the headwaters for the Alaskan North Fork Koktuli River, the South Fork Koktuli River, and the Upper Talarik Creek (UT). Because of the presence of sensitive early life stage fish, coupled with low water hardness, incidental or accidental releases of Cu and other metals from future mine operations could be harmful to sockeye salmon and other salmonids that utilize these rivers for spawning and rearing (US Environmental Protection Agency 2014). The primary

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contaminant of concern related to mining activity in this area is Cu because the concentrations of Cu in Pebble Project waste leachates are predicted to be elevated (Pebble Limited Partnership 2011; US Environmental Protection Agency 2014), and Cu is particularly toxic to fish and aquatic invertebrates (Meyer et al. 2007). Understanding the potential bioavailability and toxicity of Cu to salmonids in low-hardness waters in the Bristol Bay watershed is a critical element of any evaluation of the potential environmental consequences of mine development in this region or other regions with similar water quality.

In addition to acute mortality to aquatic biota caused by exposure to relatively low Cu concentrations, exposure to even lower Cu concentrations can result in avoidance behaviors (Hansen et al. 1999a), and adversely affect the olfactory system of salmonids through neurological impairment or olfactory inhibition (Hansen et al. 1999b; Baldwin et al. 2003; McIntyre et al. 2008; Baldwin et al. 2011; Kennedy et al. 2012). Impairment or inhibition of the olfactory system has been shown to adversely affect predator avoidance behavior in juvenile salmonids (McIntyre et al. 2012) as well as recognition of rearing water (Saucier et al. 1991). Whether the effects of Cu exposure are: 1) mortality, 2) avoidance of contaminated waters, 3) inhibition of the olfactory system during imprinting in early life stages, 4) abnormal predator avoidance behaviors, or 5) impacts to the olfactory system during navigation to natal spawning areas, an understanding of the bioavailability and toxicity of Cu to salmonids in low-hardness waters is critical to the evaluation of the potential environmental consequences of mine development. The present study focuses on our laboratory evaluation of the acute effects of Cu exposure on the survival of salmonids and other fish. The companion study to the present study details our follow-up evaluation of the effects of Cu on the salmonid olfactory system under similar water quality conditions (Morris et al. 2019 [this issue]).

The toxicity of Cu in natural waters varies as a function of water chemistry. Although a number of dissolved constituents contribute to the relative amelioration of Cu toxicity in freshwater, primary controlling variables include calcium, pH, and dissolved organic matter (DOM). These substances influence Cu toxicity through a series of geochemical processes related to competition for cation uptake sites on fish gills, as well as the formation of complexes that are less bioavailable/toxic than uncomplexed metal ions (e.g., Cu^{2+}). Specifically, Cu toxicity tends to be reduced in harder waters (i.e., higher calcium concentrations) and in waters with higher amounts of DOM. Calcium hardness derives from the weathering of rock-forming minerals (primarily carbonates and feldspars) and DOM derives largely from the decomposition of terrestrial and aquatic vegetation and biota.

To estimate the influence of site-specific water quality conditions on Cu toxicity to aquatic biota, the US Environmental Protection Agency (USEPA) recommended the use of the biotic ligand model (BLM; Di Toro et al. 2001) to calculate site-specific water quality standards (US Environmental Protection Agency 2007). The BLM consists of: 1) CHESS (Santore and Driscoll 1995), a geochemical speciation model that calculates inorganic metal speciation; 2) WHAM (Tipping 1994), a model that calculates the degree of Cu^{2+} interaction with DOM; 3) binding constants for the Cu^{2+} biotic ligand (e.g., the fish gill) complex; and 4) median lethal accumulation values for Cu on the ligand, which remain constant in the BLM for a given aquatic organism regardless of water quality. The main purpose of the BLM is to predict the concentration of total dissolved Cu that would cause toxicity to aquatic life under a range of water quality conditions, which is used to derive Cu aquatic life criteria. Although the BLM is a theoretical improvement over the hardness-based aquatic life criteria because it considers major dissolved ions, pH, and the interactions between Cu and dissolved organic carbon (DOC), the results of the model have been questioned by a number of researchers, based in part on inconsistencies between predicted and observed toxicity values that appear to be related to Cu–DOC interactions (De Schamphelaere et al. 2004; Welsh et al. 2008) or how the BLM handles metal mixtures (Chen et al. 2010).

