Literature Review of the Effects of Copper on Fish Olfaction

Report Prepared for:

Minto Explorations Ltd.
Suite 900-999 West Hastings Street
Vancouver, BC
V6C 2W2

Report Prepared by:

Minnow Environmental Inc.
101-1025 Hillside Ave.
Victoria, British Columbia
V8T 2A2

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Pierre Stecko, M.Sc., CCEP
Project Manager

Patti Orr, M.Sc.
Technical Reviewer

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EXECUTIVE SUMMARY

A comprehensive review of the scientific literature on the effects of copper in aquatic systems indicates that the sense of smell (olfaction) in fish can be impaired by copper at relatively low concentrations under certain water quality conditions. Olfaction underlies a variety of important life processes in fish, including imprinting, kin recognition, feeding, predator avoidance, mating synchronization, homing and spawning. Accordingly, potential impairment of fish olfaction should be carefully considered when evaluating the potential effects of copper on fish. Most of what is known about the effects of copper on fish olfaction is based on highly controlled laboratory environments conducted over very short time frames and under very specific water quality conditions. Determining the relevance of such findings to a particular aquatic environment requires consideration of physical, chemical and biological differences between laboratory tests and the conditions under which fish exist in nature.

A number of studies have found that copper concentrations of less than 5 ug/L can decrease the strength of electrical signals generated at the nose, cause fish to avoid copper-bearing waters, and cause fish to alter behavior due to an apparent failure of olfaction. At copper concentrations greater than 20 ug/L, copper can damage the cells of the fish nose. Recovery of the sense of smell has been observed at copper concentrations of up to 40 ug/L. At this time, it is not known whether the responses measured in such studies may result in adverse ecological effects (i.e., effects to survival, growth or reproduction). However, comparison to conventional data on the acute and chronic effects of copper to aquatic organisms indicates that the effects of copper on fish olfaction occur at concentrations higher than acute effects to cladocerans (water fleas, the most copper sensitive organisms). Because data on acute effects of copper to cladocerans were used to develop water quality guidelines for copper, this suggests that conventional guidelines are also protective of fish olfaction.

It is widely known that the effects of copper on aquatic organisms are modified by a number of substances that either bind copper and make it biologically unavailable or compete with copper for interaction with an organism. Decades of chemical and biological research indicate that, in most natural surface waters, dissolved organic carbon is the most important water quality characteristic that reduces copper bioavailability and toxicity. Few researchers have developed quantitative relationships between copper effects to fish olfaction and water quality characteristics. However, the relationships that have been developed strongly indicate that dissolved organic carbon also greatly reduces the effects of copper on fish olfaction. Application of these relationships to the waters of Minto Creek suggest that the measured concentrations of dissolved organic carbon alone in Minto Creek are sufficient to

protect fish olfaction from the potential effects of copper at copper concentrations much higher than site-specific water quality objectives developed for Minto Creek.

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1.0 INTRODUCTION

1.1 Background

The Minto Mine is operated by Minto Explorations Ltd., a wholly owned subsidiary of Capstone Mining Corporation. It is an open pit copper/gold/silver mine located approximately 240 km northwest of Whitehorse in the Yukon Territory. The mine is located in the upper reaches of the Minto Creek watershed approximately 10 km upstream (west) of the Minto Creek confluence with the Yukon River. Commercial production at the Minto Mine commenced in October 2007 and the mine has a projected mine life to 2014. Milling (concentrating) is done on site and tailings are dried and stacked at a storage facility located adjacent to the open pit and mill. Site water is managed in the water storage pond located approximately 1.5 kilometers to the east (down-gradient) of the mill.

The Minto Mine has discharged effluent to Minto Creek from the water storage pond. Direct (surface) discharge from the pond was initially projected not to be required under normal circumstances. However, due to water management challenges in both 2008 and 2009, and associated discharges of mine effluent, it became evident that discharge would be an important requirement for the management of site water. Accordingly, the Minto Mine has proposed significant changes to their water management plan and is applying for amendment of both their Water Use License and their Quartz Mining License (Minto/Access 2009). The revised water management plan has included an examination of Effluent Quality Standards proposed under the Minto Water Use License (QZ96-006). As part of this examination, Minnow Environmental was asked to evaluate the background water quality of Minto Creek and investigate options for the derivation of site-specific water quality objectives that could be applied in Minto Creek (Minnow 2009). Site-specific water quality objectives (SSWQO) were calculated (using the background concentration procedure) for three substances (aluminum, copper and iron) that are naturally found at concentrations above Canadian Water Quality Guidelines for the protection of aquatic life (CWQG; CCME 1999). In the case of copper, an additional procedure was explored for developing SSWQO (the water-effect ratio procedure), yielding an SSWQO 5.8-times the CWQG (Minnow 2009). Because the CWQG is hardness-dependent, the latter SSWQO increases with increasing hardness (e.g., at hardness between 100 and 200 mg/L would range from 13 ug/L to 24 ug/L, the lower of which is the same as the SSWQO calculated using the background concentration procedure).

In reviewing the recommended SSWQO for copper, several reviewers pointed to recent findings in the scientific literature suggesting that copper may impair olfactory responses in

fish at low concentrations (e.g., by reducing the ability of fish to sense chemical signals via their olfactory epithelium [nose]). For example, Sandahl et al. (2007) reported that short-term exposure to 2 ug/L dissolved copper significantly reduced the amplitude of electrical signals generated at the olfactory epithelium and the number of juvenile coho salmon that initiated predator avoidance behavior in response to an external alarm cue. Such findings are of interest with respect to occupation of lower Minto Creek by juvenile Chinook salmon for short periods in the summer and early fall during their out-migration from upstream tributaries to the Yukon River (Minnow/Access 2007, 2009; Minto/Access 2009). However, the relevance of the findings presented in the literature to Minto Creek must be carefully considered in terms of how the test organisms and test conditions compare to conditions in Minto Creek. Accordingly, the Minto Mine has requested that Minnow prepare this document to provide a comprehensive and up-to-date review of the scientific literature on the effects of copper on fish olfaction, as well as an analysis of the relevance of the findings to Minto Creek.

1.2 Project Objectives

The objectives of this study are: 1) to provide a comprehensive and up-to-date review of the scientific literature on the effects of copper on fish olfaction; and 2) to determine the site-specific relevance of the findings to Minto Creek. This review is important for determining if the SSWQO for copper calculated using the water-effect ratio procedure is also protective of potential olfactory responses.

1.3 Report Organization

The review of available literature on the effects of copper on fish olfaction is provided in Section 2.0 of this report. Section 2.0 includes an introduction to fish olfaction and associated physiology, a review of the types of olfactory responses observed following copper exposure and the associated effect concentrations, a review of the mechanisms of olfactory response to copper exposure, a review of factors that modify olfactory responses to copper exposure, the recovery of olfactory capacity following copper exposure, a review of the ecological relevance of the observed effects of copper on fish olfaction, and an overview of the scientific uncertainties associated with the research findings. Section 3.0 provides a comparison of the effects of copper on fish olfaction to those of “conventional” copper-effect endpoints such as survival, growth and reproduction. Because an assessment of the relevance of the potential effects of copper on fish olfaction to Minto Creek requires an understanding of the fundamental factors that influence copper bioavailability and toxicity, these are briefly reviewed in Section 3.0. Section 4.0 provides a summary of water quality data for Minto Creek, focused on the substances that are known to have the greatest

influence on the bioavailability and toxicity of copper. Section 4.0 also provides a summary of biological resources of Minto Creek. Lastly, Section 4.0 provides an integration of the literature findings and the site conditions to evaluate the site-specific relevance of the scientific research findings on the effects of copper on fish olfaction to Minto Creek. Conclusions of the literature review and analysis of site-specific relevance are provided in Section 5.0. All the references cited throughout this report are listed in Section 6.0.

2.0 REVIEW OF COPPER OLFACTORY EFFECTS

A total of 30 recent (post-1990) publications on the olfactory effects of copper on fish were reviewed, of which all but one were from the primary literature (i.e., published articles in peer-reviewed journals). Every publication was carefully reviewed to distill the key findings and conclusions (Appendix A). Reviewed publications often referred to earlier (<1990) research, some of which was also integrated into the discussion of results provided herein. This review considers:

1. Olfactory physiology;
2. Olfactory response types;
3. Olfactory response concentrations;
4. Olfactory response mechanisms;
5. Olfactory response modifiers;
6. Olfactory recovery; and
7. Ecological relevance and uncertainties.

Olfaction is the sensation of smell, and along with taste, is a form of chemoreception. Fish use olfaction for a variety of important life processes, including imprinting, kin recognition, feeding, predator avoidance, mating synchronization, homing and spawning (e.g., Chivers et al. 2002; Mirza and Chivers 2003a,b; Sandahl et al. 2004, 2007; Pyle and Mirza 2007; Tierney et al. 2010). Accordingly, consideration of potential olfaction-mediated effects warrants attention when developing safe concentrations such as guidelines or objectives for the protection of aquatic life. Although the effects of copper on fish olfaction have recently gained attention, these observations are not new. A number of studies conducted in the 1950s, 1960s and 1970s documented the effects of copper on behavior or electro-physiological responses in fish (e.g., Wisby and Hasler 1954; Sprague 1964; Sprague et al. 1965; Hara et al. 1976; Lorz and McPherson 1976, 1977). It is also noteworthy that numerous other metals (aluminum, silver, cadmium, mercury, manganese, nickel and zinc), pesticides, surfactants and hydrocarbons have been shown to affect olfactory receptor function and/or olfactory-mediated behaviours (e.g., Mirza et al. 2009; Tierney et al. 2010).

2.1 Olfactory Physiology

Teleost fish possess well developed peripheral olfactory organs (rosettes), which are paired structures that reside in bilaterally positioned olfactory chambers (Tierney et al. 2010; Figure 2.1). The olfactory epithelium of the rosettes contains ciliated olfactory sensory neurons (OSNs) embedded in a layer of mucous in direct contact with surface waters (e.g., Saucier et al. 1991b McIntyre et al. 2008a,b; Tierney et al. 2010; Figure 2.1). OSNs transduce odorant

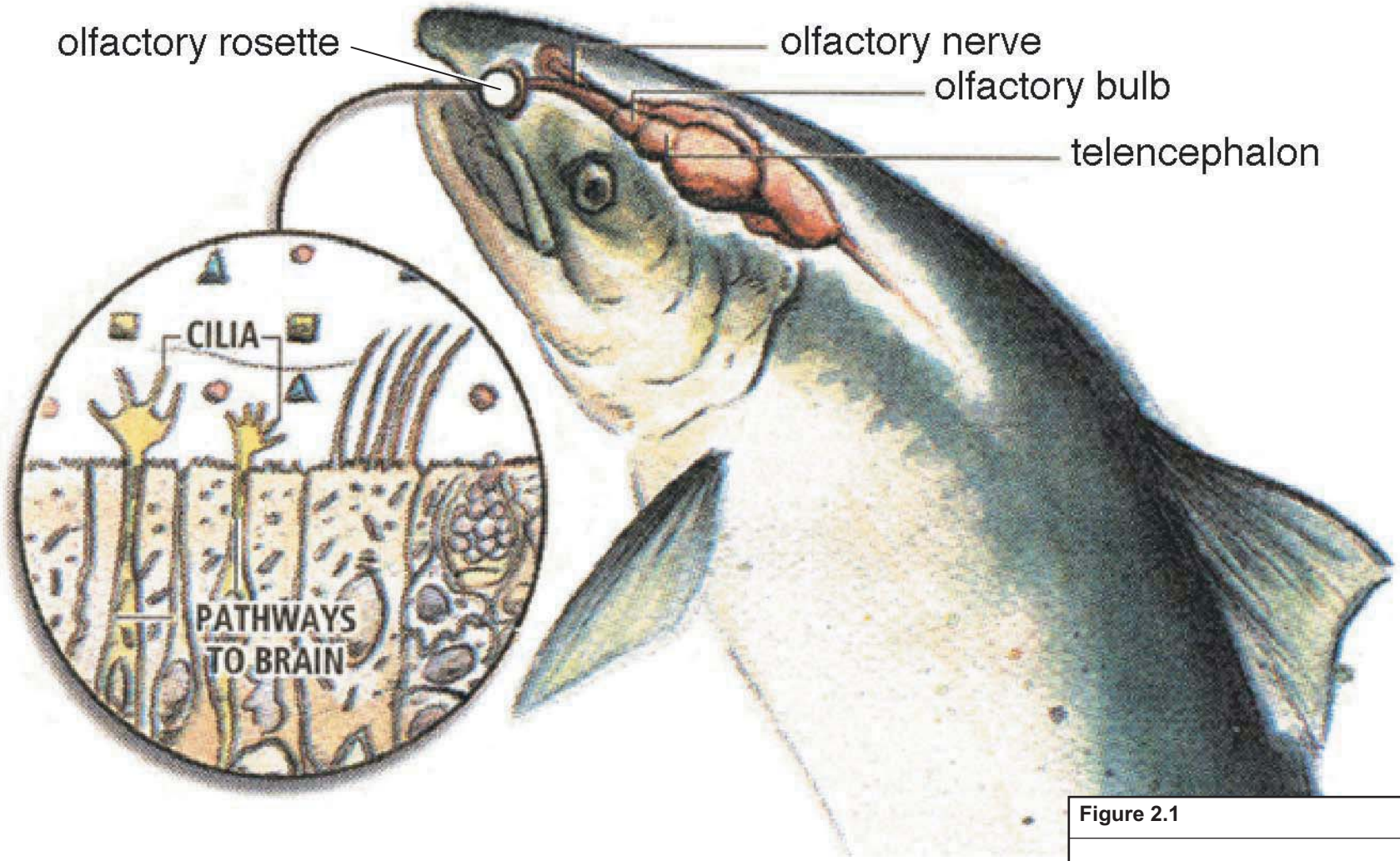

Figure 2.1

minnow environmental inc.

The salmon olfactory nervous system.

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Date: March 2010

Source: Gillermo Munro/Seattle Post-Intelligencer

binding and receptor activation into electrical signals conveyed to the brain (McIntyre et al. 2008a). The receptor (or "ligand") in the fish nose has not been identified, but copper binding in the olfactory epithelium is thought to occur on cellular surface proteins, membrane structures, and internal organelles (Green et al. 2010). It is notable that OSNs are the only vertebrate neurons capable of division and renewal (Laberge and Hara 2001; Hara 1992) and that olfactory receptor cells are continually replaced throughout the life of the vertebrate (Julliard et al. 1996).

2.2 Olfactory Response Types

Copper has been shown to interfere with the ability of fish to detect and respond to chemical signals in aquatic environments using both neuro-physiological recordings (explained below) and behavioural observations. Such studies have often been paired with histological examination of olfactory epithelial tissue to determine changes associated with copper exposure.

Neuro-physiological recordings include measurement of the electro-olfactogram (EOG) in which an electrode is placed on the surface of the olfactory rosette epithelium (Hara et al. 1976; Winberg et al. 1992; Bjerselius et al. 1993; Hansen et al. 1999c; Baldwin et al. 2003; Sandahl et al. 2004, 2006, 2007; McIntyre 2008a,b; Mirza et al. 2009; Green et al. 2010) or the electro-encephalogram (EEG) in which an electrode is placed against the surface of the olfactory bulb where olfactory sensory neurons from the rosette converge in leading to the brain (Hansen et al. 1999c; Sandahl et al. 2004). Results of EOG and EEG recordings have been shown to agree well (Sandahl et al. 2004) and the former has been more frequently used in studies of fish olfaction. EOG and EEG recordings are performed on anaesthetized and restrained fish that are typically presented with an odour stimulus under different exposure conditions.

The ability of salmonids to avoid water with high copper concentrations has been well documented. Such behavioural effects have been observed in the field (Wisby and Hasler 1954; Sprague 1964; Sprague et al. 1965; Lorz and McPherson 1976, 1977; McPherson et al. 2004), in the laboratory using preference-avoidance apparatus (e.g., Rehnberg and Schreck 1986; Saucier et al. 1991a; Saucier and Astic 1995; Woodward et al. 1995; Scherer and McNichol 1998; Hansen et al. 1999a,b; Beyers and Farmer 2001; Carreau and Pyle 2005; Mirza et al. 2009), or both (Leduc et al. 2004).

A number of studies have examined morphological changes in the tissue of the olfactory epithelium, often in conjunction with neuro-physiological of behavioural responses. These typically involve examination of the olfactory epithelium by staining and light microscopy or

scanning electron microscopy (Saucier et al. 1991b, Saucier and Astic 1995; Julliard 1996; Hansen et al. 1999c; Beyers and Framer 2001; Bettini et al. 2006; Mirza et al. 2009). Because mucous production is considered to be a first line of defense in olfactory exposures, (e.g., chemicals can be bound externally by mucous), the hypertrophy and hyperplasia of goblet cells is one early response to exposure (Saucier and Astic 1995).

2.3 Olfactory Response Concentrations

Olfactory responses to copper have been measured by a number of different methods under a variety of water quality conditions (Tables 2.1 to 2.3). The majority of studies were conducted in laboratory environments using waters with relatively low hardness. In most cases, the waters tested were well water or de-chlorinated tap water and, although the dissolved organic carbon concentrations were rarely reported, they are typically very low in such water sources. Under these test conditions, responses have been documented at copper concentrations below 10 ug/L (Tables 2.1 to 2.3). The discussion of response concentrations provided in the following paragraphs is generally focused on the lowest response concentrations. A discussion of how different water quality characteristics modify copper's influence on fish olfaction is provided in Sections 2.5 and 3.2.

Neuro-physiological responses have been documented at concentrations below 5 ug/L dissolved copper (Table 2.1). For example, in very soft water (20 mg/L hardness), Baldwin et al. (2003) found significant reductions in EOG response of juvenile coho salmon relative to controls at a dissolved copper concentration of only 1 ug/L above a background of 3 ug/L (i.e., a dissolved copper concentration of 4 ug/L is implied, but this is not explicitly stated in the paper). Based on these results, the authors determined that there was a 25% reduction of EOG response at 2.7 ug/L, 2.3 ug/L and 3.0 ug/L dissolved copper (i.e., 5.7 ug/L, 5.3 ug/L and 6.0 ug/L after consideration of background) to the odorants L-serine, taurocholic acid and an amino acid mixture, respectively. In similarly soft waters, McIntyre et al. (2008a,b,c) observed significant reductions in EOG response in juvenile coho salmon relative to controls at dissolved copper concentrations of ≤5 ug/L when dissolved organic carbon was low, but reported substantial increases in effect concentrations when dissolved organic carbon concentrations were increased (Table 2.1). A series of papers by Sandahl et al. (2004, 2006 and 2007) reported inhibition of olfactory response by low concentrations of copper in juvenile coho salmon and chum salmon fry; for coho salmon at moderate hardness, significant differences from control were observed at 2 ug/L dissolved copper (Sandahl et al. 2007) and the EC20 (concentration causing an effect in 20% of exposed organisms) was calculated as 4.4 ug/L dissolved copper. In the case of chum salmon, a significant difference from control was observed at 8 ug/L dissolved copper at low hardness and slightly acid

Table 2.1: Fish olfactory responses to copper from neuro-physiological studies.

Source	Species tested (lifestage) [a]	Effect Concentration (ug/L dCu) [b]	Effect/ Observation	Effect Statistic	Exposure Duration [c]	Temperature (°C)	pH	Dissolved Organic Carbon (mg/L)	Hardness (mg/L)
Baldwin et al. 2003	Coho salmon (juvenile)	1 (4) [d]	significant decrease in olfactory activity (EOG amplitude)	significant difference from control	30 minutes	12	7.55	nr	20
Baldwin et al. 2003	Coho salmon (juvenile)	2.7, 2.3, 3.0 (5.7, 5.3, 6.0) [d]	decrease in olfactory activity to L-serine, taurocholic acid or an amino acid mixture	Benchmark Criterion [e] (25% reduction relative to controls)	30 minutes	12	7.55	nr	20
Bjerselius et al. 1993	Atlantic Salmon (juvenile)	64 (Cu^{2+})	modified olfactory response (EOG profile distorted)	significant difference from control	< 1 minute	nr	6.6-6.7	nr	40 - 400
Brown et al. 1982 In: Hara 1992	Rainbow trout (lifestage not specified)	153	complete inhibition of olfactory bulb response	nr	2 weeks	nr	nr	nr	nr
Green et al. 2010	Fathead minnow (adult)	10	Reduction in EOG	significant difference from control	2 weeks	12	6.72 - 6.93	nr	23
Green et al. 2010	Fathead minnow (adult)	10	Reduction in EOG	significant difference from control	2 weeks	12	6.72 - 6.93	nr	46
Hansen et al. 1999b	Chinook salmon (juvenile)	25	increase in olfactory bulb activity (EEG amplitude to Cu-only water)	significant difference from control	< 10 min Cu water	12	7.67	nr	24.5
Hansen et al. 1999b	Rainbow trout (juvenile)	25	increase in olfactory bulb activity (EEG amplitude to Cu-only water)	significant difference from control	< 10 min Cu water	12	7.67	nr	24.5
Hara et al. 1976 In: Hara 1992	Rainbow trout (lifestage not specified)	8	inhibited olfactory response	nr	< 2 minutes	nr	nr	nr	nr
McIntyre et al. 2008	Coho Salmon (juvenile)	20	reduced olfactory response to L-serine (EOG amplitude decreased)	significant difference from control	30 minutes	13	7.1 - 8.6	≤ 2.76	≤ 190
McIntyre et al. 2008	Coho Salmon (juvenile)	approx. 5 [f]	reduced olfactory response	IC50	30 minutes	13	7.5	0.1	30
McIntyre et al. 2008	Coho Salmon (juvenile)	approx. 7 [f]	reduced olfactory response	IC50	30 minutes	13	7.4	1.94	27
McIntyre et al. 2008	Coho Salmon (juvenile)	approx. 30 [f]	reduced olfactory response	IC50	30 minutes	13	7.2	2.76	27
McIntyre et al. 2008	Coho Salmon (juvenile)	approx. 25 [f]	reduced olfactory response	IC50	30 minutes	13	7.1	4.11	28
McIntyre et al. 2008	Coho Salmon (juvenile)	approx. 60 [f]	reduced olfactory response	IC50	30 minutes	13	7.2	6.03	27

Table 2.1: Fish olfactory responses to copper from neuro-physiological studies.

Source	Species tested (lifestage) [a]	Effect Concentration (ug/L dCu) [b]	Effect/ Observation	Effect Statistic	Exposure Duration [c]	Temperature (°C)	pH	Dissolved Organic Carbon (mg/L)	Hardness (mg/L)
Mirza et al. 2009	Yellow perch (juvenile)	25 (from metal-contaminated lake)	increased EOG response to odorant	significant difference from control	lifespan (feral fish from contaminated lake used in lab trials)	nr	6.8 - 7.1	nr	40 - 42
Multiple references In: Hara 1992	Rainbow trout (lifestage not specified)	13	inhibition of olfactory bulb response	nr	nr	nr	nr	nr	nr
Sandahl et al. 2004	Coho salmon (juvenile)	10	inhibited olfactory response to odorants (EOG and EEG)	significant difference from control	7 days	11 - 13	7.1	nr	120
Sandahl et al. 2004	Coho salmon (juvenile)	20	distorted olfactory response (EOG profile)	significant difference from control	7 days	11 - 13	7.1	nr	120
Sandahl et al. 2004	Coho salmon (juvenile)	4.4	inhibited olfactory response to odorants (EOG and EEG)	EC20	7 days	11 - 13	7.1	nr	120
Sandahl et al. 2004	Coho salmon (juvenile)	11.1	inhibited olfactory response to odorants (EOG and EEG)	EC50	7 days	11 - 13	7.1	nr	120
Sandahl et al. 2006	Chum Salmon (fry)	8	inhibited olfactory response (EOG amplitude decreased)	significant difference from control	4 hours	9.7	6.1	nr	40
Sandahl et al. 2007	Coho salmon (juvenile)	2	inhibited olfactory response (EOG amplitude decreased)	significant difference from control	3 hours	10.8	6.7	nr	120
Winberg et al. 1992	Atlantic Salmon (juvenile)	12.7 (Cu^{2+})	olfactory EOG profile different from control	significant difference from control	< 1 minute	6 - 8	6.2	nr	nr

nr - not reported
[a] at time response was recorded
[b] if not explicitly stated in the paper, copper assumed to be dissolved if non-natural water was used for the study (e.g., dechlorinated city water, well water, de-ionized water etc)
[c] earliest time at which effect was reported. Total exposure duration for the study may have been longer.
[c] effect concentrations were reported as concentration above background; concentrations in brackets account for the background concentration of 3 ug/L copper
[e] U.S. Environmental Protection Agency. 1995. The use of the benchmark dose approach in health risk assessment. EPA 630/R-94/007. Office of Research and Development, Washington, DC.
[f] determined from a plot in the original paper; data were not provided in table form.

Table 2.2: Fish olfactory responses to copper from behavioural studies.

