Influence of urbanization on the health of juvenile salmonids in Pacific Northwest perennial streams

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2017

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Program Authorized to Offer Degree:

School of Aquatic and Fishery Sciences

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Abstract

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Increasing population and urbanization leads to stress in freshwater systems from a variety of anthropogenic influences including structural changes to habitat, temperature effects from increased runoff and reduced canopy cover, flow changes, and an increased presence of toxicants both from point- and non-point sources. Physical and chemical changes affect the biota within these urban streams at varying scales ranging from individual organisms to populations and communities creating complex interactions that present challenges for characterizing and monitoring the impact on species utilizing these freshwater habitats. Salmonids, specifically cutthroat trout (*Oncorhynchus clarkii*) and coho salmon (*Oncorhynchus kisutch*), extensively utilize small stream habitats influenced by this changing urban landscape. This study used a comprehensive fish health assessment concurrent with the U.S. Geological Survey's Pacific Northwest Stream Quality Assessment in 2015. This study quantified impacts from disease in

juvenile coho and cutthroat salmon, impacts to coho salmon growth within the context of environmental and ecological influences, and identified physiological responses in coho salmon from pollution. First, we used the previously established Geode fish health index to assess the extent that fish were diseased in these streams. Fish had elevated levels of disease in four moderately urbanized streams and had moderate disease levels in reference streams. Next, we used a bioenergetics growth modeling approach to assess the environmental factors affecting juvenile coho growth. For urban streams, we show mixed effects, whereby urban streams tended to be warmer, have earlier emergence dates and stronger early season growth. However, we also show that larger fish are under increased stress through lower growth efficiencies, especially later in the summer, when compared to fish from other streams. Finally, we related in stream contamination to physiological response in coho. We identified 52 stress genes of interest using next-generation sequencing (RNAseq) and designed a custom nanoString probe set for expression analysis using the nCounter platform. Multivariate methods were used to relate water and sediment contaminant concentrations to gene expression levels. Results indicate that elevated levels of PAHs, PCBs, and pesticides significantly correlated with increased expression of genes involved in detoxification of organic contaminants. This study presents the first time a probe-based multiplexed nanoString assay was successfully used to assess salmonids and provides an economical and comprehensive assessment tool to evaluate the exposure and physiological response of salmonids to in-stream contaminants. Together these assessments provide valuable monitoring tools to determine the relative impacts of disease, near-term environmental conditions, and contaminants to growth and physiological stress in salmonids.

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Acknowledgments

I would like to thank my faculty advisors, David Beauchamp and Steven Roberts, and my committee member Patrick Moran for the many hours they spent mentoring me on this project. They provided valuable insight and suggestions for all components of this project from inception through completion. Additional thanks to both the Roberts and Beauchamp lab for advice and assistance in both data generation and analysis. In particular, I would like to thank Jenny Gardner, Kristin Connelly, Heyjoo Ro, Evan Seamans, Adam Hansen, and Sam White. Additional support was given by many staff of the US Geological Survey, especially Kim Larsen, Lisa Wetzel, Laurie Balistrieri, and Danielle Cleveland.

Sampling and data generation for this study was a cooperation between the University of Washington and the US Geological Survey. Handling of live vertebrates in this study was performed under the auspices of the University of Washington IACUC protocol #3286-21. Any use of trade, firm, or product names are for descriptive purposes only and does not imply endorsement by the U.S. Government.

Chapter 1: Introduction to Salmonid Health Assessment: Field Collection, Disease Index, and Study Design.

Abstract

Pacific salmonids are an important commercial and cultural resource in the rapidly urbanizing Pacific Northwest Region. Salmonids are sensitive to anthropogenic influences that include changes in flow, food availability and quality, and stream temperature. This study evaluated the relative impact of disease in juvenile coho salmon and cutthroat trout at streams spanning an urban gradient. Streams were chosen based on the percentage of urban landuse within their respective watersheds. We used the previously established Geode fish health index to assess the extent that fish were diseased in these streams. Fish had elevated levels of disease in four moderately urbanized streams. Additionally, fish had moderate disease levels in reference streams. These assessments and fish collection helped to inform subsequent chapters that assessed coho for comparative growth and impacts from contaminants.

Introduction

The Puget Sound region is experiencing rapid human population growth in the city of Seattle and the surrounding area with a projected 1.2 million additional residents by 2040 (Washington State OFM 2012). This increasing population and urbanization lead to stress in freshwater systems from a variety of anthropogenic influences including structural changes to habitat, temperature effects from increased runoff and reduced canopy cover, flow changes, and an increased presence of toxicants both from point- and non-point sources (Konrad and Booth 2005). Physical and chemical changes affect the biota within these urban streams at varying scales ranging from individual organisms to populations and communities creating complex interactions that present challenges for characterizing and monitoring the impact on species

utilizing these freshwater habitats. Assessing the urban impact from water quality changes is particularly challenging owing in part to the sheer diversity of chemicals in the environment (Hamilton et al. 2004), the differential timing of their entry into waterways (Lee et al. 2002), and technological limitations in quantification (Ellis 2006).

Water quality in Pacific Northwest streams is routinely monitored by federal, state, county, and municipal agencies using routine measures of water quality such as temperature, pH, conductivity, and dissolved oxygen and more rarely measures of toxic pollutants and nutrients. Biological monitoring of ecosystem health often relies on measures of species assemblages, such as the Index of Biotic Integrity (IBI) (Karr 1991). Currently, ecosystem health is determined from the comparisons of these species assemblage to a reference condition. IBIs for fish and benthic invertebrates are widely used in the Pacific Northwest (Harris and Silveira 1999, Morley and Karr 2002, Mebane 2003, Whittier et al. 2007). Correlations between IBI scores and water quality measures are used as a metric to assess potential impacts from anthropogenic pollution. While these IBIs have been successful at identifying general patterns of ecosystem impact from toxic chemicals (Dickson et al. 1992), they are not capable of mechanistically linking specific components of water quality (physical or chemical) to biological harm, and health impacts to individuals are left unquantified (Landis et al. 2003). Modern molecular tools allow us to assess an organism's physiological response to contaminants in their environment (Hook et al. 2014), thus mechanistically linking chemical stress to individual health. Little consensus currently exists on a method to screen for the biological response to toxicants in a holistic sense that can indicate direct biological exposure from all possible chemicals in a system. With the increasing need to monitor our freshwater streams due to urbanization (Hughes et al. 2014), tools that can directly

measure exposure in organisms would benefit the understanding and identification of stress in these urban streams for the investigation of impacts on a particular family or species of animal.

In the Pacific Northwest salmon have and continue to be the focus of intense study and interest, owing to both their cultural and economic significance. Billions of dollars are spent on salmon recovery, conservation efforts, and research (Lackey, 2013). Conservation and restoration efforts in the region seek to improve habitat and reproductive success of salmon. In 2014, King County, Washington began 23 new projects to improve small stream habitat for salmon (King County 2014). Despite these large scale efforts, few direct tools are routinely utilized for monitoring the health of juvenile salmonids with regard to thermal condition, growth, disease prevalence, and toxicant exposure. More generally, the tools that do exist for measuring the toxicant effect in natural populations are finding limited use in the field (Hook et al. 2014). Salmon, therefore, are the focus of this study as they serve the dual purpose of designing a health screening tool for a commonly-found family of fish in this region's urban streams that is also applicable to monitoring the success of specific restoration projects.

Coho salmon (*Oncorhynchus kisutch*) and cutthroat trout (*Oncorhynchus clarkii*) were the target species for this study due to their widespread presence in Pacific Northwest streams. Coho inhabit small to medium sized Puget Sound lowland streams where they typically spend one year rearing before out-migrating to sea (Sandercock 1991). These small perennial streams serve as important habitat for the spawning and rearing of coho salmon. During autumn, anadromous coho make their way from the Pacific Ocean back to their natal stream. Coho utilize these smaller streams to spawn, and the fertilized eggs incubate in gravel redds during late fall and winter. Small coho salmon fry emerge from the gravel substrate during the late winter/early spring, and utilize these small stream habitats for the next year of their life, often migrating

upstream during the summer (Kahler et al., 2001). During this time, they feed predominantly on drifting invertebrates. Juvenile growth is instrumental to their survival, as it provides fish with the size necessary to survive over the winter in freshwater and then in marine environments. After 1-2 years, coho undergo a physiological change known as smoltification and migrate downstream to marine environments.

Cutthroat also rear in these streams and will spend most of their life moving in and out of these small systems. Unlike coho which spawn in the fall, cutthroat trout spawn during winter-spring, and fry emerge from the gravel in late spring or early summer. Cutthroat trout follow three general life history patterns. Many cutthroat spend their entire life in their natal stream; others move between these small stream habitats and larger fluvial or adfluvial environments, remaining in freshwater their whole life; and a third subset are anadromous and move from these freshwater systems out to marine environments, and migrating back to spawn (Trotter 1988).

Juvenile fish growth integrates both external and internal conditions. External conditions governing fish growth include prey quality and quantity (Filbert and Hawkins, 1995; Rosenfeld and Taylor, 2009), thermal regime (McCullough et al., 1999; McCullough et al., 2009; Wenger et al., 2011), and water quality (Buckley et al., 1982; Meador et al., 2006; Baldwin et al., 2009). Allometric relationships and water temperature determine rates of consumption and metabolism for fish of different sizes, and water quality has direct physiological consequences ranging from dissolved oxygen demands to contaminant metabolism. Varying availability and energetic quality of prey are resource limits on growth additionally exacerbated by density-dependent competition for limited food resources (Keely 2001). Internal conditions governing fish growth include disease and stress response each with varying ranges of energetic demand.

Growth by salmonids integrates the influence of these external and internal conditions; therefore, growth provides a useful indicator of habitat quality and ecosystem health.

These life history traits result in fish that integrate the effects of stress from anthropogenic-influenced environments over similar periods, thus allowing for better comparison of toxicant exposure among streams and for measuring integrative effects of contaminants and stress during their first year of growth. Finally, juvenile life stages are of great interest, because they are often the most sensitive period of an organism to stress (Hutchinson et al. 1998). Previous work has shown that juvenile coho are sensitive to toxicants (Barbee et al. 2008 and McIntyre et al. 2012). Furthermore, it is well documented that size achieved during critical growth periods can influence survival to adulthood (Miyakoshi et al. 2001; Duffy and Beauchamp, 2011; Thompson and Beauchamp 2014).

Objectives

This study provides a comprehensive fish health assessment that can detect disease, assess fish growth within the context of environmental and ecological influences, and identify impacts from pollution through the use of genetic biomarkers. Fish were sampled in 15 perennial streams to assess fish health conditions as a subset of the streams sampled for the Pacific Northwest Regional Stream Quality Assessment (PNSQA), part of the US Geological Survey's (USGS) National Water-Quality Assessment program (NAWQA). We first characterized the land use characteristics of each study stream's watershed, conducted fish surveys to quantifying both species composition and relative abundance, and documented the field collection of juvenile cutthroat and coho salmonids. Additionally, we used a field disease necropsy index to relate observable disease and tissue abnormalities making comparisons among streams and to the degree of urbanization within each stream. This land use characterization and field effort form

the basis of later chapters that assessed fish growth and environmental condition (Chapter 2) and genetic indicators of chemical exposure and harm (Chapter 3).

Study Sites

The PNSWA NAWQA study surveyed 88 streams within the Pacific Northwest concentrating on areas of urban impact (VanMetre, 2015). The USGS conducted water quality sampling in these streams during a ten-week period spanning April to June 2015. Once in June the streams were surveyed for fish communities, and an ecological assessment conducted (Sheibley et al. 2017, in press). In Washington, we primarily focused on streams in watersheds flowing into Puget Sound as far north as the Canadian Border, south to Olympia, and west to the Kitsap Peninsula (Figure 1). The other sub-region of focus included streams whose watersheds drain to the Columbia River, with a heavy concentration of streams in and around the Portland metropolitan area. A subset of streams for this study of fish health was chosen from this overall list of 88 streams.

Streams sampled for this fish health component spanned the gradient designated in the PNSQA study defined by the percentage of land within each stream's watershed that was developed and considered “urban.” We emphasized streams located in the Puget Sound region (Figure 1). Predominantly agricultural streams were intentionally left out of this fish health study. Of the 15 streams initially sampled for fish in June 2015, six streams were studied in more detail including a fish collection in September 2015 (Table 1). Both sampling periods included a visual necropsy assessment described below.

Methods

Fish Collection

Fish sampling was conducted during early (June and July) and late (September) periods of the summer growing season. Early season sampling for wild juvenile coho salmon and cutthroat trout coincided with the standard ecological sampling by the USGS NAWQA program, except for at May and Swamp creeks where lethal sampling occurred at a later date in July. Standard NAWQA fish survey methods were used for collection of juvenile fish (Moulton et al. 2002; Sheibley et al. 2017, in press). At each stream, a 150-m reach was designated at the location of water quality sampling. Along this reach, double-pass backpack electroshocking was used to survey fish with teams of 2-3 people to net fish alongside the electroshocker. Captured fish were held briefly in an aerated net pen in the stream until they were identified, counted, and released onsite. All fish were identified, measured for fork length (FL) (salmonids only), and counted during June, whereas, only salmonids were processed during September. During each season, a representative subsample of up to 15 coho and cutthroat from each stream reach were held alive in an aerated bucket until they could be euthanized for necropsies, liver collection, otolith extraction, diet analysis, and weight.

Field Processing

Juvenile fish were kept alive in aerated tanks of native stream water and held until individually processed. Fish were euthanized for field necropsy, tissue sampling, size measurements, and diet analysis. Fish were euthanized by blunt trauma (the potential interaction of MS-222 with gene expression precluded its use) and immediately weighed, measured, and quickly dissected for preservation of liver tissue for genetic analysis. Once the liver was removed, each fish was examined underneath a dissection microscope both externally and

internally using the methods described by Goede and Barton (1990; see Table 2 for detailed scoring criteria). During this necropsy, each major organ (skin, eyes, kidney, etc.) was rated for any of the following observable abnormalities: hemorrhaging, presence of parasites, enlargement, irregular color, or tumor growth. Using this method, each organ was scored according to specific guidelines on a 30-point scale. Determination of both individual and population health condition uses the aggregate of this score. Handling of vertebrates was conducted under the auspices of the University of Washington IACUC protocol #3286-21.

Drift Collection

Invertebrate drift sampling was conducted at all streams to determine the energetic composition of fish diets and how access to food and its energetic quality varied both among streams and during the growing season. Sampling occurred during the morning or middle of the day when invertebrate drift is less variable (Wall et al. 2015). Drift was collected three times during the growing season: during late June/early July, Mid-August, and during the final fish collection in Late-September (Table 1). The sampling pattern followed that of the overall study design with all 15 streams sampled in June and only the six long-term streams in mid- and late-summer. Drift sampling was conducted in each stream with 3- 250 μm mesh nets. Each net had a 30.5 cm x 30.5 cm opening. Nets were placed side-by-side in the thalweg of the river so that the opening was at least 2.54 cm above the water, to collect floating adult and terrestrial insects. Water velocity was measured in front of each of the three panels at a point roughly centered in front of the net using a Marsh-McBirney velocity meter both at initial deployment and when the nets were pulled. Deployment time of the drift nets was recorded, but varied, averaging around 3 hours during the June deployments and 1 hour during both August and September. Once nets

were pulled, collected drift was washed down to the cod-end of each net, placed into separate jars, one for each net, and preserved in 95% ethanol.

Necropsy Data Analysis

Necropsy scores were summed for each individual to calculate a Heath Assessment Index (HAI) score following the methods of Goede et. al. (1991); with a top score of 270 indicating a high level of disease apparent in all tissues. HAI values for individual fish from the same stream were averaged and a standard deviation (SD) and coefficient of variation (CV) calculated. Higher values indicate poor condition. The average HAI represents the overall health of fish from a particular stream. In addition to the average HAI values of fish per stream, a low CV of those values indicate that fish from the same stream are of similar health, while a high CV value indicates larger variation in condition. In this way, low CV values indicate stream conditions that affect fish in the same way, such as with poor water quality. Conversely, a high CV value indicates individual stress such as bacterial or viral infection (Goede et al 1991). Specific abnormalities accounting for the HAI score were identified by the percent detection rate. A non-parametric Kruskal-Wallis was used to test for significant differences in HAI values among streams (α=0.05), and a subsequent Dunn non-parametric posthoc pairwise comparison test determined which specific streams were significantly different (α=0.05).

Results

Fish Sampling

During June, fish were sampled at all 15 streams (Table 3). The total catch in streams ranged 13-357 fish. Salmonids represented an average 37% of fish caught in all streams, second to sculpins which were the most prevalent taxa at 56%. The remaining 7% was a mixture of Pacific lamprey, bluegill, other sunfishes, dace, shiner, sucker, and stickleback. Coho were found

in all streams except for Thornton Creek, but only in numbers large enough to sample in 10 streams: Coulter, Woodland, Jenkins, Longfellow, May, Issaquah, Swamp, Church, EF Dairy, and Harris during the early portion of the growing season in June (Table 4) and near the end of the growing season in September (Table 5). Cutthroat trout were found in all streams with large enough catches for subsampling in all streams except Longfellow. Cutthroat trout were more prevalent overall (646 cutthroats, 429 coho); only the samples at Harris, Church, Jenkins, and Coulter contained more juvenile coho than juvenile cutthroat.

Necropsy Assessment

Necropsy assessments were carried out on a total of 235 fish, 93 coho and 142 cutthroat, during the early season sampling and 105 fish, 60 coho and 45 cutthroat, during late season sampling (Figures 2 and 3). Scoring was considered separately for each species and sampling period.

Coho Necropsy

Total HAI scores for coho caught during early season were significantly higher in Woodland and Jenkins creek (Dunn, $p<0.05$, Figure 2), both urbanized watersheds. These streams had observable abnormalities in most of the tissues examined (Figure 3). Enlarged livers were common in the 4 highly urbanized streams, Woodland, Jenkins, Swamp, and Longfellow Creeks (Table 6). Other tissues did not show a pattern with increasing urbanization and total HAI scores varied significantly among streams (Kruskal-Wallis, $p<0.05$; Figure 2). Abnormalities and parasites were common among many tissues and streams, including reference streams, at moderate levels (Figure 2).

In late season, coho from Jenkins Creek had a significantly elevated total HAI score above all other streams (Dunn, $p<0.05$, Figure 2). Predominately due to abnormalities in gills,

pseudobranch inflammation, and the presence of parasites (Figure 3). Total HAI values were significantly lower when compared to early season sampling (Kruskal-Wallis, $p<0.05$). Additionally, parasites were in most coho from Coulter, but were less severe. All other abnormalities were rare or of low severity in coho from Coulter, Harris, Issaquah, May and Swamp. HAI scores were not significantly related to urban land use percentage as investigated with regression analysis during either sampling period.

Cutthroat Necropsy

Total HAI values for cutthroat trout at Burnt Bridge and Kelley Creek were significantly higher than all other streams except Woodland and East Fork Dairy Creeks (Dunn, $p<0.05$). HAI values were significantly different among streams (Kruskal-Wallis, $p<0.05$, Figure 4). External abnormalities were rare in cutthroat among fish from all streams (Table 7). There was no clear pattern associated with urbanization and abnormalities were generally variable among sites (Figure 5). Pseudobranch inflammation, parasites occurrence, and spleen abnormalities commonly occurred in fish from most streams.

Late season cutthroat had a low total HAI scores with no significant differences among streams (Kruskal-Wallis, $p<0.05$, Figure 4). Pseudobranch inflammation, spleen abnormalities, and gill abnormalities were most common, but not severe in fish from all streams (Figure 5). Parasites were only found in fish from Coulter Creek. All other tissues presented with no or low observable abnormalities. HAI scores were not significantly related to urban land use percentage as investigated with regression analysis during either sampling period.

Necropsy Species Comparisons

Overall, HAI scores in coho and cutthroat did not follow the hypothesized stress gradient from urbanization and there were no streams with high HAI scores for both species. Total HAI scores were significantly higher in cutthroat than coho in Swamp Creek (Wilcoxon rank test, $p<0.05$), but significantly lower in cutthroat in Woodland Creek ($p<0.05$) and Jenkins Creek ($p<0.05$) during early season sampling, which were both moderately urban sites. HAI scores were significantly higher in cutthroat than coho in Issaquah Creek ($p<0.05$) during late season sampling. No other significant differences were found between species in streams bearing both cutthroat and coho. These differences between coho and cutthroat from the same stream suggest that stream specific environmental condition were not the only driver of fish health. For streams sampled in both early and late seasons, HAI scores were significantly lower for both species in late season (Wilcoxon rank test, $p<0.05$).

Discussion

HAI scores from streams sampled showed no clear pattern relating to urbanized landuse within the watersheds of streams sampled. These scores instead proved to be valuable as a tool to indicate where disease/abnormalities occurred among streams. This component of fish health would have been missed if it was instead assumed to be correlated with other measured variables, and serves as valuable information to inform both the growth and contaminant components of this study. Four streams, Burnt Bridge, Kelsey Creek, Woodland Creek, and Jenkins Creek, had HAI scores averaging above 100. These were all moderate-high developed watersheds Observed disease/abnormalities in fish from these streams occurred in all tissues examined with low variability among fish (low CV) suggesting that disease stress to fish in these

streams was related to their environment (Goede et al. 1991) and not an acute problem affecting just individual fish.

More generally, these data indicate some common patterns among fish from our sample streams. First, external abnormalities (those of the fins, skin, and eyes) were rare and were of low severity, usually indicated by the presence of parasites. Second, with few exceptions, some tissues showed abnormalities in at least one fish from each stream. These were the pseudobranchs, gills, spleen, and hindgut. This suggests some fish from every stream were experiencing possible disease stress, an important consideration when putting environmentally influenced growth and contaminant response into context. Finally, many of these streams exhibited parasite infestations that affected both species similarly. Although the assessment of parasite diversity in fish has been suggested as an indicator of a healthy stream (Losee et al, 2014), the fish in this study mostly exhibited infestations of what appeared to be similar parasite-type at a high intensity. Previous work has shown parasite intensity to negatively impact both the growth and overwinter survival of juvenile coho salmon (Ferguson et al. 2012).

It was noted that abnormality/disease/parasite detection was common in many of the streams considered reference locations. Given this varied response, overall fish health needs to be considered independently of a priori "reference" streams. Fish catches in June were used to determine sampling streams for the remainder of the study. Resources were only sufficient to address one species in subsequent analyses, so only juvenile coho salmon were considered for energetics modeling and genetic analysis limiting the number of early season streams to 10. The necropsy scoring was included in chapter 2 to examine whether disease affected growth rates.

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Tables

Table 1. Study streams and sampling dates.

Stream name	USGS site #	Sampling location	Drainage area km^2	% urban[1]	Sample date(s)
East Fork Dairy Creek Near Meacham Corner, OR	14205400	N 45.68233 W 123.06955	47.05	2.4	6/30/2015[1,2,3]
Rock Creek Near Landsburg, WA	12117695	N 47.40300 W 121.89900	33.54	2.4	6/19/2015[1,2,3]
Coulter Creek Near Allyn, WA	12073895	N 47.40861 W 122.81583	46.11	4.0	6/23/2015[1,2,3] 8/15/2015[2] 9/29/2015[1,2]
Issaquah Creek Near Hobart, WA	12120600	N 47.45732 W 122.00512	9.05	15.1	6/15/2015[1,2,3] 8/16/2015[2] 9/24/2015[1,2]
Harris Creek Near Carnation, WA	12149490	N 47.69391 W 121.90026	7.76	16.8	6/22/2015[1,2,3] 8/15/2015[2] 9/24/2025[1,2]
Church Creek Near Stanwood, WA	12170000	N 48.24787 W 122.31454	29.72	24.4	6/19/2015[1,2,3]
Kelley Creek Near Portland, OR	14211499	N 45.47679 W 122.49842	33.49	39.4	6/18/15[3] 7/1/2015[1,2,3]
May Creek Near Renton, WA	12119495	N 47.52100 W 122.19700	32.12	50.9	6/20/2015[3] 7/15/2015[1,2] 8/16/2015[2] 9/23/2015[1,2]
Jenkins Creek Near Auburn, WA	12110495	N 47.33986 W 122.12967	46.42	65.8	6/16/2015[1,2,3] 8/16/2015[2] 9/21/2015[1,2]
Woodland Creek Near Lacey, WA	12080800	N 47.06361 W 122.80722	52.24	75.5	6/24/2015[1,2,3]
Mercer Creek Near Bellevue, WA	12120000	N 47.60288 W 122.18096	31.07	85.7	6/18/2015[1,2,3]
Swamp Creek Near Near, Kenmore, WA	12126910	N 47.79221 W 122.25631	19.84	89.6	6/23/2015[3] 7/9/2015[1,2,3] 8/15/2015[2] 9/17/2015[1,2]
Longfellow Creek Near West Seattle, WA	12113490	N 47.56000 W 122.36700	87.58	93.0	6/21/2015[1,2,3]
Burnt Bridge Creek Near Vancouver, WA	14211902	N 45.66123 W 122.66899	12.68	95.5	6/15/2015[3] 7/2/2105[1,2,3]
Thornton Creek Near Seattle, WA	12128000	N 47.69565 W 122.27624	67.53	96.2	6/20/2015[1,2,3]

[1]Fish Sampling for necropsy, liver, diets, otolith, and lethal takes, and salmonid FLs
[2]Drift Sampling
[3]Fish species surveys and counts

Table 2. Quantitative necropsy assessment criteria. Adapted from: Goede and Barton 1990; Adams et al 1993; Schmitt et al 1999.

Organ	Rating	Quantitative Score
Quantitative Assessment		
Fins	0-No active erosion	0
	1-Light active erosion	10
	2-Moderate active erosion with some hemorrhaging	20
	3-Severe active erosion with hemorrhaging	30
Skin	0-Normal; no aberrations	0
	1-Mild skin aberrations	10
	2-Moderate skin aberrations	20
	3-Severe skin aberrations	30
Eyes	N-No aberrations: good "clear" eye	0
	B-Generally, an opaque eye (one or both)	30
	E-Swollen, protruding eye (one or both)	30
	H-Hemorrhaging or bleeding in the eye (one or both)	30
	M-Missing one or both eyes	30
	OT-Other; any manifestation not fitting the above	30
Pseudobranchs	N-Normal; flat, containing no aberrations	0
	S-Swollen, convex in aspect	30
	L-Lithic, mineral deposits, white, somewhat amorphous spots	30
	S&L-Swollen and Lithic	30
	I-Inflamed; redness, hemorrhage, or other	30
	OT-Other; any condition not covered above	30
Gills	N-Normal; no apparent aberrations	0
	F-Frayed; erosion of tips of gill lamellae resulting in "ragged" gills	30
	C-Clubbed; swelling of the tips of the gill lamellae	30
	M-Marginate; gills with light, discolored margin along tips of the lamellae	30
	P-Pale; very light in color	30
	OT-Other; any observation not fitting above	30
Parasites	0-No observed parasites	0
	1-Few observed parasites	10
	2-Moderate parasite infestation	20
	3-Numerous parasites	30
Spleen	B-Normal; black, very dark red, or red	0
	G-Normal; granular, rough appearance of spleen	0
	D-Nodular; containing fistulas or nodules of varying sizes	30
	E-Enlarged; noticeably enlarged	30
	OT-Other; gross aberrations not fitting above categories	30
Hindgut	0-Normal; no inflammation or reddening	0
	1-Slight inflammation or reddening	10
	2-Moderate inflammation or reddening	20
	3-Severe inflammation or reddening	30
Kidney	N-Normal; firm dark red color, lying relatively flat along the length of the vertebral column	0
	S-Swollen; enlarged or swollen wholly or in apart	30
	M-Mottled; gray discoloration	30
	G-Granular; granular appearance and texture	30
	U-Urolithiasis or nephrocalcinosis; white or cream-colored mineral material	30
	OT-Other; any aberrations not fitting previous categories	30
Liver	A-Normal; solid red or light red color	0
	C-"Fatty" liver; "coffee with cream" color	30
	D-Nodules in the liver; cysts or nodules	30
	E-Focal discoloration; distinct localized color changes	30
	F-General discoloration; color change in whole liver	30
	OT- Other; deviation in liver not fitting other categories	30

Table 1. Fish counts from USGS stream surveys. Method consisted of double-pass electroshocking along a 150-meter reach.

Stream	DATE	COHO SALMON	CUTTHROAT TROUT	RAINBOW TROUT	LAMPREY SP.	SCULPIN	OTHERS[1]	TOTALS
EF Dairy	6/22/15	13	5	49	11	255	24	357
Rock	6/19/15	1	90	0	0	81	0	172
Coulter	6/23/15	127	63	0	1	89	0	280
Issaquah	6/15/15	21	50	0	0	286	0	357
Harris	6/22/15	114	18	0	0	117	0	249
Church	6/19/15	68	42	0	1	9	7	127
Kelley	6/18/15	3	39	1	2	94	74	213
May	6/20/15	7	89	0	0	244	1	341
Woodland	6/24/15	18	35	1	1	145	0	200
Jenkins	6/16/15	28	8	0	0	115	0	151
Mercer	6/18/15	2	98	3	0	26	10	139
Swamp	6/23/15	13	72	0	1	88	44	218
Burnt bridge	6/15/15	2	15	3	1	135	25	162
Thornton	6/20/15	0	21	1	0	3	3	28
Longfellow	6/21/15	12	1	0	0	0	0	13

[1] Other species include: pumpkin seed, longnose dace, bluegill, red shiner, speckled dace, largescale sucker, and three-spine stickleback.

Table 2. Fish catch data for early season sampling. Fish counts for necropsy, otolith, and liver sampling and average fork length (FL) of other caught salmonids.

Stream Species	Sampled Avg. FL	FL STDEV	#fish Sampled (lethal take)	Caught Avg. FL	FL STDEV	# Fish Caught
Burnt Bridge						
Cutthroat	75.3	7.2	10	75.3	7.2	10
Church						
Coho	57.7	3.4	10	58.8	6.1	60
Cutthroat	58.4	3.1	10	96.7	40.3	41
Coulter						
Coho	59.4	9.4	10	64.1	9.1	68
Cutthroat	51.1	8.4	10	52.0	7.1	25
East Fork Dairy						
Coho	74.9	8.9	10	72.7	7.5	19
Cutthroat	58.4	6.9	8	95.8	48.3	22
Harris						
Coho	52.4	9.3	10	52.0	8.7	76
Cutthroat	54.5	8.4	8	101.9	59.7	16
Issaquah						
Coho	62.6	5.7	10	62.6	5.7	10
Cutthroat	51.9	6.2	11	51.9	6.2	11
Jenkins						
Coho	57.3	5.6	10	58.7	5.9	23
Cutthroat	78.0	27.4	4	103.0	47.9	7
Kerry						
Cutthroat	70.0	7.9	8	85.6	35.7	14
Longfellow						
Coho	85.5	6.9	10	85.5	6.9	10
May						
Coho	71.3	4.9	3	71.3	4.9	3
Cutthroat	65.1	5.6	10	87.6	37.2	26
Mercer						
Cutthroat	58.0	11.3	10	81.4	42.3	82
Rock						
Cutthroat	62.0	25.1	13	82.2	29.3	66
Swamp						
Coho	80.5	4.9	10	80.5	4.9	10
Cutthroat	58.1	5.9	10	69.8	19.9	48
Thorton						
Cutthroat	70.9	13.2	15	94.2	38.5	25
Woodland						
Coho	65.0	11.9	10	65.0	11.9	10
Cutthroat	63.6	8.2	10	63.6	8.2	10

Table 3. Fish catch data for late season sampling. Fish counts for necropsy, otolith, and liver sampling.

Stream Species	# fish	Sampled Avg F.L.	F.L. stdev
Coulter			
Coho	10	70.9	6.9
Cutthroat	9	67.7	10.0
Harris			
Coho	10	60.9	10.6
Cutthroat	5	62.6	11.0
Issaquah			
Coho	10	78.7	7.7
Cutthroat	10	70.0	12.1
Jenkins			
Coho	10	77.4	9.2
May			
Coho	10	80.2	5.5
Cutthroat	10	74.0	12.5
Swamp			
Coho	10	82.4	4.7
Cutthroat	10	80.5	12.8

Table 6. Results of coho field based quantitative necropsy. HAI (Health Assessment Index) Score is average for all individuals from each stream; SD is standard deviation; CV is coefficient of variation. Values for each tissue/organ is percent of fish from each stream with observed abnormality.

Stream	HAI	SD	CV	Fins	Skin	Eyes	Pseudobranchs	Gills	Parasites	Spleen	Hindgut	Kidney	Liver
Early Season (June/July)													
Coulter	78	23.5	30.1	0	0	0	70	70	100	30	40	20	20
EF Dairy	70	50.5	72.1	33	0	11	33	67	56	11	44	33	33
Harris	88	35.4	40.2	0	0	0	36	91	0	55	27	27	73
Issaquah	9	20.2	225.0	0	0	10	10	10	0	0	0	0	0
Church	27	27.9	103.4	0	0	0	30	20	40	20	10	0	0
May	23	11.5	49.5	0	0	0	0	67	0	0	33	0	0
Jenkins	137	28.3	20.7	0	0	60	70	80	90	80	0	80	50
Woodland	180	63.9	35.5	60	60	70	60	70	100	80	60	60	60
Longfellow	50	24.0	48.1	10	0	0	10	10	0	40	10	0	100
Swamp	40	15.0	37.5	0	0	0	0	56	11	11	22	0	56
Late Season (September)													
Coulter	33	36.2	109.8	20	0	0	20	10	90	10	0	20	10
Harris	14	21.7	155.0	0	0	0	10	30	10	0	10	0	0
Issaquah	10	14.1	141.4	0	0	10	0	0	10	20	0	0	0
Jenkins	100	29.1	29.1	60	90	10	40	90	100	0	90	10	10
May	5	12.7	253.9	0	0	0	10	0	20	0	0	0	0
Swamp	32	25.7	80.4	30	0	0	20	20	0	10	0	30	10

Table 7. Results of cutthroat field based quantitative necropsy. HAI (Health Assessment Index) Score is average for all individuals from each stream; SD is standard deviation; CV is coefficient of variation. Values for each tissue/organ is percent of fish from each stream with observed abnormality.

	n	HAI	SD	CV	Fins	Skin	Eyes	Pseudobranchs	Gills	Parasites	Spleen	Hindgut	Kidney	Liver
Early Season														
Burnt Bridge	10	140	22.6	16.1	0	50	0	40	80	60	90	60	80	100
Church	10	29	31.4	108.4	0	0	0	40	30	10	20	10	0	0
Coulter	11	62	37.1	60.0	0	0	0	91	27	73	18	18	0	36
EF Dairy	10	93	26.7	28.7	0	0	0	60	70	70	50	70	10	70
Harris	8	64	43.7	68.6	0	0	0	50	25	0	50	75	13	50
Issaquah	11	14	24.6	180.4	0	0	0	18	0	0	18	0	0	9
Jenkins	4	23	18.9	84.1	25	0	0	0	25	100	0	0	0	0
Kelsey	10	151	50.0	33.1	10	80	0	60	80	100	70	90	80	70
May	10	52	27.0	51.9	0	0	0	70	50	30	0	60	0	20
Mercer	10	69	40.4	58.6	0	0	0	40	40	0	70	20	10	60
Rock	13	92	17.9	19.4	0	8	0	85	31	0	85	15	0	100
Swamp	10	70	17.0	24.3	20	0	0	80	40	10	60	40	0	30
Thornton	15	55	38.9	70.3	7	0	0	33	33	0	67	7	0	47
Woodland	10	93	20.0	21.5	0	20	0	80	70	100	20	40	10	40
Late Season														
Coulter	10	31	37.5	121.1	0	0	0	10	50	70	10	0	0	10
Harris	5	20	30.8	154.1	0	20	0	0	20	0	40	0	0	0
Issaquah	10	39	31.8	81.5	0	0	0	40	60	0	30	0	0	0
May	10	22	23.9	108.8	0	0	0	10	10	0	30	20	0	10
Swamp	10	36	27.6	76.6	30	0	0	40	20	0	10	0	10	30

Figures

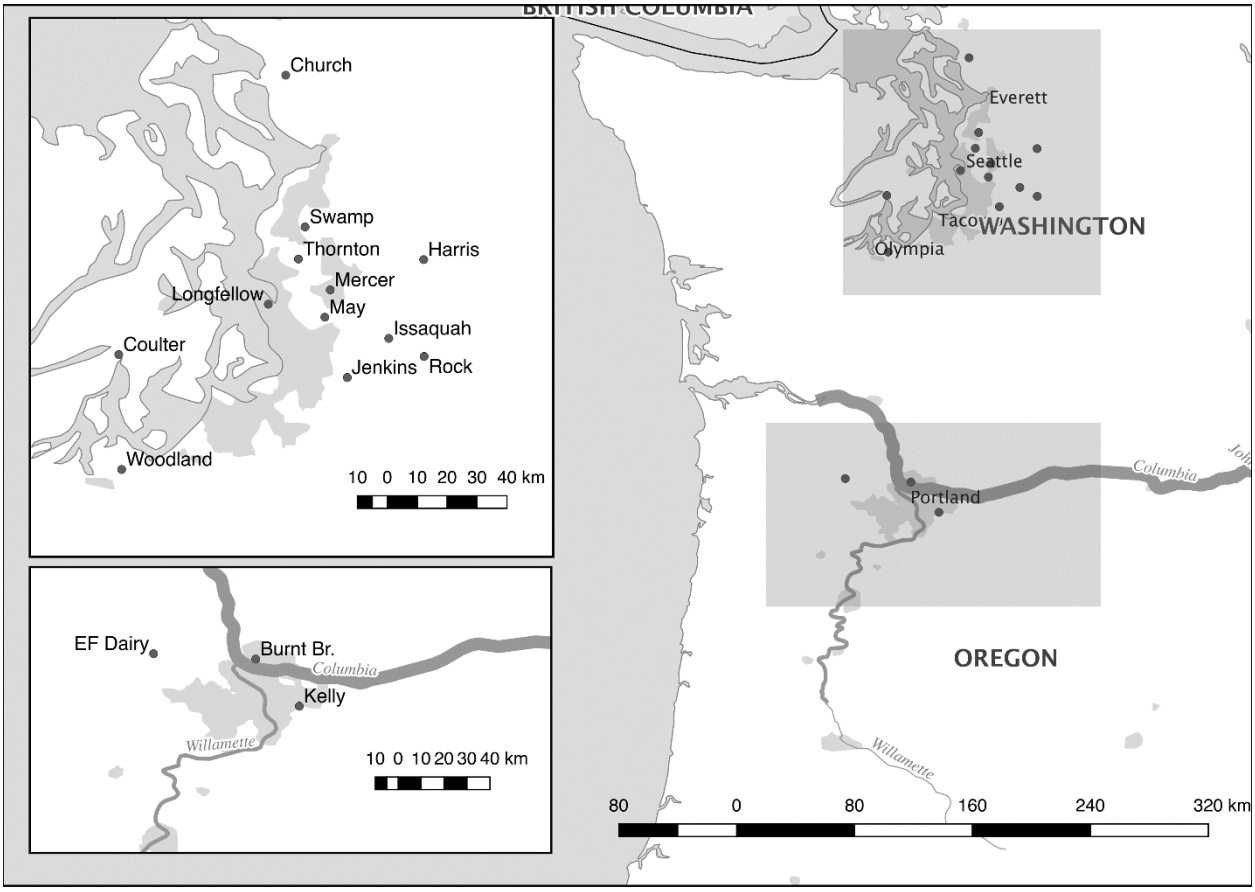

Figure 1. Sampling site locations.

