Annotated Bibliography: Effects of Zinc on Fish 2009

Abbasi, S. A. and R. Soni (1986). "An examination of environmentally safe levels of zinc (II), cadmium (II) and lead (II) with reference to impact on channelfish Nuria denricus." Environmental Pollution Series A, Ecological and Biological **40**(1): 37-51.

As a continuation of our earlier studies on the impact of heavy metals on aquatic organisms (Abbasi & Soni, 1984), the behavioural responses and survival of the teleost Nuria denricus exposed to different levels of zinc (II), cadmium (II) and lead (II) were studied. Safe concentration (SC) values of the metals were determined with the help of computer-aided long-term bioassays. Comparisons of the SC with the minimum allowable levels of the corresponding metals in drinking water, effluents, irrigation water and fisheries revealed that, except for a few cases, the existing national and international water quality standards for various water uses are higher than the SC. The disparity between the SC and the permitted levels of zinc and cadmium was especially noteworthy, indicating the need for a thorough re-evaluation of the toxicity and safe levels of these metals.

Adhikari, S. and S. Ayyappan (2004). "Behavioural role of zinc on primary productivity, plankton and growth of a freshwater teleost, Labeo rohita (Hamilton)." Aquaculture **231**(1-4): 327-336.

The role of zinc in biological production at three levels of zinc treatments was investigated. The three treatment levels were 10, 20 and 30 kg ZnSO4/ha with each treatment using three different soil bases containing 0.45, 0.75 and 1.0 ppm diethylenetriamine pentaacetic acid (DTPA)-extractable zinc. All treatments showed an increase in both plankton and primary productivity ($p<0.05$) over control (without zinc) and the maximum increase was at 10, 20 and 30 kg ZnSO4/ha for 1.0, 0.75 and 0.45 ppm DTPA-extractable zinc, respectively. In the same experiment, Labeo rohita fingerlings were stocked after 15 days of zinc treatment. All the treatments showed an increase in growth of fish ($p<0.05$) as compared with the control. Overall, maximum growth was obtained in the 30 kg ZnSO4/ha-0.45 ppm DTPA-extractable zinc, second highest followed by 10 kg-1.0 ppm, followed by third highest growth in the 20 kg-0.75 ppm treatment. Zinc from the soil was fractionated into different forms and the distribution of various forms in the soil was found in the order of water-soluble<organically bound<complexed<occluded<residual. The major portion of total zinc in the soil existed in the residual form. The amount of water-soluble, exchangeable and complexed forms of zinc diminished due to plankton and fish removal while the

addition of zinc to soil increased these three forms considerably. The contents of occluded and residual zinc in soil did not change due to plankton and fish growth or due to addition of zinc. Water-soluble, organically bound and to a less extent, the exchangeable form of zinc principally contributed to the pool of available zinc in this soil.

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.

Alsop, D. H., J. C. McGeer, et al. (1999). "Costs of chronic waterborne zinc exposure and the consequences of zinc acclimation on the gill/zinc interactions of rainbow trout in hard and soft water." Environmental Toxicology and Chemistry **18**(5): 1014-1025.

Juvenile rainbow trout were exposed to zinc in both moderately hard water (hardness = 120 mg CaCO3/L, pH = 8.0, Zn = 150 mu g/L or 450 mu g/L) and soft water (hardness = 20 mg CaCO3/L, pH = 7.2, Zn = 50 mu g/L or 120 mu g/L) for 30 d. Only the 450 mu g/L zinc-exposed fish experienced significant mortality (24% in the first 2 d). Zinc exposure caused no effect on growth rate. but growth affected tissue zinc Levels. Whole body zinc levels were elevated, but gills and liver showed no consistent increases relative to controls over the 30 d. Therefore, tissue zinc residues were not a good indicator of chronic zinc exposure. After the 30-d exposure. physiological function tests were performed. Zinc was 5.4 times more toxic in soft water (control 96 h LC50s in hard and soft water were 869 mu g/L and 162 mu g/L, respectively). AII zinc-exposed trout had acclimated to the metal, as seen by an increase in the LC50 of 2.2 to 3.9 times over that seen in control fish. Physiological costs related to acclimation appeared to be few. Zinc exposure had no effect on whole body Ca2+ or Na+ levels, on resting or routine metabolic rates, or on fixed velocity sprint performance. However, critical swimming speed (U-Cru) was significantly reduced in zinc-exposed fish, an effect that persisted in zinc-free water. Using radioisotopic techniques to distinguish new zinc incorporation, the gills were found to possess two zinc pools: a fast turnover pool (T-1/2 = 3-4 h) and a slow turnover pool (T-1/2 = days to months). The fast pool war much larger in soft water than in hard water, but at most it

accounted for <3.5% of the zinc content of the gills. The size of the slow pool was unknown, but its loading rate was faster in soft water. Chronic zinc exposure was found to increase the size of the fast pool and to increase the loading rate of the slow peal.

Alsop, D. H. and C. M. Wood (2000). "Kinetic analysis of zinc accumulation in the gills of juvenile rainbow trout: Effects of zinc acclimation and implications for biotic ligand modeling." Environmental Toxicology and Chemistry **19**(7): 1911-1918.

Juvenile rainbow trout were acclimated to hard water (Ca2+ = 1.0 mM, Mg2+ = 0.2mM; hardness = 120 mg CaCO3/L) and hard water plus 250 mu g/L Zn (3.8 mu M). After 30 d of exposure, there was no difference in the total Zn levels of the gills of Zn-exposed and control fish (similar to 70 mu g Zn/g gill). Exposure of both groups to a range of Zn concentrations (0-2,900 mu g/L Zn) for up to 7 d also had no effect on the measured total Zn levels in the gills. However, using radiolabeled Zn-65, measurement of new Zn appearance in the gills was possible. Trout were exposed to a range of Zn concentrations (with Zn-65) and the gills were sampled at times ranging from 0.5 to 72 h. The fast turnover pool of Zn in the gills increased with increasing acute Zn exposure concentration, while the maximum size of the fast pool was about ninefold larger in Zn-acclimated fish (4.14 mu g Zn/g gill) versus control fish (0.45 mu g Zn/g gill). At all sampling times, gill Zn-65 accumulation exhibited saturation kinetics, allowing calculation of binding capacity (B-max) and affinity (K-d). In both control and Zn-acclimated trout, K-d decreased rapidly (affinity increased) from 0.5 to 3 h and then remained constant up to 72 h. B-max increased rapidly from 0.5 to 3 h in both groups, then the rate of increase began to subside but was still increasing from 24 to 72 h. At all times, the K-d of Zn-acclimated fish was higher (i.e., lower affinity) and B-max was greater than controls. The stabilized K(d)s (>3 h) were approximately 280 mu g/L total Zn (log K = 5.6 as Zn2+) and 575 mu g/L total Zn (log K = 5.3 as Zn2+) in control and Zn-acclimated fish, respectively. The B-max of control fish at 0.5 h was 0.37 mu g Zn/g gill and increased to 8.63 mu g Zn/g gill by 72 h. The B-max of Zn-acclimated fish increased from 0.70 to 11.61 mu g Zn/g gill over the same time period. Preexposure to 250 mu g/L Zn appeared to have little effect on acute zinc toxicity, though the 96-h LC50s for both groups were relatively high (similar to 3,000 mu g/L Zn) in comparison to previous measurements. The relationship between gill binding constants for different metals and relative toxicity is critically assessed with respect to biotic ligand modeling.

Andres, S., F. Ribeyre, et al. (2000). "Interspecific comparison of cadmium and zinc contamination in the organs of four fish species along a polymetallic pollution gradient (Lot River, France)." The Science of The Total Environment **248**(1): 11-25.

The impact of cadmium (Cd) and zinc (Zn) discharges related to an old zinc ore treatment facility in the Lot River (France) was investigated in four fish species (the chub: Leusciscus cephalus, the roach: Rutilus rutilus, the perch: Perca fluviatilis and the bream: Abramis brama). The organisms were sampled in four stations along the polymetallic contamination gradient. Cd and Zn analysis were

carried out in five organs (gills, posterior intestine, liver, kidneys and skeletal muscle) in order to highlight the potential pathways of uptake, storage and elimination of metals. The results indicate a very strong Cd contamination in fish collected downstream from the metal source. The kidneys have the highest cadmium concentrations, but the gills and the intestine, as exchange organs, present the largest variations between the stations in close relation with the contamination gradient. Cd concentrations measured in the liver vary only slightly among the sampling stations. Unlike the trends observed for Cd, Zn levels in fish populations are strongly regulated and do not follow ambient Zn concentrations. The concentrations measured vary also according to fish species, for both Cd and Zn. This study shows that the trophic habits can explain the interspecific differences in Cd bioaccumulation. Zn levels observed for each species in non-contaminated populations also help to understand metal bioaccumulation patterns in polluted sites, suggesting that the determinism of interspecific differences is constitutive.

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.

Babich, H., C. Shopsis, et al. (1986). "In vitro cytotoxicity testing of aquatic pollutants (cadmium, copper, zinc, nickel) using established fish cell lines." Ecotoxicology and Environmental Safety **11**(1): 91-99.

The cytotoxicity of cadmium toward cultured bluegill fry (BF-2) cells was determined using several assay endpoints. The concentrations of cadmium causing a 50% decrease in colony formation, cell replication, uptake of neutral red, population growth (as determined by protein analysis), and uptake of [3H]uridine and 50% detachment of cells (as determined by protein analysis) were 0.03, 0.04, 0.08, 0.09, 0.12, and 0.21 mM cadmium, respectively. The neutral red assay was used to compare the relative sensitivities of bluegill BF-2 cells and RTG-2 cells, derived from the rainbow trout, toward four metals. The concentrations of cadmium, zinc, copper, and nickel causing a 50% reduction in the uptake of neutral red were 0.08, 0.19, 0.55, and 2.0 mM, respectively, with the BF-2 cells and 0.18, 0.64, 1.45, and >> 10.0 mM, respectively, with the RTG-2 cells. The RTG-2 cells were less sensitive to the metals, in particular to nickel.

The less stringent temperature requirements for growth, their greater sensitivity to pollutants, and their markedly shorter doubling time in vitro make the BF-2 cells the preferable cell line for ecotoxicity screening of aquatic pollutants.

Barron, M. G. and S. Albeke (2000). "Calcium control of zinc uptake in rainbow trout." Aquatic Toxicology **50**(3): 257-264.

Water hardness is known to control the uptake and toxicity of zinc and other metals through either chemical competition, biological acclimation, or both processes. The dominant process controlling zinc uptake has not been previously elucidated, nor has the effect of calcium been investigated independently of other cations. We determined zinc uptake by rainbow trout acclimated and exposed to four calcium treatments: (1) low calcium (6.5 mg Ca/l; 160 mu M) acclimation and low calcium exposure (LL) (2) low acclimation and high calcium (131 mg Ca/l; 3300 mu M) exposure (LH); (3) high acclimation and low exposure (HL) and (4) high acclimation and high exposure (HH). Trout were exposed to sublethal zinc (100 mu g Zn-65/l; 1.5 mu M) for 24 h, and whole body and gill Zn-65 levels were determined. Zinc uptake was approximately linear during the 24 h exposure period and uptake was calcium dependent. Zn-65 uptake by trout was 22 mu g/kg per h in the LL treatment and 4.5 mu g/kg per h in the HH treatment. Zn-65 uptake by trout in the LH and HL treatments was 13 and 10 mu g/kg per h, respectively. Zn-65 uptake in gills was also significantly reduced by calcium acclimation or exposure. Trout in the LH and HH treatments had five fold lower Zn-65 concentrations than LL treatment fish. The results of this study demonstrate that calcium reduces zinc uptake through both biological acclimation and chemical processes, and that the protective effects of calcium are approximately additive.

