# ANTHROPOGENICALLY-SOURCED LOW CONCENTRATION PAHS: *IN SITU* BIOAVAILABILITY TO JUVENILE PACIFIC SALMON

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# Abstract

Gill 7-ethoxyresorufin O-deethylase (EROD) activity of juvenile Chinook salmon caged in Auke Lake, AK was used as a biomarker of polycyclic aromatic hydrocarbon (PAH) exposure. Biomarker measurements in conjunction with a comprehensive sampling program that included grab water and sediment samples, and passive sampling devices were used to determine PAH concentrations, source(s), bioavailability, and resulting biological response. PAHs were detected at all lake locations except the reference site upstream of anthropogenic activity. Water samples were the best predictor of a biological response and EROD activity correlated to corresponding parts per trillion water pyrene concentrations ($r^2$=0.9662; p=0.0004). Sediment samples yielded the clearest indication of PAH sources and amalgamated contaminant magnitude, and passive samplers served as accumulators of retrospective aqueous conditions. Results suggest that salmon stocks are being exposed to chronic low-concentrations of anthrogenically-sourced PAHs during sensitive life-stages, which may be in part a contributor to their declining numbers.



**Keywords:** *PAHs, CYP1A, salmon, gill, biomarker, EROD*

*To my parents, who gave their children the gift of wilderness.*

*And,*

*to my siblings, my friends, and to Brandon for their smiles and invaluable support.*

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# 1: General Introduction

## 1.1 Biomarkers

Millions of tons of anthropogenically sourced contaminants enter aquatic systems each year, and many of these pose potential risks to aquatic organisms (Buhler and Williams, 1989; Schwarzenbach et al., 2006). Multiple influencing factors determine the bioavailability of a given xenobiotic to any particular organism (Erickson et al., 2008). These include: site conditions (e.g. organic matter [OC]), chemical properties (e.g. hydrophobicity), and biological factors (van der Oost et al., 2003; Erikson et al., 2008). Collectively, these can affect chemical(s) behaviour and contact with a site of absorption, the proportion absorbed and ultimately, the amount that reaches the site of toxic action (McElroy et al., 1989; Burgess et al., 2003; Fent, 2003; van der Oost et al., 2003; Erickson et al., 2008). Consequently, the analytically measurable quantity of a chemical in an environmental sample may not always be reflective of the fraction that interacts with a biological receptor (Fent, 2003; van der Oost et al., 2003; Schlenk et al., 2008). When contamination is suspected of producing adverse effects, evidence of *in situ* interaction with a biological receptor is an important preliminary step towards linking co-occurrence and causality (Collier, 2003; van der Oost et al., 2003; Suter et al., 2007). To this end, biological monitoring, or biomonitoring, is the practice of using organisms and organismal responses to assess interactions between environmental contaminants and receptors (van der

Oost et al., 2003). This type of monitoring can evaluate alterations at multiple biological levels ranging from exposure assessment (biomarker) to organismal (bioindicator) and population level effects (ecological indicator) (van der Oost et al., 2003).

Biomarkers were originally employed as diagnostic tool(s) for human diseases and their utility was later extended to the field of ecotoxicology (Sanchez and Porcher, 2009). As tools in environmental monitoring, biomarkers are measurable biological responses evoked following exposure to a contaminant and can serve as sub-individual indices of the relative amount of a contaminant bioavailable to organisms (Whyte et al., 2000; Altenburger et al., 2003; van der Oost et al., 2003; Hanson et al., 2006). Measured inside an ostensibly “healthy” organism, or in an excretory product, biomarkers indicate a xenobiotic-elicited physiological change and may serve as early warning signs of potentially larger-scale effects (Fent, 2003; van der Oost et al., 2003; Hanson et al., 2006; Schlenk et al., 2008).

For metabolizable xenobiotics, biotransformational enzyme activity or biotransformation products can serve as sensitive biomarkers of exposure (van der Oost et al., 2003). Some biotransformation enzymes respond in an adaptive manner and can be induced following exposure to specific xenobiotics (Schlenk et al., 2008). Cytochrome P450 1A (CYP1A) is present in multiple fish species and can be induced by various planar hydrocarbons amongst other compounds (Whyte et al., 2000). When compared to basal levels, CYP1A induction indicates that fish have been exposed to xenobiotics such as polycyclic aromatic

hydrocarbons (PAHs) and polychlorinated dibenzo *p*-dioxins (PCDDs) (Whyte et al., 2000). The extent of CYP1A induction is generally reflective of the relative concentration of inducer present and its bioavailability to an organism (van der Oost et al., 2003).

## 1.2 Cytochrome P450 1A

CYP1A is a member of the cytochrome P450 (CYPs) conserved superfamily of heme-containing isozymes present in both prokaryotes and eukaryotes (Stegeman and Hahn, 1994; Mansuy, 1998). The CYPs play an important role in synthesis or degradation of endogenous compounds including steroids and fatty acids; and, in the phase I metabolism of xenobiotics such as PAHs (Andersson and Förlin, 1992; Stegeman and Hahn, 1994; Backes and Kelley, 2003). CYP evolution is thought to have been in part, driven by the need of oraganisms to metabolize a diversity of plant toxins (Stegeman and Hahn, 1994; Whyte et al., 2000). These membrane bound enzymes are predominantly located in the endoplasmic reticulum of the liver, but are also expressed in kidney, vascular, gastrointestinal, cardiac, and gill tissue (Buhler and Williams, 1989; Porter and Coon, 1991; Stegeman and Hahn, 1994; Buhler and Wang-Buhler, 1998; Danielson, 2002; van der Oost et al., 2003; Young et al., 2005). Fish CYP1A is typically referred to at the sub-family level, due to the difficulty in enzymatically distinguishing between members (Stegeman and Hahn, 1994; Whyte et al., 2000). The fish CYP1A gene is suggested to be ancestral to the mammalian CYP1A1 and CYP1A2 genes (Stegeman and Hahn, 1994).

CYP1A induction occurs through the aryl hydrocarbon receptor (AhR) pathway (Whyte et al., 2000). The AhR is a conserved member of the basic helix-loop-helix/Per-ARNT-sim (bHLH-PAS) transcription factor family, present in teleosts and has been well characterized in the mammalian system (Nebert et al., 2000; Hahn, 2002; Hahn et al., 2005). The unbound soluble AhR is located in the cytosol complexed to multiple proteins (Hahn, 1998; Whyte, 2000; Hahn, 2002; Denison and Nagy, 2003). Upon ligand binding, the AhR translocates, separates from the protein complex and heterodimerizes with the AhR nuclear translocator (ARNT) (Miller and Ramos, 2001; Safe, 2001; Denison and Nagy, 2003; Hahn et al., 2005). The ligand/AhR/ARNT transcription factor complex can then bind to the xenobiotic response element (XRE) sequence in the regulatory region of CYP1A and other AhR-responsive genes (Safe, 2001; Dennison and Nagy, 2003; Hahn et al., 2005). Recent studies indicate that many teleosts have more than one AhR gene and that, in general, teleost AhRs undergo a similar process leading to CYP1A transcription as their mammalian counterparts (Hahn, 2002; Hahn et al., 2005).

The AhR binding affinity for a ligand generally determines its CYP1A induction capacity (Whyte et al., 2000; Billiard et al., 2002).The AhR binding site accommodates a diverse array of ligands (Dennison and Nagy, 2003). Although not typically strong ligands, various endogenous compounds such as tetrapyrroles, indoles and arachadonic acid metabolites can be AhR agonists (Dennison and Nagy, 2003). In addition, multiple planar hydrophobic exogenous compounds such as PAHs and halogenated aromatic hydrocarbons (HAHs) have

varying degrees of affinity for the AhR binding pocket, with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) being the most potent ligand characterized to date (Miller and Ramos, 2001; Dennison and Nagy, 2003).

Exposure to AhR ligands is followed by an increase in CYP1A mRNA transcript abundance, protein expression and subsequent catalytic activity (Andersson and Förlin, 1992; Stegeman and Hahn, 1994; van der Oost et al., 2003). In combination with membrane phospholipids, NADPH cytochrome P450 reductase, and often with cytochrome $b_5$, CYP mediated phase I biotransformation catalyzes the monooxygenation of relatively hydrophobic chemicals (Buhler and Williams, 1989; Stegeman and Hahn, 1994; Mansuy, 1998; Danielson, 2002; van der Oost et al., 2003). Through oxygenase activity, the CYPs may also participate in multiple other reactions including epoxidations, isomerizations, dehydrogenations, dealkylations and reductions (Stegeman and Hahn, 1994; Mansuy, 1998; Danielson, 2002). These structural modifications collectively facilitate phase II conjugation of a xenobiotic and/or increase hydrophilicity; ultimately enhancing the excretability of the compound (Buhler and Williams, 1989; Miller and Ramos, 2001; Danielson, 2002).

As a biomarker, CYP1A induction can be determined by assessing mRNA, protein abundance or through CYP enzyme activity assays (Rees et al., 2003; van der Oost et al., 2003). Ethoxyresorufin O-deethylase (EROD) activity is an established biomarker commonly utilized to assess contamination by CYP1A-inducing xenobiotics, including PAHs (Burke and Mayer, 1974; Whyte et al., 2000; Fent, 2003; van der Oost et al., 2003). This assay fluorometrically

measures the formation of resorufin, the product of CYP1A mediated dealkylation of the synthetic substrate 7-ethoxyresorufin (Burke and Mayer, 1974; Whyte et al., 2000; van der Oost et al., 2003). Some xenobiotics like TCDD induce CYP1A (i.e. are AhR agonists), but are not easily metabolized by it; while others including some PAHs, are both inducers and readily undergo biotransformation (i.e. are AhR agonists and CYP1A substrates) (Billiard et al., 2002).

In biomarker assessments, EROD activity is often measured in the liver because of this organ's high biotransformation enzyme content and capacity to metabolize xenobiotics (Buhler and Williams, 1989; Myers et al., 1998; van der Oost et al., 2003). However, recent research highlights the importance of measuring this sensitive biomarker in tissues involved in xenobiotic uptake, particularly in low PAH contamination situations (Van Veld et al., 1997; Levine and Oris, 1999; Jönssson et al., 2002; Abrahamson et al., 2007). The gill and intestine may participate in first-pass metabolism of PAHs following aqueous and food exposures, respectively (Levine and Oris, 1999; Van Veld et al., 1997). In environments where low level exposure to PAHs may be occurring, measurement of CYP1A induction in the liver may underestimate contaminant exposure if extra-hepatic metabolism, and potentially clearance, occurs in these tissues prior to systemic circulation (Levine and Oris, 1999; Jönsson et al., 2004; Jönsson et al., 2006).

