Transcriptomic profiling permits the identification of pollutant sources and effects in ambient water samples.

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Abstract

Contaminant exposure is one possible contributor to population declines of endangered fish species in the Sacramento-San Joaquin Estuary, California, including the endangered delta smelt (*Hypomesus transpacificus*). Herein we investigated transcriptional responses in larval delta smelt resulting from exposure to water samples collected at the Department of Water Resources Field Station at Hood, a site of concern, situated upstream of known delta smelt habitat and spawning sites and downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP). Microarray assessments indicate impacts on energy metabolism, DNA and RNA processing, the immune system, development and muscle function. Transcription responses of fish exposed to water samples from Hood were compared with exposures to 9% effluent samples from SRWTP, water from the Sacramento River at Garcia Bend (SRGB), upstream of the effluent discharge, and SRGB water spiked with 2 mg/L total ammonium (9% effluent equivalent). Results indicate that transcriptomic profiles from Hood are similar to 9% SRWTP effluent and ammonium spiked SRGB water, but significantly different from SRGB. SRGB samples however were also significantly different from laboratory controls, suggesting that SRWTP effluent is not solely responsible for the responses determined at Hood, that ammonium exposure likely enhances the effect of multiple-contaminant exposures, and that the observed mortality at Hood is due to the combination of both effluent discharge and contaminants arising from upstream of the tested sites. This study demonstrates that transcriptomic responses of fishes can be valuable endpoints for the detection of pollutants and their sources in surface waters at sublethal and even non-detectable concentrations.

Keywords: delta smelt; Hypomesus transpacificus; ammonium; wastewater effluent; microarray; quantitative PCR.

Introduction

Aquatic ecosystems are among the most diverse ecosystem types worldwide, however, there has been significant declines in biodiversity over the past decades; attributed to habitat destruction and degradation, flow modification, invasive species, overexploitation, and overall water quality (Kennish 2002; Dudgeon, Arthington et al. 2006; Geist 2011). The Sacramento-San Joaquin Estuary in California is an example of detrimental effects resulting within an aquatic ecosystem with intense anthropogenic impact (Lund, Hanak et al. 2010; Cloern and Jassby 2012). Endemic

to this system is a pelagic fish species that has exhibited a gradual decline in population since the 1980s (Moyle, Herbold et al. 1992; Bennett 2005) with a significant step decline recorded in 2000 (Feyrer, Nobriga et al. 2007; Sommer, Armor et al. 2007). The delta smelt (*Hypomesus transpacificus*) was classified as threatened under the Federal and State Endangered Species Act (ESA), 1993, and listed as endangered under the Californian Endangered Species Act (CESA) in 2010 (DFG 2011). It is denoted as a species with an annual life cycle, low fecundity, and a relatively limited habitat range, making this species highly susceptible to changes in the Sacramento-San Joaquin Estuary (Moyle, Herbold et al. 1992). Several factors are postulated to contribute to the decline of the delta smelt population. Habitat degradation, habitat loss, competition with introduced species, decreased food availability, for example, along with changes in abiotic water quality parameters like temperature, salinity and turbidity, have all been the subject of critical scrutiny and are considered to play a significant role in declining delta smelt numbers (Moyle, Herbold et al. 1992). Harmful effects on biota in the Sacramento-San Joaquin estuary are also likely evoked by contaminants entering the delta through anthropogenic activities such as wastewater treatment effluent, and agricultural and urban runoff (Kuivila and Foe 1995; Thompson, Hoenicke et al. 2000; Kennish 2002; Moon 2004).

The impacts of environmentally relevant concentrations of pollutants on aquatic organisms are often subtle, and thus difficult to determine, however, in the past decade researchers in the ecotoxicogenomics field have successfully evaluated sublethal effects of contaminants upon a number of species (Watanabe and Iguchi 2006; Denslow, Garcia-Reyero et al. 2007; Geist, Werner et al. 2007; Connon, Hooper et al. 2008; Garcia-Reyero, Adelman et al. 2008; Heckmann, Sibly et al. 2008; Garcia-Reyero, Kroll et al. 2009; Garcia-Reyero, Lavelle et al. 2011). Genomic responses at the individual level, often assessed through microarray technology, have been extrapolated to effects on populations (Snape, Maund et al. 2004; Miracle and Ankley 2005; Watanabe and Iguchi 2006; Connon, Hooper et al. 2008; Heckmann, Sibly et al. 2008; Fedorenkova, Vonk et al. 2010) creating a powerful tool for use in risk assessment (Hamadeh, Bushel et al. 2002; Watanabe and Iguchi 2006). Although genome sequencing for non-model, ecologically relevant species is still in the early stages (Denslow, Garcia-Reyero et al. 2007), the use of transcriptome analyses in aquatic toxicology is rapidly growing, and its application has the potential to provide information about mechanisms and modes of action for classes of chemicals, as well as provide specific signatures of toxicity (Hamadeh, Bushel et al. 2002; Denslow, Garcia-Reyero et al. 2007; Connon, Geist et al. 2012).

We have previously developed a cDNA microarray for the delta smelt (Connon, Geist et al. 2009), which was used to assess the effects of single contaminants (i.e. esfenvalerate, copper and ammonia) on larval fish (Connon, Geist et al. 2009; Connon, Beggel et al. 2011; Connon, Deanovic et al. 2011). However, the transferability of the methods applied in these studies to complex chemical mixtures commonly encountered in the field has not yet been tested. We utilize microarray and quantitative PCR analyses to assess transcription responses in delta smelt exposed to water samples from the Sacramento River. Samples were collected at the California Department of Water Resources Water Quality Monitoring Station at Hood, a test site of interest and identified as being of poor water quality (Werner, Deanovic et al. 2010), located downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP), and at the Sacramento River at Garcia Bend (SRGB), located upstream from the SRWTP effluent outlet. The SRWTP that discharges its effluent into the lower Sacramento River, which ultimately leads to delta smelt spawning and larval nursery areas. Total ammonium in the Sacramento River, downstream of the SRWTP point of discharge, has been recorded at concentrations up to 1.0 mg/L, whilst concentrations of 0.28 mg/L have been reported directly upstream from known delta smelt spawning and nursery areas (Werner, Deanovic et al. 2010). The effects of ammonia on delta smelt have previously been reported (Connon, Deanovic et al. 2011), however there is a lack of information on the effects of effluent sourced ammonia, within a complex mixture of contaminants, which is integrated into this ambient water toxicity study. The aim of this study was to investigate whether elevated ammonium entering the system would act synergistically with contaminants present in wastewater effluent discharge, and those originating upstream of the discharge point.

1. Materials and methods

Test organism

Delta smelt were obtained from the University of California Davis (UC Davis) Fish Conservation and Culture Laboratory (UCD-FCCL) in Byron, CA, USA and transported to the Aquatic Toxicology Laboratory (presently Aquatic Health Program) UC Davis in black 2.5 gal buckets at a maximum density of 150 fish per bucket. Containers were placed in coolers packed lightly with ice to maintain a temperature of 16 ±2 °C during transport. The control water utilized in this test was made from water obtained from the hatchery. Hatchery water was also used for laboratory control and low conductivity control treatments. This water was pumped

directly from the intake channel of the H.O Banks Pumping Facility near Byron, CA, and passed through a series of sedimentation beds containing natural vegetation to allow any suspended solids in the water to precipitate. The less turbid water was then exposed to an ozonation system to kill any potentially harmful microbes. Ozonated FCCL water was transported to UCD-ATL, and appropriate control waters were prepared for the test one day before fish were collected.

Water sample collection

Two exposure tests are presented in this study: a) Field station assessments on water sampled at Hood (April 30th, 2009), and b) Upstream and effluent exposures on SRGB and SRWTP water samples (June 11th, 2009). The latter was conducted to assess the effect and contribution of contaminants, including total ammonium, to sites downstream of the SRWTP outlet, and as such also included SRGB water spiked with ammonium, as detailed below.

a) Samples were collected at the California Department of Water Resources Water Quality Monitoring Station at Hood (Coordinates: 38°22'03.6"N 121°31'13.6"W; hereafter referred to as Hood) a site located approximately 8 miles downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP). Samples were taken from shore and pumped from a depth of approximately 0.5 m using a standard water pump.

b) Sacramento River water was collected at Garcia Bend, approximately 2 miles upstream from the SRWTP. This water was either spiked with a concentrated stock solution of ammonium chloride (4,000 ppm NH_4CL, Sigma-Aldrich, ACS reagent grade >99%), or diluted with SRWTP effluent of about 9% dilution to match desired ammonia concentrations of 2 $mg.L^{-1}$ total ammonium. SRWTP effluent was collected daily in form of 24h composite samples. Thus fish were exposed to SRGB water, SRWTP effluent dilution, and SRGB water with matching ammonium concentrations. Selected concentrations of total ammonium were based on effect concentrations determined in a related study (Connon, Deanovic et al. 2011).

Water samples for both exposures were collected on a daily basis, one day prior to being used for testing throughout the test. 5-gal clear low-density polyethylene (LPDE) cubitainers (total 35 gallons) were used for transport of water samples to the UCD ATL and were kept on ice in order to maintain the sample temperature at 0-6°C upon receipt at UCD ATL, where water samples were stored in an environmental chamber at 4°C.

Exposures

Fish were maintained for 48 h in test conditions prior to test initiation, and treated with gram-negative and gram-positive antibiotics (Maracyn and Maracyn-2, Virbac AH Inc., Fort Worth TX), to reduce the likelihood of disease-induced effects, and eliminate possible infections that may have been present from hatchery (Connon, Deanovic et al. 2011). Final antibiotic concentrations were 5.3 $mg \cdot L^{-1}$ Maracyn (erythromycin) and 0.26 $mg \cdot L^{-1}$ Maracyn-2 (minocycline). Use of test organisms was approved by the UC Davis Institutional Animal Care and Use Committee (Animal Use Protocol for Animal Care and Use #13361). This institution is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare. The Assurance Number is A3433-01.

