A1 - Metal Homeostasis

A1.1
Metal-omics: bad grammar or an emerging science

P Kille (Cardiff University)

The increasing availability of genomic resources and the complementary omics technologies provide a potential Pandora's box for metallo-biologist. The information contained within sequence data and that generated by omics investigation threatens to overwhelm a community accustomed to focusing on one transporter or metallo-chaperone. Using data drawn from a range of studies employing transcriptomics to investigate metal homeostasis within fish cells in culture, developing fry and adult fish, I will attempt to illustrate how the data generated can be transformed to reveal biologically relevant information. Furthermore, I will attempt to illustrate how model organism genome information is essential if we are to map comprehensively the complete set of proteins involved in metal homeostatic pathways together with the regulatory networks that detect and respond to metal imbalance. I will also discuss the benefits and limitations of comparative genomic approaches that enable us to use data derived from model organisms to interpret observation made within environmental sentinel species.

A1.2
Oxidative stress response and gene expression with acute copper exposure in zebrafish

P Craig (McMaster University), C Wood (McMaster University), G McClelland (McMaster University)

in vitro, yet the *in vivo* effects of metal-induced oxidative stress have not been extensively studied. In 2 experiments, we examined the impacts of copper (Cu) on gene expression, oxidative damage, and cell oxidative capacity in liver and gill of zebrafish. In the first experiment, soft-water acclimated zebrafish were exposed to 8 & 15 mg/L Cu for 48 h. This exposure resulted in significant increases in gene expression of cytochrome c oxidase 17 (COX 17) and catalase (CAT), associated with both increased Cu load and protein carbonyl concentrations in the gill and liver after 48 h. In the second experiment, we examined the potential protective effects of increased waterborne Ca^{2+} (3.3mM) and Na^{+} (10mM) on acute Cu toxicity. While both treatments were effective at reducing liver and/or gill Cu loads and attenuating oxidative damage at 48h, 10 mM Na+ was more protective than 3.3 mM Ca^{2+}, in accordance with previous LC50 results. There were variable changes in the maximal activities of cytochrome c oxidase (COX) and citrate synthase (CS), indicating possible alterations in cell oxidative capacity. Moreover, Cu affected COX/CS ratios in both gill and liver suggesting that Cu alters proper mitochondrial biogenic processes possibly though metallochaperones like COX 17. Overall, this study provides important steps in determining the genetic and physiological endpoints of acute Cu toxicity in a model tropical species.

A1.3
Regulation of zinc transporters in fish

C Hogstrand (King's College London)

Transport of zinc into the cytosol is mediated by proteins of the ZIP family (SLC39) and also by ECaC (TRPV6). The ZnT (SLC30) family of proteins are generally involved in transporting zinc out of the cell or into intracellular compartments. We have shown that uptake of zinc across the rainbow trout gill is increased by cortisol and the vitamin D metabolite, $1\alpha,25(OH)_2D_3$ (calcitriol) The stimulatory effect of calcitriol on zinc influx can be explained by upregulation of ECaC and ZIP1. Likewise, the hormone, stanniocalcin, reduces zinc uptake at the gill, presumably by limiting ECaC permeability. Cellular regulation of zinc is dependent on MTF1, which is an intracellular zinc sensor. MTF1 responds to an increased $[Zn^{2+}]_i$ by binding to metal response elements and inducing expression of specific genes. The originally described target for MTF1 was metallothionein (MT), which along with glutathione binds zinc and

keeps the concentration of $[Zn^{2+}]_i$ at very low levels. A change in zinc availability brings about corresponding adjustments in expression of zinc transporters in gills and intestine of zebrafish. In general, increasing zinc availability causes a downregulation of zinc importers and an upregulation of exporters. The opposite happens in zinc deficient conditions. Positive regulation by zinc of the major zinc exporter, ZnT1, is mediated by MTF1 acting on upstream promoter elements. Interestingly, we recently found that downregulation of the zinc importer, ZIP10, in intestine and gill is caused by MTF1 binding to repressor elements located in the first intron of the gene.

A1.4
Genetics and Genomics of Cadmium Exposure in the Nematode *C.elegans*

S. R. Sturzenbaum (Kings College London), Suresh C. Swain (Kings College London), Samantha Hughes (Kings College London)

Cadmium is a teratogenic, mutagenic and potentially lethal heavy metal. Therefore, it has the capacity to induce a scope of adverse effects ranging from the molecular level to the life-history of individual organisms and eventually population dynamics. Following the identification of cadmium responsive genes via complex micro-array experiments, we focused on two metallothioneins (mtl-1 and mtl-2). Initial promoter tagged GFP fusion constructs confirmed that whilst mtl-1 is constitutively expressed in the pharynx, intestinal mtl-1 and mtl-2 are induced in the presence of heavy metals. Mutant strains were isolated where mtl-1, mtl-2 or both (mtl-1 and mtl-2) were knocked out. To evaluate their involvement in the toxicogenomic response to heavy metals, the single and double knockout nematodes were subjected to increasing concentrations Cd. Compared to wild type (Bristol N2), brood size was marginally reduced in the single knockouts and significantly reduced in the double knockout nematodes in the presence and absence of Cd. Brood period was similar in all strains under control conditions but was extended when Cd was added to the media. Overall, we were able to demonstrate the link between identified molecular responses to life history parameters (and vice versa) - the ultimate goal in Environmental Genomics.

A1.5
The knockout of metallothionein and phytochelatin synthase in *C. elegans*: metal induced effects on life history and gene expression

S Hughes (Cardiff University/ King's College London), P Kille (Cardiff University), S Stürzenbaum (King's College London)

Toxicants such as heavy metals can have a major impact on life. Using the model organism *Caenorhabditis elegans*, the effect of toxic levels of heavy metals can be monitored at the individual level in terms of brood size and lifespan. *C. elegans* have two metallothionein isoforms and a phytochelatin system for metal detoxification. Phytochelatin synthase is an enzyme which generates phytochelatin, a class III metallothionein, and is located in both pharynx and gut cells. Single and combinational deletion mutants for mtl-1, mtl-2 and pcs-1 were generated. Nematodes were subjected to increasing concentrations of essential (copper and zinc) and non-essential (cadmium) heavy metals. In addition trace quantities of copper was removed from the media. Total brood size, brood period and lifespan effects were investigated. Compared to the wild type, N2, brood size was marginally reduced in the single knockouts, significantly reduced in the double mtl mutant and most severely reduced in the triple knockout. All strains displayed a decline in brood size upon metal exposure. Removal of copper also resulted in a decline in total brood size. A TaqMan qPCR approach is currently being utilised to quantify the change in relative gene expression of all 3 genes in the mutants in the presence and absence of cadmium.

A1.6

First experiments on the maternal transfer of heavy metals in the cuttlefish Sepia officinalis

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Previous studies have shown that a limited number of heavy metals accumulate in the cuttlefish embryo via the seawater once eggs are laid. However, little is known about the direct transfer of metals from the mother to the eggs. Therefore, the maternal transfer of 7 elements (^{241}Am, Ag, Cd, Co, Mn, Se, Zn) was studied following dietary exposure of a mature female cuttlefish using radiotracer techniques. Radiolabelled crabs were used as food; they were given daily during 2 weeks before the female started to spawn. Among the 7 tracers, only ^{65}Zn, ^{75}Se, and ^{110m}Ag were detected in the eggs. The radiotracer distribution in freshly laid eggs showed that ^{65}Zn and ^{75}Se were accumulated only in the vitellus. In contrast, ^{110m}Ag was found in similar amounts in the vitellus and the eggshell, indicating that Ag did not follow the same mechanisms of transfer than the two other elements. During the embryonic development, ^{75}Se and ^{65}Zn contained in the vitellus were progressively transferred to the embryo, likely to supply its metabolic needs in these essential elements during the embryogenesis. Although it has no known biological functions, Ag contained in both vitellus and eggshell was also transferred to the embryo. Overall, our results showed that transfer of the three metals from females to the eggs does actually occur, at least during the last two weeks before spawning.

Key Words: Essential metals – toxic elements – distribution – vitellus - cephalopod

A1.7
Parallel Analysis of Deletion Mutants in *Saccharomyces cerevisiae* under Zinc Deficiency

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As an essential micronutrient, zinc is required by all organisms for a variety of vital biological processes, serving as a catalytic component of numerous enzymes and a structural component of the ubiquitous zinc finger motifs found in many transcription factors. For an organism's wellbeing, intracellular zinc concentrations must be tightly regulated, as both deficiency and excess could lead to severe pathologies. During the last ten years, the baker's yeast *Saccharomyces cerevisiae* has established itself as a model organism for studying eukaryotic cells. The purpose of this study was to conduct a parallel deletion analysis (PDA) of the non-essential genes in *S. cerevisiae* under zinc deficient conditions to identify potential zinc regulator genes and zinc dependant cellular processes. In PDA or fitness profiling, yeast strains containing single deletions for almost every gene in the genome and identified with unique molecular barcodes, are pooled and exposed to an experimental condition of interest. Microarrays spotted with complementary barcodes are used to monitor the growth of each individual mutant. Depending on the function of a deleted gene in the condition under study, a mutant strain survives and competes with others or is depleted from the pool. In this study, pools of yeast homozygous diploid deletion strains were grown for five and fifteen generations in zinc deficient media. Comparison to strain growth in zinc replete media revealed a number of treatment-sensitive strains, suggesting that the deleted genes in these strains could be involved in zinc mediated functions.

