

# Tissue Contaminants and Associated Transcriptional Response in Trout Liver from High Elevation Lakes of Washington

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The consistent cold temperatures and large amount of precipitation in the Olympic and Cascade ranges of Washington State are thought to enhance atmospheric deposition of contaminants. However, little is known about contaminant levels in organisms residing in these remote high elevation lakes. We measured total mercury and 28 organochlorine compounds in trout collected from 14 remote lakes in the Olympic, Mt. Rainer, and North Cascades National Parks. Mercury was detected in trout from all lakes sampled (15 to 262  $\mu\text{g}/\text{kg}$  ww), while two organochlorines, total polychlorinated biphenyls (tPCB) and dichlorodiphenyldichloroethylene (DDE), were also detected in these fish tissues ( $<25$   $\mu\text{g}/\text{kg}$  ww). In sediments, organochlorine levels were below detection, while median total and methyl mercury were 30.4 and 0.34  $\mu\text{g}/\text{kg}$  dry weight (ww), respectively. Using fish from two lakes, representing different contaminant loading levels (Wilcox lake: high; Skymo lake: low), we examined transcriptional response in the liver using a custom-made low-density targeted rainbow trout cDNA microarray. We detected significant differences in liver transcriptional response, including significant changes in metabolic, endocrine, and immune-related genes, in fish collected from Wilcox Lake compared to Skymo Lake. Overall, our results suggest that local urban areas contribute to the observed contaminant patterns in these high elevation lakes, while the transcriptional changes point to a biological response associated with exposure to these contaminants in fish. Specifically, the gene expression pattern leads us to hypothesize a role for mercury in disrupting the metabolic and reproductive pathways in fish from high elevation lakes in western Washington.

## Introduction

Recent studies demonstrate increasing concentration of contaminants with increasing elevations, typically at remote locations far from pollutant sources (1–4). These pollutants are generally delivered via the atmosphere and deposited

through a temperature-dependent process known as “cold condensation” (5), involving both wet (rainout and washout) and dry (particulate bound) deposition (6). This process can lead to elevated concentration of long-lived, semivolatile contaminants at remote locations in the lower latitudes, particularly those at high elevations (7–9).

The consistent cold temperatures and large amount of precipitation in the Olympic and Cascade ranges of Washington State provide a regionally ideal environment for cold condensation to occur, see Figure 1. In addition, the presence of permanent snowfields and glaciers in this environment may accelerate deposition of organic chemicals through vapor scavenging (9, 10) and may serve as “sinks” for air pollutants (3, 10). Organochlorine pesticides have been demonstrated as moving globally through the atmosphere and were widely used in Washington State until the 1980s, and some still have limited applications today. Mercury transport and deposition also occurs via the atmosphere from sources both local and international (3), with coal-fired power plants identified as the largest mercury source to the atmosphere (11). In addition, pollutants that originated in central Asia have been shown to move with the jet stream and reach the Olympic peninsula in as little as 8 days (12).

Although, atmospheric deposition of contaminants in mid-latitude, high-elevation locations has recently received much attention (1, 2, 8, 13), few studies have measured contaminant levels in animals residing in lakes in the Olympic and cascade ranges of Western Washington. While trout can accumulate mercury and organochlorine pollutants in their tissues at concentrations above ambient water concentrations (14), the biological impact of this contaminant accumulation has rarely been addressed. To this end, the microarray technology recently developed in fish will allow identification of multiple targets impacted by xenobiotics in fish (15, 16). With this in mind, our objectives were to examine whether (i) contaminant levels in trout tissue in these high elevation lakes reflected one or more physical factors important in pollutant deposition, including precipitation, average temperature, and elevation, and (ii) whether differences in whole body concentrations of contaminants reflect changes in liver expression pattern of contaminant- and stressor-responsive genes in these trout. We utilized a custom-designed, low-density, targeted rainbow trout cDNA microarray consisting of 147 stress-responsive genes of known function to identify the effect of contaminant load on molecular responses in trout liver (16).

