

**Supplemental information for:**

Water chemistry alters gene expression and physiological endpoints of chronic waterborne exposure in zebrafish, *Danio rerio*.

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This file contains 29 pages of supplemental information regarding the BLM framework and feasibility of a genomic BLM approach, 6 supplemental figures, and 4 supplemental tables.

## **BLM framework and Feasibility of a Genomic BLM Approach**

Traditional Ambient Water Quality Criteria (AWQC) have been questioned for their inability to accurately predict the impact of metal pollutants in the environment. This is because the bioavailability and reactivity of metals in aquatic environments are affected by any accompanying water chemistry. Many naturally occurring anions in the water complex metal ions, changing them to metal species which are unavailable to the reactive surfaces of organisms and many naturally occurring cations compete with metal ions for binding sites on these reactive surfaces. However, the Biotic Ligand Model (BLM) is a predictive tool that does take into account both the metal species and other ions present when examining toxicological effects (1). In brief, BLM-based predictions of potential toxicity consider the water chemistry of a particular source and assume that waterborne metals bind to an organism, particularly the gill, which results in a perturbation of normal biological function. This metal binding directly correlates to the level of acute toxicity. Moreover, this method can be used for an indirect evaluation of toxicological properties of natural bodies of water. To date, BLMs to predict “acute” toxicity (i.e. mortality occurring within 48-96 h) have been developed for a number of metals (1, 2, 3). One application of the BLM in particular, to predict the acute toxicity of Cu, has been implemented as the legal basis for AWQC regulations on Cu in the United States (4). However, this BLM predicts only acute Cu toxicity, and its outputs are then factored by arbitrary coefficients (the “acute-to- chronic ratio”) to derive AWQC to protect against “chronic” Cu toxicity (i.e. deleterious effects occurring over a significant portion of the lifespan of an organism; 4). The current push in other jurisdictions is to extend the BLM so as to directly predict chronic toxicity. This will be essential for the full adoption of the BLM approach in Canada and the European Union where guidelines are designed to provide lifetime protection.

Although temperate countries have initiated the implementation of a freshwater BLM, acceptance of the BLM in tropical countries is dependent on data acquired from endemic tropical species, of which only a few are currently being studied, such as tropical algae (5). Zebrafish are an ideal model for assessment and implementation of the BLM due to their prevalence in tropical ecosystems that have the potential for heavy contamination, particularly in the Indian sub-continent, to which they are endemic.

Furthermore, due to their known softwater tolerance (6,7), well characterized reproduction & life cycle, and fully sequenced and accessible genome ([www.ensembl.org/Danio\\_rerio](http://www.ensembl.org/Danio_rerio)), they make the ideal candidate for the assessment of physiological and transcriptional endpoints of chronic metal contaminant studies.

To create a feasible BLM based on gene expression endpoints of chronic exposure to Cu, there is a requirement to directly relate the metal accumulation with a gene effect and subsequent change in a quantifiable trait (e.g. impaired growth, reproduction, ionregulatory disfunction). However, the one gene one product tenet principle does not necessarily hold true in all circumstances (e.g. 8). Furthermore, as we have observed in this study, natural exposure to contaminants (e.g. Cu) occurs in a myriad of water chemistry combinations and exposure levels, which makes accurate prediction extremely difficult without experiments that mimic the natural exposure levels. Additionally, as we have seen in a previous study, a given toxicant such as Cu may not be the direct cause of the gene expression pattern seen as the latter may in fact be more directly mediated by corticosteroid mobilization (“generalized stress response;” 9). To determine if a given gene expression pattern for one contaminant is indicative of all experimental conditions, a daunting array of experiments manipulating not only water chemistries but also multiple contaminants must be undertaken. In the present study, we have effectively provided a short list of candidate genes that are under the influence of combined treatments associated with Cu,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$ . Moreover, we show that  $\text{Na}^+$  and  $\text{Ca}^{2+}$  alone play a significant role in changing gene expression, and interpretation of metal related transcriptional expression in experiments associated with altered water chemistry requires careful interpretation, as relatively benign ions may induce significant changes in expressional patterns.

We have taken only the first step in a long process of experimentation required to make a chronic, genomic BLM realistically feasible. That being said, it may be more beneficial to identify only the key gene endpoints at lower levels of biological organization as an early biomarker of exposure which can signify impending damage to higher functions. This would only require identifying a handful of specific genes that have pathological downstream effects, and the flexibility to cover a broad range of toxicants as opposed to single contaminants. Although this approach is beneficial to the

establishment of an early detection tool for a chronic BLM, environmental regulatory agencies may not find this a rapid, cost effective assessment until further technological advancements can be made to quickly and cheaply identify gene expression endpoints in the field.

### Supplemental References

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**Figure S1.** Copper (Cu) load ( $\mu\text{g/g}$  tissue) in gill (A), gut (B), and liver (C) tissue from zebrafish exposed to ctrl (softwater), Cu ( $12\mu\text{g/L}$ ), Ca ( $3.3\text{mM}$ ), Ca+Cu ( $3.3\text{mM}$  Ca +  $12\mu\text{g/L}$  Cu), Na ( $3.3\text{mM}$ ), and Na+Cu ( $3.3\text{mM}$  Na +  $12\mu\text{g/L}$  Cu). Values are presented as means  $\pm$  SEM and treatments that do not share a common letter are significantly different from each other ( $n=10$  for all treatments,  $p<0.05$ ).

**Figure S2.** Calcium (Ca) tissue load ( $\text{mmol/kg}$  tissue) in the gut and liver of zebrafish exposed to ctrl (softwater), Cu ( $12\mu\text{g/L}$ ), Ca ( $3.3\text{mM}$ ), Ca+Cu ( $3.3\text{mM}$  Ca +  $12\mu\text{g/L}$  Cu), Na ( $3.3\text{mM}$ ), and Na+Cu ( $3.3\text{mM}$  Na +  $12\mu\text{g/L}$  Cu). Gill tissue did not contain any significant differences (data not shown). Values are presented as means  $\pm$  SEM and treatments that do not share a common letter are significantly different from each other ( $n=10$  for all treatments,  $p<0.05$ ).

**Figure S3.** Protein carbonyl levels ( $\text{nmol/mg}$  protein) in gill (A), gut (B), and liver (C) tissue from zebrafish exposed to ctrl (softwater), Cu ( $12\mu\text{g/L}$ ), Ca ( $3.3\text{mM}$ ), Ca+Cu ( $3.3\text{mM}$  Ca +  $12\mu\text{g/L}$  Cu), Na ( $3.3\text{mM}$ ), and Na+Cu ( $3.3\text{mM}$  Na +  $12\mu\text{g/L}$  Cu). Values are presented as means  $\pm$  SEM and treatments that do not share a common letter are significantly different from each other ( $n=10$  for all treatments,  $p<0.05$ ).

**Figure S4.** Catalase activity (CAT;  $\text{U/mg}$  protein) in gill (A), gut (B), and liver (C) tissue from zebrafish exposed to ctrl (softwater), Cu ( $12\mu\text{g/L}$ ), Ca ( $3.3\text{mM}$ ), Ca+Cu ( $3.3\text{mM}$  Ca +  $12\mu\text{g/L}$  Cu), Na ( $3.3\text{mM}$ ), and Na+Cu ( $3.3\text{mM}$  Na +  $12\mu\text{g/L}$  Cu). Values are presented as means  $\pm$  SEM and treatments that do not share a common letter are significantly different from each other ( $n=10$  for all treatments,  $p<0.05$ ).

**Figure S5.**  $\text{Na}^+\text{K}^+$ ATPase activity (NKA;  $\text{mmol ADP mg protein}^{-1} \text{ hr}^{-1}$ ) in gill (A), gut (B), and liver (C) tissue from zebrafish exposed to ctrl (softwater), Cu ( $12\mu\text{g/L}$ ), Ca ( $3.3\text{mM}$ ), Ca+Cu ( $3.3\text{mM}$  Ca +  $12\mu\text{g/L}$  Cu), Na ( $3.3\text{mM}$ ), and Na+Cu ( $3.3\text{mM}$  Na +  $12\mu\text{g/L}$  Cu). Values are presented as means  $\pm$  SEM and treatments that do not share a common letter are significantly different from each other ( $n=10$  for all treatments,  $p<0.05$ ).

**Figure S6.** Visual (3-D) description of principle component analysis of microarray results from the liver tissue of zebrafish exposed to ctrl (softwater), Cu ( $12\mu\text{g/L}$ ), Ca ( $3.3\text{mM}$ ), Ca+Cu ( $3.3\text{mM}$  Ca +  $12\mu\text{g/L}$  Cu), Na ( $3.3\text{mM}$ ), and Na+Cu ( $3.3\text{mM}$  Na +  $12\mu\text{g/L}$  Cu) indicating inherent physiological variability both within and between treatments, as represented by the spatial distance between coloured dots; the greater the distance apart, the higher degree of variability.