Source	Species tested (lifestage[a])	Effect Concentration (ug/L dCu)[b]	Effect/ Observation	Effect Statistic	Exposure Duration[c]	Temperature (°C)	pH	Dissolved Organic Carbon (mg/L)	Hardness (mg/L)
Beyers and Farmer 2001	Colorado pikeminnow (185 days old)	2.61, 43.3	inhibited predator avoidance response	EC1, EC50	24 hours	20	8.3	nr	117
Beyers and Farmer 2001	Colorado pikeminnow (185 days old)	18.3, 56	inhibited predator avoidance response	EC1, EC50	96 hours	20	8.3	nr	117
Carreau and Pyle 2005	Fathead minnow (juvenile)	10	inhibited predator avoidance response	significant difference from control	5 - 7 days in Cu water (embryonic development); 84 - 96 days in Cu-free water	20	7.37	2.7	18.1
Green et al. 2010	Fathead minnow (adult)	10	L-arginine avoidance	significant difference from control	2 weeks	12	6.72 - 6.93	nr	23
Hansen et al. 1999a	Rainbow trout (juvenile)	1.2 (in a metal mixture)	avoidance of metal-containing water	significant difference from control	20 minutes	12	5, 6, 7 and 8	nr	100
Hansen et al. 1999a	Rainbow trout (juvenile)	12 (in a metal mixture)	avoidance of metal-containing water	significant difference from control	45 days in 12 ug/L Cu as part of a metal mixture; 20 minute test period	12	8.0	nr	100
Hansen et al. 1999b	Rainbow trout (juvenile)	1.6	behavioural avoidance of copper	significant difference from control	20 minutes	10	7.5	nr	25.3
Hansen et al. 1999b	Rainbow trout (juvenile)	180	loss of copper avoidance	significant difference from control	20 minutes	10	7.5	nr	25.3
Hansen et al. 1999b	Rainbow trout (juvenile)	3.4	behavioural avoidance of copper	significant difference from control	25-30 day 2 ug/L Cu acclimation; 20 minute exposure	10	7.5	nr	25.3
Hansen et al. 1999b	Chinook Salmon (juvenile)	0.8	behavioural avoidance of copper	significant difference from control	20 minutes	10	7.5	nr	25.3
Hansen et al. 1999b	Chinook Salmon (juvenile)	44	loss of copper avoidance	significant difference from control	20 minutes	10	7.5	nr	25.3
Lorz and McPherson 1976, 1977	Coho Salmon (juvenile)	5 (total)	delayed and reduced downstream migration	significant difference from control	6 days	not clearly stated in report	~7	nr	~84 to ~100
Lorz and McPherson 1976, 1977	Coho Salmon (juvenile)	30 (total)	delayed and reduced downstream migration	significant difference from control	72 hours	not clearly stated in report	~7	nr	~84 to ~100
McPherson et al. 2004	Iowa darter	15	alarm cue avoidance in the field	statistical difference between conataminated and clean	10 hours	nr	8.4	nr	nr
Mebane 2000	Chinook Salmon (adult)	10 - 25	spawning migrations interupted	observation	indefinite	nr	nr	nr	40

Table 2.2: Fish olfactory responses to copper from behavioural studies.

Source	Species tested (lifestage[a])	Effect Concentration (ug/L dCu)[b]	Effect/ Observation	Effect Statistic	Exposure Duration[c]	Temperature (°C)	pH	Dissolved Organic Carbon (mg/L)	Hardness (mg/L)
Mirza et al. 2009	Yellow perch (juvenile)	9 (from metal-contaminated lake)	loss of anti-predator response to alarm cue	significant difference from control	lifespan (feral fish from contaminated lake used in lab trials)	nr	7.7 - 8.0	nr	47 - 543
Mirza et al. 2009	Yellow perch (juvenile)	25 (from metal-contaminated lake)	loss of anti-predator response to alarm cue	significant difference from control	lifespan (feral fish from contaminated lake used in lab trials)	nr	6.8 - 7.1	nr	40 - 42
Rehnberg and Schreck 1986	Coho Salmon (juvenile; 0+)	6	decreased upstream swimming and avoidance of L-serine inhibited	significant difference from control	105 minutes	12.5 - 17.4	6.72	< 2 (TOC)	30.5
Sandahl et al. 2007	Coho salmon (juvenile)	2	inhibited predator avoidance behaviour	significant difference from control	3 hours	10.8	6.7	nr	120
Saucier and Astic 1995	Rainbow trout (juvenile)	20	loss of behavioural discrimination of different types of water and lowered activity level	significant difference from control	not clearly stated in report (13 to 40 weeks)	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	40	loss of behavioural discrimination of different types of water	significant difference from control	30 weeks	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	40	complete recovery of behavioural discrimination of different types of water	significant difference from control	40 weeks in Cu water; 29 weeks in Cu-free water	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier et al. 1991a	Rainbow trout (juvenile)	22	loss of behavioural discrimination of different types of water	lowest observed effect concentration	37 - 41 weeks	7.6 - 10.1	6.50 - 6.64	nr	62 - 64
Scherer and McNicol 1998	Lake whitefish (juvenile)	1 (40)	avoidance of Cu-water	lowest observed effect concentration	10 minutes	10.2 - 11.3	7.51 - 7.78	420 uM[d]	90
Sprague et al. 1965	Atlantic salmon (adult)	20	spawning migrations interupted	lowest observed effect concentration	indefinite	nr	nr	nr	20
Sprague et al. 1965	Atlantic Salmon (juvenile)	2.4	avoidance in laboratory exposures	lowest observed effect concentration	20 minutes	nr	nr	nr	20
Timmins et al. 1972 In: Hara 1992	Goldfish and Channel catfish (lifestage not specified)	50	attraction to Cu-containing water	lowest observed effect concentration	nr	nr	nr	nr	nr
Woodward et al. 1995	Brown trout (150-d post-hatch)	6 (in a metal mixture)	avoidance	significant difference from control	20-min acclimation and 30-min test	10	8	nr	100
Woodward et al. 1996	Brown trout (150-d post-hatch)	48 (in a metal mixture)	loss of ability to avoid	significant difference from control	20-min acclimation and 30-min test	10	8	nr	100

nr - not reported

[a] at time response was recorded

[b] if not explicitly stated in the paper, copper assumed to be dissolved if non-natural water was used for the study (e.g., dechlorinated city water, well water, de-ionized water etc)

[c] lowest concentration in a series that resulted in a linear increase in avoidance; within a competing gradient (shade), avoidance was not observed at 40 ug/L copper but was observed at 72 ug/L copper

[d] molecular weight unspecified, therefore mg/L concentration could not be calculated

Table 2.3: Fish olfactory responses to copper from histopathological studies

Source	Species tested (lifestage[a])	Effect Concentration (ug/L dCu)[b]	Effect/ Observation	Effect Statistic	Exposure Duration[c]	Temperature (°C)	pH	Dissolved Organic Carbon (mg/L)	Hardness (mg/L)
Bettini et al. 2006	Tilapia mariae (adult)	20	degenerating olfactory cells	significant difference from control	4 days	19.1	7.1	nr	364
Beyers and Farmer 2001	Colorado pikeminnow (185 days old)	266	Loss of olfactory receptor cells	significant difference from control	24 hours	20	8.3	nr	117
Beyers and Farmer 2001	Colorado pikeminnow (185 days old)	60	Loss of olfactory receptor cells	significant difference from control	96 hours	20	8.3	nr	117
Brown et al. 1982 In: Hara 1992	Rainbow trout (lifestage not specified)	153	accumulation of Cu in olfactory rosettes	significant difference from control	2 weeks	nr	nr	nr	nr
Gardner and Laroche 1973 In: Hara 1992	Mummichog and Atlantic silverside (lifestage not specified)	502	irreversibly damaged olfactory sensory cells	significant difference from control	24 hours	nr	nr	nr	nr
Hansen et al. 1999c	Chinook salmon (juvenile)	25, 50	Loss of olfactory small-dendrite receptors	significant difference from control	4 hours in Cu water; 16 hours in Cu-free water	12	7.70	nr	24.5
Hansen et al. 1999c	Rainbow trout (juvenile)	200	Loss of olfactory small-dendrite receptors	significant difference from control	1 hour Cu water; 1 hour in Cu-free water	12	7.67	nr	24.5
Julliard et al. 1996	Rainbow trout (juvenile)	20	numerous pycnotic (i.e., dying) cells in the olfactory epithelium	significant difference from control	1 day	4.6 - 5.8	6.53 - 6.96	nr	63
Julliard et al. 1996	Rainbow trout (juvenile)	20	loss of intact mature olfactory receptor cells	significant difference from control	5 days	4.6 - 5.8	6.53 - 6.96	nr	63
Sandahl et al. 2006	Chum Salmon (fry)	24	decrease in the number of active olfactory receptor neurons	significant difference from control	4 hours	9.7	6.1	nr	40
Saucier and Astic 1995	Rainbow trout (juvenile)	20	degenerating olfactory epithelial cells, hyperplasia and hypertrophy of goblet cells, fewer mature neuroreceptors, olfactory knob absent	significant difference from control	40 weeks	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	40	disorganized olfactory epithelium, hyperplasia and hypertrophy of goblet cells	significant difference from control	5 weeks	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	40	disorganization of olfactory epithelium, large mucus vacuolse, cyst-like structures in olfactory epithelium	significant difference from control	25 weeks	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	40	fewer mature neuroreceptors	significant difference from control	40 weeks	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier et al. 1991b	Rainbow trout (juvenile)	22	sequential effects: 1) increase in mucous-secreting goblet cells; 2) large vacuoles and degenerating cells; 3) necrotic tissue/lesions; 4) severe damage/denudation	significant difference from control	1) 8 weeks 2) 20 weeks 3) 28 weeks 4) 36 weeks (from hatch)	7.6 - 10.1	6.50 - 6.64	nr	61.8 - 64.0

nr - not reported
[a] at time response was recorded
[b] if not explicitly stated in the paper, copper assumed to be dissolved if non-natural water was used for the study (e.g., dechlorinated city water, well water, de-ionized water etc)
[c] earliest time at which effect was reported. Total exposure duration for the study may have been longer.

conditions. Several studies documented impairment to free ionic copper at concentrations of 64 ug/L and 12.7 ug/L (Bjerselius et al. 1993 and Winberg et al. 1992, respectively). Overall, neuro-physiological studies have clearly demonstrated that copper can impair the olfactory response to alarm stimuli under certain water quality conditions.

Behavioural responses have also been observed at copper concentrations below 5 ug/L (Table 2.2.). For example, Hansen et al. (1999a) found that rainbow trout exposed to a metal mixture mimicking a metal-impacted river showed significant avoidance at a copper concentration of 1.2 ug/L (but the response cannot be attributed to copper alone). The same authors showed that chinook salmon significantly avoid copper concentrations as low as 0.8 ug/L in very soft water (dissolved organic carbon concentration was not reported) and lose their ability to avoid at a concentration of 44 ug/L (Hansen et al. 1999b). Lorz and McPherson (1976, 1977) exposed yearling coho salmon both acutely (6 days) and chronically (165 days) and tracked out-migration in the wild. They found that exposures as low as 5 ug/L caused a reduction of out-migration and that the effect was dose dependent (increased over the exposure concentrations of 5, 10, 20 and 30 ug/L). Rehnberg and Schreck (1986) found that coho salmon decreased their upstream swimming performance and avoidance of L-serine at copper concentrations as low as low as 6 ug/L. Sandahl et al. (2007) related the inhibited EOG response in coho salmon previously discussed to a decrease in predator avoidance behavior at 2 ug/L dissolved copper in moderately hard water and slightly acidic pH. Scherer and McNichol (1998) found a significant linear increase in avoidance by lake whitefish of water with copper concentrations of 1 ug/L to 40 ug/L (not tested individually relative to control). However, when shade was introduced as a competing environmental variable, avoidance (of copper and shade) did not occur at 40 ug/L, but did occur at 72 ug/L. Studying atlantic salmon, Sprague et al. (1965) observed avoidance at 2.4 ug/L copper in soft water. Avoidance of low copper levels (6 ug/L) has also been observed in brown trout at moderate hardness (Woodward et al. 1995). Overall, behavioural studies have indicated that salmonids can effectively detect and avoid copper at relatively low concentrations under certain water quality conditions.

In natural systems, there is evidence of behavioural interruption at higher copper concentrations. For example, inhibition of avoidance has been observed at copper concentrations as low as 10 ug/L in soft water (Carreau and Pyle 2005; Green et al. 2010). Delayed out-migration of coho salmon has been observed at copper concentrations as low as 5 ug/L and interruption of spawning migrations have been observed at copper concentrations of 20 ug/L in very soft water (Sprague et al. 1965). Several studies have also investigated thresholds for the apparent loss of the ability to avoid copper, perhaps because

it simply becomes toxic (see Section 3.1). Specifically, Hansen et al. (1999b) documented that rainbow trout and Chinook salmon lost their ability to avoid copper at 180 ug/L and 44 ug/L, respectively. Similar effects were reported in other species by Mirza et al. (2009), Saucier and Astic (1995), Saucier et al. (1991a) and Woodward et al. (1995).

Morphological effects of copper on the olfactory epithelium of salmonids have been assessed in a number of histopathological studies and effects were generally observed at higher concentrations than those that can impair neurophysiological function and behavior (Table 2.3). Lowest concentrations associated with morphological damage appear to be in the 20 to 25 ug/L range (Table 2.3; Saucier et al. 1991; Saucier and Astic 1995; Julliard et al. 1996; Hansen et al. 1999c; Bettini et al. 2006; Sandahl et al. 2006). Histopathological effects of copper include the general loss, damage and degeneration of olfactory cells, the loss of small dendrite receptors, hyperplasia and hypertrophy of goblet cells, disorganized olfactory epithelium, large mucus vacuoles, and cyst-like structures in the olfactory epithelium (Table 2.3). Copper concentrations associated with morphological effects on the olfactory epithelium are consistent with those documented by Linbo et al. (2006, 2009) to cause death of lateral line mechano-sensory neurons under most water quality conditions.

Despite the well-known importance of water quality conditions on the effect of copper in aquatic environments (e.g., USEPA 2007 and see Section 3.2), very few studies have considered the influence of water quality characteristics on olfactory responses. Specifically, of all the studies reviewed above, only three studies examined the influence of different water qualities (Winberg et al. 1992; Bjerselius et al. 1993; Baldwin et al. 2003; and McIntyre et al. 2008a,b,c) on olfactory response. The findings of these studies are discussed in more detail in Section 2.5.

2.4 Olfactory Response Mechanisms

Current scientific consensus seems to be that the mechanism of copper impairment of fish olfaction at low concentrations is still largely unknown (Pyle and Mirza 2007; Tierney 2010). At low concentrations, copper appears to act as a general-purpose inhibitor of fish olfaction by diminishing the sensitivity and responsiveness of olfactory sensory neurons to chemical cues, resulting in dysosmia or anosmia (a diminished ability to smell or a total absence of ability to smell; Sandahl et al. 2007). Low concentrations of copper do not appear to inhibit odorant binding to the odorant receptors, rather the Cu^{2+} ion appears to exert its effect on the transduction mechanism at the membranes of the receptor cells (Winberg et al. 1992). At higher concentrations, cellular damage and general degeneration of the sensory epithelium occurs and has been characterized in histopathological studies (e.g., Saucier et al. 1991b;

Saucier and Astic 1995; Julliard et al. 1996; Hansen et al. 1999c; Bettini et al. 2006). At high concentrations, copper accumulation is known to cause cell death via apoptosis (programmed cell death) and to specifically induce degeneration of olfactory primary neurons, leaving the other cellular constituents of the sensory epithelium apparently intact (Brown et al. 1982; Julliard et al. 1996; Hansen et al. 1999c). The precise molecular mechanisms by which copper induces apoptosis of olfactory primary neurons is unknown (Julliard et al. 1996). Saucier et al. (1991b) demonstrated that histopathological changes manifested in the following order: 1) increase in goblet cells (responsible for mucus secretion); 2) epithelial lesions (necrotic tissue); 3) cellular shrinkage (due to degeneration and necrosis) at exposure to 22 ug/L copper in low hardness water and slightly acidic pH.

2.5 Olfactory Response Modifiers

As previously indicated, it is widely known that water quality conditions have a substantial effect on the bioavailability and toxicity of copper in aquatic systems (e.g., Borgmann 1983; Winner 1985; Meador 1991; Welsh et al. 1996; Allan 2002; USEPA 2007) Some of these key relationships, determined over decades of toxicological research, are reviewed in greater detail in Section 3.2. Key factors influencing the bioavailability of copper are pH, hardness, alkalinity and dissolved organic carbon (USEPA 2007). Concentrations of aquatic ligands such as those measured as dissolved organic carbon are particularly important in determining copper bioavailability (more than for most other metals) because copper forms more stable complexes with ligands than do other divalent ions of first row transition metals in what is known as the Irving-Williams Series (Mn[II] < Fe[II] < Co[II] < Cu[II] > Zn[II]; Irving and Williams 1953). The capacity of copper to strongly bind to aquatic ligands may also explain why copper elicits a response at the fish olfactory epithelium at relatively low concentrations, as copper may have a greater capacity to bind receptor molecules in the sensory epithelium than other divalent ions.

Few of the studies on the effects of copper on fish olfaction directly evaluated the influence of different water qualities on effective concentrations. Winberg et al. (1992) and Bjerselius et al. (1993) manipulated bicarbonate and hardness, respectively to evaluate their influence on EOG response. Winberg et al. (1992) exposed atlantic salmon to 640 ug/L copper at four bicarbonate concentrations that resulted in 12.7 ug/L, 140 ug/L, 438 ug/L and 616 ug/L free ionic copper (Cu^{2+}). They found a significant correlations between Cu^{2+} concentration and EOG response and concluded that the olfactory response to copper is caused mainly by dissolved Cu^{2+} ion. Similarly, Bjerselius et al. (1993) found that calcium and magnesium ions protect the olfactory receptor function from impairment by 64 ug/L Cu^{2+}. However, it is notable that several other researchers (Baldwin et al. 2003 and Green et al. 2010) found that

calcium did not convey substantial protection. Thus, there is some uncertainty over the protective role of calcium; however the apparent differences may be due to the fact that hardness in natural systems is a combination of major ions and associated bicarbonate alkalinity, not calcium alone (calcium was added as $CaCl_2$ by Baldwin et al. 2003 and as $Ca(NO_3)_2$ by Green et al. 2010). Accordingly, when hardness is considered in surface waters, two things are occurring: 1) bicarbonate is affecting copper speciation; and 2) calcium is competing and/or affecting membrane permeability (but has little effect on copper speciation; Winberg et al. 1992).

A recent study by McIntyre et al. (2008a,b,c) examined the influence of hardness, alkalinity, pH and dissolved organic carbon on olfaction in juvenile coho salmon. This study determined that calcium, bicarbonate, and dissolved organic carbon (DOC) all provided substantial protection against the effects of copper on fish olfaction as determined by EOG response (Figure 2.2). Greatest protection was provided by dissolved organic carbon, and the McIntyre et al. (2008a) study showed that there was no difference from controls at 6 mg/L DOC when exposed to 20 ug/L copper at pH 7.1, low calcium (7.8 mg/L) and low bicarbonate (11.4 mg/L). No tests were conducted under conditions of high calcium, bicarbonate and DOC to evaluate joint protection. McIntyre et al. (2008a) also showed that pH had little influence on olfactory response over the range from 7.6 to 8.6, which might be expected as significant increases in free copper are expected only at lower pH ranges (i.e., very little free copper is expected at a pH 7.5 or above; Stumm and Morgan 1996). The results of McIntyre et al. (2008a,b,c) are strongly supported by Linbo et al. (2009) who examined the effect of copper on the lateral line of larval zebrafish under different water quality conditions. Similar to the olfactory epithelium, the lateral line has sensory neurons in direct contact with the aquatic environment (Linbo et al. 2006, 2009). Linbo et al. (2009) documented a protective role of DOC, bicarbonate and a number of major cations including calcium. As observed by McIntyre et al. (2008 a,b,c), the protective role of DOC was greatest, with an increase of 437% in the copper effect concentration over the range tested (0 - 4.3 mg/L, versus 51% and 53% for calcium and bicarbonate, respectively; Figure 2.3). Interestingly, the slopes of the relationship between effect concentrations and DOC are similar in the McIntyre et al. (2008c) and Linbo et al. (2009) studies (8.9 and 9.4, respectively) and are much greater than the other substances evaluated (≤0.1; Figures 2.2 and 2.3).

A number of authors have shown that the influence of several modifying factors, especially hardness appears to be lower at the olfactory epithelium than at the gill (e.g., McIntyre et al. 2008a,b,c; Green et al. 2010). This may be due to different binding properties at the two different ligands (i.e., the olfactory epithelial surface and the gill). It may make intuitive sense

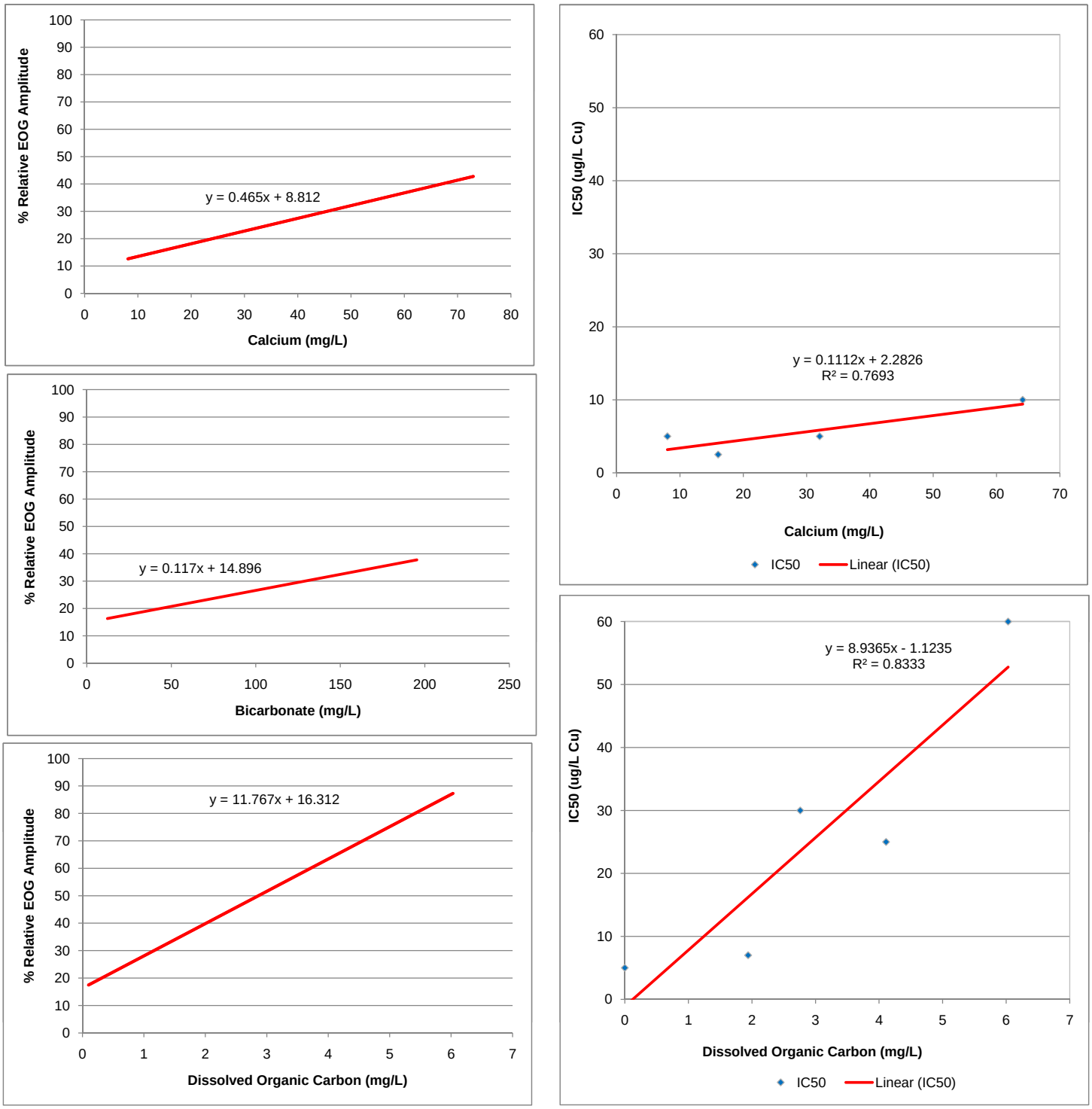
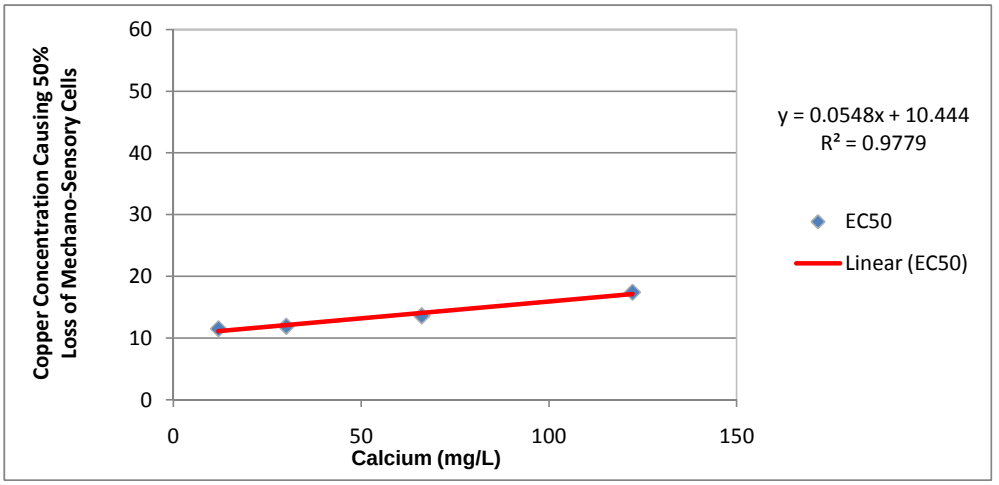
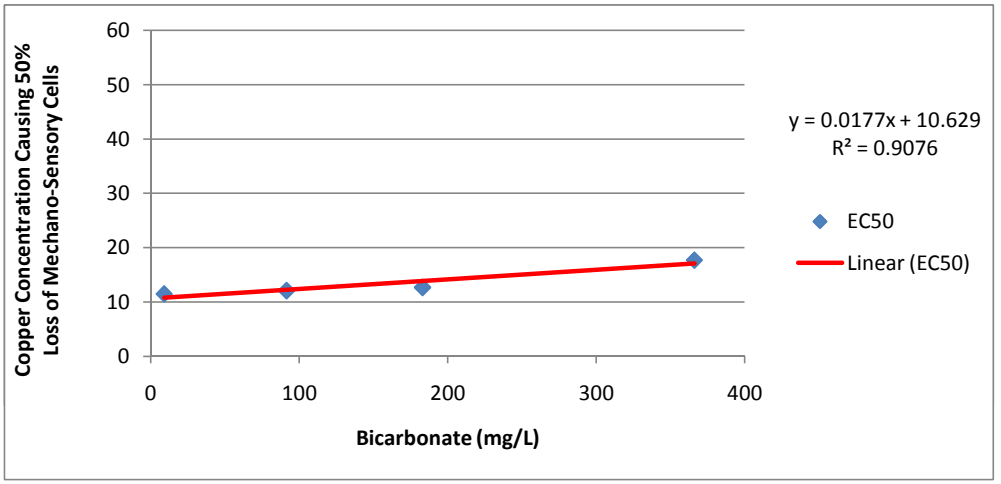


Figure 2.2: **Protective roles of calcium, bicarbonate and dissolved organic carbon on the electro-olfactogram response in juvenile coho salmon (adapted from McIntyre et al. 2008a,b,c).**





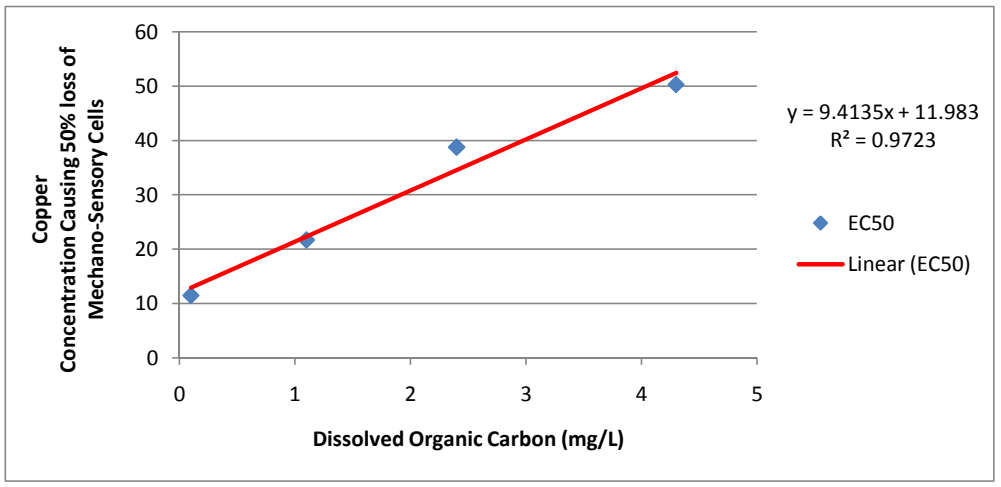


Figure 2.3: **Protective roles of calcium, bicarbonate and dissolved organic carbon on the mechano-sensory system of larval zebrafish (adapted from Linbo et al. 2009).**

that the olfactory epithelium, whose function is to perceive odorants, may have a greater binding affinity than the gill (as determined by Green et al. 2010). Accordingly, the Biotic Ligand Model does not appear to apply directly to the olfactory endpoint (McIntyre et al. 2008a; Linbo et al. 2009; Green et al. 2010), due to different strengths associated with key relationships in the two tissues. A distinct set of binding constants and endpoints for the olfactory system are likely required in order to produce a model that adequately predicts metal binding at the olfactory epithelium and protects against potential effects (Green et al. 2010).

