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Correlation of gene expression and contaminant concentrations in wild largescale suckers: A field-based study

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HIGHLIGHTS

- Hepatic tissue concentrations of OCs, PCBs, and PBDEs were measured in wild largescale suckers.
- Gene expression was analyzed by microarray in the same cohort of fish.
- The expression patterns of 69 genes correlated with tissue contaminant concentration.
- Correlated genes are candidates for use in future biomarker development.

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ABSTRACT

Toxic compounds such as organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ether flame retardants (PBDEs) have been detected in fish, birds, and aquatic mammals that live in the Columbia River or use food resources from within the river. We developed a custom microarray for largescale suckers (*Catostomus macrocheilus*) and used it to investigate the molecular effects of contaminant exposure on wild fish in the Columbia River. Using Significance Analysis of Microarrays (SAM) we identified 72 probes representing 69 unique genes with expression patterns that correlated with hepatic tissue levels of OCs, PCBs, or PBDEs. These genes were involved in many biological processes previously shown to respond to contaminant exposure, including drug and lipid metabolism, apoptosis, cellular transport, oxidative stress, and cellular chaperone function. The relation between gene expression and contaminant concentration suggests that these genes may respond to environmental contaminant exposure and are promising candidates for further field and laboratory studies to develop biomarkers for monitoring exposure of wild fish to contaminant mixtures found in the Columbia River Basin. The array developed in this study could also be a useful tool for studies involving endangered sucker species and other sucker species used in contaminant research.

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1. Introduction

The Columbia River Basin is home to many types of fish and wildlife, but this important river is threatened by the release of toxic compounds from a myriad of sources, including industrial and municipal discharges and agriculture. A recent report by the United States Environmental Protection Agency expressed concern about the levels of mercury, dichlorodiphenyltrichloroethane (DDT) and its derivatives, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ether (PBDE) flame retardants in the Columbia River and the risk they present to humans, fish, and wildlife (EPA, 2009). Studies have shown bioaccumulation of hydrophobic organic compounds and heavy metals through the food web in migratory and resident fish (Feist et al., 2005; Foster et al., 2001a, 2001b; Hinck et al., 2006; Johnson et al., 2007a, 2007b; Rayne et al., 2003; Sloan et al., 2010; Webb et al., 2006), predatory birds (Anthony et al., 1993; Buck et al., 2005; Elliott et al., 2000; Henny et al., 2004, 2008, 2011), and aquatic mammals (Elliott et al., 1999; Grove and Henny, 2008; Harding et al., 1998, 1999). To fully understand which contaminants are present at different sites in the Columbia River Basin and the risks that they present, studies that incorporate a spectrum of analyses are needed. The work described here is part of a large, interdisciplinary study investigating the

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occurrence of emerging and legacy contaminants (PBDEs and other endocrine disrupting compounds) in the lower Columbia River and their food web effects (Nilsen and Morace, this issue).

Our objective was to determine the transcriptomic effects of environmental contaminants in largescale suckers (*Catostomus macrocheilus*), a species that has previously been used to assess contaminant levels in the Columbia River Basin (Hinck et al., 2006; Rayne et al., 2003). Largescale suckers are widespread and represent an intermediate level of the food web since they both feed on contaminated prey and are themselves prey for birds and other fish (Dauble, 1986). Little is known about movement patterns of largescale suckers, but as resident fish they are thought to remain within a relatively confined home range and thus would potentially accumulate the contaminant load present in their home range (Rayne et al., 2003).

Contaminant studies in the Columbia River have generally focused on a few endpoints and indicators. Physiological endpoints such as condition factor, gonad size, and plasma triglycerides were previously correlated with contaminant exposure in fish from the Columbia River (Feist et al., 2005). A negative correlation between liver and gonad contaminant burdens and proteins such as plasma testosterone and 11-ketotestosterone was also found in white sturgeon (*Acipenser transmontanus*) in the Columbia River (Feist et al., 2005; Foster et al., 2001a). Although we have gained information about contaminant effects from these studies using individual markers of exposure, the work has been limited by the few biological markers and endpoints known to respond to contaminant exposure.

Techniques that assay a broad range of molecules, such as microarray technology, offer the potential for a more global understanding of the biological effects of contaminant exposure. Microarray analyses, which simultaneously assay the expression of thousands of genes, are powerful tools for identification of biological pathways affected by contaminant exposure and for discovery of individual genes that could be used as new indicators of contaminant exposure. Microarray technology is increasingly used in aquatic toxicology (Bartosiewicz et al., 2001; Carlson et al., 2009; Garcia-Reyero et al., 2011; Popesku et al., 2010; Sellin Jeffries et al., 2012), but its application remains limited due to the lack of DNA sequence information for ecologically-relevant species. To expand the tools available for investigating the effects of complex environmental contaminant mixtures, we developed a custom microarray for largescale suckers. We used this array to identify genes with expression patterns that correlated with hepatic levels of organochlorine (OC) pesticides, PCBs, or PBDEs in wild largescale suckers collected from the lower Columbia River.