Models have inherent uncertainty, but research comparing the results of the BLM with actual fish toxicity data has shown that the model often under predicts the toxicity of Cu to aquatic biota in the presence of DOM and/or DOC (De Schamphelaere et al. 2004; Welsh et al. 2008). One potential reason for this involves the simplified treatment of the DOM–Cu complexation in the BLM (Welsh et al. 2008). Indeed, research has shown that the site-specific nature of DOM and/or DOC (including factors such as the nature, strength, and capacity of binding sites for Cu and other metals and complexing agents) can influence the bioavailability and toxicity of Cu (MacRae et al. 1999; Marr et al. 1999; Welsh et al. 2008).

The objective of the acute bioassays described in the present study was to determine how accurately the BLM predicted the toxicity of laboratory-derived and field-collected waters with low hardness and low DOC concentrations using rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*). The goal of our project was to simulate and understand the bioavailability of Cu to salmonids in low-hardness watersheds (i.e., the Bristol Bay watershed) by investigating fish toxicity and behavior when exposed to Cu under field-relevant water quality conditions.

MATERIALS AND METHODS

Water quality and ambient criteria estimates for the Bristol Bay watershed

We compiled water quality data collected by the Pebble Limited Partnership (2011) from a total of 10 surface water sites in 2007 from the North Fork Koktuli River, South Fork Koktuli River, and Upper Talarik Creek and calculated water quality criteria for Cu using the BLM and the hardness-based method currently utilized by the Alaska Department of Environmental Conservation (2008). We confined our analysis to 2007 data from this dataset, which was the first year that all the water quality parameter inputs required for the BLM were reported (Supplemental Data, Table S1).

Bioassay exposure water

All bioassays were conducted in the Colorado Parks and Wildlife Aquatic Toxicity Laboratory in Fort Collins, Colorado, USA. The laboratory water utilized for rainbow trout and fathead minnow bioassays was a blend of dechlorinated tap water and dechlorinated tap water that was further treated in a cation exchange column (Siemens tank #W5TDICAT0045FSP). This blend produced exposure water with a hardness of approximately 30 mg/L as $CaCO_3$ —a hardness similar to many streams in the Bristol Bay watershed (e.g., Supplemental Data, Table S1) including our site water sample collected from the watershed.

Field-collected water was used for one of the fathead minnow bioassays. This water came from Upper Talarik Creek on 27 August 2013 (site UT-02; see Figure 2 in Zamzow 2011). The water was shipped on ice to the laboratory in Fort Collins and refrigerated. The remote locations in the Bristol Bay watershed required that all site water be collected using a helicopter. Therefore we conducted site water tests only with fathead minnows because of the large volume of water needed to conduct a flow-through test with rainbow trout (>4000 L) compared with a static-renewal test with fathead minnows (<25 L). Before utilizing this field water, it was filtered through glass wool to remove large particles (e.g., woody debris and macroinvertebrates).

Rainbow trout bioassay

Rainbow trout were obtained from within the Colorado Parks and Wildlife hatchery system to conduct a 96-h bioassay in a flow-through system. In our flow-through system, 2-L exposure aquaria received 30 mL/min of laboratory water, resulting in a 99% theoretical volume replacement every 5 h (calculated from Figure 2 in Weber 1993). We added 10 fish (0.49 ± 0.09 g) to each of 4 replicate aquaria over 6 exposure treatment levels. The aquaria were arranged in a single water bath in a randomized block design. Fish were not fed during the 96-h bioassay. We monitored all aquaria daily and recorded and removed all mortalities.

