



Application of novel biomarkers to determine sublethal contaminant exposure and effects in delta smelt



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Background

The toxic effects of contaminants on aquatic species are often subtle, negatively affecting ecological fitness and consequently reproduction and survival via sublethal cellular, physiological, behavioral or immunological effects.

Detecting sublethal effects of contaminants in the Sacramento-San Joaquin Delta is challenging, especially for non-model organisms such as the delta smelt (Fig 1), for which biomarker tools or toxicity testing protocols are not readily available.



Figure 1. Adult delta smelt

Objectives

To develop and apply molecular biomarkers systems, designed to assess sublethal effects of site-specific contaminants in the Sacramento-San Joaquin Delta.

Material & Methods

Laboratory investigations were conducted to evaluate chemical-related aquatic toxicity of Delta water (Fig 2) to larval delta smelt (41-dph). Exposures were carried out over 7 days.



Figure 2. Location of tested Delta water grab samples

Molecular biomarkers were developed in the delta smelt to assess effects on muscular, neurological, digestive and immune system functioning (Table 1). Gene transcription was assessed using quantitative-PCR on individual whole organisms, and normalized to β -actin transcription.

Transcription profiling was performed using Genesis software. Response similarities were clustered using Pearson's Correlation.

Neurological	Aspartacylase (ASPA) Hemopexin (Hemo) Tubulin folding Cofactor Beta (TBCB)
Muscular (structural)	Titin alpha Actin (A-Actin) Tropomyosin (TPM) Myozenin (Myo)
Muscular (activity)	Sarco-Endoplasmic Reticulum Ca2+-ATPase (SERCA) Calmodulin (CaM) Calpain Adenylate Kinase (ADK)
Immunoregulation	Tumor Necrosis Factor alpha (TNF) Transforming Growth Factor beta (TGF)
Apoptosis	Caspase (Cas-3)
Membrane integrity	Claudin 10 (CLDN10) Keratin-15 (KER-15)
Xenobiotic detection	Pregnane-X-Receptor (PXR)

Table 1. List of genes selected based on molecular function

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Results

All fish survived the highest conductivity exposures and there was non-significant mortality in low conductivity controls (12.5%). Hood, a site located below the Sacramento Regional Wastewater Treatment Plant (SRWTP), resulted in highest mortality to larval delta smelt 43.5%, 17.5% at Light 55 and 32.5% at Cache Lindsay (Fig 3).

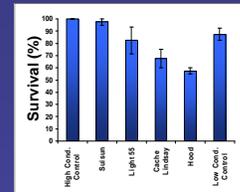


Figure 3. Toxicity responses to Delta water samples (7-d exposure)

Transcription profiles separated Delta sites based on larval delta smelt genomic responses (Fig 4). Suisun Bay exposed larvae clustered with high conductivity controls, whilst low conductivity controls correlated with freshwater samples.

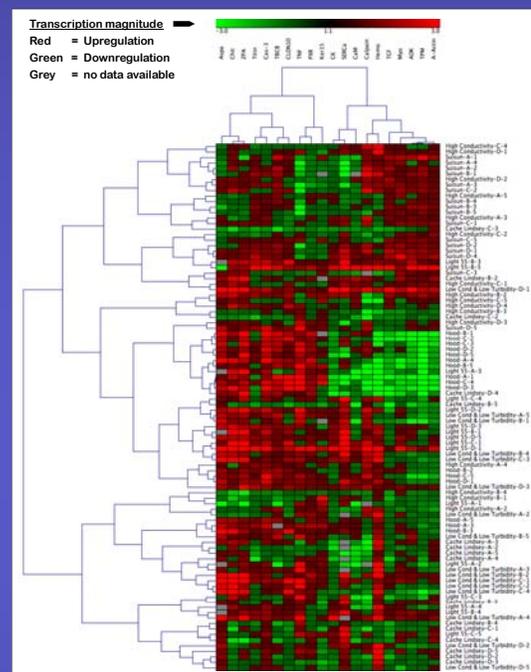


Figure 4. Transcription profiling (heatmap) of individual larval Delta smelt responses to Delta water exposure (Pearson's Correlation)

Exposure to water from Hood elicited significant transcriptional differences of genes involved predominantly in neuromuscular functions, suggesting that contaminants originating from the SRWTP effluent may impact on swimming performance, growth and development of larval delta smelt. Down-regulation of structural muscle genes may also indicate physiological damage (Further investigations are being conducted on these samples and on SRWTP effluent).

Discussion and Conclusions

- Transcription profiling is a useful to be incorporated into water quality monitoring as it identifies sublethal effects dependent on contaminant modes of action.
- Profiling can be used to aid Toxicity Identification Evaluation approaches.
- Previous studies have linked neuromuscular transcriptions changes with behavior responses such as swimming performance; a highly sensitive and ecologically relevant endpoint.