2.6 Olfactory Recovery

Numerous studies have shown that the effects of low-level copper exposure on fish olfaction are reversible (Table 2.4; Saucier et al. 1991b; Winberg et al. 1992; Bjerselius et al. 1993; Saucier and Astic 1995; Hansen et al. 1999b; Beyers and Farmer 2001; Baldwin et al. 2003; Sandahl et al. 2006). This is likely due in part to the fact that olfactory sensory neurons are capable of division and renewal (Graziadei and Monti-Graziadei 1978; Laberge and Hara 2001; Hara 1992). However, rapid response times (on the order of several hours; e.g., Winberg et al. 1992; Baldwin et al. 2003) suggest that chemical depuration and/or physiological detoxification responses may also be involved, especially copper chelation by neo-synthetized metallothioneins (Julliard et al. 1996). At higher concentrations, including those sufficient to trigger cell death in the sensory epithelium (i.e., ≥ 25 ug/L; Hansen et al. 1999c), the regeneration of olfactory neurons may take place over days or weeks (Sandahl et al. 2007). Even long-lasting exposure to 20 or 40 ug/L Cu^{2+} did not induce irreversible damage in the olfactory epithelium of rainbow trout (Saucier and Astic 1995). Saucier and Astic (1995) show that fish exposed for as long as 40 weeks are still able to discriminate olfactory cues because neurogenesis still occurred, albeit less so at 40 ug/L copper than at 20 ug/L copper. Overall, the literature strongly indicates that salmonids can recover from exposure to low to moderate concentrations of copper (≤40 ug/L). However, this does not eliminate the potential consequences of temporarily impaired olfaction.

2.7 Ecological Relevance and Uncertainties

Although the influence of copper on olfaction and behaviour has been clearly demonstrated (under test-specific water quality characteristics) and olfaction-mediated behaviours are important, there are substantial uncertainties regarding the relevance of these responses to the ecological fitness of individuals and populations (Pyle and Mirza 2007; McIntyre et al. 2008a, Tierney et al. 2010). In many cases cues for feeding or predator avoidance are predominantly visual (Tierney et al. 2010). Furthermore, a single competing gradient (shade)

Table 2.4: Recovery of fish olfactory responses following exposure to copper.

Source	Species tested (lifestage[a])	Concentration (ug/L dCu)[b]	Observation	Exposure Duration[c]	Temperature (°C)	pH	Dissolved Organic Carbon (mg/L)	Hardness (mg/L)
Baldwin et al. 2003	Coho salmon (juvenile)	5	partial recovery of olfactory activity (EOG amplitude)	30 or 60 min in Cu; 10 min in Cu-free water	12	7.55	nr	20
Baldwin et al. 2003	Coho salmon (juvenile)	10	partial recovery of olfactory activity (EOG amplitude)	30 min in Cu; 30 min in Cu-free water	12	7.55	nr	20
Baldwin et al. 2003	Coho salmon (juvenile)	20	partial recovery of olfactory activity (EOG amplitude)	30 min in Cu; 30 min in Cu-free water	12	7.55	nr	20
Bettini et al. 2006	Tilapia mariae (adult)	20	differentiation (or maturation) of new olfactory receptor	4 days in Cu-water; 3 days in Cu-free water	19.1	7.1	nr	364
Beyers and Farmer 2001	Colorado pikeminnow (185 days old)	60	regeneration of olfactory receptor cells and recovery of anti-predator response	96 hour exposure; 14 days in Cu-free water	20	8.3	nr	117
Bjerselius et al. 1993	Atlantic Salmon (juvenile)	636	partially restored olfactory response (EOG profile and amplitude)	5 minute exposure; 29 minutes in Cu-free water	nr	6.7	nr	400[g]
Hansen et al. 1999b	Chinook salmon (juvenile)	50	partial recovery of olfactory bulb activity (EEG measures)	1 hour Cu water; < 1 hour in Cu-free water	12	7.67	nr	24.5
Hansen et al. 1999b	Rainbow trout (juvenile)	300	partial recovery of olfactory bulb activity (EEG measures)	1 hour Cu water; < 1 hour in Cu-free water	12	7.67	nr	24.5
Sandahl et al. 2006	Chum Salmon (fry)	58	complete recovery of olfactory response (EOG amplitude recovery)	4 hours in Cu water; 1 day in Cu-free water	9.7	6.1	nr	40
Saucier and Astic 1995	Rainbow trout (juvenile)	20	complete recovery of behavioural discrimination of different types of water	40 weeks in Cu water; 2 weeks in Cu-free water	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	20	complete recovery of olfactory epithelium	40 weeks in Cu water; 6 weeks in Cu-free water	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	40	complete recovery of olfactory epithelium	40 weeks in Cu water; 14 weeks in Cu-free water	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier and Astic 1995	Rainbow trout (juvenile)	40	complete recovery of behavioural discrimination of different types of water	40 weeks in Cu water; 29 weeks in Cu-free water	5.5 - 9.5	6.6 - 7.6	nr	60.9
Saucier et al. 1991a	Rainbow trout (juvenile)	22	recovery of behavioural discrimination of different types of water	~39 weeks in Cu water (starting from hatch); 2 weeks in Cu-free water	7.6 - 10.1	6.50 - 6.64	nr	62 - 64
Saucier et al. 1991a	Rainbow trout (juvenile)	22	recovery of behavioural discrimination of different types of water	~39 weeks in Cu water (starting 14 days post fertilization); 10 weeks in Cu-free water	7.6 - 10.1	6.50 - 6.64	nr	62 - 64
Saucier et al. 1991b	Rainbow trout (juvenile)	22	partial morphological restoration	37 - 41 weeks in Cu; 2 weeks in Cu-free water	7.6 - 10.1	6.50 - 6.64	nr	61.8 - 64.0
Saucier et al. 1991b	Rainbow trout (juvenile)	22	increased morphological restoration	37 - 41 weeks in Cu; 10 weeks in Cu-free water	7.6 - 10.1	6.50 - 6.64	nr	61.8 - 64.0
Winberg et al. 1992	Atlantic Salmon (juvenile)	636 (12.7 Cu^{2+})	near complete recovery of olfactory EOG profile	5 min. Cu-water; 30 min. Cu-free water	6 - 8	6.2	nr	nr
Winberg et al. 1992	Atlantic Salmon (juvenile)	636 (140 - 616 ug/L Cu^{2+})	partial recovery of olfactory EOG profile and amplitude	5 min. Cu-water; 30 min. Cu-free water	6 - 8	6.2	nr	nr

nr - not reported

[a] at time response was recorded

[b] if not explicitly stated in the paper, copper assumed to be dissolved if non-natural water was used for the study (e.g., dechlorinated city water, well water, de-ionized water etc)

[c] earliest time at which recovery was reported. Total recovery duration for the study may have been longer.

has been shown to dramatically affect preference-avoidance thresholds (Scherer and McNicol 1998) and thus there is uncertainty regarding the extrapolation of olfactory study results to the real world of multiple competing influences. Lastly, few comparisons among laboratory-derived results and field-derived results have been made (Pyle and Mirza 2007). The few that have been done suggest substantially higher thresholds in the field. For example, Sprague et al. (1965) reported that adult spawning atlantic salmon avoided zinc in field conditions at about 4X the threshold found in the laboratory and Mebane et al. (1994) found that the spawning migration of Chinook salmon may have been interrupted when the fish encountered dissolved copper concentrations of about 3X-6X higher than laboratory avoidance thresholds. Fish that have spent multi-generations in contaminated environments do not show the same responses as laboratory- and aquaculture-reared fishes suggesting that caution should be taken when extrapolating results from the laboratory to the field (Mirza et al. 2009). Overall, numerous authors have indicated that a key priority for future research is to determine how a copper-induced loss of olfactory capacity (anosmia) affects individual survival and lifetime reproductive success in a field environment (McIntyre et al. 2008a; Tierney et al. 2010).

3.0 REVIEW OF "CONVENTIONAL" COPPER EFFECTS

3.1 Comparison of Olfactory Response to "Conventional" Endpoints

As apparent from Section 2.0, copper can impair fish olfaction (as assessed in neuro-physiological and behavioral studies) at low concentrations in certain water types. A key question is whether the olfactory endpoint is more sensitive than conventional toxicity endpoints that form the basis for water quality guidelines for the protection of aquatic life. Canadian water quality guidelines for the protection of aquatic life are intended to protect all life stages over indefinite exposure periods, and consider traditional endpoints (i.e., growth, reproduction and survival) as well as non-traditional endpoints (e.g., behavioural [predator avoidance, swimming ability, swimming speed, etc.] and physiological changes). However, non-traditional endpoints are only considered if their ecological relevance can be directly linked to impairment of survival, reproductive ability, or growth of individuals or populations (CCME 2007). This review of the olfactory effects of copper indicates that these links have not yet been established.

Uncertainties about the ecological relevance of reductions in olfactory response aside, if olfactory study effect concentrations are higher than those of conventional endpoints used to derive water quality guidelines, the guidelines can be considered sufficiently protective. A recent compilation of critically reviewed and quality-controlled aquatic toxicity data for copper (USEPA 2007) documents that cladocerans (water fleas) are the most sensitive organisms to both acute and chronic effects of copper (Appendix B). The lowest Species Mean Acute Value (SMAV) is for the cladoceran *Daphnia pulex* (2.7 ug/L) and the lowest SMAV for a salmonid is for rainbow trout (22.2 ug/L). These effect data are normalized to a pH of 7.5, hardness of 85 mg/L and dissolved organic carbon content of 0.5 mg/L. Chronic toxicity data indicate that cladocerans and chinook salmon can be adversely affected in 5-6 ug/L range (Appendix B). Accordingly, lowest conventional effects of copper are apparent in acute toxicity to cladocerans (i.e., at 2.7 ug/L copper). By comparison, neuro-physiological and behavioural responses to copper have been observed at concentrations as low as 2 ug/L under somewhat different water quality (i.e., a significant reduction in the amplitude of electrical signals generated by the olfactory epithelium in juvenile coho salmon and significantly reduced predator avoidance behavior; Sandahl et al. 2007). Normalization of the acute toxicity to cladocerans to the conditions reported by Sandahl et al. (2007; i.e., temperature 10.8 °C, pH 6.7 and hardness 120 mg/L) indicates a comparable acute toxicity to *D. pulex* of 1 ug/L (i.e., that copper is 2.6x more toxic to *D. pulex* under the conditions tested by Sandahl than under the USEPA [2007] normal; HydroQual 2007). Therefore, this

comparison suggests that the lowest observations of neuro-physiological and/or behavioural responses, regardless of questions of their overall ecological relevance, appear to occur at concentrations higher than those associated with acute toxicity to cladocerans when compared under comparable water chemistries. Because water quality guidelines for the protection of aquatic life in both Canada and the United States (the CCME guideline is based on USEPA 1985) are based on protecting sensitive cladocerans, available evidence indicates that the guidelines are also protective of potential olfactory effects.

3.2 Copper Effect Modifiers

As previously indicated, the bioavailability and toxicity of copper in aquatic environments are highly dependent upon water quality conditions. Accordingly, observations of responses or effects must be considered to specifically represent the associated water quality, and any attempt to extrapolate results to different water quality must carefully consider the influence of important modifying factors. This section provides a brief review of the key factors known to modify the bioavailability and toxicity of copper.

Numerous studies have shown that the bioavailability and toxicity of copper is highly dependent upon water quality characteristics such as pH, major ion concentrations (hardness), carbonates (alkalinity), dissolved organic carbon and incorporation into particulates (Di Toro et al, 2001; Paquin et al. 2002; Niyogi and Wood 2004; USEPA, 2007). It is widely recognized that these factors need to be taken into account in understanding bioavailability and the potential for adverse effects and in the development of effect-based guidelines for the protection of aquatic life. For example, the consideration of hardness in the Canadian Water Quality Guideline for copper (CCME 1999) and the application of the biotic ligand model (BLM) in the United States Water Quality Criteria for copper (USEPA 2007) reflects a recognition of copper toxicity mitigation by these water quality characteristics.

The free (hydrated) copper ion ($Cu(H_2O_6)_2^{2+}$ usually referred to as Cu^{2+}) is highly bioavailable and forms the basis for toxicity prediction (Morel 1983). Fundamental factors that reduce the fraction of total copper present as Cu^{2+} (e.g., complexation) or reduce the binding of Cu^{2+} to a biotic ligand (e.g., competition for binding at biotic ligands such as the gill membrane or olfactory epithelial membrane) reduce bioavailability and toxicity (Pagenkopf 1983; Paquin et al. 2002). Briefly, as pH increases there is greater formation of copper hydroxide and copper carbonate (compared to the free ion; Welsh et al. 1993). As previously indicated, copper has a particularly strong affinity for ligands among the divalent ions of first row transition metals (Irving and Williams 1953). Inorganic copper species are predominantly free (hydrated)

copper, copper hydroxide and copper carbonate depending on hardness and pH. At pH 6, inorganic copper is 95% hydrated copper (the free ion), whereas at pH 9 inorganic copper is 96% copper carbonate (Turner et al. 1981). Generally, the free (hydrated) copper ion is considered the most bioavailable, although copper hydroxide has also been considered bioavailable (Erickson et al. 1996). Increasing alkalinity has the effect of mitigating copper toxicity by forming less bioavailable copper carbonates, but the magnitude of this effect varies with hardness (Erickson et al. 1996), and in general, alkalinity is not independent of pH. Similarly, the influence of dissolved organic carbon is pH-dependent; as pH increases copper complexation by DOC increases due to deprotonation of surface groups and a consequent increase in the number of adsorption sites (Welsh et al. 1993).

Copper will tend toward inorganic complexation, but has a stronger tendency to form organic complexes and therefore copper is predominantly complexed by organic substances in natural waters. For example, in the presence of 2-5 mg/L DOC the free copper ion (Cu^{2+}) concentration is decreased by 5 to 7 orders of magnitude (Stumm and Morgan 1996). Dissolved organic carbon has been shown to mitigate copper toxicity by binding it and decreasing the fraction of the free metal ion (Zitko et al. 1973). Copper bound in suspended particulates is typically biologically unavailable (USEPA 2007) and the use of dissolved metal concentration to approximate bioavailability has been shown to be more accurate compared to the use of total (dissolved and particulate) metal concentrations. In short, incorporation of copper into particulate decreases the bioavailable and dissolved copper concentrations.

Hardness has often been observed to decrease copper toxicity by competition, likely at the site of toxicity (the biotic ligand; e.g., Pagenkopf 1983; Winner 1985; USEPA 1985; Santore et al. 2001; Paquin et al. 2002). At the gill, magnesium has a weaker binding strength than calcium, making calcium a more important parameter in toxicity prediction (Santore et al. 2001; Paquin et al. 2002). However, studies investigating the effect of hardness or its component cations (Ca^{2+} and Mg^{2+}) on copper toxicity have produced equivocal results, in part because factors such as pH and alkalinity often co-vary with hardness (Gensemer et al. 2002, USEPA 2007).

In summary, if site water has higher pH, contains higher concentrations of any number of ligands that bind copper (e.g., dissolved organic carbon), or of other elements that might compete with copper for uptake (e.g., hardness which represents the concentrations of calcium and magnesium), copper is expected to be less bioavailable and therefore less toxic than waters with lower pH and concentrations of these substances (e.g., Borgmann 1983; Winner 1985; Meador 1991; Welsh et al. 1996). In general, DOC and pH appear to have the greatest influence on copper bioavailability and toxicity (e.g., Hamilton and Buhl 1990;

Meador 1991; Welsh et al. 1996; Erickson et al. 1996; Di Toro et al. 2001). In waters with moderately basic pH and high concentrations of DOC, generic guidelines (Canadian water quality guidelines omit the consideration of the mitigating effect of DOC) tend to be over-protective (Stephan et al. 1985) because the data on which they are based tend to be from laboratory testing conducted in waters with low DOC concentrations.

4.0 EVALUATION OF SITE-SPECIFIC RELEVANCE

Literature findings and site conditions were evaluated together to determine whether olfactory effects on out-migrating salmon could occur in Minto Creek. Previous sections of this report have shown that that copper has been observed to influence olfaction in fish at low concentrations under certain water quality conditions (Section 2.0), and that water quality conditions have a substantial effect on the bioavailability of copper and its potential influence on fish olfaction and conventional toxicity (Sections 2.0 and 3.0). The concentrations of water quality characteristics known to have a substantial role in modifying copper bioavailablility and toxicity are reviewed for Minto Creek and used to evaluate site-specific relevance of the literature findings of olfactory effects.

4.1 Concentrations of Copper and Substances that Modify Copper Toxicity

The water quality of Minto Creek has been monitored at a number of locations since 1993. In 2005, water quality monitoring was significantly expanded to include eight stations in Minto Creek as well as consistent sampling frequencies, a comprehensive list of analytes and improved method detection limits. Water quality of Minto Creek has been discussed in detail in previous reports (e.g., Minnow 2009; Minto/Access 2009). The concentrations of copper and key substances that modify copper bioavailability and toxicity are summarized from a previous assessment of water quality (Minnow 2009) and monitoring conducted in lower Minto Creek from 2005 to 2009 (Table 4.1; Appendix C).

Concentrations of copper in Minto Creek have often been above the Canadian Water Quality Guideline for the protection of aquatic life (3 ug/L at average hardness), even under background conditions (Table 4.1a; Minnow 2009). During periods of emergency effluent discharge in 2008 and 2009, the mean copper concentration in lower Minto Creek was much higher than background (24 ug/L; Table 4.1c). In general, the concentrations of key factors that modify copper bioavailability/toxicity are high in lower Minto Creek relative to conditions in most studies of olfactory response in fish (Table 4.1 versus Tables 2.1 to 2.3). Waters of lower Minto Creek can be characterized by moderately basic pH (approximately 8 pH units), moderate hardness (mean 112 to 166 mg/L, depending upon the period considered), moderate to high alkalinity (mean 106 to 136 mg/L, depending upon the period considered) and high dissolved organic carbon (mean 12.5 to 15.1 mg/L, depending upon the period considered; Table 4.1a-c). The latter (dissolved organic carbon), in particular, is much higher than in tests of the effects of copper on fish olfaction. Specifically, most studies of fish olfaction were conducted in well water or tap water which typically has very low dissolved organic carbon concentrations (<0.5 mg/L) and studies that did report dissolved organic

Table 4.1: Concentrations of copper and key parameters that modify copper bioavailability and toxicity.

A: Summary of Background Water Quality of Minto Creek [1] (Minnow 2009)

Parameter	Units	Count	Mean	Median	10th Percentile	25th Percentile	75th Percentile
Copper	mg/L	65	0.004	0.003	0.001	0.001	0.006
Hardness	mg/L	67	112	115	69	95	131
pH (lab)	pH units	77	7.82	7.87	7.37	7.74	8.01
Alkalinity - total ($CaCO_3$)	mg/L	77	106	115	58	89	128
Dissolved Organic Carbon	mg/L	4	15.1	15.9	12.0	13.5	17.5
Calcium	mg/L	78	28.3	29.6	18.0	24.6	32.8
Magnesium	mg/L	78	9.5	9.7	5.6	7.2	11.3

[1] based on data points with less than 50 mg/L total suspended solids only

B: Summary of Water Quality of Lower Minto Creek Station W2, 2005-2009 - No Active Discharge [2]

Parameter	Units	Count	Mean	Median	10th Percentile	25th Percentile	75th Percentile
Copper	mg/L	93	0.006	0.003	0.002	0.002	0.008
Hardness	mg/L	39	136	151	66	129	161
pH (lab)	pH units	98	8.00	8.03	7.65	7.88	8.18
Alkalinity - total ($CaCO_3$)	mg/L	79	136	135	71	90	155
Dissolved Organic Carbon	mg/L	40	12.5	10.4	7.8	8.4	14.4
Calcium	mg/L	93	35.6	36.8	17.4	23.6	41.7
Magnesium	mg/L	93	12.9	11.6	5.8	8.0	13.7

[2] data collected from 2005-2009 except during emergency overflow/discharge from the Minto Mine - August 26th to September 30th 2008, June 26th to August 6th 2009 and August 13th to October 13th 2009

C: Summary of Water Quality of Lower Minto Creek Station W2, Active Discharge in 2008 and 2009 [3]

Parameter	Units	Count	Mean	Median	10th Percentile	25th Percentile	75th Percentile
Copper	mg/L	25	0.024	0.021	0.004	0.011	0.035
Hardness	mg/L	21	166	167	155	159	175
pH (lab)	pH units	94	8.11	8.10	8.00	8.10	8.20
Alkalinity - total ($CaCO_3$)	mg/L	24	126	120	108	112	140
Dissolved Organic Carbon	mg/L	6	14.5	12.4	9.9	10.6	18.0
Calcium	mg/L	25	46.7	46.9	39.1	41.5	49.4
Magnesium	mg/L	25	13.4	13.4	11.3	12.3	14.3

[3] data collected during emergency overflow/discharge from the Minto Mine - August 26th to September 30th 2008, June 26th to August 6th 2009 and August 13th to October 13th 2009

carbon had a maximum concentration of 6 mg/L. Dissolved organic carbon strongly limits free copper ion (the bioavailable form of copper) by complexation (e.g., in the presence of 2-5 mg/L dissolved organic carbon, Cu^{2+} decreased from 5 to 7 orders of magnitude; Stumm and Morgan 1996). Therefore, only a very small proportion of total copper in Minto Creek water is expected to be present as free copper ion (Cu^{2+}), substantially limiting copper bioavailability. Reduced bioavailability was confirmed in previous water-effect ratio testing (a 5.8-fold reduction in toxicity relative to laboratory waters of similar hardness).

4.2 Biological Resources of Minto Creek

As with water quality, the biological resources of Minto Creek have been well characterized (e.g., Minnow/Access 2007, 2009). Minto Creek supports a benthic community with modest density and a moderate number of taxa (approximately 6,750 organisms per square meter and 19 taxa, on average; Minnow/Access 2009). Biological productivity is constrained by several factors, most notably a predominantly sand substrate, complete glaciation in winter months, and a barrier to fish passage located approximately 1 kilometer upstream of the Yukon River (Minnow/Access 2009).

Previous fisheries assessments have documented four fish species in lower Minto Creek – Chinook salmon, arctic grayling, round whitefish and slimy sculpin. Abundances of all but Chinook salmon have been extremely low and these species have not been consistently captured. Accordingly, Chinook salmon appear to be the only species that consistently use lower Minto Creek. No spawning has been observed in Minto Creek. This is consistent with the fact that over-winter survival of eggs/alevin/fry would be unlikely due to complete winter glaciation (due to the formation of aufeis; a thick layer of ice that develops due to the freezing of groundwater). Fisheries studies undertaken to date have documented that only juvenile Chinook salmon occupy lower Minto Creek, typically in June to September. Juvenile chinook salmon of the Yukon River usually have a freshwater residence time of 1 year; 1+ juveniles are common but less abundant (Bradford et al. 2001, 2008; Duncan and Bradford 2004). Out-migrating juvenile Chinook salmon typically emerge in natal streams in spring and early summer (mid-May). They enter non-natal tributaries (such as Minto Creek) during out-migration when the temperature of non-natal streams reaches an approximate equilibration with the Yukon River, usually in late June (Bradford et al. 2001; Duncan and Bradford 2004). In one non-natal tributary to the Yukon River (Croucher Creek), juvenile Chinook salmon were observed to start entering in June, achieved a maximum density in mid-summer and continued growing to the end of August (Bradford et al. 2001). Although there are limited data on the time of occupancy of out-migrating Chinook salmon in any given tributary to the Yukon River, Bradford et al. (2001) found that juvenile Chinook salmon in Croucher Creek

were able to over-winter and that their distribution was determined by the distribution of aufeis. In Minto Creek, as in many creeks tributary to the Yukon River, there appear to be no over-wintering refugia and juvenile salmon are believed to leave these tributaries to return to larger rivers in the fall (e.g., Walker 1976). Under such circumstances, average occupancy time for any one creek may be low (e.g., Scrivener et al. 1994 found that out-migrating Chinook salmon of the Fraser River spent an average of only nine days in a studied tributary).