Fathead minnow bioassays

Fathead minnow embryos were acquired shortly after fertilization from Aquatic BioSystems (Fort Collins, CO) and incubated in laboratory water until they hatched and were used in the bioassays. We carried out 2 side-by-side bioassays using laboratory and site water spiked with Cu. Within 24 h of hatching, 10 fry were placed into 250-mL exposure beakers (200 mL of exposure water in each). Four replicate beakers were employed per treatment with 6 treatment levels for each water type. All exposure beakers were arranged in a single water bath in a randomized block design. Fish were not fed during the 96-h bioassays. Daily water exchanges were performed by transferring fry to exposure beakers with freshly prepared exposure water using glass eye droppers. We recorded and removed all mortalities during daily water renewals.

Water chemistry

Water samples were analyzed for major cations (calcium, magnesium, potassium, and sodium) and Cu (USEPA Method 6010C), major anions (sulfate and chloride; USEPA Method 300.0), and organic carbon (Standard Method 5310C). Filtered (0.45-μm pore size) and unfiltered water samples were collected and acidified (pH < 2) for cation, Cu, and organic carbon analyses. The water samples for organic carbon analyses were stored in amber bottles. Water samples collected for anion analysis were filtered (0.45-μm pore size) and stored with no preservative. At the beginning, middle, and end of each treatment of the rainbow trout bioassay (days 0, 2, and 4), water samples were collected. For cations, anion, and organic carbon analyses, water samples were collected at the beginning of the fathead minnow bioassays from a single batch of water used to prepare Cu exposure solutions each day. Water samples for Cu analysis were collected from the first batch of exposure concentrations prepared on day 0 before adding solution to the exposure beakers, and pooled samples were collected from the exposure beakers after 24 h at the first water renewal to determine if Cu concentrations decreased substantially during the static exposures. We refrigerated all water samples after

TABLE 1: Mean water quality values of surface samples collected from stations in Alaska, USA along the North Fork Koktuli River (NK), South Fork Koktuli River (SK), and Upper Talarik Creek (UT) from June–December 2007 (Pebble Limited Partnership 2011)

				Dissolved constituents (0.45-μm filter)								
Site	pH s.u.	Hardness mg/L as $CaCO_3$	Alkalinity mg/L as $CaCO_3$	Organic carbon mg/L	Calcium mg/L	Magnesium mg/L	Sodium mg/L	Potassium mg/L	Sulfate mg/L	Chloride mg/L	Copper μg/L	*n*
NK100A	6.7	19.5	22.5	1.6	5.5	1.4	2.8	0.4	2.0	0.6	0.3	7
NK100B	6.9	21.0	23.4	1.7	5.9	1.5	2.4	0.4	1.8	0.5	0.3	7
NK100C	7.3	26.0	28.0	2.0	7.1	2.0	2.7	0.5	2.0	0.5	0.2	7
SK100A	6.3	16.6	13.7	0.9	5.1	1.0	2.0	0.3	3.3	0.7	0.4	6
SK100C	6.5	18.5	13.0	1.6	5.6	1.1	2.0	0.3	7.5	0.6	1.5	5
SK100F	6.8	18.7	14.1	2.3	5.4	1.3	2.2	0.3	5.6	0.6	1.6	7
SK100G	7.0	25.0	15.1	2.2	7.1	1.8	2.7	0.4	10.6	0.6	2.6	7
UT100B	7.1	30.1	32.4	1.3	9.0	1.9	2.8	0.4	2.6	0.6	0.3	7
UT100D	7.1	39.8	39.9	2.8	10.7	3.2	3.6	0.5	5.6	0.6	0.4	7
UT119A	7.3	32.3	31.4	0.6	10.4	1.5	2.5	0.4	4.0	0.7	0.2	5

s.u. = standard units.

collection/preservation and shipped them on ice overnight to ALS Environmental for analysis.

Additional water quality parameters including temperature, pH, dissolved oxygen, hardness, and alkalinity were measured in the laboratory during testing. We monitored the water bath temperature using a temperature logger that was placed in an extra exposure tank not containing organisms, as well as with a handheld thermometer. Dissolved oxygen was measured using an optical probe and pH was checked with a meter calibrated with pH 4, 7, and 10 standards. Hardness and alkalinity values were determined by titration.