Figure S1

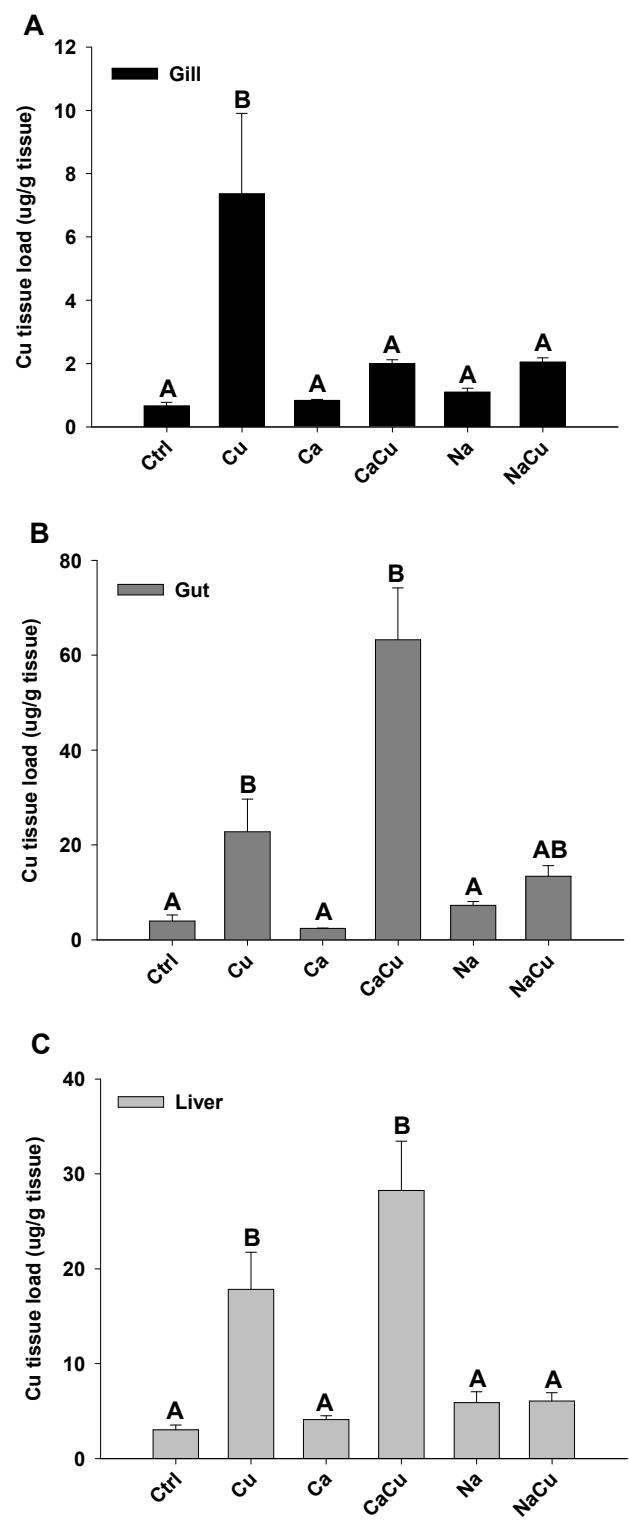


Figure S2

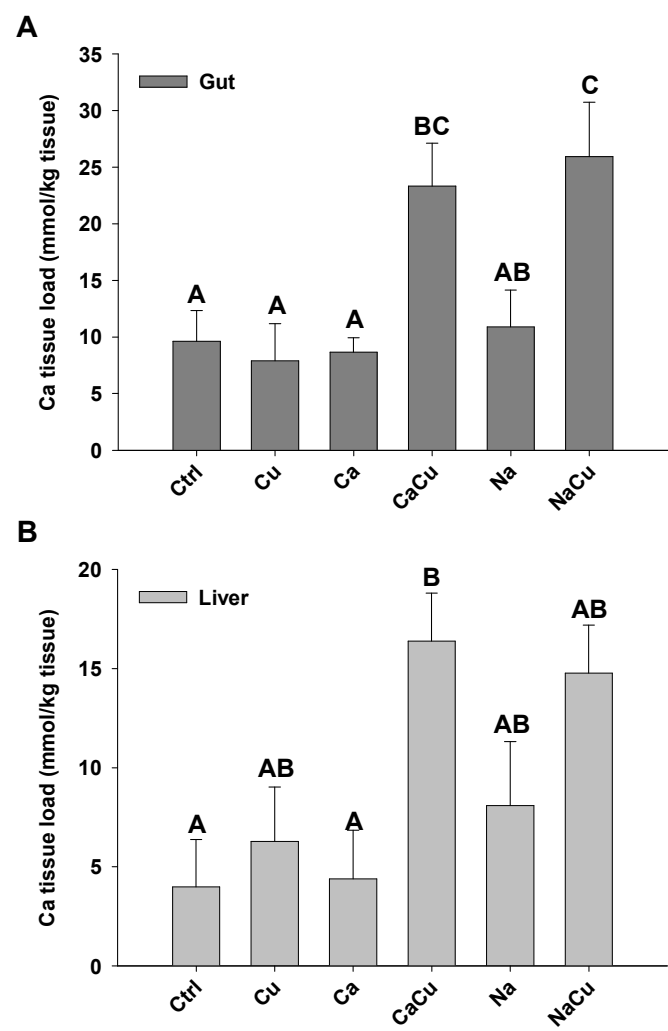
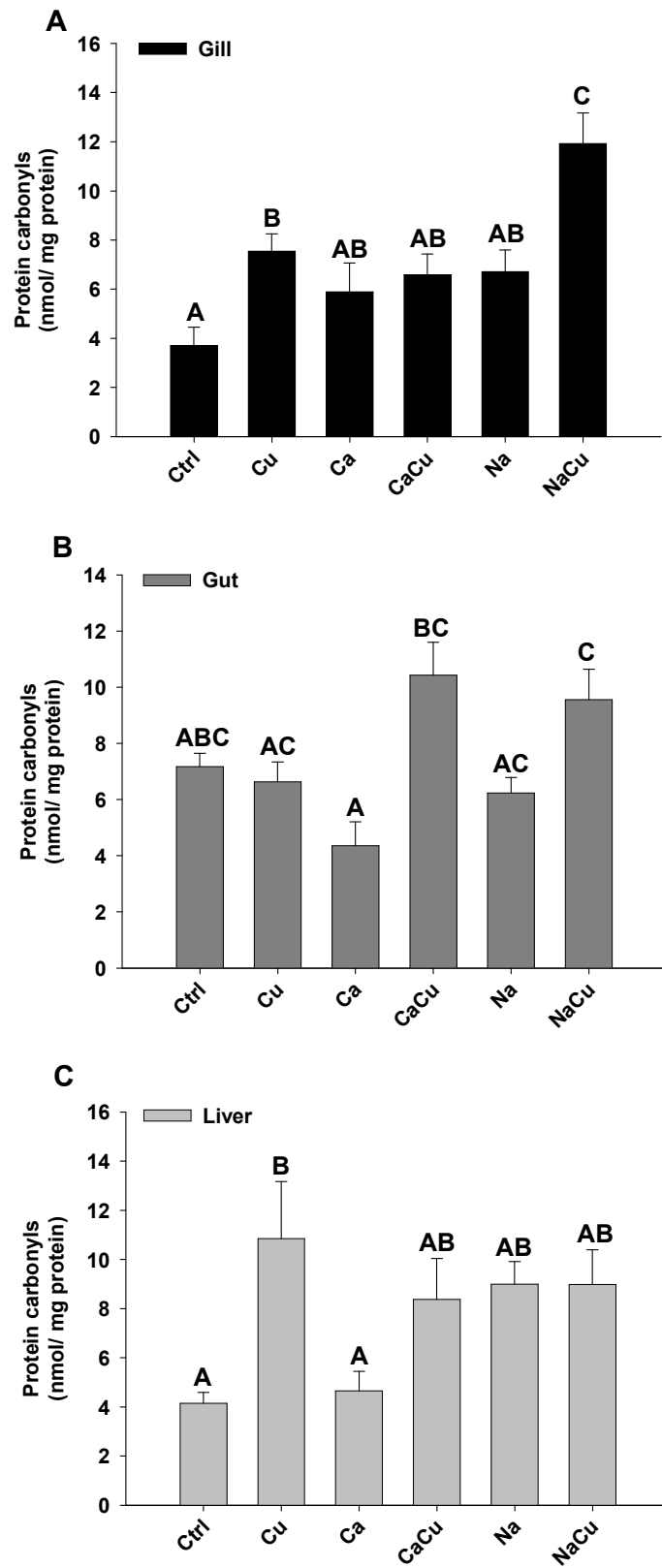


