Toxic contaminants in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) migrating through estuary, nearshore and offshore habitats of Puget Sound

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Photo of Chinook salmon fry by Richard Bell, published in “The Behavior and Ecology of Pacific Salmon,” Thomas Quinn, University of Washington Press, 2005

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ACRONYMS, ABBREVIATIONS, AND UNITS

Acronyms and abbreviations used frequently in this report are listed below, those used infrequently are excluded.

DDT	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
Ecology	Washington State Department of Ecology
EPA	U.S. Environmental Protection Agency
GC/MS	gas chromatography/mass spectrometry
GPS	global positioning system
LOQ	limit of quantitation
MDL	method detection limit
NOAA	National Oceanic & Atmospheric Administration
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
POP	persistent organic pollutant
PSEMP	Puget Sound Ecosystem Monitoring Program (formerly PSAMP)
QA/QC	quality assurance/quality control
SRM	standard reference materials
WDFW	Washington Department of Fish and Wildlife
ww	wet weight

UNITS OF MEASUREMENT

m	meter
g	gram
km	kilometer
mm	millimeters
mg/kg	milligrams per kilogram (parts per million)
ng/g	nanograms per gram (parts per billion)

SUMMARY

Juvenile Chinook salmon (*Oncorhynchus tshawytscha)* can encounter a wide range of water quality conditions, from relatively clean to highly contaminated, as they migrate from rivers into Puget Sound. During this life stage, as they transition into saltwater, they are particularly sensitive to stressors such as toxic contaminants. This study was designed to provide a synoptic assessment of contaminant exposure for major populations of juvenile Chinook salmon from Puget Sound as the fish migrate from their freshwater to marine habitats. Overall, the study estimated exposure of salmon to toxics chemicals in 1) the estuary habitats of major river systems entering Puget Sound, 2) the nearshore marine habitats associated with those rivers systems, and 3) the offshore marine habitats of the major basins of Puget Sound. The study addresses the general hypothesis that chemicals released into Puget Sound from human activities and development reduces the health and productivity of salmon and their food supply. Specifically, we hypothesized that juvenile Chinook salmon residing and feeding in the more urbanized and industrial estuary, nearshore marine, and offshore habitats of Puget Sound are exposed to higher concentrations of toxic contaminants than those in less developed habitats. In addition, we hypothesized that the elevated contaminant concentrations in the more urban areas are high enough to affect juvenile Chinook survival through reductions in growth, disease resistance, and altered hormone and protein levels.

Fish were sampled in spring and summer of 2013 from five major Puget Sound river systems and four marine basins in Puget Sound. In each river system, sampling sites included one location in the lower estuary and two locations along adjacent nearshore marine shorelines. The marine basins included fish offshore marine habitat from Admiralty Inlet, Whidbey Basin, Central Basin, and South Basin. We analyzed whole bodies for persistent organic contaminants (POPs), stomach contents for polycyclic aromatic hydrocarbons (PAHs), and gills for metals in fish collected from estuaries, nearshore marine shorelines and offshore habitats in the basins of Puget Sound. Tissue residues were compared with published adverse effects thresholds to evaluate the potential health effects on juvenile salmon from exposure to these contaminants. Finally, for the whole body analyses, we compared body burden of POPs in fish from different habitats to assess the degree to which POPs were being accumulated in the river and estuary, nearshore, or offshore habitats (i.e., routes of exposure).

The levels of organic contaminants we observed in juvenile Chinook salmon from estuary and nearshore habitats, measured as POP concentrations in whole-body fish samples or as PAH concentrations in stomach contents, supported our hypothesis that salmon residing and feeding in the more urbanized and industrialized environments are exposed to higher concentrations of contaminants than those in less developed habitats. However, for salmon collected in offshore habitats of the marine basins our hypothesis was not supported. Fish from the more developed Central Basin of Puget Sound did not have elevated POPs and PAHs concentrations compared to those from the less developed Whidbey Basin and South Basin. As juvenile Chinook salmon migrated from river systems to offshore waters of Puget Sound, all fish continued to accumulate substantial amounts of POPs, as evidenced by the higher total mass of POPs in their bodies (i.e., POP body burdens measured as ng/fish) and after four months of feeding in offshore habitats, fish from all basins had uniform concentrations of POPs (i.e., the mass of

POP compared to the mass of fish tissue measured as ng POP/g tissue ww). In general, concentrations of POPs in fish from offshore basins were lower than those measured in fish from developed river systems, indicating that the offshore was less contaminated than the developed river systems habitats. In contrast, the concentrations of POPs in the offshore habitats were sometimes higher than those from undeveloped river systems indicating that the offshore was more contaminated than the undeveloped river systems habitats. The levels of copper and lead were also elevated in gill tissues of fish from the more developed nearshore marine habitats but the concentration of cadmium, nickel and zinc were not elevated in the more urban and industrial habitats. Fish body size did not show strong association with contaminant uptake; location was consistently the primary factor associated with contaminant levels.

Levels of PCBs and PBDEs in whole body tissue samples from fish collected in the Snohomish, Green/Duwamish and Hylebos/Puyallup river systems, and PCBs in fish from the offshore habitat of the Whidbey Basin and the Central basin were high enough to potentially cause adverse effects, including reductions in growth, disease resistance, and altered hormone and protein levels. Additionally, PAHs in stomach content of Chinook salmon were elevated in salmon from the nearshore habitats of the Snohomish and Green/Duwamish systems, at concentrations high enough to potentially increase variability in growth, and to alter plasma chemistry and lipid class profiles. Moreover, approximately one-third of the salmon we sampled from Puget Sound, regardless of the degree of development, had contaminant concentrations associated with adverse effects, indicating that a significant proportion of juvenile Puget Sound Chinook salmon are at risk for some type of health impairment due to contaminant exposure, potentially affecting their marine survival.

Analysis of contaminant body burden (ng/fish) in salmon from estuary, nearshore, and offshore habitats revealed that along the migratory pathway, salmon accumulated the majority of the mass of POPs in their bodies from offshore habitats, indicating that sources of POPs to fish migrating to the Pacific Ocean is not limited to contaminant exposure in developed rivers and nearshore habitats. POP contaminant loading from urbanized river system areas and other sources is reaching non-urbanized areas offshore habitats where juvenile Chinook salmon may feed for several months, sometimes accumulating concentrations high enough to potentially impair their health. These findings suggest that that controlling the initial release of contaminants to river system and other sources may be necessary to protect offshore habitats and their associated pelagic species, including Chinook salmon.

Remediation of estuary and nearshore habitats to reduce POP exposure to juvenile Chinook salmon may also be useful to improve the health of juvenile Chinook salmon. Although juvenile Chinook salmon in estuary and nearshore habitats accumulated a lower mass of POPs (i.e., body burden measured as ng POP per fish) than salmon in offshore habitats, salmon in estuary and nearshore habitats of developed river systems often had POPs concentrations (ng POP per g of fish tissue) above adverse effects concentrations. Analysis of contaminant body burden (ng/fish) in fish from estuary and nearshore habitat of individual river systems revealed that the habitat along the migratory pathway where salmon are exposed to POPs (i.e., the route of contaminant exposure) depended on the river system and the contaminant. Thus, management efforts to reduce contaminant exposure in river systems must be prescriptive to the individual river system and contaminant of concern.

The results of this study augment previous sampling initiated as early as 1998, and will be used to establish a solid time series of contaminant conditions in juvenile Chinook salmon that can be used to fulfill the *Toxics in Fish Vital Sign* goal of tracking time trends of fish health. Future monitoring of contaminant exposure in juvenile salmon should include chemicals of emerging concern in the Puget Sound ecosystem. Additionally, the geographic scope of the monitoring should be expanded to include other river systems that contribute to the production of Puget Sound Chinook salmon, such as salmon populations from the Hood Canal, Nooksack, and Stillaguamish river systems.

INTRODUCTION

Much attention has been paid to the physical habitat alterations and climate-driven processes that may be responsible for the recent declines in marine survival of salmon (Kostow 2009, Magnusson and Hilborn 2003, Myers et al. 1998, NRC 1996, Roni et al. 2002) but alterations in habitat quality by inputs of toxic chemical contaminants can also affect salmon marine survival (Johnson et al. 2013, Meador et al. 2014). Within developed landscapes, contaminants from municipal, agricultural and industrial sources, including known chemicals of concern, enter aquatic systems via a diverse array of both point and nonpoint sources including stormwater, wastewater treatment facilities, industrial discharges and atmospheric deposition (Brown et al. 1998, Ecolgy and King County 2011). Their anadromous life-history exposes salmon and steelhead (henceforth, for simplicity, "salmon") to contaminants in freshwater, estuarine and marine waters (Cullon et al. 2009, O'Neill and West 2009). While transitioning from freshwater to saltwater, juvenile salmon integrate contaminant conditions from across the freshwater/saltwater interface. Water quality impairments in freshwater, estuarine and nearshore habitats represent a significant threat to juvenile salmon populations. During this time period, salmon are in a stage of rapid growth and development and undergo many physiological changes making them especially vulnerable to the deleterious effects of toxic chemicals, potentially reducing their survival.

Numerous studies have documented that salmon exposed to environmentally relevant concentrations of toxic chemicals experience impacts to biological functions including growth, smoltification, disease resistance and reproductive development, all of which may reduce early marine survival and overall productivity. For example, sub-lethal exposures to environmentally relevant concentrations of pesticides and copper in freshwater reduce growth of juvenile salmonids; modeling results indicate a reduction in size-dependent survival in out-migrant fish (Baldwin et al. 2009, Mebane and Arthaud 2010, Spromberg and Meador 2005). Likewise, sub-lethal polycyclic aromatic hydrocarbon (PAH) exposure in freshwater impairs immuno-competence (Bravo et al. 2011) and may subsequently reduce marine survival. Contaminant exposures that disrupt the smoltification process may alter time of entry into saltwater, as well as subsequent growth and immuno-competence. In urbanized estuaries and nearshore waters, research indicates that exposure to contaminants affects salmonid behavior, growth, immuno-competence and disease susceptibility (Arkoosh et al. 2001, Arkoosh et al. 2010, Arkoosh et al. 1998, Arkoosh et al. 1994a, Arkoosh and Collier 2002, Meador et al. 2006, Varanasi et al. 1993) and ultimately their survival (Meador 2014). Additionally, throughout freshwater, estuarine and nearshore saltwater habitats of Puget Sound, salmon eggs, alevins, fry, smolt and juveniles may be exposed to endocrine disrupting compounds that can alter their reproductive health (Peck et al. 2011).

Chinook salmon (*Oncorhynchus tshawytscha*) are valued for their importance in commercial, recreational, and aboriginal fisheries, cultural importance to First Nations, and key role in marine and freshwater food webs (Quinn 2005). Since 1999, Puget Sound Chinook salmon have been listed as "threatened" under the U.S. Endangered Species Act (USDOC 2005).

Widespread habitat degradation and loss associated with logging, agricultural land use/water diversions, dam operations, and watershed development, and high fractions of hatchery fish in many populations were major factors affecting the decline of Puget Sound Chinook salmon (Myers et al. 1998) and

continue to hinder their recovery (Ford 2011, Good et al. 2005). The role of toxic chemical exposure as a factor in the decline or as a risk factor preventing recovery is less well understood. Among Pacific salmon species, Chinook salmon have a complex and diverse life history (Quinn 2005). Ocean-type Chinook, the predominant life-history type in Puget Sound, spend considerably more time in estuaries and coastal marine waters during downstream migration than other salmon species (Quinn 2005), and thus are more susceptible to contaminant exposure.

The Puget Sound basin is the most densely populated area of Washington, and is expected to continue to grow rapidly in the future. The region contains several highly urbanized and industrialized watersheds, including areas designated as Superfund sites. Juvenile Chinook salmon migrating from freshwater to saltwater in Puget Sound *en route* to the Pacific Ocean can encounter a wide range of water quality conditions, from relatively clean to highly contaminated, depending on their migration route. Once in saltwater, they may be continually exposed to contaminants that accumulate in urbanized bays of Puget Sound and in the coastal waters of the North Pacific adjacent to developed and urbanized landscapes.

Systematic, comprehensive sampling of juvenile salmon in Puget Sound has not occurred, although studies by the Northwest Fisheries Science Center (NWFSC) indicate that juvenile Chinook salmon from Puget Sound urban populations are exposed to several persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs), often at concentrations known to cause harm (Johnson et al. 2007a, Meador et al. 2010, Olson et al. 2008, Sloan et al. 2010, Stehr et al. 2000). More limited POP exposure assessments have been completed for chum (*O. keta*), coho (O. *kisutch*) and pink (*O. gorbuscha*) salmon. Generally, concentrations of POPs in coho and pink salmon are lower than those observed for Chinook salmon from the same locations, whereas concentrations in Chinook and chum salmon are similar (Olson et al. 2008, Stehr et al. 2000). Such differences are likely related to habitat use, diet and metabolism. Juvenile salmon migrating from freshwater to saltwater habitats may also be exposed to trace metals typically present in surface runoff from impervious surfaces and industrial discharges (McIntyre et al. 2015). Assuming the estuary is an important source of contaminants for out-migrant salmonids, higher contaminant exposures in Chinook and chum salmon are consistent with the more prolonged period of estuarine exposure in these species (Quinn 2005). Over time, Chinook salmon may accumulate higher POP contaminant burdens than chum salmon because of their higher trophic status.

As a member of the Puget Sound Ecosystem Monitoring Program (PSEMP), the Washington Department of Fish and Wildlife (WDFW) assesses status and trends of the health of Puget Sound fishes and macroinvertebrates related to their exposure to toxic contaminants. This *Toxics in Biota* effort is one component of PSEMP, a multi-agency effort designed to monitor the health of the Puget Sound ecosystem. WDFW, in collaboration NWFSC, designed this current study to provide a synoptic assessment of contaminant exposure for major populations of juvenile Chinook salmon from Puget Sound as they migrate from freshwater to marine habitats. Overall, the study estimated exposure of salmon to toxic chemicals in 1) the lower reaches of the major rivers entering Puget Sound, hereafter referred to as estuaries, 2) the nearshore marine shorelines adjacent to major rivers, and 3) the offshore habitats of the basins of Puget Sound. The goals of this study were threefold: a) estimate the extent and magnitude of exposure of juvenile Chinook salmon to toxic chemicals as they migrate from their

estuaries, to marine nearshore and offshore habitats of Puget Sound, (b) assess whether contaminant concentrations are high enough to adversely affect fish health, and (c) determine which habitats types provide the greatest contaminant inputs (i.e., routes of exposure) to juvenile Chinook salmon. To meet these goals, we analyzed whole body tissue for POPs, stomach contents for PAHs, and gills for metals in fish collected from estuaries, nearshore marine shorelines and offshore habitats in the Puget Sound basin. Tissue residues were compared with published adverse effects thresholds to evaluate the potential health effects on juvenile salmon from exposure to these contaminants. Finally, for the whole body analyses, we compared the body burden of POPs in fish from different habitats to assess the degree to which POPs were being accumulated in freshwater, nearshore, or offshore habitats (i.e., routes of exposure).

The study addresses the general hypothesis that chemicals released into Puget Sound from human activities and development reduces the health and productivity of salmon and their food supply. Specifically, we hypothesized that juvenile Chinook salmon residing and feeding in the more urbanized and industrial estuary, nearshore marine, and offshore habitats of Puget Sound are exposed to higher concentrations of toxic contaminants than those in less developed habitats. In addition, it is hypothesized that the elevated contaminant concentrations in the more urban areas are high enough to affect juvenile Chinook survival through reductions in growth, disease resistance, and altered hormone and protein levels.

MATERIALS AND METHODS

Detailed sampling and analytical methods for the estuary and nearshore habitat portions of this work followed standard operating procedures described in the Quality Assurance Project Plan (QAPP) (O'Neill et al. 2013) and are summarized below along with additional sampling details pertinent to the offshore habitat portion of this study.

Study Location

Puget Sound is a deep inland fjord formed by glaciers with numerous rivers flowing into six sub-basins separated by sills, landforms, and hydrographic fronts (Burns 1985, Ebbesmeyer et al. 1988). This geomorphology results in more limited entry of oceanic water into Puget Sound and extended water residency and stratification compared to the Georgia Basin (Thomson 1994). Furthermore, freshwater inputs across the six sub-basins vary and their circulation patterns result in distinct oceanographic properties (Moore et al 2008). Thus, compared to other large estuaries, toxic chemicals that enter Puget Sound have longer residence times within the system, and this entrainment of toxics can result in biota being exposed to increased levels of contaminants for a given input (Harrison et al. 1994). For example, West et al. (2008) documented that polychlorinated biphenyl (PCB) concentrations in Puget Sound herring populations were 3 to 9 times higher than those from the nearby Strait of Georgia.

To assess contaminant exposure in juvenile Chinook salmon we focused the majority of our sampling in the estuary and adjacent nearshore marine habitats of major river systems as these habitats are the main receiving waters of contaminants entering Puget Sound. These habitats are used extensively by juvenile Chinook salmon for several months in the spring and early summer as they transition from fresh

to marine waters. While there is a continuum between estuary and nearshore marine habitats, for the purpose of this report, the estuary is defined to include the upper extent of the saltwater wedge in the river to the marine extension of the alluvial floodplain, corresponding to the large river delta geomorphic system described by Shipman (2008). The nearshore marine area is bounded by the upper limit of tidal influence and the lower limit of the photic zone. Depending on the location and season, the lower limit of the photic zone is considered to range from 5 to 20 m in depth (Redman et al. 2005).

Fish were collected from Skagit, Snohomish, Green/Duwamish, and the Nisqually river systems (Figure 1) as these rivers produce the majority of naturally produced Puget Sound Chinook salmon (Rice et al. 2011). Juvenile Chinook salmon were also collected from the Hylebos Waterway, part of the Commencement Bay Nearshore/Tideflats Superfund site, and the nearshore marine habitat of the Hylebos/Puyallup river system (Figure 1). Hylebos Creek empties into Hylebos Waterway and both the creek and the waterway have undergone extensive restoration efforts in recent years to improve juvenile salmon habitat quality. Historically, the estuary and nearshore habitats of the Hylebos Waterway/Puyallup system have been intensively studied to measure contaminant exposure in juvenile Chinook salmon and other fish species (Collier et al. 1998, Olson et al. 2008, Stehr et al. 2000). This system was included in the current study to provide a more comprehensive estimate of the extent and magnitude of contaminant exposure in out-migrating juvenile salmon. Collectively, these five river systems encompass a range of land-use practices from relatively undisturbed areas such as the Nisqually, to agricultural regions such as the Skagit, to heavily urbanized areas such as the Green/Duwamish/Elliott Bay (Table 1).

Fish were also sampled from offshore habitats (> 0.5 km from shoreline, at depths between 40 and 238 m)of four major basins of Puget Sound (Table 1), Admiralty Inlet, Whidbey Basin, South Basin, and the Central Basin), representing a continuum from less to more contaminated marine food webs respectively. After leaving the nearshore waters, juvenile Chinook salmon reside in offshore waters of Puget Sound for up to three months, putting on significant weight (Duffy and Beauchamp 2011), and potentially increasing their contaminant exposure feeding on contaminated prey.

Fish Collections

Due to their ESA listing, the numbers of Chinook salmon that we were permitted to collect were limited. Accordingly, we coordinated fish collection with other researchers who were also sampling juvenile Chinook salmon in the study area to minimize sampling effort and the number of fish taken from each system. Sample sizes and locations were selected to maximize statistical power to represent the contaminant condition of salmon by using the least number of fish.

Estuary and Nearshore Sites

As detailed in Table 1, within each river system, fish were collected at one site in the estuary habitat and at two sites in the adjacent nearshore marine habitat using a boat-deployed beach seine, fyke nets, or a lampara seine, following protocols described in (Puget Sound Estuary Program 1990, Roegner et al. 2009, Varanasi et al. 1993). Multiple hauls were completed at each site to catch the required number of fish; fish caught in all hauls were pooled to represent that site. All fish were collected in 2013, during

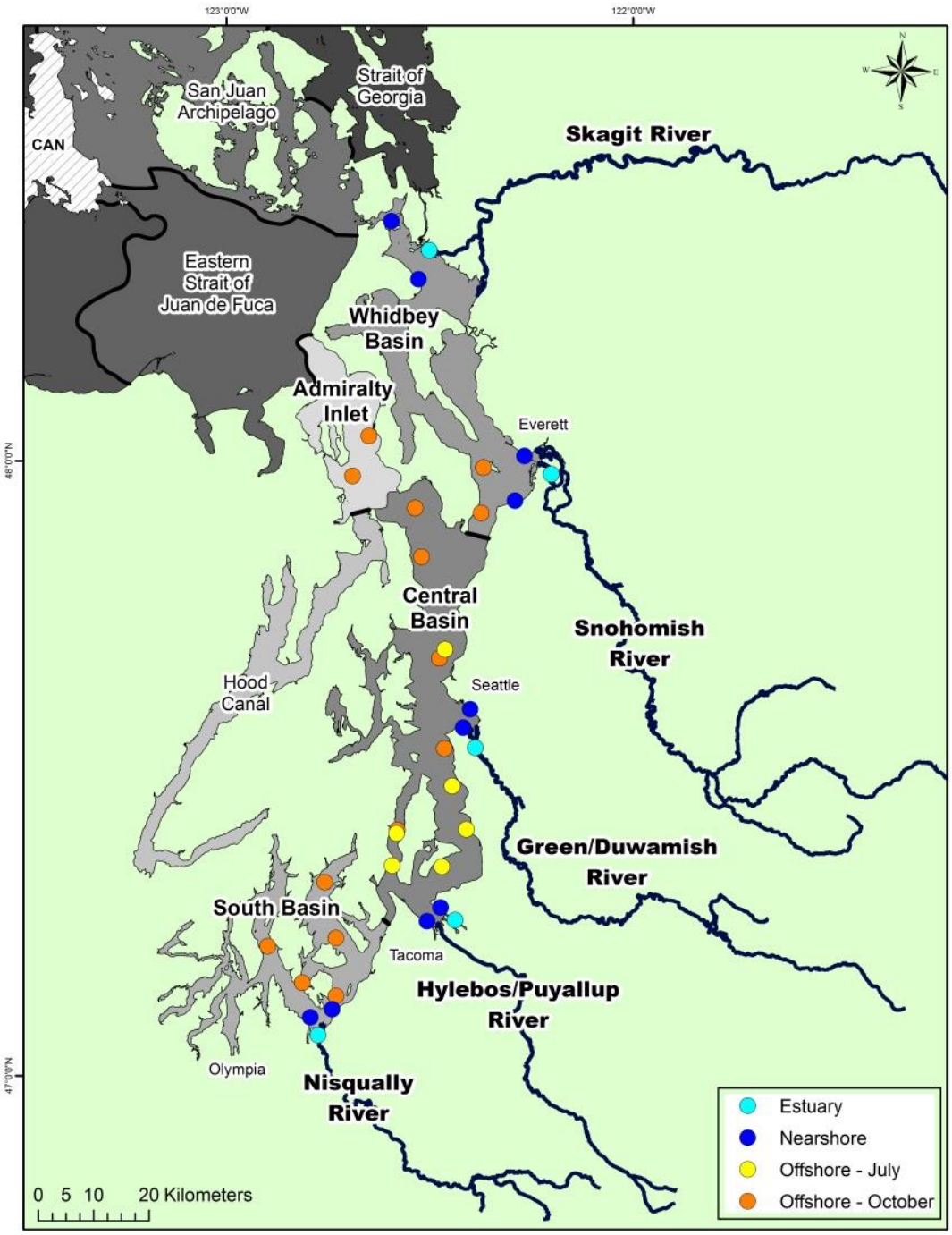

Figure 1. Locations of the estuary and nearshore habitats of five major river systems and offshore marine habitats where juvenile Chinook salmon were collected in 2013 for contaminant analyses. For the each estuary and nearshore habitat sampling sites, the circles signify the centroid of fish collection locations for that site. For the offshore habitats, circles indicate a centroid of one towing effort (i.e., mean location of the start and end of one trawl).

Table 1. Juvenile Chinook salmon sampling sites and collection information. BS = beach seine, FN = fyke nets, LS = lampara seine, MWT = mid-water trawl

System/ Marine Basin	Collection Site	Site Description	Sample Month	Number of Days Sampled	Gear	Total Number of Hauls
Skagit	Estuary	North Fork Skagit River	May	1	BS, FN	3
	Nearshore 1	Northwest Skagit Bay, Lone Tree Point, Hoypous Point	June	1	BS	3
	Nearshore 2	West Skagit Bay, Strawberry Pt.	June	1	BS	3
Snohomish	Estuary	Langus Riverfront Park, Ferry Baker Island	May	1	BS	2
	Nearshore 1	Priest Pt., North Possession Sound	June	1	BS	2
	Nearshore 2	South Possession Sound,	June, July	2	BS	6
Green/ Duwamish	Estuary	Lower Duwamish River, Kellogg Island	May	1	BS	4
	Nearshore 1	West Elliott Bay, West Seattle,	June	1	BS	6
	Nearshore 2	Myrtle Edwards Park	June	1	BS	3
Hylebos/ Puyallup	Waterway	Hylebos Waterway, 11th St. Bridge, Squally Beach	June	1	BS	3
	Nearshore 1	Skookum Wulge, Yowkwalla	June	1	BS	8
	Nearshore 2	Ruston Way, Tahoma Salt Marsh	June	1	BS	6
Nisqually	Estuary	North and South of the I-5 bridge	May	1	BS	3
	Nearshore 1	East estuary, Ketron Island, Solo Pt., East Anderson Island	June	1	LS	7
	Nearshore 2	West estuary, South Anderson Island, Hogum Bay	June	1	LS	6
Marine Basins (offshore)	Admiralty Inlet	Oak Bay and Bush Pt. area	Oct.	1	MWT	2
	Whidbey Basin	Gedney Island and Possession Sound	Oct.	1	MWT	2
	Central Basin	Brace Point, Three Tree Pt., Maury Island, SW. and W. Vashon Island, Shilshole Bay	July	2	MWT	6
	Central Basin	Alki Pt., Colvos Passage, West Pt., Apple Cove Pt., Useless Bay	Oct.	2	MWT	5
	South Basin	Case Inlet, Drayton Passage, Nisqually Reach, Carr Inlet	Oct.	1	MWT	5

the peak out-migrant time for juvenile Chinook salmon in these watersheds, as best judged by the area salmon biologists working in these systems. In general, the fish were collected from estuary habitats in mid-late May and from nearshore marine habitats approximately one month later, from mid-June to mid-July. Detailed sampling descriptions and maps of each sample location are provided in APPENDIX A: Detailed Sample Collection Methods.

Naturally produced Chinook salmon were targeted for collection; however, hatchery origin fish were collected if naturally produced Chinook salmon were unavailable at the time of collection. To determine their origins, fish were examined for the presence of an adipose fin or ventral fin clips and screened for the presence of coded-wire-tags (CWTs) using a handheld detector wand (Northwest Marine Technologies, Inc.). Fish without an adipose or ventral fin and/or containing a CWT were deemed to be of hatchery-origin, whereas all other fish were presumed to be naturally produced. However, because a small proportion (< 8%) of all juvenile Chinook salmon released from hatcheries are unmarked, some of the fish classified as "naturally produced" may be hatchery-origin fish. The unmarked hatchery fish include approximately 7% left unmarked for conservation reasons (i.e., Elwha River Chinook salmon) and an additional 1% for fish that were intended to be marked but received a poor clip or the clipped fin regenerated (Mark Kimbel, pers. comm.). All salmon retained for chemical analyses were placed in a pre-labeled plastic Ziploc® bags, placed on ice, and transported to the laboratory for processing within several hours of collection.

A total of 480 fish were collected in the estuary and nearshore habitats for chemical analyses (Table 2). At each of the river systems, except for the Hylebos/Puyallup system, between 97 and 100 fish were collected to characterize the system. At the Hylebos/Puyallup system, only 5 fish were captured in the Waterway, but 67 were collected from the nearshore habitat, similar to the other systems. Fifty-seven of the 480 fish collected for chemical analyses from the Snohomish, Green/Duwamish, Hylebos/Puyallup and Nisqually systems had CWTs (5, 20, 31, and 1, respectively; Table 3), and this information was used to confirm the hatchery origins and residence time of hatchery fish in each system. In addition to the 480 fish collected for contaminant analyses, another 50 fish with CWTs were retained to provide general information on the mix of hatchery populations present in nearshore habitats, including fish collected from Skagit, Snohomish, and Hylebos/Puyallup systems (23, 20, and 7, respectively).

Offshore Sites

Fish were collected from offshore habitats in July (Central Basin only) and October (Admiralty Inlet, Whidbey Basin, Central Basin and South Basin) of 2013 using a midwater trawl, deployed from the *CCGS W.E. Ricker*, a Canadian Department of Fisheries and Oceans (DFO) research vessel (Table 1). Multiple hauls were completed within each basin and at each haul five fish were collected to represent fish contaminant concentrations at that site. Naturally produced Chinook salmon were targeted for collection; however, hatchery origin fish were retained if naturally produced Chinook were unavailable at the time of collection. A total of 103 juvenile Chinook were collected for chemical analyses at offshore habitats, 30 fish at the Central Basin in July and between 10 and 28 fish at the each of the basins in October (Table 2). Fish were removed from nets, placed in pre-labeled plastic Ziploc® bags, frozen at -20° C, and then transported on ice to the laboratory for processing.

Table 2. Total number of juvenile Chinook salmon and composite chemical samples collected at each sampling site

System	Collection Site	Sample Months	Total Fish #	Composite Samples		
				Whole Body[1]	Gills	Stomach Contents
Skagit	Estuary	May	40	4	4	1
	Nearshore 1	June	30	5	5	1
	Nearshore 2	June	30	5	5	1
Snohomish	Estuary	May	39	4	4	1
	Nearshore 1	June	30	5	5	1
	Nearshore 2	June, July	28	5	5	5
Green/ Duwamish	Estuary	May	40	4	4	1
	Nearshore 1	June	31	5	5	1
	Nearshore 2	June	30	5	5	1
Hylebos/ Puyallup	Waterway	June	5	1	1	1
	Nearshore 1	June	30	5	5	1
	Nearshore 2	June	37	5	5	5
Nisqually	Estuary	May	40	4	4	4
	Nearshore 1	June	35	5	5	5
	Nearshore 2	June	35	5	5	5
Offshore	Admiralty Inlet	October	10	2	2	2
	Whidbey Basin	October	10	2	2	2
	Central Basin	July	30	6	6	6
	Central Basin	October	25	5	5	5
	South Basin	October	28	6	6	6
estuary/nearshore	*subtotal*		*480*	*67*	*67*	34
offshore	*subtotal*		*103*	*21*	*21*	21
All	*TOTAL*		*583*	*88*	*88*	55

[1] whole body composites did not include gills or stomach contents

Seven of the 103 fish collected for contaminant analyses, all from the July sampling in the Central Basin, had CWTs (Table 3). In addition, we retained another 54 juvenile Chinook salmon with CWTs from the offshore habitats in the remaining four Puget Sound basins that were sampled to provide general information on the mix of hatchery populations present in offshore habitats: 10 from the Whidbey Basin, 4 from Admiralty Inlet, 12 from Central Basin (July), 19 from Central Basin (October), and 9 from South Basin.