While CYP1A induction can be a sensitive biomarker of PAH exposure, modifying factors may be present in field situations. For example, PAHs almost never occur alone in aquatic systems and complex PAH mixtures can contain

both CYP1A inducers and inhibitors (Willet et al., 2001; Wassenberg et al., 2005). Furthermore, some metals such as tributyltin may be inhibitory to CYP1A activity (Whyte et al., 2000). In addition, factors such as exposure temperature and circulating hormones may confound accurate correlation of CYP1A activity to xenobiotic presence (Sleiderink et al., 1995; Whyte et al., 2000). When utilized as a biomarker in a biomonitoring study, care should be given to identify and if possible preclude potential modulating factors (Whyte et al., 2000).

## 1.3 Polycyclic Aromatic Hydrocarbons

PAHs are ubiquitous contaminants produced primarily through the incomplete combustion of organic matter (pyrogenic) and are constituents of fossil fuels (petrogenic) (Neff, 1979). Both pyrogenic and petrogenic PAHs are released into the environment largely as inadvertent by-products of anthropogenic activities (Neff, 1979; Lima et al., 2005; Dong and Lee, 2009). PAHs are a large class of related compounds; comprised of two or more fused benzene rings, they can have zero to multiple degrees of alkylation, ultimately resulting in hundreds of possible congeners and structural isomers (Harvey, 1997; Neff et al., 2005).

PAHs enter aquatic systems through various routes. On a global scale, atmospheric transportation and deposition of pyrosynthesized PAHs is the main source affecting their ubiquity (Neff, 1979; Fang et al., 2004). However, the highest concentrations of PAHs are typically found closest to localized anthropogenic developments (Neff, 1979). Associated with urbanization, runoff can be a major contributor of PAHs to receiving water bodies and sediments

(Neff, 1979). In addition, both wet and dry deposition of locally released/produced PAHs may also be a source of regional PAH contamination (McElroy et al., 1989; Manoli et al., 2000; Doong and Lin, 2004; Stout et al., 2004; Lian et al., 2009). In some systems, motorized watercraft activity, particularly 2-cycle engine use, can also contribute to contamination (Mastran et al., 2004; Lico, 2004; Rice et al., 2008). Two-cycle engines inefficiently combust fuel and characteristically release both combusted and unburnt fuel directly into the water column (Lico, 2004). Once deposited in an aquatic system, PAHs in the water column readily adsorb to the settling particulate phase and can persist in associated sediments (Burgess et al., 2003; Lima et al., 2005). While sediments can act as PAH sinks amalgamating both historic and recent inputs, PAHs can be degraded by microbial action and to a lesser degree through photooxidation (Neff, 1979; McElroy et al., 1989; Stout et al., 2001b). Pyrosynthesized PAHs are typically more refractory to degradation and less bioavailable to organisms due to their adsorption to soot during formation (Lima et al., 2005)

In PAH-contaminated aquatic systems, source identification methods can assist in characterizing the main inputs. The majority of specific PAHs created through combustion (pyrogenic) differ from those present in fossil fuels (petrogenic) reflecting the differences in the processes of PAH formation (Neff, 1979; Hansen et al., 2003;Lima et al., 2005; Neff et al., 2005). Pyrogenic PAHs are generated during rapid high temperature and low oxygen combustion conditions and can be produced during smelting, industrialization, heating, combustion of fossil fuels, and forest fires (Neff, 1979; Witt, 1995; Van Metre et

al., 2000; Lima et al., 2005; Hylland, 2006). Pyrosynthesis tends to favor formation of 3-, 4- and 5-ring parent PAHs (Lima et al., 2005). Conversely, petrogenic PAHs are typically alkylated (Neff, 1979; Neff et al., 2005) due to their biogenic precursors (i.e. ancient plant material) and the diagenic process of low temperature and pressure (Neff, 1979; Hansen et al., 2003; Neff et al., 2005). These PAHs can be discharged into aquatic environments through spills, leaks and runoff (Van Metre et al., 2000; Lima et al., 2005). A petrogenic PAH profile is generally dominated by the one (C-1), two (C-2), three (C-3) and four carbon (C-4) alkylated structural isomers over the respective parent compound, particularly the alkylated naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes and chrysenes (Neff, 1979; Wang et al., 1999; Hansen et al., 2003; Wang and Fingas, 2003; Neff et al., 2005). Comparison of the relative amount of specific isomers and other diagnostic ratios, lower- vs. higher- molecular weight PAHs, and homologue distributions can assist in determining PAHs from pyrogenic versus petrogenic sources (Stout et al., 2001b; Neff et al., 2005). Source characterization can be useful when determining the party responsible for contamination or if abatement measures are desired (Yunker et al., 2002).

PAHs can be toxic to vertebrate species. Sixteen parent compounds are included on the US Environmental Protection Agency (EPA) priority pollutant list, and seven of these are considered probable human carcinogens (Barbee et al., 2008). In teleosts, PAH exposure can cause adverse effects manifested as immunotoxicity, genotoxicity, reduced recruitment, development of blue-sac disease symptoms, and mortality in early life stages (Billiard et al., 1999; Heintz

et al., 2000; Arkoosh and Collier, 2002; Villeneuve et al., 2002; Carls et al., 2005; Rhodes et al., 2005; Reynaud and Deschaux, 2006; Barbee et al., 2008). Elicitation of adverse effects can be through both AhR/CYP1A dependent and independent pathways (Incardona et al., 2005).

In addition to being a mechanism to enhance excretion, CYP1A can also bioactivate of some PAHs; ultimately forming metabolites which are more toxic than their respective parent compound(s) (Miller and Ramos, 2001; Akcha et al., 2003; van der Oost et al., 2003; Schlenk et al., 2008). The metabolism of benzo[a]pyrene (BaP) into an adduct-forming diol-epoxide is a recognized pathway by which PAHs can be bio-transformed/bio-activated into genotoxic agents (Buhler and Williams, 1989; Miller and Ramos, 2001). PAH-DNA adducts are capable of initiating cancer and have been linked to the hepatic lesions observed in bottom-feeding fish inhabiting areas of PAH-contaminated sediments (Myers et al., 1999; Ostrander and Rotchell, 2005). In addition to formation of macromolecular adducts, bioactivated PAHs may lead to the formation of redox-cycling quinones and subsequent oxidative damage/stress (Miller and Romos, 2001; Nebert et al., 2000; Sturve et al., 2005). The ubiquity of PAHs in combination with their toxic capabilities engenders them contaminants of concern in many aquatic systems.

## 1.4 Study Objectives

Alaskan salmon stocks have generally been robust since the late 1970's due to favourable environmental conditions and conservative fisheries management practices (Mantua et al., 1997; Adkison and Finney, 2003; Quinn,

2005). However, endemic salmon populations of Auke Lake located in Southeast Alaska have declined markedly over recent decades (Appendix 1) (Taylor and Lum, 2005; Hoover, 2008; Rice et al., 2008). A 5-year monitoring study deploying passive sampling devices in the lake for 21 day intervals, found an increase in passive sampler PAH accumulation coincident with recreational watercraft use on the lake (Rice et al., 2008). The co-occurrence of PAHs and salmon declines suggests that these two events could be associated (Suter et al., 2007; Rice et al., 2008). However, without spatially and temporally related aqueous exposure concentration measurements and evidence of PAH bioavailability to salmon, a causal inference is conjectural (Collier, 2003; Suter et al., 2007). The primary objectives of the research, described herein, were to investigate PAH exposure concentrations, their *in situ* bioavailability to juvenile Pacific salmon, and to determine the associated sources of PAHs in Auke Lake. In accomplishing these goals, the findings will indicate whether PAHs, as a potential contributor to salmon declines in Auke Lake, should be further investigated (Collier, 2003; Suter et al., 2007).

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# 2: Anthropogenically-Sourced Low Concentration PAHs: *in Situ* Bioavailability to Juvenile Pacific Salmon

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Ariel Blanc designed and implemented the biomontioring study, statistically analyzed and interpreted the data, and authored the manuscript. Dr. Rice and Dr. Kennedy provided guidance and support throughout the study and during the manuscript revision process.

## 2.1 Introduction

Since the late 1970's, much of Alaska has experienced robust salmon stocks as a reflection of favourable environmental conditions and conservative fisheries management (Mantua et al., 1997; Adkison and Finney, 2003; Quinn, 2005). However, the populations of both out-migrating smolts and salmon returning to Auke Lake in Southeastern Alaska have declined markedly over recent decades (Taylor and Lum, 2005; Hoover, 2008; Rice et al., 2008). This lake system drains a predominantly undeveloped watershed, although approximately 50% of its immediate shoreline has been developed (Figure 1) (Hoover, 2008; Rice et al., 2008). The lake provides habitat for multiple species of anadromous salmon (*Oncorhynchus gorbuscha, O. keta, O. kisutch,* and *O. nerka*), resident trout (*Salvelinus malma, O. mykiss* and *O. clarki*), endemic sculpin (*Cottus asper*), prickly stickleback (*Gasterosteus sp.*) and freshwater

mussels (*Anodonta sp.*) (Moles and Marty, 2005; Hoover, 2008; Rice et al., 2008). Recent salmon declines and the virtual disappearance of stickleback and freshwater mussel suggests altered lake conditions and potentially degraded water quality (Moles and Marty, 2005; Rice et al., 2008). A previous monitoring study deploying passive sampling devices revealed increases in seasonal surface water PAHs attributable to motorized watercraft use on the lake (Rice et al., 2008). The co-occurrence of broad biota declines and PAHs, has implicated PAH contamination as a contributor to these changes.

An examination of the potential link between PAH contamination and biological effects, begins with an assessment of the concentrations and PAH bioavailability to juvenile salmonids, the organism of most concern in this system (Fent, 2003; van der Oost et al., 2003). The previously deployed passive sampling devices (low density polyethylene strips), have potential as sensitive time-integrated samplers which may perform similarly to the well described semi-permeable membrane devices (SPMDs) (Müller et al., 2001; Booij et al., 2003; Carls et al., 2004; Moles et al., 2006; Rice et al., 2008). However, unlike the aforementioned, they lack a triolein reservoir and have not yet undergone the same rigor of kinetic studies and field testing to either back-calculate aqueous exposure concentrations or substitute for an aquatic biological response (Huckins et al., 1990; Müller et al., 2001; van der Oost et al., 2003; Verweij et al., 2004). Therefore, collection of exposure media and analysis of a biological endpoint are needed to provide a more accurate indication of conditions encountered by endemic organisms. As a biological endpoint, cytochrome P450 1A (CYP1A)

induction measured by EROD activity, is a sensitive indicator, or biomarker, of exposure to certain PAHs (Whyte et al., 2000). The gill, as a major route of xenobiotic uptake can be an ideal tissue for measuring CYP1A induction in response to low aqueous concentrations of an inducer (Kennedy and Walsh, 1994; Van Veld et al., 1997; Levine and Oris, 1999; Jönsson et al., 2002). Gill EROD activity has proven to be a reliable biomarker of contaminant exposure in multiple teleost species (Jönsson et al., 2003; Mdegela et al., 2006; Abrahamson et al., 2007; Abrahamson et al., 2008; Andersson et al., 2007).