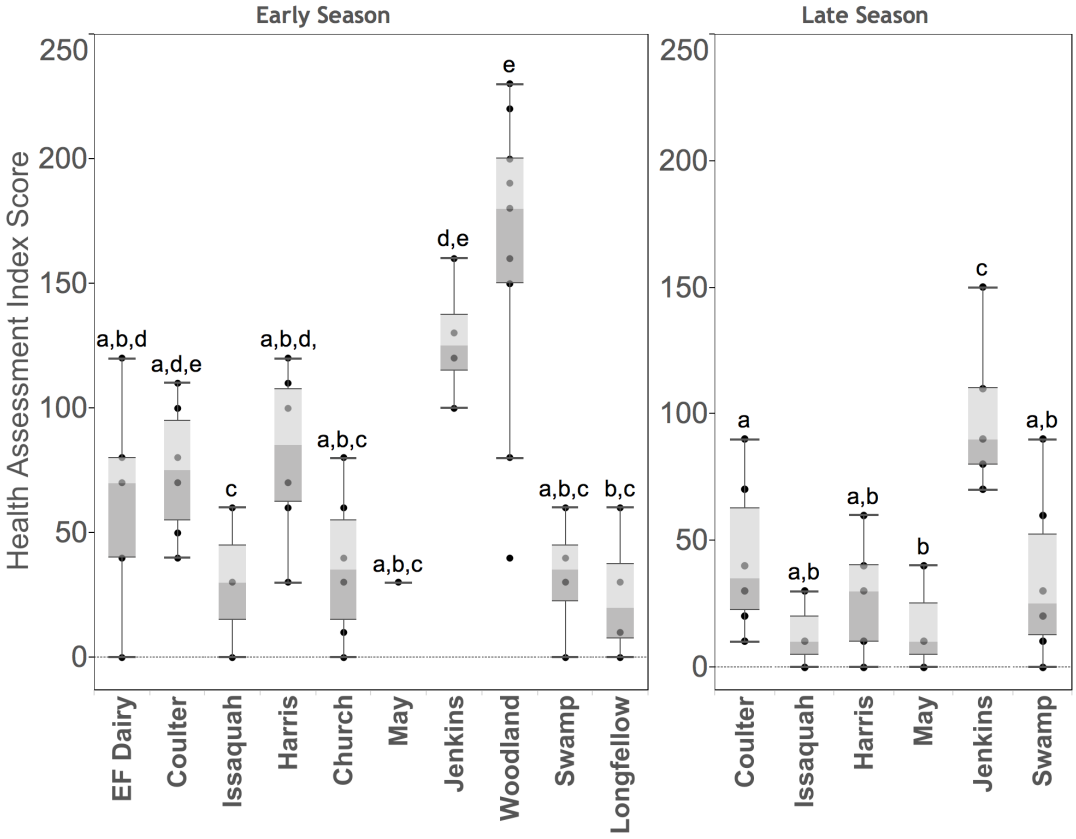

Figure 2. Boxplot of average aggregate Health Assessment Index score (HAI) by individual coho. Letters denote streams that are significantly the same (Dunn test, $p<0.05$).

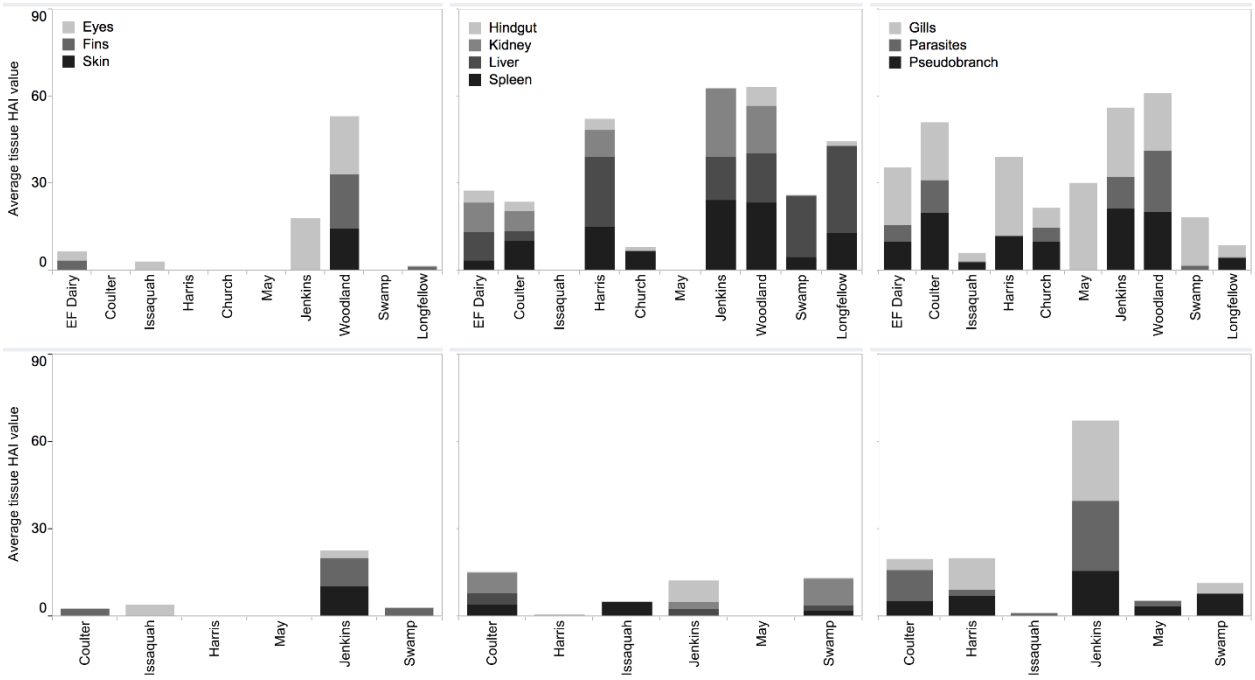

Figure 3. Average necropsy scores for all coho tissues. Sites are ordered by gradient level of urbanization from low to high (left to right). Early sampling results in top panels; late season sampling results in bottom panels.

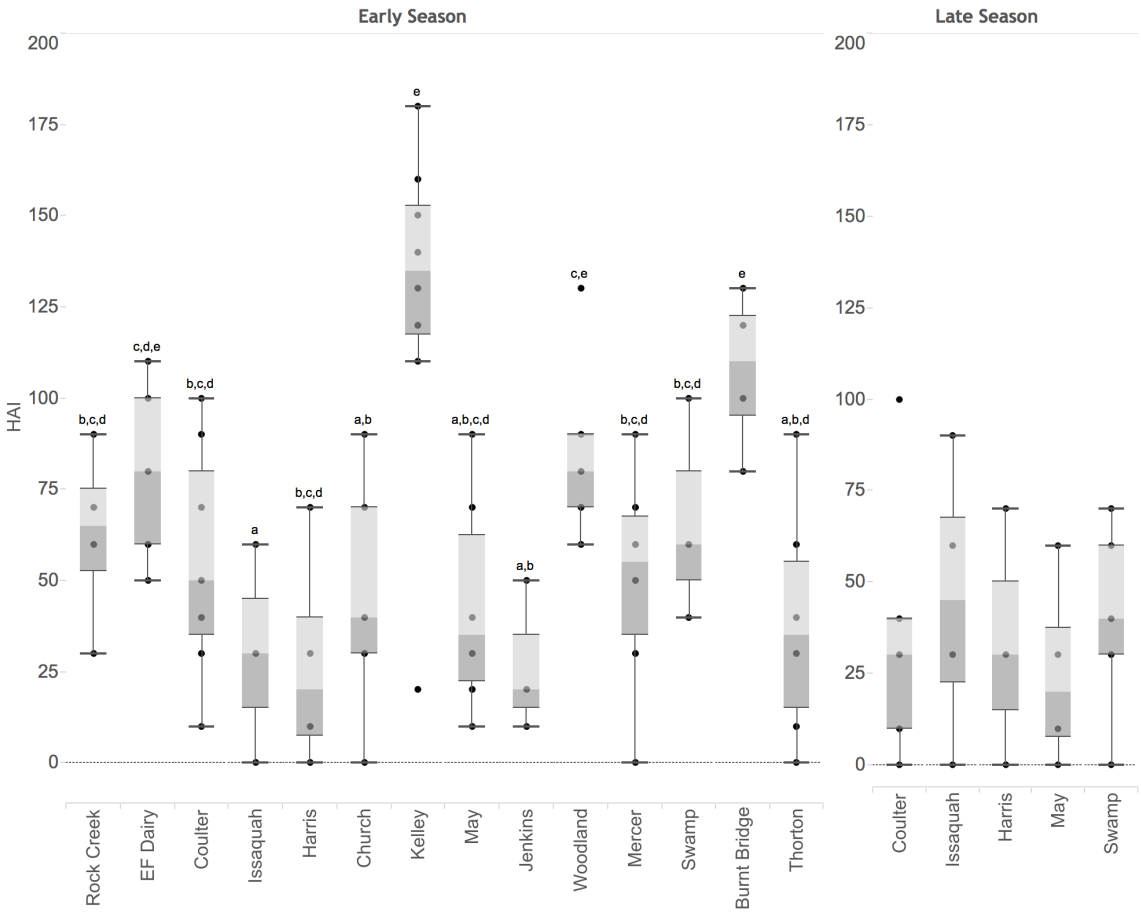

Figure 4. Boxplot of average aggregate Health Assessment Index score (HAI) by individual cutthroat. Letters denote streams that are significantly the same (Dunn test, $p<0.05$). No significant differences in HAI scores for late season cutthroat.

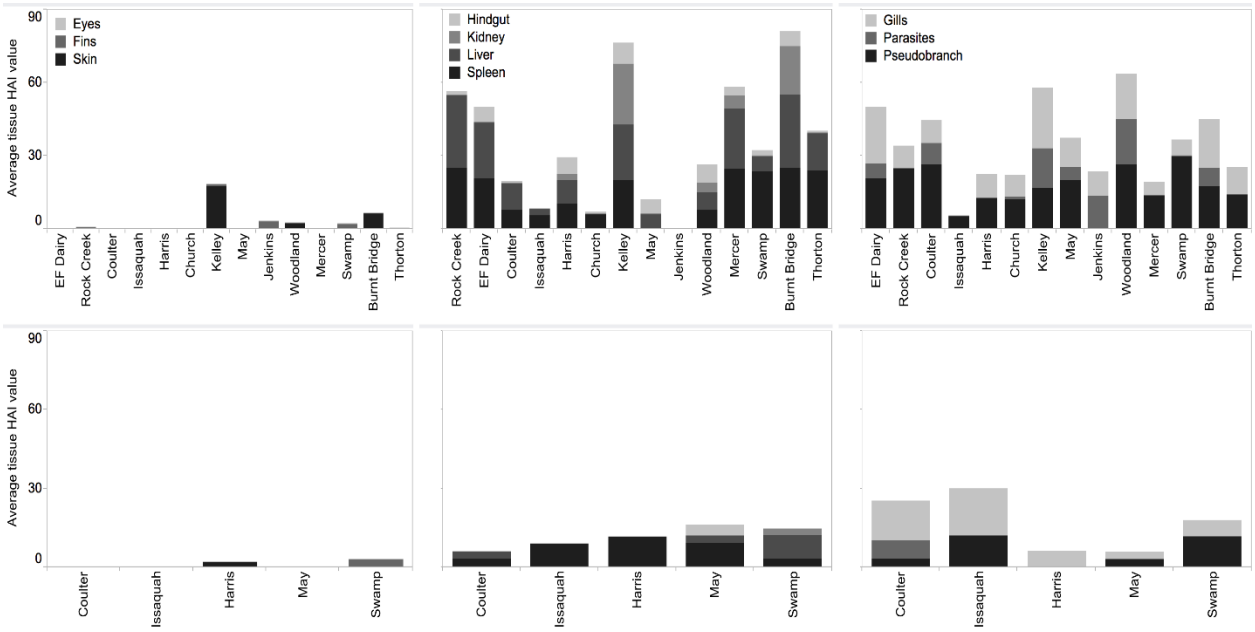

Figure 5. Average necropsy scores for all cutthroat tissues. Sites are ordered by gradient level of urbanization from low to high (left to right). Early sampling results in top panels; late season sampling results in bottom panels.

Chapter 2: Evaluating Coho Salmon Habitat Growth Potential in Tributaries Across an Urbanization Gradient Using Environmental Factors and Bioenergetics

Abstract

Salmonid growth during early life stages has a major role for survival in later life stages. Bioenergetics models can simulate changes in growth and consumption in response to environmental conditions and food availability to explore the interactions between an organism's environmental experience and utilization of available resources. Current stream assessments stop short of measuring the health or condition of species utilizing these freshwater habitats and fail to link specific stressors mechanistically to the health of organisms in the stream. Thus, there is a need to account for ecological and environmental influences on growth correctly. The bioenergetics approach evaluates these influences by considering how thermal regime, food supply, and food quality affect fish growth. This study used a bioenergetics growth modeling approach as one component of a comprehensive fish health assessment to assess the environmental factors that influence juvenile coho growth among ten streams spanning an urban gradient. For urban streams, we show mixed effects, whereby urban streams tended to be warmer, have earlier emergence dates and stronger early season growth. However, we also show that larger fish can be under increased stress through lower growth efficiencies, especially later in the summer, when compared to fish from other streams. The successful use of bioenergetics modeling to characterize salmonid growth in small perennial streams as part of a monitoring program provides a powerful assessment tool for these small urban streams.

Introduction

Increasing human population and urbanization increases stress in freshwater systems including structural changes to habitat, temperature effects from increased runoff and reduced canopy cover, flow changes, and increased toxicants (Konrad and Booth 2005). Physical and chemical changes affect the biota within urban streams at varying scales ranging from individual organisms to populations and communities creating complex interactions that present challenges for characterizing and monitoring impacts on species utilizing these freshwater habitats. Assessing the urban impact from water quality changes is particularly challenging owing in part to the sheer diversity of chemicals in the environment (Hamilton et al. 2004), the differential timing of their entry into waterways (Lee et al. 2002), and technological limitations in quantification (Ellis 2006).

Current stream monitoring programs in the Pacific Northwest emphasize measures of chemical contaminants, benthic invertebrate communities, fish counts, and species assemblages to assess "ecosystem health" (USEPA 2006; Wilmoth et al. 2015; VanMetre, 2015). This assessment stops short of measuring the health or condition of species utilizing these freshwater habitats and fails to link specific stressors mechanistically to the health of organisms in the stream. Without consideration of natural and ecological influences on growth in fish, these monitoring approaches are confined to correlating contaminant measures to species assemblages. Because fish are ectotherms and exhibit indeterminate growth, their growth history integrates the chronology of environmental and ecological conditions experienced within the habitats occupied. Bioenergetics modeling can mechanistically evaluate the relative importance of environmental and ecological influences by accounting for how thermal regime, food supply, and food quality affect fish growth (Benke et al. 1988; Goto and Wallace 2010; Black et al., 2016). By correctly

identifying factors influencing salmonid growth, management needs can be identified and prioritized. Additionally, this modeling framework can evaluate the success of previous management and restoration efforts by comparing fish energetics before and after implemented management strategies.

Juvenile fish growth integrates both external and internal conditions and thus provides a useful indicator of habitat quality and ecosystem health. External conditions governing fish growth include prey quality and quantity (Filbert and Hawkins, 1995; Rosenfeld and Taylor, 2009), thermal regime (McCullough et al., 1999; McCullough et al., 2009; Wenger et al., 2011), and water quality (Buckley et al., 1982; Meador et al., 2006; Baldwin et al., 2009). Allometric relationships and water temperature determine rates of consumption and metabolism for fish of different sizes, and water quality can impose direct physiological consequences ranging from dissolved oxygen demands to contaminant metabolism. Varying availability and energetic quality of prey can limit growth, and these effects are exacerbated by density-dependent competition for limited food resources (e.g., Keeley 2001). Internal conditions governing fish growth include disease and stress response, each with varying ranges of energetic demand.

In the Pacific Northwest salmon are the focus of intense study and interest, owing to their cultural and economic significance. Billions of dollars are spent on salmon recovery, conservation, and research (Lackey 2013). Conservation and restoration efforts in the region seek to improve habitat and reproductive success of salmon. Despite large scale efforts, few direct tools are routinely utilized for monitoring the health of juvenile salmonids with regard to thermal condition, growth, disease prevalence, and toxicant exposure.

Coho salmon (*Oncorhynchus kisutch*) became the focus of this study due to their widespread presence in Pacific Northwest streams. Coho salmon spawn in small- to medium-

sized Puget Sound lowland streams during autumn, and juveniles typically spend one year rearing in these habitats before out-migrating to sea (Sandercock 1991). Fertilized eggs incubate in gravel redds during late fall and winter, juveniles emerge during late winter or early spring, and utilize these small stream habitats for the next year of their life, feeding predominantly on drifting invertebrates while experiencing the ambient thermal regime. Generally, after one year of freshwater rearing, juvenile coho undergo a physiological change known as smoltification and migrate downstream to marine environments.

Growth during early life stages can influence survival in current and subsequent life stages for salmonids (Quinn and Peterson 1996; Beauchamp 2009; Thompson and Beauchamp 2016). Larger juveniles are more likely to survive to the smolt stage (Swales et al. 1988; Quinn and Peterson 1996), making spring and summer growth especially important. Growth integrates the combined effects of food availability, energetic quality of prey in the diet, and the environmental conditions experienced while rearing. Water temperature directly influences metabolic rates (Beauchamp 2009; McCullough et al. 2009), hatching and emergence timing (Murray and McPhail 1988; Beacham and Murray 1990), and ultimately survival when temperature thresholds are exceeded (Richter and Kolmes, 2005). Additionally, prey quality and abundance are positively correlated with growth (Rosenfeld et al. 2005; Wipfli and Baxter 2010). Growth, therefore, serves as an integrator of environmental condition with implications for individual survival to adulthood.

Bioenergetics models can simulate changes in growth and consumption in response to environmental conditions and food availability to explore the interactions between an organism's environmental experience and utilization of available resources. These models use an energy balance equation to describe the basic physiological processes associated with metabolism,

waste, consumption and growth (Ney 1993; Chipps and Wahl 2008; Hartman and Kitchell 2008). The most common application of this model uses measured temperature, diet, and growth in the field to estimate consumption rates. The model estimate of consumption can be compared to theoretical maximum consumption rates for a given body mass and thermal experience of a consumer to derive a feeding rate metric in terms of percent of maximum consumption (%Cmax). Analysis of the environmental conditions that influence %Cmax can identify constraints on fish growth (Hanson et al. 1997; Beauchamp 2009). Bioenergetics models have been corroborated for many species, but are particularly well suited for exploring growth and consumption of salmonids (Chipps & Wahl 2008). Parameterization and corroboration of the model exist for many Pacific Northwest salmonids including coho salmon (Stewart and Ibarra 1991, Ruggerone and Rogers 1992, Brodeur et al. 1992). Rice (1990) called for increased use of the bioenergetics model for quantifying stress response in fish and suggested using energetics to pinpoint the most significant source of stress for individual growth, to investigate stress at a mechanistic level, and to run simulations of different levels of a stressed condition. Beyer et al. (1999a,b) proposed the use of energetics models to predict the effects of toxicant exposure to fish.

This study used a bioenergetics growth modeling approach as one component of a comprehensive fish health assessment as part of the US Geological Survey's (USGS) National Water-Quality Assessment program (NAWQA). Additional elements of this assessment screened for disease and detected impacts from pollution through the use of a toxicogenetic biomarker assay. Fish were sampled in a subset of the streams sampled for the Pacific Northwest Regional Stream Quality Assessment (PNSQA), a region-specific part of NAWQA that assessed water and habitat quality at 88 perennial streams spanning a gradient of urban land use.

We hypothesized that growth and growth efficiency would decline with increasing urbanization. The bioenergetics modeling approach compared juvenile growth and the environmental factors affecting growth among ten coho-bearing streams selected from the initial 15 PNSQA NAWQA streams sampled during 2015. To test this hypothesis, fish growth was compared among streams across an urban gradient. Growth was also modeled for each tributary using observed age, thermal experience, and diet to determine which factors most affected growth. We then considered whether disease, thermal regime, fish density, or prey availability correlated to fish consumption or growth efficiency. Finally, we used diagnostic modeling to compare the per capita growth potential offered by each stream by simulating the growth of individuals over a fixed period, starting at the same initial body size and date, feeding at the same rate, but exposed to the unique thermal regime and diet composition observed from each stream.

Methods

We collected stream-specific data on thermal regimes, seasonal composition and relative biomass of invertebrate drift and corresponding diets, growth, and weights of juvenile (age-0) coho salmon as inputs to bioenergetics model simulations to estimate the consumption and growth efficiency of fish during discrete spring and summer seasons within each stream. Sampled streams spanned an urban gradient determined by the percentage of use within each stream's watershed that was developed (Sheibley et al. 2017, in press). The stream-specific model inputs for temporal changes in temperature, diet composition, associated energy density of prey, individual fish mass and emergence dates are in Appendix Tables A1, A2, and A3. Modeling periods were based on back-calculated days since emergence using otolith-based age

analysis. Modeling and statistical analysis were conducted using R statistical software (R Core Team, 2016), version 3.3.2.

Bioenergetics Model Inputs

Water Temperature

Mean daily water temperatures in each stream were used to represent the thermal experience of juvenile coho. Water temperature loggers (HOBO Water Temp Pro V2) were deployed at all streams, except East Fork Dairy, during the Fall/Winter of 2014 before the June sampling of juveniles to capture their thermal experience from hatching until sampling. Temperature data for East Fork Dairy was supplied by a permanent USGS gaging station (USGS site no. 14205400). The loggers recorded hourly temperatures. Temperature records were complete from April 12, 2015, until sampling in September 2015 for all streams, and from January 1, 2015, for most streams. For Swamp, Woodland, and Longfellow Creek temperature loggers were not deployed in time to capture the thermal experience during the first 1-6 weeks after emergence (based on the estimated emergence dates from otolith aging for individual fish). At these streams, early temperature values were modeled from near-by streams using a simple linear regression approach. These nearby streams were chosen based on similar habitat, elevation, size and geographical location. Temperatures from nearby streams were compared to confirm that they closely followed each other during dates when concurrent temperature data were recorded at both locations. Swamp Creek temperature predictions used Jenkins Creek data (USGS site no. 12110495); Woodland Creek temperature predictions used Crescent Creek data (USGS site no. 12072681), and Longfellow Creek temperature predictions used Miller Creek data (King Co site no. 42a).

All temperature data were edited for anomalous values and spurious spikes. Temperature calculations for bioenergetics modeling were based on mean daily temperatures derived from

hourly temperatures recorded over each 24-hour period and calculated using the HOBO data software (HOBOware pro v. 3.4) (Table A1). Additionally, degree day (DD) accumulations were calculated as the sum of mean daily temperatures over a variety of periods of interest including: 15-, 30-, 45-, and 60- days prior to fish sampling, from the stream-specific average emergence date to sampling date, and for a period from March 15th -June 30th for growth comparison modeling.

Drift Sampling

Invertebrate drift sampling occurred during the morning or middle of the day when invertebrate drift is less variable (Wall et al. 2015). Drift was collected three times during the growing season: during late June/early July, mid-August, and during the final fish collection in Late-September. All 15 streams were sampled in June, and only the six long-term streams in mid- and late-summer. Drift sampling was conducted in each stream using three 250 μm mesh nets with 30.5 cm x 30.5 cm opening. Nets were placed side-by-side in the thalweg of the river so that the opening was at least 2.54 cm above the water, to collect floating adult and terrestrial insects. Water velocity was measured in front of each of the three nets at a point roughly centered in front of the net using a Marsh-McBirney velocity meter both at initial deployment and when the nets were pulled. Deployment time varied, averaging around 3 hours during the June deployments and 1 hour during both August and September, as they appeared to be capturing sufficient mass in the shorter deployment time (Table 1). Drift nets were carefully placed upstream of all other activities and were intended to target an "undisturbed" section of the stream. Once nets were pulled, deployment time was recorded, and the collected drift was washed down to the cod-end of each net, placed into separate jars for each net, and preserved in 95% ethanol.

Drift samples were processed for all ten streams where coho were present during the early sampling, and twice more for the six streams sampled in late season. In all, 22 composite drift samples were processed. Each sample was separated by sieving into two size fractions, 500-1000 μm, and >1000 μm. Drift <500 μm was not analyzed. Invertebrates were separated from debris using dissection microscopes and sorted down to the order level. Both counts and blotted wet weights were obtained for each order. This was further condensed into nine major energetic categories based on work of McCarthy et al. (2009).

Diet Processing

Diets were analyzed to understand the quality of prey consumed by juvenile coho. Diets were analyzed for all coho used for both necropsy and genetic analysis. Stomach contents were identified using a dissecting microscope and sorted according to the same energetic categories as for the drift samples. Stomachs were removed under a dissection scope and contents were sorted based on digested and undigested material. Undigested invertebrates were identified at the order level and blotted-wet weights were recorded to the nearest 0.00001 g from each individual stomach. The proportion of each energetic category in each stomach was calculated for use as the diet proportion inputs for the bioenergetics simulations(Table A2), and in tandem with the concurrent drift proportions, was used to determine whether fish were selectively feeding.

Fish Energetic Content

Predator energy density inputs for the model were developed empirically from juvenile fish sampled in this study. The relationship between whole body wet weights and energy density (J/g) was determined by measuring the energy density of a subset of juvenile fish with a Parr Semi-micro Oxygen Bomb Calorimeter (Model no. 1425). We used 1-3 whole body samples of coho salmon that were collected from each stream at the same time as the necropsy samples.

Methods for energy density determination followed that of Trudel et al. (2005) adapted for whole bodied juvenile fish. Whole body coho from each stream were individually dried for three days at 60° C. Fish were considered dry when they lost less than 1% of weight over a 24-hour period. Dried samples were ground and homogenized using a stainless steel mortar and pestle. This homogenate was pressed into a 0.16-0.22 g pellet and combusted in the calorimeter. Energy density (Calories/g dry weight) for each pellet was calculated by the instrument using a calibration curve developed from benzoic acid standards per manufacturer protocols. This was converted to J/g dry weight and multiplied by the conversion ratio of dry to wet weight to determine the wet weight energy density (J/g wet weight) of each fish. Simple linear regression analyses were run both seasonally and for all samples together (n = 27) to determine the model of fish wet weight-to-energy density. Regression equations were not significantly different between seasons ($p > 0.05$) and the resulting regression of ED (Energy Density in J/g wet weight) as a function of WW (fish wet weight in g) was significantly and positively correlated (Figure 1; $r^2 = 0.4531$, n = 27, $p < 0.05$):

$$ED = 3814 + 239\,(WW);$$

Otolith Analysis and Fish Age Structure

Otolith deposition in juvenile salmonids occurs on a daily time-step creating visible rings with each deposit (Stevenson and Campana 1992). Extracted otoliths were cleaned with 100% ethanol to remove any organic tissue and then kept dry until analysis. A total of 138 coho otolith pairs were submitted for analysis with 92 pairs from early season and 46 from late season; 127 of these were successfully aged. Otolith microstructure analysis followed the methods of Stevenson and Campana (1992). The left otolith from each fish was first mounted in clear epoxy resin and

prepared for imaging with polishing clothes and sequentially smaller sized slurries of Silicon Carbide and Aluminum Oxide. Polished otoliths were imaged and analyzed using Image-Pro software. A standard radial axis for analysis was used for all otoliths. Checks, prominent marks indicative of early life history events such as emergence or each fish's first feeding event, were identified and increments both counted and measured along the axis from the outer edge of the otolith to these check. Each increment was counted as a day, and emergence dates calculated as the sampling day minus the number of increments between the outer edge of the otolith and the check for first feeding. Differences in emergence timing among streams were considered using an analysis of variance (ANOVA). Additionally, a simple multiple-linear regression analysis was conducted to see if age was a consistent predictor of weight and if there were seasonal differences in this relationship.

Bioenergetics modeling

Bioenergetics modeling utilized the Wisconsin Bioenergetics model (Hanson et al. 1997) parameterized for coho salmon (Stewart and Ibarra 1991). The bioenergetics model takes two forms:

1) Consumption = Growth – Respiration – Waste - Metabolism
2) Growth = Consumption – Respiration – Waste - Metabolism

Here we used growth to predict consumption rates (%Cmax). Inputs for the model include the thermal experience of the consumer on a daily time-step (Table A1), prey proportions in the diet by mass with the corresponding prey energy density (Table A2), predator energy density (equation provided above), and individual growth of the consumer (Table A3). The first form of

the equation requires the initial and final wet weight of fish during the simulation interval to predict consumption. Growth efficiency values (%GE) for all model runs were calculated as:

$$\%GE=\text{Growth (g)}/\text{Consumption (g)}*100$$

Bioenergetics modeling was used in two ways:

1) Model individual %Cmax for each sampled fish based on their specific age, diet and thermal experience ("fitted consumption simulations") and calculate %GE.

2) Using the average %Cmax fitted to observed growth in the simulations described above, simulate growth outcomes for each stream based on observed prey and thermal experience ("diagnostic modeling") over a standard period and consumption rate for each stream.

Fitted Consumption Simulations

Fitted consumption modeling was used to estimate the average feeding rate (%Cmax) and total food consumption required to satisfy the observed growth rate of juvenile coho from each stream, providing a baseline average feeding response to use in diagnostic modeling. Calculated %GE and %Cmax were then correlated with measured environmental variables using a non-parametric Spearman rank correlation to examine which environmental variables affected consumption and growth.

Consumption rates were fitted to observed growth rates for all individuals sampled in the streams (Table 2). The starting day of each simulation was unique to each individual and coincided with their respective estimated emergence date (Table A3) calculated from the otolith

analysis. The model estimated consumption rates, and growth efficiency was calculated from model output. Consumption and growth performance (%GE) were compared among streams using a Non-parametric Kruskal-Wallis rank test (α=0.05), and Tukey posthoc pair-wise comparison tests (α=0.05).

Correlation with Environmental and Individual Metrics

Pearson correlations and significance were determined for environmental and individual fish metrics for each sampling season to identify factors influencing %Cmax and %GE. Environmental variables included: degree days (DDs) (from emergence, 15- days prior to capture), individual emergence date, drift density calculated from drift sampling, the level of urbanization (% area), and fish density (coho catch, salmonid catch, and all-fish catch within standard sampling reach). Individual metrics included: fish weight (g), age (days), and Health Assessment Index (HAI score). In addition, correlations among metrics were considered in the analysis.

Diagnostic Bioenergetics Simulation

Diagnostic simulation modeling was used to answer the question: When only considering temperature and diet quality, in which stream do fish grow the most given the same consumption rate, initial body mass, and period of time? For this modeling, the %Cmax was set the same for each stream. A standard period was used for each sampling cohort based on average emergence date for all fish from each sampling period. Simulations were run for all streams with relevant sampling data for both early emergence cohorts (March 23 to June 30, 2015) and late emergence cohorts (May 12 to September 15, 2015).All diagnostic simulations used a starting emergence weight of 0.2 g (Quinn, personal communication) and consumption rate of 35% Cmax, which was the average feeding rate across all streams estimated from the earlier simulations that fitted

consumption to observed growth. Growth efficiency (%GE) was calculated for each stream using model outputs for the estimated mass of food consumed and gain in body mass over the simulation period.

Results

Bioenergetics Model Inputs

Water Temperature

Maximum temperatures were generally higher in streams with higher urban land use (Table 3), when considering streams in the Puget Sound region. Thermal experience for fish ranged from 6.1 °C to 19.1 °C. Simple linear regression equations were highly predictive of missing temperatures for Swamp ($r^2 = 0.96$), Woodland (adj. $r^2 = 0.62$), and Longfellow creeks (adj. $r^2 = 0.97$). It should be noted that in all cases some temperature predictions were estimated by extrapolating outside the observed temperature values on which the regression models were based.

Drift and Diet Sampling

Drift proportions were variable among streams, though no clear relationship was apparent between percent urbanization and biovolume or energy density of the drift (Table 1). Energy delivery in streams (J/hr) varied widely among streams and within the same stream among sampling periods (Table 1). Energy delivery of drift was lower during August and September than during early season (when comparing streams sampled during all three periods), except for Coulter and Swamp Creek. Drift energy delivery ranged from 31 J/hr (Woodland Creek) to 1127 J/hr (Issaquah Creek) in June/July; from 32 J/hr (Harris Creek) to 1018 J/hr (Issaquah Creek) in August; and 38 J/hr (May Creek) to 462 (Issaquah Creek) in September.

Average diet quality in coho varied by as much as 2033 J in June/July and 1651 J in September among streams. The average energy density of diets was lower during late season than during early season (when comparing streams sampled during all three periods), Figure 2. The energy densities of prey in the diets were significantly higher overall (t-test, $p<0.05$) than the concurrently-sampled energy density of the edible-sized drift. Additionally, the composite energy density of diets were higher than drift samples in 12 out of 16 concurrent samples (Figure 2), suggesting that drift feeding coho are selecting for higher density prey when possible.

Otolith Analysis and Fish Age Structure

For the earlier emerging cohorts, coho emergence dates spanned 75 days across all streams with the earliest fish emerging on 2/13/15 (Longfellow Creek) and the latest on 4/29/15 (Coulter Creek). The average emergence date for all streams was 3/23/15, which was then used for the diagnostic simulation modeling below. Average emergence dates for each stream varied significantly among streams (ANOVA, $P < 0.05$). Variance within a stream was as high as 46 days, Woodland Creek.

