GENE EXPRESSION IN CAGED FISH AS A FIRST-TIER INDICATOR OF CONTAMINANT EXPOSURE IN STREAMS

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Abstract—The development of sensitive, biologically based indicators of contaminant exposure (i.e., biomarkers) is an ongoing topic of research. These indicators have been proposed as a first-tier method of identifying contaminant exposure. The primary objective of this research was to implement a biomarker-based method of exposure assessment using caged fish and real-time reverse-transcriptase polymerase chain reaction (rtRT-PCR) measurements of gene expression. Primers were developed for the CYP1A, metallothionein, and vitellogenin genes in rainbow trout (Oncorhynchus mykiss), cutbow trout (Oncorhynchus clarkii), and Atlantic salmon (Salmo salar). Each of these genes has been shown to respond specifically to planar aromatic compounds, heavy metals, and environmental estrogens, respectively. Juvenile fish were placed in cages and exposed in situ at reference and contaminated sites on the Cache la Poudre River (CO, USA), the Arkansas River (CO, USA), the St. John River (NB, Canada), and two urban creeks near Dayton (OH, USA). Quantitative gene expression was determined using rtRT-PCR. Biomarker expression profiles were obtained that demonstrated differences in CYP1A, metallothionein, and vitellogenin mRNA production unique to each site, indicating that specific types of compounds were bioavailable and present in sufficient concentrations to elicit transcriptional responses in the organism. These findings support the use of a biomarker-based approach to exposure identification and assessment.

Keywords—Biomarker Cytochrome P4501A Metallothionein Vitellogenin Polymerase chain reaction

INTRODUCTION

This paper is the first in a series of papers detailing development of a series of methods for in situ multilevel, multi-point analysis of stressor exposure in freshwater streams. The following data describe the results of a series of in situ biomarker experiments conducted in five freshwater, North American streams. Future papers will detail the results of in situ toxicity testing and toxicity identification evaluation procedures, as well as in situ macroinvertebrate colonization experiments. This project was a collaborative effort between personnel from Colorado State University (Fort Collins, CO, USA), Miami University (Oxford, OH, USA), and Wright State University (Dayton, OH, USA).

A great deal of research has been conducted into the development of biomarkers of contaminant exposure [1–3]. These biomarkers have been suggested as a means of stressor identification and as sentinels of contaminant exposure, so that remedial action may be taken before detrimental effects occur at the population level [4]. The use of biomarkers has advantages over traditional analytical measurements of chemical detection. Biomarkers indicate that a compound has been taken up, distributed to the tissues, and elicited a biological response [5]. Although the biomarkers do not provide information about exact exposure concentrations and specific compound identity, they do give information as to the mechanism of action of the contaminant and can be specific to particular chemical classes such as planar aromatic compounds, heavy metals, environmental estrogens, and pesticides [3,6–8]. Furthermore, biomarkers also serve as a composite measure of exposure over time rather than a single point in time. Recently, Van der Oost et al. [4] published a thorough review of the use of biomarkers in environmental science and risk assessment.

This study sought to implement a rapid, multibiomarker approach to determine potential sources of contaminant exposure in a series of streams containing multiple stressors. This includes aquatic systems that receive inputs of industrial effluents, municipal sewage effluents, and storm water and urban runoff. Systems that are sites of historical contamination also often contain complex mixtures of chemicals. Because some biomarkers are class-specific, that is, they respond primarily to certain types of compounds, it is believed that a multibiomarker analysis of these areas can help elucidate the type or source of contamination that may be affecting local populations [1].

The goal of this study was to apply methods that measure changes in the expression of specific genes in caged fish to the field as a rapid, first-tier method for detecting chemical exposure and to test those methods in sites known to be contaminated with specific types of compounds. We hypothesized that developing this approach for use in a number of different fish species would allow us to apply the biomarkers to a wide geographic area. Because the expression of many genes are specific to a particular chemical class, these biomarkers will help determine what types of compounds are bioavailable in a particular area, thus helping to focus future on-site ecotoxicological studies.

MATERIALS AND METHODS

Chemicals

TriReagent, isopropanol, chloroform, DNase, and tris-acetate-ethylenediaminetetraacetic acid were obtained from Sig-
ma Chemical (St. Louis, MO, USA). First Strand cDNA Synthesis Kits (Reverse Transcription Kits) were obtained from Amersham-Pharmacia Biotech (Uppsala, Sweden). Quantitect SYBR Green PCR kits were obtained from Qiagen (Valencia, CA, USA).