A2 - Metal Toxicity

A2.1
Defence mechanisms of fish with different sensitivity towards heavy metals: Interaction and dynamics of proteins and hormones

M Eyckmans (University of Antwerp), R Blust (University of Antwerp)), G De Boeck (University of Antwerp)

Despite recent improvements of water quality in Belgium, there is a historic pollution of heavy metals in the aquatic environment. Different sensitivities of fish towards this metal pollution have been reported. Within this project, we will investigate the differences between rainbow trout (*Oncorhynchus mykiss*), carp (*Cyprinus carpio*) and gibel carp (*Carassius auratus gibelio*) in dealing with the effects of copper exposure. These fish species differ in their tolerance to copper, but the underlying mechanisms have not yet been revealed. Fish will be exposed to two sublethal exposure-conditions; one similar concentration for every fish species and one concentration which is the 10% LC50 96h value for each fish species. After 1h, 12h, 24h, 3days, 1 week and 1 month gill and plasma samples are taken. The intracellular proteome of gill cells as well as the hormonal balance, the role of hormonal influences and oxidative stress conditions during exposure will be evaluated by use of techniques such as iTRAQ, 2DIGE, immunoassays, Real-time PCR and a number of enzymatic assays. When proteome-profiles are constructed and quantitative and qualitative changes in expression of proteins are unravelled, we can search for differentially expressed proteins which can serve as biomarkers. With this project we want to obtain a better insight in the differences of the intracellular physiological and molecular responses in our test species and develop a sensitive biomarker for detection of metal toxicity in aquatic organisms.

A2.2
Using physiology, geochemistry and toxicology to develop prediction models for the impacts of metals in aquatic systems.

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Modeling the interactions of metal with the biological surfaces of organisms has been recently developed as a method for predicting the acute toxicity of metals in aquatic systems on a site specific (i.e. water chemistry) basis. The biotic ligand approach relies on the fact that 1. waterborne metals bind to and/or are taken up at specific sites, 2. this uptake/binding can be reliably characterized and 3. that there is a direct correlation between bioaccumulation and toxicity. As such, the development of mechanistic models has been dependent on the integration of toxicology, geochemistry and physiology. A number of biotic ligand models (BLM) have been produced (e.g. Cu, Ni, Ag, Zn, Co and Cd) and the robust nature of this approach is evidenced by its acceptance as a regulatory tool for site specific water quality criteria, for risk assessment and for setting discharge objectives. However, the growing list of BLMs are focused primarily on standard test species and tend to be from studies using waters of medium to high hardness. Therefore current studies are focused on testing the BLM approach with alternate species and in soft waters. These validation studies illustrate that mechanisms of acute metal toxicity are generally consistent although some metal interactions are not as expected. The understanding of mechanisms of chronic metal toxicity is much less advanced due to the complexities associated with chronic metal bioaccumulation. These complexities offer challenges in terms of characterizing metal uptake to sites of action and correlating accumulation with toxic impacts. Supported by NiPERA, ICA, ILZRO, CDA, Noranda-Falconbridge, Teck Cominco, NRCan and NSERC CRD program.

A2.3
The chronic effects of dietary Zn and Cu on *Daphnia magna* at multiple hierarchical levels of biological organization

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Relatively little attention has been paid to dietary metal toxicity in freshwater invertebrates. Therefore, we investigated the effects of dietary copper and zinc on the cladoceran *Daphnia magna* at multiple hierarchical levels of biological organization. First, *Pseudokirchneriella subcapitata* were exposed to different dissolved copper or zinc concentrations to obtain a range of diets contaminated with a range of internal Zn, i.e. ~100 (control) to 800 µg/g and internal Cu, i.e. ~10 (control) to 3,000 µg/g. These diets were then fed to *Daphnia magna* during 21 days and several endpoints were monitored. We considered molecular (gene expression using cDNA micro arrays and real-time PCR), physiological (molting, feeding rate), and organism level endpoints (survival, growth, reproduction). Elevated dietary Zn (>320 µg/g) decreased reproduction but did not affect survival, growth, or feeding rate. The differential expression of several molting-related genes suggested that effects on the molting cycle may be a potential indirect pathway to reproductive inhibition (because molting is closely linked with reproduction in daphnids). In contrast, the growth and reproduction of *D. magna* followed a hormetic pattern, i.e. stimulation up to 1,400 µg Cu/g and reduction at 3,000 µg Cu/g. Although the stimulation is most likely due to enhanced food assimilation, a variety of mechanisms may be involved in the toxicity, including reduced food assimilation, vitellogenesis inhibition, and molting inhibition. Thus, although the patterns of toxicity of dietary Zn and Cu toxicity are largely different at the organism level, the existence of some similarities at lower hierarchical levels cannot be excluded.

A2.4
Pb induced gene expression changes in freshwater organisms may serve environmental monitoring and reveal general mechanisms of Pb toxicity

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Despite the omission of Pb from gasoline and the reductions in use of Pb containing paint this metal remains an environmental and human health threat and is considered a priority pollutant by the USEPA. In addition to inducing anemia, Pb is known to impair cognitive abilities with an estimated multi-billion $ cost in the US alone owing to reduced IQ's. From an environmental perspective efforts are underway to develop a Biotic Ligand Model (BLM) to allow for site specific water quality criteria. Due to the nature of Pb as a calcium antagonist focus on calcium and water hardness have revealed that elevated calcium protects against acute Pb uptake and toxicity. However, calcium is much less potent in ameliorating effects of chronic exposure. In contrast, dissolved organic carbon (DOC) protects against acute and chronic Pb induced mortality. A recent study using a microarray of 5000 clones from fathead minnows (FHM) (accession #GSE8404) and QPCR revealed Pb induced gene expression changes. Glutathione S-transferase, glucose-6-phosphate dehydrogenase, ferritin and ß-globin displayed Pb induced expression changes alluding to anemia and neurological disorders as effects of chronic Pb exposure in FHM. An examination of the influence of water chemistry on Pb induced gene expression changes demonstrates promise for a "genomic" BLM. Elevated DOC reduced Pb accumulation by juvenile FHM and largely muted the Pb induced expression changes observed in Pb exposed fish in absence of DOC. Furthermore, elevated ambient calcium offered no protection against Pb accumulation or Pb induced gene expression changes in FHM.

A2.5
Integrating the variation of environmental conditions to predict non-lethal effects of metals to *Daphnia magna*

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Metals toxicity to a single species can vary widely due to water chemistry. The Biotic Ligand Model (BLM) approach permits the rational explanation of the effect of variation in water chemistry on the toxicity of metals to aquatic organisms. The BLM integrates the interaction of trace metal with solution phase ligands to predict its speciation and its subsequent interaction with receptor sites on the organism. It is expected that that changes the life-history parameters (growth, fecundity and survival) under metal exposure will be closely related with the physical and chemical properties of the exposure media.

The combined effects of varying physical and chemical parameters of the water (such as temperature, pH, conductivity, hardness, alkalinity and concentrations of major ions) on ecologically relevant endpoints (growth, fecundity and survival) of *Daphnia magna* were studied. Temperature causes major shifts in the physiological processes of *D. magna*: at 25ºC the maturity is reached earlier and by smaller individuals, resulting in higher values of fecundity over 21 days, compared to the individuals at 15ºC. Fecundity is also significantly affected by the chemical properties of the media (20 and 25ºC): high values of hardness and alkalinity favour fecundity. From all factors studied temperature is the one that affects most life-history in the absence of contaminants.

These results will be integrated into a general integrated BLM capable of predicting lethal and non-lethal effects of metals to freshwater organisms. The model will be able to deal with direct and indirect effects related physical (e.g. temperature) and chemical properties of the media (including metal speciation).

A2.6

Effect of Ca^{2+}, Na^{+} and pH on the simultaneous uptake of of Cd, Cu, Ni, Pb and Zn in the water flea *Daphnia magna* and zebrafish *Danio rerio* at environmentally relev

I Komjarova (University of Antwerp), R Blust (University of Antwerp)

The chemical characteristics of surface waters such as the concentrations of major cations (Ca^{2+}, Mg^{2+}, Na^{+}), the presence of dissolved organic matter and pH are important factors controlling trace metals accumulations and toxicity in aquatic organisms. These effects are incorporated into biotic ligand models for metals that are used to predict metal toxicity toward aquatic biota. Current biotic ligand model developments are mostly based on metal uptake and toxicity studies at metal concentrations resulting in acute or sub-chronic effects and performed in a limited number of organisms. For extrapolation purposes the models are adjusted to fit the toxicity results for other species. However, metal uptake by aquatic organisms at environmentally relevant concentrations and the effect of environmental conditions on these processes are much less documented. In this study we investigated the effects of Ca^{2+}, Na^{+} and pH on the simultaneous uptake of Cd, Cu, Ni, Pb and Zn in water flea *Daphnia magna* and zebrafish *Danio rerio* at metal concentrations below or equal to 0.1µM using a stable isotope technique. The approach allows one to separate the newly accumulated metal from the metal already present in the organisms, which is especially of interest when dealing with metals that are under homeostatic control such as Cu or Zn. The results showed that at low metal concentrations the accumulation patterns are not always the same as predicted by the biotic ligand model and the consequences of this for the environmental risk assessment of metals are discussed.