In summary, it is apparent that biological productivity of Minto Creek is constrained by a number of factors and that the key biological resource is Chinook salmon. Chinook salmon use lower Minto Creek only as juveniles as they out-migrate from natal areas upstream of Minto Creek. They likely occupy lower Minto Creek only when it is bio-energetically favourable to do so (when temperatures come up) and likely stay in lower Minto Creek for relatively short periods.

4.3 Integration of Literature Findings and Site Conditions

Quantitative relationships between water quality characteristics (modifier concentrations) and copper-induced effects on olfactory responses have been developed in one paper (McIntyre et al. 2008a,b,c), the results of which are largely supported by the research of Linbo et al. (2009) on the fish mechano-sensory system. The relationships determined in these papers (Figures 2.2 and 2.3) were used as the basis for extrapolation of potential effects on fish olfaction to Minto Creek (Table 4.2; Appendix D). This extrapolation indicates that, even at low Minto Creek dissolved organic carbon (DOC) concentrations (10th percentile), the lowest predicted IC50 for fish olfaction is 69 ug/L. This is well above concentrations previously defined as protective of lower Minto Creek (Station W2; 13 ug/L to 24 ug/L depending upon site hardness; Minnow 2009) and suggest that the previously-defined protective concentrations are also protective of potential olfactory effects. Calcium also plays a protective role, and although concentrations in Minto Creek are not sufficient to fully mitigate olfactory inhibition in salmonids at copper concentrations in the 13 to 24 ug/L range, it is reasonable to assume that the presence of calcium would add to protection provided by DOC. This is likely also true to some extent of other major cations and carbonate. Accordingly, there is a margin of safety associated with considering only the protective role of DOC, which this extrapolation suggests is protective of the potential effects of copper on fish olfaction in Minto Creek. It is worthwhile to also consider that McIntyre et al. (2008a) found 6 mg/L DOC to be fully protective of the effect of 20 ug/L copper on the olfactory response (EOG) in juvenile coho salmon.

Table 4.2: Predicted olfactory and mechano-sensory effects of copper based on site water chemistry.

Water Quality Period and Statistic		Site Water Quality (mg/L)		Copper Effect Concentrations Based on Site DOC Concentrations (ug/L copper)		Copper Effect Concentrations Based on Site Calcium Concentrations (ug/L copper)	
		Dissolved Organic Carbon	Calcium	McIntyre [2]	Linbo [3]	McIntyre [2]	Linbo [3]
Background (Minnow 2009)	10th Percentile	12.0	18.0	106	125	4.3	11.4
	25th Percentile	13.5	24.6	120	139	5.0	11.8
	Median (50th Percentile)	15.9	29.6	141	162	5.6	12.1
	Mean	15.1	28.3	134	154	5.4	12.0
Station W2 (2005-2009) - No Discharge	10th Percentile	7.8	17.4	69	85	4.2	11.4
	25th Percentile	8.4	23.6	74	91	4.9	11.7
	Median (50th Percentile)	10.4	36.8	92	110	6.4	12.5
	Mean	12.5	35.6	111	130	6.2	12.4
Station W2 (2005-2009) - Discharge	10th Percentile	9.9	39.1	88	105	6.6	12.6
	25th Percentile	10.6	41.5	94	112	6.9	12.7
	Median (50th Percentile)	12.4	46.9	110	129	7.5	13.0
	Mean	14.5	46.7	129	148	7.5	13.0

[1] median concentration causing a 50% loss in chemosensory capacity based on McIntyre et al.(2008) and Figure 2.2

[2] median concentration causing cell death in 50% of lateral line mechano-sensory cells based on McIntyre et al. (2009) and Figure 2.3

The relationships of McIntyre et al. (2008a,b,c) are highly supported by research into the effect of copper on the mechano-sensory system (Linbo et al. 2009). In addition, the slope of the McIntyre et al. (2008a,b,c) and Linbo et al. (2009) are remarkably similar (8.9 and 9.4, respectively), indicating that they are measuring fundamental copper complexation by dissolved organic carbon, which has been well characterized in the scientific literature (e.g., Irving and Williams 1953; Mantoura et al. 1978; Sposito 1987; Hering and Morel 1990; DiToro et al. 2001; Allan 2002; USEPA 2007). Even if one assumes a more sensitive olfactory endpoint than that measured by McIntyre et al. (2008a,b,c), the slope for dissolved organic carbon developed by McIntyre (an increase in effect concentration of 9 ug/L copper for every 1 mg/L increase in dissolved organic carbon) strongly indicates that effects would be expected only at copper concentrations much greater than 24 ug/L (Appendix Figure D.3). Overall, it is apparent that the effects to fish olfaction documented in the literature are not directly transferrable to Minto Creek as they would grossly over-estimate the potential for effects (i.e., effect concentrations would be expected to be higher in Minto Creek as has been previously observed with other endpoints; Minnow 2009) and that the proposed site-specific water quality objective for copper is protective of potential effects on fish olfaction under site chemistry.

Although there is evidence that the water chemistry of Minto Creek (and in fact concentrations of dissolved organic carbon alone) is protective of potential effects of copper on fish olfaction, it is worth considering that fish can avoid high concentrations of bioavailable copper and recover following exposure. The fact that Chinook salmon appeared to be attracted to Minto Creek during effluent discharge in 2009 despite elevated copper concentrations suggests that the copper may not have been in present in a detectable form.

5.0 CONCLUSIONS

Review of the literature on the effects of copper on fish olfaction clearly indicates that salmonid olfaction can be adversely affected by copper at relatively low concentrations (<5 ug/L) under certain water quality conditions. The influences of copper on fish olfaction have been observed based on electro-physiological measurements (of electrical signals from the olfactory rosettes), in laboratory-based behavioural studies, in field observations of fish behavior, and in histo-pathological examination of tissue of the olfactory epithelium of exposed fish. Olfaction underlies a variety of important life processes, including imprinting, kin recognition, feeding, predator avoidance, mating synchronization, homing and spawning. Accordingly, potential effects to fish olfaction should be considered when defining concentrations of copper in an aquatic environment. However, as has been pointed out by many authors of the primary research and became apparent in this review, there are important uncertainties as to the ecological implications of the findings of olfactory impairment by copper in fish. These include a lack of consideration of factors that modify copper bioavailability in the majority of published papers, uncertainty over the extrapolation of controlled laboratory studies to complex environments, uncertainty as to the role of avoidance and recovery, and general uncertainty as to how the measured responses translate to effects on survival, growth or reproduction. Therefore, it is currently unknown how detrimental olfactory inhibition may be even if it does occur.

The majority of studies on the effects of copper on fish olfaction were conducted in a laboratory setting using well water or de-chlorinated tap water that has different chemistry than many natural waters. Scientific understanding of the factors that influence the bioavailability and toxicity of copper is well developed, yet has received relatively little consideration in studies of olfactory effects. The few papers that explicitly consider the role of water quality characteristics on modifying the influence of copper on fish olfaction support the fact that the same substances well-known to modify copper bioavailability and toxicity (e.g., pH, hardness, alkalinity, dissolved organic carbon) are also important in mediating the effects of copper on fish olfaction, even if the relationships are not quantitatively the same (i.e., it appears that some of these factors offer somewhat lower protection to the fish nose than the fish gill). Copper has long been known to have a high affinity for ligands. This very property likely means that it binds effectively with olfactory ligands when present in free form, but also means that in waters with moderate to high ligand concentration (e.g., waters with high concentration of dissolved organic carbon) it is simply not present in free form at anything but extremely low concentrations as it strongly complexes with ligands.

The olfactory effects of copper on salmonids appear to occur at concentrations higher than acute effects to cladocerans (water fleas, which have been identified as the most sensitive species based on effects to survival, growth or reproduction). Thus, regardless of uncertainty as to the ecological relevance of olfactory responses, the olfactory endpoint does not appear to be more sensitive than conventional endpoints used to develop water quality guidelines for copper and therefore the available data suggest that conventional guidelines are also protective of the olfactory endpoint. Therefore, both water quality guidelines and site-specific objectives derived from them appear to be appropriate in protecting against adverse effects including those mediated by fish olfaction.

Application of the scientific literature-based quantitative relationships between copper effects to fish olfaction and water quality characteristics (modifying factors) to Minto Creek indicate that the concentrations of dissolved organic carbon alone in Minto Creek are sufficient to protect fish olfaction from the potential effects of copper at concentrations higher than the site-specific water quality objective of 13 to 24 ug/L copper. This does not account for the added protection likely afforded by other water quality characteristics such as alkalinity and the concentrations of major ions. Therefore, careful consideration of the literature on the olfactory effects of copper on salmonids, the factors that modify those effects, and site-specific water quality indicate that the effects to fish olfaction documented in the literature would grossly over-estimate the potential for effects in Minto Creek and that the proposed site-specific water quality objective for copper is protective of potential effects on fish olfaction under site chemistry.

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APPENDIX A

LITERATURE REVIEW OF THE CHEMO-SENSORY EFFECTS OF COPPER

APPENDIX A – REVIEW OF THE RECENT LITERATURE ON THE CHEMO-SENSORY EFFECTS OF COPPER

Articles appear in alphabetical order by first author then chronological by author.

Baldwin, D.H., J.E. Sandahl, J.S. Labenia and N.L. Scholz. 2003. Sublethal Effects of Copper on Coho Salmon: Impacts on the Nonoverlapping Receptor Pathways in the Peripheral Olfactory Nervous System. Environmental Toxicology and Chemistry 22(10): 2266-2274.

- Juvenile coho salmon
- Measurement of response via electo-olfactograms (EOG) using anaesthetized and restrained fish
- Response to 3 different odorants (l-serine, taurocholic acid [TCA], and a mixture of amino acids, l-argenine, l-aspartic acid, l-leucine, and l-serine)
- Test waters had a background concentration of 3 ug/L Cu and a hardness of 20 mg/L and some additional tests were conducted at a hardness of 120 mg/L
- Fish were subjected to eight odor pulses before the copper exposure (10 second pulses at 2 minute intervals), then exposed continuously to 10 ug/L copper for 30 to 60 minutes, and tested again post-copper to the same eight odor pulses. After this, recovery continued to be tracked with pulses of l-serine.
- A nominal concentration of 10 ug/L copper reduced the responsiveness to all three odors within 30 minutes of copper exposure by 57% to l-serine, 67% to TCA, and 35% to the amino acid mixture.
- Reductions in EOG amplitude were evident within 10 minutes of exposure and 30 minute exposures were sufficient to produce a maximum reduction in EOG amplitude
- Recovery was apparent within 30 minutes in clean water, but was still not complete within 2 hours.
- Additional tests were conducted at water hardness of 120 mg/L and 240 mg/L. The increased hardness (as $CaCl_2$) did not significantly alter the inhibition by copper.

- A series of copper concentrations (1, 2, 5, 10 and 20 ug/L) were also tested to determine a threshold dose using l-serine pulses delivered every 5 minutes during 30 minute exposures.
- Thresholds for a 25% reduction in EOG amplitude (mean ± 1 standard error) were calculated to be 2.7 ± 0.4 ug/L for l-serine, 2.3 ± 0.6 ug/L for TCA and 3.0 ± 0.7 ug/L for the amino acid mixture (nominal above background). These values equate to 5.3 to 6.0 ug/L actual when the background concentration of 3.0 ug/L copper is factored in.
- Actual data points underlying the relationships suggest a reduction in EOG amplitude of roughtly 40% to 70% at 10 ug/L copper and 60% to 80% at 20 ug/L copper (nominal above background).
- Copper has similar inhibitory effects on olfactory receptor neurons (ORNs) that respond to different classes of olfactory stimuli
- "we cannot infer specific behavioural impacts from our neurophysiological results …"
- "it should not be assumed that the neurotoxicity of copper is proportionally less in harder surface water"

Bettini, S., F. Ciani and V. Franceshini. 2006. Recovery of the Olfactory Receptor Neurons in the African *Tilapia mariae* following Exposure to Low Copper Level. Aquatic Toxicology 76: 321-328.

- Cichlid *Tilapia mariae*
- Assessed morphological changes in the olfactory mucosa
- Exposure to 20, 40 and 100 ug/L copper for 4 days
- Track recovery from 20 ug/L copper for 10 days
- Test waters had a hardness of 364 mg/L, pH was 7.1, dissolved organic carbon not reported
- The magnitude of cellular degeneration was dose-dependent, and at 20 ug/L copper, effects were more specific, mainly degeneration of the primary olfactory receptor neurons (loss of inter-cellular connections)
- Tracking recovery showed clear recovery over time, with a massive proliferative response resulting in the development and re-establishment of the olfactory tissue

- New olfactory receptor neurons require about 3 days to make contact with the external environment
- After ten days, the olfactory tissue did not present differences when compared to control tissue (the new olfactory receptor neurons have reached maturation)
- "programmed cell death (apoptosis) is considered to be one mechanism by which the normal turnover of olfactory receptor cells takes place" … this research indicates that necrosis of olfactory receptor neurons occurred

Beyers, D.W. and M.S. Farmer. 2001. Effects of Copper on Olfaction of Colorado Pikeminnow. Environmental Toxicology and Chemistry 20(4): 907-912.

- Colorado pikeminnow
- Assessed olfactory ability using a behaviour assay and then morphological changes in olfactory structures
- Behaviour assay was "fright reaction" – reaction to fright pheromone in skin homogenate
- Exposure to <10, 16.6, 33.3, 66.5, 133 and 266 ug/L copper for 24 hours and to <10, 15, 30, 60 and 120 ug/L copper for 96 hours
- Test waters had a hardness of 117 mg/L, pH was 8.3, dissolved organic carbon not reported
- Olfactory ability declined with copper concentration in both 24 and 96 hour exposures
- For copper concentrations less than 66 ug/L, olfaction was more sensitive to exposure at 24 hours than at 96 hours suggesting physiological adaptation and recovery
- Fish exposed to 60 ug/L copper for 96 hours regained olfactory ability
- EC50s for the 24 hour and 96 hour exposures were 43.3 (95% CL 28.5 and 69.0) and 56.0 (39.3 and 86.6) ug/L total copper
- Scanning electron microscopy (SEM) always detected receptor cells in the control but did not did not detect receptor cells immediately after exposure in the copper treatments
- SEM of fish exposed to 60 ug/L copper for 96 hours and then allowed a 14 day recovery confirmed regenerated olfactory receptor cells
- Authors note several potential protective mechanisms: 1) "exposure to copper has been shown to increase mucus production in fish and mucus is a strong copper chelator"; 2) "the olfactory epithelium of a number of fishes contain high levels of cytochrome P-450

mono-oxygenase whose presence suggests the ability to eliminate or sequester toxic solutes; and 3) sequestration by melanophores

- Intermittent loss and recovery of olfactory ability can potentially have a high biological cost

Bjerselius, R., S. Winberg, Y. Winberg and K. Zeipel. 1993. Ca^{2+} Protects Olfactory Receptor Function Against Acute Cu(II) Toxicity in Atlantic Salmon. Aquatic Toxicology 25: 125-138.

- Atlantic salmon (2 years old)
- Measurement of response via electo-olfactograms (EOG) using anaesthetized and restrained fish
- Olfactory stimulant was 0.0001 M L-alanine
- Test waters had variable hardness, pH was 7.6, dissolved organic carbon not reported
- Effects of calcium and magnesium were investigated - solutions tested were 400, 800, 2000, or 4000 uM of Ca^{2+} and 400 uM Ca^{2+} with 3600 uM Mg^{2+} (16, 32, 80 and 160 mg/L Ca^{2+} and 16 mg/L Ca^{2+} with 88 mg/L Mg^{2+}). High hardness usually means high bicarbonate concentrations and Cu(II) readily forms stable complexes with hydroxide and carbonate thereby possibly reducing toxicity.
- Prior to the study, juvenile Atlantic salmon were held in 6 ug/L Cu^{2+}
- Fish were exposed to 64 ug/L Cu^{2+} (Cu^{2+} was determined to be the dominant species) under water of varying Ca^{2+} and Mg^{2+} concentrations. The olfactory rosette was flushed for a total of 65 minutes (alternating solutions with and without Cu^{2+}over the time period as follows: 10 min artificial fresh water, 5 min copper solution, 30 min artificial fresh water, 14 min copper solution, 5 min artificial fresh water)
- Exposure to 64 ug/L Cu^{2+} diminished the response of Atlantic salmon to the odour and effects were observed within the first minute of exposure and became progressively worse
- Effect of Cu^{2+} on EOG profiles was less pronounced in high [160 mg/L Ca^{2+} or 88 mg/L Mg^{2+} + 16 mg/L Ca^{2+}] compared to low ionic strength solutions [80 mg/L Ca^{2+} and less]. EOG amplitudes were however suppressed to 30% - 50% of Cu-free water amplitudes.
- Both high Ca^{2+} and Mg^{2+} concentration significantly reduced the immediate effects of Cu^{2+} exposure

- 29 minutes in Cu-free water following a 5 min. exposure to Cu in high ionic strength solution (160 mg/L Ca^{2+}) partially restored the EOG profile to pre-copper exposure. Recovery in 88 mg/L mg^{2+} + 16 mg/L Ca^{2+} was not as good, suggesting that calcium plays a role greater than just contributing to ionic strength (perhaps a stabilizing effect on membranes, reducing permeability and thus copper uptake).
- Positive correlation between Ca^{2+} concentration and EOG recovery following 5 minutes of Cu^{2+}
- A significant correlation was found between the calculated free Cu^{2+} activity and the EOG response after 4 minutes of exposure indicating a reduction in effects on the olfactory epithelium due to increased ionic strength

Carreau, N.D. and G.G. Pyle. 2005. Effect of Copper Exposure During Embyonic Development on Chemosensory Function of Juvenile Fathead Minnows (*Pimephales promelas*). Ecotoxicology and Environmental Safety 61: 1-6.

- Juvenile fathead minnow
- Embryos exposed to clean water or water containing 10 ug/L copper
- After hatch, half the copper exposed embryos were transferred to clean water and half remained in the 10 ug/L copper water
- Test waters had low hardness (18.1 mg/L), pH was 7.4, alkalinity was 18.9 mg/L and dissolved organic carbon was 2.7 mg/L
- Alarm stimulus was a skin extract
- Fish behaviour was tested using a triumvirate maze at 84 to 96 days post-hatch
- Fish reared in clean water significantly avoided the alarm cue whereas those continuously reared in copper and those reared in copper then removed to clean water were unable to respond to the alarm stimulus
- Suggests that exposure to elevated copper during embryonic development is sufficient to impair chemosensory function during later life stages, whereas fish exposed during later life stages can recover
- Any event causing chemosensory dysfunction could have important ecological effects that we currently do not understand

Green, W.W., R.S. Mirza, C.M. Wood and G.G. Pyle. 2010. Copper Bindings Dynamics and Olfactory Impairment in Fathead Minnows (*Pimephales Promelas*). Environmental Science and Technology 44: 1431-1437.

- Adult fathead minnow
- 2 week acclimation to 123 uM Ca, 107 uM Na, 36 uM Mg and 1 mg/L DOC for ligand biding experiments or 168 uM Ca, 561 uM Na, 63 uM Mg (DOC not measured) for EOG and behavior experiments
- pH was 6.72 to 6.93 in all experiments
- removed olfactory rosettes to measure concentration (Cu-64)
- measured EOG response to L-Arginine
- Fish exposed to 5, 10, 15 ug/L copper
- Fish also exposed to 10 ug/L copper under different Ca concentrations – 100, 500 and 1000 uM (4, 20, 40 mg/L)
- Behaviour trials were conducted using a Y maze with a food stimulus (brine shrimp)
- Increasing Ca reduced Cu-OE binding by 200 to 600%; however, at Cu < 10 ug/L, only the highest Ca concentration (40 mg/L) significantly reduce Cu-OE binding
- As waterborne Ca increased, the Bmax decreased by up to 50%, but the log KCu-OE remained the same, indicating non-competitive inhibition
- Exposure to 10 and 15 ug/L copper reduced the EOG responses by 72% and 79%, respectively, then returned to pre-exposure within 10 min and 20 min, respectively
- Increasing Ca concentration from 4 to 40 mg/L had no protective effect against 10 ug/L copper
- Fish not exposed to Cu spent 15x more time in the food stimulus arm than those exposed to any Cu/Ca combination
- Overall, both EOG and behavioural responses were significantly reduced at 10 ug/L copper
- Authors suggest that the effect of Ca may be via noncompetitive inhibition

Hansen, J.A., D.F. Woodward, E.E. Little, A.J. DeLonay and H.L. Bergman. 1999a. Behavioral Avoidance: Possible Mechanism for Explaining Abundance and Distribution of Trout Species in a Metal-Impacted River. Environmental Toxicology and Chemistry. 18(2): 313-317.

- Juvenile rainbow trout
- Avoidance chambers
- Hardness 100 mg/L, alkalinity 100 mg/L, pH 8
- Mixture of 12 ug/L Cu, 1.1 ug/L Cd, 3.2 ug/L Pb and 50 ug/L Zn was designated 1X
- Tested 0X, 0.1 X, 0.5X 1X, 2X, 4X and 10X
- Rainbow trout significantly avoided all tested concentrations relative to the control
- Significant difference between 0.1X and all higher dilutions
- Reduced pH cause greater avoidance
- After a 45 day acclimation to 1X, rainbow trout still significantly avoided 1X water relative to control and 4X water relative to 1X
- Threshold for avoidance was less than 0.1X (1.2 ug/L Cu, 0.11 ug/L Cd, 0.32 ug/L Pb and 5.0 ug/L Zn)
- Rainbow trout also avoided lower pH waters - even a reduction from 8.0 to 7.0

Hansen, J.A., J.C.A. Marr, J. Lipton, D. Cacela and H.L. Bergman. 1999b. Differences in Neurobehavioral Responses of Chinook Salmon (Oncorhynchus tshawytscha) and Rainbow Trout (Oncorhynchus mykiss) Exposed to Copper and Cobalt: Behavioral Avoidance. Environmental Toxicology and Chemistry. 19(9): 1972-1978.

- Juvenile Chinook salmon and rainbow trout
- Some fish acclimated to 2 ug/L copper
- Test waters had 25 mg/L hardness, 25 mg/L alkalinity, pH was 7.5 and dissolved organic carbon was not reported
- Behavioural response (copper avoidance) was measured using a counter-current avoidance chamber

- Chinook salmon avoided concentrations of copper ranging from 0.8 to 22.5 ug/L, but did not respond to 44 and 340 ug/L

- Rainbow trout avoided concentrations from 1.6 to 88 ug/L, but did not respond to 180 or 360 ug/L
- The same study also acclimated Chinook and rainbow trout to 2 ug/l of copper for 30 days, and then tested their response to different concentrations of copper. Chinook did not avoid concentrations higher than those they were acclimated to, nor did they prefer clean water without copper. Rainbow trout, however, did avoid higher concentrations and showed preference for clean water over the 2 ug/l water they were acclimated to.
- Chinook salmon and rainbow trout will avoid copper at low concentrations. Effects manifest as failure to avoid were evident at 44 ug/L copper and 180 ug/L copper in chinnok salmon an rainbow trout, respectively
- Acclimation to 2 ug/L copper results in loss of avoidance in Chinook salmon but not rainbow trout
- Failure to avoid high concentrations by both species suggests that the sensory mechanism responsible for avoidance was impaired
- The sensory mechanism of Chinook salmon was more sensitive than that of rainbow trout

Hansen, J.A., J.D. Rose, R.A. Jenkins, K.G. Gerow and H.L. Bergman. 1999c. Chinook Salmon (*Oncorhynchus tshawytscha*) and Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Copper: Neurophysiological and Histological Effects on the Olfactory System. Environmental Toxicology and Chemistry. 19(9): 1979-1991.