Study Sites

Exposures were carried out at reference sites and potentially impacted sites on five streams/rivers: Wolf Creek and Hole’s Creek (Dayton, OH, USA), Arkansas River (Leadville, CO, USA), Cache la Poudre River (St. Collins, CO, USA), and St. John River (Edmundston, NB, Canada). Wolf Creek and Hole’s Creek are small streams that run through Dayton. These streams receive urban and agricultural runoff as well as some industrial effluent discharge. Polychlorinated biphenyl residues as high as 18 µg/g have been detected in fish sampled from contaminated sections of Wolf Creek [9]. Biological and water-quality assessments conducted by the Ohio Environmental Protection Agency rated much of Wolf and Hole’s Creeks as fair [9]. We chose a site on Wolf Creek near Sycamore State Park to serve as a reference area (39.79856°N, 84.33138°W). A site on Wolf Creek as it flowed through an industrial part of Dayton served as a contaminated site (39.76034°N, 84.22690°W). Two contaminated sites were chosen on Hole’s Creek (no reference area present). Contaminated site 1 was the most upstream location near a major highway (no reference area present). Contaminated site 2 was the most upstream location near a major highway (no reference area present). Contaminated site 2 was the most upstream location near a major highway (no reference area present). Contaminated site 2 was the most upstream location near a major highway (no reference area present). Contaminated site 2 was the most upstream location near a major highway (no reference area present).

The Arkansas River near Leadville (CO, USA) historically is contaminated with heavy metals as a result of historic mining activity. Discharges of highly contaminated water from the Yak Tunnel U.S. Environmental Protection Agency Superfund site flow through California Gulch and then into the Arkansas River [10]. High concentrations of metals have been shown to occur in macroinvertebrates and fish sampled downstream of the Gulch [10]. The two exposure sites on the Arkansas River were located just above (reference; 39.25685°N, 106.34395°W) and below (contaminated; 39.22168°N, 106.35571°W) California Gulch.

The Cache la Poudre River runs through the city of Fort Collins and receives urban runoff as well as discharge from a municipal wastewater treatment plant. Two sites were chosen on the river, one upstream of the city (reference; 40.62806°N, 105.16521°W) and one immediately downstream of a municipal wastewater treatment plant (contaminated; 40.54876°N, 105.00700°W).

The St. John River forms a portion of the Maine–New Brunswick border. The river flows through the city of Edmundston (NB, Canada), receiving urban runoff as well as discharge from a pulp mill, a paper mill, and three treated sewage discharges [11]. Three exposure sites were chosen on the St. John: A reference site upstream of Edmundston near Claire (NB, Canada; no samples obtained due to vandalism; see Results section), a site located in Edmundston but upstream of the pulp mill–treated effluent discharge pipe (contaminated site 1; 47.36025°N, 68.32160°W), and a site just downstream of the pulp mill–treated effluent discharge pipe (contaminated site 2; 47.35545°N, 68.27012°W).

Organisms

Juvenile, sexually immature fish (40–100 g) were obtained from hatchery sources. Rainbow trout (Oncorhynchus mykiss) used in the Wolf Creek and Hole’s Creek exposures were obtained courtesy of the London Fish Hatchery, Ohio Department of Natural Resources (London, OH, USA). Cutthroat-rainbow (cutbow) hybrid trout (Oncorhynchus clarkii x mykiss) for the Arkansas River and Cache la Poudre River exposures were obtained courtesy of the Watson-Bellevue Fish Hatchery, Colorado Division of Wildlife, Bellevue (CO, USA). Atlantic salmon (Salmo salar) for the St. John River exposures were obtained from Quality Fish Farm (Temperance Vale, NB, Canada).

Exposures and sampling

Exposures conducted at Hole’s Creek, Wolf Creek, the Arkansas River, and the Cache la Poudre River were carried out using methods similar to McClain et al. [12]. Two rigid nylon mesh cages (38 × 38 cm), each containing five fish, were placed in each stream at each of the exposure sites (total of 4 cages per stream). Based on previous studies conducted in our laboratory, an exposure duration of 48 h was chosen [12–14]. After 48 h of exposure, cages were removed, and gill and liver tissues were taken from each individual. Tissue samples were placed in sterile tubes and frozen immediately in liquid nitrogen for later analysis at Miami University.