The primary objective of this study was to characterize PAH concentrations and determine their site-specific bioavailability to juvenile salmonids; secondary to this, was to compare different physical sampling matrices as indicators of source and the biological response measured, for utilization in future site assessments of this nature. These goals were accomplished by simultaneously gathering different matrices (passive samplers, water and sediment samples) at lake locations representing potentially distinct sources of PAHs, subjecting results to basic source allocation methods, and by measuring gill EROD activities in correspondingly caged fish.

## 2.2 Materials and Methods

### 2.2.1 Fish

Three hundred yearling Chinook (*O. tshawytscha)* (approx. 12 g) were purchased from Douglas Island Pink and Chum, Inc., Juneau, AK. Fish were transported to the Auke Creek Research Station (ACRS), Juneau, AK, in

continually aerated opaque plastic containers. Fish were housed in a 3500 L holding tank (5.9-7.3 ℃, ≥ 7.3 mg/L DO, flow: approx. 20 L/min, natural photoperiod of 58°18' North, approx. 18 hours dayli ght) and fed *ad libitum* (1.5 mm EWOS Pacific diet, EWOS Ltd., Surrey, CA). Water to the ACRS was supplied from an intake pipe in Auke Lake, approximately 7 m below the surface (and thermocline) in the southern corner of the lake (Figure 1). Previous summers' water samples collected from this depth indicated that no PAHs were present above detection limits (Rice et al., 2008). Fish were acclimated for at least 2 weeks before placement in Auke Lake and feeding was suspended 2 to 4 d prior to β-napthoflavone (βNF) exposure or caging in Auke Lake.

### 2.2.2 Chemicals

Resorufin, 7-ethoxyresorufin (7-ER), dicoumarol and 95% β-napthaflavone (βNF) were purchased from Sigma Aldrich (St. Louis, MO). High performance liquid chromatography (HPLC) grade dichloromethane, hexane, pentane and pesticide grade acetone were supplied from VWR (West Chester, PA).

### 2.2.3 Lake Exposures

Five Auke Lake locations were chosen to represent areas of varying PAH contamination from different sources including motorized watercraft activity and runoff (Figure 1). The lake is divided roughly in half with motorized watercraft activity allowed in the southern portion and restricted in the northern half. Sampling locations included: the Lagoon (LGN) site at the lake outlet near a boat ramp, high motorized activity and a runoff outlet; the Central Lake (CL) site in the

southern portion experiencing high levels of motorized activity; the East Shore (ES) site within the motorized zone along an undeveloped forested shoreline; the Northwest Drainage (NWD) location in the motorized restriction zone and situated in an area receiving runoff from roadways and other developments; and, the Lake Creek Outfall (LCO) site located at the Lake Creek/ Auke Lake inlet approx. 170 m below a roadway overpass. Lake Creek (LCRef) upstream of the roadway and other anticipated anthropogenic inputs served as the reference site. The intake pipe 7 m below the lake surface supplying water to the Auke Creek Rearch Station (ACRS) was also monitored with physical sampling. All sites except the NWD were the same as in the 1999-2003 Auke Lake monitoring program (Rice et al., 2008).

At each location, two cages (approx. 32 L/cage) were placed approx. one m below the lake surface. Cages horizontally paralleled the placement of a passive sampler deployed for 21 d. Ten fish (12.03 ± 2.41g [mean ± SD]) from the ACRS were placed in each cage. Fish were sampled from one cage at 2 d, and from the other at 7 d, coinciding with the removal of the passive sampler and collection of sediment and water samples. Following retrieval from the lake, fish were transported to the Ted Stevens Marine Lab and were immediately sacrificed (by cervical dislocation and decapitated) for gill EROD activity analysis.

### 2.2.4 Gill EROD Activity

Gill filament EROD activity was measured by the method of Jönsson et al., (2002), with similar modifications as implemented by Abrahmson et al., (2008). Briefly, the right operculum of each fish was removed and the first three gill

arches excised. For fish weighing less than 10 g, the first gill arch of the left side was also removed. Arches were immediately placed in ice cold HEPES-Cortland (HC) buffer. From each fish, 2 sets of 10 gill filaments (2 mm in length) were randomly excised from the arches and placed in 2 wells of a 12-well culture plate. The HC buffer was then replaced with 0.5 mL of reaction solution containing 1 µM ER and 10 µM dicoumarol in HC buffer and placed on a shaker for a 10-min pre-incubation period at room temperature (approx. 20℃). The reaction solution was replaced with 0.7 mL of reaction solution. The samples were then incubated for 20 and 40 min, at each time point a 0.2 mL aliquot from each well was transferred to a black Corning 96-well plate. Fluorescence was measured on a Victor$^3$ plate reader (Perkin Elmer, Inc. Waltam, MA) and read at 540 nm (excitation) and 590 nm (emission). A standard resorufin curve (0 nM to 250 nM resorufin) was used to calculate the resorufin concentration in all samples. Results are expressed as picomoles reosrufin/gill filament/minute (pmr/gf/min).

### 2.2.5 βNF Exposures

As a positive control for induction, fish were exposed to βNF in a static renewal system similar to that used by Jönsson et al., (2002). Fish were exposed in transparent polyethylene bags containing 20 L of water (same water as ACRS holding tank) placed in opaque fiberglass boxes. The boxes were elevated in a 3500L holding tank which served as a water bath to ensure a constant exposure temperature. βNF was dissolved in acetone by sonication, and 0.4 mL of the βNF solution was added to each polyethylene bag, resulting in a nominal 1 µM βNF concentration. The water was lightly mixed before fish (11.70 ± 2.50 g) were

placed into each bag (n=5; 2 replicates for each treatment). The exposure bags were constantly aerated and fish were transferred to a fresh bag every 24 h for continual exposure. βNF exposure scenarios included 2, 4 and 7 d βNF exposure periods and a 2 d βNF exposure followed by 2 d in βNF-free ACRS water. These exposures were carried out at the Auke Lake surface water temperature of 13.7 ℃. In addition, a 2 d βNF exposure at the ACRS holding tank temperature of 6.0℃ was also included to assess any effect the warmer lake surface temperature might have had on EROD activity at a fixed assay temperature. Other studies with juvenile rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) employing similar exposure scenarios, have compared βNF exposed gill EROD activity to those from holding tank(s) fish as the unexposed control; these fish were shown to have comparable gill activities to those held in polyethylene bags, with and without acetone added (Jönsson et al., 2002; Jönsson et al., 2003). In the current study, βNF-exposed fish were compared to ACRS holding tank fish to evaluate induction potential.

### 2.2.6 Passive Sampling Devices

Passive samplers (low density polyethylene strips) were prepared and analyzed according to Carls et al., (2004). Briefly, after lake retrieval, passive sampler membranes were wiped with a KimWipe® to remove biofouling, spiked with deuterized surrogates, and extracted into 80:20 pentane:dichloromethane through repeated sonication. The extracts were reduced, dried with sodium sulfate, and then exchanged with hexane. The samples were then eluted through a micro silica gel/alumina column with 50:50 pentane:dichloromethane, and

exchanged with hexane. Samples were analyzed by gas chromatography – mass spectrometry (GC/MS) for 40 parent and alkylated PAHs and dibenzothiophenes (Table 1). PAH concentrations were determined by the internal standard method and are reported as ng PAH/g passive sampler with each sampling device weighing approximately 2.21 g (Short et al., 1996; Carls et al., 2004). PAHs detected below method detection limits (MDL) were assigned a value of zero. Total PAH values reported are the sum of all detected analytes.

### 2.2.7 Sediment and Water Samples

Unfiltered grab water and sediment samples collected at each location were processed according to Short et al. (1996) with minor modifications. Water samples (3.8 L) were collected from the top meter of water at the approximate depth of the caged fish and passive samplers. They were extracted with dichloromethane, spiked with deuterized surrogates, dried with sodium sulfate, and then exchanged with hexane. Sediment samples were collected from surface sediments (the approx. top 4 cm) vertically below the caged fish and passive samplers. Sediment samples were homogenized and a portion was set aside for dry weight determination. The samples were spiked with deuterized surrogates, dried with sodium sulfate, and extracted with dichloromethane by a Dionex ACE® 200 accelerated solvent extractor (Dionex Corporation, Sunnyvale, CA). They were exchanged with hexane, purified and fractionated by elution through silica gel-alumina columns with dichloromethane, exchanged with hexane, and further purified by HPLC. All samples were analyzed by GC/MS and concentrations were determined by the internal standard method. Values below the method

detection limit have been treated as zero, and all other values have been reported as ng/L (water) and ng/g dry weight (sediment).

### 2.2.8 Source Allocation

The PAH source (petrogenic or pyrogenic) for all three matrices was determined by specific indicative PAHs, composition profiles and a suite of diagnostic ratios. These included: recognized PAH distribution tendencies; relative percentages of 2- and 3-ring versus 4- to 6-ring PAHs; and, proportion of alkyl homologues (one, two, three or four carbons (C-1, C-2, C-3, C-4) versus the respective parent (C-0) compound(s). Indicators of petrogenic-source PAHs included the presence of the heterocycle dibenzothiophene; the relative alkyl homologue abundance of naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes and chrysenes in “bell-shaped” distributions (C-0<C-1<C-2<C-3) (Boehm and Farrington, 1984; Wang et al., 1999; Neff et al.; 2005); a dominance of 2- and 3- ring PAHs over 4- to 6-ring PAHs (Wang and Fingas, 2003); and, the use of diagnostic ratios which incorporate a quantitative comparison of relative petrogenic *v.* pyrogenic indicator PAHs (Table 1) (Boehm and Farrington, 1984; Stout et al., 2001b). Conversely, pyrogenic sourced PAHs were generally characterized by a predominance of 4- to 6-ring parent PAHs, with skewed homologue distributions (C-0>C-1>C-2>C-3>C-4), in addition to indicative diagnostic ratio values (Stout et al., 2001b; Neff et al., 2005; Wang and Fingas, 2005).

