Ecotoxicology and Environmental Safety 73 (2010) 849–857

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Anthropogenically sourced low concentration PAHS: *In situ* bioavailability to juvenile Pacific salmon

Ariel M. Blanc [a], Larry G. Holland [b], Stanley D. Rice [b], Christopher J. Kennedy [a,*]

[a] Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, B.C., Canada V5A 1S6
[b] Auke Bay Laboratories, Ted Steven Marine Research Institute, Juneau, AK 99801, USA

ARTICLE INFO

*Article history:*
Received 23 October 2009
Received in revised form
8 March 2010
Accepted 8 March 2010
Available online 7 April 2010

*Keywords:*
PAHs
CYP1A
Salmon
Gill
Biomarker
EROD

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 anthropogenically sourced PAHs during sensitive life-stages, which may be in part a contributor to their declining numbers.

© 2010 Elsevier Inc. All rights reserved.

## 1. Introduction

Since the late 1970s, much of Alaska has experienced robust salmon stocks as a reflection of favorable 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 (Fig. 1) (Hoover, 2008; Rice et al., 2008). The lake provides habitat for multiple species of anadromous salmon (*Oncorhynchus gorbuscha*, *Oncorhynchus keta, Oncorhynchus kisutch* and *Oncorhynchus nerka*), resident trout (*Salvelinus malma, Oncorhynchus mykiss* and *Oncorhynchus 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 PAH concentrations and their bioavailability to juvenile salmonids, the organism of most concern in this system (Fent, 2003). The previously deployed passive sampling devices (low-density polyethylene strips) (Rice et al., 2008), 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 SPMDs, they lack a triolein reservoir and have not yet undergone the rigorous kinetic studies and field testing needed to either back-calculate aqueous exposure concentrations or substitute for an aquatic biological response (Huckins et al., 1990; Müller et al., 2001; 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 and halogenated hydrocarbons (Whyte et al., 2000). The gill, as a major route of

* Coresponding author. Fax: +1 778 782 3496.
*E-mail address:* ckennedy@sfu.ca (C.J. Kennedy).

0147-6513/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2010.03.003

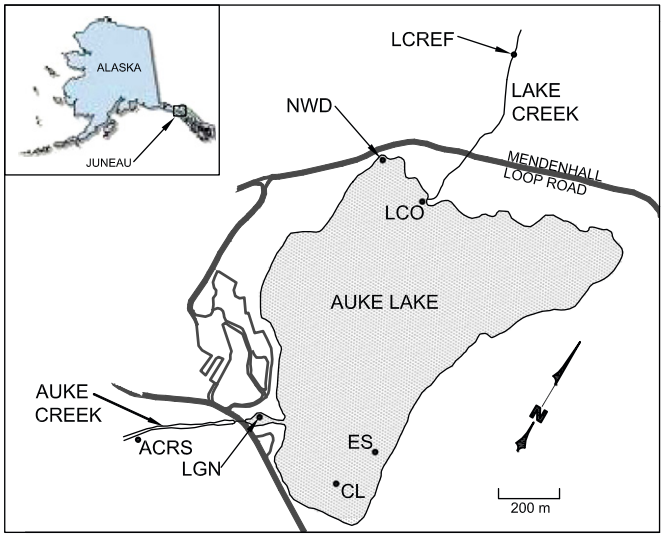


**Fig. 1.** Map of Auke Lake, AK and sampling locations. LGN—Lagoon, CL—Central Lake, ES—East Shore, NWD—Northwest Drainage, LCO—Lake Creek Outfall, LCRef—Lake Creek Reference, ACRS—Auke Creek Research Station.

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, 2008; Andersson et al., 2007).

The primary objective of this study was to characterize PAH concentrations in the lake and determine their site-specific bioavailability to juvenile salmonids; a secondary objective was to compare different physical sampling matrices as indicators of sources and biological responses to PAHs, for utilization in future site assessments of this nature. These goals were accomplished by simultaneously gathering data from 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 fish caged at the locations.

## 2. Materials and methods

### 2.1. Fish

Three hundred yearling Chinook (*Oncorhynchus* 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 °C, ≥7.3 mg/L DO, flow: approx. 20 L/min, natural photoperiod of 58°18′N, approx. 18 h daylight) 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 (Fig. 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 2–4 d prior to β-napthoflavone (βNF) exposure or caging in Auke Lake. The use of fish in this study was in accordance with the University Animal Care Committee (UACC).

### 2.2. Chemicals

Resorufin, 7-ethoxyresorufin (7-ER), dicumarol 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).

### 2.3. Lake exposures

Five Auke Lake locations were chosen to represent areas of varying PAH contamination from different sources including motorized watercraft activity and runoff (Fig. 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, a runoff outlet and in an area of high motorized watercraft activity; 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 Research 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 simultaneously placed approx. 1 m below the lake surface. Each cage contained 10 fish (12.03 ± 2.41 g [mean ± SD]) from the ACRS. Cages were positioned horizontally paralleling a passive sampling device (deployed for 21 d), and cage placement temporally corresponded with the terminal end of the sampler deployment period. Fish were sampled from one cage at 2 d, and from the other at 7 d, coinciding with the removal of the passive samplers 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 blunt force trauma and decapitation) for gill EROD activity analysis.

### 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 Abrahamson 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 (Jönsson et al., 2002). 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 dicumarol in HC buffer and placed on a shaker for a 10-min pre-incubation period at room temperature (approx. 20 °C). 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 (0–250 nM resorufin) was used to calculate the resorufin concentration in all samples. Results are expressed as picomoles reosrufin/gill filament/minute (pmr/gf/min).

