# Annotated Bibliography: Effects of Copper on Fish 2009

Alam, M. K. and O. E. Maughan (1992). "THE EFFECT OF MALATHION, DIAZINON, AND VARIOUS CONCENTRATIONS OF ZINC, COPPER, NICKEL, LEAD, IRON, AND MERCURY ON FISH." Biological Trace Element Research **34**(3): 225-236.

Acute and chronic toxicity tests for malathion, diazinon, copper (Cu), mercury (Hg), lead (Pb), zinc (Zn), nickel (Ni), and iron (Fe) were conducted. Mortalities of Barilius vagra and Cyprinus carpio (common carp) were variable but LC50-96 hr were similar for pesticides, Adult B. vagra seem to be more sensitive to malathion than juvenile carp. Both juvenile carp and adult B. vagra were extremely sensitive to diazinon. Long-term exposure to pesticides modified morphology and behavior. The LC50-96 values for Cu, Hg, and Pb were 0.3, 0.16, and 0.44, respectively, for smaller fish and 1.0, 0.77, and 1.33, respectively, for larger fish. Replicate LC50 values for Zn, Ni, and Fe were somewhat variable, and for these metals, the size of the fish seemed to affect response because LC50 values increased as fish size increased. Cooper, Pb, Zn, and Fe residues following exposure to sublethal concentrations of these metals for 15 d were significantly greater in whole juvenile common carp than in controls.

Apte, S. C., G. E. Batley, et al. (2005). "A comparison of copper speciation measurements with the toxic responses of three sensitive freshwater organisms." Environmental Chemistry **2**(4): 320-330.

Twelve natural fresh waters with similar pH and hardness, but varying dissolved organic carbon (DOC) and copper concentrations, were assessed for (a) toxicity to an alga (Chlorella sp. 12), a cladoceran (Ceriodaphnia cf. dubia) and a bacterium (Erwinnia sp.), and (b) copper speciation using a rapid Chelex extraction method, diffusive gradients in thin films (DGT) and anodic stripping voltammetry (ASV). In synthetic fresh water with no added DOC, at pH 7.0 and low hardness, the toxic responses (EC/IC50) of all three organisms to copper were similar. However, in the toxicity of copper added to natural water samples exhibited a negative linear relationship to DOC (r(2) = 0.82-0.83), with respective slopes for algae, cladocerans and bacteria decreasing in the ratio 7.4 : 3.5 : 1. The marked difference in responses in the presence of natural dissolved organic matter indicated that not all of the organisms conformed to the free ion activity model (FIAM). This was confirmed by copper ion selective electrode measurement of copper ion activity. Copper toxicity to algae in the presence of DOC was overestimated by free ion activity possibly due to surface binding of

DOC. Copper toxicity to the bacteria was greater than predicted and was shown to be a result of bioavailability of some copper complexes formed with organic matter. Cladocerans appear to more closely follow FIAM predictions. These findings have important implications for attempts to extend predictive models of metal toxicity beyond fish to more sensitive freshwater species. The measured labile copper concentrations of copper-spiked natural waters varied from 0 to 70% of total copper concentrations. There was no clear relationship between the three measurement techniques. Good correlations were obtained between both algal and bacterial growth inhibitions measured on copper-spiked natural waters and the corresponding Chelex-labile copper concentrations. A single natural water sample was manipulated to different pH and hardness values, spiked with copper, and tested using the above three organisms with the Chelex method. Toxicity test results generally agreed with studies performed in synthetic fresh waters, showing that the relationships between toxicity, pH and hardness were organism-specific. Chelex-labile copper was always over-predictive of toxicity but significantly better (P <= 0.05) than dissolved copper concentrations, as it only detects the fraction of total copper that is reactive over biologically-relevant timescales. Colloidally-bound copper and copper associated with strong ligands are not detected. The Chelex method is therefore useful as a measure where speciation is accepted in water quality regulations.

Arambasic, M. B., S. Bjelic, et al. (1995). "ACUTE TOXICITY OF HEAVY-METALS (COPPER, LEAD, ZINC), PHENOL AND SODIUM ON ALLIUM-CEPA L, LEPIDIUM-SATIVUM L AND DAPHNIA-MAGNA ST - COMPARATIVE INVESTIGATIONS AND THE PRACTICAL APPLICATIONS." Water Research **29**(2): 497-503.

The investigations of the effects of various heavy metal: Cu (as CuSO4), Pb (as Pb(NO3)(2)) and Zn (as ZnSO4), phenol and Na (as Na2SO4 and NaCl) concentrations on root length of onion bulbs (Allium cepa L.) and garden cress (Lepidium sativum L.), as well as on the survival rate of great water flea (Daphnia magna St.) showed varied sensitivity of the above test organisms. Based on IC50 Value (50% inhibitory concentration) for A. cepa L. and L. salivum L. and on LC5O value for D. magna St., the acute toxicity of the tested substances decreased after a 48-hour exposition in the following order: A. cepa L.: Cu > Pb > Zn > phenol > Na2SO4 > NaCl; L. sativum L.: phenol > Cu > Pb > Zn > Na2SO4 > NaCl; D. magna St.: Cu > Zn > phenol > Pb > Na2SO4 > NaCl.

Arnold, W. R., R. C. Santore, et al. (2005). "Predicting copper toxicity in estuarine and marine waters using the Biotic Ligand Model." Marine Pollution Bulletin **50**(12): 1634-1640.

The Biotic Ligand Model (BLM) has proven efficient in predicting the toxicity of a variety of metals to freshwater organisms. Consequently, the US EPA has proposed its use for calculating freshwater copper criteria. This study evaluates the BLM for use in estuarine and marine waters. Studies were conducted using the bivalve, Mytilus sp. and 48-h embryo-larval development chronic estimator test methods. These are the most sensitive taxa and test in the US EPA saltwater copper criteria database. Samples from five locations around the USA

were tested. There is a strong relationship between measured and BLM predicted copper EC50s (log transformed data, r(2) = 0.76, p < 0.001, n = 44). The BLM predicted within a factor 2 of measured EC50s in 41 of 44 cases. However, the BLM tends to predict lower EC50s when measured EC50s are approximately <= 10 mu g Cu/L. This may be due to limitations of the metal-dissolved organic matter interaction model. (c) 2005 Elsevier Ltd. All rights reserved.

Banerjee, S. and S. Homechaudhuri (1990). "HEMATOLOGICAL MONITORING OF A BIOINDICATOR FISH, HETEROPNEUSTES-FOSSILIS, ON EXPOSURE TO COPPER TOXICITY." Israeli Journal of Aquaculture-Bamidgeh **42**(2): 46-51.

Banks, K. E., S. H. Wood, et al. (2003). "Joint acute toxicity of diazinon and copper to Ceriodaphnia dubia." Environmental Toxicology and Chemistry **22**(7): 1562-1567.

Diazinon and copper are two contaminants that are widely found in urban streams and in municipal wastewater effluents. Because these contaminants may be found concurrently, the potential for their joint toxicity is of interest, particularly with regard to toxicity testing of wastewater effluents and the ecological implications of simultaneous exposures in urban streams. Although interactions between metals are well studied, relatively little is known about interactions between metals and organophosphate compounds such as diazinon. In this study, the interaction between copper and diazinon was evaluated using cladoceran (Ceriodaphnia dubia) in 48-h static tests within laboratory water. Using toxic units derived from concurrently established 48-h median lethal concentration values (LC50) of test organisms of each individual toxicant, the effects of the mixture of copper and diazinon on the survival of C. dubia were shown to be generally nonconcentration additive (LC50 significantly greater than one toxic unit). However, evaluation of the dose-response relationship across the entire range of effect levels revealed that the mortality induced by the mixture of copper and diazinon supported concentration additivity at higher effect levels.

Bearr, J. S., J. Diamond, et al. (2006). "Effects of pulsed copper exposures on early life-stage Pimephales promelas." Environmental Toxicology and Chemistry **25**(5): 1376-1382.

Effects of pulsed copper exposures were investigated using Pimephales promelas aged less than 24 It in short-term chronic testing (7 or 14 d) with moderately hard synthetic water. Concentrations tested were between the species mean chronic value (22 mu g/L at a hardness of 100 mg/L as CaCO3) and the 7-d continuous exposure EC50 for survival (40 mu g/L) to examine exposures that were not acutely toxic and representative of actual wastewater discharge permit exceedences. Factors tested included pulse duration, recovery time between pulses, and pulse frequency. Survival was the main endpoint affected in all treatments (analysis of variance, p < 0.05). Effects on fish biomass, independent of survival effects, were observed in only 2 of 86 treatments examined. Fish survival was negatively affected at average copper concentrations between 7 and 50% of the 7-d continuous exposure EC50.

Exposures having a 48- to 96-h recovery time between pulses had less effect on fish survival than did treatments with shorter (12-24 h) or longer (> 120 h) recovery times. Results suggest that the criteria averaging periods used in the United States, and the averaging periods typically used in wastewater discharge permit limits for copper, may not protect against effects of certain pulsed exposures.

Berntssen, M. H. G., A. K. Lundebye, et al. (1999). "Effects of elevated dietary copper concentrations on growth, feed utilisation and nutritional status of Atlantic salmon (Salmo salar L.) fry." Aquaculture **174**(1-2): 167-181.

The present experiment was conducted to study effects of elevated dietary Cu and establish upper limits of Cu in fish feed. Atlantic salmon fry were reared for 3 months on experimental diets containing either 5 (control), 35, 500, 700, 900, or 1750 nag Cu kg(-1), provided as CuSO4 . 5H(2)O. Dietary Cr2O3 was included (1%) in all experimental diets for the last two weeks in order to assess apparent digestibility of both major food components and availability of minerals (Cu, Zn and Se). Growth was significantly ($P < 0.01$) reduced after 3 months at dietary Cu concentrations of greater than or equal to 500 mg Cu kg(-1) compared to the 35 mg Ca kg(-1) group. Similarly, whole-body content of protein, glycogen and Se were significantly reduced at these dietary Cu concentrations compared to controls. Apparent digestibility (%) of the major food components and availability of Zn and Se did not differ among dietary treatments. However, apparent availability of dietary Cu was significantly reduced in fish fed experimental diets containing less than or equal to 900 mg kg(-1) compared to controls. Whole-body minus intestine Cu concentrations were only significantly increased in fish fed dietary Cu concentrations greater than or equal to 900 mg Cu kg(-1) compared to controls, indicating a strong intestinal regulating capacity of dietary Cu uptake. Dietary Cu concentrations of 500 mg Cu kg(-1) and above caused toxic responses in Atlantic salmon fry as concluded from the antagonistic interaction with Se, and reduced growth and whole-body energy stores (protein and glycogen). 

Besser, J. M., C. A. Mebane, et al. (2007). "Sensitivity of mottled sculpins (Cottus bairdi) and rainbow trout (Onchorhynchus mykiss) to acute and chronic toxicity of cadmium, copper, and zinc." Environmental Toxicology and Chemistry **26**(8): 1657-1665.

Studies of fish communities of streams draining mining areas suggest that sculpins (Cottus spp.) may be more sensitive than salmonids to adverse effects of metals. We compared the toxicity of zinc, copper, and cadmium to mottled sculpin (C. bairdi) and rainbow trout (Onchorhynchus mykiss) in laboratory toxicity tests. Acute (96-h) and early life-stage chronic (21- or 28-d) toxicity tests were conducted with rainbow trout and with mottled sculpins from populations in Minnesota and Missouri, USA, in diluted well water (hardness = 100 mg/L as CaCO3). Acute and chronic toxicity of metals to newly hatched and swim-up stages of mottled sculpins differed between the two source populations. Differences between populations were greatest for copper, with chronic toxicity values (ChV = geometric mean of lowest-observed-effect concentration and no-

observed-effect concentration) of 4.4 mu g/L for Missouri sculpins and 37 mu g/L for Minnesota sculpins. Cadmium toxicity followed a similar trend, but differences between sculpin populations were less marked, with ChVs of 1.1 mu g/L (Missouri) and 1.9 mu g/L (Minnesota). Conversely, zinc was more toxic to Minnesota sculpins (ChV = 75 mu g/L) than Missouri sculpins (chronic ChV = 219 mu Lg/L). Species-average acute and chronic toxicity values for mottled sculpins were similar to or lower than those for rainbow trout and indicated that mottled sculpins were among the most sensitive aquatic species to toxicity of all three metals. Our results indicate that current acute and chronic water quality criteria for cadmium, copper, and zinc adequately protect rainbow trout but may not adequately protect some populations of mottled sculpins. Proposed water quality criteria for copper based on the biotic ligand model would be protective of both sculpin populations tested.

Blaylock, B. G., M. L. Frank, et al. (1985). "COMPARATIVE TOXICITY OF COPPER AND ACRIDINE TO FISH, DAPHNIA AND ALGAE." Environmental Toxicology and Chemistry **4**(1): 63-71.

Bopp, S. K., H. K. Abicht, et al. (2008). "Copper-induced oxidative stress in rainbow trout gill cells." Aquatic Toxicology **86**(2): 197-204.

Copper is known to pose a serious threat to aquatic organisms. However, the mechanisms of its toxicity still remain unclear. Cu is known to exert its toxicity partly due to the formation of reactive oxygen species (ROS). The purpose of this work was therefore to link the exposure to copper at pH 6 and 7 to cellular formation of ROS and effects like cell viability and genotoxicity using the rainbow trout gill cell line RTgill-W1. To relate effects to bioavailable copper, free Cu2+ concentrations in the medium were calculated using the programm ChemEQL 3.0. 2',7'-Dichlorodihydrofluorescein-diacetate (H2DCF-DA) was used as cell-permeant indicator of ROS formation. Cell viability was assessed using the fluorogenic probe 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM). DNA strand breaks were assessed using the comet assay, and lipid peroxidation was investigated using the thiobarbituric acid-reactive substances assay (TBARS). Copper treatment resulted in a dose-dependent elevation in cytotoxicity and formation of cellular ROS. Cell viability was significantly reduced at total copper (CUT) concentrations of 5 mu M (corresponding to a free Cu2+ of 0.11 mu M at pH 7) and higher, resulting in an EC50 of Cu-T = 29.2 mu M (Cu2+ = 0.63 mu M, pH 7). Neither an impairment concerning the viability of control cells due to growth at pH 6 was observed nor significant differences for cytotoxicity in cells exposed to the same nominal Cu-T concentrations at pH 6 compared to pH 7. Cellular ROS concentrations increased significantly and decreased with loss of cell viability. After normalizing ROS formation to cell viability, ROS induction up to 25-35-fold compared to the control was detected, but mainly for rather high concentrations (Cu-T >= 100 mu M; Cu2+ >= 2.2 mu M, pH 7). ROS formation rates were slightly higher when cells were exposed to Cu at pH 6 compared to pH 7, correlating with the higher free Cu2+ concentrations. A significant induction of DNA strand breaks was noted at Cu-T of 1 and 2.5 mu

M with greater effects at pH 6 due to higher free Cu2+ concentrations than at pH 7. No effects on lipid peroxidation were observed. These results lead to the hypothesis that copper-induced loss in viability and genotoxicity in trout gill cells are partially triggered by the generation of ROS and related to the free Cu2+. (c) 2007 Elsevier B.V. All rights reserved.

Borgmann, U. (1998). "A mechanistic model of copper accumulation in Hyalella azteca." Science of the Total Environment **219**(2-3): 137-145.

A mechanistic model is proposed for describing the accumulation of copper in Hyalella azteca. The metal is assumed to diffuse passively into and out of the animal. Once inside, it binds primarily to a ligand (X), the function of which is probably to supply copper to copper-requiring essential macromolecules. Once X is saturated, no further copper is accumulated. The rate of approach to the steady-state is much faster during uptake than during depuration because the number of binding sites (X-t) is limited. Diffusion across the animal's body surface does not seem to be rate-limiting. The binding strength of copper to X (KCu-x) is stronger than the binding of copper to fish gills, but this is not necessarily a valid comparison because KCu-x is the product of several constants, including the equilibrium for diffusion across the animal's surface and the strength of the internal binding site. Prolonged exposure to elevated copper in the water gradually reduces the concentration of X-t, primarily through growth dilution. Regulation of body copper appears to be through control of the concentration of X-t, rather than through control of copper influx or efflux rates, and chronic mortality is not affected by changes in X-t. (C) 1998 Elsevier Science B.V. All rights reserved.

Borgmann, U., M. Nowierski, et al. (2005). "Effect of major ions on the toxicity of copper to Hyalella azteca and implications for the biotic ligand model." Aquatic Toxicology **73**(3): 268-287.

The effect of major ions (Ca, Mg, Na, and K) and pH on Cu toxicity (LC50) to Hyalella azteca was determined in I week exposures. The simplest equation for describing Cu toxicity is a linear relationship between the total dissolved Cu LC50 and Ca and Na in water, ignoring pH. This equation would be useful in tier one of a two-tiered approach; if the measured dissolved Cu exceeds the value predicted from the equation, the sample should either be tested for toxicity, or a more detailed chemical speciation analysis can be conducted. The data were not consistent with a single-binding-site biotic ligand model, assuming that toxicity was due to the free Cu ion alone. However, toxicity could be predicted using a two-binding-site model. This requires separate coefficients to account for the effects of Ca and Na at low and high pH values (6.5-8.4), corresponding to the different binding sites (Mg and K did not affect toxicity). The single-binding-site BLM does not allow for this. Toxicity of Cu hydroxide or carbonate complexes does not need to be invoked, but cannot be excluded, and several models invoking the toxicity of these complexes can also explain the data. The free ion LC50 is strongly dependent on pH, but the LC50 for total dissolved Cu is almost pH independent. The effects of Ca and Na on the free ion LC50 are very different

at high and low pH (contrary to single-site biotic ligand model predictions), but similar for total dissolved Cu. Published data suggest that the same model, with different coefficients, can also be applied to Daphnia and fish. A more critical evaluation of the effects of cations at both low and high pH for organisms other than Hyalella is needed to determine if the BLM needs to be adjusted to incorporate more than one binding site for other species as well. Hydrogen ions reduce the toxicity of free Cu ions to Hyalella, but Cu also reduces the toxicity of hydrogen ions. A mixture model accounting for the joint toxicity of Cu and pH, as well as their mutual antagonistic effects, is presented. (c) 2005 Elsevier B.V. All rights reserved.

Brix, K. V., D. K. Deforest, et al. (2001). "Assessing Acute and Chronic Copper Risks to Freshwater Aquatic Life Using Species Sensitivity Distributions for Different Taxonomic Groups." Environmental Toxicology and Chemistry **20**(8): 1846-1856.

Using copper as an example, we present a method for assessing chemical risks to an aquatic community using species sensitivity distributions (SSDs) for different taxonomic groups. This method fits probability models to chemical exposure and effects data to estimate the percentage of aquatic species potentially at risk and expands on existing probabilistic risk assessment methodologies. Due to a paucity of chronic toxicity data for many chemicals, this methodology typically uses an acute-chronic ratio (ACR) to estimate the chronic effects distribution from the acute effects distribution. We expanded on existing methods in two ways. First, copper SSDs were developed for different organism groups (e.g., insects, fish) that share similar sensitivities or ecological functions. Integration of exposure and effects distributions provides an estimate of which organism groups may be at risk. These results were then compared with a site-specific food web, allowing an estimation of whether key food web components are potentially at risk and whether the overall aquatic community may be at risk from the perspective of ecosystem function. Second, chronic SSDs were estimated using the relationship between copper ACRs and acute toxicity (i.e., the less acutely sensitive a species, the larger the ACR). This correction in the ACR removes concerns previously identified with use of the ACR and allows evaluation of a significantly expanded chronic data set with the same approach as that for assessing acute risks.

Brooks, K. M. and C. V. W. Mahnken (2003). "Interactions of Atlantic Salmon in the Pacific Northwest Environment Iii. Accumulation of Zinc and Copper." Fisheries Research **62**(3): 295-305.

The third paper of the potential issues of the presence of Atlantic salmon in the Pacific Northwest environment concerns the use of copper and zinc in net-pen fanning. In particular, it reviews the presence of heavy metals in the water and sediments near net-pen farms. The review ends with some conclusions on the varying degrees of risk from these metals. (C) 2003 Elsevier Science B.V. All rights reserved.

Brooks, M. L., C. J. Boese, et al. (2006). "Complexation and time-dependent

accumulation.of copper by larval fathead minnows (Pimephales promelas): Implications for modeling toxicity." Aquatic Toxicology **78**(1): 42-49.

Mechanistic models predicting copper (Cu) toxicity to aquatic biota in natural waters require organic and inorganic water chemistry, and quantified values for Cu binding by sensitive biological receptors. In bioaccumulation experiments using larval fathead minnows (FHM; Pimephales promelas), we investigated time to asymptotic accumulation of Cu and quantified the conditional stability constants (binding affinity; log Kcu-FHM) and binding-site densities of Cu-FHM complexation. Cu bioaccumulation increased rapidly, approaching an asymptote in exposures longer than 12 h, indicating that Cu loading at 24 h is an appropriate exposure duration for modeling Cu complexation by larval FHM. Results of Langmuir and Scatchard analyses of other bioaccumulation experiments produced log KCu-FHM values of 6.52, and binding-site densities of 0.39 mu mol g(-1) dry weight. These whole-body log KCu-FHM values are approximately an order of magnitude lower than those reported for adult FHM gills. However, binding-site densities for larval and adult FHM are similar. Under similar test conditions, comparable concentrations of aqueous Cu cause 50% mortality in adult and larval FHM suggesting that binding site densities determine comparable metal-tissue loadings and have greater influence on Cu bioavailability than binding affinity. 

Brooks, S. J. and C. L. Mills (2003). "The effect of copper on osmoregulation in the freshwater arnphipod Gammarus pulex." Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **135**(4): 527-537.

The influence of copper on osmoregulation in the freshwater amphipod Gammarus pulex was determined from the analysis of water permeability, haemolymph sodium concentration, sodium influx and gill Na+/K+ ATPase and Mg2+ ATPase activity. Exposure to nominal copper concentrations of 100 mug l(-1) or greater caused a significant reduction in both haemolymph sodium concentration and sodium influx within 4 h. Measurements of water permeability, expressed as the half-time of exchange of body water (t(1/2)), excluded structural gill damage as the cause of this fall in haemolymph sodium. Copper at 10 mug l(-1) or above in the assay solution significantly reduced gill Na+/K+ ATPase activity. In contrast gill Mg2+ ATPase activity was markedly less affected by copper. These differences in enzyme sensitivity were considered with respect to the potential mechanisms of copper toxicity. 

Brotheridge, R. M., K. E. Newton, et al. (1998). "Nickel, cobalt, zinc and copper levels in brown trout (Salmo trutta) from the river Otra, southern Norway." Analyst **123**(1): 69-72.

The Flat Nickel mine at Evje in southern Norway was mined extensively from 1914 to 1945 with little regard for any potential environmental effect, Much of the ore extracted was smelted at a site adjacent to the river Otra south of Evje, Recent studies have revealed heavy metal pollution in the land surrounding the smelter and in water draining from the mine leading to concern for the aquatic ecosystem in the river Otra, Brown trout were sampled from an uncontaminated

lake 9 km upstream from the smelter, from the base of the Oddebekken (a tributary draining the mine water into the Otra), from sites immediately upstream and down stream of the smelter and from a site 4 km down stream from the smelter, Fish from sites adjacent to the smelter and the base of the Oddebekken were smaller than those from the lake and down stream site, Concentrations of the metals were highest in fish sampled where the mine water entered the Otra and gradually decreased in fish further down the river, Fish from the uncontaminated lake had the lowest level of metals.

Buckley, J. A. (1983). "Complexation of Copper in the Effluent of a Sewage-Treatment Plant and an Estimate of Its Influence on Toxicity to Coho Salmon." Water Research **17**(12): 1929-1934.

Bunton, T. E. (1995). "EXCLUSION OF COPPER FROM ALTERED HEPATOCYTES IN WHITE PERCH (MORONE AMERICANA) WITH HEPATIC COPPER STORAGE." Journal of Wildlife Diseases **31**(1): 99-103.

Iron is excluded from foci of hepatocellular alteration in carcinogenesis of rodents and some fish. Among white perch (Morone americana), there is a condition of hepatic copper storage in which copper-loaded livers are produced naturally. In a group of fish collected from the Chesapeake Bay, Maryland (USA), from September to December 1990, we observed hepatic lesions which excluded copper similar to the phenomenon of iron exclusion, in a white perch with over 3,600 mu g/g wet weight hepatic copper. The lesions were of two types: one with cells morphologically different from normal hepatocytes and which had diminished to absolute exclusion of copper with the copper specific histochemical stain rubeinic acid, and a second with cells morphologically similar to normal hepatocytes which had only a partial exclusion of copper. Although the exact cause and nature of the lesions was not determined, intrinsic copper toxicity, environmental pollution, or a combination of these factors may have contributed to their development.

Bury, N. R., J. Li, et al. (1998). "Cortisol protects against copper induced necrosis and promotes apoptosis in fish gill chloride cells in vitro." Aquatic Toxicology **40**(2-3): 193-202.