Because government regulations prohibited the placement of caged salmon directly in the St. John River, a flow-through, onshore exposure method was devised. Submersible bilge pumps (Rule Industries, Gloucester, MA, USA) were used to pump water from the river into 19-L plastic onshore containers that housed the fish. Lids were secured to the containers with small holes in the top, allowing water to overflow and resulting in a flow-through exposure system. One container containing 10 juvenile Atlantic salmon was placed at each of the three exposure sites on the St. John River. Exposures were conducted for 48 h. Samples of gill and liver tissues were taken from each individual and frozen in liquid nitrogen as described above.

Basic water quality parameters such as temperature, dissolved oxygen, pH, hardness, and total ammonia were measured and recorded at each of the exposure sites (Table 1).

Primers

Primers were designed corresponding to the cytochrome P450I1A1 (CYP1A1), metallothionein, and vitellogenin mRNAs. Expression of these mRNAs and their corresponding protein products have been well-documented as indicators of exposure to planar aromatic compounds, heavy metals, and estrogenic compounds respectively [3,6,7,12–16]. Sequence information was obtained from GenBank® (National Center for Biotechnology Information, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/Genbank/) and entered into Primer3, a computer program available online, which designed optimal primers according to given specifications (Table 2) [17]. Melt curve and primer efficiency analyses were conducted for each primer for quality control purposes. Polymerase chain reaction product generated by each primer was electrophoresed on an agarose gel to ensure that amplicons of the correct size were being obtained. The expression of each biomarker gene was standardized on a sample-by-sample basis to the expression of a noninducible 18S rRNA (commercially available primer obtained from Ambion, Austin, TX, USA).
Table 1. Exposure site water-quality characteristics at Wolf Creek, USA (WC), Hole’s Creek, USA (HC), and the Arkansas River, USA (AR), Cache la Poudre River, USA (CP), and St. John River ([SJ]; Canada). ND = no data

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature (°C)</th>
<th>Dissolved O₂ (mg/L)</th>
<th>pH</th>
<th>Hardness (mg/L CaCO₃)</th>
<th>Total NH₃ (mg/L)</th>
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<tr>
<td>Dayton</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>WC reference</td>
<td>13.5</td>
<td>13.2</td>
<td>8.1</td>
<td>390</td>
<td>0.19</td>
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<td>11.1</td>
<td>8.0</td>
<td>324</td>
<td>0.56</td>
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<td>10.6</td>
<td>8.0</td>
<td>317</td>
<td>0.40</td>
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<tr>
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<td>8.0</td>
<td>309</td>
<td>0.22</td>
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<tr>
<td>Colorado</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP reference</td>
<td>10.3</td>
<td>10.9</td>
<td>8.1</td>
<td>102</td>
<td>0.84</td>
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<tr>
<td>CP contamination</td>
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<td>10.1</td>
<td>7.7</td>
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<td>6.07</td>
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<td>7.9</td>
<td>100</td>
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</tr>
<tr>
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<td>11.9</td>
<td>7.9</td>
<td>214</td>
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<tr>
<td>AR reference (summer)</td>
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<td>7.8</td>
<td>113</td>
<td>0.21</td>
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<tr>
<td>AR contamination (summer)</td>
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<td>ND</td>
<td>ND</td>
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<tr>
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<tr>
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RNA isolation and reverse-transcriptase polymerase chain reaction

Total RNA was isolated from each sample using acid guanidium isothiocyanate–phenol-chloroform extraction following the Tri-Reagent extraction protocol (Sigma Chemical) [18]. For quality control purposes, samples were quantified spectrophotometrically at 260 and 280 nm. Only samples with absorbance_{260} to absorbance_{280} ratios greater than 1.7 were used in subsequent analyses. Genomic DNA contamination was removed by treating each sample with 1 unit of DNase. The DNase activity was quenched using 20 mM of ethylenediaminetetraacetic acid.

Nonspecific reverse transcription was performed using the First Strand cDNA Synthesis Kits as per the manufacturer’s instructions (Amersham-Pharmacia Biotech). Reactions were performed on 5-µg total RNA subsamples from each isolated sample using Moloney Murine Leukemia Virus reverse transcriptase and random hexamer primers [19].