Bell, G. R., D. A. Higgs, et al. (1984). "The effect of dietary ascorbate, zinc, and manganese on the development of experimentally induced bacterial kidney disease in sockeye salmon (Oncorhynchus nerka)." Aquaculture **36**(4): 293-311.

Seven groups of juvenile sockeye salmon (Oncorhynchus nerka) were each reared on a different test diet to determine the influence of nutritional status on their resistance to experimentally induced bacterial kidney disease (BKD). Six test diets were formulated so that the prophylactic efficacy of two levels of vitamin C (supplied as Na L-ascorbate-2-sulfate) in combination with low levels of manganese (Mn) without zinc (Zn) and high levels of Mn with Zn supplement could be evaluated. The Oregon Moist Pellet diet served as a reference standard. Mortality curves were clearly distinct and mean survival times were significantly different ($P<0.05$) for each of three dosages of R. salmoninarum inoculated, irrespective of diet. Survival time was found to be inversely related to dietary ascorbate level at each dosage of the bacterium but only when diets contained low levels of Zn and Mn. None of the dietary variations exerted a significant effect on the production of serum antibodies against an injected bacterial vaccine. Seawater challenge and injections of Prednisolone acetate failed to initiate an outbreak of BKD in any of the uninoculated dietary stocks where, historically, naturally infected fish might have been expected.

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.

Bowen, L., I. Werner, et al. (2006). "Physiological and behavioral effects of zinc and temperature on coho salmon (Oncorhynchus kisutch)." Hydrobiologia **559**: 161-168.

Pacific salmon species including the U.S. federally endangered coho salmon (Oncorhynchus kisutch) and the U.S. federally threatened steelhead trout (Oncorhynchus mykiss) have declined at an alarming rate in the last 40 years. Two of the main causes for the decline in coastal coho populations include increases in temperature and contaminant loads in coastal watersheds. Zinc, in particular, is one of the most common contaminants in aquatic systems. Using an experimental mesocosm design, we examined physiological, biochemical, and behavioral responses of coho salmon to excess dietary zinc and increased temperatures, with the ultimate goal of relating results to wild populations of coho salmon and steelhead in the Navarro River, California. Fish were obtained from a hatchery and divided into four treatments: low water temperature-no dietary zinc, high temperature-no zinc, low temperature-zinc, and high temperature-zinc. Each treatment had four replicate tanks. Zinc concentrations in liver increased during exposure to a high zinc diet. Iron concentrations in liver increased during simultaneous exposure to high zinc diet and increased temperature, and growth

was reduced in this experimental treatment. Expression of hsp-70 was not significantly different between treatments, but showed decreasing trends with high dietary zinc and high temperature. Feeding rate increased with exposure to a high zinc diet. Comparison with steelhead trout samples from the Navarro River, California, showed levels of zinc, iron, and hsp-70 greater than those found in the experimental Coho salmon. All comparisons between the hatchery coho salmon and wild steelhead should be viewed with caution due to the differences between species, the laboratory and natural environment, and the genetic differences between wild and hatchery fish.

Bradley, R. W., C. Duquesnay, et al. (1985). "ACCLIMATION OF RAINBOW-TROUT, SALMO-GAIRDNERI RICHARDSON, TO ZINC - KINETICS AND MECHANISM OF ENHANCED TOLERANCE INDUCTION." Journal of Fish Biology **27**(4): 367-379.

Bringolf, R. B., B. A. Morris, et al. (2006). "Influence of dissolved organic matter on acute toxicity of zinc to larval fathead minnows (Pimephales promelas)." Archives of Environmental Contamination and Toxicology **51**(3): 438-444.

We conducted laboratory toxicity tests in support of the development of a biotic ligand model (BLM) to predict acute toxicity of zinc (Zn) to fathead minnows (Pimephales promelas). To test the effect of dissolved organic matter (DOM) on Zn toxicity, we exposed larval fathead minnows to Zn in water containing elevated concentrations of dissolved organic carbon (DOC) in 96-h static-renewal toxicity tests. We tested DOM isolated from four surface waters: Cypress Swamp, Delaware; Edisto River, South Carolina; Suwannee River, Georgia; and Wilmington, Delaware, wastewater treatment effluent. The DOM isolates from the Edisto River and Wilmington wastewater treatment effluent contained elevated concentrations of NaCl (20-110x control NaCl) due to the use of a Na+-exchange resin to remove Ca2+ and Mg2+ during the DOM isolation process. Therefore, we also performed Zn toxicity tests in which we added up to 20 mM NaCl to exposure solutions containing Cypress Swamp and Suwannee River DOM. A threshold concentration of 11 mg DOC/L was needed to decrease Zn toxicity, after which the 96 h Zn LC50 was positively correlated with DOC concentration. Elevated NaCl concentrations did not alter Zn toxicity in the presence of DOM. In conjunction with data from other studies with fish and invertebrates, results of this study were used to calibrate Version 2.1.1 of the Zn BLM. BLM-predicted LC50s for our exposure waters containing elevated DOM concentrations were within the range of acceptable deviation relative to the observed LC50s (i.e., 0.5-2x observed LC50s); however, BLM-predicted LC50s for our exposure waters containing < 1 mg DOC/L were 2-3x lower than the observed LC50s (i.e., the BLM over-predicted the toxicity). Therefore, the current composite-species BLM for Zn could be improved for fathead minnows if that species were modeled separately from the other species used to calibrate Version 2.1.1.

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.

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.

Chapman, G. A. (1978). "EFFECTS OF CONTINUOUS ZINC EXPOSURE ON SOCKEYE SALMON DURING ADULT-TO-SMOLT FRESHWATER RESIDENCY." Transactions of the American Fisheries Society **107**(6): 828-836.

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.

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.

Cuvin-Aralar, M. L. A. (1994). "Survival and heavy metal accumulation of two Oreochromis niloticus (L.) strains exposed to mixtures of zinc, cadmium and mercury." The Science of The Total Environment **148**(1): 31-38.

Two Nile tilapia strains of Oreochromis niloticus (L.) (Cichlidae, Teleostei) fingerlings were exposed to mixtures of zinc, cadmium and mercury. The two strains used were Chitralada or NIFI (originally from the National Inland Fisheries Institute, Thailand) and CLSU (from the Freshwater Aquaculture Center of the Central Luzon State University, The Philippines). Short-term (10 days) exposure to a metal mixture of 5 mg 1-1 zinc (Zn), 0.5 mg 1-1 cadmium (Cd) and 0.02 mg l-1 mercury (Hg) gave significantly higher survival percentage in the NIFI strain compared with the CLSU strain. Similar exposure conditions using larger and older fingerlings of the two strains also showed a slightly higher survival percentage in the NIFI strain but the difference was not significant. Prolonged exposure of the fingerlings to a lower concentration of the metal mixture (1.0 mg l-1 Zn, 0.1 mg l-1 Cd, 0.01 mg l-1 Hg) also resulted in similar survival percentages between the two strains at the end of the 60 days run. Whole body accumulation of Zn was significantly higher in CLSU than in NIFI after 14-day exposure to the low concentration metal mixture. There was no significant difference in the accumulation of Cd and Hg between the two strains. Of the three metals, Hg had the highest bioaccumulation factor (BF) which was ~900-1000, followed by Cd with 255-280 and Zn with 180-195 times the nominal concentration in the water. Concentration of Cd and Hg in fish tissues increased with exposure period while the concentration of Zn was maintained in NIFI and decreased in CLSU between the 6th and 14th day of exposure, suggesting that Zn (an essential element) accumulation maybe regulated by both strains.

Davies, P. E. (1985). "The toxicology and metabolism of chlorothalonil in fish. IV. Zinc coexposure and the significance of metallothionein in detoxication in Salmo gairdneri." Aquatic Toxicology **7**(4): 301-306.

Rainbow trout, Salmo gairdneri, were subjected to various regimes of exposure to zinc (0.36 mg/l) and 14C-chlorothalonil (14C-TCIN, 10 [mu]g/l). Gel column chromatography of hepatic cytosol after exposure showed no binding of 14C-TCIN to metallothionein proteins with or without pre-exposure or with coexposure to zinc. 14C-TCIN did not induce the production of metallothionein with or without 96 h preexposure to 0.36 mg/l zinc, whereas induction did occur with zinc coexposure over 156 h. 14C-TCIN exposure did not affect cytosolic zinc levels, but exposure to zinc did reduce the level of cytosolic 14C-TCIN over the observed protein molecular weight range. It appears that metallothionein does not play a significant role in TCIN detoxication at this sublethal exposure level.

De Schamphelaere, K. A. C. and C. R. Janssen (2004). "Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (Oncorhynchus mykiss): Comparison with other fish species and development of a biotic ligand model." Environmental Science &

Technology **38**(23): 6201-6209.

In this study, the effects of modifying Ca (0.2-4 mM), Mg (0.05-3 mM), Na (0.75-5 mM), and pH (5.5-7.5) on the chronic toxicity of zinc to juvenile rainbow trout (Oncorhynchus mykiss) were investigated using standard 30-d assays in which survival and growth were monitored. Survival was observed to be a more sensitive end point than growth, and mortality mainly occurred during the initial stages of the exposure. This suggested that the mode of action of zinc toxicity was mainly of an acute nature. A review and analysis of existing literature demonstrated similar results for most other fish species investigated. Overall, up to a 30-fold variation of zinc toxicity was observed, as indicated by no observed effect concentrations varying between 32.7 and 974 mug of Zn L-1. Increased concentrations of Ca2+, Mg2+, Na+, and H+ (within the tested ranges) resulted in a reduction of chronic zinc toxicity by a factor of 12, 3, >2, and 2, respectively. This suggests the major importance of Ca competing with zinc and protecting against zinc toxicity, which seems to be a ubiquitous concept in fish species (and probably also invertebrate). On the basis of the toxicity data obtained, a chronic biotic ligand model (BLM) was developed that takes into account both chemical speciation of zinc and competition between zinc and the above-mentioned cations. The developed model was able to predict chronic effect concentrations with an error of less than a factor of 2 in most cases. Hence, it was concluded that the chronic Zn BLM can reduce toxicity variability due to bioavailability to a considerable extent and that the BLM can become an important tool in criteria setting and risk assessment practice of zinc and zinc substances.

De Schamphelaere, K. A. C., S. Lofts, et al. (2005). "Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface waters." Environmental Toxicology and Chemistry **24**(5): 1190-1197.

Bioavailability models predicting acute and/or chronic zinc toxicity to a green alga (Pseudokirchneriella subcapitata), a crustacean (Daphnia magna), and a fish (Oncorhynchus mykiss) were evaluated in a series of experiments with spiked natural surface waters. The eight selected freshwater samples had varying levels of bioavailability modifying parameters: pH (5.7-8.4), dissolved organic carbon (DOC, 2.48-22.9 mg/L), Ca (1.5-80 mg/L), Mg (0.79-18 mg/L), and Na (3.8-120 mg/L). In those waters, chronic zinc toxicity (expressed as 10% effective concentrations [EC10]) varied up to 20-fold for the alga (72-h EC10 from 27.3 to 563 mu g Zn/L), and approximately sixfold for the crustacean (2 1 -d EC 10 from 59.2 to 387 jig Zn/L), and fivefold for the fish (30-d LC 10, lethal concentration for 10% of the organisms, from 185 to 902 mu g Zn/L). For P. subcapitata a refined bioavailability model was developed by linking an empirical equation, which predicts toxicity expressed as free Zn2+ activity as a function of pH, to the geochemical speciation model WHAM/Model V. This model and previously developed acute and/or chronic biotic ligand models for D. magna and O. mykiss generally predicted most effect concentrations by an error of less than a factor of two. In waters with pH > 8, however, chronic toxicity to D. magna was underestimated by a factor 3 to 4. Based on the results of this validation exercise and earlier research, we determined applicability ranges for pH (6-8) and Ca (5-

160 mg/L) in which all three developed models are valid. Within these ranges, all three models may be considered useful tools for taking into account bioavailability in regulatory assessments of zinc.