For the late-emerging cohorts, coho emergences dates across all streams spanned 77 days for these fish with the earliest emerging on 4/5/15 (Swamp Creek) and the latest on 6/21/15 (Harris Creek). The average emergence date for all late season streams was 5/12/15, which was also used as a starting date for simulation modeling. The emergence dates within a stream varied as much as 48 days, Coulter Creek. Average emergence dates among streams were significantly different (ANOVA, $p < 0.05$).

Juvenile coho salmon that were sampled in the streams in June emerged significantly earlier than fish sampled from the same streams in September (ANOVA, $p < 0.05$, Table 3).

Average emergence shifted by 45 days from 3/27/15 (range 2/26/15-4/29/15) for juveniles sampled in June to 5/12/15 (range 4/5/15-6/21/15) for juveniles sampled in September. The large variability from early to late season drove the decision to model early and late emerging fish separately. The earlier emerging cohorts likely had moved out of the sampled habitats between our June and September sampling periods. Overall, fish age was a reasonable predictor of fish weight (r^2= 0.49); although the relationship was much stronger when considering seasons separately (r^2= 0.71) (Figure 3). Seasonal differences in growth suggest different growing conditions between seasons. Variance in these relationships suggested different growing conditions among streams.

Fitted Consumption Bioenergetics Models

From early season sampling, streams differed significantly in terms of feeding rate, final body mass, and growth efficiency (Figure 4). The two most urbanized streams, Swamp and Longfellow, had the earliest emergence dates and held the largest juvenile coho salmon, but also required the highest consumption rates, but lowest growth efficiency (Figure 4). There were five levels of significantly different growth efficiencies across the ten streams sampled (Tukey posthoc, $p<0.05$): Coulter and East Fork Dairy Creeks, both reference streams, had the highest %GE at a mean of 33.2% and 31.2%; Swamp and Longfellow Creeks, urban sites, had the lowest %GE at a mean of 16.6% and 24.1%.

During late season consumption rates spanned a similar range when compared to the early season sampling though %GE were generally lower (Figure 4). Both %Cmax and %GE were significantly different among streams (Tukey post hoc, $p<0.05$). Among late season streams %Cmax was highest in Swamp Creek at 41.7% and lowest in Harris creek at 29.1%. Swamp and Harris Creek had the lowest %GE at 16.7% and 16.9%, while Coulter had the highest at 25.3%.

Correlations

Growth efficiency was correlated with fish age, urbanization, and stream temperature whereas %Cmax was poorly correlated with all environmental variables except prey energy density in early season (Table 4); in late season (Table 5) correlations with %Cmax and %GE were not significant. During the early season, %GE was negatively correlated with average fish age, %Urbanization, and average DDs experience for fish from each stream during early season and all were significant (Spearman rank, $p<0.05$). In late season %Urbanization and degree days accumulated for the 15 days prior to catch were negatively correlated, though not significantly. In both early and late season, %Cmax negatively correlated with prey energy density, though only early season was significant (Spearman rank, $p<0.05$).

Individual metrics that correlated with %Cmax and %GE differed between early and late seasonal cohorts. Significant correlations with %Cmax were positive with age and weight during the early and late seasons, and negative with %GE and HAI score during the early season only. In contrast, %GE was significantly negatively correlated age and weight for the early season cohort, but not the late cohort. This suggests differential pressures on growth efficiency between spring and summer.

Diagnostic Growth Bioenergetics Modeling

Diagnostic simulations indicated considerable variability among streams in the amount of growth and growth efficiency associated with a standard consumption rate of 35% over specified periods for early and late emerging cohorts, given the observed thermal regimes, diets and corresponding composite energy densities for each stream and simulation period (Table 6 and Figure 5). Early emerging cohorts consistently experienced higher growth efficiency than late emerging cohorts from the same stream. When combined with sampling season as a factor,

percent urban land use within each watershed was a strong predictor of DDs experienced by fish during diagnostic simulation periods (r^2= 0.935; Figure 6a). DD accumulation was higher in the late season cohort evident by the higher intercept in regression analysis. The level of urbanization was also negatively correlated with growth efficiency with significantly lower expected growth efficiency during summer than the cooler spring simulations ($p<0.05$; Figure 6b).

Discussion

Juvenile coho grew less efficiently in the most urbanized of the small perennial streams sampled, but these streams also contained the largest fish. This was contrary to the hypothesis that anthropogenic influences in urban streams would result in lower growth. These apparently contradicting findings were explained by earlier emergence in warm urbanized streams and higher consumption rates which allowed for a longer period of growth and the greater food intake required to overcome the lower growth efficiency in order to achieve larger sizes. Additionally, emergence dates were highly variable both within and among streams and highly correlated with urbanization. This variability has interesting consequences for both growth, shown here, and potential implications for subsequent life history stages.

This study showed that degree day accumulation was higher in streams in urbanized watersheds during both spring and summer. Temperatures in urban watersheds in the region are impacted by urban landuse (Sun et al. 2015). The higher temperatures observed in our study potentially lead to changes in individual metabolic rate (Lee et al. 2003) and stream invertebrate communities (Sponseller et al. 2001). Reduced growth efficiencies in urbanized streams in the early season and across the remainder of the streams during late season are likely partially attributable to increased temperatures as evident from high negative correlations. Fish from these

streams experienced various periods when ambient temperatures would depress growth under average feeding rates. Though these same higher temperatures in the earlier part of the spring would mean higher growth than in cooler streams. The diagnostic simulations, which restricted impacts on growth to the effects of prey energy density and temperature, confirmed less growth in Swamp and Longfellow Creek given the same amount of feeding effort among streams. Furthermore, this lower growth efficiency was inversely correlated with temperature metrics.

Although fish had to work harder in urban streams to achieve the same amount of growth, they were larger. Their larger size was attributable to earlier emergence times the year, and thus longer growing periods. Previous work has shown emergence timing is dependent on temperature during incubation (Steel et al. 2012). Within stream variation in emergence timing was larger than expected. Emergence dates for coho from the same stream, when considering fish from both sampling periods, spanned as much as three months. As our study showed, earlier emergence afforded a longer period of access to food and thus growth. This could be particularly important, as larger coho have a greater chance of over winter survival (Quinn and Peterson 1996). Kahler et al. (2001) reported that some coho salmon migrate upstream after emergence. While emigration from the initial rearing area might explain the large shift to later emergence dates observed in fish sampled later in the season at the same location, some other size-or time-selective process like predation or flow induced displacement are also possible.

Previous work by (Weber et al. 2014) concluded that trout consumption rates could be predicted by invertebrate drift density. We did not observe this in our study. Drift density was poorly correlated with consumption rates for both early and late season cohorts. It could be that consumption rates in these small streams were influenced by more interacting factors then can be modeled with their simple regression approach. Flow over the summer in Harris Creek, for

example, was reduced and ponded in sections, limiting the access to drift not easily characterized by our short drift sampling period. Additionally, increasing fish densities can depress prey availability in small streams (Rosenfeld et al. 2005). As a monitoring tool, it would be useful to predict consumption rates and subsequent growth capacity in a stream as suggested by (Weber et al. 2014), but more work is needed to determine the appropriate temporal and spatial dimensions for drift sampling that can account for the variability in these small perennial streams. However, consumption rates in our study did correlate negatively with prey energy in diets, suggesting that fish increased consumption to make up for low energy food. This could provide a potential predictor of fish consumption.

Summary

The successful use of bioenergetics modeling to characterize salmonid growth in small perennial streams as part of a monitoring program provides a useful tool to determine which streams have a reduced capacity for salmonid growth and identify which environmental factors are limiting. Our study highlights the importance of age, temperature, and prey quality on growth for juvenile salmonids. For urban streams, we show mixed effects, whereby urban streams tended to be warmer, have earlier emergence dates and stronger early season growth. However, depressed growth efficiency suggested that larger fish could be under increased stress, especially later in the summer, when compared to fish from other streams. This relationship between thermal experience and altered life history is ripe for continued research to connect subsequent impacts to both growth and predation pressure.

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Tables

Table 1. Summary of invertebrate drift biomass measured for each stream and sampling dates for all invertebrates retained after filtering the sample through 500-micron mesh (All drift weight (g)) and 1-mm mesh (Drift weight > 1mm (g)). The mean delivery rate of invertebrates to each drift sampler is reported in terms of g/h and J/h and is considered an index of foraging opportunity for drift-feeding fish.

Stream	Sampling date	Start time	End time	Hours	Average sampling rate (cfs)	All drift weight (g)	Drift weight >1mm (g)	G/hr	J/hr
June sampling									
EF Dairy	6/30/15	1056	1500	4.1	1.93	0.83	0.286	0.20	733
Coulter	6/23/15	930	1435	5.1	0.77	0.17	0.120	0.03	119
Issaquah	6/15/15	955	1240	2.8	0.99	0.81	0.497	0.29	1127
Harris	6/22/15	829	1330	5.0	0.45	0.39	0.293	0.08	348
Church	6/19/15	900	1300	4.0	0.30	0.22	0.129	0.06	208
May	7/14/15	1400	1450	0.8	0.49	0.15	0.133	0.18	945
Jenkins	6/24/15	1158	1630	4.5	1.17	0.70	0.320	0.15	491
Woodland	6/16/15	900	1600	7.0	0.73	0.05	0.013	0.01	31
Swamp	7/09/15	1050	1430	3.7	0.75	0.08	0.046	0.02	72
Longfellow	6/21/15	846	1235	3.8	0.19	0.52	0.389	0.14	500
August sampling									
Coulter	8/22/15	1610	1647	0.62	1.75	0.06	0.049	0.10	378
Issaquah	8/26/15	940	1025	0.75	0.99	0.16	0.144	0.21	1018
Harris	8/25/15	1225	1325	1.00	0.11	0.01	0.004	0.01	32
May	8/26/15	1125	1210	0.75	0.38	0.01	0.012	0.01	37
Jenkins	8/25/15	1530	1615	0.75	0.62	0.06	0.045	0.08	269
Swamp	8/25/15	1005	1050	0.75	0.50	0.14	0.105	0.19	783
September sampling									
Coulter	9/29/15	1410	1514	1.07	1.86	0.11	0.053	0.10	353
Issaquah	9/23/15	1158	1258	1.00	1.21	0.12	0.072	0.12	462
Harris	9/21/15	1225	1325	1.00	0.27	0.06	0.005	0.06	214
May	9/22/15	1323	1428	1.08	0.66	0.01	0.040	0.01	38
Jenkins	9/20/15	1235	1335	1.00	0.66	0.04	0.016	0.04	142
Swamp	9/17/15	1448	1548	1.00	0.37	0.02	0.005	0.02	73

Table 2. Fitted consumption bioenergetics modeling summary. Growth simulations run for each individual fish starting on their respective day of emergence through the day they were sampled. Initial body mass was assumed to be 0.2g at emergence. Averages are calculated from model outputs for all individuals from a specific stream.

STREAM	Avg. emergence date	N	Avg final weight (g)	Avg prey energy density[1] (J)	Avg. Degree days	Avg. %Cmax	Avg prey consumed (g)	Growth efficiency (%)
Early season (June/July)								
EF Dairy	3/20/15 (14.3)	9	4.5 (1.4)	5505	1207	31.0 (2.1)	13.9 (4.5)	31.2 (0.8)
Coulter	4/04/15 (12.1)	10	2.3 (1.1)	3892	864	36.4 (4.5)	6.2 (2.9)	33.2 (1.9)
Issaquah	3/18/15 (13.5)	10	2.7 (.8)	4291	984	34.5 (1.7)	9.1 (3.1)	27.2 (0.7)
Harris	4/06/15 (14.8)	9	1.7 (1.0)	5269	904	24.9 (3.7)	4.9 (2.9)	31.0 (3.3)
Church	4/06/15 (8.12)	10	1.8 (.3)	4912	901	28.9 (1.6)	5.4 (1.6)	30.5 (1.3)
May	3/14/15	3	3.8 (0.4)	5495	1572	26.7 (0.8)	13.5 (1.4)	26.6 (0.9)
Jenkins	3/28/15 (11.3)	10	2.2 (.7)	4658	990	31.0 (2.3)	7.2 (2.2)	27.9 (1.3)
Woodland	3/15/15 (17.4)	10	3.6 (1.6)	4693	1272	31.5 (3.3)	12.4 (6.1)	27.4 (1.4)
Swamp	3/18/15 (8.5)	7	6.1 (1.4)	3472	1513	51.5 (4.1)	36.5 (13.3)	16.6 (1.9)
Longfellow	2/28/15 (9.0)	10	8.1 (2.2)	4374	1391	44.7 (3.9)	32.9 (9.8)	24.1 (0.6)
Late season (September)								
Coulter	5/26/15 (15.5)	6	3.68 (1.54)	4252	1572	31.3 (2.6)	13.8 (6.3)	25.3 (1.0)
Issaquah	5/05/15 (10.9)	4	5.55 (1.55)	4197	1994	36.8 (3.2)	27.3 (10.1)	19.4 (0.4)
Harris	5/31/15 (16.6)	6	2.57 (1.93)	4435	1559	29.1 (4.5)	13.4 (9.4)	16.6 (2.7)
May	5/03/15 (13.3)	6	5.49 (1.20)	5314	2052	29.3 (0.8)	22.5 (7.0)	23.7 (0.9)
Jenkins	5/12/15 (15.8)	4	5.58 (2.61)	4591	2000	34.2 (2.1)	20.3 (5.0)	20.2 (1.8)
Swamp	4/08/15 (9.2)	4	5.87 (0.68)	3709	2382	41.7 (2.1)	34.5 (7.6)	16.7 (0.7)

[1] Avg. prey energy density: Calculated based on the average of the daily energy density value of diets over the period defined by the average emergence date through sampling date.

Table 3. Summary of emergence dates, sampling dates, and stream temperature metrics. Degree days were calculated without using a base temperature, as suggested by Chezik et al. 2015 and are for the period specified.

Stream	% Urban	Avg. Emergence date	Fish sampling date	Period min temp (°c)	Period max temp (°c)	DD[1]_15	DD[1]_30	DD[1]_45	Growth_DD[2]	Simulation_DD[3]
Early season sampling (June)										
EF dairy	2.4	3/20/15	6/30/15	7.4	18.6	242	460	650	1207	1197
Coulter	4.0	4/04/15	6/23/15	8.0	13.1	186	362	529	864	1084
Issaquah	15.1	3/18/15	6/15/15	7.8	15.3	208	399	567	984	1178
Harris	16.8	4/06/15	6/22/15	8.3	14.3	196	393	578	904	1164
Church	24.4	4/06/15	6/19/15	8.3	15.7	217	421	603	901	1236
May	50.9	3/14/15	7/15/15	9.1	16.7	239	462	675	1572	1257
Jenkins	65.8	3/28/15	6/16/15	9.4	16.4	219	423	606	990	1281
Woodland	75.5	3/15/15	6/24/15	11.1	13.7	196	394	589	1272	1269
Swamp	89.8	3/18/15	7/9/15	9.2	18.1	259	488	713	1513	1320
Longfellow	93.0	2/28/15	6/21/15	6.1	17.3	243	467	669	1391	1349
Late season sampling (September)										
Coulter	4.0	5/26/15	9/29/15	9.8	14.2	166	343	530	1572	1586
Issaquah	15.1	5/5/15	9/24/15	10.0	16.9	191	392	613	1994	1816
Harris	16.8	5/31/15	9/24/15	11.4	14.9	195	395	602	1559	1698
May	50.9	5/3/15	9/23/15	10.7	16.7	201	413	638	2052	1854
Jenkins	65.8	5/12/15	9/21/15	12.0	19.1	212	428	669	2000	1931
Swamp	89.8	4/8/15	9/17/15	9.2	18.1	213	445	687	2382	1974

[1]DD_: Degree days calculated based on the number of days specified prior to sampling date.
[2]Growth_DD: Degree days calculated from date of average emergence to sampling date.
[3]Simulation_DD: Degree days calculated from 3/12/15 to 6/30/15; the period used for bioenergetics simulation modeling

Table 4. Bioenergetics Spearman rank correlation matrices for early season. Stream based metrics include age, land use, prey energy, degree days, and fish counts (upper table). Individual metrics include age, weight, and HAI score (lower table). Values in bold indicate significance ($p<0.05$).

	%Cmax_Avg	%GE_Avg	Avg_Age	%Urban	PreyE	DD_15	DD_all	#Coho	#Salmonids	#Fish
%GE_Avg	-0.43									
Avg_Age	0.52	**-0.73**								
%Urban	0.39	**-0.79**	0.52							
PreyE	**-0.87**	0.35	-0.18	-0.32						
DD_15	0.25	-0.61	**0.73**	0.44	0.02					
DD_all	0.19	**-0.78**	**0.90**	0.62	0.09	**0.70**				
#Coho	-0.16	0.71	**-0.89**	-0.47	-0.19	**-0.75**	**-0.94**			
#Salmonids	-0.33	0.45	-0.52	-0.53	0.02	-0.45	-0.52	0.55		
#Fish	-0.19	0.27	0.07	**-0.74**	0.21	-0.15	-0.03	-0.05	0.34	
Drift_density	-0.01	-0.33	0.13	0.15	0.08	-0.04	0.16	-0.35	-0.04	-0.07

	%Cmax	%GE	Age	Weight
%GE	**-0.36**			
Age	**0.53**	**-0.54**		
Weight	**0.70**	**-0.42**	**0.91**	
HAI	**-0.22**	0.15	-0.07	-0.14

%Cmax_Avg: Average %Cmax from fitted consumption model for each stream.
%GE_Avg: Average %GE from fitted consumption model for each stream.
Avg_Age: average age of fish from each stream.
%Urban: % land area within watershed that is developed for "urban" (non-agricultural) use
PreyE: Average prey energy density for each stream during the standard period used for diagnostic modeling
DD_15: Accumulated degree days
DD_all: Accumulated degree days for each stream calculated from average fish age for each stream.
#Coho: Number of coho found in survey reach
#Salmonids_count: Number of salmonids found in survey reach
#Fish: Number of fish found in survey reach
Drift_density: amount of drift/hr (g/hr)
%Cmax: %Cmax from fitted consumption model for each coho.
%GE: %GE from fitted consumption model for each coho.
Age: Age of fish calculated based on emergence date determined from otolith.
Weight: Wet weight of individual coho.
HAI: HAI score for each individual.

Table 5. Bioenergetics Spearman rank correlation matrices for late season. Stream based metrics include age, land use, prey energy, degree days, and fish counts (upper table). Individual metrics include age, weight, and HAI score (lower table). Values in bold indicate significance ($p<0.05$).

	%Cmax	%GE	Avg_Age	%Urban	PreyE	DD_15
%GE	-0.37					
Avg_Age	0.66	-0.26				
%Urban	0.37	-0.54	0.60			
PreyE	-0.71	0.54	-0.26	0.03		
DD_15	0.37	-0.54	0.60	**1.00**	0.03	
DD_all	0.60	-0.20	**0.94**	0.77	-0.09	0.77

	%Cmax	%GE	Age	Weight
%GE	-0.25			
Age	**0.54**	-0.22		
Weight	**0.60**	0.07	**0.82**	
HAI	0.26	-0.07	0.08	-0.08

%Cmax_Avg: Average %Cmax from fitted consumption model for each stream.
%GE_Avg: Average %GE from fitted consumption model for each stream.
Avg_Age: average age of fish from each stream.
%Urban: % land area within watershed that is developed for "urban" (non-agricultural) use
PreyE: Average prey energy density for each stream during the standard period used for diagnostic modeling
DD_15: Accumulated degree days
DD_all: Accumulated degree days for each stream calculated from average fish age for each stream.
%Cmax: %Cmax from fitted consumption model for each coho.
%GE: %GE from fitted consumption model for each coho.
Age: Age of fish calculated based on emergence date determined from otolith.
Weight: Wet weight of individual coho.
HAI: HAI score for each individual.

Table 6. Diagnostic bioenergetics model results. Simulation was based on two periods, early and late season, based on average emergence timing for each sampled cohort. Fish simulated had a starting weight of 0.2 g and a %Cmax of 35% (average consumption rate for all fish from both periods).

Stream	Avg. prey energy density[1]	Degree days	Growth (g)	Growth efficiency (%)
Spring simulation period: 3/23/15 – 6/30/15				
EF Dairy	5505	1197	9.48	40.8
Coulter	3892	1084	4.12	31.9
Issaquah	4291	1178	5.07	32.4
Harris	5269	1164	9.58	41.2
Church	4912	1236	7.52	36.3
May	5495	1257	10.50	40.3
Woodland	4693	1269	7.24	36.2
Jenkins	4658	1281	6.60	33.4
Swamp	3472	1320	2.41	21.8
Longfellow	4374	1349	4.85	28.4
Summer simulation period: 5/12/15 – 9/15/15				
Coulter	4164	1586	32.4	8.05
Issaquah	4206	1816	25.5	6.33
Harris	4641	1698	25.3	9.08
May	5357	1854	31.4	12.98
Jenkins	4599	1931	26.8	7.58
Swamp	3759	1974	25.8	4.74

[1] Avg. prey energy density: Calculated based on the average of the daily energy density value of diets over the period.

Figures

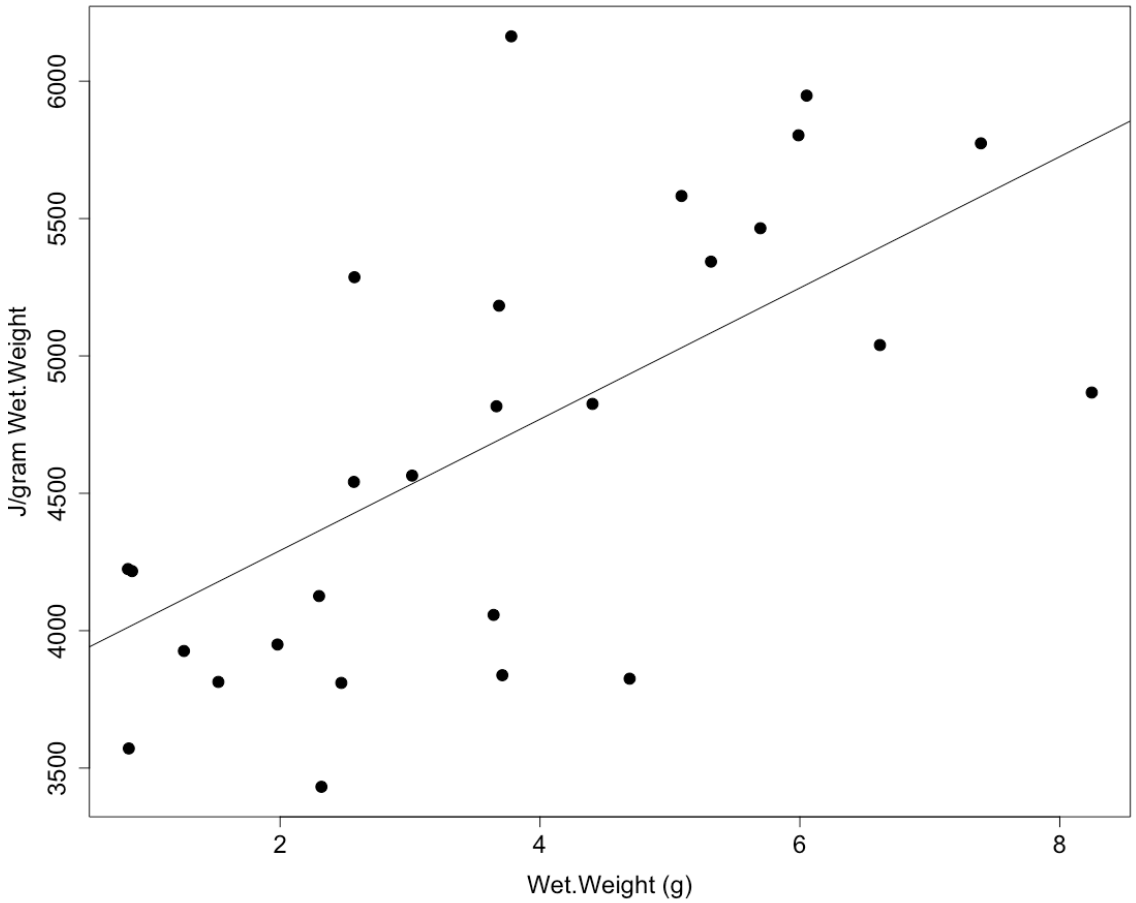

Figure 1. Predator Energy density regression. Simple linear regression was used to develop a predator energy density equation for the bioenergetics model using the measured wet-weight of sampled coho and their energy density from bomb calorimetry (n=23).

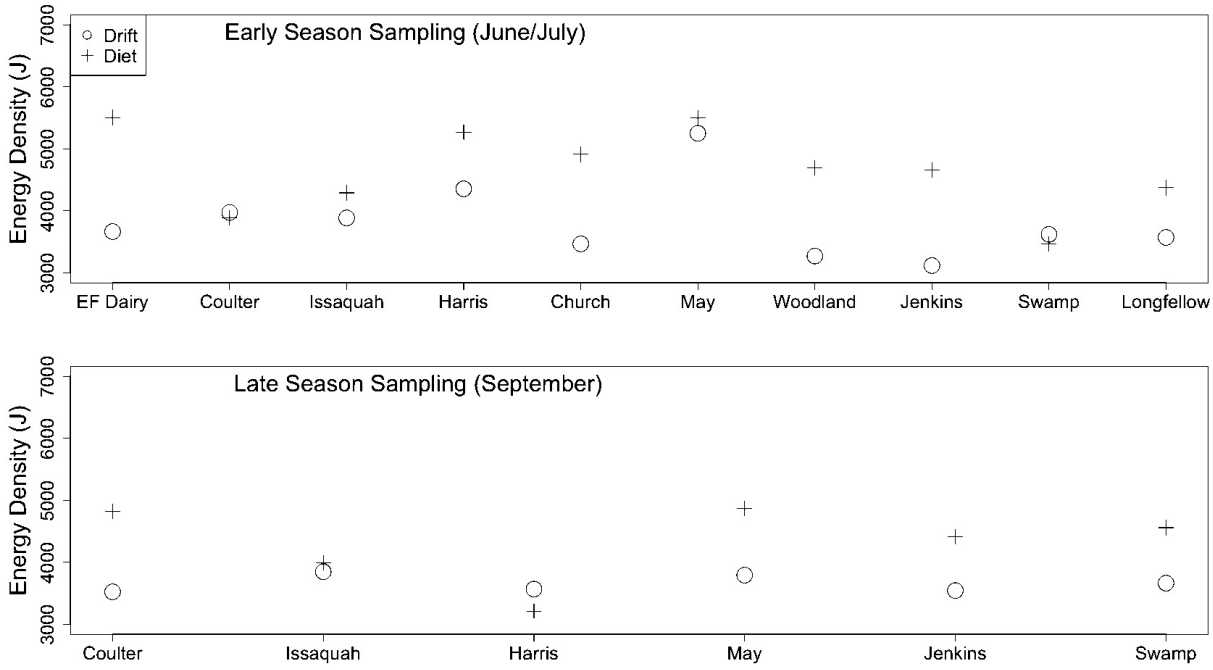

Figure 2. Average energy density of both diet and drift samples measured on concurrent days. Averaged energy density values for drift include all invertebrates retained by a 500um sie**ve.** Diet energy density values are calculated from pooled diet samples from all sampled coho from a specific stream.

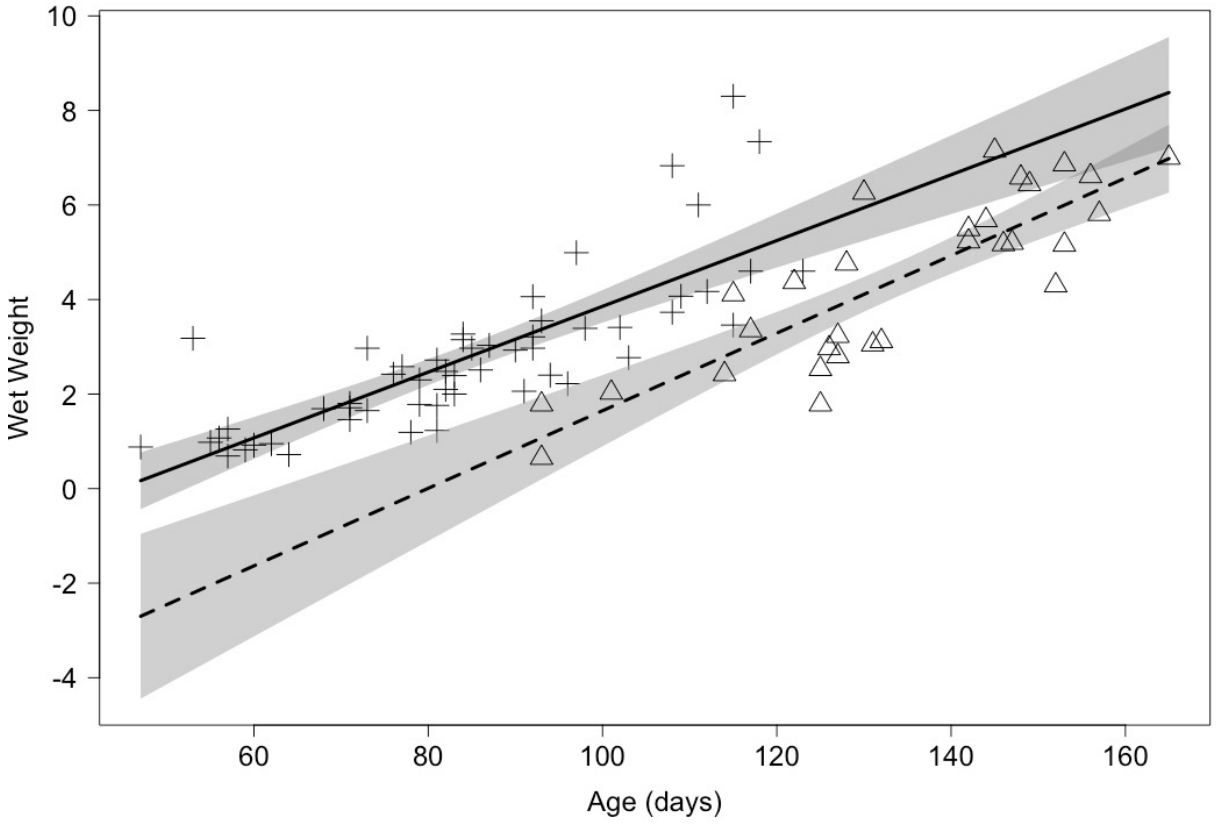

Figure 3. Regression of fish age to wet weight (adj. $r^2 = 0.71$) for early and late sampling season. "Age" is defined as days since emergence until the day fish were sampled. Seasons are significantly different (p-value=3.01e-12). Early season coho denoted by plus-symbol and solid line; late season coho denoted by triangles and dotted-line.

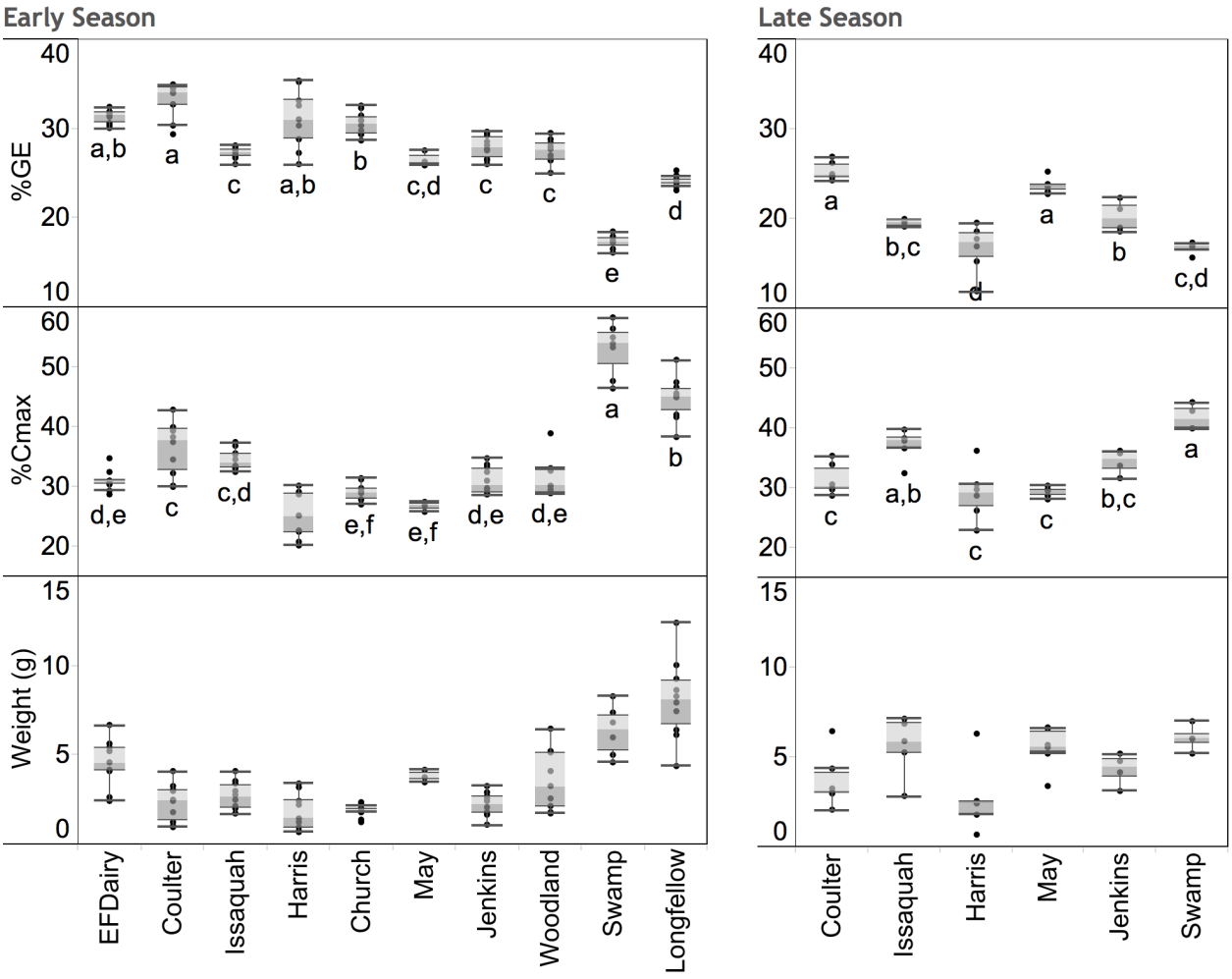

Figure 4. Boxplot of calculated growth efficiency, fitted consumption rate, and total fish growth for individual coho from fitted-bioenergetics modeling of fish from each stream. Letters indicate significant difference among streams (posthoc Tukey multiple pairwise comparison test, α=0.05).

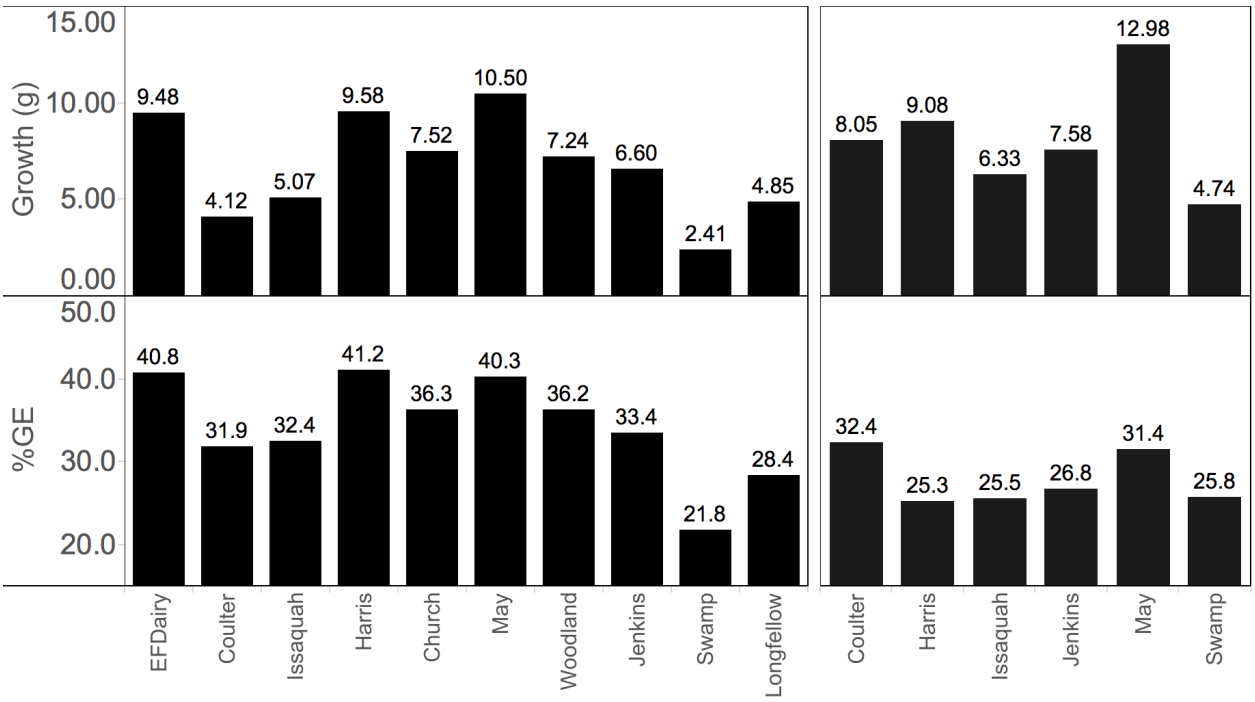

Figure 5. Diagnostic modeling output. Calculated growth efficiency (bottom panel) and total amount of growth (top panel) when feeding at 35% of maximum consumption in each stream during a standard period. Early season 3/23/15 – 6/30/15; late season 5/12/15 – 9/15/15.