For all tests, tests were conducted in 10-L aquaria filled with 7-L of water using 41 days post hatch (dph) and 47-dph-old delta smelt, for Hood, and SRWTP and SRGB samples, respectively. Experimental control fish for both exposures were maintained in hatchery water from UCD-FCCL. Electrical conductivity (EC, adjusted to 20°C) was modified using deionized water, to match that of the respective test controls. Delta smelt are known to be affected by turbidity levels (Hobbs, Bennett et al. 2006; Feyrer, Nobriga et al. 2007), thus the turbidity of controls was adjusted to match that of the field water samples using Nanno 3600™, a concentrated *Nannochloropsis* algae solution ($68 \cdot 10^9$ $cells \cdot ml^{-1}$; Reed Mariculture, Inc. Campbell, CA). Twelve larval fish were placed into each of four replicate aquaria per experimental treatment. Animals were maintained at 17 ± 1.2°C, a light:dark cycle of 16h:8h, and fed "ad libitum" three times a day during the acclimation and testing period with live *Artemia franciscana*. At test initiation, water in aquaria was drained to approximately 2 L, replenished with respective water samples, and exposed in a flow-through system at a rate of 1.44-L per day. Fish were exposed for 7 days and EC, pH, temperature, dissolved oxygen (DO), turbidity and total ammonium were measured daily. Total ammonia was measured using a Hach (Loveland, CO) AmVer Ammonia Test'N Tube Reagent Set, "low range" test kit (0-2.5 mg/L N). Unionized ammonia concentrations for all samples were calculated using measured total ammonia-N, temperature, EC and pH. Mortality was recorded on a daily basis, and any dead fish were removed. All tests were terminated at the same time of day (noon), when surviving fish were counted, euthanized with buffered(neutral pH, using sodium bicarbonate ($NaHCO_3$)) tricaine methanesulfonate (MS-222, Sigma, St. Louis, MO, USA), rinsed in deionized water, snap-frozen, and stored at -80°C for subsequent genomic analyses.

Microarray screening on field station assessments (Hood)

Microarray assessments were carried out on fish exposed to water samples from Hood. Subsequent analyses of SRGB and SRWTP tests were conducted using qPCR on genes identified as responding significantly from the microarray study (see below). We utilized a delta smelt cDNA microarray with 8,448 expressed sequence tags (ESTs), the development of which is described in (Connon, Geist et al. 2009). In brief, purified PCR fragments ranging in size from 1-4 kb, were pin-printed in duplicate onto epoxysilane coated glass slides (Schott-Nexterion, USA). PCR fragments were printed without knowledge of sequence annotation, thus only genes that were differentially expressed following exposures were sequenced for identification. Genomic assessments were conducted between larvae exposed to water from the Sacramento River at Hood and control water (detailed above). Total RNA was extracted from whole, individual fish, using Trizol Reagent (Invitrogen) following manufacturer's guidelines. RNA concentrations were determined using a NanoDrop ND1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA), total RNA 260/280 and 260/230 ratios ranged between 1.88 and 2.15 and 1.70 and 2.10, respectively. Total RNA integrity was verified through electrophoresis on a 1% agarose gel. Total RNA from 3 fish per replicate, per treatment, was pooled, resulting in four biological replicates and four controls. A total of 500ng total RNA for each was amplified using a SuperScript^{TM} Indirect RNA Amplification System (Invitrogen). Resulting amplified RNA (aRNA) was labeled with Alexa fluor dyes ® 555 and 647 (Invitrogen) as per manufacturer's instructions. Two color microarray assessments were carried out using 1µg of amplified aRNA for each control and exposed sample, including dye swaps for each (total 4 slides), which were hybridized for 16 hours at 42°C. Slides were scanned using a GenePix 4000B scanner (Axon Instruments). Microarray data, experimental design and hybridization details are available for download through the Gene Expression Omnibus repository (www.ncbi.nlm.nih.gov) accession number GSE 40991.

Data was analyzed using LIMMA GUI (Linear model for microarray analysis graphical user interface; (Robert Gentleman 2005), written in the R-programming language (available through Bioconductor http://www.Bioconductor.org). Data was normalized using print-tip Lowess and between arrays applying average intensity quantile normalization methods, with background correction. A linear model fit was computed using the duplicates on the arrays and least-squares method, with Benjamini Hochberg false discovery rate adjustment (Benjamini and Hochberg 1995). Only a small proportion of features on the cDNA microarray were previously sequenced (Connon, Geist et al. 2009; Connon, Beggel et al. 2011; Connon, Deanovic et al. 2011), so genes

that were differentially expressed following exposure to ambient water from Hood in this study, were sequenced at the CA&ES Genomic Facility, UC Davis. Sequences were annotated according to homologies to protein database searches using translated nucleotide sequences and direct nucleotide queries (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were only annotated if they were found to have a BLASTx match with the expected value smaller than $1x10^{-5}$ and a score above 30, and each annotation was individually checked for homology. Differentially expressed genes were functionally classified according to the Kyoto Encyclopedia of Genes and genomes (KEGG - http://www.genome.jp/kegg/kegg2.html) into functional groups. Additional information was gathered from literature and the Gene Ontology Database (GO - http://www.uniprot.org/uniprot) to aid classification.

Quantitative polymerase chain reaction (PCR) assessments

Specific genes of interest identified through microarray assessments in this study, as well as previous studies conducted on delta smelt (Connon, Geist et al. 2009; Connon, Beggel et al. 2011; Connon, Deanovic et al. 2011) were selected (Table 1) to conduct comparative quantitative PCR (qPCR) studies amongst Hood, SRWTP effluent, SRGB plus ammonia, SRGB controls and UCD-FCCL controls. A total of 12 fish per treatment; three from each replicate, were assessed by qPCR. All RNA extractions were performed as indicated above. Complementary DNA (cDNA) was synthesized using 2 µg total RNA, with 50 units of Superscript III (Superscript III Reverse Transcriptase, Invitrogen, Carlsbad, CA, USA), 600 ng random primers, 10 units of RNaseOut, and 1 mM dNTPs (Invitrogen) to a final volume of 20 µL. Reactions were incubated for 50 min at 50 °C followed by a 5 min denaturation step at 95 °C. Samples were diluted with the addition of 130 µL nuclease-free water to a total volume of 150 µL for subsequent real-time PCR assessments. Primers and probes for qPCR analyses were designed using Roche Universal Probe Library Assay Design Center (https://www.roche-applied-science.com). Primers were obtained from Eurofins MWG Operon (http://www.eurofinsdna.com), and TaqMan probes were supplied by Roche or Applied Biosystems (Table 1). The assessed efficiency of the primer-probe systems ranged between 91 and 109%. TaqMan Universal PCR Mastermix (Applied Biosystems) was used in qPCR amplifications. SDS 2.2.1 software (Applied Biosystems) was applied to quantify transcription and qPCR data was analyzed using the ($Log_2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen 2001). Differences in transcription were calculated relative to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH); identified using GeNorm (Vandesompele, De Preter et al. 2002) as a suitable reference gene for this assessment. Data were assessed separately for Hood and SWRTP

tests, relative to respective controls. Statistical significance was tested using Shapiro Wilk normality test on $Log_2^{(-\Delta\Delta Ct)}$ data, followed by Mann Whitney U test to test for significant differences in fold-change. The full suite of gene responses, combining data from Hood, and SRWTP and SRGB tests, and respective controls, were then subjected to principal component analysis (PCA) using Genesis software version 1.7.5 36 (Sturn, Quackenbush et al. 2002) on delta Ct data relative to GAPDH $Log_2^{(\Delta Ct)}$ to assess genomic profiling similarities between samples.

Results

Water physicochemistry

Water physicochemical parameters (EC, pH, temperature, DO, and turbidity) remained stable throughout the test and there were no significant differences between treatment groups (Table 2). Nominal total ammonium concentration spiked into SRGB water samples were consistent with measured concentrations. Total ammonium concentrations at Hood, SRGB and laboratory control samples were below the estimated limit of uncertainty of the Hach (Loveland, CO) AmVer Ammonia Test'N Tube Reagent Set (0.7 mg/L) used and are thus not comparable.

Mortality

Delta smelt mortality after 7-d exposure to water samples from the Sacramento River at Hood was 44.7%, significantly exceeding the mortality in the controls of 14.8% ($p < 0.01$). The control treatment included a low EC (179 $\mu S \cdot cm^{-1}$) and low turbidity (5 NTU) adjusted water, these two parameters combined, though predominantly the low EC, may have contributed to the mortality observed, since no mortality was recorded for those fish maintained in non-adjusted culture water from UCD-FCCL (1167 $\mu S \cdot cm^{-1}$ and 11 NTU). There were no significant differences in mortality amongst SRGB (26.5%), SRGB plus 2 mg/L total ammonium (26.1%), and 9% SRWTP effluent (25%).

Microarray assessment (Hood field station)

Microarray analysis of delta smelt larvae exposed to ambient water collected from Hood identified 103 genes responding significantly to the treatment (cut-off $p < 0.05$). Eighty eight genes were down-regulated and only 15 were up-regulated. A total of 94 genes were assigned to a function/pathway, whereas 9 genes remained unknown. The differentially transcribed genes,

annotation and functional classifications are presented in Table 3, and supplementary information (Table S1).

Metabolic pathways responsible for pancreatic secretion, protein digestion and absorption, fatty acid metabolism, pentose phosphate pathways, glycolysis and gluconeogenesis, as well as the starch and sucrose pathway, were affected by exposure. Genes involved in these metabolic pathways included, among others, *intestinal fatty acid binding protein 2b* (FABP2), *carboxypeptidase b* (CPB1) and *aminopeptidase N* (ANPEP), a gene coding for an enzyme that acts as a catalyst in the amino acid cleavage reaction of protein or peptide substrates (Taylor 1993). Energy supply pathways were affected along with metabolism, as indicated by the down regulated transcription of genes such as *vacuolar proton pump subunit H* (ATPeV54kD), which is involved in the oxidative phosphorylation pathway (Saraste 1999).