Sample Processing

Fish Biometrics

Prior to tissue collection for chemical analyses, individual fish were measured for fork length to the nearest millimeter (mm) and, weighed to the nearest gram (g). All fish were necropsied the day of collection with the exception of fish from the Nisqually estuary habitat, the nearshore site 2 from Snohomish system, and the offshore habitats; these fish were all frozen at 20° (C) prior to processing for contaminant analyses. While processing the fish, scales were removed for age analysis and CWTs were

Table 3. Mark type and origin data for all juvenile Chinook used for analytical chemistry samples. AI = adipose fin was intact, AC = adipose fin was clipped, CWT = coded wire tag

			Mark Type				Origin	
System	Collection Site	N	Unmarked AI	Marked AI/CWT	Marked AC	Marked AC/CWT	Percent naturally spawned (%)[a]	Percent hatchery fish (%)
Skagit	Estuary	40	40	0	0	0	100	0
	Nearshore 1	30	30	0	0	0	100	0
	Nearshore 2	30	30	0	0	0	100	0
	Total	**100**	**100**	**0**	**0**	**0**	**100**	**0**
Snohomish	Estuary	39	39	0	0	0	100	0
	Nearshore 1	30	2	0	28	0	6.7	93
	Nearshore 2	28	6	0	17	5	21	79
	Total	**97**	**47**	**0**	**45**	**5**	**52**	**48**
Green/ Duwamish	Estuary	40	20	20	0	0	50	50
	Nearshore 1	31	24	0	7	0	77	23
	Nearshore 2	30	28	0	2	0	93	6.7
	Total	**101**	**72**	**20**	**9**	**0**	**71**	**29**
Hylebos/ Puyallup	Waterway	5	0	3	2	0	0	100
	Nearshore 1	30	10	20	0	0	33	67
	Nearshore 2	37	20	8	9	0	54	46
	Total	**72**	**30**	**31**	**11**	**0**	**42**	**58**
Nisqually	Estuary	40	40	0	0	0	100[b]	0
	Nearshore 1	35	7	0	27	1	20	80
	Nearshore 2	35	2	0	33	0	5.7	94[b]
	Total	**110**	**49**	**0**	**60**	**1**	**45**	**55**
Offshore (July)	Central Basin	30	2	2	21	5	7	93
Offshore (Oct.)	Admiralty Inlet	10	10	0	0	0	100	0
	Whidbey Basin	10	6	0	4	0	60	40
	Central Basin	25	17	0	8	0	68	32
	South Basin	28	22	0	6	0	79	21
	Total (Oct)	**73**	**55**	**0**	**18**	**0**	**75**	**25**

[a] assumes that clipping error is minimal and otolith-only marking is limited; [b] one fish collected at each of these locations was a yearling;

removed for reading, if present. In addition, fin snips were removed and preserved in ethanol for subsequent genetic stock identification should funding become available in the future.

Tissue Samples for Chemical Analyses

The following tissue samples were collected for chemical analyses: stomach contents for measurement of PAHs; gill tissue for copper, zinc, lead, nickel and cadmium; whole bodies less stomach contents and gills (hereafter referred to as whole body samples) for measurement of POPs including PCBs, polybrominated diphenyl ether (PBDEs), dichloro-diphenyl-trichloroethanes (DDTs), and other organochlorine pesticides. With the exception of the Hylebos Waterway site, 4-6 composite samples of whole bodies and gills, and 1-6 composite samples of stomach contents (Table 2) were created, as described in Scholz et al. (2011), Stehr et al. (2000), Stein et al. (1995). Composite samples, rather than individual fish, were analyzed to reduce analytical costs.

Each whole body and gill tissue sample was comprised of 4-10 fish .Each composite contained a minimum of 4 fish per composite. The maximum number of fish per composite was also limited to 10 to minimize the number of fish sacrificed for the study. The number of fish per whole body and gill composite varied, depending on fish size and number collected at each site. Smaller fish were typically collected in the estuary habitats, necessitating more fish per composite to provide a sufficient tissue mass for chemical analyses. Larger fish were collected at the nearshore and offshore habitats and required fewer fish per composite. Stomach content samples were originally composited to match the compositing scheme of the whole body and gills samples. However, there was insufficient mass in the majority of composite samples for the nearshore and offshore sites, which made it necessary to combine several composites to make larger, super-composites representing the entire site (Table 2). In addition, the stomach content composite sample for fish collected in the Snohomish Nearshore 2 area was not analyzed because due to insufficient tissue mass.

All tissue samples were placed into pre-cleaned I-Chem® jars and maintained on ice during the necropsy procedure, then stored at -20° (C) until the samples could be homogenized for chemical analyses. To avoid any metal contamination associated with processing, only ceramic and titanium utensils were used for resection and sample collection, and all utensils and surfaces were cleaned between composites as detailed in the QAPP for this study (O'Neill et al 2013).

Prior to chemical analyses, whole body and gill samples were homogenized to ensure that tissues were representative of the sample. Whole body juvenile Chinook samples were thawed overnight and then ground the following day into a homogeneous mixture using a Bamix® hand mixer. The composite samples were then re-frozen and sent to the National Oceanic and Atmospheric Association (NOAA) NWFSC for POPs chemical analysis (Table 2). The gill samples were thawed, removed from their vial, and finely chopped using a ceramic knife on a pre-cleaned Teflon cutting board. Samples were then re-frozen and delivered for trace metals analysis to the Washington Department of Ecology's (Ecology) Manchester Laboratory (Table 2).

Chemical Analyses

The primary contaminants of concern for this study are commonly detected chemicals typically found in the lower reaches of rivers and estuaries of Puget Sound. Juvenile Chinook salmon may be exposed to these chemicals as they migrate from fresh water habitats to Puget Sound marine waters and the coastal Pacific Ocean. These contaminants include a number of POPs (i.e., PCBs; PBDEs; organochlorine pesticides DDTs, chlordanes, hexachlorocyclohexanes [HCHs], hexachlorobenzene [HCB], aldrin, dieldrin, mirex, endosulfans), PAHs, and five trace metals (cadmium, copper, lead, nickel, and zinc) as detailed in Table 4. Additionally, the lipid content of all whole body samples was measured.

POPs

Concentrations of POPs in whole body samples of juvenile Chinook salmon were analyzed according to Sloan et al. 2014, consistent with previous WDFW/PSEMP studies. This method comprises three steps: (a) extraction, (b), cleanup by silica/aluminum columns and size-exclusion high-performance liquid chromatography (SEC HPLC), and (c) quantitation of chlorinated hydrocarbons (CHs) and aromatic hydrocarbons (AHs) using gas chromatography /mass spectrometry (GC/MS) with selected-ion monitoring (SIM). Samples were extracted using accelerated solvent extraction (ASE with methylene chloride), which provided an extract that was used for AH, CH recovery, and gravimetric lipid evaluation. This method also included alterations to typical GC/MS methods to stabilize the instrument and improve accuracy such as chemical ionization filaments (to increase source temperature), employing a cool on-column injection system in the GC, a guard column before the analytical column, and point-to-point calibration to improve data fit over the full range of GC/MS calibration standards (Sloan et al. 2014). As part of a performance-based quality assurance program (Sloan et al. 2014), a method blank and a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM 1974c) were analyzed with each batch of whole body samples. Concentrations of individual analytes measured in SRM 1974c were in excellent agreement with the certified and reference values published by NIST. In addition, the method blank and surrogate recovery quality control samples all met established laboratory criteria outlined in the QAPP for this project (O'Neill et al. 2013) except for minor deviations (discussed in APPENDIX B: Data Quality Control Check (POPs, PAHs, and trace metals)) that did not compromise the usability of the results.

PAHs

Stomach content samples collected from juvenile Chinook salmon were analyzed for individual 42 individual PAHs (Table 4) by GC/MS according to methods outlined in Sloan et al. (2014). Briefly, each sample was weighed and mixed with drying agents (magnesium sulfate and sodium sulfate), transferred to a 33-ml accelerated solvent extraction (ASE) cell, and the surrogate standard was added to the top of each sample cell. Samples were extracted with two cell volumes of dichloromethane on an ASE at 2,000 psi and 100°C and the combined extract (≈50 ml) was collected in a 60-ml collection tube. Each sample extract was then filtered through a gravity flow column containing silica gel and alumina to remove polar compounds and the extract was then further cleaned up using HPLC with size exclusion chromatography to remove lipids and other interfering biogenic compounds. The volume of the cleaned up extracts was reduced and a GC internal standard was then added to determine the recovery of the

Table 4. All analytes measured in the three juvenile Chinook tissue matrices and their associated CAS numbers. Summations are labeled when applicable; bolded PCB congeners contributed to the estimated total PCBs calculation and 42 PAHs (22 Low Molecular Weight PAHs and 20 High Molecular Weight PAHs) listed were included in the summations. PAH homologs do not have CAS numbers associated with them.

	Individual Analyte	CAS No.		Individual Analyte	CAS No.
46 PCB Congeners	PCB 17	37680-66-3	$\sum_6$DDTs	*o,p'*-DDD	53-19-0
	PCB 18	37680-65-2		*o,p'*-DDE	3424-82-6
	PCB 28	7012-37-5		*o,p'*-DDT	789-02-6
	PCB 31	16606-02-3		*p,p'*-DDD	72-54-8
	PCB 33	38444-86-9		*p,p'*-DDE	72-55-9
	PCB 44	41464-39-5		*p,p'*-DDT	50-29-3
	PCB 49	41464-40-8	$\sum_{11}$PBDEs	BDE 28	41318-75-6
	PCB 52	35693-99-3		BDE 47	5436-43-1
	PCB 66	32598-10-0		BDE 49	243982-82-3
	PCB 70	32598-11-1		BDE 66	189084-61-5
	PCB 74	32690-93-0		BDE 85	182346-21-0
	PCB 82	52663-62-4		BDE 99	60348-60-9
	PCB 87	38380-02-8		BDE 100	189084-64-8
	PCB 95	38379-99-6		BDE 153	68631-49-2
	PCB 99	38380-01-7		BDE 154	207122-15-4
	PCB 101 (90)	37680-73-2		BDE 155	35854-94-5
	PCB 105	32598-14-4		BDE 183	207122-16-5
	PCB 110	38380-03-9	$\sum_3$HCHs	α-hexachlorocyclohexane	319-84-6
	PCB 118	31508-00-6		β-hexachlorocyclohexane	319-85-7
	PCB 128	38380-07-3		γ-hexachlorocyclohexane	58-89-9
	PCB 138 (163, 164)	35065-28-2	$\sum_8$Chlordanes	α-chlordane	56534-02-2
	PCB 149	38380-04-0		cis-nonachlor	5103-73-1
	PCB 151	52663-63-5		β-chlordane	5103-74-2
	PCB 153 (132)	35065-27-1		heptachlor	76-44-8
	PCB 156	38380-08-4		heptachlor-epoxide	1024-57-3
	PCB 158	74472-42-7		nonachlor III	130939-67-2
	PCB 170	35065-30-6		oxychlordane	27304-13-8
	PCB 171	52663-71-5		trans-nonachlor	39765-80-5
	PCB 177	52663-70-4	HCB	Hexachlorobenzene	118-74-1
	PCB 180	35065-29-3	Misc. Pesticides	Aldrin	309-00-2
	PCB 183	52663-69-1		Dieldrin	60-57-1
	PCB 187 (159, 182)	52663-68-0		α-endosulfan	959-98-8
	PCB 191	74472-50-7		Mirex	2385-85-5
	PCB 194	35694-08-7	Metals	Cadmium	7440-43-9
	PCB 195	52663-78-2		Copper	7440-50-8
	PCB 199	52663-75-9		Lead	7439-92-1
	PCB 205	74472-53-0		Nickel	7440-02-0
	PCB 206	40186-72-9		Zinc	7440-66-6
	PCB 208	52663-77-1			
	PCB 209	2051-24-3			

Continued.

Table 4 (continued). All analytes measured in the three juvenile Chinook tissue matrices and their associated CAS numbers. Summations are labeled when applicable; bolded PCB congeners contributed to the estimated total PCBs calculation and 42 PAHs (22 Low Molecular Weight PAHs and 20 High Molecular Weight PAHs) listed were included in the summations. PAH homologs do not have CAS numbers associated with them.

	Individual Analyte	CAS No.		Individual Analyte	CAS No.
Low Molecular Weight PAHs (LMW PAHs)	naphthalene (NPH)	91-20-3	High Molecular Weight PAHs (HMW PAHs)	fluoranthene (FLA)	206-44-0
	C1-naphthalenes (C1NPH)	-		pyrene (PYR)	129-00-0
	C2-naphthalenes (C2NPH)	-		C1-fluoranthenes/pyrenes (C1FLA)	-
	C3-naphthalenes (C3NPH)	-		C2-fluoranthenes/pyrenes (C2FLA)	-
	C4-naphthalenes (C4NPH)	-		C3-fluoranthenes/pyrenes (C3FLA)	-
	1-methylnaphthalene (MN1)[a]	90-12-0		C4-fluoranthenes/pyrenes (C4FLA)	-
	2-methylnaphthalene (MN2)[a]	91-57-6		benz[*a*]anthracene (BAA)	56-55-3
	2,6-dimethylnaphthalene (DMN)[a]	28804-88-8		chrysene (CHR)	218-01-9
	2,3,5-trimethylnaphthalene (TMN)[a]	2245-38-7		C1-benzanthracenes/chrysenes (C1CHR)	-
	acenaphthylene (ACY)	208-96-8		C2-benzanthracenes/chrysenes (C2CHR)	-
	acenaphthene (ACE)	83-32-9		C3-benzanthracenes/chrysenes (C3CHR)	-
	fluorene (FLU)	86-73-7		C4-benzanthracenes/chrysenes (C4CHR)	-
	C1-fluorenes (C1FLU)	-		benzo[*b*]fluoranthene (BBF)	205-99-2
	C2-fluorenes (C2FLU)	-		benzo[*k*]fluoranthene (BKF)	207-08-9
	C3-fluorenes (C3FLU)	-		benzo[*e*]pyrene (BEP)	192-97-2
	dibenzothiophene (DBT)	132-65-0		benzo[a]pyrene (BAP)	50-32-8
	C1-dibenzothiophenes (C1DBT)	-		perylene (PER)	198-55-0
	C2-dibenzothiophenes (C2DBT)	-		indeno[*1,2,3-cd*]pyrene (IDP)	193-39-5
	C3-dibenzothiophenes (C3DBT)	-		dibenz[*a,h*]anthracene (DBA)	53-70-3
	C4-dibenzothiophenes (C4DBT)	-		benzo[*ghi*]perylene (BZP)	191-24-2
	phenanthrene (PHN)	85-01-8			
	anthracene (ANT)	120-12-7			
	C1-phenanthrenes/anthracenes (C1PHN)	-			
	C2-phenanthrenes/anthracenes (C2PHN)	-			
	C3-phenanthrenes/anthracenes (C3PHN)	-			
	C4-phenanthrenes/anthracenes (C4PHN)	-			
	1,7-dimethylphenanthrene (DMP)[a]	483-87-4			
	7-Isopropyl-1-methylphenanthrene (Retene)[a]	483-65-8			

[a] analytes were not included in the summation for LMWPAHs or $\sum_{42}$PAHs

surrogate standard. The sample extracts were then analyzed for PAHs on a low-resolution quadrupole GC/MS system equipped with a 60 m DB-5 GC capillary column and an electron impact mass spectrometer in selected ion monitoring mode. The instrument was calibrated using sets of up to ten multi-level calibration standards of known concentrations. As part of a performance-based quality

assurance program (Sloan et al. 2014), a method blank and a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM 1974c) were analyzed with each batch of stomach content samples. The data quality control checks for chemical analyses of PAH met the criteria outlined in the QAPP for this project (O'Neill et al. 2013) except for minor deviations (discussed in APPENDIX B: Data Quality Control Check (POPs, PAHs, and trace metals)) that did not compromise the usability of the results.

Lipid Determination

The amount of total, nonvolatile extractable lipid (reported as percent lipid) in whole body samples of Chinook salmon were determined by gravimetric analysis, according to Sloan et al. 2014. We measured whole body lipid content of salmon because it affects contaminant uptake and toxicity (Elskus et al. 2005). For lipophilic contaminants like POPs, the tissue concentration that causes a toxic response is typically directly related to the amount of lipid in the animal (Lassiter and Hallam 1990, van Wezef et al. 1995).

Trace Metals

Analyses for cadmium, copper, lead, nickel, and zinc (Table 4) were conducted at the Ecology's Manchester Environmental Laboratory in Manchester WA, following EPA methods 200.8. As detailed in APPENDIX B: Data Quality Control Check (POPs, PAHs, and trace metals), the method blank and surrogate recovery quality control samples, matrix spikes, and internal standards for these analyses all met established laboratory criteria.

Data Quality

There were no analytical issues that compromised data quality or the ability to analyze data. Minor deviations from the study plan (see Deviations from the QAPP, below) likely had a trivial effect on data interpretation.

Deviations from the QAPP

The overall sampling design described in the QAPP was expanded to include fish from the Hylebos/Puyallup river system and offshore marine habitats of Puget Sound as previously described in the methods section. Inclusion of these additional sampling locations enhanced the geographic scope and spatial assessment of contaminant exposure in out-migrant juvenile Chinook salmon.

The terminology used in the QAPP to describe the types of sampling locations was changed to more accurately reflect the terminology used by salmon researchers within the region. The QAPP refers to "river" and "estuary" sampling location, however, in the current report these sites are referred to as "estuary" and "nearshore marine" habitats, respectively. These terms were modified at the request of salmon biologist in the region to better reflect the salmon habitat that was actually sampled.

Chemical analyses of POPs and PAHs in fish tissue followed methods outlined in Sloan et al. 2014 rather than (Sloan et al. 2004). Metals analyses were completed at Ecology's Manchester Environmental Laboratory instead of the King County Environmental Laboratory, however, the analytical methods outlined in the QAPP were used by the Manchester Laboratory.

Analyses of stable isotopes were not completed because the instrument necessary to run these analyses was no longer operational. Stable isotope analysis was planned as an in-kind match by the NWFS; additional funds were not available to complete the analyses.

The number of composite samples collected for analysis of POPs and metals varied slightly from sample numbers described in the QAPP (i.e., 4 composites in estuary samples rather than 5) due to the size and availability of fish available for analyses. Additionally, as anticipated in the QAPP, PAH analysis was constrained by collection of a sufficient mass of stomach content material.

Data Analysis

Fish Biometrics

Condition factor of each fish was calculated based on Fulton's formula of,

$$K = \left(\frac{W}{L^3}\right) \times 100{,}000$$

where, K is Fulton's condition factor, W is the weight of the fish in grams (g) and L is the fork length of the fish in millimeters (mm); the value was multiplied by 100,000 to scale the condition factor close to one (Ricker, 1975).

To provide an overview of the variation in the size and condition of juvenile Chinook salmon among systems, habitat types (i.e., estuary, nearshore and offshore)and individual sampling locations, fish length, weight and condition factor of individual fish were analyzed by ANOVA or Kruskal-Wallis one-way analysis of variance depending on whether test assumptions were met (SigmaPlot 2008). All measurements were compared among systems (all fish within the system pooled, n = 5), among the five estuary habitats, among five nearshore habitats and then among sites within each system (i.e., estuary, nearshore 1, and nearshore 2).

For each composite whole body and gill sample, the mean length and, weight, and condition factor of fish that contributed to the sample were calculated. Mean composite length and weight, along with the percent of naturally produced fish in the composite sample, were considered as potential covariates affecting spatial differences in POP accumulation as discussed below.

POPs

Summed analytes are the sum of all detected values within each group, with zeroes substituted for non-detected (< LOQ) analytes within each group. In most cases, summed totals were dominated by substantial concentrations of a number of individual analytes; substituting zero for non-detects would not have substantially altered comparison results for the summed analytes. An estimated sum total PCB (TPCBs) concentration was calculated by summing the detected values for 17 commonly detected congeners (noted in bold text in Table 4) and multiplying the result by two (Lauenstein and Cantillo 1993). This method has been demonstrated to closely approximate the true PCB concentration (Lauenstein and Cantillo 1993) and is the standard method used by WDFW. Analyte data are presented as summed values for PBDEs, DDTs, Chlordanes, and HCHs. Summed PBDEs ($\sum_{11}$PBDEs) were calculated

by adding the congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183 (Table 4). Summed DDTs ($\sum_6$DDT) were calculated by summing the concentrations of o,p'-DDD, o,p'-DDE , o,p'-DDT, p,p'-DDD, p,p'-DDE ,and pp'-DDT (Table 4). Summed HCHs ($\sum_3$HCHs) were calculated by summing values for α-hexachlorocyclohexane, β-hexachlorocyclohexane and γ-hexachlorocyclohexane (Table 4). Sum chlordanes ($\sum_8$chlordanes) were calculated by summing the values for α-chlordane, cis-nonachlor, β-chlordane, heptachlor, heptachlor-epoxide, nonachlor III, oxychlordane, and trans-nonachlor (Table 4). In cases where all analytes in a group were not detected, the greatest limit of quantitation (LOQ) for any analyte in the group was used as the summation concentration, and the value was censored as "not detected" with a "U" qualifier. All statistical analyses were performed using wet weight (ww) POP concentrations.

POP Accumulations

A General Linear Model (GLM; SYSTAT 2009) was used to measure the statistical significance of differences in natural logarithm transformed POP concentrations in juvenile Chinook salmon among the Puget Sound river systems and between two habitat types (i.e., estuary and nearshore) within these river systems. At four of the five river systems with sufficient sample sizes in both the estuary and nearshore habitats, POP concentrations were compared among river systems (all habitats within a system combined), between estuary and nearshore habitat types (all systems within a habitat type combined), and among habitat types within systems. The Hylebos/Puyallup system was excluded from this analysis because the "estuary" habitat sampled in this system only included one sample from the Hylebos Waterway and was not considered representative of the Puyallup and Hylebos rivers and associated estuary habitat. Additional GLMs were completed to compare the variation of POPs among the five nearshore habitats. The Hylebos/Puyallup nearshore sites were included in these GLMs to provide an expanded geographic assessment of POPs in nearshore habitats.

GLMs were also used to evaluate spatial variation in accumulation of specific POP classes or analytes for whole body fish samples collected from the offshore habitats, and then to compare POPs accumulation in offshore habitats with fish collected from the river systems. All POP concentrations were log transformed prior to analyses. To compare POP concentrations among the three different habitats types (i.e., estuary, nearshore marine, and offshore), excluding potential basin differences, samples from Whidbey, Central and South basins were pooled within each of the three habitat types. Similarly, to compare POP concentrations among basins (Whidbey, Central, and South), excluding potential habitat differences, samples from estuary, nearshore and offshore habitats were pooled within each basin. For these comparisons, fish collected from the Skagit and Snohomish river systems were included in the Whidbey Basin, and fish from the Hylebos/Puyallup nearshore habitats were included in the Central Basin. Offshore samples from Admiralty Inlet were excluded from these analyses because the corresponding estuary and nearshore habits in that basin were not sampled. The offshore habitat samples collected in July were excluded because they were only collected in the Central Basin. The Hylebos Waterway sample was also excluded from these analyses because it was not representative of the Hylebos/Puyallup estuary habitat. To compare POP concentrations among basin and habitat combined, the subset of samples selected for statistical comparisons was limited to the Green/Duwamish and Nisqually systems and their associated offshore habitat (i.e., Central and South

basins respectively) because sample size in the remaining study basins was insufficient to conduct this analysis.

Covariates that might affect POP concentrations in different systems and habitats include average lipid content, average fish size (fork length), and percent of natural produced fish in a composite sample. These covariates were evaluated prior to their inclusion in the GLM analyses described above using visual examination of scatterplots and with linear regressions to determine whether the effects of a covariate could be eliminated *a priori* to performing GLM analyses on data subsets, and to ensure that auto-correlated covariates were not included together in GLM runs. Based on these evaluations, fish length was the only covariate tested as factor explaining the variation of POPs among samples.

Multiple comparisons testing (Tukey's Honestly-Significant-Difference Test, SYSTAT 2009) was used to conduct pairwise comparisons of among systems as a whole (estuary and habitat combined), between habitat types, among estuary systems, among nearshore systems, for all significant results. Test results were considered statistically significant at probability (p) levels of ≤ 0.05 (alpha threshold = 0.05). Mean POP concentration were calculated as geometric means and are noted on all tables and figures as geometric means, however, in the text are referred to as means.

Effects of POP Exposure on Fish Health

To assess the extent to which the marine survival of Puget Sound juvenile Chinook salmon may be affected by POP exposure, measured concentrations from fish in the current study were compared to literature based contaminant concentrations documented to cause adverse health effects in juvenile salmon (Arkoosh et al. 2013, Arkoosh et al. 2010, Arkoosh et al. in press, Beckvar 2005, Meador et al. 2002).

Critical tissue residue levels above which multiple adverse effects are likely to occur (i.e., adverse effects thresholds) have been estimated for both PCBs and DDTs. An adverse health effects threshold for juvenile salmon of PCBs of 2,400 ng PCBs/g lipid was estimated by Meador et al (2002) based on a wide range of toxicological studies on juvenile trout and salmon with effects ranging from enzyme induction to mortality. A salmon specific adverse effects threshold for DDTs has not been developed. However, based on literature values for end-points including, growth, reproduction and survival, Beckvar et al. (2005) estimated that concentrations above 600 ng/g ww or 6,000 ng/g lipid (adjusted for lipid content as recommended by Johnson et al. (2007b) may cause adverse effects in a variety of fish species, including juvenile Pacific salmon.

PBDE accumulation in juvenile salmon associated with dietary exposure to individual PBDE congeners and mixtures of PBDE congeners have been documented to alter immune function and endocrine hormone levels, as well as increase disease susceptibility (Arkoosh et al. 2013, Arkoosh et al. 2010, Arkoosh et al. in press). In contrast to PCBs and DDTs, critical tissue residue levels of PBDEs associated with disease susceptibility and endocrine hormone levels are more complex, showing non-monotonic responses rather than a threshold concentration above which adverse effects occur (Arkoosh et al. 2013, Arkoosh et al. 2010, Arkoosh et al. in press). For example, in laboratory studies where Chinook salmon were exposed to 5 dietary doses of PBDE mixtures (PBDE 47 and 99), resulting in whole body

mean (±SD) concentrations of 115 (±18.4), 538 (±72.3), and 2,012 (±520), 3,695 (±506) and 8,698 (±855) ng PBDE/g lipid, increased diseased susceptibility was measured in fish with tissue concentrations of 538 (±72.3), 2,012 (±520)and 8,698 (±855) ng PBDE/g lipid, but not at the lowest (115 (±18.4)ng/g lipids) and intermediate concentrations (3,695 (±506)ng/g lipids). The highest measured concentration of 8,698 ±855 ng/g lipid) in whole-body fish, was considerably higher than concentrations typically measured in field caught juvenile Chinook salmon from the Pacific Northwest (Sloan et al. 2010). Although Arkoosh et al. (2013) did not test PBDE 47 and PBDE 99 mixtures between concentrations 538 ng/g (±72.3) and 2,012 (±520) (i.e., > 610 ng/g lipid and < 1,492 ng/g lipid) for potential effects on disease susceptibility, an independent study by Arkoosh et al. (2010) measured increased disease susceptibility in fish with ∑PBDE concentrations that include much of that range (i.e., 1600ng/g lipid (±660ng/g lipid). Thus, we concluded from Arkoosh et al.'s 2010 and 2013 studies that the concentrations of mixtures of PBDE 47 and PBDE 99 between 538 ng/g (±72.3) and 2,012 (±520) are associated with increase disease susceptibility. Accordingly, in this study field caught Chinook salmon with PBDE 47 + 49 concentrations ≥ 466 and ≤ 2532 ng/g lipid were assumed to have increased disease susceptibility (i.e., 538 ng/g lipid minus 1SD of 72.3 ng/g lipid = 466 ng/g lipid and 2,012 ng/g lipid plus 1 SD of 520 ng/g lipid = 2,532 ng/ g lipid).

Arkoosh et al. 2013 also reported altered thyroid levels associated with exposure to PBDE 47 and 99. Fish were fed a mixture of PBDE 47 and 99 at 5 difference exposure concentrations which resulted in body mean (±SD) concentrations of 115 (±18.4), 538 (±72.3), and 2,012 (±520), 3,695 (±506) and 8,698 (±855) ng PBDE/g lipid. Altered thyroid levels were observed in fish with PBDE tissue concentrations of 2,012 and 8,695 ng PBDE/g lipid but not the remaining tissue levels. For the purpose of this report, relevant concentrations of mixtures of PBDE 47 and PBDE 99 associated with altered thyroid hormone levels were concluded to be within the exposures measures where Arkoosh et al. 2013 observed altered thyroid levels (i.e., 2,012 ng/g lipid ±1SD of 520 ng/g lipid = ≥ 1,492 ng/ g lipid and ≤ 2,532 ng/g lipid).

Accordingly, to estimate the proportion of samples that had PBDE concentrations that were within the adverse effects concentrations, the sum of PBDE 47 and 99 for each whole body sample was calculated. These data were used to identify the sample concentrations that fell within the concentration range (adjusted to two significant figures) of the PBDE mixture (PBDE 47 and 99) associated with increased disease susceptibility (≥ 470 ng/g lipid and ≤ 2,500 ng/ g lipid) or altered thyroid hormone levels (≥ 1,500 ng/g lipid and ≤ 2,500 ng/g lipid).

Estimating Major Routes of Contaminant Exposure

Total body burdens (ng/fish) of POP classes in out-migrating smolts at estuary and nearshore habitats within a system were compared to assess the percent of the total POP class accumulation acquired while rearing in the freshwater/estuarine and nearshore habitats. POP body burdens in each composite sample were calculated as:

POP class body burden (ng/fish) = whole body POP class concentration (ng/g) x mean composite fish weight (g)

For each system, the average contribution from freshwater and the estuary to fish in nearshore habitats was calculated as the mean POP body burden (ng/fish) of fish collected in the estuary, divided by the mean POP body burden of nearshore fish. Within each system, the maximum contribution from freshwater and estuary habitat was calculated as the 95th-percentile POP body burden (ng/fish) of estuary fish divided by the mean POP body burden of nearshore fish.

Likewise, for each offshore habitat, the average contribution from freshwater, estuary and nearshore habitats to fish feeding in the offshore was calculated as the mean POP body burden (ng/fish) of fish collected in the nearshore, divided by the mean POP body burden of fish collected from offshore habitat of that basin. For each basin, the maximum contribution from freshwater, estuary and nearshore habitat was calculated as the 95th-percentile POP body burden (ng/fish) of nearshore fish divided by the mean POP body burden of offshore fish.

PAHs

PAH Accumulations

The summed concentrations of 42 PAH analytes ($\sum_{42}$PAHs;Table 4) from juvenile Chinook salmon stomach contents were compared among estuary habitats, nearshore habitats, river systems (estuary + nearshore habitats pooled), and basins (estuary, nearshore, and offshore habitats pooled) using parametric GLMs (SYSTAT 2009) on natural logarithm transformed data. Statistical comparisons among river estuary habitats were not possible due to limited sample sizes (n = 1 for all but the Nisqually system). Statistical comparisons among nearshore habitats were limited to the Snohomish, Hylebos/Puyallup and Nisqually nearshore due to limited sample sizes from the Skagit and Duwamish nearshore habitats (n = 2 for each). Statistical comparisons among systems (estuaries + nearshore habitats) included the Skagit, Snohomish, Green/Duwamish, and Nisqually systems; the Hylebos/Puyallup system was not included because the Hylebos Industrial Waterway sample (n = 1) was not considered a good representation of that system's river estuary. In addition, an among-sampling site comparison was made for the Nisqually system.