- Juvenile Chinook salmon and rainbow trout
- Examination of olfactory epithelial structure (histology) and measurement of response via olfactory bulb electo-encephalograms (EEG) using anaesthetized and restrained fish
- Response to l-serine following exposure to a range of copper concentrations (0, 25, 50, 100, 200 and 300 ug/L at water hardness of 25 mg/L).
- Test waters had 25 mg/L hardness, pH was 7.5 and dissolved organic carbon was not reported

- In hour-long exposures to copper during EEG testing, fish were tested for a response to l-serine every 5 minutes, along with a pre-copper period and a post-copper period
- The number of olfactory receptors was significantly reduced in Chinook salmon exposed to ≥50 ug/L copper and in rainbow trout exposed to ≥200 ug/L copper for 1 hour
- The number of olfactory receptors was significantly reduced in both species exposed to 25 ug/L copper for 4 hours, but the effect was greater in Chinook salmon
- Microscopy indicated that the loss of receptors was from cellular necrosis (ruptured plasma membranes, on the exposed dendrite, swollen mitochondria, and no cilia or microvilli projecting from the dendrite)
- EEG response to l-serine initially reduced by all copper concentrations but were virtually eliminated in Chinook salmon exposed to ≥50 ug/L copper and in rainbow trout exposed to ≥200 ug/L copper
- Chinook salmon showed a 50% reduction in response at 25 ug/L, with the degree of reduction increased with increasing concentration until the response was completely eliminated at 200 ug/L. Recovery was apparent 60 min following copper exposure, but only in fish exposed to 25 and 50 ug/L
- Rainbow trout responded immediately to copper exposure, with reductions in response to serine of 50-65% of controls at concentrations of 25 to 100 ug Cu./L. By the end of the 60-minute post-copper exposure, fish were already returning to control-level responses at these concentrations. However, exposure concentrations of 200 and 300 ug/l eliminated the response to serine by the end of the 60-minute exposure period, and recovery was much slower
- The rate of decline in EEG response increased with increasing copper concentration and EEG recovery rates were slower in fish exposed to higher copper concentrations
- Chinook salmon more sensitive (response more easily reduced) than rainbow trout, perhaps due to fewer goblet cells and therefore lower mucus production in the olfactory epithelium
- The olfactory system appears to be stimulated by copper and that stimulation may lead to avoidance responses. Higher concentrations may overload, overstimulate or damage the olfactory system, leading to olfactory impairment
- "The rate and degree of olfactory bulb EEG response decline following the initial exposure of copper probably depends on hardness, alkalinity and other water parameters that influence copper speciation and, thus, the bioavailable copper concentration." "Increased hardness, alakalinity, pH, and dissolved organic carbon

concentrations have all been shown to reduce toxicity in studies of lethality and metal binding on fish gills". Evidence that at least some of these are important to the EEG / EOG. "likelihood that bioavailable copper is important in olfactory neuroelectrical measurements … "

- "Low bioavailable copper concentrations likely result in slower depressions, whereas relatively higher concentrations have a more rapid effect"
- Lower concentrations of copper may be impairing ion pumps or blocking ion channels, as has been shown in other fish epithelial tissue. Because signal transduction depends on calcium in the external water contacting the cilia and microvilli of receptors, copper may be blocking calcium channels or it may be depolarizing receptors
- Prolonged exposure and exposure to higher concentrations caused irreversible physical damage (necrosis). Which seems to be dependent on the binding of copper to an epithelial binding site (i.e., an olfactory receptor protein which is a ligand)

Hecht, S.A., D.H. Baldwin, C.A. Mebane, T. Hawkes, S.J. Gross, and N.L. Scholz. 2007. An Overview of Sensory Effects on Juvenile Salmonids Exposed to Dissolved Copper: Applying a Benchmark Concentration Approach to Evaluate Sublethal Neurobehavioral Toxicity. National Oceanic and Atmospheric Administration – National Marine Fisheries Service Technical Memorandum NMFS-NWFSC-83. October 2007.

- This paper is a review paper and therefore overlaps considerably with this independent review. Accordingly, the points below do not duplicate the findings of original research paper, rather focus on data interpretation and conclusions.
- Impairment of sensory function can occur following 10 minutes of exposure and last for hours to days depending on concentration and duration. Neurotoxic effects (to olfactory system) happen in a matter of minutes.
- Direct exposure to dCu can impair and destroy olfactory sensory neurons (exact mechanism not known)
- Statistically significant decline in antipredator behaviour of juvenile coho at 5, 10, and 20 ug/L dCu
- Copper-induced reductions in juvenile salmonid olfactory physiology and behavior are significantly correlated
- In some cases, dose-dependent physiological recovery of neuron function may occur within a few hours at low dCu (i.e., <25 ug/L)

- Olfactory neuron cell death can take weeks-months for recovery (i.e, concentrations greater or equal to 25 ug/L)
- Acclimation of the olfactory system to copper may not occur
- Hardness, alkalinity, and DOC alter the bioavailability of dCu to fish gills. Ambient levels of these parameters may have less influence on olfactory responses.
- Hardness of 240 mg/L did not protect pacific salmonids olfactory system from 13 ug/L dCu during a 30 min. exposure.
- Need large increases in alkalinity to get protection from dCu
- DOC is the most protective against dCu compared to alkalinity and hardness
- Salmonids will actively avoid water containing dCu if they can detect it (these waterways may be part of a migratory route). Except, one study found that Chinook salmon no longer avoid copper following a 20-day exposure to concentration of 2 ug/L.
- Adding 4 and 8 mg/L DOC and altering pH 6.5 to 8.5 did not affect the avoidance behavior of Chinook
- Lab and field experiments with salmonids have shown disruption in d/s migration by juveniles, loss of homing ability and loss of avoidance response to even acutely lethal concentrations of copper following a long-term sublethal copper exposure (22 ug/L; 37 – 41 weeks)
- The lateral line is sensitive to dCu in zebrafish (greater or equal to 20 ppb; 3 hours). No salmonid studies.

Julliard, A.K., D. Saucier and L. Astic. 1996. Time-Course of Apoptosis in the Olfactory Eplithelium of Rainbow Trout Exposed to a Low copper Level. Tissue & Cell 28(3) 367-377.

- Yearling rainbow trout
- 20 ug/L Cu^{2+} for 15 days
- Hardness 62-64 mg/L, pH 6.53-6.96, Ca 20.2 mg/L, DOC not measured
- Copper dosed as CuSO4
- Removed olfactory rosettes and fixed for examination
- Ultrastructural observations

- Apoptosis after 1 day, peak at day 5 (apoptosis = programmed cell death)
- An increased number of dying receptor cells was observed throughout the width of the olfactory epithelium in fish exposed to 20 ug Cu2+/L
- Mature sensory cells with dendritic knobs protruding from their apical surface became absent from the olfactory epithelium on Day 5
- The precise molecular mechanisms by which copper induces apoptosis of olfactory primary neurons are actually unknown

Leduc, O.H.C., J.M. Kelly and G.E. Brown. Detection of Conspecific Alarm Cues by Juvenile Salmonids Under Neutral and Weakly Acidic Conditions: Laboratory and Field Tests. Oecologia 139: 318-324.

- Juvenile rainbow trout and small *in-situ* brook trout
- Weak acid (pH 6.0) in lab (RT) and field (BT)
- Measured response of con-specific alarm cue (skin extract)
- In lab, measured anti-predator behavior (swimming, freezing, shelter use, dashing)
- In field, measured number of feeding attempts and number of aggressive interactions
- In both laboratory and field settings, there was no response to con-specific alarm cue under weakly acidic conditions
- The researchers concluded that the loss of chemical alarm cues was due to changes in the alarm cue itself (protonation), rather than physiological stress or olfactory receptor damage

Linbo, T.L., C.M. Stehr, J.P. Incardona and N.L. Scholz. 2006. Dissolved Copper Triggers Cell Death in the Peripheral Mechanosensory System of Larval Fish. Environmental Toxicology and Chemistry. 25(2): 597-603.

- Larval zebrafish (Danio rerio) – a tropical freshwater minnow
- Examination of the peripheral mechanosensory system, or lateral line using a variety of observational tools
- Mechano-sensory information is transduced by hair cell; can measure hair cell death.
- Exposure to a range of copper concentrations (0 to 65 ug/L)

- Test waters had 150 mg/L hardness, pH was 7.0 to 7.4, alkalinity was 5 mg/L and dissolved organic carbon was not reported
- Fish were exposed for 5 hours to nominal concentrations of 0, 5, 15, 25, 30, 40, 50, or 65 ug/L dissolved copper
- Additional time to effect exposures were at nominal concentrations of 1, 5, 25 and 50 ug/L assessed at 0, 1/2 , 1, 2, 4 and 6 hours
- Regeneration was assessed by transfer to clean water and examination of fish at 24, 48 and 72 hours
- Dissolved copper caused a dose-dependent loss of neurons in the lateral line at concentrations ≥20 ug/L
- EC50s ranged from 27.4 to 29.8 ug/L dissolved copper and EC20s ranged from 19.4 to 21.8 ug.L dissolved copper. Because the EC20s are near the lower end of the 95% CI for controls, the EC20s serve as approximate indicators of the threshold
- After 1 hour of exposure to 50 ug/L copper, sensory neurons were largely absent, indicating a rapid effect
- Following transfer to clean water, the lateral line regenerated over the course of 2 days, with evidence of regeneration after 24 hours
- In larvae exposed to 50 ug/L copper for 3 days, the lateral line did not recover
- The basic architecture and function of the lateral line is highly conserved across teleosts

Linbo, T.L., D.H. Baldwin, J.K. McIntyre and N.L. Scholz. 2009. Effects of Water Hardness, Alkalinity, and Dissolved Organic Carbon on eth Toxicity of Copper to the Lateral Line of Developing Fish. Environmental Toxicology and Chemistry. 28(7): 1455-1461.

- Larval zebrafish (Danio rerio) – a tropical freshwater minnow
- Examination of the peripheral mechanosensory system, or lateral line using a variety of observational tools
- Exposure to a range of copper concentrations (0, 5, 10, 20 and 40 ug/L) for 3 hours under varying water chemistries
- Chemistry of test waters was varied for this experiment 45 to 320 mg/L hardness, pH was 6.97 to 8.47, alkalinity was 4.5 to 300 mg/L and dissolved organic carbon was 0.1 to 4.3 mg/L

- Reductions in copper toxicity were observed for all of the water quality parameters, but all except for the influence of DOC were moderate
- EC50s increased approximately 50% from soft water (45 mg/L $CaCO_3$) to hard water (320 mg/L $CaCO_3$) regardless of which cation was varied
- EC50s increased by an average of 437% across the DOC range tested (0 to 4.3 mg/L) from approximately 12 ug/L to approximately 50 ug/L copper
- The reduction in copper neurotoxicity by DOC was greater than for hardness, sodium of alkalinity over the ranges tested
- Comparison to the BLM suggests that water chemistry parameters are less influential on the mechanosensory system compared to the fish gill and this is consistent with findings of other on the olfactory system
- Comparisons suggest that copper may be available and toxic to zebrafish lateral line neurons in inorganic complexes (e.g., copper carbonate) that are biologically unavailable to the gill
- "it should be noted that the intended use of the BLM as a regulatory tool may result in site-specific criteria that are protective against toxicity to the olfactory and lateral line systems of fish. This is because the criteria mode of the BLM considers toxicity data for the most sensitive aquatic species (e.g., C. dubia). Relative to fish, the parameter for critical copper accumulation (LA50) is much lower for C. dubia. The net effect is that the BLM-derived criteria are below our calculated EC50s for copper toxicity to the lateral line in waters with different chemical compositions. In all cases, even the CMC is below the respective EC50 for copper-induced toxicity to the lateral line.

Lurling, M. and M. Scheffer. 2007. Info-Disruption: Pollution and the Transfer of Chemical Information Between Organisms. TRENDS in Ecology and Evolution 22(7): 374-379.

- Review paper
- Many chemicals have been shown to disrupt the transfer of chemical information, from heavy metals and pesticides to seemingly harmless substances such as surfactants
- Form a new class of chemical threats which could have far-reaching implications for ecosystem functioning
- Chemical information transfer is ubiquitous among animals and plants (algae, plants, invertebrates, vertebrates)

- Communication with conspecifics and natural enemies to tune behavior
- Heavy metals have been shown to impair predator avoidance behavior in fish, aquatic snails, amphibians and water fleas
- Pesticides: diazinon impaired response of Chinook salmon to alarm cues; atrazine and diuron decreased anti-predator behavior in goldfish; endosulfan and carbaryl inhibited crest development in Daphnia; carbaryl inhibited the development of antipredator morphology in the cladoceran *Bosmina*; pentachlorophenol made water fleas negatively phototactic and the rotifer *Brachionus* reverse its anti-predator response
- A surfactant has been observed to invoke an anti-predator response in green algae
- Natural chemical information transfer, which is vital in regulating predator-prey and symbiotic interactions, as well as mating and other intraspecific interactions, can be disrupted by pollutants ranging from pesticides and heavy metals to surfactants and environmental acidification
- Concentrations required to disrupt chemical information systems are typically low compared with concentrations needed to invoke other adverse effects
- Outstanding challenges include assessing effects on individuals (what are the costs and consequences for growth and reproduction) and assessing effects on populations and ecosystems

McIntyre, J.K., D.H. Baldwin, J.P. Meador and N.L. Scholz. 2008. Chemosensory Deprivation in Juvenile Coho Salmon Exposed to Dissolved Copper under Varying Water Chemistry Conditions. Environmental Science and Technology. 42: 1352-1358. With Supporting Information (http://pubs.acs.org) and the Correction in Environmental Science and Technology. 2008. 42: 6774-6775.

Note that this paper is a key paper due to its consideration of the effect of water quality on chemosensory response. However, a significant correction was issued without a corresponding re-examination of the findings and conclusions by the authors.

- Juvenile coho salmon
- Measurement of response via electo-olfactograms (EOG) using anaesthetized and restrained fish
- Response to the odorant l-serine
- Fish were reared in water with 0.20 ug/L dissolved copper

- Test waters were low ionic strength artificial fresh water and were varied by adding calcium as $CaCl_2$, alkalinity as NaHCO3, DOC as fulvic acid and natural organic matter, and adjusting pH using HCl.
- Exposure to 20 ug/L dissolved copper for 30 minutes
- Amplitude of EOG recording was significantly reduced in response to the natural odorant 0.00001 M L-serine in the presence of 20 ug/L dissolved copper Cu (82% reduction in low ion water)
- 20 ug/L copper reduced the EOG in all tested waters except the high DOC water
- The high DOC water (6 mg/L DOC; 10 mg/L fulvic acid) did not differ significantly from the controls when exposed to 20 ug/L copper at pH 7.2.
- Changes in pH (7.1 to 8.6) had no influence on amplitude
- Water hardness, alkalinity and DOM all reduced the effect of copper on EOG response, but the effect of hardness (calcium) and alkalinity were considered relatively minor, whereas the relationship with DOM was more than an order of magnitude greater over the ranges tested
- The protective effects of DOM are much greater than the protection afforded by either water hardness or alkalinity
- Comparison between gill and nose using BLM demonstrate that ligands for copper likely distinct
- Investigated the extent to which the BLM may extend to the salmon olfactory system. Originally reported that the influence of calcium was 3-fold greater in the gill, the influence of bicarbonate was 80-fold greater in the gill and the influence of dissolved organic carbon was 20-fold greater in the gill. HOWEVER, this was an error! The actual numbers are 2-fold, 41-fold and 4-fold, indicating that calcium and DOC are very important to the olfactory endpoint
- Prior to the correction, McIntyre took the lower protective roles of calcium, bicarbonate and DOC as evidence that ligands in the salmon olfactory epithelium may have a relatively higher affinity for copper (than gill) and that biocarbonate-bound copper is bioavailable to the OSNs
- A key priority for future research is to determine how a copper induced loss of olfactory capacity affects life history traits that individual survival and lifetime reproductive success

McPherson, T.D., R.S. Mirza and G.G. Pyle. 2004. Responses of Wild Fishes to Alarm Chemicals in Pristine and Metal-Contaminated Lakes. Canadian Journal of Zoology 82: 694-700.

- Iowa darters (*Etheostoma exile*)
- Field study
- Minnow trapping using clean traps, traps treated with Iowa dater skin extract and traps treated with swordtail (phylogenetically distant) skin extract
- Minnow trapping in clean and contaminated lakes in the Sudbury area
- In the clean lakes, darters avoided traps with the alarm stimulus relative to controls, but did not in the contaminated lakes
- No effects were observed on other species
- Wild Iowa darter inhabiting contaminated lakes exhibit an impaired response to the alarm cue
- It is suspected that free metal cations present in metal-contaminated lakes disrupt olfaction by damaging olfactory epithelium, leading to an impairment of olfactory function
- Protective mechanisms leading to recovery of olfactory ability include increased mucus production, induction of detoxifying mechanisms, metal sequestration by melanophores

Mirza, R.S., W.W. Green, S. Connor, A.C.W. Weeks, C.M. Wood and G.G. Pyle. 2009. Do You Smell What I Smell? Olfactory Impairment in Wild Yellow Perch from Metal Contaminated Waters. Ecotoxicology and Environmental Safety. 72: 677-683.

- Yellow perch
- Electro-olfactograms, behavior and histology
- L-alanine and skin extract
- North Bay dechlorinated tap water
- EOGs measured following dechlorinated tap water (blank), L-alanine, yellow perch skin extract, rainbow trout skin extract
- Measured behaviors were movement, foraging, group cohesion and area avoidance
- Yellow perch from contaminated lakes had greater EOG, but no associated anti-predator behavior contrary to clean lake fish

- Yellow perch from reference lakes significantly decreased activity, but not those from contaminated lakes
- Olfactory rosettes and olfactory bulb removed and examined
- No differences in neuron density at rosettes or bulb
- Wild yellow perch from contaminated lakes can detect chemical stimuli, but olfactory signal processing is disrupted
- "Fish that have spent multi-generations in contaminated environments do not show the same responses as laboratory- and aquaculture-reared fishes suggesting that caution should be taken when extrapolating results from the laboratory to the field under certain ecological contexts"
- "A lack of behavioural response may be due to effects of metal accumulation within the olfactory system"
- "If we had only conducted the electrophysiological study, then we may have drawn a false conclusion that yellow perch from contaminated lakes were not olfactory impaired"

Pyle, G.G. and R.S. Mirza. 2007. Copper-Impaired Chemosensory Function and Behavior in Aquatic Animals. Human and Ecological Risk Assessment. 13: 492-505.

- Review paper
- The mechanism of copper impairment of fish olfaction is unknown
- Previous studies examining the effects of metals on chemosensation have been conducted primarily under controlled laboratory conditions and do not necessarily reflect natural contaminant exposure conditions
- Review of chemo-sensation in aquatic animals … findings of studies by his group using daphnia, leeches, fish
- Copper inhibits the number and length of neck spines in Daphnia pulex (Hunter and Pyle 2004) … suggests that D. pulex may be more susceptible to predation in contaminated environments
- 10 ug/L copper decreases the time spent by leeches in food cue arm of a Y maze versus the control arm … suggests that copper has a deleterious effect on chemosensory function in leeches and that leeches exposed to relatively low waterborne copper are unable to detect food cues or to discriminate between clean or metal-contaminated food sources

- In the laboratory, fathead minnow exposed to copper (10 ug/L) at the embryo stage could not avoid an alarm cue (Carreau and Pyle 2005) … suggest that copper may disrupt important developmental stages in the embryonic olfactory system that can translate to severe chemosensory impairment later in life
- In the field, Iowa darter avoided traps treated with an alarm cue in a clean lake but not in a contaminated lake (McPherson et al. 2004)
- Olfactory receptor neurons are the only vertebrate neurons capable of division (Laberge and Hara 2001)
- Impaired olfaction in copper-exposed fish is transient in adults
- Copper ma act on the signal transduction pathway inhibiting the signal from being propagated from the sensory epithelium to the brain
- Propose the development of a chronic chemosensory-based Biotic Ligand Model

Rehnberg, B.C. and C.B. Schreck. 1986. Acute Metal Toxicology of Olfaction in Coho Salmon: Behaviour, Receptors and Odor-Metal Complexation. Bulletin of Environmental Contamination and Toxciology 36: 579-586.

- Juvenile coho salmon
- Measurement of avoidance response to the odorant l-serine
- Test waters had alkalinity 25.5 mg/L, hardness 30.5 mg/L, pH 6.72 and DOC <2 mg/L
- Exposure to 10^{-7},10^{-6} and 10^{-5} M (6.35, 63.5 and 635 ug/L) dissolved copper for 1 hour 45 minutes
- Also tested 10^{-7},10^{-6} and 10^{-5} M mercury and zinc
- Tested the response to a 10-minute exposure to L-serine
- Avoidance of serine was inhibited at all the copper concentrations tested (6.35 to 635 ug/L)
- Mercury also inhibited avoidance at all concentrations tested; Zn had no effect
- Tested binding of the metals to serine and concluded that the observed inhibitory effects on serine detection could not be explained by the formation of nonstimulatory metal-serine complexes

- Tested binding of the metals to olfactory tissue homogenate and concluded that the inhibitory effects seen in behavioural assays could not be explained by interaction at the serine receptor

Sandahl, J.F., D.H. Baldwin, J.J. Jenkins and N.L. Scholz. 2004. Odor-Evoked Field Potentials as Indicators of Sublethal Nerotoxicity in Juvenile Coho Salmon (*Oncorhynchus kisutch*) Exposed to Copper, Chlorpyrifos or Esfenvalerate. Canadian Journal of Fisheries and Aquatic Science. 61: 404-413.

- Juvenile coho salmon (10-12 months)
- Exposed for 7 days to 5, 10 and 20 ug/l copper concentrations
- Test waters had hardness 120 mg/L, 0.4 ug/L coppper
- Response to the odorant l-serine (amino acid) and taurocholic acid (TCA; a salmonid bile salt) following 5s deliveries
- Measurement of response via electo-olfactograms (EOG) and electro-encephalograms (EEG) using anaesthetized and restrained fish
- EOG and EEG results agreed well
- Copper inhibited the response of Coho salmon to the odors by 50% when exposed to 10 ug/l, and 90% when exposed to 20 ug/l, but fish exposed to 5 ug/l were not significantly different from the controls (but were 20% lower)
- Note that measured concentrations were 70% to 72% of measured and results reported as nominal
- Chlorpyrifos also inhibited the olfactory response to both odorants in the sensory epithelium (EOG) and bulb (EEG); no inhibition by esfenvalerate
- Benchmark concentrations were also estimated. The BMC20 was 4.4 ug/L and the BMC50 was 11.1 ug/L
- The response observed after 7 days of exposure was equal to the response seen after only 30 minutes, implying that the olfactory receptors were not progressively saturated with the additional exposure
- Similar copper exposures lasting more than a few hours are known to trigger cell death among primary receptor neurons. It is therefore likely that diminished EOG and paired EEG responses of copper-exposed animals in this study were due a consequence of peripheral degeneration or loss of receptor neurons

- Presumably, for salmonids, a reduction or loss of olfactory sensitivity could interfere with imprinting, kin recognition, predator avoidance, homing, spawning, and other aspects of Pacific salmon biology that are reliant on olfaction
- Pacific salmon are unable to navigate back to their natal streams when olfactory function is lost (Wisby and Hasler 1954)

Sandahl, J.F., G. Miyasaka, N. Koide, and H. Ueda. 2006. Olfactory Inhibition and recovery in Chum Salmon (*Oncorhynchus keta*) Following Copper Exposure. Canadian Journal of Fisheries and Aquatic Science 63: 1840-1847.

- Juvenile chum salmon (2-3 months)
- Test waters had hardness 40 mg/L, pH 6.1, conductivity 140 uS/cm
- Exposed to copper concentrations of 3, 8, 24, and 58 ug/L for 4 hours
- Response to the odorant L-serine and hatchery outlet stream water was assessed
- Then placed in clean water to track recovery over 7 days
- Measurement of response and recovery was via electo-olfactograms (EOG) using anaesthetized and restrained fish
- Also assessed effects to the olfactory epithelium using a dye
- Copper reduced the olfactory responses of chum fry to both odorants in a concentration-dependent manner
- During recovery, EOG responses increased in all exposure groups and were statistically similar to the control group after 1 day, indicating that the toxic effect of copper on the electrical properties of the neurons was generally reversible
- The number of labeled cells increased after 4h exposures to 3 and 8 ug/L copper, but decreased after 4h exposures to 24 and 58 ug/L copper, perhaps indicative of a physiological need (uptake) at lower concentrations
- After 10 days, the mean number of labeled cells in the 24 ug/L exposure showed only a partial return to the control group number
- “Depending upon the conditions of exposure, inhibitory effects can be either reversible or irreversible (Moran et al. 1992; Julliard et al. 1996; Hansen et al. 1999)

- "Irreversible damage caused by high or prolonged exposures to copper can target cellular surface proteins, membrane structure, or internal organelles and can eventually lead to cell death (Brown et al. 1982)"
- "Heavy meals can inhibit the electrical properties of olfactory neurons, presumably by blocking ligand-gated and/or voltage-gated ion channels. This effect can be eversible, as indicated by time-course EOG recordings in salmon (Baldwin et al. 2003). However, more severe, irreversible damage to a neuron ultrastructure by metals, including the GPCR (G protein-coupled receptors)-rich cilia and microvilli, the cellular membrane, and supportive organelles, can result in extended reductions in neuronal electrical activity"
- "Metals, like copper, my act as odorant agonists, alter a receptor protein's steric properties, alter transduction, or second messenger pathways, or affect processes involving GPCR endocytosis and recycling"
- "The degree of copper toxicity to the olfactory system is dependent on both exposue concentration and exposure time (e.g., Saucier and Astic 1995). The combination of these parameters (in addition to other parameters such as water chemistry, acclimation to test water, etc.) will influence the degree of impact on target sites, and ultimately whether the toxic effects are reversible (short-term) or irreversible (long-term)"
- "The initial targets of copper on olfactory receptor neurons are believed to be proteins associated with odorant receptors, ion channels, and intracellular transduction mechanisms (Rehnberg and Schreck 1986; Winberg et al. 1992; Bjerselius et al. 1993). Longer exposures to copper at relatively hish concentrations can damage cilia and microvilli located at the cellula surface, rupture the cellular membrane, or critically impair the protein-rich mitochondria and organelles of the cytoplasm (Moran et al. 1992; Hansen et al. 1999)."
- "Severe damage to olfactory receptor neurons can lead to necrosis or apoptosis, requiring the entire regeneration of receptor cells (Moran et al. 1992; Julliard, et al. 1996; Hansen et al. 1999)"
- "Although copper can be highly toxic to the olfactory neuron, it is also an essential trace metal. Copper is required for the proper functioning of several enzymes and has been shown to play an important role in odorant binding for some receptor proteins. Copper is also necessary in the synthesis of certain neuropeptides within the olfactory system (Rutkoski et al. 1999). Fish olfactory neurons may contain a metal-sequestering mechanism" (would explain the bi-phasic dye uptake in this study as also observed by Tjalve et al. 1986)

- "How salmonids can acclimate, and indeed thrive, in some natural waters with relatively high copper concentrations is an important question …"

Sandahl, J.F., D.H. Baldwin, J.J. Jenkins and N.L. Scholz. 2007. A Sensory System at the Interface between Urban Stormwater Runoff and Salmon Survival. Environmental Science and Technology. 41: 2998-3004.

