Marine Pollution Bulletin xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

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

In situ biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway

Matthew A. Kelley [a], Annika Gillespie [a], Guo-Dong Zhou [b], Shu Zhang [b], James P. Meador [c], Bruce Duncan [d], Kirby C. Donnelly [a], Thomas J. McDonald [a,*]

[a] *Department of Environmental and Occupational Health, School of Rural Public Health, Texas A&M Health Science Center, 101 Adriance Road, College Station, TX 77843, United States*
[b] *Institute of Biosciences and Technology, Texas A&M Health Science Center, 2121 W. Holcombe Blvd, Houston, TX 77030, United States*
[c] *Ecotoxicology and Environmental Fish Health Program, Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 2725 Montlake Boulevard East, Seattle, WA 98112, United States*
[d] *US Environmental Protection Agency, Region 10, 1200 Sixth Avenue, Seattle, WA 98101, United States*

ARTICLE INFO

Keywords:
Sediment
Lower Duwamish Waterway
Chinook salmon
Caged exposure

ABSTRACT

Contaminated sediments may have wide-ranging impacts on human and ecological health. A series of *in situ* caged exposure studies using juvenile Chinook salmon was conducted in the Lower Duwamish Waterway (LDW). Chemical analysis of sediment, water, and fish tissue were completed. Additionally, in 2004, DNA adducts in hepatic and gill tissues were measured. Gills contained significantly higher DNA adducts at stations B2 and B4, prompting further analysis of gills in 2006 and 2007. Fluorescent aromatic compounds (FACs) in bile, and CYP1A1 in hepatic tissue were also measured during 2006 and 2007, respectively. FACs in field-caged fish were comparable or significantly higher than wild-caught fish LDW fish and significantly higher than lab fish after only 8–10 days, demonstrating the equivalency of exposure to that of migrating salmon. Furthermore, selected biomarkers appear to be capable of detecting spikes in contamination between sampling years, emphasizing the need for multiple year data collection.

1. Introduction

The Lower Duwamish Waterway (LDW) refers to a portion of the Lower Duwamish River in Washington State covering approximately 8.85 km (USEPA and WSDOE, 2008). The LDW represents a transition zone or estuary, as it receives fresh water from the Green River, which is the primary source of water for the Duwamish River (Windward Environmental LLC, 2007), and salt water from the Puget Sound, as the LDW flows into Elliott Bay (USEPA and WSDOE, 2008). The LDW has been heavily modified from its original state to facilitate use as a navigational corridor (Windward Environmental LLC, 2007). In addition to historical releases of contamination from industrial sources, combined sewer overflows (CSOs) and storm drains discharge into the LDW (USEPA, 2001). As a result, in some areas of the LDW, contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalates, and metals exceed levels of concern in sediments (Windward Environmental LLC, 2003a) and in 2001, the LDW was declared a Superfund site. Today the LDW is characterized mostly by industrial activities such as shipping, boat manufacturing, marina operations, and airplane part manufacturing activities, among others (USEPA, 2001); however, the residential areas of Georgetown and South Park are also located along or nearby the shores of the LDW.

Contaminated sediments and water, such as those present in the LDW, may have wide-ranging impacts on human and ecological health. These impacts can include direct impacts on population health and indirect impacts due to the limited productivity of contaminated sediments. Assessment of toxicity and risk related to contaminated sediments is particularly challenging because sediments typically contain complex mixtures of toxicants. Previous studies that sampled fish from the LDW have documented elevated prevalence of fin erosion disease (Wellings et al., 1976), hepatic lesions (McCain et al., 1977; Pierce et al., 1978), immunosuppression (Arkoosh et al., 1991), fluorescent aromatic compounds (FACs) in bile (Krahn et al., 1986b), and DNA adducts (Varanasi et al., 1989a). However, in spite of the contamination, a robust food-web remains in place in the LDW (Windward Environmental LLC, 2007).

Potential study designs to assess the current state of contamination in the LDW and evaluate potential health effects in migratory fish include laboratory studies, field studies using wild-caught fish, and *in situ* studies using caged fish. Laboratory studies are unable to duplicate true field conditions and thus may provide misleading

* Corresponding author. Address: Department of Environmental and Occupational Health, Texas A&M Health Science Center, School of Rural Public Health, 1266 TAMU, SRPH Building A, 101 Adriance Road, College Station, TX 77843, United States. Tel.: +1 979 458 4267; fax: +1 979 845 0885.
E-mail address: tmcdonald@srph.tamhsc.edu (T.J. McDonald).

0025-326X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2011.07.026

conclusions (Hanson and Larsson, 2007; Martin-Diaz et al., 2008). Similarly, although biomarkers have been demonstrated to be effective indicators of exposure (Van Der Oost et al., 2003), biomarker data collected from field studies using wild-caught fish are subject to high inter-individual variability due to mobility of fish and varied exposure histories (Hanson and Larsson, 2007). In order to reduce this variability and to more closely resemble true field exposure conditions, a series of 8–10 days *in situ* caged fish exposure studies were conducted in the LDW each July, over a 4-year period (2004–2007). Despite the advantages of *in situ* caged exposures, such as integration of natural variation in exposure conditions, disadvantages include potential cage loss and altered food availability for test organisms (Chappie and Burton, 2000). Juvenile Chinook salmon were chosen as the test species based on both their ecological significance (currently listed as Threatened under the Endangered Species Act in Washington State) and economic importance in the Pacific Northwest.

In spite of the rapid metabolism of PAHs by fish, a small number of studies have been conducted examining the relationship between PAHs in fish tissue and levels of PAHs present in sediment, though it is worth noting that numerous other exposure assessment methods were typically used concurrently (Meador et al., 1995). In some instances, body burden of parent PAHs in tissue have been related to other biomarkers. For example, Goksøyr et al. (1994) related CYP1A1 induction, as measured by 7-ethoxyresorufin-*O*-deethylase (EROD) activity, to levels of parent PAHs in tissue of caged Atlantic cod. Following metabolism, PAH metabolites accumulate in bile (Varanasi et al., 1989b), thus many studies have utilized analysis of FACs to evaluate the relationship between contaminated sediment and ecological receptors. For example, Collier and Varanasi (1991) detected elevated levels of FACs in bile from English sole (*Parophrys vetulus*) following administration of either a PAH-rich Duwamish Waterway sediment extract or Benzo(a)pyrene (BaP). Recently, Meador et al. (2008) reported a very high correlation ($r^2 = 0.87$) between dietary dose and biliary FACs. In addition, FAC analysis of bile has also been examined in relationship to adverse effects in fish. Krahn et al. (1986b) reported a correlation between hepatic lesions in English sole at various Puget Sound locations, including the Duwamish Waterway, and concentrations of FACs in bile. Further, Meador et al. (2008) found that biliary FACs provide a good surrogate for exposure and resulting growth effects in fish.

DNA adducts have frequently been used to study PAH-related contamination, though unlike the current study, this analysis typically utilizes hepatic tissue instead of gill tissue (Van Der Oost et al., 2003). PAH–DNA adducts are formed when reactive PAH metabolites covalently bind with DNA. The ^{32}P-Postlabeling DNA adduct method was developed by Reddy and Randerath (1986). This method provided advantages over other methods of DNA adduct measurement because it required a small amount of DNA, as little as 10 μg, and was highly sensitive in detection of DNA adducts as detection limits are as low as 1 adduct per 10^9 nucleotides (Reddy and Randerath, 1986). DNA adducts have previously been used in field studies effectively. Stein et al. (1992) found a correlation between levels of PAHs present in sediment and levels of hepatic DNA adducts in several species of Puget Sound flatfish. Further, in an *in situ* biomarker study using caged coho salmon (*Oncorhynchus kisutch*), Barbee et al. (2008), determined that DNA adducts provided a sensitive marker of genotoxicity of PAHs in sediments.

The CYP4501A subfamily has shown utility as a biomarker due to its role in biotransformation of contaminants (Sarkar et al., 2006). Following exposure to contaminants such as PAHs and PCBs, CYP1A is induced via the aryl hydrocarbon receptor (AhR), thus induction of CYP1A has often been used as a biomarker for pollution monitoring in various species of fish (Sarkar et al., 2006). ELISA or Western blots are among the various methods which can be used to measure CYP1A protein levels immunologically (Bucheli and Fent, 1995; Van Der Oost et al., 2003).

Objectives of this study included characterization of the extent of PAH and PCB contamination present in sediment and water samples from the LDW during the exposure period, as well as measurement of various biomarkers. These biomarkers included (1) body burden of PAHs and PCBs present in fish tissue, (2) DNA adducts in gill tissue composites, (3) CYP1A1 induction in hepatic tissue (2007 only), and (4) fluorescent aromatic compounds (FACs) in bile (2006 only). Further, the relationship between biomarker responses in caged fish and levels of contaminants present in environmental media (sediment and water) was examined. Use of multiple biomarkers provided additional lines of evidence to further delineate the relationship between contaminated sediment and juvenile salmon migrating through the LDW. Additionally, levels of contaminants in sediment were compared with sediment quality thresholds (SQTs) in order to evaluate the likelihood of adverse effects, and source allocation of PAHs was performed using PAH ratios to determine if LDW PAH contamination was of either petrogenic or pyrogenic origin, or potentially a combination of both.

2. Materials and methods

2.1. Collection of environmental samples

Sediment samples from each field site were collected from a boat using a petite ponar grab sampler (WILDCO, Buffalo, NY). From each study year, one sediment sample was collected at each station. Upon collection, the sediment samples were homogenized in stainless steel bowls and transferred to glass I-CHEM certified 1L sampling jars with Teflon lined lids (VWR, West Chester, PA). During the 2004 study, three water samples were collected at cage deployment and three additional water samples were collected at cage retrieval, however minimal variation was observed, and in subsequent study years a minimum of two water samples were collected at each station. These water samples were collected at each site using a Beta bottle sampling device (WILDCO, Buffalo, NY) placed just above the sediment surface within the exposure zone of the deployed cages on the day of cage deployment and immediately following cage retrieval. Samples were stored in 1 L I-CHEM certified amber bottles with Teflon lids (VWR, West Chester, PA).

2.2. Caged in situ exposure with juvenile Chinook salmon

Juvenile ocean-type Chinook salmon (*Oncorhynchus tshawytscha*) were obtained as eggs or fry from the University of Washington hatchery and reared at the NOAA Fisheries marine lab in Mukilteo, WA. They were raised in freshwater and acclimated to seawater in June of each year. Fish were held in chilled aerated tanks during transport to the LDW. While being held on the EPA research boat during transport to the exposure sites, fish were gradually acclimated to water conditions present in the LDW through addition of LDW water to the tanks in 5–10 min intervals.