A2.7

Effects of copper toxicity on three species of scleractinian corals

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Most corals thrive in a narrow range of water quality and temperature regimes and as such can be considered sentinels of our oceans' health. Globally, coral reefs have been declining at an accelerating rate. Caribbean reefs, in particular, have suffered an estimated 80% loss of reef cover in the last 30 years. Land-based sources of pollution and global warming have been identified as major stressors linked to these declines. Contaminants, such as metals, although noted as a concern have not

been closely monitored in these sensitive ecosystems, nor have the potential impacts been characterized. There is a need to develop biomonitoring tools to assess potential effects of metal exposure. In this study, three species of laboratory-reared scleractinian corals, *Acropora cervicornis, Pocillopora damicornis,* and *Montastraea faveolata* were exposed to copper (ranging from 0-25 µg/L) for four weeks. At the end of the exposure period mortality, growth, copper accumulation, carbonic anhydrase activity, zooxanthellae density and electron transport rate were measured. The three coral species exhibited significantly different sensitivities to copper, with effects occurring at copper concentrations as low as 10 µg/L. The relationships between physiological/toxicological endpoints and copper accumulation within and between species will be presented as a means to elucidate the potential mechanism for effects and explain observed differences in sensitivity.

A2.8
Ca and Mg physiology determining metal bioavailability and acclimation in cladocerans: lab and field evidence.

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Several aspects of the role of Ca and Mg physiology in metal toxicity and acclimation will be discussed. In a first series of laboratory experiments, the mechanism(s) of Zn toxicity towards *Daphnia magna* was investigated. Analogous to Zn-induced hypocalcaemia in fish, it was found that elevated Zn levels decreased Ca body burdens in daphnids early during exposure. These effects were accompanied by decreased filtration rates and energy reserves. From day 14 onwards acclimation mechanisms were observed involving restoration of Ca balance and increasing ingestion rates. Therefore the link between Ca homeostasis and filtration rate was investigated into more detail. Preliminary experiments reveal that when Ca supply through the medium is limiting, filtration rate is increased in an attempt to retrieve more Ca from the food. Daphnids fed on food low in Ca were not able to recover to the same extent, indicating the importance of food source in this kind of experiments. The importance of Ca and/or Mg physiology and acclimation toward metal sensitivity of natural populations has led to a second series of experiments investigating Ni sensitivity of cladoceran populations (N=11) originating from habitats with different hardness levels. Acute and chronic Ni toxicity tests reveal no significant differences between the intrinsic sensitivity of soft vs. hard water organisms. Biotic ligand modeling exercises revealed that increased water hardness had a stronger protective effect against acute, but not chronic Ni toxicity to soft water organisms Physiological processes related to presence/absence of food and potential acclimation during long-term exposure may explain the differences between acute and chronic results but clearly more research is needed in this area.

A2.9
Do acid volatile sulfides (AVS) control the bioavailability of metals to benthic macro-invertebrates?

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Several studies showed that acid volatile sulfides (AVS) control the acute toxicity of sediment-bound divalent cationic metals. Few studies however investigated the effect of AVS on the bioavailability of metals to sediment-dwelling invertebrates.
In the present study 14 watercourses in Flanders were selected based on very low levels of dissolved metals but high concentrations of sediment-bound metals. At each site three sediment samples were taken and midge larvae and/or oligochaete worms were collected. The AVS and simultaneously extracted metals (SEM) of the sediment were measured with the “purge and trap” method and total metal levels were determined in sediment and organisms. In addition, organic content and clay content were measured.
Arsenic, Cd, Co, Cr, Cu, Ni, Pb and Zn were measured in sediments and organisms. Accumulated levels were compared between depurated and and non-depurated organisms. The mean AVS levels ranged from 0.76 to 211 µmol/g sediment dry weight. At all but two sites SEM-AVS was < 0,

suggesting no metal bioavailability. Despite this, at all sites high levels of at least one metal were found in depurated organisms, indicating that sulfide-bound metals were at least partially bioavailable to sediment-dwelling macro-invertebrates.
Only in very few cases one or more of the sediment characteristics contributed to the variation, as explored by multiple regression analysis. In none of the cases AVS contributed to the variation, indicating that AVS did not control the bioavailability of the metals to the investigated invertebrates.

A2.10
The endogenous use of silicon for amelioration of aluminium toxicity in the freshwater snail, *Lymnaea stagnalis*.

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We previously showed that, in the presence of exogenous silicon, the pond snail *Lymnaea stagnalis* does not exhibit the behavioural toxicity associated with aluminium exposure. Levels of Si in the digestive gland (DG), the major detoxificatory organ of molluscs, increased in proportion to Al accumulation, which led us to propose that Si is used intracellularly to detoxify Al by forming non-toxic aluminosilicates. However, we could not eliminate the possibility that Al-Si interactions occurred in the water prior to their uptake. This has been addressed here by exposing snails to Al (500 ug l^{-1}) and Si (7 mg l^{-1}) sequentially, thus preventing ex-vivo associations. Pre-loading snails with Si for 20 days abolished behavioural toxicity in response to subsequent Al exposure, and recovery from Al-induced toxicity was faster when Si was subsequently added to the water column. NMR spectroscopy showed a change in Al speciation in the DG in the presence of Si, while EELS demonstrated structural similarities of the Al-Si entities in the excretory granules of the digestive gland cells to allophane, an amorphous aluminosilicate . High resolution TEM with EDX and EELS elemental mapping showed co-localisation of Al and Si in DG granules. Oxygen mapped to the same regions as Al and Si, whereas P and S, common ligands utilised in metal detoxification, did not. These results support our hypothesis that Si is exploited endogenously by snails to specifically detoxify Al.

A2.11
Effects of metals on macroinvertebrate communities in upland streams of Northern England

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The effects of metal speciation on toxicity to aquatic organisms under laboratory conditions is well established and models, including the Biotic Ligand Model (BLM), have been developed to predict how toxic effects vary with water chemistry under laboratory conditions. There is now increased interest in applying the outcomes of the BLM and related models to setting safe concentrations of metals in the field. In order to address these issues, we sampled water chemistry water chemistry and benthic macroinvertebrate communities in 33 streams in Northern England in locations where historic mining is known to have occurred, and in locations where no mining has occurred and metal contamination is expected to be absent. The objectives of the work were to determine (i) whether field effects of metals on macroinvertebrate communities can be detected in the field, (ii) whether an approach based on the BLM is useful in explaining any field effects, and to consider the implications of the results are for setting metal Environmental Quality Standards for UK freshwaters. Expected numbers of macroinvertebrate taxa in the streams were predicted using RIVPACS. In eight sites the macroinvertebrate community was notably impoverished, with fewer than 60% of the predicted taxa actually found. To account for the effects of chemistry on metal toxicity a conceptual

'toxicity binding model' was developed, essentially a field version of the BLM. This model could be best fitted to the results by considering zinc, copper, lead and aluminium to be exerting toxic effects.

A2.12
Toxic Effects of Titanium Dioxide Nanoparticles and Carbon Nanotubes to Rainbow Trout (*Oncorhynchus mykiss)*.

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There is limited data on the ecotoxicity of nanomaterials to fish, and some of these materials are metals or contain large quantities of trace metals during manufacuture. We used a body systems approach to conduct a detailed toxicological investigation on the effects of single walled carbon nanotubes (SWCNT) and also titanium dioxide nanoparticles to rainbow trout. Dispersion and characterisation of nanoparticles was also performed. A semi-static test system was used to expose rainbow trout to either a freshwater control, solvent control, 0.1, 0.25 or 0.5 mg l^{-1} SWCNT for up to 10 days. SWCNT exposure caused a dose-dependent rise in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), and mucus secretion. No major haematological or blood disturbances were observed in terms of red and white blood cell counts, haematocrits, whole blood haemoglobin, and plasma Na^+ or K^+. Tissue metal levels (Na^+, K^+, Ca^{2+}, Cu, Zn and Co) were generally unaffected. Some dose-dependent changes in brain and gill Zn or Cu were observed. SWCNT exposure caused statistically significant increases in Na^+K^+-ATPase activity in the gills and intestine. Thiobarbituric acid reactive substances (TBARS) showed dose-dependent decreases in the gill, brain and liver during SWCNT exposure compared to controls. Total glutathione levels in the gills and livers also increased. Pathologies were observed in the brain and liver during SWCNT exposure. Stress-induced drinking also resulted in precipitated SWCNT in the gut lumen and intestinal pathology. For the titanium dioxide experiment, fish were exposed to control, 0.1, 0.5 or 1.0 mg l^{-1} TiO_2 nanoparticles for 14 days, and similar measurements to those above were made. Exposure to titanium dioxide nanoparticles caused some gill pathologies including oedema and thickening of the lamellae. No major haematological or blood disturbances were observed and tissue metal levels were generally unaffected. Some dose-dependent changes in tissue Zn or Cu were observed. Exposure to titanium dioxide nanoparticles caused statistically significant decreases in Na^+K^+-ATPase activity in the gills and intestine, and a trend of decreasing enzyme activity in the brain. TBARS increased in the gill, intestine and brain, but not the liver. TiO_2 particle exposure caused increases in the total glutathione levels in the gills, but depletion of hepatic glutathione. Pathologies were noted in the liver and intestine, but with limited effects in the brain.