Altered transcription of genes assigned to genetic information processing is likely an indication of effects on protein biosynthesis, in particular transcription and translation. The messenger RNA surveillance pathway was potentially affected by exposure, as indicated by the down-regulation of *Eukaryotic peptide chain release factor subunit 1* (eRF-1), along with a down-regulation of *60S ribosomal export protein* (NMD3). Up-regulation of *Nei endonuclease VIII-like 1* (NeiL1) is indicative of base excision repair induction, suggesting oxidative DNA damage resulting from exposure (Bandaru, Sunkara et al. 2002; Dou, Mitra et al. 2003; Vartanian, Lowell et al. 2006; Das, Boldogh et al. 2007).

Effects on the immune system were also highlighted through the microarray assessments. Pathways associated with biological defense were affected, in particular with antigen processing and presentation, complement activation and intestinal immune network for immunoglobin A (IgA) production. Genes such as *Major Histocompatibility Complex 2* (MHC2), *Beta-2-Microglobulin* (B2M), *Complement factor BF-2* (BF-2) and *Complement regulatory protein* (CRRY), are associated with the immune system and were significantly down-regulated on exposure (Braciale, Morrison et al. 1987; Germain and Margulies 1993; Xie, Wang et al. 2003; Kim and Song 2006; Zipfel and Skerka 2009).

Neuromuscular system effects were also apparent as indicated by *Tubulin Cofactor Beta* (TBCB) (Grynberg, Jaroszewski et al. 2003; Lopez-Fanarraga, Carranza et al. 2007); implicated in nerve development and cell differentiation, and *Taxilin beta-like* (TXLNB); a gene promoting motor nerve regeneration(Itoh, Fujimori et al. 2005), both of which were down-regulated. Interestingly, *Atrogin-1* (MAFbx32), which is known to be highly expressed during muscle atrophy (Gomes, Lecker et al. 2001), was significantly up-regulated following exposure. Furthermore, *Transgelin* (TAGLN) a gene responsible for muscle development was down-

regulated, along with *Troponin 1* (TNN1), a muscle filament involved in regulation of striated muscle contraction, through alpha-actin, and tropomyosin binding(Assinder, Stanton et al. 2009; Lehman, Galińska-Rakoczy et al. 2009). Both *Alpha-Actin* (ACTA) and *Tropomyosin* were also affected by exposure, up and down-regulated respectively. *Parvalbumin Typ 1* (PvalbT1), involved in muscle relaxation after contraction and calcium ion binding, was significantly up-regulated, along with *Calmodulin 2* (CAM2) (Celio and Heizmann 1982; Heizmann 1984; Chin and Means 2000). *Ictacalcin* (ICN) also involved in calcium ion binding and calcium homeostasis was significantly down-regulated (Porta, Bettini et al. 1996).

Genes associated with bone structure and development, were also influenced by exposure, as suggested by the down-regulation of *Collagen Type XI* (ColXI) and *secreted protein, acidic, cysteine-rich* (*osteonectin*) (SPARC), both of which are involved in collagen binding in vertebral development and ossification (Delany and Hankenson 2009; Wargelius, Fjelldal et al. 2010).

Comparative qPCR (Field station, upstream and effluent samples)

A set of 22 genes identified as indicated above, were used for comparative qPCR studies amongst Hood, SRWTP effluent, SRGB plus ammonium, and respective controls (Figure 1a and b). The response profile relative to controls, between the 9% effluent, 2 mg/L ammonium spiked SRGB water, and Hood are comparable. Of significance is down-regulation of Collagen XI (ColX1) which, regardless of ammonium concentration, responded similarly to effluent and ammonia spiked SRGB water. The majority of the assessed genes that responded significantly ($p < 0.05$); 14 of 22, corresponded to microarray data in their up or down regulation. Creatine kinase (CK) and sarcoendoplasmic reticulum calcium ATPase (SERCA), genes involved in muscular activity, were significantly down-regulated ($p < 0.01$) on exposure to Hood while aspartoacylase (ASPA) a gene associated in nerve signaling, was significantly down-regulated ($p < 0.05$) on exposure to Hood, but up-regulated on exposure to ammonium spiked SRGB water ($p < 0.05$) and 9% effluent ($p < 0.01$).

Correlating transcriptional responses resulting from each treatment, by means of PCA, highlights the similarity of responses between Hood, SRWTP effluent and ammonium spiked SRGB water (Figure 2), differentiating significantly ($p < 0.05$) to the respective SRGB and laboratory controls. Furthermore, the laboratory control differs significantly ($p < 0.05$) from the SRGB control,

Discussion

This study utilized a transcriptional profiling approach to assess and monitor the impacts of contaminants in the environment. As demonstrated in the current study, the use of transcription profiling to assess the impact of complex mixtures will unlikely identify specific contaminants responsible for toxicity, especially without integration of toxicity identification and evaluation (TIE), but it does show promise towards the identification of contaminant sources.

Increased mortality following exposure to water from the Sacramento River at Hood could not be attributed to the physicochemical parameters of this site, when compared to the matching conductivity and turbidity controls, implying that other stressors, potentially contaminants, were responsible for the observed mortality. These contaminant impacts are highlighted in the microarray assessment, identifying effects on important molecular pathways in delta smelt exposed to water collected from the Hood field station.

One of the most characterized molecular pathways is that of PPAR metabolic regulation, a pathway of nuclear receptors that function as transcription factors that regulate gene expression, playing an essential role in numerous diverse physiological process including cell differentiation, development, metabolism of carbohydrates, lipids and proteins, and is activated by signals that control energy and nutrient homeostasis (Mandard, Müller et al. 2004; Puigserver 2005). The PPARs are master regulators of suites of other genes, thus it is high likely that changes in their transcriptional activity could have big effects on their numerous target genes. A key factor at the starting point of the PPAR signaling pathway, by which all three known PPA receptors, PPARα, PPARβ/δ, and PPARγ (Michalik, Auwerx et al. 2006) are affected, is the Intestinal FABP2; involved in fat digestion and absorption (Kaikaus, Bass et al. 1990). Potential adverse effects on digestion are conditioned by effects on energy supply may be translated into impaired growth, reduced fitness, significant malnutrition and starvation. Furthermore, dietary protein deficiencies have been reported to affect the immune system and increase susceptibility to contaminants (Banerjee 1999). Furthermore the PPAR signaling pathway reportedly a target of endocrine disruption, and plays an important role in fatty acid metabolism (Casals-Casas, Feige et al. 2008).

There are further indications that exposure may have adversely affected energy and metabolism, as indicated by the down-regulation of ATPeF0E and ATPeV54kD, which function as proton pumps in the oxidative phosphorylation pathway (Saraste 1999). Closely linked with the effects on metabolism is the production of reactive oxygen species, which can potentially evoke DNA damage (TPA Devasagayam and Saroj S Ghaskadbi 2004). In a healthy organism,

the generation of pro-oxidants in the form of reactive oxygen species is effectively kept in check by the various levels of antioxidant defense. However, following exposure to adverse physicochemical, environmental or pathological agents, this delicately maintained balance is shifted in favor of pro-oxidants resulting in 'oxidative stress' (TPA Devasagayam and Saroj S Ghaskadbi 2004), further potentiating the toxicity of contaminants (Banerjee 1999). NeiL1, which is involved in base excision and repair in DNA bubble formation during replication (Dou, Mitra et al. 2003; Hu, de Souza-Pinto et al. 2005; Das, Boldogh et al. 2007), was up-regulated on exposure. This increase in transcription could potentially indicate that genetic information processing is adversely affected. The base excision repair aspect is not the only indicator of potential effects on genetic information processing. The altered transcription of eRF-1 and NMD3, involved in the mRNA surveillance pathway, and RNA transport (Czaplinski, Ruiz-Echevarria et al. 1998; Kashima, Yamashita et al. 2006), also contributes to this hypothesis. Effects on these pathways may indicate changes in protein biosynthesis, in particular transcription, translation, and RNA degradation and can lead to metabolic impairments.

Effects on development were also highlighted in this study. Down-regulation of *collagen XI* has been associated with vertebral deformities, specifically in the development of vertebral compact bone (Wargelius, Fjelldal et al. 2010). Bone structure might also be negatively affected by the down-regulation of SPARC/Osteonectin BM-40. Studies on bone structure have revealed decreased numbers of osteoblasts and osteoclasts, as well as decreased bone-formation rate and a loss of osteonectin in SPARC-Null mice (Delany and Hankenson 2009), and an increased collagen maturity (Boskey, Moore et al. 2003). These findings indicate potential effects on bone formation and bone remodeling, and impaired bone structure accompanying a lack of SPARC/Osteonectin BM-40 in the delta smelt. Further a lack of SPARC/Osteonectin BM-40 was shown to have adverse effects on wound healing in mice (Basu, Kligman et al. 2001), suggesting that similar effects may impact on exposed delta smelt.

Effects upon muscle function may be indicative of subsequent effects on swimming performance. Tropomyosin is involved in muscle contraction, interacting with calcium and binding to actin filaments during the contraction cycle. Accumulation of calcium in muscular tissue contributes to muscle degradation, muscular dystrophy and muscle fiber necrosis (Olive, Rivera et al. 1994). Elevated Ca^{2+}- level in the muscle cells are likely indicated by changes in calmodulin and parvalbumin regulation, since calmodulin is a Ca^{2+}-binding protein (Chin and Means 2000) and parvalbumin is involved in the removal of calcium from myofibrils, and facilitation of muscle relaxation (Rewal, Wen et al. 2005), and is localized in fast contracting

muscles (Freund 1989). Along with likely muscular atrophy, as suggested by the up-regulation of the ubiquitin mediated *Atrogin-1* (MAFbx), it is highly indicative that these effects would impact on both muscle development and function.