Figure S3



**Figure S4**

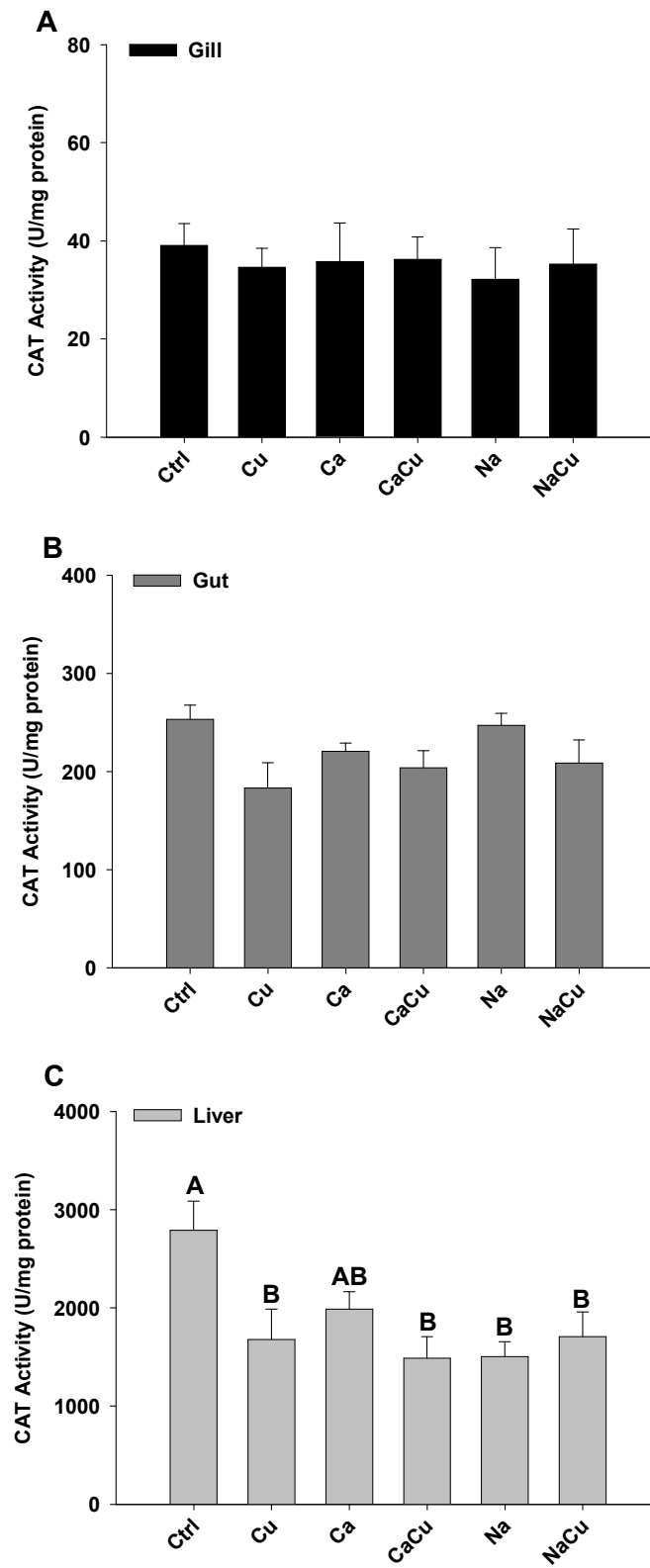


Figure S5

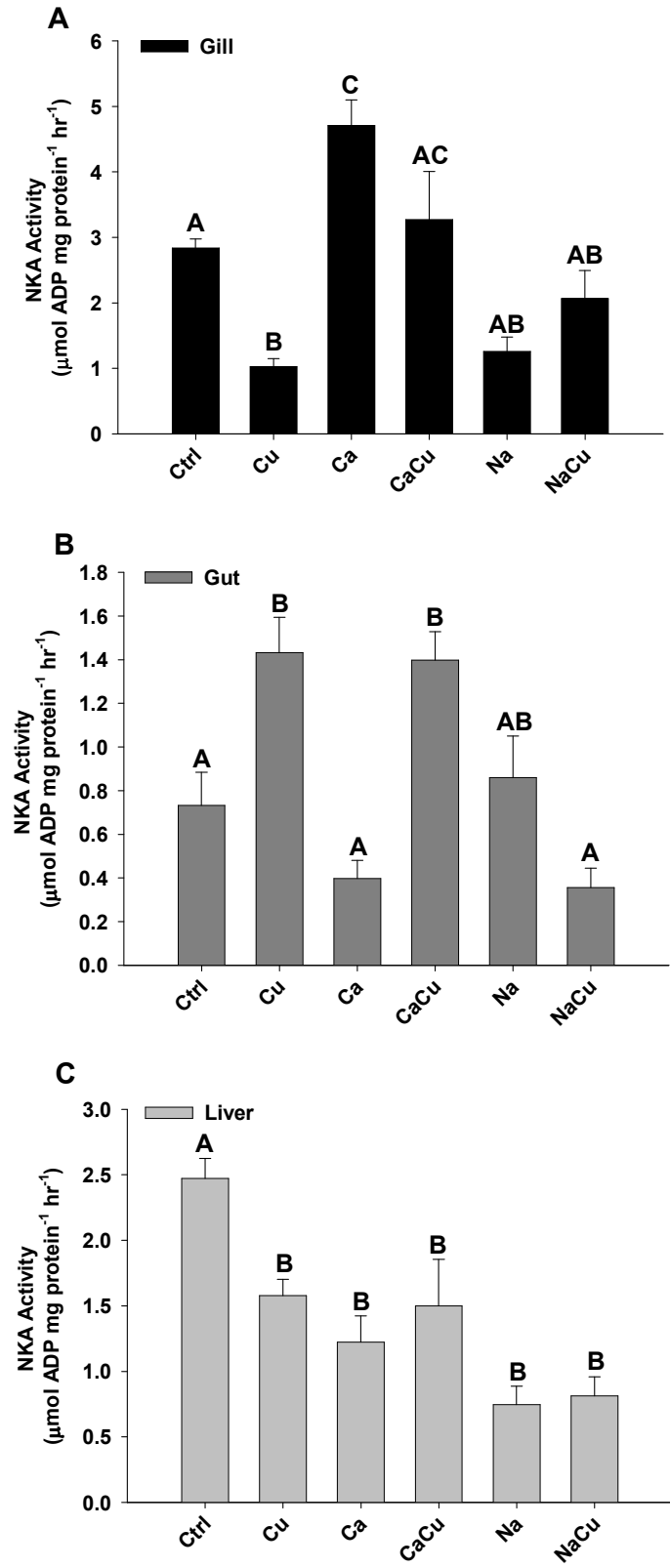
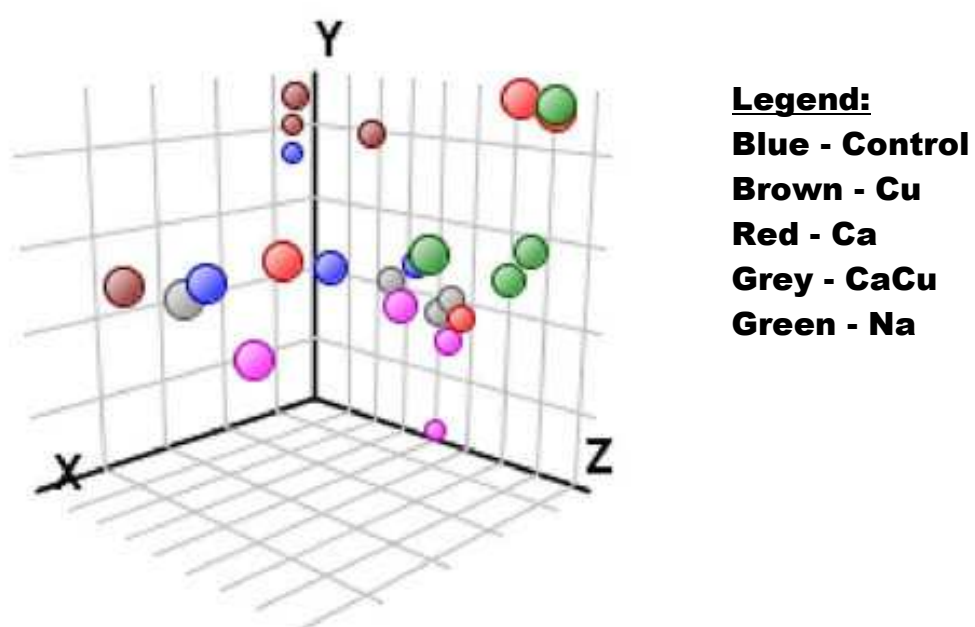


Figure S6



**Table S1:** Water chemistry parameters (means  $\pm$  SEM; n = 21) of each exposure group, showing essential competitive ions, pH, and water hardness averaged over the 21 day exposure period. Values with a \* indicate a significant difference from all other treatments (p<0.05).

| Parameter                                  | Treatment       |                 |                     |                    |                     |                    |
|--------------------------------------------|-----------------|-----------------|---------------------|--------------------|---------------------|--------------------|
|                                            | Ctrl            | Cu              | Ca                  | Ca+Cu              | Na                  | Na+Cu              |
| Na <sup>+</sup> ( $\mu$ M)                 | 84.3 $\pm$ 1.7  | 85.9 $\pm$ 2.3  | 86.1 $\pm$ 1.2      | 81.5 $\pm$ 2.2     | 3137.3 $\pm$ 141.9* | 3012.7 $\pm$ 93.3* |
| Mg <sup>2+</sup> ( $\mu$ M)                | 26.8 $\pm$ 0.7  | 25.9 $\pm$ 0.6  | 26.0 $\pm$ 0.5      | 26.3 $\pm$ 0.5     | 26.0 $\pm$ 0.7      | 25.3 $\pm$ 0.6     |
| Ca <sup>2+</sup> ( $\mu$ M)                | 60.0 $\pm$ 3.1  | 61.6 $\pm$ 3.6  | 3307.8 $\pm$ 136.5* | 3357.2 $\pm$ 88.4* | 65.7 $\pm$ 1.5      | 66.5 $\pm$ 2.2     |
| Cu <sup>2+</sup> ( $\mu$ g/L)              | 3.2 $\pm$ 0.2   | 12.5 $\pm$ 0.7* | 3.0 $\pm$ 0.3       | 11.4 $\pm$ 0.8*    | 2.7 $\pm$ 0.3       | 12.0 $\pm$ 0.4*    |
| pH                                         | 7.04 $\pm$ 0.02 | 7.02 $\pm$ 0.02 | 7.03 $\pm$ 0.02     | 7.03 $\pm$ 0.02    | 7.06 $\pm$ 0.04     | 7.06 $\pm$ 0.04    |
| CaCO <sub>3</sub> (mg Ca <sup>2+</sup> /L) | 8.7 $\pm$ 0.3   | 8.8 $\pm$ 0.5   | 334 $\pm$ 30.6*     | 339 $\pm$ 40.2*    | 9.1 $\pm$ 0.5       | 9.1 $\pm$ 0.7      |

**Table S2:** Forward (F) and reverse (R) primers used for real-time qPCR validation of microarray results.

| Gene         | Primer                                                         | Accession #  | Amplicon size (bp) |
|--------------|----------------------------------------------------------------|--------------|--------------------|
| atp7a        | F: 5'-GGCTCGACTTCTCGCAGCT-3'<br>R: 5'-ATTCCGCATTTTCACTGCCT-3'  | NM_001042720 | 50                 |
| ctr-1        | F: 5'-GAATCAGGTGAACGTGCGCT-3'<br>R: 5'-CCATCAGATCCTGGTACGGG-3' | AY077715     | 51                 |
| ECaC         | F: 5'-ACTTGGTCAACCGCAGAAAG-3'<br>R: 5'-CAGATTCCACTTGAGCGTGA-3' | AY383562     | 197                |
| ef1 $\alpha$ | F: 5'-GTGCTGTGCTGATTGTTGCT-3'<br>R: 5'-TGTATGCGCTGACTTCCTTG-3' | NM_131263    | 201                |
| esr1         | F: 5'-AGAAACACAGCCGGCCCTA-3'<br>R: 5'-TGGTGAGCAGGGACATCATG-3'  | NM_152959    | 51                 |

**Table S3:** Results of K-means clustering showing 4 distinct cluster patterns (A,B,C,D) of expression containing genes significantly different from control treatment ( $p < 0.05$ ) with an expressional direction greater than 2-fold. Complete results (all significantly regulated genes) of this microarray experiment were submitted to the public archive ArrayExpress (<http://www.ebi.ac.uk/microarray-as/ae/>) in accordance with Microarray Gene Expression Data Society (MGED) recommendations (Accession # E-MEXP-2288).

(A) Cluster-1

| Probe Set ID      | Unigene ID | Gene Title                                                                   | Cu   | Ca   | Na   | Ca+Cu | Na+Cu |
|-------------------|------------|------------------------------------------------------------------------------|------|------|------|-------|-------|
| Dr.813.1.S1_at    | Dr.813     | acetyl-CoA acetyltransferase 2                                               | -    | down | down | down  | down  |
| Dr.3267.1.S1_at   | Dr.3267    | claudin h                                                                    | up   | -    | -    | -     | -     |
| Dr.3099.1.A1_at   | Dr.77826   | coronin, actin binding protein, 2A                                           | up   | -    | -    | -     | -     |
| Dr.1603.1.A1_at   | Dr.77119   | Cytochrome P450, family 51                                                   | down | down | down | down  | down  |
| Dr.1603.1.A1_x_at | Dr.77119   | Cytochrome P450, family 51                                                   | down | down | down | down  | down  |
| Dr.20850.1.S1_at  | Dr.20850   | fatty acid binding protein 7, brain, a                                       | down | down | down | down  | -     |
| Dr.1041.1.S1_at   | Dr.76172   | fucosidase, alpha-L- 1, tissue                                               | up   | -    | -    | -     | -     |
| Dr.20230.1.S1_at  | Dr.76342   | glia maturation factor, beta                                                 | -    | -    | down | down  | -     |
| Dr.7311.1.S1_at   | Dr.76519   | guanine nucleotide binding protein-like 3 (nucleolar)                        | -    | -    | down | -     | -     |
| Dr.8000.1.S1_at   | Dr.79923   | glutathione peroxidase 1b                                                    | up   | -    | -    | -     | -     |
| Dr.13321.1.S2_at  | Dr.76367   | hypoxia induced gene 1                                                       | -    | -    | down | down  | down  |
| Dr.13321.1.S1_at  | Dr.76367   | hypoxia induced gene 1 /// similar to putative growth hormone like protein-1 | -    | -    | down | down  | -     |
| Dr.2051.1.S1_at   | Dr.77183   | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)                   | down | down | down | down  | down  |
| Dr.25191.1.S1_at  | Dr.75844   | isocitrate dehydrogenase 1 (NADP+), soluble                                  | -    | -    | down | -     | -     |
| Dr.6064.1.A1_at   | Dr.6064    | hypothetical protein LOC100000433                                            | up   | up   | -    | up    | -     |
| Dr.13618.1.A1_at  | Dr.23709   | similar to bloodthirsty                                                      | -    | down | down | -     | -     |
| Dr.3310.1.A1_at   | Dr.78055   | similar to LOC495252 protein                                                 | up   | -    | -    | -     | -     |
| Dr.17283.1.A1_at  |            | hypothetical LOC558794                                                       | down | down | down | down  | down  |
| Dr.4267.1.A1_at   |            | hypothetical LOC558794                                                       | down | down | down | down  | down  |
| Dr.6321.1.A1_at   | Dr.6321    | hypothetical LOC559001                                                       | -    | down | down | down  | down  |
| Dr.2871.1.A1_at   | Dr.132256  | similar to Tmc4 protein                                                      | up   | -    | -    | -     | -     |
| Dr.17419.1.A1_at  | Dr.75992   | hypothetical LOC564534                                                       | -    | -    | -    | down  | down  |
| Dr.23441.1.S1_at  | Dr.78880   | zgc:85644 /// hypothetical protein LOC791831                                 | up   | -    | -    | -     | -     |