Upon arrival at each site, water quality parameters were measured using a Hydrolab multiprobe (Hydrolab-Hach Company, Loveland, CO) to ensure that conditions were acceptable for salmon survival. However, close examination of this data revealed potential inaccuracies in the Hydrolab data. Alternatively, representative water quality parameters collected by King County monitoring stations near our sampling sites are displayed in Table 1. Acceptable conditions were further verified upon cage retrieval, excluding one notable exception. During the 2004 sampling period, 100%

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

Table 1
Physicochemical characteristics of the field sites.[a]

Site	B2–B4	K1	Reference controls
Depth of cage (m)	2–4	2–4	NR
Temperature (°C)	13.8 (0.7)	13.5 (0.3)	11
Salinity (‰)	26.7 (2.3)	27.2 (0.8)	28
Dissolved oxygen (ppm)	6.2 (1.0)	6.9 (0.8)	9.0

[a] GPS coordinates for each site are listed in materials and methods. All measurements based on CTD data for several depths (2.0–4.0 m) and times on 16 July and 20 August 2007 (http://green.kingcounty.gov/marine/CTD.aspx). CTD stations were downriver between 180 and 530 m from sites B2–B4 and 1250 m from site K1. Values are means and SD for $n = 24$ (B2–B4) and $n = 10$ (K1) individual measurements. NR: not relevant, control fish were maintained in holding tanks at the Mukilteo lab with flow-through filtered seawater.

mortality was experienced at our intended field-reference site near North Wind's Weir. Based on historical water quality data from the King county stations, we learned that salinity can be very low at the surface, therefore we concluded that a sudden change in percent salinity was likely responsible for the severe mortality event. In contrast, at all other sampling stations during all other study years, divers reported healthy fish upon cage retrieval. On rare occasions, fish mortality (less than 1% of exposed fish) was experienced when cages were tied off to the dock waiting to be processed.

The cage deployment process proceeded following collection of sediment and water samples and involved net transfer of fish into a 45.7 × 30.5 × 20.3 cm plastic-coated, wire mesh pinfish trap cage (Model 1264; FRABILL, Inc., Jackson, WI) modified to prevent fish escape. Weights were attached to the bottom of the cages to keep the cage location stationary. With the help of the US EPA Region 10 dive team, either 2 or 4 cages, each containing 10–15 fish, depending on total number of fish available during each study year, were deployed at each sampling location. During the 2004, 2005 and 2006 studies, a 10 day exposure was employed. However, following notation of empty stomachs in exposed fish and blood analyses indicating stress (data not shown), the exposure period was reduced to 8 days in 2007. Cages were deployed by slowly lowering the cage apparatus (Fig. 1) with fish enclosed to the sediment surface, resulting in minimal sediment disturbance. If necessary, USEPA SCUBA divers carefully placed the cage on the sampling location. Following the end of the exposure period, fish were retrieved from the sampling stations in the same order in which they were placed and sacrificed by administration of MS-222 followed by severing of the spinal cord. All animal care procedures were conducted in accordance with institutional animal care and use committees (IACUC) for Texas A&M University.

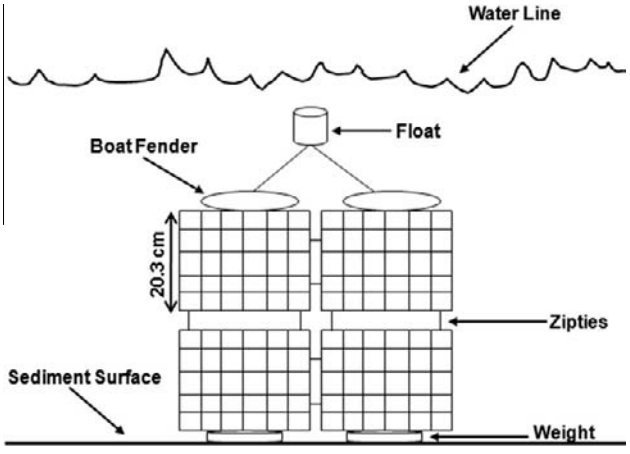

Fig. 1. Diagram of caging apparatus deployed at various locations in the Lower Duwamish Waterway for 8–10 days each July from 2004 to 2007. Typically 10–15 juvenile Chinook salmon (depending on study year) were included in each of four cages deployed at each site.

The search for an appropriate uncontaminated reference site in the LDW was particularly challenging due to the extent of contamination present, thus, for ^{32}P-postlabeling and CYP1A1 induction, reference fish were caged at the Mukilteo lab on clean water during the 2007 study period (flow-through seawater which has been sand-filtered and UV-sterilized) for 7 days without feeding. One treatment group of reference fish were fed during caging, while another was subjected to starvation during the exposure. No significant differences were noted between the two groups (data not shown), thus the reference fish which were starved were utilized as the control group because their conditions more closely resembled that of the field-caged fish. Lengths and weights were collected following sacrifice, with mean length ranging from 8.2 to 12.7 cm and mean weight ranging from 8.0 to 22.6 g depending on study year. Mean length and weight for fed hatchery caged fish were 10 cm and 8.8 g, respectively. Mean length and weight for starved hatchery caged fish were 9.2 cm and 7.8 g, respectively. Additionally, for whole body tissue analysis for PAHs and PCBs, $t = 0$ fish were sacrificed each year on the day the study began were used as a reference. For FACs, previously published values for laboratory controls and wild-caught LDW fish were used for comparison.

With the exception of station K1, which was first sampled during the 2005 study period, sampling stations were selected based on chemical analysis results of sediment and water sampling conducted in July 2003 on the LDW (Gillespie, 2006). Fig. 2 displays sampling locations and GPS coordinates for each site are as follows K1: 47.55678 W122.34446, B2: N47.52925 W122.31320, B3: N47.52848 W122.31193, B4: N47.52663 W122.31023. Station K1 is located approximately at river kilometer 1.4–1.5, near the West bank of the LDW. Stations B2, B3 and B4 are located between river kilometer 5.5 and 5.8 approximately, with stations B2 and B3 being closer to the East banks of the LDW and station B4 being closer to the West bank of the LDW. Boeing Plant 2, South Park Marina and the neighborhood of South Park are all located in close vicinity to the sampling stations. These locations are contained within seven areas of the LWD Superfund Site that that were recommended for early action remediation (Windward Environmental LLC, 2003b).

2.3. Sample extraction

For sediment samples, approximately 10 g of oven-dried sample were extracted using methylene chloride in a Dionex (Dionex Corp., Sunnyvale, CA) Model 200 Accelerated Solvent Extractor (ASE) according to US EPA standard extraction method 3535 (USEPA, 1996a). Sediment extracts were also treated with copper in order to remove sulfur-related interference. For water samples, liquid:liquid extractions were performed with methylene chloride according to US EPA standard extraction method 3510C (USEPA, 1996b). Whole body fish tissue samples (excluding gills and liver which were removed for biomarker analysis) were first composited in a stainless steel blender according to exposure station (treatment group). Next, blended fish tissue composites were weighed wet and then lyophilized overnight under vacuum using a Labconco Free Zone 12 L Freeze Dry System (Labconco Corp., Kansas City, MO). Freeze-dried fish tissue composites were then re-weighed prior to extraction to determine the percent moisture of each composite. In addition, fish tissue composites were then ground into a powder-like consistency with a mortar and pestle to ensure optimal extraction.

Tissue samples were then extracted with 40 mL of methylene chloride using the ASE according to US EPA standard extraction method 3535 (USEPA, 1996a). A surrogate spike solution with

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

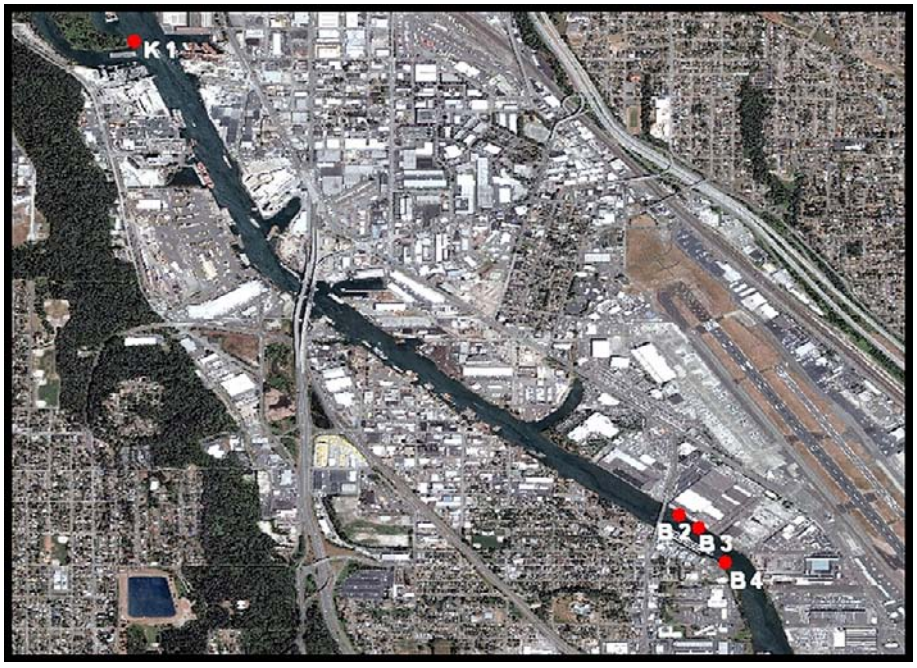

Fig. 2. Water and sediment samples were collected at locations in the Lower Duwamish Waterway, followed by deployment of cages containing juvenile Chinook salmon.

deuterated PAHs was added to each sample to correct for PAH recovery efficiencies. For both sediment and tissue samples, the ASE was operated at 1500 psi, and each sample was solvent flushed in two cycles at 50% cell volume and 60 s purge time. The stainless steel extraction cells were heated to 100 °C with 2 min pre-heat, 5 min heat, and 5 min static times for each cycle. Sample filters (Part No. 049458) were grade D28 with a 1.983 cm diameter. A Zymark TurboVap LV evaporative solvent reduction apparatus (Zymark Corp., Hopkinton, MA) at 45 °C for 13 min was used to concentrate the extracts to one mL. Extracts were further reduced to 0.5 mL under purified nitrogen. Tissue extracts were further purified using silica gel/alumina long columns, followed by gel permeation cleanup using HPLC.