Microarray analysis also pointed to potential effects on immune system. The *major histocombatibility complex class II* (MHCII) and *β-microglobulin* (B2M) proteins play important roles in antigen processing and presentation pathway. MHCII occurs in macrophages, where it is also referred to as antigen presenting cells, and in B cells. It is directly linked with the T cell receptor signaling pathway (Markmann, Lo et al. 1988; Cresswell 1994), which in turn controls the cytokine production and the activation of other immune cells. B2M is a small protein normally found on the surface of many cells, including lymphocytes and is known to be involved in cell protection (Tanaka, Ebata et al. 2005). Catabolism of B2M takes place almost exclusively in the kidney and its excretion is an indication of long term nephrotoxicity (Sørensen, Nissen et al. 1985). Reduced transcription levels of B2M are known to compromise the immune system (Tay, Welsh et al. 1995). B2M was observed to be down-regulated in microarray and qPCR data, although not to statistical significance. An important aspect of the immune defense of an organism is the complement cascade. The complement factor BF-2 and CRRY contribute significantly to the complement cascade and influence the *C3 convertase* (Ponnuraj, Xu et al. 2004; Milder, Gomes et al. 2007) which is a central step in this system (Sarma and Ward 2011). Complement factor BF-2 is a serine protease, which activates the *C3 convertase* (Ponnuraj, Xu et al. 2004; Milder, Gomes et al. 2007), while CRRY reduces the C3/C5 convertase activity, via *decay accelerating factor* (DAF) and membrane *cofactor protein* (MCP), mediate cell lysis (Nangaku, Quigg et al. 1997). Furthermore several functions such as cell lysis, chemotaxis, phagocyte recruitment, inflammation and B-cell receptor signaling pathway are associated with BF-2 (Walport 2001; Carroll 2004).

It has previously been demonstrated that contaminants alone can have severe impacts on the immune system (Clifford, Eder et al. 2005), and can function as predisposing factors, which accompanied with low levels of pathogen infections, can lead to high mortalities. The measured immune system responses could be resultant of synergistic effects of contaminants with undetermined pathogens. Fish in the present study were treated with antibiotics, which should reduce the risk of infection, though possible infections arising from exposure to ambient water samples should not be ruled out entirely.

A number of contaminants, including pyrethroids, heavy metals and fluoranthenes, have previously been detected at the Hood site (Werner, Deanovic et al. 2010). Several sources of agricultural and urban contaminants originate upstream from the site. Effluent discharge from the

Sacramento Regional Wastewater Treatment Plant (SRWTP) is one of the largest known sources of ammonium to the lower Sacramento River. Inflow from the American River, has been reported to carry a number of pesticides such as bifenthrin, which is heavily applied for land use control in urban areas of Sacramento (Weston, Holmes et al. 2009; Weston and Lydy 2010), and upstream of the confluence with the American River, there are vast agricultural regions that also contribute pollutants to the Sacramento River.

Transcriptional differences were significant between delta smelt exposed to Hood and upstream at SRGB, however, both the addition of 95% SRWTP effluent to water from SRGB as well as the addition of ammonium to the SRGB resulted in significant similarities to Hood. These responses suggest that ammonium originating from the wastewater treatment has a significant impact on the delta smelt. Ammonium concentrations at Hood were low, and the upregulation of the ammonium transporter gene on fish exposed to Hood was non-significant, corroborating this and suggesting that other contaminants may be responsible for the observed transcriptional differences.

We have previously reported that ammonium affects cell membrane permeability (Connon, Deanovic et al. 2011), potentially enhancing the uptake and effects of multiple-contaminant exposure. It is therefore debatable that effects of contaminants present upstream (SRGB site) may be enhanced by the addition of ammonia, and that SRWTP effluent contributes further contaminants to the Sacramento River, but that this discharge is not solely responsible for the resulting mortality at Hood. In fact, PCA supports this hypothesis through the clustering of Hood with SRWTP effluent and ammonium spiked SRGB samples (Figure 2), and the significant differentiation between transcriptional responses in SRGB exposed fish to those from the laboratory control.

The findings of this investigation indicate that contaminants originating upstream of Hood are a potential cause for delta smelt growth and development abnormalities, leading to the recorded mortality. We have measured transcription indicators of impacts on the energy metabolism, DNA and RNA processing, development of bone and muscle and on the immune system. Previous studies have indicated the presence of numerous contaminants at this site that arise from anthropogenic activities (Werner, Deanovic et al. 2010) but further investigations upstream from Hood and SRGB are required to determine the source and classes of contaminants in the Sacramento River, and these investigations should include broad-scale chemical analyses. Although SRWTP effluent discharge is reportedly a major source of contaminants, urban and agricultural activities throughout the area, and upstream of the tested sites, are likely to

contribute to the complex mixture of compounds adversely affecting the delta smelt habitat and population dynamics.

This study has highlighted how transcriptomic assessments can successfully be utilized to investigate not only the effects of complex contaminant mixtures, within ambient water samples, but also demonstrated the use of these techniques towards the identification of contaminant sources. This enhances the potential of future applications of transcriptomic techniques in complement with existing TIE approaches, aiding the determination of contaminants, or contaminant classes responsible for toxicity.

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References:

Assinder, S. J., J.-A. L. Stanton, et al. (2009). "Transgelin: An actin-binding protein and tumour suppressor." The International Journal of Biochemistry & Cell Biology **41**(3): 482-486.

Bandaru, V., S. Sunkara, et al. (2002). "A novel human DNA glycosylase that removes oxidative DNA damage and is homologous to Escherichia coli endonuclease VIII." DNA Repair **1**(7): 517-529.

Banerjee (1999). "The influence of various factors on immune toxicity assessment of pesticide chemicals." Toxicology Letters **107**(1-3): 21-31.

Basu, A., L. Kligman, et al. (2001). "Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40)." BMC Cell Biology **2**(1): 15.

Benjamini, Y. and Y. Hochberg (1995). "Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing." Journal of the Royal Statistical Society. Series B (Methodological) **57**(1): 289-300.

Bennett, W. A. (2005). "Critical assessment of the delta smelt population in the San Francisco Estuary, California." San Francisco Estuary and Watershed Science **3**(2).

Boskey, A. L., D. J. Moore, et al. (2003). "Infrared Analysis of the Mineral and Matrix in Bones of Osteonectin-Null Mice and Their Wildtype Controls." Journal of Bone and Mineral Research **18**(6): 1005-1011.

Braciale, T. J., L. A. Morrison, et al. (1987). "Antigen Presentation Pathways to Class I and Class II MHC-Restricted T Lymphocytes." Immunological Reviews **98**(1): 95-114.

Carroll, M. C. (2004). "The complement system in regulation of adaptive immunity." Nat Immunol **5**(10): 981-986.

Casals-Casas, C., J. N. Feige, et al. (2008). "Interference of pollutants with PPARs: endocrine disruption meets metabolism." International Journal of Obesity **32**(Suppl. 6): S53-S61.

Celio, M. R. and C. W. Heizmann (1982). "Calcium-binding protein parvalbumin is associated with fast contracting muscle fibres." Nature **297**(5866): 504-506.

Chin, D. and A. R. Means (2000). "Calmodulin: a prototypical calcium sensor." Trends in Cell Biology **10**(8): 322-328.

Clifford, M. A., K. J. Eder, et al. (2005). "Synergistic effects of esfenvalerate and infectious hematopoietic necrosis virus on juvenile chinook salmon mortality." Environmental Toxicology and Chemistry **24**(7): 1766-1772.

Cloern, J. E. and A. D. Jassby (2012). "Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay." Reviews of Geophysics **50**(4): n/a-n/a.

Connon, R., J. Geist, et al. (2009). "Linking mechanistic and behavioral responses to sublethal esfenvalerate exposure in the endangered delta smelt; *Hypomesus transpacificus* (Fam. Osmeridae)." BMC Genomics **10**(1): 608.

Connon, R., H. L. Hooper, et al. (2008). "Linking Molecular and Population Stress Responses in Daphnia magna exposed to cadmium." Environmental science & technology **42**(6): 2181-2188.

Connon, R. E., S. Beggel, et al. (2011). "Linking molecular biomarkers with higher level condition indicators to identify effects of copper exposures on the endangered delta smelt (*Hypomesus transpacificus*)." Environmental Toxicology and Chemistry **30**(2): 290-300.

Connon, R. E., L. A. Deanovic, et al. (2011). "Sublethal responses to ammonia exposure in the endangered delta smelt; *Hypomesus transpacificus* (Fam. Osmeridae)." Aquatic Toxicology **105**(3–4): 369-377.

Connon, R. E., J. Geist, et al. (2012). "Effect-Based Tools for Monitoring and Predicting the Ecotoxicological Effects of Chemicals in the Aquatic Environment." Sensors **12**(9): 12741-12771.

Cresswell, P. (1994). "Assembly, Transport, and Function of MHC Class II Molecules." Annual Review of Immunology **12**(1): 259-291.

Czaplinski, K., M. J. Ruiz-Echevarria, et al. (1998). "The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs." Genes & Development **12**(11): 1665-1677.

Das, A., I. Boldogh, et al. (2007). "The Human Werner Syndrome Protein Stimulates Repair of Oxidative DNA Base Damage by the DNA Glycosylase NEIL1." Journal of Biological Chemistry **282**(36): 26591-26602.

Delany, A. and K. Hankenson (2009). "Thrombospondin-2 and SPARC/osteonectin are critical regulators of bone remodeling." Journal of Cell Communication and Signaling **3**(3): 227-238.

Denslow, N. D., N. Garcia-Reyero, et al. (2007). "Fish `n` chips: the use of microarrays for aquatic toxicology." Molecular bioSystems **3**(3): 172-177.

DFG, D. O. F. A. G. (2011). State & Federally listed endangered & threatened animals of California**:** 1-13.

Dou, H., S. Mitra, et al. (2003). "Repair of Oxidized Bases in DNA Bubble Structures by Human DNA Glycosylases NEIL1 and NEIL2." Journal of Biological Chemistry **278**(50): 49679-49684.

Dudgeon, D., A. H. Arthington, et al. (2006). "Freshwater biodiversity: importance, threats, status and conservation challenges." Biological Reviews **81**(02): 163-182.

Fedorenkova, A., J. A. Vonk, et al. (2010). "Ecotoxicogenomics: Bridging the Gap between Genes and Populations." Environmental science & technology **44**(11): 4328-4333.

Feyrer, F., M. L. Nobriga, et al. (2007). "Multidecadal trends for three declining fish species: habitat patterns and mechanisms in the San Francisco Estuary, California, USA." Canadian Journal of Fisheries & Aquatic Sciences **64**(4): 723-734.