Dean, R. J., T. M. Shimmield, et al. (2007). "Copper, zinc and cadmium in marine cage fish farm sediments: An extensive survey." Environmental Pollution **145**(1): 84-95.
The diet of cage-farmed Atlantic salmon contains a range of trace metals, some of which have toxic properties, e.g. zinc, copper and cadmium. A survey of metal concentrations (ICP-MS analysis) in surface sediments of ca. 70 stations was carried out in both May and December 2000 around a Scottish fish farm. Additionally, at 13 stations on 2 orthogonal transects centered on the farm, sediments were analysed at 1 cm intervals to 8 cm depth. Maximum concentrations in surface sediments were 921, 805 and 3.5 [mu]g g-1 for Zn, Cu and Cd, respectively, and were found at stations near the fish farm. The calculated losses from the farm (feed input minus fish output) were 87.0%, 4.3% and 14.0% of the background-corrected inventories for Zn, Cu and Cd, respectively, indicating that for Cu and Cd at least, the feed is not the only source.

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.

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.

Eaton, J. G. (1973). "Chronic toxicity of a copper, cadmium and zinc mixture to the fathead minnow (Pimephales promelas rafinesque)." Water Research **7**(11): 1723-1736.

Fathead minnows were exposed to a series or concentrations of a copper, cadmium and zinc mixture during a 12.5 month chronic test in water of 200 mg 1-1 total hardness. The metal concentrations in the mixture were selected on the basis of results obtained during previous chronic exposures to each of the metals individually in the same water. Strict summation of the chronic toxicities of the metals was not indicated when they were tested in combination. Toxic effects of the mixture attributable to copper appeared to be increased, but that attributable to cadmium was reduced. The effects thought to be due to zinc were similar in degree to those observed in the single chronic exposure. Summation of effects resulting from a mixture containing about the same proportions of copper, cadmium and zinc occurred at a much higher, acutely lethal concentration. A lethal threshold was attained in the mixture when each metal was present at a concentration of 0.4 or less of its individual lethal threshold.

Eddy, F. B. and C. Talbot (1985). "Sodium balance in eggs and dechorionated embryos of the atlantic salmon Salmo salar L. exposed to zinc, aluminium and acid waters." Comparative Biochemistry and Physiology Part C: Comparative Pharmacology **81**(2): 259-266.

1. 1. Ionic regulation of eyed eggs of Atlantic salmon was studied using intact eggs and embryos from which the chorion and perivitelline fluid had been removed, a preparation which remained in good condition for at least 2 weeks. 2. 2. Both intact eggs and embryos in freshwater showed similar Na4 influx rates (0.0064 and 0.01[mu]Mg -1 hr-1, respectively) but Na+ efflux rates (0.0024 and 0.0064[mu]M g-1 hr-1, respectively) were greater in embryos, suggesting that the perivitelline fluid which is known to bind cations has an important function in preventing Na+ loss from the yolk and embryo. 3. 3. Over 90% of the egg Na+ is located in the yolk and embryo but only about 10% is exchangeable while the chorion contains about 8% Na+ which is non-exchangeable. 4. 4. Both eggs and embryos in acid water at pH 4, in Zn2+ 5 mM 1-1 and in aluminium 1 mM l-1 showed greatly reduced Na+ uptake but eggs in 10[mu]M 1-1 aluminium or 100[mu]M 1-1 Zn2+ showed normal Na+ balance while embryos were normal in 10¬M 1-1 aluminium but showed reduced Na+ uptake in 100[mu]M 1-1 Zn2+. 5. 5. It is concluded that the chorion and perivitelline fluid have a capacity to protect the embryo from metal ions and acid water.

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.

Farmer, G. J., D. Ashfield, et al. (1979). "Effects of zinc on juvenile Atlantic salmon Salmo salar: Acute toxicity, food intake, growth and bioaccumulation." Environmental Pollution (1970) **19**(2): 103-117.

The effects of long-term zinc exposure on juvenile salmon were assessed by

measuring changes in their voluntary food intake, growth and rate of zinc accumulation. The food intake of salmon surviving concentrations as great as the 21-day LC50 was initially reduced but returned to amounts observed for control fish within 10 to 20 days. After 45 days' exposure, the food intake of these individuals was greater than that of the controls. Growth rates of salmon exposed to zinc (maximum concentration estimated to cause mortality <=50% during an acute bioassay) and offered rations of 2.0 or 3.5% dry body weight per day were not reduced during a three-month period. However, caloric content of salmon at the higher concentrations showed a small decrease during the experimental period. Zinc concentration of salmon feeding to satiation once daily and exposed to zinc increased during the first two months of exposure before reaching a maximum, while zinc concentration of salmon offered sub-excess rations (2.0 and 3.5% per day) increased linearly during a three-month period.

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.

Fletcher, G. L. and M. J. King (1978). "Copper, zinc, calcium, magnesium and phosphate in the gonads and livers of Sockeye salmon (Oncorhynchus nerka) during spawning migration." Comparative Biochemistry and Physiology Part A: Physiology **60**(2): 127-130.

1. 1. Concentrations and total amounts of Cu2+. Zn2+, Ca2+, Mg2+ and PO3-4 were measured in gonads and livers of Sockeye salmon during spawning migration. 2. 2. Total ovarian Zn2+, Ca2+, Mg2+ and PO3-4 increased during migration, while total testicular Zn2 + declined. 3. 3. Salmon do not eat during migration; therefore the essential elements incorporated by the ovary must have been absorbed from the water or released from storage within the body. 4. 4. It is hypothesized that almost all of the ovarian Zn2+ and most of the Ca2+, Mg2+ and PO3-4 was obtained from body stores other than the liver.

Franco, J. L., T. Posser, et al. (2008). "Biochemical alterations in juvenile carp (Cyprinus carpio) exposed to zinc: Glutathione reductase as a target." Marine Environmental Research **66**(1): 88-89.

The aim ofthis study was to investigate biochemical changes injuvenile carp (Cyprinus carpio) exposed to zinc chloride (10, 30 and 100 VM) for a period of 48 h. Zinc exposure caused a concentration-dependent reduction in glutathione reductase'(GR) activity in gills, liver and brain. Gill glutathione S-transferase (GST) was reduced when animals were exposed to the highest concentration of 100 pM zinc. The phosphorylation of p381APK increased in the brain of fish exposed to zinc 100 liM, while phosphorylation of the extracellular signal-regulated protein kinase 112 (ERKI/2) and c-Jun N-teriminal protein kinase 1/2 UNK1 /2) remained unchanged. Expression of proteins HSP60 and HSP70 were not affected by zinc exposure. Considering the significant concentration-dependent inhibition of GR in all tissues analyzed, this enzyme could be a potential biomarker of exposure to zinc, which has to be confirmed. (C) 2008

Gheorghiu, C., J. Cable, et al. (2007). "Effects of waterborne zinc on reproduction, survival and morphometrics of Gyrodactylus turnbulli (Monogenea) on guppies (Poecilia reticulata)." International Journal for Parasitology **37**(3-4): 375-381.

Recent reviews indicate that pollutants in the surrounding macroenvironment directly influence the population dynamics, distribution and dispersal of fish ectoparasites, often leading to increased parasitism. The aim of the current study was to explore the effects of sublethal concentrations of waterborne zinc (up to 240 mu g Zn/L) on survival, reproduction and morphometrics of Gyrodactylus turnbulli, a viviparous monogenean infecting the skin and fins of the guppy, Poecilia reticulata. Parasite survival and reproduction on the fish were recorded daily for individual parasites maintained in isolated containers. Both survival and reproduction were reduced in 30 and 120 mu g Zn/L, compared with 0, 15, and 60 mu g Zn/L indicating direct toxic effects of Zn on the parasite. However, as generation time was unaffected by Zn, we attribute the reduced reproduction to the shorter lifespan. Parasite survival off the fish was monitored hourly. Average lifespan of the detached parasites decreased linearly from 19.5 h in 0 mu g Zn/L to 17.3 h in 240 mu g Zn/L, further supporting the direct toxic effect of Zn to the parasite. In addition, temporal dynamics of parasite morphometrics were monitored from mini-epidemics sampled after 1, 5, 10, and 15 days exposure to various Zn concentrations. All morphological parameters decreased significantly in response both to concentration and duration of exposure to waterborne Zn. Together these data clearly indicate that concentrations as low as 120 mu g Zn/L are directly toxic to G. turnbulli. (c) 2006 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

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.

Glover, C. N., N. R. Bury, et al. (2004). "Intestinal zinc uptake in freshwater rainbow trout: evidence for apical pathways associated with potassium efflux and modified by calcium." Biochimica et Biophysica Acta (BBA) - Biomembranes **1663**(1-2): 214-221.

Understanding the mechanisms of intestinal zinc uptake in fish is of considerable interest from both nutritional and toxicological perspectives. In this study, properties of zinc transport across the apical membrane of freshwater rainbow trout intestinal epithelia were examined using right-side-out brush border membrane vesicles (BBMV's). Extravesicular calcium was found to have complex actions on zinc uptake. At a low zinc concentration of 1 [mu]M, calcium (0.1-2 mM) significantly stimulated zinc uptake. In contrast, calcium inhibited zinc uptake at higher zinc levels (100 [mu]M). Lanthanum and cadmium in the external medium did not block zinc uptake, suggesting that interactions between zinc and calcium were not exerted at a calcium channel. Copper also failed to exercise any inhibitory action. Zinc association with the BBMV's was enhanced by an outward potassium gradient. This stimulatory effect was only present at a zinc concentration of 100 [mu]M. The potassium channel blocker, tetraethylammonium chloride inhibited zinc uptake at this relatively high zinc concentration, suggesting the presence of a low affinity zinc uptake pathway linked to potassium efflux. The present study provides evidence that the mechanism of intestinal zinc uptake in rainbow trout is pharmacologically very different from that of the piscine gill and the mammalian intestine.

Glover, C. N. and C. Hogstrand (2003). "Effects of dissolved metals and other hydrominerals on in vivo intestinal zinc uptake in freshwater rainbow trout." Aquatic Toxicology **62**(4): 281-293.

For aquatic organisms, zinc is both an essential nutrient and an environmental contaminant. The intestine is potentially the most important route of zinc absorption, yet little is known regarding this uptake pathway for zinc in fish. A recently developed in vivo perfusion system was used to investigate the effect of luminal composition upon intestinal zinc uptake in freshwater rainbow trout (Oncorhynchus mykiss). Perfusate cadmium and copper had specific, yet distinct, antagonistic effects upon lumen to tissue zinc movement. Copper significantly reduced the proportion of zinc taken up from the perfusate, and concomitantly limited the passage of zinc into the circulation and beyond. Conversely, cadmium decreased subepithelial zinc accumulation, with rates falling to 29 nmol g-1 h-1 from the control (zinc alone) values of 53 nmol g-1 h-1. Calcium had a similar action to copper, also reducing post-intestinal zinc accumulation from 0.06 to 0.02 nmol g-1 h-1, an effect attributed to interactions between calcium and the zinc uptake pathway. In addition to these effects, luminal composition also had a marked influence upon epithelial response to

zinc. Calcium, copper and magnesium all greatly reduced zinc-induced mucus secretion. Cadmium, a toxic metal, significantly increased mucus secretion. It is proposed that these modifications were related to the essentiality of each element, and their potential mechanisms of uptake. Despite changes at the epithelium, the post-epithelial accumulation of zinc was dependent mainly upon the nature of the competing cation. Intestinal saline ion substitution experiments suggested a potential link of potassium ion efflux to zinc uptake. The effect of pH buffering of luminal solutions was also investigated.