- Juvenile coho salmon (4 to 5 months)
- Alarm cue was con-specific skin extract (tested different concentrations), l-serine and TCA
- Test waters had hardness 120 mg/L, 0.3 ug/L copper, pH 6.7 (6.5 – 7.1)
- Treatments: 0, 2, 5, 10, 20 ug/L (nominal) copper chloride in 30L aquaria; 3 hour exposure
- Behavioural tests in response to skin extract followed by measurement of EOG at the olfactory rosette in response to skin extract, l-serine and TCA
- Behavioural responses - swim speed and freezing behaviour recorded anti-predator responses to the alarm cue by computer-assisted video analysis
- Concentration-dependent increase in EOG amplitude in response to skin extract (0.1 to 10 ug protein/L)
- At 2 ug/L copper, the reduction in swimming speed was significant but fewer fish became motionless
- At 5 to 20 ug/L Cu, there was no change in swim speed following alarm cue
- Dissolved copper inhibited EOG responses to all three odorants in a concentration-dependent manner … at 20 ug/L, EOG responses to all three odorants were nearly abolished
- At 2 ug/L copper, EOG response was reduced by approximately 40% over the entire range of odour concentrations
- Olfactory inhibition and diminished alarm response was highly correlated ($r2 = 0.94$; $p < 0.01$) … fish with reduced olfactory sensitivity showed reduced anti-predator behaviour

Saucier, D. and L. Astic. 1995. Morpho-Functional Alterations in the Olfactory System of Rainbow Trout (Oncorhynchus mykiss) and Possible Acclimation in Response to Long-Lasting Exposure to Low Copper Levels. Comparative Biochemistry and Physiology 112A: 273-284.

- Yearling rainbow trout (140 mm)
- Test waters had hardness 60.9 mg/L and pH 6.6-7.6
- Fish exposed to 20 ug/L or 40 ug/L copper for 40 weeks
- Assessed histopathological alterations and olfactory discrimination by behavioural tests (Y maze)
- Histopathological alterations in the olfactory epithelium of the 20 ug/L copper group were moderate and did not evolve with time
- After return to well water, it took roughly 6 weeks for the 20 ug/L copper group to present an olfactory epithelium similar to the controls
- Histopathological alterations in the olfactory epithelium of the 40 ug/L copper group were more severe and intensified with exposure duration
- After return to well water, it took 10 weeks before morphological recovery was evident, even though some mucus vacuoles persisted in the sensory mucosa. It took 14 weeks to return to a control-like olfactory epithelium.
- Fish exposed to 20 ug/L copper were unable to discriminate their own rearing water from well water at 13 and 19 weeks of exposure at the same concentration as did the controls. However, when the flow rate in the rearing tank was reduced, thereby concentrating the odor, fish responded by preferring that portion of the behavior test tank.
- After 30 and 40 weeks of exposure, the concentration of rearing water had to be increased again for fish to show attraction. After just 2 weeks in clean water, the fish responded comparably to the controls, indicating recovery.
- Fish exposed to 40 ug/L copper responded similarly to the controls at weeks 13 and 19 but, by week 30, they were unable to detect their own rearing water at the concentrations tested. In addition, it required a total of 29 weeks in clean water before these fish were able to recover completely to control levels even though some improvement was evident after 2 weeks..
- At high copper concentrations, the olfactory epithelium may be unable to regenerate and maintain olfactory function due to the increasing number of degenerating OSNs

Saucier, D., L. Astic and P. Rioux. 1991a. The Effects of Early Chronic Exposure to Sublethal Copper on the Olfactory Discrimination of Rainbow Trout, *Oncorhynchus mykiss*. Environmental Biology of Fishes. 30: 345-351.

- Rainbow trout (eggs and alevins)
- Test waters had hardness 61.8-64 mg/L, pH 6.50-6.64, 20.2 mg/L calcium
- Exposed to 22 ug/L copper (20-25 ug/L) copper for 41 weeks (eggs) or 37 weeks (alevins)
- Behaviour assessed using a rectangular two-choice apparatus
- After 8 months (between 29 and 33 weeks) of exposure, fish were evaluated for 10 min to determine if they were able to discriminate their own rearing water (which control fish were attracted to), or heterospecific water containing a largemouth bass (which control fish avoided), over clean well water
- Controls significantly preferred their own odour
- Exposed fish did not prefer their own odour over well water or heterospecific water
- The degree of response was greater in fish exposed from eggs (embryos) compared with those exposed from alevins … embryos showed no preference among the 3 compartments whereas alevins stayed longer in the central section than in the heterospecific odour arm.
- Following exposure, fish were placed in clean water, and after 2 or 10 weeks were again tested to see if they were able to detect their own rearing water. After two weeks in clean water, the fish exposed as alevins stayed longer in the central section than in their own odour arm which was significantly preferred over the foreign odour arm. The fish exposed as embryos preferred the central section over their own odour arm whereas there was no significant difference between the 2 stimulus arms.
- It took the embryo group 10 weeks in clean water to recover to control levels … to prefer their own odour.
- Exposed fish behaved as if they had lost their discrimination capability.

Saucier, D., L. Astic, P. Rioux and F. Godinot. 1991b. Histopathological Changes in the Olfactory Organ of Rainbow Trout (*Oncorhynchus mykiss*) Induced by Early Chronic Exposure to a Sublethal Copper Concentration. Canadian Journal of Zoology 69: 2239-2245.

- Rainbow trout (embryos and alevins)
- Test waters had hardness 61.8-64 mg/L, pH 6.50-6.64, 20.2 mg/L calcium
- Exposed to 22 ug/L copper (20-25 ug/L) copper for 41 weeks (embryos) or 37 weeks (alevins)
- Histopathology
- Morphological alterations were identical in both embryos and alevins and appeared at the same post-hatching periods
- Histopathological changes manifested in the following order: 1) increase in goblet cells; 2) epithelial lesions (necrotic tissue); 3) cellular shrinkage (due to degeneration and necrosis)
- Suggest an initial increase in mucous production which does not appear sufficient to ensure long-term protection
- By the 2nd week and more so by the 5th week post-exposure, some morphological restoration was observed in both exposed groups
- By the 10th week post-exposure, a few epithelial lesions and shrinkage were still noted, but were confined to the lower part of the lamellae
- Despite the incomplete morphological recover, functional recovery was achieved (Saucier et al. 1991a)

Scherer, E. and R.E. McNicol. 1998. Preference-Avoidance Responses of Lake Whitefish (*Coregonus clupeaformis*) to Competing Gradients of Light and Copper, Lead and Zinc. Water Research 32(3) 924-929.

- Lake whitefish (14 months old)
- Test waters had hardness 90 mg/L, pH 7.51-7.78, DOC 430 uM, Cu < 1 ug/L
- Tested preference/avoidance of competing gradients (shade and metals) in a countercurrent trough under either uniform illumination or shaded in one half

- Fish exposed to 1, 10, 20 and 40 ug/L copper under uniform illumination and 1, 10, 20, 36, 40 and 72 ug/L copper under ½ shade
- Under uniform light conditions, there was a significant linear increase in avoidance with copper concentration, with avoidance evident at the lowest concentration (1 ug/L)
- When copper was dosed into the shaded part of the trough, avoidance was suppressed … avoidance did not occur at 40 ug/L, but did occur at 72 ug/L
- "Nothing can be determined from single-factor tests about the relative strength or protective value of responses in view of natural gradients and conditions the organism would be simultaneously exposed and responding to in actual field situations"
- "If laboratory preference-avoidance tests are to be considered ecologically meaningful, then the relative strength of such responses vis a vis competing natural gradients needs to be assessed in tests analogous or similar to those here described"

Tierney, K.B., D.H. Baldwin, T.J. Hara, P.S. Ross, N.L. Scholz and C.J. Kennedy. 2010. Olfactory Toxicity in Fishes. Aquatic Toxicology 96: 2-26.

- Review paper … the points below do not duplicate the findings of original research paper, rather focus on new information and interpretation/conclusions
- "Numerous studies spanning several species have shown that ecologically relevant exposures to common pollutants such as metals and pesticides can interfere with fish olfaction and disrupt life history processes that determine individual survival and reproductive success"
- "Sensory neurons interface almost directly with the aquatic environment, typically protected only in a covered by mucous. In such an exposed situation, dissolved contaminants can interact with the olfactory neurons as readily as odorants"
- "Contaminants can act as signals, modify odorant perception, and/or act on the nervous system and/or other physiologic responses (i.e., not directly through olfaction), all of which potentially alter normal olfactory responses"
- "Most teleost fish possess well developed peripheral olfactory organs. These organs, or rosettes, are paired structures that reside in bilaterally positioned olfactory chambers. Once an odorant is taken into the olfactory chamber, either actively or passively depending on the fish an odorant, olfaction begins with an interaction between an odorant molecule, or ligand, and an olfactory sensory neuron (OSN) located in he olfactory epithelium (OE)."

- "Physiological changes in the electrical properties of OSNs have predominantly been characterized using an extracellular recording technique known as an electro-olfactogram (EOG)"
- "The generator potential needs to be of sufficient magnitude to evoke an action potential. For this reason, factors that educe the generator potentials conceivable cause fewer action potentials, and so can disrupt olfactory information."
- "Olfaction can serve as the foundation for many complex behaviours, including alarm and avoidance response, feeding, migration, kin and conspecific recognition, and mating synchronization"
- "The changes in olfactory function can be categorized as: 1) anosmia, or an inability to smell; 2) hyposmia, or a reduced capacity to smell; and 3) dysosmia, where olfactory information is processed incorrectly. Most chemical contaminants cause some degree of hyposmia or, at higher exposure concentrations, functional anosmia"
- Effects on olfaction have been observed following exposure to a wide range of chemicals: pH, metals (aluminum, cadmium, copper, mercury), pesticides (2,4-D, atrazine, carabaryl, carbofuran, chlorothalonil, chlorpyrifos, cypermethrin, diazinon, endosulfan, glyphosate, IPBC, linuron, mancozeb, roundup, simazine, trifluralin), surfactants (SLS, alkyldimethyl-3,4-dichloro-benzyl ammonium chloride, B-hydroxyethylbenzyl coco imidazolinium chloride, hydroxyethylbenzyl stearyl imidazolinium chloride, branched sodium dodecylbenzene sulfonate, calcium dodecylbenzene sulfonate, di-hydrogenated tallow dimethyl ammonium chloride, di-coco dimethyl ammonium chloride, lauryldimethylbenzyl ammonium chloride …), hydrocarbons and morpholine
- "Data from both rosette (EOG) and bulbar (EEG) recordings typically vary proportionally with each other"
- "Metals are well known for their effectiveness as blockers of ion channels (reviewed in Florea and Busselberg 2006). In studies of fish olfactory toxicity, copper has received the most attention. This metal can acuse a concentration-dependent decrease in EOG/EEG, with negative effects occurring at concentrations below 10 ug/L."
- "Findings suggest that copper exposure can have a general effect on olfactory tissue, as suggested by Thompson and Hara (1977)."
- "The ameliorative effects of DOC were more pronounced; DOC levels above ~6.0 mg/L were protective against copper toxicity."

- "While there may be modest differences in copper sensitivity among species, the available evidence suggests that copper is a general-purpose olfactory toxicant for all freshwater fish."
- "Disruption of physiological function may occur at exposure concentrations that are lower than those that cuase overt physical damage."
- "In many cases, food cues are visual"
- "Fish avoid many metals. Specifically, arsenic, cadmium, chromium, cobalt, copper, iron, mercury, nickel, selenium, and zinc are avoided to varying degrees."
- "Since copper can impair the the olfactory epithelium within minutes (Baldwin et al. 2003), conceivably a copper plume could impair neurological detection rapidly enough to prevent on olfactory-mediated behavioral response."
- Lots of questions … e.g., Is OSN or behavioral response a better indicator of toxicity? At what point of OSN impairment does contaminant avoidance fail?
- "The significance of measures of olfactory toxicity to organismal survival and beyond remains poorly understood."
- "With the number of contaminants and complexity of some of their negative effects, determining the importance of impaired olfaction and other altered physiological conditions can ultimately only be decided on a case-by-case basis and over time, in-situ."
- "Future toxicity studies would benefit by linking the effects of exposure to growth, reproduction or recruitment, which together represent levels of survivorship relevant to population. Relating the importance of a decrease in olfactory neuron response froma short-term pesticide exposure to a population-level impact is a challenge, but not one that is insurmountable"
- "The ramifications for extrapolating neurological and physiological data to behavioral and ecological impacts are straightforward: lower order measures (e.g., EOG) may underestimate the impact of toxicity to higher order biological responses (e..g., mating). Additionally, other toxic effects, such as those independent of olfaction and with possibly unknown mechanisms of action, need to be considered when determining organismal toxicity. Ultimately, regulations will need to be constructed that set contaminant levels lower than where negative effects are observed in olfactory-based responses."

Winberg, S., R. Bjerselius, E. Baatrup and K.B. Doving. 1992. The Effect of Cu(II) on the Electro-Olfactogram of the Atlantic Salmon (*Salmo salar* L.) in Artificial Freshwater of Varying Inorganic Carbon Concentrations. Ecotoxicology and Environmental Safety. 24: 167-178.

- Atlantic salmon (1-yr old)
- Test waters had pH 6.2, alkalinity 0.04 mM, Ca^{2+} 1 mg/L, Cu^{2+} 5 ug/L
- Measured the EOG response to the olfactory stimulant 790 uM L-alanine
- 640 ug/L copper chloride in solutions of 334, 34, 3.4 or 0 mg/L bicarbonate. Concentrations of Cu^{2+} were 2%, 22%, 69% and 97% of the total [copper] at each [bicarbonate], respectively. The dominant species at 2% and 22% Cu^{2+} was copper carbonate.
- Based on measured pH, this yielded free copper (Cu^{2+}) of 0.2 uM (12.7 ug/L), 2.2 uM (140 ug/L), 6.9 uM (438 ug/L) and 9.7 uM (616 ug/L)
- Exposures were over a 1 hour period with intermittent exposure to artificial freshwater and the test water
- L-alanine was presented for 10-second periods every second minute
- Response to L-alanine decreased with increasing concentration of free copper (Cu^{2+})
- At all bicarbonate + $CuCl_2$ solutions, the shape of the EOG response to L-alanine differed from control. The shape of the EOG recording tended to appear very different from control at the two lowest bicarbonate solutions while the two highest bicarbonate solutions showed greater similarity in EOG shape to control.
- At the highest bicarbonate concentration, the amplitude of the EOG varied from 60% of control to 160% over the response period
- Hyperpolarization observed at 3.4 mg/L and 0 mg/L biocarbonate
- Significant negative correlation between Cu^{2+} concentration
- Partial restoration of the EOG response after 5 min. of irrigation with artificial freshwater (5 ug/L)
- EOG of salmon is affected primarily by the activity of Cu^{2+}. Specifically, Cu^{2+} affected different stages of the transduction mechanism in the olfactory receptor cell.
- “Among the copper species tested, the toxic effect is caused mainly by dissolved Cu^{2+} ion.”

- "The results suggest that Cu^{2+} exerts its toxic effects on the transduction mechanisms of the olfactory receptor cells"
- "In most natural waters, only a small fraction of total copper is present as the free cupric ion (Cu^{2+}). Copper readily forms stable complexes with inorganic ligands (OH-, carbonate) and organic ligands (humic substances, amino acids)."
- "The observed reduction of copper toxicity in hard water (i.e., with high concentgrations of calcium and magnesium) is an effect of the high bicarbonate concentration (high alkalinity) in hard water rather than an effect of hardness per se."

Woodward, D.F. J.A. Hansen, H.L. Bergman, E.E. Little and A.J. DeLonay. 1995. Brown Trout Avoidance of Metals in Water Characteristic of the Clark Fork River, Montana. Canadian Journal of Fisheries and Aquatic Science 52: 2031-2037.

- Brown trout (150 days post-hatch)
- Avoidance of a metals mixture (cadmium, copper, lead and zinc) and acidification in cylindrical avoidance chambers
- Hardness 100 mg/L, alkalinity 100 mg/L, pH 8
- Mixture of 12 ug/L Cu, 1.1 ug/L Cd, 3.2 ug/L Pb and 50 ug/L Zn was designated 1X
- Tested 0X, 0.1 X, 0.5X, 1X, 2X, 4X and 10X
- 20-min acclimation followed by 30-min test phase
- The 0.5X, 1X, 2X, 4X and 10X mixtures were all significantly avoided (and less than 20% of time spent in the test water)
- Greatest avoidance was observed at 2X and 1X, when fish spent 8% and 13%, respectively, of their time in the test water
- Lower avoidance at 4X and 10X may be indicative of impaired perceptive acuity
- A reduction in pH from 8.0 to 7.0, 6.0 or 5.0 all resulted in significant avoidance in 0X water, but in 1X water (which was avoided anyway), only moderate and insignificant additional avoidance was observed (but number of trips into test waters were significantly reduced with reductions in pH)
- "The lowest concentration of copper avoided in our experiment (0.5X) was 6.5 ug/L"

APPENDIX B

CONVENTIONAL TOXICITY DATA

Appendix Table B.1: Ranked freshwater species mean acute values (USEPA 2007). **

** at water quality BLM-normalized to 20°C, pH 7.5, DOC 0.5 mg/L, Ca = 14.0 mg/L, Mg = 12.1 mg/L, Na = 26.3 mg/L, K = 2.1 mg/L, SO4 = 81.4 mg/L, Cl = 1.90 mg/L, Alkalinity = 65.0 mg/L and S = 0.0003 mg/L

Rank	Species Scientific Name	Species Common Name	Species Mean Acute Value (ug/L)
1	*Daphnia pulicaria*	Cladoceran	2.73
2	*Ceriodaphnia dubia*	Cladoceran	5.93
3	*Daphnia magna*	Cladoceran	6.00
4	*Lithoglyphus virens*	Snail	6.67
5	*Gammarus pseudolimnaeus*	Amphipod	9.60
6	*Scapholeberis sp.*	Amphipod	9.73
7	*Actinonaias pectorosa*	Mussel	11.33
8	*Hyalella azteca*	Amphipod	12.07
9	*Juga plicifera*	Snail	12.31
10	*Ptychocheilus oregonensis*	Northern pikeminnow	14.61
11	*Physa integra*	Snail	20.41
12	*Oncorhynchus mykiss*	Rainbow trout	22.19
13	*Etheostoma rubrum*	Fountain darter	22.74
14	*Oncorhynchus kisutch*	Coho salmon	22.93
15	*Oncorhynchus tshawytscha*	Chinook salmon	25.02
16	*Oncorhynchus apache*	Apache trout	32.54
17	*Oncorhynchus clarkii*	Cutthroat trout	32.97
18	*Oncorhynchus gorbuscha*	Pink salmon	40.13
19	*Bufo boreas*	Boreal toad	47.49
20	*Lumbriculus variegatus*	Worm	48.41
21	*Utterbackia imbecillus*	Mussel	52.51
22	*Oncorhynchus nerka*	Sockeye salmon	54.82
23	*Poeciliopsis occidentalis*	Gila topminnow	56.15
24	*Gila elegans*	Bonytail chub	63.22
25	*Salvelinus confluentus*	Bull trout	68.31
26	*Pimephales promelas*	Fathead minnow	69.63
27	*Scaphirhynchus platorynchus*	Shovelnose sturgeon	69.63
28	*Xyrauchen texanus*	Razorback sucker	78.66
29	*Etheostoma lepidum*	Greenthroat darter	82.80
30	*Etheostoma flabellare*	Fantail darter	124.3
31	*Ptychocheilus oregonensis*	Colorado pikeminnow	132.2
32	*Etheostoma nigrum*	Johnny darter	178.3
33	*Acrocheilus alutaceus*	Chiselmouth	216.3
34	*Chironomus decorus*	Midge	1,987
35	*Lepomis macrochirus*	Bluegill sunfish	2,231
36	*Campeloma decisum*	Snail	3,573
37	*Acroneuria lycorias*	Stonefly	20,636
38	*Notemigonus crysoleucas*	Golden shiner	107,860

Appendix Table B.2: Ranked freshwater genus mean acute values (USEPA 2007). **

** at water quality BLM-normalized to 20°C, pH 7.5, DOC 0.5 mg/L, Ca = 14.0 mg/L, Mg = 12.1 mg/L, Na = 26.3 mg/L, K = 2.1 mg/L, SO4 = 81.4 mg/L, Cl = 1.90 mg/L, Alkalinity = 65.0 mg/L and S = 0.0003 mg/L

Rank	Species Scientific Name	Common Name	Species Mean Acute Value (ug/L)
1	*Daphnia*	Cladoceran	4.05
2	*Ceriodaphnia*	Cladoceran	5.93
3	*Lithoglyphus*	Snail	6.67
4	*Gammarus*	Amphipod	9.60
5	*Scapholeberis*	Cladoceran	9.73
6	*Actinonaias*	Mussel	11.33
7	*Hyalella*	Amphipod	12.07
8	*Juga*	Snail	12.31
9	*Physa*	Snail	20.41
10	*Oncorhynchus*	Salmon/trout	31.39
11	*Ptychocheilus*	Pikeminnow	43.94
12	*Bufo*	Toad	47.49
13	*Lumbriculus*	Worm	48.41
14	*Utterbackia*	Mussel	52.51
15	*Poeciliopsis*	Topminnow	56.15
16	*Gila*	Chub	63.22
17	*Salvelinus*	Trout	68.31
18	*Pimephales*	Minnow	69.63
19	*Scaphirhynchus*	Sturgeon	69.63
20	*Xyrauchen*	Sucker	78.66
21	*Etheostoma*	Darter	80.38
22	*Acrocheilus*	Chiselmouth	216.3
23	*Chironomus*	Midge	1,987
24	*Lepomis*	Sunfish	2,231
25	*Campeloma*	Snail	3,573
26	*Acroneuria*	Stonefly	20,636
27	*Notemigonus*	Shiner	107,860

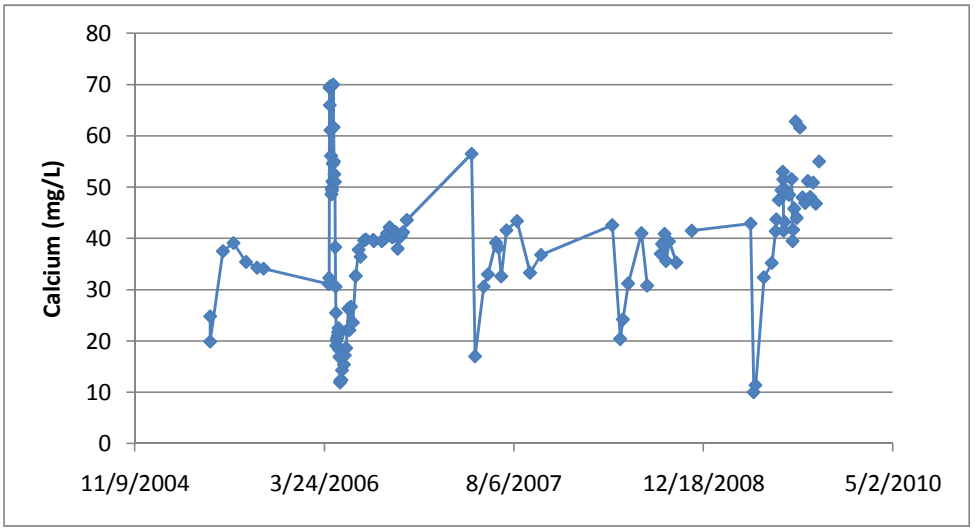
Appendix Table B.3: Ranked freshwater species mean chronic values (USEPA 2007). **

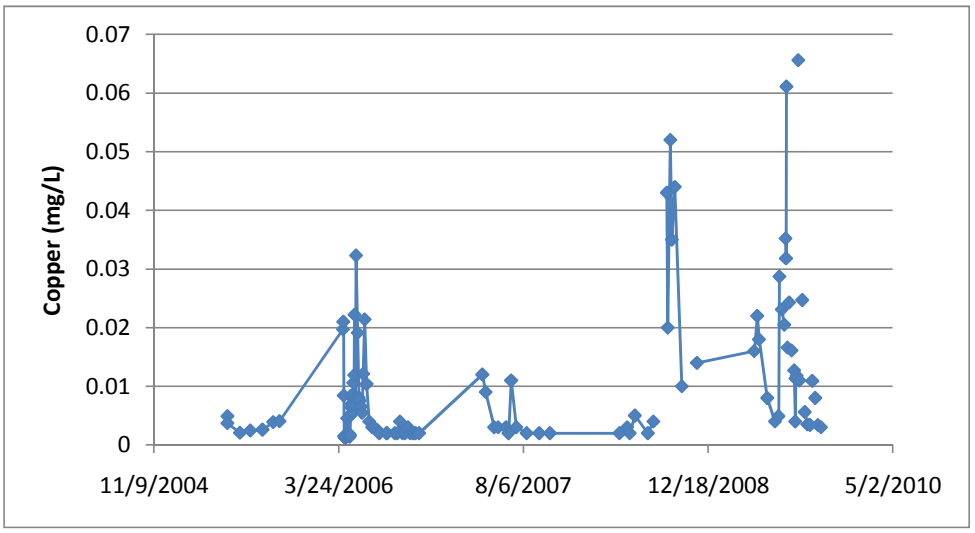
** not BLM-normalized

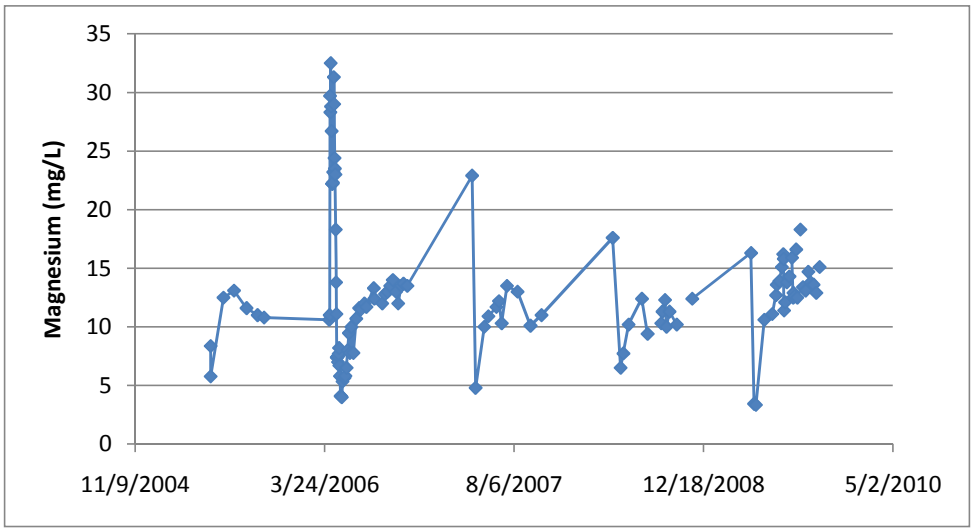
Rank	Species Scientific Name	Species Common Name	Species Mean Chronic Value (ug/L)
1	*Brachionus calyciflorus*	Rotifer	3.54
2	*Daphnia pulex*	Cladoceran	5.68
3	*Oncorhynchus tshawytscha*	Chinook salmon	5.92
4	*Clistoronia magnifica*	Caddisfly	7.67
5	*Pimephales promelas*	Fathead minnow	9.38
6	*Campeloma decisum*	Snail	9.77
7	*Salvelinus fontinalis*	Brook trout	12.5
8	*Daphnia magna*	Cladoceran	14.1
9	*Pimephales notatus*	Bluntnose minnow	18.0
10	*Ceriodaphnia dubia*	Cladoceran	19.3
11	*Catostomus commersoni*	White sucker	20.9
12	*Oncorhynchus mykiss*	Rainbow trout	23.8
13	*Lepomis macrochirus*	Bluegill sunfish	27.2
14	*Salmo trutta*	Brown trout	29.9
15	*Salvelinus namaycush*	Lake trout	30.9
16	*Esox lucius*	Northern pike	60.4