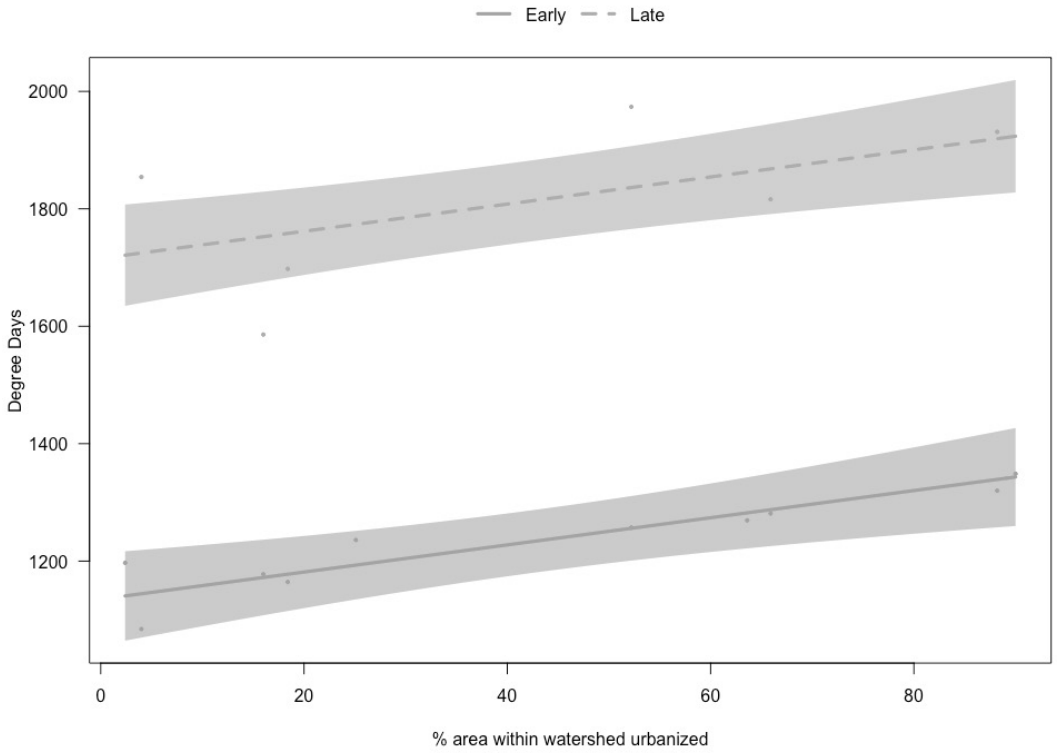

Figure 6a. Relationship between urbanization and degree days over simulation model periods. Both season and percent urbanization are significant terms in the regression model; adj. r^2=0.935.

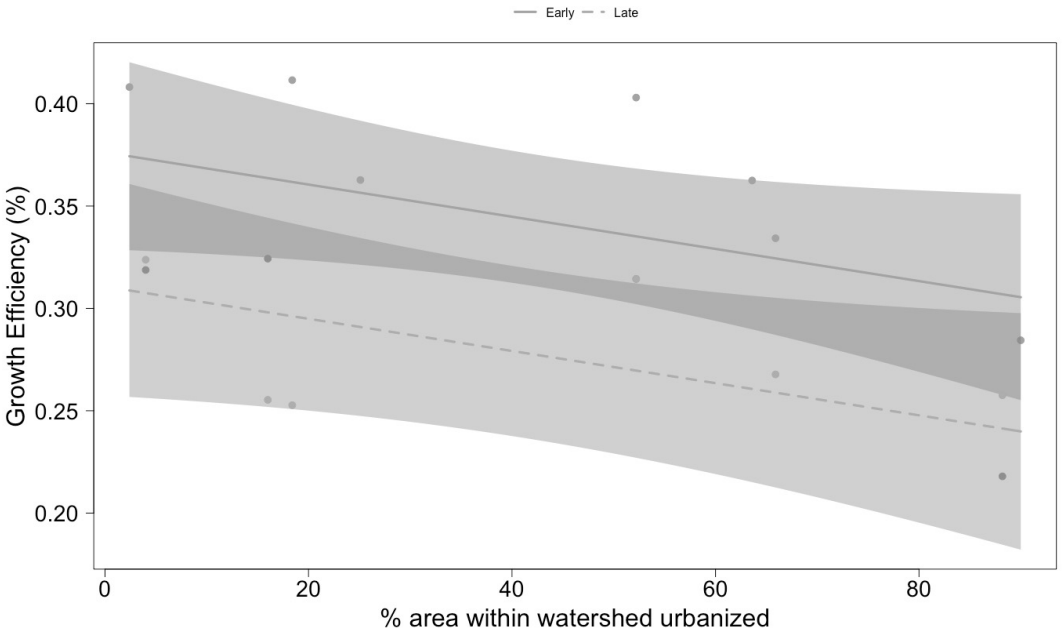

Figure 6b. Relationship between percent urban land use within the watershed and growth efficiency. Growth efficiency is based on diagnostic bioenergetics model using a standard consumption rate of 35% over the period of time for each stream. Both season and percent urbanization are significant terms in the model; adj. r^2=0.4593.

Chapter 3: Assessment of Toxicant Impact to Coho Salmon Using a Novel Toxicogenetic Biomarker Assay

Abstract

Coho salmon (*Oncorhynchus kisutch*) are a culturally and commercially significant Pacific salmon species that spend the first year of life in their small natal streams. This extended rearing time makes them an ideal species to use for monitoring chemical impacts on fish found in urban streams. The purpose of this study was to characterize the hepatic gene expression of juvenile coho from 10 streams in the Pacific Northwest where watersheds spanned a gradient of urban land-use intensity and to relate expression to concurrently measured contaminant concentrations. Next-generation sequencing (RNAseq) was used to construct the transcriptome of this non-model species using sequencing data from 24 individuals originating from 4 different streams. Differential expression analysis of this transcriptome data was used to identify 52 stress genes of interest for comparison of expression profiling among fish from all 10 streams. Derived sequences were used to design custom nanoString probes for expression analysis of identified genes using the nCounter platform. Multivariate methods were used to relate water contaminant concentrations to gene expression levels. Results indicate that elevated concentrations of PAHs, PCBs, and pesticides are significantly correlated to increased expression of genes involved in detoxification of organic contaminants. This study presents the first time a probe-based multiplexed nanoString assay was successfully used to assess salmonids and provides an economical and comprehensive assessment tool to evaluate the exposure and physiological response of salmonids to in-stream contaminants.

Introduction

Urbanization presents unique ecological challenges with the shift in the population from rural areas to major cities and their surrounding suburbs (U.S. Census Bureau, 2010). This move, to densely populated urban areas, is disproportionately concentrated in the coastal areas of the United States (U.S. Census Bureau, 2010). Here, access to internationally serviced ports has created a logistical and financial incentive for their development (Los Angeles, San Francisco Bay, Columbia River, and the Puget Sound). The proximity to ocean connected shipping channels, an abundance of fresh water, and miles of coastline attract large segments of the human population. Many of these same features support biologically diverse landscapes with an abundance of fish, mammals, and birds that exploit this confluence of marine and freshwater environments.

As urbanization increases, the ecological impacts to freshwater streams are greater (Walsh 2005; Gessner et al. 2014). In particular, urban streams face increased loading of contaminants due to increased runoff from extensive impervious surfaces and the general increased use and presence of chemicals within residential, commercial, and industrial landscapes (Paul and Meyer, 2001). The severity of these impacts depends not only on the level of urbanization within a watershed but with the interface of geological and population gradients and historical landuse within them (Allan, 2004).

In the Pacific Northwest, steep coastal geology has resulted in a preponderance of small 1st and 2nd order streams that quickly flow off the cascade foothills and through larger streams and rivers, eventually flowing into Puget Sound. In much of this region, these waters flow through urbanized areas and pick up contaminants from storm water running over and through a combination of paved surfaces, outfalls, and landscaped environments. These varied sources

contribute to a toxic cocktail of chemicals that often include: current-use pesticides, petroleum and its by-products (PAHs and mercury), flame retardants (PBDEs), trace metals, nutrients, contaminants of emerging concern (pharmaceuticals and personal care products), and the continual burden of legacy contaminants (PCBs, organochlorines, lead, and mercury) (Paul and Meyer 2001; Walsh et. al. 2005). This mix is found in dissolved freshwater concentrations, sorbed to sediments carried in the water column or settled on the stream bed, and transported in the tissue of biota (algae, plants, invertebrates, amphibians, fish, and birds) living in and around these freshwater streams. The risks these contaminants pose to aquatic life are complex and numerous and include the synergistic, additive, and antagonistic effects conferred from these mixtures (Eggen et al. 2004; Laetz, 2009; Backhaus and Faust, 2012). With these different contaminants, multiple species of interest, and their interactions, the task of monitoring for and assessing the impact of these pollutants is clearly complex.

The continual exposure of fish to contaminants make them an indicator of contaminant impacts to aquatic ecosystems (Hahn et al. 2016). Just as a passive sampler set in a stream collects chemicals over time, fish are continuously exposed to contaminants including those that have bio-accumulated in their prey (Johnson et al. 2007). Recently, molecular level bioindicators or biomarkers are being used to monitor for current and recent contaminant impact to individuals (Hanh et al. 2016). These base level molecular responses have been linked to whole organism effects through adverse outcome pathways (Ankley et al., 2010), and give insight on both chronic and emerging problems facing fish.

This study seeks to address this monitoring complexity using a tiered approach. Previously described work had assessed growth impacts of temperature and food availability to juvenile coho salmon using these urban habitats (Chapter 2) and looked for visible

manifestations of disease with a careful and through visual necropsy assessment (Chapter 1). In this section, the direct impact of contaminants and other stressors on fish was considered using a genetic signaling assessment that correlates discrete measures of water and sediment chemistry with expression levels of stress and detoxification genes. Seeking a method that was both broad in scope and specific in diagnosis, differential gene expression using transcriptomics was used as a biomarker screening for potential toxicant exposure and physiological stress (Qian Xi et al. 2014). Response to other stressors such as temperature and disease were also considered. Current transcriptome sequencing technologies allow the exploration of a multitude of pathways from a single sample. Many of these pathways are highly sensitive and unique to chemical classes (e.g. metals, pesticides, hydrocarbons, etc.). Gene expression data were paired with water chemistry characterization, to link specific chemicals that directly affect fish at a sublethal level, resulting in an early indicator of chemical exposure (Roberts et al. 2005).

Study Objectives

This chapter had three primary research objectives:

- Measured sediment and water chemistry in the 15 "fish health" streams from the larger PNSQA study (described in Chapter 1) to understand if increasing urbanization leads to higher levels of contaminants.
- Conducted gene expression analysis with liver tissue collected from juvenile coho salmon within these streams; used to evaluate genes that are stress induced, regulate growth, and that are a response to contaminant exposure.
- Evaluate correlations among observed chemistry concentrations and expression patterns using multivariate techniques.

Chemistry monitoring occurred in cooperation with the U.S. Geological Survey's Pacific Northwest Stream Quality Assessment (PNSQA) program. Gene expression studies were carried out using a novel nanoString probe-based assay. This study was the first time a nanoString probe based assay was used on a Salmonid species.

Methods

Water quality sampling and chemistry analysis

Different measurements of water and sediment chemistry were measured during the spring of 2015 for 15 streams representing a gradient in degree of urbanization in Washington and Oregon (Table 1). Chemical concentrations in water and sediment were measured directly using advanced analytical methods with low detection limits.–The USGS PNSQA regional study analyzed water and sediment samples for numerous chemical constituents during the 10 weeks preceding fish sampling in June. Analysis of discrete water samples during this period included pharmaceuticals, pesticides, and organic wastewater indicators; bed sediment analysis included wastewater indicator compounds, polycyclic aromatic hydrocarbons (PAHs), and other semi-volatile compounds. What follows is a summarized description of the field collection and analytical methods for the relevant PNSQA data set. Sheibley et. al. (2017, in press) contains a comprehensive description of USGS methods for the PNSQA study with methods for additional chemical analytes and ecological data sets not used for the present study.

Discrete Water Quality Sampling

Discrete water quality sampling was conducted during the PNSQA USGS survey of 88 streams throughout the study region (VanMetre et al., 2015). Discrete water samples were collected weekly at the non-reference streams for a total of 10 samples per stream. Data collection in reference streams occurred during the last four weeks of data collection only.

Discrete water sampling was conducted utilizing an isokinetic, equal-width-increment (EWI) sampler across the entire stream (Davis, 2005) whenever possible. In a few cases, streams did not meet EWI sampling requirements, and a simple grab sample from the "centroid" of flow was used (Sheibley et. al. 2017, in press). All water sampling was conducted using "parts-per-billion" protocol (U.S. Geological Survey, 2006).

Analytes for discrete water quality samples included pharmaceuticals, pesticides, and organic wastewater indicators (OWIs). The analysis occurred at the USGS National Water Quality Lab in Lakewood, CO. Pharmaceutical were analyzed by direct aqueous injection liquid chromatography tandem mass spectrometry (DAI LC–MS/MS) (Furlong et al., 2014) and included 112 human-use pharmaceuticals listed in. Pesticide analysis was by DAI LC-MS/MS (Sandstrom et al., 2015) and included 229 pesticides and pesticide degradates chose that were based on common use and the probability of occurrence. OWI analysis used capillary column gas chromatography/mass spectrometry and included 75 compounds (Zaugg et al., 2006a).

Results for the discrete water samples collected from all 10 weeks were compared to observed coho gene expression levels. Multivariate analysis and comparisons to gene expression used screened chemistry values by removing analytes not found in any streams including concentrations below detection levels that were considered "non-detects". Given the very low detection limits of the methods described above, non-detections were assumed to have a concentration of zero. Chemical data were summarized by detection occurrence and max concentration for each detected analyte.

Bed sediment chemistry sampling

Streambed sediments were collected once during the ecological survey using established USGS methods (Radtke, 2005; Shelton and Capel, 1994). Approximately 6 to 10 L of fine grain

sediments were collected from depositional areas along the ecological survey reach using stainless steel cylinders and stainless steel spatulas. All collected sediments were homogenized and placed on ice. This bulk sample, one for each stream, was homogenized, put on ice, immediately shipped to various laboratories and refrigerated at 4º C until analysis (Sheibley 2017). Multivariate analyses compared concentrations of polycyclic aromatic hydrocarbons (PAHs), organochlorine insecticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and other semi-volatile compounds with levels of site urbanization and gene expression. PAHs and other semi-volatile compounds were analyzed by solid-phase extraction GC/MS (Zaugg and others 2006b). Organochlorines, PCBs, and PBDEs were determined by a custom method using pressurized liquid extraction, solid-phase extraction, and final analysis by electron capture negative ionization mode (GC/ECNIMSS). Results for the discrete bed sediment samples were used in comparison to observed coho gene expression levels. Chemistry values were screened before multivariate analysis and comparison to gene expression. Analytes were removed that were not found in any stream. Concentration found below detection levels were considered non-detects (concentration of zero). Concentrations of the 18 PCBs, 9 PBDEs and 5 chlordane congeners were respectively summed for analysis. Chemical data was summarized by detection occurrence for chemical classes and measured concentration.

Transcriptional Response

Hepatic tissue transcriptional response of juvenile coho from 10 streams sampled during early season sampling (June/July 2015) and six streams from late season sampling (September 2015) was investigated to see if fish showed signs of physiological stress through the differential expression of genes involved in contaminant metabolism, stress response, and growth. Hepatic tissue was chosen due to the liver's role in metabolism and removal of toxic contaminants. We

conducted differential gene expression analysis initially at the whole transcriptome level using next-generation whole transcriptome sequencing (RNAseq) on 24 individual fish from 4 streams for the identification of gene targets and their sequences; Appendix B. Analysis of 93 individuals from all coho bearing streams over the two sampling seasons utilized a more targeted custom designed nanoString probe-based assay. The assessment of coho for gene expression used liver tissues collected during the USGS regional assessment. Livers were preserved in RNAlater solution and stored at -20º C to minimize RNA degradation (Olsvik et al. 2007). The methods for evaluating transcriptional response were broken down into four steps: 1) high coverage transcriptome sequencing (RNAseq) for 24 individuals from 4 streams (6 per stream) spanning the gradient of urban land use (see Appendix C); 2) differential expression analysis of sequenced individuals (see Appendix C); 3) targeted gene expression analysis for the remaining samples using a nanoString probe-based assay, and 4) multivariate analysis. These methods were adapted from Blazer (2015) and Qian Xi (2014) with additional insight unique to the study provided by University of Washington Geneticist Dr. Steven Roberts (personal communication).

nanoString Probes and "sequencing" of stress targets

A custom multiplexed probe-based on nanoString assay was developed to target 48 specific genes of interest. Genes were selected for the assay by cross-referencing the list of stress genes in Wiseman et al. (2007) with differentially expressed genes observed in the RNAseq analysis. It also included additional genes traditionally used as biomarkers in toxicology research since it was recognized that DEGs could differ in fish from streams not included in the RNAseq dataset. Additionally, the assay included four reference genes: Beta-actin, Elongation Factor 1 and 1b, and S20 for normalization (Olsvik et al. 2005).

nanoString nCounter expression assays require specific 100 bp probe sets for the quantification of gene targets. These probes are designed using the defined sequence of the mRNA target of interest. In the case of coho, no standard use RefSeq accession numbers were available because the species is not fully sequenced. Probes were therefore designed based on a combination of rainbow trout (a closely-related salmonid) sequences and sequences from the RNAseq derived transcriptome assembly, presented in Appendix C. First, a nanoString bioinformaticist designed potential probes based on rainbow trout RefSeq records for the genes of interest. These derived probes were then "blasted" against the assembled and annotated coho transcriptome using BLASTn (Camacho et al., 2008) to determine if they would find the same target genes. If a probe blasted at 95% or greater to a coho contig, then no change was made, if less than a 95% match the probe was redesigned using a transcriptome derived RNAseq sequence. As a final check, probes designed by RNAseq sequences were blasted against the annotated transcriptome to confirm they targeted matching sequenced coho genes. In this way, there was some guarantee that designed probes accounted for both evolutionary differences between the two species yet still hit specified target genes. The attempt was to also account for potential errors in Illumina sequencing and Trinity transcriptome assembly. Final probes (Table 2) were designed by nanoString Inc. Seattle, WA who also provided the necessary reagents. Institute for Systems Biology (ISB) in Seattle, WA analyzed the RNA samples on the nCounter platform using this custom-designed assay.

RNA Isolation for nanoString

Samples from remaining fish for nanoString analysis were processed using the same RNAzol protocol described for next-gene sequencing. All RNA samples used for sequencing were analyzed using the nanoString probe based expression assay. Six fish from each of the

remaining coho bearing streams sampled during the early season had RNA extracted from their preserved liver tissue. Additionally, RNA was extracted from liver tissue from six fish for each stream sampled in the late season sampling. RNA integrity for these remaining samples was checked using an Aligent Bioanalyzer in the School of Aquatic Fisheries and Sciences and had a RIN>7. Samples were diluted to 100ng/L and transferred on dry ice to the ISB for analysis using the nanoString nCounter system following standard protocols (nanoString Technology, Inc. 2013). The nCounter system uses a probe based quantification that is similar to qPCR, but with the added ability to multiplex many genes into a single assay whereby mRNA that hybridizes with supplied probes are directly imaged and counted (Geiss et al., 2008). Data was transferred from ISB, which we analyzed using the nSolver software (nanoString Technology, Inc. 2013).

Data Screening and Analysis

We analyzed the data using the nanoString nCounter software, version 3.0.22. The raw un-normalized counts can be retrieved on the OSF repository (Spanjer, 2017; osf.io/ywh3m). Fish were analyzed separately for June and September samples. Expression levels were quantified using transcript counts and were considered the total counts of each gene analyzed for the sample. These counts were first normalized by a negative control background subtraction to account for detection errors in the nCounter system. Next, positive control spikes added to each sample allowed for normalization to account for sample preparation errors. Finally, the expression levels of "house-keeping" reference genes were used to normalize expression among samples. These "house-keeping" genes were considered to be equally expressed among all individual samples and to account for differences in starting tissue amounts of RNA. The downstream analysis used these final normalized counts. Furthermore, genes were excluded from analysis if they had a count of less than 20 after negative background subtraction. This cutoff

value was determined from the highest negative control probe count and is the method suggested by the manufacturer. Data analysis used multivariate methods to determine general expression patterns and how those patterns related to water chemistry, and comparisons to RNAseq counts for fish subject to both expression assays.

Multivariate analysis

Multivariate analysis was used to explore patterns of chemistry and gene expression, how they relate to one-another, and how both chemistry and gene expression relate to levels of urbanization. All analyses were carried out using the software Primer-E 7 (Clarke and Gorley, 2015). Several steps were performed, not necessarily in the order described. Cluster analysis was used to group streams based on their pattern of measured chemistry. These groupings were then used to classify streams for a factor analysis performed on gene expression data. The function ANOSIM (Clarke and Warwick 2001) in Primer allows for pair-wise tests of similarity among predefined groups (based on *a priori* landuse and not following cluster analysis) and reports an R statistic as a measure of similarity between pairs measured on a scale of 0 (very similar) to 1 (completely different) (Clarke and Gorley 2006). The function SIMPER (Clarke 1993) was then used to identify which variables (in this case genes) were most influential on global gene expression. Gene expression data was grouped both by stream location (to determine differences among streams) and by group chemistry clusters (to see what genes were dissimilar based on chemistry profiles). Finally, chemical concentration and gene expression were both visualized using nonmetric dimensional scaling (NMDS) and component loadings considered (e.g. which gene or chemical most influence dissimilarity among samples). Both data sets were transformed to account for broad ranges of values. Chemistry data were square root transformed to account for right-skewed distributions. Gene expression was more drastically normalized around each

gene's global mean expression value. Although measured in the same units, the magnitude of impact per transcript is not considered equitable among different genes (e.g. ten transcripts of CYP1A are not relatable to 200 transcripts of HS70 in absolute terms when considering the impact on the individual). Dissimilarity matrices were calculated using simple Euclidean distances.

Results

Chemistry

Discrete Water-quality Samples

Multivariate analysis of chemistry data used the maximum water concentration during the 10-weeek sampling period. The following summaries of stream chemistry are based on maximum concentrations of each analyte during the sampling period and are by no means comprehensive of all analytes detected. Table B1-B3 summarizes the maximum values for the sampling period. The total number of detections for both water and sediment samples were summarized (Figure 1).

Organic Waste Water Indicator

Weekly composites samples analyzed for 75 organic wastewater indicators resulted in the detection of 75 chemicals. Concentrations were very low overall with a maximum range of 0.002 to 1.89 ng/L across all chemicals. Cholesterol, indole, and beta-sitosterol were the most ubiquitous among streams, while many OWIs above detection limits were found only in a single stream.

Pesticides

Pesticides were the most common class of chemical found in discrete water quality samples, with 62 different pesticide and pesticide degradates found among all 15 stream locations. Maximum concentrations of pesticides ranged from 0.0048 ng/L to 2500 ng/L, and the

median maximum concentration among all streams of 4.34 ng/L. Prometon, metolachlor, and 2_4-D were the most commonly found among all streams, While the highest concentrations were triclopyr, 2_4-D, N_3_4-dichlorophenylNmethylurea, oryzalin, sulfometuron-methyl, imazamox, and diuron.

Pharmaceuticals

Monthly composites analyzed for 112 human-use pharmaceuticals resulted in the detection of 7 chemicals in these 15 streams. Of these, metformin, nicotine, and methyl benzotriazole were the most ubiquitous; followed by caffeine, carbamazepine, and cotinine; acetaminophen was less common. Max concentrations ranged from 0.64 to 1125ng/L, with a median maximum concentration across all streams of 11.7 ng/L. Concentrations greater than 30 ng/L were limited to caffeine, metformin, nicotine, and methyl benzotriazole.

Sediment Chemistry Samples

Polycyclic Aromatic Hydrocarbon

There were 30 different PAHs detected in the 15 surveyed streams. Perylene was the most commonly detected, found in all streams, followed by 2,6-dimethlnaphthalene, pyrene, and chrysene. Concentrations ranged from 1.2 to 1930 µg/kg. 18 of these PAHs were found in fewer than 50% of surveyed streams. The sum of total PAHs per stream sediment sampled ranged from 30.0 to 2987 µg/kg. Concentrations for all PAHs are listed in Table B4.

Halogenated Compounds

Halogenated compounds including PCBs, PBDEs, Chlordanes, DDT and its degradates, were found in 14 streams with none found in Issaquah Creek. Concentrations ranged from 0.1 to 62.4 µg/kg. Summed-PCBs, summed-chlordanes, and dieldrin were most commonly found. All

other compounds were found in fewer than 50% of surveyed streams. Concentrations for all halogenated compounds are listed in Table B5.

Multivariate Analysis of Patterns among Chemicals

Discrete Water Samples

The relatively high pesticide and pharmaceutical concentrations at May, Swamp, Issaquah, and Longfellow creek set them apart from reference streams. One of the measured values for caffeine and one for imazamox was more than ten times any other chemical measured in a single stream and subsequently heavily influenced resulting ordination; necessitating their subsequent removal. Reference streams were, in fact, the lowest in chemical concentrations, see Figure 2. East Fork Dairy, Coulter, and Jenkins grouped together with low levels of chemicals measured. Roughly three tiers of Urbanization are apparent from Figure 2. Medium and low tiered urban streams also generally clustered together, with Harris, Woodland, and Church having moderate levels of chemicals measured. May, Swamp, Issaquah, and Longfellow clustered together, with their distance from the center reflecting higher chemical values (Figure 2). Furthermore, the confirmation of Coulter and EF Dairy as reference streams supported the use of Coulter as a reference. While it was expected that "Urban tier" level would largely correlate with a pattern of increased chemical detections, it was interesting to note that Issaquah, a tier 1 stream, and May, a tier 3 stream, clustered with the two highest urban streams. Conversely, Jenkins, a tier 4 stream, clustered most closely with the two reference condition streams. Thus, while imperfect, at over two thirds of the study sites, land use intensity was a reasonable predictor of dissolved contaminant levels in streams.

A visual exploration of chemicals driving these relationships is shown in an NMDS plot (Figure 3) overlaid with vectors of chemical variables that correlated with streams positioned in

ordination space. A cut-off correlation of r ≥0.6 was selected to look at the chemicals with the largest concentrations driving relationships among streams. The herbicides: 2,4-dichlorophenoxyacetic acid (2,4-D) and triclopyr; insecticides: fipronil and carbendazim; pharmaceuticals: metformin, methyl benzotriazole (degradant of benzotriazole), and cotinine (tobacco byproduct); and p-cresol (coal tar by-product) appear the most influential in the grouping of sites. P-cresol and herbicides showed more relation to May and Issaquah creeks, while pharmaceuticals and insecticides exerted more influence on Swamp Creek. Several pharmaceuticals were also found in Kelly Creek, but coho salmon were not collected there for study. Longfellow exhibits the highest influence from all chemicals combined.

Sediment Samples

Ordination and clustering for sediment chemistry was done separately for PAH and halogenated compounds because both the concentration ranges and relative toxicity of these compounds are different. Clustering confirmed that the reference streams EF Dairy and Coulter Creek had the lowest concentrations of halogenated compounds and relatively low levels of PAHs in Coulter Creek. EF Dairy did have more PAHs detected, though this was still much lower rate than the two highest sites. For halogenated compounds, Longfellow had the most contamination, farthest from all other sites (Figure 4). This was followed by Woodland Creek. The rest of the sites clustered far from concentrations measured at Longfellow and Woodland Creeks suggesting three levels of contamination: low, med, and high. For PAHs Jenkins Creek had far more PAHs than any other stream (Figure 5) and clustered with Longfellow Creek, the next highest stream. The remaining sites had more moderate levels of PAHs but clustered dependent on which chemicals were detected.

The NMDS ordination (see Figure 6, and Figure 7) reflects these varying levels of contamination. For PAHs no specific chemical dominates the ordination. It is evident that the different PAHs co-vary at sites, with increased levels of all contaminants at Jenkins Creek. Longfellow has a different contaminant profile evident by its distance from the remaining sites and Jenkins Creek. Halogenated compounds show similar co-variability. With all detected chemicals influencing the ordination of Longfellow and Woodland Creeks away from the rest of the streams. The sum of PCBs has slightly more influence on the ordination of Woodland than Longfellow Creek in relation to the rest of the concentrations. As with the discrete water quality samples, Longfellow shows a consistent pattern of contamination and in regards to PAHs Jenkins Creek is disproportionately elevated.

nanoString

The nanoString assay resulted in differential expression among fish from different streams. The predominant finding was for high upregulation of several gene targets in fish from Longfellow Creek and a more nuanced response in fish from remaining streams. The nanoString assay was successful for all submitted coho samples resulting in expression values for 57 fish from early season sampling and 36 fish from late season sampling. Not all genes had expression values above a low cut-off threshold. After removing genes with low expression, 38 genes were carried forward for analysis from early season sampling and 32 in late season sampling. Average expression values spanned many orders of magnitude. Several lowly expressed genes had an average expression value across all samples of less than 10, in contrast to the case of Hemopexin, with an average count of 115,460. Normalization accounted for these substantial variations in expression magnitude before multivariate analysis. Table 3 and 4 summarize results for genes used in multivariate analysis. The nanoString assay and RNAseq results were significantly

correlated ($p<0.05$) with an overall Pearson's correlation value of 0.932 (Figure 8) for 28 genes with low to high expression levels (individuals with >2 transcripts in the nanoString assay), n=24, from four sites with six individual fish per site.

nanoString Multivariate

Multivariate Gene Expression Analysis Early Season

Analysis of similarities (ANOSIM) in Primer-E identified genes that were significantly different among factors (either streams or chemistry) from both the early and late season samples. Since the clearest division in discrete water chemistry among streams was for two main clusters, the chemical group ANOSIM analysis used only low and high factors resulting in a global r-statistic of 0.15, which is a low but significant (0.1% as likely to be observed by random permutations of the data) correlation. The top 5 up-regulated genes in the "high" discrete chemistry group included: hba-1, cyt, G6PD, COX6A, and Arg and included both vitellogenin genes, CYP1A/CYP2K5, and GST-pi/M-GST. The top 5 downregulated genes in the high chemistry group were HMG-T2, NCX, IGF-I, TGF-β, APOA4, CK-1, Galectin. This analysis was repeated for clusters derived from both sediment PAH and halogenated compound data. For sediment PAH data the global r-statistic was not significant and subsequently SIMPER was not performed, suggesting that although PAHs were highly elevated at Jenkins Creek no correlated gene expression was observed. Streams were placed into 3 clusters for ANOSIM analysis based on halogenated compounds resulting in a global r-statistic of 0.508 which was significant, 0.1% as likely to be observed by chance. Subsequent SIMPER analysis revealed that this was driven almost completely by expression levels at Longfellow Creek with upregulation of the same genes as previously described.

The ANOSIM/SIMPER analysis on a stream-by-stream basis provided additional insight since the global statistic from discrete chemistry analysis was small. ANOSIM on a pair-wise comparison produced significant results with a global R-statistic of 0.486 with a significance equal to 0.1% as likely to be observed by chance. Each of the streams considered as part of the discrete water quality high chemistry group was examined in more detail in comparison to Coulter (reference stream) using the SIMPER function. For May vs. Coulter, there was an averaged square difference of 89 units with very few up-regulated genes, including HSP70 and VTG-C; down-regulated genes accounted for all other differences including Cys, MTA, NCX, IGF-1, and CK-1. For Swamp vs. Coulter, there was an average squared difference of 70units. The top five up-regulated genes in fish from Swamp were Cyt, VTG-C, G6PD, IGF-1, and COX6A. There were only four down-regulated genes, which were MTA, CK-1, CBLN, and NCX. For Issaquah vs. Coulter, there was an average squared difference of 59units. The top 5 up-regulated genes in fish from Issaquah were VTG-C, M-GST, IGF-1, Arg, and COX6A. The top 5 down-regulated genes were MTA, CK-1, NCX, Cys, and hba-1. Longfellow vs. Coulter had the largest difference in expression with a squared difference of 154. The top 5 up-regulated genes compared to Coulter were hba-1, CYP1A1, COX6A, GST-pi, and M-GST. Only two genes were down-regulated which were IGF-1, HPX, CK-1, and NCX. From this later pair-wise comparison, it was apparent that the expression observed in Longfellow drove many of the differences found in the group ANOSIM/SIMPER analysis. An NMDS run helped to visualize these relationships and had an ordination plot, used, overall stress in the solution was equal to 0.16 (Figure 9 and 10, with overlaid gene influence broken into two groups). The CYP genes, GST, VTG, and ER genes profoundly affected the ordination of fish from Longfellow and to a lesser extent Swamp. Overall many individuals had a more nuanced response that were less

evident from the global ordination. One exception was the fish from May that show almost universal downregulation of genes compared to individuals from all other streams.

Multivariate Gene Expression Analysis Late Season

Late season genetic samples were tested using ANOSIM to determine if expression significantly differed among stream. This analysis resulted in a global R-statistic equal to 0.24 with a significance of 0.1%. Although significant, this is a low R-statistic, suggesting only a few genes are indeed differentially expressed among streams. These relationships were further investigated using SIMPER for pairwise comparison between streams. Based on the chemistry found in the earlier chemistry sampling comparisons were again made to Coulter with Issaquah, May, and Swamp Creeks. Swamp had an average squared distance from Coulter of 49 units. The top up-regulated genes were CYP1A, HPX, ER-β, Arg, and THR-β, and HSP70. Top down-regulated genes were Galectin, CK-1, HMG-CoA, COS6, and Ubiquinol. May had an average squared distance from Coulter of 60 units. The top up-regulated genes were CYP1A, Cyt, G3PD, HS70b, CK-1, and Galectin. Top down-regulated genes were Galectin, CK-1, HMG-CoA, and COX6A. Issaquah had an average squared distance from Coulter of 73units. The top up-regulated genes were TGF-β, CBLN, COX6A, Galectin, and HMG-T2. Top down-regulated genes were G3PD, HMG-CoA, HSP70, and HSP70b.

Discussion

This study successfully utilized a transcriptomic approach to assess expression patterns in wild juvenile coho salmon in 10 small perennial streams spanning an urban gradient using both next generation sequencing and a custom probe based nanoString assay. To our knowledge this is the first study to use the nanoString nCounter platform in salmonids. nanoString results were strongly correlated to those from RNA sequencing performed on a subset of the individual fish.

Expression was shown to vary among streams with elevated expression of stress-related genes in the most consistently elevated contaminated stream, Longfellow Creek. Overall contamination in the studied streams were low, but expression results alongside previously studied impacts on growth and fish disease suggest juvenile coho experience additional stressors in urbanized streams not seen for fish from our reference streams.

Contaminants and Gene Response

This study successfully utilized a transcriptomic approach to assess expression patterns in wild juvenile coho salmon in 10 small perennial streams spanning an urban gradient using both next generation sequencing and a custom probe based nanoString assay. To our knowledge this is the first study to use the nanoString nCounter platform in salmonids. nanoString results were strongly correlated to those from RNA sequencing performed on a subset of the individual fish. Expression was shown to vary among streams with elevated expression of stress-related genes in the most consistently elevated contaminated stream, Longfellow Creek. Overall contamination in the studied streams was low, but expression results alongside previously studied impacts on growth and fish disease suggest juvenile coho experience additional stressors in urbanized streams not seen for fish from our reference streams.