Although the diagnostic ratios employed herein have primarily been applied to sediment samples, the use of some of these has also been extended

for relative water and passive sampling device source allocation purposes (Menzie et al., 2002; Stein et al., 2006; Sower and Anderson, 2008). Passive sampling devices can have dissimilar affinities, rates of sampling and retention capabilities for freely dissolved PAHs (Müller et al., 2001; Carls et al., 2004; Rusina et al., 2007; Sower and Andersson, 2008). For this reason, only the diagnostic ratios evaluating the relative abundance of PAH isomers (with theoretically similar partitioning properties) were used for passive sampler PAH allocation in this study (Table 1) (Müller et al., 2001; Carls et al., 2004; Sower and Andersson, 2008).

### 2.2.9 Statistics

Statistical analysis were performed by JMP© 7.0.2. Data were assessed for normality (Shapiro-Wilks test) and for homoscedasticity. Where variances differed, the data were log transformed and reanalyzed. For each caging location, a one-way ANOVA followed by Tukey's multiple comparison test was used to determine statistical difference between basal (0 d), 2 d and 7 d gill filament EROD activity. For the 2 d and 7 d time points, a one-way ANOVA followed by Dunnett's multiple comparison test was used to asses differences between the EROD activity at all Auke Lake locations *v.* the Lake Creek reference site. βNF-treatments were compared by a one-way ANOVA followed by Tukey's multiple comparsion test. Gill EROD values are displayed as mean and 95% confidence intervals (CI) and significance was determined by a probability (p) value $< 0.05$. Linear regression analysis was used to explore the relationship between mean 7 d EROD activity at each site and the corresponding

total and single PAHs in passive samplers, water and sediment samples; regression analysis $r^2$ and p values are reported.

## 2.3 Results

### 2.3.1 PAHs in Water: Concentration and Composition

PAHs were detected in all Auke Lake locations; however, none were detected in the reference site upstream of the lake. The LGN sample contained the highest concentration of total PAHs (399.51 ng/L), followed by the LCO (236.65 ng/L), the ES (107.80 ng/L), the ACRS (85.59 ng/L), the CL (54.48 ng/L), and the NWD (30.54 ng/L) (Figure 2). PAH composition was dominated by 2- and 3-ring compounds. These made up 100% of the total PAHs detected at the ACRS, and between 91-99% at all other sites. Alkylated naphthalene and phenanthrene/anthracene homologues were detected at greater concentrations than their respective parent compounds at all locations; with the alkylated naphthalenes comprising > 55% total PAHs at all sites except the LGN (49.5%). Dibenzothiophenes were detected in the LGN (30.49 ng/L), LCO (9.13 ng/L) and ACRS (1.27 ng/L) samples. Of the 4- to 6-ring PAHs detected in water samples, pyrene was the only PAH detected in all water samples: LGN (2.26 ng/L), CL (1.76 ng/L), ES (1.56 ng/L), NWD (1.50 ng/L), and LCO (1.46 ng/L) (Figure 3). Sample diagnostic ratios are displayed in Table 1.

### 2.3.2 PAHs in Passive Samplers: Concentrations and Composition

The CL site, an area of high recreational activity by motorized watercraft, accumulated the most PAHs (1,514.4 ng/g device). Other lake sites had

significant concentrations although somewhat lower: LCO (775.25 ng/g device), NWD (514.81 ng/g device), ES (437.75 ng/g device), and LGN (407.79 ng/g device). The two lowest concentrations were at the Auke Creek research station, which draws water below the thermocline (81.21 ng/g device), and the LCRef did not accumulate any PAHs over the three week sampling period (Figure 2).

Two- and 3-ring PAHs dominated the composition of PAHs in the passive samplers, but much higher proportions of 4- to 6-ring PAHs were detected compared to the water grab samples. Two- and 3-ringed PAH percentages ranged from 57.9% (2- and 3-ring PAHs at the ACRS) to 83.9% (ES). At all Auke Lake locations where detected, the alkylated naphthalenes, fluorenes, and phenanthrene/anthracenes homologue concentrations were found in substantially higher concentrations than their respective parent compounds. Additionally, dibenzothiophenes were detected in the NWD and CL passive samplers. Of the 4- to 6-ring PAHs detected, chrysene(s) and a distinct fluoranthene, pyrene and C-1 fluoranthenes/pyrenes cluster accumulated in all passive samplers (Figure 3). Diagnostic ratios for isomers are shown in Table 1.

### 2.3.3 PAHs in Sediment: Concentration and Composition

Sediments collected from the LGN contained over 10,000 ng/g total PAHs, a 10-fold increase over all other sites, followed by the NWD (506.1 ng/g), the ES (425.59 ng/g), the ACRS (288.4 ng/g), and the CL (136.85 ng/g) sites. PAHs were not detected at the LCRef or LCO sites (Figure 2). Sediments from these two sites were comprised of gravel and pebbles. The NWD sediment contained the lowest percent of 4- to 6-ring PAHs (49.85%) with alkylated

pyrenes/fluoranthenes and chrysenes dominating. The other locations sediment samples contained an increasing percentage of 4- to 6-ring PAH: the ACRS (53.66%), the CL (56.01 %), the LGN (58.04 %), and the ES (65.35 %). The LGN, ACRS, and NWD sediments contained parent and alkylated dibenzothiophenes (Figure 3). Sediment total PAH concentrations are the sum of all detected PAHs with the exception of perylene. Diagnostic ratios are depicted in Table 1.

### 2.3.4 Gill EROD Activity

Juvenile Chinook salmon gill EROD activity was responsive to aqueous CYP1A inducers, but was complicated by the holding conditions at the ACRS where the water source was from minus 7 m in the lake. All βNF exposures resulted in significantly increased EROD activity compared to unexposed fish. After 2 d, βNF exposures at both 6.0 and 13.7℃ EROD activities were approximately 2-fold higher than measured in uninduced (basal) fish held in ACRS water (0.033 ± 0.007 pmr/gf/min) (Figure 4). βNF exposures of 2 and 7 d at 13.7℃ resulted in similar EROD activities indic ating that maximum induction was likely reached within the first 2 d. Lastly, EROD activity of 2 d βNF-exposed fish did not decline significantly once transferred to ACRS (βNF-free) water for 2 d.

EROD activities increased at all lake locations following 7 d of caging and were significantly greater than the upstream reference location (LCRef). The LCRef EROD activity declined significantly from the basal activity during the 7 d. The LGN had the highest gill EROD activity (0.059 ± 0.014 pmr/gf/min), a 7-fold

higher activity than fish caged at the reference location (LCRef) (0.009 ± 0.003 pmr/gf/min) (Figure 5).

The EROD activities after 2 d of exposure in the lake were variable, lower at some locations and higher at others. The LGN EROD activity (0.010 ± 0.002 pmr/gf/min) had declined significantly from the 0 d, while the ES's (0.047 ± 0.007 pmr/gf/min) had increased significantly.

### 2.3.5 Correlation of Gill EROD Activity with Sampling Matrices and Specific PAHs

Correlations with sampling matrices total PAHs were positive, but weak; 7-d mean gill EROD activity was the most closely correlated to total PAHs in water samples (water $r^2$=0.3119, p=0.2494; passive sampler$^2$=0.2172, p=0.3515; sediment $r^2$=0.2292, p=0.3368). Although anticipated, summation of 4- to 6-ring PAHs did not yield a more meaningful relationship to EROD activity (water $r^2$=0.2935, p=0.2669; passive sampler $r^2$=0.2409, p=0.3229; sediment $r^2$=0.2289, p=0.3371).

Stronger correlations between gill EROD activity and specific individual PAHs were found. Aqueous pyrene was significantly correlated with gill EROD activity ($r^2$=0.9662, p=0.0004) as were C-2 fluorenes ($r^2$= 0.6747, p=0.0450) (Figures 6 and 7). Additionally, benzo(ghi)perylene accumulated in passive samplers was significantly correlated with mean EROD activity ($r^2$=0.6823, p=0.0428).

## 2.4 Discussion

Juvenile Chinook salmon caged in the lake had gill EROD activity significantly greater than the upstream controls, demonstrating that the method served as an appropriate and sensitive measure for biomonitoring low concentration PAHs; ng/L pyrene concentrations were correlated with increased gill EROD activity after 7 d of caging. Furthermore, PAHs in the lake are from localized anthropogenic activities and runoff is likely a chronic source.

EROD activities significantly increased over baseline following 2 d exposures to βNF, a model CYP1A inducer. The levels of EROD induction in the present study is comparable to that using Atlantic salmon (*Salmo salar*) parr and smolts, indicating that juvenile Chinook salmon are responsive to CYP1A inducers similar to other salmonid species (Jönsson et al., 2003). Throughout the 7-d of biomonitoring, fish caged in the lake showed an overall increase in EROD activity, while reference site activities substantially declined from holding site activities. This broadly suggests that CYP1A inducer(s) (i.e. PAHs) were present and bioavailable in the lake, but were absent in upstream water.

Fish held in Auke Creek Research Station (ACRS) water had “basal” EROD activities at least one order of magnitude above values reported previously for non-induced rainbow trout, and both Atlantic salmon parr and smolts (Jönsson et al., 2002; Jönsson et al., 2003; Jönsson et al., 2006). The reason for the elevated gill EROD activity observed is not known, but the likely explanation is the presence of an inducer in the ACRS water, since ACRS water, passive sampler and sediment samples all contained measurable PAH concentrations.

This occurrence highlights the importance of ensuring clean holding tank water in biomonitoring studies.

Although co-located, water and passive samplers conveyed distinct source and magnitude of contamination information. Grab water samples provide a snapshot of actual PAH concentrations in time and space and all samples reflected petrogenic sources. The samples were dominated by alkyl naphthalenes and phenanthrenes/anthracenes; and in some samples, dibenzothiophenes were detected. The prevalence of alkylated 2- and 3-ring PAHs combined with diagnostic ratio values, suggests that PAHs in the water column were predominantly petrogenic in nature. The LGN sample had the highest aqueous PAH concentration suggesting elevated recent or continual input(s) at the site. Passive samplers are time-integrated PAH accumulators through a chosen deployment period (Carls et al., 2004; Rice et al., 2008). All lake passive samplers primarily accumulated 2- and 3-ring PAHs dominated by naphthalene, fluorene, phenanthrene/anthracene alkyl-homologue dominance indicating that accumulated PAHs were largely of a petrogenic source (Boehm and Farrington, 1984; Neff et al., 2005; Iqbal et al., 2008). However, pyrogenic sourced PAHs were also present indicated by distinct fluoranthene and pyrene cluster accumulated in all passive samplers and diagnostic ratio values supported pyrogenic sources (Van Metre, 2000; Doong and Lin, 2004).