### 2.5. 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 (the same water as ACRS holding tank) placed in opaque fiberglass boxes. The boxes were elevated in a 3500 L 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 °C. In addition, a 2 d βNF exposure at the ACRS holding tank temperature of 6.0 °C 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 (*O. mykiss*) and Atlantic salmon (*Salmo salar*) employing similar exposure scenarios have monitored gill EROD activity of βNF-exposed fish using fish from holding tank(s) as the unexposed control; the holding tank fish were shown to have gill EROD activities comparable to those held in polyethylene bags, with and without acetone added (Jönsson et al., 2002, 2003). In the current study, βNF-exposed fish were compared to ACRS holding tank fish to evaluate induction potential.

### 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 deuterated surrogates, and extracted into 80:20 pentane:dichloromethane through repeated sonication. The extracts were reduced, dried with sodium sulfate, and then transferred to hexane. The samples were applied to and eluted through a micro silica gel/alumina column with 50:50 pentane:dichloromethane, reconcentrated and transferred to hexane. Extracts were analyzed by gas chromatography equipped with a mass selective detector (GC/MS) for 40 parent and alkylated PAHs and dibenzothiophenes. 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). Concentrations of analytes in reference samples were within 15% of the National Institute of Standards and Technology (NIST) standards, and precision expressed as coefficient of variation was usually $<20\%$, depending on the specific analyte. PAHs detected below method detection limits (MDL) were assigned a value of zero. Total PAH values reported are the sum of all detected analytes.

### 2.7. 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 deuterated surrogates, dried with sodium sulfate, and then transferred to 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 deuterated surrogates, dried with sodium sulfate, and extracted with dichloromethane by a Dionex ACE® 200 accelerated solvent extractor (Dionex Corporation, Sunnyvale, CA). Extracts were transferred to hexane, purified and fractionated by elution through silica gel-alumina columns with dichloromethane, transferred to hexane, and further purified by HPLC. Extracts were analyzed by GC–MS, and PAH concentrations were determined by the internal standard method (Short et al., 1996). Quality assurance and control samples yielded results similar to those for the passive sampling devices (discussed above). 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).

### 2.8. 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 homologs (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-sourced PAHs included the presence of the heterocycle dibenzothiophene(s); the relative alkyl homolog 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 4–6-ring PAHs (Wang and Fingas, 2003); and, the use of diagnostic ratios which incorporate a quantitative comparison of relative petrogenic vs. pyrogenic indicator PAHs (Table 1) (Boehm and Farrington, 1984; Stout et al., 2001b). Conversely, pyrogenic-sourced PAHs were generally characterized by a predominance of 4–6-ring parent PAHs, with skewed homolog 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, 2003).

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

**Table 1**
PAH source-indicative diagostic ratios for water, sediment and passive sampling devices deployed in Auke Lake, AK.

| | P0/ANT[a] | FLU/PYR[b] | C1/C0[c] | ANT/(ANT+P0)[d] | FLU/(FLU+PYR)[e] | BAP/(BAP+C0)[f] | ICP/(ICP+BZP)[g] | BAP/BZP[h] | (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)[i] |
|---|---|---|---|---|---|---|---|---|---|
| *Water* | | | | | | | | | |
| 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.*PAHs*: ACE, acenaphthene; ACN, acenapthylene; ANT, anthracene; BAA, benzo[a]anthracene; BAP, benzo[a]pyrene; BBF, benzo[b]fluoranthene; BEP, benzo[e]pyrene; BZP, benz[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.

[a] Petrogenic > 5; pyrogenic < 5: Neff et al. (2005).
[b] Petrogenic < < < 1; pyrogenic approaches ≥ 1: Neff et al. (2005).
[c] Petrogenic > 1; pyrogenic < 1: Neff et al. (2005).
[d] Petrogenic < 0.1; pyrogenic > 0.1: reviewed by Yunker et al. (2002) and Bucheli et al. (2004).
[e] 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).
[f] Petrogenic < 0.2; petrogenic or pyrogenic = 0.2–0.35; pyrogenic > 0.35: reviewed by Yunker et al. (2002) and Bucheli et al. (2004).
[g] 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).
[h] Non-vehicle < 0.6; vehicle > 0.6: reviewed by Yunker et al. (2002) and Bucheli et al. (2004).
[i] Petrogenic = 0.3; pyrogenic = 0.7: Hwang et al. (2003) and reviewed by Bucheli et al. (2004).

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 Anderson, 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 Anderson, 2008).

### 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 and 7 d gill filament EROD activity. For the 2 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 vs. 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.

## 3. Results

### 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) (Fig. 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% and 99% at all other sites. Alkylated naphthalene and phenanthrene/anthracene homologs 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 4–6-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) (Fig. 3). Sample diagnostic ratios are displayed in Table 1.

### 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 (1514.4 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 lowest concentrations was 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 3-week sampling period (Fig. 2).

Two- and 3-ring PAHs dominated the composition of PAHs in the passive samplers, but much higher proportions of 4–6-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 homolog 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 4–6-ring PAHs detected, chrysene(s) and a distinct fluoranthene, pyrene and C-1 fluoranthenes/pyrenes cluster accumulated in all passive samplers (Fig. 3). Diagnostic ratios for isomers are shown in Table 1.