Contaminants and Gene Response

We characterized contaminant concentrations for many different analytes. For discrete samples, we measured the largest concentrations of pesticides and pharmaceuticals. Pharmaceuticals continue to be an area of concern for urban streams (Lower Columbia River Estuary Partnership 2007; Rounds et al. 2009) and the concentrations of nicotine and caffeine in storm water receiving streams is well documented (Sauvé et al. 2012; Wicke et al. 2016). Metformin, found in most the streams sampled, is a common type 2 diabetes medicine that is

persistent and commonly found in urban streams (Bradley et al. 2016). The many different pesticides detected are a symptom of urban streams (Hoffman et al. 2000; Weston et al. 2011), where residential and landscaping use of pesticides is highly variable. Although not seen in high concentrations in the streams studied here, this mix of different chemicals is not well understood and a continued area for research into impacts on fish.

Our sediment chemistry showed a diverse mix of polycyclic aromatic hydrocarbons. These were particularly elevated at Jenkins Creek. Sediment sampling at this site was in proximity to a heavily used railway, and the stream had trestle pilings driven into the stream not far from sampling. Although PAHs have been shown to induce the aryl hydrocarbon receptor-cytochrome P-450 (AhR-CYP1A) pathway (Salaberria et al. 2014), this was not observed in fish from Jenkins Creek in this study. However, Longfellow Creek did have both elevated levels of PAHs and up-regulated levels of genes involved in PAH pathways, namely CYP and GST genes. This finding at Longfellow is consistent with our understanding of *Oncorhynchus* species responses to xenobiotic detoxification pathways, in the case of CYP and GST, and in well-described pathways of endocrine disruption from environmental estrogens, in VTG and ER. The disruption of cardiac sodium-calcium exchange via the consistently downregulated NCX gene in this gene is a topic discussed by Incardona (2015) following crude oil exposures.
Halogenated compounds in sediment were low. The exceptions were at Woodland and Longfellow Creek both with elevated levels of PCBs. Longfellow also had elevated levels of chlordane and DDT and its degradates. These elevated levels of halogenated compounds in Longfellow might also explain the increased expression of cytochrome, glutathione, and estrogen receptor genes in fish from this stream (Stein et al. 1995; Browne 2010).

The nanoString results from the streams studied revealed a few patterns. Longfellow, the most urbanized and polluted coho bearing stream, had many stress genes upregulated when compared to the reference stream Coulter Creek. These included the cytochrome P450, vitellogenin, and cytochrome c oxidase at equal-to or greater-than a log2 fold-change of 2. The references streams varied in their response. Coulter Creek had relatively low expression levels for all genes in the assay. EF Dairy Creek near Portland, Oregon was the southernmost stream. Here the upregulation of five genes including heat shock 70 suggests that expression levels were likely influenced by higher temperatures in this stream. The magnitude of differential expression was low with log2 fold changes below 2, except for genes in fish at the streams mentioned above. In part, this could be due to the relatively large amount of individual biological variation captured in this study by the use of multiple biological replicates. Additionally, the levels of PAHs and halogenated compounds in sediments observed in this study were relatively low and previous studies have concentrated on chemical doses through food, water concentration, or injection (Norrgren et al. 1999; Stagg et al. 2000, Åkerblom et al. 2000; Rees et al. 2003). It is unclear how closely sediment concentrations match fish exposure, and more work detailing the relationship between sediment and tissue concentration of these chemicals is needed. The potential for gene induction from the pesticide concentrations measured seems unlikely, as previous work show gene induction from pesticides occur with water concentrations well above one ppb (µg/L) (Wheelock et al. 2005 and Olsvik et al. 2010). In our study, single pesticides were well below 1 ppb, though further work is needed to understand the impact on mixtures approaching these concentrations.

nanoString Assay

The nanoString assay used in this study is highly customizable with the ability to characterize up to 800 target genes without amplification or conversion to cDNA. Additionally, the results represent counts of mRNA transcripts within each sample instead of the fluorescence values obtained through traditional qPCR allowing for simplified interpretation and statistical techniques. It has previously been used on bass fish (Hahn et al. 2016) and has widespread clinical and research use in the human health field. The fact that the results correlated well with counts from RNAseq data confirm its ability to accurately characterize expression making it a cost effective add-on for ecological applications.

Summary

The results of our study indicate that juvenile coho are subjected to increased exposure to contaminants in non-reference streams. These contaminants are found as a complex mixture. As such, it is challenging to attribute an observed biological response to any one chemical. The weight of evidence from our transcriptomic characterization suggests that fish in Longfellow Creek exhibit clear signs of physiological stress and upregulation of known xenobiotic metabolism genes. The remaining streams showed more variability of chemical concentrations from one type to another, and among gene expression. It is likely fish in these streams were not subjected to high enough concentrations of contaminants during our study to elicit consistent genetic responses. The low variability corroborates this among expression levels between streams sampled in late season when levels of contaminants are thought to be low because of minimal precipitation and stormwater runoff. Additional laboratory studies that confirmed physiologic responses of fish to sublethal levels of contaminant mixtures would aid the use of transcriptomics in field studies.

Data Reference

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Tables

Table 1. Summary of discrete and integrated chemistry sampling and gene expression assays by stream at each of the Pacific Northwest Stream Quality Assessment (PNSQA) fish health streams in 2015. Values in the table represent the number of samples collected for each parameter type. Ref, reference; tier 1-5, urban gradient stream with tier 1 with least urbanization and tier 5 with the greatest urbanization; X, sample was collected or parameter was measured; --, not sampled

			Early season discrete measurements					Gene expression assays		
Stream id	Site type	State	Pesticides	Organic Waste Indicators	Pharmaceuticals	Suspended Sediment Chemistry	Streambed chemistry	Early Season RNAseq	Early Season nanoString Assay	Late Season nanoString Assay
Coulter	Ref	WA	4	--	--	4	X	X	X	X
EF Dairy	Ref	OR	4	--	--	4	X	--	X	--
Rock	Ref	WA	4	--	--	4	X	--	--	--
Issaquah	Tier 1	WA	10	3	3	10	X	X	X	X
Church	Tier 2	WA	10	3	3	10	X	--	X	--
Harris	Tier 2	WA	10	3	3	10	X	--	X	X
Kelley	Tier 3	OR	10	3	3	10	X	--	--	--
May	Tier 3	WA	10	3	3	10	X	--	X	X
Jenkins	Tier 4	WA	10	3	3	10	X	X	X	X
Woodland	Tier 4	WA	10	3	3	10	X	--	X	--
Burnt	Tier 5	WA	10	3	3	10	X	--	--	--
Longfellow	Tier 5	WA	10	3	3	10	X	--	X	--
Swamp	Tier 5	WA	10	3	3	10	X	X	X	X
Thornton	Tier 5	WA	10	3	3	10	X	--	--	--

Table 2. nanoString custom code set with associated target sequences. *denotes reference genes.

Gene	Abv.	Accession	Position	Target Sequence
***Beta-Actin**	ACTB	DN25949.1	1310-1409	AATGCTTCTAAACAGACTGTACCCAATCCCAAACGACCG ACCCAGCCACCCGACTACCACTTCAGCTCTGCCACCAGAC ACACCACCCAGAGGGAGAGAG
***Elongation Factor 1A**	EF1a	XM_014177562.1	1496-1595	GGCTGCCAGCACTGGCAAGGTGACCAAGTCCGCCGTTAA GGCCGCCAAAGCCAAATGAATTCCTGCTCCAGACATCCA GCAACAACAAGGCGTGTCACCG
***Elongation factor 1Ab**	EF1ab	NM_001124339.1	638-737	TGGTTGGCATGGGGACAACATGCTGGAAGCCAGCGCCAA TATGGGCTGGTTCAAGGGATGGAAGGTCGAACGTAAGGA TGGTAACGCCAACGGTGTGACT
***S20**	S20	NM_001124364.1	186-285	TGGAGCTAAGGAGAAGAACCTCAAGGTGAAGGGTCCAGT CCGTATGCCCACCAAGACTCTGCGCATCACCACCAGAAA GACACCCTGTGGAGAAGGCTCC
Alpha tubulin subunit	TUBA	DN27715.1	111-210	TTTAAGTAGGCTACAACCATATTTGCACAGGTGGGGTCAG TTCCATGACGCAACTCCCACATTGACTGGTAGAACTCACT CACGGAGAACTCATTAACAG
Alpha-globin IV	hba-1	NM_001124551.1	155-254	CCCGGCTCTGGGCCAGTCAAGAAGCATGGAGGCATCATC ATGGGTGCAATTGGTAAAGCAGTCGGACTGATGGACGAC CTCGTGGGGGGAATGAGTGCTC
Apolipoprotein A-IV	APOA4	DN27594.1	20-119	CTCTGGGTCCAGGGGTTCATTGGGACAGGTCCAGGGTGA GTTGTAAGGACATAACTGGGCTGGGTCAAGTAGACAGGT GCTGAGTGGTGCACTGCGGTCT
Arginase	Arg	BK001403.1	770-869	CTACCTCATCCACGAGCTGCACTCCAAGATTCCAGTTCTG CCCAACTTCTCCTGGATAAAGCCATGTGTATCAGCCAAAG ACATAGTCTACATTGGCTTG
Aryl hydrocarbon receptor	Ahr	DN13107.1	380-479	TTGGACCTGGCCAGCGATGATCTGAGGGCTTCCCATGTGG TTGTTTGGTGCCTGAGCACAGGATCCAGCCATGTTGGTGC AAAATGTAACAGCCCCGTTC
Atrial natriuretic peptide	anp	NM_001124211.1	366-465	AGTAAAGCTGTGTCTGGGTGCTTCGGAGCTAGGATGGAC CGCATCGGGACCTCGAGCGGTTTAGGCTGCAGTCCTAAA AGACGTAGCTAGTCAGTTGATG
B-type natriuretic peptide	BNP	NM_001124226.1	193-292	AAATGTACTTCTCCACCGACTTCAAAAGTCCTTTCCTGAG CTGAGTGAGGTTGAGCGAGTCTCCACAGAAAAGTCGGAT GATGTCACTCCTGAAGCCATG
Beta-2-microglobulin	B2M	DN26679.1	717-816	CTTGAAACTGGCACCATGGCAACAAAGTGGTACCAAAAA AACTAAAACTAAAAAAGCCCCAGTTTGTCATCTCAGCTCT AGTGAAAGGCTGGCACGGATT
Cardiac sodium-calcium exchanger	NCX	NM_001124598.1	1697-1796	TACTTTTGAAGAACCGGTAATGACCATAAGCGAAAGTAT CGGCATGATGGAAGTAAAGGTTCTTCGTACCTCAGGAGC GCGGGGTCTAGTGGTCGTACCC
Cellular tumor antigen p53	p53	NM_001124692.1	495-594	GTTCCAGCTACGTTTCCTCCAGTCCAGCACAGCCAAGTCC GTCACTTGCACATACTCGCCAGACCTGAACAAGTTGTTCT GCCAGTTGGCGAAGACTTGT
Chemokine CK-1	CK-1	NM_001124254.1	242-341	ACTGGACCAAGAGGGTACAACGCTGTCTGCGCAAGCGTC AGGAGAAGAAATCCCAACTGAAGAAAAGGGTCTGAACTC ATGTTGCCATGGAGAGGGCTTT
Cytochrome P450 1A1	CYP1A1	DN25489.1	1924-2023	GGTATGTAGGGGGATTCCAAACAAGTGTGTATTGGATAA AGGACTTTCACAAACTCATAGAGTTGATTAGTTTCTATGA TCAGCATACCACAGGGCCTCC
Cystatin	Cys	NM_001124704.1	369-468	CGCCCCTGGATGAGCGATATCCAGATGGTCAAGAACCAG TGTGAAAGTTAAGACCCAGTGAAGAGAACTTCAATCAAT GTCTAGTCTACCCAATAACTAC
Cytochrome c oxidase subunit 6A mitochondrial (Cytochrome c oxidase polypeptide VIa)	COX6A	DN24715.1	137-236	ATCTGCTGCATCACACGCAGGCCATGAGGGAGGATCAGC TAGGACCTGGAAGATCCTGTCGTTTGTTTTGGCCCTACCT GGTGTGGCCGTCTGCATCGCC
Cytochrome P450 2K1	CYP2K1	DN27225.1	339-438	GGAGGAGATCAGCAGGGTTATAGGAAGTCGTCAAACCTT GGTAGAGGACAGGAAGAACCTGCCCTACACTGATGCAGT GATCCATGAGACCCAGAGACTG
Cytochrome P450 2K5	CYP2K5	NM_001124742.1	2506-2605	TGGGGTGGCTGAAACAGTGCCAATCACATCCAAACATGA GGAAACCAGGTGTTTGGTGTCGTTCCAGTTGTCCCATTCC GGTCATCACTATGAGCCATCC
Cytochrome P450 2U1	CYP2UI	DN21173.1	1487-1586	CTGGGGTTGGCTGGGTCTAACGTCTCTCTGTGTCTGGTGA TGATCTGCTTCAGGAAGGCAGTTATGTCCCTTTCCACTTG CCGTACCTCTCTGAAGACAC

Cytochrome P450 3A27	CYP3A27	DN18454.1	175-274	TAGTCGGTTCCCCTCCTGTCTTCGTGTCGTTGCCTTTCTGA GAGTCGATCATCAGTTGTAAGAAATCCACCCGACTAGTTG AGTTCCCAGCGTCACGTCC
Cytoglobin	cyt	NM_0011243 90.1	225-324	TGCCCAGCTCAGGAAGCACTCACGCAGGGTGATGAACGC CATCAACACCCTGGTGGAGAACCTCCATGACGGGGACAA GATGGTGTCTGTTCTGAAGCTG
Estrogen receptor-alpha	ER-α	DN31838.1	66-165	CTGTGGAGTCCCTCCACAACAGCTCGGCAGTGGAAAGCA TGCTGGACAACATCACCGACGCCCTCATCCACCACATCAG CCATTCAGGAGCCTCTGTGCA
Estrogen receptor-beta	ER-β	NM_0011245 70.1	1659-1758	GGCATGCAGCACCTCTCTAGCATGAAGAAGAAGAATGTC GTGCTGCTCTATGACCTGCTCTTGGAAATGCTGGACGCCA ACACAACCCACAGCAGCCGTA
Galectin	Galectin	NM_0011606 62.1	281-380	GGAGGATTCCCCTTCAACCAGGGAGAGAAGTTTAAGATA AATATCACCTTCACCAAGGAGCAGTTCCTAGTTTCTTTTC CTGACGGCTCTGAGATCCACT
Glucose-6-phosphate 1-dehydrogenase	G6PD	EF551311.1	361-460	CGTGACCTGCAGAGCTCAGAGGAGCTGTCCACCCACCTCT CTTCCCTGTTTACTGAGGATCAGATCTACCGCATAGACCA CTACCTGGGCAAGGAGATGG
Glyceraldehyde-3-phosphate dehydrogenase	G3PD	NM_0011242 46.1	737-836	GGCCTTCCGTGTGCCCGTGGCTGACGTATCAGTGGTGGAA CTAACCTGCCGCCTGTCCCGGCCTGGCAGCTACGCTGAAA TCAAAGGGGCTGTCAAGAAG
Growth arrest and DNA-damage-inducible protein GADD45 alpha	GADD45-α	XM_0141899 62.1	776-875	ATCTAGATGAGATACAAAAGAGGATGGATTTCCACTGGG CTGGTGGAGTTAAACTCTCGGCTATGGCAGTCGAGGCGA GATGAGGCACATTTTATGTTCG
Growth arrest and DNA-damage-inducible protein-beta	GADD45-β	NM_0011605 37.1	938-1037	AAGACGAACTGTAGAGAAGACTCACTGCTGTTTGCCCAG CCATTTTGGAGCAACCGTGGGCGGCAGCATAGCGTGGGA ACTGATTTGCAGTTTCGTTTAT
GST-pi	GST-pi	AB026119.1	327-426	CCAGGAATACGATACTGGTAAAGACCAATACATCAAAGA CCTTCCTAACCACCTCGACAAGTTTGAAGCTGTGATGGCC AAAAACAAGACTGGTTTTCTC
Heat shock 70 isoform b	HSP70b	AB196461.1	472-571	AAGCGCACCCTGTCCTCCAGCACCCAGGCCAGCATCGAG ATCGACTCTTTGTACGAGGGAATCGACTTCTACACCTCCA TCACCAGGGCTCGCTTTGAGG
Heat shock protein 70	HSP70	NM_0011605 20.1	264-363	CACAGTGTTTGATGCTAAGCGGCTGATAGGGCGGAAGTTT GACGACAGTGTCGTCCAGGCAGACATGAAACACTGGCCG TTTACAGTGATCAACGACTCG
Heat shock protein HSP 90-beta	HSP90	DN19651.1	322-421	CAAGAGCAACACTGGTCCACTGGCAGCAGGAGTTAAGAT AATTATTTTGTTATACCATAAAAGGACACCGACCAGGTTT CTCCAGTAAAAAAAAGGCAAC
Hemopexin	HPX	Z68112.1	543-642	TACAATGTGAAGACGAAGAAGGTTGAGGAGAAAAAGTTT GAGGGAATGCCCAACTGCACCAGTGCCTTCCGCTTCATGG AGCATTACTACTGTTTCCATG
HMG-CoA reductase	HMG-CoA	AB218825.1	211-310	GTACTGTCGTGATTCACTTCCTGGATAAAGAACTCACTGG CCTCAACGAGGCCTTACCTTTCTTCCTGCTTCTGATCGACC TCTCCAAGGCATGCGCTCT
HMG-T2	HMG-T2	DN27811.1	936-1035	ACAAGGAGAGTTGGTATGACACAAAACCAAGACAACCTT CAGACATGCTTCTACCAAATTGATATTGACAGGGGAATG CTGATTCTGTGTAGTGTGAAGG
Insulin-like growth factor I (IGF-I) (Somatomedin)	IGF-1	DN27843.1	374-473	GTCAAATGGGATGCGGCCATAGAGGTCATCCTGGCTTTCA ATTCACCTGGCAATATCGTCCGTAGCTATATGTGAATGGA CAAGATGGAGGCCTAATCTT
Insulin-like growth factor II (IGF-II) (Erythrotropin)	IGF-II	NM_0011246 97.1	990-1089	TCACCCAGACACTCAAACATACGTTCACATTCTTCCTGTC CTGACTCTCACTCTTGCGCTCTCTCTTTCATTCACCGACAC AAAAGACACCAACACAATC
M-GST	M-GST	XM_0141917 25.1	587-686	GATGAGGTTCTCAGCTCCCCTTCCTGCTACCATTCCCCAG TGAGGTACTGCAACCCCAATCTAATAGTAGTAGAATGTCC CTTGTGGGCATCCATACATG
Metallothionein A	MTA	M18103.1	70-169	AAAATGGATCCTTGTGAATGCTCCAAAACTGGATCTTGCA ACTGCGGTGGATCCTGCAAGTGCTCCAACTGCGCATGCAC CAGTTGTAAGAAAGCAAGTT
Metallothionein B	MTB	KC679073.1	511-610	CCCCACTACTAATTACCAGTTGGGGGCGTTTAAGTGGATT TCTCTCCAGTTGTATGTGGTAAATTACCTTCCCACTTGATT ATCACATAGGGTGCCCAGC
Microsomal glutathione S-transferase 3	MGST-3	BT073167.1	411-510	AAGAGGATGGAGGGTGCCTATGGGTACATTGGTTATTTTG GAGTCATCATTCTTTCCATCGCAGTGGCTTTACAGTTGCTT GGAGTCTTGTGAACCAGCT

potassium voltage-gated channel	KCN	DN53833.1	6-105	GGCCAGTAACGGCTGTCTGCGGGCACTGGCCATGAGGTT TAAGACCACCCACGCCCCTCCGGGCGACACCCTGGTCCA CATGGGAGACGTCCTCTCAGCT
Precerebellin	CBLN	DN27394.1	114-213	GAACAATGTTTGAGTCCAGAAAGCTGGCACGGGTTTCCA ACAAAATGAAGCGATACAAGCTACTGATCCTGGAGGTGA GTGAAGTCAGGTGGACTAGCTC
Somatotropin-1 (Growth hormone 1)	GH1	NM_001124689.1	348-447	ATATCTCTTTCCGTCTGATTGAATCCTGGGAGTACCCTAG CCAGACCCTGATCATCTCCAACAGCCTAATGGTCAGAAAT GCCAACCAGATCTCTGAGAA
Somatotropin-2 (Growth hormone 2)	GH2	DN14604.1	1435-1534	TACCGCCTGGTATCCATCAAAGGAATGGAGCTGGCATAA TCTACTCAATATCTAACAGCAACATAAGCGCTGCATCTGT GGTATGTGCACTGTACGCATG
Thyroid Hormone Receptor-alpha	THR-α	AF302245.1	26-125	CCGCCTACTCCTGTAAGTACGATGGCTGCTGCATCATCGA CAAGATCACCCGCAACCAGTGCCAGCTGTGCCGCTTCAA GAAGTGCATCGCAGTGGGCAT
Thyroid Hormone Receptor-beta	THR-β	AF405205.1	101-200	CTTGTGAAGACCAGATTATCCTATTGAAAGGCTGCTGCAT GGAGATCATGTCTTTACGGGCGGCCGTTCGCTATGACCCC GAGAGCGAGACGCTGACGCT
Transforming Growth Factor-Beta	TGF-β	DN19337.1	240-339	TTTTGTGGCGGACCTCTGTCTTGAGTGTTCTCTCAAGTTTA GCATGGGGAAGTGCATCTAGTGAATGACACAGCATCGGT CTTGGAACATTTTAAATAGT
Tubulin alpha 8 like 3-2	tba1a	NM_001141467.2	1483-1582	AACAGACTAATATTGGAAATCATTCCATAGAGTAAGCAC ATTCCACCCAGCTACAAGAAGAACCTTCCTGCCTCACATC AGCCAGTTTGAATAAAATATG
Tubulin alpha-1C chain	tba1c	GDQG01038898.1	431-530	GGAGCAGGCCGTTCCCCACTTCATGGTCAAGTAAAGGCA GTTCTATCTCTCAGTGATACGTAAGCCGACCGAGGACATA TCCCTCCTTTGAACTTTCTCC
Ubiquinol-cytochrome c reductase	UQCRB	AF465782.1	920-1019	TCATTGGCAGCTATGACATCACTTTTGGTGGTGGAAAGCA CCTGAGCAGCCGCCTTGCTCGTCTGGCCTCAGAGGAGAGT CTGTGCCACAGCTTTCAGGC
Vitellogenin	VTG-C	NM_001124375.1	1605-1704	CTATAACAACATCTACTGGTCAGACCTCGGCACCAAGAC CATTGAAGTGGCCAACTTCAACGGCACCAAGCGGAAGGT TCTCTTCAGCAGTGGTTTGAAA
Vitellogenin C	VTG	DN57264.1	146-245	GCGAACATCTACCAGACGGACAGTGCAACAAGTGAGGAT GTTGTTGTTCTTTTCCAACTCCTGCGTGTGACATCGCTAGA AAACCACGAGGTTCTGTGGA

Table 3. Early season gene used in multivariate analysis of expression levels.

Gene name	Avg count	Min count	Max count	%cv
Cardiac sodium-calcium exchanger	13.55	1.53	37.1	70.81
Insulin-like growth factor i (igf-i) (somatomedin)	19.18	1.04	39.55	51.27
Metallothionein b	15.86	1.47	39.55	60.82
Apolipoprotein a-iv	19.04	4.33	40.33	48
VTG- vitellogenin	15.79	1	49.56	92.03
Somatotropin-1 (growth hormone 1)	6.17	1	49.97	178.91
Cellular tumor antigen p53 (fragment)	24.13	4.24	53.16	57.97
Growth arrest and dna-damage-inducible protein-alpha	12.63	1	59.23	122.37
Hmg-t2	38.65	19.23	68.47	36.2
Thyroid hormone receptor-alpha	41.55	11.7	84.03	43.13
Precerebellin	14.27	1	127.06	176.27
Glucose-6-phosphate 1-dehydrogenase	68.91	24.31	158.76	62.44
Estrogen receptor-alpha	29.56	1	180.49	162.36
Vitellogenin c	47.95	1.11	205.48	108.94
Transforming growth factor- beta	64.2	25.78	213.06	67.05
Thyroid hormone receptor-beta	79.86	24.39	247.14	55.36
Glyceraldehyde-3-phosphate dehydrogenase	78.36	2.19	357.28	95.01
Cytoglobin	84.78	1	366.13	126.07
Chemokine ck-1	114.63	10.69	462.69	85.65
M-gst	322.5	133.49	621.19	39.27
Growth arrest and dna-damage-inducible protein-beta	218.61	17.39	888.13	108.55
Ubiquinol-cytochrome c reductase	493.3	268.94	911.92	33.14
Galectin	165.52	1.47	1652.07	197.15
Insulin-like growth factor ii (igf-ii) (erythrotropin)	767.83	147.56	1659.04	60.25
Arginase	684.76	203.76	1849.84	55.01
Tubulin alpha	702.87	164.79	2232.05	80.25
Hmg-coa reductase	747.03	1	4216.96	134.71
Heat shock protein 70	1633.39	150.2	6028.26	87.99
Alpha-globin iv	1510.85	38.94	8242.58	152.36
Cytochrome c oxidase subunit 6a mitochondrial	4300.31	861.7	12531.16	78.9
Cytochrome p450 2k5	3648.76	521.65	14201.74	74.54
Cystatin	9261.89	2474.97	15660.11	34.48
Chtochrome p450 1a1	6563.53	489.52	22550.79	91.52
Cytochrome p450 2k1	11575.95	5571.53	23004.52	36.09
Microsomal glutathione s-transferase 3	15697.96	4680.29	29373.36	42.69
Gst-pi	13541.99	3550.56	30816.33	61.29
Heat shock 70b	23455.09	15329	32588.04	21.89
Metallothionein a	57168.7	15037	249173.47	83.46
Hemopexin-like protein (fragment)	230767.08	124002.48	342134.88	26.05

Table 4. Late season gene used in multivariate analysis of expression levels.

Gene name	Avg count	Min count	Max count	%cv
Cellular tumor antigen p53	6.34	1	32.77	98.05
Cytoglobin	8.59	1	51.41	144.32
Estrogen receptor-alpha	9.83	1	193.97	326.94
Glucose-6-phosphate 1-dehydrogenase	10.79	1.49	32.77	64.2
Hmg-t2	12.58	2.75	30.5	56.58
Precerebellin	13.22	1	124.37	168.9
Glyceraldehyde-3-phosphate dehydrogenase	15.38	1.08	48.97	65.01
Vitellogenin c	20.74	1	169.84	140.46
Transforming growth factor- beta	22.18	3.83	100.46	85.33
Thyroid hormone receptor-beta	22.22	4.36	45.3	44.36
Hmg-coa reductase	51.57	1	358.26	156.78
Growth arrest and dna-damage-inducible protein-beta	53.69	2.8	295.82	117.07
Galectin	57.96	11.56	304.58	103.16
Tubulin-alpha	92.79	21.93	432.61	82.27
M-gst	93.88	24.69	202.6	45.71
Chemokine ck-1	96.24	4.74	323.79	78.25
Insulin-like growth factor ii (igf-ii) (erythrotropin)	157.76	44.32	555.83	59.45
Ubiquinol-cytochrome c reductase core i protein	173.83	96.35	338.14	27.99
Alpha-globin iv	175.02	8.6	1206.09	131.14
Arginase	177.19	68.85	358.71	37.48
Estrogen receptor-beta	259.07	136.34	516.35	32.28
Heat shock protein 70	299.7	64.86	953.46	56.98
Cytochrome c oxidase subunit 6a mitochondrial	1367.9	483.16	2924.45	37.92
Cytochrome p450 2k5	1543.6	423.45	3752.66	47.97
Chtochrome p450 1a1	1739.51	225.9	4119.57	50.43
Gst-pi	3861.87	1663.39	8546.55	45.56
Cystatin	4078.12	1113.34	6370.2	31.15
Cytochrome p450 2k1	5044.57	2906.19	14743.83	40.65
Beta-actin	5686.69	3471.59	8829.98	20.41
Microsomal glutathione s-transferase 3	5730.22	1127.14	12097.25	45.97
Heat shock 70b	9772.48	4857.53	14132.02	24.07
Metallothionein a	16564.47	6162.96	35941.86	34.09
Hemopexin	115460.84	61949	167069.55	21.92

Figures

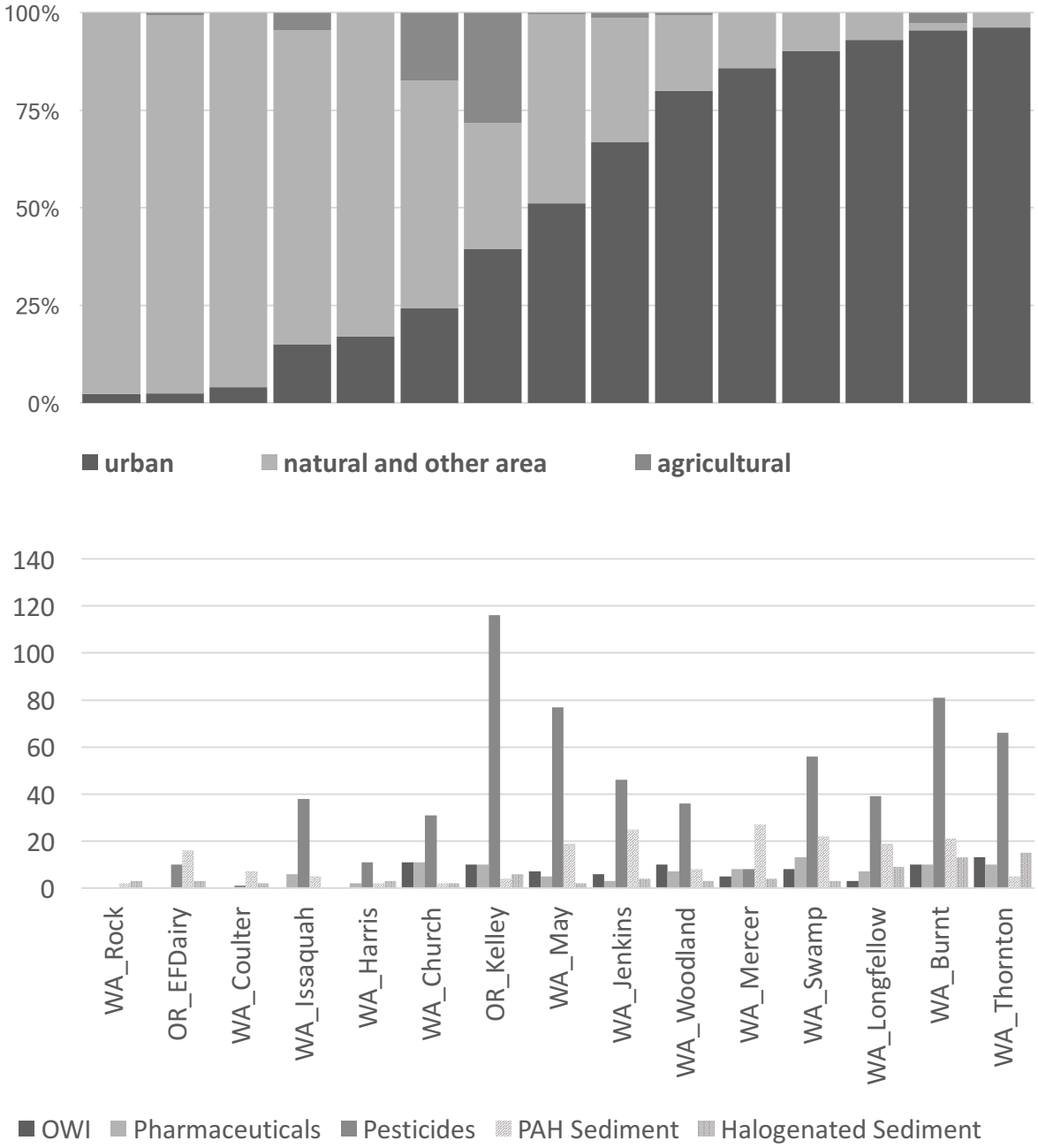

Figure 1. Landuse by stream location and chemical detects by stream location. Landuse is based on the percentage area within a watershed that the defined land use type. Detections span the entire ten week sampling period, but each compound is only counted once.

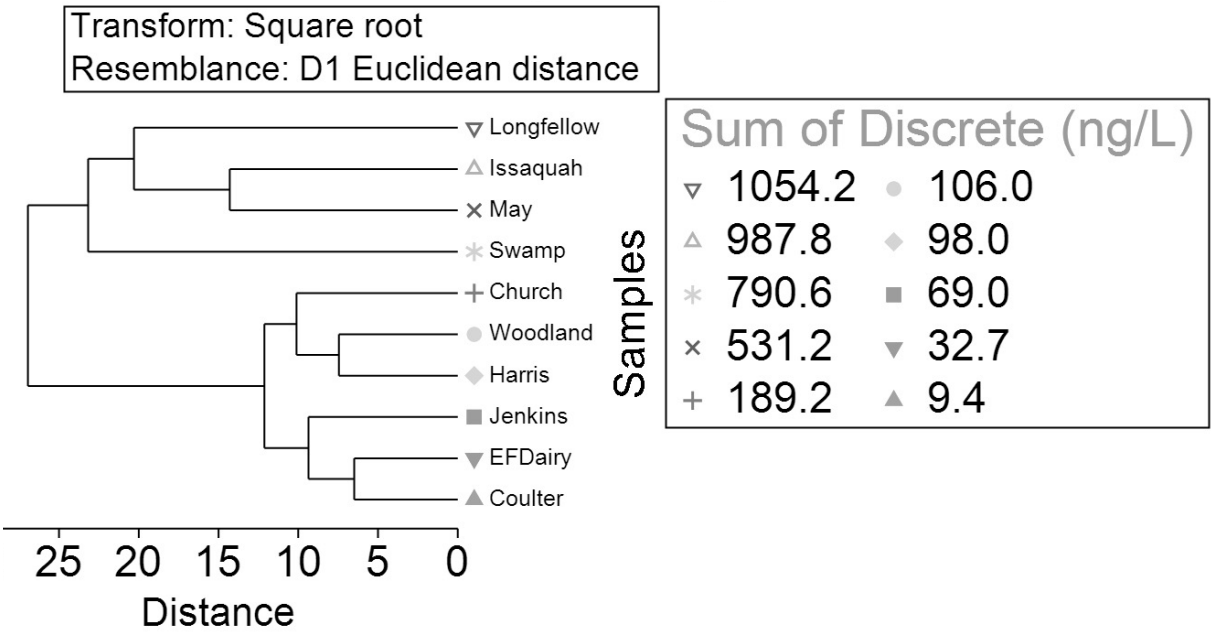

Figure 2. Hierarchical clustering of streams bases on maximum chemistry measurements of OWI, pesticides, and pharmaceuticals (discrete chemistry samples).

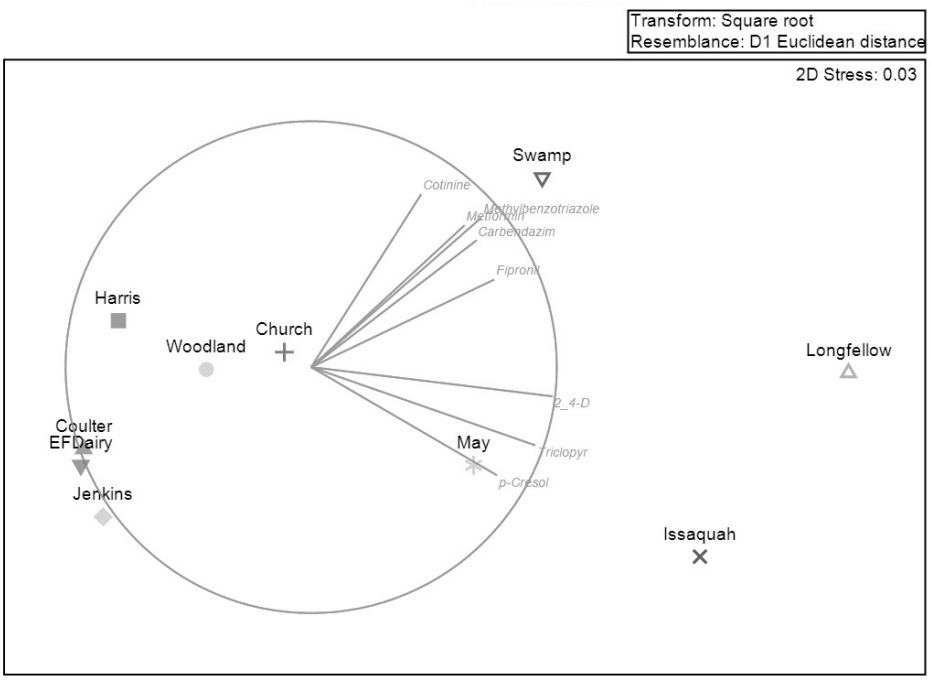

Figure 3. NMDS plot of streams based on maximum chemistry measurements of OWI, pesticides, and pharmaceuticals overlaid with chemicals that correlate to ordination with a Pearson's correlation value >0.6.