The central lake (CL) passive sampler accumulated the highest concentration of PAHs. This is similar to a previous Auke Lake study which found increased accumulation of PAHs at this site that was attributable to high

recreational motorized watercraft a source of both unburnt fuel and combustion related hydrocarbons (Tjärnlund et al., 1996; Lico, 2004; Rice et al., 2008). However, the concentrations accumulated were several-fold less than measured in previous years, likely due to colder temperatures, high precipitation and anecdotally-related lower watercraft use on the lake (Rice et al., 2008).

Sediments can act as PAH sinks, amalgamating both historic and recent PAH contamination. Sediment samples indicated that runoff is an underlying source of PAHs to the lake. The lagoon (LGN) and northwest drainage (NWD) sites proximal to shorelines, roadways and other anthropogenic activities had the highest total PAH concentrations. Sediments at these locations characteristically accumulated both petrogenic and pyrogenic sourced PAHs including dibenzothiophenes, bell-shaped parent alkyl-naphthalene and phenanthrene/anthracene distributions, and several petrogenically indicative diagnostic ratios (Stout et al., 2001a). In contrast, the East shore (ES) and central lake (CL) samples indicated predominantly pyrogenic sources. These sediments characteristically contained a high proportion of 4- to 6-ring PAHs, sloped homologue distributions, and several diagnostic ratios suggestive of pyrogenic-sources (Stout et al., 2001b; Neff et al., 2005).

When considered together, the matrices reveal a holistic explication of the contamination. They indicate that lake-wide petrogenic-sourced PAHs predominate in the water column and are notable in sediments closest to anthropogenic activities implicating this association as a likely source. Those sediment and water samples proximal to shorelines had the highest

concentration of total PAH and no PAHs were detected in the sampling matrices upstream of urbanization, implying that PAHs are from local sources, including runoff from developed surfaces. Runoff is nationally recognized as a major source of PAHs to receiving water bodies and sediments and it characteristically contains both petrogenic and pyrogenic PAHs (Cole et al., 1984; Hoffman et al., 1984; Latimer et al., 1990; Stout et al., 2001a; Menzie et al., 2002; Stout et al., 2004; Stein et al., 2006). In addition, pyrogenic PAHs primarily comprised contamination in other sediments and were present in the water intermittently or at low concentrations indicated by passive sampler accumulation of pyrogenic PAHs over 21 d. Sources of these PAHs could include both wet and dry deposition of PAHs from localized combustion and other activities and direct input from recreational watercraft use (Tjärnlund et al., 1996; Manoli et al., 2000; Lico, 2004; Lima et al., 2005; Rice et al., 2008). However, the aforementioned were likely not the predominant sources during this study as it was conducted in a low watercraft-use summer and if deposition from localized combustion was the major source, a more pronounced pyrogenic presence would be expected in the water samples (Fernandes et al., 1997; Stein et al., 2006; Wang et al., 2008). It is unknown to what degree atmospherically transported PAHs might contribute to lake concentrations (Stout et al., 2001b; Fang et al., 2004).

Comparison of the sampling matrices to corresponding EROD activities, demonstrated that water samples were the most consistent predictor of the biological response. This would be expected, as grab water samples most accurately reflected PAH concentrations encountered by the pelagic species.

Although water samples were dominated by 2- and 3-ring PAHs which are not typically considered strong CYP1A inducers, stronger inducers were likely present but below the detection capabilities of the grab water sampling technique (Barron et al., 2004). The passive samplers accumulate over time, and hence are more sensitive at detecting low concentrations, particularly when they are episodic (Carls et al., 2004). Had the passive sampler deployment and salmonid biomonitoring time periods been of equal length, the responses might have exhibited a better correlation.

Of the PAHs detected in water samples, a strong correlation existed between pyrene concentration and respective EROD activities. Other studies have also found pyrene capable of induction. CYP1A was induced in fish hepatoma cell lines following *in vitro* pyrene exposure (Fent and Bätsher, 2000), and, *in vivo* studies have found induction in zebrafish (*Danio rerio*) embryos, and nile tilapia (*Oreochromis niloticus*) (Zapata-Pérez et al., 2002; Incardona et al., 2005). In addition, another biominitoring study noted a significant correlation between SPMD-estimated pyrene concentrations (ng/L) and 1-hydroxypyrene metabolites in caged carp (*Cyprinus carpio*) bile (Verweij et al., 2004).

The lowest water total PAH and corresponding pyrene concentrations associated with significantly increased gill EROD activity (ng/L) were orders of magnitude below the current Alaskan Water Quality Standard for total PAH of 10 µg/L (Heintz et al., 1999; ADEC, 2009). Other studies have found that total PAH concentrations in the ng/L or µg/L level are capable of adversely affecting early-life stage salmonids and other teleost species (Heintz et al., 1999; Heintz et al.,

2000; Carls et al., 2005, Incardona et al., 2009). Specifically, one study found decreased post-emergence growth with possible recruitment implications occurring at concentrations below those inducing CYP1A in the exposed embryos (Carls et al., 2005). Thus, in some situations, CYP1A induction in response to chronic low concentration PAHs may serve as an early warning sign of organismal and potentially population level effects in anadromous Pacific salmon (Heintz et al., 2000; Carls et al., 2005).

The magnitude of sediment PAHs at the most contaminated site appeared to reflect localized biological effects. Although sediment PAHs did not correlate well to lake-wide CYP1A induction, the LGN sediment and water samples had the highest total PAH and pyrene concentrations detected in the lake and fish at this location correspondingly had the highest EROD activity. These sediments contained PAH concentrations notably elevated (at least 10-fold) over all other lake sites. Multiple individual and the sum of the 10 PAHs specified by the freshwater *Consensus-based Sediment Quality Guidelines* were above their threshold-effects concentration(s), but below their respective probable effects concentration(s) (MacDonald et al., 2000). In addition, prickly sculpin collected from this area exhibited lower fitness metrics and higher incidence of hepatic lesions than sculpin from more remote reference lakes (Moles and Marty, 2005). Interpreted together, PAH concentrations in LGN sediments are of a magnitude potentially capable of adversely affecting benthos, and are likely bioavailable to resident epibenthic organisms (MacDonald et al., 2000; Moles and Marty, 2005). Furthermore, it is becoming increasingly evident that contaminated sediments

may serve as reservoirs of PAHs capable of compromising juvenile salmon survival potential with possible latent population level effects (Arkoosh et al., 1998b; Arkoosh et al., 2001; Arkoosh and Collier, 2002; Meador et al., 2006; Spromberg and Meador, 2006: Johnson et al., 2008)

## 2.5 Conclusion

The sampling matrices employed herein detected low levels of PAH and represent varying degrees of temporal integration; collectively they yield a comprehensive picture of site conditions over time. Water samples most consistently predicted the pelagic species biomarker response; sediments yielded the most accurate indication of potential PAH sources and the aggregated magnitude of contamination; and, passive samplers accumulated a greater degree of larger molecular weight PAHs than were measured in water samples, thus providing a retrospective indication of PAHs present throughout the deployment period. During preliminary site assessments, it is valuable to consider the different strengths of these matrices and determine what information is desired to characterize site conditions.

Elevated EROD activity was detected in all caged fish within the lake after 7 days, and correlated highly with pyrene concentrations in the grab water samples. While EROD alone cannot be directly equated to adverse organismal or population effects, in this study it established a convincing link between low-level PAH contamination, bioavailability and a biochemical response in anadromous Pacific salmonids (Fent, 2003). Given the chronic nature of exposure, these results are consistent with declining salmonid populations, but

are not proof that they are a cause. The physical samples indicated that runoff is likely a continual contributor of PAH in Auke Lake. It then follows that in high watercraft use summers, salmon would be exposed to even greater PAH concentrations. Ongoing unabated runoff into salmon rearing habitat, in addition to other PAH generating activities, could be perpetuating water quality alterations and providing chronic exposures of bioavailable PAHs to the declining salmon species during sensitive life-stages.

## 2.6 Figures

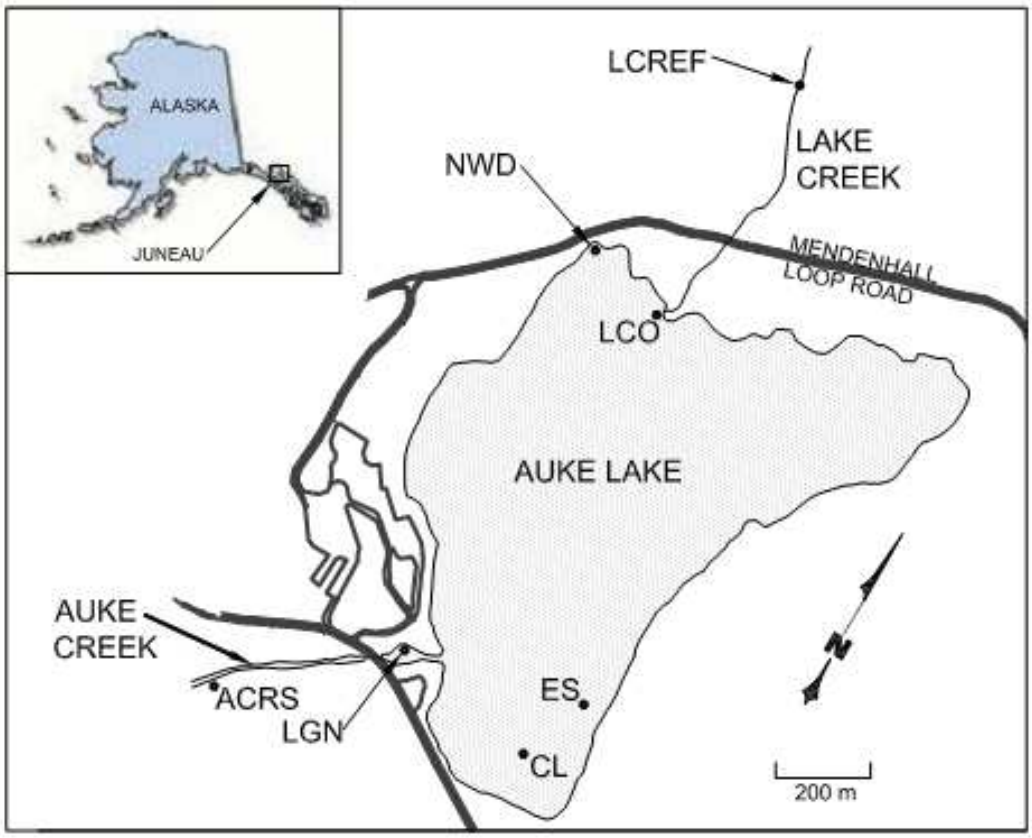



Figure 1 Map of Auke Lake, AK and sampling locations. LGN (Lagoon), CL (Central Lake), ES (East Shore), NWD (Northwest Drainage), LCO (Lake Creek Outfall), LCRef (Lake Creek Reference), ACRS (Auke Creek Research Station).

