Science of the Total Environment 468–469 (2014) 688–698

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

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HIGHLIGHTS

- Ammonia present in effluent is likely acting synergistically with other contaminants.
- Transcriptomic profiling differentiates between upstream, downstream and effluent discharge.
- Transcription profiling can identify sources of contamination.

ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form 22 August 2013
Accepted 24 August 2013
Available online 21 September 2013

Editor: Christian E.W. Steinberg

Keywords:
Delta smelt
Hypomesus transpacificus
Ammonium
Wastewater effluent
Microarray
Quantitative PCR

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 repair mechanisms 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.

1. Introduction

Aquatic ecosystems are among the most diverse ecosystem types worldwide, however, there have 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 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 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 et al., 1992; Bennett, 2005) with a significant step decline recorded in 2000 (Feyrer et al., 2007; Sommer 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 known as a species with an annual life cycle, low fecundity, and a relatively limited habitat range, making it highly susceptible to

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http://dx.doi.org/10.1016/j.scitotenv.2013.08.081

changes in the Sacramento-San Joaquin Estuary (Moyle et al., 1992). Habitat degradation, habitat loss, competition with introduced species, decreased food availability, 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 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 et al., 2000; Kennish, 2002; Kuivila and 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 effects of contaminants upon a number of species (Watanabe and Iguchi, 2006; Denslow et al., 2007; Geist et al., 2007; Connon et al., 2008; Garcia-Reyero et al., 2008, 2009, 2011; Heckmann et al., 2008). Genomic responses at the individual level, often assessed through microarray technology, have been extrapolated to effects on populations (Snape et al., 2004; Miracle and Ankley, 2005; Watanabe and Iguchi, 2006; Connon et al., 2008; Heckmann et al., 2008; Fedorenkova et al., 2010) creating a powerful tool for use in risk assessment (Hamadeh et al., 2002; Watanabe and Iguchi, 2006). Although genome sequencing for non-model, ecologically relevant species is still in the early stages (Denslow 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 et al., 2002; Denslow et al., 2007; Connon et al., 2012).

We have previously developed a cDNA microarray for the delta smelt (Connon et al., 2009), which was used to assess the effects of single contaminants (i.e. esfenvalerate, copper and ammonia) on larval fish (Connon et al., 2009, 2011a, 2011b). 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 et al., 2010b), 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 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 et al., 2010a). The effects of ammonia on delta smelt have previously been reported (Connon et al., 2011b), 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 ammonium entering the system as part of a contaminant mixture present in wastewater effluent, or Sacramento River water, would exert greater toxicity than as a single substance. Therefore we compare exposures to wastewater effluent, with upstream river water samples, with and without added ammonium, and contrast responses to down-stream water sample exposures.

2. Materials and methods

2.1. 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. Hatchery water was also used for laboratory control and low electrical conductivity (EC, adjusted to 20 °C) 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 control waters were prepared for the test one day before fish were collected.

2.2. Water sample collection

Results of two exposure experiments are presented here: a) Exposure to water sampled from the Sacramento River at Hood, on April 30th, 2009, and b) Upstream and effluent exposures on SRGB and SRWTP water samples, beginning on 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) Water from the Sacramento River at 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) was collected as a single grab sample. This site is located approximately 8 miles downstream of the SRWTP. The sample was 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 (SRGB), approximately 2 miles upstream from the SRWTP effluent discharge. This water was tested unaltered (as control), mixed with SRWTP effluent to a final total ammonium concentration of 2 mg L^{-1} (averaging 9% effluent), or spiked with a concentrated stock solution of ammonium chloride (4,000 ppm NH_4CL, Sigma-Aldrich, ACS reagent grade >99%), to match the ammonium concentration of the SRWTP effluent. Final total ammonium concentrations were 1.9 ± 0.23 mg L^{-1} for SRWTP effluent samples and 1.87 ± 0.26 (SD) for spiked SRGB during the 7-d experimental period. SRGB and effluent samples were collected daily, one day prior to being used for testing. SRWTP effluent was collected in form of 24-h composite samples.

Twenty liter, clear low-density polyethylene (LPDE) cubitainers (total 700 liters) were used for collection and transport of water samples to the UCD ATL and were kept on ice to maintain sample temperature at 0–6 °C until receipt at UCD ATL, where water samples were stored in an environmental chamber at 4 °C.

2.3. Exposures

Fish were maintained for 48 h in test conditions prior to test initiation, and treated with antibiotics (Maracyn and Maracyn-2, Virbac AH Inc., Fort Worth TX), to reduce the likelihood of disease-induced effects, and eliminate possible infections (Connon et al., 2011b). Final antibiotic concentrations were 5.3 mg L^{-1} Maracyn (erythromycin) and 0.26 mg 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.

Tests were conducted in 10-L aerated aquaria filled with 7-L of water from Hood, SRWTP and SRGB samples, and respective controls. Larval