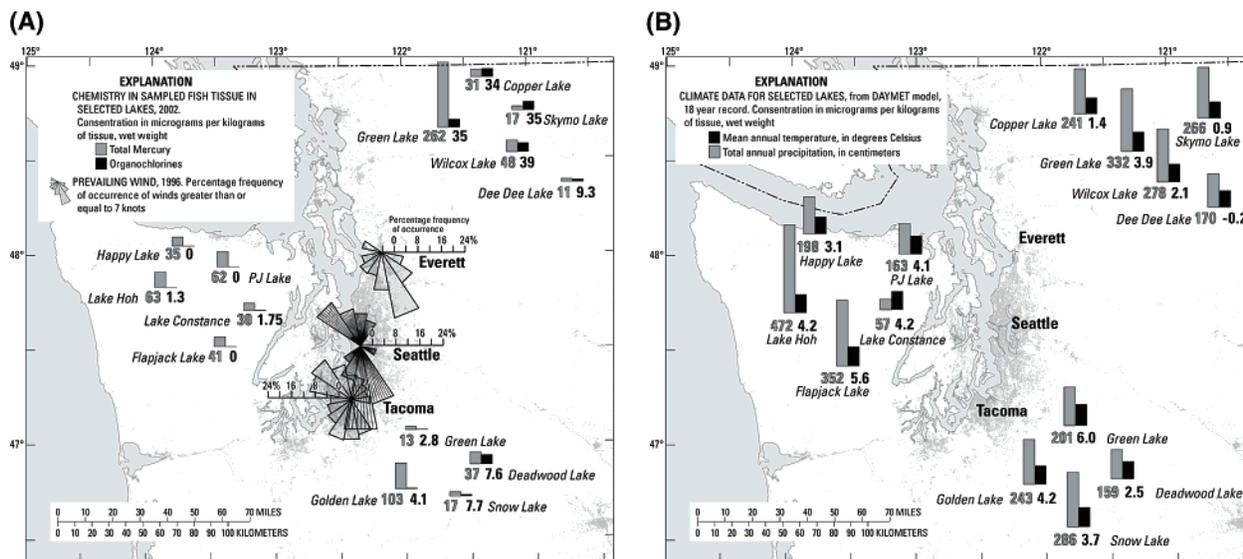
## Materials and Methods

**Lake Selection.** Lake basins were selected by collecting and prioritizing independent (non-nested) basins of the three National Parks from ArcGIS files, which addressed physical, climatic, and biological qualities of the basins (i.e., elevation, geographic orientation, land cover, percent permanent snow and ice, and fish species presence). Candidate lake basins were ranked using a priority system requiring, first, basins containing self-sustaining (not actively stocked) populations of trout and, second, those with the highest lake elevations from the available pool in each park. Meteorological parameters for these basins, including mean annual temperature (MAT) and total annual precipitation (TAP), were generated from the DAYMET (Daily Surface Weather Data and Climatological Summaries, www.daymet.org) weather model with a 1 km grid size (Figure 1). Fourteen lakes, ranging between 1010 and 2070 m elevation, were selected in Washington’s three National Parks, and these lakes were generally in remote locations and classified as oligotrophic,

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**FIGURE 1.** Fish tissue contaminants, total mercury and organochlorines, and climate data, total annual precipitation (TAP), and mean annual temperature (MAT) for 14 lakes sampled in 2002. Fish tissue concentration map includes overlay of wind rose diagrams from major metropolitan centers.

and ranged between 3.5 and 16 km from the nearest access roads.

**Sampling and Chemistry.** Sampling was carried out in the above lakes from July through September 2002. A small, lightweight gillnet and angling were used to collect resident cutthroat trout (*Salmo clarkii*), predominately brook and rainbow trout. Fish were caught via angling for microarray samples (2003) and/or with a gill net for contaminant or chemical analysis. Lengths, weights, gross abnormalities, and sex were recorded, and composite samples of five whole male fish were taken, as available, for chemical analysis and placed immediately on dry ice. Lake surface water quality parameters, including temperature, conductivity, and pH, were also recorded.

Sampling in September and October 2003 involved collecting fish tissue, in five of the original lakes with the highest tissue contaminant concentrations, as well as collecting sediment samples. Sediment samples were collected by compositing multiple (minimum of five) grab samples from several locations around the lake, at 0.5–1 m depths, using a clean hands/dirty hands technique (17, 18). Sediment subsamples for organochlorine and mercury analyses were taken on-site and frozen immediately on dry ice. Subsamples of sediments were taken for measurement of acid volatile sulfides (AVS), organic carbon content (measured as loss on ignition), pore-water sulfides, and percent solids were characterized from each composite sediment sample as described in Supporting Information (SI); see also Table 1 in SI.

Chemical analysis for organochlorine compounds were sent to the U.S. Geological Survey's (USGS) National Water Quality Laboratory (Denver, CO) for chemical analysis of organochlorines by a gas chromatography with electron capture detector (GC-ECD) (19, 20). Mercury samples were sent to the USGS Wisconsin Mercury Lab (Middleton, WI) for analysis of total mercury by atomic fluorescence spectroscopy (AFS) (20). Whole fish tissues were analyzed only for total mercury, as previous studies have shown that >90% of mercury in fish tissue is in the methylmercury form (21). A detailed description of the contaminants evaluated, their respective detection limits, and the detection frequency of each compound can be found in SI, Table 2.