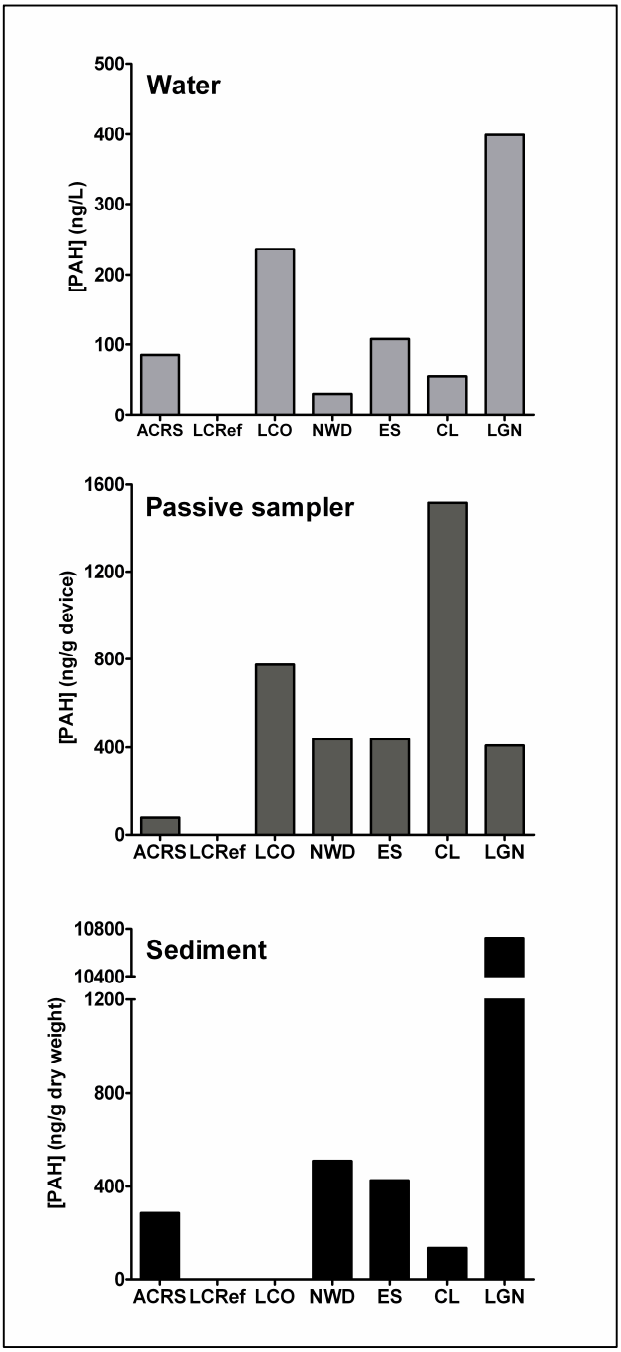
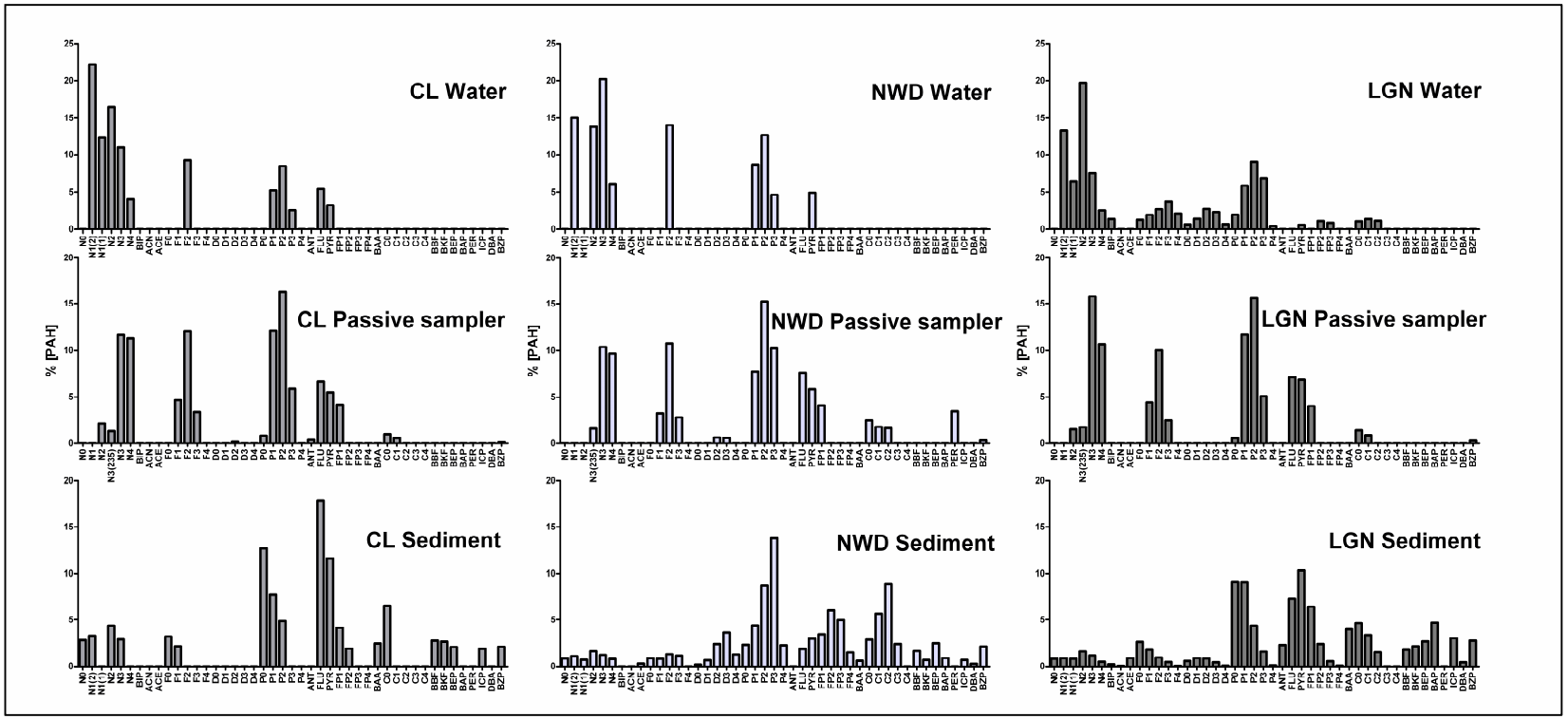



Figure 2 Total PAH concentrations in water (ng/L), passive sampling devices (ng/g sampling device) and sediments (ng/g dry weight) at sampling locations. ACRS (Auke Creek Research Station); CL (Central Lake), ES (East Shore), LCO (Lake Creek Outfall), LCRef (Lake Creek Reference), LGN (Lagoon), NWD (Northwest Drainage

Figure 3 Individual PAH profiles at selected sites in water, passive samplers and sediment (as % of total PAH). CL (Central Lake), LGN (Lagoon), NWD (Northwest Drainage). ACE: acenapthene; ACN: acenapthylene; ANT: anthracene; BAA: benzo[a]anthracene; BAP: benzo[a]pyrene; BBF: benzo[b]fluoranthene; BEP: benzo[e]pyrene; BIP: biphenyl; BKF: benzo[k]fluoranthene; BZP: benzo[ghi]perylene; C0: chrysene; C1: C-1 chrysenes; C2: C-2 chrysenes; C3: C-3 chrysenes; C4: C-4 chrysenes; D0: dibenzothiophene; D1: C-1 dibenzothiophenes; D2: C-2 dibenzothiophenes; D3: C-3 dibenzothiophenes; D4: C-4 dibenzothiophenes; DBA: dibenzo[ah]anthracene; F0: fluorene; F1: C-1 fluorenes; F2: C-2 fluorenes; F3: C-3 fluorenes; F4: C-4 fluorenes; FLU: fluoranthene; FP1: C-1 fluoranthenes/pyrenes; FP2: C-2 fluoranthenes/pyrenes; FP3: C-3 fluoranthenes/pyrenes; FP4: C-4 fluoranthenes/pyrenes; ICP: indeno[123-cd]pyrene; N0: naphthalene; N1: C-1 naphthalenes; N1(1): 1-methylnaphthalene; N1(2): 2-methylnaphthalene; N2: C-2 naphthalenes; N3: C-3 naphthalenes; N3(235): 2,3,5-trimethylnaphthalene; N4: C-4 naphthalenes P0: phenanthrene; P1: C-1 phenanthrenes/anthracenes; P2: C-2 phenanthrenes/anthracenes; P3: C-3 phenanthrenes/anthracenes; P4: C-4 phenanthrenes/anthracenes; PER: perylene; PYR: pyrene.


CL Water
NWD Water
LGN Water
CL Passive sampler
NWD Passive sampler
LGN Passive sampler
CL Sediment
NWD Sediment
LGN Sediment
% [PAH]

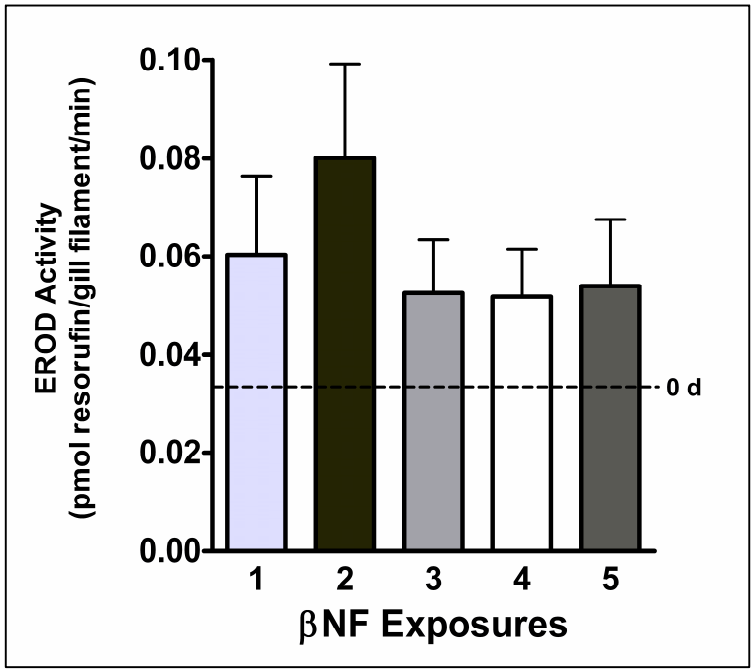



Figure 4 Mean (+95% CI) gill EROD activities (pmol resorufin/gill filament/min) [n=10] following various βNF exposure scenarios. No significant difference between βNF exposure scenarios was found ($p<0.05$). 1 (2 d βNF [13.7℃]), 2 (2 d βNF [6℃]), 3 (4 d βNF [13.7℃]), 4 (2 d βNF 2 d ACRS [13.7℃], 5 (7 d βNF [13.7℃]). Dashed line indicates 0 d or basal mean gill EROD activity (n=12).