In order to distinguish between toxic actions of copper (Cu) and the indirect actions of the metal mediated via the stress hormone cortisol, a 24 h in vitro gill filament culture was used to investigate the effects of this heavy metal and hormone, singly and in combination, on apoptosis and necrosis of chloride cells in the cichlid fish, tilapia (Oreochromis, mossambicus). Cell death was identified after fluorescent double-labelling using a confocal laser scanning microscope. Incubation of filaments with 50 mu M and 100 mu M CuSO4 caused an approximate 5- and 16-fold increase, respectively, in chloride cell necrosis when compared to control, but had no significant effect on apoptosis. A 12 h incubation with 0.28 mu M cortisol prior to exposure to 100 mu M CuSO4 reduced necrosis by about 75%. The apparent protection provided by cortisol against copper toxicity could be blocked by the glucocorticoid receptor blocker RU 486.

Incubation with 0.83 mu M cortisol induced apoptosis to the same extent as that of camptothecin, a topoisomerase I inhibitor. We conclude that Cu directly causes necrosis of chloride cells, whilst cortisol protects against copper toxicity at lower concentrations, and induces apoptosis at higher concentrations, typical for severely stressed fish. (C) 1998 Elsevier Science B.V.

Butcher, J., J. Diamond, et al. (2006). "Toxicity models of pulsed copper exposure to Pimephales promelas and Daphnia magna." Environmental Toxicology and Chemistry **25**(9): 2541-2550.

Semiempirical models are useful for interpreting the response of aquatic organisms to toxicants as a function of exposure concentration and duration. Most applications predict cumulative mortality at the end of the test for constant exposure concentrations. Summary measures, such as the median lethal concentration, are then estimated as a function of concentration. Real-world exposures are not constant. Effects may depend on pulse timing, and cumulative analysis based only on integrated exposure concentration is not sufficient to interpret results. We undertook a series of pulsed-exposure experiments using standard toxicological protocols and interpreted the results (mortality, biomass, and reproduction) using a dynamic generalization of a Mancini/Breck-type model that includes two compartments, one for internal concentration as a function of exposure and one for site-of-action concentration or accumulated damage as a function of the internal dose. At exposure concentrations near the effects level, the model explained approximately 50% of the variability in the observed time history of survival, 43% of the change in biomass, and 83% of the variability in net reproduction. Unexplained variability may result from differences in organism susceptibility, amplified by the effects of small sample sizes in standard tests. The results suggest that response is sensitive to prior conditions and that constant-exposure experiments can underestimate the risk from intermittent exposures to the same concentration. For pulsed exposures, neither the average nor the maximum concentration alone is an adequate index of risk, which depends on both the magnitude, duration, and timing of exposure pulses. Better understanding about the impacts of pulsed exposures will require use of experimental protocols with significantly greater numbers of replicates.

Carbonell, G. and J. V. Tarazona (1994). "Toxicokinetics of Copper in Rainbow-Trout (Oncorhynchus-Mykiss)." Aquatic Toxicology **29**(3-4): 213-221.

Rainbow trout (Oncorhynchus mykiss) received 80 mug copper kg-1 body weight as an intravenous injection of copper sulfate. Copper levels were analyzed by atomic absorption spectrometry in plasma samples collected before and at regular times after the copper injection. Plasma copper concentration-time curves were analyzed according to standard pharmacokinetic compartmental procedures. A two-compartment model was adapted to derive toxicokinetic parameters, including a time-independent component to assess the basal plasma copper levels before the copper injection. The volume of the central compartment 43 ml kg-1 was similar to that reported for rainbow trout plasma. Steady-state distribution volume was estimated in 178 ml kg-1. Distribution and final

elimination half-lives were 7.2 and 195.5 min, respectively. Steady-state distribution volume, the area under the curve, the copper clearance and the medium retention time calculated by the two-compartment model were similar to those estimated using non-compartmental equations. These toxicokinetic data suggest a rapid elimination of copper from the exchangeable pool. Thus, during long-term waterborne copper exposures, plasma copper concentration would achieve a steady-state situation after a few hours or days; further accumulation of copper would be related to the binding of copper to non-exchangeable ligands which would not have toxicological consequences. The large number of factors associated to the capability of the fish to offer and produce non-exchangeable binding sites would explain the variety of responses on copper accumulation described in the literature.

Carreau, N. D. and G. G. Pyle (2005). "Effect of copper exposure during embryonic development on chemosensory function of juvenile fathead minnows (Pimephales promelas)." Ecotoxicology and Environmental Safety **61**(1): 1-6.

Fish rely on chemosensation to alert them of nearby predators. Recent evidence suggests that metals disrupt this chemical communication system. Our objective was to determine the chemical alarm response of juvenile fathead minnows after embryonic copper (Cu) exposure. Embryos were randomly assigned to one of two treatments: clean water or water containing 10 mu g/L Cu. Once hatched, half of the Cu-exposed embryos were transferred to clean water (after hatch), while the other half remained in the Cu-contaminated water. Fish were tested using a triumvirate maze at the age of 84-96 d post-hatch. Fish reared in clean water significantly avoided the alarm cue. However, fish reared under continuous Cu exposure and those that were only exposed to Cu during embryonic development were unable to respond to the chemical alarm stimulus. Fish from all treatments did not respond to two control stimuli. Results from this study suggest that fish exposed to elevated Cu concentrations during embryonic development is sufficient to impair chemosensory function during later life stages. This could result in an inability to detect nearby predators by olfaction, which could lead to important ecological perturbations in populations inhabiting metal-contaminated systems. 

Carvalho, C. D., H. S. S. de Araujo, et al. (2004). "Hepatic metallothionein in a teleost (Prochilodus scrofa) exposed to copper at pH 4.5 and pH 8.0." Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology **137**(2): 225-234.

Copper accumulation and induction of metallothionein (MT) were investigated in the liver of juvenile Prochilodus scrofa exposed to copper (96 h-LC50) at pH 4.5 (LC50 = 98 +/- 0.8 mug Cu l(-1)) and pH 8.0 (LC50 = 16 +/- 0.5 mug Cu l(-1)) at 20 degreesC. No significant difference was found in copper accumulated in the liver at either pH. The ratio between copper accumulation in the liver after 96 h exposure and the copper in the water at pH 4.5 and 8.0 was 5.1 and 34.4, respectively. MT in the liver of fish exposed to copper was isolated by DEAE-Sepharose chromatography and identified by SIDS-PAGE. No MT was identified in the controls. MT concentration was 126.8 +/- 21.5 and 167.4 +/- 25.6 mug g(-

1) wet mass in fish exposed to copper at pH 4.5 and pH 8.0 (P>0.05), respectively. The results indicate that the pH of the water is a determining factor in copper toxicity, and the increased concentration of MT in the liver of R scrofa exposed to copper suggests the possibility of using MT as a biomarker to evaluate the biological response of this species to copper contamination in aquatic environments. (C) 2003 Elsevier Inc. All rights reserved.

Carvalho, C. D. and M. N. Fernandes (2008). "Effect of copper on liver key enzymes of anaerobic glucose metabolism from freshwater tropical fish Prochilodus lineatus." Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **151**(3): 437-442.

We investigated the effect of copper on liver key enzymes of the anaerobic glucose metabolism (hexokinase, HK; phosphofructokinase, PFK; pyruvate kinase, PK; lactate dehydrogenase, LDH) as well as of the pentose pathway (glycose-6-phosphate dehydrogenase, G6PDH) from the fish Prochilodus lineatus. The fish were acclimated at either 20 degrees C or 30 degrees C at pH 7.0, transferred to water at pH 4.5 or 8.0, and exposed to 96 h-CL50 copper concentrations. Copper accumulation in liver was higher in fish acclimated at 20 degrees C and maintained in water pH 8.0. Three-way analysis of variance revealed a significant effect of temperature on all enzymes, a significant effect of pH on all enzymes except for PK, and a significant effect of copper on only PFK, and LDH in pH 4.5 at 20 degrees C and, at 30 degrees C, on PFK and PK at pH 4.5 and 8.0, HK at pH 4.5 and G6PDH at pH 8.0. There were significant interactions between treatments for many enzymes. These changes suggest that the activity of enzymes in question is modified by a change in ambient water. At least at 30 degrees C, the overall reduction in the glycolytic enzyme activities of copper-exposed fish seems to reduce energy availability via glucose metabolism, thereby contributing to enhance copper toxic effects. (C) 2007 Elsevier Inc. All rights reserved.

Carvalho, C. S. and M. N. Fernandes (2006). "Effect of temperature on copper toxicity and hematological responses in the neotropical fish Prochilodus scrofa at low and high pH." Aquaculture **251**(1): 109-117.

Copper sulfate has been widely used to control algae and pathogens in fish culture ponds. However, its toxic effects on fish depend not only on its concentration in water but also on water quality. The susceptibility of the neotropical freshwater fish Prochilodus scrofa to copper was evaluated at two temperatures with low and high water pH. Juvenile fish were acclimated at 20 and 30 degrees C and exposed to copper (static bioassay system) in water with pH 4.5 and 8.0. The 96 h-LC50 were determined at each temperature and pH, as were the hematological parameters. The 96 h-LC50 for copper (98 and 88 mu g Cu. L-1 in water with pH 4.5 and 16 and 14 mu g Cu L-1 in water with pH 8.0 for fish kept at 20 and 30 degrees C, respectively) was significantly dissimilar in pH 4.5 and 8.0, but no difference was found between 20 and 30 degrees C in the same water pH. At 20 degrees C, regardless of the water pH, the hematocrit (Hct) increased while the red blood cells (RBC) and hemoglobin (Hb)

concentration decreased compared to control pH 7.0. Copper exposure in water pH 4.5 and 8.0 causes an increase on the Hct, RBC and Hb concentrations in relation to the controls pH 4.5 and 8.0. At 30 degrees C, the changes on the blood parameters depended on the water pH and, after copper exposure at low and high pH, the blood changes indicated more complex responses. The changes in hematological parameters of the fish, regardless of the pH and water temperature, indicate ionoregulatory or respiratory disturbances that imply an increase in energy consumption to restore homeostasis instead of other physiological functions such as weight gain and growth. 

Chapman, G. A. (1978). "TOXICITIES OF CADMIUM, COPPER, AND ZINC TO 4 JUVENILE STAGES OF CHINOOK SALMON AND STEELHEAD." Transactions of the American Fisheries Society **107**(6): 841-847.

Chapman, G. A. and D. G. Stevens (1978). "ACUTELY LETHAL LEVELS OF CADMIUM, COPPER, AND ZINC TO ADULT MALE COHO SALMON AND STEELHEAD." Transactions of the American Fisheries Society **107**(6): 837-840.

Clearwater, S. J., A. M. Farag, et al. (2002). "Bioavailability and Toxicity of Dietborne Copper and Zinc to Fish." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **132**(3): 269-313.

To date, most researchers have used dietborne metal concentrations rather than daily doses to define metal exposure and this has resulted in contradictory data within and between fish species. It has also resulted in the impression that high concentrations of dietborne Cu and Zn (e.g. >900 mg kg(-1) dry diet) are relatively non-toxic to fish. We reanalyzed existing data using rations and dietborne metal concentrations and used daily dose, species and life stage to define the toxicity of dietbome Cu and Zn to fish. Partly because of insufficient information we were unable to find consistent relationships between metal toxicity in laboratory-prepared diets and any other factor including, supplemented metal compound (e.g. CuSO4 or CuCl2), duration of metal exposure, diet type (i.e. practical, purified or live diets), or water quality (flow rates, temperature, hardness, pH, alkalinity). For laboratory-prepared diets, dietbome Cu toxicity occurred at daily doses of > 1 mg kg(-1) body weight d(-1) for channel catfish (Ictalurus punctatus), 1-15 mg kg(-1) body weight d(-1) (depending on life stage) for Atlantic salmon (Salmo salar) and 35-45 mg kg(-1) body weight d(-1) for rainbow trout (Oncorhynchus mykiss). We found that dietborne Zn toxicity has not yet been demonstrated in rainbow trout or turbot (Scophthalmus maximus) probably because these species have been exposed to relatively low doses of metal (<90 mg kg(-1) body weight d(-1)) and effects on growth and reproduction have not been analyzed. However, daily doses of 9-12 mg Zn kg(-1) body weight d(-1) in laboratory-prepared diets were toxic to three other species, carp Cyprinus carpio, Nile tilapia Oreochromis niloticus, and guppy Poecilia reticulata. Limited research indicates that biological incorporation of Cu or Zn into a natural diet can either increase or decrease metal bioavailability, and the relationship

between bioavailability and toxicity remains unclear. We have resolved the contradictory data surrounding the effect of organic chelation on metal bioavailability. Increased bioavailability of dietbome Cu and Zn is detectable when the metal is both organically chelated and provided in very low daily doses. We have summarized the information available on the effect of phosphates, phytate and calcium on dietbome Zn bioavailability. We also explored a rationale to understand the relative importance of exposure to waterborne or dietbome Cu and Zn with a view to finding an approach useful to regulatory agencies. Contrary to popular belief, the relative efficiency of Cu uptake from water and diet is very similar when daily doses are compared rather than Cu concentrations in each media. The ratio of dietbome dose waterborne dose is a good discriminator of the relative importance of exposure to dietbome or waterborne Zn. We discuss gaps in existing data, suggest improvements for experimental design, and indicate directions for future research. (C) 2002 Elsevier Science Inc. All rights reserved.

Collyard, S. A., G. T. Ankley, et al. (1994). "INFLUENCE OF AGE ON THE RELATIVE SENSITIVITY OF HYALELLA-AZTECA TO DIAZINON, ALKYLPHENOL ETHOXYLATES, COPPER, CADMIUM, AND ZINC." Archives of Environmental Contamination and Toxicology **26**(1): 110-113.

Laboratories testing Hyalella azteca use a wide range of ages (or sizes) of the amphipod in their studies. The objective of this study was to investigate age-specific differences in sensitivity of the amphipod to contaminants with varying toxic modes of action. Hyalella azteca, ranging in age from <1 to 26 d, were tested in 96-h water-only exposures with the organophosphate pesticide diazinon, a mixture of alkylphenol ethoxylates (nonionic surfactants), copper sulfate, cadmium chloride, and zinc sulfate. Overall age-specific differences in sensitivity to the five test chemicals were relatively small; 96-h LC50 values typically varied by 50% or less among the various age classes of H. azteca. When differences in sensitivity were observed, trends were apparently related to the contaminant tested rather than to the age of the amphipods, i.e., no particular age class consistently was the most sensitive to the toxicants.

Craig, P. M., C. M. Wood, et al. (2007). "Oxidative stress response and gene expression with acute copper exposure in zebrafish (Danio rerio)." American Journal of Physiology-Regulatory Integrative and Comparative Physiology **293**(5): R1882-R1892.

In fish, environmental pollution is one factor that induces oxidative stress, and this can disturb the natural antioxidant defense system. Oxidative stress has been well characterized in vitro, yet the in vivo effects of metal-induced oxidative stress have not been extensively studied. In two experiments we examined the impacts of copper (Cu) on gene expression, oxidative damage, and cell oxidative capacity in liver and gill of zebrafish. In the first experiment, soft water-acclimated zebrafish were exposed to 8 and 15 mu g/ l Cu for 48 h. This exposure resulted in significant increases in gene expression of cytochrome c oxidase subunit 17 (COX- 17) and catalase, associated with both increased Cu load and protein carbonyl concentrations in the gill and liver after 48 h. In addition, we examined the potential protective effects of increased waterborne Ca2+ (3.3 mM) and Na+

(10 mM) on acute Cu toxicity. While both treatments were effective at reducing liver and/ or gill Cu loads and attenuating oxidative damage at 48 h, 10 mM Na (+) was more protective than 3.3 mM Ca2+. There were variable changes in the maximal activities of COX and citrate synthase ( CS), indicating possible alterations in cell oxidative capacity. Moreover, Cu affected COX-to-CS ratios in both gill and liver, suggesting that Cu alters normal mitochondrial biogenic processes, possibly though metallochaperones like COX-17. Overall, this study provides important steps in determining the transcriptional and physiological endpoints of acute Cu toxicity in a model tropical species.

Das, B. K. and N. Das (2005). "Impacts of quicklime (CaO) on the toxicity of copper (CuSO4, 5H(2)O) to fish and fish food organisms." Chemosphere **61**(2): 186-191.

Static bioassays of 96 h duration were conducted in the laboratory using fry of common carp (Cyprinus carpio), adult tubificid worm (Branchiura sowerbyi) and adult copepod plankton (Cyclops viridis) to determine LC50 values of Cu and CaO to these organisms and effects of interaction between Cu and CaO. Ninety-six hour LC50 values of Cu to fry of common carp, worm and copepod were found to be 1.40 mg l(-1), 0.08 mg l(-1) and 0.03 mg l(-1) respectively. CaO up to 500 mg l(-1) did not produce any mortality of the fry of common carp up to 96 h. But 96 h LC50 values of CaO to worm and copepod were 83.00 mg l(-1) and 27.80 mg l(-1) respectively. When common carp fry, worm and the copepod were exposed to respective LC50 dose of Cu in presence of varying concentration of CaO, mortality of the organisms significantly reduced and was found inversely correlated with the doses of CaO [y=48.36-0.807x, r=-0.99 (n=7) for fish; y=44.46-0.146x, r=-0.97 (n=7) for worm; y=49.46-0.66x, r=-0.99 (n=7) for the copepod]. The present results indicate that CaO is non-toxic to fish and is capable of reducing the toxicity of Cu to fish while CaO and Cu are antagonistic to each other for the worm and the copepod. Potential of using CaO as antitoxic agent for Cu in water is discussed. 

Dautremepuits, C., S. Betoulle, et al. (2004). "Immunology-related perturbations induced by copper and chitosan in carp (Cyprinus carpio L.)." Archives of Environmental Contamination and Toxicology **47**(3): 370-378.

Copper is used in treatment mixtures to control fungal diseases in vineyards. Its concentrations are relatively high in some aquatic ecosystems, and the main problem observed in this study was the antioxidant stress induced by this heavy metal. Copper toxicologic effects in aquatic organisms have prompted the demand for alternative use of low-toxicity molecules in culture treatments. Chitosan is a polymer with antifungal property similar to copper and may be an interesting biopesticide. Thus, it is necessary to investigate the potential toxicity of chitosan for aquatic animal health, either alone or in conjunction with copper. In this study, carp were exposed to two sublethal chitosan concentrations (75 and 150 mg/L) or to two sublethal copper concentrations (0.1 and 0.25 mg/L) or to a mixture of chitosan plus copper (75 mg/L and 0.1 mg/L, respectively). The results of the present study show that exposure to copper at environmentally realistic levels or to chitosan at sublethal concentrations may significantly

stimulate various aspects of immune functions in carp such as nonspecific cellular immunity, represented by total immunoglobulin level, ceruloplasmin activity, and oxidative activity of phagocytes. This acute-phase inflammatory response induced separately by the two treatments was not observed, especially on phagocyte oxidative activity, when carp were exposed to the copper-chitosan mixture. This fact could be explained by a possible chelation of copper by chitosan decreasing the biodisponibility of the two products for immune cells. Thus, the immunotoxicologic impact of copper and chitosan on fish immune response would be less pronounced with the combined treatments than with separate treatments in an aquatic environment.

Dautremepuits, C., S. Paris-Palacios, et al. (2004). "Modulation in hepatic and head kidney parameters of carp (Cyprinus carpio L.) induced by copper and chitosan." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **137**(4): 325-333.

Copper is used in treatment mixtures to control fungal diseases in vineyards plants. High concentrations of copper are inducing antioxidant stress in some aquatic ecosystems, and potential bioaccumulation in aquatic organisms has prompted the demand for alternative use of low toxic molecules in culture treatments. Chitosan is a biomolecule with antifungal and heavy metal ion chelating properties that may be used as a biopesticide. In this study, we investigate the potential toxicity of chitosan for aquatic animal health, alone or associated with copper. Carp (Cyprinus carpio L.) were exposed to different chitosan concentrations (from 37.5 to 375 mg/l) or to two sublethal copper concentrations (0.1 and 0.25 mg/l) or to chitosan and copper (75 and 0.1 mg/l, respectively). Antioxidant enzyme activities were enhanced in chitosan treated fish after 4 days and depressed after 8 days. This phenomenon indicated a non-negligible toxicity of chitosan in fish physiology. However, the mixture copper-chitosan seems to induce a lower degree of oxidative stress than each fungicide alone. These observations show that chitosan is a potentially noxious molecule for some fish and any industrial and/or agricultural uses of this compound will have to address this problem. 

Dave, G. and R. Q. Xiu (1991). "TOXICITY OF MERCURY, COPPER, NICKEL, LEAD, AND COBALT TO EMBRYOS AND LARVAE OF ZEBRAFISH, BRACHYDANIO-RERIO." Archives of Environmental Contamination and Toxicology **21**(1): 126-134.

The toxicity of mercury (HgCl2), copper (CuCl2:5 H2O), nickel (NiSO4:6 H2O), lead (Pb(CH3COO)2:3 H2O) and cobalt (CoCl2:6 H2O) was studied under standardized conditions in embryos and larvae of the zebrafish, Brachydanio rerio. Exposures were started at the blastula stage (2-4 h after spawning) and the effects on hatching and survival were monitored daily for 16 days. Copper and nickel were more specific inhibitors of hatching than cobalt, lead, and mercury. Nominal "no effect" concentrations determined from the dose-response relationships (ZEPs, Zero Equivalent Points) for effect on hatching time were 0.05-mu-g Cu/L, 10-mu-g Hg/L, 20-mu-g Pb/L, 40-mu-g Ni/L and 3,840-mu-g Co/L, and those for effect on survival time were 0.25-mu-g Cu/L, 1.2-mu-g Hg/L,

30-mu-g Pb/L, 80-mu-g Ni/L, and 60-mu-g Co/L. The "no effect" concentrations for Ni, Hg and Pb are consistent with previously reported MATC values for sensitive species of fish. The "no effect" concentrations for copper are 1-2 orders of magnitude lower than previously reported values. The major reason for the latter discrepancy was considered to be the absence of organics that can complex copper ions in the reconstituted water that we used, which had a hardness of 100 mg/L (as CaCO3) and a pH of 7.5-7.7. Unexposed controls were started with embryos from different parental zebrafishes and the parental-caused variability in early embryo mortality, median hatching time and median survival time were estimated.

De Boeck, G., K. Van der Ven, et al. (2007). "Sublethal copper exposure induces respiratory stress in common and gibel carp but not in rainbow trout." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **144**(4): 380-390.

Rainbow trout, common carp, and gibel carp were exposed to sublethal Cu levels (1.0 or 1.7 [M) for I week. In rainbow trout, arterial oxygen tension (PaO2) remained normal and there was no indication of anaerobic metabolism. PaO2 was considerably lower in common and gibel carp and Cu exposure decreased this further. The decrease was transient for common carp but persistent in gibel carp and coincided with an elevation in arterial carbon dioxide tension (PaCO2) indicating that all gas exchange was compromised in both cyprinid species. The disturbed gas exchange resulted in acidosis, which was respiratory and metabolic for common carp but mainly respiratory for gibel carp. Gibel carp produced ethanol as end product of their alternative anaerobic pathway. The hypothesis that hypertrophy and hyperplasia, resulting in increased diffusion distances, are reducing PaO2 appeared invalid. Hypoventilation seems a more likely cause. Ionoregulatory parameters responded more uniform among species. Fast and pronounced decreases in plasma sodium and chloride developed for all three species, independent of the observed gill damage. Rainbow trout lost 20% of their plasma Na in the first 3 days, while common and gibel carp had only lost 13 and 16% respectively at that time. This difference might be crucial when challenged with Cu exposure and allow a fish to survive the first shock phase and supports it the hypothesis that sodium turnover is a key factor in predicting Cu toxicity. 

De Boeck, G., A. Vlaeminck, et al. (1997). "Effects of Sublethal Copper Exposure on Copper Accumulation, Food Consumption, Growth, Energy Stores, and Nucleic Acid Content in Common Carp." Archives of Environmental Contamination and Toxicology **33**(4): 415-422.

Juvenile common carp were exposed for 28 days to three different sublethal copper concentrations (0.20 mu M, 0.55 mu M, and 0.80 mu M). Food consumption was monitored on a daily basis during the exposure period, while growth, copper accumulation, energy stores, and nucleic acid contents were assessed weekly. Copper exposure to 0.80 mu M affected both growth and feeding behavior in common carp. At 0.55 mu M, growth was affected despite normal food consumption. Even at the lowest copper concentration (0.20 mu M),

metabolic demand for the fish increased, challenging the carp with an increased demand for food. Copper accumulation mainly occurred in the liver, reaching an equilibrium between uptake and excretion after 1 month of exposure. Substantial biochemical changes were observed at the two highest copper exposure concentrations, but the correlation between growth rate and RNA:DNA ratio was poor considering the substantial differences in growth rate. The use of the RNA:DNA ratio as a sensitive biomarker is questioned.