Real-time PCR reactions were performed using the QuantiTect SYBR-Green PCR kit per kit instructions (Qiagen). Reaction volumes included 0.5 µl of cDNA reverse transcription product, 10 µl of kit MasterMix (contains nucleotides, buffer, Taq enzyme, and SYBR Green dye), 1 µl of 15-µM primer, and 8.5 µl of molecular-grade water. The mixture was placed in a RotorGene thermocycler (RotorGene RG-3000, Corbett Research, Queensland, Australia) and incubated at 95 (20 s), 60 (20 s), and 72°C (20 s) to denature the cDNA, anneal the primers, and extend the product, respectively. Reactions were carried out for a total of 50 cycles.

Using computer software (RotorGene 5, Corbett Research, Australia), fluorescence was plotted against cycle number, generating a curve for each sample showing increasing fluorescence with cycle number. A threshold fluorescence value that was above background fluorescence and fell within the linear range of all samples then was chosen. Depending on the amount of gene-specific cDNA in each sample, it took a particular number of cycles to reach that threshold level of fluorescence (C₇ value). The C₇ value is inversely proportional to the expression (amount of mRNA) of each gene. A method of comparison of C₇ values, the 2⁻ΔΔC₇ method, was used for the data analysis [20].

Table 2. Real-time reverse transcriptase–polymerase chain reaction primer sequences

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene</th>
<th>Primer sequences</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncorhynchus mykiss</td>
<td>Cytochrome P4501A</td>
<td>5’-gtcgtgacgccagaactcaaa-3’</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-cttcatggtgaggccgtact-3’</td>
<td></td>
</tr>
<tr>
<td>O. mykiss</td>
<td>Metallothionein</td>
<td>5’-atcctgcaagtctgaact-3’</td>
<td>[27]</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>Vitellogenin</td>
<td>5’-aggaatggactgcattgtg-3’</td>
<td></td>
</tr>
<tr>
<td>Salmo salar</td>
<td>Cytochrome P4501A</td>
<td>5’-tgtgatggccagaactcaaa-3’</td>
<td>[29]</td>
</tr>
<tr>
<td>S. salar</td>
<td>Metallothionein</td>
<td>5’-aggaatggactgcattgtg-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-cttcatggtgaggccgtact-3’</td>
<td></td>
</tr>
<tr>
<td>S. salar</td>
<td>Vitellogenin</td>
<td>5’-ttctgatagctgccggatg-3’</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-ctcagggccccactttcct-3’</td>
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</table>
Gene expression in caged fish

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**Fig. 1.** Mean relative (A) cytochrome P4501A (CYP1A) and (B) metallothionein (Mt) gene expression (± 1 standard error) in rainbow trout (n = 10 fish per site) exposed to Wolf Creek (WC) and Hole’s Creek ([HC]; Dayton, OH, USA) water (letters denote statistical differences in gene expression; α = 0.05). □ Wolf Creek reference; ▣ Wolf Creek contaminated; ■ Hole’s Creek contaminated 1; ▪ Hole’s Creek contaminated 2.

**Fig. 2.** Mean relative cytochrome P4501A (CYP1A) and metallothionein (Mt) expression (± 1 standard error) in cutbow trout (n = 10 fish per site) exposed to Arkansas River water (USA) in (A) May and (B) August (* = statistically different from reference site fish; α = 0.05). □ Reference site; ■ contaminated site

Relative gene expression

\[2^\Delta \Delta C_T = 2^\Delta C_T - (\Delta C_T,\text{control gene})\]

The calibrator refers to the relative expression (\(\Delta C_T\)) of the gene of interest in some particular sample. For these studies, we used the mean relative expression of the biomarker gene in the control (reference site) samples as the calibrator and adjusted all samples to that mean. Therefore, the mean expression of each gene in the controls equals one, and expression of the treatments is given relative to a value of one.

**Statistical analysis**

Statistical analyses were performed using JMPIN 4 statistical software (SAS Institute, Cary, NC, USA). To achieve homogeneity of variance, all gene expression data (\(2^\Delta \Delta C_T\) values) were log transformed. A one-way analysis of variance followed by a Tukey’s post hoc test was used to determine site-to-site differences in mean relative gene expression (\(\alpha = 0.05\)).

**RESULTS**

**Wolf Creek and Hole’s Creek**

Gill and liver CYP1A expression varied significantly (\(p < 0.01\)) between the reference site on Wolf Creek and the contaminated sites on Wolf Creek and Hole’s Creek (Fig. 1A). Gill metallothionein expression was higher at Hole’s Creek contaminated site 1 compared to all other sites (Fig. 1B). Although there were no statistically significant differences in liver metallothionein expression between the sites, the general pattern was the same with the highest mean expression at Hole’s Creek contaminated site 1. No individuals sampled during this study expressed detectable amounts of vitellogenin mRNA in the liver.