2.4. Chemical analysis

Sediment extracts, water extracts, and extracts from fish tissue composites were all analyzed for PAHs and other semivolatile organic compounds (SVOCs) using USEPA method 8270C (USEPA, 1996c) and for total PCBs and PCB homologs following USEPA method 680 (Alford-Stevens et al., 1985). Water samples are not filtered prior to extraction, thus sediment particles are sometimes present in samples. Analysis was performed using an Agilent 5980 gas chromatograph coupled to an Agilent 5972 mass selective detector in selected ion monitoring mode. A DBS-MS 60 m × 0.25 mm ID × 0.25 mm film thickness column (Agilent Technologies, Palo Alto, CA) was utilized. The injection port was maintained at 300 °C and the transfer line at 280 °C. The temperature program was as follows: 60 °C for 6 min increased at 12 °C/min to 180 °C and then increased at 6 °C/min to 310 °C and held for 11 min for a total run time of 47 min. Total PAHs (tPAHs) reported include 51 PAHs and alkylated homologs. Reporting limits for individual PAHs were 10 ng/L in water, 0.1–0.5 ng/dry g in sediment and 0.6–6.5 ng/g wet in fish tissue. Low-molecular-weight PAHs (LMW PAHs) reported include naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene (AN), and phenanthrene (PH), while high-molecular-weight PAHs (HMW PAHs) reported include fluoranthene (FL), pyrene (PY), benz(a)anthracene (BaA), chrysene (CH), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), BaP, Indeno(1,2,3-c,d)pyrene (IP), dibenz(a,h)anthracene (DBahA), and benzo(g,h,i)perylene (BghiP). Carcinogenic PAHs reported include BaA, CH, BbF, BkF, BaP, IP, and DBahA. PCBs were detected by level of chlorination and all homolog groups were summed for total PCBs (tPCBs) reported. Reporting levels for PCB homolog groups were 5 ng/L in water, 0.8–4.5 ng/dry g in sediment and 2.0–13.5 ng/g wet in fish tissue.

2.5. ^{32}P-postlabeling

Similar to Barbee et al. (2008), hepatic tissue and gills were removed from fish and composited according to exposure location. Hepatic and gill samples were typically composites from 4 to 6 fish from each in-water exposure site. During the 2004 study, adduct analysis using hepatic and gill tissue composites was performed. Relative adducts labeled in hepatic composites were lower than relative adducts labeled in gill tissue composites. Thus, in subsequent study years, the study design called for utilization of hepatic samples for other analyses and gill tissue composites were utilized for adduct analyses. Juvenile Chinook salmon which were caged in clean water at the Mukilteo lab were utilized as a reference (control) for statistical comparisons in this assay. These reference fish were caged and exposed for 7 days at the Mukilteo lab and were not fed during this exposure time period, thus effects due to stress from caging or starvation during the experiment should be experienced in both experimental fish and reference fish. In 2005, the exposure was conducted, as in other study years, however due to various issues including thawing and some deterioration of samples during shipment delays, DNA extracted from the gill samples was of questionable quality. Rather than attempt the postlabeling analysis with questionable DNA, study investigators decided to exclude the 2005 salmon from DNA adduct analysis.

DNA was isolated by solvent extraction combined with enzymatic digestion of the protein and RNA (Randerath et al., 1986). Isolated DNA was stored at −80 °C, until postlabeling assay. DNA adducts were quantified using nuclease p1-enhanced bisphosphate ^{32}P-postlabeling (Reddy and Randerath, 1986). Briefly, DNA (10 μg)

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

was enzymatically degraded to normal (Np) and modified (Xp) deoxyribonucleoside 3′-monophosphates with micrococcal nuclease and spleen phosphodiesterase at pH 6.0 and at 37 °C for 3.5 h. After treatment of the mixture with nuclease P1 to convert normal nucleotides to nucleosides, modified nucleotides (Xp) were converted to 5′-^{32}P-labeled deoxyribonucleoside 3′,5′-bisphosphates (pXp) by incubation with carrier-free [γ-^{32}P]ATP and T4 polynucleotide kinase at pH 9.0. Radioactively labeled modified nucleotides were mapped by multidirectional anion-exchange thin-layer chromatography (TLC) on polyethyleneimine (PEI)-cellulose sheets (Mabon et al., 1996). After removal of orthophosphate and traces of radioactive impurities by one-dimensional development with 2.3 M sodium phosphate, pH 5.75 overnight (D1), bulky labeled DNA adducts retained in the lower (2.8 × 1.0 cm) part of the D1 chromatogram were, after brief autoradiography on Cronex 4 X-ray film, contact-transferred to fresh thin-layer sheets and resolved by two-dimensional TLC. The non-polar DNA adducts were separated with solvents 3.82 M lithium formate, 6.75 M urea, pH 3.35 and 0.72 M sodium phosphate, 0.45 M Tris–HCl, 7.65 M urea, pH 8.2 in the first and second dimensions, respectively. ^{32}P-labeled adducts were visualized by screen-enhanced autoradiography at −80 °C using Kodak XAR-5 film and were determined with the aid of an Instant Imager (Packard Instruments) (Zhou et al., 1999). Radioactive labeled DNA adducts were then quantified using instant imager software and statistical analysis was performed using SigmaPlot software version 11.0 (Systat Software, Inc., San Jose, CA). RAL values were calculated according to the following:

$$\mathrm{RAL} = \text{Sample count rate[cpm]} \div (\text{DNA} - \text{P[pmol]} \leftarrow \\ * \text{Spec.Act.ATP[cpm/pmol]}) \leftarrow$$

where DNA-P represents the amount of DNA assayed (expressed as pmol DNA monomer units) and specific activity (Spec. Act.) of [γ-^{32}P]ATP used in the labeling reaction. Thus, for 10 μg DNA, and ATP with a specific activity of 4.5 × 10^6 cpm/pmol, 145 cpm corresponds to an RAL value of 10^9 (i.e., an estimated level of one modification in 10^9 DNA nucleotides).

2.6. FACs in bile

FACs in bile were measured by high-performance liquid chromatography (HPLC) using the method employed by Krahn et al. (1986a) with minor adaptation. For a more detailed description please refer to Meador et al. (2008). Results were quantified and reported as phenanthrene (PHN) equivalents. Bile samples were analyzed using an HPLC equipped with a Waters (Milford, MA, USA) 600 Multisolvent Delivery System pump, an autosampler, and three PerkinElmer (Norwalk, CT, USA) fluorescence detectors. A 30- to 42-μm reverse-phase Vydac C18 guard column (0.20 × 2 cm) (Alltech, Los Altos, CA, USA) and a Phenomenex (Torrance, CA, USA) Synergy™ Hydro RP C18 reverse-phase analytical column (4.6 × 150 mm) were used for this analysis. Each sample run utilized 3–5 μl of thawed, untreated bile and was eluted with a HPLC linear mobile phase gradient at a flow rate of 1.0 ml/min. Initially, the gradient ranged from 100% solvent A (water containing 5 μl/L of acetic acid) to 100% solvent B (methanol) over an 8 min period. This was followed by 13 min of 100% solvent B before the gradient returned to 100% solvent A over a period of 3 min.

Samples were run with three different types of quality control samples including HPLC control standard, a bile reference material and a method blank set. Biliary FACs (PHN) were determined through measurement of fluorescence at the 260/380 nm ex/em wavelength pair. The PHN signal was chosen based on the results of Meador et al. (2008) who analyzed dietary exposure studies with salmonids that were fed a range of high and low molecular weight PAHs mimicking stomach contents of field collected fish. They determined that the PHN signal exhibited the highest correlation with PAH-dose (μg/g fish/day) and was therefore appropriate for field-exposed fish.

In this system, FACs are known to elute at >9 min and total area under the curve was integrated for each wavelength pair. In order to account for feeding state and concentration of water in bile, following quantification, analytes were normalized to protein as described by Lowry et al. (1951). This step was deemed necessary as fish experiencing starvation have been shown excrete bile less frequently than feeding fish and thus exhibit comparatively elevated biliary FAC values and protein content (Collier and Varanasi, 1991).

2.7. CYP1A1 expression

Western blot analysis was used to determine the relative levels of CYP1A1 expression in liver samples of *in situ* caged juvenile Chinook salmon. CYP1A1 was only measured during the 2007 study period and reference fish were caged at the Mukilteo lab for comparison. In order to obtain the required amount of liver tissue for analysis, typically the hepatic tissue from three fish from the same in-river exposure site were composited for each sample. Microsomes were prepared by homogenizing whole livers with ice-cold homogenizing buffer (pH 7.4), composed of 10 mM Tris and 0.25 M sucrose. Following homogenization, several centrifugal steps were completed and microsomes were suspended in buffer and stored at −80 °C until use. Protein concentration was measured through use of Biorad Protein Assay Dye Reagent Concentrate (catalog No. 500-0006) followed by measurement on a SpectraMax 340 (Molecular Devices, Sunnyvale, CA) at the 600 (nm) wavelength.

The separating gel was a standard 10% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) preparation. Samples were electrophoresed and proteins were detected by incubation with 1:1000 polyclonal primary antibody CYP1A1 (H-70, Santa Cruz Biotechnology, Santa Cruz, CA) followed by blotting with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:5000 dilution). Blots were then exposed to chemiluminescent substrate PerkinElmer Life Sciences) and placed in Kodak X-Omat AR autoradiography film. Band intensities (integrated density) were determined by a scanning laser densitometer (Sharp Electronics Corp., Mahwah, NJ) using Zero-D Scanalytics software (Scanalytics Corp., Billerica, MA) and Image J software (NIH, Bethesda, MD).

2.8. Statistics

For statistical comparisons, in this study we considered a p value <0.05 to indicate statistically significant differences between mean values. ANOVA was typically used to assess statistically significant differences between treatment groups. In regard to tissue composites, total PAHs detected from each station from all study years were compared to fish tissue composites from Chinook salmon which were sacrificed prior to the exposure (time = 0) from all study years. A Kruskal–Wallis one way ANOVA was performed on ranks using SigmaPlot software (San Jose, CA) because the data failed to meet the assumption of a normal distribution. Results indicated that a significant difference between the treatment groups was present, thus a posthoc test using Dunn's method was conducted in order to make multiple comparisons versus the control.

In regard to DNA adduct analysis, levels of total RAL in composites from each station from each sampling year were compared for significant difference using one way ANOVA. The data failed to satisfy the assumption of a normal distribution, thus the one way ANOVA was conducted using ranked data. In addition, the

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

Student–Newman–Keuls post hoc test was selected for multiple comparisons versus the control (caged Mukilteo lab fish). It is also of note that statistical analysis of biliary FACs was performed using ANOVA followed by administration of Fisher's LSD post hoc test.

3. Results

3.1. Sediment analysis

Levels of contaminants measured in sediment samples from July 2004 to July 2007 varied among stations, although means are not significantly different. These results are presented by study year in Fig. 3. When overall mean of the four sampling years is considered, station B2 had the highest mean concentration of total PAH (tPAH) (2.7 ppm). Sediment from station K1, originally intended to be a reference station, exhibited the next highest mean tPAH concentration (1.8 ppm). An important distinction between these two sampling stations is that mean total PCBs (tPCBs) were present at station B2 at high levels (1.2 ppm), whereas station K1 samples contained only 16 ppb. In general, HMW PAHs were more predominant in the sediment samples across stations.