A2.13
The effects of salinity on acute zinc toxicity to the euryhaline fish, *Fundulus heteroclitus*.

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Zinc is an essential element for most aquatic organisms; however, it may be toxic at elevated concentrations, particularly when exposed through the water. Presumably the ionic zinc (Zn^{2+}) form is the most toxic. It is well known that the toxicity of Zn^{2+} varies with water chemistry and that its bioavailability is controlled by ligand interactions and competing ions. A biotic ligand model (BLM) has been developed to predict the toxicity of zinc to aquatic organisms in freshwater. Although this model works well, minimal research has been directed toward development of a zinc BLM for use in marine and estuarine waters. We conducted a study using the euryhaline fish, *Fundulus heteroclitus*, to investigate the effects of changing salinity on acute zinc toxicity. *F. heteroclitus* (7-d old) were exposed to a range of zinc concentrations in six different salinities for 96 h and survival was monitored. Additionally, adult *F. heteroclitus* were exposed to zinc in three different salinity waters for 96 h. Physiological parameters such as plasma ion concentrations were measured to assess impairment. The overall goal of this study was to better understand zinc toxicity in different salinity

waters and to generate acute zinc toxicity data in a range of salinities, which may be used in development of estuarine and marine BLMs.

A2.14
Population Genetic Traits and Phenotypes of Brown Trout Inhabiting a Metal Contaminated River in North West Cornwall

C Durrant (King's College London), N Bury (King's College London), C Hogstrand (King's College London)

This study investigates the population genetic dynamics of brown trout *Salmo trutta* L. resident in the River Hayle (Cornwall, South West England). Due to metal influx from disused mine adits the river has elevated metal concentration (copper, zinc, lead, tin arsenic). A marsh region downstream of the adit acts as a "sponge" for waterborne metals, thus the river is divided into three sections; a pristine upper section a heavily contaminated middle section devoid of life and a lower section (below the marsh) with raised Cu and Zn levels. However, metal levels in the middle region still exceed the Environmental Quality Standards (EQS). Electrofishing surveys show that brown trout populations thrive both above and below the contaminated central region. It is our hypothesis that this region may potentially present a barrier to gene flow preventing genetic information being passed between upper and lower regions and resulting in a land locked upper region. Via the use of microsatellites for 9 loci, we will establish whether metal pollution effects genetic diversity and sub-population interactions and isolation.

A2.15
Copper accumulation kinetics in gills of the yellow clam *Mesodesma mactroides*

S Martins (FURG, Brazil), A Bianchini (FURG, Brazil)

Several physiological functions, such as growth, burrowing activity, metabolism and ionorregulation can be disrupted in clams exposed to high environmental copper concentrations. The aim of this study was to determine the kinetics of copper accumulation in the yellow clam *Mesodesma mactroides,* a bivalve playing an important ecological role in sandy beaches of Brazil, Uruguay and Argentina. Juvenile clams were acclimated (1 week) at fixed salinity (30 ppt) and temperature (20ºC) and then individually exposed (N=6 per treatment) to different copper concentrations (0.049 to 1.25 mg dissolved Cu/L) for 3 h. Kinetic parameters for gill copper accumulation was determined considering the Michaelis-Menten saturation model. Results showed that copper accumulates in gills at concentrations as lower as 0.197 µg dissolved Cu/L. Km and Vmax values were 0.039 µg dissolved Cu/L and 34.3 µg/g dry weight, respectively. These results, together with data for other marine invertebrates and vertebrates, will be applied in the Biotic Ligand Model (BLM) framework to calibrate a BLM version for marine conditions.

A2.16
DIETARY COPPER EFFECT ON REPRODUCTION OF THE EURYHALINE COPEPOD *ACARTIA TONSA*

M Lauer (FURG), A Bianchini (FURG)

Acartia tonsa is a cosmopolite euryhaline copepod often used in ecotoxicological studies. The main goal of the present study was to evaluate dietary copper effect on copepod reproduction. Both males and females acclimated to different salinities (5, 15, 30) were separately exposed to dietary copper (algae) for a 12-h period a day. The diatom *Thalassiosira weissflogii* cultivated in different salinities (5, 15, 30*)* was pre-exposed to copper (160 µg/L) for 24 h and used as food source. After copper exposure, copepods were transferred and kept in a new experimental medium without food for 12 h. A control treatment for each salinity (copepods fed non copper contaminated algae for 12 h) was also tested. After 6 days of copper exposure, couples (3♀:1♂) were formed with the surviving copepods and fed with non copper contaminated algae. Eggs and nauplii generated over 24 h were counted. Compared to the respective control, a reduction of 39.0, 40.4 and 40.5% in egg production was registered for salinities 5, 15 and 30, respectively. These results show that dietary copper has a significant effect on copepod reproduction and that this effect is not affected by salinity at the copper exposure condition tested. These findings can be

useful in the scope of the development of a Biotic Ligand Model for estuarine and marine conditions. Financial support: International Copper Association.

A2.17
Phenoloxidase activation detected during the embryonic development of the European Cuttlefish, *Sepia officinalis* and response to Ag and Cu exposure

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In the present work, we examined the phenoloxidase (PO) activity and the activation of the prophenoloxidase (proPO) system by several modulators at different developmental stages of the egg and embryo of the cuttlefish *Sepia officinalis* (Linnaeus). The study of PO-like activity was driven during the last stages of the embryonic development (26 to 30 according to Lemaire (1970)) on 1) eggs placed in natural seawater (controls) and 2) eggs exposed to dissolved Ag (1 $\mu g.l^{-1}$) and Cu (250 $\mu g.l^{-1}$). Detection of PO-like activity was carried out by measurement of L-Dopa transformation in dopachromes. Transformation was monitored by UV spectrophotometry at 490 nm on 96 well microplates. Purified trypsin, laminarin and bacterial lipopolysaccharides were tested as activators of PO activity at different concentrations (0.1, 1 and 10 mM). As well as, several PO inhibitors phenylthiouera (10 mM), b-2-mercaptoethanol (3.5 mM), sodium diethyldithiocarbonate (10 mM), ethylenediaminetetraacetic acid (EDTA) (5 mM) and tropolone (16 mM). The L-Dopa transformation was higher for the final developmental stages than the earlier ones. Modulators effects varied between the stage 26 and 30 of Lemaire suggesting a change of the PO protein. Ag exposure led to an inhibition of the PO-like activity whereas Cu induced an activation of the L-Dopa transformation during the first 10 days of exposure, and then, an inhibition until the hatching. Ag reduces the PO activity as soon as it penetrates within the egg. In contrast, Cu first activated the PO expression during 12 days days, then, it produced its inhibition.

A2.18
Metal induced oxidative stress in *Chlamydomonas reinhardtii*

I Szivak (EAWAG, Swiss Federal Inst. of Aquatic Science and Technology), L Sigg (EAWAG, Swiss Federal Inst. of Aquatic Science and Technology), R Behra (EAWAG, Swiss Federal Inst. of Aquatic Science and Technology)

The toxic effects of heavy metals appear to be partly related to the production of reactive oxygen species (ROS), which can cause oxidative damage to cells. This study assessed metal induced ROS production in the fresh water alga *Chlamydomonas reinhardtii* by using an optimized fluorescence assay, which allowed for quantifiable ROS detection using flow-cytometry (FCM).
The ability of essential and environmentally significant metals to induce ROS production was examined. *C. reinhardtii* were exposed for 2.5 h to redox active metals, such as Fe^{3+}, Cu^{2+}, Ag^{+}, Cr^{3+}, Cr(VI), and the metalloid arsenic(III) and (V), and non-redox active metals, such as Zn^{2+}, Pb^{2+} and Cd^{2+}. ROS production was measured by FCM as a function of metal concentration under controlled speciation. The DFFDA dye (2',7'-difluorodihydrofluorescein diacetate) was used as a cell-permeable indicator for ROS, which becomes fluorescent in presence of a wide variety of ROS including hydroxyl radicals ($OH^{\cdot}$).
As expected, redox active metals increased the intracellular ROS level, which can be explained by their ability to catalyse directly $OH^{\cdot}$ radical production via the Fenton and the Haber/Weiss reaction. The effective free metal ion concentrations ranged from 10^{-6} to 10^{-8} M except in the case of Fe^{3+}, which was effective at lower concentrations (10^{-14} M).
Results indicate that non-redox active metals also increased ROS at a similar free ion concentration range as most redox active metals. However, these metals may have indirectly increased the level of ROS. Possible mechanisms may include interactions with anti-oxidant defence systems and with ROS producing physiological processes.