To statistically compare chemical concentrations in stomach contents between habitat types (estuary, nearshore, and offshore) within Puget Sound data from the Whidbey Basin, Central Basin, and South Basin were pooled for each habitat type (Table 5). Also, differences between basins were investigated using pooled habitat type data within each basin (Table 6). For consistency, all figures display the arithmetic mean PAH concentrations (rather than a mix of geometric and arithmetic mean) and 95% confidence intervals, when available.

Effects of PAH Exposure on Fish Health

To evaluate the potential for adverse effects, measured PAH concentrations in stomach contents were compared to a published adverse effects threshold based on growth in juvenile Chinook salmon for PAHs (Meador et al. 2006). The threshold is based on the summed concentrations of 17 PAHs ($\sum_{17}$PAHs). Table 7 compares the PAH concentrations fed to juvenile Chinook by Meador et al. (2006) with those detected in this study. Four of the individual PAHs included in the analytes fed to juvenile Chinook by Meador et al. (2006) were not analyzed in this study. As such, values from this study are considered to

Table 5. Samples pooled for comparison of $\sum_{42}$PAHs in stomach contents between three habitat types (see Figure 15, AB comparisons); comparison of basins within nearshore area only also performed (see Figure 15, roman numerals).

Habitat Type	Basin	Sample Locations	n	Total n
Estuary (May)	Whidbey Basin	Skagit estuary	1	7
		Snohomish estuary	1	
	Central Basin	Duwamish estuary	1	
	South Basin	Nisqually estuary	4	
Nearshore (June)	Whidbey Basin	Skagit nearshore	2	25
		Snohomish nearshore	5	
	Central Basin	Duwamish nearshore	2	
		Hylebos/Puyallup	6	
	South Basin	Nisqually nearshore	10	
Offshore (October)	Whidbey Basin	Whidbey Basin	2	13
	Central Basin	Central Basin	5	
	South Basin	South Basin	6	

Table 6. Samples pooled for comparison of $\sum_{42}$PAHs in stomach contents between three basins (see Figure 18).

Basin	Habitat Type	Sample Locations	n	Total n
Whidbey Basin	Estuary (May)	Skagit estuary	1	11
		Snohomish estuary	1	
	Nearshore (June)	Skagit nearshore	2	
		Snohomish nearshore	5	
	Offshore (October)	Whidbey Basin offshore	2	
Central Basin	Estuary (May)	Duwamish estuary	1	14
	Nearshore (June)	Duwamish nearshore	2	
		Hylebos/Puyallup nearshore	6	
	Offshore (October)	Central Basin offshore	5	
South Basin	Estuary (May)	Nisqually estuary	4	20
	Nearshore (June)	Nisqually nearshore	10	
	Offshore (October)	South Basin offshore	6	

be a conservative estimate for this comparison. To compare PAH concentrations in pellets fed to juvenile Chinook salmon in the laboratory by Meador et al. (2006) with those measured in food consumed the juvenile Chinook in this study, dry weight pellet concentrations from Table 2 in Meador et al. (2006) were converted to ww concentrations using a 0.1 conversion factor, based on the fact that fish pellets are 90% solids (James Meador, personal communication, 2014). The calculated wet weight values were converted from µg/g to ng/g to match the stomach content concentrations. The converted threshold values calculated from Table 2 (Treatments 1-5) in Meador et al. 2006 are as follows: 1 = 3,800; 2 = 12,200; 3 = 32,400; 4 = 95,100; 5 = 117,100 ng/g ww. Fish in the highest two doses of thee PAH treatments had significantly lower reductions in fish weight than those in the control treatment. In the remaining, PAH treatments, the fish showed altered growth rates, with much more variable size

Table 7. Polycyclic aromatic hydrocarbons (PAHs) used to compare to adverse effects threshold for growth with those calculated for juvenile Chinook by Meador et al. 2006. NA = not available.

Polycyclic Aromatic Hydrocarbons (PAHs)	
From Table 1 in Meador et al. 2006	Matching 17 PAHs used in this study
naphthalene (NPH)	naphthalene (NPH)
2-methylnaphthalene (2MN)	2-methylnaphthalene (MN2)
dimethylnaphthalene (DimethNPH)	dimethylnaphthalene (DMN)
dibenzothiophene (Dbnzthiop)	dibenzothiophene (DBT)
acenaphthene (ACE)	acenaphthene (ACE)
Fluorene	fluorene (FLU)
Dimethfluorene	*NA*
phenanthrene (PHN)	phenanthrene (PHN)
9-ethylphenanthrene (EthPHN)	*NA*
9-ethyl-10-methylphenanthrene (EthMePHN)	*NA*
methyl isopropyl phenanthrene (Retene)	7-Isopropyl-1-methylphenanthrene (Retene)
anthracene (ANTH)	anthracene (ANT)
fluoranthene (FLA)	fluoranthene (FLA)
pyrene (PYR)	pyrene (PYR)
Methyl pyrene (MePYR)	*NA*
benz[*a*]anthracene (B*a*A)	benz[*a*]anthracene (BAA)
chrysene (CHR)	chrysene (CHR)
benzo[*a*]pyrene (B*a*P)	benzo[*a*]pyrene (BAP)
benzo[*k*]fluoranthene (B[*k*]FLA)	benzo[*k*]fluoranthene (BKF)
benzo[*ghi*]perylene (BZP)	benzo[*ghi*]perylene (BZP)
dibenzanthracene (DibenzANTH)	dibenz[a,h]anthracene (DBA)

distributions than fish in the control fish and altered lipid profiles. These threshold values were compared to the summed $\sum_{17}$PAH values.

Trace Metals

Five samples from the Skagit system were qualified as estimates for copper concentrations due to a method blank contamination. Insufficient tissue sample was available for re-analysis for these five samples so for each sample the method blank was subtracted from the measured concentrations and that new value was used for statistical analyses. Additionally, for all metals, if a sample was measured below method detection limits (< MDL), then that MDL value was used for the data analyses.

A General Linear Model (GLM; SYSTAT 2009) was used to measure the statistical significance of differences in metal concentrations in gills samples of juvenile Chinook salmon among the river systems of Puget Sound, among estuary habitats, among nearshore habitats and between two habitat types of in these river systems as was described for the analysis of POPs in whole boy samples collected from the five river system, except the metals concentrations were not ln transformed.

RESULTS

Fish Biometrics and Phenotypic Traits

A total of 583 juvenile Chinook salmon were collected for chemical analysis, 480 from estuary and nearshore habitats and 103 at offshore sites (Table 2). All juvenile Chinook salmon from estuary and nearshore habitats were sub-yearlings, except two yearlings from the Nisqually River system. Similarly, all fish caught in offshore waters were sub-yearlings, except two fish caught in October, one in the Central basin and one in the South basin.

Most of the Chinook salmon used for chemical analyses were naturally produced fish (61%), rather than hatchery-produced, but this varied by system. Among the river systems, the percent of wild fish ranged from 100% for the Skagit, 71% for the Green/Duwamish, 48% for the Snohomish, 45% for the Nisqually and 42% for the Hylebos/Puyallup (Table 3). Additionally, the percent of wild fish often varied considerably among estuary and nearshore habitats within each system. The majority of fish caught in the offshore system in October were naturally produced (100% in Admiralty Inlet, 60% in the Whidbey Basin, 68 % in the Central Basin and 79% in the South Basin), but only 7% of fish caught in July in the Central Basin were naturally produced (Table 3).

Of the 480 fish collected from estuary and nearshore habitats for chemical analyses , 57 had CWTs indicating they were of hatchery origin within their respective rivers (with the exception of the single hatchery fish from the Nisqually River for which the CWT was lost): 5 fish collected in the Snohomish nearshore were from the Wallace River Hatchery, 20 fish collected in the Green/Duwamish estuary were from the Soos Creek Hatchery, 3 fish collected in the Hylebos Waterway and 28 in the Puyallup nearshore were from the White River Hatchery. Additionally, we retained another 54 fish with CWTs that were not used for chemical analyses, including fish from the Skagit, Snohomish, Green/Duwamish, and Hylebos/Puyallup systems, that were to affirm the movement patterns of fish. Twenty-two of the fish collected in the Skagit nearshore originated from the Marblemount Hatchery on the Skagit River, while one originated from the Samish Hatchery on the Samish River. Similar to the fish collected for chemical analyses, all 20 Snohomish fish caught in the nearshore originated from the Wallace River Hatchery and all seven fish collected in the Puyallup nearshore originated from the White River Hatchery. Unfortunately, the CWT data for the Green/Duwamish fish (n = 5) were lost and the origin of those fish are unknown. Overall, based on the CWT information for the chemistry fish and non-chemistry fish combined, 96% of fish collected in the Skagit nearshore originated from the Skagit River while 100% of fish collected in the Snohomish, Green/Duwamish, and Hylebos/Puyallup nearshore originated from their respective rivers.

In contrast, the origin of all the offshore fish with CWTs showed a substantial mix of fish from different river systems. Of the fish collected from Admiralty Inlet, 50% originated from Whidbey Basin rivers, 25% from Central Basin rivers, 25% from South Basin rivers. Seventy percent of fish collected in Whidbey Basin originated there, while 10% and 20% originated from Central and South Basin, respectively. The majority of fish collected in Central Basin in July originated from South Basin rivers (62%), 29% from Central Basin and 9.5% from Whidbey Basin rivers. Conversely, in October, most fish collected in Central Basin originated from Central Basin (59%) and Whidbey Basin rivers (30%), but only 12% from South

Basin rivers. Finally, only 11% of the fish collected in South Basin in October originated there, while 44% and 33% originated from Central Basin and Whidbey Basin rivers, respectively. An additional 11% of the South Basin caught fish originated outside the Puget Sound, specifically from a hatchery on the Chilliwack River in British Columbia.

Mean fish size at individual sites ranged from 35 to 201 mm and from 0.50 to 116 g and generally increased as fish moved from estuary (60.5 mm and 2.60 g) to the nearshore (84.2 mm and 6.03 g) and the offshore habitats (144 mm and 37.2 g). Mean size of fish collected at the two nearshore habitat sites within each system generally were not significantly different from each other (Table 8). However, for river systems, fish size within a given habitat type (nearshore or estuary) varied among river systems (Table 8). Among offshore habitats, fish size was similar among basins (Table 9). Overall, fish size may in part be affected by whether the fish were naturally produced (generally smaller) or of hatchery origin (larger). The percent of natural origin fish in whole body composite samples collected for chemical analyses was negatively correlated with the average size of fish in samples collected from estuary and nearshore habitats combined ($r^2 = 0.39$, $F = 42.49$, $p < 0.0001$) and for samples collected from offshore habitats ($r^2 = 0.33$, $F = 7.81$, $p = 0.0152$).

The lowest average condition factor among fish in river systems was measured in the Green/Duwamish system (0.912), though it was not significantly lower than those in the Snohomish and Hylebos/Puyallup systems, but significantly lower than those in the Skagit and Nisqually systems (Table 8). The fish from the Snohomish and Hylebos/Puyallup systems had intermediate condition factors, similar to fish from the other estuary/nearshore systems. Fish collected from offshore systems in October generally had higher condition factors than those in estuary/nearshore systems and varied slightly among basins (Table 9).

The percent lipid measured in whole juvenile Chinook salmon ranged from 0.59% to 4.6%. Overall, mean lipid content in fish from the offshore habitat (0.96%) was similar to levels in fish from the estuary habitats, both of which were significantly lower than levels measured in the nearshore habitat (1.5%; ANOVA, $F= 4.171$, $p < 0.017$, $n = 86$, Tukey's post hoc pairwise comparisons). The lower mean lipid levels in the offshore fish were largely influenced by very low levels in fish from the Central basin sampled in July (0.65%). Indeed, when the six composite samples collected in the offshore habitat in July were removed from the comparison of lipids among habitat types, there was no longer a significant difference in lipid levels among fish from estuary, nearshore and offshore habitats (ANOVA, $F = 1.746$, $p < 0. 181$, $n = 860$). Variation in lipid levels among samples was also affected in part by whether fish in the composite samples were naturally produced or of hatchery origins. Samples composed of 100% hatchery origin fish had significantly higher lipid levels than those that contained 100% naturally produced fish (mean = 1.77 vs. 1.21; Mann Whitney t-test = 297, $p = 0.021$, $n = 63$).

Table 8. Mean size (length and weight) and condition factor of juvenile Chinook salmon organized by the three collection sites within each system; by estuary (estuary only), by pooled nearshore marine habitat sites (pooled nearshore) and by each system. In addition, the results of a multitude of statistical analyses are represented by superscript letters to the right of the mean values. Values with the same letter are not significantly different ($p > 0.05$).

System[1]	Collection Site	n	Mean Fork Length (mm)			Mean Weight (g)			Mean Condition Factor		
			Site[1]	Estuary Only[2] Pooled Nearshore[3]	System[4]	Site[1]	Estuary Only[2] Pooled Nearshore[3]	System	Site[1]	Estuary Only[2] Pooled Nearshore[3]	System[4]
Skagit	Estuary	40	53.8^{A}	53.8^{A}	66.2^{A}	1.56^{A}	$1.56^{A,B}$	3.25^{A}	0.96^{A}	0.96^{A}	0.997^{B}
	Nearshore 1	30	75.3^{B}	74.5^{A}		4.52^{B}	4.37^{A}		1.02^{B}	1.02^{C}	
	Nearshore 2	30	73.8^{B}			4.21^{B}			1.02^{B}		
Snohomish	Estuary	39	50.3^{A}	50.3^{A}	$73.4^{B,C}$	1.31^{A}	1.31^{A}	$4.64^{B,C}$	0.91^{A}	0.91^{A}	$0.950^{A,B}$
	Nearshore 1	30	90.2^{B}	88.9^{C}		6.42^{B}	6.87^{B}		0.88^{A}	$0.98^{A,B}$	
	Nearshore 2	28	87.5^{B}			7.35^{B}			1.09^{A}		
Green/ Duwamish	Estuary	40	79.7^{A}	79.7^{C}	$81.3^{B,C}$	4.80^{A}	$4.80^{B,C}$	$4.99^{B,C}$	0.93^{B}	0.93^{A}	0.912^{A}
	Nearshore 1	31	84.5^{B}	82.3^{B}		5.35^{A}	5.11^{A}		0.86^{A}	$0.90^{A,B}$	
	Nearshore 2	30	80.1^{A}			4.86^{A}			0.93^{B}		
Hylebos/ Puyallup	Waterway	5	77.2^{A}	$77.2^{B,C}$	76.8^{B}	4.12^{A}	$4.12^{B,C}$	4.51^{B}	0.85^{A}	0.85^{A}	$0.966^{A,B}$
	Nearshore 1	30	78.5^{A}	76.7^{A}		4.57^{A}	4.54^{A}		0.93^{A}	0.98^{B}	
	Nearshore 2	37	75.3^{A}			4.52^{A}			1.02^{A}		
Nisqually	Estuary	40	58.1^{A}	$58.1^{A,B}$	83.1^{C}	2.70^{A}	2.70^{B}	6.69^{C}	1.20^{A}	1.20^{A}	1.01^{B}
	Nearshore 1	35	92.8^{B}	97.4^{C}		7.28^{B}	8.97^{B}		0.89^{A}	0.90^{A}	
	Nearshore 2	35	102^{B}			10.67^{B}			0.92^{A}		

[1] letters represent the results of the comparison of the three collection sites within each system (ANOVA or Kruskal-Wallis test)

[2] letters represent the results of between river comparisons only (Kruskal-Wallis)

[3] letters represent the results of between pooled estuary sites only (ANOVA or Kruskal-Wallis test)

[4] letters represent the results of between study location comparison (ANOVA or Kruskal-Wallis test)

Table 9. Mean size (length and weight), and condition factor of juvenile Chinook salmon collected in offshore habitats of four major basins of Puget Sound. All fish were collected in the month of October with the exception of some fish collected from Central Basin in July. In addition, the results of a multitude of statistical analyses are represented by superscript letters to the right of the mean values. Values with the same letter are not significantly different (p > 0.05). Fish collected in Central Basin in July were not included in the statistical analyses.

Basin	N	Mean Fork Length (mm)[1]	Meant Weight (g)	Mean Condition Factor
Admiralty Inlet	10	145^{A}	39.7^{A}	1.25^{B}
Whidbey Basin	10	153^{A}	40.7^{A}	1.09^{A}
Central Basin (July)	*30*	*118*	*17.1*	*1.04*
Central Basin (October)	25	156^{A}	49.3^{A}	1.24^{B}
South Basin	28	157^{A}	45.7^{A}	$1.18^{A,B}$

[1] letters represent the results of the length, weight, and condition factor post hoc pairwise comparison among the four basins (ANOVA or Kruskal-Wallis test)

POPs in Whole Body Samples

Overall, among the POPs evaluated, TPCBs dominated the chemical classes by concentrations measured in whole body samples from all locations, ranging from 5.3 to 90 ng/g ww. $\sum_{11}$PBDE concentrations ranged from 0.94 to 40 ng/g ww and were roughly one-third of TPCB concentrations in the same sample; however, four of the 88 samples had $\sum_{11}$PBDE concentrations that were greater than the TPCB concentration. Of the organochlorine pesticides analyzed, $\sum_{6}$DDTs were detected in all samples, ranging from 1.0 to 6.9 ng/g ww. $\sum_{8}$Chlordanes were detected in 83% of the samples, with values ranging 0.10 ng/g ww (LOQ) to 3.6 ng/g ww. HCB was detected in 61% of the samples, ranging in values from 0.11 ng/g ww (LOQ) to 11 ng/g ww. Dieldrin was detected in 32% of the samples, but at very low concentrations ranging from 0.12 ng/g ww (LOQ) to 1.9 ng/g ww. Of the three HCH isomers, lindane was never detected and α-HCH and β-HCH were only detected in the Hylebos/Puyallup system at 3% and 4%, respectively. Due to the low number of detected values, $\sum_{3}$HCHs were not analyzed statistically. Three other pesticides, endosulfan sulfate, aldrin, and mirex and were never detected. Summary statistics were calculated for each collection location as geometric means (all POPs), medians, and 25th and 75th percentiles (APPENDIX C: Summary Statistics of Persistent Organic Pollutants Measured in Juvenile Chinook Salmon Whole Body Tissue).

Of the 46 PCB congeners tested, 15 (including four co-eluting) were detected in every sample (PCBs 28, 101/90[1], 110, 118, 138/163/164, 149, 153/132, 180, and 187/159/182) and another seven were detected in over 90% of the samples (PCBs 18, 31, 33, 52, 70, 95, and 99). Four PCB congeners were not detected in any samples (PCBs 191, 205, 208, and 209) and nine were detected in less than 50% of the samples (PCBs 17, 82, 156, 158, 171, 194, 195, 199, and 206). The remaining 11 congeners were detected in between 50% and 90% of the samples (Table 10).

Of the 11 PBDE congeners tested, two (PBDE 47 and 99) were detected in every sample, and another (PBDE 100) was detected in greater than 90% of the samples. Two congeners (PBDE 155 and 183) were

[1] Co-eluting congeners are expressed as congener numbers separated by a slash mark. The leftmost congener is dominant and concentration decreases as the co-eluters are listed from left to right.

Table 10. The frequency of detection (%) of the 46 PCB congeners measured in 88 juvenile Chinook salmon whole body (less gills and stomach contents) samples. The bolded PCB congeners contributed to the estimated total PCBs (TPCBs) calculation. Numbers in parentheses indicate coeluting congeners.

PCB congener	Homolog group	Frequency of detection (%)
PCB 17	Tri	42
PCB 18	Tri	95
PCB 28	Tri	100
PCB 31	Tri	99
PCB 33	Tri	90
PCB 44	Tetra	76
PCB 49	Tetra	75
PCB 52	Tetra	93
PCB 66	Tetra	83
PCB 70	Tetra	95
PCB 74	Tetra	52
PCB 82	Penta	13
PCB 87	Penta	85
PCB 95	Penta	92
PCB 99	Penta	99
PCB 101 (90)	Penta	100
PCB 105	Penta	88
PCB 110	Penta	100
PCB 118	Penta	100
PCB 128	Hexa	84
PCB 138 (163, 164)	Hexa	100
PCB 149	Hexa	100
PCB 151	Hexa	65
PCB 153 (132)	Hexa	100
PCB 156	Hexa	33
PCB 158	Hexa	42
PCB 170	Hepta	65
PCB 171	Hepta	25
PCB 177	Hepta	57
PCB 180	Hepta	100
PCB 183	Hepta	63
PCB 187 (159, 182)	Hepta	100
PCB 191	Hepta	0
PCB 194	Octa	30
PCB 195	Octa	2.0
PCB 199	Octa	40
PCB 205	Octa	0
PCB 206	Nona	11
PCB 208	Nona	0
PCB 209	Deca	0

not detected in any of the samples and the remaining six were detected in fewer than 50% of the samples (PBDEs 28, 49, 66, 85, 153, and 154; Table 11).

Table 11. The frequency of detection (%) of 11 PBDE congeners measured in 88 juvenile Chinook salmon whole body (less gills and stomach contents) samples.

PBDE Congeners	Frequency of Detection (%)
BDE 28	7
BDE 47	100
BDE 49	47
BDE 66	9
BDE 85	2
BDE 99	100
BDE 100	98
BDE 153	30
BDE 154	27
BDE 155	0
BDE 183	0

Of the six DDT isomers tested, only *p'p'*-DDE was detected in every sample and *o'p'*-DDE was not detected in any samples. The remaining four isomers were detected in < 50% of the samples (*o,p'*-DDD, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDT; Table 12).

Table 12. The frequency of detection (%) of organochlorine pesticides measured in 88 juvenile Chinook salmon whole body (less gills and stomach contents) samples.

DDT Isomers	Frequency of Detection (%)	*HCH Isomers*	Frequency of Detection (%)
o,p'-DDD	13	α-hexachlorocyclohexane	2
o,p'-DDE	0	β-hexachlorocyclohexane	3
o,p'-DDT	2	γ-hexachlorocyclohexane	0
p,p'-DDD	48		
p,p'-DDE	100	***Chlordane analytes***	
p,p'-DDT	36	α-chlordane	24
		cis-nonachlor	30
Hexachlorocyclobenzene	61	β-chlordane	10
		heptachlor	0
Miscellaneous Pesticides		heptachlor-epoxide	2
Aldrin	0	nonachlor III	1
Dieldrin	32	oxychlordane	48
α-endosulfan	0	trans-nonachlor	81
Mirex	0		

Chlordanes were detected in all but 14 of the estuary, nearshore, and offshore samples, four from the Nisqually system, six from the Skagit system, two from Admiralty Inlet and two from the Central Basin. Trans-nonachlor and oxychlordane were the most often detected compounds (81% and 48%, respectively) followed by cis-nonachlor (30%), α-chlordane (24%) and β-chlordane (10%). Heptachlor epoxide and nonachlor were seldom detected (2% and 1%, respectively) and heptachlor was never detected in any of the samples (Table 12).

HCB was detected in 61% of the samples from estuary, nearshore, and offshore habitats (Table 12), but the number of detections varied among river systems and basins (APPENDIX C: Summary Statistics of Persistent Organic Pollutants Measured in Juvenile Chinook Salmon Whole Body Tissue, Table C 5). Detected HCB values ranged from 0.11 to 0.37 ng/g ww for all samples with the exception of one sample from the Snohomish estuary, where 11 ng/g ww was detected. However, only 26% of the samples with detected values (14 of 54 samples) were above the range of non-detected value (0.11– 0.29 ng/g ww).

The only organochlorine pesticide detected was dieldrin and it was found in 32% of the samples (Table 12) however, the number of detections varied considerably among river systems and basins. Dieldrin was most commonly detected in the Green/Duwamish system (12/67 samples) and the Hylebos/Puyallup system (10/67 samples). Dieldrin was only detected in 4/16 samples from the Snohomish system, 1/14 from the Skagit system, 1/2 from Whidbey Basin and was not detected in any samples from the Nisqually system, Admiralty Inlet, Central Basin (July), Central Basin (October) or South Basin. Detected dieldrin values ranged from 0.12 to 1.9 ng/g ww, however, only 50% of the detected values were above the range of non-detected value (0.10 – 0.28 ng/g ww).

POPs Accumulation in Estuary and Nearshore Marine Habitats

This section presents a more detailed assessment of the accumulation of specific POPs classes or analytes in estuary and nearshore habitats of the five river systems. For these analyses, data for the two nearshore sites within each system were pooled. First, the variation observed in the Skagit, Snohomish, Green/Duwamish and Nisqually systems, the four systems with balanced sampling efforts among estuary and nearshore habitats across systems, is described. The Hylebos/Puyallup system was not included in this analysis because there were too few estuary samples to adequately represent the estuary habitat in this river system. Next, the variation in each POP measured in fish among all five systems for just the nearshore habitats is described; the Hylebos/Puyallup system was included in this analysis because there were enough nearshore samples to adequately represent that habitat in this system.

TPCBs

Excluding the Hylebos/Puyallup system, overall, most (77%) of the variation in TPCBs in juvenile salmon was related to the system in which they were collected; system*habitat interaction (i.e. system-specific differences between estuary and nearshore habitats) accounted for an additional 7.7% of the variation (GLM on ln TPCBs with system, habitat, fish length and interaction terms; n = 56; $r^2 = 0.854$; $F_{system} = 61.461$, df = 3, 49, $p < 0.001$; $F_{system*habitat} = 8.562$, df = 3, 49, $p < 0.001$). In general, TPCB levels were not significantly different between estuary and nearshore habitats and were not correlated with fish length. Significantly different mean TPCBs concentrations were measured among each of the systems; post hoc pairwise comparisons indicated that the lowest concentrations were in the Skagit system, and were progressively higher in fish from the Nisqually, Snohomish and the Green/Duwamish systems (7.1, 13, 16, and 46 ng/g ww, respectively). The mean TPCB concentrations in fish from the Green/Duwamish system were approximately six times higher than those in the Skagit system and twice those in the Snohomish system (Figure 2).

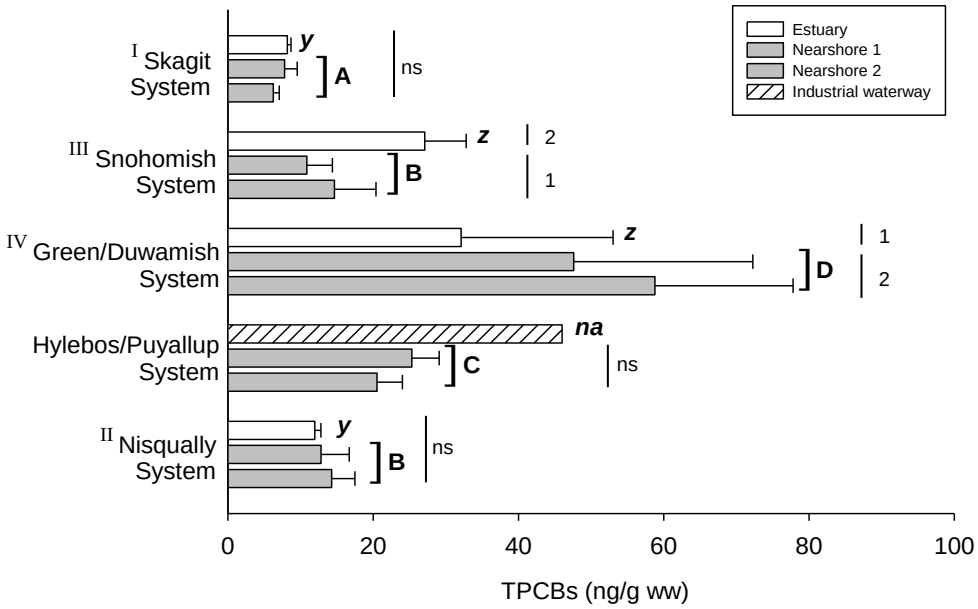

Figure 2. Comparison of geometric means (+ 95% confidence intervals) of estimated total PCBs (TPCBs; ng/g ww) measured in juvenile Chinook salmon whole bodies (less gills and stomach contents) collected from five major river systems, in Puget Sound, WA. Pairwise comparisons of the systems (Roman numerals, excluding Hylebos/Puyallup), four estuary habitats (white bars, *y-z*), five nearshore marine habitats (gray bars, A-D) and the estuary and nearshore habitat within systems (numbers) are also shown. Similar letters or numbers signify no significant difference (p > 0.05). na = not analyzed, ns = not significant.

Although TPCB concentrations did not differ overall between fish from estuary and nearshore habitats in systems as a whole, TPCB concentrations in fish from estuary and nearshore habitats varied among systems. In the Skagit and Nisqually systems, post hoc pairwise comparisons indicated that TPCBs concentrations were similar in juvenile Chinook salmon caught in estuary and nearshore habitats within each system (Figure 2). However, within the Snohomish system, fish collected in the nearshore habitat had significantly lower mean TPCB concentrations than those in the estuary habitat (13 and 27 ng/g ww, respectively; Figure 2). Within the Green/Duwamish system, mean TPCBs concentrations also differed between habitats, but in contrast to the Snohomish system, TPCBs were higher in fish from the nearshore than those in the estuary (53 and 32 ng/g ww, respectively; Figure 2).

Similar to the results examining the systems as whole units, among the four estuary habitats, post hoc pairwise comparisons indicated that mean TPCB concentrations in fish from the Skagit and Nisqually estuaries (8.2 and 12 ng/g ww, respectively), were similar to each other and significantly lower than mean levels in fish from the Snohomish and Green/Duwamish estuaries (27 and 32 ng/g ww, respectively; Figure 2). Likewise, fish from the Skagit nearshore had significantly lower mean TPCB concentrations than those from other nearshore habitats (7.0 ng/g ww), followed by relatively low and similar concentrations in Nisqually and Snohomish nearshore (14 and 13 ng/g ww, respectively), and significantly higher concentrations in Chinook salmon from the Green/Duwamish nearshore (53 ng/g) system, over seven times higher than the Chinook salmon from the Skagit nearshore (Figure 2).

In a separate comparison of TPCB concentrations among nearshore habitats that included fish from the Hylebos/Puyallup system, most (85%) of the variation in TPCBs in juvenile salmon in nearshore habitats

was related to the system where they were collected (GLM on ln TPCBs with system, fish length and interaction terms; n = 50, r^2 = 0.853, F_{system} = 65.118, df = 4, 45, p < 0.001; Figure 2). The TPCB concentrations were not correlated with fish length. Post hoc pairwise comparisons indicated that the mean TPCBs in the Hylebos/Puyallup fish from the nearshore (23 ng/g ww) were significantly higher than those in the Snohomish nearshore, but lower than those in the Green/Duwamish. All other pair-wise comparisons of nearshore habitats among river systems had results that were similar to pairwise comparisons reported for nearshore habitats that excluded the Hylebos/Puyallup nearshore fish.