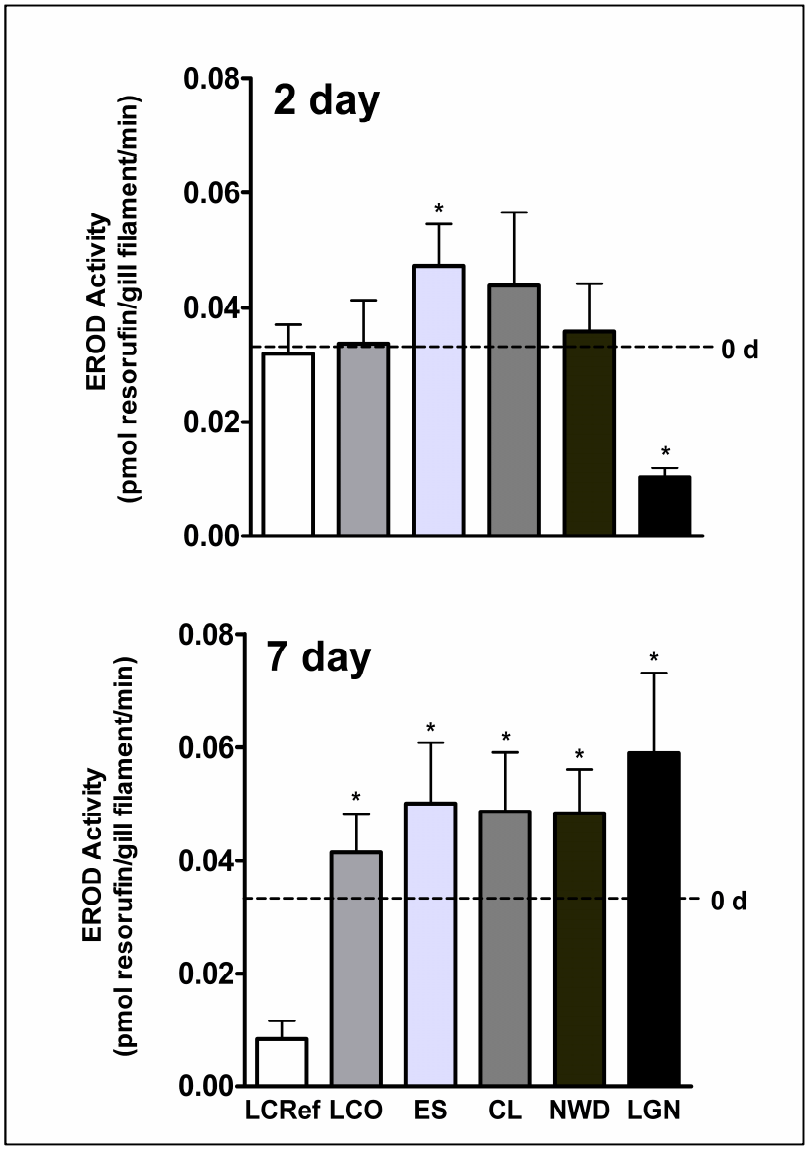



Figure 5 Mean (+95% CI) gill EROD activity (pmol resorufin/gill filament/min) [n=9-10] following 2- and 7-d caging at Auke Lake and Lake Creek Reference (LCRef) locations. * indicates locations that differ significantly from the LCRef site ($p<0.0001$). CL (Central Lake), ES (East Shore), LCO (Lake Creek Outfall), LCRef (Lake Creek Reference), LGN (Lagoon), NWD (Northwest Drainage). Dashed line indicates 0 d mean gill EROD activity (n=12).

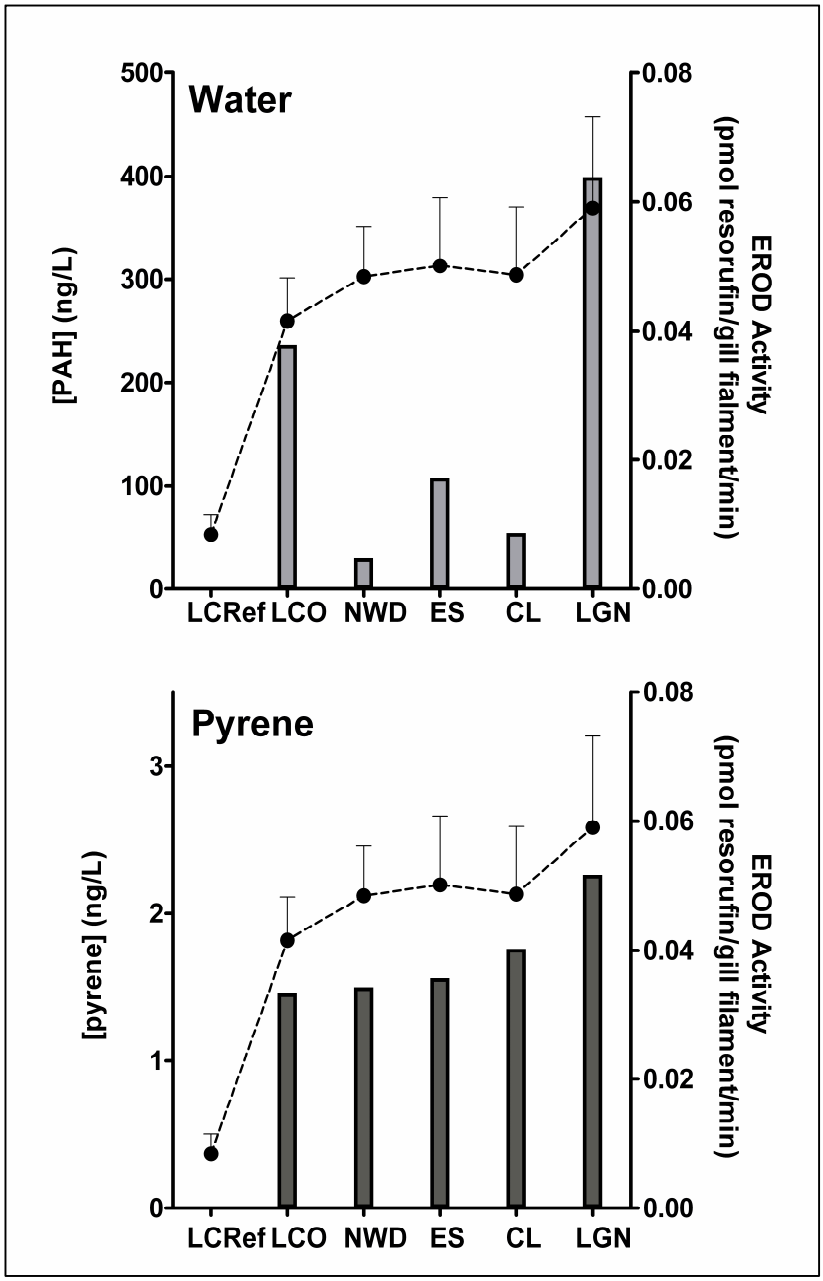



Figure 6 Mean (+95% CI ) 7 d gill EROD activity (pmol resorufin/gill filament/min) (●) and total PAH and pyrene (ng/L) (■) at sampling locations. CL (Central Lake), ES (East Shore), LCO (Lake Creek Outfall), LCRef (Lake Creek Reference), LGN (Lagoon), NWD (Northwest Drainage).

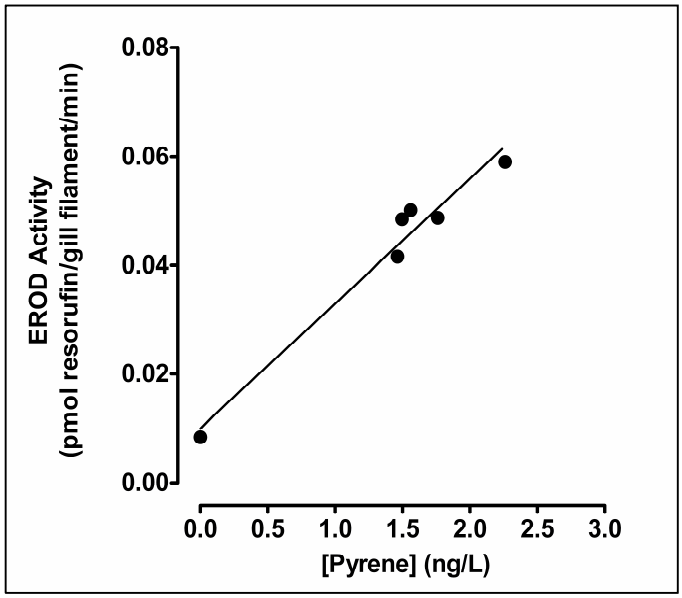



Figure 7 Site location aqueous pyrene concentrations (ng/L) and 7-d mean gill EROD activities (pmol resorufin/gill filament/min). Linear regression ($r^2$=0.9662; p=0.0004).