A2.19

Effects of Mercury on Na^+,K^+-ATPase intensity and activity in the Branchial Cavity Organs of *Palaemon elegans* (Decapoda, Crustacea)

S Khodabandeh (Tarbiat Modares University), R Talaiy (TMU)

The toxic effects of mercury on human and animal systems are well documented; however, there have been a limited number of studies that examined the effects of mercury on the osmoregulatory enzymes of crustacean. Na^+,K^+-ATPase is an enzyme embedded in the cell membrane and responsible for the active transport of sodium and potassium ions. In the present study, the effects of mercury on Na^+,K^+-ATPase intensity and activity were investigated in the branchial cavity organs of *Palaemon elegans,* caught from the Caspian Sea. The experiment began with the transfer of samples from the stock tank to the 6 experimental aquariums (3 control group and 3 aquariums containing 50 µg $HgCl_2.L^{-1}$). The study was performed through immunofluorescence light microscopy by using a mouse monoclonal antibody $IgGa_5$.

In the control group, very strong Na^+,K^+-ATPase immunofluorescence cells (ionocytes) were detected in two layers of the epipodites epithelium and the branchiostegite inner epithelium. A weak immunofluorescence also observed on the gill lamellae. Na^+,K^+-ATPase enzyme intensity and activity were also significantly higher in the branchiostegite and epipodites compared to gill lamellae. Na^+,K^+-ATPase intensity and activity decreased significantly from controls to mercury exposed *Palaemon.* Mercury exposure decreases *P. elegans* osmoregulatory capability by increases levels of necrosis and apoptosis of immunofluorescence cells and disturbs the Na^+/K^+ pump. The results are discussed in relation to effects of heavy metal contaminations on osmotic and ionic regulation in crustacean.

A2.20

Tissue accumulation and toxicity of mercury in the golden grey mullet, *Liza aurata*, fry

S Khodabandeh (Tarbiat Modares University), E Ghanizadeh Kazerooni (TMU), M Shahriyari Moghaddam (TMU), A Esmaili Sari (TMU)

Heavy metals pollution and their bioaccumulation potential in aquatic organisms present a growing risk for wildlife and humans. The osmoregulatory system provides the most extensive interface of a fish with water dissolved pollutants. Tissue accumulation and toxicity of mercuric chloride were investigated in mullet fry (1.5-2 g), exposed to different concentrations of mercuric chloride (0, 50, 100 and 350 ppb) for 96 hours. No mortality was observed on 0, 50 and 100 ppb exposed groups following 96 hours. Hg concentration was 549.5, 1893.1 and 3364.7 ppb in the gills of control, 50 and 100 ppb groups, respectively. Mass mortality observed in 350 ppb exposed group following 2 hours and Hg concentration in the gills was 15300.2 ppb. In all mercuric chloride treated groups, lifting epithelial cells from lamellae, lamellae fusion and hyperplasia on lamellae surface were observed. There was a tendency of gill tissue recovery in 50 and 100 ppb groups after 24 hours. Fishes exposed to 350 ppb also showed gills cells necrosis. The kidneys are also disorganized and tubule cells decrease in number and change in size. Glomerulus's capillary dilation and vacuoleation of the proximal tubule cells of kidney were observed in the all mercury treated groups.

A2.21

Histopathological and Pathomorphological Effects of Mercuric Chloride on the Gills of Persian Sturgeon, *Acipenser persicus*, Fry

Z Khoshnood (Tarbiat Modares University), S Mosafer (TMU), M Shahriyari Moghaddam (Zabul University), S Khodabandeh (TMU)

Mercury contamination remains as a severe problem in both fresh and sea water fish. The toxic effects of mercury on fish are multidirectional and manifested by numerous changes in the morphological, physiological and biochemical processes of their body systems. Fish gills are the most important organ for the uptake of inorganic mercury. *Acipenser persicus*, young stages (2-3 g) were exposed to freshwater contain 15 ppb of mercury chloride ($HgCl_2$). Following 48 hours exposure, the

gills of six samples were removed and fixed for histological and ultrastructural studies. Light microscopic and scanning electronmicroscopic analyses showed a higher occurrence of histophathological lesions such as epithelial lifting, lamellae fusion and lamellae surface hyperplasia. Gill epithelial showed occasional necrosis which is almost complete and blood comes out of the capillaries. Ultrastructuraly, we observed mucous and chloride cells deformation, numerous apical vesicles appearance in the chloride cells, mitochondria and endoplasmic reticulum swollen and lesions. We conclude that, exposure of *A. persicus* to this concentration of mercury first stimulate chloride cells activity to mercury removal from the cytoplasm and the inhibitory interactions by involvement of mitochondrial and endoplasmic reticulum and finally caused gill damage that can interferes with ion and osmoregulation processes.

A2.22

The effects of soluble pollutants from highway runoff on UK fish and invertebrates.

N Bury (King's College London), R Hurle (King's College London), T Bark (King's College London), S Mustow (White Young Green), P Whitehouse (Environment Agency), M Whitehead (Highways Agency)

Monitoring and control of pollution from diffuse sources is difficult, but the EC Water Framework Directive (2000/60/EC) requires operational objectives to be set in all river basins to achieve 'good status' of surface waters. Run-off from roads contains a mixture of chemicals, but the ecological impact of run-off in receiving streams is unclear. The current study, funded by the Highways Agency, will present results from our tests to determine the toxicity of various soluble pollutants identified in run-off zinc, copper, cadmium, aluminium, salt, potassium acetate, ammonia, cyanide, pyrene, fluroanthene, diesel, crank case oil, glyphosate and diuron)] on endogenous invertebrate (*Gamarus pulex, Hydropsyche siltalai, Chronomus riparius, Lymnaea pereger, Spharium corneum, Eropdella octoculata and Baetis rhodani*)and fish (Brown trout, Bullheads, Minnow, Roach, and Sticklebach). The aim is for the toxicity test results to be linked to a predictive model for in pipe chemical concentrations based on traffic-flow, carriageway drainage area, and rain event to the dilution in the receiving waters and effects that concentration will have on the biota representative of the receiving water type. The model will thus be site-specific.

A2.23

Bioaccumulation, histopathology and weight changes in the blue crab, *Callinectes amnicola* exposed to sublethal doses of heavy metals.

A Otitoloju (University of Lagos), O Elegba (University of Lagos), A Osibona (University of Lagos)

On the basis of the 96-h LC_{50} values, Copper with LC_{50} value of 0.018mM was found to be 2 times more toxic than Lead (0.041mM) against the lagoon crab, *Callinectes amnicola*. The exposure of the lagoon crab to sublethal concentrations (1/100th and 1/10th of 96-h LC_{50} values) of Cu and Pb compound respectively, resulted in the bioaccumulation of the test metals to varying degrees in the selected organs that were dependent on the type of metal and concentration of metal compound in the test media. The degree of metal (Cu and Pb) accumulation was generally in the following order: gills > flesh > heptopancrease. Exposure of the crabs to sublethal concentrations of the metals also caused varying pathological changes such as the disruption of the gill filament and degeneration of glandular cells with multifocal areas of calcification in the hepatopancreas. A reduction in the weight of the exposed animals over a 14 day period of observation was also recorded. The significance of these results and the need to use *C. amnicola* in monitoring programmes aimed at establishing the total environmental level of heavy metals in aquatic ecosystems were discussed.

A2.24

Assimilation of silver, zinc and cadmium from natural metal-contaminated diets by zebrafish

D Boyle (King's College London), C Hogstrand (King's College London), N Bury (King's College London)

In this study we investigated the assimilation efficiencies (AEs) of three metals (Ag, Cd and Zn) from *Nereis diversicolor*, a polychaete worm, to zebrafish (*Danio rerio*) during a short term exposure to a natural metal-contaminated diet. Two populations of *N. diversicolor* which exhibit contrasting metal partitioning strategies and tissue metal concentrations were fed to zebrafish for 21 days. On days 0, 7, 14 and 21 of the dietary exposure zebrafish were isolated and fed radiolabelled *N. diversicolor* (Ag, Cd and Zn (Day 0 only)) to assess metal AEs. Calculated AEs were 1-6% for Ag, 8-16% for Cd and 33-46% for Zn. There was no significant difference in AEs of metals between zebrafish fed *N. diversicolor* from either site or a significant effect of previous dietary metal exposure. Results are discussed with regards to metal accumulation at the intestine of zebrafish and the expression of metal specific transporters (e.g. Ctr1, DMT1) and metallothionein in the intestine during short term dietary metal exposure. Thus, dietary uptake of Ag, Cd and Zn in zebrafish from *N. diversicolor* was independent of pre-exposure and/or metal partitioning in the worms.