$\sum_{11}$PBDEs

Excluding the Hylebos/Puyallup system, most (39.4%) of the variation in $\sum_{11}$PBDEs in juvenile salmon was related to the river system in which they were collected; however, system specific differences between estuary and nearshore habitats accounted for an additional 25% of the variation (GLM on ln $\sum_{11}$PBDEs with system, habitat, fish length and interaction terms; n = 56, r^2 = 0.644; F_{system} = 21.682, df = 3, 49, p < 0.001; $F_{system*habitat}$ = 11.459, df = 3, 49, p < 0.001). In general, $\sum_{11}$PBDE levels were not significantly different between estuary and nearshore habitats and were not correlated with fish length. Post hoc pairwise comparisons indicated that mean $\sum_{11}$PBDE concentrations among fish from the Skagit, Nisqually and Green/Duwamish systems were similar to each other (2.0, 2.4, and 4.2 ng/g ww, respectively) and were all lower than those in the Snohomish system (8.2 ng/g ww). Mean $\sum_{11}$PBDE concentrations in the Snohomish system fish were four times higher than those in the Skagit system, 3.5 times those in the Nisqually system, and twice those in the Duwamish system (Figure 3).

Although mean $\sum_{11}$PBDE concentrations did not differ between fish from estuary and nearshore habitats overall (5.3 and 3.6 ng/g ww), there were differences in some systems. Post hoc pairwise comparisons indicated that mean $\sum_{11}$PBDE concentrations in the Skagit and Nisqually systems were similar between estuary and nearshore marine habitats. However, in the Snohomish system, the mean $\sum_{11}$PBDE levels were significantly lower in fish collected from the nearshore than in fish caught in the estuary (5.0 and 29 ng/g ww; Figure 3). In contrast, within the Green/Duwamish system, the mean $\sum_{11}$PBDE levels were significantly higher in fish collected from the nearshore than those in the estuary (4.8 and 2.9 ng/g ww; Figure 3).

Similar to the results obtained when examining the systems as whole units, post hoc pairwise comparisons indicated that among estuary habitats mean $\sum_{11}$PBDE concentrations in fish from the Skagit, Nisqually and Green/Duwamish systems were similar to each other (1.8, 1.5, and 2.9 ng/g ww, respectively) and were all lower than those measured for the Snohomish system (29 ng/g ww). A different pattern of $\sum_{11}$PBDE concentration was observed among fish in nearshore habitats: post hoc pairwise comparisons indicated that mean $\sum_{11}$PBDEs were significantly lower in fish from the nearshore Nisqually habitat (1.5 ng/g ww) than all other nearshore habitats, except those in the Skagit system. Mean $\sum_{11}$PBDE concentrations in fish from nearshore habitats from the Skagit, Snohomish and the

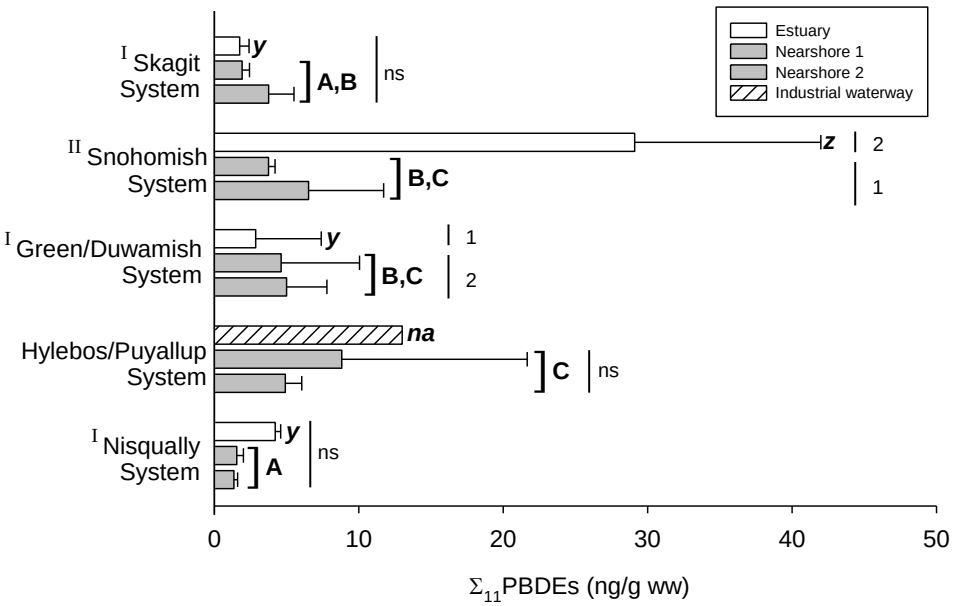

Figure 3. Comparison of geometric means (+ 95% confidence intervals) of $\sum_{11}$PBDEs (ng/g ww) measured in juvenile Chinook salmon whole bodies (less gills and stomach contents) collected from five major river systems, in Puget Sound, WA. Pairwise comparisons of the systems (Roman numerals, excludes Hylebos/Puyallup), four estuary habitats (white bars, *y-z*), five nearshore marine habitats (gray bars, A-D) and the estuary and nearshore habitat within systems (numbers) are also shown. Similar letters or numbers signify no significant difference (p > 0.05). na = not analyzed, ns = not significant.

Green/Duwamish systems were similar to each other (2.7, 5.0, and 4.8 ng/g ww, respectively).

In a separate comparison of $\sum_{11}$PBDE concentrations among nearshore habitats that included fish from the Hylebos/Puyallup system, most (50%) of the variation in nearshore habitats was related to the system in which they were collected (GLM on ln $\sum_{11}$PBDEs with system, fish length and interaction terms; n = 50, r^r = 0.496, F_{system} = 11.072, df = 4, 45, p < 0.001; Figure 3). $\sum_{11}$PBDE concentrations were not correlated with fish length. Post hoc pairwise comparisons indicated that the mean $\sum_{11}$PBDE concentrations in fish from the Hylebos/Puyallup nearshore (6.6 ng/g ww) were significantly higher than those in the Nisqually and Skagit nearshore habitats, but similar to levels measured in nearshore habitats for all other systems. All other system pair wise comparisons were the same as those reported for comparison among nearshore habitats that excluded the Hylebos/Puyallup nearshore fish.

$\sum_6$DDTs

Excluding the Hylebos/Puyallup system, most (68%) of the variation in $\sum_6$DDT concentrations in juvenile Chinook salmon was related to the system in which they were collected; habitat and system specific differences between estuary and nearshore habitats accounted for an additional 5.7% and 5.3% of the observed variation (GLM on ln $\sum_6$DDTs with system, habitat, fish length and interaction terms; n = 56, r^2 = 0.79; F_{system} = 42.290, df = 3, 48, p < 0.001; $F_{habitat}$ = 13.867, df = 1, 48, p < 0.001; $F_{system*habitat}$ = 4.051, df = 3, 48, p = 0.012). $\sum_6$DDT concentrations were not correlated with fish length. Post hoc pairwise comparisons indicated that the mean $\sum_6$DDTs concentrations were lowest in juvenile Chinook salmon collected from the Skagit and Nisqually systems (1.5 and 1.8 ng/g ww, respectively), similar to each other and significantly lower than those of Snohomish and Green/Duwamish system (2.3 and 3.9 ng/g

ww). The $\sum_6$DDT concentrations measured in the Green/Duwamish system (3.9 ng/g ww) were significantly higher than all other systems, more than 2.5 times higher than the Skagit system (Figure 4).

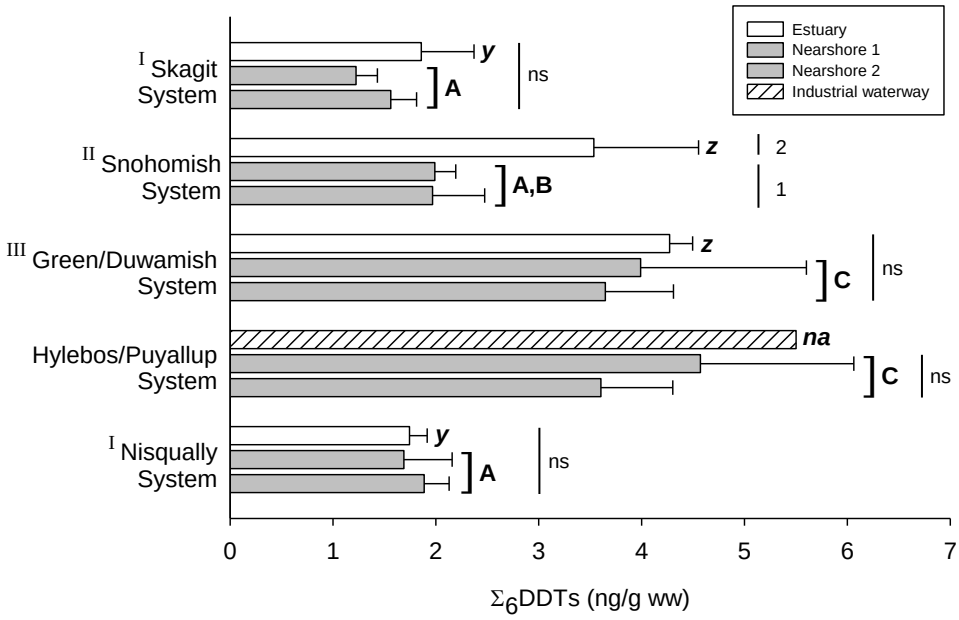

Figure 4. Comparison of geometric means (+ 95% confidence intervals) of $\sum_6$DDTs (ng/g ww) measured in juvenile Chinook salmon whole bodies (less gills and stomach contents) collected from five major river systems, in Puget Sound, WA. Pairwise comparisons of the systems (Roman numerals, excludes Hylebos/Puyallup), four estuary habitats (white bars, *y-z*), five nearshore marine habitats (gray bars, A-D) and the estuary and nearshore habitat within systems (numbers) are also shown. Similar letters or numbers signify no significant difference (p > 0.05). na = not analyzed, ns = not significant

Mean $\sum_6$DDT concentrations were significantly higher in fish from pooled estuary samples from all systems than pooled nearshore habitats from all systems (2.8 and 2.4 ng/g ww, respectively). This difference also was observed in each river systems, but only in the Snohomish system was this difference significant (3.5 and 2.0 ng/g ww, respectively; Figure 4).

Among the estuary habitats in the four main river systems (excluding the Puyallup), post hoc pairwise comparisons indicated that concentrations of mean $\sum_6$DDTs in juvenile Chinook salmon from the Skagit and Nisqually estuaries (1.9 and 1.7 ng/g ww, respectively) were similar to each other and significantly lower than those in the Snohomish and Green/Duwamish estuaries (3.5 and 4.3 ng/g ww, respectively; Figure 4). $\sum_6$DDT concentrations were also similar between the Snohomish and Green/Duwamish estuaries. Among the nearshore habitats in the four river systems, mean $\sum_6$DDT concentrations were lowest in juvenile Chinook salmon from the Skagit River (1.4 ng/g ww) , which were similar to those from the Nisqually (1.8 ng/g ww), but significantly lower than those from the Snohomish and Green/Duwamish nearshore habitats (2.0 and 3.8 ng/g ww). Mean $\sum_6$DDT concentrations in fish collected in the Snohomish nearshore (2.0 ng/g ww) were similar relative to the Nisqually nearshore fish, but significantly lower than levels observed in fish from the Green/Duwamish nearshore (3.8 ng/g ww).

In a separate comparison of $\sum_6$DDT concentrations among nearshore habitats that included the fish from the Hylebos/Puyallup system, most (78%) of the variation in $\sum_6$DDTs in juvenile salmon in nearshore habitats was related to the nearshore system in which they were collected (GLM on ln $\sum_6$DDTs with system, fish length and interaction terms; n = 50, r^2 = 0.778, F_{system} = 39.526, df = 4, 45, p < 0.001; Figure 4). The $\sum_6$DDT concentrations were not correlated with fish length. Post hoc pairwise comparisons indicated that mean $\sum_6$DDT concentrations in the fish from the Hylebos/Puyallup nearshore were similar to those from the Green/Duwamish nearshore (4.1 and 3.8 ng/g ww, respectively), and significantly higher than those in other nearshore habitats. All other system pair wise comparisons were the same as those reported for comparison among nearshore habitats that excluding the Hylebos/Puyallup nearshore fish.

$\sum_8$Chlordanes

Overall, most (80%) of the variation in $\sum_8$chlordane concentrations in juvenile salmon was related to the system in which they were collected (GLM on ln $\sum_8$chlordanes with system, habitat, fish length and interaction terms; n = 56, r^2 = 0.799, F_{system} = 68.825, df = 3, 52, p < 0.001). Mean $\sum_8$chlordane levels were not significantly different between estuary and nearshore habitats (2.0 and 2.4 ng/g ww, respectively). Additionally, $\sum_8$chlordanes were not correlated with fish length. As was observed for TPCBs, post hoc pairwise comparisons indicated that the mean $\sum_8$chlordane concentration was lowest in the Skagit system, and progressively higher in fish from the Nisqually, Snohomish and the Green/Duwamish systems (0.16, 0.25, 0.51, and 1.9 ng/g ww, respectively). The mean $\sum_8$chlordane concentrations in fish from the Green/Duwamish system were approximately 12 times higher than those in the Skagit, eight times greater than in the Nisqually, and four times than levels in the Snohomish systems (Figure 5).

In a separate comparison of $\sum_8$chlordane concentrations among the estuary habitats from the four systems, almost all (97%) of the variation in $\sum_8$chlordane was related to the river system where they were collected (GLM on ln $\sum_8$chlordanes with system, fish length and interaction terms; n = 16, r^2 = 0.967, F_{system} = 116.063, df = 3, 12, p < 0.001; Figure 5). The $\sum_8$chlordane concentrations in estuary habitats were not correlated with fish length. Post hoc pairwise comparisons indicated that the mean $\sum_8$chlordanes concentrations in the fish collected from Skagit and Nisqually estuary habitats (0.15 and 0.22 ng/g ww, respectively) were similar to each other and significantly lower than those measured in fish from the Snohomish estuary (0.64 ng/g ww), which were also significantly lower than those measured in the Green/Duwamish estuary (1.5 ng/g ww). Overall, mean $\sum_8$chlordane concentrations in the fish from the Green/Duwamish estuary were 10 times higher than levels in Skagit River fish, almost seven times the levels in the Nisqually estuary fish, and more than twice the levels in the Snohomish estuary fish (Figure 5).

In a separate comparison of $\sum_8$chlordane concentrations that included fish from the Hylebos/Puyallup system, almost all (83%) of the variation in in nearshore habitats was related to the river system where they were collected (GLM on ln $\sum_8$chlordanes with system, fish length and interaction terms; n = 50, r^2 = 0.83, F_{system} = 54.805, df = 4, 45,p < 0.001; Figure 5). The $\sum_8$chlordane concentrations in nearshore habitats were not correlated with fish length. Post hoc pairwise comparisons indicated that the mean $\sum_8$chlordane concentrations in fish collected from nearshore marine shorelines of the Skagit and

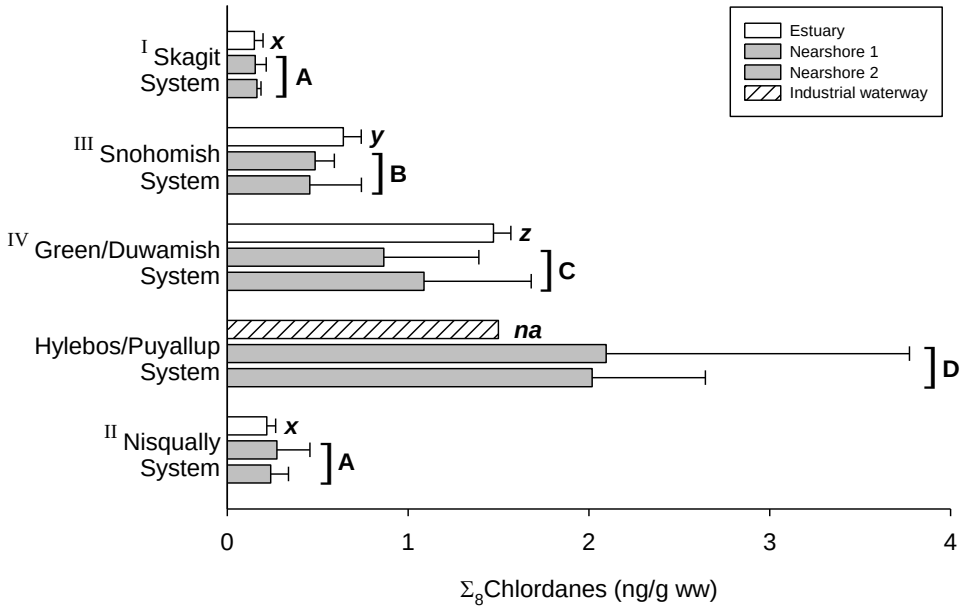

Figure 5. Comparison of geometric means (+ 95% confidence intervals) of $\sum_8$chlordanes (ng/g ww) measured in juvenile Chinook salmon whole bodies (less gills and stomach contents) collected from five major river systems, in Puget Sound, WA. Pairwise comparisons of the systems (Roman numerals, excludes Hylebos/Puyallup), four estuary habitats (white bars, *y-z*), and the five nearshore marine habitats (gray bars, A-D) are also shown. Similar letters or numbers signify no significant difference (p > 0.05). na = not analyzed, ns = not significant. The estuary and nearshore habitat within systems were not statistically analyzed because several of the systems had few detected values.

Nisqually systems (0.16 and 0.26 ng/g ww, respectively), were similar to each other and significantly lower than those measured in fish from the more developed Snohomish nearshore system (0.47 ng/g ww). Intermediate mean $\sum_8$chlordane concentrations were measured in Green/Duwamish nearshore fish (0.97 ng/g ww), significantly higher than levels in fish from the Snohomish nearshore and significantly lower than those from Hylebos/Puyallup nearshore (2.1 ng/g). Mean $\sum_8$chlordanes concentrations measured in fish collected from the Hylebos/Puyallup nearshore system were roughly 13 times higher than fish collected in the Skagit estuary (Figure 5).

HCB

Statistical analyses were not completed for HCB because it was not detected in most (39%) of the samples and only 26% of the samples with detected values (14 of 54 samples) were above the range of non-detected values (0.11– 0.29 ng/g ww). The average detected concentrations are shown in Figure 6.

Dieldrin

The large number of non-detected values for dieldrin limited the types of spatial comparisons that could be done for this compound. However, there were clear differences in the detection limits between habitat types within a system and among the river systems. For example, dieldrin was detected in all four of the estuary samples from the Green/Duwamish system but not in samples collected from any of the other estuary systems (APPENDIX C: Summary Statistics of Persistent Organic Pollutants Measured in Juvenile Chinook Salmon Whole Body Tissue, Table C 4). Likewise, among samples collected from nearshore habitats, dieldrin was not detected in the Nisqually samples (n = 10), 1/10 of Skagit samples, 3/10 of Snohomish samples, 8/10 of the Green/Duwamish samples, and 9/10 of the Hylebos/Puyallup

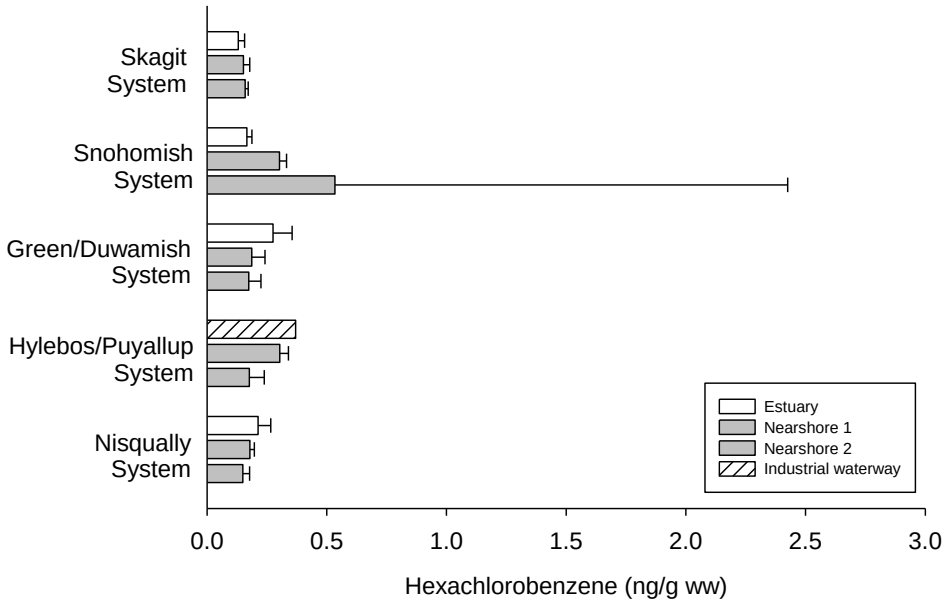

Figure 6. Comparison of geometric means (+ 95% confidence intervals) of hexachlorobenzene (HCB; ng/g ww) measured in juvenile Chinook salmon whole bodies (less gills and stomach contents) collected from five major river systems, in Puget Sound, WA. Statistical comparisons were not made because of the large number of non-detected values (see text for details).

system. Statistical analyses for dieldrin were limited to a comparison among the Skagit, Snohomish, Green Duwamish and Nisqually systems, the four systems with balanced sampling among estuary and nearshore systems.

Excluding the Hylebos/Puyallup system, overall, most (48%) of the variation in dieldrin measured in juvenile salmon was related to the system in which they were collected (GLM on ln dieldrin with system, habitat, fish length and interaction terms; n = 56, F_{system} = 16.15, df = 3, 51, $p < 0.001$). Dieldrin levels were not correlated with fish length. Mean dieldrin levels were not significantly different between estuary and nearshore habitats (1.97 and 2.36 ng/g ww, respectively). Post hoc pairwise comparisons indicated mean dieldrin levels measured in fish from the Green/Duwamish system (0.29 ng/g ww) were significantly higher than those measured in Skagit system, Snohomish and Nisqually systems, which were all similar to each other (0.13, 0.15 and 0.17 ng/g ww, respectively; Figure 7).

POPs Accumulation in Offshore Habitats

In all four offshore habitats, TPCBs, $\sum_{11}$PBDEs and $\sum_{6}$DDTs were detected in every whole body tissue sample. TPCBs concentrations ranged from 8.3 to 37 ng/g ww and were generally higher than $\sum_{11}$PBDEs levels (range = 1.2 to 5.0 ng/g ww), followed by $\sum_{6}$DDTs (range = 0.63 to 2.6 ng/g ww). Other POPs classes or analytes were detected less frequently and at lower concentrations.

Spatial variation in accumulation of specific POP classes or analytes for whole body fish samples collected from the offshore habitats are presented below. As detailed in the methods, for each POP class or analyte, spatial comparisons included variation in POP concentrations in fish 1) among offshore habitat sites, 2) among habitats, pooling samples from basins by offshore, nearshore and estuary habitats, 3) among basins, pooling samples from all habitat types by Whidbey, Central and South basins,

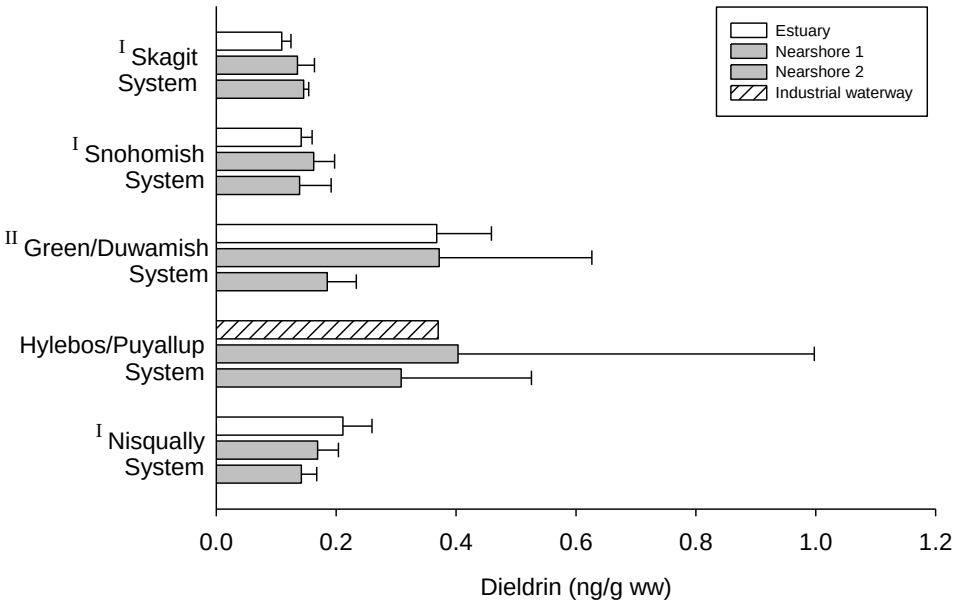

Figure 7. Comparison of geometric means (+ 95% confidence intervals) of the organochlorine pesticide, dieldrin (ng/g ww), measured in juvenile Chinook salmon whole bodies (less gills and stomach contents) collected from five major river systems, in Puget Sound, WA. Statistical analyses were limited to pairwise comparisons of the systems (Roman numerals, excludes Hylebos/Puyallup) because of the large number of non-detected values (see text for details). Similar roman numerals signify no significant difference (p > 0.05).

and 4) among basin and habitat combined, limited to the subset of samples from the Green/Duwamish and Nisqually systems and their associated offshore habitat (i.e., Central and South basins respectively).

TPCBs

Among offshore habitats sampled in October, the mean TPCB concentration was lower in fish collected from Admiralty Inlet (8.8 ng/g ww) than those from the Whidbey, Central and South basins (22, 23, and 24 ng/g ww, respectively; Figure 8). Of these, the concentrations of TPCBs in the Central and South basins, the only sites with sufficient samples for statistical analyses, were not significantly different from each other (n= 11, r^2 = 0.01, F = 0.055, df = 1, 9, p = 0.82; Figure 8). The offshore Central Basin was sampled in July as well as October, and the mean TPCB concentrations were similar (19 and 23 ng/g ww, respectively; n = 11, r^2 = 0.277, F = 0.750, df = 1, 9, p = 0.409; Figure 8).

A comparison of TPCB concentrations among habitat types (all samples from the Whidbey, Central and South basins pooled by habitat type, except as described in the Methods), revealed that mean TPCB concentrations in fish collected from the offshore habitat as a unit (24 ng/g ww) were higher but not significantly different than those from the estuary (17 ng/g ww) and nearshore habitats (17 ng/g ww) as units (GLM; n=79, r^2 = 0.033, $F_{habitat}$ = 1.281, df = 2, 76, p = 0.284; Figure 8). In this analysis, fish length was not correlated with TPCB levels and the length*habitat interaction was not significant.

A comparison of TPCB concentrations by basin (all samples from estuary, nearshore, and offshore, habitats pooled within basins, except as described in the Methods), revealed the variation in TPCBs were

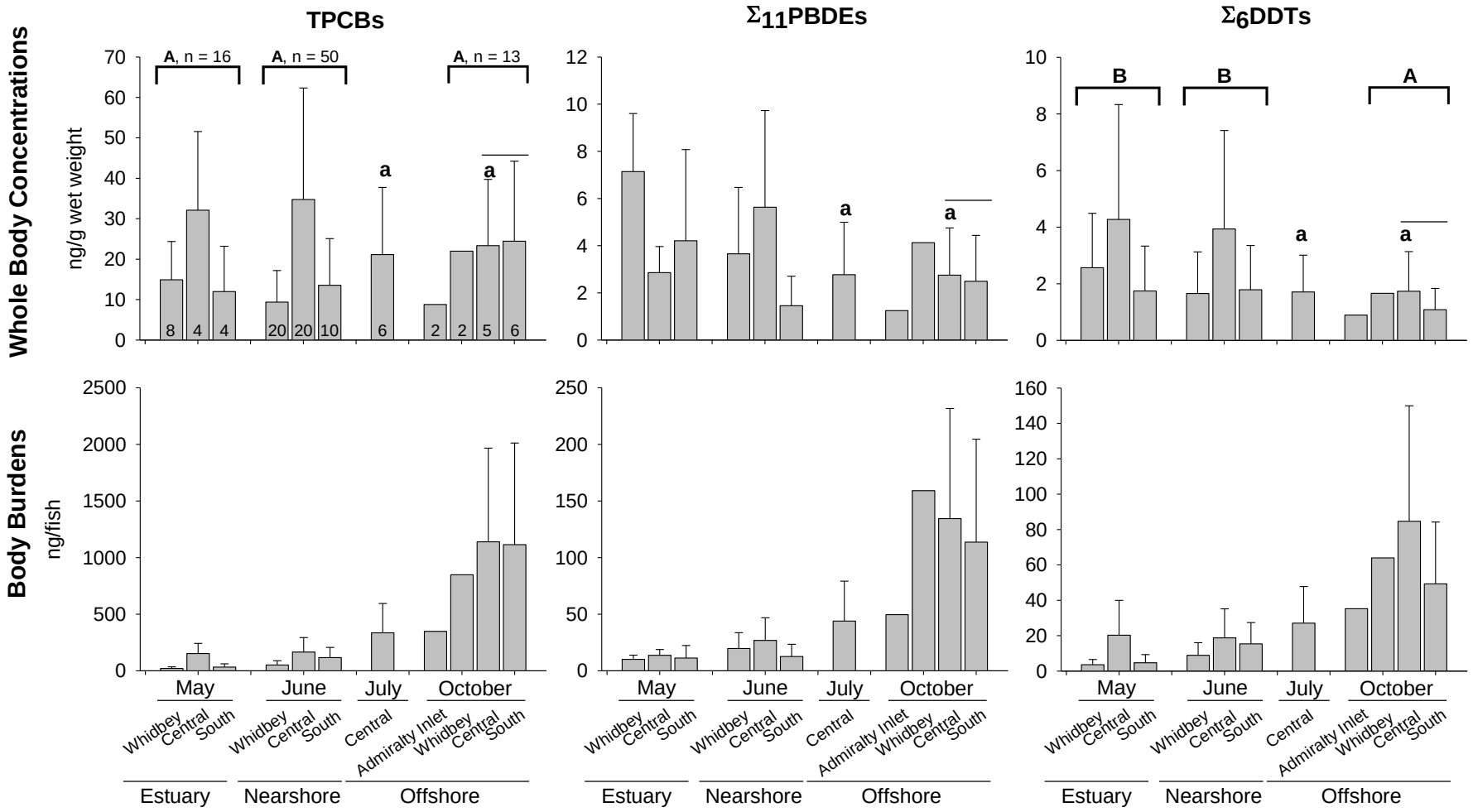

Figure 8. Comparison of geometric means (+95% confidence intervals) of three different POP concentrations (ng/g ww) and body burdens (ng/g fish) measured in juvenile Chinook salmon whole body samples (less gills and stomach contents) collected from estuary, nearshore and offshore habitats within four major Puget Sound basins. Numbers within the bars of the TPCB figure indicate sample size. Similar letters signify no significant difference (p> 0.05) in pairwise comparisons (GLM and Tukey's Honestly Significant Difference Test). The bars show pooled samples used for statistical comparisons between habitat types (upper case letters) and between July and Oct samples for the Central Basin (lower case letters). POPs in offshore habitats in Oct were not significantly different between Central and South basins (shown with a horizontal solid line.)

mostly associated with location basin differences, and to a lesser extent with differences in fish size among basins. Basin as a factor accounted for 47.3% of the observed in TPCB concentration and the basin*length interaction term accounted for an additional 5.2 % of the variation (GLM on ln TPCBs with basin, fish length and interaction terms; n = 79; $r^2 = 0.473$; $F_{basin} = 11.872$, df = 2, 76, $p < 0.001$; $F_{basin*length} = 4.062$, df = 2, 76, p = 0.21). Fish length was not significantly correlated with TPCBs. Post hoc pairwise comparisons indicated mean TPCBs concentrations in fish collected from the South Basin (16 ng/g ww) were significantly lower than those collected from the Central Basin (32 ng/g ww; Figure 9). Mean TPCB concentrations in fish from the Whidbey Basin (11 ng/g ww) were similar to those from the South Basin, but significantly lower than those from the Central Basin (Figure 9).