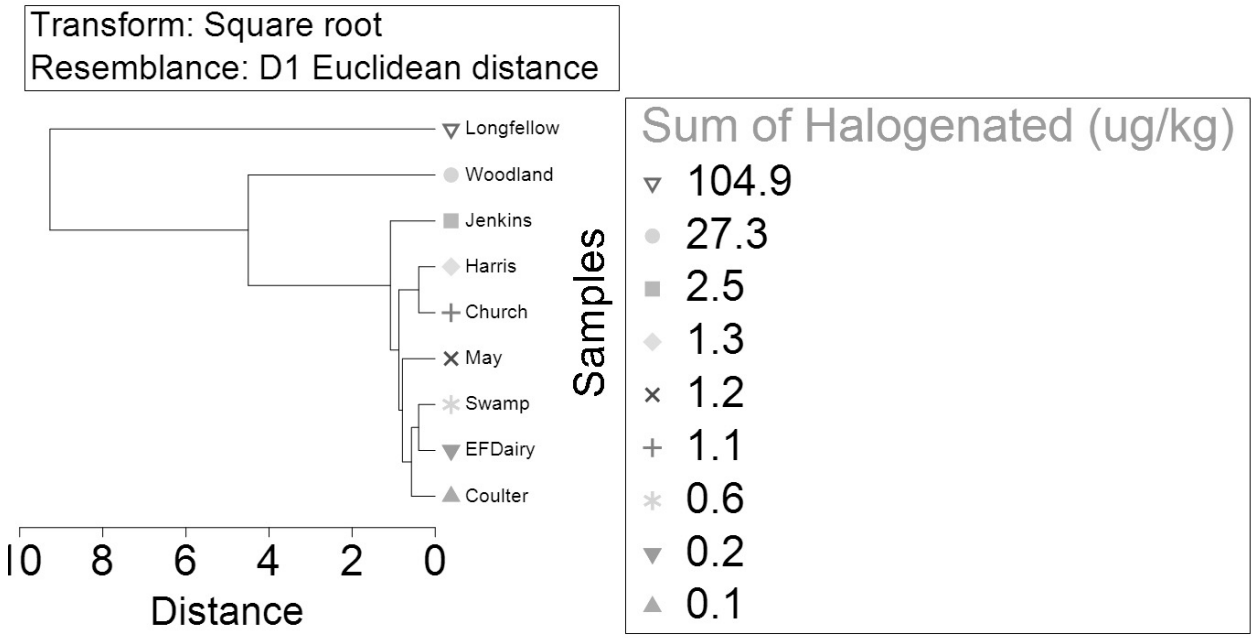

Figure 4. Hierarchical clustering of streams bases on measurements of halogenated compounds in stream bed sediments.

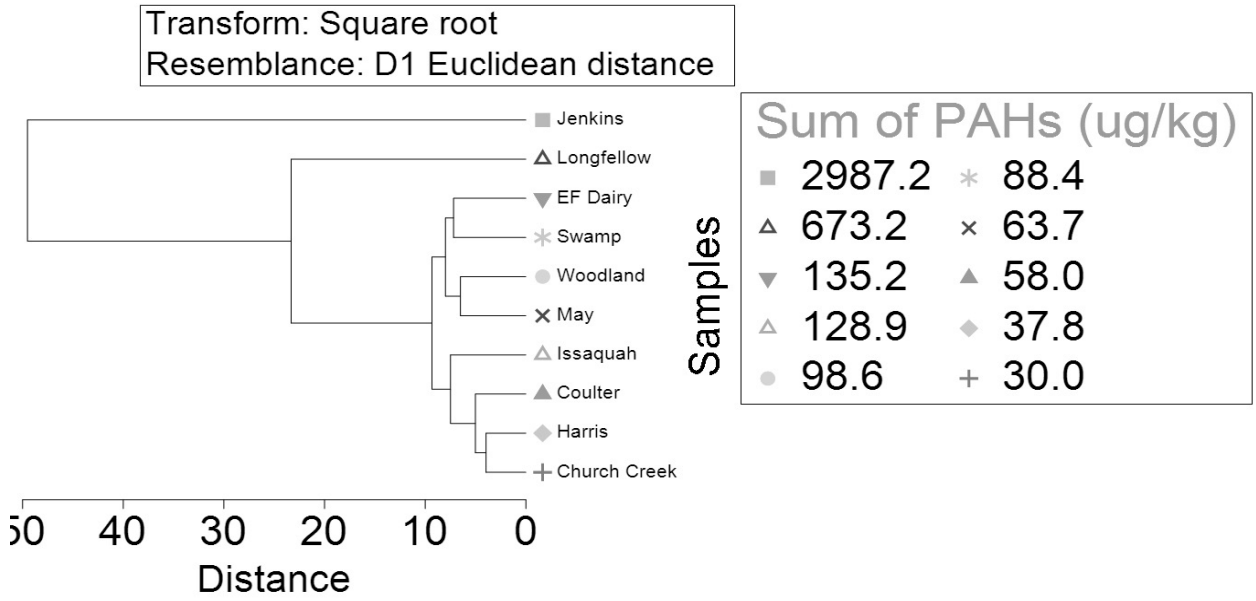

Figure 5. Hierarchical clustering of streams bases on measurements of polycyclic aromatic hydrocarbons in stream bed sediments.

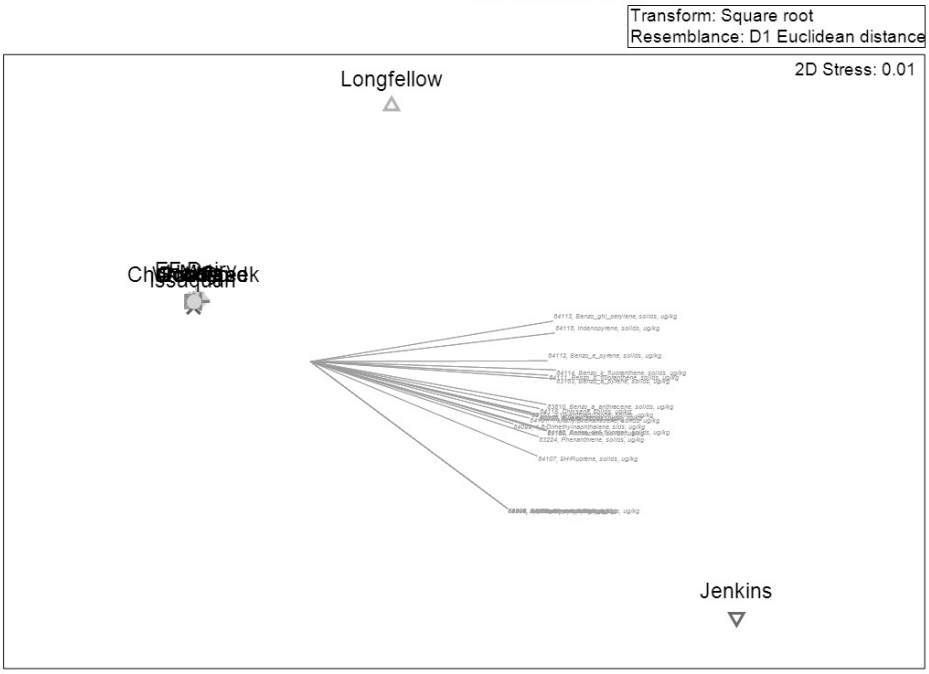

Figure 6. NMDS plot of streams based on sediment chemistry measurements of PAHs overlaid with chemicals that correlate to ordination with a Pearson's correlation value >0.6.

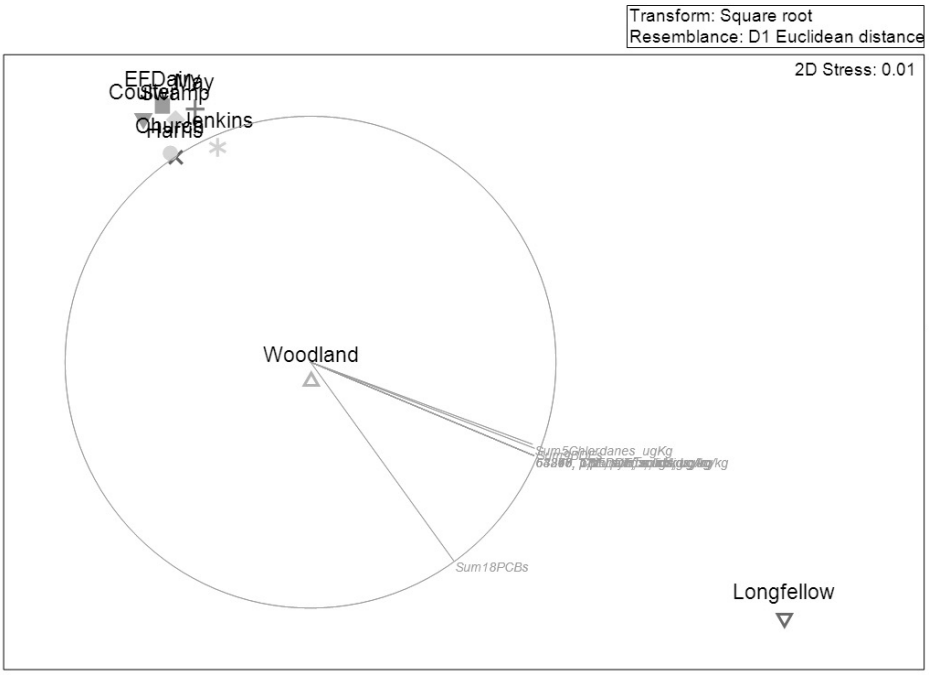

Figure 7. NMDS plot of streams based on sediment chemistry measurements of halogenated compounds overlaid with chemicals that correlate to ordination with a Pearson's correlation value >0.6.

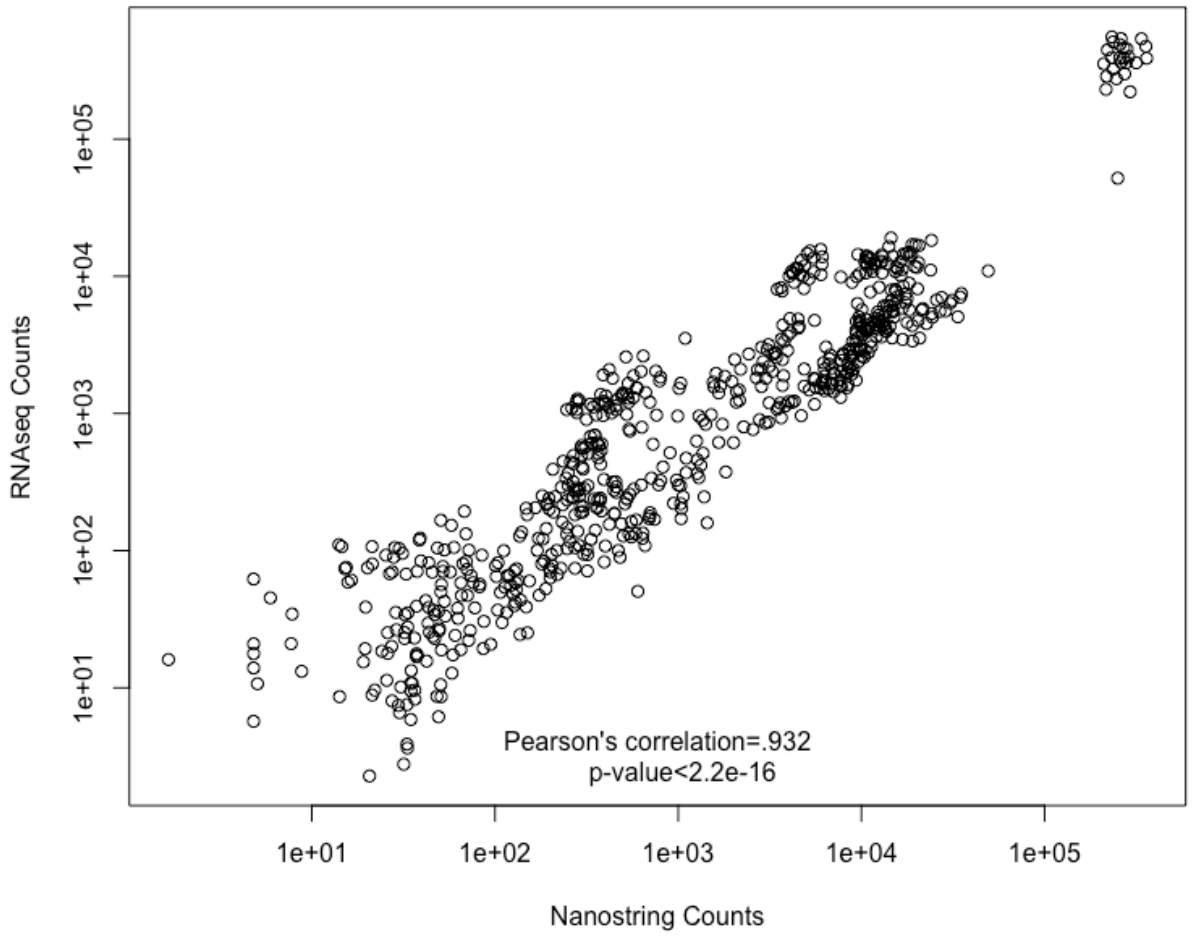

Figure 8. Correlation between gene expression technology using RNAseq and nanoString counts. Coho salmon, n= 24, from four streams with six individuals per site.

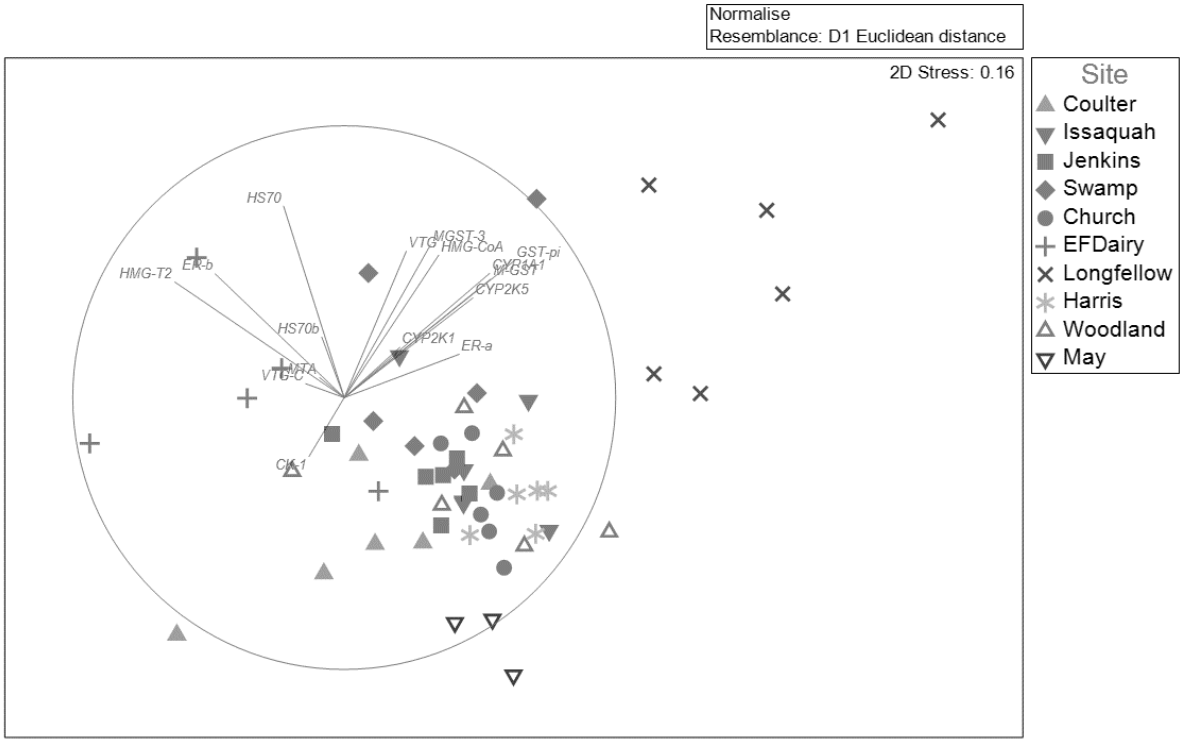

Figure 9. NMDS plot of streams based on genes measured in early season overlaid with genes that correlated to ordination with a Pearson's correlation value >0.6 (only half displayed, the rest below).

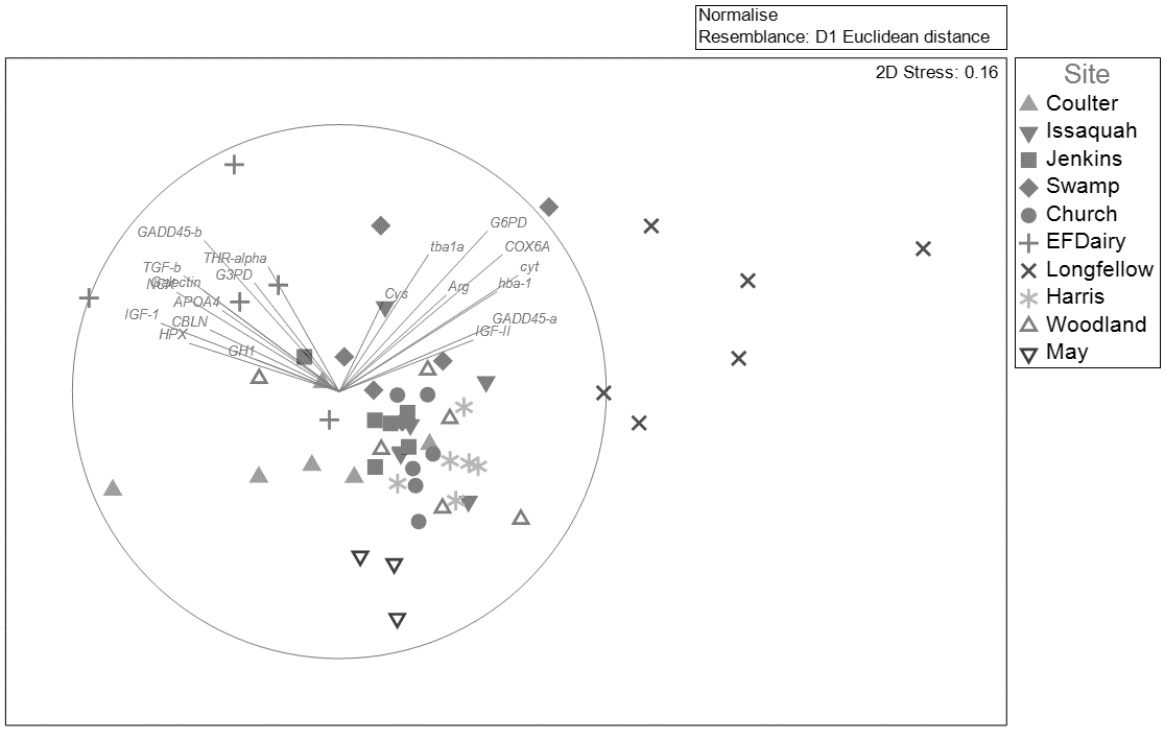

Figure 10. NMDS plot of streams based on genes measured in early season overlaid with genes that correlated to ordination with a Pearson's correlation value >0.6 (only half displayed, the rest above)

Study Conclusions

Disease

- HAI scores from streams sampled showed no clear pattern relating to urbanized landuse within the watersheds of streams sampled. These scores instead proved to be valuable as a tool to indicate where disease/abnormalities occurred among streams.
- Four streams, Burnt Bridge, Kelsey Creek, Woodland Creek, and Jenkins Creek, had HAI scores averaging above 100. These were all moderate-high developed watersheds
- Observed disease/abnormalities in fish from these streams occurred in all tissues examined with low variability among fish (low CV) suggesting that disease stress to fish in these streams was related to their environment.
- Fish from every stream were experiencing possible disease stress, an important consideration when putting environmentally influenced growth and contaminant response into context.
- It was noted that abnormality/disease/parasite detection was common in many of the streams considered reference locations.

Fish Growth (Bioenergetics)

- Juvenile coho grew less efficiently in the most urbanized of the small perennial streams sampled, but these streams also contained the largest fish. These apparently contradicting findings were explained by earlier emergence in warm urbanized streams and higher consumption rates which allowed for a longer period of growth and greater food intake.
- Emergence dates were highly variable both within and among streams and highly correlated with urbanization. This variability has interesting consequences for both growth and potential implications for subsequent life history stages.
- Reduced growth efficiencies in urbanized streams in the early season and across the remainder of the streams during late season are likely partially attributable to increased temperatures as evident from high negative correlations.
- The successful use of bioenergetics modeling to characterize salmonid growth in small perennial streams as part of a monitoring program provides a useful tool to determine which streams have a reduced capacity for salmonid growth and identify which environmental factors are limiting.

Contaminants and Physiological Response

- The results of our study indicate that juvenile coho are subjected to increased exposure to contaminants in non-reference streams.

- The weight of evidence from our transcriptomic characterization suggests that fish in Longfellow Creek exhibit clear signs of physiological stress and upregulation of known xenobiotic metabolism genes.
- Remaining streams showed more variability of chemical concentrations from one type to another, and among gene expression; likely indicating that fish in these streams were not subjected to high enough concentrations of contaminants during our study to elicit consistent genetic responses.
- The use of transcriptomics in field studies would be greatly aided by additional laboratory studies that confirmed physiologic responses of fish to sublethal levels of contaminant mixtures.
- The results from our nanoString assay correlated well with counts from the RNAseq data confirming its ability to accurately characterize gene expression and making it a cost effective add-on for ecological applications.

Appendix A, Bioenergetics Modeling Inputs

Table A1. Input temperature in (ºC) for all streams used for bioenergetics modeling. Italicized and bold temperature values were estimated based on regression model with near-by stream.

Date	Church	Coulter	EF Dairy	Harris	Issaquah	Jenkins	Longfellow	May	Swamp	Woodland
2/1/15	6.2	7.3	6.9	5.7	7.0	7.9	***8.0***	6.8	***6.6***	***10.8***
2/2/15	6.9	7.8	8.0	6.2	7.4	8.3	***8.5***	7.5	***7.4***	***10.9***
2/3/15	7.2	8.0	7.8	6.5	7.6	8.5	***8.7***	7.7	***7.6***	***11.0***
2/4/15	6.6	8.1	8.3	6.3	7.3	8.1	***8.4***	7.4	***7.2***	***10.9***
2/5/15	8.1	8.4	8.8	7.0	8.2	8.9	***9.4***	8.5	***8.5***	***11.3***
2/6/15	9.7	9.1	9.4	7.9	8.9	9.4	***10.4***	9.4	***9.6***	***11.6***
2/7/15	9.4	9.1	9.3	7.9	8.6	9.2	***9.9***	9.2	***9.3***	***11.5***
2/8/15	9.5	9.1	9.2	8.2	8.7	9.2	***10.0***	9.4	***9.6***	***11.5***
2/9/15	9.7	9.1	9.2	8.3	8.9	9.4	***10.1***	9.5	***9.7***	***11.6***
2/10/15	9.9	9.3	9.2	8.8	9.0	9.8	***10.5***	10.0	***10.2***	***11.6***
2/11/15	8.7	8.6	8.9	8.1	8.4	9.0	***9.7***	9.0	***9.0***	***11.3***
2/12/15	9.3	9.1	8.7	8.6	9.3	9.8	***10.6***	9.9	***10.2***	***11.6***
2/13/15	9.5	9.0	8.6	8.9	9.2	9.9	***10.6***	10.2	***10.5***	***11.6***
2/14/15	9.6	9.3	8.9	9.0	9.2	10.0	***10.9***	10.2	***10.5***	***11.8***
2/15/15	7.2	8.2	8.5	7.8	7.5	8.5	***8.9***	8.3	***8.3***	***11.4***
2/16/15	6.7	8.0	8.0	7.2	7.1	8.0	***8.8***	7.9	***7.8***	***11.3***
2/17/15	5.9	7.7	7.7	6.6	7.0	8.0	***8.4***	7.3	***7.1***	***11.1***
2/18/15	6.3	7.2	7.8	6.5	7.3	8.1	***8.4***	7.4	***7.2***	***10.9***
2/19/15	7.5	7.9	8.6	7.4	8.4	9.2	***9.6***	8.4	***8.3***	***11.3***
2/20/15	8.3	8.6	8.4	7.9	8.7	9.8	***10.0***	9.2	***9.3***	***11.6***
2/21/15	7.4	8.0	8.0	7.6	7.9	9.1	***9.2***	8.4	***8.5***	***11.3***
2/22/15	5.9	7.2	6.6	6.4	6.5	7.9	***7.8***	6.9	***6.6***	***10.7***
2/23/15	5.1	6.6	5.8	5.7	6.0	7.3	***6.9***	6.0	***5.6***	***10.5***
2/24/15	5.4	6.6	6.2	5.8	6.3	7.5	***7.0***	6.2	***5.8***	***10.6***
2/25/15	7.0	7.1	7.8	6.4	7.5	8.6	***8.3***	7.3	***7.2***	***10.8***
2/26/15	7.7	8.2	8.3	7.4	8.3	9.5	***9.6***	8.5	***8.5***	***11.3***
2/27/15	8.0	8.4	8.8	7.8	8.3	9.5	***9.5***	9.0	***9.1***	***11.4***
2/28/15	6.3	7.7	7.6	7.0	7.2	8.6	***8.3***	7.9	***7.8***	***10.9***
3/1/15	5.6	6.7	6.6	6.1	6.4	7.7	***7.4***	6.8	***6.6***	***10.5***
3/2/15	6.4	7.5	7.6	6.8	7.3	8.6	***8.2***	7.6	***7.5***	***10.9***
3/3/15	5.0	6.6	6.5	6.0	6.0	7.7	***6.7***	6.3	***6.0***	***10.3***
3/4/15	4.2	6.0	6.0	5.3	5.4	7.3	***6.0***	5.6	***5.1***	***10.1***
3/5/15	4.8	6.4	6.6	5.7	6.2	7.8	***6.9***	6.3	***5.9***	***10.4***
3/6/15	5.5	6.8	7.4	6.3	6.9	8.4	***7.7***	7.0	***6.7***	***10.7***
3/7/15	6.0	7.0	7.8	6.6	7.1	8.6	***7.9***	7.2	***7.0***	***10.7***
3/8/15	6.3	7.3	8.3	6.8	7.4	8.9	***8.4***	7.7	***7.5***	***10.9***
3/9/15	6.8	7.5	8.5	7.1	7.7	9.1	***8.5***	8.1	***8.0***	***11.0***
3/10/15	7.9	7.9	8.4	7.3	7.6	9.1	***9.2***	8.3	***8.3***	***11.2***
3/11/15	9.2	8.6	9.2	8.2	8.7	10.0	***10.4***	9.5	***9.6***	***11.6***
3/12/15	10.3	9.2	10.3	8.9	9.7	10.9	***11.3***	10.4	***10.7***	***11.9***
3/13/15	9.6	9.0	9.7	8.9	9.3	10.5	***11.1***	10.1	***10.4***	***11.8***
3/14/15	10.3	9.7	10.1	9.6	9.9	11.1	***12.0***	11.1	***11.5***	***12.1***
3/15/15	9.2	8.9	9.6	9.1	9.1	10.3	***10.3***	10.0	***10.3***	***11.6***
3/16/15	8.8	8.5	8.5	8.8	8.8	9.9	***10.0***	9.8	***10.0***	***11.7***
3/17/15	8.4	8.4	8.5	8.5	8.5	9.6	***10.0***	9.4	***9.6***	***11.7***
3/18/15	9.2	8.6	9.5	9.2	9.0	10.1	***10.5***	10.1	***10.3***	***11.9***
3/19/15	9.2	8.7	8.9	9.1	9.1	10.1	***10.9***	10.2	***10.4***	***11.9***
3/20/15	9.5	9.0	9.2	9.7	9.5	10.6	***11.3***	10.6	***10.9***	***12.0***
3/21/15	9.7	9.3	9.7	9.9	9.5	10.7	***11.2***	10.7	***11.0***	***12.1***
3/22/15	8.4	8.3	8.8	8.9	8.5	9.7	***10.3***	9.7	***9.9***	***11.6***
3/23/15	8.7	8.0	8.5	8.5	8.3	9.3	***9.8***	9.1	***9.2***	***11.5***
3/24/15	9.4	8.7	8.7	8.6	8.3	9.5	***10.1***	9.5	***9.6***	***11.4***
3/25/15	9.6	9.1	9.3	8.7	8.5	9.7	***10.6***	9.8	***10.0***	11.6
3/26/15	11.3	10.1	9.9	10.0	10.0	11.0	11.9	11.4	***11.9***	12.6
3/27/15	11.3	10.0	10.1	10.6	10.3	11.5	11.9	12.0	***12.6***	12.8
3/28/15	11.4	10.0	9.9	11.0	10.3	11.5	12.0	12.1	***12.7***	12.8
3/29/15	11.1	9.9	9.9	10.8	10.0	11.2	11.6	11.6	***12.2***	12.5
3/30/15	12.0	10.7	9.9	11.2	10.8	11.8	12.5	12.2	***12.9***	12.9
3/31/15	11.4	10.0	9.5	10.9	9.9	11.5	11.6	11.6	***12.1***	12.5
4/1/15	10.6	8.6	8.2	9.8	8.4	10.2	10.6	10.0	***10.3***	11.7
4/2/15	10.2	8.6	7.9	9.7	8.6	10.1	10.7	10.1	***10.3***	11.7
4/3/15	9.5	8.3	7.5	9.3	8.2	9.7	9.9	9.5	***9.7***	11.3
4/4/15	8.7	8.1	7.4	9.0	8.3	9.4	9.6	9.1	***9.2***	11.1

4/5/15	8.3	8.0	7.6	8.5	7.8	9.6	9.5	9.1	***9.2***	11.2
4/6/15	8.5	8.7	8.3	8.5	8.2	9.7	10.2	9.5	***9.7***	11.7
4/7/15	8.3	8.8	8.9	8.3	8.3	9.9	10.3	9.5	***9.6***	11.8
4/8/15	9.6	9.0	9.3	9.4	9.1	10.7	10.9	10.4	***10.8***	12.0
4/9/15	9.6	9.0	8.7	9.7	9.5	11.0	11.3	10.7	***11.1***	12.0
4/10/15	9.4	8.8	8.5	9.8	9.2	10.9	11.1	10.7	***11.0***	11.8
4/11/15	9.0	8.8	8.9	9.7	9.2	10.7	10.9	10.4	***10.7***	11.6
4/12/15	9.2	8.7	8.3	9.3	8.8	10.5	10.7	10.2	10.2	11.6
4/13/15	8.9	8.4	7.8	8.9	8.4	10.2	10.2	9.6	9.4	11.2
4/14/15	8.9	8.6	7.6	8.3	8.0	9.6	9.8	9.1	9.1	11.1
4/15/15	9.6	8.8	7.7	8.3	8.1	9.8	9.8	9.5	9.5	11.3
4/16/15	9.5	8.8	9.1	8.9	8.8	10.4	10.3	10.0	10.1	11.7
4/17/15	10.5	9.4	10.4	9.8	9.9	11.4	11.3	11.1	11.3	12.3
4/18/15	10.9	9.7	10.8	10.3	10.3	11.9	11.8	11.7	11.7	12.6
4/19/15	10.8	9.8	11.0	10.7	10.6	12.1	11.9	11.9	12.0	12.8
4/20/15	11.3	10.4	11.6	11.3	11.2	12.4	12.3	12.3	12.5	13.1
4/21/15	11.2	10.2	10.9	11.3	10.6	12.1	12.3	12.1	12.0	12.7
4/22/15	10.2	9.5	9.1	10.6	10.1	11.4	11.1	11.0	10.9	12.2
4/23/15	9.9	9.1	8.3	10.2	9.5	11.1	10.7	10.5	10.6	11.8
4/24/15	9.1	9.2	8.7	9.8	8.9	10.4	10.7	10.1	10.4	11.6
4/25/15	8.5	9.1	9.2	9.6	9.2	10.6	11.0	10.2	10.5	11.7
4/26/15	9.2	8.9	8.6	9.2	9.0	10.6	10.4	10.1	10.4	11.6
4/27/15	12.0	10.6	11.4	11.4	11.8	12.7	13.0	12.6	13.0	13.1
4/28/15	12.2	10.7	11.2	11.7	11.2	12.7	12.9	12.4	12.8	13.0
4/29/15	11.5	10.1	9.6	11.2	10.6	11.8	12.0	12.0	12.3	12.5
4/30/15	12.1	10.2	10.4	11.7	11.0	11.9	12.4	11.9	12.9	12.6
5/1/15	12.2	10.3	11.5	11.8	11.2	12.3	12.9	12.3	13.1	13.0
5/2/15	11.5	10.5	11.2	11.6	11.3	12.5	12.9	12.2	12.8	13.0
5/3/15	11.0	10.1	11.1	11.2	10.9	12.1	12.2	11.7	12.3	12.9
5/4/15	11.1	10.2	10.6	11.2	10.7	12.0	12.2	11.7	12.3	12.7
5/5/15	11.2	10.3	10.3	10.9	10.7	12.1	12.3	11.5	11.6	12.3
5/6/15	11.0	9.7	10.2	10.4	10.0	11.4	11.8	10.7	11.1	12.4
5/7/15	11.1	9.6	10.6	10.9	10.6	11.7	12.0	11.1	11.9	12.6
5/8/15	11.7	10.1	11.5	11.4	11.4	12.4	12.7	11.9	12.8	12.9
5/9/15	12.4	10.6	12.3	12.1	12.1	13.0	13.3	12.5	13.6	13.2
5/10/15	12.2	10.9	12.0	12.5	12.4	13.3	13.9	12.9	14.0	13.3
5/11/15	12.2	10.7	11.2	12.2	11.4	12.6	13.2	12.3	13.2	12.7
5/12/15	11.9	10.6	10.3	11.8	10.8	12.1	12.8	12.0	13.0	12.5
5/13/15	11.9	10.5	10.5	11.5	10.6	12.0	12.4	11.7	12.4	12.4
5/14/15	11.9	10.6	10.9	11.4	11.2	12.2	12.4	12.0	12.4	12.6
5/15/15	12.2	10.6	10.9	11.4	10.9	12.2	13.1	12.2	12.8	12.6
5/16/15	12.5	10.7	10.7	11.7	10.9	12.3	13.4	12.3	13.1	12.5
5/17/15	12.6	10.8	11.2	11.8	11.2	12.5	13.5	12.6	13.4	12.7
5/18/15	13.4	11.5	12.3	12.7	12.4	13.2	14.4	13.5	14.3	13.3
5/19/15	12.9	11.8	12.0	13.4	12.7	13.9	15.0	14.5	14.7	13.4
5/20/15	13.3	11.7	12.7	13.6	12.9	13.7	14.8	13.7	14.9	13.3
5/21/15	13.6	12.0	13.3	13.8	13.6	14.4	15.3	14.2	15.4	13.7
5/22/15	13.2	11.5	12.4	13.4	12.6	14.1	15.0	13.7	14.7	13.2
5/23/15	13.1	11.1	12.0	12.8	11.9	12.8	13.9	12.9	13.7	12.8
5/24/15	12.4	11.1	12.1	12.6	11.9	12.4	14.0	12.9	13.6	12.7
5/25/15	12.6	11.0	11.7	12.5	11.7	12.2	13.8	12.7	13.4	12.6
5/26/15	13.2	11.2	12.1	12.6	12.0	12.6	14.5	13.2	13.9	12.9
5/27/15	13.8	11.7	13.1	13.2	12.9	13.1	15.3	13.6	14.6	13.3
5/28/15	14.4	12.1	14.2	13.7	13.9	14.1	15.9	14.3	15.3	13.6
5/29/15	14.6	12.4	14.8	14.1	14.6	15.0	16.4	14.8	15.9	13.6
5/30/15	13.6	12.0	14.3	13.8	14.2	14.5	15.8	14.1	15.1	13.2
5/31/15	13.2	11.7	14.7	13.3	13.6	13.9	15.2	13.9	14.8	13.2
6/1/15	13.2	11.4	13.4	12.9	12.5	13.1	14.4	13.2	13.9	12.6
6/2/15	13.1	11.5	12.4	12.8	12.2	12.6	14.4	13.1	13.8	12.6
6/3/15	13.1	11.4	12.3	12.9	12.0	12.7	14.6	13.2	14.4	12.5
6/4/15	13.3	11.6	12.7	12.9	12.6	12.8	14.9	13.5	14.6	13.1
6/5/15	13.9	11.9	14.3	13.3	13.5	13.6	15.6	14.0	15.3	13.2
6/6/15	14.6	12.3	15.5	13.6	14.3	14.4	16.1	14.6	15.9	13.3
6/7/15	15.7	12.9	16.3	14.2	15.0	15.1	17.0	15.1	16.8	13.7
6/8/15	15.6	13.1	17.0	14.3	15.3	15.6	17.3	15.5	16.9	13.7
6/9/15	15.0	13.0	16.4	14.0	15.1	15.4	17.0	15.2	16.4	13.5
6/10/15	14.1	12.7	14.9	13.5	14.6	14.8	16.5	14.8	15.9	13.1
6/11/15	13.6	12.3	14.5	13.3	14.5	15.3	16.4	14.4	15.7	12.9
6/12/15	13.2	12.0	14.2	12.9	13.6	15.6	15.8	13.8	14.8	12.6
6/13/15	12.7	11.7	13.6	12.2	12.9	15.0	15.1	13.3	14.4	12.6