**Arkansas River**

Two exposures, one in spring and one in summer, were carried out on the Arkansas River. In the spring exposure, there were no differences in CYP1A expression in the gill or liver tissues (Fig. 2A). Also, there were no significant differences in metallothionein expression in the gill or liver tissues. No individuals sampled during this study expressed detectable amounts of vitellogenin mRNA in the liver.

During the summer exposure, there were differences in biomarker expression between the reference and contaminated sites (Fig. 2B). Metallothionein expression in the gill was higher at the contaminated site than at the reference site (\(p < 0.0002\)). A similar pattern was observed in metallothionein expression in the liver, but the difference was not statistically significant (\(p = 0.06\)). Neither the gill nor liver had differences in CYP1A expression. None of the fish sampled following this exposure expressed detectable levels of vitellogenin mRNA in the liver.

**Cache la Poudre River**

The expression of CYP1A in the gill (\(p < 0.01\)) and liver tissues (\(p < 0.01\)) was elevated significantly at the Cache la Poudre contaminated site when compared to the reference site.
Neither the gill nor liver tissues had differences in metallothionein expression. No individuals exposed during this study expressed detectable levels of vitellogenin mRNA in the liver.

St. John River

Due to vandalism of the exposure system at the upstream reference site, biomarker values of the fish at the two downstream sites were compared to controls obtained directly from the hatchery. Differences in biomarker expression were observed between the reference fish (obtained from a local hatchery) and fish exposed at the two contaminated sites. Detectable amounts of vitellogenin mRNA were expressed by all fish sampled from all sites, including the hatchery fish (Fig. 4A). Hatchery fish and fish exposed just downstream of the pulp mill wastewater-treatment effluent pipe expressed the highest levels of vitellogenin ($p < 0.01$).

The CYP1A expression in the liver was higher in the reference fish and the fish downstream of the pulp mill wastewater treatment effluent than in the fish exposed to contaminated site 1 (Fig. 4A; $p < 0.01$). However, CYP1A expression in the gill tissue was lowest in the hatchery fish and increased greatly at the contaminated sites (Fig. 4B; $p < 0.01$).

No difference was found in liver metallothionein expression between sites (Fig. 4A). Metallothionein expression in the gill was increased slightly in fish exposed at contaminated site 1 compared to those exposed at contaminated site 2 (Fig. 4B; $p = 0.04$).

**DISCUSSION**

The goal of this study was to apply in situ methods using caged fish and molecular techniques as a rapid, first-tier approach to biomarker and exposure assessment across a wide geographic area. Sites known from the literature to be contaminated with specific types of compounds were chosen to test these methods. We were able to conduct successfully 48-h caged-fish exposures at sites in a number of North American streams across a wide geographic area. Comparisons of gene expression between upstream reference and downstream contaminated sites yielded differences indicating a biological response to potential contaminant exposure. Differing expression profiles were seen in each river, suggesting that organisms were being exposed to different types of contaminants or stressors and that the biomarkers were specific to different stressors. Increased CYP1A expression in fish exposed at contaminated sites on Hole’s Creek, Wolf Creek, the Cache la Poudre River, and the St. John River suggested exposure to planar aromatic compounds. These compounds could have originated from a number of sources, including urban runoff and municipal or industrial effluents. Hewitt et al. [21] documented accumulation of aryl hydrocarbon receptor ligands in liver tissue of fish exposed to pulp mill effluent from the St. John River site downstream of California Gulch was indicative of heavy metals exposure likely originating from the Yak Tunnel Superfund site [10]. Fish exposed just downstream of the pulp mill on the St. John River demonstrated increased vitellogenin expression relative to fish exposed just upstream of that site. Treated, bleached sulfite mill effluent released at this site previously had been shown to feminize male fathead minnows [22]. High vitellogenin expression in control salmon sampled during this exposure could be from a dietary estrogen in the hatchery food [23].