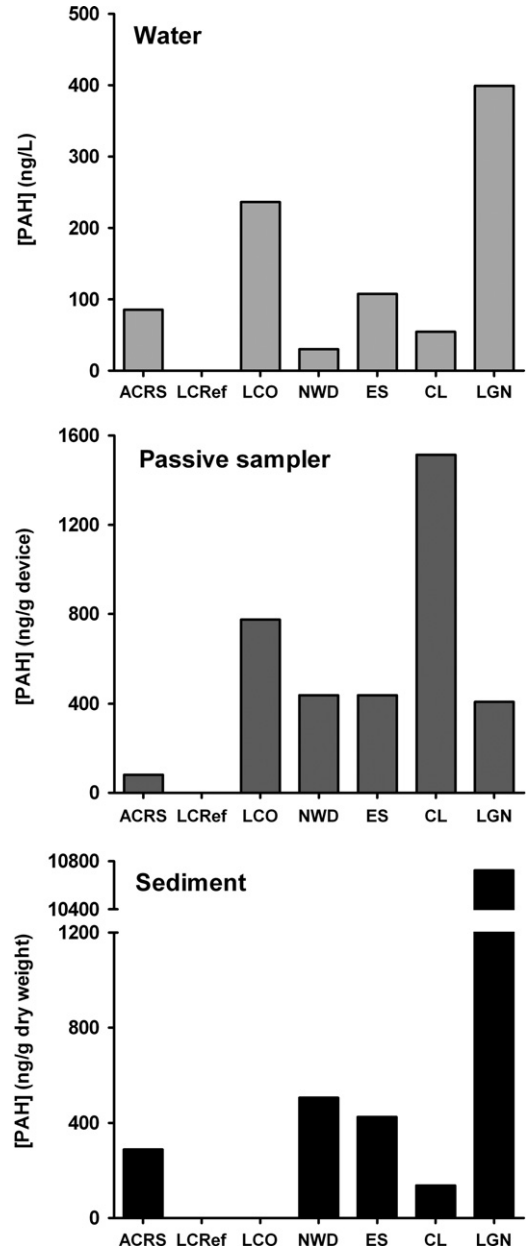


**Fig. 2.** Total PAH concentrations in water (ng/L), passive sampling devices (ng/g sampling device) and sediments (ng/g dry weight) at sampling locations. ACRS—Auke Creek Research Station, CL—Central Lake, ES—East Shore, LCO—Lake Creek Outfall, LCRef—Lake Creek Reference, LGN—Lagoon, NWD—Northwest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

### 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 (Fig. 2). Sediments from these two sites were comprised of gravel and pebbles. The NWD sediment contained

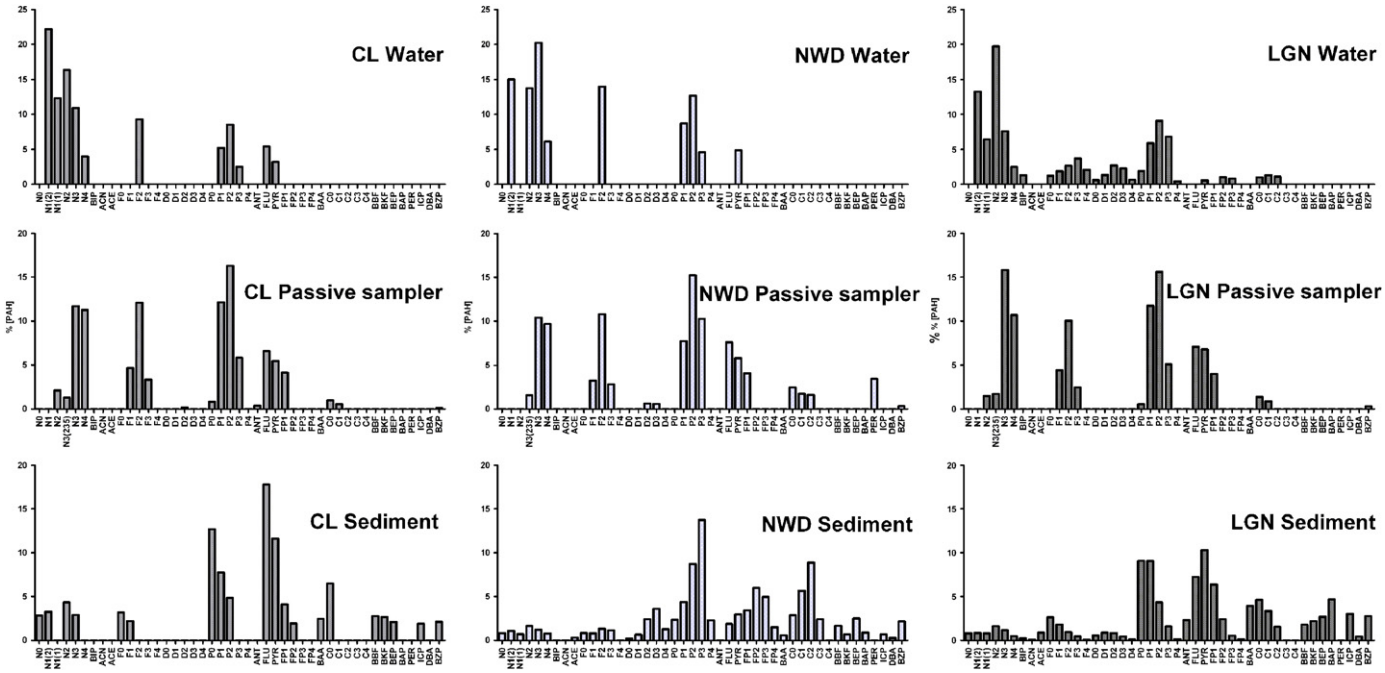


**Fig. 3.** Individual PAH profiles at selected sites in water, passive samplers and sediment (as % of total PAH). CL—Central Lake, LGN—Lagoon, NWD—Northwest Drainage. ACE: acenapthene; ACN: acenapthylene; ANT: anthracene; BAA: benzo[a]anthracene; BAP: benzo[a]pyrene; BBF: benzo[b]fluoranthene; BEP: benzo[e]pyrene; BIP: biphenyl; BKF: benzo[k]fluoranthene; BZP: benz[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.

the lowest percent of 4–6-ring PAHs (49.85%) with alkylated pyrenes/fluoranthenes and chrysenes dominating. The other locations sediment samples contained an increasing percentage of 4–6-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 (Fig. 3). Sediment total PAH concentrations are the sum of all detected PAHs with the exception of perylene. Diagnostic ratios are depicted in Table 1.