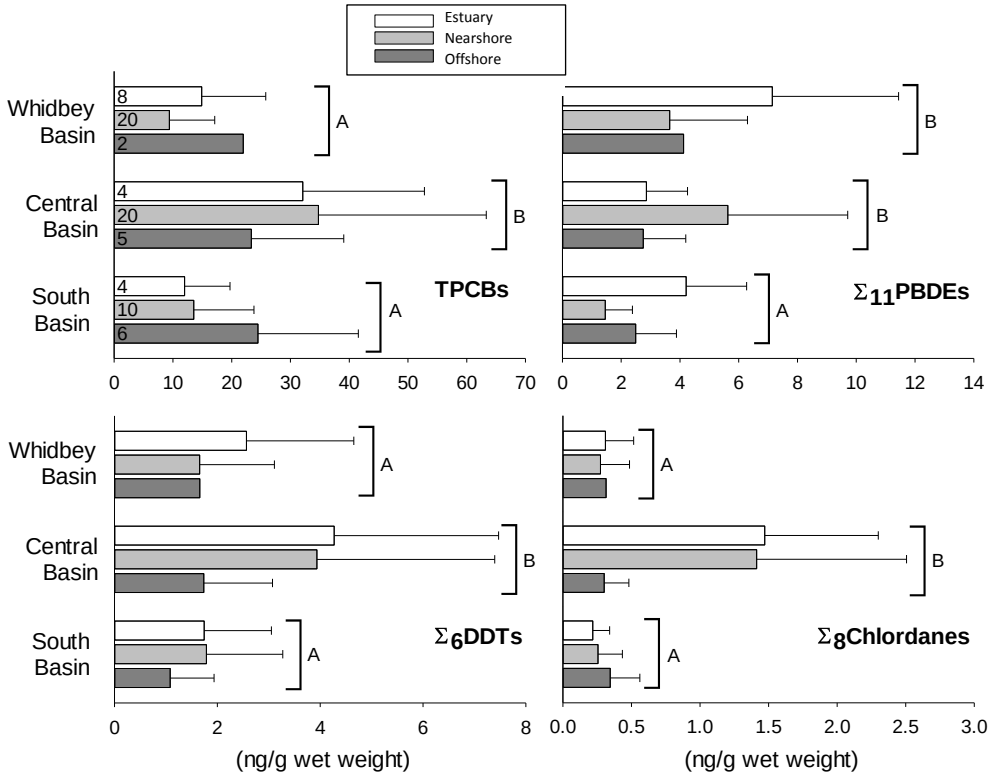

Figure 9. Comparison of geometric means (+95% confidence intervals) of four POPs (ng/g ww) measured in juvenile Chinook salmon whole body samples (less gills and stomach contents) collected from estuary, nearshore and offshore habitats within major Puget Sound basins. Numbers within the bars of the TPCB figure indicate sample size. Similar letters signify no significant difference (p > 0.05) in pairwise comparisons (GLM and Tukey's Honestly Significant Difference Test), between basin samples pooled across habitats.

The comparison of TPCB concentrations among basins and habitat types combined indicated that most of the variation in TPCBs in was attributed to location, including 49.3% associated with basin differences and 22.6% associated with basin*habitat type interaction (GLM on ln TPCBs with basin, habitat, fish length and interaction terms; n = 39, $r^2 = 0.719$, $F_{basin} = 39.705$, df = 1, 35, $p < 0.001$; $F_{basin*habitat} = 14.04$, df = 2, 35, $p < 0.001$). Post hoc pairwise comparisons indicated that mean TPCB concentrations were significantly higher in fish from the Central Basin (represented by the Green/Duwamish river system and the offshore habitat) than those from South (represented by Nisqually River system and the offshore habitat; Figure 10). As noted previously, TPCB concentrations in fish from the offshore habitats of the

Central and South basins were not significantly different from each other, indicating that overall basin wide differences (i.e. for all three habitat types within a basin pooled), were due to differences in the estuary and nearshore habitats between these basins. Indeed, post hoc pairwise comparisons indicated that within the Central Basin, mean TPCB concentrations in fish from estuary and nearshore habitats of

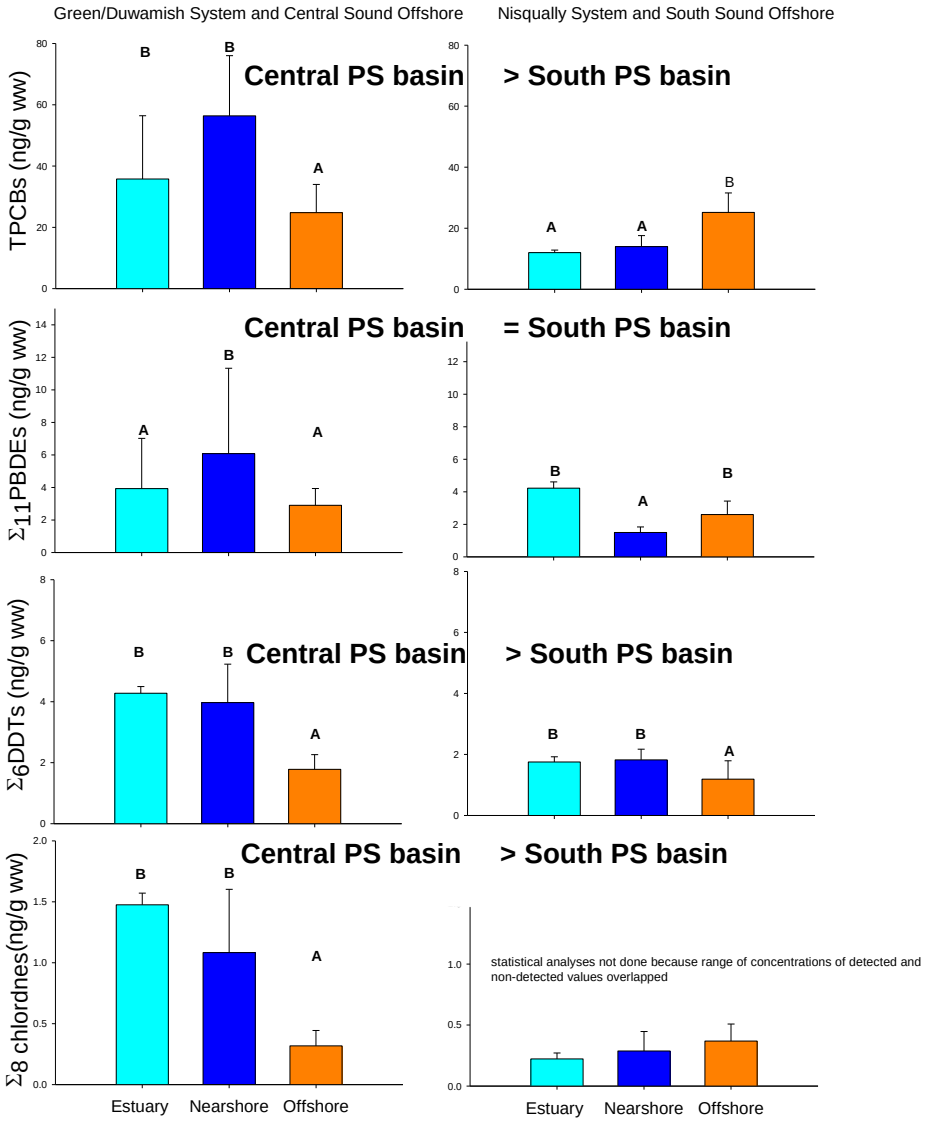

Figure 10. Comparison of geometric means (+95% confidence intervals) of four POPs (ng/g ww) measured in whole body juvenile Chinook salmon (less gills and stomach contents) collected from Central and South basins of Puget Sound in October 2013. Pairwise comparisons (GLM and Tukey's Honestly Significant Difference Test) between the two basins and the three habitat types within each basin (A-B) are shown. Similar letters signify no significant difference (p >0.05). Note that statistical analyses were not performed for $\sum_8$chlordanes among habitat types within South Basin because of a high number of non-detected values. Light blue = estuary fish, dark blue = nearshore fish, and orange = offshore fish

the Green/Duwamish river system were not significantly different from each other, but fish in both habitats had significantly higher TPCBs concentrations than those from offshore habitat in the Central Basin (Figure 10). In contrast, within the South Basin, fish from the estuary and nearshore habitats of the Nisqually system also had similar TPCBs concentrations but in this case, they both had significantly

lower TPCB concentrations than fish in the offshore habitat of South Basin (Figure 10). For habitat types combined across these two basins, mean TPCBs were not significantly different among fish collected from estuary, nearshore, and offshore habitats (20, 27, 24 ng/g ww, respectively). Also, fish length was not correlated with TPCB levels.

$\sum_{11}$PBDEs

Among offshore habitat samples collected in October, the lowest mean $\sum_{11}$PBDEs concentration was measured in fish from Admiralty Inlet (1.2 ng/g ww), with uniformly higher concentrations in fish from the Whidbey, Central and South basins (4.1, 2.8, and 2.6 ng/g ww, respectively; Figure 8). As was observed for TPCBs, mean $\sum_{11}$PBDEs concentrations in the offshore habitat of the Central and South basins were similar to each other (2.6, and 2.8 ng/g ww, respectively; n = 11, r^2 = 0.02, F = 0.232, df = 1, 9, p = 0.64; Figure 8).

A comparison of $\sum_{11}$PBDE concentrations among habitat type, (all samples from the Whidbey, Central and South basins pooled by habitat type, except as described in the Methods), indicated that $\sum_{11}$PBDEs levels were determined by both habitat type and fish length, accounting for 18.9% of the variation among samples (GLM on ln$\sum_{11}$PBDEs with habitat type, fish length and interaction terms; n = 79; r^2 = 0.189; $F_{habitat}$ = 4.687, df = 2, 75, p = 0.012; F_{length} = 13.313, df = 1, 75, p < 0.001). However, further visual analyses (not shown) revealed only a weak inverse relationship between $\sum_{11}$PBDEs concentration and fish length. $\sum_{11}$PBDE concentrations were highest in the smaller estuary fish (mean, 5.0 ng/g ww and mean, 60.4 mm), intermediate in mid-sized nearshore fish (mean, 3.6 ng/g ww and mean, 83.9 mm) and lowest in in the offshore fish that were also much larger (mean, 2.8 ng/g ww and mean, 156 mm), confounding the interpretation of these results. It was not possible to test if the lower $\sum_{11}$PBDE concentrations detected in fish from offshore habitats compared to those in estuary and nearshore habitats (Figure 8) were associated with location (i.e. habitat type) or with fish size. Accordingly, we did not complete post-hoc pairwise comparisons for this habitat comparison.

A comparison of $\sum_{11}$PBDE concentrations among basins as units, (all samples from estuary, nearshore, and offshore, habitats pooled within basins, except as described in the Methods) revealed that basins accounted for 15.2% of the observed variation in $\sum_{11}$PBDEs (GLM on ln$\sum_{11}$PBDEs with basin, fish length and interaction terms; n = 79, F_{basin} = 6.802, df = 2, 76, p = 0.002). Fish length was not significantly correlated with $\sum_{11}$PBDEs and the basin*interaction term was also not significant. Mean $\sum_{11}$PBDEs levels in fish collected from the South Basin (2.1 ng/g ww) were significantly lower than those collected from the Central and Whidbey basins (4.5 and 4.4 ng/g ww, respectively, Figure 9). Fish from the Whidbey Basin had mean $\sum_{11}$PBDE levels that were not significantly different than those from the Central Basin (Figure 9).

The comparison of $\sum_{11}$PBDE concentrations among habitat and basins combined indicated that most (40.4%) of the variation in $\sum_{11}$PBDEs in juvenile salmon was related to location, specifically to the basin specific difference in $\sum_{11}$PBDEs accumulation in the estuary, nearshore, and offshore habitats (i.e., basin*habitat interaction; GLM on ln $\sum_{11}$PBDEs with basin, habitat, fish length and interaction terms; n = 39, r^2 = 0.404, $F_{basin*habitat}$ = 12.209, df = 2, 36, p < 0.001). In the Central Basin (represented by the Green/Duwamish river system and the associated offshore habitat) and South Basin (represented by the

Nisqually river systems and the associated offshore habitat), mean $\sum_{11}$PBDE levels were not significantly different between the Central and South basins as units (3.7 and 2.1 ng/g ww; Figure 10), or among habitat types as units (estuary = 3.5, nearshore = 2.7, and offshore = 2.6 ng/g ww). $\sum_{11}$PBDE concentrations were not correlated with fish length. However, post hoc pairwise comparisons indicate that within the Central Basin, the concentration of $\sum_{11}$PBDEs in fish from estuary and offshore habitats were not significantly different from each other but they were both significantly less those measured in fish from the nearshore habitat (Figure 10). Within the South Basin, fish from the offshore and estuary habitats also had similar $\sum_{11}$PBDEs concentrations, but in contrast to the Central Basin, both were significantly higher than those observed in fish from the nearshore habitat (Figure 10).

$\sum_6$DDTs

The concentrations of $\sum_6$DDTs in fish in the offshore habitats ranged from 0.63 to 2.6 ng/g ww. Among offshore habitats sampled in October, the mean $\sum_6$DDTs concentrations were lowest in fish collected from Admiralty Inlet (0.89 ng/g ww), and uniformly higher in fish collected from the Whidbey, Central and South basins (1.7, 1.7, and 1.4 ng/g ww, respectively). The $\sum_6$DDTs concentrations in the offshore habitat of the Central and South basins, the only sites with sufficient samples sizes to complete statistical analyses, were not significantly different from each other (n = 11, r^2 = 0.02, F = 4.245, df = 1, 9, p = 0.069; Figure 8). Within the Central Basin, fish collected in July and October had similar mean $\sum_6$DDTs concentrations (1.6, and 1.7 ng/g ww, respectively; n = 11, r^2 = 0.02, F = 0.188, df = 1, 9, p = 0.675; Figure 8).

A comparison of $\sum_6$DDT levels among habitat types (all samples from the Whidbey, Central and South basins pooled by habitat type, except as described in the Methods), indicated that location differences associated with habitat type only accounted for 17.5% of the observed variation in $\sum_6$DDT concentrations among samples (GLM on ln$\sum_6$DDTs with habitat type, fish length and interaction term`s; n = 79, r^2 = 0.175, $F_{habitat}$ = 8.069, df = 2, 76, p < 0.001). Fish length and fish and habitat*length interaction terms were not significantly correlated with $\sum_6$DDT levels. Post hoc tests indicated that mean $\sum_6$DDTs in fish from estuary and nearshore habitats were similar to each other (2.7 and 2.4 ng/g ww, respectively) and both had significantly higher mean $\sum_6$DDT levels than fish in the offshore habitats (1.4 ng/g ww; Figure 8). However, because the fish in offshore habitats were also larger than fish from the estuary and nearshore habitats, we cannot rule out that fish size was a factor affecting the variation in$\sum_6$DDT levels among fish samples.

A comparison of $\sum_6$DDTconcentrations among basins as units (all samples from estuary, nearshore, and offshore, habitats pooled within basins, except as described in the Methods) revealed that basin and length accounted for 45.6% and 16.7% of the observed variation in $\sum_6$DDT levels among whole body fish samples (GLM on ln$\sum_6$DDTs with basin, fish length and interaction terms; n = 79, r^2 = 0.623, F_{basin} = 45.439, df = 2, 75, p < 0.001; F_{length} = 33.003, df = 1, 75, p < 0.001). Central Basin fish had significantly higher mean $\sum_6$DDT levels than those collected from South Basin and were also greater than those from the Whidbey Basin (3.5, 1.5, and 1.9 ng/g ww, respectively). Fish from South Basin and the Whidbey Basin had similar $\sum_6$DDTs concentrations (Figure 9).

The comparison of $\sum_6$DDT concentrations among the basins combined indicated that the most (75.6%) of the variation in $\sum_6$DDTs in juvenile salmon was related to location, specifically to the basin differences (46.3%) and an additional 29.2% in habitat differences (GLM on ln $\sum_6$DDTs with basin, habitat, fish length and interaction terms; n = 39, r^2 = 0.756, F_{basin} = 62.11, df = 1, 35, p < 0.001; $F_{habitat}$ = 21.11, df = 2, 35, p < 0.001). Post hoc analysis indicated that $\sum_6$DDT levels were significantly higher in fish from the Central Basin (represented by the Green/Duwamish river system and the associated offshore habitat) than the South Basin (represented by the Nisqually river systems and the associated offshore habitat; Figure 10). Likewise, post hoc tests indicate that when habitats are considered as units, $\sum_6$DDT levels in fish from estuary and nearshore habitats were not significantly different from each other, but they had significantly higher $\sum_6$DDT levels than those measured in fish from the offshore habitat (Figure 10). Fish length was not a significant factor affecting $\sum_6$DDT concentrations in these groups and no other interaction terms were significant.

$\sum_8$Chlordanes

Among offshore habitats sampled in October, the mean $\sum_8$chlordanes concentration was lower in fish collected from Admiralty Inlet (0.89 ng/g ww) than those from the Whidbey, Central and South basins 1.7, 1.7, and 1.4 ng/g ww, respectively). Of these, the concentrations of $\sum_8$chlordanes in the Central and South basins, the only sites with sufficient samples sizes to complete statistical analyses, were not significantly different from each other (n = 11, r^2 = 0.04, F = 0.36, df = 1, 9, p = 0.56; Figure 11). Within the Central basin, fish collected in July and October also had similar mean $\sum_8$chlordanes concentrations. (1.6, and 1.7 ng/g ww, respectively; n = 11, r^2 = 0.221, F = 2.557, df = 1, 9, p = 0.144; Figure 11).

A comparison of $\sum_8$chlordane levels among habitat types (all samples from the Whidbey, Central and South basins pooled by habitat type, except as described in the Methods), indicated that the variation in $\sum_8$chlordane levels among samples was not explained by location difference associated with habitat type alone (Figure 11). A GLM on ln$\sum_8$chlordanes with habitat type, fish length and interaction terms indicated that the best fit model included habitat type and habitat*length, which collectively only accounted for 15.9 % of the variation, (n = 79, r^2 = 0.159, $F_{habitat}$ = 5.593, df = 2, 73, p < 0.01; $F_{habitat*length}$ = 5.352, df = 2, 73, p < 0.01), however, neither factor was significant on its own. Further visual examination of the relationship between mean fish length and habitat types with mean $\sum_8$chlordane concentrations (not shown) revealed that the intermediate sized fish from the nearshore habitats generally had higher concentrations (0.52 ng/g ww and mean, 83.9 mm) than the smaller fish from the estuary habitat (0.42 ng/g ww and mean, 60.4 mm) and the larger fish from the offshore habitat (0.32 ng/g ww and 156 mm). However, fish length was not correlated with $\sum_8$chlordane concentrations in any habitat type, suggesting that neither habitat, fish length, nor the habitat*length interaction were important variables explaining the observed variation in $\sum_8$chlordane concentrations. Thus, post hoc tests were not run for this habitat comparison.

A comparison of $\sum_8$chlordane levels among basins as units, with samples from estuary, nearshore and offshore habitats within a basin pooled indicated that overall, basin and the basin*length interaction accounted for 49.3% and 15.1% of the observed variation in $\sum_8$chlordanes among whole body fish samples (GLM on ln$\sum_8$chlordanes with basin, fish length and interaction terms; n = 79, r^2 = 0.644, F_{basin} = 35.423, df = 2, 76, p < 0.001; $F_{basin*length}$ =15.705, df = 2, 76, p < 0.001). In each basin, $\sum_8$chlordane

concentrations were generally higher in larger fish sampled in the offshore habitats; however, the positive relationship between fish size and $\sum_8$chlordanes was more evident in the South and Whidbey Basins than for fish from the Central Basin. Post hoc tests in this more general basin-wide comparison indicated mean $\sum_8$chlordanes levels in fish collected from the Whidbey Basin were similar to those from South Basin and less than those from the Central Basin (0.29, 0.27, and 1.1 ng/g ww, respectively; Figure 9).

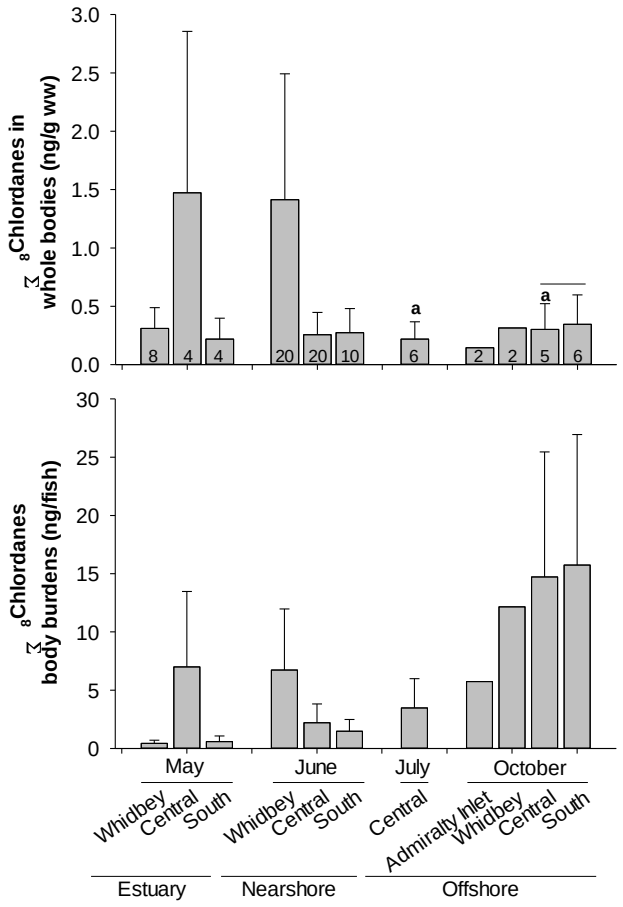

Figure 11. Comparison of geometric means (+95% confidence intervals) of $\sum_8$chlordanes concentrations (ng/g ww) and body burdens (ng/g fish) measured in juvenile Chinook salmon whole body samples (less gills and stomach contents) collected from estuary, nearshore and offshore habitats within four major Puget Sound basins. Numbers within the bars of the top figure indicate sample size. Pairwise comparisons (GLM and Tukey's Honestly Significant Difference Test) indicate no significant difference ($p > 0.05$) between July and Oct. offshore samples from the Central Basin (noted by lower case letter a) and between Oct. offshore samples in the Central and South (shown with a horizontal solid line), the only offshore sites with sufficient samples sizes for statistical comparisons. Post hoc pairwise comparisons were not run to compare $\sum_8$chlordanes among habitat types because there was a significant habitat*length interaction (see text for details).

A comparison of $\sum_8$chlordanes in whole body samples of juvenile salmon among basins and habitat types combined was not completed for the offshore habitats of the Green/Duwamish and Nisqually systems, as had been done for the other POPs. In the South Basin, detected $\sum_8$chlordane concentrations in fish samples were very low, often less than the LOQ for other samples within this basin, such that comparison of these data among habitats would not have provided meaningful information. In contrast,

detected $\sum_8$chlordane levels in whole body fish samples within the Central Basin were consistently detected above the LOQ range, indicating that juvenile Chinook salmon from the Central Basin had significantly greater concentrations than those from South Basin (Figure 10). A comparison of $\sum_8$chlordanes in samples among the habitat types of the Central Basin indicated that most (70.4%) of the variation in $\sum_8$chlordane levels was related to habitat differences (GLM on ln $\sum_8$chlordanes habitat, fish length and interaction terms; n = 19, $r^2 = 0.704$, $F_{habitat} = 19.007$, df = 2, 16, $p < 0.001$). Post hoc test indicated that, like $\sum_6$DDTs, $\sum_8$chlordane levels were significantly lower in fish from offshore habitats of the Central Basin than those in the estuary and nearshore habitat of the Green/Duwamish system, which were also similar to each other (Figure 10).

HCB

HCB was detected in 10 of the 21 offshore habitat samples (five in South Basin, four in the Central Basin, and one in the Whidbey Basin). Detected concentrations ranged from 0.14 – 0.35 ng/g www, however, only four of the samples with detected values (19% of all samples) were above the range of non-detected value (0.13 – 0.22 ng/g ww). Because HCB was only detected above the LOQ range in 19% of the samples, we did not make statistical comparisons among basins or among estuary, nearshore and offshore habitats.

Dieldrin

Dieldrin was only detected in one sample from the offshore habitats, a sample from the Whidbey Basin, (0.12 ng/g ww). The LOQ values in the remaining 20 offshore habitats samples ranged from 0.11 ng/ g to 0.17 ng/g ww and therefore, statistical comparisons among basins or among estuary, nearshore, and offshore habitats were not completed.

PAHs in Salmon Stomach Contents

PAH Accumulation in Estuary and Nearshore Habitats

Though PAHs were found in stomach contents of juvenile Chinook salmon from all sites, the number of individual PAHs detected among sites varied from 10 to 100%. Overall, the $\sum_{42}$PAHs concentrations ranged from 2.1 to 32,000 ng/g ww, with lowest and highest values occurring at the Nisqually and Snohomish nearshore sites, respectively. Low molecular weight (LMW) PAHs were detected more frequently than the high molecular weight (HMW) PAHs in fish stomachs, with the mean LMW:HMW concentration ratio at 2.3 and 1.2 in estuaries and nearshore habitats, respectively (Figure 12). Though one of the four composite samples taken from the Snohomish nearshore 2 site was a high outlier, with PAH concentrations two orders of magnitude higher than the other three replicates ($\sum_{42}$PAHs replicate 3 = 32,400 ng/g ww; mean for other three replicate samples from that site = 170 ng/g ww ±SD = 86.6), the result was not considered a spurious measurement and was retained in the analyses described below. In addition, summary statistics were calculated for each collection location as means, medians, and 25th and 75th percentiles (APPENDIX D: Summary Statistics of Polycyclic Aromatic Hydrocarbons Measured in Juvenile Chinook Salmon Stomach Contents).

The $\sum_{42}$PAH concentrations in stomach contents taken from the four systems tested as individual units (Skagit, Snohomish, Green/Duwamish, and Nisqually) differed significantly from one another (n = 26, r^2 =

0.725, F = 19.378, df = 3, 22, p < 0.001). Pairwise testing revealed mean $\sum_{42}$PAH concentrations in stomach contents taken from the Nisqually and Skagit systems (17 and 35 ng/g ww, respectively) were significantly lower than those collected from the Snohomish and Green/Duwamish systems (5,800 and 4,300 ng/g ww, respectively; Figure 12). Though not included in the statistical analysis, stomach contents taken from the Hylebos/Puyallup system (mean, 440 ng/g ww) appeared to have $\sum_{42}$PAH concentrations somewhat intermediate to the other systems. Within the Nisqually system, the only

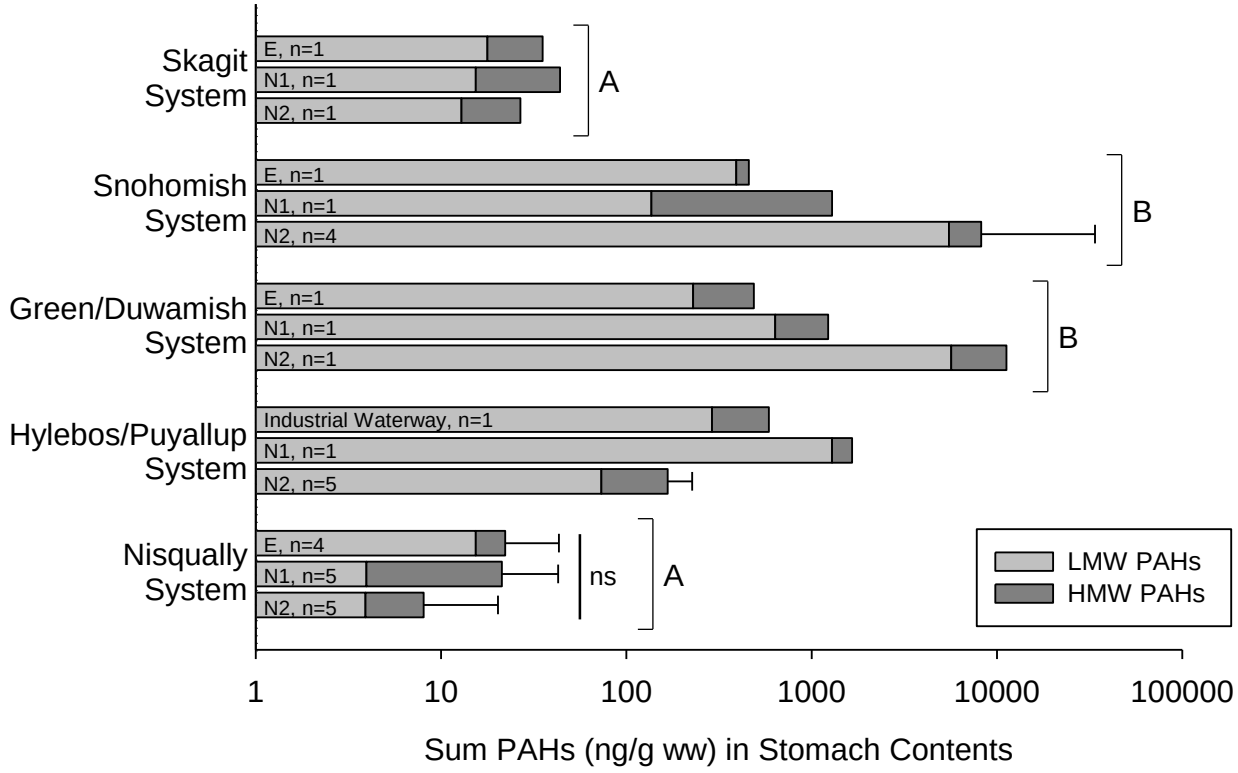

Figure 12. Comparison of means (+95% confidence intervals) of summed polycyclic aromatic hydrocarbons ($\sum_{42}$PAHs, ng/g ww) from juvenile Chinook salmon stomach contents collected from five major river systems (estuary and nearshore marine sites depicted separately) in Puget Sound, WA (E = estuary, N1 = nearshore marine 1, N2 = nearshore marine 2). Similar letters signify no significant difference (p > 0.05) in pairwise comparisons between systems, and ns = no significant difference among habitats within the one system, Nisqually, where sample size allowed a statistical comparison (GLM and Tukey's Honestly-Significant-Difference Test). LMW = low molecular weight, HMW = high molecular weight

system with enough replication to allow for within-system statistical comparison, there were no significant differences (n = 14, r^2 = 0.340, F = 2.839, df = 2, 11, p = 0.101; Figure 12) in stomach contents taken from the estuary habitat (mean, 22.2 ng/g ww) or either of the two nearshore habitats sampling sites (means, 21 and 8.1 ng/g ww, respectively).