**Transcriptional Response.** Juvenile fish from Skymo (average body mass 119 g, average total length 237 mm) and Wilcox (average body mass 150 g, average total length 244 mm) Lakes were collected by angling in 2003 for the

microarray analysis (discussed below). Liver samples were dissected immediately on-site using an aseptic technique and frozen immediately on dry ice. The remainder of the carcass was used in the five fish composite samples in 2003.

Microarray experiments were designed to comply with minimum information about microarray experiment (MIAME) guidelines, provided online (<http://www.mged.org/Workgroups/MIAME/miame.html>). A custom-made cDNA microarray consisting of 147 stress-responsive rainbow trout genes with known function was employed to screen for transcriptional responses in the liver of these fish (15). The trout array cross-hybridized with 100% efficiency with the cutthroat trout samples, and therefore pooled liver RNA from hatchery reared trout was used as the reference sample for each slide. Briefly, each microarray slide was hybridized with RNA from an independent liver sample and the reference RNA pool for a total of eight slides (five Wilcox Lake and three Skymo Lake). Total RNA was extracted from livers using the RNeasy extraction kit (Qiagen, ON). The quality of the total RNA obtained was checked by gel electrophoresis on a 1% agarose gel containing ethidium bromide, and the concentration was determined spectrophotometrically at 260/280 nm using Cary 50 UV-vis spectrophotometer (Varian Inc, ON).

Fifty micrograms of intact total RNA (either lake sample or reference RNA) were indirectly labeled following the Institute for Genomic Research protocol (<http://www.tigr.org/tdb/microarray/protocolsTIGR.shtml>) with the red fluorescent dye ester cyanine 5 (Cy5) and green fluorescent dye ester cyanine 3 (Cy3) (Amersham Biosciences, Piscataway, NJ). Dye swapping was carried out by switching dye labeling of sample and reference RNA between slides. The details of cDNA synthesis and labeling protocol, hybridization, post-processing, and image analysis are provided in the SI. The raw dataset has been deposited into the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>, series GSE6886).

**Quantitative Real-Time PCR (qPCR).** To validate the reliability of the microarray results, a few genes that were significantly different between lakes were randomly picked for gene quantification by means of quantitative real-time PCR (qPCR) according to established protocol (24) and is provided in SI.

**Statistical Analyses.** The contaminant loading pattern in the 14 lakes sampled in 2002 was related to environmental variables hypothesized to be factors controlling contaminant

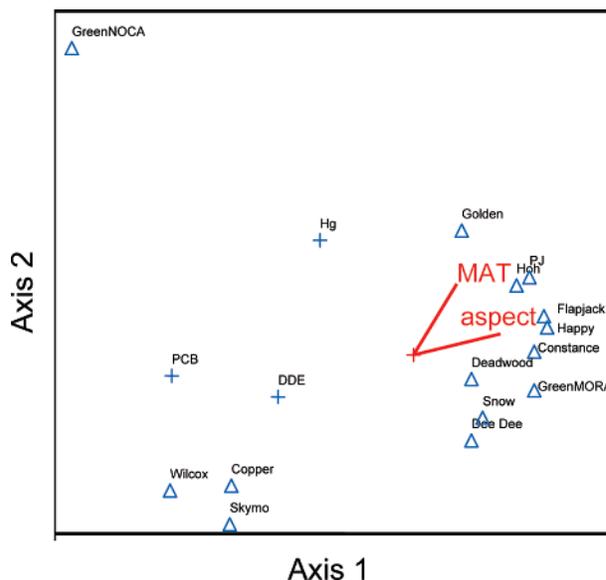
deposition. Those factors included basin-wide characteristics such as mean annual temperature, lake level elevation, total annual precipitation, two-dimensional basin area, three-dimensional basin area, the ratio of 3D area to 2D surface areas, lake surface area, total basin relief, and average basin drainage aspect (0–360). Fish specific variables that could also affect tissue concentrations, namely length, weight, % males, and % lipids, were also included with the environmental variables as potential explanatory variables. An ordination of tissue contaminant concentrations and environmental variables, including fish parameters, was conducted using principal component analysis (PCA); (PC-Ord version 4 software, Gleneden, OR). Fish tissue concentrations of the contaminants, all in  $\mu\text{g}/\text{kg}$  ww, for each of the sites were compared to 13 environmental and fish explanatory variables at each site; using a square root transformation and an Euclidean distance measure. Student's *t*-test was used to determine the significant differences in transcriptional responses observed with either microarray or qPCR between Wilcox Lake (five independent samples) and Skymo Lake (three independent samples). A *P*-value of  $<0.05$  was considered statistically significant.