De Schamphelaere, K. A. C., D. G. Heijerick, et al. (2002). "Refinement and field validation of a biotic ligand model predicting acute copper toxicity to Daphnia magna." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **133**(1-2): 243-258.

A previously developed biotic ligand model (BLM) was validated for its capacity to predict acute 48-h EC50 values of copper to Daphnia magna in 25 reconstituted media with different PH values and concentrations of artificial dissolved organic carbon, Ca, Mg and Na. Before the BLM validation, fitting of measured (with a copper ion-selective electrode) and calculated (with the BLM) Cu2+-activity was performed by adjusting the WHAM model V (i.e. the metal-organic speciation part of the BLM) copper-proton exchange constant to pK(MHA) = 1.9. Using this value, the 48-h EC50 values observed agreed very well with BLM-predicted EC50 values for tests performed at pH<8, but not at all for tests performed at pH > 8. Additional experiments demonstrated that this was due to toxicity of the CuCO3 complex, which is the most abundant inorganic copper species at pH > 8. This was incorporated into the initial BLM by allowing the binding Of CuCO3 (next to Cu2+ and CuOH+) to the biotic ligand of D. magna. The affinity of CuOH+ and CuCO3 for the biotic ligand was approximately five- and 10-fold lower than that of Cu2+, respectively. With the refined BLM, 48-h EC50 values could be accurately predicted within a factor of two not only in all 25 reconstituted media, but also in 19 natural waters. This validated and refined BLM could support efforts to improve the ecological relevance of risk assessment procedures applied at present. 

De Schamphelaere, K. A. C. and C. R. Janssen (2002). "A biotic ligand model predicting acute copper toxicity for Daphnia magna: The effects of calcium, magnesium, sodium, potassium, and pH." Environmental Science & Technology **36**(1): 48-54.

The extent to which Ca2+, Mg2+, Na+, K+ ions and pH independently mitigate acute copper toxicity for the cladoceran Daphnia magna was examined. Higher activities of Ca2+, Mg2+, and Na+ (but not K+) linearly increased the 48-h EC50 (as Cu2+ activity), supporting the concept of competitive binding of these ions and copper ions to toxic action or transport sites at the organism-water interface (e.g. fish gill, the biotic ligand). The increase of the EC50 (as Cu2+ activity) with increasing H+, however, seemed to suggest cotoxicity of CuOH+ rather than proton competition. Based on the biotic ligand model (BLM) concept, we developed a methodology to estimate stability constants for the binding of CU2+, CuOH+, Ca2+, Mg2+, Na+, and H+ to the biotic ligand, solely based on toxicity

data. Following values were obtained: log K-CuBL = 8.02, log K-CuOHBL = 7.45, log K-CaBL = 3.47, log K-MgBL = 3.58, log K-NaBL = 3.19, and log K-HBL similar to 5.4. Further, we calculated that on average 39% of the biotic ligand sites need to be occupied by copper to induce a 50% acute effect for D. magna after 48 h of exposure, Using the estimated constants, a BLM was developed that can predict acute copper toxicity for D. magna as a function of water characteristics. The presented methodology can easily be applied for BLM development for other organisms and metals. After validation with laboratory and natural waters (including DOG), the developed model will support efforts to improve the ecological relevance of presently applied risk assessment procedures.

De Schamphelaere, K. A. C., J. L. Stauber, et al. (2005). "Toward a biotic ligand model for freshwater green algae: Surface-bound and internal copper are better predictors of toxicity than free Cu2+-ion activity when pH is varied." Environmental Science & Technology **39**(7): 2067-2072.

The freshwater green microalgae Chlorella sp. and Pseudokirchneriella subcapitata (P. subcapitata) were chronically (48 and 72 h, respectively) exposed to copper at various pH levels, i.e., pH 6-7.5 and pH 5.9-8.5, respectively. Concentrations resulting in 50% inhibition of exponential growth rate (EC50) were determined as dissolved Cu, estimated chemical activity of the free Cu2+ ion (as pCu = - log{Cu2+ activity as molarity}), and as external (surface-bound) Cu and internal Cu in the algal cells. With increasing pH, EC50(dissolved) decreased from 30 to 1.1 mu g of Cu L-1 for Chlorella sp. and from 46 to 18 mu g of Cu L-1 for P. subcapitata. The pH effect on copper toxicity was even more obvious when expressed as Cu2+ activity. The EC50(pCu) increased on average 1.4 pCu unit per pH unit for Chlorella sp. and 1.1 pCu unit per pH unit for P. subcapitata, thus indicating a marked increase of Cu2+ toxicity at higher pH (more than 1 order of magnitude per pH unit). In contrast, it was found that EC50 values expressed as surface bound or external copper (EC50(external)) and as internal copper (EC50(internal)) did not vary substantially when pH was increased. External Cu was operationally defined as the Cu fraction removable from the algal cell by short-term contact with ethylenediaminetetraacetic acid; internal copper was defined as the nonremovable fraction. For Chlorella, sp. the EC50(external) varied between 5 and 10 fg of Cu/ cell (factor of 2 difference) and the EC50(internal) between 25 and 40 fg of Cu/cell (factor of 1.6 difference). For P. subcapitata the EC50(external) varied between 10 and 28 fg of Cu/cell (factor of 2.8 difference) and the EC50internal between 42 and 71 fg of Cu/cell (factor of 1.7 difference). Because the observed variation in EC50(external) and EC50(internal) is much less than the variation in EC50(Cu)(2+), it is concluded that both external and internal copper are better predictors of copper toxicity than Cu2+ when pH is varied. From the perspective of toxicity modeling, this observation is the first step toward considering the use of the cell surface as the algal biotic ligand for Cu in a similar way as fish gills fulfill this role in the biotic ligand model for predicting metal toxicity to fish species.

Demayo, A., M. C. Taylor, et al. (1982). "EFFECTS OF COPPER ON HUMANS, LABORATORY AND FARM-ANIMALS, TERRESTRIAL PLANTS, AND AQUATIC LIFE." Crc Critical Reviews in Environmental Control **12**(3): 183-255.

Dethloff, G. M. and H. C. Bailey (1998). "Effects of Copper on Immune System Parameters of Rainbow Trout (Oncorhynchus Mykiss)." Environmental Toxicology and Chemistry **17**(9): 1807-1814.

Agricultural, urban, industrial, and mining sources release metals; into waterways. The effects of sublethal concentrations of metals on integrated physiological processes in fish, such as immunocompetency, are not well understood. The objective of this study was to determine the physiological effects of a range of sublethal copper concentrations (6.4, 16.0, and 26.9 mu g Cu/L) on Shasta-strain rainbow trout (Oncorhynchus mykiss) exposed in sofi water. Trout were sampled after 3, 7, 14, and 21 d of exposure to copper. The percentage of monocytes was consistently elevated at 26.9 mu g Cull,, and the percentage of lymphocytes was decreased. A consistent increase in the percentage of neutrophils occurred at 26.9 and 6.4 mu g Cult. Respiratory burst activity was decreased for all concentrations at all sampling days, but a significant reduction occurred only at 14 and 21 d of exposure to copper. B-like cell proliferation was decreased by exposure to the higher copper concentrations. Proliferation results, however, had high variability. T-like cell proliferation and phagocytosis were not altered. Hepatic copper concentration was consistently elevated in trout exposed to 26.9 mu g Cu/L; no correlation was found between hepatic copper concentration and the Immune system responses investigated. Consistent alterations in immunological parameters suggest that these parameters could serve as indicators of chronic metal toxicity in natural systems.

Dethloff, G. M., H. C. Bailey, et al. (2001). "Effects of Dissolved Copper on Select Hematological, Biochemical, and Immunological Parameters of Wild Rainbow Trout (Oncorhynchus Mykiss)." Archives of Environmental Contamination and Toxicology **40**(3): 371-380.

Rainbow trout (Oncorhynchus mykiss) were sampled from a creek in the western Sierra Nevada, Plumas County, CA, that receives run-off from a 40-ha copper (Cu) tailings pile. Reference sites included a site upstream of the Cu input and another site located on a nearby creek. Hepatic Cu concentrations were significantly elevated in trout from sites with elevated dissolved Cu concentrations compared with concentrations in trout from reference sites. Trout at the Cu-contaminated sites also exhibited decreased hematocrit (Hct), leukocrit (Lct), and percentage of lymphocytes in blood compared to trout from reference sites. The percentage of monocytes in blood and respiratory burst activity were affected by gender and age, respectively. Condition factor, percentage of neutrophils in blood, muscle glycogen acid protein, and plasma acetylcholinesterase were not affected by dissolved Cu concentration or gender. Age also did not appear to be a factor. The data from this study support the use of immune system parameters to assess alterations in salmonids experiencing prolonged exposure to low-level Cu contamination and illustrate the variability in

physiological responses of wild fish caused by demographic features. Overall, of the parameters measured, Hct, Let, and percentage of lymphocytes in blood appeared to offer robust measures for assessing effects of metals on wild fish and did not appear affected by select demographic features.

Dethloff, G. M., D. Schlenk, et al. (1999). "Alterations in Physiological Parameters of Rainbow Trout (Oncorhynchus Mykiss) With Exposure to Copper and Copper/Zinc Mixtures." Ecotoxicology and Environmental Safety **42**(3): 253-264.

Rainbow trout (Oncorhynchus mykiss) mere exposed to sublethal concentrations of copper (Cu, 14 mu g/liter) and zinc (Zn, 57 and 81 mu g/liter) for a 21-day period. The four treatments included a control, a Cu control, a Cu and low-Zn treatment and a Cu and high-Zn treatment. Selected parameters [e.g., hemoglobin (Hb), hematocrit (Hct), plasma glucose, lactate and cortisol, differential leukocyte count, respiratory burst, tissue metal concentrations, hepatic metallothionein (MT), brain acetylcholinesterase (AChE)] mere evaluated at 2, 7, 14, and 21 days of exposure, Whole blood and plasma parameters mere not altered by exposure to metals. The percentage of lymphocytes was consistently decreased in the three metal treatments, while percentages of neutrophils and monocytes mere increased. Respiratory burst activity was elevated in all metal treatments. Gill Zn concentration mas highly variable, with no significant alterations occurring. Gill Cu concentration mas elevated above control levels in all metal treatments, Gill Cu concentration in the two Cu/Zn treatments nas also elevated above levels in the Cu control, Hepatic metal concentrations and MT levels were not altered from control values. Measurements of brain AChE indicated an elevation in this parameter across metal treatments. In general, alterations in physiological parameters appeared to be due to Cu, with Zn having no interactive effect, (C) 1999 Academic Press.

Dethloff, G. M., D. Schlenk, et al. (1999). "The Effects of Copper on Blood and Biochemical Parameters of Rainbow Trout (Oncorhynchus Mykiss)." Archives of Environmental Contamination and Toxicology **36**(4): 415-423.

Metals are released into aquatic systems from many sources, often at sublethal concentrations. The effects of sublethal concentrations of metals on fish are not entirely understood. The objective of this study was to determine the hematological and biochemical effects of a range of copper concentrations (6.4, 16.0, 26.9 mu g Cu/L) on rainbow trout (Oncorhynchus mykiss) over a prolonged period of time. Trout were exposed to copper, and, at intervals of 3, 7, 14, and 21 days, selected parameters were evaluated. Hemoglobin, hematocrit, plasma glucose, and plasma cortisol levels were elevated in trout exposed to 26.9 mu g Cu/L at day 3 and then returned to levels comparable to control fish. Plasma protein and lactate levels were not significantly altered in trout from any copper treatment. Hepatic copper concentration and hepatic metallothionein mRNA expression were consistently elevated in trout exposed to 26.9 mu g Cu/L. Both of these parameters stabilized by day 3, with only hepatic copper concentration showing a further increase at day 21. Hepatic copper concentration and hepatic metallothionein mRNA expression appear to be robust indicators of copper

exposure. Most blood-based parameters evaluated appear to be associated with a transitory, nonspecific stress response. The return of elevated hematological and biochemical parameters to control levels after 3 days and the stabilization of hepatic metallothionein mRNA expression and copper concentration over a similar time period suggested acclimation to dissolved copper at 26.9 mu g/L. Further analysis of the data on blood-based parameters indicated that certain parameters (hemoglobin, hematocrit, plasma glucose, plasma cortisol) may be useful in field monitoring.

Eaton, J. G. (1973). "CHRONIC TOXICITY OF A COPPER, CADMIUM AND ZINC MIXTURE TO FATHEAD MINNOW (PIMEPHALES-PROMELAS-RAFINESQUE)." Water Research **7**(11): 1723-1736.

Erickson, R. J., C. F. Kleiner, et al. (1997). "Effect of acclimation period on the relationship of acute copper toxicity to water hardness for fathead minnows." Environmental Toxicology and Chemistry **16**(4): 813-815.

The acute (96-h) toxicity of copper sulfate to fathead minnows was tested in a 2 x 2 factorial experiment, with factors being low (ca. 1 meq/L) and high (ca. 4 meq/L) water hardness during an acclimation period and low and high hardness during the exposure period. Acclimation hardness was found to have no significant effects on copper lethality, except that the 24-h LC50 at high exposure hardness was 44% higher for fish sub ect to low acclimation hardness than fish subject to high acclimation hardness. In contrast, exposure hardness was found to have major effects, with LC50s being two to three times greater at high exposure hardness than at low, regardless of the acclimation hardness. These results suggest that the extent of the acclimation period is not a general, severely confounding factor for previous reports of the effects of hardness on copper toxicity to fish, although it may assume some importance for some species and test conditions.

Evans, R. D., G. C. Balch, et al. (2006). "Uptake and elimination of lead, zinc, and copper by caddisfly larvae (Trichoptera : Hydropsychidae) using stable isotope tracers." Archives of Environmental Contamination and Toxicology **51**(1): 35-42.

Stable isotopes of Pb, Zn, and Cu were used in laboratory experiments to determine the uptake and elimination of these metals by stream-dwelling caddisfly (Trichoptera: Hydropsychidae) larvae. For Pb and Cu, larvae were exposed to environmentally realistic levels (2.5 and 4.5 mu g . L-1, respectively) of one isotope for 9 days followed by a 9-day exposure to either the same isotope, to a second stable isotope of the same metal, or to RW containing no added isotope (two phases in total). For zinc, the exposure concentration was 15 mu g . L-1, and the experiment lasted for a total of 27 (i.e., three phases) rather than 18 days to see if uptake and elimination changed during the extended time period. The uptake clearances (k(u)) determined for the various metals averaged 7.8, 1.4, and 0.6 L . g dw(-1) . d(-1) for Pb, Zn, and Cu, respectively, if the total metal concentration in the water was used in the calculations. The clearance rate constants (k(e)) were less variable, averaging 0.15 d(-1) for Pb, 0.22 d(-1) for Zn,

and approximately 0.1 d(-1) for Cu and were similar in both the presence (i.e., elimination) and absence (i.e., depuration) of metal in the water. These values are also comparable with those reported in the literature for other aquatic invertebrates. The use of stable isotopes thus allowed simultaneous measurement of uptake and clearance (elimination and depuration) of these metals at environmentally realistic concentrations and could be of great benefit for determining partitioning, assimilation efficiency, and pathways of these and other metals in the environment.

Farag, A. M., D. Skaar, et al. (2003). "Characterizing aquatic health using salmonid mortality, physiology, and biomass estimates in streams with elevated concentrations of arsenic, cadmium, copper, lead, and zinc in the Boulder River watershed, Montana." Transactions of the American Fisheries Society **132**(3): 450-467.

Abandoned tailings and mine adits are located throughout the Boulder River watershed in Montana. In this watershed, all species of fish are absent from some tributary reaches near mine sources; however, populations of brook trout Salvelinus fontitalis, rainbow trout Oncorhynchus mykiss, and cut-throat trout O. clarki are found further downstream. Multiple methods must be used to investigate the effects of metals released by past mining activity because the effects on aquatic life may range in severity, depending on the proximity of mine sources. Therefore, we used three types of effects-those on fish population levels (as measured by survival), those on biomass and density, and those at the level of the individual (as measured by increases in metallothionein, products of lipid peroxidation, and increases in concentrations of tissue metals)-to assess the aquatic health of the Boulder River watershed. Elevated concentrations of Cd, Cu, and Zn in the water column were associated with increased mortality of trout at sites located near mine waste sources. The hypertrophy (swelling), degeneration (dying), and necrosis of epithelial cells observed in the gills support our conclusion that the cause of death was related to metals in the water column. At a site further downstream (lower Cataract Creek), we observed impaired health of resident trout, as well as effects on biomass and density (measured as decreases in the kilograms of trout per hectare and the number per 300 m) and effects at the individual level, including increases in metallothionein, products of lipid peroxidation, and tissue concentrations of metals.

Finlayson, B. J. and K. M. Verrue (1982). "TOXICITIES OF COPPER, ZINC, AND CADMIUM MIXTURES TO JUVENILE CHINOOK SALMON." Transactions of the American Fisheries Society **111**(5): 645-650.

Fitzgerald, G. P. and S. L. Faust (1963). "FACTORS AFFECTING TOXICITY OF COPPER TO ALGAE AND FISH." American Journal of Botany **50**(6P2): 629-&.

Giguere, A., P. G. C. Campbell, et al. (2006). "Sub-cellular partitioning of cadmium, copper, nickel and zinc in indigenous yellow perch (Perca flavescens) sampled along a polymetallic gradient." Aquatic Toxicology **77**(2): 178-189.

Sub-cellular metal distributions were studied in indigenous yellow perch (Perca

flavescens) collected from eight lakes located along a cadmium (Cd), copper (Cu), nickel (Ni) and zinc (Zn) concentration gradient. Ambient dissolved metal concentrations were measured to evaluate exposure and total hepatic metal concentrations were determined as a measure of metal bioaccumulation. Metal partitioning among potentially metal-sensitive fractions (cytosolic enzymes, organelles) and detoxified metal fractions (metallothionein) was determined after differential centrifugation of fish liver homogenates. Major proportions of hepatic Cd and Cu were found in the heat-stable cytosolic peptides and proteins fraction (HSP), a fraction including metallothioneins, whereas the potentially metal-sensitive heat-denaturable proteins fraction (HDP) was the largest contributor to the total Ni and Zn burdens. The concentrations of Cd, Cu and Ni (but not Zn) in each sub-cellular fraction increased along the metal contamination gradient, but the relative contributions of each fraction to the total burden of each of these metals remained generally constant. For these chronically exposed fish there was no threshold exposure concentration below which binding of Cd or Ni to the heat-denaturable protein fraction did not occur. The presence of Cd and Ni in the HDP fraction, even for low chronic exposure concentrations, suggests that metal detoxification was imperfect, i.e. that P. flavescens was subject to some metal-related stress even under these conditions. (c) 2005 Elsevier B.V. All rights reserved.

Gravenmier, J. J., D. W. Johnston, et al. (2005). "Acute toxicity of copper to the threespine stickleback, Gasterosteus aculeatus." Environmental Toxicology **20**(2): 150-159.

This study focuses on characterizing the acute toxicity of copper in freshwater to the threespine stickleback, Gasterosteus aculeatus, a small and widely distributed eurylhaline fish. The threespine stickleback is used as an effluent monitoring species in both Canada and the United States, yet in some locations natural populations are listed as threatened or endangered. Four 96-h static renewal acute toxicity tests were performed in moderately hard water using U.S. EPA methods with adult fish (mean wet weight 0.41g/fish). The geometric mean of the 24-, 48-, 72- and 96-h LC(50)s based on measured concentrations of total copper (estimated dissolved copper in parentheses) in the test solutions were 382.2 (366.9), 278.7 (267.6), 256.6 (246.3), and 227.2 (218.1) mu g Cu/L, respectively. Conservative estimates of acute toxicity thresholds, made using LC1 values, for adult threespine sticklebacks over 24-, 48-, 72- or 96-h exposure periods in moderately hard water are approximately 114.3 (109.7), 78.3 (75.2), 67.0 (64.3), and 52.4 (50.3) mu g Cu/L, respectively. Test results were normalized to a range of water hardness from very soft to very hard using two U.S. EPA methods, the water hardness and the Biotic Ligand Model normalization procedures. Subsequently, interspecies sensitivity comparisons were made with aquatic animal species used in both the current and proposed U.S. EPA copper water quality criteria documents. Information reported in this study may be useful in effluent toxicity identification evaluations, ecological risk assessments and criteria development where copper is a concern. (c) 2005 Wiley Periodicals. Inc.

Grosell, M., J. Blanchard, et al. (2007). "Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates." Aquatic Toxicology **84**(2): 162-172.

The present paper presents original data and a review of the copper (Cu) toxicity literature for estuarine and marine environments. For the first time, acute Cu toxicity across the full salinity range was determined. Killifish, Fundulus heteroclitus, eggs were hatched in freshwater (FW), 2.5, 5, 10, 15, 22 and 35 ppt (seawater, SW) and juveniles were allowed to acclimate for 7 days prior to acute toxicity testing. Sensitivity was highest in FW (96 h LC50: 18 mu g/l), followed by SW (96 h LC50: 294 mu g/l) with fish at intermediate salinities being the most tolerant (96 h LC50 > 963 mu g/l at 10 ppt), This approximately 50-fold, non-linear variation in sensitivity could not be accounted for by Cu speciation or competition among cations but can be explained by physiology. The relative Na+ gradient from the blood plasma to the water is greatest in FW followed by SW and is smallest at 10 ppt. Regression of Cu toxicity versus the equilibrium potential for Na+, which reflects the relative Na+ gradient, revealed that 93% of the variation can be attributed to Na+ gradients and thus osmoregulatory physiology. Examination of the existing literature on acute Cu toxicity in SW (defined as > 25 ppt) confirmed that early life stages generally are most sensitive but this pattern may be attributable to size rather than developmental stage. Regardless of developmental stage and phylogeny, size clearly matters for Cu sensitivity. The existing literature on the influence of salinity on acute Cu toxicity as well as studies of mechanisms of Cu toxicity in fish and invertebrates are reviewed. 

Grosell, M., C. Nielsen, et al. (2002). "Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **133**(1-2): 287-303.

The mechanisms of acute copper and silver toxicity in freshwater organisms appear similar. Both result in inhibition of branchial sodium (and chloride) uptake initiating a cascade of effects leading to mortality. The inhibition of the branchial Na/K-ATPase in the basolateral membrane is generally accepted as the key component responsible for the reduced sodium uptake. We propose that branchial carbonic anhydrase and the apical sodium channel may also be important targets for both copper and silver exposure. Several attempts have been made to predict metal sensitivity. A prominent example is the geochemical-biotic ligand model. The geochernical-biotic ligand modeling approach has been successful in explaining variations in tolerance to metal exposure for specific groups of animals exposed at different water chemistries. This approach, however, cannot explain the large observed variation in tolerance to these metals amongst different groups of freshwater animals (i.e. Daphnia vs. fish). Based on the detailed knowledge of physiological responses to acute metal exposure, the present review offers an explanation for the observed variation in tolerance. Smaller animals are more sensitive than large animals because they exhibit higher sodium turnover rates. The same relative inhibition of sodium uptake

results in faster depletion of internal sodium in animals with higher sodium turnover. We present a way to improve predictions of acute metal sensitivity, noting that sodium turnover rate is the key predictor for variation in acute copper and silver toxicity amongst groups of freshwater animals. We suggest that the presented sodium turnover model is used in conjunction with the Biotic Ligand Model for risk management decisions. (C) 2002 Elsevier Science Inc. All rights reserved.

Gupta, A. K. and V. K. Rajbanshi (1991). "TOXICITY OF COPPER AND CADMIUM TO HETEROPNEUSTES-FOSSILIS (BLOCH)." Acta Hydrochimica Et Hydrobiologica **19**(3): 331-340.

Static bioassay tests were conducted to determine the acute toxic effects of copper, cadmium and their mixtures on Heteropneustes fossilis in two different seasons. Median lethal concentrations (24, 48, 72 and 96 h LC50) revealed that copper is more toxic to fish even at very low concentrations in comparison to cadmium, tested separately or in combination with cadmium. The data indicate that the level of tolerance of the fish to metallic ions tested was temperature specific. Generally, fishes were found to be more susceptible to metallic ions at higher temperatures as revealed by the threshold concentration, MATC and LC50 values. The behavioural changes of the test fishes were also observed in reference to different concentrations of the metallic ions and temperatures.

Hall, L. W., M. C. Scott, et al. (1998). "Ecological risk assessment of copper and cadmium in surface waters of Chesapeake Bay watershed." Environmental Toxicology and Chemistry **17**(6): 1172-1189.