|                    |           |                                                                   |      |      |      |      |      |
|--------------------|-----------|-------------------------------------------------------------------|------|------|------|------|------|
| Dr.16696.1.S1_at   | Dr.121313 | hypothetical protein LOC796134 /// si:dkey-286j15.3               | -    | -    | down | -    | down |
| Dr.11310.1.S1_at   | Dr.11310  | tubulin, alpha 1 /// similar to alpha-tubulin isotype M-alpha-2   | up   | -    | -    | -    | -    |
| Dr.6695.1.S1_at    | Dr.6695   | 3'-phosphoadenosine 5'-phosphosulfate synthase 2                  | down | -    | down | -    | down |
| Dr.13544.1.S1_at   | Dr.84415  | programmed cell death 6                                           | -    | -    | -    | -    | down |
| Dr.6619.1.S1_at    | Dr.77295  | phosphogluconate hydrogenase                                      | -    | -    | down | -    | down |
| Dr.12110.1.S1_at   | Dr.12110  | sterol-C4-methyl oxidase-like                                     | -    | down | down | down | down |
| Dr.11087.1.A1_at   | Dr.80934  | secretogranin III                                                 | up   | -    | -    | -    | -    |
| Dr.22702.1.A1_at   | Dr.81058  | si:dkey-193c22.1                                                  | -    | -    | down | -    | down |
| Dr.22146.1.A1_at   | Dr.79887  | solute carrier family 6 (neurotransmitter transporter), member 19 | up   | down | down | down | down |
| Dr.7815.1.S1_at    | Dr.79639  | synaptosome-associated protein 25a                                | up   | -    | -    | -    | -    |
| Dr.24558.1.S1_at   | Dr.84122  | signal recognition particle 9                                     | -    | down | down | down | down |
| Dr.24214.1.S1_at   | Dr.132384 | sulfotransferase family, cytosolic sulfotransferase 1             | down | down | down | down | down |
| Dr.4767.1.A1_a_at  | Dr.76728  | transmembrane emp24 protein transport domain containing 3         | -    | -    | down | -    | -    |
| Dr.3773.1.S1_at    | Dr.77112  | UDP-N-acetylglucosamine pyrophosphorylase 1                       | up   | -    | down | -    | -    |
| Dr.1129.1.A1_at    |           | wu:fa99h02                                                        | -    | -    | down | -    | -    |
| Dr.23582.1.A1_at   |           | wu:fb13f03                                                        | up   | -    | -    | -    | -    |
| Dr.25291.4.S1_at   | Dr.104591 | wu:fb18f06                                                        | up   | -    | -    | -    | -    |
| Dr.1659.1.A1_at    |           | wu:fc02a12                                                        | -    | -    | -    | down | down |
| Dr.4920.1.A1_at    |           | wu:fc27g01                                                        | up   | -    | -    | -    | -    |
| Dr.3698.1.A1_at    |           | wu:fd02f07                                                        | down | down | down | down | down |
| Dr.7168.1.S1_at    |           | wu:fd56d05                                                        | -    | down | down | down | down |
| Dr.6154.1.A1_at    |           | wu:fj19d05                                                        | -    | -    | down | down | down |
| Dr.7862.1.A1_at    |           | wu:fj55d04                                                        | -    | down | down | down | down |
| Dr.20787.2.A1_a_at | Dr.84956  | zgc:103549                                                        | -    | -    | down | -    | down |
| Dr.21447.1.A1_at   | Dr.44087  | zgc:112255                                                        | -    | -    | -    | -    | down |
| Dr.13622.1.A1_at   | Dr.84494  | zgc:112474                                                        | -    | down | down | -    | down |
| Dr.4218.1.A1_at    | Dr.74233  | zgc:162396                                                        | -    | -    | -    | -    | down |
| Dr.11583.1.A1_at   | Dr.75327  | Zgc:162964                                                        | -    | -    | down | -    | -    |
| Dr.7273.1.A1_at    | Dr.80345  | zgc:66484                                                         | -    | -    | down | -    | -    |
| Dr.25285.1.S1_at   | Dr.78528  | zgc:73223                                                         | -    | -    | down | -    | -    |
| Dr.13512.1.S1_at   | Dr.82983  | zgc:73376                                                         | -    | -    | down | -    | -    |
| Dr.10016.1.A1_at   | Dr.82081  | zgc:77556                                                         | -    | -    | -    | -    | down |

|                  |           |                          |      |      |      |      |      |
|------------------|-----------|--------------------------|------|------|------|------|------|
| Dr.5934.1.A1_at  | Dr.113528 | zgc:85981                | up   | -    | -    | -    | -    |
| Dr.9746.8.S1_at  | Dr.76432  | zgc:86706                | -    | -    | down | down | down |
| Dr.4911.1.A1_at  | Dr.77249  | zgc:92000                | -    | -    | down | -    | down |
| Dr.20008.2.A1_at | Dr.32396  | zgc:92061                | up   | up   | -    | -    | -    |
| Dr.14685.1.S1_at | Dr.81719  | zgc:92379                | -    | -    | -    | down | -    |
| Dr.12321.1.A1_at |           |                          | -    | down | down | down | down |
| Dr.13438.1.A1_at | Dr.123250 | Transcribed locus        | -    | -    | -    | -    | down |
| Dr.18183.1.A1_at |           |                          | up   | -    | -    | -    | -    |
| Dr.18374.1.A1_at |           |                          | up   | -    | -    | -    | -    |
| Dr.21698.1.A1_at | Dr.78933  | CDNA clone IMAGE:6034266 | down | down | down | down | down |
| Dr.26534.1.A1_at | Dr.76999  | Transcribed locus        | down | down | down | down | down |
| Dr.7806.1.A1_at  | Dr.124155 | Transcribed locus        | -    | -    | down | -    | -    |
| Dr.9875.1.A1_at  | Dr.81956  | Transcribed locus        | -    | down | down | down | down |

(B) Cluster 2

| Probe Set ID      | Unigene ID | Gene Title                                                 | Cu   | Ca   | Na   | Ca+Cu | Na+Cu |
|-------------------|------------|------------------------------------------------------------|------|------|------|-------|-------|
| Dr.24206.1.S1_at  | Dr.72266   | chaperonin containing TCP1, subunit 4 (delta)              | -    | -    | -    | -     | up    |
| Dr.13284.2.S1_at  | Dr.108026  | choline kinase alpha                                       | down | -    | -    | -     | -     |
| Dr.11504.1.A1_at  | Dr.80698   | ecdysoneless homolog (Drosophila)                          | -    | -    | -    | -     | up    |
| Dr.10717.1.S2_at  | Dr.119569  | estrogen receptor 1                                        | down | down | down | -     | up    |
| Dr.7972.1.S1_at   | Dr.77165   | GTP binding protein 4                                      | -    | -    | -    | -     | up    |
| Dr.6932.3.S1_at   | Dr.76671   | high-mobility group box 3a                                 | -    | up   | -    | -     | up    |
| Dr.1149.1.A1_a_at | Dr.35374   | heterogeneous nuclear ribonucleoprotein A0                 | -    | -    | -    | -     | up    |
| Dr.19560.1.S2_at  | Dr.115835  | insulin induced gene 1                                     | down | -    | -    | -     | -     |
| Dr.11707.2.A1_at  | Dr.132454  | spermine oxidase /// hypothetical LOC554383                | -    | -    | -    | up    | -     |
| Dr.236.1.S1_at    | Dr.75518   | similar to LOC494737 protein                               | -    | -    | down | -     | -     |
| Dr.11399.2.S1_at  |            | hypothetical protein LOC791639                             | -    | -    | -    | -     | up    |
| Dr.12594.1.S1_at  | Dr.114931  | X-box binding protein 1 /// hypothetical protein LOC793034 | down | -    | -    | -     | -     |
| Dr.2850.1.S1_at   | Dr.132178  | methionine adenosyltransferase II, alpha                   | down | down | down | -     | -     |
| Dr.18414.1.S1_at  | Dr.18414   | programmed cell death 4a                                   | -    | -    | -    | -     | up    |

|                    |           |                                                          |      |      |      |    |    |
|--------------------|-----------|----------------------------------------------------------|------|------|------|----|----|
| Dr.6191.1.S1_at    | Dr.78102  | pim-1 oncogene                                           | -    | -    | -    | up | -  |
| Dr.73.1.A1_at      | Dr.73     | sb:cb360                                                 | down | -    | -    | -  | -  |
| Dr.7467.1.S1_at    | Dr.115188 | serine incorporator 5                                    | -    | -    | -    | up | -  |
| Dr.9272.1.A1_at    | Dr.47567  | si:dkey-30h14.2                                          | down | -    | down | -  | -  |
| Dr.5307.1.S1_at    | Dr.5307   | solute carrier family 20, member 1b                      | down | -    | down | -  | -  |
| Dr.6847.1.S1_a_at  | Dr.79948  | solute carrier family 31 (copper transporters), member 1 | down | -    | -    | -  | up |
| Dr.11296.1.S1_at   | Dr.106021 | solute carrier family 43, member 1                       | down | down | down | -  | -  |
| Dr.24245.3.S1_a_at | Dr.76177  | staphylococcal nuclease domain containing 1              | -    | -    | up   | -  | up |
| Dr.3648.1.S1_at    | Dr.35540  | translocating chain-associating membrane protein 1       | down | -    | -    | -  | -  |
| Dr.25607.1.S1_at   | Dr.104447 | wu:fi34d08                                               | -    | -    | up   | -  | up |
| Dr.14725.1.A1_at   | Dr.84733  | zgc:112350                                               | -    | -    | -    | -  | up |
| Dr.8914.1.S1_at    | Dr.80042  | zgc:113054                                               | down | down | down | -  | -  |
| Dr.25243.1.S1_at   | Dr.39091  | zgc:113200                                               | -    | -    | up   | -  | up |
| Dr.9003.1.A1_at    |           | zgc:123010                                               | -    | -    | down | -  | -  |
| Dr.774.1.S1_at     | Dr.75933  | zgc:136866                                               | -    | -    | -    | -  | up |
| Dr.22015.1.A1_at   | Dr.108327 | zgc:158135                                               | -    | -    | down | -  | -  |
| Dr.25593.2.A1_at   | Dr.105866 | Zgc:171426                                               | -    | -    | -    | -  | up |
| Dr.13813.1.A1_at   | Dr.83439  | zgc:171663                                               | -    | -    | -    | up | -  |
| Dr.9070.1.A1_at    | Dr.9070   | zgc:63770                                                | -    | -    | -    | -  | up |
| Dr.23925.1.A1_at   | Dr.80026  | Transcribed locus                                        | down | down | down | -  | -  |
| Dr.24938.1.S1_a_at |           |                                                          | down | up   | down | -  | up |
| Dr.24938.1.S1_x_at |           |                                                          | down | up   | -    | -  | up |