## Results

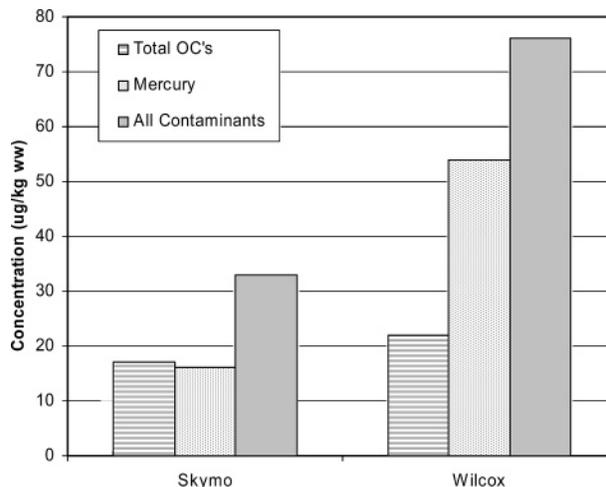
**Physical Characteristics of the Lakes.** At the time of sampling, water quality parameters of the 14 lakes sampled were relatively similar (temperature 7–14 °C, pH 6.36–8.05, 8.55, and conductivity 3–117  $\mu\text{S}/\text{cm}$ ) and can be characterized as cold, low conductivity lakes at circum neutral pH. MAT for these lakes ranged from  $-0.2$  to 6 °C, and TAP from 57 to 472 cm per year (Figure 1). Lake basins were located in the Cascades or North Cascades, Level III Ecoregion. Physical habitat was similar at most sites, with the basins dominated by fir and hemlock forests below tree line, and moss and lichen communities above. All basins were in remote locations without vehicle access or permanent structures.

**Chemical Analysis.** The 2002 median mercury tissue concentration for the 14 lakes sampled was 41  $\mu\text{g}/\text{kg}$  ww (std dev 36,  $n = 14$ ), with only two lakes exceeding 100  $\mu\text{g}/\text{kg}$  ww (Figure 1). Percent lipids were also evaluated in each tissue sample, and contaminant concentration on a percent lipid basis is also provided in SI Table 3; however, changes in ranking were negligible. The highest mercury tissue concentration observed was 262  $\mu\text{g}/\text{kg}$  ww, measured at Green Lake in the North Cascades (GreenNOCA) in 2002. Organochlorine compounds in tissue were generally low, with 26 of the 28 analytes not detected (see SI, Table 2). Only total polychlorinated biphenyls (PCBs) and *p,p'*-dichlorodichlorophenylethylene (DDE) were detected in tissues, but their concentrations were consistently low and often below the method reporting limit (MRL). Without inclusion of the nondetected compounds, the median sum concentration of all organochlorines detected in tissues was 12.6  $\mu\text{g}/\text{kg}$  ww (std dev 15.4,  $n = 14$ ).

Five of the original fourteen lakes sampled in 2002 were resampled in 2003, namely Green Lake in the North Cascades (GreenNOCA), Green Lake at Mt. Rainier (GreenMORA), and Skymo, Wilcox, and Deadwood Lakes. Concentrations between the two sampling years for either mercury or total organochlorine tissue residues were not significantly different (two-sided *T*-test,  $P < 0.05$ ). Bottom sediments were also sampled in 2003; however, no sediment samples had detectable organochlorine levels. Method reporting limits are listed in Supporting Information (see SI, Table 2). Total mercury in sediments (STHg) and methyl mercury in sediments (SMHg) were measured in all five lake sediments, although it should be noted that the reporting limits with the AFS method (18) for total mercury and methyl mercury are lower than for the organochlorines. STHg and SMHg median



**FIGURE 2.** Ordination following principal component analysis of fish tissue contaminant concentrations, untransformed ( $\mu\text{g}/\text{kg}$  wet weight), and square root transformed environmental and fish condition variables. Vectors shown only for those variables with correlation coefficients  $>0.3$ . (MAT, mean annual temperature).