Freund, T. F. (1989). "GABAergic septohippocampal neurons contain parvalbumin." Brain research **478**(2): 375-381.

Garcia-Reyero, N., I. Adelman, et al. (2008). "Gene expression profiles of fathead minnows exposed to surface waters above and below a sewage treatment plant in Minnesota." Marine Environmental Research **66**(1): 134-136.

Garcia-Reyero, N., K. Kroll, et al. (2009). "Gene expression responses in male fathead minnows exposed to binary mixtures of an estrogen and antiestrogen." BMC Genomics **10**(1): 308.

Garcia-Reyero, N., C. M. Lavelle, et al. (2011). "Behavioral and genomic impacts of a wastewater effluent on the fathead minnow." Aquatic Toxicology **101**(1): 38-48.

Geist, J. (2011). "Integrative freshwater ecology and biodiversity conservation." Ecological Indicators **11**(6): 1507-1516.

Geist, J., I. Werner, et al. (2007). "Comparisons of tissue-specific transcription of stress response genes with whole animal endpoints of adverse effect in striped bass (Morone saxatilis) following treatment with copper and esfenvalerate." Aquatic Toxicology **85**(1): 28-39.

Germain, R. N. and D. H. Margulies (1993). "The Biochemistry and Cell Biology of Antigen Processing and Presentation." Annual Review of Immunology **11**(1): 403-450.

Gomes, M. D., S. H. Lecker, et al. (2001). "Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy." Proceedings of the National Academy of Sciences **98**(25): 14440-14445.

Grynberg, M., L. Jaroszewski, et al. (2003). "Domain analysis of the tubulin cofactor system: a model for tubulin folding and dimerization." BMC Bioinformatics **4**(1): 46.

Hamadeh, H. K., P. R. Bushel, et al. (2002). "Prediction of Compound Signature Using High Density Gene Expression Profiling." Toxicological Sciences **67**(2): 232-240.

Heckmann, L.-H., R. Sibly, et al. (2008). "Systems biology meets stress ecology: linking molecular and organismal stress responses in *Daphnia magna*." Genome Biology **9**(2): R40.

Heizmann, C. W. (1984). "Parvalbumin, and intracellular calcium-binding protein; distribution, properties and possible roles in mammalian cells." Cellular and Molecular Life Sciences **40**(9): 910-921.

Hobbs, J. A., W. A. Bennett, et al. (2006). "Assessing nursery habitat quality for native smelts (Osmeridae) in the low-salinity zone of the San Francisco estuary." Journal of Fish Biology **69**(3): 907-922.

Hu, J., N. C. de Souza-Pinto, et al. (2005). "Repair of Formamidopyrimidines in DNA Involves Different Glycosylases." Journal of Biological Chemistry **280**(49): 40544-40551.

Itoh, S., K. E. Fujimori, et al. (2005). "Long-term effects of muscle-derived protein with molecular mass of 77 kDa (MDP77) on nerve regeneration." Journal of Neuroscience Research **81**(5): 730-738.

Kaikaus, R. M., N. M. Bass, et al. (1990). "Functions of fatty acid binding proteins." Cellular and Molecular Life Sciences **46**(6): 617-630.

Kashima, I., A. Yamashita, et al. (2006). "Binding of a novel SMG-1–Upf1–eRF1–eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay." Genes & Development **20**(3): 355-367.

Kennish, M. J. (2002). "Environmental threats and environmental future of estuaries." Environmental Conservation **29**(01): 78-107.

Kim, D. D. and W.-C. Song (2006). "Membrane complement regulatory proteins." Clinical Immunology **118**(2–3): 127-136.

Kuivila, K. M. and C. G. Foe (1995). "Concentrations, transport and biological effects of dormant spray pesticides in the San Francisco Estuary, California." Environmental Toxicology and Chemistry **14**(7): 1141-1150.

Lehman, W., A. Galińska-Rakoczy, et al. (2009). "Structural Basis for the Activation of Muscle Contraction by Troponin and Tropomyosin." Journal of Molecular Biology **388**(4): 673-681.

Livak, K. J. and T. D. Schmittgen (2001). "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-[Delta][Delta]CT Method." Methods **25**(4): 402-408.

Lopez-Fanarraga, M., G. Carranza, et al. (2007). "Tubulin cofactor B plays a role in the neuronal growth cone." Journal of neurochemistry **100**(6): 1680-1687.

Lund, J., E. Hanak, et al. (2010). Comparing Futures for the Sacramento-San Joaquin Delta. San Francisco, California, Public Policy Institute of California.

Mandard, S., M. Müller, et al. (2004). "Peroxisome proliferator-activated receptor a target genes." Cellular and Molecular Life Sciences **61**(4): 393-416.

Markmann, J., D. Lo, et al. (1988). "Antigen presenting function of class II MHC expressing pancreatic beta cells." Nature **336**(6198): 476-479.

Michalik, L., J. Auwerx, et al. (2006). "International Union of Pharmacology. LXI. Peroxisome Proliferator-Activated Receptors." Pharmacological Reviews **58**(4): 726-741.

Milder, F. J., L. Gomes, et al. (2007). "Factor B structure provides insights into activation of the central protease of the complement system." Nat Struct Mol Biol **14**(3): 224-228.

Miracle, A. L. and G. T. Ankley (2005). "Ecotoxicogenomics: linkages between exposure and effects in assessing risks of aquatic contaminants to fish." Reproductive Toxicology **19**(3): 321-326.

Moon, K. M. K. a. G. E. (2004). "Potential Exposure of Larval and Juvenile Delta Smelt to Dissolved Pesticides in the Sacramento–San Joaquin Delta, California." American Fisheries Society Symposium **39**: 229-241.

Moyle, P. B., B. Herbold, et al. (1992). "Life History and Status of Delta Smelt in the Sacramento-San Joaquin Estuary, California." Transactions of the American Fisheries Society **121**(1): 67 - 77.

Nangaku, M., R. Quigg, et al. (1997). "Overexpression of Crry protects mesangial cells from complement- mediated injury." Journal of the American Society of Nephrology **8**(2): 223-233.

Olive, M., R. Rivera, et al. (1994). "Parvalbumin immunocytochemistry and calcium deposition in muscle fiber necrosis and subsequent regeneration following intramuscular injection of metoclopramide." Muscle & nerve **17**(5): 494-499.

Ponnuraj, K., Y. Xu, et al. (2004). "Structural Analysis of Engineered Bb Fragment of Complement Factor B: Insights into the Activation Mechanism of the Alternative Pathway C3-Convertase." Molecular Cell **14**(1): 17-28.

Porta, A. R., E. Bettini, et al. (1996). "Molecular cloning of ictacalcin: a novel calcium-binding protein from the channel catfish, Ictalurus punctatus." Molecular Brain Research **41**(1-2): 81-89.

Puigserver, P. (2005). "Tissue-specific regulation of metabolic pathways through the transcriptional coactivator PGC1-[alpha]." Int J Obes Relat Metab Disord **29**(S1): S5-S9.
Rewal, M., Y. Wen, et al. (2005). "Role of Parvalbumin in Estrogen Protection From Ethanol Withdrawal Syndrome." Alcoholism: Clinical and Experimental Research **29**(10): 1837-1844.
Robert Gentleman, V. J. C., Wolfgang Huber, Rafael A. Irizarry, Sandrine Dudroit (2005). Bioinformatics and Computational Biology Solutions Using R and Bioconductor, Springer.
Saraste, M. (1999). "Oxidative Phosphorylation at the fin de siècle." Science **283**(5407): 1488-1493.
Sarma, J. and P. Ward (2011). "The complement system." Cell and Tissue Research **343**(1): 227-235.
Snape, J. R., S. J. Maund, et al. (2004). "Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology." Aquatic Toxicology **67**(2): 143-154.
Sommer, T., C. Armor, et al. (2007). "The collapse of pelagic fishes in the upper san francisco estuary." Fisheries **32**(6): 270-277.
Sørensen, P., M. Nissen, et al. (1985). "Beta-2-microglobulin excretion: An indicator of long term nephrotoxicity during <i>cis</i>-platinum treatment?" Cancer chemotherapy and pharmacology **14**(3): 247-249.
Sturn, A., J. Quackenbush, et al. (2002). "Genesis: cluster analysis of microarray data." Bioinformatics **18**(1): 207-208.
Tanaka, T., T. Ebata, et al. (2005). "[beta]2-Microglobulin required for cell surface expression of blastocyst MHC." Biochemical and biophysical research communications **332**(1): 311-317.
Tay, C., R. Welsh, et al. (1995). "NK cell response to viral infections in beta 2-microglobulin-deficient mice." The Journal of Immunology **154**(2): 780-789.
Taylor, A. (1993). "Aminopeptidases: structure and function." The FASEB Journal **7**(2): 290-298.
Thompson, B., R. Hoenicke, et al. (2000). "An Overview of Contaminant-Related Issues Identified by Monitoring in San Francisco Bay." Environmental Monitoring and Assessment **64**(1): 409-419.
TPA Devasagayam, J. T., KK Boloor, Ketaki S Sane, and R. L. Saroj S Ghaskadbi (2004). "Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects." The Journal of the Association of Physicians of India **52**: 794-804.
Vandesompele, J., K. De Preter, et al. (2002). "Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes." Genome biology **3**(7): research0034.0031 - research0034.0011.
Vartanian, V., B. Lowell, et al. (2006). "The metabolic syndrome resulting from a knockout of the NEIL1 DNA glycosylase." Proceedings of the National Academy of Sciences of the United States of America **103**(6): 1864-1869.
Walport, M. J. (2001). "Complement." New England Journal of Medicine **344**(14): 1058-1066.
Wargelius, A., P. G. Fjelldal, et al. (2010). "Collagen type XI {alpha}1 may be involved in the structural plasticity of the vertebral column in Atlantic salmon (Salmo salar L.)." J Exp Biol **213**(7): 1207-1216.
Watanabe, H. and T. Iguchi (2006). "Using ecotoxicogenomics to evaluate the impact of chemicals on aquatic organisms." Marine Biology **149**(1): 107-115.
Werner, L. A. Deanovic, et al. (2010). Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008-2010. Davis California, Aquatic Toxicology Laboratory, School of Veterinary Medicine, University of California Davis, California**:** 1-217.