This ecological risk assessment was designed to characterize risk of copper and cadmium exposure in the Chesapeake Bay watershed by comparing the probability distributions of environmental exposure concentrations with the probability distributions of species response data determined from laboratory studies. The overlap of these distributions was a measure of risk to aquatic life. Dissolved copper and cadmium exposure data were available from six primary data sources covering 102 stations in Is basins in the Chesapeake Bay watershed from 1985 through 1990. Highest environmental concentrations of copper (based on 90th percentiles) were reported in the Chesapeake and Delaware (C and D) Canal, Choptank River, Middle River, and Potomac River, the lowest concentrations of copper were reported in the lower and middle mainstem Chesapeake Bay and Nanticoke River. Based on the calculation of 90th percentiles, cadmium concentrations were highest in the C and D Canal, Potomac River, Upper Chesapeake Bay, and West Chesapeake watershed. Lowest environmental concentrations of cadmium were reported in the lower and middle mainstem Chesapeake Bay and Susquehanna River. The ecological effects data used for this risk assessment were derived primarily from acute copper and cadmium laboratory toxicity tests conducted in both fresh water and salt water, chronic data were much mon limited. The 10th percentile (concentration protecting 90% of the species) for all species derived from the freshwater acute copper toxicity database was 8.3 mu g/L. For acute saltwater

copper data, the 10th percentile for all species was 6.3 mu g/L copper, The acute 10th percentile for all species in the freshwater cadmium database was 5.1 mu g/L cadmium. The acute 10th percentile for all saltwater species was 31.7 mu g/L cadmium. Highest potential ecological risk from copper exposures was reported in the C and D Canal area of the northern Chesapeake Bay watershed. Relatively high potential ecological risk from copper exposure was also reported in Middle River. Moderate potential ecological risk from copper exposure was reported in selected locations in the Choptank and Potomac Rivers. Potential ecological risk from copper exposure was either low or data were insufficient to assess ecological risk in the other 14 basins. Potential ecological risk from cadmium exposures was much lower than for copper. Highest potential ecological risk from cadmium exposure was reported in the C and D Canal. Low to moderate potential ecological risk: for the most sensitive trophic group (fish) was reported in the Potomac River, upper mainstem bay, West Chesapeake watershed, Choptank River. and Chester River. In the other 12 basins, ecological risk was either judged to be low or insufficient data were available for determining risk.

Handy, R. D. (1992). "THE ASSESSMENT OF EPISODIC METAL POLLUTION .2. THE EFFECTS OF CADMIUM AND COPPER ENRICHED DIETS ON TISSUE CONTAMINANT ANALYSIS IN RAINBOW-TROUT (ONCORHYNCHUS-MYKISS)." Archives of Environmental Contamination and Toxicology **22**(1): 82-87.

Rainbow trout, Oncorhynchus mykiss, were fed on a commercial feed enriched with either Cd or Cu for 32 days and allowed to recover on normal food for 12 days. The body burden of fish fed Cd-enriched diet increased from about 0.05 to 0.39-mu-g/g wet weight, reflected by elevation in the Cd content of gill, liver, blood plasma, kidney, skin, mucus, and whole gut. Muscle Cd content did not alter. Most organs remained contaminated with Cd after 12 days depuration. The body burden of animals fed Cu-enriched diet increased from about 1.2 to 1.5-mu-g/g wet weight, with the gill, whole gut, blood plasma, skin, and mucus contaminated after 32 days, no changes in the liver and muscle occurred. All organs, except the gill and kidney, returned to control values after 12 days depuration. In exposed animals, 76 and 53% of the Cd or Cu body burden, respectively, were contained in the gut, unlike waterborne exposure. Differences in the body distribution of toxicants after intermittent waterborne and dietary exposure may identify the principal routes of pollutant uptake. Ratios of toxicant concentrations in gill/liver may establish the exposure status of fish, but cannot be used to differentiate dietary and waterborne contamination. Analysis of mucus may identify waterborne Cu exposure, dietary and waterborne contributions to mucus Cd content are more difficult to establish.

Handy, R. D. (2003). "Chronic effects of copper exposure versus endocrine toxicity: two sides of the same toxicological process?" Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **135**(1): 25-38.

Chronic sub-lethal exposure to copper (Cu) causes a series of cellular and physiological changes in fish that enable the animal to survive. Copper is also an

endocrine disrupting metal in the aquatic environment, and has a number of normal neuro-endocrine roles in vertebrates. This paper explores whether the chronic effects of Cu exposure can be explained by the effects of Cu on neuro-endocrine functions in fish. Chronic Cu exposure involves complex physiological adjustments in many body systems, including increased oxygen consumption, reduced mean swimming speed, upregulation of ionic regulation, decreasing lymphocyte levels and increasing neutrophils, altered immunity, modulation of Cu-dependent and independent enzyme activities, and proliferation of epithelial cells in gills or intestine. These responses can occur with exposure via the food or the water and can be rationalised into three major categories: (1) up-regulation of enzymes/metabolism (2) altered haematopoietic responses and (3) altered cellularity (cell type, turnover or size) in tissues. Some of these responses can be explained by stimulation of general stress responses, including the adrenergic response and stimulated cortisol release via the hypothalamic-pituitary-interrenal axis. This can occur despite evidence of vacuolation and foci of necrosis in the brain, and increased macrophage activity, in the kidney of fish exposed to dietary Cu. In addition to generic stress responses, Cu regulates specific neuro-endocrine functions, including the loss of circadian rhythm during dietary Cu exposure that involves the failure to respond to circulating melatonin and a loss of circulating serotonin. We conclude that the chronic physiological effects of Cu and apparent endocrine disrupting effects of Cu are two sides of the same toxicological process. 

Handy, R. D., D. W. Sims, et al. (1999). "Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (Oncorhynchus mykiss) during chronic dietary exposure to copper." Aquatic Toxicology **47**(1): 23-41.

The aim of this study was to obtain a holistic view of the sublethal toxic responses, and compensations, of fish exposed to Cu in the diet. Rainbow trout were fed to appetite on either a Cu-loaded diet (500 mg Cu kg(-1) dw) or a control diet (11.9 mg Cu kg(-1) dw) for 3 months. Nutritional performance, blood and muscle chemistry, histology, respiration, and swimming behaviour were measured. Copper concentration in intestine and liver of exposed fish was 16 and 2.5 times greater than in controls respectively, confirming oral Cu exposure. [Cu] remained low in the gills of fish irrespective of Cu treatment (< 0.096 mu mol g(-1) dw). Hepatic and intestinal metallothionien levels increased 8-and 1.5-fold, respectively, in Cu-treated fish compared to controls. Growth rate, appetite, food conversion ratios, carcass composition, oxygen consumption and ventilation rate in exposed fish were not different from controls. There were no treatment related changes in serum chemistry (glucose, triglycerides, protein, Na, K, Hb) or the mineral composition of skeletal muscle (Ca, Mg, Zn, Na, or K). However, quantitative histology showed a 9% increase in gill secondary lamellae length, together with lipid depletion in the livers of copper treated fish compared to controls. Routine swimming activity was affected significantly by oral Cu exposure. Time spent swimming was 35% lower in Cu-exposed fish after 3 months, while total distance moved was reduced by only 21% because mean

swimming speed was 12% higher in contaminated fish. Maximum speeds attained by exposed fish were not different compared to controls. Exposed fish lowered activity overall by decreasing time spent swimming at low (1-17 cm s(-1)) and medium speeds (17-40 cm s(-1)), whilst increasing proportionately time spent fast swimming (40-58 cm s(-1)). These results are discussed in terms of sub-lethal oral Cu affecting the partitioning of energy utilisation between locomotion and maintenance metabolism by trout in a physiological drive towards invariant growth rates. 

Hansen, J. A., J. Lipton, et al. (2002). "Relationship between exposure duration, tissue residues, growth, and mortality in rainbow trout (Oncorhynchus mykiss) juveniles sub-chronically exposed to copper." Aquatic Toxicology **58**(3-4): 175-188.

We conducted a 56-day sub-chronic test on the effects of Cu on rainbow trout (Oncorhynchus mykiss) fry at a nominal water hardness of 100 mg 1(-1) (as CaCO3). Response measures were growth, whole body Cu concentrations, and mortality. Significant mortality was observed in fish exposed to 54.1 mug Cu 1(-1) (47.8%) and 35.7 mug Cu 1(-1) (11.7%). Growth was dose-dependent over the range of Cu treatments (0-54 mug Cu 1(-1)), and was modeled as a function of Cu exposure concentration and exposure duration. Calculated inhibition concentrations (based on change in wet weight through a 56-day Cu exposure) were IC50 = 54.0 mug Cu 1(-1), IC20 = 21.6 mug Cu 1(-1), IC10 = 10.8 mug Cu 1(-1), and IC01 = 1.1 mug Cu 1(-1). Measured whole body Cu was also dose-dependent, and growth of trout fry was readily modeled as a function of tissue Cu and exposure duration. This model was virtually identical to a model previously developed for rainbow trout exposed to Cu at a hardness of 25 mg 1(-1). Following the 56-day exposure period, we performed a 96-h acute challenge to Cu and Cd to evaluate the effects of Cu acclimation on acute Cu and Cd toxicity. Sensitivity to Cu was dependent on the 'acclimation dose'; trout previously held in control aquaria (i.e. not acclimated to Cu) suffered over 80% mortality, whereas trout previously exposed to 35.7 mug Cu 1(-1) for 56 day suffered 20% mortality. These fish also showed somewhat reduced sensitivity to Cd, suggesting acclimation to Cu can enhance tolerance to other metals. Finally, the relationship between growth response and hardness (derived from several studies) appeared to have a different slope than the hardness relationship previously observed for lethality responses. 

Hansen, J. A., J. D. Rose, et al. (1999). "Chinook Salmon (Oncorhynchus Tshawytscha) and Rainbow Trout (Oncorhynchus Mykiss) Exposed to Copper: Neurophysiological and Histological Effects on the Olfactory System." Environmental Toxicology and Chemistry **18**(9): 1979-1991.

Olfactory epithelial structure and olfactory bulb neurophysiological responses were measured in chinook salmon and rainbow trout in response to 25 to 300 mu g copper (Cu)/L. Using confocal laser scanning microscopy, the number of olfactory receptors was significantly reduced in chinook salmon exposed to greater than or equal to 50 mu g Cu/L and in rainbow trout exposed to greater than or equal to 200 mu g Cu/L for 1 h. The number of receptors was significantly

reduced in both species following exposure to 25 mu g Cu/L for 4 h. Transmission electron microscopy of olfactory epithelial tissue indicated that the loss of receptors was from cellular necrosis. Olfactory bulb electroencephalogram (EEG) responses to 10(-3) M L-serine were initially reduced by all Cu concentrations but were virtually eliminated in chinook salmon exposed to greater than or equal to 50 mu g Cu/L and in rainbow trout exposed to greater than or equal to 200 mu g Cu/L within 1 h of exposure. Following Cu exposure, EEG response recovery rates were slower in fish exposed to higher Cu concentrations. The higher sensitivity of the chinook salmon Olfactory system to Cu-induced histological damage and neurophysiological impairment parallels the relative species sensitivity observed in behavioral avoidance experiments. This difference in species sensitivity may reduce the survival and reproductive potential of chinook salmon compared with that of rainbow trout in Cu-contaminated waters.

Hansen, J. A., P. G. Welsh, et al. (2002). "Effects of Copper Exposure on Growth and Survival of Juvenile Bull Trout." Transactions of the American Fisheries Society **131**(4): 690-697.

Metal toxicity from mine wastes has been identified as a potential contributor to declining populations of the federally threatened bull trout Salvelinus confluentus. Although several studies have been published on the effects of long-term Cu exposure on growth in salmonid fish, none have been conducted with bull trout. We evaluated the effects of a 60-d Cu exposure on bull trout in water at 220 mg/L hardness (as CaCO3) and pH 7.9. The endpoints measured were growth, whole-body Cu concentration, and mortality. Exposure to Cu at 179 mug/L resulted in 7% mortality (compared with a mortality of 2% in controls), but no effects of Cu exposure on growth were observed at any Cu concentration tested. Measured tissue residues of Cu in these bull trout were higher than those associated with reduced growth in rainbow trout Oncorhynchus mykiss, indicating that bull trout may be more tolerant of long-term Cu exposure than rainbow trout. This greater tolerance to Cu may be associated with a lower rate of Cu accumulation or the slower overall. growth rate in bull trout than in rainbow trout. Our results indicate that accumulation of whole-body Cu does not predict toxicological effects in this species.

Hara, T. J., Y. M. C. Law, et al. (1976). "Effects of Mercury and Copper on Olfactory Response in Rainbow-Trout, Salmo-Gairdneri." Journal of the Fisheries Research Board of Canada **33**(7): 1568-1573.

Hashemi, S., R. Blust, et al. (2007). "The effect of food rations on tissue-specific copper accumulation patterns of sublethal waterborne exposure in Cyprinus carpio." Environmental Toxicology and Chemistry **26**(7): 1507-1511.

Common carp (Cyprinus carpio) were fed to two different food rations, 0.5% body weight (low ration [LR]) and 5% body weight (high ration [HR]), and were exposed to sublethal (1 mu M) copper levels for 28 d in softened Antwerp (Belgium) city tap water (Ca2+, 79.3 mg/L; Mg2+, 7.4 mg/L; Na+, 27.8 mg/L; pH

7.5-8.0). Copper accumulations in the liver, gills, kidney, anterior intestine, posterior intestine, and muscle were determined. Copper accumulation in the gills, liver, and kidney of LR fish was significantly higher than in HR fish. The only time copper uptake in HR fish was significantly higher than in LR fish was in the posterior intestine after two weeks of exposure. No difference was found between the two rations in the anterior intestine. Copper accumulation in the liver of both feeding treatments occurred in a time-dependent manner and did not reach steady state in any treatment. On the contrary, copper concentration in the gills reached a steady state for both HR and LR fish within the first week of exposure. No copper accumulation was found in muscle tissues of either treatment. Copper concentration dropped to control levels in all tissues, except liver tissue, two weeks after the exposure ended. Our studies indicated that copper uptake was influenced by the food ration in carp. The difference in copper accumulation probably is related to the amount of dietary NaCl and different rates of metallothionein synthesis. Low food availability provides less Na+ influx and leads to increased brachial uptake of Na+ and copper. In addition, it has been shown that starved animals show increased levels of metallothionein, possibly causing higher copper accumulation.

Herbert, D. W. M. and J. M. Vandyke (1964). "TOXICITY TO FISH OF MIXTURES OF POISONS .2. COPPER-AMMONIA + ZINC-PHENOL MIXTURES." Annals of Applied Biology **53**(3): 415-&.

Hernandez, P. P., V. Moreno, et al. (2006). "Sub-lethal concentrations of waterborne copper are toxic to lateral line neuromasts in zebrafish (Danio rerio)." Hearing Research **213**(1-2): 1-10.

in teleosts. the lateral line system is composed of neuromasts containing hair cells that are analogous to those present in the inner ear of all vertebrates. In the zebrafish embryo and early larva, this system is composed of the anterior lateral line (ALL). which covers the head, and the posterior lateral line (PLL), present in the trunk and tail. The mechanosensory hair cells found in neuromasts can be labeled in Vivo using fluorescent dyes such as 4-di-2-Asp (DiAsp) or FM 1-43. We have studied the effects of water-borne copper exposure on the function of the lateral line system in zebrafish larvae. our results show that transient incubation of post-hatching larvae for 2 h with non-lethal concentrations of copper (1-50 mu M CuSO4) induces cellular damage localized to neuromasts, apoptosis, and loss of hair cell markers. This effect is specific to copper, as other metals did not show these effects. Since hair cells in fish can regenerate, we followed the reappearance of viable hair cells in neuromasts after copper removal. In the PLL, we determined that there is a threshold concentration of copper above which regeneration does not occur, whereas, at lower concentrations, the length of time it takes for viable hair cells to reappear is dependent oil the amount of copper used during the treatment. The ALL behaves differently though, as regeneration can Occur even after treatments with concentrations of copper an order of magnitude higher than the one that irreversibly affects the PLL. Regeneration of hair cells is dependent oil cell

division within the neuromasts as damage that precludes proliferation prevents reappearance of this cell type. (C) 2005 Published by Elsevier B.V.

Hollis, L., L. Muench, et al. (1997). "Influence of dissolved organic matter on copper binding, and calcium on cadmium binding, by gills of rainbow trout." Journal of Fish Biology **50**(4): 703-720.

Complexation of Cu by 5 mg C l(-1) dissolved organic matter (DOM) from a marsh kept Cu from binding to gills of small rainbow trout Oncorhynchus mykiss in 9-day exposures to 0.5 mu m Cu in soft water. The protective effect of DOM occurs because the formation of Cu-DOM complexes reduces the amount of free Cu in the water, so the disruptive effects of Cu on ionoregulation, such as inhibited Na uptake, cannot develop. The Cu-DOM complexes themselves do not bind to the gills. Calcium (1100 mu M) reduced the accumulation of Cd by trout gills in short, 2-h exposures through competition for gill binding sites but not over longer, 7-day exposures to 0.14 mu M Cd. However, the protective effect of Ca against Cd toxicity persisted throughout the longer experiment, likely due to the decrease in the electrochemical gradient for diffusive loss of Ca from the fish to the water. Rainbow trout and fathead minnows Pimephales promelas accumulated Cu and Cd on their gills in a similar manner; thus, binding constants for metal-gill interactions determined for one species of fish can be generalized to other fish species. When literature binding constants determined for fathead minnows were applied to our studies with rainbow trout, computer modelling of Cu-gill and Cu-DOM interactions simulated our results well. In contrast Cd-gill and Ca-gill modelling predicted the initial competitive effect of Ca against Cd accumulation by trout gills, but did not predict the longer-term accumulation of Cd by trout gills. (C) 1997 The Fisheries Society of thr British Isles.

Huang, S. B., Z. J. Wang, et al. (2003). "Measuring the bioavailable/toxic concentration of copper in natural water by using anodic stripping voltammetry and Vibrio-qinghaiensis sp.Nov.Q67 bioassay." Chemical Speciation and Bioavailability **15**(2): 37-45.

Bioassays were carried out in the culture media for Vibrio-qinghaiensis sp.Nov-Q67 and the influences of alkalinity and different concentrations of chloride, ethylene diamine tetraacetic acid (EDTA) and natural derived fulvic acid (FA) on the labile concentration and toxicity of Cu were investigated. The labile concentration of Cu was obtained by differential pulse anodic stripping voltammetry with a double acidification method (DAM-DPASV). Changes in water alkalinity and chloride concentrations did not affect the labile concentration of Cu, but increases of alkalinity and concentrations of chloride reduced the toxicity on Q67 In the presence of EDTA and FA, both labile concentration of Cu and toxicity were reduced. By excluding Cu-carbonate complexes and Cu-chloride from labile concentration, a bioavailable concentration of Cu (or [Cu*]) was obtained and used to predict the acute toxicity of Cu on Q67. For natural waters, the labile concentration of Cu was measured by DAM-DPASV and [Cu*] was calculated by a MINTEQ A2 software based on composition of waters. This procedure was tested for Guanting Reservoir waters by spiking different concentration Cu. The results showed that [Cu*] was a good indicator for Cu toxicity and could be used

in field conditions.

Hutchinson, T. H., T. D. Williams, et al. (1994). "TOXICITY OF CADMIUM, HEXAVALENT CHROMIUM AND COPPER TO MARINE FISH LARVAE (CYPRINODON-VARIEGATUS) AND COPEPODS (TISBE BATTAGLIAI)." Marine Environmental Research **38**(4): 275-290.

For comparative purposes, the toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (Cyprinodon variegatus) and copepods (Tisbe battagliai) has been evaluated. Toxicity to fish larvae was measured in terms of survival and growth over 7 days, whilst toxicity to copepods was assessed in tel ms of survival and reproduction after 8 days exposure. For fish larvae, 96 h LC(50) values (based on mean measured concentrations of total metal ion) were 1.23 mg Cd/litre, 31.6 mg Cr6+/litre and >0.22 mg Cu/litre. Subchronic values (SChVs) for larval fish survival and growth after 7 days were 0.75 mg Cd/litre, 24.0 mg Cr6+/litre and 0.16 mg Cu/litre. For copepod nauplii and adults, 96 h LC(50) values were as follows. 0.46 mg Cd/litre and 0.34 mg Cd/litre, respectively, 1.60 Cr6+/litre, and 5.9 mg Cr6+/litre respectively; and 0.064 mg Cu/litre and 0.088 mg Cu/litre, respectively. SChVs foR naupliar survival and adult survival or reproduction after 8 days were 0.024 mg Cd/litre, 0.42 mg Cr6+/litre and 0.008 mg Cu/litre.

Johnson, A., E. Carew, et al. (2007). "The effects of copper on the morphological and functional development of zebrafish embryos." Aquatic Toxicology **84**(4): 431-438.

Waterborne copper exposure can exert a variety of physiological effects in fish, including the disruption of sensory system function, which has wide-reaching implications for fish behaviour. In developing fish larvae, copper is known to affect key parameters, such as survival and growth and more recently has been shown to interfere with the octavolateral system. The present study aimed to take a combined view of morphological (e.g. length, yolk sac area) and functional (e.g. heart beat, behaviour) processes to understand the complex effect of copper on fish development. In the first of two experiments, zebrafish embryos were exposed to a range of copper concentrations (11-1000 mu g l(-1)) from fertilisation for a 72 h period. The greatest mortality was seen between 5 and 24 h post-fertilisation (hpf) and was more pronounced at the higher copper concentrations. Copper also had an inhibitory effect on hatching. Length and yolk sac area of individuals were recorded across treatments at 72 hpf and elevated copper was found to slow development. Individuals from the higher copper treatments had the fastest heart rates at 28 hpf suggesting that stress responses were induced in the embryos during copper exposure. In the second experiment, embryos were exposed in a similar manner to two copper concentrations, based on those from Experiment 1 that resulted in < 50% mortality. At 120 hpf, embryos exposed to both copper concentrations possessed significantly fewer functional neuromasts, an effect which was associated with a reduced ability to orientate in a current. Therefore, although mortality at these copper concentrations was low initially, and then almost non-existent after 24 hpf, the inability of copper-exposed larvae to orientate in a water current as a result of lateral line dysfunction is likely

to seriously compromise survival. (c) 2007 Elsevier B.V. All rights reserved.

Jonsson, M. E., C. Carlsson, et al. (2006). "Effects of copper on CYP1A activity and epithelial barrier properties in the rainbow trout gill." Aquatic Toxicology **79**(1): 78-86.

The effects of copper on P-naphthoflavone (beta NF)-induced ethoxyresorufin O-deethylase (EROD) activity were studied in rainbow trout (Oncorhynchus mykiss) gill filaments (after in vivo exposure) and in gill cells cultured as both primary cultures and as polarised epithelia, i.e. with water in the apical compartment and culture medium in the basolateral compartment. In the in vivo study beta NF and copper were added to the water, in primary cultures both chemicals were added to the culture medium and in cultured epithelia copper was added to the apical water whilst beta NF was added to the basolateral culture medium. In primary cultures this investigation was repeated with and without foetal bovine serum (FBS) supplementation of the culture media. Gill barrier properties, specifically polyethylene glycol (PEG-4000) permeability (i.e. paracellular permeability), sodium efflux and transepithelial electrical resistance (TER) were also investigated in cultured gill cell epithelia after apical treatment with copper. Two micromolar copper had no effect on EROD activity in gill filaments in vivo irrespective of whether EROD was induced by 0.01, 0.1 or 1.0 mu M beta NF Similarly, 0.5-100 mu M copper had no effect on EROD induction in cultured epithelia. In primary cultures copper did reduce EROD induction but the effective concentration was dependent on whether the cells were supplemented with FBS, i.e. EROD activity was reduced by all copper concentrations of 5 and above if FBS was included, but only by 1000 mu M if FBS was omitted. In cultured epithelia PEG-4000 permeability increased, whilst sodium efflux and TER were unaffected following treatment with 75 mu M copper. Based on these results we conclude that the branchial monooxygenase system is a less sensitive target for copper than the barrier properties of the gill. Indeed, these data suggest the apical membrane of the gill epithelial cells minimises the uptake of waterborne copper and therefore protects the intracellular environment, including the CYP1A system. This could enable the freshwater fish gill to retain their potential of first-pass metabolism of waterborne organic compounds whilst simultaneously being exposed to waterborne copper. (c) 2006 Elsevier B.V. All rights reserved.

Judy, R. D. (1979). "ACUTE TOXICITY OF COPPER TO GAMMARUS-FASCIATUS SAY, A FRESHWATER AMPHIPOD." Bulletin of Environmental Contamination and Toxicology **21**(1-2): 219-224.

Kallanagoudar, Y. P. and H. S. Patil (1997). "Influence of water hardness on copper, zinc and nickel toxicity to Gambusia affinis (B&G)." Journal of Environmental Biology **18**(4): 409-413.

The response of the fresh water fish Gambusia affinis to lethal toxicity of copper, nickel and zinc in the water of different hardness (50, 150 and 300 mg/l CaCO3) was investigated. Results revealed that copper was found to be more toxic to male, female and fries than nickel and zinc in all the water hardness. Toxicity of the metals was reduced with the increase in the hardness. Males were slightly

more tolerant than female and fries were highly sensitive to all the metals. All the three metals induced mucus secretion. Experimental data suggest that water hardness gives protection to fish exposed to metals with reduced lethality of the metals in hard water.