It is important to note that, although biomarkers can be helpful in determining exposure, they are subject to variation inherent in living organisms. We believe that our experimental approach comparing upstream reference sites to downstream contaminated sites was the most appropriate way to examine the data. Because of probable variation in species sensitivity and physiology, as well as differences in stream characteristics, cross-stream comparisons were not appropriate in this study. A number of environmental factors such as temperature, handling stress, diet, and water chemistry may affect an organism’s baseline physiology and, in turn, expression of some bio-

**Fig. 3.** Mean relative cytochrome P4501A (CYP1A) and metallothionein (Mt) expression ($\pm$ 1 standard error) in cutbow trout ($n = 10$ fish per site) exposed to Cache la Poudre River (CO, USA) water (* = significantly different from reference site fish; $\alpha = 0.05$). [Reference site; - contaminated site]

**Fig. 4.** Mean relative cytochrome P4501A (CYP1A), metallothionein (Mt), and vitellogenin (VTG) expression ($\pm$ 1 standard error) in Atlantic salmon ($n = 10$ fish per site) (A) liver and (B) gill exposed to St. John River (NB, Canada) water (letters denote statistical differences in gene expression; $\alpha = 0.05$). [Reference fish; - contaminated site 1; - contaminated site 2]
markers. For example, on the Arkansas River, differences in metallothionein expression were observed during the summer exposure but not the spring. Seasonal changes in precipitation, surface runoff, and water chemistry could result in changes in the bioavailability of metals in this system, thus influencing biomarker expression. However, metallothionein expression also has been shown to be sensitive to temperature, and it is possible that differences in water temperature at the Arkansas River sites in spring (7°C) versus summer (13.1°C) also influenced levels of inducible metallothionein-specific mRNA [24,25]. With the exception of the Cache la Poudre River, no significant differences in noncontaminant water-quality parameters were observed between reference and contaminated (upstream/downstream) sites. Two parameters, hardness and total ammonia both, were elevated significantly at the contaminated site downstream of the wastewater treatment plant relative to the reference site. Only the CYP1A biomarker was upregulated at the Cache la Poudre contaminated site, and the authors know of no studies documenting a direct, noncontaminant-related effect of ammonia or hardness on CYP1A. The lack of differences in noncontaminant water-quality parameters between sites and the specificity of the biomarkers provide strong evidence of exposure to particular groups of bioavailable compounds at each of the contaminated sites. Tissue-specific differences in biomarker expression also were observed, indicating a need to analyze tissues from multiple organs when conducting these types of studies to account for biosynthetic differences between tissues and also confounding factors arising from potential differences in route of exposure. Tissue-specific differences in CYP1A expression (St. John River) and in metallothionein expression (Arkansas River) were noted between sites. Because the gill tissues are in direct contact with the water column, they are able to metabolize or detoxify some compounds before they reach systemic circulation, resulting in a low induction response in the liver [14]. Also, differences in the biochemistry and mode of action of specific compounds, as well as differences in tissue sensitivity and biosynthetic potential, could result in these differences. Because vitellogenin, to our knowledge, is not expressed in the gill, hepatic tissue still would need to be sampled as the major site of vitellogenin biosynthesis. Thus, failure to sample multiple organs could result in false-negative or incomplete exposure assessments. We suggest that, at a minimum, gill and liver tissues be analyzed routinely. However, investigators will need to consider potential targets of their contaminant of concern when selecting tissues.

An advantage to assessments using molecular techniques is that reverse-transcriptase polymerase chain reaction (PCR) methods require small amounts of tissue allowing many genes to be assessed using a single sample. Furthermore, because the same technique is used to measure the expression of all genes, this is a relatively efficient way of assessing many biomarker endpoints. We were able to determine the expression of three genes indicative of three chemical classes in a single sample while retaining sufficient amounts of mRNA in storage to analyze further genes in the future. With the advent and refinement of microarray technology in environmental science, it might be possible to use nearly identical methods to measure the expression of thousands of genes in each of these samples and determine other physiological pathways affected by the exposures.

The use of caged fish and measurement of gene induction using reverse-transcriptase PCR was shown to be a sensitive, effective, first-tier tool for assessing contaminant exposure. We were able to cage fish in a number of streams across North America and obtain good quality tissue samples for molecular analysis. Different exposure sites gave varying genetic response profiles, both within a river and among rivers. These profiles indicated that organisms in each river were being exposed to a unique set of stressors that affected different physiological pathways. By identifying the nature of some of those stressors and taking careful consideration of environmental and biological variation, the use of biomarkers can enable future studies investigating the potential impact of those stressors on populations to be more focused and efficient.

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