Unfortunately, there was not enough replication between the estuary samples to allow for statistical comparison among the various systems (Figure 13). However, visual inspection suggests fish from the Skagit and Nisqually estuary habitats may be exposed to lover overall $\sum_{42}$PAHs in their diets than fish from the Snohomish and Green/Duwamish estuary habitats, or from the Hylebos Waterway.

Among nearshore habitats, similar to the systems as a whole, sum $\sum_{42}$PAH concentrations in stomach contents of juvenile Chinook salmon taken from the three nearshore habitats (Snohomish, Hylebos/Puyallup, and Nisqually) were significantly different from one another (n = 21, r^2 = 0.672, F =

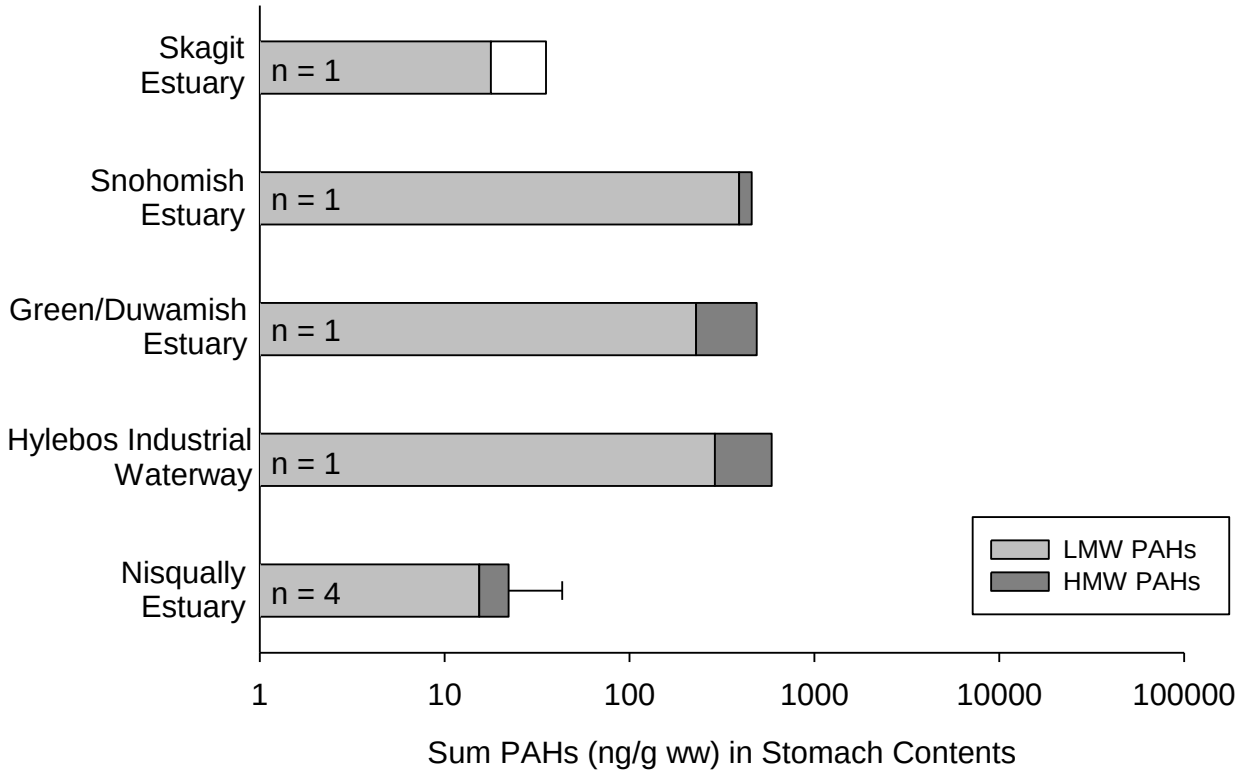

Figure 13. Comparison of means (+95% confidence intervals) of summed polycyclic aromatic hydrocarbons ($\sum_{42}$PAHs, ng/g ww) from juvenile Chinook salmon stomach contents collected within five estuary habitats in Puget Sound, WA. n = number of composites, LMW = low molecular weight, HMW = high molecular weight. Low sample size precluded a statistical comparison.

18.398, df = 2, 18, p < 0.001; Figure 14). Mean $\sum_{42}$PAH concentrations in fish stomach contents from the Nisqually nearshore (15 ng/g ww) were significantly lower than those from the Snohomish and Hylebos/Puyallup nearshore areas (6,800 and 420 ng/g ww, respectively). Though not included in the statistical analysis, mean $\sum_{42}$PAH concentration in the Skagit nearshore stomach content samples (35 ng/g ww) were closer to those taken from the Nisqually nearshore, while the Green/Duwamish nearshore concentration (6,300 ng/g ww) was closer to the Snohomish and Hylebos/Puyallup nearshore concentrations(Figure 14).

Taken together, mean $\sum_{42}$PAH concentrations in stomach contents of juvenile Chinook salmon collected in estuary habitats (250 ng/g ww; excluding the Hylebos Industrial Waterway sample = 590 ng/g ww) were about 10 times lower than those in fish from nearshore habitats (2,700 ng/g ww), though these two groups (all estuaries – excluding Hylebos Industrial Waterway vs. all nearshore habitats) were not significantly different from each other (n = 32, r^2 = 0.007, F = 0.212, df = 1, 30, p = 0.649).

PAH Accumulation in Offshore Habitats

PAHs were detected in the stomach contents of Chinook salmon taken from all of the offshore habitats of each basin sampled (Admiralty Inlet, Whidbey Basin, Central Basin, and South Basin), though the

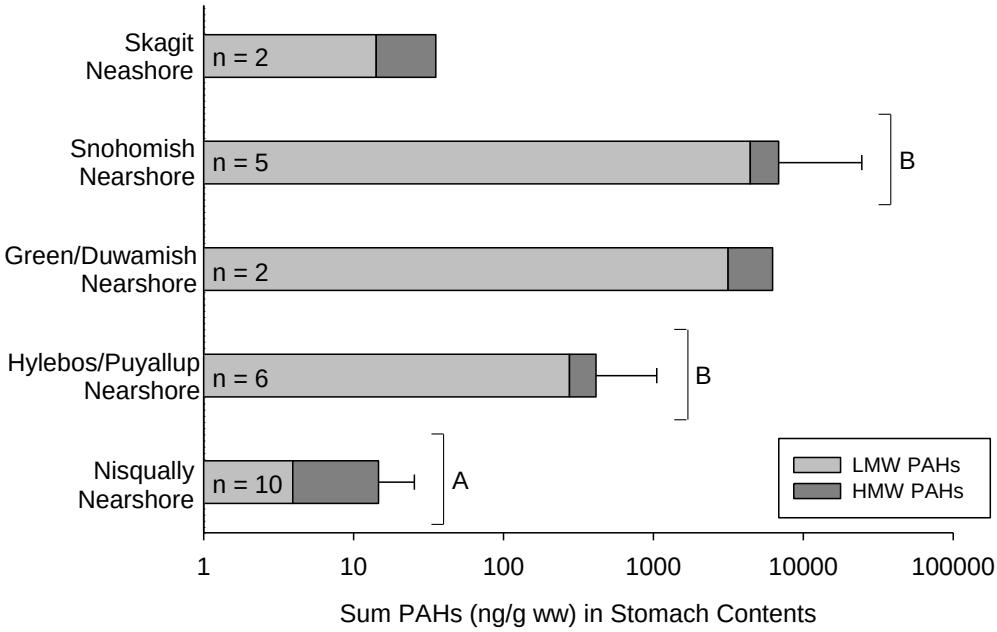

Figure 14. Comparison of means (+95% confidence intervals) of summed polycyclic aromatic hydrocarbons ($\sum_{42}$PAHs, ng/g ww) from juvenile Chinook salmon stomach contents collected within five nearshore habitats in Puget Sound, WA. n = number of composites, LMW = low molecular weight, HMW = high molecular weight. Similar capital letters signify no significant difference (p > 0.05) in pairwise comparisons between nearshore areas where sample size was sufficient to conduct a statistical comparison (GLM and Tukey's Honestly-Significant-Difference Test). LMW = low molecular weight, HMW = high molecular weight.

number of individual PAHs detected the samples varied from 5 to 83%. Overall, the $\sum_{42}$PAHs concentrations in offshore habitats ranged from 2.0 ng/g ww in samples from South Basin to 230 ng/g.ww in fish from the Whidbey Basin. Mean concentrations are presented in Figure 15. Due to a lack of detection for many of the HMW PAHs, the ratio of LMW:HMW PAHs could only be calculated for eight of the 20 offshore samples. For these eight samples, the concentrations of LMW PAHs was greater than HMW PAHs in all but one offshore Chinook stomach content sample (Central Basin, sample 13CPS-TS14, LMW:HMW = 0.79) and the mean ratio was 2.99.

Due to a shortage of replicates (Admiralty Inlet and Whidbey Basin, n = 2 for both), the only statistical comparison made between the offshore samples was a t-test between the Central Basin and South Basin stomach contents; no significant difference was detected (t = 0.821, df = 9, p = 0.433; Figure 15). However, when data were pooled to investigate differences between the three habitat types (Table 5), we found significantly higher mean$\sum_{42}$PAHs levels in fish gut contents from nearshore areas (2,000 ng/g ww) relative to offshore areas (21.0 ng/g ww; n = 45, r^2 = 0.235, F = 6.442, df = 2, 42, p = 0.004; Figure 15). Mean $\sum_{42}$PAH concentrations in stomach contents taken from estuaries (150 ng/g ww) were intermediate between the nearshore and offshore areas. However, mean $\sum_{42}$PAH levels in stomach content samples collected from the nearshore areas of the Whidbey and Central basins (4,900 and 1,900

ng/g ww, respectively) were significantly higher than levels detected in samples from the nearshore area of South Basin (15 ng/g ww; n = 25, r^2 = 0.570, F = 14.586, df = 2,22, p < 0.001; Figure 15). These data suggest that the Whidbey and Central basins are mostly responsible for the differences between the nearshore and offshore habitats in the former analysis.

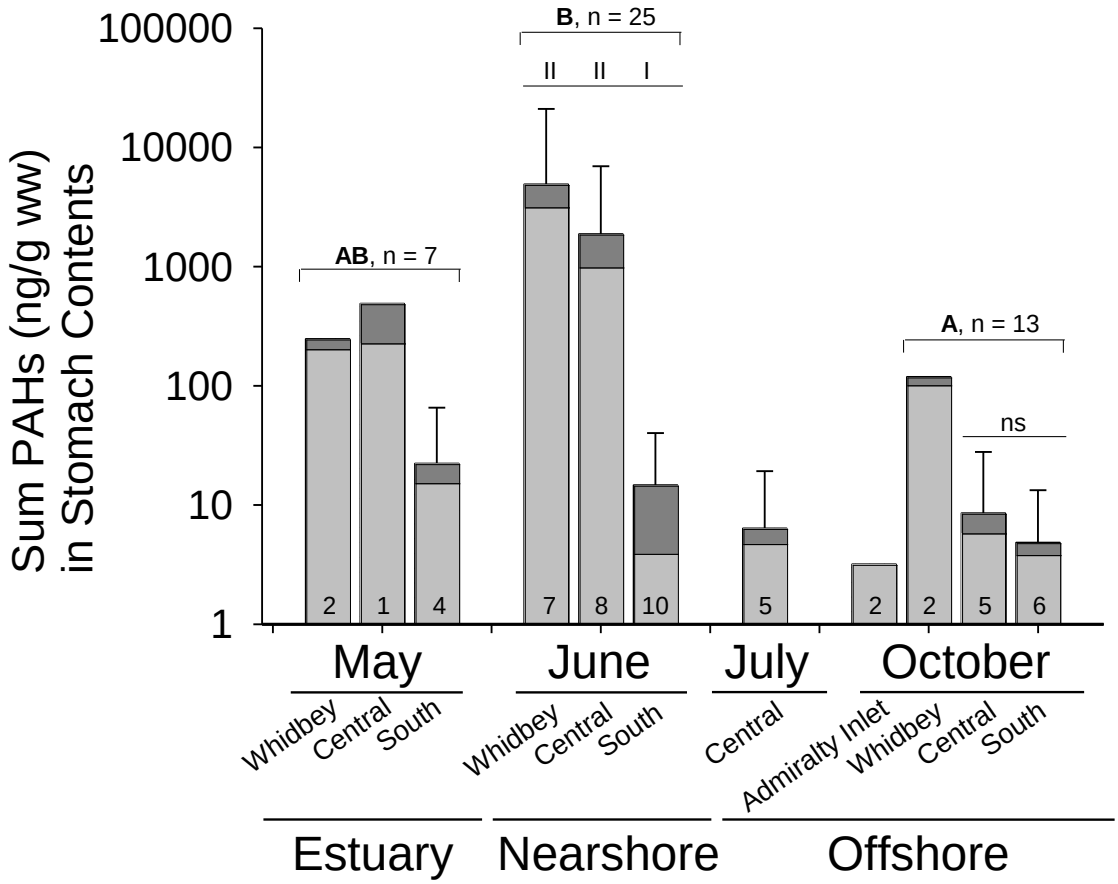

Figure 15. Comparison of means (+95% confidence intervals) of summed PAHs ($\sum_{42}$PAHs; ng/g ww) from juvenile Chinook salmon stomach contents collected from four basins during four months in Puget Sound, WA. Numbers in bars indicate sample size, LMW- PAHs are light grey, and HMW-PAHs are dark grey. Similar letters or Roman numerals signify no significant difference (p > 0.05) between habitat types (letters) and between basins (Roman numeral) in pairwise comparisons (GLM and Tukey's Honestly-Significant-Difference Test). ns = no significant difference (p > 0.05) among offshore habitats in the Central and South Basins, the only Oct. offshore sites with sufficient sample numbers for statistical comparisons.

Additional habitat comparisons were made within two basins, Central Basin and South Basin. In the Central Basin mean $\sum_{42}$PAH levels in stomach contents taken from fish in nearshore areas (June, 1,900 ng/g ww) were significantly higher than fish taken in the offshore area in both July and October (6.4 and 8.5 ng/g ww, respectively; n = 18, r^2 = 0.790, F = 28.158, df = 2, 15, p < 0.001; Figure 16). In the South Basin, mean $\sum_{42}$PAH levels in stomach contents taken from estuaries in May (22 ng/g ww) were significantly higher than offshore samples in October (4.8 ng/g ww), while nearshore samples collected in June (mean, 15 ng/g ww) were not significantly different from those collected in the estuary or the offshore habitats (n = 20, r^2 = 0.304, F = 3.709, df = 2, 17, p < 0.046; Figure 17).

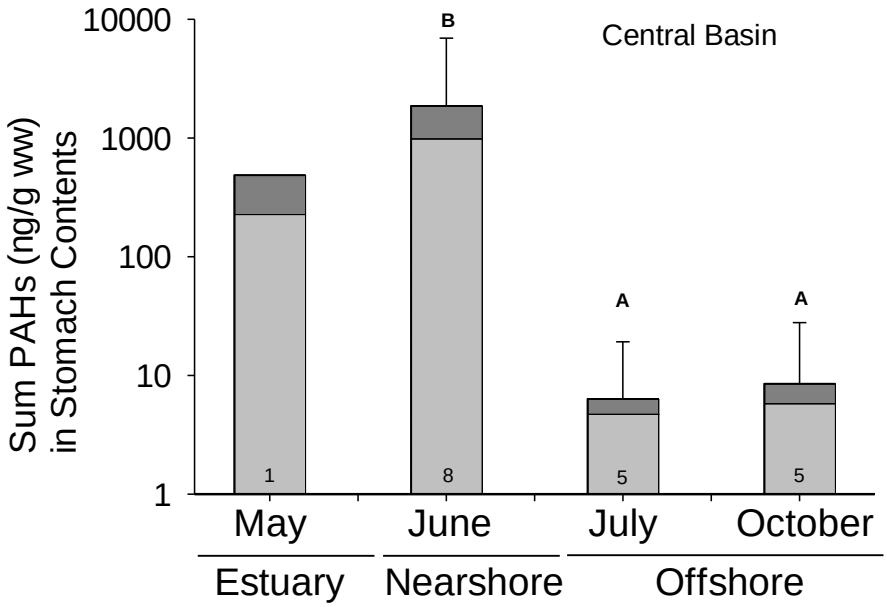

Figure 16. Comparison of means (+95% confidence intervals) of summed PAHs ($\sum_{42}$PAHs; ng/g ww) from juvenile Chinook salmon stomach contents collected from estuary, nearshore and offshore habitats of the Central Puget Sound during four months. Numbers in bars indicate sample size; LMW- PAHs are light grey, and HMW-PAHs are dark grey. Similar letters signify no significant difference (p > 0.05) in pairwise comparisons (GLM and Tukey's Honestly-Significant-Difference Test) for those habitats with sufficient sample size to conduct the statistical test.

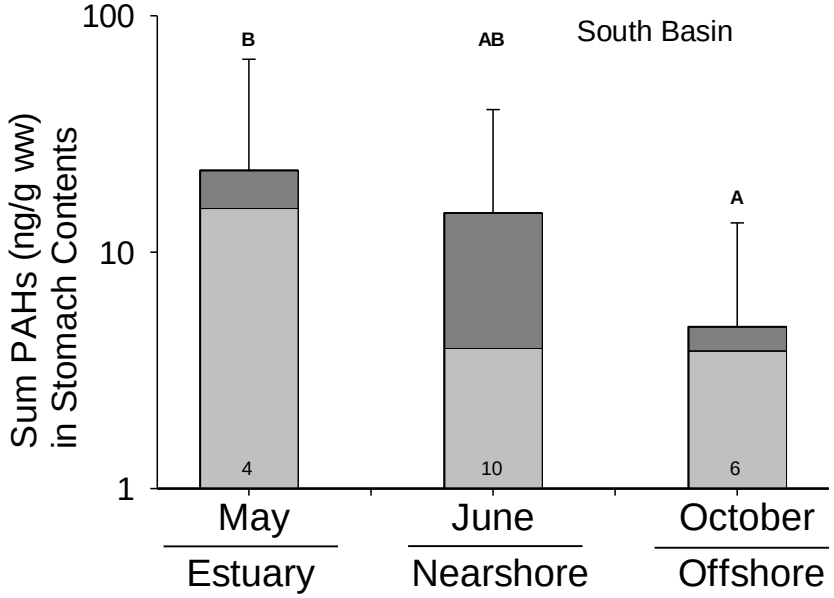

Figure 17. Comparison of means (+95% confidence intervals) of summed PAHs ($\sum_{42}$PAHs; ng/g ww) from juvenile Chinook salmon stomach contents collected from estuary, nearshore and offshore habitat of South Puget Sound during three months. Numbers in bars indicate sample size; LMW- PAHs are light grey, and HMW-PAHs are dark grey. Similar letters signify no significant difference (p > 0.05) in pairwise comparisons (GLM and Tukey's Honestly-Significant-Difference Test) for habitat types with sufficient sample size to conduct the statistical test.

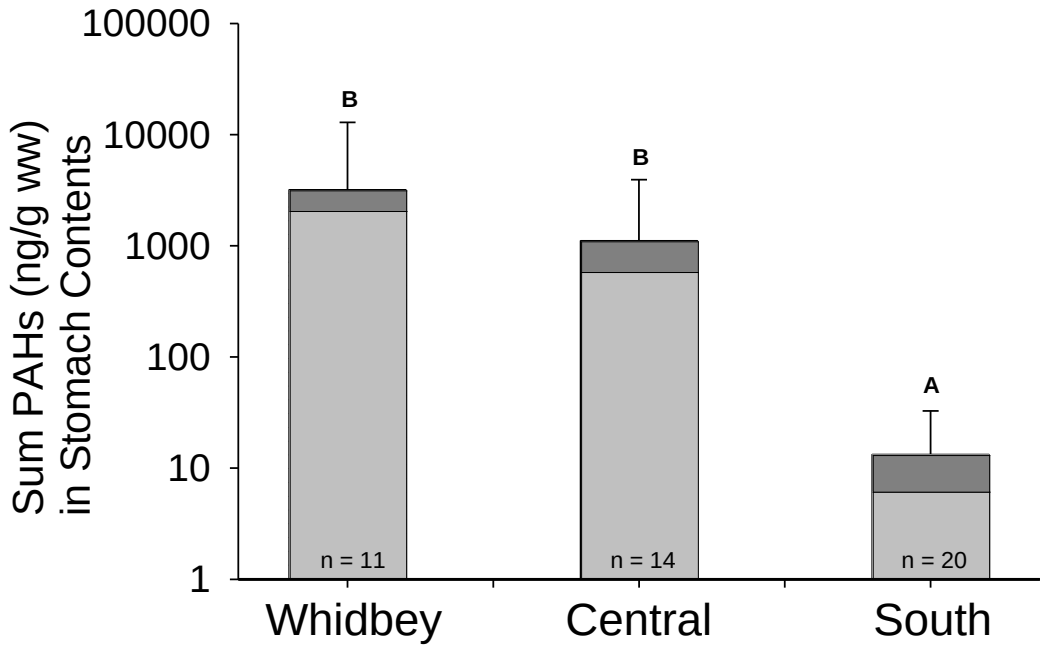

Figure 18. Comparison of means (+95% confidence intervals) of summed PAHs ($\sum_{42}$PAHs; ng/g ww) from juvenile Chinook salmon stomach contents collected within three basins (estuary + nearshore areas + offshore areas pooled for each basin) in Puget Sound, WA. Numbers in bars indicate sample size; LMW- PAHs are light grey, and HMW-PAHs are dark grey. Similar letters signify no significant difference (p > 0.05) between basins in pairwise comparisons.

Trace Metals in Salmon Gills in Estuary and Nearshore Habitats

A total of 67 composite gill tissue samples were analyzed for copper, cadmium, lead, zinc and nickel from the estuary/nearshore systems; offshore samples were not analyzed for trace metals. Summary statistics were calculated for each collection location as means, medians, and 25th and 75th percentiles (APPENDIX E: Summary Statistics of Trace Metals Measured in Juvenile Chinook Salmon Gill Tissue).

Cadmium

Cadmium was detected in all but nine gill samples (n = 67), four from the Green/Duwamish system and five from the Nisqually system (Table 13). Detected cadmium levels ranged from 0.012 to 0.10 mg/kg ww. Excluding the Hylebos/Puyallup system, most (74%) of the variation in cadmium concentrations among samples was related to location, measured as system differences (GLM on Cd with system, habitat, fish length and interaction terms; n = 56, $r^2 = 0.741$, $F_{system} = 49.66$, df = 3, 52, $p < 0.001$; Figure 19). Cadmium concentrations did not vary significantly among habitat types, among habitats within systems, and were not correlated with fish length. Generally, cadmium concentrations were higher in fish from river systems within the Whidbey Basin (i.e., represented by the Skagit and Snohomish system) than those in the Central Basin (represented by the Green/Duwamish system) and the South Basin (represented by the Nisqually system). The lowest mean cadmium concentrations were measured in fish gills collected in the Nisqually and Green/Duwamish systems (0.016, and 0.016 mg/kg ww) which were not significantly different from each other. Intermediate mean cadmium concentrations were detected in fish gills from the Skagit system (0.037 mg/kg ww), significantly higher than the Nisqually and Green/Duwamish systems, but significantly less than those in the Snohomish system (0.069 mg/kg

Table 13. The frequency of detection (%) of five trace metals measured in 67 samples of juvenile Chinook salmon gill tissue (estuary and nearshore fish only). Fish collected in the offshore basins did not have trace metals analysis performed on their gill tissue.

Metal	Frequency of Detection (%)
Zinc	100
Cadmium	91
Copper	100
Lead	90
Nickel	100

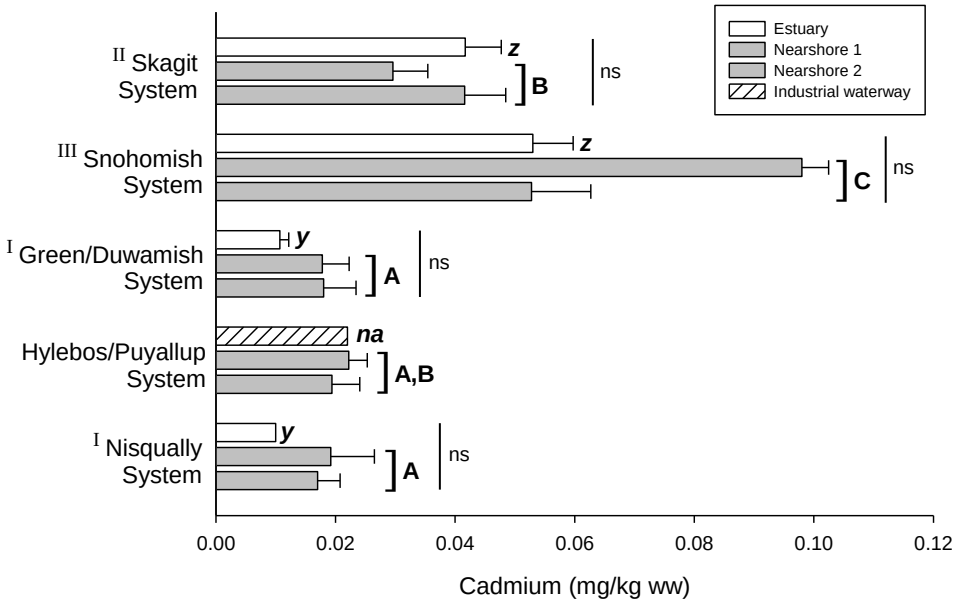

Figure 19. Comparison of means (+ 95% confidence intervals) of cadmium (mg/kg ww), measured in the gills of juvenile Chinook salmon across five major river systems in Puget Sound, WA. Pairwise comparisons of four systems (Roman numerals at left), four estuaries (white bars, *y-z*), five nearshore marine shorelines (gray bars, A-C) are shown. Similar letters or numbers signify no significant difference (p > 0.05). Cadmium was not significantly different between any of the three collection sites within systems (shown as vertical bars with ns = not significant). na = not analyzed.

ww; Figure 19). Overall, cadmium levels in gill samples from the Snohomish system were four times higher than those measured in fish gills from the Nisqually and Duwamish systems and almost twice as high as those in the Skagit system.

In a separate comparison of cadmium among nearshore habitats that included fish from the Hylebos/Puyallup system, cadmium concentrations in fish gill tissues were also significantly different among systems, and were not correlated with fish length (GLM on Cd with system, fish length and interaction terms; n = 50, $r^2 = 0.767$, $F_{system} = 36.95$, df = 4 ,45, $p < 0.001$). Mean cadmium levels in Chinook salmon gills collected in the nearshore habitats followed a similar pattern to the system comparison, with significantly lower concentrations in fish gills from South Basin (Nisqually, 0.018 mg/kg ww), and the Central Basin (Green/Duwamish, and Hylebos/Puyallup; 0.018 and 0.021 mg/kg ww,

respectively) than in the Snohomish nearshore habitats (0.075 mg/kg ww) within the Whidbey Basin. Mean cadmium levels in fish gills collected in the Hylebos/Puyallup nearshore were not significantly different than those in the Green/Duwamish, Nisqually, or the Skagit nearshore (0.036 mg/kg ww), but were significantly different than the Snohomish nearshore (Figure 19).

Copper

Copper was detected in all samples with values ranging from 0.37 to 0.85 mg/kg ww (Table 13). Excluding the Hylebos/Puyallup system, 16% of the variation in copper was related to location measured as system differences (GLM on copper with system, habitat, fish length and interaction terms; n = 56, r^2 = 0.231, F_{system} = 3.303, df = 3, 52, p = 0.027). The lowest mean copper concentration was measured in gills from juvenile Chinook salmon collected in the Snohomish system (0.51 mg/kg ww), similar to those in fish from the Skagit and the Nisqually systems (0.56 and 0.58 mg/kg ww, respectively). Fish collected in the Green/Duwamish system (0.62 mg/kg) had significantly higher mean copper levels than those from the Snohomish systems. No other significant differences in copper among systems were observed (Figure 20). Mean copper concentrations were not significantly different between estuary and nearshore habitats as units (0.56 and 0.60 mg/kg ww, respectively) and were not correlated with fish length. In addition, within each of the Skagit, Snohomish, Green/Duwamish and Nisqually systems, mean copper levels were similar in estuary and nearshore habitats (0.64 vs. 0.53, 0.54 vs. 0.50, 0.57 vs. 0.64, and 0.51 vs. 0.60, respectively; Figure 20). Copper concentrations in gill tissues did not differ significantly among the four estuaries (Figure 20) or the four nearshore habitats.

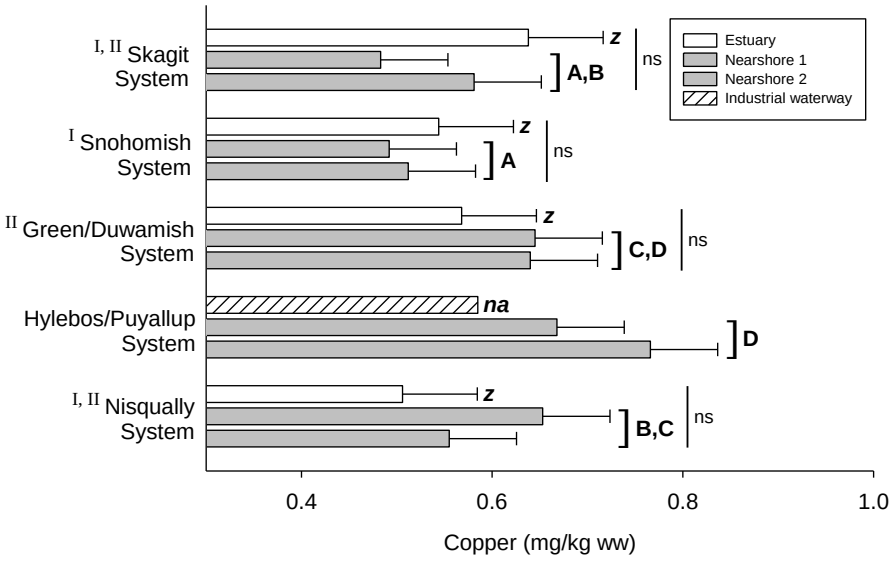

Figure 20. Comparison of means (+ 95% confidence intervals) of copper (mg/kg ww), measured in the gills of juvenile Chinook salmon across five major river systems in Puget Sound, WA. Pairwise comparisons of the five systems (Roman numerals at left), four estuaries (white bars, *y-z*), five nearshore marine shorelines (gray bars, A-D) and the three collection sites within systems (all ns = not significant from each other) are also shown. Similar letters or numbers signify no significant difference (p > 0.05). na = not analyzed.

In contrast, in a separate comparison of copper among nearshore habitats that included the fish from the Hylebos/Puyallup system, concentrations of copper in fish gill tissues were significantly different among nearshore habitat systems, accounting for 48% of the observed variation, but was not correlated

with fish length (GLM on copper with system, fish length and interaction terms; n = 50, r^2 = 0.475, F_{system} = 10.189, df = 4, 45, p < 0.001). Mean copper concentrations in Hylebos/Puyallup nearshore samples (0.72 mg/kg ww) were significantly higher than all other nearshore habitats except the Duwamish system (0.64 mg/kg ww; Figure 20). In addition, gill tissue from fish collected in the Green/Duwamish contained mean copper levels that were significantly higher than fish from the Snohomish and Skagit systems (0.50 and 0.53 mg/kg ww, respectively) but not the Nisqually system (0.60 mg/kg ww; Figure 20).