3.2. Source allocation of PAHs in LDW sediments

Based on the ratios of PAHs in sediment samples (Table 2), contamination in the LDW likely comes from both petrogenic and pyrogenic sources and varies both spatially and temporally. Neff et al. (2005) assert that a phenanthrene to anthracene (PH/AN) ratio of less than 5 is typical of pyrogenic assemblages, while a PH/AN ratio of greater than 5 is typical of petrogenic assemblages. In addition, pyrogenic assemblages typically have a fluoranthene to pyrene (FL/PY) ratio which approaches or exceeds 1, while FL/PY ratios which are substantially below 1 are typical of petrogenic assemblages (Neff et al., 2005).

Chemical analysis of sediment samples collected during the 2004 sampling period indicates that both petrogenic and pyrogenic sources were present as the PH/AN ratios are all above 5 and the FL/PY ratios are all above 1, thus indicating a mixed exposure. Data from our other study years (2005–2007) indicates that the contamination is coming from a variety of sources as ratios vary between stations and even from the same station from year to year. These results highlight the dynamic nature of this estuary.

3.3. Water analysis

Total PAHs measured in water samples varied substantially among sampling years (Fig. 4). The highest mean total PAHs were measured in 2005. Water samples were also analyzed for PCBs each year; however PCBs were only detected in water samples during the 2005 study. The mean range of total PCBs (tPCBs) detected in 2005 LDW water samples ranged from 0.2 to approximately 6 ppb (data not shown). During the 2005 study, station K1, where minimal levels of tPCBs in sediment (25 ppb) were detected, had the highest concentration of tPCBs in water (6 ppb).

Table 2
Ratios of the PAH isomers phenanthrene to anthracene (PH/AN) and fluoranthene to pyrene (FL/PY) in sediments collected from various locations in the Lower Duwamish Waterway between July 2004 and July 2007 are presented below. Source interpretations were completed using guidelines published by Neff et al. (2005). $n = 1$ for each site/year combination with the exception site K1, which was not sampled in 2004.

Year	Site	PH/AN	FL/PY	Source
2004	K1	NA	NA	NA
	B2	9.0	1.3	Petrogenic/pyrogenic
	B3	12.0	1.7	Petrogenic/pyrogenic
	B4	9.8	1.6	Petrogenic/pyrogenic
2005	K1	8.5	1.5	Petrogenic/pyrogenic
	B2	1.3	1.5	Pyrogenic
	B3	0.2	1.0	Pyrogenic
	B4	4.8	1.2	Pyrogenic
2006	K1	6.0	1.3	Petrogenic/pyrogenic
	B2	8.0	1.1	Petrogenic/pyrogenic
	B3	6.0	0.6	Petrogenic
	B4	12.3	1.2	Petrogenic/pyrogenic
2007	K1	2.3	1.4	Pyrogenic
	B2	17.2	1.8	Petrogenic/pyrogenic
	B3	2.6	1.3	Pyrogenic
	B4	4.5	1.3	Pyrogenic

3.4. Tissue composite analysis

Total PCBs were not detected in any of the fish tissue samples for all years sampled. Mean concentrations of total PAHs in Chinook salmon tissue samples from each sampling year are shown in Fig. 5. Among fish that were caged at the Mukilteo lab, one treatment was fed and another was not, in order to allow examination of the effects of feeding on levels of PAHs in tissue and biomarker responses. For samples from the B2, B3 and B4 treatment groups, means and standard error of the means from each sampling year from July 2004 to July 2007 are displayed. However, analysis of the fed and unfed caged lab fish was completed only for the July 2007 exposures. In addition, results for the K1 treatment group are shown only for the 2005 and 2006 sampling events. In 2004 station K1 was not sampled, and in 2007divers were unable to locate the cage deployed at this station.

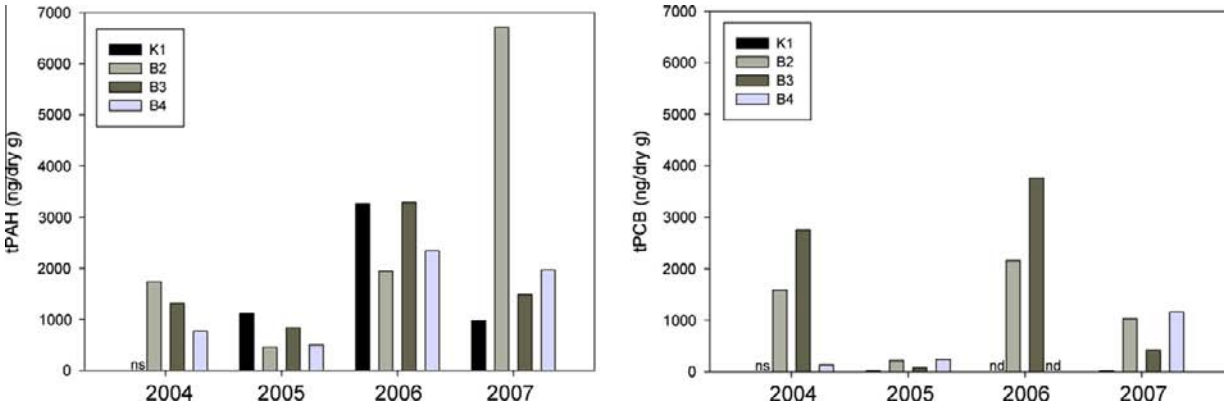

Fig. 3. Total PAHs and total PCBs detected in Lower Duwamish Waterway sediments collected from July 2004 to July 2007. For each station $n = 1$ per year, with the exception of station K1, that was not sampled (ns) in 2004.

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

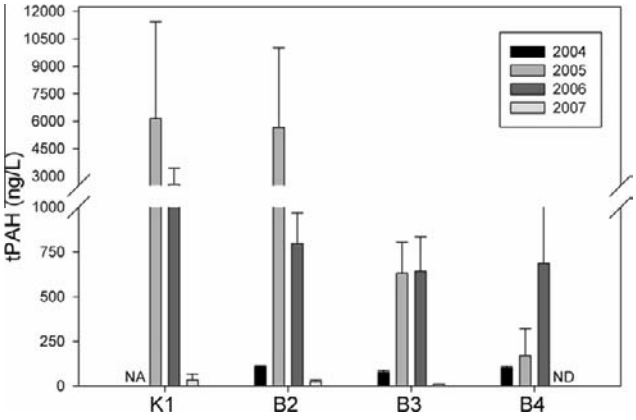

Fig. 4. Yearly mean and SEM total PAHs detected in water samples from the Lower Duwamish Waterway during 2004–2007. Although the number of samples collected at each station varied by sampling year, a minimum of two water samples were collected. NA = not sampled, ND = not detected.

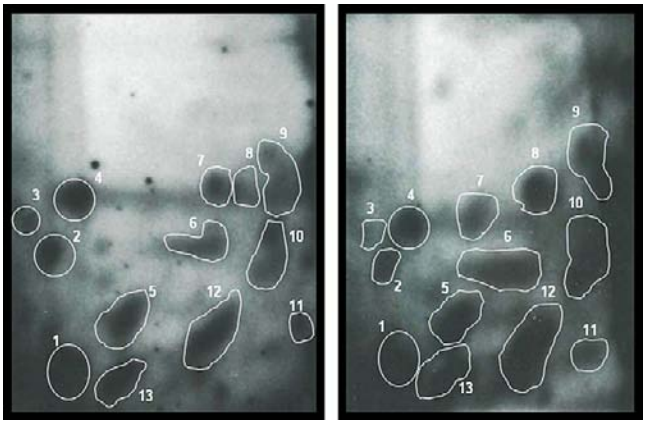

Fig. 6. Representative autoradiograms of individual DNA adducts in gill tissue composites from juvenile Chinook salmon exposed to Lower Duwamish Waterway sediments at station B3 (left) and B4 (right).

Results of the statistical analysis revealed that levels of total PAHs detected in tissue composites from the juvenile Chinook salmon which were exposed at stations K1, B2, B3 and B4 during the 2005 sampling period were significantly higher when compared to levels in tissue composites taken from salmon which were sacrificed prior to exposure (time = 0). Although additional instances of elevated levels of total PAHs in salmon composites were noted, ANOVA did not reveal any other significant differences among individual stations and control fish.

3.5. DNA adduct analysis

DNA adducts were quantified as relative adducts labeling (RAL) per 10^9 nucleotides. Typical autoradiograms of DNA adducts detected by the nuclease P1-enhanced ^{32}P-postlabeling assay are presented in Fig. 6. During the 2004 study, levels of DNA adducts detected in gill tissue composites were significantly elevated compared to hepatic tissue composites at stations B2 and B4, while station B3 was comparable (Fig. 7A). Thus, in 2006 and 2007, only gill tissue composites were utilized for the assay. Levels of DNA adducts recorded from gill composites from fish exposed during the 2004 and 2006 study periods were all elevated compared to the reference fish (Fig. 7B), while levels of DNA adducts recorded from gill composites of fish exposed during the 2007 study period were all similar to levels of adducts in gill composites from reference fish (Fig. 7B). Adducts in gill composites from fish exposed at all stations in the LDW during the 2004 and 2006 study periods were all significantly higher than adducts in the gill composites from reference fish. In contrast to the 2004 and 2006 LDW DNA adduct data, the 2007 DNA adduct data revealed no instances of elevated levels of DNA adducts compared to controls.

3.6. FACs in bile

Quantification of the PHN signal resulted in values of 11.0 and 13.6 μg/mg protein for composites from station K1 and B3, respectively. In addition, data published by Meador et al. (2008) provides a comparison to put the data in perspective (Table 3). The comparison indicates that juvenile Chinook salmon caged in the LDW for

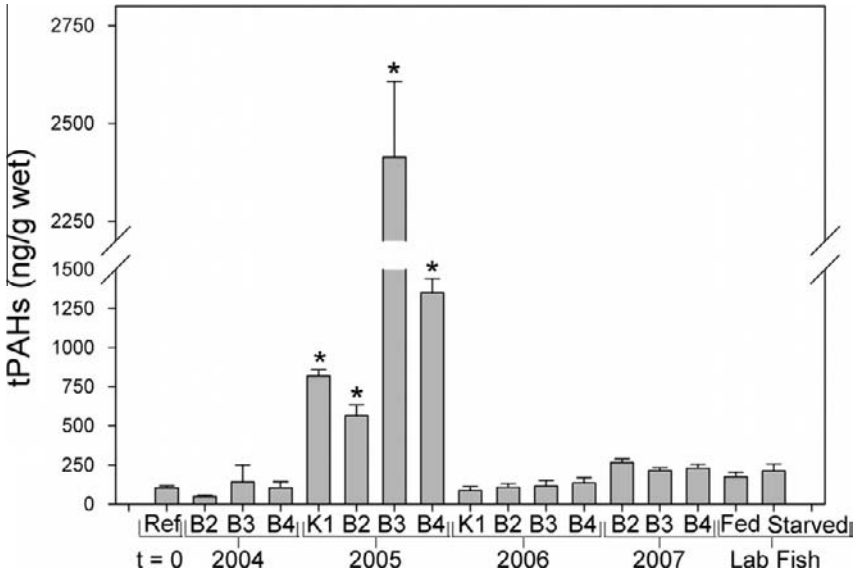

Fig. 5. Mean and SEM total PAHs detected in juvenile Chinook salmon tissue composites, illustrating the significant spike across stations in 2005, following an 8–10 days caged exposure (depending on study year) in the Lower Duwamish Waterway, compared to fish that were not exposed ($t = 0$). Fish that were caged at the Mukilteo lab ± feeding were also included. Typically 3–8 fish per were used per composite and $n = 4$ for each treatment group. *Statistically significant difference from control.