Hammock, D., C. C. Huang, et al. (2003). "The effect of humic acid on the uptake of mercury(II), cadmium(II), and zinc(II) by Chinook salmon (Oncorhynchus tshawytscha) eggs." Archives of Environmental Contamination and Toxicology **44**(1): 83-88.

The Chinook salmon (Oncorhynchus tshawytscha) is endangered or threatened in several of its ranges. The uptake of metals by Chinook salmon eggs and how humic acid (HA) affects the uptake is a subject of interest. Humic acid (0, 0.001, 0.01, and 0.05g/l) reduces the uptake of the metal ions Hg(II), Cd(II), and Zn(II), (1.0 muM) by eggs. HA is more effective in reducing the uptake of Hg than that of Cd or Zn. At [HA] = 0.001 g/L Hg uptake is reduced by 44% compared to no HA, while Cd and Zn uptakes are slightly or not reduced. Once the metals are taken up by the eggs, Hg migrates more slowly from the chorion to the yolk than either Zn or Cd. In experiments in which the metal contents of the chorion and yolk were measured at up to 24 h and five days after uptake, the order of migration was Cd > Zn > Hg. This observation is important when discussing the effects of metals on biological processes in the yolk because when Hg is taken up by eggs, a smaller percentage reaches the yolk than does Cd and Zn.

Hansen, J. A., P. G. Welsh, et al. (2002). "Relative Sensitivity of Bull Trout (Salvelinus Confluentus) and Rainbow Trout (Oncorhynchus Mykiss) to Acute Exposures of Cadmium and Zinc." Environmental Toxicology and Chemistry **21**(1): 67-75.

Bull trout (Salvelinus confluentus) were recently listed as threatened in the United States under the federal Endangered Species Act. Present and historical habitat of this species includes waterways that have been impacted by metals released from mining and mineral processing activities. We conducted paired bioassays with bull trout and rainbow trout (Oncorhynchus mykiss) to examine the relative sensitivity of each species to Cd and Zn independently and as a mixture. A total of 15 pairs of acute toxicity bioassays were completed to evaluate the effects of different water hardness (30 or 90 mg/L as CaCO3), pH (6.5 or 7.5), and temperature (8 or 12 degreesC) on Cd and Zn toxicity. For both species, the acute toxicity of both Cd and Zn was greater than previously observed in laboratory studies. Bull trout were about twice as tolerant of Cd and about 50% more tolerant of Zn than were rainbow trout. Higher hardness and lower pH water produced lower toxicity and slower rates of toxicity in both species. Elevated temperature significantly increased the sensitivity of bull trout to Zn but decreased the sensitivity (not significantly) of rainbow trout to Zn. At a hardness of 30 mg/L, the toxicity values (i.e., median lethal concentration; 120-h LC50) for both species were lower than the current U.S. national water quality criteria for

protection of aquatic life, indicating that current national criteria may not be protective of sensitive salmonids-including the threatened bull trout-in low calcium waters.

Harper, D. D., A. M. Farag, et al. (2008). "Effects of acclimation on the toxicity of stream water contaminated with zinc and cadmium to juvenile cutthroat trout." Archives of Environmental Contamination and Toxicology **54**(4): 697-704.

We investigated the influence of acclimation on results of in situ bioassays with cutthroat trout in metal-contaminated streams. Cutthroat trout (Oncorhynchus clarki) were held for 21 days (1) in live containers at a reference or "clean" site having dissolved metals near detection limits (0.01 mu g/L cadmium [Cd] and 2.8 mu g/L zinc [Zn]; hardness 32 mg/L as CaCO3) and (2) at a site in a mining-impacted watershed having moderately increased metals (0.07 mu g/L Cd and 38 to 40 mu g/L Zn; hardness 50 mg/L as CaCO3). The 96-hour survival of each treatment group was then tested in situ at five sites from September 5 to 9, 2002, and each group exhibited a range of metal concentrations (0.44 to 39 mu g/L arsenic [As], 0.01 to 2.2 mu g/L Cd, and 0.49 to 856 mu g/L Zn). Survival was 100% at three sites for both treatments. However, a higher percentage of metal-acclimated fish survived at the site with the second highest concentrations of Cd and Zn (0.90 and 238 mu g/L, respectively) compared with fish acclimated at the reference site (100% vs. 55%, respectively). Survival was 65% for acclimated fish and 0% for metal-naive fish at the site with the largest metal concentrations (2.2 mu g/L Cd and 856 mu g/L Zn). Water collected from the site with the largest concentrations of dissolved metals (on October 30, 2002) was used in a laboratory serial dilution to determine 96-hour LC50 values. The 96-hour LC50 estimates of naive fish during the in situ and laboratory experiments were similar (0.60 mu g Cd/L and 226 mu g Zn/L for in situ and 0.64 mu g Cd/L and 201 mu g Zn/L for laboratory serial dilutions). However, mortality of naive cutthroat trout tested under laboratory conditions was more rapid in dilutions of 100%, 75%, and 38% site water than in situ experiments.

Heier, L. S., I. B. Lien, et al. (2009). "Speciation of lead, copper, zinc and antimony in water draining a shooting range-Time dependant metal accumulation and biomarker responses in brown trout (Salmo trutta L.)." Science of the Total Environment **407**(13): 4047-4055.

The speciation of Pb, Cu, Zn and Sb in a shooting range run-off stream were studied during a period of 23 days. In addition, metal accumulation in gills and liver, red blood cell ALA-D activity, hepatic metallothionine (Cd/Zn-MT) and oxidative stress index (GSSG/ tGSH levels) in brown trout (Salmo trutta L.) exposed to the stream were investigated. Fish, contained in cages, were exposed and sampled after 0, 2, 4, 7, 9, 11 and 23 days of exposure. Trace metals in the water were fractionated in situ according to size (nominal molecular mass) and charge properties. During the experimental period an episode with higher runoff occurred resulting in increased levels of metals in the stream. Pb and Cu were mainly found as high molecular mass species, while Zn and Sb were mostly present as low molecular mass species. Pb, Cu and Sb

accumulated on gills, in addition to Al origination from natural sources in the catchment. Pb, Cu and Sb were also detected at elevated concentration in the liver. Blood glucose and plasma Na and Cl levels were significantly altered during the exposure period, and are attributed to elevated concentrations of Pb, Cu and Al. A significant suppression of ALA-D was detected after 11 days. Significant differences were detected in Cd/Zn-MT and oxidative stress (tGSH/GSSG) responses at Day 4. For Pb the results show a clear link between the HMM (high molecular mass) positively charged Ph species, followed by accumulation on gills and liver and a suppression in ALA-D. Thus, high flow episodes can remobilise metals from the catchment, inducing stress to aquatic organisms. (C) 2009 Elsevier B.V. All rights reserved.

Heijerick, D. G., K. A. C. De Schamphelaere, et al. (2002). "Predicting acute zinc toxicity for Daphnia magna as a function of key water chemistry characteristics: Development and validation of a biotic ligand model." Environmental Toxicology and Chemistry **21**(6): 1309-1315.

The individual effect of different major cations (Ca2+, Mg2+, Na+, K+, and H+) on the acute toxicity of zinc to the waterflea Daphnia magna was investigated. The 48-h median effective concentration (EC50) in the baseline test medium (i.e., a standard medium with very low ion concentrations) was about 6 muM (Zn2+). An increase of Ca2+ (from 0.25 mM to 3 mM), Mg2+ (from 0.25 mM to 2 mM), and Na+ activity (from 0.077 mM to 13 mM) reduced zinc toxicity by a factor of 6.3, 2.1, and 3.1, respectively. No further toxicity reduction was observed when Ca2+ and Mg2+ activities exceeded 3.0 and 2.0 mM, respectively. Both K+ and H+ did not significantly alter zinc toxicity (expressed as Zn2+ activity). From these data, conditional stability constants for Ca2+ (log K = 3.24), Mg2+ (log K = 2.97), Na+ (log K = 2.16), and Zn2+ (log K = 5.31) were derived and incorporated into a biotic ligand model (BLM) predicting acute zinc toxicity to D. magna in surface waters with different water quality characteristics. Validation of the developed BLM using 17 media with different pH, hardness, and dissolved organic carbon (DOC) content resulted in a significant correlation coefficient (R-2 = 0.76) between predicted and observed 48-h EC50. Eighty-eight percent of the predictions were within a factor of 1.3 of the observed 48-h EC50.

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-&.

Hilmy, A. M., N. A. El Domiaty, et al. (1987). "The toxicity to Clarias Lazera of copper and zinc applied jointly." Comparative Biochemistry and Physiology Part C: Comparative Pharmacology **87**(2): 309-314.

1. The acute toxicity to juvenile Clarias lazera of a mixture of copper and zinc over a 96 hr exposure period was determined. Fish were exposed to the summation of half the 96 hr tlm value of each toxicant. 2. Percentage survival was much reduced indicating that the metals potentiate each others lethal action. 3. Comparison between metal residues in fish exposed to copper and zinc or to

their mixture showed that the uptake of one metal was decreased by the presence of the other. 4. Toxic effects of the mixture on the physiological parameters studied were mainly attributable to copper, indicating that the presence of zinc did not influence the mode of action of copper.

Holcombe, G. W., D. A. Benoit, et al. (1979). "LONG-TERM EFFECTS OF ZINC EXPOSURES ON BROOK TROUT (SALVELINUS-FONTINALIS)." Transactions of the American Fisheries Society **108**(1): 76-87.

Hughes, G. M. and R. J. Adeney (1977). "The effects of zinc on the cardiac and ventilatory rhythms of rainbow trout (Salmo Gairdneri, Richardson) and their responses to environmental hypoxia." Water Research **11**(12): 1069-1077.

Recordings were made of the cardiac and ventilatory rhythms of rainbow trout subjected to solutions of 40 and 10 ppm zinc in an experimental circulation. The higher concentration produces clearly-defined increases in ventilatory and coughing frequency and a decrease in heart rate; the percentage coupling between the two rhythms usually increased. During exposure to 10 ppm added zinc the effects are not so readily discernible but are comparable in type. The addition of 10 ppm zinc has a significant effect on the responses of rainbow trout to hypoxia before and after this treatment. It is concluded that zinc interferes with some of the mechanisms involved in the uptake of oxygen at the gills and consequently can significantly affect the ability of fish to respond to the additional stress of oxygen lack in its environment.

Ibrahim, A., B. M. MacKinnon, et al. (2000). "The influence of sub-lethal levels of zinc on smoltifying Atlantic salmon Salmo salar and on their subsequent susceptibility to infection with Lepeophtheirus salmonis." Contributions to Zoology **69**(1-2): 119-128.