(C) Cluster 3

| Probe Set ID         | Unigene ID | Gene Title                         | Cu | Ca | Na | Ca+Cu | Na+Cu |
|----------------------|------------|------------------------------------|----|----|----|-------|-------|
| AFFX-Dr-acta1-5_x_at | Dr.75552   | actin, alpha 1, skeletal muscle    | -  | -  | up | -     | up    |
| Dr.14922.1.A1_at     | Dr.81194   | abhydrolase domain containing 4    | -  | -  | up | -     | -     |
| AFFX-Dr-acta1-M_at   | Dr.75552   | actin, alpha 1, skeletal muscle    | -  | up | -  | -     | -     |
| Dr.19542.1.A1_at     | Dr.13893   | autocrine motility factor receptor | -  | -  | up | -     | -     |
| Dr.5195.1.A1_at      | Dr.5195    | Apolipoprotein L, 1                | -  | -  | up | -     | -     |

|                    |           |                                                                      |      |    |    |      |      |
|--------------------|-----------|----------------------------------------------------------------------|------|----|----|------|------|
| Dr.2022.1.A1_at    | Dr.77297  | arginase, type II                                                    | down | -  | up | down | down |
| Dr.10283.1.A1_at   | Dr.78270  | BCL2/adenovirus E1B interacting protein 3-like                       | -    | -  | up | -    | -    |
| Dr.959.2.S1_at     | Dr.75609  | basic transcription factor 3                                         | -    | -  | up | -    | -    |
| Dr.21005.1.S1_s_at | Dr.88584  | complement component c3b /// complement component c3c                | -    | up | up | -    | -    |
| Dr.941.1.S1_at     | Dr.32745  | cell cycle associated protein 1                                      | -    | -  | up | -    | up   |
| Dr.1357.1.S1_at    | Dr.75181  | chaperonin containing TCP1, subunit 8 (theta)                        | -    | -  | up | -    | up   |
| Dr.26344.3.S1_at   | Dr.104797 | cell division cycle 42                                               | -    | -  | up | -    | -    |
| Dr.637.1.S1_at     | Dr.75081  | cadherin 2, neuronal                                                 | -    | -  | up | -    | -    |
| Dr.12605.1.S1_at   | Dr.75267  | cyclin-dependent kinase inhibitor 1b (p27, kip1)                     | -    | -  | up | -    | -    |
| Dr.535.2.A1_at     |           | carboxyl ester lipase, like                                          | up   | up | up | -    | up   |
| Dr.7190.1.A1_at    | Dr.80131  | coiled-coil-helix-coiled-coil-helix domain containing 3              | -    | -  | up | -    | -    |
| Dr.15261.1.A1_at   | Dr.89300  | cytoplasmic polyadenylation element binding protein 4                | -    | -  | up | -    | -    |
| Dr.10433.1.S1_at   | Dr.116325 | cryptochrome 2a                                                      | -    | -  | up | -    | -    |
| Dr.25214.1.A1_at   | Dr.87867  | cytochrome P450, family 24, subfamily A, polypeptide 1, like         | down | -  | up | down | down |
| Dr.25208.2.S1_at   | Dr.77160  | cytochrome P450, family 3, subfamily A, polypeptide 65               | -    | up | up | -    | -    |
| Dr.664.2.S1_at     | Dr.114326 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 /// similar to Ddx5 protein | -    | -  | up | -    | -    |
| Dr.20598.1.S1_at   | Dr.20756  | eukaryotic translation initiation factor 3, subunit D                | -    | -  | up | -    | up   |
| Dr.1710.2.S1_at    | Dr.33806  | eukaryotic translation initiation factor 4E family member 3          | -    | up | up | -    | -    |
| Dr.10306.1.S1_at   | Dr.121185 | ets variant gene 6 (TEL oncogene)                                    | -    | up | up | -    | -    |
| Dr.7171.2.S1_a_at  | Dr.33603  | glutamic pyruvate transaminase (alanine aminotransferase) 2          | -    | up | up | -    | -    |
| Dr.24246.1.S1_at   | Dr.24246  | H1 histone family, member X                                          | up   | up | up | up   | -    |
| Dr.6382.1.A1_at    | Dr.138736 | hedgehog interacting protein                                         | -    | -  | up | -    | -    |
| Dr.14662.1.S1_at   | Dr.118179 | HNF1 homeobox b                                                      | -    | up | up | -    | -    |
| Dr.12882.1.A1_at   | Dr.43052  | heparan sulfate 6-O-sulfotransferase 1a                              | -    | up | up | -    | -    |
| Dr.25548.1.S1_at   | Dr.76602  | heat shock protein 90kDa beta (grp94), member 1                      | -    | -  | up | -    | -    |
| Dr.7103.1.S1_at    | Dr.7103   | inhibitor of DNA binding 3                                           | -    | up | up | -    | -    |
| Dr.8587.1.A2_at    | Dr.76315  | insulin-like growth factor binding protein 1                         | -    | up | up | -    | -    |
| Dr.12583.1.S1_at   | Dr.12583  | insulin-like growth factor binding protein 3                         | -    | -  | up | -    | -    |
| Dr.25874.1.A1_at   | Dr.66408  | im:7151086                                                           | -    | -  | up | -    | -    |
| Dr.374.1.S1_at     | Dr.35325  | interferon regulatory factor 2 binding protein 2                     | -    | up | up | -    | -    |
| Dr.16331.2.S1_at   | Dr.114298 | Similar to Mitogen-activated protein kinase 3                        | -    | -  | up | -    | -    |
| Dr.7722.1.A1_at    |           | similar to complement C3-H1                                          | -    | up | up | up   | -    |

|                    |           |                                                                         |      |    |      |      |      |
|--------------------|-----------|-------------------------------------------------------------------------|------|----|------|------|------|
| Dr.25379.1.S1_at   | Dr.118237 | hypothetical LOC567732                                                  | -    | up | up   | -    | -    |
| Dr.25331.1.S1_at   | Dr.75455  | hypothetical LOC567732                                                  | -    | up | up   | -    | -    |
| Dr.7111.1.S1_a_at  | Dr.115516 | similar to Probable RNA-dependent helicase p72                          | -    | -  | up   | -    | -    |
| Dr.10226.1.S1_at   | Dr.77293  | similar to phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 | down | -  | -    | down | down |
| Dr.1815.1.A1_at    | Dr.132528 | similar to microfilament and actin filament cross-linker protein        | -    | up | up   | -    | -    |
| Dr.16652.1.S1_at   | Dr.75158  | hypothetical LOC562726                                                  | -    | -  | up   | -    | -    |
| Dr.10476.1.A1_at   | Dr.75427  | hypothetical LOC567461                                                  | down | -  | -    | -    | -    |
| Dr.1964.1.A1_at    | Dr.132290 | hypothetical LOC569591                                                  | up   | up | up   | -    | -    |
| Dr.96.1.A1_at      | Dr.107751 | similar to complement protein component C7-1                            | -    | -  | up   | -    | -    |
| Dr.17464.1.A1_at   | Dr.74715  | hypothetical protein LOC571260                                          | -    | -  | up   | -    | -    |
| Dr.26268.1.A1_at   | Dr.87685  | similar to putative scavenger receptor MARCO                            | -    | up | up   | -    | -    |
| Dr.17059.1.S1_at   | Dr.104967 | similar to meningioma-expressed antigen 5                               | -    | up | up   | up   | -    |
| Dr.16658.1.S1_at   | Dr.143695 | hypothetical protein LOC792055                                          | -    | -  | up   | -    | -    |
| Dr.16095.1.S1_at   | Dr.16095  | insulin-like growth factor binding protein-1b                           | -    | -  | up   | up   | -    |
| Dr.10442.1.A1_at   | Dr.117624 | makorin, ring finger protein, 1 /// similar to Makorin RING zinc-finger | -    | -  | up   | -    | -    |
| Dr.15836.1.A1_at   | Dr.77432  | lyric-like                                                              | -    | -  | up   | -    | -    |
| Dr.15836.2.A1_at   | Dr.77432  | lyric-like                                                              | -    | -  | up   | -    | -    |
| Dr.608.1.A1_at     | Dr.75475  | v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B        | -    | up | -    | -    | -    |
| Dr.16754.1.S1_at   | Dr.86078  | mitogen-activated protein kinase kinase kinase 7 interacting protein 1  | -    | -  | up   | -    | -    |
| Dr.17570.1.S1_at   | Dr.116082 | MAP kinase-interacting serine/threonine kinase 2b                       | -    | up | up   | -    | -    |
| Dr.23065.1.S1_at   | Dr.81717  | muscle, skeletal, receptor tyrosine kinase                              | -    | up | -    | up   | -    |
| Dr.16048.1.S1_at   | Dr.78260  | myelocytomatosis oncogene b                                             | down | -  | down | -    | -    |
| Dr.12684.1.S1_at   | Dr.7230   | nuclear factor (erythroid-derived 2)-like 2                             | down | -  | -    | -    | -    |
| Dr.3986.1.S1_at    | Dr.77152  | nuclear receptor subfamily 2, group F, member 2                         | -    | up | up   | -    | -    |
| Dr.11921.1.S1_at   | Dr.11921  | nuclear receptor subfamily 5, group A, member 5                         | -    | up | up   | -    | up   |
| Dr.10500.1.A1_at   | Dr.76399  | oxysterol binding protein-like 9                                        | -    | -  | up   | -    | -    |
| Dr.12233.1.S1_at   | Dr.24504  | poly A binding protein, cytoplasmic 1 a                                 | -    | up | up   | up   | up   |
| Dr.763.1.A1_at     | Dr.7296   | protein phosphatase 1, catalytic subunit, beta isoform, like            | -    | up | up   | -    | up   |
| Dr.19303.1.A1_at   | Dr.86354  | protein phosphatase 1, regulatory (inhibitor) subunit 13 like           | -    | -  | up   | -    | -    |
| Dr.17113.1.A1_a_at | Dr.143803 | phosphatase and tensin homolog A                                        | -    | -  | up   | -    | -    |
| Dr.14305.1.A1_at   | Dr.82680  | sal-like 1a (Drosophila)                                                | -    | up | up   | -    | -    |
| Dr.26405.1.S1_at   | Dr.74531  | syndecan 4 like                                                         | -    | -  | up   | -    | -    |