Khangarot, B. S., V. S. Durve, et al. (1981). "TOXICITY OF INTERACTIONS OF ZINC - NICKEL, COPPER - NICKEL AND ZINC - NICKEL - COPPER TO A FRESH-WATER TELEOST, LEBISTES-RETICULATUS (PETERS)." Acta Hydrochimica Et Hydrobiologica **9**(5): 495-503.

Koivisto, S. and M. Ketola (1995). "EFFECTS OF COPPER ON LIFE-HISTORY TRAITS OF DAPHNIA-PULEX AND BOSMINA-LONGIROSTRIS." Aquatic Toxicology **32**(2-3): 255-269.

Daphnia magna, and to a lesser extent D. pulex, are commonly used in standardised laboratory toxicity tests. Neither of these species is common in lakes inhabited by fish which is explained by strong predation pressure by fish. They also exhibit different life-history strategies compared to lake-inhabiting species like Bosmina. Daphnids produce many small (relative to adult size) juveniles whereas the opposite is true for Bosmina. The large neonate size allows an earlier maturation of Bosmina compared to Daphnia. In the present study the effects of copper (10-30 ppb) on life-history traits of D. pulex and B. longirostris were compared. The only significant copper effect on D. pulex was a minor delay of maturation. On the contrary, negative impacts of the same copper concentrations were found on the survival, growth, maturation age, and fecundity of B. longirostris. The population growth rate (r) of B. longirostris decreased with increasing copper concentration. The study showed that B. longirostris was about two times more sensitive to copper stress than D. pulex.

Kolok, A. S. and D. L'Etoile-Lopes (2005). "Do copper tolerant fathead minnows produce copper tolerant adult offspring?" Aquatic Toxicology **72**(3): 231-238.

The objective of this study was to determine if the relative Cu tolerance of fathead minnow parents determines the relative Cu tolerance of their adult offspring. It was hypothesized that the adult offspring of Cu-tolerant minnows would inherit Cu tolerance from their parents. The relative Cu tolerance of 96 adult fish was determined based upon their reduction in swim performance following a sublethal exposure to 150 mu g Cu/l. Control, Cu-tolerant and Cu-susceptible lines of fish were produced and fish within each line were allowed to breed. The offspring were raised to adults, then exposed to one of two sublethal Cu concentrations (150 or 225 mu g Cu/l) for 8 days. There were no significant differences in relative Cu tolerance, as measured by reduction in swim performance, among the three lines of fish at either dose. However, significant differences in whole body Na+ occurred among the fish lines after exposure to 150 mu g Cu/l, but not after exposure to 225 mu g Cu/l. Significant differences in whole body Cu occurred between Cu-tolerant and Cu-susceptible fish lines after exposure to either Cu dose. The offspring did not inherit the relative Cu tolerance of their parents, however, the selection lines had diverged from each other,

particularly with respect to their whole body Cu concentrations after exposure. (c) 2005 Elsevier B.V. All rights reserved.

Lang, D. W., G. H. Reeves, et al. (2006). "The influence of fall-spawning coho salmon (Oncorhynchus kisutch) on growth and production of juvenile coho salmon rearing in beaver ponds on the Copper River Delta, Alaska." Canadian Journal of Fisheries and Aquatic Sciences **63**(4): 917-930.

Lauren, D. J. and D. G. McDonald (1987). "ACCLIMATION TO COPPER BY RAINBOW-TROUT, SALMO-GAIRDNERI - PHYSIOLOGY." Canadian Journal of Fisheries and Aquatic Sciences **44**(1): 99-104.

Leblanc, G. A. (1985). "EFFECTS OF COPPER ON THE COMPETITIVE INTERACTIONS OF 2 SPECIES OF CLADOCERA." Environmental Pollution Series a-Ecological and Biological **37**(1): 13-25.

Lewis, M. (1978). "ACUTE TOXICITY OF COPPER, ZINC AND MANGANESE IN SINGLE AND MIXED SALT-SOLUTIONS TO JUVENILE LONGFIN DACE, AGOSIA-CHRYSOGASTER." Journal of Fish Biology **13**(6): 695-700.

Li, J. S., J. L. Li, et al. (2007). "The effects of copper, iron and zinc on digestive enzyme activity in the hybrid tilapia Oreochromis niloticus (L.) x Oreochromis aureus (Steindachner)." Journal of Fish Biology **71**(6): 1788-1798.

The present experiment was conducted to study effects of Cu, Fe and Zn on activities of digestive enzymes of the hybrid tilapia Oreochromis niloticus x Oreochromis aureus. The acidic protease activities increased 65.5 and 55.1% by addition of homogenates of digesta-containing stomach with copper (75 mg l(-1)) and zinc (50 mg l(-1)) respectively. Addition of Cu and Zn increased the activities of protease in the hepatopancreas homogenates by 132.7 and 38.1% respectively, and reduced the activity of protease in the digesta-containing intestine homogenates by 11.0 and 13.8% respectively. Addition of Fe (50 mg l(-1)) increased the acidic protease activity by 96.7% but did not alter the activities of protease in the intestine and hepatopancreas. Addition of Cu markedly inhibited activities of amylase in intestine and hepatopancreas homogenates, while Zn addition showed no effects. Addition of Fe reduced activities of amylase in the intestine homogenates by 47.9% but had no effect on amylase activities in the hepatopancreas. When Cu (75 mg kg(-1)), Fe (50 mg kg(-1)) and Zn (50 mg kg(-1)) were supplemented to basal diet for 3 weeks, the activities of amylase in hepatopancreas homogenates increased 125.3, 215.6 and 70.0%, respectively, the activities of amylase in intestine increased 79.8, 74.6 and 48.5%, respectively, and the activities of lipase in intestine increased 90.5, 149.8 and 84.0%, respectively. Supplementation of Cu, Fe or Zn into diet had no effects on activity of protease in all digestive organs. Therefore, the results suggest that effects of Cu, Fe and Zn on activity of digestive enzymes in vitro were different from those seen in vivo, and that the positive effects of Cu, Fe and Zn supplemented to fish diet would be valuable information for formulating fish feed.

Linbo, T. L., D. H. Baldwin, et al. (2009). "EFFECTS OF WATER HARDNESS, ALKALINITY, AND DISSOLVED ORGANIC CARBON ON THE TOXICITY OF COPPER TO THE LATERAL LINE OF DEVELOPING FISH." Environmental Toxicology and Chemistry **28**(7): 1455-1461.

Conventional water chemistry parameters such as hardness, alkalinity, and organic carbon are known to affect the acutely lethal toxicity of copper to fish and other aquatic organisms. In the present study, we investigate the influence of these water chemistry parameters on short-term (3 h), sublethal (0-40 mu g/L) copper toxicity to the peripheral mechanosensory system of larval zebrafish (Danio rerio) using an in vivo fluorescent marker of lateral line sensory neuron (hair cell) integrity. We studied the influence of hardness (via CaCl2, MgSO4, or both at a 2:1 molar ratio), sodium (via NaHCO3 or NaCl), and organic carbon on copper-induced neurotoxicity to zebrafish lateral line neurons over a range of environmentally relevant water chemistries. For all water parameters but organic carbon, the reductions in copper toxicity, although statistically significant, were small. Increasing organic carbon across a range of environmentally relevant concentrations (0.1-4.3 mg/L) increased the EC50 for copper toxicity (the effective concentration resulting in a 50% loss of hair cells) from approximately 12 mu g/L to approximately 50 mu g/L. Finally, we used an ionoregulatory-based biotic ligand model to compare copper toxicity mediated by targets in the fish gill and lateral line. Relative to copper toxicity via the gill, we find that individual water chemistry parameters are less influential in terms of reducing cytotoxic impacts to the mechanosensory system.

Linbo, T. L., C. M. Stehr, et al. (2006). "Dissolved Copper Triggers Cell Death in the Peripheral Mechanosensory System of Larval Fish." Environmental Toxicology and Chemistry **25**(2): 597-603.

Dissolved copper is an increasingly common non-point source contaminant in urban and urbanizing watersheds. In the present study, we investigated the sublethal effects of dissolved copper on the peripheral mechanosensory system, or lateral line, of larval zebrafish (Danio rerio). Zebrafish larvae were exposed to copper (0-65 mu g/L), and the cytotoxic responses of individual lateral line receptor neurons were examined using a combination of in vivo fluorescence imaging, confocal microscopy, scanning electron microscopy, and conventional histology. Dissolved copper triggered a dose-dependent loss of neurons in identified lateral line neuromasts at concentrations >= 20 mu g/L. The onset of cell death in the larval mechanosensory system was rapid (< 1 h). When copper-exposed zebrafish were transferred to clean water, the lateral line regenerated over the course of 2 d. In contrast, the lateral line of larvae exposed continuously to dissolved copper (50 mu g/L) for 3 d did not recover. Collectively, these results show that peripheral mechanosensory neurons are vulnerable to the neurotoxic effects of copper. Consequently, dissolved copper in nonpoint source storm-water runoff has the potential to interfere with rheotaxis, schooling, predator avoidance, and other mechanosensory-mediated behaviors that are important for

the migration and survival of fish.

Liu, R. Q., D. Y. Zhao, et al. (2006). "Fate and transport of copper applied in channel catfish ponds." Water Air and Soil Pollution **176**(1-4): 139-162.

A pilot-scale study and field measurements at commercial ponds were conducted to investigate the environmental fate of copper (Cu) applied as an algaecide in commercial catfish ponds. In the pilot study, a total of 774 g Cu(II) was applied to an experimental catfish pond over a period of 16 summer weeks. More than 90% of Cu applied became associated with suspended sediment particles within a few minutes of addition, and then nearly all Cu applied was transferred to the sediment phase within about 2 days. At the end of the study, the peak Cu content in the sediment increased from an initial concentration of 25 similar to 35 mg kg(-1) to about 200 mg kg(-1), and the applied Cu was able to reach a sediment depth of about 16 cm. Meanwhile, Cu concentration in the catfish body decreased from 12.7 +/- 2.81 mg kg(-1) to 6.15 +/- 2.54 mg kg(-1). Copper mass balance indicated that virtually all Cu applied was retained in the sediment. Only 0.01% of the total Cu applied was taken up by fish and 0.1% remained in pond water. Data from 3 commercial fishponds of different ages (1-25 years) and with different sediment types (acidic, neutral and calcareous) supported the pilot-scale observation. Both pilot-testing results and field measurements revealed that Cu is predominantly accumulated within the top sediment layer and barely reached the bottom soil regardless of the pond age and the type of the sediments. Field monitoring of groundwater quality suggested that the copper leaching into the groundwater surrounding the ponds was insignificant.

Luider, C. D., J. Crusius, et al. (2004). "Influence of natural organic matter source on copper speciation as demonstrated by Cu binding to fish gills, by ion selective electrode, and by DGT gel sampler." Environmental Science & Technology **38**(10): 2865-2872.

Rainbow trout (Oncorhynchus mykiss, 2 g) were exposed to 0-5 muM total copper in ion-poor water for 3 h in the presence or absence of 10 mg C/L of qualitatively different natural organic matter (NOM) derived from water spanning a large gradient in hydrologic residence time. Accumulation of Cu by trout gills was compared to Cu speciation determined by ion selective electrode (ISE) and by diffusive gradients in thin films (DGT) gel sampler technology. The presence of NOM decreased Cu uptake by trout gills as well as Cu concentrations determined by ISE and DGT. Furthermore, the source of NOM influenced Cu binding by trout gills with high-color, allochthonous NOM decreasing Cu accumulation by the gills more than low-color autochthonous NOM. The pattern of Cu binding to the NOM measured by Cu ISE and by Cu accumulation by DGT samplers was similar to the fish gill results. A simple Cu-gill binding model required an NOM Cu-binding factor (F) that depended on NOM quality to account for observed Cu accumulation by trout gills; values of F varied by a factor of 2. Thus, NOM metal-binding quality, as well as NOM quantity, are both important when assessing the bioavailability of metals such as Cu to aquatic organisms.

Lundebye, A. K., M. H. G. Berntssen, et al. (1999). "Biochemical and physiological

responses in Atlantic salmon (Salmo salar) following dietary exposure to copper and cadmium." Marine Pollution Bulletin **39**(1-12): 137-144.

Three experiments were conducted with Atlantic salmon (Salmo salar) to assess the effects of dietary exposure to copper and cadmium. The results presented here provide an overview, details of each experiment will be published in full elsewhere. In the first experiment, salmon parr exposed for four weeks to 35 and 700 mg Cu kg(-1) diet had significantly elevated intestinal copper concentrations, cell proliferation (PCNA) and apoptosis rates compared to control fish. No differences were observed in gib or plasma copper concentrations among the groups, In contrast to the controls, the Cu exposed groups did not grow significantly during the exposure period. The second experiment (three months exposure) was conducted to assess the effects of dietary copper (control, 35, 500, 700, 900 or 1750 mg Cu kg(-1) diet) on growth and feed utilization in salmon fingerlings, Growth was significantly reduced after three months exposure to dietary Cu concentrations above 500 mg kg-l. Similarly, copper body burdens were significantly higher in fish exposed to elevated dietary copper concentrations (above 35 mg Cu kg(-1) diet). In the third experiment, salmon parr were exposed to one of six dietary cadmium concentrations (0, 0.5, 5, 25, 125 or 250 mg Cd kg(-1) diet) for four months. Cadmium accumulated in the liver > intestine > gills of exposed fish. Rates of apoptosis and cell proliferation in the intestine increased following exposure to dietary cadmium. Exposure to elevated concentrations of dietary cadmium had no effect on growth in salmon parr. Results from these studies indicate that cellular biomarkers have potential as early warning signs of negative effects on the overall fitness of an organism. (C) 1999 Elsevier Science Ltd. All rights reserved.

MacRae, R. K., D. E. Smith, et al. (1999). "Copper binding affinity of rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis) gills: Implications for assessing bioavailable metal." Environmental Toxicology and Chemistry **18**(6): 1180-1189.

In this study, we determined the conditional stability constant (log K') of copper for the gills of rainbow trout (Oncorhynchus mykiss; RBT) and brook trout (Salvelinus fontinalis; BT). Using toxicity-based complexation bioassays, which measure the effect of competing organic ligands on copper toxicity, the RBT gill copper log K' range was 6.4 to 7.2. Using a Scatchard analysis of gill Cu accumulation, the RBT log K' was 7.50 and the BT log K' was 7.25. The close agreement in RBT log K' values between these two methods suggests that measurement of gill copper accumulation is an acceptable alternative for determining a toxicity-based gill copper binding affinity. Our results also suggest that there is either a single gill copper binding component or, more realistically, multiple components with similar binding properties that function collectively to define a single toxicologically relevant copper conditional stability constant. These results suggest analytical approaches to measuring bioavailable metal concentrations, such as geochemical modeling where biological ligands are included in speciation calculations, may adequately simulate complex biological ligands. A method to convert gill copper accumulation to a bioavailable water

criterion is also discussed.

March, F. A., F. J. Dwyer, et al. (2007). "An evaluation of freshwater mussel toxicity data in the derivation of water quality guidance and standards for copper." Environmental Toxicology and Chemistry **26**(10): 2066-2074.

The state of Oklahoma has designated several areas as freshwater mussel sanctuaries in an attempt to provide freshwater mussel species a degree of protection and to facilitate their reproduction. We evaluated the protection afforded freshwater mussels by the U.S. Environmental Protection Agency (U.S. EPA) hardness-based 1996 ambient copper water quality criteria, the 2007 U.S. EPA water quality criteria based on the biotic ligand model and the 2005 state of Oklahoma copper water quality standards. Both the criterion maximum concentration and criterion continuous concentration were evaluated, Published acute and chronic copper toxicity data that met American Society for Testing and Materials guidance for test acceptability were obtained for exposures conducted with glochidia or juvenile freshwater mussels. We tabulated toxicity data for glochidia and juveniles to calculate 20 species mean acute values for freshwater mussels. Generally, freshwater mussel species mean acute values were similar to those of the more sensitive species included in the U.S. EPA water quality derivation database. When added to the database of genus mean acute values used in deriving 1996 copper water quality criteria, 14 freshwater mussel genus mean acute values included 10 of the lowest 15 genus mean acute values, with three mussel species having the lowest values. Chronic exposure and sublethal effects freshwater mussel data available for four species and acute to chronic ratios were used to evaluate the criterion continuous concentration. On the basis of the freshwater mussel toxicity data used in this assessment, the hardness-based 1996 U.S. EPA water quality criteria, the 2005 Oklahoma water quality standards. and the 2007 U.S. EPA water quality criteria based on the biotic ligand model might need to be revised to afford protection to freshwater mussels.

Markich, S. J., A. R. King, et al. (2006). "Non-effect of water hardness on the accumulation and toxicity of copper in a freshwater macrophyte (Ceratophyllum demersum): How useful are hardness-modified copper guidelines for protecting freshwater biota?" Chemosphere **65**(10): 1791-1800.

Several nations have adopted hardness-modified copper (Cu) guidelines for protecting freshwater biota. However, there is a lack of good quality data and mechanistic understanding on the effects of true water hardness (calcium (Ca) and magnesium (Mg)) on the bioavailability and toxicity of Cu to freshwater biota, particularly macrophytes. This study determined the effect of true water hardness (35, 90 and 335 mg CaCO3/1, added as Ca and Mg chloride in a 1: 1 mole ratio) on the cell surface binding affinity (log K), accumulation and toxicity (96 h growth (biomass and stern length) and photosynthetic pigment inhibition) of Cu in the free-floating submerged macrophyte, Ceratophyllum demersum, in a synthetic freshwater with constant alkalinity (16 mg CaCO3/1) and pH (7.0). There were no significant ($P > 0.05$) differences in the cell surface binding affinity, accumulation or toxicity of Cu in C demersum with a 10-fold increase in water hardness from

35 to 335 mg CaCO3/1. The mean 96 h EC50 values (and 95%, confidence intervals) for biomass, the most sensitive endpoint, were 8.4 (7.6-9.2), 8.9 (8.0-9.43) and 9.9 (9.1-10.7) mu g/l Cu for 35, 90 and 335 mg CaCO3/1, respectively. Speciation calculations indicated only very small (1-6%) differences in the percentage distribution (i.e. bioavailability) of Cu over the hardness range. These collective results indicate no apparent competition between Cu and Ca/Mg for binding sites on the cell surface. Given that the mechanism of Cu uptake (via Cu-specific and Na-linked transporters) is fundamentally different to that of Cd, Ni, Pb and Zn (via Ca transporters), for which other hardness-dependent algorithms have been developed, it is doubtful whether a hardness-modified Cu guideline value will be sufficiently protective of sensitive freshwater biota, such as C demersum, particularly in medium-hard fresh surface waters with low levels of dissolved organic carbon. The biotic ligand model offers a more flexible and mechanistic approach for deriving site-specific Cu (metal) guidelines for protecting freshwater biota. 

Marr, J. C. A., J. A. Hansen, et al. (1998). "Toxicity of cobalt and copper to rainbow trout: application of a mechanistic model for predicting survival." Aquatic Toxicology **43**(4): 225-238.

We conducted 14-day laboratory toxicity tests with rainbow trout to evaluate, (1) the acute toxicity of Co and Cu, and (2) the effects of selected Co concentrations on the toxicity of Cu in Co/Cu mixtures. A one-compartment, uptake-depuration model was used to estimate incipient lethal levels (ILLs) and quantify differences in lethality responses among the Co-only, Cu-only and Co/Cu exposures. Additionally, we compared mortality percentages observed in Co/Cu mixtures to those predicted from two models of joint toxicity. Co was a slower-acting and less potent toxicant than was Cu. For Co, the ILL for 50% mortality was 346 mu g l(-1); for Cu, the ILL for 50% mortality was 14 mu g l(-1). Moreover, in Co/Cu mixtures, Co acted as an antagonist during the first 48-96 h, but later acted as an additive or slightly synergistic toxicant-making it difficult to predict short-term mortality of fish in Co/Cu mixtures. Lethality thresholds for Cu were reduced by 11-26% and 37-45%, respectively, in the presence of 50 and 250 mu g l(-1) Co, although those differences were not statistically significant. The ordering of ILLs for the metal exposures tested was: 250 mu g l(-1) Co + Cu > 50 mu g l(-1) Co + Cu > Cu-only > Co-only. 

Marr, J. C. A., J. Lipton, et al. (1999). "Bioavailability and acute toxicity of copper to rainbow trout (Oncorhynchus mykiss) in the presence of organic acids simulating natural dissolved organic carbon." Canadian Journal of Fisheries and Aquatic Sciences **56**(8): 1471-1483.

Copper bioavailability and toxicity to early life stage rainbow trout (Oncorhynchus mykiss) were evaluated by laboratory toxicity testing performed using organic acid mixtures. Geochemical modeling was used to design exposure solutions that simulate dissolved organic carbon (DOC) of a natural aquatic system and to determine the fractions of total Cu present as inorganic species (e.g., Cu2+) and as individual Cu-organic complexes. Failure time modeling indicated that

mortality was best predicted by a combination of total inorganic Cu and distinct Cu-organic complexes. The Cu-organic complexes that contributed to toxicity are characterized as low-affinity Cu-ligands, and our results support the hypothesis that Cu toxicity in nature is a function of the binding characteristics of individual ligands. Estimates of time-independent median lethal concentration thresholds determined at widely varying equivalent concentrations of DOC (0-16 mg/L) were constant (7.9-8.6 mu g Cu/L) when modeled using the sum of inorganic Cu and Cu bound to the two low-affinity ligands as predictors of toxicity. Our results indicate that Cu bound to organic complexes may be available to fish and that acute toxicity of Cu is determined by the binding affinities of specific DOC components relative to Cu-binding affinity of fish gill.

McCoy, C. P., T. M. Ohara, et al. (1995). "LIVER AND KIDNEY CONCENTRATIONS OF ZINC, COPPER AND CADMIUM IN CHANNEL CATFISH (ICTALURUS-PUNCTATUS) - VARIATIONS DUE TO SIZE, SEASON AND HEALTH-STATUS." Veterinary and Human Toxicology **37**(1): 11-15.

Significant differences in liver and kidney concentrations of zinc (Zn), copper (Cu) and cadmium (Cd) were detected in normal Mississippi farm-raised channel catfish (Ictalurus punctatus) collected at different times of the year. These seasonal differences were not solely due to variation in fish size. Comparing the concentration of each metal in liver vs kidney indicated that Cd was lower in liver for all seasons studied, Cu was higher in liver for all seasons studied, and Zn was higher in the liver in the winter-killed (winter mortality syndrome) and the spring fish groups. Metal concentration was associated with body weight, as indicated by significant Pearson correlation coefficients for kidney Cd (all seasons and fall), liver Cu (summer), liver Zn (all seasons and winter), and kidney Zn (all seasons and winter). The adjusted means were not dramatically changed as compared to the raw data. Differences were noted when seasonal values obtained from normal fish were compared to tissues of moribund fish afflicted with winter mortality syndrome. Zinc was reduced in liver and kidney of these moribund fish.

McIntyre, J. K., D. H. Baldwin, et al. (2008). "Chemosensory deprivation in juvenile coho salmon exposed to dissolved copper under varying water chemistry conditions." Environmental Science & Technology **42**(4): 1352-1358.

Dissolved copper is an important nonpoint source pollutant in aquatic ecosystems worldwide. Copper is neurotoxic to fish and is specifically known to interfere with the normal function of the peripheral olfactory nervous system. However, the influence of water chemistry on the bioavailability and toxicity of copper to olfactory sensory neurons is not well understood, Here we used electrophysiological recordings from the olfactory epithelium of juvenile coho salmon (Oncorhynchus kisutch) to investigate the impacts of copper in freshwaters with different chemical properties. In low ionic strength artificial fresh water, a short-term (30 min) exposure to 20 mu g/L dissolved copper reduced the olfactory response to a natural odorant (10-5 M L-serine) by 82%. Increasing water hardness (0.2-1.6 mM Ca) or alkalinity (0.2-3.2 mM HCO3-) only slightly

diminished the inhibitory effects of copper. Moreover, the loss of olfactory function was not affected by a change in pH from 8.6 to 7.6. By contrast, olfactory capacity was partially restored by increasing dissolved organic carbon (DOC; 0.1-6.0 mg/L). Given the range of natural water quality conditions in the western United States, water hardness and alkalinity are unlikely to protect threatened or endangered salmon from the sensory neurotoxicity of copper. However, the olfactory toxicity of copper may be partially reduced in surface waters that have a high DOC content.

McKim, J. M., J. G. Eaton, et al. (1978). "METAL TOXICITY TO EMBRYOS AND LARVAE OF 8 SPECIES OF FRESHWATER FISH .2. COPPER." Bulletin of Environmental Contamination and Toxicology **19**(5): 608-616.

McNulty, H. R., B. S. Anderson, et al. (1994). "AGE-SPECIFIC TOXICITY OF COPPER TO LARVAL TOPSMELT ATHERINOPS-AFFINIS." Environmental Toxicology and Chemistry **13**(3): 487-492.