6/14/15	13.7	11.9	14.1	12.4	13.5	15.5	15.5	13.7	14.9	12.7
6/15/15	14.7	12.3	15.5	13.1	14.4	16.4	16.1	14.5	15.6	12.9
6/16/15	13.3	12.3	14.7	13.0	14.3	16.4	15.9	14.3	15.3	12.9
6/17/15	13.5	12.2	14.2	12.7	14.2	16.1	15.8	14.0	15.1	12.8
6/18/15	13.9	12.4	15.4	12.8	14.5	16.4	15.8	14.2	15.0	12.9
6/19/15	14.2	12.4	15.0	12.9	14.3	15.6	16.1	14.4	15.2	13.0
6/20/15	13.4	12.0	14.6	12.7	13.7	14.5	15.9	14.0	15.0	12.7
6/21/15	13.7	12.4	15.3	12.6	13.8	14.3	15.9	14.2	15.1	12.9
6/22/15	13.8	12.5	15.1	12.6	13.8	14.3	16.1	14.1	15.2	12.9
6/23/15	13.8	12.3	15.1	12.7	14.1	15.2	16.1	14.1	15.2	12.8
6/24/15	15.1	12.4	15.9	13.4	15.2	16.6	16.6	14.6	15.6	12.7
6/25/15	15.7	12.8	16.8	13.4	15.0	16.8	17.0	15.1	16.2	13.0
6/26/15	17.1	13.4	18.4	14.0	15.9	16.5	18.0	15.9	17.3	13.3
6/27/15	17.7	14.2	18.6	14.3	16.3	16.8	18.5	16.1	17.4	13.2
6/28/15	17.1	13.2	17.8	14.1	15.4	16.0	18.0	15.5	16.6	12.6
6/29/15	18.5	13.5	18.1	14.8	16.3	16.5	18.5	16.3	17.7	13.2
6/30/15	17.7	13.7	18.4	14.9	16.7	16.7	18.9	16.4	18.1	13.2
7/1/15	17.8	13.8	18.6	14.3	16.5	16.6	18.8	16.8	17.9	13.2
7/2/15	18.3	13.9	19.1	14.4	16.7	17.2	19.1	16.6	18.0	13.3
7/3/15	18.4	14.1	19.0	14.5	16.9	17.4	19.3	16.7	18.1	13.4
7/4/15	17.5	14.0	18.7	14.2	16.8	17.3	19.2	16.5	17.9	13.3
7/5/15	17.0	13.9	19.2	13.8	16.2	16.6	18.7	15.9	17.5	13.3
7/6/15	16.6	13.8	18.7	13.9	16.4	16.8	18.7	16.2	17.5	13.2
7/7/15	15.7	13.5	18.3	13.6	16.1	16.4	18.2	15.7	16.8	13.1
7/8/15	16.1	13.5	18.5	13.8	16.3	16.0	18.0	15.9	16.9	13.1
7/9/15	16.3	13.7	18.7	14.1	16.5	19.1	18.3	16.1	17.2	13.1
7/10/15	16.5	13.4	17.7	13.5	15.3	18.3	18.0	15.3	16.5	12.6
7/11/15	16.6	12.9	16.1	13.3	14.5	16.6	17.2	14.9	15.8	12.4
7/12/15	17.0	13.1	16.6	13.7	15.1	16.0	17.6	15.3	16.5	12.8
7/13/15	16.7	13.2	16.9	13.6	15.3	16.0	18.0	15.4	16.4	12.9
7/14/15	15.9	13.0	16.9	13.3	15.2	15.7	17.8	15.2	16.2	13.0
7/15/15	15.4	12.9	16.8	13.1	15.1	15.5	17.7	15.0	16.0	13.0
7/16/15	16.3	13.2	16.8	13.3	15.5	16.0	17.7	15.2	16.4	13.0
7/17/15	15.8	12.9	16.6	12.9	14.9	15.6	17.3	14.8	16.1	12.9
7/18/15	17.4	13.2	17.8	13.4	15.6	16.2	17.9	15.4	16.8	13.1
7/19/15	18.0	13.7	18.7	14.2	16.3	17.1	18.8	16.2	17.7	13.4
7/20/15	17.9	13.7	18.3	14.1	16.2	17.1	18.9	16.1	17.5	13.3
7/21/15	16.5	13.3	16.9	13.3	15.3	16.2	18.0	15.3	16.0	12.6
7/22/15	15.2	12.7	15.4	12.9	14.9	15.7	17.3	14.9	15.5	12.4
7/23/15	15.8	13.0	15.8	12.9	14.8	15.8	17.2	14.8	15.7	12.8
7/24/15	14.5	12.8	16.1	12.7	14.2	15.2	16.9	14.4	15.2	12.5
7/25/15	15.0	12.7	16.1	13.0	14.2	15.3	16.9	14.5	15.0	12.7
7/26/15	14.8	12.2	15.2	13.2	13.9	14.7	16.3	14.3	15.5	12.6
7/27/15	14.9	11.9	14.7	13.0	13.6	14.3	16.0	14.0	15.2	12.6
7/28/15	15.7	12.3	16.0	13.3	14.4	15.0	16.8	14.5	16.0	12.9
7/29/15	16.2	12.8	17.3	13.8	15.0	15.8	17.3	15.0	16.6	13.0
7/30/15	16.8	13.1	18.5	14.1	15.5	16.2	17.8	15.4	16.9	13.1
7/31/15	16.5	13.3	19.0	14.2	15.7	16.6	18.1	15.6	17.0	13.2
8/1/15	16.4	13.2	18.4	14.1	15.7	16.7	18.0	15.5	16.8	13.1
8/2/15	16.5	13.2	17.7	13.7	15.3	16.4	18.1	15.5	16.8	12.9
8/3/15	16.4	13.4	17.5	13.9	15.3	16.4	18.2	15.6	16.9	13.1
8/4/15	15.7	13.3	17.1	13.7	15.4	16.2	17.9	15.5	16.5	13.1
8/5/15	14.3	12.7	16.5	12.8	14.3	15.3	16.8	14.4	15.0	12.8
8/6/15	15.6	12.6	15.9	13.3	14.7	15.7	16.9	14.8	15.6	12.7
8/7/15	15.3	12.6	16.8	13.4	14.6	15.5	16.9	14.7	15.7	12.9
8/8/15	16.0	13.0	17.0	13.8	14.8	16.0	17.4	15.1	15.9	12.9
8/9/15	16.7	13.1	16.7	14.3	15.5	16.4	17.4	15.3	16.4	13.0
8/10/15	16.5	13.2	17.7	14.2	15.6	16.7	17.7	15.4	16.5	13.2
8/11/15	16.8	13.3	18.1	14.0	15.6	16.6	17.8	15.5	16.5	13.2
8/12/15	16.4	13.2	18.5	13.9	15.5	16.6	17.5	15.3	16.1	13.0
8/13/15	16.4	13.5	18.4	14.3	15.5	16.5	18.0	15.5	16.5	13.2
8/14/15	15.6	13.0	17.1	13.6	14.6	15.8	17.3	15.3	16.1	12.7
8/15/15	15.5	12.6	16.5	14.2	14.1	15.1	16.9	15.0	16.4	12.7
8/16/15	14.9	12.4	16.2	13.8	14.3	15.1	16.8	14.7	15.9	12.9
8/17/15	14.5	12.5	17.1	13.9	14.5	15.3	16.6	14.7	15.9	12.8
8/18/15	15.4	12.8	17.7	14.1	14.8	15.7	17.0	15.0	16.2	13.0
8/19/15	16.6	13.0	18.7	14.6	15.3	16.4	17.5	15.4	16.7	13.2
8/20/15	16.0	13.1	18.1	14.1	15.0	16.3		15.2	16.1	13.1
8/21/15	15.4	12.8	16.5	13.3	13.9	15.2		14.4	15.5	12.5
8/22/15	14.3	12.1	15.7	12.9	13.5	14.4		13.8	14.8	12.4

8/23/15	14.0	12.0	16.4	12.9	13.7	14.6	14.0	14.9	12.5
8/24/15	14.1	11.9	16.1	12.9	13.8	14.6	14.0	14.7	12.6
8/25/15	13.4	11.7	15.5	12.4	13.6	14.4	13.7	14.3	12.4
8/26/15	13.6	11.9	15.9	12.6	13.6	14.5	13.9	14.7	11.6
8/27/15	14.5	12.3	16.2	13.3	14.3	15.2	14.3	15.2	
8/28/15	15.5	12.6	16.7	13.5	14.5	15.7	14.6	15.6	
8/29/15	16.0	12.6	16.4	13.9	14.5	15.7	15.2	16.1	
8/30/15	15.4	12.9	15.7	14.5	14.1	15.1	15.7	16.3	
8/31/15	15.0	12.6	15.1	14.4	13.4	14.4	15.2	15.5	
9/1/15	15.0	12.6	15.6	14.4	13.4	14.4	15.0	15.3	
9/2/15	14.4	12.2	15.2	14.1	13.6	14.1	14.6	15.3	
9/3/15	13.6	11.1	13.3	13.3	12.6	13.0	13.6	14.5	
9/4/15	12.8	11.1	12.1	12.4	12.2	12.8	12.6	13.6	
9/5/15	12.3	10.6	11.9	12.0	11.7	12.5	12.3	13.0	
9/6/15	12.8	11.1	11.7	12.3	12.1	13.1	13.2	13.7	
9/7/15	13.2	11.6	12.9	13.1	12.9	13.9	13.9	14.1	
9/8/15	14.0	11.6	14.2	13.2	13.0	14.2	13.8	14.4	
9/9/15	14.5	11.9	15.5	13.3	13.5	14.8	14.0	14.9	
9/10/15	14.6	12.0	15.9	13.2	13.5	14.6	14.1	15.1	
9/11/15	14.8	11.9	16.5	13.5	13.7	14.9	14.3	15.2	
9/12/15	14.7	12.1	17.2	13.8	13.9	15.2	14.4	15.3	
9/13/15	14.5	12.1	16.0	13.4	13.6	15.0	14.1	14.8	
9/14/15	12.8	11.5	13.1	12.5	12.7	13.5	12.9	13.6	
9/15/15	12.5	10.4	11.7	12.0	12.0	12.9	12.4	13.1	
9/16/15	12.3	10.8	12.8	11.5	11.9	12.7	12.1	12.8	
9/17/15	13.0	11.3	13.6	12.2	12.4	13.5	12.9	13.2	
9/18/15	13.7	11.5	13.3	12.9	12.5	13.4	12.9		
9/19/15	14.4	12.0	13.8	13.4	12.9	14.1	13.4		
9/20/15	15.3	12.5	14.7	14.1	13.5	15.0	14.3		
9/21/15	13.5	11.9	14.3	13.4	13.2	16.7	13.4		
9/22/15	11.3	10.7	12.3	15.6	11.4	19.0	11.6		
9/23/15	11.3	10.2	11.8	19.3	11.0	19.1	14.4		
9/24/15	12.9	10.9	12.8	20.8	11.7	21.0	21.0		
9/25/15	13.2	11.2	13.8	23.1	12.2	23.1	23.2		
9/26/15	12.0	11.1	13.2		11.8				
9/27/15	10.1	9.9	11.7		10.6				
9/28/15	9.8	9.8	11.7		10.3				
9/29/15	10.1	9.9	12.0		10.4				
9/30/15	10.1	10.3	12.2		10.6				

Table A2. Diet proportions and energy density used as inputs for bioenergetics modeling. Model interpolates proportions between sampling dates.

		Diet proportions (energy densities)								
Stream	Date	Aquatic larvae soft (2746 J)	Aquatic larvae rigid (4272 J)	Aquatic Nymphs (3076 J)	Aquatic Other (2789 J)	Winged Insect (4225 J)	Coleo-ptera (6387 J)	Hymen-optera (5134 J)	Hemip-tera (5210 J)	Other (5000 J)
EF Dairy	6/30/15	0.00	0.23	0.02	0.00	0.07	0.55	0.09	0.03	0.00
Coulter	6/23/15	0.05	0.14	0.17	0.07	0.56	0.01	0.01	0.01	0.00
	9/29/15	0.06	0.01	0.03	0.00	0.00	0.00	0.21	0.01	0.68
Issaquah	6/15/15	0.13	0.43	0.07	0.00	0.02	0.01	0.03	0.15	0.15
	9/24/15	0.01	0.03	0.48	0.00	0.27	0.10	0.06	0.05	0.01
Harris	6/22/15	0.02	0.20	0.08	0.00	0.03	0.41	0.07	0.19	0.00
	9/24/15	0.05	0.02	0.48	0.34	0.00	0.00	0.11	0.00	0.00
Church	6/19/15	0.03	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.95
May	7/15/15	0.06	0.25	0.02	0.00	0.00	0.61	0.06	0.00	0.00
	9/21/15	0.06	0.29	0.16	0.00	0.04	0.40	0.03	0.00	0.02
Jenkins	6/16/15	0.00	0.33	0.04	0.00	0.00	0.01	0.00	0.01	0.61
	9/21/15	0.07	0.19	0.19	0.01	0.02	0.01	0.00	0.48	0.03
Woodland	6/24/15	0.02	0.19	0.08	0.00	0.13	0.07	0.00	0.01	0.49
Swamp	7/9/15	0.06	0.28	0.62	0.00	0.00	0.00	0.04	0.00	0.00
	9/17/15	0.08	0.10	0.04	0.05	0.00	0.00	0.04	0.00	0.69
Longfellow	6/21/15	0.16	0.05	0.03	0.09	0.00	0.00	0.00	0.02	0.65

Table A3. Individual fish metrics used as input for bioenergetics models. Fitted-consumption simulations started on emergence date and ended on the sampling date for each individual fish.

SampleID	Stream	Emergence Date	Sampling Date	Weight (g)
		Early Season		
13ACH	Church	4/11/15	6/19/15	1.78
17ACH	Church	4/11/15	6/19/15	1.38
18ACH	Church	4/5/15	6/19/15	1.95
1ACH	Church	3/20/15	6/19/15	2.18
2ACH	Church	3/30/15	6/19/15	1.86
3ACH	Church	4/18/15	6/19/15	1.22
4ACH	Church	4/8/15	6/19/15	1.8
5ACH	Church	4/6/15	6/19/15	2.32
6ACH	Church	4/13/15	6/19/15	1.99
9ACH	Church	4/3/15	6/19/15	1.9
10ACO	Coulter	4/5/15	6/23/15	1.78
11ACO	Coulter	3/30/15	6/23/15	2.97
12ACO	Coulter	4/2/15	6/23/15	2.48
13ACO	Coulter	3/23/15	6/23/15	3.21
16ACO	Coulter	3/23/15	6/23/15	4.06
3ACO	Coulter	4/1/15	6/23/15	2.39
4ACO	Coulter	4/29/15	6/23/15	0.98
5ACO	Coulter	4/6/15	6/23/15	1.19
6ACO	Coulter	3/28/15	6/23/15	3.03
8ACO	Coulter	4/22/15	6/23/15	0.95
10AEF	EFDairy	3/25/15	6/30/15	4.11
12AEF	EFDairy	4/7/15	6/30/15	2.63
19AEF	EFDairy	3/7/15	6/30/15	5.19
3AEF	EFDairy	3/21/15	6/30/15	4.17
5AEF	EFDairy	3/15/15	6/30/15	6.64
6AEF	EFDairy	3/14/15	6/30/15	4.54
7AEF	EFDairy	3/1/15	6/30/15	5.59
8AEF	EFDairy	3/16/15	6/30/15	5.39
9AEF	EFDairy	4/15/15	6/30/15	2.44
1AHA	Harris	4/19/15	6/22/15	0.72
2AHA	Harris	4/26/15	6/22/15	0.69
3AHA	Harris	4/23/15	6/22/15	0.92
4AHA	Harris	3/30/15	6/22/15	3.15
5AHA	Harris	4/2/15	6/22/15	1.23
6AHA	Harris	4/12/15	6/22/15	1.46

7AHA	Harris	3/16/15	6/22/15	3.39
8AHA	Harris	4/7/15	6/22/15	2.42
9AHA	Harris	3/18/15	6/22/15	2.22
14AIS	Issaquah	4/5/15	6/15/15	1.71
15AIS	Issaquah	3/15/15	6/15/15	2.97
16AIS	Issaquah	3/4/15	6/15/15	2.77
1AIS	Issaquah	3/21/15	6/15/15	2.51
4AIS	Issaquah	3/5/15	6/15/15	3.41
5AIS	Issaquah	3/24/15	6/15/15	2
6AIS	Issaquah	3/25/15	6/15/15	2.1
7AIS	Issaquah	4/8/15	6/15/15	1.69
8AIS	Issaquah	2/26/15	6/15/15	4.07
9AIS	Issaquah	3/14/15	6/15/15	3.55
10AJE	Jenkins	3/31/15	6/16/15	2.58
11AJE	Jenkins	3/24/15	6/16/15	3.27
1AJE	Jenkins	3/18/15	6/16/15	2.93
3AJE	Jenkins	4/21/15	6/16/15	1.07
4AJE	Jenkins	3/17/15	6/16/15	2.06
5AJE	Jenkins	3/27/15	6/16/15	2.72
6AJE	Jenkins	4/6/15	6/16/15	1.8
7AJE	Jenkins	3/27/15	6/16/15	1.76
8AJE	Jenkins	4/4/15	6/16/15	1.65
9AJE	Jenkins	3/14/15	6/16/15	2.4
10ALO	Longfellow	2/25/15	6/21/15	8.3
1ALO	Longfellow	3/7/15	6/21/15	7.46
2ALO	Longfellow	3/3/15	6/21/15	6.4
3ALO	Longfellow	2/13/15	6/21/15	10
4ALO	Longfellow	3/5/15	6/21/15	6.11
5ALO	Longfellow	2/22/15	6/21/15	8.65
6ALO	Longfellow	2/20/15	6/21/15	12.41
7ALO	Longfellow	3/11/15	6/21/15	4.39
8ALO	Longfellow	3/13/15	6/21/15	7.9
9ALO	Longfellow	2/24/15	6/21/15	9.27
11AMA	May	3/23/15	7/15/15	3.46
12AMA	May	3/30/15	7/15/15	3.73
13AMA	May	3/26/15	7/15/15	4.17
15ASW	Swamp	3/20/15	7/9/15	6
1ASW	Swamp	3/13/15	7/9/15	7.34
2ASW	Swamp	3/23/15	7/9/15	6.83
3ASW	Swamp	3/8/15	7/9/15	4.6
4ASW	Swamp	3/16/15	7/9/15	8.3
5ASW	Swamp	3/14/15	7/9/15	4.6
8ASW	Swamp	4/3/15	7/9/15	4.99
15AWO	Woodland	3/9/15	6/24/15	4.1
16AWO	Woodland	3/19/15	6/24/15	2.58
17AWO	Woodland	2/22/15	6/24/15	5.1
1AWO	Woodland	2/25/15	6/24/15	3.22
3AWO	Woodland	4/9/15	6/24/15	1.72
4AWO	Woodland	2/23/15	6/24/15	5.18
5AWO	Woodland	4/4/15	6/24/15	2.11
7AWO	Woodland	3/14/15	6/24/15	6.44
8AWO	Woodland	4/6/15	6/24/15	1.77
		Late Season		
4ACO929	Coulter	5/21/15	9/29/15	3.05
5ACO929	Coulter	5/25/15	9/29/15	3.23
6ACO929	Coulter	6/20/15	9/29/15	2.03
7ACO929	Coulter	5/30/15	9/29/15	4.37
8ACO929	Coulter	5/3/15	9/29/15	6.44
10ACO929	Coulter	5/26/15	9/29/15	2.97
1AHA924	Harris	5/31/15	9/22/15	2.42
2AHA924	Harris	6/21/15	9/22/15	1.78
3AHA924	Harris	5/15/15	9/22/15	6.26
5AHA924	Harris	6/21/15	9/22/15	0.65
8AHA924	Harris	5/20/15	9/22/15	2.53
10AHA924	Harris	5/20/15	9/22/15	1.78
1AIS924	Issaquah	5/20/15	9/24/15	2.8
2AIS924	Issaquah	4/24/15	9/24/15	6.86
5AIS924	Issaquah	5/5/15	9/24/15	5.23
8AIS924	Issaquah	5/2/15	9/24/15	7.15
1AJE921	Jenkins	4/21/15	9/21/15	5.16

2AJE921	Jenkins	5/16/15	9/21/15	4.76
8AJE921	Jenkins	5/29/15	9/21/15	4.11
9AJE921	Jenkins	5/12/15	9/21/15	3.12
1AMA923	May	4/28/15	9/23/15	6.58
3AMA923	May	5/4/15	9/23/15	5.49
5AMA923	May	4/29/15	9/23/15	5.2
7AMA923	May	4/20/15	9/23/15	6.61
8AMA923	May	5/2/15	9/23/15	5.68
10AMA923	May	5/29/15	9/23/15	3.35
1ASW917	Swamp	4/18/15	9/17/15	4.3
10ASW917	Swamp	4/5/15	9/17/15	7
16ASW917	Swamp	4/24/15	9/17/15	5.17
19ASW917	Swamp	4/13/15	9/17/15	5.81

Appendix B, Chemistry Tables.

All chemistry data was retrieved from the US Geological Survey National Water Information System at waterdata.usgs.gov

Table B1. Organic waste water indicators, ng/L. Maximum measured during 10-week sampling period April-June 2015.

Stream USGS Site no.	3_4-dichlorophenyl isocyanat	3-beta-coprostanol	3-methyl-1h-indole	4-nonylphenol (all isomers)	4-nonylphenol diethoxylate	5-methyl-1h-benzotriazole	9_10-anthraquinone	Beta-sitosterol	Bisphenol a	Camphor	Carbazole	Cholesterol	Diethyl phthalate	Fluoranthene	Fyrol fr 2	Hhcb
Burnt 14211902	-	-	0	-	-	-	0.02	0.44	-	-	-	0.41	0.15	0.01	-	-
Church 12170000	-	-	-	0.09	-	-	-	-	-	-	-	0.23	0.26	-	-	-
Coulter 12073895	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EF Dairy 14205400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Harris 12149490	-	-	-	-	-	-	-	-	-	-	-	0.18	-	-	-	-
Issaquah 12120600	-	-	-	-	-	-	-	0.28	-	-	-	0.18	0.16	-	-	-
Jenkins 12110495	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-
Kelly 14211499	-	-	-	-	-	-	-	-	-	-	-	0.37	-	-	-	0.01
Longfellow 12113490	-	-	0	-	-	-	-	0.39	-	-	-	0.32	-	-	-	-
May 12119495	0.02	-	0	-	-	-	-	0.32	-	-	0	0.44	-	0	-	-
Mercer 12120000	-	0.17	0.01	-	0.31	0.06	0.02	0.53	-	-	-	0.58	-	0.01	0.04	0.01
Rock 12117700	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Swamp 12126910	-	-	-	0.1	-	-	-	-	-	-	-	0.39	-	-	-	-
Thorton 12128000	0.02	0.14	0.01	-	-	0.04	0.02	0.65	-	0.03	-	1.89	-	0.01	0.08	0.01
Woodland 12080800	-	-	0	-	-	-	-	0.31	0.03	-	-	0.78	0.16	-	-	-

Table B1, cont. Organic waste water indicators, ng/L. Maximum measured during 10-week sampling period April-June 2015.

Stream USGS Site no.	Isophorone	N_n-diethyl-m-toluamide	Naphthalene	P-cresol	Pentachlorophenol	Phenanthrene	Prometon	Pyrene	Tbep	Tetrachloroethene	Tribromomethane	Tributyl phosphate	Isophorone
Burnt 14211902	-	0.01	-	-	-	-	-	0.01	-	-	-	-	-
Church 12170000	-	-	-	-	-	-	-	-	-	-	0.01	-	-
Coulter 12073895	-	-	-	-	-	-	-	-	-	-	-	-	-
EF Dairy 14205400	-	-	-	-	-	-	-	-	-	-	-	-	-
Harris 12149490	-	0.01	-	-	-	-	-	-	-	-	-	-	-
Issaquah 12120600	-	0.01	-	0.05	-	-	-	-	-	-	-	-	-
Jenkins 12110495	-	-	-	-	-	-	-	-	-	0.04	0.01	-	-
Kelly 14211499	-	0.01	-	-	-	-	-	-	-	-	0.01	-	-
Longfellow 12113490	-	-	-	0.02	-	-	-	-	-	-	-	-	-
May 12119495	0	0.01	0.03	-	-	0.01	-	0	-	-	0.01	-	0
Mercer 12120000	0.01	0.01	-	-	0.17	-	-	0.01	-	-	-	0.01	0.01
Rock 12117700	-	-	-	-	-	-	-	-	-	-	-	-	-
Swamp 12126910	0.01	0.01	-	-	-	-	0.06	-	-	-	0.01	0.02	0.01
Thorton 12128000	0	-	-	0.02	-	-	-	0.01	-	0.11	0.01	0.01	0
Woodland 12080800	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B2. Pharmaceuticals in surface water, ng/L. Maximum measured during 10-week sampling period April-June 2015.

Stream USGS Site no.	Acetominophen	Caffeine	Carbamazepine	Cotinine	Metformin	Nicotine	Methylbenzotriazole
Burnt 14211902	-	59.21	1.98	1.92	5.76	73.28	120.1
Church 12170000	5.67	11.09	0.64	2.82	22.95	74.3	8.99
Coulter 12073895	-	-	-	-	-	-	-
EF Dairy 14205400	-	-	-	-	-	-	-
Harris 12149490	-	-	-	-	2.4	76.68	-
Issaquah 12120600	-	1125	-	-	3.85	58.66	10.73
Jenkins 12110495	-	18.22	0.99	-	-	4.04	-
Kelly 14211499	6.13	65.21	0.87	2.4	30.23	33.65	6.75
Longfellow 12113490	-	18.91	-	1.82	14.12	-	82.68
May 12119495	-	-	7.45	-	5.67	62.75	-
Mercer 12120000	22.1	25.52	-	3.17	39.64	30.98	76.38
Rock 12117700	-	-	-	-	-	-	-
Swamp 12126910	-	17.92	2.84	4.65	27.95	89.29	142.0
Thorton 12128000	-	10.62	-	1.09	7.69	23.82	76.12
Woodland 12080800	-	7.44	3.79	-	-	59.24	6.72

Table B3. Pesticides in surface water, ng/L. Maximum measured during 10-week sampling period April-June 2015.

Stream USGS Site no.	2_4-d	2chloro4isopropylamino_triazine	2hydroxy4isopropylamino-triazine	2hydroxy4isopropylaminoethylamino-triazine	3_4-dichlorophenylurea	4-hydroxychlorothalonil	Acetochlor	Aldicarbsulfone	Atrazine	Azinphos-methyl	Azoxystrobin	Bromacil	Carbaryl	Carbendazim	Chlorpyrifos	Chlorsulfuron	Demethylhexamzinone	Demethylnorfluraz
Burnt 14211902	258.2	6.54	1.02	5.61	141.9	-	-	-	5.64	-	1.94	2.93	3.96	29.52	-	-	-	-
Church 12170000	43.78	-	-	4.67	-	-	-	-	3.18	-	-	-	10.72	3.51	-	-	0.56	-
Coulter 12073895	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EF Dairy 14205400	-	-	-	-	-	-	-	-	-	-	-	0.76	-	-	7.94	-	6.86	-
Harris 12149490	-	-	-	-	-	-	3.28	-	-	-	-	10.53	-	-	-	-	-	-
Issaquah 12120600	679.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.84	-
Jenkins 12110495	-	-	-	-	-	11.81	-	-	4.89	-	-	-	-	-	-	-	-	-
Kelly 14211499	217.5	-	0.9	21.69	-	-	-	-	5.48	-	1.33	-	4.69	12.09	-	-	6.69	46.44
Longfellow 12113490	576.0	-	-	0.63	-	-	-	-	-	-	-	-	142.2	37.64	-	-	-	-
May 12119495	263.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mercer 12120000	74.91	-	-	7.15	-	-	-	-	1.89	-	-	2.02	7.48	13.27	-	33.95	-	-
Rock 12117700	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Swamp 12126910	197.9	-	-	4.6	-	-	-	20.29	0.85	-	-	-	12.9	16.44	-	75.31	-	-
Thorton 12128000	-	-	-	2.64	-	-	-	-	2.43	-	-	-	-	23.59	-	14.57	-	-
Woodland 12080800	15.29	-	-	-	-	-	-	-	-	3.85	-	-	-	-	-	-	1.67	-

Table B3, cont. Pesticides in surface water, ng/L. Maximum measured during 10-week sampling period *April-June 2015.*

Stream USGS Site no.	Desulfinylfipronil	Diazinon	Diflubenzuron	Dimethenamid	Diuron	Ethoprop	Fipronilamide	Fipronilsulfide	Fipronilsulfone	Fipronil	Hexazinonetransfl	Hexazinone	Hydroxysimazine	Imazamox	Imidacloprid	Malathion	Metalaxyl	Methoxyfenozide
Burnt 14211902	1.89	1.49	-	-	25012	3.07	-	1.77	2.03	2.91	-	-	5.6	-	-	-	-	-
Church 12170000	-	-	-	-	-	-	-	-	-	-	-	-	3.48	-	-	-	-	-
Coulter 12073895	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EF Dairy 14205400	-	-	-	-	-	-	-	-	-	-	-	7.55	-	-	-	9.63	-	-
Harris 12149490	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Issaquah 12120600	-	-	-	-	-	-	-	-	-	-	-	1.58	-	-	-	-	-	-
Jenkins 12110495	-	-	-	0.23	17.95	-	3.33	-	2.09	-	-	0.68	-	-	-	-	-	-
Kelly 14211499	-	-	-	-	4.35	-	-	-	-	-	-	3.82	34.24	-	-	-	0.37	-
Longfellow 12113490	-	-	-	-	15.17	-	-	-	1.42	2.64	-	-	-	-	-	-	0.89	-
May 12119495	-	-	1.65	-	9.99	-	-	-	2.06	0.68	-	-	1.96	-	-	-	-	-
Mercer 12120000	-	-	-	-	3.94	-	-	1.24	1.21	1.97	-	-	6.59	-	-	-	-	-
Rock 12117700	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Swamp 12126910	-	2.05	-	-	6.11	-	-	0.71	1.72	1.1	-	-	5.5	-	-	-	-	-
Thorton 12128000	-	-	-	-	15.36	-	-	-	-	1.49	-	-	3.3	-	20.17	-	-	0.42
Woodland 12080800	-	-	-	-	-	-	-	-	0.8	-	-	2.78	-	2678	-	-	-	-

Table B3, cont. Pesticides in surface water, ng/L. Maximum measured during 10-week sampling period April-June 2015.

Stream USGS Site no.	Metolachlorsulfonic	Metolachlor	Myclobutanil	N_3_4-dichlorophenylmethylurea	Norflurazon	Oryzalin	Piperonylbutoxide	Prometon	Propanil	Propiconazole	Simazine	Sulfentrazone	Sulfometuron-methyl	Tebuconazole	Tebupirimfosoxon	Tebuthiuron104	Tebuthiuron108	Tebuthiuron	Trans-permethrin	Triclopyr
Burnt 14211902	-	2.85	-	1053	-	38.34	-	2.29	-	13.56	26.82	-	1794	2.93	-	0.71	12.84	9.07	-	357.3
Church 12170000	-	-	-	0.83	-	-	-	1.52	-	-	-	-	-	-	-	-	-	1.04	-	-
Coulter 12073895	-	-	-	-	-	-	9.41	-	-	-	-	-	-	-	-	-	-	-	-	-
EF Dairy 14205400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Harris 12149490	-	4.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Issaquah 12120600	-	4.34	-	-	-	-	-	-	-	-	-	-	6.48	-	-	-	-	-	-	221.2
Jenkins 12110495	-	-	-	1.02	-	-	-	2.24	-	-	5.44	-	6.08	-	-	-	-	8.07	-	-
Kelly 14211499	-	-	-	2.25	12.64	1056.11	-	2.62	-	5.02	11.21	9.21	2.07	-	0.32	-	-	2.69	-	229.7
Longfellow 12113490	-	6.63	-	10.89	-	-	-	4.2	6.36	17.71	-	-	-	4.22	-	-	-	1.52	-	126.6
May 12119495	18.03	5.16	2.04	4.11	-	-	-	5.27	-	3.29	1.00	-	12.82	8.05	-	-	-	-	1.27	95.44
Mercer 12120000	-	5.89	-	1.76	-	-	-	3.25	-	8.18	-	-	64.04	4.21	-	-	-	3.86	-	30.23
Rock 12117700	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Swamp 12126910	-	8.27	-	3.8	-	-	-	5.24	-	-	-	-	139.0	-	-	-	-	-	-	21.32
Thorton 12128000	-	5.56	-	5.84	-	-	2.21	3.92	-	5.37	-	-	33.23	-	-	-	-	0.46	0.83	36.41
Woodland 12080800	-	8.48	-	-	-	-	-	0.32	-	-	-	-	-	-	-	-	-	1.8	-	-

Table B4. Polycyclic aromatic hydrocarbons in sediment, µg/kg.

Stream USGS Site no.	Date	1-Methylphenanthrene	1-Methylpyrene,	1,2-Dimethylnaphthalene	1,6-Dimethylnaphthalene	2-Methylanthracene	2,3,6-Trimethylnaphthalene	2,6-Dimethlnaphthalene	9,10-Anthraquinone,	9H-Fluorene	Acenaphthene	Anthracene, solids,	Benzo_a_anthracene	Benzo_a_pyrene,	Benzo_b_fluoranthene,	Benzo_def_fluorene	Benzo_e_pyrene,	Benzo_ghi_perylene	Benzo_k_fluoranthene,
Burnt 14211902	7/1/15	6.4	12.4	-	-	-	-	12.4	45.7	5.5	-	17.6	59.6	58.2	114	13.6	67.8	57.5	42.2
Church 12170000	6/19/15	-	-	-	3.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coulter 12073895	6/23/15	-	-	-	-	-	-	15.5	-	-	-	-	-	-	-	-	-	-	-
EF Dairy 14205400	6/30/15	-	-	-	4.3	-	-	49.2	-	-	-	-	9.1	-	-	-	-	-	-
Harris 12149490	6/22/15	-	-	-	-	-	-	-	11.8	-	-	-	-	-	-	-	-	-	-
Issaquah 12120600	6/15/15	7.5	-	-	7.9	-	-	7.1	-	-	-	-	-	-	-	-	-	-	-
Jenkins 12110495	6/16/15	54	30.7	12.7	42.6	-	-	37.2	148	68.4	69	87.1	156	127	161	61.3	85.8	41.1	60
Kelly 14211499	7/2/15	4.6	7.4	-	-	-	-	4.2	21.2	-	-	7.7	24	33	42.2	4.8	32.5	32.6	13.2
Longfellow 12113490	6/21/15	10.9	-	-	8.2	-	-	6.9	31.9	4.2	-	11.2	36.8	46.2	64.7	8.1	43.1	36.4	25.3
May 12119495	7/14/15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mercer 12120000	6/18/15	114	92.1	-	18.5	225	19.3	29.4	142	147	67.5	960	640	306	488	281	259	128	208
Rock 12117700	6/19/15	126	-	26.8	127	-	49.3	43.4	-	-	-	-	20.4	-	29.6	-	30.5	-	-
Swamp 12126910	7/9/15	-	-	-	-	-	-	33.4	-	-	-	-	-	-	12.4	-	-	-	-
Thorton 12128000	6/20/15	9.5	-	-	7.1	4.4	-	12.4	73.2	8.4	-	14.3	55.9	70.4	112	14.3	69	70.8	37.3
Woodland 12080800	6/24/15	3.3	-	-	-	-	-	11.3	18.3	-	-	-	-	-	-	-	9	-	-

Table B4, cont. Polycyclic aromatic hydrocarbons in sediment, µg/kg.