|                  |          |                                                                       |      |    |    |      |      |
|------------------|----------|-----------------------------------------------------------------------|------|----|----|------|------|
| Dr.10320.1.S1_at | Dr.78523 | serum/glucocorticoid regulated kinase 1                               | down | -  | -  | -    | down |
| Dr.7568.1.A1_at  | Dr.78433 | si:ch211-132p20.4                                                     | down | -  | -  | down | down |
| Dr.22971.1.A1_at | Dr.81479 | si:ch211-235e18.3                                                     | down | -  | -  | down | down |
| Dr.17322.1.A1_at | Dr.17322 | si:ch211-81a5.3                                                       | -    | -  | -  | down | -    |
| Dr.19979.1.S1_at | Dr.77248 | si:dkey-261e22.2                                                      | -    | -  | up | -    | up   |
| Dr.24312.1.S1_at | Dr.82478 | solute carrier family 16 (monocarboxylic acid transporters), member 1 | -    | -  | up | -    | -    |
| Dr.20663.1.S1_at | Dr.76663 | solute carrier family 25, member 33                                   | down | -  | -  | down | down |
| Dr.12410.1.S1_at | Dr.32057 | sprouty (Drosophila) homolog 4                                        | -    | up | -  | -    | -    |
| Dr.4713.1.S1_at  | Dr.75888 | staufen, RNA binding protein, homolog 2 (Drosophila)                  | -    | -  | up | -    | -    |
| Dr.8233.1.S1_at  | Dr.8233  | T-box 2b                                                              | down | -  | -  | down | down |
| Dr.10250.1.A1_at | Dr.10250 | L-threonine dehydrogenase                                             | -    | up | up | down | down |
| Dr.578.2.S1_a_at | Dr.32598 | thyrotroph embryonic factor                                           | -    | up | -  | -    | -    |
| Dr.12632.1.S1_at | Dr.78052 | transcription factor binding to IGHM enhancer 3a                      | -    | up | up | -    | -    |
| Dr.5123.2.S1_at  | Dr.77214 | tight junction protein 2b (zona occludens 2)                          | -    | -  | -  | -    | -    |
| Dr.18844.1.S1_at | Dr.18844 | T-cell leukemia, homeobox 1                                           | -    | -  | up | -    | -    |
| Dr.4590.1.A1_at  | Dr.79310 | transducer of ERBB2, 1a                                               | down | -  | -  | -    | down |
| Dr.2710.1.S1_at  | Dr.2710  | Similar to target of myb1 (chicken)                                   | -    | up | up | -    | -    |
| Dr.4186.1.S1_at  | Dr.4186  | tumor protein D52-like 2                                              | -    | -  | up | -    | -    |
| Dr.17866.1.S1_at |          | wu:fa12f04                                                            | -    | -  | up | -    | -    |
| Dr.17775.1.A1_at | Dr.76322 | wu:fa99c11                                                            | -    | -  | up | up   | -    |
| Dr.4738.1.A1_at  |          | wu:fb12g02                                                            | -    | -  | up | -    | -    |
| Dr.3479.1.A1_at  |          | wu:fb37e01                                                            | up   | up | up | -    | -    |
| Dr.2518.1.A1_at  |          | wu:fb51f10                                                            | -    | -  | up | -    | -    |
| Dr.3649.1.A1_at  |          | wu:fb76b09                                                            | -    | -  | up | -    | -    |
| Dr.11357.1.S1_at |          | wu:fc17e06                                                            | -    | -  | up | -    | -    |
| Dr.17714.1.S1_at |          | wu:fc55g02                                                            | -    | up | up | -    | -    |
| Dr.5309.1.A1_at  | Dr.76654 | wu:fc66f06                                                            | -    | -  | up | -    | -    |
| Dr.22087.1.A1_at |          | wu:fd12f01                                                            | -    | up | up | -    | -    |
| Dr.6952.1.A1_at  |          | wu:fe25f07                                                            | -    | up | -  | -    | -    |
| Dr.24042.1.A1_at |          | wu:fj17e06                                                            | -    | -  | up | -    | -    |
| Dr.7230.1.A1_at  |          | wu:fj67e03                                                            | -    | up | up | -    | -    |
| Dr.9920.1.A1_at  |          | wu:fk63e10                                                            | -    | -  | up | up   | -    |

|                  |           |                                         |    |    |    |    |    |
|------------------|-----------|-----------------------------------------|----|----|----|----|----|
| Dr.3139.1.S1_at  | Dr.76498  | zinc finger protein 36, C3H type-like 2 | -  | up | -  | -  | -  |
| Dr.4213.1.S1_at  | Dr.140544 | zgc:111986                              | -  | -  | up | -  | -  |
| Dr.25598.1.A1_at | Dr.75936  | zgc:114051                              | up | up | up | -  | -  |
| Dr.2204.1.A1_at  | Dr.77881  | zgc:136474                              | -  | up | up | -  | -  |
| Dr.3936.1.A1_at  | Dr.106159 | zgc:162730                              | -  | up | up | -  | -  |
| Dr.14046.1.S1_at | Dr.75179  | zgc:55886                               | -  | up | up | -  | -  |
| Dr.8025.1.S1_at  | Dr.115955 | zgc:56324                               | -  | up | up | -  | -  |
| Dr.9112.1.S1_at  | Dr.77889  | zgc:63976                               | -  | -  | up | -  | -  |
| Dr.25776.1.S1_at | Dr.85899  | zgc:63987                               | -  | up | up | -  | -  |
| Dr.5325.1.A1_at  | Dr.4035   | zgc:65870                               | -  | up | up | up | -  |
| Dr.701.1.S1_at   | Dr.114995 | zgc:77155                               | -  | up | up | -  | up |
| Dr.3518.1.A1_at  | Dr.140561 | zgc:77551                               | -  | -  | up | -  | -  |
| Dr.15501.1.S1_at | Dr.15501  | zgc:85866                               | -  | up | -  | -  | -  |
| Dr.10045.1.S1_at | Dr.76599  | zgc:86598                               | -  | -  | up | -  | -  |
| Dr.11473.2.S1_at | Dr.132698 | zgc:92578                               | -  | -  | up | -  | up |
| Dr.1593.1.A1_at  | Dr.77305  | Transcribed locus                       | -  | -  | up | -  | -  |
| Dr.16319.1.A1_at | Dr.16319  | Transcribed locus                       | up | up | up | up | -  |
| Dr.16387.1.A1_at | Dr.123601 | Transcribed locus                       | -  | -  | up | -  | -  |
| Dr.18657.1.S1_at | Dr.86964  | Transcribed locus                       | -  | up | up | -  | -  |
| Dr.19004.1.S1_at | Dr.133759 | Transcribed locus                       | -  | -  | up | -  | -  |
| Dr.26107.1.A1_at |           |                                         | -  | up | up | -  | -  |
| Dr.26218.1.A1_at | Dr.134514 | Transcribed locus                       | -  | -  | -  | up | -  |
| Dr.26372.1.A1_at | Dr.122837 | Transcribed locus                       | -  | -  | up | -  | up |
| Dr.9217.1.A1_at  | Dr.105658 | Transcribed locus                       | -  | -  | up | up | -  |

(D) Cluster 4

| Probe Set ID     | Unigene ID | Gene Title                                     | Cu | Ca | Na | Ca+Cu | Na+Cu |
|------------------|------------|------------------------------------------------|----|----|----|-------|-------|
| Dr.3910.1.S1_at  | Dr.107967  | acyl-Coenzyme A dehydrogenase family, member 8 | -  | -  | -  | -     | down  |
| Dr.19974.1.S1_at | Dr.76282   | Rho GDP dissociation inhibitor (GDI) gamma     | up | -  | -  | -     | -     |
| Dr.9277.1.S1_at  | Dr.67666   | arrestin domain containing 3                   | up | -  | up | up    | -     |
| Dr.7102.1.S1_at  | Dr.78272   | chromosome 20 open reading frame 149, like     | up | up | up | up    | up    |

|                    |           |                                                                      |      |    |      |      |      |
|--------------------|-----------|----------------------------------------------------------------------|------|----|------|------|------|
| Dr.5504.1.S1_at    | Dr.76887  | cystathionine-beta-synthase                                          | -    | -  | -    | down | down |
| Dr.20083.1.A1_at   | Dr.21399  | cyclin G2                                                            | -    | -  | -    | -    | down |
| Dr.10688.1.S1_at   | Dr.105878 | cyclin-dependent protein kinase 5                                    | -    | -  | -    | down | down |
| Dr.23612.1.A1_at   | Dr.41241  | cadherin EGF LAG seven-pass G-type receptor 1a                       | up   | -  | -    | -    | -    |
| Dr.9.1.S1_at       | Dr.75198  | hypothetical LOC563969                                               | up   | up | up   | -    | -    |
| Dr.3560.1.A1_at    | Dr.76994  | cystathionase (cystathionine gamma-lyase)                            | up   | -  | -    | down | down |
| Dr.10070.1.A1_at   | Dr.10070  | fructose-1,6-bisphosphatase 1, like                                  | -    | -  | up   | -    | -    |
| Dr.25520.1.A1_at   | Dr.84666  | F-box protein 25                                                     | up   | -  | -    | -    | -    |
| Dr.1544.1.A1_at    | Dr.17618  | glutamic-oxaloacetic transaminase 2a                                 | -    | -  | -    | -    | down |
| Dr.17145.1.S1_at   | Dr.85820  | potassium channel tetramerisation domain containing 12.1             | -    | -  | -    | down | down |
| Dr.1368.4.A1_at    | Dr.117367 | hypothetical protein LOC100005355                                    | up   | -  | up   | -    | -    |
| Dr.16935.1.A1_a_at | Dr.16935  | Hypothetical LOC558832                                               | up   | -  | up   | -    | -    |
| Dr.4661.1.A1_at    | Dr.132403 | hypothetical LOC559003                                               | -    | -  | -    | -    | down |
| Dr.6172.1.A1_at    | Dr.81026  | hypothetical LOC567192                                               | up   | -  | -    | -    | -    |
| Dr.3974.1.A1_at    | Dr.86160  | similar to PAM                                                       | -    | up | up   | -    | -    |
| Dr.12107.1.A1_at   | Dr.12107  | N-myc downstream regulated gene 1 /// hypothetical protein LOC792085 | up   | -  | -    | -    | -    |
| Dr.24726.1.S1_at   | Dr.77225  | microtubule-associated protein 1 light chain 3 beta                  | up   | -  | up   | -    | -    |
| Dr.23324.1.A1_at   | Dr.81778  | matrix metalloproteinase 14 (membrane-inserted) beta                 | -    | -  | up   | -    | -    |
| Dr.9142.1.S1_at    | Dr.77491  | myosin, heavy polypeptide 10, non-muscle                             | -    | -  | up   | -    | -    |
| Dr.2973.1.A1_at    | Dr.2973   | neutrophil cytosolic factor 1                                        | up   | -  | up   | -    | -    |
| Dr.23465.1.S1_at   | Dr.76653  | neuroepithelial cell transforming gene 1                             | up   | up | -    | -    | -    |
| Dr.3216.1.A1_at    | Dr.105888 | nucleoside phosphorylase                                             | down | -  | -    | down | down |
| Dr.26132.1.S1_at   | Dr.37928  | phosphoenolpyruvate carboxykinase 1 (soluble)                        | down | up | down | down | down |
| Dr.1519.1.S1_at    | Dr.116775 | RAB6A, member RAS oncogene family                                    | -    | -  | -    | down | -    |
| Dr.13041.1.A1_at   | Dr.108840 | retinol dehydrogenase 12, like                                       | up   | -  | -    | -    | down |
| Dr.14097.1.S1_at   | Dr.33203  | Similar to Myelin basic protein                                      | up   | -  | -    | -    | -    |
| Dr.18513.2.S1_a_at | Dr.82774  | saccharopine dehydrogenase b                                         | -    | -  | up   | -    | -    |
| Dr.26392.1.A1_at   | Dr.74531  | syndecan 4 like                                                      | -    | -  | up   | -    | -    |
| Dr.11588.1.S1_at   | Dr.117332 | sideroflexin 2                                                       | -    | -  | -    | -    | down |
| Dr.9611.1.A1_at    | Dr.107310 | si:ch211-241e15.2                                                    | up   | -  | -    | -    | -    |
| Dr.20551.1.A1_at   | Dr.41221  | si:dkey-222b8.2                                                      | -    | -  | -    | -    | down |
| Dr.2788.1.S1_at    | Dr.76517  | si:dkeyp-86b9.2                                                      | -    | -  | -    | -    | down |