### 3.4. Gill EROD activity

Juvenile Chinook salmon gill EROD activity was responsive to aqueous CYP1A inducers. 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 °C EROD activities were approximately 2-fold higher than measured in uninduced (basal) fish held in ACRS water ($0.033 \pm 0.007$ pmr/gf/min) (Fig. 4). βNF exposures of 2 and 7 d at 13.7 °C resulted in similar EROD activities indicating 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.

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 ($0.010 \pm 0.002$ pmr/gf/min) had declined significantly from the 0 d, while the ES's ($0.047 \pm 0.007$ pmr/gf/min) had increased significantly.

Following 7 d of caging, EROD activities increased at all lake locations 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 ($0.059 \pm 0.014$ pmr/gf/min), a 7-fold higher activity than fish caged at the reference location (LCRef) ($0.009 \pm 0.003$ pmr/gf/min) (Fig. 5).

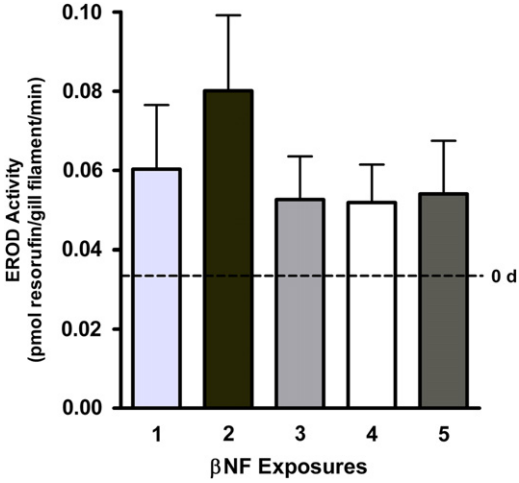


**Fig. 4.** Mean (+95% CI) gill EROD activities (pmol resorufin/gill filament/min) [$n=10$] following various βNF exposure scenarios. No significant difference between βNF exposure scenarios was found ($p<0.05$). 1 (2 d βNF [13.7 °C]), 2 (2 d βNF [6 °C]), 3 (4 d βNF [13.7 °C]), 4 (2 d βNF 2 d ACRS [13.7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

### 3.5. Correlation of gill EROD activity with sampling matrices and specific PAHs

Correlations of gill EROD with total PAHs in the sampling matrices were positive, but non-significant; 7-d mean gill EROD activity was the most closely correlated to total PAHs in water

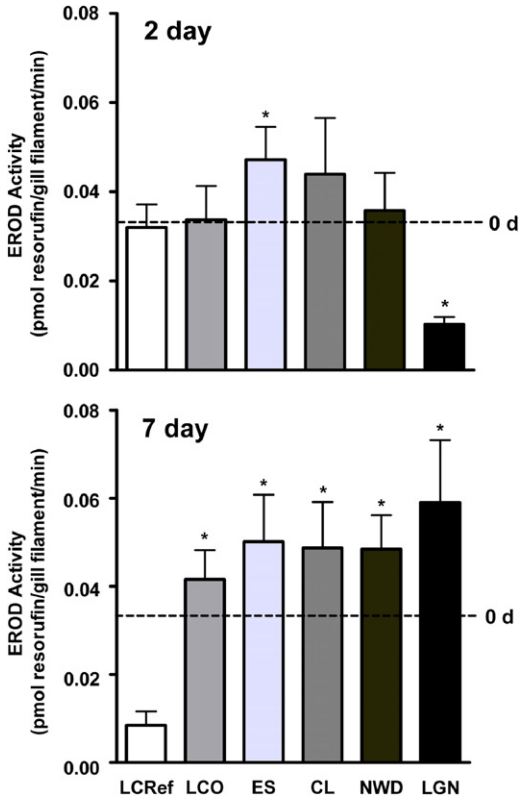


**Fig. 5.** Mean (+95% CI) gill EROD activity (pmol resorufin/gill filament/min) [$n$=9–10] following 2- and 7-d caging at Auke Lake and Lake Creek Reference (LCRef) locations. * indicates locations that differ significantly from the LCRef site ($p<0.0001$). CL—Central Lake, ES—East Shore, LCO—Lake Creek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

samples (water $r^2$=0.31, $p$=0.25; passive sampler $r^2$=0.22, $p$=0.35; sediment $r^2$=0.23, $p$=0.34). Although anticipated, summation of 4–6-ring PAHs did not yield a more meaningful relationship to EROD activity (water $r^2$=0.30, $p$=0.27; passive sampler $r^2$=0.24, $p$=0.32; sediment $r^2$=0.23, $p$=0.34).

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) (Figs. 6 and 7) as were C-2 fluorenes ($r^2$=0.6747, $p$=0.0450). Additionally, benzo(ghi)perylene accumulated in passive samplers was significantly correlated with mean EROD activity ($r^2$=0.6823, $p$=0.0428).