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

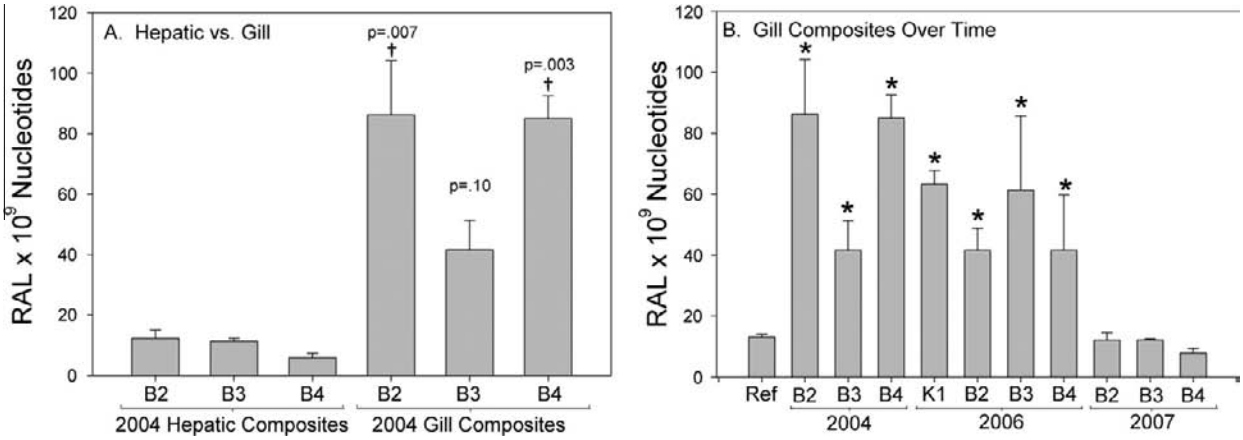

Fig. 7. Relative Adduct Labeling (RAL) detected by ^{32}P-postlabeling of fish hepatic versus gill composites from various Lower Duwamish Waterway locations from 2004 (A), and RAL detected in gill composites from 2004, 2006 to 2007 (B). Juvenile Chinook salmon were caged in the Lower Duwamish Waterway for 8–10 days depending on study year. Gill tissue composites typically included 4–6 fish per composite. For all treatment groups $n = 4$ composites. *Statistically significant difference from control.

Table 3
Mean and standard error of FACs detected in bile composites from juvenile Chinook salmon which were caged in the Lower Duwamish Waterway at various locations for 10 days in July 2006. Comparison data was provided by Meador et al. (2008) and includes composites of reference lab fish from 2001, 2003, to 2004, as well as fish that were wild-caught in the LDW near Kellogg Island in 2001 and 2004. For B3 $n = 5$ composites, for K1 and Kellogg Island Wild-caught $n = 3$ composites, and for Mukilteo Lab $n = 14$ composites.

Site	PHN FACs µg/mg protein	Standard Error
B3 (Caged)	13.6[a,b]	0.75
K1 (Caged)	11.0[a]	2.00
Kellogg Island Wild-caught (2001 and 2004)	8.8[a]	1.01
Ref (Mukilteo Lab)	4.5	0.74

PHN FACs = phenanthrene equivalent fluorescent aromatic compounds.
[a] PHN FACs significantly elevated compared to controls caged at the Mukilteo Lab.
[b] PHN FACs significantly elevated compared to Kellogg Island wild-caught fish.

Fig. 8. Western blot analysis using hepatic tissue composites (typically 2–4 livers per composite based on weight) to evaluate CYP1A1 expression in juvenile Chinook salmon following a caged exposure in the LDW in July 2007.

10 days exhibited higher mean levels of biliary FACs than both reference lab fish from various years and from juvenile Chinook salmon that were captured in the LDW near Kellogg Island. This indicates that caged fish were exposed to PAHs in water from gill ventilation.

3.7. CYP1A1 induction

The western blot displaying CYP1A1 induction is presented in Fig. 8. Results indicate the highest levels of CYP1A1 induction were present in juvenile Chinook salmon that were exposed in the LDW at stations B3 and B4 (integrated density of 34 and 26). However, neither station was significantly elevated relative to controls (integrated density of 21). In addition, juvenile Chinook salmon exposed in the LDW at sampling station B2 produced a CYP1A1 response that was lower than lab fish that were caged in clean water at the Mukilteo lab (integrated density of 17).

4. Discussion

A series of *in situ* exposure studies have been conducted using caged juvenile Chinook salmon in the LDW over a four-year period. Examination of the data revealed that the biomarker responses among field-exposed fish were often elevated compared to controls. For example, DNA adduct analysis revealed significantly elevated levels compared to controls, with the exception of the 2007 study period, when the lowest levels of contaminants were detected in water samples. Additionally, tissue body burden tPAHs were significantly elevated during the 2005 study period when some of the highest levels of tPAHs were detected in water samples and the only study period when tPCBs were detected in water samples. Further, FACs were significantly elevated compared to controls. For example, field-caged fish from station B3 exhibited significantly higher FACs compared to both Mukilteo lab fish and wild-caught fish from the LDW, after only a 10 day exposure. Previous studies have established that juvenile salmonids typically exhibit a residence time of up to 2 months in the Duwamish estuary (Meyer et al., 1981). More specifically, McCain et al. (1990) estimated that juvenile Chinook salmon captured during their study migrated through the Duwamish Waterway for an exposure period ranging from 1 to 6 weeks. Finally, CYP1A1 induction levels were comparable among stations; however this analysis was only completed during the 2007 study period when contaminants in water were detected at their lowest level.

Previous research conducted on the LDW has documented varying degrees of contamination. For example, examination of a small selection of studies (Collier et al., 1986; Malins et al., 1982, 1987; Stein et al., 1992; Varanasi et al., 1985), reported levels of tPAHs and tPCBs as high as 12 and 0.8 ppm, respectively in LDW sediments. Analysis of sediment samples collected from 2004 to 2007 detected elevated concentrations of PAHs and PCBs in the LDW and the range of contaminant concentrations observed were comparable with those detected by previous studies.

Interestingly, tissue residue analysis of tPAHs in fish tissue composites revealed a spike during the 2005 sampling period compared to other sampling years. This spike was somewhat consistent with levels of tPAHs detected in water samples, as the highest levels of tPAHs detected in water samples over the course of the longitudinal study were associated with the 2005 sampling period. However, it is of note that only two of the four stations (K1 and B2) were substantially higher than all other study years. Thus measurement of PAHs in fish tissue following an *in situ* exposure does appear to be useful as an indicator of exposure. One potential

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

explanation for the spike is that stirred up sediment in the water column prior to sampling may have resulted in increased bioavailability of contaminants during the LDW 2005 sampling period. In support of this hypothesis, the only incidences in which total PCBs were detected in water samples occurred during the 2005 sampling period. This detection is likely the result of increased presence of sediment particles in the water as the samples were not filtered prior to extraction.

In contrast, PCBs were not detected in any of the fish tissue composites that were analyzed, which is a major limitation of the study. The absence of feeding during the caged exposure offers a potential explanation for this result, however the major factor is likely the method chosen for PCB analysis. A similar caged exposure conducted for 8 days in the LDW in 2009 using juvenile Chinook salmon detected total PCBs in the 108–138 ppb range for field-exposed fish, while controls ranged from 78 to 88 ppb (unpublished data). These analyses were conducted utilizing gas chromatography with electrochemical detection. Future studies would benefit from the use of more sensitive congener-specific methods of PCB analysis.

Biomarkers such as DNA adduct analysis and biliary FACs, appear capable of serving as additional indicators of exposure. For instance, measurement of PAH metabolites in bile is indicative of recent PAH exposures (Van Der Oost et al., 2003). Analysis of the PHN signal from biliary FACs in this study in combination with previously collected data provided by Meador et al. (2008) (Table 3) revealed that juvenile Chinook salmon caged at sites K1 and B3 in the LDW, as well as wild Chinook salmon captured in the vicinity of Kellogg Island, exhibited significantly higher PHN FACs than control fish from the Mukilteo lab. In addition, juvenile Chinook salmon caged in the LDW at site B3 exhibited a significantly higher PHN signal than the wild-caught fish from the LDW. This elevated short-term biomarker of PAH exposure indicates the potential for adverse health effects in migrating salmon even with a residence time in the LDW as short as 10 days.

Although many studies have documented upregulation of cytochrome P450 and FACs in fish following exposure to PAHs in water, no clear link between this response and long-term effects in individuals is evident (Lee and Anderson, 2005). However, upregulation of FACs is a clear indicator of exposure and inverse regression analysis can be utilized to relate biomarker responses to predict dose. For example, we used the station B3 mean PHN FACs value of 13.6 μg/mg protein in the equation provided by Meador et al. (2008) to predict dose. Based on the 90% upper confidence level for the inverse regression equation a predicted dose of 10.4 μg/g fish/day is obtained. A recent analysis by Kane Driscoll et al. (2010) showed that doses in this range are predicted to elicit adverse effects in fish. Interestingly, a laboratory study that exposed juvenile Chinook salmon to a dietary PAH-mixture that was modeled from PAHs detected in the stomach contents of LDW juvenile Chinook salmon reported increased FACs with increased dietary dosing (Palm et al., 2003). Field studies have also shown associations between FACs and adverse effects. For instance, Johnson et al. (1993) noted that concentrations of FACs in bile were correlated with probability of ovarian development. Moreover, a study conducted in Eagle Harbor, Washington, a PAH-contaminated site, noted reductions in FACs, hepatic DNA adducts and risk of lesions following placement of a sediment cap in remediation efforts (Myers et al., 2008).

In addition to biliary FAC analysis, investigators intended to analyze the stomach contents of caged salmon. It was hypothesized that a limited amount of feeding on small aquatic organisms that flow by the cages would occur. However, upon dissection it was noted that stomach contents of the fish were empty. Thus, the biomarker responses observed in this study are predominantly related to water column exposures resulting from gill ventilation. Consequently, without dietary uptake, the exposure for fish to contaminants in this system was underestimated, especially for PCBs.