Lead

Lead was detected in all but seven gill samples (n = 67), two from the Nisqually system and five from the Snohomish system (Table 13). Detected values ranged from 0.019 to 0.48 mg/kg ww. Overall, the only significant factor accounting for variation in lead concentration among samples was location, in particular system-specific habitat differences (i.e., system*habitat factor) accounting for 21% of the total variation (GLM on lead within system, habitat, fish length and interaction terms; n = 56, r^2 = 0.215, $F_{system*habitat}$ = 4.747, df = 3, 52, p = 0.005). Lead concentrations did not vary significantly among systems, among habitat types, and were not correlated with fish length. Post hoc pairwise comparison indicated that mean lead gill concentrations were similar between fish caught in estuary and nearshore habitats within each of the Nisqually (0.041 and 0.033 mg/kg ww), Skagit (0.093 and 0.047 mg/kg ww) and Snohomish (0.15 and 0.026 mg/kg ww) systems. In contrast, mean lead levels in fish gills from the Green/Duwamish estuary (0.069 mg/kg ww) were lower than those from the nearshore habitat (0.12 mg/kg ww; Figure 21). Among the four estuary habitats, the lowest mean lead levels were measured in fish gills from the Green/Duwamish estuary (0.069 mg/kg ww), similar to those from the Nisqually and Skagit estuaries (0.041 and 0.093 mg/kg w ww), but significantly lower than those from the Snohomish estuary (0.15 mg/kg ww; Figure 21). No other significant differences in lead levels were measured in fish gills among the estuary habitats. In contrast to the estuary comparison, among the nearshore habitats, mean lead concentrations in fish gills from the Green/Duwamish nearshore habitat (0.12 mg/kg ww) were significantly higher than those from the Snohomish (0.026 mg/kg ww) but were similar to those from the Skagit and Nisqually nearshore habitats (0.047 and 0.033 mg/kg ww; comparison not shown in Figure 21). No other significant differences in mean lead levels were measured in fish gill tissue among the four nearshore habitats.

In a separate comparison of mean lead levels in gill tissue among nearshore habitats that included the fish from the Hylebos/Puyallup system, system difference explained about 37% of the variation among samples, (GLM on lead with system, fish length and interaction terms; n = 50, r^2 = 0.574, F_{system} = 15.14, df = 4, 45, p < 0.001), and length was not correlated with lead levels. As with the four system nearshore comparisons above, mean lead concentrations in gill tissues were also highest in fish from the Green Duwamish nearshore habitat (0.12 mg/kg ww), similar to those from the Hylebos/Puyallup nearshore (0.086 mg/kg ww), but significantly higher than those from Skagit, Nisqually and Snohomish nearshore habitats (0.047, 0.033, and 0.026 mg/kg ww; Figure 21). No other significant differences were measured in lead in fish gill tissue among the five nearshore habitats.

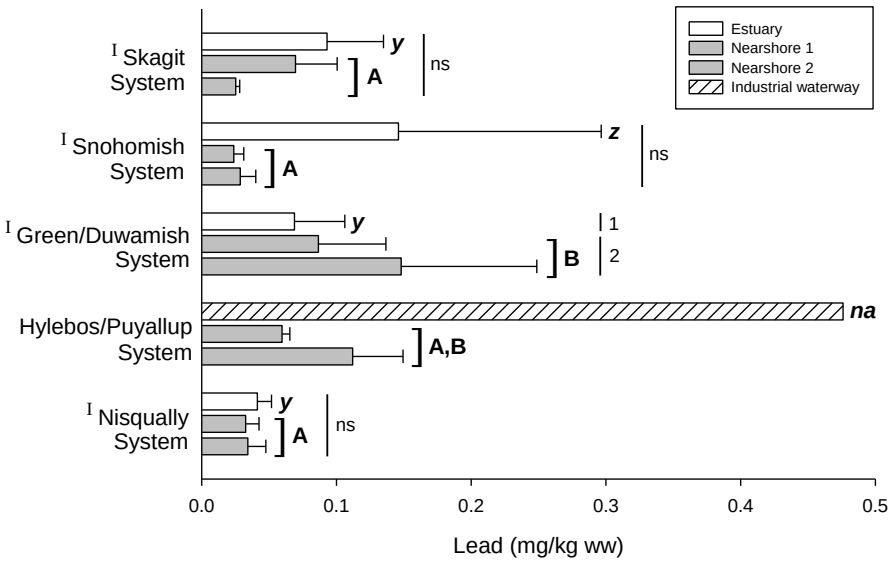

Figure 21. Comparison of means (+ 95% confidence intervals) of lead (mg/kg ww), measured in the gills of juvenile Chinook salmon collected from five major river systems in Puget Sound, WA. Pairwise comparisons of the five systems (Roman numerals at left), four estuaries (white bars, y,*z*), five nearshore marine shorelines (gray bars, A-B), and the three collection sites within systems (Arabic numerals) are also shown. Similar letters or numbers signify no significant difference (p > 0.05). ns = not significant; na = not analyzed.

Nickel

Nickel was detected in all gill tissue samples (n = 67), with detected values ranging from 0.028 to 0.23 mg/kg ww (Table 13). Excluding the Hylebos/Puyallup system, most (88%) of the variation in nickel was related to location, including the system, habitat, or system specific habitat differences between estuary and nearshore habitats (GLM on nickel with system, habitat, fish length and interaction terms; n = 56, r^2 = 0.88, F_{system} = 68.84, df = 3, 48, p < 0.001; $F_{habitat}$ = 100.953, df = 1, 48, p < 0.001; $F_{system*habitat}$ = 49.949, df = 3, 48, p < 0.001). Nickel concentrations were not correlated with fish length. Nickel concentrations varied significantly among systems; post hoc pairwise comparisons indicated that mean nickel gill concentrations in the Skagit system (0.11 mg/kg ww) were significantly higher than those in fish from Snohomish, Nisqually, and Green/Duwamish systems (0.051, 0.052, and 0.058 mg/kg ww), which were similar to each other.

Overall, mean nickel concentrations were significantly higher in gill tissue from fish caught in estuaries than those in the nearshore (0.098 and 0.054 mg/kg, respectively), however, this pattern was driven by the higher nickel concentrations in the Skagit estuary. Within the Skagit system, mean concentrations of nickel in fish gill tissue from the estuary (0.20 mg/kg ww) were significantly higher than levels in the nearshore habitat (0.061 mg/kg ww). In each of the Snohomish, Green/Duwamish, and Nisqually systems, mean nickel concentrations were higher in fish gills from the estuary relative to nearshore habitats (0.067 vs. 0.045, 0.064 vs. 0.055, and 0.059 vs. 0.049, respectively); however, these differences were not statistically significant (Figure 22). Similar to the whole system comparison, mean nickel concentrations in gill tissues differed significantly among the four estuaries. Significantly higher mean nickel concentrations were detected in the Skagit estuary (0.20 mg/kg ww), approximately three times

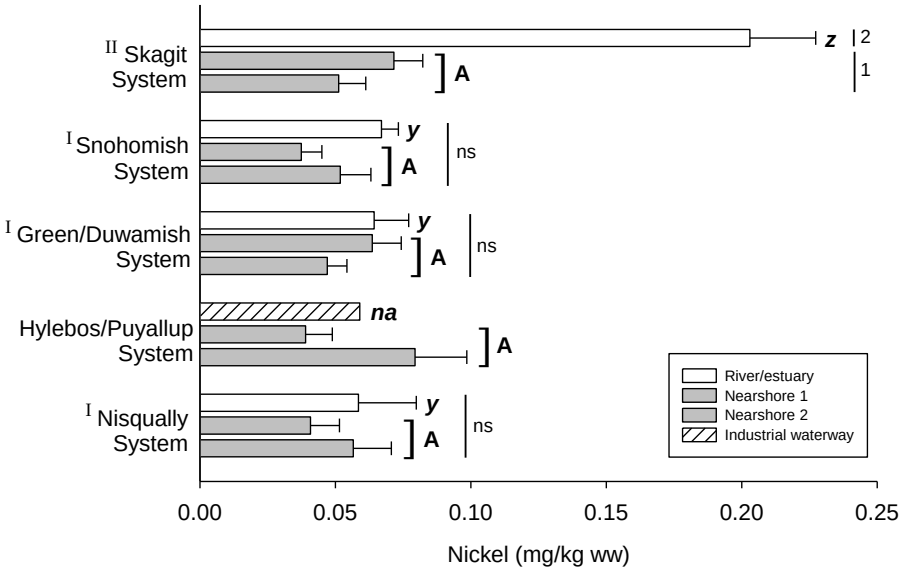

Figure 22. Comparison of means (+ 95% confidence intervals) of nickel (mg/kg ww), measured in the gills of juvenile Chinook salmon collected from five major river systems in Puget Sound, WA. Pairwise comparisons of the five systems (Roman numerals at left), four estuaries (white bars, *y-z*), five nearshore marine shorelines (gray bars, all A), and the three collection sites within systems (Arabic numbers) are also shown. Similar letters or numbers signify no significant difference (p > 0.05). ns = not significant; na = not analyzed.

the values measured in the Nisqually, Duwamish and Snohomish estuary fish (0.059 to 0.067 mg/kg ww; Figure 22). Nickel concentrations did not vary significantly among the four nearshore sites.

In a separate comparison of nickel among nearshore habitats that included fish from the Hylebos/Puyallup system, mean concentrations of nickel in fish gill tissues were also not significantly different among system, and were not correlated with fish length (GLM on nickel with system, fish length and interaction terms; n = 50, r^2 = 0.29, F_{system} = 1.911, df = 4, 40, p = 0.127; F_{length} = 0.318, df = 1, 40, p = 0.576; $F_{system*length}$ = 1.709, df = 4, 40, p = 0.169; Figure 22).

Zinc

Zinc was detected in all gill samples with values ranging from 22.3 to 39.2 mg/kg ww (n = 67; Table 13). Excluding the Hylebos/Puyallup system, most (61%) of the variation in zinc concentration among samples was related to location, including the system, habitat, or system specific habitat differences between estuary and nearshore habitats (GLM on zinc with system, habitat, fish length and interaction terms; n = 56, r^2 = 0.611, F_{system} = 7.332, df = 3, 48,p < 0.001; $F_{habitat}$ = 19.477, df = 1,48, p < 0.001; $F_{system*habitat}$ = 6.299, df = 3, 48, p < 0.001). Significantly lower mean zinc concentrations were measured in fish gills from the Duwamish system (27 mg/kg ww) compared to all other systems except the Nisqually (31 mg/kg ww). Mean zinc concentrations in fish gills from the Skagit and Snohomish systems (33 and 32 mg/kg ww, respectively; Figure 23) were not significantly different from each other, but the fish gills from the Skagit had significantly higher zinc levels than gill tissue from the Nisqually. No other significant differences in mean zinc concentrations among river systems were observed.

Overall, mean zinc concentrations were significantly lower in fish gills collected in estuaries than those in the nearshore (28 and 33 mg/kg ww, respectively). Likewise, within the Nisqually system, gill tissue in

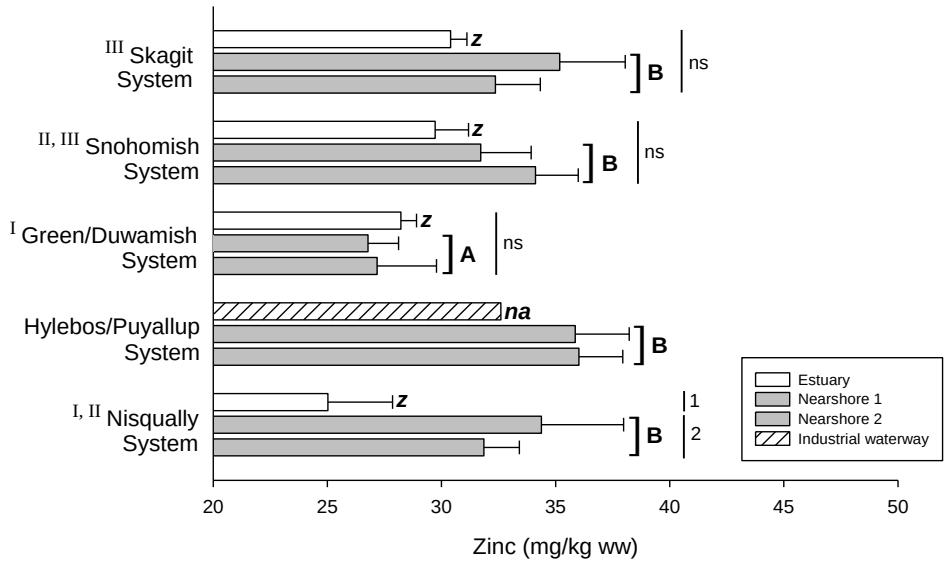

Figure 23. Comparison of means (+ 95% confidence intervals) of zinc (mg/kg ww), measured in the gills of juvenile Chinook salmon collected from five major river systems in Puget Sound, WA. Pairwise comparisons of the five systems (roman numerals), four estuaries (white bars, *y-z*), five nearshore marine shorelines (gray bars, A-B), and the three collection sites within systems (Arabic numerals) are also shown. Similar letters or numbers signify no significant difference ($p > 0.05$). na = not analyzed, ns = not significant .

fish from the estuary habitat (25 mg/kg ww) had significantly lower mean zinc concentrations than those from the nearshore habitat (33 mg/kg ww; Figure 23). Gill tissue from fish collected from the Skagit, Snohomish, and Green/Duwamish systems, each also had lower mean zinc concentrations in estuary fish compared to nearshore (30 vs. 34, 30 vs. 33, and 28 vs. 27 mg/kg ww, respectively), however, these differences were not statistically significant (Figure 23).

Among the four estuary habitats, mean zinc concentrations were similar in fish gill tissues from the Nisqually, Green/Duwamish, Snohomish, and Skagit systems (25, 28 and 30, and 30 mg/kg ww; Figure 23). In contrast to the estuary comparison, among the nearshore habitats, mean zinc concentrations in fish gills from the Green/Duwamish system (27 mg/kg ww) were significantly lower than all nearshore marine habitats, (33 to 36 mg/kg ww) which were not significantly different from each other.

In a separate comparison of zinc among nearshore habitats that included fish from the Hylebos/Puyallup system, zinc levels in fish gill tissues were also best modeled by systems differences, accounting for 57.4% of the observed variation among samples (GLM on Zn with system, fish length and interaction terms; n = 50, $r^2 = 0.368$, $F_{system} = 6.55$, df = 4, 45, $p < 0.001$). Zinc levels were not correlated with fish length among nearshore habitats. Mean zinc concentrations in fish gills from the Green/Duwamish nearshore habitat were significantly lower than all other nearshore habitats and no other significant difference were observed (Figure 23).

Effects of Contaminant Exposure on Fish Heath Assessment

Measured concentrations of TPCBs, and $\sum_{11}$PBDEs in whole body samples and $\sum_{17}$PAHs in stomach content samples of juvenile Chinook salmon from estuary, nearshore and offshore habitats in the Whidbey and Central Basins were often at concentrations documented to cause adverse health effects

in juvenile salmon (Table 14). Measured concentrations of $\sum_6$DDTs never exceeded adverse effects threshold in any sample (5,000 ng DDT/g lipid; Beckvar et al. 2005).

Overall, 28.4% of the 88 whole body composite salmon samples analyzed had TPCB levels above the 2,400 ng PCB/g lipid adverse effects threshold for juvenile salmon (Meador et al. 2002). All samples exceeding this PCB threshold were collected in the estuary and nearshore habitats of the Green/Duwamish and Hylebos/Puyallup systems in the Central Basin (78.6% and 18.2%, respectively), the Snohomish system in the Whidbey Basin (21.4%), or in the adjacent offshore habitats of the Central and Whidbey basins (72.7% and 50%, respectively; Table 14). Within the Green/Duwamish system, 25% of the estuary samples and 100% of the nearshore habitat samples collected from the Elliott Bay shoreline exceeded the PCB threshold. Within the Hylebos/Puyallup system, the one whole body composite sample collected from the Hylebos Waterway did not exceed the PCB threshold, but 20% of the samples collected from the nearshore habitat of the Commencement Bay shoreline did. In the adjacent offshore habitat of the Central Basin, 83% of samples collected in July and 60% of the samples collected in October exceeded the PCB adverse effects threshold. Within the Snohomish system, 50% of the whole body samples from the estuary habitat and 10% of those from the nearshore habitat exceeded the PCB threshold. While, in the adjacent offshore habitat of the Whidbey Basin, 50% of the whole body samples exceeded the PCB threshold. Fish collected from estuary and nearshore habitats of the Nisqually and Skagit systems, and the offshore habitats of the Admiralty Inlet and South Basin did not contain TPCBs above this PCB adverse effects threshold (Table 14).

Overall, 13.6 % of the whole body samples of juvenile Chinook salmon had PBDE concentrations in the range of concentrations known to cause increased disease susceptibility (≥ 470 and ≤ 2,500 ng/g lipid of the sum of PBDE 47 and PBDE 99) as determined by Arkoosh et al. (2013). Like the PCB results, all samples that had measured PBDEs levels within the disease susceptibility effects concentration range were collected in the Snohomish, Green/Duwamish, and Hylebos/Puyallup systems (35.7%, 7.1 % and 45.5%, respectively; Table 14). Within the Snohomish system, 100% of the estuary samples and 10% of the nearshore samples collected from the Port Gardner shoreline had PBDE concentrations above the disease susceptibility effects concentration. Within the Green/Duwamish system, 10% of the samples from the nearshore habitat had PBDE concentrations above the disease susceptibility effects concentration range, but none of the estuary samples exceeded this threshold. Within the Hylebos/Puyallup system, 100% and 40% of samples from the estuary and nearshore habitats contained PBDE concentrations above the disease susceptibility effects concentration range. Only one of the whole body salmon samples (16.7%) collected in the offshore habits, a sample from the Central Basin in July, had measured PBDE levels within the disease susceptibility effects concentration range (Table 14).

Table 14. Percentage of samples exceeding POPs and PAHs adverse effects concentrations for juvenile Chinook salmon

		Whole Bodies				Stomach Contents		
System	Habitat	n	% samples > PCB threshold[1]	% samples within range of PBDE levels associated with increased disease susceptibility[2]	% samples within range of PBDE levels associated with altered thyroid levels[3]	n	% samples > 3,800 ng PAH/g ww for altered growth[4]	% samples >12,200 ng PAH/g ww for altered growth & plasma chemistry[4]
Skagit	Estuary	4	0	0	0	1	0	0
	Nearshore	10	0	0	0	2	0	0
	Total	*14*	*0*	*0*	*0*	*3*	*0*	*0*
Snohomish	Estuary	4	50	100	75	1	0	0
	Nearshore	10	10	10	0	5	20	20
	Total	*14*	*21.4*	*35.7*	*21.4*	*6*	*16.7*	*16.7*
Green/ Duwamish	Estuary	4	25	0	0	1	0	0
	Nearshore	10	100	10	0	2	50	0
	Total	*14*	*78.6*	*7.1*	*0*	*3*	*33.3*	*0*
Hylebos/ Puyallup	Industrial waterway	1	0	100	0	1	0	0
	Nearshore	10	20	40	0	6	0	0
	Total	*11*	*18.2*	*45.5*	*0*	*7*	*0*	*0*
Nisqually	Estuary	4	0	0	0	4	0	0
	Nearshore	10	0	0	0	10	0	0
	Total	*14*	*0*	*0*	*0*	*14*	*0*	*0*
Offshore	Admiralty Inlet	2	0	0	0	2	0	0
	Whidbey Basin	2	50	0	0	2	0	0
	Central Basin (July)	6	83	16.7	0	5	0	0
	Central Basin (Oct.)	5	60	0	0	5	0	0
	South Basin	6	0	0	0	6	0	0
	Total	*21*	*42.9*	*4.76*	*0*	*20*	*0*	*0*
	Overall Total	*88*	*28.4*	*13.6*	*3.4*	*53*	*3.77*	*1.89*

[1] 2400 ng/g lipid, Meador et al. 2002

[2] $\geq$470 ng/g lipid and $\leq$ 2500 ng/g lipid, derived from Arkoosh et al. 2013 and Arkoosh et al 2010

[3] $\geq$1,500 ng/g lipid and $\leq$ 2,500 ng/g lipid, derived from Arkoosh et al. 2013

[4] Meador et al. 2006

Few (3.4%) of the 88 whole body samples of juvenile Chinook salmon had PBDEs in the range of concentrations known to cause altered thyroid levels (≥ 1,500 and ≤ 2,500 ng/g lipid of the sum of PBDE49 and PBDE 99; Arkoosh et al. 2013). All of these samples were detected in fish from the Snohomish estuary, constituting 75% of the samples collected at the location (Table 14).

Additionally, a few (3.7%) of the 53 stomach content samples had measured PAH levels within the range of two PAH concentrations documented to alter growth (> 3,800, > 12,200 ng PAHs/g ww; Meador et al. 2006). Although the PAH levels in stomach contents of our juvenile Chinook from all the estuary habitats were below dietary effects levels, 20% and 50% of samples taken from the Snohomish and Green/Duwamish nearshore habitats approached or exceeded PAH dietary doses observed to affect growth (>3,800 ng PAHs/g ww). One additional sample from the Snohomish nearshore, 20% of all Snohomish samples, had PAH concentrations that exceeded the higher effects level dose (>12,200 ng PAHs/g ww) suggesting with even greater certainty that the diet of those fish could affect growth rate (Table 14). This sample also had PAHs concentrations at levels associated with altered plasma chemistry, including lower levels of albumin and lipase. None of our samples exceeded the third treatment dietary dose of 32,400 ng PAHs/g ww. Meador et al. (2006) noted that had exposure to dietary PAHs at levels equivalent to >3,800, >12,200 and >32,400 ng/g ww (treatments 1, 2 and 3, respectively) continued for a longer than 53 days, the fish from those treatments would likely have exhibited significantly reduced growth. Since the number of summed PAHs (17 total) from our juvenile Chinook stomach contents were less than what Meador et al. used in their dietary feeding experiment (21 total), our comparison against these thresholds is likely conservative and may underestimate the proximity of the PAH levels measured in juvenile Chinook stomach contents to Meador's lower thresholds for altered growth.

Routes of POP Contaminant Exposure

Routes of Contaminant Exposure in Estuary and Nearshore Habitats

Within each river system, we compared mean body burden (ng/fish) of three POPs classes (i.e., TPCBs, $\sum_{11}$PBDEs, and $\sum_{6}$DDTs) measured in fish from nearshore habitats with those in fish from the estuary of the same system to ascertain the average portion of the measured POP body burden that was accumulated in freshwater and/or estuary habitat of that system (Table 15). The maximum contribution of POPs from the freshwater and/or estuary habitats to those measured in the nearshore was also estimated based on 95th percentile POP body burden (ng/fish) measured in fish from the estuary rather than the mean value (see Methods for additional detail). $\sum_{8}$Chlordanes, HCB, and dieldrin were excluded from this analysis because they were infrequently detected or detected in low concentrations. Also, for this analysis, the two nearshore sites in a system were each compared against the estuary sample from that system to provide a measure of the variability of the route of exposure. The Hylebos/Puyallup system was not included in this analysis because the one estuary sample collected was not considered to adequately represent the estuary within that system.

TPCBs: Within each of the Skagit, Snohomish and Nisqually systems the TPCBs body burdens (ng/g fish) in fish from nearshore habitats were 2 to 4.5 times higher than levels measured in the estuaries of their respective systems. These data indicate that most of the TPCBs measured in fish from nearshore habitats of less developed river systems were accumulated in the nearshore habitats of those systems. The TPCBs body burdens (ng/g fish) in fish from the nearshore of the Green/Duwamish system were only 1.6 – 1.9 times higher than those measured in the estuary, and the majority (54-61%) of the TPCBs were accumulated from the freshwater (i.e., area above tidal influence) and/or the estuary habitat within that system. Fish in the nearshore

Table 15. A comparison of geometric mean body burdens of POPs (ng/fish) in juvenile Chinook whole body (less gills and stomach contents) samples collected from the estuary and two nearshore sites. Bold text signifies that the majority of the contaminant was accumulated in the freshwater habitat of the system.

SKAGIT SYSTEM								
	Estuary		Nearshore 1	*% POP from freshwater and estuary for Nearshore 1*		Nearshore 2	*% POP from freshwater and estuary for Nearshore 2*	
	Geometric mean	95th Percentile	Geometric mean	*Based on the mean*	*Based on 95th percentile*	Geometric mean	*Based on the mean*	*Based on 95th percentile*
TPCBs	13	14	35	*37*	*40*	26	***54***	***54***
$\sum_{11}$PBDEs	2.7	3.8	8.6	*32*	*45*	16	*25*	*25*
$\sum_{6}$DDTs	2.9	3.6	5.4	***53***	***66***	6.4	***56***	***56***
SNOHOMISH SYSTEM								
	Estuary		Nearshore 1	*% POP from freshwater and estuary for Nearshore 1*		Nearshore 2	*% POP from freshwater and estuary for Nearshore 2*	
	Geometric mean	95th Percentile	Geometric mean	*Based on the mean*	*Based on 95th percentile*	Geometric mean	*Based on the mean*	*Based on 95th percentile*
TPCBs	35	40	70	*50*	*57*	110	*33*	*37*
$\sum_{11}$PBDEs	37	51	24	***155***	***211***	47	***79***	***107***
$\sum_{6}$DDTs	4.5	5.0	13	*36*	*39*	14	*32*	*35*
GREEN/DUWAMISH SYSTEM								
	Estuary		Nearshore 1	*% POP from freshwater and estuary for Nearshore 1*		Nearshore 2	*% POP from freshwater and estuary for Nearshore 2*	
	Geometric mean	95th Percentile	Geometric mean	*Based on the mean*	*Based on 95th percentile*	Geometric mean	*Based on the mean*	*Based on 95th percentile*
TPCBs	150	300	250	***61***	***121***	280	***54***	***106***
$\sum_{11}$PBDEs	14	33	24	***56***	***134***	24	***56***	***135***
$\sum_{6}$DDTs	20	21	21	***97***	***100***	18	***115***	***119***
NISQUALLY SYSTEM								
	Estuary		Nearshore 1	*% POP from freshwater and estuary for Nearshore 1*		Nearshore 2	*% POP from freshwater and estuary for Nearshore 2*	
	Geometric mean	95th Percentile	Geometric mean	*Based on the mean*	*Based on 95th percentile*	Geometric mean	*Based on the mean*	*Based on 95th percentile*
TPCBs	32	36	93	*35*	*38*	145	*22*	*24*
$\sum_{11}$PBDEs	11	12	11	***100***	***103***	14	***81***	***83***
$\sum_{6}$DDTs	4.7	4.8	12	*39*	*39*	19	*24*	*25*

Green/Duwamish estuary were exposed to and accumulated TPCBs in the nearshore, but less so than in the freshwater portions of the river and the estuary (Table 15).

$\sum_{11}$PBDEs: Within the Snohomish and Nisqually systems, the $\sum_{11}$PBDEs body burdens (ng/g fish) in fish from nearshore habitats were only 0.6 to 1.4 times higher than those measured in the estuaries, indicating that most,

if not all, of the $\sum_{11}$PBDEs measured in fish from nearshore habitats were accumulated in freshwater and/or estuary habitats of the system, (79 - 155% and 81 – 100%, respectively, based on mean concentrations). Values greater than 100% indicate that the fish in the nearshore habitat had lower mean concentrations PBDE concentrations than those in the estuary habitat. Similarly, $\sum_{11}$PBDEs body burdens (ng/g fish) in fish from nearshore habitats within the Green/Duwamish system were 1.8 times higher than those from the estuary and the majority of $\sum_{11}$PBDEs were accumulated in the freshwater and/or habitats of these systems (56%). In contrast, $\sum_{11}$PBDEs body burdens (ng/g fish) in fish from nearshore habitats within the Skagit system were 3 – 5.6 times higher, indicating the majority of $\sum_{11}$PBDEs were accumulated in the nearshore habitats of this system (68 -75%; Table 15).

$\sum_6$DDTs: As was observed for TPCBs, within the Snohomish and Nisqually system, $\sum_6$DDT body burdens (ng/g fish) in fish from nearshore habitats were 2.6 – 4 times higher than those detected in the estuary of those systems, indicating the majority of $\sum_6$DDT were accumulated in the nearshore habitats of these systems (64-68% and 61-81%, respectively). Within the Skagit system, the majority of $\sum_6$DDTs in the nearshore fish were accumulated in the freshwater and/or estuary (53 -56%), but a substantial amount continued to be accumulated in the nearshore (44 -47%). Unlike the Snohomish and the Nisqually systems, $\sum_6$DDTs body burdens in fish from the Green/Duwamish nearshore habitats were very similar to those measured in the estuary habitat, indicating that most, if not all, of the $\sum_6$DDTs were accumulated while the fish were in the freshwater and/or estuary portion of the system, (97-115%; Table 15).

Based on the comparison of POP body burdens in fish from the estuary and nearshore habitats of the same system, the major route of contaminant exposure or "source" for juvenile Chinook salmon in the Skagit system appears to be the nearshore habitat. Significant amounts of $\sum_6$DDT were also accumulated in fish from the nearshore Skagit habitat; however, the majority of $\sum_6$DDT was accumulated while fish were in the freshwater system, either the lower river or the estuary. In both the Snohomish and Nisqually systems, the nearshore is also the major route of exposure for TPCBs and $\sum_6$DDTs in juvenile Chinook salmon migrating through that system. However, in stark contrast, the freshwater habitat in these systems is the main source of $\sum_{11}$PBDEs for fish migrating through these systems. In contrast to all other systems, the Green/Duwamish was the only system for which the majority of TPCBs in juvenile Chinook salmon were accumulated in the estuary habitat. Most of the $\sum_6$DDTs, and the majority of $\sum_{11}$PBDEs accumulated in juvenile Chinook in this system were associated with their time in the estuary.

Routes of Contaminant Exposure: Offshore vs. Nearshore Habitats

Within each basin, we compared mean body burden (ng/fish) of TPCBs, $\sum_{11}$PBDEs, and $\sum_6$DDTs in fish from offshore habitats with those of fish from the nearshore habitat of the same basin to ascertain the average portion of the measured POP body burden measured in the offshore habitat that was accumulated in the freshwater, estuary and nearshore habitat (Table 16). The maximum contribution of POPs from the freshwater, estuary and nearshore habitats to those measured in the offshore was also estimated based on 95th percentile POP body burden (ng/fish) measured in fish from the nearshore rather than the mean value (see Methods for additional detail). $\sum_8$Chlordanes, HCB, and dieldrin were excluded from the analysis because they were infrequently detected or detected at low concentrations. Fish samples from the Skagit and Snohomish nearshore habitats were combined and compared to fish collected in the offshore Whidbey Basin, fish collected from the Green/Duwamish and the Hylebos/ Puyallup nearshore habitats were compared to fish collected in the

Table 16. A comparison of geometric mean body burdens of POPs (ng/fish) in juvenile Chinook whole body (less gills and stomach contents) samples collected from the nearshore and offshore sites within the three major basins in Puget Sound.Bold text signifies that the majority of the contaminant was accumulated in freshwater and nearshore habitats.