**FIGURE 3.** Two-year average contaminant levels from five fish composite samples, collected from Skymo and Wilcox Lakes. Transcriptional responses in fish liver between these lakes were performed using trout cDNA microarray.

concentrations were 30.4 and 0.34  $\mu\text{g}/\text{kg}$  dw, respectively (SI, Table 4).

**Relationship to Environmental Conditions.** Fish tissues collected from the Olympic sites consistently showed no organochlorine detections (Figure 1A) while the two sites with the highest concentrations were on the western slope of the cascades. An ordination of the observed contaminant pattern in the composite samples to general characteristics of each basin, as well as relevant characteristics of the composite, is presented in Figure 2. Two variables were effective in the ordination model: average basin aspect and mean annual temperature (MAT). Each is shown as arrows in Figure 2. The first axis had an Eigen value of 2.03 and explained 68% of the variability in the multivariate model.

Fish from Wilcox Lake had higher total contaminants and mercury concentration, but relatively similar organochlorine concentration, compared to Skymo Lake fish (Figure 3). The 2-year average mercury concentration in fish from the two lakes was over 3-fold different, with Skymo Lake fish having

**TABLE 1. The Gene Name, Medline Accession Number, Gene Ontology (GO) Accession Number, and the Biological Process of the Genes That Were Significantly Different in Wilcox Lake Compared to Skymo Lake (45 of the 147 genes)<sup>a</sup>**

Gene name	Medline accession no.	GO accession no.	Biological process	Gene expression (% Skymo Lake)
20β-HSD	AF100933	GO:0006633	fatty acid biosynthesis	55 ± 15
AhR	AF065138/AF065137	GO:0042221	response to chemical stimulus	850 ± 43
β-globin2	AB015451	GO:0015671	oxygen transport	55 ± 4
chicken type2 GnRH	AF125973	GO:0009755	hormone mediated signaling	587 ± 112
COX2	AJ238307	GO:0019371	cyclooxygenase pathway	430 ± 56
CPSIII	U65893	GO:0006807	nitrogen compound metabolism	403 ± 95
CXCR	AJ001039	GO:0007411	axon guidance	507 ± 109
CYP2K1v2	AF045052	GO:0043390	aflatoxin B1 metabolism	813 ± 144
CYP2K5	AF151524	GO:0043390	aflatoxin B1 metabolism	520 ± 43
cystatin B	AY594696	GO:0006508	proteolysis	590 ± 99
G-6-Pase	AF120150.1	GO:0006094	gluconeogenesis	344 ± 68
glucokinase	AF053331	GO:0001678	cell glucose homeostasis	38 ± 8.3
glutathione peroxidase	AY622862	GO:0006979	response to oxidative stress	1363 ± 190
GnRH1	AF110992	GO:0007275	development	12 ± 4
GnRH2	AF110993	no accession no.	development	37 ± 8
GnRH-R	AJ272116	GO:0007186	GPCR protein signaling pathway	1286 ± 202
GS01	AF390021	GO:0006542	glutamine biosynthesis	446 ± 33
GS03	AF390023	GO:0006542	glutamine biosynthesis	883 ± 47
GS04	AF390024	GO:0006542	glutamine biosynthesis	957 ± 73
Hepatic GLUT2	AF321816	GO:0015758	glucose transport	606 ± 148
Hsp70constitutive	AB176854/AF176855	GO:0009408	response to heat	863 ± 160
Hsp70inducible	AB176854/AF176855	GO:0009408	response to heat	717 ± 43
IGF type I receptor lb	AF062500	GO:0048009	IGF receptor signaling pathway	753 ± 217
IGF-1	M95183	GO:0007517	muscle development	406 ± 52
matrix metalloproteinase II	AB021698	GO:0006508	proteolysis	1047 ± 52
MCH	X73837	GO:0007610	behavior	314 ± 56
MHC-fast	Z48794.1	GO:0030048	actin filament-based movement	730 ± 115
MHC-I	AJ007847	GO:0006955	immune response	412 ± 98
Mx2	U47945	GO:0045087	innate immune response	369 ± 42
MyoD	Z46924	GO:0007517	muscle development	308 ± 47
P450side chain cleavage	S57305	GO:0006704	glucocorticoid biosynthesis	830 ± 87
PEPCK	AF246149	GO:0006094	gluconeogenesis	481 ± 70
POMC-A	X69808	GO:0007218	neuropeptide signaling pathway	11 ± 6
pyruvate kinase	AF113695	GO:0006096	glycolysis	509 ± 31
rtSox23	AB007906	GO:0009653	morphogenesis	199 ± 27
Sox 9	AB006448	GO:0051216	cartilage development	406 ± 56
Sox P1	D83256	GO:0045165	cell fate commitment	177 ± 13
Sox-LZ	D61688	GO:0035051	cardiac cell differentiation	167 ± 33
StAR protein	AB047032	GO:0006694	steroid biosynthesis	729 ± 218
SUMO-1	AB036430	GO:0016925	protein sumoylation	556 ± 80
TDNT	U53366	GO:0006259	DNA metabolism	400 ± 64
TGF-beta	X99303	GO:0007275	development	282 ± 36
thyroid hormone receptor	AH007700	GO:0016481	negative regulation of transcription	319 ± 23
VEP-alpha	AF231706.1	GO:0007339	binding of sperm to Zone pellucida	553 ± 75
Vtg1	X92804	no accession no.	reproduction-development	21 ± 4