In contrast to FAC measurement, DNA adducts provide a more cumulative marker of PAH exposure that is representative of longer-term exposures (Van Der Oost et al., 2003). Biomarkers such as DNA adducts are also useful as representatives of the biologically effective dose, or the dose that reaches the target tissues (Poirier and Weston 1996). Moreover, although DNA adducts provide a clear indicator of exposure to carcinogens, a complex array of factors may alter the number of adducts observed in individuals, complicating attempts to quantitatively link environmental contamination and DNA adduct levels (Dunn, 1991). In gill tissue composites, DNA adducts were elevated compared to controls in 2004 and 2006, while adduct levels were lowest in 2007 when ambient exposure was detected at the lowest levels of the exposure period. Unfortunately, the quality of DNA extracted from the 2005 fish was not acceptable for DNA adduct analysis as a result of thawing due to unexpected delays during shipment of fish from Washington State to Texas A&M University. Results from previously published field studies provide some support for the finding of elevated DNA adduct levels in LDW fish. Stein et al. (1993) found that three species of benthic flatfish exhibited higher levels of DNA adducts compared to fish collected from an uncontaminated location.

Western blot analysis of hepatic tissue was performed using samples from the 2007 sampling period and revealed non-significant elevations in CYP1A1 induction at stations B3 and B4 compared to reference fish. Among the potential explanations for the lack of significant induction is that the lowest levels of contaminants in waters were detected during the 2007 sampling year. In addition, Browne et al. (2010) were supplied a sub-set of fish from this experiment in 2007 and measured hepatic expression of seven different genes following the caged exposure in the LDW. Results of the study failed to identify any site-related differences in gene expression compared to controls, providing further information suggesting low bioavailability of contaminants in the water column during the 2007 sampling period, compared to other study years.

Although advantages of *in situ* biomarker studies are numerous, a major disadvantage is altered food availability during the exposure (Chappie and Burton, 2000). This altered food availability may cause physiological changes in fish. Martin et al. (2001) found alterations in expression of certain liver proteins in rainbow trout (*Oncorhynchus mykiss)* that were subjected to starvation for 14 days. Changes in growth hormone expression in rabbitfish (*Siganus guttatus*) have also been documented after as little as 3 days of starvation (Ayson et al., 2007). However, the consequences of altered feeding status on biomarker response can be difficult to estimate. For example, a 30 day starvation experiment using rainbow trout showed alterations in EROD activity after 30 days (Hanson and Larsson, 2007). In contrast, a longer-term study, also utilizing rainbow trout, found no difference in EROD activity based on no, half or full rations for 9 weeks, though the study did report a significant reduction in glutathione *S*-transferase (GST) after 6 weeks (Gourley and Kennedy, 2009).

As pointed out in Browne et al. (2010), starved salmonids have been shown to exhibit reduced metabolism (Brett, 1995). Additionally, starved fish have been shown to exhibit reduced activity level. Sogard and Olla (1996) demonstrated that activity level in walleye pollock (*Theragra chalcogramma*) was significantly reduced with starvation. These findings have important implications in this study as many PAHs require metabolic activation to exert genotoxicity (Pisoni et al., 2004). In addition, reduced activity likely alters gill ventilation rate and contaminant uptake (Browne et al., 2010; Meador et al., 2008). Thus, biomarker responses in this study are likely to underestimate the true exposure of migrating salmon, as

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

hydrophobic compounds, such as PCBs are predominantly accumulated via ingestion (Gobas et al., 1999). This is supported by the lack of measurable PCB concentrations in tissue.

Further, the complex nature of sediment contamination poses a formidable challenge. For example, knowledge of the effects of chemical–chemical interactions in these complex mixtures is still limited and data are sometimes highly variable between studies. Along with mixture interactions, many sources of pollution in the LDW remain active, producing an ever-changing state of contamination. Source allocation of PAHs appears to indicate that both point and non-point sources contribute to the toxicant burden, thus resulting in a more complex mixture of mutagenic and non-mutagenic PAHs. These data are consistent with the geographical location and site history for the LDW, which indicate that despite pollution reduction efforts, contamination likely continues to enter the estuary from a variety of remaining sources. Sources such as combined sewer overflows, storm drains, accidental spillage, and direct disposal or discharge have historically contributed to the contamination in the LDW (USEPA, 2001). Boating activities in the LDW also may contribute to disturbance of the top few cm of sediment (Windward Environmental LLC, 2007). Proximity to Interstate 5 (a heavily traveled roadway) also provides the opportunity for addition of contaminants through both runoff and atmospheric transport.

Further complexity is present due to the ability of PCBs to alter effects of other chemicals as documented by previous studies. For example, Collier et al. (1985) noted that exposure of coho salmon to PCBs substantially altered the biological fate of 2,6-dimethylnaphthalene (DMN). Shelton et al. (1983) showed that PCBs can either enhance or inhibit carcinogenesis induced by aflatoxin exposure in rainbow trout. Stein et al. (1984) exposed English sole (*P. vetulus*) to sediments spiked with radiolabeled PCBs and BaP for 10 days. Results indicated that simultaneous exposure increased the amount of BaP-derived radioactivity in bile. The authors suggest that previous research has demonstrated that PCBs induce mixed function oxidases (MFO), which are responsible for metabolism of PAHs, thus potentially explaining why the results indicated increased metabolism of PAHs upon coexposure with PCBs. As previously stated, many PAHs require metabolic activation in order to exert genotoxicity (Pisoni et al., 2004), thus increased metabolism of PAHs in the presence of PCBs would be expected to promote DNA adduct formation.

However, contrasting results were obtained in a recent study conducted by Gillespie (2006) using a sediment extract from the LDW. Mice were exposed dermally to 100 nmol BaP alone or 100 nmol BaP in combination with LDW sediment extract. Although the LDW sediment extract contained a complex mixture of PCBs and PAHs, tPCBs were the predominant contaminant group present in the sediment extract (approximately 20-fold higher than levels of tPAHs). Results indicated that administration of BaP alone resulted in the highest levels of DNA adduct formation, while adduct levels decreased in a dose-dependent manner upon addition of increasing quantities of the predominantly PCB-containing LDW sediment extract. Due to potential chemical–chemical interactions, the method of DNA adduct analysis utilized in this study may be most appropriate for use at sites at which PAHs are the sole or predominant contaminant of concern.

The focus of our study was primarily PAHs and PCBs, although many additional contaminants are present in the LDW. These contaminants could potentially have affected results even though analysis was not performed on these chemicals. Data published in the LDW Final Remedial Investigation Report (Windward Environmental LLC, 2010), was used to compile a table listing additional contaminants that were detected within approximately 50 and 100 m of our sampling stations (Table 4). Close examination of the data reveals that in the vast majority of samples, levels of contaminants, including various metals, detected near our sampling sites fell below both cleanup screening levels (CSLs) and sediment quality standards (SQSs). In light of this information, it is unlikely that these contaminants played a prominent role in the observed biomarker responses. It is also of note that these SQSs and CSLs were derived based on toxicity in invertebrate organisms and are thus not directly applicable to vertebrate fish.

Other important limitations of the study include similar levels of contamination between sampling stations. The lack of an adequate exposure gradient was therefore not optimal for evaluation of utility of the biomarkers employed in this study. Sampling sites were selected based on previous data collection, discussions with public agencies, and the presence of both PAHs and PCBs in sediment, in order to evaluate biomarker response in the presence of complex mixtures. Ideally, sampling locations would have provided a more diverse exposure gradient in which varying levels of contaminant would be encountered and evaluated. This would provide a more accurate depiction of the relationship between levels of DNA adducts present in the exposed test species and levels of contaminants present in contaminated water and sediment. Collection of additional sediment and water samples each study year may have also been beneficial in differentiating levels of exposure between stations.

Additionally, although it is possible that the increase in contaminants detected in 2005 water samples was related to elevated contaminant input to the LDW, relative to other study years, another potential explanation is that a disturbance of the sediment resulted in elevated contaminants in the water column. Water samples were not filtered prior to extraction, thus contaminants that were sorbed onto sediment particles in the water column were also extracted during sample processing. This hypothesis of altered results due to increased presence of sediment in the water column is supported by analysis of water samples from the 2005 study period that detected PCBs in the majority of samples, contradicting every other study period that produced only non-detects in this regard (data not shown).

Given the complex nature of these exposures, it is difficult to speculate on why the 2005 spike in tissue–PAHs was only consistent with elevations in water–PAHs at two of the four stations. It may simply be an issue related to the heterogeneity of water sampling or it could be related to a host of unknown factors. If the exposure to water column contaminants in 2005 was indeed elevated relative to other sampling years due to disturbance of the sediment, metabolic pathways may have simply become overwhelmed, resulting in greater retention of parent PAHs in tissue during the 2005 sampling period. Further, data collected during the Lower Duwamish Waterway Group's LDW Remedial Investigation detailed higher levels of PCBs present in salmon tissue collected shortly following dredging events (Windward Environmental LLC, 2007). Future studies would benefit from filtration of water samples and perhaps comparison of filtered and unfiltered water samples. Incorporation of passive sampling devices, such as solid-phase microextraction samplers, would also aid in more accurately assessing the contaminants in the water column. Devices such as these would integrate changes in the exposure over time, as opposed to the current study design that simply took a snapshot of the water column prior to and at the conclusion of the exposure.

Further, the lack of a true in-river reference site (sampling station K1 was originally intended for this purpose but high levels of PAHs were detected) was not ideal and in selected instances divers were unable to locate deployed cages. An additional factor that was not accounted for in the study design is that exposure among fish in bottom cages likely was not identical to that of fish placed in top cages. When fish were randomly selected for composites by station no differentiation was made between top or bottom cages. Finally,

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

Table 4
Relative sediment toxicity to organisms. Contaminants detected in close proximity to sampling stations were compiled from the Lower Duwamish Waterway Group's Final Remedial Investigation Report (Windward Environmental LLC, 2010). Levels of contaminants were then compared to Washington State sediment standards and cleanup levels.