The age-specific sensitivity of topsmelt (Atherinops affinis) larvae to copper was assessed. A series of 7-d growth and survival experiments were conducted using cohorts of larval fish isolated into different age groups of 0, 1, 3, 5, 7, 9, 11, 15, and 20 d post-hatch. Fish aged 0, 3, and 5 d were less sensitive to copper chloride than fish greater-than-or-equal-to 7 d old. The median lethal concentration (LC50) for copper ranged from 365 mug L-1 in 0-d larvae, to 137 mug L-1 in 20-d larvae. NOECs remained relatively constant for all ages: 180 mug L-1 for 1- and 3-d-old fish, 100 mug L-1 for all other cohorts. Regression analysis indicated a significant negative correlation between LC50 and gill surface area (GSA; R2 = -0.793) and cutaneous surface area (CSA; R2 = -0.760. Although these correlations were expected because both morphometrics increase with age, the relationships between increasing respiratory surface area and LC50 may indicate that the increase in sensitivity with larval age is related to an increase in copper uptake, either cutaneously or branchially. GSA increased more than sevenfold between hatch and 20 d, whereas CSA increased only threefold throughout the same period.

Meyer, J. S., R. C. Santore, et al. (1999). "Binding of nickel and copper to fish bills predicts toxicity when water hardness varies, but free-ion activity does not." Environmental Science & Technology **33**(6): 913-916.

Based on a biotic-ligand model (BLM), we hypothesized that the concentration of a transition metal bound to fish gills ([M-gill]) will be a constant predictor of mortality, whereas a free-ion activity model is generally interpreted to imply that the chemical activity of the aquo ("free") ion of the metal will be a constant predictor of mortality. In laboratory tests, measured [Ni-gill] and calculated [Cu-gill] were constant predictors of acute toxicity of Ni and Cu to fathead minnows (Pimephales promelas) when water hardness varied up to 10-fold, whereas total aqueous concentrations and free-ion activities of Ni and Cu were not. Thus, the BLM, which simultaneously accounts for (a) metal speciation in the exposure water and (b) competitive binding of transition-metal ions and other cations to

biotic ligands predicts acute toxicity better than does free-ion activity of Ni or Cu. Adopting a biotic-ligand modeling approach could help establish a more defensible, mechanistic basis for regulating aqueous discharges of metals.

Miller, P. A., R. P. Lanno, et al. (1993). "Relative Contributions of Dietary and Waterborne Copper to Tissue Copper Burdens and Waterborne-Copper Tolerance in Rainbow-Trout (Oncorhynchus-Mykiss)." Canadian Journal of Fisheries and Aquatic Sciences **50**(8): 1683-1689.

In a 42-d study, rainbow trout (Oncorhynchus mykiss) were fed a diet containing either 13 or 684 mg Cu.kg(-1) and simultaneously exposed to waterborne-Cu concentrations of 5, 32, 55, or 106 mu g.L(-1) (low-Cu diet) or 13, 38, 62, or 127 mu g.L(-1) (high-Cu diet). There were no significant effects on mortality, growth, condition factor, or food conversion efficiency. Elevated dietary Cu increased Cu concentrations in liver ($p < 0.001$), kidney ($p < 0.001$), gill($p = 0.005$), and digesta ($p < 0.001$). Increasing waterborne-Cu concentrations elevated Cu concentrations in liver ($p = 0.018$) and kidney ($p = 0.002$) but not in gill ($p = 0.930$) or digesta ($p = 0.519$). Waterborne-Cu exposure increased Zn concentrations in liver ($p = 0.025$) but decreased those in kidney ($p = 0.045$). For fish on the high-Cu diet, diet provided 99, 85, and 63% of the Cu in the liver for the 38, 62, and 127 mu g.L(-1) waterborne-Cu treatments, respectively. Based on Cu tolerance (incipient lethal level for Cu), dietary and waterborne Cu partitioned into functionally different compartments. Although both waterborne-Cu ($p < 0.00001$) and dietary-Cu ($p = 0.019$) preexposure increased Cu tolerance, waterborne Cu had a much greater impact.

Monteiro, S. M., E. Rocha, et al. (2009). "A stereological study of copper toxicity in gills of Oreochromis niloticus." Ecotoxicology and Environmental Safety **72**(1): 213-223.

Stereological methods were used to estimate the volumetric density (V-v) of the filamentar epithelium (FE, 39%), lamellae (L, 28%), central venous sinus (CVS, 14%), central axis (16%), mucous cells (MC, 2%) and chloride cells (CC, 1%) in the gill filament of control Nile Tilapia. The relative volumes of FE and L, and the relative volumes of CVS and central axis, varied inversely under exposure to copper, with high copper toxic levels declanching a chronic defence mechanism that was, nevertheless, overcome, and low copper toxic levels causing adaptation within a moderate acute phase type of response. Copper also induced a decrease of the V-v (MC, gill filament) due to reduction of surface MC, despite the marked increase of stem MC at chronic exposure to high copper toxic levels. Diminution of the numerical density of filamentar CC was responsible for the decreased V-v (CC, gill filament), although lamellar CC significantly increased at chronic exposure to low copper toxic levels. The present results demonstrate that cell relative volumes, mean volumes and numerical densities are dependent on the variations of the FE and L, which without a quantitative approach may be misinterpreted, thus stressing the importance of using stereological tools for analyzing histopathological patterns. 

Mount, D. R., A. K. Barth, et al. (1994). "DIETARY AND WATERBORNE EXPOSURE

OF RAINBOW-TROUT (ONCORHYNCHUS-MYKISS) TO COPPER, CADMIUM, LEAD AND ZINC USING A LIVE DIET." Environmental Toxicology and Chemistry **13**(12): 2031-2041.

In two 60-d exposures, rainbow trout fry were fed brine shrimp (Artemia sp.) enriched with Cu, Cd, Pb, and Zn both individually and as a mixture combined with As. Dietary concentrations fed to trout were selected based on metal concentrations measured in invertebrates collected from the Clark Fork River (CFR), Montana. In addition to dietary exposure, treatments also included simultaneous exposure to a mixture of waterborne metals at sublethal concentrations. Fish in all treatments showed increased tissue metal concentrations from water and/or dietary exposure. Despite these accumulations, trout showed no effects on survival or growth from dietary concentrations as high as 55 mu g Cd/g dry weight, 170 mu g Pb/g dry weight, or 1,500 mu g Zn/g dry weight (corrected for depuration). Dietary Cu concentrations up to 350 mu g Cu/g dry weight did not reduce survival or growth. Fish fed Cu concentrations higher than those typical of CFR invertebrates (660 and 800 mu g Cu/g dry weight; corrected for depuration) showed about 30% mortality with no effect on growth; waterborne Cu released from Artemia may have contributed to this mortality. Trout exposed to diets with a mixture of Cu, Cd, Pb, Zn, and As dose to that measured in CFR invertebrates showed tower weight than did control fish after 35 d, but this difference was no longer present after 60 d.

Nussey, G., J. H. J. vanVuren, et al. (1996). "Acute toxicity tests of copper on juvenile Mozambique tilapia, Oreochromis mossambicus (Cichlidae), at different temperatures." South African Journal of Wildlife Research **26**(2): 47-55.

In the Olifants River, copper is one of several metals which pose a threat to the conservation status of the river. The LC(50) values attained during this study, can be used as an indication of the levels at which copper becomes lethal to O. mossambicus and must only be seen as the limits within which the concentration of copper can be regarded as lethal for this species in the Olifants River. Acute toxicity tests were performed to determine the lethal copper concentrations for juvenile Oreochromis mossambicus at representative mean summer (29 degrees C) and winter temperatures (19 degrees C) of the Olifants River, Kruger National Park, Northern Province, South Africa. Fish were exposed to various copper concentrations (0.0, 1.0, 1.7, 1.8, 2.0, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 3.0, 3.5, 4.0 mg/l) for 96 h in experimental flow-through systems at pH levels which ranged between 7.4 and 8.1, and mortalities were monitored. Dosage-survival curves of percentage survival versus actual copper concentrations were plotted after 24, 48, 72 and 96 h, and thereafter the LC(50) or median lethal concentration (the concentration of the test material that will kill or immobilize 50% of the test organisms over a predetermined length of time, usually 24 to 96 h) values were calculated for both summer and winter temperatures. Time survival curves of percentage survival versus time were also plotted, after which the LT(50) or median survival time (the time required for half the fish to die at a specific toxicant concentration) values were calculated for the two temperatures. Toxicity curves were constructed using both the LC(50) and LT(50) values to determine

the incipient LC(50) values (ILC(50) - the lethal concentration for 50% of the test organism in a long-term exposure or lethal threshold concentration). The LC(50) values (lethal copper concentration) of 2.61 mg/l and 2.78 mg/l as well as the ILC(50) values (lethal threshold concentration) of 2.95 mg/l and 3.32 mg/l for copper at 29 +/- 1 degrees C and 19 +/- 1 degrees C respectively, are considerably higher than the copper concentrations occurring in the water of the Olifants River, during summer (0.055 +/- 0.016 mg/l) and winter (0.085 +/- 0.032 mg/l). Thus the fish in the Olifants River are not exposed to acute lethal copper concentrations.

Pelgrom, S., L. P. M. Lamers, et al. (1994). "INTERACTIONS BETWEEN COPPER AND CADMIUM DURING SINGLE AND COMBINED EXPOSURE IN JUVENILE TILAPIA OREOCHROMIS-MOSSAMBICUS - INFLUENCE OF FEEDING CONDITION ON WHOLE-BODY METAL ACCUMULATION AND THE EFFECT OF THE METALS ON TISSUE WATER AND ION CONTENT." Aquatic Toxicology **30**(2): 117-135.

Juvenile tilapia (Oreochromis mossambicus) were exposed for 96 h to ranges of sublethal concentrations of Cu or Cd, under both fed and non-fed conditions. Exposure to one metal (Cu or Cd) not only resulted in an increased whole body content of the metal exposed to, but also influenced the concentration of the other metal present in the fish. Furthermore, the total amount of Cu and Cd accumulated during exposure to heavy metals was influenced by the nutritional state of the fish. Besides exposure to either Cu or Cd, fish were also exposed to mixtures of Cu and Cd. Results indicated that accumulation during Cu/Cd co-exposure cannot be predicted by simple addition of the effects of single metal exposures. Obviously, complex interaction mechanisms are involved, as was concluded e.g. from the significantly decreased whole body Cd-content of Cu/Cd-co-exposed fish compared to the Cd-content of Cd-exposed fish. This phenomenon was observed in both fed and non-fed fish. Because ionic homeostasis is known to be affected by heavy metals, in this study also whole body water, calcium and sodium content in Cu and/or Cd-exposed fish were determined. The results indicate that also with respect to these parameters the two metals interact. The effects on water and ion appear to be dissociated. The data reveal previously unrecognized effects of interaction of the metals on whole body metal content, water and ion regulation.

Peres, I. and J. C. Pihan (1991). "COPPER LC50 TO CYPRINUS-CARPIO - INFLUENCE OF HARDNESS, SEASONAL-VARIATION, PROPOSITION OF MAXIMUM ACCEPTABLE TOXICANT CONCENTRATION." Environmental Technology **12**(2): 161-167.

Acceptable concentrations of heavy metals in the aquatic environment are normally assessed through the evaluation of the lethal concentration in acute intoxication. Research work has been carried out to identify values of 48 h copper LC50 for Cyprinus carpio under well defined experimental conditions and to discuss limitations rising from the definition of maximum acceptable toxicant concentrations as a fraction of LC50. Results have shown a fair stability of the biological material used and that, for a water hardness of 50 mg.l-1, sensitivities

of Cyprinus carpio and Salmo gairdneri are similar, the former being more resistant for hardness values higher than 50 mg. l-1. Since Cyprinus carpio can be taken as a representative species for second class European rivers and provided that its LC50 is in the same order of magnitude of other species it is proposed as the standard organism for evaluation of maximum acceptable concentration in copper.

Pinho, G. L. L., M. S. Pedroso, et al. (2007). "Physiological effects of copper in the euryhaline copepod Acartia tonsa: Waterborne versus waterborne plus dietborne exposure." Aquatic Toxicology **84**(1): 62-70.

The physiological effects of waterborne and waterborne plus dietborne copper exposure were determined in the euryhaline copepod Acartia tonsa at different salinities (5, 15 and 30 ppt). Copepods were exposed (48 h) to a reported 48-h LC50 for copper (CuCl2), which had been previously determined under the same experimental conditions. Whole body copper accumulation, ion concentrations (Na+, Cl-, Mg2+,), and Na+, K+-ATPase activity were the endpoints measured in all experimental groups. Feeding rate was also measured in fed experimental groups. In copper-exposed copepods, whole body copper accumulation was dependent on salinity, decreasing as salinity increased. However, it was similar in copepods exposed to waterborne and waterborne plus dietborne copper, irrespective the salinity tested. Waterborne copper exposure induced a disturbance of the whole body Na+ concentration in all salinities tested. This effect was characterized by an increased whole body Na+ concentration in seawater (salinity 30 ppt) and a decreased whole body Na+ concentration at lower salinities (5 and 15 ppt). The ionoregulatory imbalance in low salinity (5 ppt) was associated with an inhibition of the whole body Na+, K-ATPase activity, as observed in freshwater fish and crustaceans. When copepods were exposed to waterborne plus dietborne copper, the physiological effects described were only observed at a low salinity (5 ppt) and were associated with a marked inhibition of the feeding rate. Taken altogether, the data suggest that the physiological effects induced by waterbome copper exposure in A. tonsa acclimated to higher salinities (15 and 30 ppt) are due to a combined effect of food restriction and copper exposure. Differential physiological responses to waterborne and waterbome plus dietborne copper cannot be ascribed to differences in whole body copper burden. (c) 2007 Elsevier B.V All rights reserved.

Playle, R. C., D. G. Dixon, et al. (1993). "COPPER AND CADMIUM-BINDING TO FISH GILLS - ESTIMATES OF METAL GILL STABILITY-CONSTANTS AND MODELING OF METAL ACCUMULATION." Canadian Journal of Fisheries and Aquatic Sciences **50**(12): 2678-2687.

Fathead minnows (Pimephales promelas) were exposed to 17 mug Cu . L-1 or 6 mug Cd . L-1 in synthetic soft water in the presence of competing ligands. Measured gill metal concentrations correlated with free metal ion concentrations, not with total metal. Langmuir isotherms were used to calculate conditional metal-gill equilibrium constants and the number of binding sites for each metal.

Log K(Cu-gill) was estimated to be 7.4 and the number of Cu binding sites on a set of gills (70 mg, wet weight) was approximately 2 x 10(-8) mol (approximately 30 nmol . g wet weight-1). Log K(Cd-gill) was approximately 8.6, and the number of Cd binding sites on minnow gills was approximately 2 x 10(-10) mol (approximately 2 nmol . g wet weight-1). Stability constants for H+ and Ca interactions at metal-gill binding sites and for metal interactions with dissolved organic carbon (DOC) were estimated using these metal-gill constants. All stability constants were entered into the MINEQL+ aquatic chemistry program, to predict metal accumulation on fish gills using metal, DOC, and Ca concentrations, and water pH. Calculated metal accumulation on gills correlated well with measured gill metal concentrations and with LC50 values. Our approach of inserting biological data into an aquatic chemistry program is useful for modelling and predicting metal accumulation on gills and therefore toxicity to fish.

Playle, R. C., D. G. Dixon, et al. (1993). "COPPER AND CADMIUM-BINDING TO FISH GILLS - MODIFICATION BY DISSOLVED ORGANIC-CARBON AND SYNTHETIC LIGANDS." Canadian Journal of Fisheries and Aquatic Sciences **50**(12): 2667-2677.

Adult fathead minnows (Pimephales promelas) were exposed to 17 mug Cu . L-1 or 6 mug Cd . L-1 for 2 to 3 h in synthetic softwater solutions at pH 6.2 containing either naturally-occurring, freeze-dried dissolved organic carbon (DOC) or synthetic ligands such as EDTA. After exposures, gills were assayed for bound Cu or Cd. As a first approximation, lake of origin or molecular size fraction of DOC did not influence Cu binding to gills, while DOC concentration did. DOC concentrations greater-than-or-equal-to4.8 mg . L-1 prevented Cu from accumulating on fathead gills. At the relatively low concentrations used, neither Cu nor Cd interfered with binding of the other metal on gills, suggesting different gill binding sites. Cadmium accumulation on gills was more sensitive to increased concentrations of Ca and H+ than was Cu. Surprisingly, Cd bound to gills to the same or greater extent than did Cu: for synthetic ligands, Cd binds less well than Cu. This result corroborates previously published observations that Cd, unlike Cu, is taken up at gills through high affinity Ca channels. Accumulation of Cd on fish gills was never associated with C-14-labelled EDTA or C-14-citrate, indicating that free metal interacts with the gill while metal-ligand complexes usually do not.

Playle, R. C., R. W. Gensemer, et al. (1992). "COPPER ACCUMULATION ON GILLS OF FATHEAD MINNOWS - INFLUENCE OF WATER HARDNESS, COMPLEXATION AND PH OF THE GILL MICROENVIRONMENT." Environmental Toxicology and Chemistry **11**(3): 381-391.

Water pH in the gill micro-environment of adult fathead minnows (Pimephales promelas Rafinesque) was measured by means of opercular catheters and latex masks. Synthetic soft water of pH < 5.7 was rendered more basic as it passed over the gills and water of pH > 5.7 was made more acidic, so that pH of the gill micro-environment stayed at approximately 5.4 to 5.9 over the inspired pH range 4.8 to 6.3. Copper would therefore be > 99% Cu2+ at the gills. To measure Cu

accumulation on gills, the target organ for Cu toxicity, adult fathead minnows were exposed for 2 to 3 h to 16-mu-g/L Cu in synthetic soft water (Ca2+ and Na+ approximately 50-mu-eq/L) at pH 4.8 and 6.3. Gill Cu concentrations were about 1.7-mu-g Cu/g wet tissue for the inspired pH 4.8 and 6.3 exposures. Added Ca2+ of 2,100 or 4,000-mu-eq/L reduced gill Cu accumulation during exposures at pH 4.8 but not at pH 6.3. EDTA eliminated Cu deposition at both pH 4.8 and pH 6.3 when equimolar with Cu, but reduced Cu deposition (by 50%) when half equimolar only in the pH 4.8 exposures. These results can be explained by Ca2+ and H+ competition with Cu for gill binding sites and by complexation of Cu by EDTA. Added CaCO3 did not reduce gill Cu, although both competition by Ca2+ and complexation by CO3(2-) were expected. Water chemistry calculations suggested that because little CO3(2-) is available at pH < 7, CO3(2-) from CaCO3 is less likely to affect Cu deposition on fish gills than is Ca2+ from CaCO3.

Pyle, G. and C. Wood (2008). "Radiotracer studies on waterborne copper uptake, distribution, and toxicity in rainbow trout and yellow perch: A comparative analysis." Human and Ecological Risk Assessment **14**(2): 243-265.

Rainbow trout (Oncorhynchus mykiss) are often used to estimate important biotic ligand model (BLM) parameters, such as metal-binding affinity (log K) and capacity (Bmax). However, rainbow trout do not typically occupy metal-contaminated environments, whereas yellow perch (Perca flavescens) are ubiquitous throughout most of North America. This study demonstrates that dynamic processes that regulate Cu uptake at the gill differ between rainbow trout and yellow perch. Rainbow trout were more sensitive to acute aqueous Cu than yellow perch, and toxicity was exacerbated in soft water relative to similar exposures in hard water. Whole body Na loss rate could account for acute Cu toxicity in both species, as opposed to new Cu uptake rate that was not as predictive. Time course experiments using radiolabelled Cu (64Cu) revealed that branchial Cu uptake was rather variable within the first 12 h of exposure, and appeared to be a function of Cu concentration, water hardness, and fish species. After 12 h, new branchial Cu concentrations stabilized in both species, suggesting that metal exposures used to estimate BLM parameters should be increased in duration from 3 h to 12+ h. In rainbow trout, 71% of the new Cu bound to the gill was exchangeable (i.e., able to either enter the fish or be released back to the water), as opposed to only 48% in yellow perch. This suggests that at equal exposure concentrations, proportionally more branchial Cu can be taken up by rainbow trout than yellow perch, which can then go on to confer toxicity. These qualitative differences in branchial Cu handling between the two species emphasize the need to develop BLM parameters for each species of interest, rather than the current practice of extrapolating BLM results derived from rainbow trout (or other laboratory-reared species) to other species. Data reported here indicate that a one-size-fits-all approach to predictive modeling, mostly based on rainbow trout studies, may not suffice for making predictions about metal toxicity to yellow perch - that is, a species that inhabits metal-contaminated lakes around northern Canadian industrial operations.

Radhakrishnaiah, K., P. Venkataramana, et al. (1992). "EFFECTS OF LETHAL AND SUBLETHAL CONCENTRATIONS OF COPPER ON GLYCOLYSIS IN LIVER AND MUSCLE OF THE FRESH-WATER TELEOST, LABEO-ROHITA (HAMILTON)." Journal of Environmental Biology **13**(1): 63-68.

Blood glucose level increased in L. rohita after 1, 2 and 3 days of exposure to lethal (1.0 mg/1) and 1, 15 and 30 days of exposures to sublethal (0.2 mg/1) concentrations of copper, with a corresponding decrease in its liver glycogen content and increase in the activities of liver glycogen phosphorylase and glucose-6-phosphatase. Muscle glycogen also decreased alongwith an increase in its glycogen phosphorylase activity. Further, elevation in LDH and suppression in SDH activities were observed in liver and muscle of the fish at both the concentrations of the metal. All these changes were significant and in the order of 1 < 2 < 3 days with the lethal concentration as against the order of 1 > 15 > 30 days with the sublethal concentration. The observation suggest a stimulated glycolysis in the liver and muscle of copper intoxicated teleost, Labeo rohita.

Reddy, A. S., M. V. Reddy, et al. (2008). "Impact of copper on the oxidative metabolism of the fry of common carp, Cyprinus carpio (Linn.) at different pH." Journal of Environmental Biology **29**(5): 721-724.

In order to evaluate the impact of copper on the energetics of a fish, the levels of glucose, glycogen, pyruvate and lactate, the rate of tissue oxygen consumption and the activities of glycogen phosphorylase, isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) were estimated in the whole body of the fry of Cyprinus carpio immediately after 1,7,15 and 30 days on exposure to a sublethal concentration of copper 0.08 mgl(-1) at pH 7.5 (normal), 6.0 (weak acidic) and 9.0 (weak alkaline). A progressive increase in glucose level and glycogen phosphorylase activity with the corresponding decrease in glycogen level over the time of exposure atpH 7.5 indicated glycogenolysis. Increase in the rate of oxygen consumption, pyruvate level and ICDH and SDH activities at days 1 and 7 (day 1>7) followed by their decrease at days 15 and 30 (day 15<30) at pH 7.5 indicated an initial elevation in the energetics of the fish fry with a gradual suppression of it on prolonged exposure. During this period the animal might have relied more on energetically less efficient glycolysis as evident by the progressive increase in the level of lactate and LDH activity. The degree of glycogenolysis was relatively more at pH 6.5 than at pH 7.5. At that pH, a progressive decrease in glucose level with an increase in the pyruvate and lactate levels and in LDH activity and a decrease in the rate of oxygen consumption and ICDH and SDH activities revealed greater reliance of the fish on anaerobic glycolysis than on oxidative metabolism. At pH 9.0 also the fish fry initially exhibited glycogenolysis, but gradually it came to normal on day 30 (day 1>7>15>30). Decrease in the glucose level, increase in pyruvate level, rate of oxygen consumption, and ICDH and SDH activities at all the days of exposure suggested an elevation in oxidative metabolism, but it also came to normal on prolonged exposure. Even the lactate level and LDH activity initially increased but gradually reached to normal on day 30. These results

indicated that copper suppresses the energetics of the fish fry at pH 6.0, elevates at pH 9.0 relative to the changes at pH 7.5 suggesting that the toxicity of copper is dependent on pH of the water.

Rehwoldt, R., G. Bida, et al. (1971). "ACUTE TOXICITY OF COPPER, NICKEL AND ZINC IONS TO SOME HUDSON RIVER FISH SPECIES." Bulletin of Environmental Contamination and Toxicology **6**(5): 445-&.

Richards, J. G., B. K. Burnison, et al. (1999). "Natural and commercial dissolved organic matter protects against the physiological effects of a combined cadmium and copper exposure on rainbow trout (Oncorhynchus mykiss)." Canadian Journal of Fisheries and Aquatic Sciences **56**(3): 407-418.

Environmentally realistic concentrations of a natural dissolved organic matter (DOM) (8 mg C/L as dissolved organic carbon (DOC)) protected against the acute respiratory and ionoregulatory effects of 0.2 mu M Cd and 0.8 mu M Cu on rainbow trout (Oncorhynchus mykiss). The protection afforded by low natural DOC was the same as that afforded by similar or higher concentrations of commercial DOG. Trout exposed to the metals alone experienced large decreases in arterial Pot, increases in arterial P-CO2, increases in blood lactate, decreases in plasma concentrations of Cl, and developed pronounced haemoconcentration. There were no deleterious effects of 31 mg C/L commercial DOC on any measured aspect of trout physiology except for an increase in plasma Cl, which was probably due to elevated aqueous Cl concentrations associated with the DOM addition. No concentration of DOC used in the present study prevented Cd from being bound by trout gills, and some of these fish showed hypocalcemia; however, Cu was kept off the gills of trout exposed to metals plus DOM. Computer modelling using metal-gill binding constants simulated well the accumulation of Cd and the lack of Cu accumulation by trout gills in the presence of DOM.