<sup>a</sup> Original number are log<sub>2</sub> ratios, but gene expression values are shown as % Skymo Lake; *P* < 0.05, Student's *t*-test.

approximately one-third the mercury body burden of Wilcox Lake fish. A one-sample *T*-test indicates that the 2-year average mercury concentration from Skymo Lake, the low mercury lake, was significantly lower than the population of all lakes sampled in this study (*P*-value 0.005, *n* = 14), while the higher mercury lake, Wilcox, was not significantly higher than the population (*P*-value 0.63, *n* = 14). Consequently, the fish from these two lakes were compared for gene expression patterns associated with contaminant exposure; discussed below.

**Transcriptional Responses.** There was a clear difference in the gene expression pattern between the Wilcox Lake fish and the Skymo Lake fish (Table 1). Of the 147 genes, 45 genes (31%) were significantly different between the two lakes (two-sided, *T*-test, *P* < 0.05). The majority of those genes were upregulated (38 genes; 84%), while only 16% (7 genes) were downregulated in the Wilcox Lake fish compared to the Skymo Lake fish. The significantly regulated genes were assigned a biological process category according to the gene ontology annotation database ([www.geneontology.org](http://www.geneontology.org)) (Table 1) and can be classified broadly into six main

physiological categories: (1) general cellular maintenance and housekeeping, (2) immune function, (3) stress response, (4) metabolism and growth, (5) reproduction, and (6) xenobiotic response. In the more contaminated Wilcox Lake fish, genes involved in xenobiotic metabolism, particularly aryl hydrocarbon receptor (AhR) and cytochrome P450 metabolizing enzymes, as well as several genes involved in the stress-response, intermediary metabolism, and endocrine response were significantly elevated (Table 1). The notable exception was a significant lowering of gonadotropin releasing hormone (GnRH) and pro-opiomelanocortin (POMC) transcripts in the liver of fish collected from Wilcox Lake compared to Skymo Lake (Table 1). In addition, several immune responsive and housekeeping genes were also significantly affected in fish from Wilcox Lake compared to Skymo Lake.

To validate the transcriptional responses observed with the microarray, mRNA abundance of four upregulated (AhR, Phosphoenol pyruvate carboxykinase (PEPCK), Steroidogenic acute regulatory (StAR) protein and heat shock protein 70 (hsp70) and two downregulated (Glucokinase and beta-

globin2) genes were determined using qPCR. The gene expression pattern between the two lakes seen with qPCR was similar to that seen with the microarray analysis (SI, Figure 1).

## Discussion

To the authors knowledge this is the first study to examine the changes in gene expression in response to contaminant loading in field-collected fish. With respect to national data compilations for the United States (27), the overall tissue concentrations measured in these lakes were typical to low for insectivorous or omnivorous trout species. However, a clear difference in liver transcript levels in trout from two lakes with different contaminant loading suggests for the first time a biological impact at the transcriptional level associated with anthropogenic stressors in high altitude remote lakes.

**Contaminant Load.** The largest component of the contaminant load evaluated in this study was mercury, while DDE and PCBs were detected in some fish tissue samples but were typically at or below the detection limit (SI Table 2). None of the tissue contaminant concentrations exceeded criteria for the protection of human health; however, the highest mercury concentration approached EPA's recommended human health criteria of 300  $\mu\text{g}/\text{kg}$  ww. Some of these detections, particularly for mercury, do fall within the range where tissue residue guidelines for the protection of wildlife consumers have been set (4). These mercury levels are considerably lower than the ranges commonly reported in freshwater sediments (10–753  $\mu\text{g}/\text{kg}$  dw as THg) and or in soils (0.3–22  $\mu\text{g}/\text{kg}$  dw as MHg) (12). The sediment data here indicate that with one exception, methyl to total mercury ratios were low, median of 0.8%. Along with high acid volatile sulfides levels, high organic matter content, and low biota to sediment bioaccumulation factors suggests that contaminants in sediments are unlikely to be responsible for the observed fish tissue concentrations.