Statistical analysis and model calculations

We used the BLM, Ver 2.2.3 (Biotic Ligand Model 2005) to estimate median lethal concentration (LC50) and water quality criteria values utilizing water chemistry from laboratory and site water samples. To fit dose–response curves, we used the drc package in R (Ritz and Streibig 2005). We fit a 3-parameter log-logistic model for each endpoint of each test (Ritz 2010). This produced estimates for the inflection point, the steepness of the line tangent to the curve at the inflection point, and the lower limit of the curve. This lower limit can be thought of as the modeled mortality when the dose is equal to zero. To obtain effect concentrations and confidence intervals (CIs) based on the profile-likelihood method (Venzon and Moolgavkar 1988; Faraggi et al. 2003), we reparameterized the log-logistic model for each desired effect level (US Environmental Protection Agency 2013) and optimized the model using the bbmle R package (Bolker 2013). Effect concentrations calculated using these methods were adjusted for the modeled control mortality.

RESULTS

Water quality and aquatic criteria estimates for the Bristol Bay watershed

We calculated BLM-derived and hardness-based acute Cu water quality criteria, also known as criterion maximum concentration (CMC), using chemistry from a total of 65 surface water samples collected from 10 sites along the North Fork Koktuli River, South Fork Koktuli River, and Upper Talarik Creek from June to December 2007. Average water quality values for each site are listed in Table 1. Biotic ligand model CMCs ranged from 0.05 to 17.5 μg Cu/L and hardness-based CMCs ranged from 2.3 to 6.1 μg Cu/L for the 65 samples. The ratio of BLM to hardness-based CMC for each sample increased as a function of increasing pH and DOC (Figure 1).

Bioassay water chemistry

Exposure water chemistry and measured Cu concentrations for rainbow trout and fathead minnow bioassays are reported in Tables 2 and 3, and Supplemental Data, Tables S2 and S3. The chemistry of the site water was similar to the mean water chemistry reported for Upper Talarik Creek in the USEPA assessment (2014). Compare Table 3 "Site water" in the present study with Tables 8 to 10 in the USEPA assessment (2014).

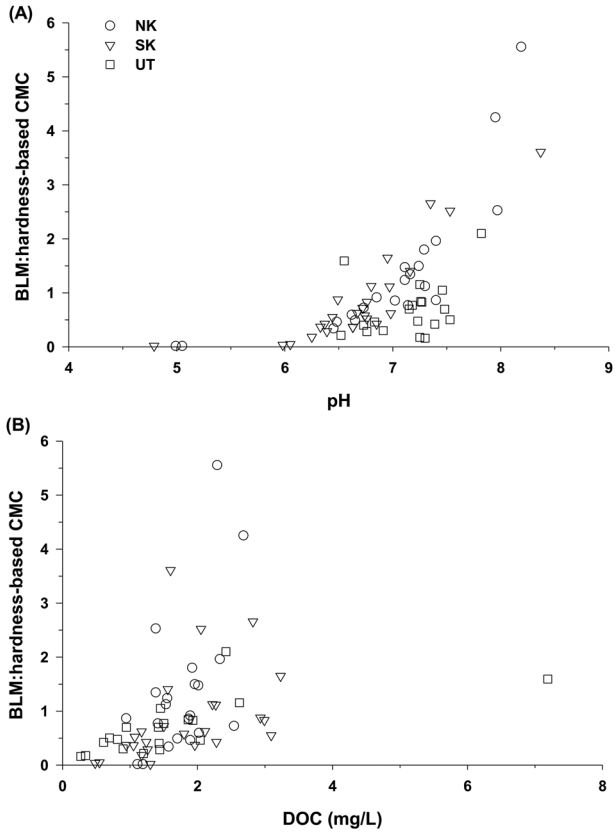

FIGURE 1: Ratio of biotic ligand model (BLM)-derived and hardness-based acute copper water quality criteria, also known as criterion maximum concentration (CMC), compared with (**A**) pH and (**B**) dissolved organic carbon (DOC) for 65 water samples collected in Alaska from the North Fork Koktuli River (NK), the South Fork Koktuli River (SK), and Upper Talarik Creek (UT) in 2007 (Pebble Limited Partnership 2011; Supplemental Data, Table S1).