|                    |           |                                        |      |      |    |      |      |
|--------------------|-----------|----------------------------------------|------|------|----|------|------|
| Dr.4948.1.A1_at    | Dr.77323  | solute carrier family 26, member 5     | up   | up   | up | -    | -    |
| Dr.4351.1.A1_at    | Dr.105401 | striatin, calmodulin binding protein 3 | -    | -    | -  | -    | down |
| Dr.578.1.A1_at     | Dr.32598  | thyrotroph embryonic factor            | -    | -    | -  | down | -    |
| Dr.20029.1.A1_at   | Dr.33137  | tissue factor pathway inhibitor a      | up   | up   | up | -    | -    |
| Dr.12134.2.A1_at   | Dr.104473 | wu:fb34a04                             | -    | -    | -  | -    | down |
| Dr.8747.1.A1_at    |           | wu:fc16h10                             | up   | up   | up | -    | -    |
| Dr.1854.1.A1_at    |           | wu:fc96c10                             | down | -    | -  | down | down |
| Dr.23743.1.S1_at   | Dr.72340  | wu:fj58g06                             | -    | -    | -  | -    | down |
| Dr.7226.1.S1_at    |           | wu:fj65h10                             | -    | -    | -  | down | -    |
| Dr.9564.1.A1_at    |           | wu:fj88f05                             | -    | -    | -  | -    | down |
| Dr.7535.1.A1_at    |           | wu:fp56f09                             | -    | -    | -  | -    | down |
| Dr.11661.1.S1_at   | Dr.114303 | yippee-like 3                          | up   | -    | up | -    | -    |
| Dr.5605.4.S1_at    | Dr.104496 | zgc:123210                             | up   | -    | -  | -    | -    |
| Dr.24907.1.A1_at   | Dr.33774  | zgc:136656                             | up   | -    | -  | -    | -    |
| Dr.7099.2.A1_at    | Dr.81135  | zgc:153424                             | -    | -    | -  | -    | down |
| Dr.2433.2.A1_at    | Dr.37700  | zgc:153863                             | -    | down | -  | -    | down |
| Dr.10914.1.A1_at   | Dr.10914  | zgc:154020                             | up   | up   | -  | up   | -    |
| Dr.12072.1.A1_at   | Dr.123501 | zgc:154085                             | -    | -    | up | -    | -    |
| Dr.18410.1.S1_at   | Dr.18410  | zgc:56136                              | up   | -    | up | up   | -    |
| Dr.12459.1.A1_at   | Dr.82369  | zgc:63561                              | -    | -    | -  | -    | down |
| Dr.13966.1.S1_at   | Dr.116215 | zgc:63767                              | up   | -    | up | -    | -    |
| Dr.26476.1.S1_at   | Dr.88906  | zgc:64119                              | up   | up   | up | -    | -    |
| Dr.3873.1.A1_at    | Dr.76245  | zgc:73347                              | up   | -    | up | -    | -    |
| Dr.23613.1.A1_at   | Dr.23613  | zgc:77033                              | -    | -    | -  | -    | down |
| Dr.5385.1.A1_at    | Dr.77527  | zgc:77259                              | -    | -    | up | -    | -    |
| Dr.20525.1.A1_a_at | Dr.79390  | zgc:77868                              | up   | -    | -  | -    | -    |
| Dr.5399.1.S1_at    | Dr.32015  | zgc:91854                              | -    | -    | -  | -    | down |
| Dr.14577.1.A1_at   | Dr.83602  | zgc:92090                              | -    | -    | -  | -    | down |
| Dr.11062.1.A1_at   | Dr.82842  | zgc:92294                              | up   | -    | -  | -    | -    |
| Dr.12194.1.A1_at   | Dr.42256  | Transcribed locus                      | up   | -    | -  | -    | -    |
| Dr.12232.1.A1_at   | Dr.83076  | CDNA clone IMAGE:7250984               | -    | -    | -  | down | down |
| Dr.13160.1.A1_at   | Dr.83987  | Transcribed locus                      | up   | -    | -  | -    | -    |

|                  |           |                                                              |    |    |    |    |      |
|------------------|-----------|--------------------------------------------------------------|----|----|----|----|------|
| Dr.15287.1.S1_at | Dr.143198 | Transcribed locus, Lsm3 protein [Homo sapiens]               | -  | -  | -  | -  | down |
| Dr.17.1.A1_at    | Dr.17     | Transcribed locus                                            | up | -  | up | -  | -    |
| Dr.18025.1.A1_at | Dr.18025  | Transcribed locus, cAMP responsive element binding protein 1 | -  | -  | up | -  | -    |
| Dr.18880.1.A1_at | Dr.84419  | Transcribed locus                                            | up | -  | -  | -  | -    |
| Dr.5569.1.S1_at  | Dr.5569   | Transcribed locus                                            | up | up | up | -  | -    |
| Dr.7870.1.A1_at  | Dr.7870   | Transcribed locus                                            | up | -  | up | up | -    |
| Dr.906.1.S1_at   | Dr.82153  | Transcribed locus                                            | up | -  | -  | -  | -    |

**Table S4:** Unique (A) and shared (B) effects of Ca and Na in the presence of Cu compared to copper alone indicating the significant changes to gene expression in the liver of zebrafish. Na or Ca associated Cu exposure either enhanced or prevented the expression of genes compared to Cu alone, and the direction of regulation is indicated. Fold change is based upon change in expression from Ctrl values. The gene list was compiled based on a >2 difference in gene expression.

(A)

| Probe Set ID     | Fold Change<br>Cu | Fold Change<br>Ca+Cu | Effect    | Regulation | Gene Title                                    |
|------------------|-------------------|----------------------|-----------|------------|-----------------------------------------------|
| Dr.813.1.S1_at   | -1.4              | -3.8                 | Enhanced  | Decrease   | acetyl-CoA acetyltransferase 2                |
| Dr.16095.1.S1_at | 1.7               | 4.5                  | Enhanced  | Increase   | insulin-like growth factor binding protein-1b |
| Dr.23065.1.S1_at | 1.3               | 5.0                  | Enhanced  | Increase   | muscle, skeletal, receptor tyrosine kinase    |
| Dr.9920.1.A1_at  | 1.5               | 3.5                  | Enhanced  | Increase   | wu:fk63e10                                    |
| Dr.5325.1.A1_at  | 1.6               | 3.8                  | Enhanced  | Increase   | zgc:65870                                     |
| Dr.9277.1.S1_at  | 6.6               | 3.0                  | Prevented | Decrease   | si:ch211-235e18.3                             |
| Dr.22971.1.A1_at | -5.0              | -2.7                 | Prevented | Increase   | arrestin domain containing 3                  |
| Dr.9611.1.A1_at  | 3.7               | -1.3                 | Prevented | Increase   | si:ch211-241e15.2                             |
| Dr.2871.1.A1_at  | 4.3               | 1.0                  | Prevented | Increase   | similar to Tmc4 protein                       |

| Probe Set ID     | Fold Change<br>Cu | Fold Change<br>Na+Cu | Effect    | Regulation | Gene Title                        |
|------------------|-------------------|----------------------|-----------|------------|-----------------------------------|
| Dr.10688.1.S1_at | -1.1              | -3.4                 | Enhanced  | Decrease   | cyclin-dependent protein kinase 5 |
| Dr.1603.1.A1_at  | -2.8              | -5.0                 | Enhanced  | Decrease   | Cytochrome P450, family 51        |
| Dr.17283.1.A1_at | -2.4              | -4.5                 | Enhanced  | Decrease   | hypothetical LOC558794            |
| Dr.3216.1.A1_at  | -2.5              | -6.4                 | Enhanced  | Decrease   | nucleoside phosphorylase          |
| Dr.9564.1.A1_at  | -1.1              | -3.3                 | Enhanced  | Decrease   | wu:fj88f05                        |
| Dr.9746.8.S1_at  | 1.0               | -3.1                 | Enhanced  | Decrease   | zgc:86706                         |
| Dr.5399.1.S1_at  | 1.0               | -3.1                 | Enhanced  | Decrease   | zgc:91854                         |
| Dr.9277.1.S1_at  | 6.6               | 2.0                  | Prevented | Increase   | arrestin domain containing 3      |
| Dr.2871.1.A1_at  | 4.3               | -1.7                 | Prevented | Increase   | similar to Tmc4 protein           |
| Dr.2433.2.A1_at  | 1.3               | -3.5                 | Prevented | Increase   | zgc:153863                        |

|                  |     |      |           |          |           |
|------------------|-----|------|-----------|----------|-----------|
| Dr.13966.1.S1_at | 3.2 | 1.1  | Prevented | Increase | zgc:63767 |
| Dr.14577.1.A1_at | 1.6 | -7.6 | Prevented | Increase | zgc:92090 |

**(B)**

| Probe Set ID      | Fold Change Cu | Fold Change Ca+Cu | Fold Change Na+Cu | Effect    | Regulation | Gene Title                         |
|-------------------|----------------|-------------------|-------------------|-----------|------------|------------------------------------|
| Dr.21698.1.A1_at  | -2.4           | -12.1             | -8.0              | Enhanced  | Decrease   | CDNA clone IMAGE:6034266           |
| Dr.12232.1.A1_at  | -1.3           | -4.7              | -5.3              | Enhanced  | Decrease   | CDNA clone IMAGE:7250984           |
| Dr.1603.1.A1_x_at | -2.9           | -5.3              | -8.3              | Enhanced  | Decrease   | Cytochrome P450, family 51         |
| Dr.4267.1.A1_at   | -2.4           | -5.0              | -4.8              | Enhanced  | Decrease   | hypothetical LOC558794             |
| Dr.6321.1.A1_at   | -1.8           | -7.9              | -5.0              | Enhanced  | Decrease   | hypothetical LOC559001             |
| Dr.13321.1.S2_at  | -1.4           | -4.1              | -3.5              | Enhanced  | Decrease   | hypoxia induced gene 1             |
| Dr.3698.1.A1_at   | -2.0           | -7.6              | -21.3             | Enhanced  | Decrease   | wu:fd02f07                         |
| Dr.7168.1.S1_at   | -2.0           | -5.6              | -5.9              | Enhanced  | Decrease   | wu:fd56d05                         |
| Dr.13284.1.A1_at  | -4.2           | 1.2               | -1.2              | Prevented | Decrease   | choline kinase alpha               |
| Dr.13284.2.S1_at  | -4.1           | 1.1               | -1.3              | Prevented | Decrease   | choline kinase alpha               |
| Dr.10717.1.S2_at  | -9.0           | 1.8               | 2.4               | Prevented | Decrease   | estrogen receptor 1                |
| Dr.10717.1.S1_at  | -7.8           | 2.2               | 2.8               | Prevented | Decrease   | estrogen receptor 1                |
| Dr.8000.1.S1_at   | 5.9            | 1.3               | -1.3              | Prevented | Decrease   | insulin induced gene 1             |
| Dr.6064.1.A1_at   | 14.6           | 3.2               | 1.5               | Prevented | Decrease   | myelocytomatosis oncogene b        |
| Dr.19560.1.S2_at  | -4.1           | -1.8              | -1.9              | Prevented | Decrease   | solute carrier family 43, member 1 |
| Dr.16048.1.S1_at  | -4.2           | -1.3              | -1.6              | Prevented | Decrease   | zgc:113054                         |
| Dr.2973.1.A1_at   | 5.2            | 1.7               | -1.1              | Prevented | Increase   | glutathione peroxidase 1b          |
| Dr.11296.1.S1_at  | -4.3           | 1.2               | 1.3               | Prevented | Increase   | hypothetical protein LOC100000433  |
| Dr.11310.1.S1_at  | 4.0            | 1.5               | 1.3               | Prevented | Increase   | neutrophil cytosolic factor 1      |
| Dr.23582.1.A1_at  | 3.6            | 1.2               | -1.0              | Prevented | Increase   | tubulin, alpha 1                   |
| Dr.1659.1.A1_at   | 1.5            | -4.2              | -3.8              | Prevented | Increase   | wu:fb13f03                         |
| Dr.8914.1.S1_at   | -3.4           | 1.1               | 1.2               | Prevented | Increase   | wu:fc02a12                         |
| Dr.20008.2.A1_at  | 9.2            | 1.6               | -1.2              | Prevented | Increase   | zgc:92061                          |