However, the concentrations of mercury seen in the fish tissues in some of these lakes are well within the limits reported previously to impact fish reproduction in laboratory studies (28, 29). Indeed, there was clearly a difference in the levels of contaminants in fish tissues between lakes, as seen between the Wilcox Lake and Skymo Lake fish (Figure 3). Wilcox Lake fish had a greater than 3-fold higher contaminant load than Skymo Lake fish (Figure 3), even though both these lakes were in the same region (Figure 1). While food chain length has been demonstrated to be important in accounting for differences in fish tissue concentrations (30 and therein) gut contents observed in these fish and previous studies (31, 32, and therein) indicate that the food webs are short with consistently similar prey items.

These points further support the suggestion from the PCA ordination of local, watershed-level characteristics and fish characteristics in Figure 2 that small scale basin-specific features may be affecting the contaminate delivery. While temperature has been described as the most important variable controlling volatility of these contaminants and is a key condition in the cold condensation phenomena (9), the importance of average basin aspect, or orientation toward or away from the dominate wind patterns, was unexpected. A deposition pattern that draws more from local sources than from global sources is suggested by the prominence of the average basin aspect variable in the ordination model. Given the proximity of all these sites to the Pacific Ocean and what might be considered a "global" source signal, a more uniform or even higher contaminant load in the wetter Olympic mountains to the west (Figure 1) might be expected, but was not observed. Qualitative comparison of elevated contaminant levels, urban centers, and the dominant wind pattern from the southeast, see Figure 1, further support the

idea that local sources may be important in the elevated contaminant levels.

Other local or geologic sources of mercury were considered. Local sources of mercury are available through the US Environmental Protection Agency toxic release inventory (<http://www.epa.gov/triexplorer/>), which indicates that mercury releases in Washington are fairly consistently distributed along the western slope of the Cascade range; the general location of the major regional highway and the urban corridor. The 2003 inventory showed that no other counties in Washington State reported mercury releases. Geologic sources of mercury from the parent mineralogy are also possible. However, upon review of geologic maps of the 14 study basins presented here, none indicated a predominance of mercury-type minerals.

Regional sources of contaminant deposition was suggested in another recent study (13), while also noting that temperature was not an effective predictor of contaminant deposition. While several authors have shown that local as well as global sources can contribute to atmospheric deposition (1, 2, 8, 13), this is the first study to show average basin aspect and/or average slope orientation as a factor influencing contaminant levels. Taken together, our results support the idea that current, atmospheric deposition from local urban centers of Puget Sound, and temperature may be responsible for the different contaminant levels in these fish tissues. However, further work to quantify deposition rates with direct measurements per basin, such as described in ref 13, would be needed to quantify source contributions.

**Transcriptional Response.** Using a selective transcriptomics approach with a low-density trout cDNA array, enriched with stress-related genes, we show liver gene expression pattern in feral trout from two high elevation lakes in western Washington. The gene expression patterns of selected genes with the array were similar to that seen with qPCR, confirming the reliability of the array results. The clear difference in liver transcriptional response between the higher tissue contaminant fish (Wilcox Lake) relative to the lower contaminant fish (Skymo Lake) suggest a biological impact associated with contaminant exposure in these high mountain lakes. The significantly higher gene expression (81% of significant genes) in the higher contaminant tissue leads us to propose a higher metabolic demand associated with this transcriptional upregulation in Wilcox Lake fish. As the main contaminant detected in the tissue sample was mercury, we hypothesize that this metal is involved in the transcriptional responses seen in this high elevation lake. This is interesting given that the mercury concentration detected, even in the lake with the higher tissue concentration, was well below the criteria for the protection of human health (4, <http://www.ec.gc.ca/ceqg-rcqe/>). However, the liver transcriptional responses suggest a potential impact of low level contaminant concentration on fish metabolism, while effects associated with specific gene changes observed in this study remain to be tested. Likewise, the variability of field-collected organisms can preclude strict standardization of subjects, as in the lab, and further suggests the need for large sample sizes; a noted limitation to this study.