Rainbow trout bioassay

Average control mortality in our rainbow trout bioassay was low (5 ± 10%) and we observed a dose–response relationship

TABLE 2: Water quality parameters measured in exposure tanks during 96-h rainbow trout bioassay in laboratory water

Parameter	Units	Average ($n = 18$)	SD
Temperature	°C	11.9	0.2
pH	s.u.	6.45	0.12
Dissolved oxygen	mg/L	8.6	0.2
Hardness	mg/L as $CaCO_3$	31	0.6
Alkalinity	mg/L as $CaCO_3$	14	1.1
Dissolved constituents (0.45-μm filter)			
Organic carbon	mg/L	0.8	0.04
Calcium	mg/L	10.1	0.16
Magnesium	mg/L	1.1	0.01
Sodium	mg/L	2.3	0.13
Potassium	mg/L	0.5	0.02
Sulfate	mg/L	4.8	0.79
Chloride	mg/L	9.2	0.79

SD = standard deviation; s.u. = standard units.

TABLE 3: Water quality parameters measured in exposure beakers during 96-h fathead minnow bioassays in laboratory and site water

		Laboratory water			Site water		
Parameter	Units	Average	SD	*n*	Average	SD	*n*
Temperature[a]	°C	24.8	0.5	1169	24.8	0.5	1169
pH (stock solution[b])	s.u.	6.91	0.13	3	7.11	0.08	3
pH (composite[c])	s.u.	7.04	0.22	24	7.18	0.17	24
Dissolved oxygen (stock solution)	mg/L	7.2	0.4	8	7.3	0.5	8
Dissolved oxygen (composite)	mg/L	6.5	0.2	24	6.3	0.2	24
Hardness	mg/L as $CaCO_3$	33	0.8	2	30	0.0	2
Alkalinity	mg/L as $CaCO_3$	26	2.8	2	34	1.8	2
Dissolved constituents (0.45-μm filter)							
Organic carbon	mg/L	1.0	–	1	1.4	–	1
Calcium	mg/L	10.4	–	1	9.5	–	1
Magnesium	mg/L	1.0	–	1	1.9	–	1
Sodium	mg/L	6.1	–	1	3.2	–	1
Potassium	mg/L	0.5	–	1	0.5	–	1
Sulfate	mg/L	11.8	–	1	2.5	–	1
Chloride	mg/L	5.0	–	1	0.7	–	1

[a]Measured with a temperature logger in the water bath.
[b]Measurements were taken from stock solutions immediately before addition to beakers for each renewal.
[c]Calculations were taken from composite water samples of replicate beakers collected after each 48-h renewal period.
SD = standard deviation; s.u. = standard units.

with increasing Cu concentration (Figure 2A). We used the BLM to estimate LC50 values based on water chemistry measured during this test using the lowest, middle, and highest pH values measured during the test to capture the range of possible BLM LC50 estimates over the course of the exposure. The calculated LC50 for this test was 16 μg Cu/L (95% CI: 12, 21). The BLM LC50 estimation for this test using the average standard pH (6.45) was 28 μg Cu/L—1.8 times higher than the actual LC50 (Supplemental Data, Table S4 and Figure 3). The default critical Cu value (i.e., median lethal accumulation) in the BLM for rainbow trout is 3.70 nmol Cu/g wet weight. We also ran the BLM in speciation mode to determine the critical Cu value that corresponded to our calculated LC50 (16 μg Cu/L); this revised critical Cu value was 1.36 nmol Cu/g wet weight.