Werner, L. A. Deanovic, et al. (2010). "Monitoring acute and chronic water column toxicity in the Northern Sacramento–San Joaquin Estuary, California, USA, using the euryhaline amphipod, Hyalella azteca: 2006 to 2007." Environmental Toxicology and Chemistry **29**(10): 2190-2199.

Weston, D. P., R. W. Holmes, et al. (2009). "Residential runoff as a source of pyrethroid pesticides to urban creeks." Environmental Pollution **157**(1): 287-294.

Weston, D. P. and M. J. Lydy (2010). "Urban and Agricultural Sources of Pyrethroid Insecticides to the Sacramento-San Joaquin Delta of California." Environmental Science & Technology **44**(5): 1833-1840.

Xie, J., Y. Wang, et al. (2003). "β2-Microglobulin as a negative regulator of the immune system: high concentrations of the protein inhibit in vitro generation of functional dendritic cells." Blood **101**(10): 4005-4012.

Zipfel, P. F. and C. Skerka (2009). "Complement regulators and inhibitory proteins." Nat Rev Immunol **9**(10): 729-740.

Tables and Figures.

Table 1. Primers and probes used for quantitative PCR assessments of gene transcription in delta smelt (*Hypomesus transpacificus*).

Gene Name	Gene Code	Primer 5'→3'	Primer 3'→5'	Roche Probe No. #
Alpha Actin	a-actin	cctgcctcgtcgtactcctg	catcctggcttccctgtcc	*11*
Adenylate Kinase	Adk	ctgtcttctggggacctgttg	ctcctttctgcataattgcctgt	*36*
Amylase	Amy	gatcaccatgttcttgatctgacg	ccatcaatcctgaccaaacctg	*99*
Aspartoacylase	Aspa	cagagccttcacgacagaaa	tgaacctcatagggcaggtc	*22*
Fbxo32 (Atrogin)	Atrogin	ggaagcaccaaagagcgtca	ggcgctgcagaaatccaa	*7*
Calmodulin	Calm2	ttccttattcgacatggatggc	gcagacccagtgactgcatg	*17*
Caspase 3	Casp3	gagaaccggtatgaaccaacg	tccaagcttcccaaacactttc	*159*
Creatine Kinase	CK	cgatcggcgttggagatg	gccaagttcaacgagattctgg	*163*
Collagen XI	ColXI	ccaaaatcgatcaggttccaat	tggttggcatccccaaag	*#*
Estrogen Receptor 1	ESR1	tccaggagctgtctctccat	gagaccgatcatgagcacct	*72*
Keratin 15	Krt15	ccagcaaaaccagttactcctcc	cctgatgagcctccatacctca	*38*
Myosin Light Chain 2	Mlc2	catgggagaccgcttcacc	tgtcgatgggagcttcacg	*10*
Ammonium transporter	NH4 transp	caggctgtcttatcgcttacgg	cagcgtcatgactaacagctgaa	*61*
Pregnane X Receptor	PXR	tgaggcggtggagaagag	gaggcggtggagaagag	*144*
Sarcoendoplasmic Reticulum – Calcium ATPase	SERCa	catgatcattgggggagca	tgctgtgatgacaacgaggac	*148*
Tubulin Cofactor Beta	Tbcb	gactcctgcagctggtatgga	ccagcttctgcaggaacttgtc	*78*
Transforming Growth Factor beta	TGF-b	caacggcatagtgcatgtgg	gaatgtgtgcacgttgttggt	*76*
Thyroid Hormone Receptor alpha	THR-a	gcgtggacaagatcgagaag	tgtgcttgcggtagttgatg	*62*
Transmembrane protein 4 sub-family 4	Tms4sf4	ccctggctctcatctccatc	ccatctttggcatacttcacc	*64*
Tumor Necrosis factor alpha	TNF-a	ctttttccgctgttccatgttc	gttaccagcatacgcagtgtcc	*2*
Tropomyosin	Tpm	tcccttaacagacgcatccag	cagtagccagacgctcctgtg	*101*
Zona Pellucida	Zpa	catgcggctgagtttggataa	tgccattgatagcatcaacttca	*106*
*Glyceraldehyde 3-phosphate dehydrogenase**	GAPDH	tccacgagaaagacccaact	cacgccagtagactcaacca	*159*

Custom design (FAM-caacgtcatggtcaatg-BHQ Applied Biosystems), *reference gene.

Table 2. Physicochemical water parameters determined during the tests. Values represent mean and standard deviations (SD) over the 7 d test period.

	Control		Hood		SRGB		SRGB plus 2 mg/L NH_4-N		SRWTP 9% effluent	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Temperature °C	17.0	1.2	16.9	1.2	16.9	0.2	17.0	0.3	16.9	0.4
EC (µS/cm) (20°C adjustment)	179	36	150	30	172	59	221	59	219	60
DO (mg/l)	9.6	0.2	9.8	0.2	9.6	0.2	9.5	0.3	9.6	0.3
pH	7.95	0.14	7.97	0.06	7.89	0.10	7.83	0.08	7.83	0.12
Turbidity (NTU)	7	1	5	4	7	5	6	5	6	5
Measured Ammonia Nitrogen (mg/L)	-		-		-		1.90	0.24	1.96	0.16
Unionized Ammonia (mg/L)	-		-		-		0.038	0.007	0.039	0.010

SRGB: Sacramento River at Garcia Bend, SRWTP: Sacramento Regional Wastewater Treatment Plant, EC: Electric Conductivity, DO: Dissolved Oxygen, NTU: Nephelometric Turbidity Units.
"-": Below estimated limit of uncertainty.

Table 3. Microarray assessment and functional classification of delta smelt (*Hypomesus transpacificus*) genes responding to Sacramento River water from Hood, as determined by Kyoto Encyclopedia for Genes and Genomes (KEGG) pathway analysis.

Functional Category	Upregulated Transcripts	Downregulated Transcripts
1 Metabolism		
1.1 Carbohydrate Metabolism		
Pentose Phosphate Pathway [PATH:ko00030]		TALDO1, PRPS, PGI
Glycolysis Glyconeogenesis [PATH:ko00010]		PGI
Starch and sucrose Pathway [PATH:ko00500]		PGI
Amino sugar and nucleotide Metabolism [PATH:ko00520]		CYB5R3, PGI
1.2 Energy Metabolism		SQRDL *
Oxidative Phosphorylation [PATH:ko00190]		ATPeF0E, ATPeV54kD
1.3 Lipid Metabolism		
Arachidonic acid Metabolism [PATH:ko00590]		PTGES
Sphingolipid Metabolism [PATH:ko00600]		ASAH1
Glycerophospholipid metabolism [PATH:ko00564]		PCYT2
1.4 Nucleotide Metabolism		
Purine Metabolism [PATH:ko00230]		PRPS
1.5 Aminoacid Metabolism		
Tryptophan Metabolism [PATH:ko00380]		TPH
Glycine, Serine and Threonin Metabolism [PATH:ko00260]	GATM	Setd8b, GAMT,GNMT
Arginine and Proline Metabolism [PATH:ko00330]	GATM	GAMT
1.6 Metabolism of Other Amino Acids		
Glutathione Metabolism [PATH:ko00480]		ANPEP
Phosphonate and phosphinate metabolism [PATH:ko00440]		PCYT2
1.7 Metabolism of Cofactors & Vitamins		
Porphyrin and chlorophyll metabolism [PATH:ko00860]		FTH1, FTL
1.8 Enzyme Families		
Peptidases		NPEPL1
2 Genetic Information Processing		
2.1 Transcription		
Spliceosome [PATH:ko03040]		HSPA1_8, BUD31
Transcription factors [BR:ko03000]		CEBPD
2.2 Translation		
mRNA surveillance pathway [PATH:ko03015]		eRF-1
RNA transport [PATH:ko03013]		NMD3
Ribosome biogenesis in eukaryotes [PATH:ko03008]		NMD3, NOB1, WDR43, HSR1
Translation factors [BR:ko03012]		eEF-2
2.3 Folding Sorting and Degradation		
Proteasome [PATH:ko003050]	PSMC4	PSMA3, PSMB4, PSME3
Protein Processing in endoplasmic reticulum [PATH:ko04141]		HSPA1_8, SAR1, UBE2G1, TBCB
Ubiquitin mediated proteolysis [PATH:ko04120]		UBE2G1, UBE2L3
SNARE interactions in vesicular transport [PATH:ko04130]		SNAP29
Ubiquitin system [BR:ko04121]	ZFAND2B, FBXO32 MAFbx	KLHL31
Chaperones and folding catalysts [BR:ko03110]		CCT5, CCT8, DNAJC7, TXNIP
2.4 Replication and Repair		
Base excision repair [PATH:ko03410]	NeiL1	
DNA repair and recombination proteins [BR:ko03400]	UBE2V	
Chromosome [BR:ko03036]		H1_5
3 Environmental Information Processing		
3.1 Signal Transduction		EMP55*, LRG1 *
MAPK signaling Pathway [PATH:ko04010]		HSPA1_8, RAP1B
Phosphatidylinositol signaling system [PATH:mcc04070]	CaM2	
Calcium signaling pathway [PATH:mcc04020]	CaM2	
Calcium ion binding *	PvalbT1 *	PvalbT2 *, PvalbT3 *, EPD-1 *, ICN *, SPARC *
Wnt-signaling Pathway [PATH:ko04310]		RHOA
TGF-beta signaling Pathway [PATH:ko04350]		RHOA
3.2 Signal Molecules and Interactions		
Cell adhesion molecules (CAMs) [PATH:ko04514]		MHC2, CNTN1
GTP-binding proteins [BR:ko04031]		RAB27A
Protein binding *		IST1 *, BSDC1 *, FAHD1 *, HIGD1A *, LCN1 *, SDS22 *, ATG101 *, EMP55*
4 Cellular Processes		
4.1 Transport and Catabolism		
Lysosome [PATH:ko04142]	CTSD	ASAH1, ATPeV54kD
Endocytosis [PATH:ko04144]		HSPA1_8, RHOA, VPS28, VPS4
Phagosome [PATH:ko04145]		MHC2, ATPeV54kD
Peroxisome [PATH:ko04146]		PXMP2
Regulation of autophagy [PATH:ko04140]		GABARAP, ATG101*
4.2 Cell Motility		
Regulation of actin cytoskeleton [PATH:ko04810]		RHOA, TNNI1 *
Cytoskeleton proteins [BR:ko04812]		MLC1, Krt4, TNNI1, TNNI2