Smoltifying Atlantic salmon were treated for 6 weeks in freshwater with 0. 200. and 400 ppb zinc (as Zn SO4). After 6 weeks salmon were transferred to a salt water seapen and exposed to infection with Lepeophtheirus salmonis for 14 weeks. Zinc treatment resulted in some physiological changes consistent with increased stress, such as decreased leukocrit values, and increased plasma cortisol levels. Plasma glucose levels were significantly elevated in fish previously treated with zinc, but this was only evident after fish had been in a seapen, 14 weeks subsequent to treatment. Improved conversion of tetraiodothyronine to triiodothyronine, as well as no changes in interrenal cell nuclear diameters suggested that overall stress effects were low. Fish exposed to 200 ppb and 400 ppb zinc showed gill pathology. infection intensity with L. salmonis was significantly higher on salmon previously exposed to 400 ppb zinc. This research suggests that for smoltifying Atlantic salmon, the No Observed Pathological effect (NOPE) level for exposure for ZNSO(4) may be around 150 ppb.

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.

Kargin, F. and H. Y. Cogun (1999). "Metal interactions during accumulation and elimination of zinc and cadmium in tissues of the freshwater fish Tilapia nilotica." Bulletin of Environmental Contamination and Toxicology **63**(4): 511-519.

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.

Krause, P. R. and R. N. Bray (1994). "Transport of cadmium and zinc to rocky reef communities in feces of the blacksmith (Chromis punctipinnis), a planktivorous fish." Marine Environmental Research **38**(1): 33-42.

The blacksmith (Chromis punctipinnis), an abundant planktivorous reef fish off southern California, releases fecal material as it forages in the water column during the day and shelters in reefs at night. This behavior results in direct transportation of cadmium and zinc to reef communities. Cadmium and zinc concentrations, measured in fish feces after digestion in weak hydrochloric acid to better assess metal levels potentially available to detritivores, averaged 24·2 [mu]g cadmium and 368 [mu]g zinc g-1 dry weight of feces. Concentrations of both metals varied significantly among the five sampling dates. Fresh feces spiked with 109Cd and 65Zn adsorbed additional metals for 9 and 6 h, respectively, but the increase was negligible (<0·1%). The total amount of weak-acid-leachable cadmium and zinc egested by sheltering blacksmiths is approximately 4·4 [mu]g and 66·8 [mu]g m-2 night-1, respectively, and may represent more cadmium and almost as much zinc as is transported by the passive settlement of particulate material from the water column. This transport mechanism between planktonic and benthic communities via feces of reef fishes is probably widespread in temperate and tropical seas.

Lange, A., O. Ausseil, et al. (2002). "Alterations of tissue glutathione levels and metallothionein mRNA in rainbow trout during single and combined exposure to cadmium and zinc." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **131**(3): 231-243.

The objective of this study was to assess the effects of Cd and Zn exposure of rainbow trout (Oncorhynchus mykiss) on (a) hepatic glutathione (GSH) levels; and (b) hepatic and branchial metallothionein (MT) mRNA expression. Juvenile

rainbow trout were exposed to waterborne Cd (nominal concentrations: 1.5 or 10 [mu]g Cd l-1), Zn (150 or 1000 [mu]g Zn l-1) or Cd/Zn mixtures (1.5 [mu]g Cd l-1 with 200 [mu]g Zn l-1 or 10 [mu]g Cd l-1 with 1000 [mu]g Zn l-1). After 14 and 28 days of treatment, hepatic concentrations of total glutathione, oxidized glutathione (GSSG) and cysteine were determined by means of fluorometric high performance liquid chromatography (HPLC). Branchial and hepatic expression of MT mRNA was measured by means of semi-quantitative RT-PCR. Exposure of trout to Zn did not result in significantly elevated tissue levels of Zn, whereas Cd accumulation factors changed significantly with time and concentration. Despite of the absence of Zn accumulation, hepatic GSH but not MT mRNA levels were significantly altered in Zn-exposed fish. Cd, on the contrary, affected mainly the MT response but not GSH. Also tissue specific differences in the regulation of the two thiol pools were expressed. The thiol response after exposure to metal mixtures could not be explained by simple addition of the effects of the individual metals. The results indicate that cellular thiol pools show different reaction patterns with respect to specific metals and metal mixtures. Under conditions of long-term, low dose metal exposure, the function of GSH appears to go beyond that of a transitory, first line defense.

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. (C) 2007 The Authors.

Lourdes, M. and A. Cuvinaralar (1994). "SURVIVAL AND HEAVY-METAL ACCUMULATION OF 2 OREOCHROMIS-NILOTICUS (L) STRAINS EXPOSED TO MIXTURES OF ZINC, CADMIUM AND MERCURY." Science of the Total Environment **148**(1): 31-38.

Two Nile tilapia strains of Oreochromis niloticus (L.) (Cichlidae, Teleostei) fingerlings were exposed to mixtures of zinc, cadmium and mercury. The two strains used were Chitralada or NIFI (originally from the National Inland Fisheries Institute, Thailand) and CLSU (from the Freshwater Aquaculture Center of the Central Luzon State University, The Philippines). Short-term (10 days) exposure to a metal mixture of 5 mg l-1 zinc (Zn), 0.5 mg l-1 cadmium (Cd) and 0.02 mg l-1 mercury (Hg) gave significantly higher survival percentage in the NIFI strain compared with the CLSU strain. Similar exposure conditions using larger and older fingerlings of the two strains also showed a slightly higher survival percentage in the NIFI strain but the difference was not significant. Prolonged exposure of the fingerlings to a lower concentration of the metal mixture (1.0 mg l-1 Zn, 0.1 mg l-1 Cd, 0.01 mg l-1 Hg) also resulted in similar survival percentages between the two strains at the end of the 60 days run. Whole body accumulation of Zn was significantly higher in CLSU than in NIFI after 14-day exposure to the low concentration metal mixture. There was no significant difference in the accumulation of Cd and Hg between the two strains. Of the three metals, Hg had the highest bioaccumulation factor (BF) which was approximately 900-1000, followed by Cd with 255-280 and Zn with 180-195 times the nominal concentration in the water. Concentration of Cd and Hg in fish tissues increased with exposure period while the concentration of Zn was maintained in NIFI and decreased in CLSU between the 6th and 14th day of exposure, suggesting that Zn (an essential element) accumulation maybe regulated by both strains.

Maage, A. and K. Julshamn (1993). "Assessment of zinc status in juvenile Atlantic salmon (Salmo salar) by measurement of whole body and tissue levels of zinc." Aquaculture **117**(1-2): 179-191.

Duplicate groups of 40 g Atlantic salmon were fed dry pelleted diets based on cod muscle meal as a protein source for 8 weeks. The basal diet contained 17 mg Zn/kg to which 0, 10, 20, 40 or 80 mg Zn/kg diet, respectively, were added as $ZnSO_4 \cdot 7H_2O$. After 8 weeks, 15 fish from each dietary treatment were marked and then they were fed the basal diet for another 4 weeks. Fish were sampled after 2, 4, 8 and 12 weeks. Blood samples were withdrawn and serum was analyzed for zinc concentration and alkaline phosphatase (AP) activity. Liver, kidney, vertebrae and intestine were removed from ten fish in each dietary treatment. A further ten fish were used for whole body mineral analyses. There were no significant differences in growth due to different dietary zinc

concentrations and this, together with data on alkaline phosphatase and iron levels, showed that the basal diets contained sufficient zinc to prevent overt zinc deficiency symptoms. Zinc concentrations in the whole body and tissues, however, were significantly influenced by dietary zinc level. On the basis of the dietary zinc concentration required to maintain whole body zinc and serum zinc concentrations within the normal range, it was concluded that the dietary zinc requirement of juvenile Atlantic salmon is between 37 and 67 mg Zn/kg dry diet under our test conditions. Hence, until more data are available, juvenile Atlantic salmon diets based almost entirely on marine fish protein should contain > 67 mg Zn/kg dry diet.

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.

Mebane, C. A., D. P. Hennessy, et al. (2008). "Developing acute-to-chronic toxicity ratios for lead, cadmium, and zinc using rainbow trout, a mayfly, and a midge." Water Air and Soil Pollution **188**(1-4): 41-66.

In order to estimate acute-to-chronic toxicity ratios (ACRs) relevant to a coldwater stream community, we exposed rainbow trout (Oncorhynchus mykiss) to cadmium (Cd), lead (Pb), and zinc (Zn) in 96-h acute and 60+ day early-life stage (ELS) exposures. We also tested the acute and sublethal responses of a mayfly (Baetis tricaudatus) and a midge (Chironomus dilutus, formerly C. tentans) with Pb. We examine the statistical interpretation of test endpoints and the acute-to-chronic ratio concept. Increasing the number of control replicates by 2 to 3x decreased the minimum detectable differences by almost half. Pb ACR estimates mostly increased with increasing acute resistance of the organisms (rainbow trout ACRs <approximate to mayfly < Chironomus). The choice of test endpoint and statistical analysis influenced ACR estimates by up to a factor of four. When calculated using the geometric means of the no- and lowest-observed

effect concentrations, ACRs with rainbow trout and Cd were 0.6 and 0.95; Zn about 1.0; and for Pb 3.3 and 11. The comparable Pb ACRs for the mayfly and Chironomus were 5.2 and 51 respectively. Our rainbow trout ACRs with Pb were about 5-20x lower than earlier reports with salmonids. We suggest discounting previous ACR results that used larger and older fish in their acute tests.

Memmert, U. (1987). "Bioaccumulation of zinc in two freshwater organisms (Daphnia magna, crustacea and Brachydanio, rerio, pisces)." Water Research **21**(1): 99-106.

Daphnia magna and Brachydanio rerio are important test organisms in toxicity tests. The bioaccumulation of zinc in these species was investigated in two semistatic experiments in synthetic freshwater with a zinc concentration of 250 [mu]g 1-1. Fishes were fed with polluted or unpolluted Daphnia magna to determine the significance of zinc accumulation from contaminated natural food. Daphnia magna accumulates zinc to a high extent within days. Uptake from food particles substantially contributes to the zinc accumulation in filter-feeding Daphnia; their zinc content strongly depends on the total but not on the dissolved zinc concentration in water. Zinc concentration of Brachydanio rerio increases only to a small extent during the 5 weeks accumulation time. They accumulate no additional zinc from the food source. In unpolluted and polluted Brachydanio a significant negative correlation exists between whole body zinc concentration and body dry weight.

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.

Ní Shúilleabháin, S., C. Mothersill, et al. (2004). "In vitro cytotoxicity testing of three zinc

metal salts using established fish cell lines." Toxicology in Vitro **18**(3): 365-376.

The utilisation of fish cell lines has proven to be a valuable, rapid and cost-effective tool in the ecotoxicological assessment of chemicals and environmental samples. The main objective of this study was to investigate the value of multiple endpoint measurements in evaluating the cytotoxicity of three divalent zinc salts in three established fish cell lines (EPC, CHSE and RTG-2) and the potential for their employment as effective screening tools for zinc contaminated environmental samples. A significant stimulatory effect was detected with the neutral red assay in EPC and RTG-2 cells exposed to the lower doses of some zinc compounds. Significant (p[less-than-or-equals, slant]0.01) lactate dehydrogenase release was detectable only with the highest exposure concentration of ZnCl2. Toxicity ranking based on IC50 values calculated from the neutral red and coomassie blue assay data found that in general, ZnCl2 was the most cytotoxic metal compound to the cell lines employed. Differential cell sensitivities were observed to be dependant on the particular compound tested and the endpoint employed. It was found that the use of light microscopy in the identification of cell morphological changes was a valuable adjunct in verifying the results of colorimetric tests. In conclusion, careful consideration should be given to study design and statistics applied and use of a battery style approach is recommended for toxicological screening studies.