**Table S5:** Unique (A) and shared (B) effects of Ca and Na in the presence of Cu compared to the respective ion alone indicating the significant changes to gene expression in the liver of zebrafish. Na or Ca either enhanced or prevented the expression of genes compared to lone ion exposure, and the direction of regulation is indicated. The gene list was compiled based on a >2-fold difference in gene expression.

(A)

| Probe Set ID      | Fold Change Na | Fold Change Na+Cu | Effect    | Regulation | Gene Title                                                   |
|-------------------|----------------|-------------------|-----------|------------|--------------------------------------------------------------|
| Dr.5504.1.S1_at   | -1.3           | -3.4              | Enhanced  | Decrease   | cystathionine-beta-synthase                                  |
| Dr.3216.1.A1_at   | -1.5           | -6.4              | Enhanced  | Decrease   | nucleoside phosphorylase                                     |
| Dr.6847.1.S1_a_at | 1.7            | 3.8               | Enhanced  | Decrease   | zgc:153863                                                   |
| Dr.2433.2.A1_at   | -1.2           | -3.5              | Enhanced  | Increase   | solute carrier family 31 (copper transporters), member 1     |
| Dr.2022.1.A1_at   | 2.1            | -4.8              | Prevented | Decrease   | Cytochrome P450, family 51                                   |
| Dr.9277.1.S1_at   | 4.7            | 2.0               | Prevented | Decrease   | Cytochrome P450, family 51                                   |
| Dr.535.2.A1_at    | 6.4            | 3.6               | Prevented | Decrease   | fatty acid binding protein 7, brain, a                       |
| DrAffx.2.58.A1_at | 3.9            | -1.1              | Prevented | Decrease   | solute carrier family 43, member 1                           |
| Dr.1603.1.A1_x_at | -13.5          | -8.3              | Prevented | Decrease   | sterol-C4-methyl oxidase-like                                |
| Dr.1603.1.A1_at   | -10.1          | -5.0              | Prevented | Decrease   | Transcribed locus                                            |
| Dr.15261.1.A1_at  | 3.1            | 1.1               | Prevented | Decrease   | zgc:73223                                                    |
| Dr.20850.1.S1_at  | -5.3           | -1.8              | Prevented | Increase   | arginase, type II                                            |
| Dr.24246.1.S1_at  | 6.1            | 1.6               | Prevented | Increase   | arrestin domain containing 3                                 |
| Dr.14662.1.S1_at  | 4.0            | 1.8               | Prevented | Increase   | carboxyl ester lipase, like                                  |
| Dr.16652.2.A1_at  | 3.6            | 1.2               | Prevented | Increase   | cytochrome P450, family 24, subfamily A, polypeptide 1, like |
| Dr.25331.1.S1_at  | 4.1            | 1.4               | Prevented | Increase   | cytoplasmic polyadenylation element binding protein 4        |
| Dr.8587.1.A2_at   | 4.3            | -1.0              | Prevented | Increase   | H1 histone family, member X                                  |
| Dr.16095.1.S1_at  | 3.4            | -1.1              | Prevented | Increase   | HNF1 homeobox b                                              |
| Dr.374.1.S1_at    | 3.3            | 1.1               | Prevented | Increase   | hypothetical LOC562726                                       |
| Dr.7722.1.A1_at   | 5.6            | 2.0               | Prevented | Increase   | hypothetical LOC567732                                       |
| Dr.96.1.A1_at     | 4.7            | 1.5               | Prevented | Increase   | Insulin-like growth factor binding protein 1                 |
| Dr.2710.1.S1_at   | 3.5            | 1.3               | Prevented | Increase   | Insulin-like growth factor binding protein-1b                |
| Dr.4948.1.A1_at   | 4.1            | 1.3               | Prevented | Increase   | interferon regulatory factor 2 binding protein 2             |
| Dr.11296.1.S1_at  | -3.4           | 1.3               | Prevented | Increase   | Similar to complement C3-H1                                  |

|                  |      |      |           |          |                                              |
|------------------|------|------|-----------|----------|----------------------------------------------|
| Dr.12110.1.S1_at | -4.5 | -2.4 | Prevented | Increase | Similar to complement protein component C7-1 |
| Dr.23925.1.A1_at | -4.5 | -1.0 | Prevented | Increase | Similar to target of myb1 (chicken)          |
| Dr.18657.1.S1_at | 3.3  | -1.2 | Prevented | Increase | solute carrier family 26, member 5           |
| Dr.16319.1.A1_at | 4.4  | 1.4  | Prevented | Increase | Transcribed locus                            |
| Dr.9217.1.A1_at  | 5.5  | 1.3  | Prevented | Increase | Transcribed locus                            |
| Dr.17775.1.A1_at | 5.0  | 1.2  | Prevented | Increase | Transcribed locus                            |
| Dr.3479.1.A1_at  | 4.3  | 1.4  | Prevented | Increase | wu:fa99c11                                   |
| Dr.22087.1.A1_at | 3.7  | -1.3 | Prevented | Increase | wu:fb37e01                                   |
| Dr.9920.1.A1_at  | 7.4  | 1.4  | Prevented | Increase | wu:fd12f01                                   |
| Dr.25598.1.A1_at | 3.7  | 1.7  | Prevented | Increase | wu:fk63e10                                   |
| Dr.5325.1.A1_at  | 3.6  | 1.4  | Prevented | Increase | zgc:114051                                   |
| Dr.25285.1.S1_at | -4.6 | -1.6 | Prevented | Increase | zgc:65870                                    |
| Dr.14577.1.A1_at | 1.8  | -7.6 | Prevented | Increase | zgc:92090                                    |

| Probe Set ID     | Fold<br>Change<br>Ca | Fold<br>Change<br>Ca+Cu | Effect    | Regulation | Gene Title                                    |
|------------------|----------------------|-------------------------|-----------|------------|-----------------------------------------------|
| Dr.21698.1.A1_at | -8.9                 | -12.1                   | Enhanced  | Decrease   | CDNA clone IMAGE:6034266                      |
| Dr.6321.1.A1_at  | -2.6                 | -7.9                    | Enhanced  | Decrease   | hypothetical LOC559001                        |
| Dr.13321.1.S2_at | -1.6                 | -4.1                    | Enhanced  | Decrease   | hypoxia induced gene 1                        |
| Dr.7168.1.S1_at  | -3.3                 | -5.6                    | Enhanced  | Decrease   | wu:fd56d05                                    |
| Dr.9920.1.A1_at  | 1.2                  | 3.5                     | Enhanced  | Increase   | wu:fk63e10                                    |
| Dr.10717.1.S2_at | -3.9                 | 1.8                     | Prevented | Decrease   | estrogen receptor 1                           |
| Dr.16095.1.S1_at | -1.3                 | 4.5                     | Prevented | Decrease   | Insulin-like growth factor binding protein-1b |
| Dr.3936.1.A1_at  | 3.9                  | 1.3                     | Prevented | Increase   | zgc:162730                                    |
| Dr.26476.1.S1_at | 3.8                  | 1.6                     | Prevented | Increase   | zgc:64119                                     |
| Dr.20008.2.A1_at | 5.4                  | 1.6                     | Prevented | Increase   | zgc:92061                                     |

**(B)**

| <b>Probe Set ID</b> | <b>Fold<br/>Change<br/>Na</b> | <b>Fold<br/>Change<br/>Ca</b> | <b>Fold<br/>Change<br/>Na+Cu</b> | <b>Fold<br/>Change<br/>Ca+Cu</b> | <b>Effect</b> | <b>Regulation</b> | <b>Gene Title</b>                                           |
|---------------------|-------------------------------|-------------------------------|----------------------------------|----------------------------------|---------------|-------------------|-------------------------------------------------------------|
| Dr.12232.1.A1_at    | -1.4                          | -1.5                          | -5.3                             | -4.7                             | Enhanced      | Decrease          | CDNA clone IMAGE:7250984                                    |
| Dr.3698.1.A1_at     | -17.4                         | -4.7                          | -21.3                            | -7.6                             | Enhanced      | Decrease          | wu:fd02f07                                                  |
| Dr.2051.1.S1_at     | -17.2                         | -9.3                          | -5.3                             | -7.2                             | Prevented     | Decrease          | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)  |
| Dr.21005.1.S1_s_at  | 4.3                           | 3.5                           | 1.3                              | 1.3                              | Prevented     | Increase          | complement component c3b /// complement component c3c       |
| Dr.25208.1.A1_at    | 6.2                           | 3.5                           | 1.0                              | 1.4                              | Prevented     | Increase          | cytochrome P450, family 3, subfamily A, polypeptide 65      |
| Dr.25208.2.S1_at    | 6.8                           | 4.1                           | 1.3                              | 1.4                              | Prevented     | Increase          | cytochrome P450, family 3, subfamily A, polypeptide 65      |
| Dr.7171.2.S1_a_at   | 5.3                           | 4.5                           | 1.9                              | 1.5                              | Prevented     | Increase          | glutamic pyruvate transaminase (alanine aminotransferase) 2 |
| Dr.17570.1.S3_at    | 6.0                           | 4.1                           | 1.8                              | 1.1                              | Prevented     | Increase          | MAP kinase-interacting serine/threonine kinase 2b           |
| Dr.26268.1.A1_at    | 5.9                           | 5.0                           | 1.5                              | 1.9                              | Prevented     | Increase          | similar to putative scavenger receptor MARCO                |