Fathead minnow laboratory and site water bioassays

Control mortality was also low (3 ± 5%) in each fathead minnow bioassay and we observed a dose–response relationship for each test (laboratory water and site water; Figure 2B,C). We used the BLM to estimate LC50 values based on water chemistry measured during each test, using the lowest, middle, and highest pH values measured during the test to capture the range of possible BLM LC50 estimations over the course of these exposures. The LC50 values for the laboratory and site water bioassays were 29 and 79 μg Cu/L (95% CIs: 23, 35 and 58, 125), respectively. The BLM LC50 estimates for the laboratory and site water bioassays employing the usual pH from each (7.04 and 7.18, respectively) were 67 and 100 μg Cu/L, respectively. These BLM estimates are 2.3 and 1.3 times higher than the actual LC50 values (Supplemental Data, Table S4 and Figure 3).

The default critical Cu value (i.e., median lethal accumulation) in the BLM for fathead minnows is 5.48 nmol Cu/g wet weight. As with our rainbow trout analysis, we also ran the BLM in speciation mode to determine the critical Cu value that corresponded to our measured LC50 values for the laboratory and site water bioassays (29 and 79 μg Cu/L, respectively). These revised critical Cu values were 1.15 and 3.55 nmol Cu/g wet weight, respectively.

DISCUSSION

The purpose of these experiments was to ascertain whether the BLM could accurately estimate adverse concentrations of Cu to salmonids in low-hardness waters such as those typically found in the Bristol Bay watershed. The results of the acute testing with rainbow trout and fathead minnows showed that the BLM consistently underpredicted toxicity in all tests. In fact, the BLM underpredicted the toxicity of Cu to rainbow trout and fathead minnows in low-hardness laboratory water tests by 1.8- and 2.3-fold, respectively (see Supplemental Data, Table S4). In addition, the BLM underpredicted the toxicity of Cu to fathead minnows in field-collected water by 1.3-fold. These comparisons are based on the LC50 derived from the dose–response relationship for each test compared with the BLM-estimated LC50 value for each test using the average pH measured during those tests. Nevertheless, there is variability associated with the derived LC50 values from the dose–response curves (Figure 1) and there was variability in the pH measured during each test (Tables 2 and 3); thus it is important to compare more than just these average values. A more holistic comparison for each of these tests is depicted in Figure 3, illustrating that there was a much higher degree of variability associated with the LC50 estimate for the fathead minnow site water test than for the other 2 tests for both the dose–response estimate (caused by variability in the biological response) and from the BLM estimate (because of variability in test water pH).

Many scientists conducting research related to refining and evaluating the BLM generally apply an acceptability criterion of 2