Indeed, genes involved in a number of physiological processes including immune function, stress adaptation, reproduction, and metabolism were upregulated in the Wilcox Lake fish compared to the Skymo Lake fish (SI Figure 1; Table 1). Also, several neuroendocrine genes involved in the functioning of the hypothalamus–pituitary–gonadal (HPG) axis were upregulated in the contaminated Wilcox Lake fish liver (Table 1). For instance, an upregulation of GnRH (GnRH-R) and a concomitant downregulation of GnRH-1 and GnRH-2 in the Wilcox Lake fish implicate a role for the contaminants in disrupting the HPG axis in fish. The reduction in transcript levels of GnRH seen with higher

mercury levels in fish tissues (Wilcox lake fish compared to Skymo Lake fish) supports the recent finding showing an inverse correlation between luteinizing hormone releasing hormone (LHRH) and MeHg in rat hypothalamus (33). The effect of mercury on the hypothalamus may be responsible for the reduced steroid levels seen in mercury treated fish (28). Although liver is not involved in the functioning of the HPG axis, the changes in transcript levels of neuroendocrine-related gene in the liver lead us to propose a similar mechanism of action by xenobiotics in the brain of fish. The mRNA abundance of brain-specific genes in the liver is a novel observation and may potentially be used as a biomarker of neuroendocrine disruption by mercury in fish.

Recently, reproductive endpoints, including impaired oogenesis, have been suggested to be a sensitive indicator of methylmercury contamination in fish (34). Also, reduced spawning success in Fathead minnows was reported by Hammerschmidt et al. (29) at carcass mercury concentrations as low as 200  $\mu\text{g}/\text{kg}$  ww (assuming 80% ww). Furthermore, a dose-dependent reduction in spawning success as well as a suppression of testosterone and 17- $\beta$ -estradiol (E2) in male and female Fathead minnows, respectively, was observed at mercury body burdens around 900  $\mu\text{g}/\text{kg}$  ww (28). Considered together, the HPG axis appears to be sensitive to methylmercury poisoning in fishes. The significant changes in the transcript levels of GnRH and GnRH-R, key hormone and signaling molecules, respectively, that are primary regulators of the gonadal axis, in fish from the higher contaminant lake supports the notion that mercury may be a key contaminant impacting fish in high elevation lakes.

In addition to neuroendocrine genes involved in reproduction, several genes involved in intermediary metabolism and stress response were upregulated in the Wilcox Lake fish liver, suggesting a higher metabolic demand in these fish. This increased metabolic demand may be due to the stress associated with contaminant exposure (26, 35, 36). The higher transcript abundance of hsp70 genes, a sensitive indicator of cellular stress response, in the liver of Wilcox Lake fish further supports the contention that these fish are responding to the stress associated with contaminant loading, by eliciting a heat shock response (26, 35). Also, several immune responsive genes and genes involved in endocrine signaling, including growth and metabolism, were also impacted, suggesting immunomodulatory and cell signaling impairment due to metal exposure. Indeed, even in fish exposed to low mercury concentrations, a significant mercury accumulation was found in liver, kidney, brain, and gonads and modulated several physiological processes (36–38). While the specific role of mercury in the transcriptional response seen in this study is unclear, several studies have shown that heavy metal and organochlorine contamination impairs stress response, growth, and energy metabolism in teleostean fish (35, 39). Taken together, these results suggest that anthropogenic chemical accumulation in high elevation lakes impact fish homeostasis by altering multiple physiological processes. Even though low levels of contaminants were observed in these high altitude lakes, changes in the gene expression patterns may act as an early warning indicator of xenobiotic effects on the aquatic species living in these pristine environments. While the majority of gene changes were liver-specific, the observation that extrahepatic genes (neuroendocrine and steroidogenic genes) were also expressed in the liver suggests the power of gene array for identifying novel pathways, while their biological significance remains to be determined.

In conclusion, we report the presence of mercury and a few organochlorine compounds in trout tissue from remote, high elevation lakes of western Washington State. The contaminant levels were generally low and below any human consumption advisory level. However, the gene expression

pattern in fish suggests a biological impact at multiple levels (e.g., growth, reproduction, stress response, metabolism, immune response) associated with this contaminant level in remote lakes. Additionally, much work is needed in characterizing the baseline variability of gene responses observed in these field-collected specimens. On the basis of the low sediment concentrations and bioavailability and the influence of basin aspect in an ordination model, we hypothesize that atmospheric sources, particularly from neighboring urban centers, is a likely source to the observed fish tissue contamination.

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## Supporting Information Available

The Supporting Information for this manuscript includes more methods discussion and five tables and one figure. In particular, a brief description of the other sediment parameters collected, including a table with those results, more description of the microarray processing procedures including information on the fish used, analytical detection limits, and some discussion and results of the qPCR work, including primers used. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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