Stream USGS Site no.	Date	Bis(2-ethylhexyl)phthalate	Carbazole	Chrysene	Dibenzothiophene	Fluoranthene	Indenopyrene	Naphthalene	Perylene	Phenanthrene,	Phenanthridine	Pyrene
Burnt 14211902	7/1/15	347	22	79.4	-	181	49.3	-	56.9	69.4	-	155
Church 12170000	6/19/15	-	-	-	-	-	-	-	26.1	-	-	-
Coulter 12073895	6/23/15	-	-	-	-	-	-	-	42.5	-	-	-
EF Dairy 14205400	6/30/15	-	-	11.3	-	22.8	-	-	16.3	-	-	22.2
Harris 12149490	6/22/15	-	-	-	-	-	-	-	26	-	-	-
Issaquah 12120600	6/15/15	-	-	-	-	-	-	-	86.2	20.2	-	-
Jenkins 12110495	6/16/15	-	77.1	213	-	422	45.4	117	48.8	436	20	386
Kelly 14211499	7/2/15	-	7.8	27.4	-	65.8	25.8	-	17	33.3	-	80.8
Longfellow 12113490	6/21/15	-	-	53.3	-	88.7	33.7	-	27.1	52.8	-	83.8
May 12119495	7/14/15	-	-	8.8	-	20.2	-	-	17.7	-	-	17
Mercer 12120000	6/18/15	-	119	1100	61.2	1930	130	148	742	916	-	1450
Rock 12117700	6/19/15	-	-	70.7	19.5	31.4	-	50.7	25.1	265	-	36.1
Swamp 12126910	7/9/15	-	-	8.9	-	-	-	-	20.3	-	-	13.4
Thorton 12128000	6/20/15	243	25.2	90.5	-	185	66.2	-	56.9	82.9	-	163
Woodland 12080800	6/24/15	-	-	9.8	-	17.7	-	-	14.1	-	-	15.2

Table B5. Halogenated compounds in sediments, µg/kg.

Stream USGS Site no.	Date	Chlorpyrifos	Cyfluthrin	DCPA	Desulfinylfipronil	Dieldrin,	Fipronil sulfide	lambda-Cyhalothrin	p,p'-DDD	p,p'-DDE,	p,p'-DDT,	Pendimethalin	Pentabromotoluene	Sum_18_PCBs	Sum_5_Chlordanes	Sum_9_PBDEs	TBE	Tetradifon	Trifluralin
Burnt 14211902	7/1/15	0.1	-	-	0.04	0.68	0.04	-	6.2	2.9	1.6	1.25	-	1.72	1.09	2.31	0.05	-	0.05
Church 12170000	6/19/15	-	-	-	0.01	-	-	-	-	-	-	-	-	1.05	-	-	-	-	-
Coulter 12073895	6/23/15	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	0.06	-	-	-
EF Dairy 14205400	6/30/15	-	-	-	-	0.08	-	-	-	-	-	-	-	0.04	0.09	-	-	-	-
Harris 12149490	6/22/15	-	-	-	-	-	-	-	-	-	-	-	0.12	1.11	0.03	-	-	-	-
Issaquah 12120600	6/15/15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Jenkins 12110495	6/16/15	-	-	-	-	0.54	-	-	-	-	-	-	-	1.57	0.35	0.06	-	-	-
Kelly 14211499	7/2/15	-	-	0.06	-	2.82	-	-	2.97	6.05	-	-	-	0.2	1.08	-	-	-	-
Longfellow 12113490	6/21/15	0.12	-	-	-	0.61	-	-	-	6.03	25.1	-	-	62.04	5.43	5.38	0.17	-	0.02
May 12119495	7/14/15	-	-	-	-	-	-	-	-	-	-	-	-	0.41	0.79	-	-	-	-
Mercer 12120000	6/18/15	-	-	-	-	-	-	-	-	-	2.04	-	-	0.21	0.94	0.16	-	-	-
Rock 12117700	6/19/15	-	-	-	-	0.01	-	-	-	-	-	-	-	0.27	0.03	-	-	-	-
Swamp 12126910	7/9/15	-	-	-	-	0.1	-	-	-	-	-	-	-	0.32	0.18	-	-	-	-
Thorton 12128000	6/20/15	0.05	1.27	0.01	0.04	1.3	-	0.23	8.74	5.58	6.02	-	-	7.81	5.98	0.48	0.12	0.24	0.14
Woodland 12080800	6/24/15	-	-	-	-	0.2	-	-	-	-	-	-	-	26.78	0.33	-	-	-	-

Appendix C, RNAseq Analysis

Coho salmon (*Oncorhynchus kisutch*) is a non-model organism without a fully sequenced genome. To determine gene targets for the nanoString assay used in Chapter 3 a fully sequenced and annotated transcriptome was needed. Additionally, we were able to use the differential expression of genes observed in a smaller subset of streams to target genes that we wanted investigate in more detail with the nanoString assay (see chapter 3). The methods and results for this RNAseq analysis are detailed here.

Methods

RNA Isolation

Hepatic RNA was isolated from 24 fish for RNAseq analysis, six each from Coulter, Issaquah, Jenkins, and Swamp Creeks. Fish were sampled and processed under the auspices of the University of Washington IACUC protocol 3286-21. Liver tissue was immediately transferred into 1.5mL snap-tubes and homogenized using Teflon pestles in RNAzol RT solution upon removal from the freezer. Isolation followed manufacturer methods (Molecular Research Center Inc. 2015). 50uL of 0.1% DEPC treated water was used to dissolve RNA after isolation. RNA concentration and quality for each sample was checked using a Nanodrop and further treated with Turbo DNA-free to remove genomic DNA contamination. Resulting total RNA samples were diluted to 100ng/L and transferred to the University of Washington Genomics department for library preparation and sequencing. The facility ran RNA samples on an Aligent Bioanalyzer 2100 to check for degradation. All 24 samples had a RIN >7. An Illumina RNA TrueSeq kit was used to create sequencing libraries resulting in insert sizes with less than 300

base pairs. Libraries (n=24) were multiplexed with unique barcodes and run over four lanes on an Illumina NextSeq500 resulting in paired-end reads 150bp in length.

Sequencing and Transcriptome Assembly

Transcriptome Assembly

Raw read files were combined from individual fish across the four lanes of sequencing into two fastq files for an individual, one for each read direction. These FASTQ files were checked for quality with FastQC v0.11.5 and parsed with TrimGalore! v.0.4.2 (Krueger, 2016) using the default settings to remove adapter sequences and errant reads retaining only the paired reads. Trinity v2.06 (Grabherr et al. 2011) was used on the National Center for Genome Analysis Support Galaxy server ‘’(Afgan et al. 2016) to assemble the transcriptome. The assembly used default settings for paired-end reads and did not specify strandedness. The Trinity assembler combines short reads into full-length sequence transcripts known as contigs. The resulting assembled transcriptome was assessed for completeness using TransRate (Smith-Unna et al., 2016)

Annotation

Contigs were identified and annotated by searching for closely matching protein sequences from closely related salmonid species including rainbow trout (*Oncorhynchus mykiss*) and Atlantic Salmon (Salmo salar) for general annotation. We performed an addition annotation using the UniProt Swiss-Prot database for use in Gene Ontology (GO) analysis (too few annotated salmon genes had assigned GO terms for this analysis). The transcriptome was annotated using BLASTx (Camacho et al. 2008) using a custom salmonid UniProt database downloaded from uniprot.org. This database was generated by downloading all protein sequences for any salmonid species and included sequences from both the Swiss-Prot and

TrEMBL databases (Uniprot Consortium, 2017). The BLASTx search was limited to the single closest match for each contig with only a single high-scoring segment pair (HSP) reported. The assembled transcriptome, blast annotations, and custom blast database can be retrieved from the OSF repository (Spanjer, 2017).

Differential Expression Analysis

DESeq2 (Love et al. 2014) package in R v3.3.2 (R Core Team 2016) was used for differential expression analysis among the four creeks with fish from the same creek treated as biological replicates (n=6). First, reads matching each contig for individual fish were counted by pseudo-aligning back to the assembled transcriptome using kallisto v0.43.0 (Bray et al. 2016). The counts from different fish were merged into a matrix and analyzed for differential expression among streams using DESeq2. As part of the DESeq2 pipeline, counts were normalized based on sequencing depth, and a negative binomial generalized linear model was used to test for differential expression. Coulter Creek was considered a reference stream for all pairwise comparisons. Differential expression results were significant if the adjusted p-value ≤ 0.1.

Enrichment Analysis

Enrichment analysis was performed to determine functional over-representation in differentially expressed genes (DEG) for each of the three pairwise comparisons (padj ≤ 0.1). The Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang et al. 2009) used uploaded UniProt accession codes for all annotated genes in the transcriptome as "background" to determine enrichment in each DEG set (significant enrichment determined at the $p<0.1$ level). Gene lists consisted of accession codes from separate pairwise comparisons. The resulting enriched functional annotation set was retrieved and used to assess gene functions of differentially expressed genes.

Results

RNAseq

Transcriptome and Annotation

The NextSeq500 produced >400 million paired reads across four lanes of sequencing. Trimmed and quality checked FASTQ files for each coho can be found in the National Center for Biotechnology Information Sequence Read Archive under BioProject PRJNA397730. The Trinity Assembly produced ~356k contigs with a mean length of 824 nucleotides and a total length of 293,356,238 nucleotides (Table C1). 303,905 contigs were annotated.

Differential Expression

Ordination of normalized count data across all fish used principal component analysis (Figure C1). Although the first two axes explain less than 10% of variation across samples, the separation of individuals by stream location suggests that environmental conditions unique to each sampling location influenced expression. Differential expression varied by pairwise comparison as well (Figure C2). As compared to Coulter, Issaquah had 1169 DEGs, Jenkins Creek had 188 DEGs, and Swamp Creek 850 DEGs. Figure C3 shows the comparison of down- and up- regulated DEGs across all streams and suggests that Jenkins and Swamp, the two urbanized streams, cluster closer together based on DEGs. Functional analysis of DEGs showed a range of functional enrichment depending on location. Figures C4-C6 for each stream shows the top 15 enriched terms for each of the three Gene Ontology categories based on corrected p-values. In the Issaquah vs. Coulter comparison, genes involved in “immune response” and “oxidation-reduction process” topped the biological process enrichment list; in Jenkins vs. Coulter genes involved in “oxidation-reduction,” “transport," and “mRNA” processing topped

the biological process enrichment list; and similarly for Swamp vs. Coulter, "oxidation-reduction" was at the top followed by "metabolic processes."

Data References

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Tables

Table C1. Summary statistics for Trinity assembled hepatic coho salmon (*Oncorhynchus kisutch*) transcriptome. Transcriptome was assembled from >600 million 150bp PE short reads from total RNA isolated from 24 individual juveniles (year-0) fish livers sampled at 4 different small perennial streams that drain into the Salish Sea.

TRANSRATE METRIC	
Assembly	CohoTranscriptome.fasta
N seqs	356147
Smallest (nt)	201
Largest (nt)	14674
N bases	293356238
Mean length	823.69426
N-under 200 nt	0
N-over 1k nt	83000
N-over 10k nt	82
N-with orf	73387
Mean-orf-percent	49.45142
90 percentile length	307
70 percentile length	726
50 percentile length	1544
30 percentile length	2550
10 percentile length	4304
%Gc content	45.823%

Figures

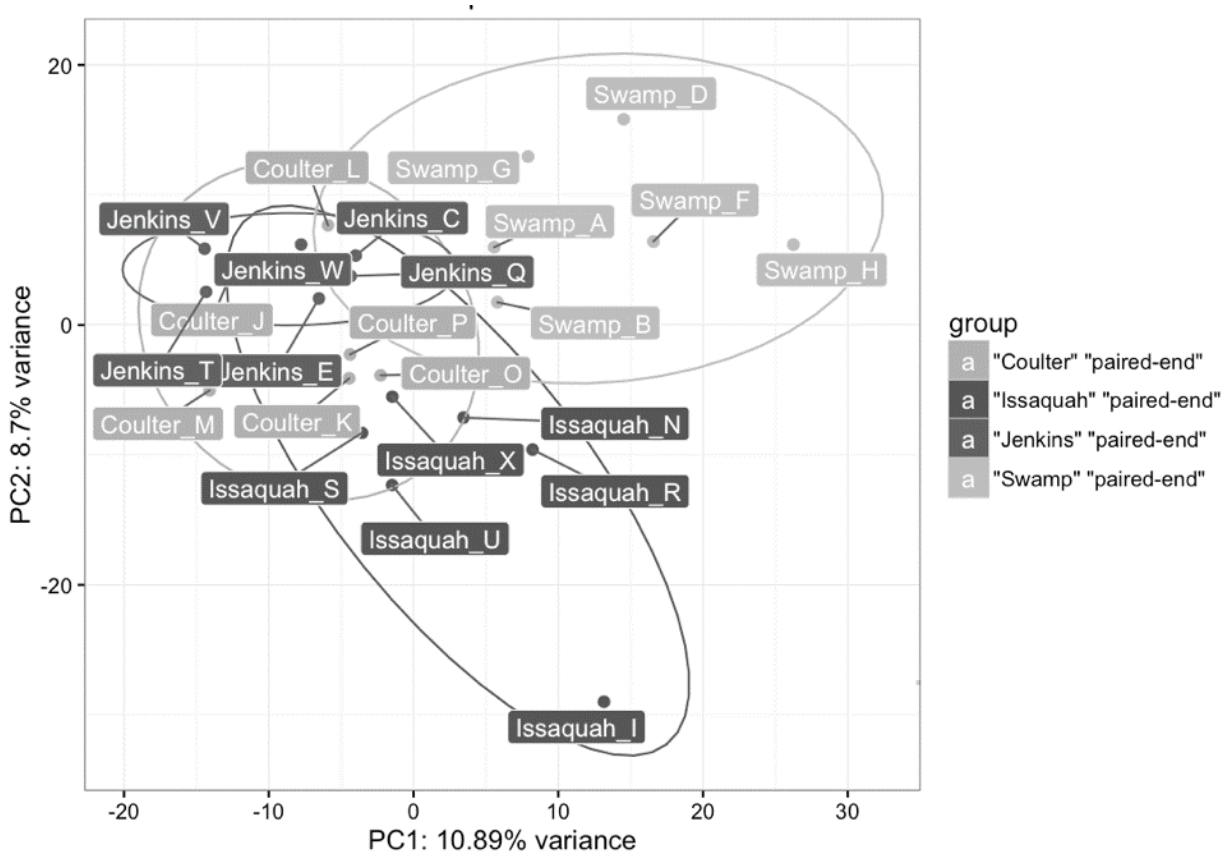

Figure C1. Principal Component Analysis of raw Kalisto mapped counts for 24 juvenile coho salmon (*Oncorhynchus kisutch*) grouped by sample location. All reads that mapped back to the assembled transcriptome were used to investigate how samples varied in gene expression by location, thus showing that some variance in gene expression is likely due to sampling location.

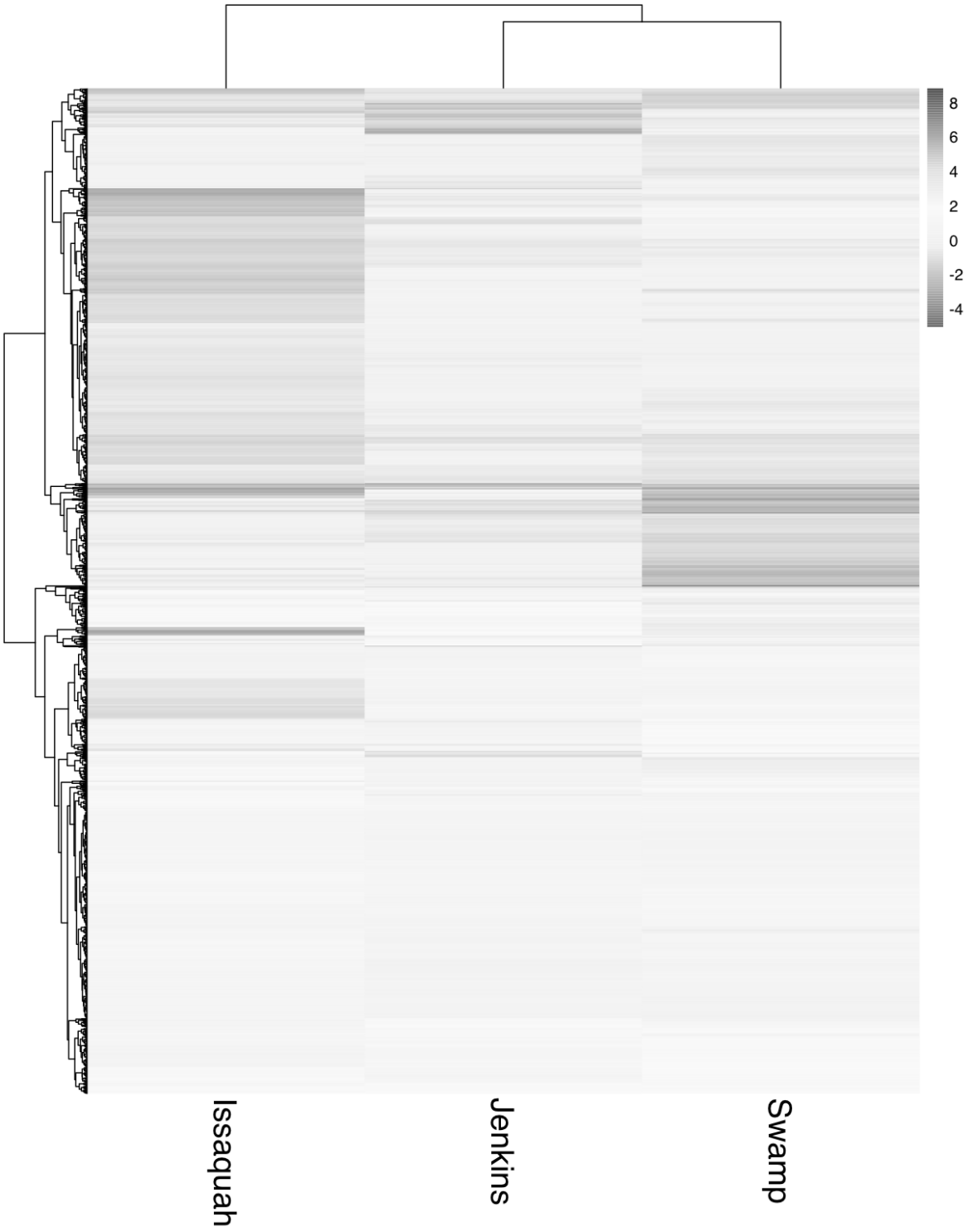

Figure C2. Log2 fold change for differentially expressed genes (DEGs) as compared to reference stream (Coulter Creek). All comparisons are pairwise to the reference stream at padj=0.05. If a gene was significant for any of the three comparisons, the log2 fold change is included for all three streams in this heatmap. Contigs are roughly even split by up/down regulation. Fish from Jenkins and Swamp are more similar in their DEGs than Issaquah.

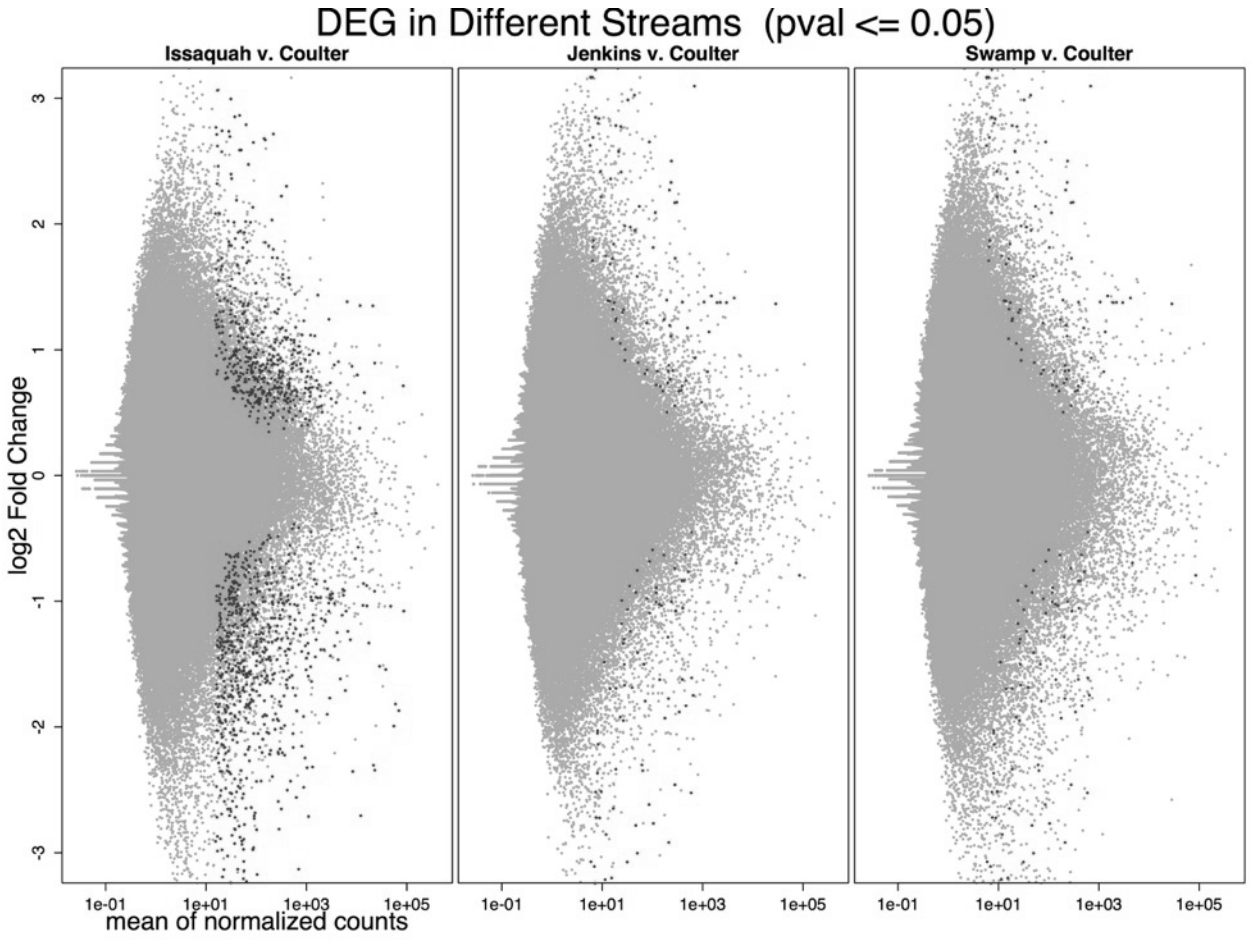

Figure C3. Volcano plot showing proportion of significant (padj ≤0.05) differentially expressed genes (DEGs) as compared to total expression pattern among all streams. Issaquah shows a much higher proportion of significant DEGs than Jenkins or Swamp.

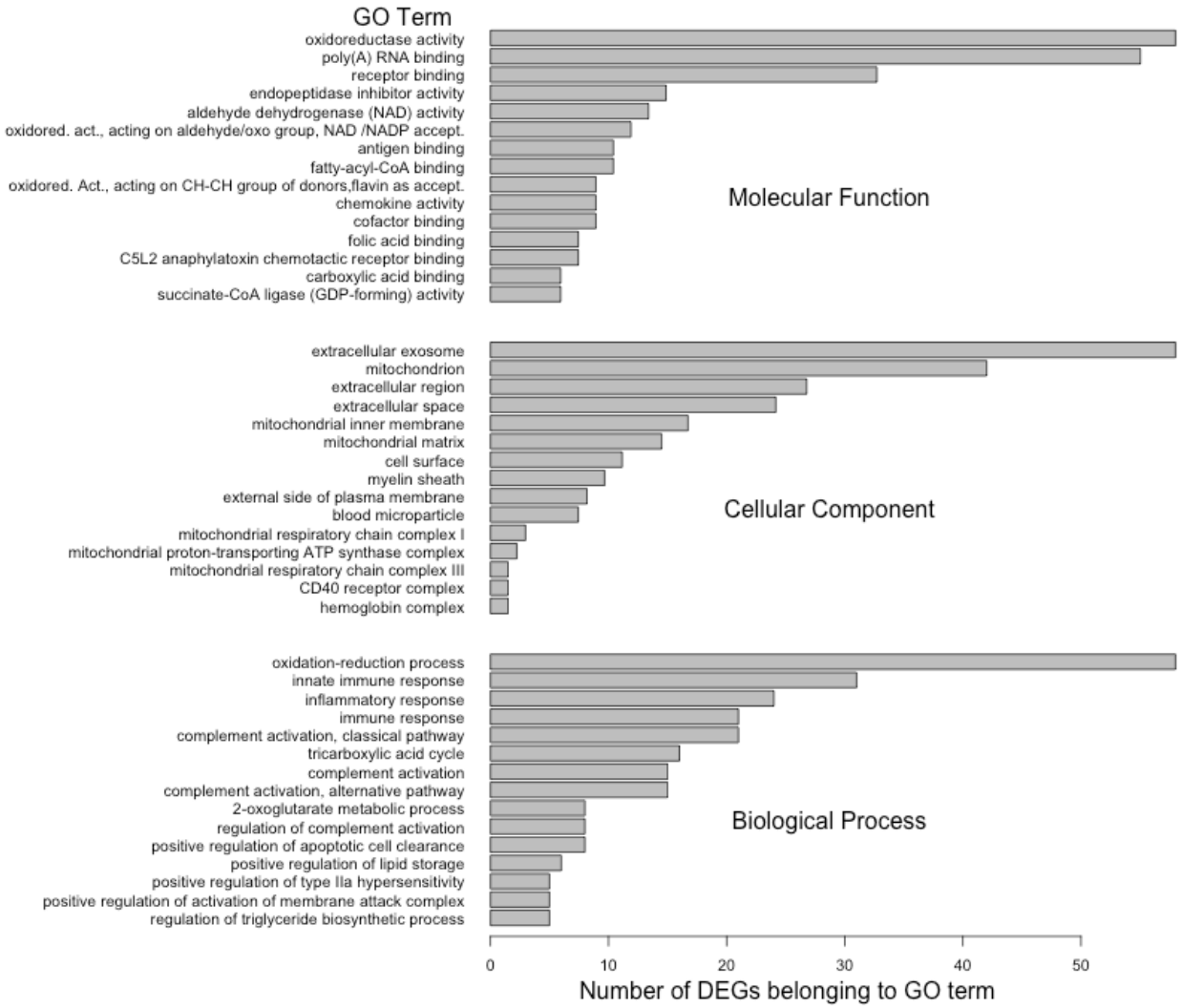

Figure C4. Top Gene Ontology terms for significant differentially expressed genes, from RNA sequencing, between Issaquah and Coulter Creeks. Top 15 terms for each GO category (molecular function, cellular component, and biological process). Top 15 terms for each were determined by lowest enrichment pvalue reported by DAVID as compared to GO terms representing whole transcriptome. Bar length indicates the number of DEGs belonging to each term

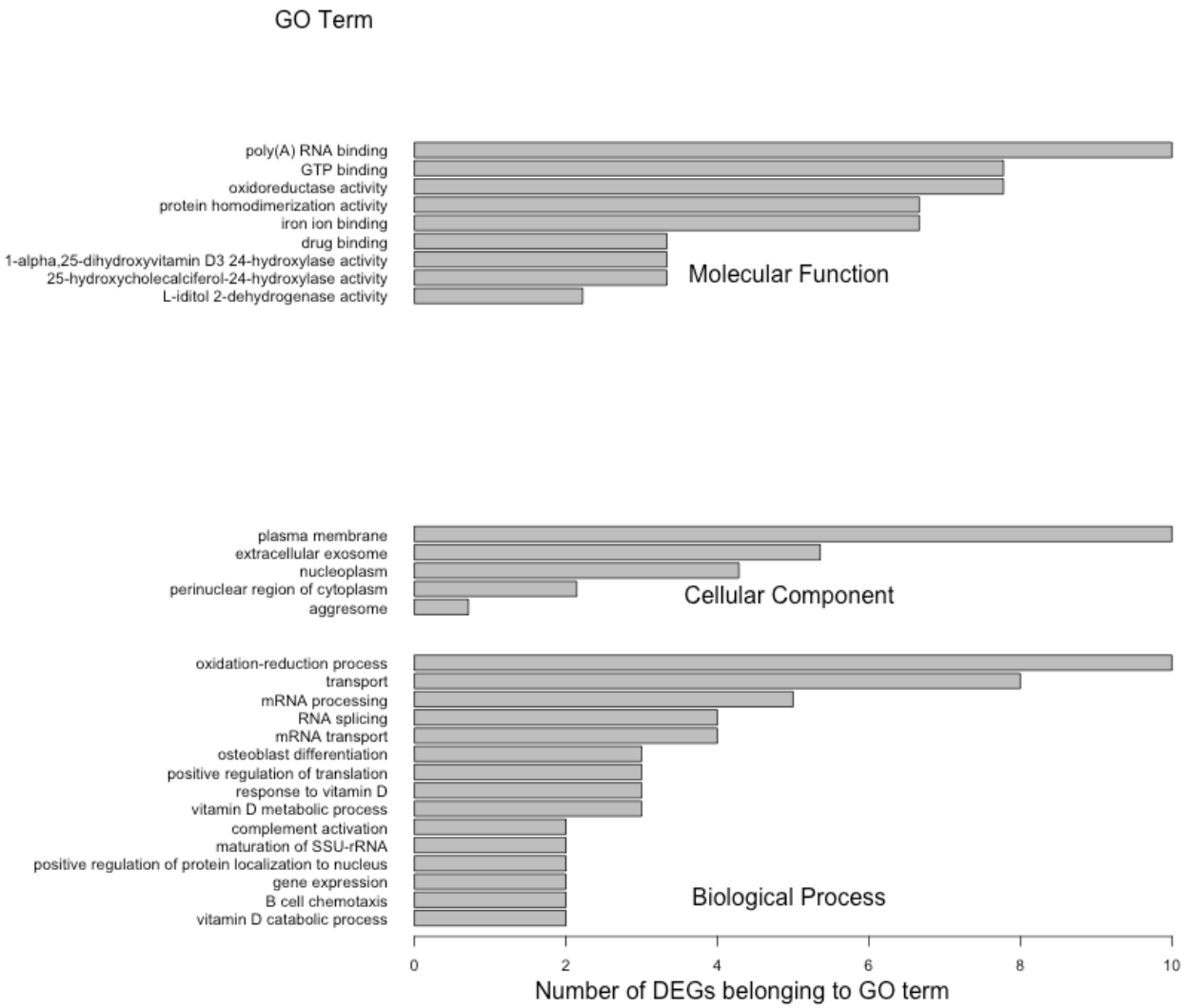

Figure C5. Top Gene Ontology terms for significant differentially expressed genes, from RNA sequencing, between Jenkins and Coulter Creeks. Top 15 terms for each GO category (molecular function, cellular component, and biological process). Top 15 terms for each were determined by lowest enrichment pvalue reported by DAVID as compared to GO terms representing whole transcriptome. Bar length indicates the number of DEGs belonging to each term

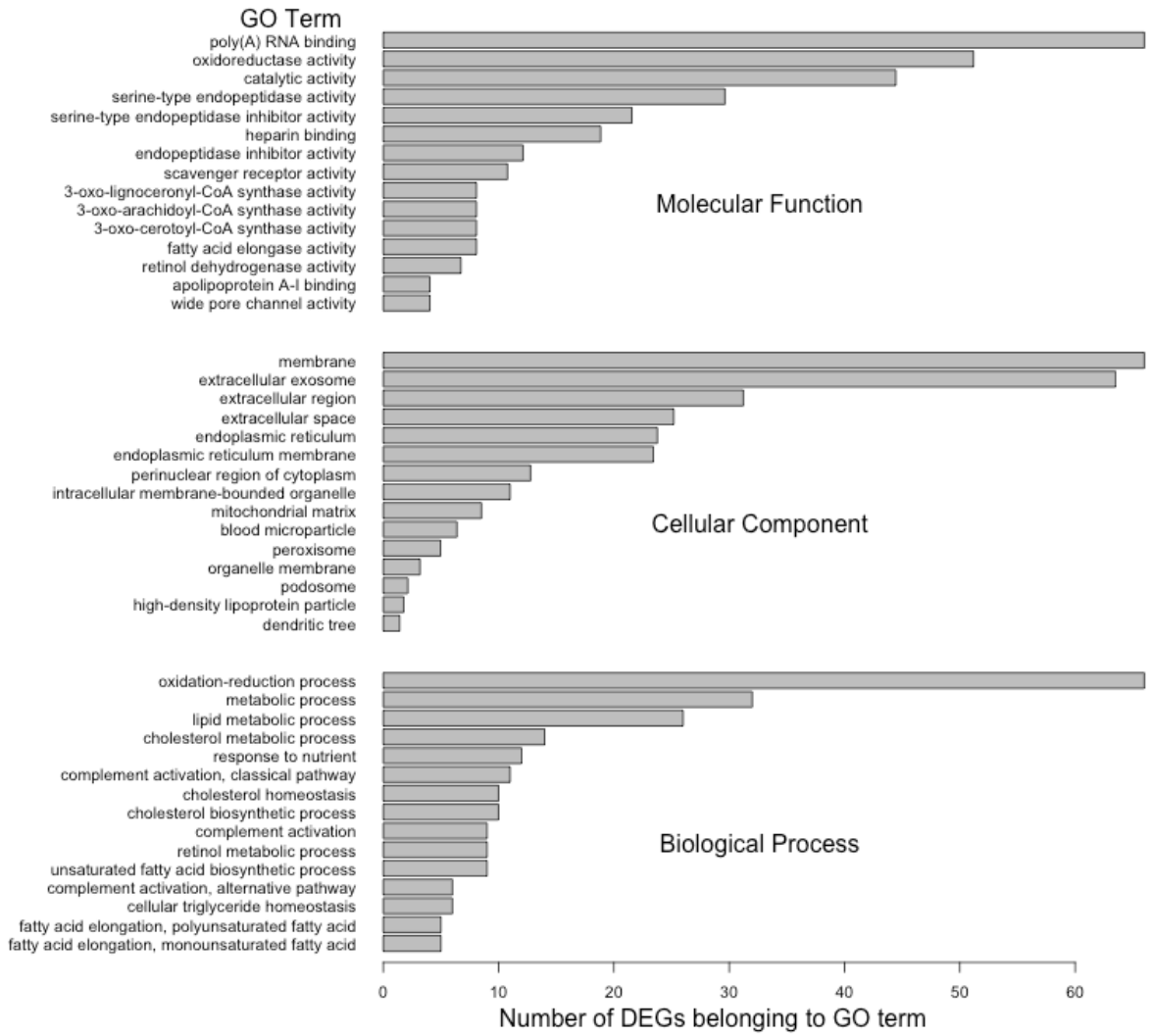

Figure C6. **Top Gene Ontology terms for significant differentially expressed genes between Swamp and Coulter Creeks.** Top 15 terms for each GO category (molecular function, cellular component, and biological process). Top 15 terms for each were determined by lowest enrichment pvalue reported by DAVID as compared to GO terms representing whole transcriptome. Bar length indicates the number of DEGs belonging to each term