Muscle Atrophy *	FBXO25_32 MAFbx32 *	
Cell matrix adhesion and Matrix structural constituent *		EPD-1 *, ColXI *
4.3 Cell Growth and Death		
Oocyte meiosis [PATH:mcc04114]	CaM2	
Apoptosis *		MLC1 *, CHAC1, NUPR1 *
cell cycle, cell division *		IST1 *, TXNIP *, WDR82 *
4.3 Cell Communication		
Focal adhesion [PATH:ko04510]		RHOA, RAP1B
Adherens junction [PATH:ko04520]		RHOA
Tight junction [PATH:ko04530]		RHOA
5 Organismal Systems		
5.1 Immune System		BF-2 *
Hematopoietic Cell Lineage [PATH:ko04640]		ANPEP
Antigen processing and presentation [PATH:ko04612]		PSME3, MHC2, HSPA1_8, B2M
T cell receptor signaling pathway [PATH:ko04660]	UBE2V *	RHOA, HSR1 *, RAB27A *, TXNIP *
Chemokine signaling pathway [PATH:ko04062]		RHOA, RAP1B
Leukocyte transendothelial migration [PATH:ko04670]		RHOA, RAP1B
Intestinal immune network for IgA production [PATH:ko04672]		MHC2
Complement and coagulation cascades		CRRY
5.2 Endocrine System		
Renin-Angiotensin System [PATH:ko04614]		ANPEP, THOP1
PPAR signaling pathway [PATH:ko03320]	FABP2	UCP1
Insulin signaling pathway [PATH:mcc04910]	CaM2	
GnRH signaling pathway [PATH:mcc04912]	CaM2	
Melanogenesis [PATH:mcc04916]	CaM2	
5.3 Circulatory System		
Vascular smooth muscle contraction [PATH:ko04270]	CaM2	RHOA
Cardiac muscle contraction [PATH:ko04260]	ActA	TNNI1, Tpm
Relaxation after contraction *	PvalbT1 *	PvalbT2 *, PvalbT3 *
calcium homeostasis *		ICN *
5.4 Digestive System		
Pancreatic secretion [PATH:ko04972]		RHOA, RAP1B, CPB1
Salivary secretion [PATH:mcc04970]	CaM2	
Gastric acid secretion [PATH:mcc04971]	CaM2	
Fat digestion and absorption [PATH:ko04975]	FABP2	
Protein digestion and absorption [PATH:ko04974]		CPB1
Proteolysis *		BF-2 *, LCN1 *
5.5 Nervous System		
Neurotrophin signaling pathway [PATH:ko04722]	CaM2	RHOA, RAP1B
Long-term potentiation [PATH:ko04720]	CaM2	RAP1B
Axon guidance [PATH:ko04360]		RHOA, CNTN1*, St8sia4*
5.6 Sensory System		
Phototransduction [PATH:mcc04744]	CaM2	ARRDC2
Olfactory transduction [PATH:mcc04740]	CaM2	
Sensory perception of taste *		LCN1 *
5.7 Development		
Cell differentiation *		MLC1 *, Tbcb *
Ossification, Collagen binding *		SPARC *
Muscle organ -, Nervous system development *		TAGLN *, Tbcb *, TXLNB*
5.8 Environmental Adaptation		
Stress response, heat response *		HSPB8*, HIGD1A *
Response to oxidative stress	NeiL1	
Facilitate necrotic cell death under different types of stress*		HEBP2 *
6 Not Assigned		
	CG057 , apo14kDa , SLC6A9	FUNDC1 , TNNT3B, apo14kDa, GATLS1, NAT8L, TMEM106B, PWP1

* Denotes genes for which no KEGG pathways were identified, and where functional categories were attributed from KEGG Brite functional hierarchies, gene ontology and related litertature. Abbreviations; Gene names: **ActA**: Alpha Actin; **ANPEP**: Aminopeptidase N; **Apo14kDa**: 14kDa Apolipoprotein; **Arrdc2**: Arrestin domain-containing protein 2; **ASAH1**: N-acylsphingosine amidohydrolase; **ATG101**: Autophagy-related protein 101; **ATPeF0E**: ATP synthase e chain, mitochondrial; **ATPeV54kD**: Vacuolar proton pump subunit H; **B2M**: Beta-2 microglobulin; **BF-2**: Complement factor Bf-2; **BSDC1**: BSD domain containing 1; **BUD31**: BUD31 homolog; **CaM2**: Calmodulin-2; **CCT5**: T-complex protein 1 subunit epsilon; **CCT8**: T-complex protein 1 subunit theta; **CEBPD**: CCAAT/enhancer-binding protein delta; **CG057**: CG057 protein; **CHAC1**: Cation transport regulator-like protein 1; **CNTN1**: Contactin 1a precursor; **ColXI**: Collagen type XI alpha1 short isoform; **CPB1**: Carboxypeptidase B; **CRRY**: Complement regulatory protein **CTSD**: Cathepsin D; **CYB5R3**: NADH-cytochrome b5 reductase; **DNAJC7**: DnaJ homolog subfamily C member 7; **eEF-2**: Elongation factor 2; **EMP55**: 55 kDa Erythrocyte membrane protein; **EPD-1**: Ependymin-1; **eRF-1**: Eukaryotic peptide chain release factor subunit 1; **FABP2**: Intestinal fatty acid binding protein 2b; **FAHD1**: Fumarylacetoacetate hydrolase domain-containing protein 1; **MAFbx32** : F-box only protein 32 Muscle atrophy F-box protein, Atrogin1; **FTH1**: Ferritin, heavy subunit; **FTL**: Ferritin, middle subunit; **FUNDC1**: FUN14 domain-containing protein 1; **GABARAP**: Gamma-aminobutyric acid receptor-associated protein-like 1; **GAMT**: Guanidinoacetate N-methyltransferase; **GATM**: Glycine amidinotransferase, mitochondrial; **GATSL1**: GATS-like protein 1; **GNMT**: Glycine N-methyltransferase; **GPI**: Phosphoglucose isomerase-2; **H1_5**: H1 histone family, member 0; **HEBP2**: Heme-binding protein 2; **HIGD1A**: HIG1 domain family member 1A; **HSPA1_8**: Heat shock cognate 71 kDa protein; **HSPB8**: Heat shock 22kDa protein 8; **HSR1**: GTP-binding protein HSR1; **ICN**: Ictacalcin; **IST1**: KIAA0174-like protein, IST-homolog1; **KLHL31**: Kelch-like protein 31; **KRT4**: Krt4 protein; **LCN1**: Lipocalin precursor; **LRG1**: Leucine-rich alpha-2-glycoprotein; **MCL1**: Myeloid leukemia differentiation protein homologue; **MHC2**: Major histocompatibility complex 2; **NAT8L**: N-acetyltransferase 8-like protein; **NEIL1**: Nei endonuclease VIII-like 1; **NMD3**: 60S Ribosomal export protein NMD3; **NOB1**: RNA-binding protein NOB1; **NPEPL1**: Aminopeptidase-like 1; **NUPR1**: Nuclear protein 1; **PCYT2**: Ethanolamine-phosphate cytidylyltransferase; **PRPS**: Phosphoribosyl pyrophosphate synthetase 1A isoform 1; **PSMA3**: Proteasome subunit alpha type 7; **PSMB4**: Proteasome subunit beta type 4; **PSMC4**: 26S protease regulatory subunit 6B; **PSME3**: Proteasome activator complex subunit 3; **PTGES**: Prostaglandin E synthase 3; **PvalbT1**: Parvalbumin Typ1; **PvalbT2**: Parvalbumin Typ2; **PvalbT3**: Parvalbumin Typ3; **PWP1**: Periodic tryptophan protein 1 homolog; **PXMP2**: Epithelial membrane protein 2; **RAP1B**: Ras-related protein Rap-1b precursor; **RAB27A**: Ras-related protein Rab-27A; **RHOA**: Transforming protein RhoA precursor; **SAR1**: SAR1 gene homolog A; **SDS22**: Protein phosphatase 1 regulatory subunit 22; **SLC6A9**: Novel protein similar to vertebrate solute carrier family 6 (neurotransmitter transporter, glycine); **SNAP29**: Synaptosomal-associated protein 29; **SPARC**: SPARC precursor; **SQRDL**: Sulfide:quinone oxidoreductase; **St8sia4**: Alpha-2,8-polysialyltransferase IV; **TAGLN**: Transgelin; **TALDO1**: Transaldolase; **TBCB**: Tubulin folding cofactor B; **THOP1**: Thimet oligopeptidase; **TMEM106B**: Transmembrane protein 106B; **TNNI1**: Troponin1; **TNNI2**: Troponin, slow skeletal Muscle; **TNNT3B**: Troponin T3b, skeletal, fast isoform 2; **TPH**: Tryptophan hydroxylase; **TPM**: Tropomyosin; **TXLNB**: Taxilin-Beta-like; **TXNIP**: Thioredoxin-interacting protein; **UBE2G1**: Ubiquitin-conjugating enzyme E2 G1; **UBE2L3**: Ubiquitin-conjugating enzyme e2 l3; **UBE2V**: Ubiquitin-conjugating enzyme E2 variant 1; **UCP1**: Mitochondrial uncoupling protein 2; **VPS28**: Vacuolar protein sorting-associated protein 28 homolog; **VPS4**: Vacuolar protein sorting-associating protein 4B; **WDR43**: WD repeat-containing protein 43; **WDR82**: WD repeat domain containing 82 isoform 1; **ZFAND2B**: AN1-type zinc finger protein 2B.