APPENDIX C

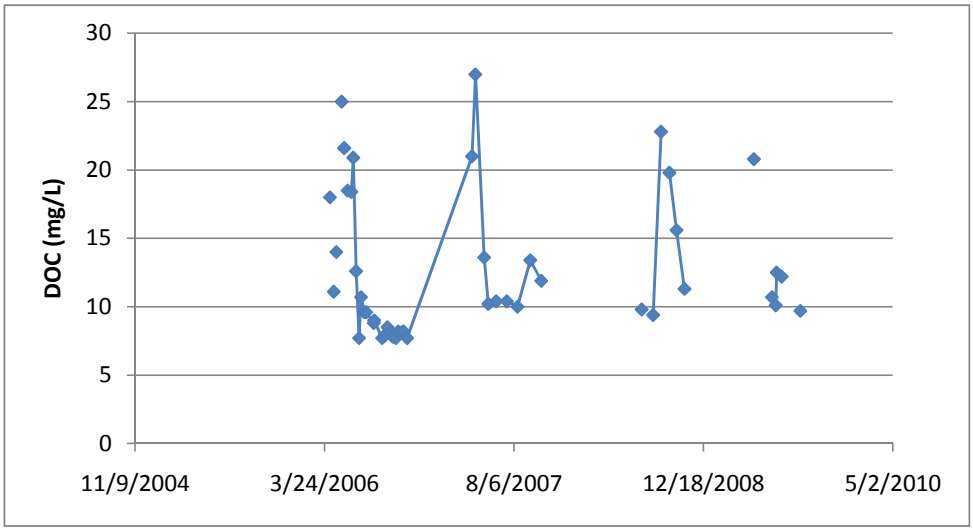
MINTO CREEK WATER QUALITY

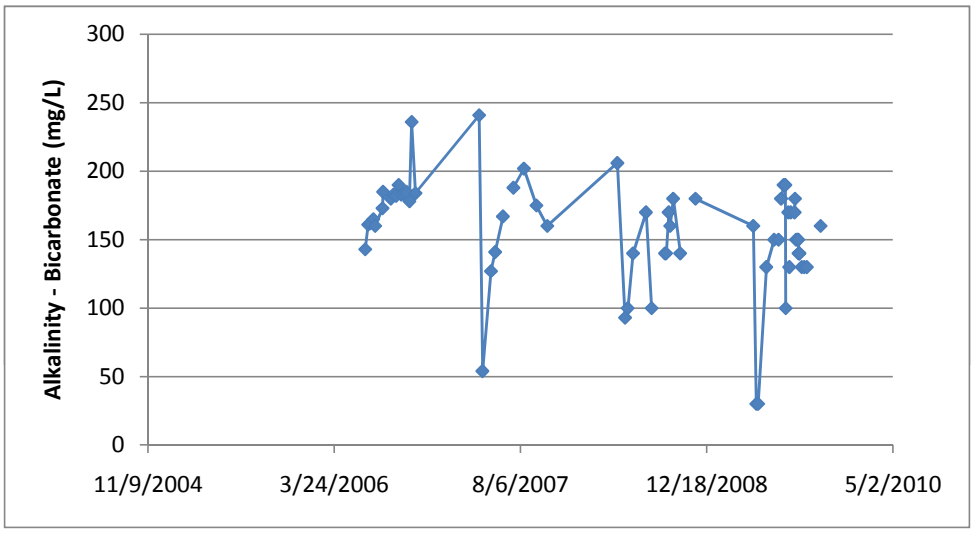


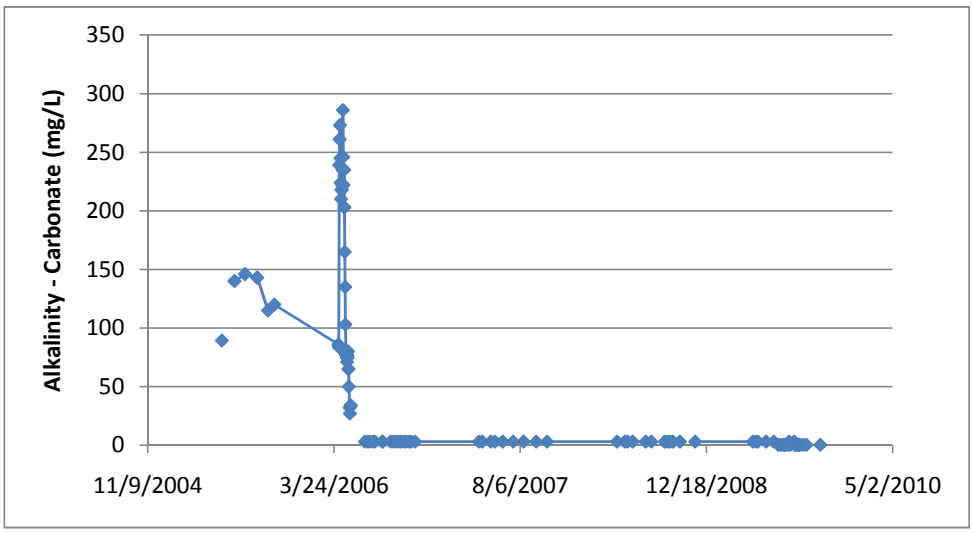




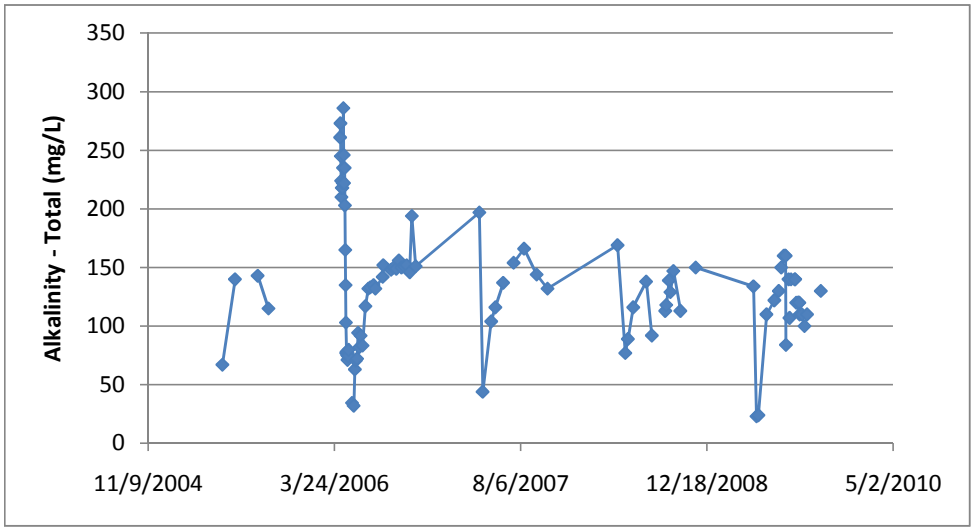
Appendix Figure C.1: Plots of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.

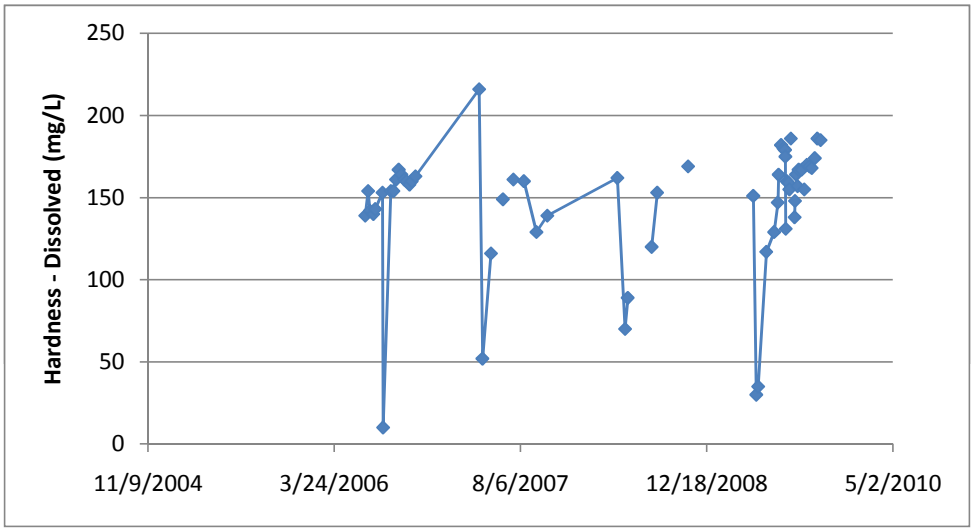


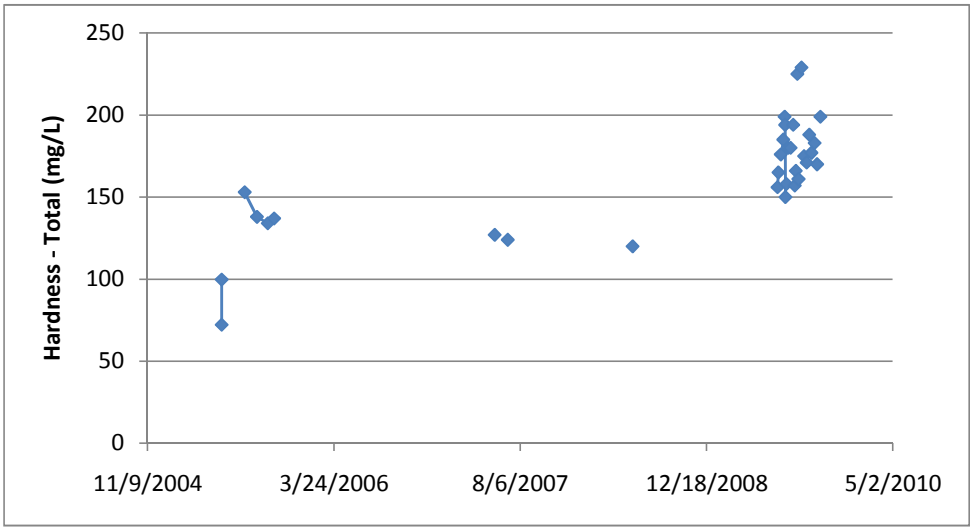




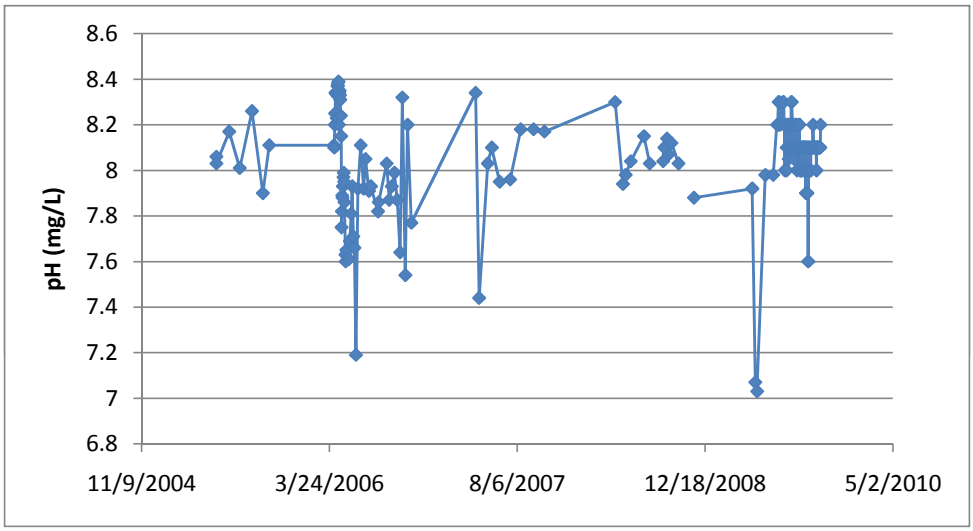
Appendix Figure C.1: Plots of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.







Appendix Figure C.1: Plots of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.



Appendix Figure C.1: Plots of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.

Appendix Table C.1: Concentrations of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.

Sample Date	Ca-T	Cu-T	Mg-T	C-DOC	Alk-Bicrb	Alk-Carb	Alk-T	Hard-D	Hard-T	pH-L
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	pH units
5/27/2005	24.8	0.0049	8.37			89.3			99.8	8.06
5/27/2005	19.9	0.0037	5.78				67.1		72.2	8.03
6/30/2005	37.5	0.00207	12.5			140	140			8.17
7/28/2005	39.1	0.00245	13.1			146			153	8.01
8/30/2005	35.4	0.0026	11.6			143	143		138	8.26
9/28/2005	34.3	0.0039	11			115	115		134	7.9
10/15/2005	34.1	0.00404	10.8			120			137	8.11
4/5/2006	31.1	0.0197	10.6			86				8.11
4/6/2006	32.3	0.021	11			84				8.1
4/7/2006	69.4	0.0084	29.7	18		239				8.2
4/8/2006	66	0.0015	28.3			261	261			8.25
4/9/2006	69.7	0.0013	32.5			273	273			8.34
4/10/2006	61.1	0.00126	28.8			245	245			8.34
4/11/2006	56.1	0.0012	26.7			224	224			8.34
4/12/2006	48.6	0.00125	22.2			210	210			8.23
4/13/2006	49.8	0.00146	22.2			218	218			8.33
4/14/2006	49.4	0.00139	22.2			218	218			8.37
4/15/2006	51.1	0.00162	22.3			222	222			8.38
4/16/2006	54.6	0.00453	23.2			235	235			8.37
4/17/2006	70	0.0019	31.3	11.1		286	286			8.39
4/18/2006	61.7	0.00152	29			246	246			8.2
4/19/2006	55	0.00146	24.4			222	222			8.34
4/20/2006	52.5	0.00185	23.5			235	235			8.35
4/21/2006	51	0.00175	23			203	203			8.33
4/22/2006	38.3	0.00184	18.3			165	165			8.31
4/23/2006	30.6	0.00137	13.8			135	135			8.24
4/24/2006	25.5	0.00164	11.1	14		103	103			8.15
4/25/2006	19.1	0.00501	7.4			77	77			7.75
4/26/2006	20.1	0.00833	7.4			78	78			7.82
4/27/2006	20.6	0.0064	7.5			75	75.2			7.89
4/28/2006	19	0.007	7			71	71.2			7.88
4/29/2006	21.1	0.0064	7.5			77	76.7			7.93
4/30/2006	21.7	0.0055	7.7			75	74.5			7.97
5/1/2006	22.5	0.0069	8.2			80	79.8			7.99
5/2/2006	18.4	0.0086	6.7			65				7.95
5/3/2006	16.9	0.0106	5.8			50				7.86
5/5/2006	11.9	0.0119	4.1			32				7.63
5/6/2006	12.3	0.0222	4.1			27				7.6
5/8/2006	12.4	0.00803	4	25		34				7.65
5/10/2006	14.3	0.0323	5.34				34.5			7.65
5/14/2006	15.4	0.0191	5.65	21.6			32.1			7.61
5/17/2006	17.2	0.00806	5.81				63.1			7.69
5/20/2006	18.6	0.00753	6.51				70.6			7.81
5/23/2006	22.1	0.00653	8.01	18.5			72.2			7.93
5/26/2006	26.3	0.00546	9.47				94.3			7.71
5/29/2006	22.1	0.0121	7.77				82			7.66
6/2/2006	26.7	0.0214	9.99	18.4			91.8			7.19
6/7/2006	23.6	0.0104	7.78	20.9			83.4			7.92
6/15/2006	32.7	0.004	10.7	12.6	143	<6	117	139		8.11
6/23/2006	37.8	0.003	11.6	7.7	161	<6	132	154		7.92
6/28/2006	36.4	0.003	11.5	10.7	162	<6	133	142		8.05
7/7/2006	39.6	<0.005	12	9.6	165	<6	135	140		7.91
7/12/2006	39.8	0.002	11.7	9.6	160	<6	132	143		7.93
7/31/2006	39.7	0.002	13.3	8.8	173	<6	142	153		7.82
8/2/2006	39.5	0.002	12.4	9	185	<6	152	10		7.86
8/23/2006	39.5	0.002	12	7.7	180	<6	148	154		8.03
8/30/2006	39.9	0.002	12.8	7.9	183	<6	150	154		7.87

Appendix Table C.1: Concentrations of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.

Sample Date	Ca-T	Cu-T	Mg-T	C-DOC	Alk-Bicrb	Alk-Carb	Alk-T	Hard-D	Hard-T	pH-L
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	pH units
9/6/2006	41	0.004	13	8.5	182	<6	149	161		7.93
9/13/2006	42.2	0.002	13.5	8.1	190	<6	156	167		7.99
9/20/2006	40.2	0.002	14	7.8	183	<6	150	164		7.87
9/28/2006	41.3	0.003	13	7.7	184	<6	151	161		7.64
10/4/2006	38	0.002	12	8.2	185	<6	152	160		8.32
10/12/2006	40.4	0.002	13.5	8.1	178	<6	146	158		7.54
10/18/2006	41.2	0.002	13.7	8.2	236	<6	194	160		8.2
10/28/2006	43.6	0.002	13.5	7.7	184	<6	151	163		7.77
4/17/2007	56.5	0.012	22.9	21	241	<6	197	216		8.34
4/26/2007	17	0.009	4.8	27	54	<6	44	52		7.44
5/19/2007	30.6	0.003	10	13.6	127	<6	104	116		8.03
5/30/2007	33	0.003	10.9	10.2	141	<6	116		127	8.1
6/20/2007	39.2	0.003	11.7	10.4	167	<6	137	149		7.95
6/27/2007	38.2	0.002	12.2							
7/4/2007	32.6	0.011	10.3						124	
7/18/2007	41.6	0.003	13.5	10.4	188	<6	154	161		7.96
8/15/2007	43.4	0.002	13	10	202	<6	166	160		8.18
9/18/2007	33.3	0.002	10.1	13.4	175	<6	144	129		8.18
10/17/2007	36.8	0.002	11	11.9	160	<6	132	139		8.17
4/22/2008	42.6	0.002	17.6		206	<6	169	162		8.3
5/13/2008	20.4	0.003	6.51		93	<6	77	70		7.94
5/20/2008	24.2	0.002	7.73		100	<6	89	89		7.98
6/3/2008	31.2	0.005	10.2		140	<6	116		120	8.04
7/8/2008	41	0.002	12.4	9.8	170	<6	138			8.15
7/23/2008	30.8	0.004	9.4		100	<6	92	120		8.03
8/7/2008				9.4				153		
8/28/2008	37	0.043	10.3	22.8	140	<6	113			8.04
8/31/2008	38.9	0.02	11.3		140	<6	118			8.1
9/7/2008	40.9	0.052	12.3		170	<6	139			8.14
9/11/2008	35.6	0.035	10		160	<6	129			8.07
9/19/2008	39.4	0.044	11.3	19.8	180	<6	147			8.12
10/8/2008	35.3	0.01	10.2	15.6	140	<6	113			8.03
10/29/2008				11.3				169		
11/18/2008	41.5	0.014	12.4		180	<6	150			7.88
4/22/2009	42.9	0.016	16.3		160	<6	134	151		7.92
4/30/2009	10	0.022	3.44	20.8	30	<6	23	30		7.07
5/5/2009	11.4	0.018	3.34		30	<6	24	35		7.03
5/27/2009	32.4	0.008	10.6		130	<6	110	117		7.98
6/17/2009	35.2	0.004	11.1	10.7	150	<6	122	129		7.98
6/26/2009	41.4	0.0049	12.7	10.1				147	156	8.2
6/27/2009										
6/28/2009										
6/29/2009	43.7	0.0287	13.6	12.5	150	<0.5	130	164	165	8.2
6/30/2009										
6/30/2009										
7/1/2009										8.3
7/2/2009										8.2
7/3/2009										8.3
7/4/2009										
7/5/2009	47.5	0.0231	13.9		180	<0.5	150	182	176	8.2
7/6/2009										
7/7/2009										
7/8/2009										
7/8/2009										
7/9/2009										
7/9/2009										
7/10/2009										

Appendix Table C.1: Concentrations of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.

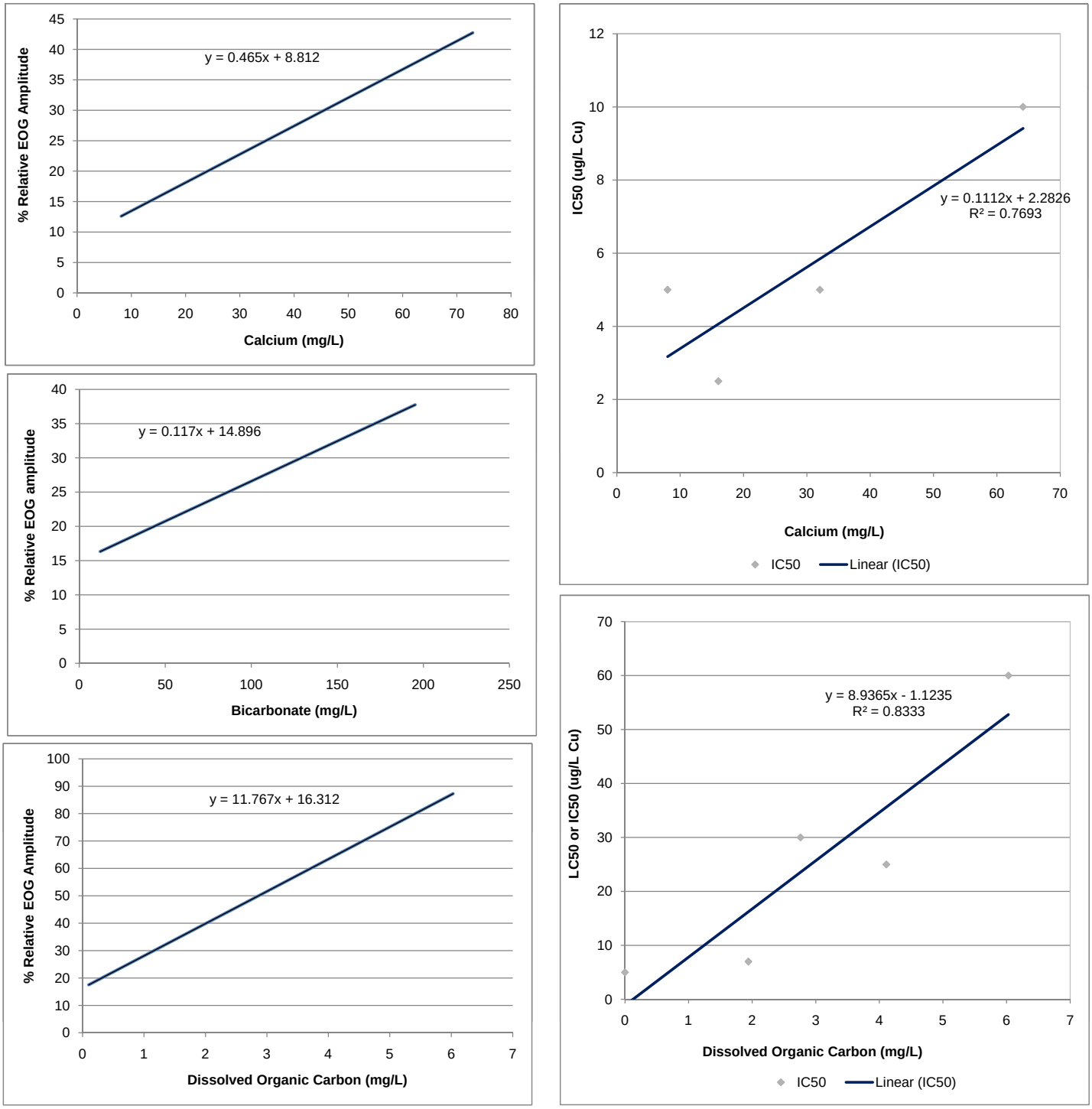
Sample Date	Ca-T	Cu-T	Mg-T	C-DOC	Alk-Bicrb	Alk-Carb	Alk-T	Hard-D	Hard-T	pH-L
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	pH units
7/10/2009										
7/11/2009										
7/11/2009										
7/12/2009										
7/12/2009	49.4	0.0205	15.1	12.2	190	<0.5	160	180	185	8.3
7/13/2009										
7/14/2009										
7/15/2009										8.3
7/16/2009	53	0.0352	16.2		190	<0.5	160	179	199	8.2
7/17/2009	51.5	0.0318	15.8		190	<0.5	160	175	194	8.2
7/18/2009	41.5	0.0611	11.4		100	<0.5	84	131	150	8
7/19/2009										8.2
7/20/2009										
7/21/2009	43.2	0.0166	12.1					160	158	
7/22/2009										8.1
7/22/2009										8
7/23/2009										
7/24/2009										
7/24/2009										8.2
7/25/2009										
7/25/2009	49.3	0.0243	13.8		170	<0.5	140	159	180	8.2
7/26/2009										8.1
7/26/2009										8.2
7/27/2009										8.2
7/28/2009					130	<6	107	155		8.05
7/29/2009										8.1
7/30/2009										8.1
7/31/2009										8.1
8/1/2009	48.5	0.0161	14.3		170	<0.5	140	186	180	8.2
8/1/2009										
8/2/2009										8.2
8/2/2009										
8/3/2009										8.1
8/3/2009										
8/4/2009										8.3
8/5/2009										8.3
8/8/2009	51.6	0.0127	15.9						194	8.2
8/9/2009										
8/11/2009	39.5	0.004	12.5		170	<6	140	138		8.05
8/12/2009	41.7	0.0113	12.9		180	<0.5	140	148	157	8.1
8/14/2009										8.1
8/15/2009	45.8	0.0118	12.6		150	<0.5	120	164	166	8.2
8/16/2009										8.2
8/17/2009										8.2
8/18/2009										8.2
8/19/2009	62.8	0.0656	16.6		150	<0.5	120	157	225	8
8/20/2009					150	<0.5	120			8.1
8/21/2009					150	<0.5	120			8.1
8/22/2009	44	0.011	12.5		140	<0.5	120	167	161	8.1
8/23/2009					140	<0.5	120			8.1
8/24/2009					140	<0.5	110			8.2
8/25/2009										8.2
8/26/2009										8.2
8/27/2009										8
8/28/2009										8.1
8/29/2009										8
8/30/2009	61.6	0.0247	18.3		130	<0.5	110	167	229	8

Appendix Table C.1: Concentrations of copper and key copper bioavailability/toxicity modifiers in lower Minto Creek (Station W2), 2005-2009.