## 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 appear to be primarily 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 were comparable to βNF-exposed Atlantic salmon (*S. 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

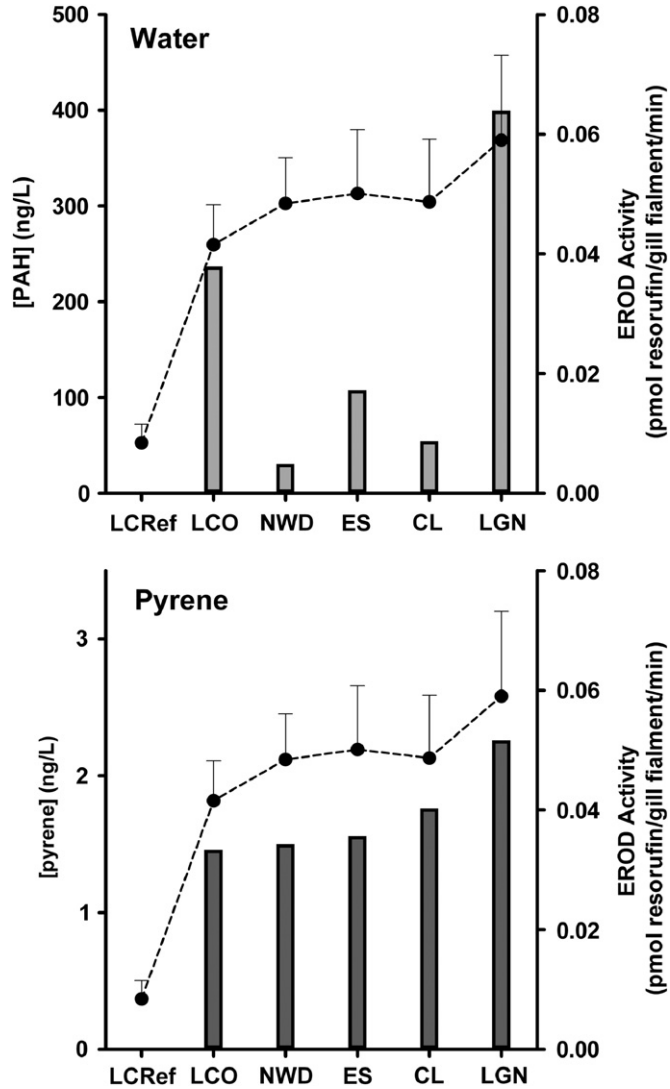


**Fig. 6.** Mean (+95% CI) 7 d gill EROD activity (pmol resorufin/gill filament/min) (●) and total PAH and pyrene (ng/L) (■) at sampling locations. CL—Central Lake, ES—East Shore, LCO—Lake Creek Outfall, LCRef—Lake Creek Reference, LGN—Lagoon, NWD—Northwest Drainage.

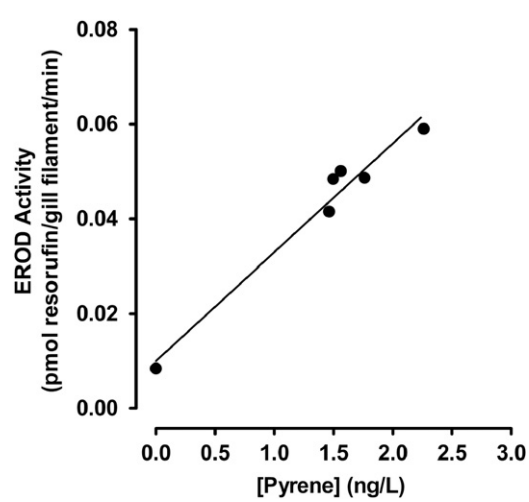


**Fig. 7.** Site location aqueous pyrene concentrations (ng/L) and 7-d mean gill EROD activities (pmol resorufin/gill filament/min). Linear regression ($r^2$=0.9662; $p$=0.0004).

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) (e.g. PAHs) were present and bioavailable in 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, 2003, 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 information on sources and magnitude of PAH contamination. Grab water samples provide a snapshot of actual PAH concentrations; all samples reflected petrogenic sources as they 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. In contrast to water samples, the 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-homolog 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 an associated diagnostic ratio values supported pyrogenic sources (Van Metre et al., 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, possibly 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 contained a high proportion of 4–6-ring PAHs, sloped homolog distributions, and several diagnostic ratios suggestive of pyrogenic-sources (Stout et al., 2001b; Neff et al., 2005). It is unknown to what extent grain size and organic carbon content influenced sediment PAH concentrations. Future Auke Lake studies should account for these parameters.

When considered together, the matrices provide a comprehensive picture of PAH contamination in Auke Lake. 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. The water and sediment 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, 2004; Menzie et al., 2002; Stein et al., 2006). In addition, sediment contamination at sites more distally located from developed shorelines was comprised primarily of pyrogenic PAHs. These were also present in the water intermittently or at low concentrations, as 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 hydrophobic chemicals 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. Pyrene has been previously shown capable of CYP1A induction. Following *in vitro* pyrene exposure, CYP1A was induced in fish hepatoma cell line (Fent and Bätscher (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 values of total PAH in water 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, 2000; Carls et al., 2005, Incardona et al., 2009). Specifically, one study found decreased pink salmon

(*O. gorbuscha*) 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, 2001; Arkoosh and Collier, 2002; Meador et al., 2006; Spromberg and Meador, 2006: Johnson et al., 2008)

## 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; passive samplers accumulated a greater degree of larger molecular weight PAHs than were measured in water samples, likely providing a retrospective indication of PAHs present throughout the deployment period; and sediments yielded the most accurate indication of potential PAH sources and the aggregated magnitude of contamination. 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 activities were measured in all fish after 7 d of caging in the lake, and were strongly correlated with pyrene concentrations in the grab water samples. While EROD activity alone cannot be directly equated to adverse organismal or population effects, in this study it provides a convincing link between low-level aqueous PAH contamination and bioavailability to anadromous Pacific salmon. The sediment samples indicated that runoff is likely a continual contributor of PAH in Auke Lake. It then follows that in addition to other likely local PAH sources, and specifically to PAHs generated during 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.