Ní Shúilleabháin, S., C. Mothersill, et al. (2006). "Cellular responses in primary epidermal cultures from rainbow trout exposed to zinc chloride." Ecotoxicology and Environmental Safety **65**(3): 332-341.

In this study, we examined the effects of zinc chloride (ZnCl2) (0-200 mg/L) on primary epidermal cultures from Oncorhynchus mykiss. Increases in the rate and amount of mucus released were detected post-exposure, as was a dose-dependent increase in the synthesis of acidic glycoproteins. The cytotoxicity of ZnCl2 to the cultures was significantly increased (P[less-than-or-equals, slant]0.05) when exposures were conducted in serum-free medium as opposed to medium containing serum. Significant increases in the levels of apoptosis and necrosis were observed with increasing exposure concentration, but these were seen to decrease over time. The in vitro cytological and pathological changes observed in this study were found to be in accordance with previously published in vivo studies on the effects of heavy metals on the integument. This model system may help to further elucidate the effects of ecotoxicants on the external innate immune system of fish.

Niyogi, S., G. G. Pyle, et al. (2007). "Branchial versus intestinal zinc uptake in wild yellow perch (Perca flavescens) from reference and metal-contaminated aquatic ecosystems." Canadian Journal of Fisheries and Aquatic Sciences **64**(11): 1605-1613.

Zinc is an essential micronutrient for freshwater fish but can be toxic to them at elevated concentrations. Therefore, the regulation of zinc uptake is important in maintaining homeostasis when fish are chronically exposed to elevated zinc in nature. This study examined the kinetics of in vivo branchial and in vitro intestinal zinc uptake in wild yellow perch (Perca flavescens) from metal -contaminated

and reference lakes in northern Ontario. The results showed that the branchial zinc uptake involves high -affinity transport sites, whereas the intestinal zinc uptake involves low-affinity transport sites. Interestingly, significant alterations in the branchial zinc uptake (reduced affinity, increased maximum transport rate) but no apparent changes in the intestinal zinc uptake characteristics were observed in the metal-impacted yellow perch population relative to the reference population. Subsequently, no differences in zinc concentrations of gill, liver, and whole body were recorded between reference and metal-impacted yellow perch populations. Overall, our study indicated,that the gill, not the gut, likely plays a critical role in maintaining the zinc homeostasis in wild fish under chronic exposure.

Paulauskis, J. D. and R. W. Winner (1988). "Effects of water hardness and humic acid on zinc toxicity to Daphnia magna Straus." Aquatic Toxicology **12**(3): 273-290.

The effects of water hardness and humic acid (HA) on the acute and chronic toxicity of zinc to Daphnia magna were evaluated. Increases in either water hardness or HA concentration resulted in proportional decreases in both acute (3-day) and chronic (50-day) zinc toxicity. The effect of either of these variables (hardness or HA) was independent of the other in acute tests. In chronic tests, HA reduced the toxic effect of zinc on reproduction more in soft water than in hard water. In addition to reducing the chronic toxicity of zinc, our data suggest that an increase in water hardness also changes the mode of chronic toxic action of zinc. As previously shown for other metals, this study indicates that a reliance on only reproductive and survival endpoints in short-duration chronic tests may lead to an underestimation of the chronic toxicity of zinc.

Rahel, F. J. (1981). "SELECTION FOR ZINC TOLERANCE IN FISH - RESULTS FROM LABORATORY AND WILD POPULATIONS." Transactions of the American Fisheries Society **110**(1): 19-28.

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-&.

Roy, R. and P. G. C. Campbell (1995). "Survival time modeling of exposure of juvenile atlantic salmon (Salmo salar) to mixtures of aluminum and zinc in soft water at low pH." Aquatic Toxicology **33**(2): 155-176.

The toxicity of mixtures of aluminum and zinc to juvenile Atlantic salmon (Salmo salar) was studied in low ionic strength waters similar to those encountered during spring snowmelt in salmon rivers on the Canadian Precambrian Shield. Fish were exposed to environmentally realistic concentrations of inorganic monomeric Al singly (<= 15 [mu]M), and to mixtures of Al and a sublethal concentration of zinc (1.7 to 0.8 [mu]M), at three acidic pH levels (pH 4.5, pH 4.8 and pH 5.2). For Al-only and Zn-only exposures, the LC-50(M) values exhibited a marked pH dependence (higher values at low pH); even when expressed in terms of the free-metal ions (Al3+ or Zn2+), to take into account the influence of

pH changes on metal speciation, the LC-50(Mz+) values were still different at each pH. Modification of the free-ion activity model, to include competition between the proton and the free-metal ions at the gill surface, can explain part of this pH dependence, but there remains some residual dependence on the H+-concentration. as yet unexplained. Fish mortality data at each pH exposure were also modeled with a Cox proportional hazards model. In all cases, the presence of Zn reduced survival times in the Al + Zn mixtures, despite the use of sub-lethal Zn concentrations (only 0.1-0.2 toxic units). Survival time modeling indicated a simple additive mode for the toxicity of the Al + Zn mixture; models with the best fit included a covariate term for Al (either as Al3+ or as inorganic monomeric Al) and a classification code variable for Zn.

Roy, R. and P. G. C. Campbell (1995). "SURVIVAL-TIME MODELING OF EXPOSURE OFJUVENILE ATLANTIC SALMON (SALMO-SALAR) TO MIXTURES OF ALUMINUM AND ZINC IN SOFT-WATER AT LOW PH." Aquatic Toxicology **33**(2): 155-176.

The toxicity of mixtures of aluminum and zinc to juvenile Atlantic salmon (Salmo salar) was studied in low ionic strength waters similar to those encountered during spring snowmelt in salmon rivers on the Canadian Precambrian Shield. Fish were exposed to environmentally realistic concentrations of inorganic monomeric Al singly (less than or equal to 15 mu M), and to mixtures of Al and a sublethal concentration of zinc (1.7 to 0.8 mu M), at three acidic pH levels (pH 4.5, pH 4.5 and pH 5.2), For Al-only and Zn-only exposures, the LC-50(M) values exhibited a marked pH dependence (higher values at low pH), even when expressed in terms of the free-metal ions (Al3+ or Zn2+), to take into account the influence of pH changes on metal speciation, the LC-50(M(z+)) values were still different at each pH. Modification of the free-ion activity model, to include competition between the proton and the free-metal ions at the gill surface, can explain part of this pH dependence, but there remains some residual dependence on the H+-concentration, as yet unexplained. Fish mortality data at each pH exposure were also modeled with a Cox proportional hazards model. In all cases, the presence of Zn reduced survival times in the Al+Zn mixtures, despite the use of sub-lethal Zn concentrations (only 0.1-0.2 toxic units). Survival time modeling indicated a simple additive mode for the toxicity of the Al+Zn mixture; models with the best fit included a covariate term for Al (either as Al3+ or as inorganic monomeric Al) and a classification code variable for Zn.

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.

Sanchez-Bayo, F. and K. Goka (2005). "Unexpected effects of zinc pyrithione and imidacloprid on Japanese medaka fish (Oryzias latipes)." Aquatic Toxicology **74**(4): 285-293.

Biological effects of the biocide zinc pyrithione (Zpt), used in anti-dandruff shampoos and antifouling paints and the agricultural insecticide imidacloprid on Japanese medaka fish (Oryzias latipes) were assessed in experimental rice fields. Both chemicals are toxic to medaka, in particular Zpt, which also causes teratogenic effects such as spinal cord deformities in embryos at very low, sublethal concentrations. Rates of malformation in medaka fry from paddies

treated twice a week with anti-dandruff shampoo (0.18-0.37 mu L/L each time) over a period of 4 months were within the natural background, perhaps due to the quick dissipation rate of this chemical in the environment. Both Zpt and imidacloprid caused stress syndrome in juvenile medaka, with fish from Zpt-shampoo fields having a significantly lower weight to body length ratio than those from control fields. As it often happens with stressed fish, a massive infestation by a Trichodina ectoparasite was observed in medaka from imidacloprid fields. However, despite their high stress levels, fish from the Zpt fields did not suffer such infestation, supposedly because the disinfectant action of this biocide.

Santore, R. C., R. Mathew, et al. (2002). "Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow, and Daphnia magna." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **133**(1-2): 271-285.

The Biotic Ligand Model has been previously developed to explain and predict the effects of water chemistry on the toxicity of copper, silver, and cadmium. In this paper, we describe the development and application of a biotic ligand model for zinc (Zn BLM). The data used in the development of the Zn BLM includes acute zinc LC50 data for several aquatic organisms including rainbow trout, fathead minnow, and Daphnia magna. Important chemical effects were observed that influenced the measured zinc toxicity for these organisms including the effects of hardness and pH. A significant amount of the historical toxicity data for zinc includes concentrations that exceeded zinc solubility. These data exhibited very different responses to chemical adjustment than data that were within solubility limits. Toxicity data that were within solubility limits showed evidence of both zinc complexation, and zinc-proton competition and could be well described by a chemical equilibrium approach such as that used by the Zn BLM.

Sappal, R., J. Burka, et al. (2009). "Bioaccumulation and subcellular partitioning of zinc in rainbow trout (Oncorhynchus mykiss): Cross-talk between waterborne and dietary uptake." Aquatic Toxicology **91**(4): 281-290.

Zinc homeostasis was studied at the tissue and gill subcellular levels in rainbow trout (Oncorhynchus mykiss) following waterborne and dietary exposures, singly and in combination. Juvenile rainbow trout were exposed to 150 or 600 [mu]g l-1 waterborne Zn, 1500 or 4500 [mu]g g-1 dietary Zn, and a combination of 150 [mu]g l-1 waterborne and 1500 [mu]g g-1 dietary Zn for 40 days. Accumulation of Zn in tissues and gill subcellular fractions was measured. At the tissue level, the carcass acted as the main Zn depot containing 84-90% of whole body Zn burden whereas the gill held 4-6%. At the subcellular level, the majority of gill Zn was bioavailable with the estimated metabolically active pool being 81-90%. Interestingly, the nuclei-cellular debris fraction bound the highest amount (40%) of the gill Zn burden. There was low partitioning of Zn into the detoxified pool (10-19%) suggesting that sequestration and chelation are not major mechanisms of cellular Zn homeostasis in rainbow trout. Further, the subcellular partitioning of Zn did not conform to the spill-over model of metal toxicity because

Zn binding was indiscriminate irrespective of exposure concentration and duration. The contribution of the branchial and gastrointestinal uptake pathways to Zn accumulation depended on the tissue. Specifically, in plasma, blood cells, and gill, uptake from water was dominant whereas both pathways appeared to contribute equally to Zn accumulation in the carcass. Subcellularly, additive uptake from the two pathways was observed in the heat-stable proteins (HSP) fraction. Toxicologically, Zn exposure caused minimal adverse effects manifested by a transitory inhibition of protein synthesis in gills in the waterborne exposure. Overall, subcellular fractionation appears to have value in the quest for a better understanding of Zn homeostasis and interactions between branchial and gastrointestinal uptake pathways.

Sappal, R. and C. Kamunde (2009). "Internal bioavailability of waterborne and dietary zinc in rainbow trout, Oncorhynchus mykiss: Preferential detoxification of dietary zinc." Aquatic Toxicology **93**(2-3): 166-176.