2. Materials and methods

2.1. Site descriptions

To facilitate collection of fish with a range of contaminant burdens, we chose three sites in the lower Columbia River with different levels of urbanization and contaminant point sources (see Nilsen et al. (this issue) for map of study sites). The sites are as follows: Skamania (river km 225), Columbia City (river km 132), and Longview (river km 106). The Skamania site is the furthest upstream and has no known contaminant point sources nearby. It is 10 km downstream of Bonneville Dam, the lower-most dam on the Columbia River, but upstream of large urban areas, including Portland, Oregon, and Vancouver, Washington. The Columbia City site is located between Columbia City and St. Helens, Oregon. There are four environmental cleanup areas near the upstream end of this site. Other potential sources of contamination include the St. Helens wastewater treatment plant and upstream input from Lake River, which receives storm water runoff from Vancouver, Washington, via Vancouver Lake. Finally, the Longview site is farthest downstream and located directly in the Port of Longview at Longview, Washington, and across from Rainier, Oregon, the location of the now decommissioned Trojan Nuclear Power Plant. The port has waterfront industrial property, and effluents from Three Rivers Regional and City of Rainier wastewater treatment plants also feed into the river.

2.2. Fish and tissue collection

Largescale suckers were collected from the three sites by shoreline boat electrofishing on March 23, 2010 and May 4–6, 2011. In 2010, we collected four fish from Skamania and three fish from Longview and euthanized them in an overdose of MS-222 (200 mg/L) buffered with an equal amount of sodium bicarbonate. We dissected the whole liver, brain, heart, kidney, and gonad and preserved a 130 mg portion of each in RNAlater (Ambion, Austin, Texas). In 2011, we collected 6–8 fish from each site and euthanized them as described. We did not test the effects of MS-222 on gene expression in this study because all of our fish were anesthetized in the same manner, and thus all would be similarly affected by anesthetic exposure. We measured fork length to the nearest 0.5 cm and weighed fish to the nearest gram. Gonads were extracted and weighed separately to the nearest 0.1 g, and liver samples were preserved as described above. Small portions (1–2 g) of the livers were also placed into a glass jar, manually homogenized with a metal spatula, and then placed on ice for transport back to the lab where they were frozen at −20 °C until analyzed for contaminants. We calculated the gonadosomatic index (GSI) for each fish by dividing gonad weight by total fish weight and multiplying by 100.

2.3. Contaminant analysis

Liver samples were analyzed using high-pressure solvent extraction, column cleanup, and concentration followed by quantification by gas chromatography–mass spectrometry (GC–MS) for 58 compounds, including DDTs and other OC pesticides, PCBs, and PBDEs (Table S1). Samples were analyzed by the U.S. Geological Survey's National Water Quality Laboratory (Denver, Colorado) as described (Nilsen et al., this issue). For quality control, the lab ran method blanks, reagent spikes, and surrogate compounds. Quality control results and individual contaminant data for each fish are included in Table S2. When a contaminant was detected in the method blank only values four times greater than the blank value were reported as detections. For statistical analyses, contaminant concentration values below the method detection limit were set to zero and included in the analyses. We identified PCB congeners by their IUPAC number.

2.4. Microarray development

We used total RNA from the liver, brain, heart, kidney, and gonad of the seven largescale suckers (3 males and 4 females) collected in 2010 to construct a normalized cDNA library, which we sequenced using the 454 GS-FLX (Roche 454 Life Sciences, Branford, Connecticut) sequencing platform as described in Garcia-Reyero et al. (2008) and the Illumina Genome Analyzer IIx (Illumina Inc., San Diego, California) sequencing platform following the manufacturer's protocols. The assembled cDNA contigs yielded a total of 158,403 non-redundant sequences, which we annotated by conducting large-scale homology searches of the National Center for Biotechnology Information (NCBI) nr and nt databases using an in-house computational pipeline implementing the basic local alignment search tool (blastx and blastn; http://blast.ncbi.nlm.nih.gov/Blast.cgi). About 55,700 of the sequences queried had a hit with high sequence similarity (e-value $\leq 10^{-5}$). We also characterized the assembled sequences with respect to functionally annotated genes by BLAST searching against the NCBI reference sequences (RefSeq) for human (*Homo sapiens*) and zebrafish (*Danio rerio*). We found 43,436 human homologs and 54,386 zebrafish homologs. We designed a 15K-oligonucleotide microarray with 14,708 unique probes, 50 replicate probes, and 536 control probes using the eArray service from Agilent. Replicate probes were included to ensure the consistency of expression measurements across each array, and the control probes were used to

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