## Acknowledgments

We would like to extend special thanks to the following Auke Bay Laboratory staff: Marie Larsen and Josie Lunasin for their work in the chemistry lab; Adam Moles, John Joyce and Lawrence Schaufler for facilitating study logistics; and, to Josh Fibble for his assistance in the field. In addition we would also like to thank Brandon Hausmann at Wilson Engineering, Bellingham, WA for his contribution mapping. Funding was provided by the National Marine Fisheries Service.

## References

Abrahamson, A., Brandt, I., Brunström, B., Sundt, R.C., Jørgensen, E.H., 2008. Monitoring contaminants from oil production at sea by measuring gill EROD activity in Atlantic cod (*Gadus morhua*). Environ. Pollut. 153, 169–175.

Abrahamson, A., Andersson, C., Jönsson, M.E., Fogelberg, O., Örberg, J., Brunström, B., Brandt, I., 2007. Gill EROD in monitoring of CYP1A inducers in fish—a study in rainbow trout (*Oncorhynchus mykiss*) caged in Stockholm and Uppsala waters. Aquat. Toxicol. 85, 1–8.

Adkison, M.D., Finney, B.P., 2003. The long-term outlook for salmon returns to Alaska. Alaska Fishery Res. Bull. 10, 83–94.

Alaska Department of Environmental Conservation (ADEC), 2009. 18 AAC 70 Water Quality Standards.

Andersson, C., Katsiadaki, I., Lundstedt-Enkel, K., Örberg, J., 2007. Effects of 17α-ethynylestradiol on EROD activity, spiggin and vitellogenin in three-spined stickleback (*Gasterosteus aculeatus*). Aquat. Toxicol. 83, 33–42.

Arkoosh, M.R., Collier, T.K., 2002. Ecological risk assessment paradigm for salmon: analyzing immune function to evaluate risk. Hum. Ecol. Risk. Assess. 8, 265–276.

Arkoosh, M.R., Casillas, E., Huffman, P., Clemons, E., Evered, J., Stein, J.E., Vranasi, U., 1998b. Increased susceptibility of juvenile Chinook salmon from a contaminated estuary to *Vibrio anguillarum*. Trans. Am. Fish. Soc. 127, 360–374.

Arkoosh, M.R., Clemons, E., Huffman, P., Kagley, A.N., Casillas, E., Adams, N., Sanborn, H.R., Collier, T.K., Stein, J.E., 2001. Increased susceptibility of juvenile Chinook salmon to vibriosis after exposure to chlorinated and aromatic compounds found in contaminated urban estuaries. J. Aquat. Anim. Health 13, 257–268.

Barron, M.G., Heintz, R., Rice, S.D., 2004. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor agonists in fish. Mar. Environ. Res. 58, 95–100.

Boehm, P.D., Farrington, J.W., 1984. Aspects of the polycyclic aromatic hydrocarbon geochemistry of recent sediments in the Georges Bank region. Environ. Sci. Technol. 18, 840–845.

Booij, K., Hofmans, H.E., Fischer, C.V., Weerlee, E.M.V., 2003. Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes. Environ. Sci. Technol. 37, 361–366.

Bucheli, T.D., Blum, F., Desaules, A., Gustafsson, Ö, 2004. Polycyclic aromatic hydrocarbons, black carbon, and molecular markers in soils of Switzerland. Chemosphere 56, 1061–1076.

Carls, M.G., Heintz, R.A., Marty, G.D., Rice, S.D., 2005. Cytochrome P4501A induction in oil-exposed pink salmon *Oncorhynchus gorbuscha* embryos predicts reduced survival potential. Mar. Ecol. Prog. Ser. 301, 253–265.

Carls, M.G., Holland, L.G., Short, J.W., Heintz, R.A., Rice, S.D., 2004. Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices. Environ. Toxicol. Chem. 23, 1416–1424.

Cole, R.H., Frederick, R.E., Healy, R.P., Rolan, R.G., 1984. Preliminary findings of the priority pollutant monitoring project of the nationwide urban runoff program. J. Water Pollut. Control Fed. 56, 898–908.

Doong, R., Lin, Y., 2004. Characterization and distribution of polycyclic aromatic hydrocarbon contaminations in surface sediment and water from Gao-ping River, Taiwan. Water Res. 38, 1733–1744.

Fang, G., Chang, C., Wu, Y., Fu, P.P., Yang, I., Chen, M., 2004. Characterization, identification of ambient air and road dust polycyclic aromatic hydrocarbons in central Taiwan, Taichung. Sci. Total Environ. 327, 135–146.

Fent, K., 2003. Ecotoxicological problems associated with contaminated sites. Toxicol. Lett. 140–141, 353–365.

Fent, K., Bätscher, R., 2000. Cytochrome P4501A induction potencies of polycyclic aromatic hydrocarbons in a fish hepatoma cell line: demonstration of additive interactions. Environ. Toxicol. Chem. 19, 2047–2058.

Fernandes, M.B., Sicre, M.-A., Boireau, A., Tronczynski, J., 1997. Polyaromatic hydrocarbon (PAH) distributions in the Seine River and its estuary. Mar. Pollut. Bull. 34, 857–867.