Internal bioavailability of zinc (Zn) in the liver and intestine of juvenile rainbow trout (Oncorhynchus mykiss) was investigated following exposure to 150 or 600 mu g l(-1) waterborne Zn, 45 or 135 mu g dietary Zn g(-1) fish day(-1), and a combination of 150 mu g l(-1) waterborne and 45 mu g dietary Zn g(-1) fish day(-1) for 40 days. At the organ/tissue level the concentrations of Zn in the intestine were 15-25 times those in the liver, and a transient partially additive accumulation was observed in the intestine. At the subcellular level Zn distribution was ubiquitous with the accumulation pattern in the liver being heat stable proteins (HSP) > mitochondria > nuclei-cell debris > heat denaturable proteins (HDP) > microsomes-lysosomes (M-L) = NaOH resistant fraction, while in the intestine it was nuclei-cell debris > HSP > NaOH resistant fraction > mitochondria > M-L = HDP. The majority of cellular Zn was biologically available in both tissues with the estimated putative metabolically active pools (MAP) being 65-78% in the liver and 59-75% in the intestine. We show, for the first time, preferential streaming of dietary Zn into the metabolically detoxified pool (MDP) and that of waterborne Zn to the MAP. Specifically, in the liver the cellular Zn load shifted to MAP in the waterborne Zn and combined exposures, and to the MDP in the dietary Zn exposures. In the intestine the proportion of cletoxified Zn increased in the dietary Zn-exposed fish but was unchanged in the waterborne and combined exposures despite elevated concentrations. Under the experimental conditions used in the present study, uptake from the food drove the accumulation of Zn in the intestine while uptake from both sources was important in the liver, consistent with its central location. Further, additive accumulation in the MOP (hepatic and intestinal), intestinal HSP, and hepatic HDP was revealed. Overall these data suggest that fish are better insulated from dietary than waterborne Zn toxicity.

Sastry, K. V. and S. Subhadra (1984). "Effect of cadmium and zinc on intestinal absorption of xylose and tryptophan in the fresh water teleost fish." Chemosphere **13**(8): 889-898.

The effect of cadmium and of zinc on the rate of uptake of a pentose sugar

xylose and an aminoacid tryptophan by the intestine of a teleost fish, was studied under two experimental conditions. In the first, four concentrations of cadmium or zinc (1.0 mM, 0.1 mM, 0.01 mM and 0.001 mM) mixed with the nutrient solution were filled in the intestinal sacs, and the rate of absorption was recorded after 1 h at 23°C. In the second experiment fish were exposed by bath to a sublethal concentration of cadmium (0.26 mg/1) or zinc (4 mg/1) for 15 and 30 days and the rate of absorption of the two nutrients was measured. The activity of intestinal Na+, K+ activated adenosine triphosphatase was also assayed. The two heavy metals at all the four concentrations decreased the rate of intestinal transport of nutrients. Increase in the concentration of each of the heavy metals decreased the uptake of nutrients, but the decreases were not linear. The rate of intestinal absorption of the two nutrients was also reduced by exposure of fish to the heavy metals . The activity of Na+, K+ ATPase decreased with all four concentrations of cadmium and zinc and was diminished in fish exposed for 15 and 30 days. Of the two heavy metals, cadmium was more effective in reducing the rate of transport of xylose and tryptophan.

Sauer, G. R. and N. Watabe (1989). "Ultrastructural and histochemical aspects of zinc accumulation by fish scales." Tissue and Cell **21**(6): 935-943.

The effect of zinc exposure on the ultrastructure of the scales and scale associated cells of the estuarine teleost, Fundulus heteroclitus was investigated in laboratory experiments. The Timm sulfide silver stain indicated that in the calcified region of the scales, Zn was colocalized with the calcium phosphate mineral crystals. X-ray diffraction analysis showed that Zn did not have an effect on crystal structure. The scale osteoblasts of Zn-exposed fish showed an increase in the number of lysosome-like structures contained by the cytoplasm. In Zn-exposed animals, X-ray microanalysis revealed that these structures contain greatly increased levels of zinc and sulfur relative to controls. In all specimens, the lysosomes contained higher levels of Zn than either the surrounding cytoplasm or adjacent scales. The findings suggest that osteoblast lysosomes may be involved in the accumulation of Zn and other metals by fish scales by the enzymatic degradation of metallothioneins or other metal-binding proteins. This could represent an important mechanism for the detoxification of excess heavy metal ions taken up from the environment and the metabolism of essential metals by calcified tissues.

Saunders, R. L. and J. B. Sprague (1967). "Effects of copper-zinc mining pollution on a spawning migration of atlantic salmon." Water Research **1**(6): 419-432.

Pollution from a base metal mine on a tributary of the Northwest Miramichi River caused many adult Atlantic salmon, which were on their normal upstream spawning migration, to return prematurely downstream through a counting fence on that river during summer and early autumn. These observations gave an opportunity to document avoidance reactions of salmon to pollution, which has seldom been done in the fishes' natural environment. Downstream returns of salmon rose from between 1 and 3 per cent during 6 years before pollution to between 10 and 22 per cent during 4 years of pollution. Early runs (June-July) of

salmon to the headwaters were delayed and reduced in number. Chemical analyses of river water showed levels of Cu2+ and Zn2+ which varied with rates of river discharge. During some periods Cu2+ + Zn2+ concentrations exceeded lethal levels for immature salmon, as established in another (laboratory) study. The threshold concentration for 50 per cent survival of fish under specified temperature conditions is designated as 1·0 toxic unit. Adult salmon in nature showed avoidance reactions at about 0·35-0·43 toxic unit of Cu2+ + Zn2+. A level of 0·8 toxic unit may have blocked all upstream movement. Of the salmon returning downstream because of pollution, about 31 per cent reascended, 62 per cent were not seen again and 7 per cent were taken by angling and commercial fishing below the counting fence. Estimated losses from the stock available in the upper part of the river from 1960 to 1963 varied from 8 to 15 per cent of the total run. There is no evidence that successive year-classes of salmon are growing accustomed to the pollution.

Schlenk, D. and C. D. Rice (1998). "Effect of zinc and cadmium treatment on hydrogen peroxide-induced mortality and expression of glutathione and metallothionein in a teleost hepatoma cell line." Aquatic Toxicology **43**(2-3): 121-129.

Cellular thiols such as glutathione (GSH) and metallothioneins (MTs) have been shown to protect cells from metal toxicity by nature of their ability to sequester metals. Thiols are also scavengers of reactive oxygen species and protect against oxidative stress. Few studies have examined the role of thiols in protection from oxidative stress in aquatic species. To examine the role of MT and GSH in oxidative stress in fish, Poeciliopsis hepatoma cells (PLHC-1) were treated with 0.1, 1.0 and 10 mM of the oxidant, hydrogen peroxide (H2O2), for 24 h. When cells were pretreated with 10 mu M ZnSO4 for 24 h prior to H2O2 treatment, survival was not signifcantly different than controls at the 0.1 mM H2O2 concentration. However, 10 mu M ZnSO4 provided no protection at the 1.0 and 10 mM H2O2 concentrations. When cells were pretreated with IO mu M CdCl2 24 h prior to H2O2, significant protection was afforded at the 0.1 and 1.0 mM H2O2 concentrations. Low-molecular weight Cd-109-binding proteins (indicative of (MTs)) were measured in each group of cells 24 h after 0.1, 1.0 and IO mu M ZnSO4 or CdCl2 treatments and was significantly induced by CdCl2 at the 1 and 10 mu M concentrations. Significant induction of MT-like proteins were only observed at the 10 mu M ZnSO4 concentration and H2O2 failed to induce MT-like proteins in the cells. Cellular GSH was also measured following metal and H2O2 treatments. Treatment of cells with 10 mu M CdCl2 led to significant increases in GSH, whereas ZnSO4 failed to change GSH content. H2O2 treatments of I and 10 mM led to a reduction of GSH. These data indicate differential protection afforded by the two metals and suggests that metal-induced GSH and MT may provide better protection against oxidative damage than induction of MT alone.

Schmitt, C. J., W. G. Brumbaugh, et al. (2007). "Accumulation of metals in fish from lead-zinc mining areas of southeastern Missouri, USA." Ecotoxicology and Environmental Safety **67**: 14-30.

Sellers Jr, C. M., A. G. Heath, et al. (1975). "The effect of sublethal concentrations of copper and zinc on ventilatory activity. Blood oxygen and pH in rainbow trout (Salmo gairdneri)." Water Research **9**(4): 401-408.

Changes in buccal and opercular pressure amplitude, as well as ventilation and coughing frequency were monitored in rainbow trout using catheterization of respiratory cavities and pressure transducers. One or more of the ventilatory parameters measured were found to change under toxicant stress at concentrations of copper or zinc at or below the LC 50. Possible synergistic effects were indicated when the two metal ions were tested together. Serial analyses of arterial PO2 and pH in fish exposed to copper and zinc individually at concentrations approximating the LC 50 showed that environmental zinc produced a sharp decrease in both PO2 and pH. Copper, however, caused little effect other than a transient increase in pH. The toxic action of the two metals in low concentrations thus may not be the same.

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.

Somasundaram, B., P. E. King, et al. (1984). "The effects of zinc on postfertilization development in eggs of Clupea harengus L." Aquatic Toxicology **5**(2): 167-178.

The uptake of zinc and its effects on postfertilization development in eggs of Clupea harengus L. were examined. 30-50% of accumulated zinc was bound to the chorion and the presence of small amounts of zinc in the larva at hatching suggested that the greater part accumulates in the perivitelline space. The highest level of zinc uptake occurred at the beginning of tissue initiation. Although the volumes of the eggs were not altered significantly, development was accelerated at low concentrations (0.1, 0.5 and 2.0 ppm) and slowed at 6.0 ppm. These effects were discussed in terms of larval viability.

Sprague, J. B. (1968). "Avoidance reactions of rainbow trout to zinc sulphate solutions." Water Research **2**(5): 367-372.

Rainbow trout (Salmo gairdnerii Richardson) showed strong avoidance reactions to sublethal concentrations of zinc sulphate. The threshold avoidance level was 5.6 [mu]g/l of zinc added to laboratory water. This is only 0.01 of the lethal threshold concentration. There were no significant differences in threshold avoidance levels at 9.5° and 17°C., nor when background of zinc in the water was increased during acclimation and/or testing, from 3 [mu]g/l to 13 [mu]g/l.

Stubblefield, W. A., B. L. Steadman, et al. (1999). "Acclimation-induced changes in the toxicity of zinc and cadmium to rainbow trout." Environmental Toxicology and Chemistry **18**(12): 2875-2881.

Adults and juvenile rainbow trout exposed for 21 d to sublethal levels of zinc or cadmium exhibited significant changes in their respective incipient lethal levels

(ILL). Acclimation resulted in exposure-dependent changes in both tolerance (ILL concentration) and resistance (time to ILL) in both size classes of fish for each metal. The ILLs for adult rainbow trout exposed to zinc increased from 695 mu g/L at 131 h for nonacclimated fish to 2,025 mu/L at 168 h for fish previously exposed to 0.5 ILL (324 mu g/L zinc). The ILLs for cadmium-exposed fish increased from 6 mu g/L at 187 h for nonacclimated fish to 122 mu g/L at 266 h for Fish acclimated to 0.5 ILL (10.2 mu g/L cadmium). Similar, although somewhat less dramatic, acclimation responses were observed for juveniles with both zinc and cadmium. Juveniles were found to be approximately three times less sensitive to the toxic effects of the metals than were adult fish.

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, M. C., A. Demayo, et al. (1982). "EFFECTS OF ZINC ON HUMANS, LABORATORY AND FARM-ANIMALS, TERRESTRIAL PLANTS, AND FRESH-WATER AQUATIC LIFE." Crc Critical Reviews in Environmental Control **12**(2): 113-181.