A2.25
Induction of metallothionein in Atlantic Limpet *Patella* ulyssiponensis (Gmelin, 1791) after exposure to mercury

A Cardoso (Unidade Biotecnologia Ambiental), A Mota (Centro de Química Estrutural), J Santos Oliveira (Unidade Biotecnologia Ambiental)

Metallothionein (MT) is a metal-inducible protein a crucial role in detoxification process of certain metals (Cd, Cr and Hg). Specimens were collected in the Portuguese coast from April to July 2006. The limpets were contaminated with 20 and 100 mg $Hg.L^{-1}$ for six days. Hg in the total homogenate tissues as well as Hg and MT in the cytosol fraction increased with the contamination level, following the order gills > digestive gland = gonads > foot. Cytosolic Hg and MT relation was significant ($p<0.05$) on all tissues after exposed to 20 and 100 mg $Hg.L^{-1}$. The Hg and MT concentration were founded in gills suggested if we excluded dietary this are the most important tissues for the Hg accumulated. An experimental linearly correlation was obtained between the MT and the Hg content in the cytosol, independently of the organ and the Hg concentration. Our results are dependent of Hg concentration. The results showed that MT in the limpet P. ulyssiponensis response to Hg concentrations.

A2.26
The Metabolic Cost of Resistance to Copper in *Nereis diversicolor* Measured As Scope for Growth

C Pook (University of Plymouth), C Lewis (University of Plymouth), T Galloway (University of Plymouth)

The Scope For Growth [*SfG*] assay was developed to integrate the diverse physiological responses of bivalve molluscs to stress through an assessment of the balance between their energy intake, uptake and expenditure. This assay has the advantage of integrating effects at the sub-individual level into an individual-specific index of health with the potential for extrapolations from this index to predict higher order effects upon the population. Here the technique has been adapted for application to the common euryhaline polychaete, *Nereis diversicolor*. A population of this species found at Restronguet Creek in the Fal estuary in the South West of the United Kingdom is shown to have developed resistant to acute copper toxicity and the energetics of this population are compared and contrasted here with the natural variability in S*f*G of populations from sites relatively free from anthropogenic contamination. The S*f*G assay revealed significant differences in resting metabolic rate and in food intake between the three control populations and the resistant population but no overall difference in S*f*G. This suggests that there is an energetic "cost" associated with the resistance phenomenon that must be met through increased acquisition of energy in the form of food. Despite this burden, health of the resistant population- measured as S*f*G- is unaffected overall, suggesting that the trade-off between acquisition and expenditure of energy that is entailed is not detrimental to the health of the individual. The mechanisms of resistance to copper toxicity are discussed with reference to their effect upon physiological energetics.

A2.27

Accumulation of proline concentration, carbohydrates and lipid peroxidation in helianthus shoots-leaves as affected by Mn toxicity and hormones

A Giannakoula (Department of Crop Production, Technological Educational Institute of Thessaloniki, PO Box 141, Sindos 54100, Greece), I Ilias (TECHNOLOGICAL INSTITUTE OF AGRICULTURE)

The objective of this research was to investigate the effects of metal toxicity and growth retardants Prohexadione-Calcium on the growth and physiological parameters of helianthus (*Helianthus annus* L.) grown under a growth chamber environment. Main stem length of helianthus plants decreased in a quadratic pattern as the concentration of Mn and Prohexadione-Ca increased. High concentrations (200 mgl^{-1}) resulted in shorter plants than control plants. The application of 200 mgl^{-1} of Mn resulted in diminution of the leaf chlorophyll concentration, in both cultivars and also, significantly affected variable fluorescence Fv, maximum quantum yield of photosystem II (PSII) photochemistry (Fv/Fm), and the others chlorophyll fluorescence parameters (Fo, Fm, Fv/Fo). Since chlorophyll content and and variable fluorescence decreased significantly but Fo did not change significantly the decrease of $t_{1/2}$ indicates negative changes on the acceptor side of PSII, which also may related to the diminution of the Calvin cycle. Furthermore, high accumulation of proline concentration, carbohydrates and lipid peroxidation was induced after exposure of plants to Mn treatment.

A3 - Metal Nutrition

A3.1
Mineral Prospecting in Fish-Are Requirements Underestimated?

S Davies (University of Plymouth)

There is a paucity of information concerning mineral nutrition to enhance the health and welfare of farmed fish. This presentation embraces new developments and applications in the applied fish nutrition of trace elements in aquaculture to meet intensive production demands.
Due to increasing growth rates and production related stress type diseases, there is growing evidence of morphological deformities, reduced immuno-competence and disease resistance in various fish species due to reduced macro and trace-elemental status. Particular interest has focused on phosphorous (P), zinc (Zn), and selenium (Se) in aqua feeds from inorganic and organically complexed sources.
The bioavailability of P from raw materials and purified sources can vary appreciably causing changes in tissue and whole body levels with dependence on P digestibility from the gastro-intestinal tract. Investigations report P digestibility and net retention for rainbow trout fed graded levels of P inclusion in experimental diets.
Research findings for trout report differences in the fate of zinc and selenium from inorganic and organic sources producing differences in their relative partition in tissues and organs. Marker enzymes such as alkaline phosphatase as a bio-indicator of Zn levels in rainbow trout have been measured and compared.
The importance of selenium as a vital component in the cellular antioxidant defence mechanism of fish is also of primary concern. Selenium status in trout fed natural selenoproteins and inorganic selenite, and relationship to glutathione peroxidase and also thioredoxin reductase activity in selected tissues has been determined. Their significance to homeostatic regulation, physiological function and importance to fish health is stated.

A3.2
Trophic transfer and dietary toxicity of Cd from oligochaetes to rainbow trout

T Ng (McMaster University)

The oligochaete *Lumbriculus variegatus* was exposed to aqueous Cd at 0, 5, 20 and 200 mg l^{-1} for a week, then fed to the rainbow trout *Oncorhynchus mykiss*. After a month of dietary exposure to Cd, the trout accumulated 1.43 ng g^{-1} to 395 ng g^{-1} Cd in the whole bodies, with 80 % in the gut, followed by carcass > liver = kidney > gill. Cd concentration increased in the kidney and liver over time in the two highest Cd exposed fish, but it was constant in the other organs. Ca influx from the water was unaffected by the dietary Cd treatments, but growth of the fish exposed to the highest Cd was reduced 50 % in the later weeks of exposure. Despite of that, the fish was efficient in detoxifying Cd by storing Cd in the metallothionein-like proteins of the gut. This study demonstrated a greater Cd retention (1 – 5 %) and sublethal toxicity in the rainbow trout from the biologically incorporated diet than from commercial diets in previous studies. Trophic transfer of Cd to trout apparently did not relate to the percentage of Cd in subcellular fractions of the oligochaetes (funded by NSERC CRD grant, ICA/CDA, NiPERA, ILZRO/ IZA, Noranda-Falconbridge Inc., Teck-Cominco, Inco.).

A3.3
The effects of a natural metal-contaminated diet on metal bioavailability and reproduction in fish

D Boyle (King's College London), C Hogstrand (King's College London), A Lundebye (NIFES), H Amlund (NIFES), K Brix (EcoTox), N Bury (King's College London)

In this study we investigated metal uptake and toxicity through the food-chain in fish fed a natural metal-contaminated diet. Zebrafish were fed *Nereis diversicolor*, a polychaete worm, collected from a metal-impacted region with elevated As, Cu and Zn levels for 68 days. Reproductive output was reduced in zebrafish fed the high metal diet: cumulative

number of eggs produced and cumulative spawning events were reduced by 48% and 30% respectively, compared to controls. mRNA expression levels of vitellogenin, an egg yolk protein, were also reduced whereas metallothionein, an indicator of Cu and Zn exposure, was unchanged. Reproductive impairment in zebrafish was associated with elevated tissue As concentrations. No significant Cu or Zn accumulation was observed in fish fed the high metal diet. Chemical analysis of arsenic content and speciation revealed markedly different As handling strategies in *N. diversicolor* from different geochemical regions. Inorganic arsenicals were the dominant species in *N. diversicolor* (58%), a metal-impacted site, but accounted for <1% of total As in control *N. diversicolor*. This suggests that reproductive impairment in zebrafish fed the metal-enriched diet was caused by As accumulation and toxicity and metal speciation is integral to determining dietary metal bioavailability and toxicity in fish.

A3.4
Essential and non-essential metals in feed ingredients and their consequence for farmed fish.

A Lundebye Haldorsen (NIFES), H Amlund (NIFES), M Berntssen (NIFES)

Farmed fish rely on formulated feeds that cover their nutritional requirements to support optimal growth rates. Feeds are formulated to contain adequate levels of essential metals using mineral mixes to supplement the levels present in the feed ingredients. In addition to containing essential metals, feed ingredients have background contamination of non-essential metals that may occur naturally, or be due to environmental pollution. Marine feed ingredients typically contain different pollutants, for example fish oils contain lipid soluble persistent organic pollutants, in addition to lipid-soluble forms of elements such as arsenic (as arsenolipids). The main source of non-essential metals in fish feed is from fish meal, which can contain considerable levels of arsenic, cadmium, lead and mercury, primarily bound to protein. Contaminated mineral mixes have also been shown to affect cadmium concentrations in fish feed, but not reflected in edible fish tissue. Data will be presented on levels of non-essential and essential metals in feed ingredients and feed, and the potential consequence for feed and food safety will be addressed.