Heintz, R.A., Rice, S.D., Wertheirmer, A.C., Bradshaw, R.F., Thrower, F.P., Joyce, J.E., Short, J.W., 2000. Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. Mar. Ecol. Prog. Ser. 208, 205–216.

Heintz, R.A., Short, J.W., Rice, S.D., 1999. Sensitivity of fish embryos to weathered crude oil: Part II. increased mortality of pink salmon (*Oncorhynchus gorbuscha*)

embryos incubating downstream from weathered *Exxon Valdez* crude oil. Environ. Toxicol. Chem. 18, 494–503.
Hoffman, E.J., Mills, G.L., Latimer, J.S., Quinn, J.G., 1984. Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. Environ. Sci. Technol. 18, 580–587.
Hoover, C.L., 2008. Auke Creek Weir Studies: 2006, Fishery Data Series No. 08-51. Alaska Department of Fish and Game, Department of Sport Fish and Commercial Fisheries, Anchorage, AK.
Huckins, J.N., Tubergen, M.W., Manuweera, G.K., 1990. Semipermeable membrane devices containing model lipid: a new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioconcentration potential. Chemosphere 20, 533–552.
Hwang, H., Wade, T.L., Sericano, J.L., 2003. Concentrations and source characterization of polycyclic aromatic hydrocarbons in pine needles from Korea, Mexico, and United States. Atmos. Environ. 37, 2259–2267.
Incardona, J.P., Carls, M.G., Day, H.L., Sloan, C.A., Bolton, J.L., Collier, T.K., Sholtz, N.L., 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. Environ. Sci. Technol. 43, 201–207.
Incardona, J.P., Carls, M.G., Teraoka, H., Sloan, C.A., Collier, T.K., Scholz, N.L., 2005. Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. Environ. Health Perspect. 113, 1755–1762.
Iqbal, J., Overton, E.B., Gisclair, D., 2008. Polycyclic aromatic hydrocarbons in Louisiana rivers and coastal environments: source fingerprinting and forensic analysis. Environ. Forensics 9, 63–74.
Johnson, L.L., Arkoosh, M.R., Bravo, C.F., Collier, T.K., Krahn, M.M., Meador, J.P., Myers, M.S., Reichert, W.L., Stein, J.E., 2008. The effects of polycyclic aromatic hydrocarbons in fish from Puget Sound, Washington. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. Taylor and Francis Group, LLC, United States of America, pp. 877–923.
Jönsson, M.E., Abrahamson, A., Brunström, B., Brandt, I., 2006. Cytochrome P4501A induction in rainbow trout gills and liver following exposure to waterborne indigo, benzo[a]pyrene and 3,3'4,4',5-pentachlorobiphenyl. Aquat. Toxicol. 79, 226–232.
Jönsson, M.E., Abrahamson, A., Brunström, B., Brandt, I., Ingebrigtsen, K., Jørgensen, E.H., 2003. EROD activity in gill filaments of anadromous and marine fish as a biomarker of dioxin-like pollutants. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 136, 235–243.
Jönsson, M.E., Brandt, I., Brunström, B., 2002. Gill filament-based EROD assay for monitoring waterborne dioxin-like pollutants in fish. Environ. Sci. Technol. 36, 3340–3344.
Kennedy, C.J., Walsh, P., 1994. The effect of temperature on the uptake and metabolism of benzo[a]pyrene in isolated gill cells of the gulf toadfish, *Opsanus beta*. Fish Physiol. Biochem. 13, 93–103.
Latimer, J.S., Hoffman, E.J., Hoffman, G., Fasching, J.L., Quinn, J.G., 1990. Sources of petroleum hydrocarbons in urban runoff. Water Air Soil Pollut. 52, 1–21.
Levine, S.L., Oris, J.T., 1999. CYP1A expression in liver and gill of rainbow trout following waterborne exposure: implications for biomarker determination. Aquat. Toxicol. 46, 279–287.
Lico, M.S., 2004. Gasoline-related organics in Lake Tahoe before and after prohibition of carbureted two-stroke engines. Lake Reservoir Manage. 20, 164–174.
Lima, A.L.C., Farrington, J.W., Reddy, C.M., 2005. Combustion-derived polycyclic aromatic hydrocarbons in the environment—a review. Environ. Forensics 6, 109–131.
MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31.
Manoli, E., Samara, C., Konstantinou, I., Albanis, T., 2000. Polycyclic aromatic hydrocarbons in the bulk precipitation and surface waters of Northern Greece. Chemosphere 41, 1845–1855.
Mantua, N.J., Hare, S.R., Zhang, Y., Wallace, J.M., Francis, R.C., 1997. A Pacific interdecadal climate oscillation with impact on salmon production. Bull. Am. Meteor. Soc. 78, 1069–1079.
Mdegela, R., Myburgh, J., Correia, D., Braathen, M., Ejobi, F., Botha, C., Sanvik, M., Utne Skaare, J., 2006. Evaluation of the gill filament-based EROD assay in African sharptooth catfish (*Clarias gariepinus*) as a monitoring for waterborne PAH-type contaminants. Ecotoxicology 15, 51–59.
Meador, J.P., Sommers, F.C., Ylitalo, G.M., Sloan, C.A., 2006. Altered growth and related physiological responses in juvenile Chinook salmon (*Oncorhyndhus tshawytscha*) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). Can. J. Fish. Aquat. Sci. 63, 2364–2376.
Menzie, C.A., Hoeppner, S.S., Cura, J.J., Freshman, J.S., LaFrey, E.N., 2002. Urban and suburban storm water runoff as a source of polycyclic aromatic hydrocarbons (PAHs) to Massachusetts estuarine and coastal environments. Estuaries 25, 165–176.