Saiki, M. K., D. T. Castleberry, et al. (1995). "Copper, Cadmium, and Zinc Concentrations in Aquatic Food-Chains From the Upper Sacramento River (California) and Selected Tributaries." Archives of Environmental Contamination and Toxicology **29**(4): 484-491.

Metals enter the Upper Sacramento River above Redding, California, primarily through Spring Creek, a tributary that receives acid-mine drainage from a US EPA Super-fund site known locally as Iron Mountain Mine. Waterweed (Elodea canadensis) and aquatic insects (midge larvae, Chironomidae; and mayfly nymphs, Ephemeroptera) from the Sacramento River downstream from Spring Creek contained much higher concentrations of copper (Cu), cadmium (Cd), and zinc (Zn) than did similar taxa from nearby reference tributaries not exposed to acid-mine drainage. Aquatic insects from the Sacramento River contained especially high maximum concentrations of Cu (200 mg/kg dry weight in midge larvae), Cd (23 mg/kg dry weight in mayfly nymphs), and Zn (1,700 mg/kg dry weight in mayfly nymphs). Although not always statistically significant, whole-body concentrations of Cu, Cd, and Zn in fishes (threespine stickleback,

Gasterosteus aculeatus; Sacramento sucker, Catostomus occidentalis; Sacramento squaw-fish, Ptychocheilus grandis; and chinook salmon, Oncorhynchus tshawytasch) from the Sacramento River were generally higher than in fishes from the reference tributaries.

Saiki, M. K., B. A. Martin, et al. (2001). "Copper, cadmium, and zinc concentrations in juvenile chinook salmon and selected fish-forage organisms (aquatic insects) in the upper Sacramento River, California." Water Air and Soil Pollution **132**(1-2): 127-139.

This study assessed the downstream extent and severity of copper (Cu), cadmium (Cd), and zinc (Zn) contamination from acid mine drainage on juvenile chinook salmon (Oncorhynchus tshawytscha) and aquatic insects over a roughly 270-km reach of the Sacramento River below Keswick Reservoir. During April-May 1998, salmon were collected from four sites in the river and from a fish hatchery that receives water from Battle Creek. Salmon from river sites were examined for gut contents to document their consumption of various invertebrate taxa, whereas salmon from river sites and the hatchery were used for metal determinations. Midge (Chironomidae) and caddisfly (Trichoptera) larvae and mayfly (Ephemeroptera) nymphs were collected for metal determinations during April-June from river sites and from Battle and Butte creeks. The fish hatchery and Battle and Butte creeks served as reference sites because they had no history of receiving mine drainage. Salmon consumed mostly midge larvae and pupae (44.0%, damp-dry biomass), caddisfly larvae (18.9%), Cladocera (5.8%), and mayfly nymphs (5.7%). These results demonstrated that insects selected for metal determinations were important as fish forage. Dry-weight concentrations of Cu, Cd, and Zn were generally far higher in salmon and insects from the river than from reference sites. Within the river, high metal concentrations persisted as far downstream as South Meridian (the lowermost sampling site). Maximum concentrations of Cd (30.7 mug g(-1)) and Zn (1230 mug g(-1)), but not Cu (87.4 mug g(-1)), in insects exceeded amounts that other investigators reported as toxic when fed for prolonged periods to juvenile salmonids.

Sanders, B. M., J. Nguyen, et al. (1995). "Induction and subcellular localization of two major stress proteins in response to copper in the fathead minnow Pimephales promelas." Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology **112**(3): 335-343.

In the present study we characterize the stress response induced by copper in the fathead minnow, Pimephales promelas, The fathead minnow epithelial cell line ATCC CCL 42 was used to examine the induced synthesis and subcellular localization of the two major stress proteins, stress70 and cpn60, Western blot analysis demonstrated increased stress70 in cells exposed to 400 and 500 mu M Cu, Two-dimensional analysis revealed three isoforms of stress70, one of 70 kDa and two of 72 kDa, at the highest Cu concentration, Chaperonin60 abundance did not change over the same range of Cu concentrations, Indirect immunofluorescence microscopy revealed that stress70 localized in the cytoplasm, particularly in the paranuclear region, Chaperonin60 was localized in mitochondria, Further, when we examined the stress response elicited by Cu in

fathead minnow larvae in vivo, we found that Cu induced the stress response at nominal Cu concentrations that were more than an order of magnitude lower that in the cell culture, This disparity between the concentration of Cu, which induced the stress response in cells in culture and in vivo, may be the result of differences in Cu complexation that alter its availability, uptake and toxicity.

Santore, R. C., D. M. Di Toro, et al. (2001). "Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia." Environmental Toxicology and Chemistry **20**(10): 2397-2402.

The biotic ligand model (BLM) was developed to explain and predict the effects of water chemistry on the acute toxicity of metals to aquatic organisms. The biotic ligand is defined as a specific receptor within an organism where metal complexation leads to acute toxicity. The BLM is designed to predict metal interactions at the biotic ligand within the context of aqueous metal speciation and competitive binding of protective cations such as calcium. Toxicity is defined as accumulation of metal at the biotic ligand at or above a critical threshold concentration. This modeling framework provides mechanistic explanations for the observed effects of aqueous ligands, such as natural organic matter, and water hardness on metal toxicity, In this paper, the development of a copper version of the BLM is described. The calibrated model is then used to calculate LC50 (the lethal concentration for 50% of test organisms) and is evaluated by comparison with published toxicity data sets for freshwater fish (fathead minnow, Pimephales promelas) and Daphnia.

Sarnowski, P. and M. Witeska (2008). "The Effects of Copper and Cadmium in Single Exposure or Co-Exposure on Growth of Common Carp (Cyprinus Carpio L.) Larvae." Polish Journal of Environmental Studies **17**(5): 791-796.

The effects of copper and cadmium in single or co-exposure (each at the concentration of 0.2 mgxdm(-3), and in mixture -0.1 mgxdm(-3)) on growth of common carp larvae (in terms of body length and perimeter area) during the first 30 days post hatching were evaluated. Body length increased in a similar rate during the entire experimental period, while the increase of body perimeter area became faster after the shift into exogenous feeding, and then during swim bladder inflation. Copper was more toxic to the fish comparing cadmium or a mixture of both metals which indicates a possible antagonism of cadmium against copper toxicity. Body perimeter area was a more sensitive indicator of heavy metal intoxication compared to body length, and may be Used as an approximation of body mass for very small fish that cannot he accurately weighed alive.

Saucier, D. and L. Astic (1995). "MORPHOFUNCTIONAL 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 a-Physiology **112**(2): 273-284.

Morpho-functional changes in the olfactory system of Oncorhynchus mykiss were

investigated at different times during a 40-week exposure to either 20 mu g Cu-2+/l (20-mu g group) or 40 mu g Cu-2+/l (40-mu g group), Histopathological alterations in the olfactory epithelium of the 20-mu g group were moderate, whereas those in the 40-mu g group were more severe and intensified with time exposure, The 20-mu g group exhibited some olfactory discrimination ability but presented higher sensitivity thresholds than controls. The 40 mu g group became unresponsive with time, even to a water inflow 4 times more odor concentrated, The time frame for morpho-functional recovery after returning to well water was dose dependent, Morpho-functional alterations in response to a 20 mu g Cu-2+/l exposure appeared less important and the functional recovery shorter in adults compared to what has been previously observed in younger fish. Fish acclimation in conditions of long-lasting exposure to each copper dose is discussed.

Schjolden, J., J. Sorensen, et al. (2007). "The toxicity of copper to crucian carp (Carassius carassius) in soft water." Science of the Total Environment **384**(1-3): 239-251.

Crucian carp (Carassius carassius) were exposed to a Cu rich medium (pH 6.6, conductivity 25 mu S/cm, 2.91 mg Ca2+/l, approximately 300 mu g Cu2+/l). Untreated department water (pH 6.6, conductivity 25 mu S/cm, 2.91 mg Ca2+/l) acted as control. Mortality in crucian carp was first observed after 13 days of exposure to the Cu rich medium. There were, however, significant changes in haematocrit, plasma chloride, plasma sodium and water content in muscle in fish exposed to the Cu rich medium after two days. After 14 days of exposure to copper, haematocrit increased to 52 +/- 2% (control: between 37 and 40%), plasma chloride decreased to 45 +/- 5 mmol/l (control: 99-106 mmol/l), plasma sodium decreased to 81 +/- 6 mmol/l (control: 116-137 mmol/l), and water content in muscle increased to 83.0 +/- 0.36/o (control: 78.7-79.9%). No apparent changes in blood ethanol, and minor changes in plasma lactate were observed in copper exposed fish. Analyses of the gills revealed an increasing concentration of copper on the gills from fish exposed to Cu rich water. After 14 days, the concentration of copper accumulated in the gill was 12.8 +/- 4.1 mu g Cu/g wet weight (control: 0.91-1.19 mu g Cu/g wet weight). A reduction of the respiratory area in fish exposed to copper was observed, in terms of both lamellar and filamental fusion. The normoxic O-2 uptake did not change, but the critical oxygen tension was elevated to 6.12 +/- 1.04 mg O-2/l after a 6 day exposure to copper (control: 1.03 +/- 0.05 Mg O-2/l). This study shows that crucian carp has a higher tolerance to copper compared to other freshwater fish species. Our results suggest that this tolerance is based on the ability of crucian carp to avoid becoming hypoxic as well as an extreme tolerance to severe loss of plasma ions. 

Schwartz, M. L., P. J. Curtis, et al. (2004). "Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (Oncorhynchus mykiss)." Environmental Toxicology and Chemistry **23**(12): 2889-2899.

Natural organic matter (NOM) was concentrated from various sites across

Canada using a portable reverse-osmosis unit to obtain a range of NOM types, from mainly allochthonous (terrestrially derived) to mainly autochthonous (aquatically derived) NOM. The addition of NOM to Cu exposures in ion-poor water always decreased Cu toxicity to rainbow trout (Oncorhynchus inykiss, similar to1 g) over a 96-h period, and the degree of protection varied with respect to NOM source. A good correlation was found between the specific absorbance coefficient (SAC) and time to reach 50% mortality (LT50; $p < 0.001$), indicating that more optically dark, allochthonous-like NOM decreases Cu toxicity better than does optically light, more autochthonous-like NOM. A similar, good relationship between NOM source and Pb toxicity was seen ($p < 0.001$), once confounding effects of Ca binding to NOM were accounted for. No significant relationship between Cd toxicity and NOM optical quality was seen ($p = 0.082$), and in toxicity tests with Cd the presence of some of the NOM sources increased Cd toxicity compared to Cd-only controls. Specific absorbance coefficients were used as a proxy measurement of NOM aromaticity in our study, and fluorescence indices were run on some NOM samples to obtain percent aromaticity for each sample. A good correlation was found between SAC and percent aromaticity, indicating that the simple SAC measurement is a reasonable indication of NOM aromaticity and of metal binding by NOM.

Sciera, K. L., J. J. Isely, et al. (2004). "Influence of multiple water-quality characteristics on copper toxicity to fathead minnows (Pimephales promelas)." Environmental Toxicology and Chemistry **23**(12): 2900-2905.

Water quality influences the bioavailability and toxicity of copper to aquatic organisms. Understanding the relationships between water-quality parameters and copper toxicity may facilitate the development of site-specific criteria for water quality and result in better protection of aquatic biota. Many studies have examined the influence of a single water-quality parameter on copper toxicity, but the interactions of several characteristics have not been well studied in low-hardness water. The goal of the present research was to examine the interactions among water-quality characteristics and their effects on copper toxicity to larval fathead minnows (Pimephales promelas). The effects of dissolved organic carbon (DOC) concentration, DOC source, pH, and hardness on acute copper toxicity were determined using a complete factorially designed experiment. Hardness, pH, DOC, and interaction of pH and DOC all significantly affected copper toxicity. A predictive model based on these data described 88% of the variability in copper toxicity. This model also explained 58% of the variability in copper toxicity for an independent dataset of South Carolina (USA) waters. The biotic ligand model underpredicted the acute copper toxicity to fathead minnows when compared with observed values.

Shaw, B. J. and R. D. Handy (2006). "Dietary copper exposure and recovery in Nile tilapia, Oreochromis niloticus." Aquatic Toxicology **76**(2): 111-121.

There are few reports of dietary copper (Cu) toxicity to warm water species of freshwater fish, and little is known about recovery from dietary Cu exposure. In this study Nile tilapia (Oreochromis niloticus) were fed to satiation on a Cu-

loaded diet (2000 mg Cu kg(-1) dry weight (dw) feed), or a control diet (3 mg Cu kg(-1) dw feed), for 42 days. All fish were then fed the control diet for a further 21 days to assess recovery. Nutritional performance, haematology, histology, and tissue ion content (Cu, Na+, and K+) were measured. No mortalities occurred during the experiment. Dietary copper exposure was confirmed by elevated Cu concentrations in the intestine (30-fold), liver (three-fold) and gills (2.7-fold) of Cu-exposed fish compared to controls after 42 days (ANOVA, $P < 0.05$). Copper-exposed fish showed a reduction in food intake, and weight gain by day 21 of exposure, compared to controls (ANOVA, $P < 0.05$) and this persisted throughout the experiment. There were no treatment-dependent effects on food conversion ratio or hepatosomatic index, and all fish showed normal tissue Na+ and K+, and haematology throughout the experiment. Gill and intestine did not show overt pathology, but fatty change was observed in the liver of Cu-fed fish during exposure. The recovery phase on normal food was characterised by a reduction in intestinal and branchial Cu levels back to control values. However, the liver of the Cu-fed fish showed a further 1.7-fold rise in Cu content and marked hepatic lipidosis (increased intracellular fat stores) post-exposure, suggesting redistribution of Cu to the liver and delayed hepatotoxicity. 

Singh, M. (1995). "HEMATOLOGICAL RESPONSES IN A FRESH-WATER TELEOST CHANNA-PUNCTATUS TO EXPERIMENTAL COPPER AND CHROMIUM POISONING." Journal of Environmental Biology **16**(4): 339-341.

The effect of copper sulfate and potassium dichromate poisoning on haematological parameters of a fresh water fish Channa punctatus has been studied RBC, Hb%, PVC and MCHC values were significantly decreased while WBC, MCV & MCH were increased considerably upon treating the fish with both metallic compounds independently. Chromium proved more toxic than copper. Possible reasons for changes have been discussed.

Smith, M. J. and A. G. Heath (1979). "ACUTE TOXICITY OF COPPER, CHROMATE, ZINC, AND CYANIDE TO FRESHWATER-FISH - EFFECT OF DIFFERENT TEMPERATURES." Bulletin of Environmental Contamination and Toxicology **22**(1-2): 113-119.

Sola, F., J. Isaia, et al. (1995). "EFFECTS OF COPPER ON GILL STRUCTURE AND TRANSPORT FUNCTION IN THE RAINBOW-TROUT, ONCORHYNCHUS-MYKISS." Journal of Applied Toxicology **15**(5): 391-398.

Effects of copper were studied in freshwater adapted rainbow trout using the perfused head preparation. In its monovalent chemical form, copper at millimolar concentrations had no significant effects on Na+ and water transport. By contrast, the divalent form produced an increase in gill perfusion pressure, a significant reduction in Na+ influx and water fluxes and reversed Na+ net flux. Observations by light microscopy showed important cell damage (oedema, mucus production, cellular desquamation). By electron microscopy there was smoothing of apical membranes, swelling of the tubular system and destruction

of mitochondria. The Na, K-ATPase activity was totally suppressed and residual ATPase activity largely inhibited by 1 mM Cu2+. There was inhibition of the Na,K-ATPase activity with an IC50 Of similar to 10 mu M of total copper (free and bound cupric fractions). As active sodium transport is located on the secondary lamellae, our results show that its entry mechanism is inhibited at that level by cupric ions only. Results are discussed in relation to hydromineral balance of the trout.

Stouthart, X., J. L. M. Haans, et al. (1996). "Effects of water pH on copper toxicity to early life stages of the common carp (Cyprinus carpio)." Environmental Toxicology and Chemistry **15**(3): 376-383.

Carp eggs were exposed immediately after fertilization to Cu concentrations of 0.3 and 0.8 mu mol/L at water pH 7.6 or pH 6.3. Mortality, the incidence of spinal cord deformation, heart rate, tail movements, hatching success, and whole-body content of K, Na, Mg, Ca, and Cu were determined over time. Light microscopical preparations of eggs (48 h after fertilization) and larvae (168 h after fertilization) were studied. At pH 7.6, Cu did not affect egg mortality, heart rate, tail movements, and whole-body K and Mg content. Hatching success increased only in the 0.3 mu mol/L Cu group. Exposure to 0.8 mu mol/L Cu increased larval mortality and larval deformation and decreased whole-body Na and Ca content. At pH 6.3, exposure to 0.8 mu mol/L Cu increased egg mortality and decreased heart rate and tail movements. Furthermore, premature hatching, a concentration-dependent increase of larval mortality, and larval deformation was observed. Exposure to 0.3 and 0.8 mu mol/L Cu decreased the whole-body content of K, Na, Mg, and Ca. Uptake of Cu after hatching increased two-fold at pH 6.3 compared to the pH 7.6 groups. At pH 6.3, all Cu-exposed larvae were unable to fill their swim bladder. Also, after 168 h the yolk sac remained largely unabsorbed in the 0.3 and 0.8 mu mol/L Cu group. Exposure to 0.8 mu mol/L Cu resulted in coagulation of proteins in eggs and yolk sacs. No significant changes in any of the assessed parameters were observed in control groups of pH 6.3 and pH 7.6.

Swales, S., A. W. Storey, et al. (2000). "Temporal and spatial variations in fish catches in the fly river system in Papua New Guinea and the possible effects of the Ok Tedi copper mine." Environmental Biology of Fishes **57**(1): 75-95.

Biological monitoring of fishes in the Fly River system in Papua New Guinea has been carried out in relation to the input into the system of mine wastes from the Ok Tedi copper mine. A total of 86 fish species representing 32 families has been recorded from sites in the main river channel since the commencement of monitoring operations in 1983. Catfish in the families Ariidae (16 spp.) and Plotosidae (9 spp.) were the dominant groups overall, although Nematolosa herrings were the most numerous species, forming over 37% of the catch. However, barramundi, Lates calcarifer, comprised the greatest biomass, forming over 30% of the overall catch. Fish catch biomass at most sites showed considerable temporal and spatial variation over the period of sampling. However, significant reductions in biomass, ranging from 65% to 96%, were

recorded at most sites in the Ok Tedi, middle and upper Fly. The greatest declines in biomass were recorded in the Ok Tedi at sites closest to the mine, although reductions up to 73% were also recorded at sites in the middle Fly. Barramundi, which formed a high proportion of catch biomass at many sites, particularly in the middle Fly, declined in number at most sites following peak numbers in the early 1990's. The main causative factors involved in the overall declines in fish catches, including both mine-related and non-mine-related factors, are discussed. It is concluded that loss of fish habitat through increased river bed aggradation, due to the input of mined waste rock and tailings, is likely to be the main causal factor. However, other mine-related factors, such as elevated levels of dissolved and particulate copper, and other non-mine-related factors, such as introduced species, may also be involved in declining fish catches.

Tao, S., T. Liang, et al. (1999). "Synergistic effect of copper and lead uptake by fish." Ecotoxicology and Environmental Safety **44**(2): 190-195.

Paracheirodon innesi was exposed (simultaneously or sequentially) to copper and lead in varying concentrations in a synthetic water solution. Metal accumulations in fish gills and the fish (without gills) were measured at the end of the exposure period. Results of the exposure experiment indicated that lead seems to facilitate the uptake of copper and vice versa when the exposure process is simultaneous. In the case of sequential exposure first to copper for 7 days then to lead for 20 days, lead accumulations in the fish and the gills were enhanced by copper, indicating a possible alteration of the physiological absorption process. The synergistic effects observed during either simultaneous or sequential exposure experiments can be described using logistic equations. (C) 1999 Academic Press.

Tao, S., G. J. Liu, et al. (2002). "Estimation of conditional stability constant for copper binding to fish gill surface with consideration of chemistry of the fish gill microenvironment." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **133**(1-2): 219-226.

Binding-site concentration and conditional stability constants for copper and fish gill surface interactions were calculated based on the data from the literature. Six scenarios were modeled by including or excluding pH and alkalinity differences between the fish gill microenvironment and the bulk solution and the presence of free mucus in the calculation. We demonstrate that changes in pH or alkalinity, or both, for model input had only a slight influence on the calculated results because of the small difference in pH and alkalinity between the gill microenvironment and the bulk solution under the specific experimental conditions. Inclusion of free mucus in the model, however, led to a large change in the final results. For example, with consideration of free mucus and changes in pH and alkalinity in the model, the calculated site concentration and the stability constant were 0.022 mumol/g wet tissue and log K = 8.77, respectively, compared to 0.026 mumol/g wet tissue with log K = 7.78 without free mucus and pH/alkalinity change. 

Tao, S., W. X. Liu, et al. (2006). "Short-term dynamic change of gill copper in common carp, Cyprinus carpio, evaluated by a sequential extraction." Archives of Environmental Contamination and Toxicology **51**(3): 408-415.

Dynamic changes in Cu speciation and its binding to fish gills were investigated by exposing common carp (Cyprinus carpio) to a 1 mg/1 Cu solution for 7 hours. Cu speciation in the bulk solution and fish gill microenvironment was calculated using general chemical equilibrium modeling. A sequential extraction procedure using distilled water, magnesium dichloride (1.0 mol/1), and acetic acid (10%) was used to characterize the Cu associated with the fish gills. Cu residual in the gill tissue was measured after the sequential extraction. Changes in total Cu concentration, pH, and dissolved organic carbon (Doc) in the bulk solution were recorded during the experimental period and calculated for the fish gill microenvironment. Cu-hydroxide species and Cu were dominant Cu species in both bulk solution and the fish gill microenvironment, whereas increased Cu-mucus was found in the fish gill microenvironment. DOC in the exposure medium, assumed to arise from mucus release, also increased and complexed Cu in solution. Forty-three percent of the Cu associated with the gills was readily water extractable, with an additional 22% exchangeable with Mg2+ or protons. Only 35% of the Cu accumulated within the gill tissues. The binding of Cu to the fish gills reached apparent equilibrium after 3 hours of exposure. Furthermore, the amount of water-extractable Cu within the gills showed significant correlation to the concentration of Cu predicted to be complexed with calculated free mucus in the gill microenvironment.

Tao, S., A. M. Long, et al. (2002). "Copper speciation in the gill microenvironment of carp (Cyprinus carpio) at various levels of pH." Ecotoxicology and Environmental Safety **52**(3): 221-226.

The fish gill microenvironment of Cyprinus carpio under stress of copper exposure was investigated. pH and other parameters including free copper activity, alkalinity, and inorganic and organic carbons in the surrounding water (inspired water) and in the gill microenvironment (expired water) were measured or calculated at various levels of pH and varying total copper concentrations. The chemical equilibrium calculation (from MINEQA2) and complexation modeling (mucus-copper) were coupled to calculate both species distribution. The results indicate that the pH in the fish gill microenvironment was different from that in the surrounding water with a balance point around 6.9. The secretion of both CO, and mucus was affected in both linear and nonlinear ways when the fish were exposed to elevated concentrations of copper. The complexation capacity of the gill mucus was characterized by a conditional stability constant (log k(Cu-mucus)) of 5.37 along with a complexation equivalent concentration (LCu-mucus) of 0.96 mmol Cu/mg C. For both the fish microenvironment and the surrounding water, the dominant copper species shifted from Cu2+ to CuCO30 and to Cu(OH)(2)(0) when the pH of the surrounding water changed from 6.12 to 8.11. The change in copper speciation in the gill microenvironment is smaller than that in the surrounding water due to the pH buffering capacity of the fish gills. (C) 2002

Elsevier Science (USA).