Early Action area =	K1 Not an early action area	B2 Boeing Plant 2		B3 Boeing Plant 2		B4 T117					
Distance from station	No stations in 100 m, used nearest 3 within approx. 175 m	<50 m	<100 m	<50 m	<100 m	<50 m	<100 m				
Contaminant of concern								SQS	CSL	Units	Maps
Total PCBs	–	++	++	+	++	++	++	192[a]	1040[a]	ng/g	Map 4–20
BEHP	–	–	+	–	++	–	–	752[a]	1248[a]	ng/g	Map 4–44
HPAH	–	–	+	–	–	–	+	15360[a]	84800[a]	ng/g	Map 4–61
LPAH	–	–	–	–	–	–	++	5920[a]	12480[a]	ng/g	Map 4–62
BBP	–	–	–	–	++	–	–	78.4[a]	1024[a]	ng/g	Map 4–63
Phenol	–	–	–	–	–	–	++	420	1200	ng/g	Map 4–64
Benzoic acid	–	–	–	–	–	–	–	650	650	ng/g	Map 4–65
Arsenic	–	–	–	–	–	–	–	57	93	µg/g	Map 4–29
Cadmium	–	+	+	–	++	–	–	5.1	6.7	µg/g	Map 4–50
Chromium	–	–	–	–	+	–	–	260	270	µg/g	Map 4–51
Copper	–	–	–	–	–	–	–	390	390	µg/g	Map 4–52
Lead	–	–	–	–	–	–	–	450	530	µg/g	Map 4–53
Mercury	–	–	–	–	–	–	–	0.41	0.59	µg/g	Map 4–54
Silver	–	–	–	–	–	–	–	6.1	6.1	µg/g	Map 4–56
Zinc	–	–	–	–	+	–	–	410	960	µg/g	Map 4–58
Sediment toxicity ($\sum$ + 's)	0	3	5	1	10	2	7				

SQS = Washington State sediment quality standard, CSL = Washington State cleanup screening level.
Code: – = <SQS, + = >SQS, ++ = >CSL.
Web link to access maps: http://ldwg.org/assets/phase2_ri/final%20ri/Final_LDW_RI_Map_Folio_2_Section_4_Maps.pdf.
[a] = SQS or CSL was originally derived from data normalized to organic carbon. SQS or CSL has been converted using median total organic carbon of 1.6% (Meador et al., 2008).

Table 5
PAH and PCB chemical analysis data from sediment samples collected in the Lower Duwamish Waterway during July 2004–July 2007 are compared sediment quality thresholds or guidelines. All units are in ppb. For K1 $n = 3$ and $n = 4$ for all other sites.

Site	LDW 2004–2007 sediment means				Horness et al. (1998) and Johnson et al. (2002)	Meador et al. (2010)
	K1	B2	B3	B4	Sediment quality threshold	Sediment guideline
Total PAHs	1782	2712	1730	1395	230–2800	–
Total PCBs	16	1248	1752	383	–	12–106

bulk sediment chemistry data, including factors such as total organic carbon, which have the potential to alter bioavailability were not analyzed in the study and could have potentially aided in accounting for potential differences in bioavailability between stations.

Numerous sediment quality guidelines (SQGs) are available to serve as screening tools to help estimate the likelihood of toxicity among aquatic organisms and to identify areas of concern. However, the scientific basis for many of these SQGs primarily involves the use of benthic invertebrates which are far more likely to bioaccumulate PAHs, thus bringing the applicability of these SQGs to fish into question (Johnson et al., 2002). In contrast, Horness et al. (1998) have produced a set of sediment quality thresholds (SQTs) based on hockey stick regression analysis of liver lesion prevalence in English sole (*P. vetulus*). The method was later expanded upon to include additional endpoints by Johnson et al. (2002). In regard to sediment-associated PCBs, Meador et al. (2010) have provided a sediment guideline for juvenile Chinook salmon based on tPCB bioaccumulation. A comparison of sediment samples collected in the LDW from 2004 to 2007 to these assessment tools is presented in Table 5. Note that mean levels of contaminants at each of the stations sampled in the LDW exceeded the lower range of the SQT or guideline.

In addition, examination of the data in relation to SQTs, such as the one provided by Horness et al. (1998), indicate that fish exposed to the PAH contamination present in the LDW would likely exhibit an elevated prevalence of hepatic lesions. This prediction would be consistent with data generated from previous studies conducted in part on the LDW (McCain et al., 1977; Pierce et al., 1978). For example, several studies conducted by the National Marine Fisheries Service have demonstrated that juvenile salmon from the Duwamish exhibited reduced growth and impaired immune function compared to specimens from less contaminated areas (USEPA, 2001). Among these studies, Varanasi et al. (1993), in addition to the above mentioned effects, reported elevated concentrations of PCBs in tissue and elevated FACs. Additionally, the study noted that when held in the laboratory and monitored for survival, juvenile Chinook salmon from the Duwamish exhibited reduced survival compared to juvenile Chinook from a non-urban estuary.

The SQTs utilized in this study are preferable to other available methods as they link concentrations of PAHs in sediment with adverse effects in resident fish, that readily metabolize PAHs, rather than with toxicity in benthic organisms, that are not capable of metabolizing PAHs (Johnson et al., 2002). In addition, both urban and non-urban sampling locations were included in the analysis, so the results are applicable to urban waterways such as the LDW. Disadvantages of the approach include that salmon spend less time in residence in the LDW, have less contact with sediment, and consume fewer benthic invertebrates than English sole, which were used to generate the SQT. Therefore salmon exposure may be considerably lower than English sole (Johnson et al., 2002).

5. Conclusions

Although each of the biomarkers employed in this 4 year *in situ* study offer advantages and disadvantages, cumulatively, they

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026

provide valuable information for use in environmental monitoring of contaminated sites. In the case of the LDW, the biomarkers provided a sensitive measure of exposure assessment, as response in field-caged fish was often elevated compared controls. It also appears that the biomarkers employed may aid in detection of spikes in contaminant levels across study years. The considerable variation observed in sediment and water samples, as well as biomarker responses, emphasizes the challenges associated with characterization of contaminated waterways. This is especially true at sites such as the LDW, where contamination is ongoing, despite improvements in source control in recent years. This study illustrates the importance of longitudinal studies in order to accurately assess the contamination. Although use should be considered on a site-specific basis, in addition to traditional toxicity measures, caged *in situ* exposure studies provide supplemental data which would enable site managers to make better informed management decisions.

Disclaimer

The views and opinions expressed in this manuscript do not necessarily reflect those of the US Environmental Protection Agency or the National Atmospheric and Atmospheric Administration. In addition, although USEPA Region 10 provided research support for this work, their effort was not a part of the negotiated risk assessment for the Lower Duwamish Waterway Superfund site.

Acknowledgements

This work was supported by an NIEHS Superfund Research Program (SRP) Grant to Texas A&M University (P42 ES04917). Additional funding for the study was provided in part from the National Oceanic and Atmospheric Administration, Oceans and Human Health Program (NA05NS4781253), and the Region 10 office of the USEPA. The efforts of Shirley Wang during sample collection and Dr. Ling Yu He during sample extraction are much appreciated. The authors also wish to show their appreciation to members of USEPA, Region 10, including but not limited to Allison Hiltner, Grechen Schmidt, Doc Thompson, the EPA Region 10 Dive Team, as well as boat captains and staff at the USEPA Manchester Lab. In addition, the authors wish to thank Guy Crow of South Parking Marina for allowing use of his facility during sample collection. The efforts of Frank Sommers at NOAA are also appreciated.

References

Alford-Stevens, A., Bellar, T.A., Eichelberger, J.W., Budde, W.L., 1985. Determination of pesticides and PCBs in water and soil/sediment by GC/MS (CD ROM). United States Environmental Protection Agency Office of Research and Development, Cincinnati, Ohio.

Arkoosh, M.R., Casillas, E., Clemons, E., McCain, B., Varanasi, U., 1991. Suppression of immunological memory in juvenile chinook salmon (*Oncorhynchus tshawytscha*) from an urban estuary. Fish Shellfish Immunol. 1, 261–277.

Ayson, F.G., de Jesus-Ayson, E.G.T., Takemura, A., 2007. MRNA expression patterns for GH, PRL, SL, IGF-I and IGF-II during altered feeding status in rabbitfish, *Siganus guttatus*. Gen. Com. Endocrinol. 150 (2), 196–204.

Barbee, G.C., Barich, J., Duncan, B., Bickham, J.W., Matson, C.W., Hintze, C.J., Autenrieth, R.L., Zhou, G.D., McDonald, T.J., Cizmas, L., Norton, D., Donnelly, K.C., 2008. In situ biomonitoring of PAH-contaminated sediments using juvenile coho salmon (*Oncorhynchus kisutch*). Ecotoxicol. Environ. Saf. 71, 454–464.

Browne, E., Kelley, M., Zhou, G.D., He, L.Y., McDonald, T., Wang, S., Duncan, B., Meador, J., Donnelly, K., Gallagher, E., 2010. In situ biomonitoring of juvenile Chinook salmon (*Onchorhynchus tshawytscha*) using biomarkers of chemical exposures and effects in a partially remediated urbanized waterway of the Puget Sound, WA. Environ. Res. 110, 675–683.

Bucheli, T.D., Fent, K., 1995. Induction of cytochrome P450 as a biomarker for environmental contamination in aquatic ecosystems. Crit. Rev. Environ. Sci. Technol. 25, 201–268.

Chappie, D.J., Burton Jr., G.A., 2000. Applications of aquatic and sediment toxicity testing *in situ*. Soil Sed. Contam. 9, 219–245.

Collier, T.K., Gruger, E.H., Varanasi, U., 1985. Effect of Aroclor 1254 on the biological fate of 2, 6-dimethylnaphthalene in coho salmon (*Oncorhynchus kisutch*). B. Environ. Contam. Toxicol. 34, 114–120.

Collier, T.K., Stein, J.E., Wallace, R.J., Varanasi, U., 1986. Xenobiotic metabolizing enzymes in spawning English sole (*Parophrys vetulus*) exposed to organic-solvent extracts of marine sediments from contaminated and reference areas. Comp. Biochem. Physiol. C 84, 291–298.

Collier, T.K., Varanasi, U., 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. Arch. Environ. Contam. Toxicol. 20, 462–473.

Dunn, B.P., 1991. Carcinogen adducts as an indicator for the public health risks of consuming carcinogen-exposed fish and shellfish. Environ. Health Perspect. 90, 111–116.

Gillespie, A.M., Environmental toxicity of complex chemical mixtures [PhD Dissertation]. Texas A&M University, College Station, Texas, 2006.

Gobas, F.A.P.C., Wilcockson, J.B., Russell, R.W., Haffner, G.D., 1999. Mechanism of biomagnification in fish under laboratory and field conditions. Environ. Sci. Technol. 33, 133–141.

Goksøyr, A., Beter, J., Husoy, A.M., Larsen, H.E., Westrheim, K., Wilhelmsen, S., Klungsoyr, J., 1994. Accumulation and effects of aromatic and chlorinated hydrocarbons in juvenile Atlantic cod (*Gadus morhua*) caged in a polluted fjord (Sørfjorden, Norway). Aquat. Toxicol. 29, 21–35.

Gourley, M.E., Kennedy, C.J., 2009. Energy allocations to xenobiotic transport and biotransformation reactions in rainbow trout (*Oncorhynchus mykiss*) during energy intake restriction. Comp. Biochem. Physiol. C 150 (2), 270–278.

Hanson, N., Larsson, A., 2007. Influence of feeding procedure on biomarkers in caged rainbow trout (*Oncorhynchus mykiss*) used in environmental monitoring. J. Environ. Monitor. 9, 168–173.

Horness, B.H., Lomax, D.P., Johnson, L.L., Myers, M.S., Pierce, S.M., Collier, T.K., 1998. Sediment quality thresholds: estimates from hockey stick regression of liver lesion prevelance in English sole (*Pleuronectes vetulus*). Environ. Toxicol. Chem. 17, 872–882.