A3.5
The Genotoxicity of UV light in EPC A1 cell while in the presence of organic, inorganic and nanoparticulate* Zinc, an in vitro approach. *Results pending

D Leeming (University of Plymouth), A Jha (University of Plymouth), S Davies (University of Plymouth)

This study aimed to investigate the effect of Zinc supplementation in different forms; organic, inorganic and nanoparticulate, on the genotoxicity of UVA and UVB to carp epithelial cells. Zinc, being redox inert and capable of exhibiting variable co-ordination numbers, bonds non-specifically and stably to a wide range of compounds. It is utilized as both a structural and functional prosthetic group in many enzymes, some of which i.e. superoxide dismutase (SOD) or metalothionein, are known free radical scavengers. The results show a significant decrease in DNA single strand breaks in cells supplemented with both organic and inorganic Zinc for both of the UV exposures compared to the non zinc supplemented cells. Indicating that zinc supplementation allows cells to resist or repair the damage caused by UV. Furthermore, the different forms of zinc generated different levels of damage remediation as DNA damage in cells supplemented with the organic form reduce DNA damage to a greater extent than the inorganic form. UVA exposed cells display comparable trends, with a negative correlation between the Zinc concentration and % tail DNA. The evident effect of Zinc supplementation could not be explained by increases in SOD activity however the activities of numerous zinc dependant enzymes including metalothionein or polymerases still need to be investigated in order to deduce whether the cells are either resisting or repairing damage. The implications of these results and of any further investigations provide justification for carrying out whole fish trials and are potentially relevant for dermatological pharmaceutical/cosmetic research into antioxidant activity.

A4 - Metal Speciation

A4.1
Kinetics of internal and external metal binding: explanations for differences in availability and toxicity of metals across species.

R Blust (University of Antwerp)

The accumulation and toxicity of metals by aquatic and other organisms is function of the external and internal bioavailability of the metals The external availability determines the rate of metal uptake and the internal availability the potential to interact with different types of ligands and binding sites. The higher the internal availability of a specific metal the more likely it is that toxic effects will occur. The internal availability depends on the rate of metal uptake and the rates of internal complexation and elimination from the organism. The external availability of metals strongly depends on the environmental conditions and the functional organisation of the exchange surfaces. This explains why the effects of environmental conditions on metal uptake explain much of the variation in metal accumulation and toxicity on a species specific level. The internal availability strongly depends on the internal organisation of the organisms and the way metals are being processed. This is why metal sensitivity varies widely among species even under comparable exposure conditions. The present work explores to what extend differences in external and internal availability contribute to the observed differences in metal accumulation and toxicity within and among species with special emphasis on the kinetics of the processes. The meaning of metal body and tissue concentrations in terms of external and internal bioavailability is evaluated. A kinetic model is used to show under which conditions a relationship between external exposure, accumulation and toxicity can or cannot be expected for different types of organisms.

A4.2
Cyanobacteria in a freshwater reservoir – does metal concentration influence bloom occurrence?

M Baptista (CIIMAR)

Environmental assessments considering potential impacts of toxic agents should take into account the overall status of the aquatic environment.
In this work we analysed the metal content of Torrão reservoir (north Portugal), at two different locations: Marco de Canavezes - seasonally subjected to toxic *Microcystis aeruginosa* blooms – and Amarante, where no blooms have been recorded. Particulate and total dissolved Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn, as well as sediment metal content, were monitored monthly, throughout two years.
The bloom occurrence affected the reservoir metal speciation. The overall sediment metal content in Marco was higher than in Amarante. Sediment quality guidelines (SQGs) show that the average metal concentration in Torrão is not likely to be harmful, but during the blooming, Cu and Pb sediment content was higher than the threshold effect concentration, indicating possible harmful effects. For both particulate and dissolved metal in the water, the overall water metal content in Amarante is higher than in Marco.
Scum-forming and individual-colonies-forming blooms yielded different results, with the first occurring simultaneously with a significant increase of metal levels (Cu, Fe, Ni and Zn), in both water and sediment. Concentration of strong organic ligands (evaluated as Cu-binding ligands) significantly increased during both types of blooming. The change in the metal content was attributed to the influence of cyanobacteria on the metal bioavailability. Such changes were locally and temporarily confined to the bloom event.

A4.3
Uptake of Platinum Group Elements by the Marine Phytoplankton *Chlorella stigmatophora*

L Shams (School of Earth, Ocean and Environmental Science, University of Plymouth), A Turner (School of Earth, Ocean and Environmental Science, University of Plymouth), M Brown (School of Biological Science, University of Plymouth), G

Millward (School of Earth, Ocean and Environmental Science, University of Plymouth)

Little information is known about the accumulation of platinum group elements (PGE: Rh, Pd and Pt) by primary producer organisms in aquatic systems. This study aims to establish the nature and extent of uptake of PGE by the marine phytoplankton, *Chlorella stigmatophora*. Algal cells were cultured in media with and without nutrient trace metals and EDTA, and were spiked with PGE concentrations of 5 – 30 µg L^{-1} in the pH range 6.5 – 8.5 and incubated for 24 h before being isolated by filtration. Filtrates, EDTA-washed and acid-digested algae were analyzed by ICP-MS to discriminate aqueous, adsorbed and internalized PGE. Uptake by the algae followed the order Rh > Pd >> Pt and could be explained, at least qualitatively, by what little is known about the aqueous speciation of these elements in sea water. Palladium showed the greatest tendency to be internalized by the algae (up to 90% of total sorbed metal), compared with Rh (up to 67%) and Pt (up to 39%). Uptake of Rh was most sensitive to pH and to the presence of competing and complexing nutrient metals. The results are discussed in terms of the availability, transport and fate of these metals in aquatic systems.

A4.4
Metal bioaccumulation, distribution and storage strategies of *Gammarus pulex* exposed to a copper and zinc contamination gradient

F Khan (King's College London), N Bury (King's College London), C Hogstrand (King's College London)

This study investigated whether organisms from a metal contaminated watershed (Cu^{2+} and Zn^{2+}) exhibited differing metal handling strategies when compared to previously unexposed organisms. An experiment was designed in which *Gammarus pulex* collected from a metal clean site (River Cray, Kent) and the upper reaches (Drym) of the River Hayle, Cornwall, a river in a region with a history of mining, were transplanted to four sites along the R. Hayle that exhibit a metal gradient. Organisms were sampled at t=0, 1, 2, 4, 16 and 32d post transplantation. In both sets of gammarids the heat stable cytosolic fraction and the insoluble mineralized or granular fraction contained greatest concentrations of copper and zinc. These two fractions were further investigated to determine metal detoxification strategies. SEM/x-ray microanalysis confirmed the presence of calcium-phosphate rich granules present in organisms from the Cray transplanted to metal rich area for a period of 16 days. These granules contained trace concentrations of copper. Gel filtration chromatography showed copper and zinc eluting with a 7.5kDa protein suggesting the induction of a metal-binding protein. Marked increase in total copper and zinc metal body burden were observed between organisms transplanted from the Cray compared to the Drym gammarids at sites with elevated metals. Results suggest that within the same species differing metal handling strategies may exist depending on prior exposure history – the *G. pulex* population originating from a region with elevated background metals accumulated less metal.

A4.5
Intracellular metal speciation and the trophic transfer of metals in invertebrates

P Rainbow (Natural History Museum)

Aquatic invertebrates take up trace metals both from solution and from food, and the latter route can be a very significant contributor to total metal uptake. The assimilation efficiencies of different invertebrates for different trace metals from different foods vary greatly and there is a need to seek general principles underlying this variation. One important factor is the form of chemical binding (speciation) of trace metals in the food. Operational schemes for the subcellular fractionation of accumulated trace metals typically allow the identification of up to 5 subdivided metal-binding fractions including both soluble and insoluble forms. Attempts have been made to relate the assimilation of trace metals by invertebrates to one or more specific fractions, as a basis for understanding the principles controlling the uptake of metals from the diet. Results are presented on the assimilation of trace metals by filter-feeding herbivores from phytoplankton, by a decapod crustacean from polychaetes, and by a gastropod mollusc from bivalves to this end. As might be predicted, variation in the biology of prey and predators prevents simple generalisations, but still

casts light on the factors affecting metal uptake from the diet.