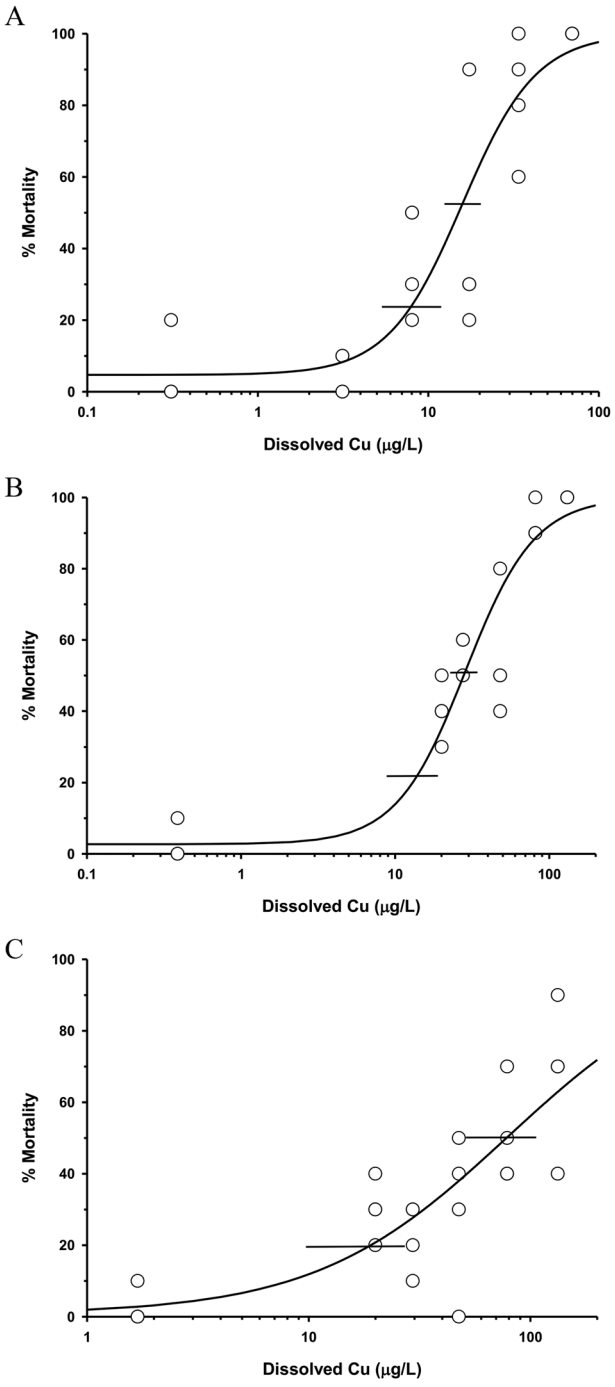

FIGURE 2: Dose–response relationship of (A) rainbow trout and (B) fathead minnows exposed to laboratory water spiked with copper (Cu), and (C) fathead minnows exposed to Alaskan site-collected water (Upper Talarik Creek [UT-02]) spiked with Cu for 96 h with 4 replicates at each exposure concentration. Horizontal bars indicate the 95% confidence intervals for the median lethal concentration (upper bar) and the 20% lethal concentration (lower bar) estimates.

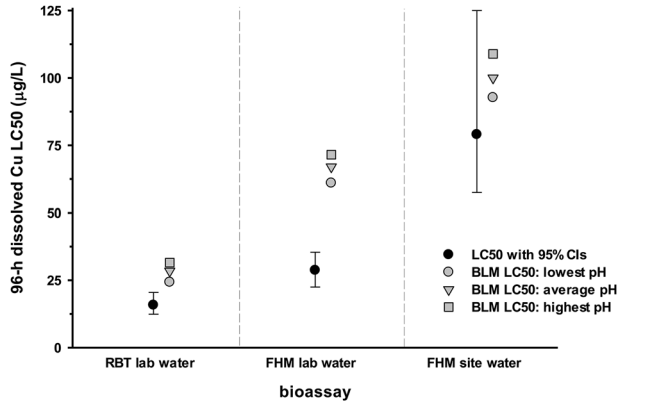

FIGURE 3: Estimated median lethal concentration (LC50) values for rainbow trout (RBT) and fathead minnow (FHM) bioassays using laboratory (lab) or Alaskan Pebble Project area site-collected water (Upper Talarik Creek [UT-02]) based on dose–response relationships for each bioassay (black circles; see Figure 2). Median lethal concentration values estimated using the biotic ligand model (BLM) with water chemistry measured during each bioassay and the lowest (gray circles), average (gray inverted triangles), and highest (gray squares) pH values calculated during each bioassay. CIs = confidence intervals.

times, whereby the BLM LC50 estimates are considered "acceptable" if they are within a factor of ± 2 times the LC50 estimates derived from the regression analysis of the dose–response data. Only the results of our fathead minnow test using laboratory water are outside of this acceptability criterion, with the BLM LC50 estimate at 2.3 times higher than the LC50 calculated using our bioassay data. If one is applying this 2-times acceptability criterion to our data, the modeling results appear to be quite satisfactory. Moreover, one could also argue that the BLM estimates for our fathead minnow test using site water were the most "accurate" of the 3 tests, given that they were within the 95% CI of the calculated LC50 for this test and not for the fathead minnow or rainbow trout laboratory water tests (Figure 3). However, this fathead minnow site water test also had a very wide CI range (58, 125 μg Cu/L, or a total of 67 μg Cu/L) compared with the fathead minnow laboratory water test CI range (23, 35 μg Cu/L, or a total of 12 μg Cu/L) and the rainbow trout laboratory water test CI range (12, 21 μg Cu/L, or a total of 9 μg Cu/L). Although it may be statistically significant, this significance may also just be a consequence of the high variability in this LC50 estimate. Regardless of whether the BLM-estimated LC50 values for our tests are within a factor of ± 2 times our calculated bioassay LC50 values, the BLM LC50 values are all higher, and thus consistently under predicting toxicity.