Johnson, L., Casillas, E., Sol, S., Collier, T., Stein, J., Varanasi, U., 1993. Contaminant effects on reproductive success in selected benthic fish. Mar. Environ. Res. 35 (1–2), 165–170.

Johnson, L.L., Collier, T.K., Stein, J.E., 2002. An analysis in support of sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish. Aquat. Conservation 12, 517–538.

Kane Driscoll, S.B., McArdle, M.E., Menzie, C.A., Reiss, M., Steevens, J.A., 2010. A framework for using dose as a metric to assess toxicity of fish to PAHs. Ecotoxicol. Environ. Saf. 73, 486–490.

Krahn, M.M., Moore, L.K., MacLeod Jr., W.D., 1986a. Standard analytical procedures of the NOAA National Analytical Facility, 1986. Metabolites of aromatic compounds in fish bile. NOAA Technical Memorandum NMFS F/NWC-102. National Oceanic and Atmospheric Administration, Seattle, WA.

Krahn, M.M., Rhodes, L.D., Myers, M.S., Moore, L.K., MacLeod, W.D., Malins, D.C., 1986b. Associations between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Arch. Environ. Contam. Toxicol. 15, 61–67.

Lee, R.F., Anderson, J.W., 2005. Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. Mar. Pollut. Bull. 50 (7), 705–723.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mabon, N., Moorthy, B., Randerath, E., Randerth, K., 1996. Monophosphate ^{32}P-postlabeling assay of DNA adducts from 1,2:3,4-diepoxybutane, the most genotoxic metabolite of 1,3-butadiene: in vitro methodological studies and in vivo dosimetry. Mutat. Res. Genet. Toxicol. 371, 87–104.

Malins, D.C., McDain, B.B., Brown, D.W., Sparks, A.K., Hodgins, H.O., Chan, S.-L., Chemical contaminants and abnormalities in fish and invertebrates from Puget Sound. NOAA Technical Memorandum OMPA-19. Office of Marine Pollution Assessment, Boulder, CO, 1982.

Malins, D.C., McCain, B.B., Myers, M.S., Brown, D.W., Krahn, M.M., Roubal, W.T., Schieve, M.H., Landahl, J.T., Chan, S.-L., 1987. Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. Environ. Health Perspect. 71, 5–16.

Martin, S.A.M., Cash, P., Blaney, S., Houlihan, D.F., 2001. Proteome analysis of rainbow trout (*Oncorhynchus mykiss*) liver proteins during short term starvation. Fish Physiol. Biochem. 24, 259–270.

Martin-Diaz, M.L., DelValls, T.A., Riba, I., Blasco, J., 2008. Integrative sediment quality assessment using a biomarker approach: review of 3 years of field research. Cell Biol. Toxicol. 24, 513–526.

McCain, B.B., Pierce, K.V., Wellings, S.R., Miller, B.S., 1977. Hepatomas in marine fish from an urban estuary. B. Environ. Contam. Toxicol. 18, 1–2.

Meador, J., Ylitalo, G.M., Sommers, F.C., Boyd, D.T., 2010. Bioaccumulation of polychlorinated biphenyls in juvenile chinook salmon (*Oncorhynchus tshawytscha*) outmigrating through a contaminated urban estuary: dynamics and application. Ecotoxicology 19, 141–152.

Meador, J.P., Buzitis, J., Bravo, C.F., 2008. Using fluorescent aromatic compounds in bile from juvenile salmonids to predict exposure to polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 27, 845–853.

Meador, J.P., Stein, J.E., Reichert, W.L., Varanasi, U., 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev. Environ. Contam. Toxicol. 143, 79–165.

Meyer, J.H., Pearce, T.A., Patlan, S.B., 1981. Distribution and food habits of juvenile salmonids in the Duwamish Estuary Washington, 1980.. Department of the Interior, Fisheries Assistance Office, US Fish and Wildlife Service, Olympia, Washington: United States.

Myers, M.S., Anulacion, B.F., French, B.L., Reichert, W.L., Laetz, C.A., Buzitis, J., Olson, O.P., Sol, S., Collier, T.K., 2008. Improved flatfish health following remediation of a PAH-contaminated site in Eagle Harbor, Washington. Aquat. Toxicol. 88 (4), 277–288.

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.

Palm Jr., R.C., Powell, D.B., Skillman, A., Godtfredsen, K., 2003. Immunocompetence of juvenile chinook salmon against *Listonella anguillarum* following dietary exposure to polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 22 (12), 2986–2994.

Pierce, K.V., McCain, B.B., Wellings, S.R., 1978. Pathology of hepatomas and other liver abnormalities in English sole (*Parophrys vetulus*) from the Duwamish River estuary, Seattle, Washington. J. Natl. Cancer Inst. 60, 1445–1453.

Pisoni, M., Cogotzi, L., Frigeri, A., Corsi, I., Bonacci, S., Iacocca, A., Lancini, L., Mastrototaro, F., Focardi, S., Svelto, M., 2004. DNA adducts, benzo(a)pyrene monooxygenase activity, and lysosomal membrane stability in *Mytilus galloprovincialis* from different areas in Taranto coastal waters (Italy). Environ. Res. 96, 163–175.

Randerath, K., Reddy, M.V., Disher, R.M., 1986. Age- and tissue-related DNA modifications in untreated rats: detection by ^{32}P-postlabeling assay and possible significance for spontaneous tumor induction and aging. Carcinogenesis 7, 1615–1617.

Reddy, M.V., Randerath, K., 1986. Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts. Carcinogenesis 7, 1543–1551.

Sarkar, A., Ray, D., Shrivastava, A.N., Sarker, S., 2006. Molecular biomarkers: their significance and application in marine pollution monitoring. Ecotoxicology 15, 333–340.

Shelton, D.W., Coulombe, R.A., Pereira, C.B., Casteel, J.L., Hendricks, J.D., 1983. Inhibitory effect of Aroclor 1254 on aflatoxin-initiated carcinogenesis in rainbow trout and mutagenesis using a Salmonella/trout hepatic activation system. Aquat. Toxicol. 3, 229–238.

Sogard, S.M., Olla, B.L., 1996. Food deprivation affects vertical distribution and activity of a marine fish in a thermal gradient: potential energy-conserving mechanisms. Mar. Ecol. Prog. Ser. 133, 43–55.

Stein, J., Collier, T.K., Reichert, W.L., Casillas, E., Hom, T., Varanasi, U., 1992. Bioindicators of contaminant exposure and sublethal effects: studies with benthic fish in Puget Sound, Washington. Environ. Toxicol. Chem. 11, 701–714.

Stein, J.E., Collier, T.K., Reichert, W.L., Casillas, E., Hom, T., Varanasi, U., 1993. Bioindicators of contaminant exposure and sublethal effects in benthic fish from Puget Sound, WA, USA. Mar. Environ. Res. 35 (1–2), 95–100.

Stein, J.E., Hom, T., Varanasi, U., 1984. Simultaneous exposure of English sole (*Parophrys vetulus*) to sediment-associated xenobiotics: Part 1 – Uptake and disposition of 14C-polychlorinated biphenyls and 3H-benzo[a]pyrene. Mar. Environ. Res. 13, 97–119.

USEPA, Method 3535: Pressurized fluid extraction (PFE). SW-486, Test methods for evaluation solid waste, physical/chemical methods. United States Environmental Protection Agency, Washington, DC, 1996a.

USEPA, Method 3510C: Separatory funnel liquid-liquid extraction. SW-846, Test methods for evaluating solid waste, physical/chemical methods. United States Environmental Protection Agency, Washington, DC, 1996b.

USEPA, Method 8270C: Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS). SW-846, Test methods for evaluating solid waste, physical/chemical methods. United States Environmental Protection Agency, Washington, DC, 1996c.

USEPA, NPL site narrative for Lower Duwamish Waterway. United States Environmental Protection Agency Seattle, 2001.

USEPA, WSDOE, What is in the Lower Duwamish Waterway? Lower Duwamish Waterway Superfund site. United States Environmental Protection Agency and Washington State Department of Ecology, Seattle, Washington, 2008, p. 6.

Van Der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish accumulation and biomarkers in environmental risk assessment: a review. Environ. Technol. Pharmcol. 13, 57–149.

Varanasi, U., Casillas, E., Arkoosh, M.R., Hom, T., Misitani, D.A., Brown, D.W., Chan, S.L., Collier, T.K., McCain, B.B., Stein, J.E., 1993. Contaminant Exposure and Associated Biological Effects in Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) from Urban and Nonurban Estuaries of Puget Sound: NOAA Technical Memorandum NMFS-NWFSC-8. National Marine Fisheries Service, Seattle.

Varanasi, U., Reichert, W.L., Stein, J.E., 1989a. ^{32}P-postlabeling analysis of DNA Adducts in liver of wild English sole (*Parophrys vetulus*) and winter flounder (*Pseudopleuronectes americanus*). Cancer Res. 49, 1171–1177.

Varanasi, U., Reichert, W.L., Stein, J.E., Brown, D.W., Sanborn, H.R., 1985. Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. Environ. Sci. Technol. 19, 836–841.

Varanasi, U., Stein, J.E., Nishimoto, M., 1989b. Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish. In: Varanasi, U. (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press Inc., Boca Raton, Florida, pp. 93–149.

Wellings, S.R., Alpers, C., McCain, B.B., Miller, B.S., 1976. Fin erosion disease of starry flounder (*Platichthys stellatus*) and English sole (*Parophrys vetulus*) in the estuary of the Duwamish River, Seattle, Washington. J. Fish. Res. Board of Canada 33, 2577–2586.

Windward Environmental LLC, 2003a. Final Lower Duwamish Waterway phase I remedial investigation report. Prepared for the Lower Duwamish Waterway Group, Seattle, WA.

Windward Environmental LLC, 2003b. Lower Duwamish remedial investigation, task 5: identification of candidate sites for early action. Technical memorandum: data analysis and candidate site identification. Prepared for the Lower Duwamish Waterway Group, Seattle, WA.

Windward Environmental LLC, 2007. Lower Duwamish Waterway Remedial Investigation. Prepared for the Lower Duwamish Waterway Group, Seattle, WA.

Windward Environmental LLC, 2010. Lower Duwamish Waterway Remedial Investigation Report Final. Prepared for the Lower Duwamish Waterway Group, Seattle, WA.

Zhou, G.D., Hernandez, N.S., Randerath, E., Randerath, K., 1999. Acute elevation by short-term dietary restriction or food deprivation of type I I-compound levels. Nutr. Cancer 35, 87–95.

Please cite this article in press as: Kelley, M.A., et al. *In situ* biomonitoring of caged, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Lower Duwamish Waterway. Mar. Pollut. Bull. (2011), doi:10.1016/j.marpolbul.2011.07.026