A4.6
Differential bioaccumulation behaviour of nine radiotracers (^{54}Mn, ^{60}Co, ^{65}Zn, ^{109}Cd, ^{110m}Ag, ^{134}Cs, ^{203}Hg, ^{210}P

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Cuttlefish eggs were exposed to ^{54}Mn, ^{60}Co, ^{65}Zn, ^{109}Cd, ^{110m}Ag, ^{134}Cs, ^{203}Hg, ^{210}Pb, and ^{241}Am dissolved in seawater and then placed in depuration conditions in order to assess their uptake and loss kinetics and their corresponding distributions among the eggcompartments. With the exception of ^{134}Cs, all the tracers were efficiently taken up, reaching load/concentration factors (LCR) ranging between 150 and 1700. Radiotracers activities increased in the eggs with time except for ^{54}Mn and ^{109}Cd, showing a saturation steady-state and decreased, respectively, after one month of development. Surprisingly, ^{134}Cs uptake kinetics followed the egg weight kinetic, suggesting it was incorporated into the eggs with water. Interestingly, ^{60}Co, ^{109}Cd, ^{210}Pb, and ^{241}Am were mainly associated to the eggshell during the whole experiment, indicating that the eggshell acted as an efficient shield against their penetration all along the embryonic development. In contrast ^{54}Mn, ^{65}Zn, ^{110m}Ag, ^{134}Cs and ^{203}Hg crossed the eggshell after one month of development. Since this time on, these elements were accumulated in the embryo which contained up to more than 40% of the whole egg ^{110m}Ag burden at the end of the exposure period (day 50). In depuration conditions, it is noteworthy that ^{54}Mn, ^{65}Zn, ^{110m}Ag, ^{203}Hg activities continued to increase in the embryo despite that no radiotracer source was available any longer. This suggests that the metal was progressively translocated from the eggshell to the embryo. Our experiments showed that the shell of cuttlefish eggs have selective properties against trace element penetration and incorporation leading to different uptake kinetics of these metals.

A4.7
Trace metals in aquatic systems impacted by iron mining and ore processing in Brazil: geochemistry, bioavailability and bioaccumulation

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The impact of mining to watersheds depends mainly on the type of the mining, processing of ores and environmental factors. This study aims to assess the environmental impact from iron mining and related activities based on local geochemical conditions and fate of iron and trace metals in water resources, their bioavailability and potential bioaccumulation in aquatic organisms. Fe, Mn, Al, Cr, Zn, Cu, Cd, As, Hg, Ni and Pb were determined in water, sediments, benthic invertebrates and fish from tailing dams, rivers (Doce River basin) and from a coastal lagoon (Mãe-Bá Lagoon) influenced by an iron mining in Brazil. Sediment characteristics and water physicochemical parameters were also measured. Normalization of sediment data with Clay+silt content was applied to identify the enriched sites and the anthropogenic sources. Trace metal bioavailability was assessed by a water speciation model (CHEAQS) and AVS (acid volatile sulphide) analysis. Results of the monitoring studies are presented and the outcome of a multivariate statistical analysis are used to explain relationships between anthropogenic sources, abiotic and biotic compartments, bioavailability and bioaccumulation.

A4.8
The impact of salinity on the uptake rate of Cd, Cu, Ni, Pb and Zn in the European sea bass

N Celis (University of Antwerp), G De Boeck

(University of Antwerp), R Blust (University of Antwerp)

The amount of free metal ions available in the water and thus the interactions between the metal and aquatic organisms can be influenced by several geochemical factors such as competition and organic or inorganic complexation which are key determinants for waterborne metal uptake in saltwater environments. However for marine and estuarine environments results are rather scarce and not so straightforward as they seem to be for freshwater systems. The aim of this study was to simultaneously determine the influx of Cd, Cu, Ni, Pb and Zn in the European sea bass (*Dicentrarchus labrax*, L.) thereby using a stable isotope technique which is fairly new in the field of ecotoxicology and to assess the effect of salinity on these metal uptake rates. Sea bass are euryhaline teleosts and common predators in estuarine systems that are characterized by the occurrence of salinity gradients. First, fish were acclimated to 3 different salinities of 1, 12 and 35 ppt representing hypo-, iso-, and hyperosmotic conditions, respectively. Following the acclimation period bass were exposed to environmentally realistic metal concentrations for 24 h after which influxes were determined by means of quadrupole ICP-MS. The results show the effect of salinity (bioavailability and competition) on metal uptake rates and are analysed within a risk assessment perspective for the further development of site specific metal effects models.

1 - Metal Chemistry

1.1
Dynamic speciation and bioavailability of metals in aqueous systems

R Town (University of Southern Denmark)

Metal biouptake is an interfacial process involving consumption of a given species, often the free metal ion. Many studies report that metal biouptake is well described by equilibrium modeling (e.g. FIAM, BLM). Predictions of biouptake are subsequently derived from equilibrium-based computations of the free metal ion concentration, with other metal complex species considered not bioavailable. However, more often than not the metal concentrations employed are sufficiently high such that the unsupported flux of free metal ion exceeds the maximum biouptake flux. That is, the possible contribution of metal complex species to the uptake flux is never called into play. Dynamic analysis reveals that the FIAM applies only if (i) mass transfer towards the biosurface is not flux-limiting and (ii) depletion of the medium is negligible. These conditions may not always be met at low, environmentally relevant, free metal concentrations, or in biofilms in which depletion of the medium may easily occur with larger species. Considerations on the impact of metal speciation on bioavailability must be concerned with kinetics of complex formation/dissociation reactions. Lability describes the ability of complexes to restore equilibrium with the free metal, or any target species. On the level of an interfacial flux, lability derives from volume reaction rate constants as compared to the diffusion rate at the relevant time scale. It will vary with the mass transport conditions, spatial scales and time. Several different dynamic speciation analysis techniques are available to probe metal complexation kinetics. Deployment of a suite of techniques may provide different measures of labile complexes. For similar reasons different organisms may experience different bioavailabilities in one and the same medium.

1.2
Dynamic metal sorption by charged polysaccharides

E Rotureau (Wageningen University)

The dynamics of metal sorption by a gel-like polysaccharide is investigated by means of the electrochemical technique of stripping chronopotentiometry at scanned deposition potential (SSCP). The measured response reflects the flux properties (limiting diffusive or kinetic fluxes) of metallic species towards a macroelectrode and allows the determination of their stability constants and lability features in aqueous solution. The soft colloidal ligand studied here is a functionalised carboxymethyl-dextran which has been well characterised in terms of its electrohydrodynamic properties as function of pH and ionic strength. This study reveals a time-dependence of the metal ion complexation that was not observed in the case of hard-particle complexing agents (carboxylated latex nanospheres). The decrease of the SSCP signal with time after addition of the ligand to the metal solution identifies strong differences in the dynamic nature (lability) of the successive metal complexes formed. This decrease is relatively fast during the first minutes (formation of 1:1 complexes), followed by an ongoing gradual change over hours (slow formation of complexes with higher coordination number). Apparently, the second class of complexes requires a slow conformational reorganisation of the macromolecule that probably becomes the limiting step in the multidentate complexation reaction. The data allow for the determination of the relevant parameters for the metal adsorption and release kinetics constants and thus provide knowledge of the time-dependent stability and lability of metal species in biogels.

1.3
Metal speciation modelling for environmental biologists: perspectives from a geochemist

A Turner (University of Plymouth)

In both biology and geochemistry, it is generally assumed that the free aqueous ion of a metal is the principal, and sometimes only, reactive species, a

premise that is the foundation of many practical and theoretical models. In this presentation, equilibrium speciation modelling is briefly reviewed, and is exemplified by simulations of metal speciation in natural waters using the Windermere Humic Aqueous Model (WHAM). Within realistic constraints and parameterisations, it is shown that the activity of the free ion is sometimes underestimated and sometimes overestimated. The assumptions and limitations resulting in such estimates are addressed. Particular emphasis is placed on extremely slow reaction (complexation-dissociation) kinetics, the presence of additional reactive (e.g. hydrophobic) species, and the possible existence of independent pools of metal that exhibit intra-, but not inter-equilibrium properties. A model for the latter effect is presented in both biological and geochemical contexts, and it is shown that many observations often attributed to experimental artefact are consistent with this model.

1 - Welcome Evening and Inaugural Lecture

1.1
Whence metal ecotoxicology? Exploiting integrative concepts

S N Luoma (US Geological Survey/ The Natural History Museum), P S Rainbow (The Natural History Museum)

Studies of metal contamination have evolved from different perspectives. Alliances occur among applied ecotoxicology, geochemistry, experimental biology and observations from nature. But differences in how the science is viewed through the lenses of these different disciplines are also common and explain some of the contradictions and controversies in managing metal contamination. For example, environmental quality standards are largely derived from toxicity testing. A holistic view of the state of knowledge shows instances of (sometimes severe) over-protection and under-protection in regulations, but such validation studies are rare. In this talk we suggest that, as experimental biology pushes forward mechanistic knowledge of metal exposure and effects, integration among disciplines becomes more feasible. For example, the biotic ligand model (BLM) integrates geochemical modeling with toxicity testing. Better knowledge of trans-membrane transport properties of metals present opportunities to reduce uncertainties that presently limit the BLM's application to natural waters. Knowledge of basic bioaccumulation properties and the basic mechanisms whereby organisms detoxify metals begin to explain differences in metal sensitivity among species. From such knowledge it is possible to begin to predict how metal effects will be manifested ecologically, and narrow uncertainties about ecological effects of metals in natural waters. Examples will be shown, integrated across several levels of organization, how knowledge of basic biology helps unravel the complexities that confound understanding of the role of metal and metalloid contamination in aquatic environments. Such understanding is not only necessary but can be realistically applied to managing risks from metal contaminants.

1 - Metal Speciation (Cont.)