Tao, S., Y. Wen, et al. (2001). "Simulation of acid-base condition and copper speciation in the fish gill microenvironment." Computers & Chemistry **25**(3): 215-222.

pH, alkalinity, and mucus content in the fish gill microenvironment of carp (Cyprinus carpio) were measured by exposing fish to copper at various water pH levels using an apparatus which separates inspired acid expired water. The relationship between pH levels inside and outside of the gill microenvironment, between pH and alkalinity, and between mucus secretion, pH, and copper exposure concentration were modeled. Copper speciation in the surrounding water and in the fish gill microenvironment was simulated using MINTEQA2 chemical equilibrium calculation software. The results of the modeling for pH, alkalinity, and mucus calculation were then adopted as inputs for purposes of parameter identification in the speciation modeling. The differences observed in the copper species distribution between that of the fish gill microenvironment and the surrounding water were based on the speciation modeling. The change in copper bioavailability for fish uptake was also examined. The results indicate the presence of an experimental pH balance point at 6.9, where the pH in the fish gill microenvironment is identical to that of the surrounding water. The observed deviation range in pH levels between that found at the gills and that of the surrounding water varied from -0.4 to 0.8 units. A sinusoidal model was developed for calculation of gill pH based on the pH of the surrounding water. Models calculating alkalinity either in the gill microenvironment or in the surrounding water and for estimating mucus secretion were also developed. The results of the chemical equilibrium calculations demonstrate that, within a pH range of 6-9, the dominant species of copper in bulk solution shifted from free ions to that of the hydroxo complex. With respect to the fish gill microenvironment, the dominant species found under acidic conditions were the mucus-copper complex and free ions. Because of the influence of mucus complexation and pH change, bioavailable copper species in the fish gill microenvironment were significantly lower than that in the bulk solution, especially under acidic conditions. (C) 2001 Elsevier Science Ltd. All rights reserved.

Taylor, D., B. G. Maddock, et al. (1985). "THE ACUTE TOXICITY OF 9 GRAY LIST METALS (ARSENIC, BORON, CHROMIUM, COPPER, LEAD, NICKEL, TIN, VANADIUM AND ZINC) TO 2 MARINE FISH SPECIES - DAB (LIMANDA-LIMANDA) AND GRAY MULLET (CHELON-LABROSUS)." Aquatic Toxicology **7**(3): 135-144.

Taylor, L. N., J. C. McGeer, et al. (2000). "Physiological effects of chronic copper exposure to rainbow trout (Oncorhynchus mykiss) in hard and soft water: Evaluation of chronic indicators." Environmental Toxicology and Chemistry **19**(9): 2298-2308.

Effects of chronic copper exposure on a suite of indicators were examined: acute toxicity, acclimation, growth, sprint performance, whole-body electrolytes, tissue residues, and gill copper binding characteristics. Juvenile rainbow trout were exposed for 30 d to waterborne copper in hard water (hardness = 120 mg/L as

CaCO3, pH = 8.0, Cu = 20 and 60 mug/L) and soft water (hardness = 20 mg/L as CaCO3, pH = 7.2, Cu = 1 and 2 mug/l). Significant acclimation to the metal occurred only in fish exposed to 60 mug/L, as seen by an approx. twofold increase in 96-h LC50 (153 vs 91 mug Cu/L). Chronic copper exposure had little or no effect on survival, growth, or swimming performance in either water hardness, nor was there any initial whole-body electrolyte loss (Na+ and Cl-). The present data suggest that the availability of food (3% wet body weight/day, distributed as three 1% meals) prevented growth inhibition and initial ion losses that usually result from Cu exposure. Elevated metal burdens in the gills and livers of exposed fish were measures of chronic copper exposure but not of effect. Initial gill binding experiments revealed the necessity of using radiolabeled Cu (Cu-64) to detect newly accumulated Cu against gill. background levels. Using this method, we verified the presence of saturable Cu-binding sites in the gills of juvenile rainbow trout and were able to make estimates of copper-binding affinity (log K-gill=Cu) and capacity (B-max). Furthermore, we showed that both chronic exposure to Cu and to low water calcium had important effects on the Cu-binding characteristics of the gills.

Taylor, L. N., C. M. Wood, et al. (2003). "An evaluation of sodium loss and gill metal binding properties in rainbow trout and yellow perch to explain species differences in copper tolerance." Environmental Toxicology and Chemistry **22**(9): 2159-2166.

The main objective of the study was to use a species comparison approach in order to understand sensitivity and tolerance differences to copper. We hypothesized that species differences in toxicity would be reflected by differences in copper binding to high-affinity sites on the gill. Specifically, the strength of copper binding (affinity, logK) and maximum number of binding sites (saturation, B-max) for copper at the gill surface would vary among different species of fish. Two species that are different in their copper sensitivity are the rainbow trout (Oncorhynchus mykiss) and yellow perch (Perca flavescens). We explicitly compared acute toxicity (median lethal concentrations via 96-h LC50s) and whole-body Na+ loss in both organisms in two distinct water chemistries (i.e., hard and soft water). For both species, the copper binding sites at the gill surface were characterized for their affinity and saturability. The binding properties of the gill were quite similar between the two species in each water chemistry. Based on estimations of the free cupric ion concentration, the affinity, or logK, was 8.4 for both species in soft water, whereas in hard water, the affinity was higher (similar to9.7). The B-max value in soft water was 1.88 nmol/g for rainbow trout and yellow perch, while in hard water, saturation occurred at 3.63 nmol/g for rainbow trout and 9.01 nmol/g for yellow perch. More importantly, the amount of copper bound to the gills at 50% mortality (i.e., lethal accumulation; the LA50) was different between the two species (yellow perch LA50s were nine times higher than those of rainbow trout in soft water and hard water), indicating that the copper binding to the yellow perch gill must not have been 'biologically reactive.' According to 96-h LC50s, yellow perch were less sensitive to copper than were rainbow trout; however, the difference between the two species was similar in hard water (1.05 vs 4.16 muM) and soft water (similar to0.10 vs 0.44

muM). Perch were more tolerant because they lost less sodium upon exposure to copper; yet this mechanism of tolerance was not reflected by the amount of copper at the gill surface. The influence of water chemistry on the binding properties of the gill demonstrates the dynamic nature of the gill in maintaining ionoregulatory homeostasis, a key issue in the future development of the chronic biotic ligand model.

Vanvuren, J. H. J., M. Vandermerwe, et al. (1994). "THE EFFECT OF COPPER ON THE BLOOD-CHEMISTRY OF CLARIAS-GARIEPINUS (CLARIIDAE)." Ecotoxicology and Environmental Safety **29**(2): 187-199.

Environmental stressors, both natural and humanly induced, could cause changes in cellular function which alter the physiology of organ systems in fish. The need to comprehend and predict the condition which stress metals will pose on fish, and extrapolate the effect of pollutants from laboratory to population levels, have forced scientists to search for physiological and biochemical indicators of health and sublethal toxicant effects. Hematological evaluation of fish blood provides valuable facts concerning the physiological response of fish to changes in the external environment. Furthermore, hematological Variables are well known for their clinical value in prognosis and diagnosis. Sublethal responses after exposure to toxicants can be determined by commonly applied techniques. Measurements for a number of hematological and carbohydrate metabolic variables were recorded at winter and summer temperatures after fish, acclimated for 3 months to experimental conditions, were exposed to sublethal concentrations of copper for 96 hr in a continuous-flow experimental system. Controls were run at the same time to establish essential baseline hematological values. Fish were exposed to the mean copper concentration as was found in the Olifants River, Kruger National Park, during summer(0.05 +/- 0.032 mg liter(-1)) and winter (0.085 +/- 0.032 mg liter(-1)) to establish the effect thereof on the variables mentioned. The results proved that the concentration of copper in the river exerts a physiological effect on Clarias gariepinus at 21 +/- 1 and 28 +/- 1 degrees C which manifests in changed blood chemistry. Pathological conditions, such as erythrocytopenia, leucocytosis, hyperglycemia, and hyperprotonemia, are evident. The fish physiologically adapted to the environmental change, which does not necessarily reflect a State of normality. (C) 1994 Academic Press, Inc.

Varanka, Z., I. Rojik, et al. (2001). "Biochemical and morphological changes in carp (Cyprinus carpio L.) liver following exposure to copper sulfate and tannic acid." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **128**(3): 467-478.

As a consequence of human activity various toxicants reach the aquatic ecosystems; humics may interact with them and may change their toxicity. Many fish are exposed to a considerable concentration of humics and pollutants. Because of paucity of data on the biochemical action of tannins in the presence of the fungicide CuSO4 a comparative study was undertaken. The alterations of redox-parameters in carp liver were monitored and tissue necrosis was followed by measuring the plasma transaminase activities and by electron microscopy.

Tannic acid, a representative phenolic/humic compound, exerted prooxidant effects in carp, which may be partially due to formation of prooxidant intermediates/end-products via its biotransformation. Alternatively, tannic acid may partially inhibit the antioxidant enzymes of fish. The response to CuSO4 was more severe. Although tannic acid alone acted as a prooxidant in fish, electron micrographs demonstrated that it reduced the necrotizing effect of copper, which may be due to the complexing activity of tannic acid with the biomolecules of the hepatocytes and to the H2O2-degrading activity of tannin-CuSO4 combination. Our results indicate that the heavy metal-detoxifying capacity of tannin may be significant; however, tannin-exposure alone or combined with metals may be toxic for fish due to enzyme inhibition and oxidative stress induction. 

Vinot, I. and J. C. Pihan (2005). "Circulation of copper in the biotic compartments of a freshwater dammed reservoir." Environmental Pollution **133**(1): 169-182.

This study concerns a chronic copper release in an aquatic ecosystem: Mirgenbach reservoir; which is characterized by high salinity, conductivity and hardness, a eutrophic state and a high temperature. To study the bioavailability of copper in the biotic compartments, the sampling covered the entire food chain (phyto- and zooplankton, macroalgae, aquatic plants, crustaceans, mollusks, and fish). Of the organisms present, the filter feeder Dreissena polymorpha, the detritivorous Bithynia tentaculata and Orconectes limosus were most contaminated by copper. The level of copper found in fish was the lowest. Body copper concentrations recorded in the present study show large variability between species even in some that are closely related. In most cases, however, the metal handling strategy, feeding habits, morphology and ecology can, at least partially, explain the metal content recorded. Pollution factors have been used to assess the state of contamination of the food chain. This study showed finally that the copper in the lake is bioavailable and bioaccumulated by organisms up to high levels and some effects of long-term toxicity of copper on benthic community and planktonic biomass were pointed out. 

Vutukuru, S. S., S. Chintada, et al. (2006). "Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, Esomus danricus." Fish Physiology and Biochemistry **32**(3): 221-229.

Static-renewal bioassays [Methods for acute toxicity tests with fish, macro-invertebrates and amphibians: USEPA, ERS, EPA 660/3 75-009 (1975)] were carried out on Esomus danricus exposed to sub-lethal (0.55 mg/l) and lethal (5.5 mg/l) concentrations of copper. The 96-h median lethal concentration (LC50) was 5.5 mg/l. Biochemical stress responses, such as visceral superoxide dismutase (SOD) and catalase (CAT) activities, were measured during this 96-h period. Malondialdehyde, a product of lipid peroxidation, was present at elevated levels in the visceral tissue of copper-exposed fish. Copper was found to be highly toxic to the fish and induced significant declines ($p < 0.05$-$0.001$) in all of the biochemical profiles studied, demonstrating a linear and positive correlation with

both the concentration and duration of exposure to copper. In E. danricus, CAT appeared to be more sensitive to copper exposure ($p < 0.001$) than SOD at both lethal and sub-lethal levels. These results indicate that antioxidant responses can be employed as biomarkers of oxidative stress for this species in aquatic environments contaminated with copper.

Welsh, P. G., J. Lipton, et al. (2008). "Influence of flow-through and renewal exposures on the toxicity of copper to rainbow trout." Ecotoxicology and Environmental Safety **69**(2): 199-208.

We examined changes in water chemistry and copper (Cu) toxicity in three paired renewal and flow-through acute bioassays with rainbow trout (Oncorhynchus mykiss). Test exposure methodology influenced both exposure water chemistry and measured Cu toxicity. Ammonia and organic carbon concentrations were higher and the fraction of dissolved Cu lower in renewal tests than in paired flow-through tests. Cu toxicity was also lower in renewal tests; 96 h dissolved Cu LC50 values were 7-60% higher than LC50S from matching flow-through tests. LC50 values in both types of tests were related to dissolved organic carbon (DOC) concentrations in exposure tanks. Increases in organic carbon concentrations in renewal tests were associated with reduced Cu toxicity, likely as a result of the lower bioavailability of Cu-organic carbon complexes. The biotic ligand model of acute Cu toxicity tended to underpredict toxicity in the presence of DOC. Model fits between predicted and observed toxicity were improved by assuming that only 50% of the measured DOC was reactive, and that this reactive fraction was present as fulvic acid. 

Welsh, P. G., J. L. Parrott, et al. (1996). "Estimating acute copper toxicity to larval fathead minnow (Pimephales promelas) in soft water from measurements of dissolved organic carbon, calcium, and pH." Canadian Journal of Fisheries and Aquatic Sciences **53**(6): 1263-1271.

We tested an empirical model that predicts copper (Cu) toxicity to larval fathead minnow (Pimephales promelas) from surface water pH and dissolved organic carbon (DOC) concentrations with an independent data set of 18 softwater lakes. These lakes ranged widely in concentrations of DOC (1.7-14.8 mg L(-1)), pH (5.5-6.9), and calcium (Ca) (1.9-4.6 mg L(-1)), all factors known to modify metal toxicity. Acute median lethal concentrations (96-h LC(50)s) for total Cu were 5.3-169.5 mu g . L(-1) in the 18 lakes. Although measured Cu toxicity was correlated with predicted toxicity (r(2) = 0.78), the model overestimated the actual Cu toxicity; however, this could be corrected by including Ca in a modified model: log 96-h Cu LC(50) = 0.981 + 0.192 pH + 0.136 (pH x log DOC) + 0.166 Ca (r(2) = 0.84). Similar parameters were observed when the analysis was restricted to the 18 study lakes (r(2) = 0.85). Toxicity was also expressed as log 96-h Cu LC(50) = 0.033 + 0.025 pH + 0.015(pH x log colour) + 0.078 hardness (r(2) = 0.83) because colour and hardness are easily measured surrogates for DOC and Ca concentrations. This research demonstrates that acute lethality of Cu to larval fathead minnows varies in a predictable way in soft water, on the basis of DOG,

pH, and Ca concentrations.

Welsh, P. G., J. F. Skidmore, et al. (1993). "EFFECT OF PH AND DISSOLVED ORGANIC-CARBON ON THE TOXICITY OF COPPER TO LARVAL FATHEAD MINNOW (PIMEPHALES-PROMELAS) IN NATURAL LAKE WATERS OF LOW ALKALINITY." Canadian Journal of Fisheries and Aquatic Sciences **50**(7): 1356-1362.

The impacts of pH and dissolved organic carbon (DOC) on the acute toxicity of Cu to larval fathead minnow (Pimephales promelas) were determined using natural soft water from two Precambrian Shield lakes in south-central Ontario. By artificially manipulating the pH and DOC levels of the water, we demonstrated that both acidification and the removal of DOC increased the toxicity of Cu. The 96-h Cu LC50s were determined over a pH range from 5.4 to 7.3 and a DOC concentration range from 0.2 to 16 mg-L-1. The LC50s ranged from a low of 2 mug.L-1 (pH 5.6, DOC 0.2 mg.L-1) to a high of 182 mug.L-1 (pH 6.9, DOC 15.6 mg.L-1). A multiple regression model (log(10)96-h Cu LC50 = -0.308 + 0.192 pH + 0.136 (pH.log10DOC)) was used to describe the relationship between Cu toxicity, pH, and DOC. The model was significant ($p < 0.00001$) and explained 93% of the variability in the toxicity data. These results suggest that current water quality objectives for Cu, and possibly for other metals, may not be sufficiently protective of aquatic life in soft, moderately acidic water containing low levels of DOC.

Wilson, R. W. and E. W. Taylor (1993). "DIFFERENTIAL RESPONSES TO COPPER IN RAINBOW-TROUT (ONCORHYNCHUS-MYKISS) ACCLIMATED TO SEA-WATER AND BRACKISH-WATER." Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology **163**(3): 239-246.

Rainbow trout, Oncorhynchus mykiss, acclimated to 33% sea water (12 mg.ml-1 salinity) experienced significant (10 meq.l-1) increases in plasma [Na+] and [Cl-] within 5 h of exposure to 6.3 mumol copper.l-1 indicating severe impairment of branchial ionoregulatory capacity. All plasma ion levels subsequently stabilised once the transbranchial [Na+] gradient was reduced to zero. The similar ionic strength of the external medium and their body fluids appeared to protect trout maintained in 33% sea water from further ionoregulatory stress and any secondary physiological disturbances during exposure to copper. Despite three- and fourfold greater transbranchial [Na+] and [Cl-] gradients, trout acclimated to full-strength sea water (35 mg.ml-1 salinity) suffered no major changes in plasma Na+, Cl-, K+, or Ca2+, blood gases or haematology during 24 h exposure to 6.3 mumol copper.l-1. This reduction in toxicity in full strength sea water cannot be explained by differences in copper speciation. We suggest that during acute exposure to water-borne copper, active NaCl extrusion is unaffected due to the basolateral location of the gill Na+/K+-ATPase, but that ionoregulatory disturbances can occur due to gill permeability changes secondary to the displacement of surface-bound Ca2+. However, in full strength sea water the three-fold higher ambient [Ca2+] and [Mg2+] appear to be sufficient to prevent any detrimental permeability changes in the presence of 6.3 mumol copper.l-1. Plasma [NH4+] and [HCO3-] were both significantly elevated during exposure to

copper, indicating that some aspects of gill ion transport (specifically the apical Na+/NH4+ and Cl-/HCO3- exchanges involved in acid/base regulation and nitrogenous waste excretion) are vulnerable to inhibition in the presence of waterborne copper.

Wilson, R. W. and E. W. Taylor (1993). "THE PHYSIOLOGICAL-RESPONSES OF FRESH-WATER RAINBOW-TROUT, ONCORHYNCHUS-MYKISS, DURING ACUTELY LETHAL COPPER EXPOSURE." Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology **163**(1): 38-47.

Acutely lethal (24 h) exposure of adult rainbow trout (Oncorhynchus mykiss) to 4.9 mumol copper . l-1 in fresh water (pH 7.9, [Ca2+]almost-equal-to 0.8 mEq . l-1) caused a rapid decline of plasma Na+ and Cl- and arterial O2 tension, and initially a pronounced tachycardia. The internal hypoxia probably resulted from histopathologies observed in the gills of fish exposed to copper, such as cell swelling, thickening and curling of the lamellae, and haematomas. Copper cannot therefore be considered purely as an ionoregulatory toxicant during acutely lethal conditions. Mortality during exposure to copper could not simply be explained by the plasma ionic dilution, nor by the internal hypoxia, since arterial O2 content remained relatively unchanged. Secondary to the ionoregulatory and respiratory disturbances were a number of deleterious-physiological responses which included a massive haemoconcentration (haematocrit values as high as 60%) and a doubling of the mean arterial blood pressure. The time-course of these changes suggest that cardiac failure was the final cause of death. In this respect copper exposure resembles low pH exposure in freshwater trout (Milligan and Wood 1982). Copper and H+ appear to be similar in both the primary site of their toxic action (the gills) and the secondary physiological consequences which result from acutely lethal exposures. Furthermore, the acute toxicity syndrome observed may be common to many metals which cause ionoregulatory and/or respiratory problems in freshwater fish.

Wong, P. P. K., L. M. Chu, et al. (1999). "Study of toxicity and bioaccumulation of copper in the silver sea bream Sparus sarba." Environment International **25**(4): 417-422.

The toxicity and bioaccumulation of copper were studied in fingerlings (mean body weight = 9.4+/-2.1 g) and subadults (mean body weight = 85.5+/-27.1 g) of the silver sea bream Sparus sarba. Test fish were obtained from local fish culture sites. Static tests over 96 h showed that subadults were not more tolerant to copper than the much smaller fingerlings. The 24-h, 48-h, 72-h, and 96-h LC50 for fingerlings were 2.01 mg Cu L-1, 1.28 mg Cu L-1, 1.17 mg Cu L-1, and 1.03 mg Cu L-1, respectively. The values for subadults were 2.36 mg Cu L-1, 1.52 mg Cu L-1, 1.34 mg Cu L-1, acid 1.24 mg Cu L-1, respectively. Copper concentrations corresponding to 13%, 25%, and 40% of the 96-h LC50 value were used to study the effects of copper exposure on the growth rate of S. sarba in 30-d bioassays, The growth rate of fingerlings was higher than that of subadults by approximately a hundred-fold. For both fingerlings and subadults, reduced growth was observed at 0.15 mg Cu L-1 (similar to 40% of the 96-h

LC50 value). Growth appeared to be a more sensitive endpoint for toxicity tests than mortality. Fish exposed to copper for 30 d contained more copper than control animals. The highest copper concentrations for both fingerlings and subaduits were found in the intestine. The order of copper concentration was intestine > liver > gonad > gills, skin and muscle. (C) 1999 Elsevier Science Ltd.

Wu, S. M., Y. C. Ho, et al. (2007). "Effects of Ca2+ or Na+ on metallothionein expression in tilapia larvae (Oreochromis mossambicus) exposed to cadmium or copper." Archives of Environmental Contamination and Toxicology **52**(2): 229-234.

The objectives of this study were to try to determine the reasons of the external Ca2+ and Na+ enhancement of Cd2+ and Cu2+ resistance in fish. Tilapia larvae at 3 days posthatch were exposed to (A) 0 (control), 40 mu g/L Cd2+, 40 mu g/L Cd2+ + 2 mM Ca2+ (Cd/hyper-Ca), and 2 mM Ca2+ or (B) 0 (control), 75 mu g/L Cu2+, 75 mu g/L Cu2+ + 0.52 mM Na+ (Cu/hyper-Na), and 0.52 mM Na+. After 48 hours, results indicated that (1) Cd/hyper-Ca and Cu/hyper-Na treatments showed decreased growth inhibition induced by the metals; (2) metal accumulation in Cd/hyper-Ca-treated larvae was lower compared with those exposed only to Cd; and (3) metallothionein (MT) expression was significantly higher in Cu/hyper-Na-treated larvae than in the group treated with Cu only. Taking all of this into account, either supplementary Ca2(+) or Na+ in ambient water may help fish to maintain Ca2(+) or Na+ homeostasis, which could decrease metal accumulation and its detrimental effects. Consequently, the fish increase MT expression and retard the growth inhibition caused by metals.

Wurts, W. A. and P. W. Perschbacher (1994). "EFFECTS OF BICARBONATE ALKALINITY AND CALCIUM ON THE ACUTE TOXICITY OF COPPER TO JUVENILE CHANNEL CATFISH (ICATALURUS-PUNCTATUS)." Aquaculture **125**(1-2): 73-79.

Three experiments were conducted to evaluate the relative importance of calcium hardness and bicarbonate alkalinity to the acute response of juvenile channel catfish (Ictalurus punctatus) exposed to a toxic concentration of copper sulfate. A preliminary bioassay revealed 28 mg.1(-1) copper sulfate caused 50% mortality within 48 h (48-h LC50) in juvenile channel catfish placed in water with calcium hardness and bicarbonate alkalinity, set at 75 mg.1(-1) CaCO3. Catfish were then exposed to 28 mg.1(-1) copper sulfate concentrations in environments where hardness or alkalinity concentrations were varied. Bicarbonate alkalinities above 75 mg.1(-1) CaCO3, with calcium hardness held at 20 mg.1(-1) CaCO3, significantly reduced catfish mortalities from 97-100% to 63-70%. Copper-induced mortalities were 100% for all fish placed in calcium hardness treatments (20-250 mg.1(-1) CaCO3) in which bicarbonate alkalinity was held at 20 mg.1(-1) CaCO3. When bicarbonate alkalinity was held constant at 75 mg.1(-1) CaCO3 and calcium hardness was varied from 20 to 250 mg.1(-1) CaCO3, copper related catfish mortalities displayed high variability and means ranged from 6.7 to 60%. Mortalities decreased as calcium concentrations increased. Although differences in mortalities were not statistically significant, the latter hardness findings appear to suggest a biologically significant calcium effect on copper toxicity in the presence of sufficient alkalinity concentrations.

Zahner, H. M., E. J. VanGenderen, et al. (2006). "Whole-body sodium concentration in larval fathead minnows (Pimephales promelas) during and following copper exposure." Environmental Toxicology and Chemistry **25**(6): 1635-1639.

This research used whole-body sodium concentration to characterize exposure and recovery of larval fathead minnows (Pimephales promelas) from acute pulsed copper exposures. Whole-body sodium was chosen because the acute mechanism of copper toxicity to fishes is putative disruption of ion regulation, resulting in a loss of sodium and eventually leading to mortality. Whole-body sodium response in larval fathead minnows exposed to copper was both concentration and duration dependent. The loss of sodium to approximately 70% of control levels occurred within 12 h of exposure. Organisms demonstrated an ability to recover whole-body sodium within 48 h after exposure to concentrations below 0.47 mu M Cu2+ for 3, 6, or 9 h. However, at higher concentrations, organisms required more than 48 h to recover. Whole-body sodium concentrations and mortality for all continuous exposures were strongly correlated. These results may facilitate development of a physiologically based model to predict the response of organisms to copper in receiving streams.

Zaroogian, G., S. Anderson, et al. (1992). "INDIVIDUAL AND COMBINED CYTOTOXIC EFFECTS OF CADMIUM, COPPER, AND NICKEL ON BROWN CELLS OF MERCENARIA-MERCENARIA." Ecotoxicology and Environmental Safety **24**(3): 328-337.