Our bioassay results along with our analysis of BLM-derived and hardness-based CMC values measured using low-hardness water chemistry indicate that there is a great deal of uncertainty regarding the application of existing water quality criteria to such areas. For instance, in field samples the ratio of BLM to hardness-based CMC for each sample increased as a function of increasing pH and DOC (Figure 1). This increase is not surprising, given the influence both pH and DOC have on toxicity estimates in the BLM, where an increase of either parameter reduces the concentration of free Cu (Cu^{2+}) and therefore reduces the theoretical toxicity of Cu. Nonetheless, our analysis also indicates that the ratio between the BLM and hardness-based CMCs spans more than 3 orders of magnitude (0.02–5.6) within the ranges of pH and DOC measured in these field-collected samples. This shows the vast differences in CMC values one

would expect to calculate throughout the watershed using site chemistry, which presents an interesting dilemma when considering which water quality criteria to apply in the Bristol Bay watershed.

One alternative to applying off-the-shelf water quality criteria models (i.e., hardness-based or BLM) could be to conduct site-specific testing to provide additional data (i.e., LC50 values) that could be used to calibrate the BLM for broader application throughout the Bristol Bay watershed. These tests could be accomplished on site (i.e., streamside testing) or in a laboratory. Each option poses logistical challenges; however, on-site testing provides access to large quantities of actual site water, with the major drawback likely being the requirement to use older life stage fish collected on site. The laboratory testing option could be accomplished using site waters or more accessible regional water that closely matches the chemistry of the Bristol Bay watershed streams, along with site-specific salmonid species and strains (i.e., tests conducted with field-spawned salmon embryos reared in the laboratory). The LC50 values from such tests can be employed to calibrate the BLM model so that toxicity approximations are more accurate. For example, replacing the default critical Cu (median lethal accumulation) values in the BLM with test-specific values derived by running the BLM in speciation mode results in BLM LC50 values that match the regression-derived values. This calibration would allow for a watershed-level evaluation of the potential toxicity of Cu to local salmonids by taking into account slight variations in water chemistry throughout the watershed. Whereas calibrating the BLM LC50 estimate to regression-derived values based on bioassay data can be accomplished by acquiring test-specific critical Cu values, calibrating the BLM water quality criteria estimate in such a way cannot be done. Consequently, one approach to verifying that the BLM water quality criteria are protective for a specific species and life stage in the watershed would be to compare the BLM CMC values with low effect thresholds from site-specific tests such as 20% lethal concentration values, benchmark dose estimates, or lowest-observed-effect concentrations.

Generally speaking, site-specific testing and calibration are useful for many different water types. Our study and subsequent analysis illustrate that these methods may be especially relevant for low-hardness waters. Accordingly—to reduce uncertainty associated with estimating adverse effect levels of Cu to salmonids and other major aquatic species in low-hardness waters—we recommend that site-specific testing using water sources and species relevant to the area be conducted to obtain Cu criteria and/or calibrate existing models such as the BLM that may be utilized in the future. This is also relevant because the Alaska Department of Environmental Conservation is currently considering developing guidance pertaining to use of the BLM on a site-specific basis as part of its 2018 to 2020 Triennial Review process of existing state water quality standards. Finally—given research carried out by Colorado Parks and Wildlife indicating that elevated temperatures increase the toxicity of Cu to salmonids (Brinkman et al. 2013)—we also recommend conducting additional research using water sources and species relevant to the area of interest exposed to Cu under higher thermal regimes resulting from changes in stream hydrology caused by mining activity (e.g., decreased stream flows) and/or global climate change (Overeem et al. 2011; Wobus et al. 2011; Matell et al. 2013; Wobus et al. 2015, 2016).

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