Tort, L. and L.-H. Madsen (1991). "The effects of the heavy metals cadmium and zinc on the contraction of ventricular fibres in fish." Comparative Biochemistry and Physiology Part C: Comparative Pharmacology **99**(3): 353-356.

1. The force-frequency relationship has been determined in strips of ventricular fibres of sea bass and dogfish after treatment with several concentrations of cadmium and zinc. 2. At increasing metal concentrations a reduction of twitch force is observed in the heart fibres, the reduction being similar in the two species for each metal. 3. Cadmium shows a more powerful effect since comparable reduction is obtained at 10 times lower concentration. 4. The removal of metal concentrations in previously-treated strips does not allow the initial force to recover. 5. The results are discussed in relation to the consequences of metal exposure on the heart function and the repercussion on fish performance.

van Dyk, J. C., G. M. Pieterse, et al. (2007). "Histological changes in the liver of Oreochromis mossambicus (Cichlidae) after exposure to cadmium and zinc." Ecotoxicology and Environmental Safety **66**(3): 432-440.

The toxic effects, of two heavy metals, cadmium (Cd) and zinc (Zn), on the histology of the liver of the southern African freshwater fish Oreochromis mossambicus, were investigated. The goal was to identify whether metal concentrations and exposure period influence the degree and nature of histological changes in the liver of exposed fish. Selected fish were exposed to a mixture of 5% concentrations of the LC50 of cadmium and zinc and to a mixture of 10% concentrations of the LC50 of cadmium and zinc, over both short- and long-term exposure periods. Similar histological changes occurred in the livers of specimens exposed to both 5% and 10% concentrations, indicating a definite

toxic response to both the metal concentrations. These histological changes included hyalinization, hepatocyte vacuolation, cellular swelling, and congestion of blood vessels. The intensity of these histological changes was, however, influenced by the extent of the exposure period.

Van Hoof, F. and M. Van San (1981). "Analysis of copper, zinc, cadmium and chromium in fish tissues. A tool for detecting metal caused fish kills." Chemosphere **10**(10): 1127-1135.

In order to find the causative agent in frequently occuring fish kills in a Belgian river a series of toxicity tests has been conducted in which rudd (Scardinius erythrophtalmus) were exposed to acute lethal and subacute non lethal concentrations of copper, chromium, cadmium and zinc. The concentrations of these metals in gills, opercle, kidney, liver and muscle were measured. Metal levels in gills were the most valuable indicator of acute lethal exposure. This information was compared with levels found in rudd from a surface water storage reservoir and from the river Meuse. Fish collected after fish kills in the river Meuse were analysed. In one case copper could be identified as one of the toxicants concerned by fish tissue analysis. Metal levels in fish tissues can give valuable additional information concerning the cause of kills provided that background information is available about metal levels in water and normal tissue levels.

Vangen, B. and G.-I. Hemre (2003). "Dietary carbohydrate, iron and zinc interactions in Atlantic salmon (Salmo salar)." Aquaculture **219**(1-4): 597-611.

Atlantic salmon (initial weight 312 g) were fed four experimental diets containing either low (5%) or high (10%) levels of wheat starch, combined with either a low (6 mg kg-1) or high (30 mg kg-1) iron and zinc level, in order to elucidate interactions between dietary starch and iron plus zinc. No difference in specific growth rate or protein productive value as a consequence of the variations in dietary starch was observed. Protein efficiency ratio was significantly lower in fish fed with 5% compared to 10% starch diets, indicating higher deposition of nonprotein components as a consequence of the higher starch level. Dietary iron plus zinc significantly influenced final weight, condition factor and hepatosomatic index, with lower values in fish fed with higher mineral levels. The present dietary manipulations did not affect blood haematology, with all values being within normal ranges for Atlantic salmon. Plasma levels of glucose, protein, cholesterol or triacylglycerols were not affected by the dietary treatments. Low activities of plasma aspartate amino transferase and alanine amino transferase indicated, along with no mortality, no negative effects on liver function or fish health due to dietary treatments. Glycogen levels in whole body, liver and spleen were increased in fish fed with 10% compared to 5% starch diets. Liver iron concentration was affected by both dietary starch and mineral levels. Whole body homogenates showed significantly higher zinc levels in fish fed with higher iron and zinc diets, whereas plasma zinc levels were affected by dietary starch, iron and zinc levels, which showed a significant interaction effect.

Verma, Y. (2005). "Retardation of fin regeneration in freshwater fish Oreochromis mossambicus by zinc." Acta Hydrochimica Et Hydrobiologica **32**(6): 429-433.

Regeneration of partially amputated caudal fin was studied in freshwater fish Oreochromis mossambicus exposed to sublethal concentrations of zinc (5.0 mg L-1 and 10.0 mg L-1) under ambient laboratory conditions over a period of 20 days. Caudal fin regeneration was measured on 5th, 10th, 15th and 20th day of exposure and after amputation. Significant ($p < 0.05$) retardation in fin regeneration was observed on day 5th and 10th in fish exposed to the nominal concentration of 5.0 mg L-1 Zn, while retardation was found highly significant ($p < 0.01$) at all the observations in 10.0 mg L-1. The maximum inhibition in caudal fin regeneration (20.8% and 24.3%) was found during the initial observation at both of the concentrations (5.0 mg L-1 and 10.0 mg L-1) of zinc exposure. Later on the regeneration rate was almost as good as in the control group. Thus in this study fin regeneration was significantly inhibited at all time points following Zn exposure as a detrimental effect of Zn to fish. This study demonstrates that fish caudal fin regeneration is a simple assay, sensitive and easy to perform, and can serve as a model to determine the toxicity of pollutants in aquatic environment.

Wiklund, A. K. E., T. Borjesson, et al. (2006). "Avoidance response of sediment living amphipods to zinc pyrithione as a measure of sediment toxicity." Marine Pollution Bulletin **52**(1): 96-99.

An avoidance test was developed using non-cultured individuals of the sediment dwelling amphipod Monoporeia affinis. As test substance we used zinc pyrithione, an antifouling agent and a common shampoo ingredient. The toxicity to Daphnia and fish is well known but sediment toxicity of this very hydrophobic compound is less known. The preference of juvenile M. affinis was tested in jars, each including 12 petri dishes. In each replicate, half of the petri dishes contained sediment mixed with six concentrations ranging from 0 to 10 mu g zinc pyrithione per L sediment and half of the petri dishes contained the corresponding sediment-substance mixture plus an extra food addition. The amphipods significantly avoided petri dishes with the three highest concentrations of zinc pyrithione and the calculated EC50 was 9.65 mu g L-1 sediment. No difference in mortality was observed between concentrations. Using the avoidance behaviour in sediment toxicity testing is a simple and cost-effective screening for toxicants. (c) 2005 Elsevier Ltd. All rights reserved.

Witeska, M. and B. Kosciuk (2003). "The changes in common carp blood after short-term zinc exposure." Environmental Science and Pollution Research **10**(5): 284-286.

Blood zinc level, hematological parameters and blood cell morphology were evaluated in common carp immediately after 3 h exposure to 20 mg dm(-3) of zinc (Zn0), and in 24, 48 and 96 hours after the end of it (Zn24, Zn48, Zn96). Blood zinc level in the non-exposed fish was 8 mg dm(-3), reached a maximum of 20 mg dm(-3) in Zn48, while it dropped to 9 mg dm(-3) in Zn96. Zinc caused a stress reaction in fish indicated by an increase in hematocrit value in Zn0, and elevated plasma glucose level and trombocytosis which persisted until the end of the experiment. Zinc-exposed fish showed an increased frequency of abnormal

erythrocytes, and a compensatory release of immature erythrocytes to the blood stream. In zinc-treated fish, leukocyte count initially increased and subsequently decreased significantly below the control level due to a drop in lymphocyte number. Lymphocyte viability was reduced, and abnormal lymphocytes appeared. A decreased count of juvenile neutrophiles, and reduced phagocyte activity also occurred. The results indicate possible zinc-induced disturbances in both specific and non-specific immune mechanisms.

Zhang, L. and W.-X. Wang (2007). "Gastrointestinal uptake of cadmium and zinc by a marine teleost Acanthopagrus schlegeli." Aquatic Toxicology **85**(2): 143-153.

Gastrointestinal metal uptake represents a potential route for metal bioaccumulation in marine fish. Drinking of seawater for osmoregulation causes constant waterborne exposure of the gastrointestinal tract. Tissue specific Cd and Zn accumulation and distribution were investigated in juvenile black sea bream (Acanthopagrus schlegeli) exposed to waterborne Cd (5.7 nM) and Zn (2.6 nM) for 4 h-7 days. The intestine accumulated a large portion of the Cd (43-58%) and Zn (18-28%), and had the highest Cd (>1.0 nmol g-1) and Zn (>1.8 nmol g-1) concentrations of all body fractions, suggesting that the intestines were the major uptake sites for these waterborne metals. Among all the segments of the gastrointestinal tract, the anterior intestine played the most important role in Cd and Zn uptake. A gastrointestinal injection assay was conducted to distinguish waterborne metal uptake by the intestines and the gills. The intestine contained over 90% of the Cd in the body after depuration for 3-7 days, suggesting that little waterborne Cd entered the rest of the body through the intestine, and that Cd may exert its toxic effects on the gastrointestinal system. In contrast, intestine retained less than 20% of the total Zn after depuration, suggesting that Zn tended to be transported from the intestine to the internal tissues via the cardiovascular system. The uptake kinetics of waterborne Cd and Zn by the intestines and the gills were determined as a first-order and saturated pattern, respectively, over a wide range of ambient metal concentrations (6.2 nM-4.5 [mu]M for Cd, and 13 nM-15 [mu]M for Zn). An in vitro intestinal perfusion assay investigated the effects of intestinal metal composition and drinking rate on uptake. The presence of EDTA significantly reduced intestinal Zn uptake to 11%, while cysteine improved it by 59%. The intestinal Cd and Zn uptake rates were unaffected by the perfusion rate.

Zhang, L. and W.-X. Wang (2007). "Waterborne cadmium and zinc uptake in a euryhaline teleost Acanthopagrus schlegeli acclimated to different salinities." Aquatic Toxicology **84**(2): 173-181.

Metal uptake and toxicity in marine fish are usually much lower than those in freshwater fish, but the underlying mechanisms remain unclear. In this study, we investigated Cd and Zn uptake by the euryhaline black sea bream (Acanthopagrus schlegeli) over a salinity range from 0 to 35 psu. Cd and Zn uptake increased as salinity decreased. The gills were the most sensitive organs in response to salinity change, and played a more important role in Cd and Zn uptake at a lower salinity. Cd and Zn uptake in the viscera contributed to 34-36%

of the overall accumulation at full salinity (35 psu), but decreased to 13-16% in freshwater despite the increase of uptake rate. Water permeability, drinking, and major ion uptake (Ca) in the fish at different salinities were also concurrently examined. The overall water uptake was comparable, whereas the drinking rate decreased at lowered salinities. In contrast, the Ca uptake increased significantly with decreasing salinity. The responses of Cd and Zn uptake to salinity challenge were correlated with the Ca uptake, suggesting that they may be taken up through the Ca uptake pathway. At a constant salinity, Cd and Zn uptake increased with reducing Ca concentration, indicating the competitive effect of Ca on metal uptake. Ca channel blockers (verapamil and lanthanum) significantly reduced the uptake of Cd, Zn, and Ca when the fish were acclimated in freshwater, but had no impact on their uptake in marine water. Furthermore, the chloride cell number in the gills could not explain the lower Cd and Zn uptake in seawater. Our results indicated that both ambient physicochemical factors and the physiological responses of fish resulted in difference of metal uptake in marine and freshwater environments.

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