Moles, A., Marty, G.D., 2005. Physiological changes in prickly sculpin (*Cottus asper*) inhabiting a lake used by jet-propelled watercraft. Bull. Environ. Contam. Toxicol. 74, 1151–1158.
Moles, A., Holland, L., Andersson, O., 2006. Assessment of the significance of direct and indirect pollution inputs to a major salmon-producing river using polyethylene membrane devices. Environ. Toxciol. Chem. 25, 2011–2017.
Müller, J.F., Manomanii, K., Mortimer, M.R., McLachlan, M.S., 2001. Partitioning of polycyclic aromatic hydrocarbons in the polyethylene/water system. Fresen. J. Anal. Chem. 371, 816–822.
Neff, J.M., Stout, S.A., Gunster, D.G., 2005. Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: identifying sources and ecological hazard. Integr. Environ. Assess. Manage. 1, 22–33.
Quinn, T.P., 2005. The Behavior and Ecology of Pacific Salmon and Trout.. University of Washington Press, Washington, DC.
Rice, S.D., Holland, L.G., Moles, A., 2008. Seasonal increases in polycyclic aromatic hydrocarbons related to two-stroke engine use in a small Alaskan lake. J. Lake Reservoir Manage. 24, 10–17.
Rusina, T.P., Smedes, F., Klanova, J., Booij, K., Holoubek, I., 2007. Polymer selection for passive sampling: a comparison of critical properties. Chemosphere 68, 1344–1351.
Short, J.W., Jackson, T.J., Larsen, M.L., Wade, T.L., 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the Exxon Valdez oil spill. Am. Fish. Soc. Symp. 18, 140–148.
Sower, G.J., Anderson, K.A., 2008. Spatial and temporal variation of freely dissolved polycyclic aromatic hydrocarbons in an urban river undergoing superfund remediation. Environ. Sci. Technol. 42, 9065–9071.
Spromberg, J.A., Meador, J.P., 2006. Relating chronic toxicity responses to population-level effects: a comparison of population-level parameters for three salmon species as a function of low-level toxicity. Ecol. Model 199, 240–252.
Stein, E.D., Tiefenthaler, L.L., Schiff, K., 2006. Watershed-based sources of polycyclic aromatic hydrocarbons in urban storm water. Environ. Toxicol. Chem. 25, 373–385.
Stout, S.A., Magar, V.S., Uhler, R.M., Ickes, J., Abbot, J., Brenner, R., 2001a. Characterization of naturally-occurring and anthropogenic PAHs in urban sediments—Wycoff/Eagle harbor superfund site. Environ. Forensics 2, 287–300.
Stout, S.A., Uhler, A.D., Boehm, P.D., 2001b. Recognition of and allocation among multiple sources of PAH in urban sediments. Environ. Claims J. 13, 141–158.
Stout, S.A., Uhler, A.D., Emsbo-Mattingly, S.D., 2004. Comparative evaluation of background anthropogenic hydrocarbons in surficial sediments from nine urban waterways. . Environ. Sci. Technol. 38, 2987–2994.
Taylor, S.G., Lum, J.L., 2005. Annual Report, Auke Creek Weir 2004: Operations, Fish Counts, and Historical Summaries. National Marine Fisheries Service, Auke Bay Laboratory, Juneau, AK.
Tjärnlund, U., Ericson, G., Lindesjöö, E., Petterson, I., Åkerman, G., Balk, L., 1996. Further studies of the effects of exhaust from two-stroke outboard motors on fish. Mar. Environ. Res. 42, 267–271.
Van Metre, P.C., Mahler, B.J., Furlong, E.T., 2000. Urban sprawl leaves its PAH signature. Environ. Sci. Technol. 34, 4046–4070.
Van Veld, P.A., Vogelbein, W.K., Cochran, M.K., Goksøyr, A., Stegeman, J.J., 1997. Route-specific cellular expression of cytochrome P4501A (CYP1A) in fish (*Fundulus heteroclitus*) following exposure to aqueous and dietary benzo[a]-pyrene. Toxicol. Appl. Pharmacol. 142, 348–359.
Verweij, F., Booij, D., Satumalay, K., van der Molen, N., van der Oost, R., 2004. Assessment of bioavailable PAH, PCB and OCP concentrations in water, using semipermeable membrane devices (SPMDs), sediments and caged carp. Chemosphere 54, 1675–1689.
Wang, J., Nie, Y., Luo, X., Zeng, E.Y., 2008. Occurrence and phase distribution of polycyclic aromatic hydrocarbons in riverine runoff of the Pearl River Delta, China. Mar. Pollut. Bull. 57, 767–774.
Wang, Z., Fingas, M.F., 2003. Development of oil hydrocarbon fingerprinting and identification techniques. Mar. Pollut. Bull. 47, 423–452.
Wang, Z., Fingas, M.F., Shu, Y.Y., Sigouin, L., Landrialult, M., Lambert, P., Trupin, R., Campagna, P., Mullin, J., 1999. Quantitative characterization of PAHs in burn residue and soot samples and differentiation of pyrogenic PAHs from petrogenic PAHs—the 1994 Mobile burn study. Environ. Sci. Technol. 33, 3100–3109.
Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillett, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30, 347–570.
Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org. Geochem. 33, 489–515.
Zapata-Pérez, O., Gold-Bouchot, G., Ortega, A., López, T., Albores, A., 2002. Effect of pyrene on hepatic cytochrome P4501A (CYP1A) expression in Nile tilapia (*Oreochromis niloticus*). Arch. Environ. Contam. Toxicol. 42, 477–485.