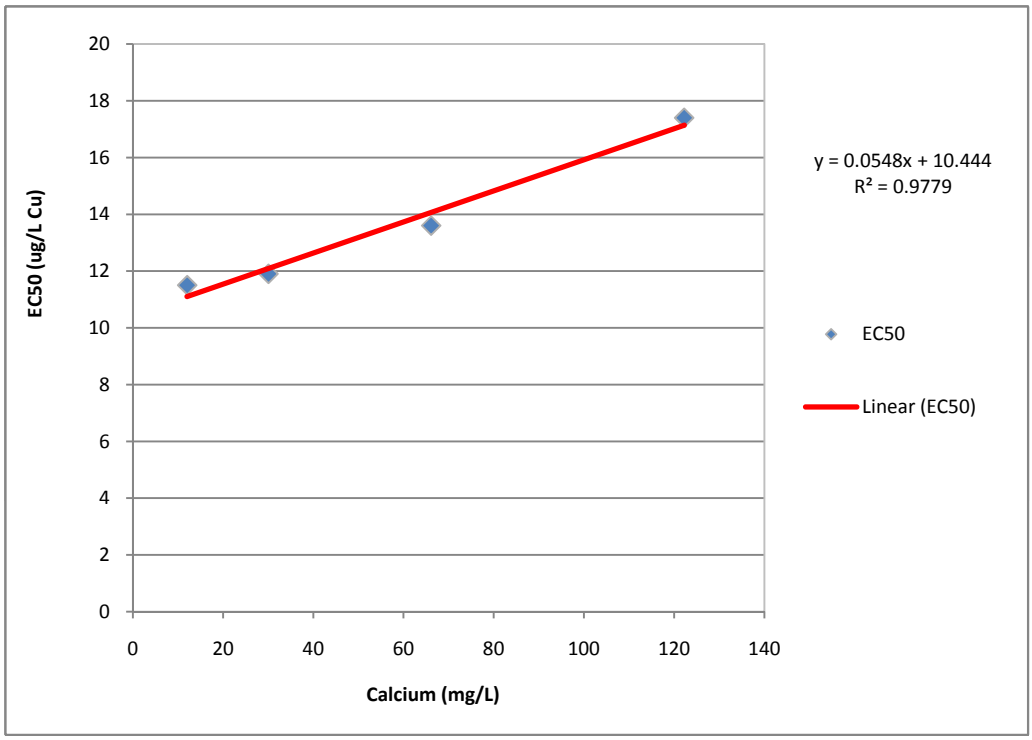
Sample Date	Ca-T	Cu-T	Mg-T	C-DOC	Alk-Bicrb	Alk-Carb	Alk-T	Hard-D	Hard-T	pH-L
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	pH units
8/31/2009				9.7						8
9/1/2009										8.1
9/2/2009										8.1
9/3/2009										8.1
9/4/2009										8.1
9/5/2009										8.1
9/6/2009	48	0.0056	13.4		130	<0.5	100	155	175	8
9/7/2009										8.1
9/8/2009										8.1
9/9/2009										8.1
9/10/2009										8.1
9/11/2009										8.1
9/12/2009										7.9
9/13/2009	46.9	0.0035	13.1		130	<0.5	110	170	171	8.1
9/14/2009										8
9/15/2009										8.1
9/16/2009										7.9
9/17/2009										8.1
9/18/2009										7.6
9/19/2009										8.1
9/20/2009	51.2	0.0034	14.7					169	188	8.1
9/21/2009										8
9/22/2009										8
9/23/2009										8
9/24/2009										8.1
9/25/2009										8
9/26/2009	48.1	0.0109	13.8					168	177	8.1
9/27/2009										8.1
9/28/2009										8.1
9/29/2009										8.1
9/30/2009										8.1
10/1/2009										8.2
10/2/2009										8.1
10/3/2009										8.1
10/4/2009	50.9	0.008	13.6					174	183	8.1
10/5/2009										8.1
10/6/2009										8.1
10/7/2009										8.1
10/8/2009										8.1
10/9/2009										8.1
10/10/2009										8
10/11/2009	46.8	0.0034	12.9					186	170	8.1
10/12/2009										8.1
10/13/2009										8.1
10/14/2009										8.1
10/15/2009										8.1
10/16/2009										8.1
10/17/2009										8.1
10/18/2009										8.1
10/19/2009										8.1
10/20/2009	55	0.003	15.1		160	<0.5	130	185	199	8.1
10/21/2009										8.2

APPENDIX D

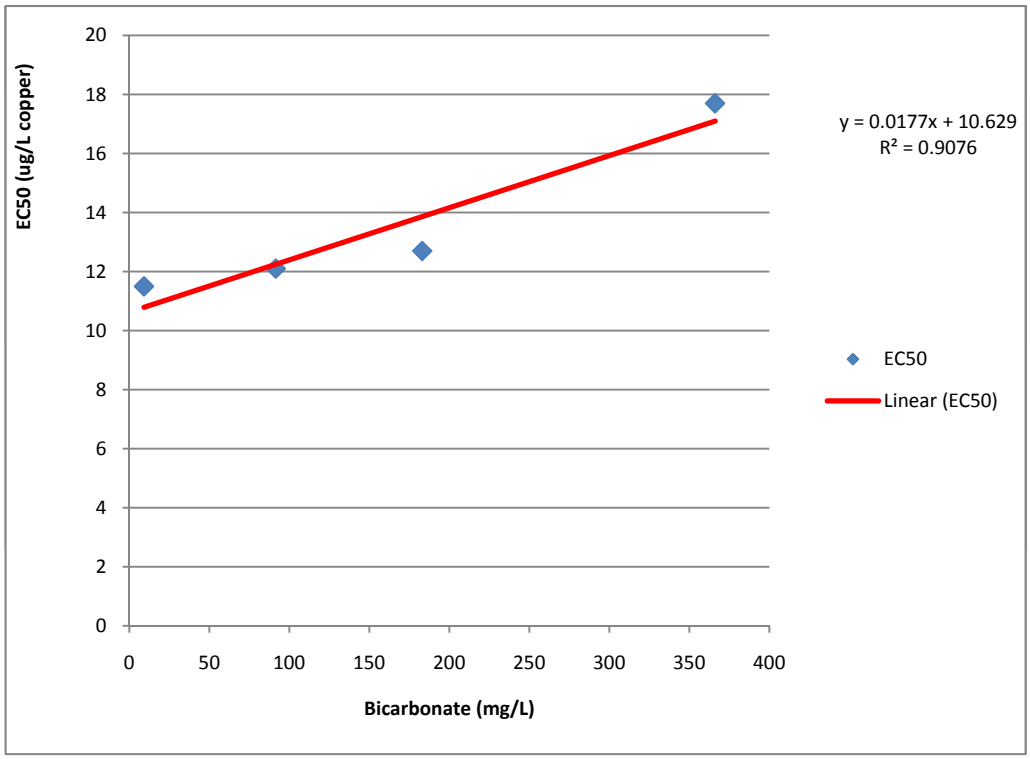
QUANTITATIVE RELATIONSHIPS BETWEEN OLFACTORY EFFECTS OF COPPER AND WATER QUALITY



Appendix Figure D.1: Protective roles of calcium, bicarbonate and dissolved organic carbon on the electro-olfactogram response in juvenile coho salmon (McIntyre et al. 2008a,b,c).

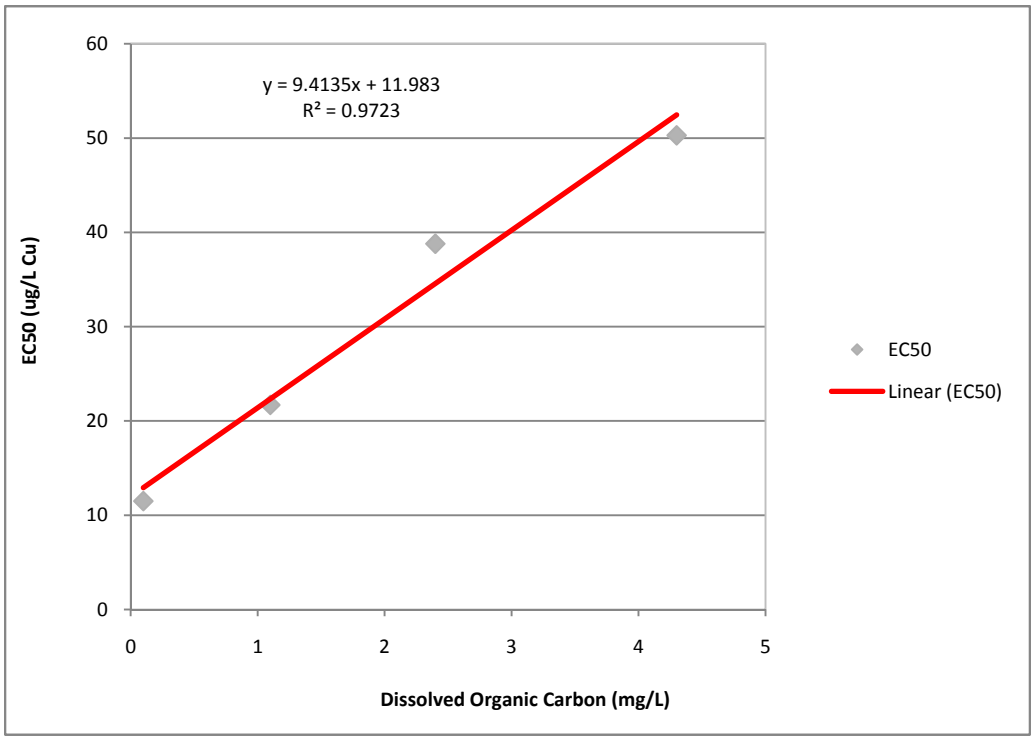


A) Relationship Between Effect Concentrations and Calcium (from Linbo 2009)

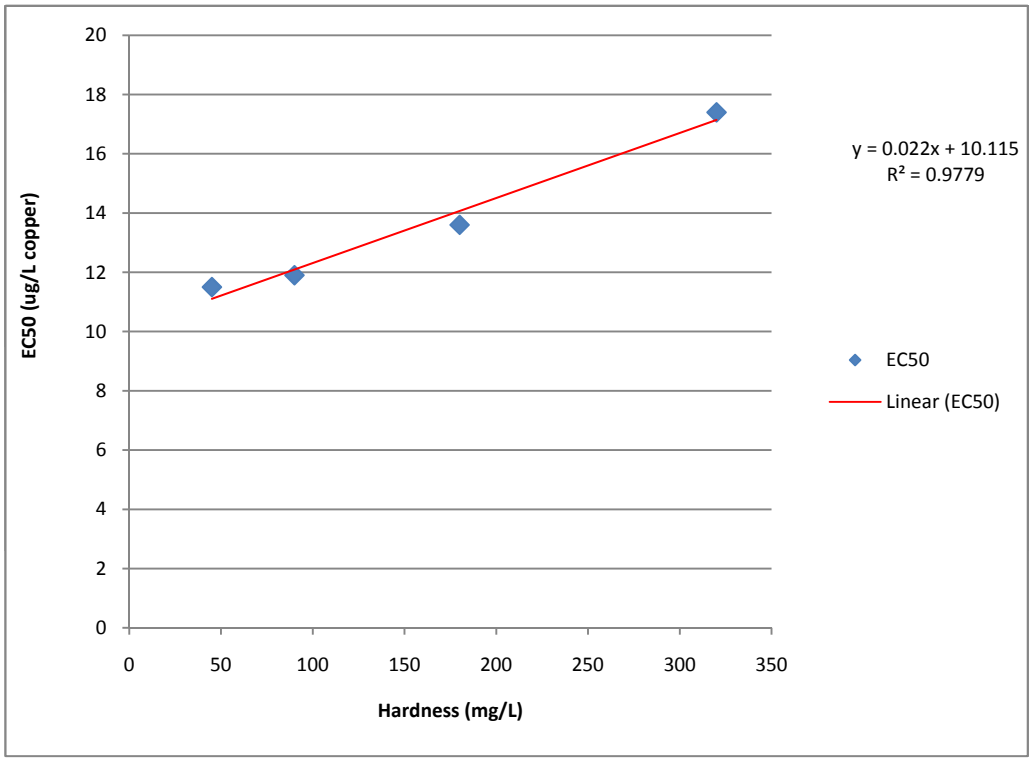


B) Relationship Between Effect Concentrations and Bicarbonate (from Linbo 2009)

Appendix Figure D.2: Protective roles of water quality on the effects of copper on mechano-sensory cells of larval zebrafish.

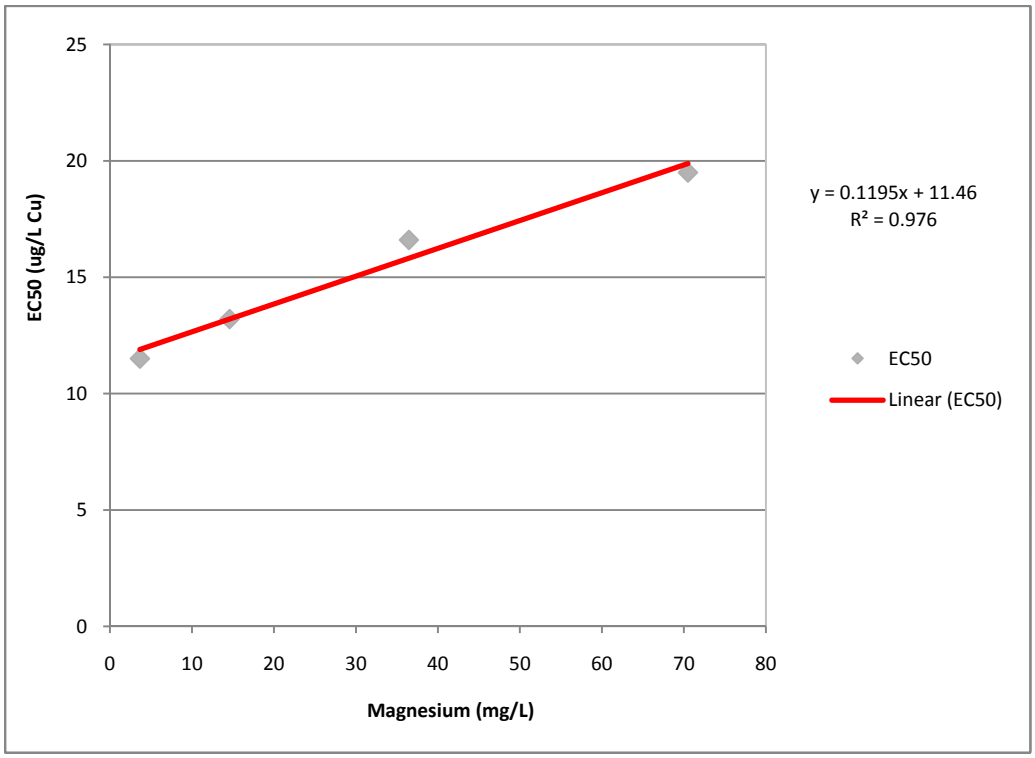


C) Relationship Between Effect Concentrations and DOC (from Linbo 2009)

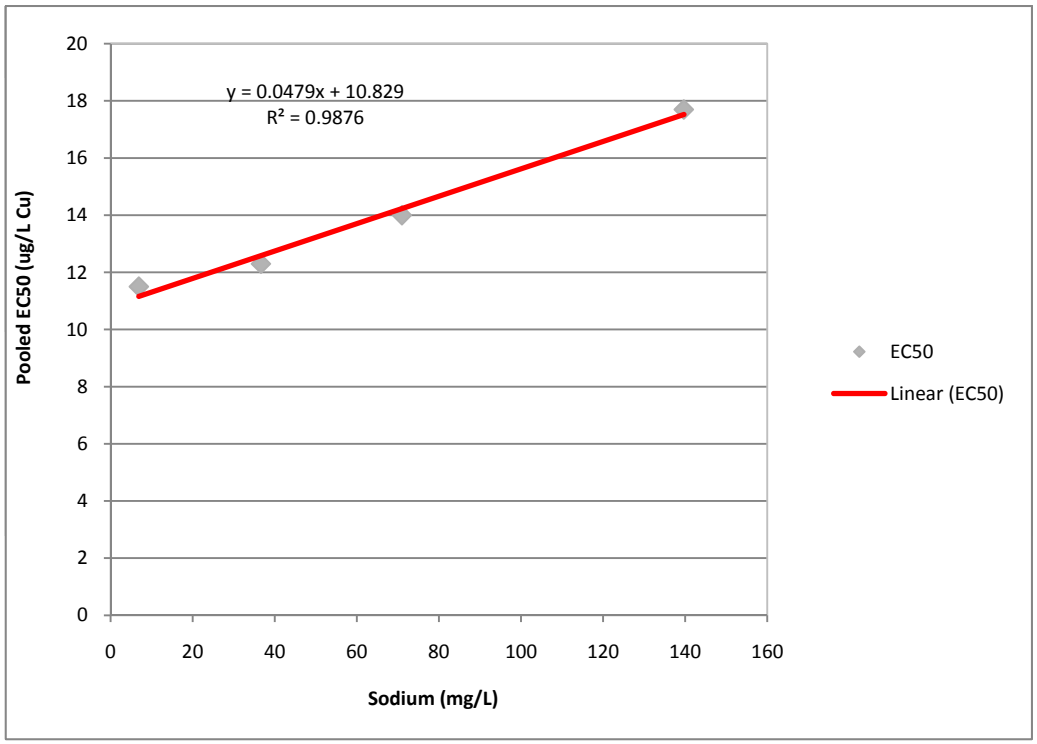


D) Relationship Between Effect Concentrations and Hardness (from Linbo 2009)

Appendix Figure D.2: Protective roles of water quality on the effects of copper on mechano-sensory cells of larval zebrafish.

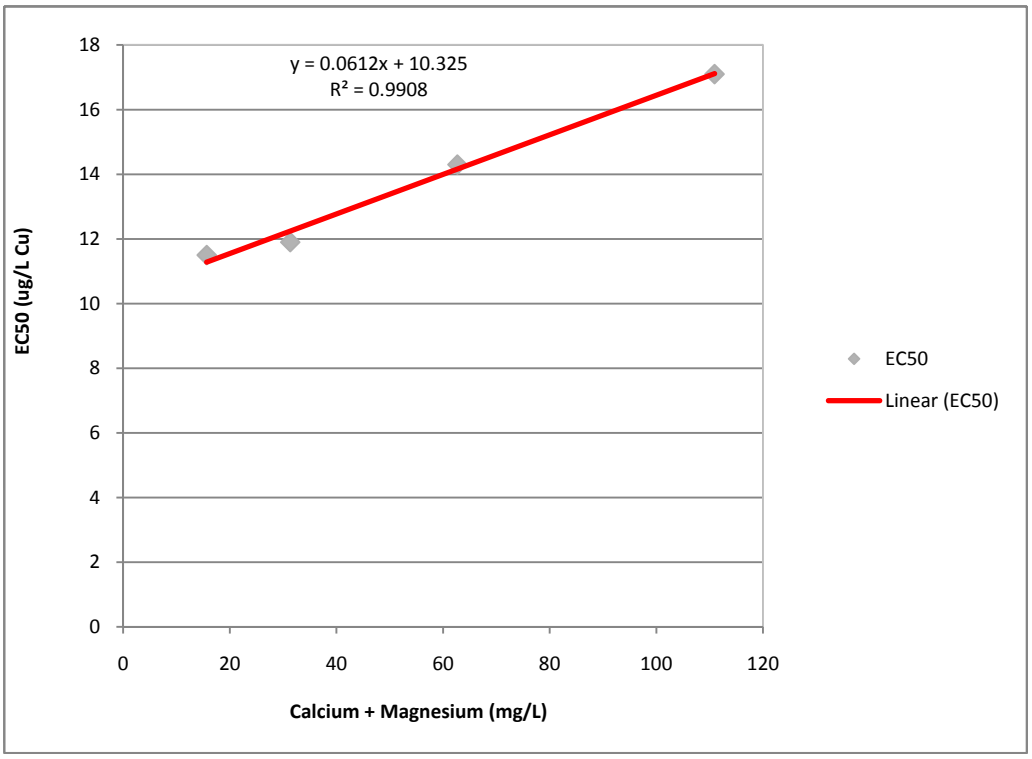


E) Relationship Between Effect Concentrations and Magnesium (from Linbo 2009)



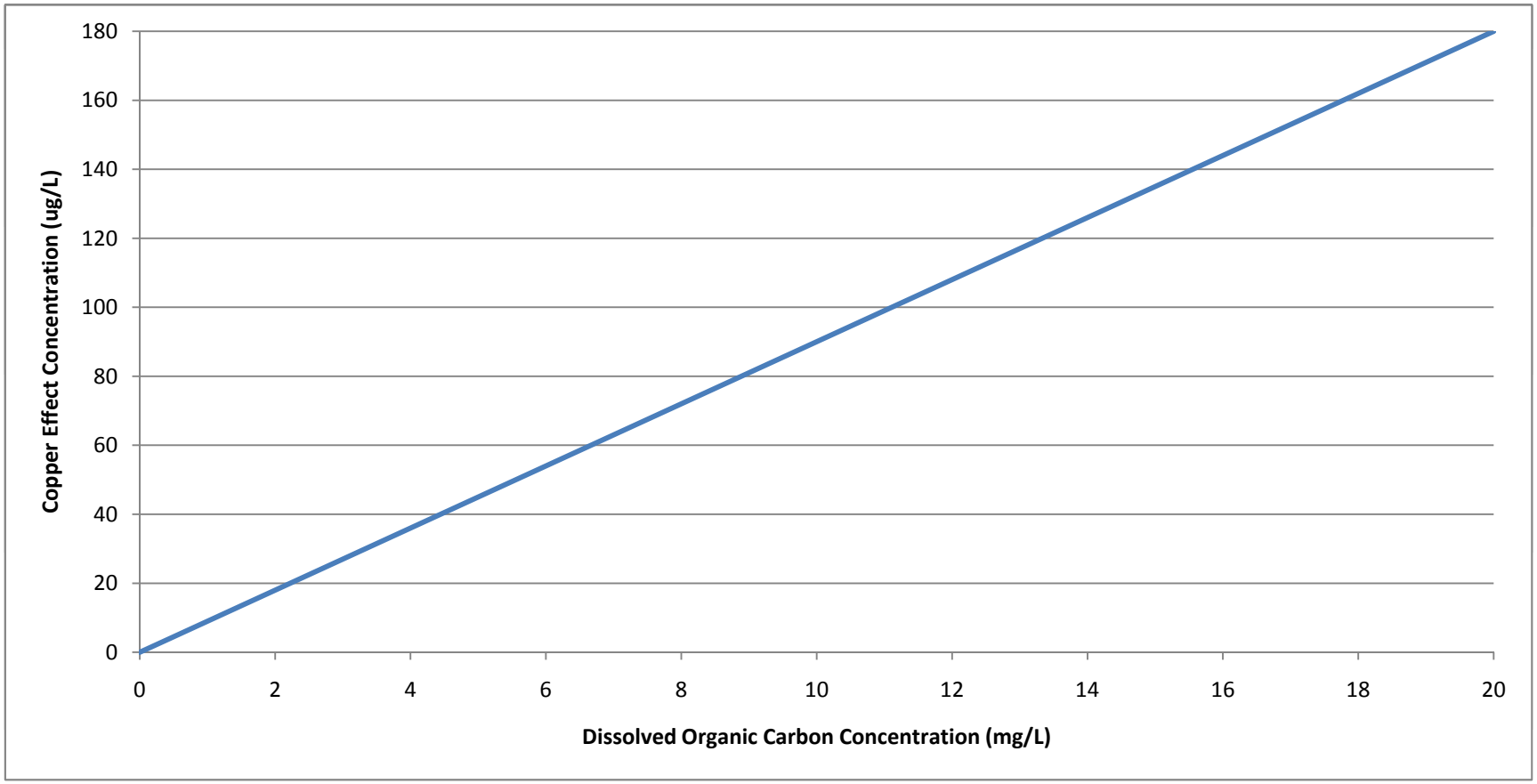
F) Relationship Between Effect Concentrations and NaCl Sodium (from Linbo 2009)

Appendix Figure D.2: Protective roles of water quality on the effects of copper on mechano-sensory cells of larval zebrafish.



G) Relationship Between Effect Concentrations and Magnesium (from Linbo 2009)

Appendix Figure D.2: Protective roles of water quality on the effects of copper on mechano-sensory cells of larval zebrafish.



Appendix Figure D.3: Hypothetical relationship between copper effect concentration (IC50 or EC50) and dissolved organic carbon concentration with an origin of 0,0 and a slope of 9 (copper effect concentration [ug/L]:dissolved organic carbon concentration [mg/L]) as determined by McIntyre et al. (2008) and Linbo et al. (2009).