## 2.7 Tables

Table 1 PAH source indicative diagnostic ratios for water, sediment and passive sampling devices deployed in Auke Lake, AK

| WATER | P0/ ANT[1] | FLU/ PYR[2] | C1/ C0[3] | ANT/ (ANT+P0)[4] | FLU/ (FLU+PYR)[5] | BAP/ (BAP+C0)[6] | ICP/ (ICP+ BZP)[7] | BAP/ BZP[8] | (FLU+PYR+BAA+C0+BBF+BKF+BEP+BAP+ICP +BZP) / (N0+ACN+ACE+F0+P0+ANT+FLU+PYR+ BAA+C0+BBF+BKF+BAP+ ICP+DBA+BZP)[9] |
|---|---|---|---|---|---|---|---|---|---|
| LCRef | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| LCO | NA | 0 | NA | 0 | 0 | NA | NA | NA | 0.12 |
| CL | NA | 1.68 | NA | NA | 0.63 | NA | NA | NA | 1.00 |
| LGN | NA | 0 | 1.30 | 0 | 0 | 0 | NA | NA | 0.34 |
| ES | NA | 0 | NA | NA | 0 | NA | NA | NA | 1.00 |
| NWD | NA | 0 | NA | NA | 0 | NA | NA | NA | 1.00 |
| ACRS | NA | NA | NA | 0 | NA | NA | NA | NA | 0 |
| **PASSIVE SAMPLER** | | | | | | | | | |
| LCRef | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| LCO | NA | 1.61 | NA | 0 | 0.62 | NA | NA | NA | NA |
| CL | 2.22 | 1.21 | NA | 0.31 | 0.55 | NA | NA | NA | NA |
| LGN | NA | 1.04 | NA | 0 | 0.51 | NA | NA | NA | NA |
| ES | NA | 1.33 | NA | 0 | 0.57 | NA | NA | NA | NA |
| NWD | NA | 1.31 | NA | NA | 0.67 | NA | NA | NA | NA |
| ACRS | NA | 1.48 | NA | 0 | 0.7 | NA | NA | NA | NA |
| **SEDIMENT** | | | | | | | | | |
| LCRef | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| LCO | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| CL | NA | 1.53 | 0 | 0 | 0.61 | 0.28 | 0.47 | 0 | 0.75 |
| LGN | 3.94 | 0.70 | 0.72 | 0.20 | 0.41 | 0.46 | 0.52 | 1.69 | 0.76 |
| ES | NA | 1.35 | 0 | 0 | 0.57 | 0.24 | 0.50 | 0 | 0.83 |
| NWD | NA | 0.63 | 1.96 | 0 | 0.39 | 0.17 | 0.24 | 0.40 | 0.89 |
| ACRS | 5.47 | 1.13 | 0.35 | 0.16 | 0.53 | 0.35 | 0.52 | 1.67 | 0.73 |

Sampling locations: ACRS: Auke Creek Research Station; CL: Central Lake; ES: East Shore; LCO: Lake Creek Outfall; LCRef: Lake Creek Reference; LGN: Lagoon; NWD: Northwest Drainage.

PAHs: ACE: acenaphthene; ACN: acenapthylene; ANT: anthracene; BAA: benzo[a]anthracene; BAP: benzo[a]pyrene; BBF: benzo[b]fluoranthene; BEP: benzo[e]pyrene; BZP: benzo[ghi]perylene; C0: chrysene; C1: C-1 chrysenes; DBA: dibenzo[ah]anthracene; FLU: fluoranthene; F0: fluorene; ICP: indino[123-cd]pyrene; P0: phenanthrene; P1: C-1 phenanthrenes/anthracenes; PYR: pyrene. NA: not applicable.

[1] Petrogenic > 5; Pyrogenic < 5 : Neff et al., 2005.

[2] Petrogenic <<<< 1; Pyrogenic approaches ≥ 1 : Neff et al., 2005.

[3] Petrogenic > 1; Pyrogenic < 1 : Neff et al., 2005.

[4] Petrogenic < 0.1; Pyrogenic > 0.1 : reviewed by Yunker et al. (2002) and Bucheli et al. (2004).

[5] Petrogenic < 0.4; combustion (gas/diesel/crude oil) = 0.4-0.5; combustion (wood/coal/grass) > 0.5 : reviewed by Yunker et al. (2002) and Bucheli et al. (2004).

[6] Petrogenic < 0.2; petrogenic or pyrogenic = 0.2 -0.35; Pyrogenic > 0.35 : reviewed by Yunker et al. (2002) and Bucheli et al. (2004).

[7] Petrogenic < 0.20; combustion (vehicle/crude oil) = 0.2-0.5; Combusition (grass/wood/coal) > 0.5 : reviewed by Yunker et al. (2002) and Bucheli et al. (2004).

[8] Non-vehicle < 0.6; Vehicle > 0.6 : reviewed by Yunker et al. (2002) and Bucheli et al. (2004).

[9] Petrogenic = 0.3; Pyrogenic = 0.7 : Hwang et al. (2003) and reviewed by Bucheli et al. (2004).

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# 3: General Conclusion

## 3.1 Overview

In order to retrospectively determine if PAHs could be participating in adverse effects on salmon populations, this study quantified and spatially and temporally characterized PAH concentrations and simultaneously assessed their *in situ* bioavailability to juvenile salmon via a sensitive gill EROD biomarker assay (Jönsson et al., 2002). Overall, the present research indicates that juvenile salmonids endemic to Auke Lake, AK are being exposed to locally generated/released anthropogenically sourced, bioavailable PAHs.

## 3.2 PAH Sources

Source identification techniques are key components of PAH contamination studies and can assist in determining source(s) and in focusing management options (Zhang et al., 2005). In the current study, basic source identification methods were employed to determine if PAHs in the sediments, water and accumulated in passive sampling devises were predominantly petrogenic or pyrogenic in nature (Yunker et al., 2002).

The source identification techniques revealed that different areas of the lake experience PAHs inputs from different sources. A distinct petrogenic PAH signature was identified in sediments in areas receiving runoff. These PAHs could be the result of uncombusted fuels released onto impervious surfaces

subsequently entering the lake through runoff events (Menzie et al., 2002; Stout et al., 2004). Furthermore, sediment samples at runoff locations had the highest PAH concentrations suggesting that runoff contributes to lake contamination. Sediment sample profiles of sites situated away from developed shorelines were indicative of pyrogenic PAHs. These PAHs are likely the product of localized combustion and subsequent wet and dry deposition in the lake (Dickhut et al., 2000, Van Metre et al., 2000; Stout et al., 2004). Although activity was anecdotally lower than in previous summers, recreational watercraft use is also likely a contributing source of both pyrosynthesized and fuel related PAHs to the lake (Rice et al., 2008). The passive sampling device deployed at the area of highest activity accumulated the highest concentration of PAHs. This was similar, although several-fold less in concentration, to the initial monitoring study indicating that even relatively low watercraft use contributed to PAHs in the water column (Rice et al., 2008). No PAHs were detected in any of the sampling matrices collected at the reference location upstream of anthropogenic developments/activities. This indicates that PAHs sources are likely localized and that atmospheric transport of remotely emitted PAHs and deposition of these in the lake, is likely not a major contributor to total PAH contamination.

At each biomonitoring site, water, passive sampler and sediment samples were collected to determine which sampling matrices correlated most closely to the biological receptor response in addition to comparing the PAH information each matrices revealed. Of the sampling matrices, water samples' total PAH, and of the PAHs specifically pyrene, was most closely correlated to the biomarker

response. This relationship was expected as water was the main exposure media, and the biomarker was measured in respiratory tissue. However, it should be noted that the correlation alone does not unequivocally prove causality. To confirm low (ng/L) pyrene as a gill filament EROD inducer, a future laboratory study could expose salmonids to concentrations of pyrene similar to and less than those measured in this study and analyze corresponding gill filament EROD activity.

The water samples PAH profiles were dominated by lower molecular weight, petrogenic compounds. Although accumulating PAHs from the aqueous phase, passive sampling device PAH concentrations and profiles were not reflective of the associated water samples. The sampling devises captured a greater proportion of higher molecular weight, pyrogenic PAHs than were represented in the grab water samples. The disparities between passive and water sampler PAH concentration and profiles could be attributable to several factors, including time integration, the partitioning behaviour of individual PAHs into the devices, and turbulence effects (Müller et al., 2001; Carls et al., 2004; Booij et al., 2005; Vrana et al., 2005). Sediment samples were not relatable to water or passive sampler total PAHs or biological response except at the most contaminated site at which both water PAH and EROD activity were also the highest. Sediments can act as sinks, accumulating PAHs over time. In relation to this, sediment samples were the best indicator of time-integrated source and are the matrices typically used for source identification in PAH contaminated aquatic systems (Sower and Anderson, 2008). PAH water solubility tends to decrease

with increasing ring number and alkylation (Neff, 1979). In regards to this, the distribution of PAHs between the different matrices was as expected and generally reflected the PAHs relative water solubilities/hydrophobicities.

Taken together, the current study indicates that different sampling matrices can reveal disparate information regarding source and magnitude of PAH contamination. Each of these matrices has strengths: water is most representative of pelagic species exposure concentrations; passive samplers are capable of accumulating and magnifying pulse or very low concentration aqueous PAHs; and, sediments are the ultimate repository of PAHs and can reveal useful time-integrated indication of PAH source(s) and magnitude. When utilized in monitoring or biomonitoring studies, attention to the specific information desired will assist in determining which type of sampling matrices is the most appropriate.

Auke Lake is experiencing PAH contamination from multiple localized sources. Implementation of more rigorous runoff abatement measures in addition to limiting the number and hours of two-cycle engine use may decrease the amount of PAHs entering the lake

## 3.3 Low Concentration PAHs

Compared to the current Alaskan water quality standards, the total PAH concentrations detected in the current study were orders of magnitude below the level of concern (ADEC, 2008). Nevertheless, salmonids caged at PAH contaminated areas in Auke Lake showed CYP1A induction above the levels

measured in fish caged at the reference site where no PAHs were detected in any of the sampling matrices. This indicates that the relatively low levels of PAH in Auke Lake (ng/L) are bioavailable and capable of eliciting a biological response. Furthermore, the PAH concentrations associated with gill EROD induction were within the relative range of concentrations previously shown capable of adversely affecting embryonic salmonids (Heintz et al., 1999; Heintz et al., 2000; Carls et al., 2005). Embryonic salmonid exposure to low aqueous PAH concentrations can cause abated post-emergence growth (Heintz et al., 2000; Carls et al., 2005). In addition, juvenile salmonid dietary exposure to environmentally relevant PAH concentrations has been shown to cause an overall decrease in stored lipids and biomass (Meador et al., 2005). These types of PAH exposures potentially lead to compromised growth during a salmonids first marine year (Meador et al., 2005). Reduced growth during this life-stage may disadvantage juvenile salmonids' ability to capture prey, escape predation and ultimately its probability of surviving (Beamish and Mahnkin, 2001; Beamish et al., 2004; Moss et al., 2005; Meador et al., 2005). Lower survival in the first marine year survivability could potentially result in population level effects (Spromberg and Meador, 2006). It is unknown if the concentrations detected in the current study are of a magnitude capable of eliciting such effects. Future studies could assess whether the chronic low concentration PAHs encountered in Auke lake are capable of compromising salmonid growth potential (Meador et al., 2005). Additionally, might the sub-lethal PAH exposures early in the salmonids' life attenuate their ability to adapt/respond to other natural and

anthropogenic freshwater and marine stressors ultimately jeopardizing fitness and recruitment (Arkoosh et al., 1998b; Arkoosh et al., 2001; Arkoosh and Collier, 2002; Jacobson et al., 2003; Johnson et al., 2008)?

The current study indicates that anthropogenically sourced PAHs in Auke Lake, AK are bioavailable to anadromous Pacific salmon. Whether PAHs alone are the sole cause of the recent population declines is unknown and defining this relationship requires further investigation.

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# Appendix 1


Aerial map of Auke Lake and vicinity, Juneau, Alaska (Google™ Earth)