Figure 1. Fold-change in transcription of 22 genes that responded significantly in juvenile delta smelt exposed for 7 days to ambient water samples collected at (A) the lower Sacramento River at the Department of Water Resources Field Station Hood, (B) the Sacramento River at Garcia Bend (SRGB) and 9% effluent from the Sacramento Regional Wastewater Treatment Plant (SRWTP). *, **, and *** represent statistical significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively. # represents statistical significance at $p < 0.05$ between SRGB spiked with 2 mg/L total ammonium and 9% SRWTP effluent containing 2 mg/L total ammonium. a-actin: *Alpha Actin*, Adk: *Adenylate Kinase*, Amy: *Amylase*, Aspa: *Aspartoacylas*e, Atrogin: *Fbxo32 (Atrogin)*, Calm2: *Calmodulin*, Casp3: *Caspase 3*, CK: *Creatine Kinase*, ColXI: *Collagen XI*, ESR1: *Estrogen Receptor 1*, Krt15: *Keratin 15*, Mlc2: *Myosin Light Chain 2*, NH4 transp: *Ammonium transporter*, PXR: *Pregnane X Receptor*, SERCa: *Sarcoendoplasmic Reticulum –Calcium ATPase*, Tbcb: *Tubulin Cofactor Beta*, TGF-b: *Transforming Growth Factor beta*, THR a: *Thyroid Hormone Receptor alpha*, Tms4sf4: *Transmembrane protein 4 sub-family 4*, TNF-a*: Tumor Necrosis factor alpha*, Tpm: *Tropomyosin*, Zpa: *Zona Pellucida*.

Figure 2. Principal component analysis of transcriptional responses in juveniles delta smelt exposed for 7 days to ambient water samples collected at the lower Sacramento River at the Department of Water Resources Field Station Hood, the Sacramento River at Garcia Bend (SRGB) and 9% effluent from the Sacramento Regional Wastewater Treatment Plant (SRWTP), and SRGB water spiked with 2 mg/L total ammonium, and UCD-FCCL culture water. Letters a, b, and c upper and lower-case represent significant differences between samples (alpha = 0.05) as determined by PC1 and PC2, respectively.

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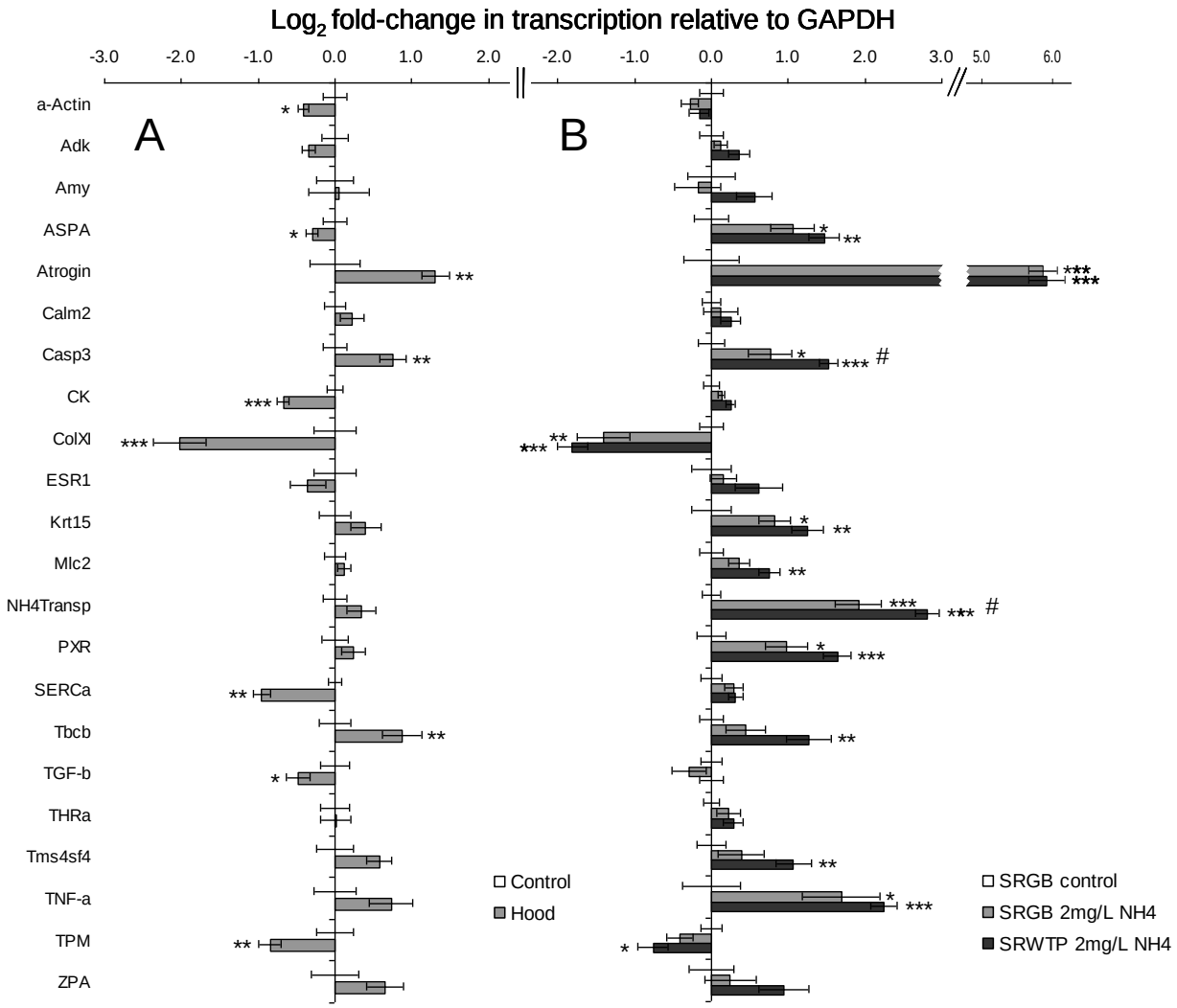

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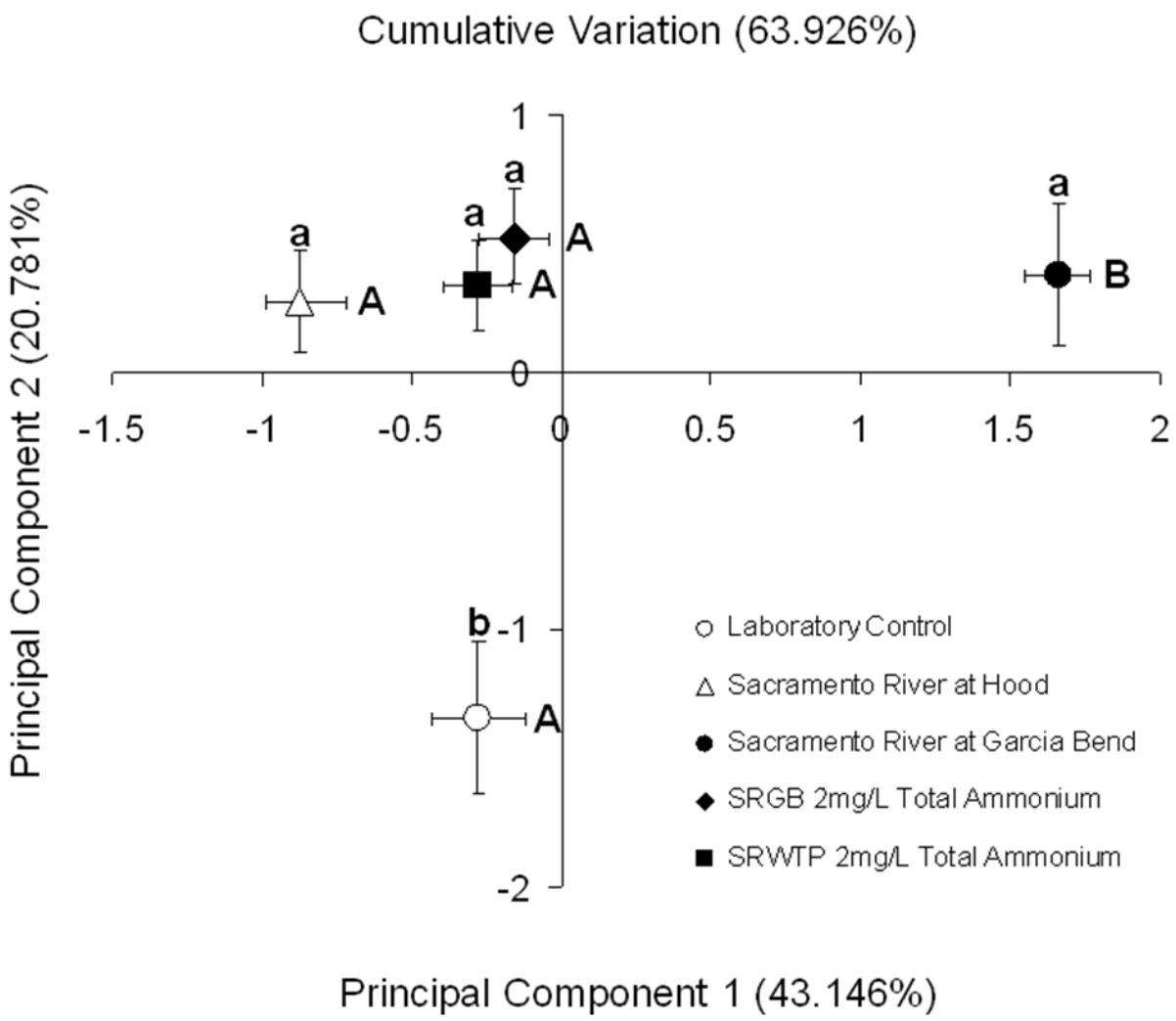

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Conflict of Interest:

All authors declare that there is no conflict of interest, nor potential for conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, our work.