WHIDBEY BASIN					
	Nearshore		Offshore	*% from freshwater, estuary and nearshore*	
	Geometric mean	95th Percentile	Geometric mean	*Based on the mean*	*Based on 95th percentile*
TPCBs	51	120	850	*6.0*	*14*
$\sum_{11}$PBDEs	20	62	160	*12*	*39*
$\sum_{6}$DDTs	8.9	18	64	*14*	*27*
CENTRAL BASIN					
	Nearshore		Offshore	*% from freshwater, estuary and nearshore*	
	Geometric mean	95th Percentile	Geometric mean	*Based on the mean*	*Based on 95th percentile*
TPCBs	170	360	1100	*15*	*32*
$\sum_{11}$PBDEs	27	91	130	*20*	***68***
$\sum_{6}$DDTs	19	28	85	*22*	*33*
SOUTH BASIN					
	Nearshore		Offshore	*% from freshwater, estuary and nearshore*	
	Geometric mean	95th Percentile	Geometric mean	*Based on the mean*	*Based on 95th percentile*
TPCBs	120	230	1100	*11*	*21*
$\sum_{11}$PBDEs	13	17	110	*11*	*15*
$\sum_{6}$DDTs	15	26	50	*31*	*52*

offshore Central Basin, while fish collected from the Nisqually nearshore were compared to fish collected in the offshore of South Basin.

Overall, the comparisons of body burdens (ng/g fish) of TPCBs, $\sum_{11}$PBDEs, and $\sum_{6}$DDTs in juvenile Chinook collected in the offshore basins compared to those in the nearshore indicate that fish continue to accumulate these chemicals in offshore waters. Moreover, the offshore habits are the major source of POPs to salmon on their migratory route to the Pacific Ocean (Table 16.

TPCBs: Within the Whidbey, Central, and South basins, the TPCBs body burdens (ng/fish) of fish collected in those offshore areas were approximately seven to 17 times higher than those measured in their respective nearshore habitats (Table 16). This indicates that the fish continue to accumulate TPCBs as they move into the offshore environment. Most notably, fish collected in the offshore habitats of the Whidbey and South basin accumulated higher percentages of their PCB body burden from the offshore habitat (approximately 94% and 89% than those fish in the offshore habitat of the Central Basin, approximately 85%; Table 16). Fish in the offshore habitats have similar PCB body burdens among marine basins but because fish emerging from the nearshore habitat of the Central Basin have relatively higher PCB body burden than those fish from the nearshore habitats of the Whidbey and South basins, fish from the Central Basin accumulate less of their total PCB body burden from the offshore habitat.

$\sum_{11}$PBDEs: Within each of the three basins, the $\sum_{11}$PBDEs body burdens (ng/fish) of fish collected in the offshore basins were five to nine times higher than those measured in their respective nearshore habitats (Table 16), indicating that the fish continue to accumulate flame retardants as they move into the offshore environment. The most notable accumulation was measured in fish collected in the offshore Whidbey Basin, which had eight times higher $\sum_{11}$PBDEs body burdens than fish collected in the Skagit and Snohomish nearshore habitats (Table 16).

$\sum_{6}$DDTs: Within each of the three basins, the $\sum_{6}$DDT body burdens (ng/fish) of fish collected from the offshore basins were three to seven times higher than those measured in their respective nearshore habitats (Table 16), indicating that the fish continue to accumulate $\sum_{6}$DDTs as they move into the offshore environment. Fish collected in the Whidbey Basin had seven times higher $\sum_{6}$DDTs body burdens than fish collected in the Skagit and Snohomish nearshore habitats (Table 16).

DISCUSSION

The levels of organic contaminants we observed in juvenile Chinook salmon from estuary and nearshore habitats, measured as POP concentrations in whole-body fish samples or as PAH concentrations in stomach contents, supported our hypothesis that salmon residing and feeding in the more urbanized and industrialized environments are exposed to higher concentrations of contaminants than those in less developed habitats. However, for salmon collected in offshore habitats of the marine basins our hypothesis was not supported - , fish from the more developed Central Basin of Puget Sound did not have elevated POPs and PAHs concentrations compared to those from the less developed Whidbey Basin and South Basin. As juvenile Chinook salmon migrated from river systems to offshore waters of Puget Sound, all fish continued to accumulate substantial amounts of POPs, as evidenced by the higher total mass of POPs in their bodies (i.e., POP body burdens measured as ng/ fish) and after four months of feeding in offshore habitats, fish from all basins had uniform concentrations of POPs (i.e., the mass of POP compared to the mass of fish tissue measured as ng POP/g tissue ww). In general, concentrations of POPs in fish from offshore basins were lower than those measured in fish from developed river systems, indicating that the offshore was less contaminated than the developed river systems habitats. In contrast, the concentrations of POPs in the offshore habitats were sometimes higher than those from undeveloped river systems indicating that the offshore was more contaminated than the undeveloped river systems habitats. The levels of copper and lead were also elevated in gill tissues of fish from the more developed nearshore marine habitats but the concentration of cadmium, nickel and zinc were not elevated in the more urban and industrial habitats. Fish body size did not show strong association with contaminant uptake; location was consistently the primary factor associated with contaminant levels. In the sections that follow, we discuss 1) the spatial pattern in contaminant exposure in juvenile Chinook salmon, 2) the potential effects of contaminant exposure on salmon health, and 3) where in the salmon's migratory pathway are fish accumulating contaminants.

Spatial Patterns of Contaminant Exposure

POPs in whole body salmon samples

In all five river systems (which included estuary and nearshore marine habitats), TPCBs, $\sum_{11}$PBDEs, $\sum_{6}$DDTs were detected in every whole body tissue sample of juvenile Chinook salmon. TPCB concentrations were generally higher than those of $\sum_{11}$PBDEs, followed by $\sum_{6}$DDTs. Organochlorine pesticides, including, $\sum_{8}$chlordanes, hexachlorobenzene, and dieldrin were also detected, but at lower frequencies and concentrations. Juvenile

Chinook salmon entering Puget Sound from the more developed river systems accumulated higher concentrations of these POPs than those migrating thorough less developed river systems. In particular, juvenile Chinook salmon from the more developed Snohomish and Green/Duwamish river systems accumulated higher concentrations of POPs than those migrating thorough the less developed Skagit and Nisqually systems. Fish from the urbanized Hylebos/Puyallup system generally also had POP concentrations that were intermediate between those of the Snohomish and Green/Duwamish system, but were not included in the system-wide comparison because too few estuary samples were collected.

Although POP concentrations were elevated in salmon from more developed river systems, we also observed additional spatial variability in contaminant exposure within habitats of these developed river systems that were specific to the system and the particular POP class or analyte evaluated. For example, concentrations of TPCBs and $\sum_{11}$PBDEs in fish within the Snohomish system were always higher in fish from the estuary relative to those from the nearshore habitat. However, in the Green/Duwamish system, the concentrations of TPCBs and $\sum_{11}$PBDEs were always higher in fish from nearshore habitat than the estuary habitat. In contrast to TPCBs and $\sum_{11}$PBDEs, $\sum_{6}$DDTs concentrations were consistently higher in fish collected from estuary habitats than nearshore habitats, regardless of river system, but only in the Snohomish system was this difference statistically significant.

Spatial variation in POP exposure was less apparent in fish in offshore habitats. Levels of all three contaminant classes (TPCBs, $\sum_{11}$PBDEs and $\sum_{6}$DDTs) in juvenile Chinook salmon were similar regardless of whether they were collected in the offshore habitat of the Whidbey Basin, Central Basin and South Basin. The only difference was in Admiralty Inlet, where the concentrations of all three contaminants were much lower than the offshore habitats in other basins.

The similar concentrations of POPs in juvenile Chinook salmon among offshore habitats of the Whidbey Basin, Central Basin and South Basin could be related, in part, to the mixing of salmon from multiple river systems with low and high contaminant levels, resulting in less variable averages in the mixed collections from offshore habitats. Examination of the hatchery origins for all fish with CWTs collected from the offshore habitat (including the fish processed for chemical analyses and other fish that were not processed) indicated substantial mixing of fish from different river systems, consistent with previous studies of juvenile Chinook salmon in Puget Sound (Brennan et al. 2004, Fresh et al. 2006, Rice et al. 2011). However, the mixing of populations with high and low contaminant levels is insufficient to explain the concentrations observed in offshore samples. If the fish had obtained all contaminants in freshwater and estuarine habitats and none in offshore habitats, they would have retained the same body burdens (ng/fish) but the concentrations (ng/g) would have decreased as the fish added mass without additional contaminants. This was not the case. For example the concentration of TPCBs in fish from offshore habitats of the Whidbey, Central and South basins as a unit was higher (but not significantly) than fish in estuary and nearshore habitats as units, yet the total body burdens increased seven to 15 times, indicating that fish in offshore habitats continued to accumulate POPs as they fed in offshore habitats for several months. Furthermore, the relatively high TPCB concentrations in juvenile salmon in the offshore marine habitats indicate that contaminant exposure was not limited to developed estuarine and nearshore habitats; fish from undeveloped river systems were exposed as they moved into offshore habitats. For example, within the South Basin, fish from the offshore habitat had significantly higher TPCB concentrations than fish from the estuary and nearshore habitats of the Nisqually system. In contrast, within the Central Basin, TPCB concentrations in fish from the offshore habitat were lower than those in fish from the estuary and nearshore

habitats of the Green/Duwamish and Hylebos river systems. Unlike TPCBs, concentration of $\sum_{11}$PBDEs and $\sum_{6}$DDTs and $\sum_{8}$chlordanes were always lower in fish from offshore habitats.

The body burden data from offshore sites in the Whidbey, Central and South basins indicated fish continued to uptake contaminant as they fed and grew, implying that salmon prey in offshore waters is contaminated. Previous studies have noted that plankton, and small schooling pelagic fish sampled in offshore waters of Puget Sound are contaminated with POPs (West et al. 2011a, West et al. 2011b). Johnson et al (2007b) concluded that elevated TPCB and $\sum_{6}$DDT levels in juvenile Chinook salmon captured in estuaries and nearshore marine habitats are likely derived from consuming contaminated prey in those habitats; however, additional uptake from the water column via ventilation cannot be ruled out.

The low concentrations POPs in fish from Admiralty Inlet suggest that these fish did not originate in Puget Sound, but migrated in from other locations, potentially from the northern Salish Sea or the Strait of Georgia. Alternatively, the fish sampled from Admiralty Inlet may have the migrated there from the more contaminated Whidbey, Central and South basins but only includes the subset of fish with low POPs concentrations that survived to migrate out of Puget Sound, through Admiralty Inlet. Additional sampling would be needed to confirm this hypothesis.

The spatial variability in POP concentrations in river systems was not associated with fish size or other potential covariates such as fish origin (i.e. hatchery vs. naturally produced). For all statistical comparisons of POPs among river systems, among estuaries and among nearshore habitats, fish length was never correlated with concentrations of specific POP classes and did not account for significant amounts of the observed variation among samples. For example, similar sized fish were collected from estuaries of the Skagit, Nisqually and Snohomish river systems, but $\sum_{11}$PBDEs were only elevated in fish from the Snohomish estuary. Larger fish were collected from the Green/Duwamish estuary, but $\sum_{11}$PBDE concentrations in that system were lower than those in the Snohomish estuary. Likewise, among nearshore habitats, fish from both the Nisqually and Skagit systems had uniformly low $\sum_{11}$PBDE levels compared to other nearshore habitats. However, some of the largest fish sampled from the nearshore habitat were collected from the Nisqually, while those of the Skagit were some of the smallest. Statistical comparisons of POPs between estuary and nearshore habitats indicated that the best fit model did not include fish length as a significant factor affecting concentrations of specific POP classes or analytes; however, these comparisons were confounded because larger fish were generally measured in nearshore habitats compared to estuary habitats. Of the POP classes or analytes we measured, only $\sum_{6}$DDTs showed consistent differences between estuary and nearshore habitats; fish sampled from estuaries always had higher concentrations than fish in the nearshore, although only in the Snohomish system was this difference statistically significant.

In the current study, we did not statistically test whether the percent of naturally produced fish in samples was correlated with the observed POP concentrations in juvenile Chinook salmon in estuary and nearshore habitats. However, the percent of naturally produced fish in composite fish samples was correlated with fish length (hatchery fish being larger than naturally produced fish), and as discussed above, fish length was not a significant factor affecting POP concentrations in juvenile Chinook salmon. The low POP concentrations in salmon from the Nisqually River system in both hatchery and wild fish also suggested that rearing history did not affect POP concentrations in a manner that would create or mask variation among sites. Hatchery produced salmon collected in the field could accumulate higher POP concentrations than naturally produced salmon through exposure to POP-contaminated hatchery feed prior to release. However, POP concentrations in

hatchery feed have tended to decline in recent years (Johnson et al. 2010, Maule et al. 2007) to levels that would not mask the concentrations measured herein. Alternatively, naturally produced salmon could be exposed to higher POP concentrations than hatchery produced salmon because naturally produced fish tend to spend more time in estuaries than hatchery fish. Although previous studies that compared contaminant exposure in hatchery and naturally produced fish in Puget Sound and the Columbia River suggested that hatchery rearing can be an important contributor to contaminant levels in fish from non-urban areas, but for those fish that migrate through urban estuaries, the contribution is likely less significant and much more variable (Johnson et al. 2013, Johnson et al. 2010, Lower Columbia Estuary Partnership 2007, Meador et al. 2010).

Previous studies of contaminants in juvenile Chinook salmon have also documented elevated levels of POPs and PAHs exposures in fish sampled from more developed estuary and nearshore habitats including juvenile Chinook salmon from urban rivers and estuaries of Puget Sound (Stehr et al. 2000, Johnson et al. 2007a, Olson et al. 2008, Meador et al. 2010, Sloan et al. 2010) and urbanized regions of the lower Columbia River and the Washington and Oregon coast (Johnson et al. 2013, Johnson et al. 2007b, Sloan et al. 2010, Yanagida et al. 2012). TPCB concentrations in whole body samples of juvenile Chinook salmon from our study were similar to those measured in Puget Sound since the early 2000's, but lower than levels measured in juvenile Chinook salmon prior to 2000 (Johnson et al. in prep). Fish from three of the four sites within Puget Sound that have been monitored prior to 2000, including the estuaries of the Snohomish and Green/Duwamish, and the Nisqually River, currently all have significantly lower TPCB concentrations (Johnson et al. in prep). Limited long term monitoring has also been conducted in the nearshore marine shoreline adjacent to the Puyallup River, but consistent declines in PCBs are not evident at this site. Similarly, $\sum_{11}$PBDE concentrations measured in fish in our study are similar to concentrations measured in 2006 (Sloan et al. 2010). In contrast to TPCBs and $\sum_{11}$PBDEs, the $\sum_{6}$DDT concentrations measured in juvenile Chinook salmon are generally lower than concentrations measured in previous Puget Sound studies (Johnson et al. in prep).

PAHs in salmon stomach contents

Similar to the patterns observed for POPs in whole body samples, juvenile Chinook salmon from the Snohomish, Green/Duwamish and Hylebos/Puyallup river systems appeared to have the greatest dietary exposure to $\sum_{42}$PAHs, with the highest exposure occurring in the nearshore habitats. Though lack of replication did not allow for statistical comparison among the estuary habitats, or among all of the nearshore habitats, $\sum_{42}$PAH levels in stomach contents among both habitat types followed the pattern observed among river systems. Salmon feeding in offshore habitats had stomach content $\sum_{42}$PAH levels that were less than those in nearshore habitats, but similar to those in estuary habitats. Among the offshore habitats, fish stomach contents from the Whidbey Basin had higher $\sum_{42}$PAH concentrations than those in the Central and South Basins; however, low samples sizes prevented a statistical comparison. In addition, fish diets in the South Basin appear to be less contaminated overall with $\sum_{42}$PAHs than those in the Whidbey and Central basins, due in large part to the variation $\sum_{42}$PAHs in prey consumed in the nearshore habitats of these basins rather than the offshore or estuary habitats. Overall, these results indicate offshore habitats generally do not provide a significant source of dietary $\sum_{42}$PAHs to juvenile Chinook salmon.

$\sum_{42}$PAHs concentrations in stomach contents of juvenile Chinook salmon observed in this study were similar to levels measured in previous studies at most sites except for the Duwamish estuary site (Kellogg Island) and the nearshore habitat of the Hylebos/Puyallup system (Commencement Bay nearshore), which both showed declining trends (Johnson et al. in prep). As detailed in Johnson et al. (in prep), PAH concentrations in stomach

contents at the Kellogg Island site between 1986 and 1999 ranged from 14,000 to 29,000 ng/g ww. In contrast, concentrations measured in 2006 were less than 1000 ng/g ww, similar to those measured in the current study. Likewise, in Commencement Bay, PAHs in stomach contents ranged from 3000 – 4000 ng/g ww between 1995 and 2002, but ranged between 100 and 600 ng/g ww in the current study (Johnson et al. in prep).

Metals in gill samples

Unlike the spatial patterns observed for POPs and PAHs, metal exposure, measured as concentrations of cadmium, copper, lead, nickel and zinc in gill tissues, were not strongly associated with the degree of development of the sampling locations, except for elevated levels of copper and lead on nearshore marine habitats of the developed systems. Average metal concentrations were highest for zinc (32 mg/kg ww), followed by copper, lead, nickel and cadmium (0.60, 0.074, 0.064, and 0.032 mg/kg ww, respectively).

Cadmium is a naturally occurring heavy metal, but environmental concentrations have increased in the Puget Sound basin over background levels mostly due to manufacturing releases, combustion of fossil fuels and the use of phosphorous fertilizers (Ecology and King County 2011). Cadmium is a persistent bioaccumulative toxic contaminant; however, the extent to which cadmium is accumulated by fish is determined by the cadmium source, exposure level, distance from contamination source, and the presence of other ions, especially zinc and calcium. Gill tissue is a suitable matrix to assess environmental exposures cadmium because kidney, gills and liver tissue tend to accumulate the highest levels of cadmium during aqueous or dietary exposures (see review by Sorensen 1991). In this study, cadmium concentrations were 2 – 4 times higher in gill tissue of juvenile Chinook salmon from the Snohomish and Skagit river systems within the Whidbey Basin, than those in the Central and South Basins. No other spatial patterns were observed. The distinct spatial enrichment of cadmium in gills of fish from the river systems in the Whidbey Basin, especially the Snohomish, suggests there may be a natural elevated source of cadmium in this basin.

Copper is an essential trace element involved in many functions in vertebrates and invertebrates. It is widely used in building materials (e.g., copper roofs and treated lumber), automobile parts (e.g., brake pads), and pesticides (Davis et al. 2001) and consequently copper is often a pervasive contaminant in urban and agricultural watersheds. Sources of copper to the Puget Sound environment include inputs from urban lawn and garden use of pesticides; leachate from plumbing components, vehicle brake pads and tire wear, and leachate from anti-fouling paints (Ecology and King County 2011). Generally, copper is not considered to be toxic to humans or wildlife at environmentally relevant concentrations, but can be highly toxic to aquatic organisms, including juvenile salmon, even at low environmental concentrations (Ecology and King County 2011). In freshwater, short-term-exposure to copper reduces the olfactory capacity of salmon and, therefore, their ability to detect important olfactory cues from nearby prey and predators (Baldwin et al. 2003, McIntyre et al. 2012, McIntyre et al. 2008, Sandahl et al. 2007). In addition to these behavioral effects, modeling by Mebane and Arthaud (2010) suggests that body size reductions due to chronic early life stage exposure to sublethal copper concentrations could reduce juvenile salmon survival and population recovery trajectories.

In fish, the liver actively processes and stores large copper loads, but the gills do not (Sorensen 1991). Consequently, high copper levels in gill tissues would only be expected if the fish were exposed to high enough environmental concentrations to overwhelm the liver's capacity to detoxify. Among nearshore habitats, we observed higher mean copper concentrations in gill tissues of fish from the highly developed Hylebos/Puyallup and Green/Duwamish systems (0.72 and 0.64 mg/kg ww, respectively) compared to the other nearshore habitats of less developed systems (0.50 to 60 mg/kg ww); however, only levels in the Hylebos/Puyallup system

were significantly higher. It is unknown if the elevated copper in gills of fish from the Hylebos/Puyallup nearshore habitat is associated with the degree of development in this system or with differences in water chemistry between this and other systems.

Lead is a naturally occurring metal, but is also produced by human activities and is a known persistent bioaccumulative toxic chemical (Ecology 2009). Historically, lead was used in gasoline and paints but current sources to fresh and marine water of Puget Sound include: ammunition and hunting shot use, loss of fishing sinkers and wheel weights, roofing material leaching and aviation fuel combustion (Ecology and King County 2011). Exposure to lead in the environment can be measured in kidney, gill, and liver tissue because these tissues tend to accumulate the highest levels of lead during aqueous or dietary exposure (see review by Sorensen 1991). For salmon in particular, fish gill tissues are the major and most efficient site of calcium and or lead update (Varanasi and Gumar 1978). Lead uptake in fish is affected by environmental concentrations, exposure time, diet, pH, salinity temperature and other parameters. In this study, we observed that levels in gill tissues of juvenile Chinook salmon did not show spatial difference among river systems overall or among estuaries of different river systems. However, among nearshore habitats of river systems, lead levels were generally higher in the more urbanized nearshore habitats of the Hylebos/Puyallup and Green/Duwamish systems, suggesting that juvenile Chinook salmon may have greater exposure to lead in developed nearshore habitats.

Nickel occurs naturally in the environment at low levels, but can be toxic to fish at high concentrations. The primary sources of nickel emissions to the environment are from human activities, including the combustion of coal and oil for heat or power generation, the incineration of waste and sewage sludge, nickel mining and primary production, steel manufacture, electroplating, and cement manufacturing (EPA 1984). In our study, the highest nickel levels in gill samples were measured in fish from the Skagit estuary, approximately 2.5 to 5.5 times higher than any other sampling locations; no other spatial difference among river systems or habitat types were observed. These data indicate that juvenile salmon migrating down the Skagit River are exposed to a unique source of nickel not experienced by salmon from other river systems. The source of elevated nickel concentrations in the Skagit estuary habitat is unknown, but could possibly include natural sources. A nickel deposit exists near Mt. Vernon, at Devil's Mountain, and the ore is well exposed for two miles along the side of the mountain (Lucas 1975).

Zinc is generally found in large quantities in vertebrates and is an essential element in fish, necessary for many biochemical processes including digestion of proteins and carbohydrates, and regulation of the release of carbon dioxide at the gills lamellae (see review in Sorensen 1991). Within Puget Sound the largest source of zinc to the environment was estimated to be leachate from rooftops, particularly those with galvanized components; other sources include galvanized materials, tire wear, brake pad wear, and the agricultural application of fertilizers and micronutrients. Uptake of zinc by fish can be affected by environmental factors such as the exposure concentration, the duration of exposure, and water hardness, but can also be affected by biological attributes like fish size and trophic position (Sorensen, 1991). In our study, zinc concentrations were generally higher in fish collected from nearshore habitats than estuary habitats, potentially associated with difference in fish size between these two habitats or changes in water hardness as the fish move from freshwater to marine waters. As discussed in the POP section above, statistical comparisons of zinc levels in gill tissue between estuary and nearshore habitats indicated that the best fit model included habitat types as a significant factor affecting zinc concentrations, but did not include fish length. However, this comparison was confounded because larger fish

were generally measured in nearshore habitats compared to estuary habitats. The only other spatial pattern of note was the lower zinc concentration in fish from Green/Duwamish river system, in which fish in the nearshore habitat had significantly lower zinc levels than fish from all other nearshore habitats, possibility indicating a zinc deficiency in Green/Duwamish nearshore fish. A few previous contaminant monitoring studies have observed depleted levels of some elements in marine organisms when organic contaminants are elevated in their tissues (de Goeij et al. 1974; Mearns et al. 1991). A mechanism for this sort of metal-depletion phenomenon has been proposed by Brown et al. (1987).

Potential Effects of Contaminant Exposure on Marine Survival

The results of this study indicated that juvenile Chinook salmon residing and feeding in the more urbanized and industrial estuary, nearshore marine, and offshore habitat are exposed to high enough concentrations of TPCBs, $\sum_{11}$PBDEs and $\sum_{42}$PAHs to affect their survival through reduction in growth, disease resistance, and altered hormone and protein levels, and potentially mortality. Fish from the Snohomish, Green/Duwamish and Hylebos/Puyallup river systems, as well as fish from multiple river-systems that had moved offshore and were caught in the Whidbey and Central Basins of Puget Sound, were most likely to be adversely affected by contaminant exposure. $\sum_{6}$DDT levels in Puget Sound salmon were all below an adverse effects threshold concentration estimated from peer-reviewed studies (Beckvar 2005).

Most (78.6%) of the salmon samples from the Green/Duwamish river had TPCB levels that exceeded a PCB adverse effects threshold concentration for juvenile salmon (2,400 ng/g lipid; Meador et al. 2002). Below this 2,400 ng/g lipid threshold, sub-lethal adverse effects from PCB contamination are less likely to occur; however, above this threshold, multiple adverse effects have been reported. Indeed, over 20% of the fish from the Green/Duwamish river-system exceeded the PCB adverse effects threshold by a factor 2 – 2.5 times, at concentrations reported to be associated with increased enzyme activity, altered thyroid hormone levels and increased mortality (Meador et al. 2002. Fewer salmon samples from the Snohomish and Hylebos/Puyallup river system exceeded the PCB adverse effects threshold (21.4 and 18.2%, respectively). As the fish moved offshore, they continued to be exposed to PCBs, such that in the offshore habitat of Whidbey Basin and Central Basin in October, over half of the fish samples exceeded the threshold.

Although mean TPCB concentrations in fish from the offshore habitats of the South Basin were similar to those in the Central Basin on the basis of wet weight (24 and 23 ng/g respectively), South Basin salmon had higher mean lipid levels than the fish sampled from the Central Basin (1.3% vs. 0.94%, respectively), resulting in higher mean PCB concentration in Central than South Basin salmon on a lipid weight basis (2,253 vs 1,955 ng/g lipid). For lipophilic contaminants like PCBs, the tissue concentration causing a toxic response is directly related to the lipid content (Lassiter and Hallam 1990, van Wezef et al. 1995), hence we conclude that Central Basin salmon were more likely to be impaired by PCB exposure than South Basin salmon.

All of the samples with PBDE levels exceeding a health effects threshold were collected from the Snohomish, Green/Duwamish, and Hylebos/Puyallup river systems, except for one of the offshore samples. These fish had PBDE tissue residues in the range of concentrations demonstrated to increase disease susceptibility based on PBDE dietary exposure studies conducted on post smolt stage salmon (Arkoosh et al. 2013, Arkoosh et al. 2010, Arkoosh et al. in press). It is likely therefore that the greatest risk juvenile salmon faced related to PBDE exposure was in the urban river systems.

We observed PAH levels in stomach contents of salmon from two marine nearshore habitats that may have been high enough to affect fish health. Although none of the stomach contents of the juvenile Chinook salmon sampled had PAH concentrations at or above levels that significantly reduce growth (Meador et al. 2006), PAH levels in salmon stomach contents from the Snohomish and Green/Duwamish nearshore marine habitats approached or exceeded PAH doses observed to alter plasma chemistry and lipid class profiles (Meador et al. 2006). However, Meador et al. (2006) noted that if fish used in their experiment that showed altered growth, and had been exposed to the dietary PAH concentrations that they tested for a longer time, their growth would likely have been reduced. Since the number of summed PAHs (17 total) from our juvenile Chinook stomach contents were less than what Meador et al. used in their dietary feeding experiment (21 total), our comparison against these thresholds is conservative and may underestimate the proximity of the juvenile Chinook stomach contents to Meador's lower thresholds. Additionally, even if PAHs are below toxicity thresholds, they may contribute to immunosuppressive or growth-altering impacts of other contaminants that are present in environmental mixtures (e.g., see Loge et al. 2005).

In total, approximately one third of the salmon sampled from Puget Sound, regardless of the degree of development, had contaminant concentrations associated with adverse effects, indicating that a significant proportion of Puget Sound Chinook salmon are at risk for some type of health impairment due to contaminant exposure. Moreover, the types of health impairments that Puget Sound salmon likely experienced, can affect their marine survival. For example, adequate energy reserves and normal growth are vital to juvenile fish survival, and also strongly influence reproductive potential of adult fish. The immune system plays an important role in the survival of individuals and, therefore, the productivity of the population (Segner et al. 2012).

The effects of contaminant exposure on the health and marine survival of juvenile Chinook salmon is likely greater than that inferred from a comparison of individual contaminants and a limited number of adverse effect endpoints we have evaluated. The salmon are undoubtedly exposed to more toxic chemicals in the urbanized estuaries and nearshore marine habitats of Puget Sound than were assessed in this study and some of these contaminants may also be present in the offshore habitat. Moreover, juvenile salmon are exposed to complex mixtures of contaminants, potentially exacerbating the effects of the measured contaminant exposure of individual contaminants on their health and survival. For example, within the Snohomish system, 21.4%, of the fish samples exceeded a PCB adverse effects threshold, 35.7% had PBDE concentrations at levels documented to increase disease susceptibility, 21.4% had PBDE concentrations at levels documented to alter for thyroid hormone levels, and 16.7 % had elevated PAH concentrations in their stomach contents that may alter growth rates. Currently, there are very limited data on the toxicity of environmentally relevant contaminant mixtures that salmon are exposed to as they migrate through developed habitats; however, there is a high likelihood for additive adverse effects (Meador 2006). In a laboratory study exposing coho salmon to pesticides, Laetz et al. (2009) demonstrated synergistic adverse effects of exposures to pesticide mixtures compared to individual pesticides.

Several studies in Puget Sound have documented that growth is impaired for out-migrant juvenile Chinook salmon exposed to contaminants mixtures in urban estuaries and bays of Puget Sound (Varanasi et al. 1993). The growth rates of juvenile Chinook salmon collected from urban estuaries (e.g., Hylebos and Duwamish Waterways) and held in the laboratory for 90 days were lower than those for fish from the corresponding hatcheries or from nonurban estuaries. Furthermore, concentrations of plasma hormones involved in the regulation of growth in fish, such as thyroxine (T4), triiodothyronine (T3), and insulin-like growth factor (IGF),

were altered in salmon from urban estuaries in comparison with hormone levels in hatchery or non-urban fish (Casillas et al., unpublished data). Thus exposure to contaminants may interfere with the endocrine modulation of growth in juvenile salmon, reducing overall growth.

Arkoosh et al. (1998) provided a particularly compelling example of the importance of environmentally relevant contaminant mixtures on fish health. In that study, hatchery Chinook salmon collected from an urban and a non-urban estuary in Puget Sound and their corresponding hatcheries were each exposed to a naturally occurring pathogen. Mixtures of contaminants present in the urbanized habitats of Puget Sound suppressed the immune system, rendering those juvenile Chinook salmon more vulnerable to naturally occurring pathogens. Chinook salmon collected from the urban estuary were more susceptible to bacteria-induced mortality from naturally occurring marine pathogens than were fish from the corresponding hatchery upstream from the urban- estuary, and fish from a nonurban estuary and its corresponding hatchery (Arkoosh et al. 1998). Laboratory exposure studies with sediment extracts and contaminant model mixtures demonstrated that contaminants such as PCBs and PAHs, apart from other estuarine variables specifically associated with the Duwamish and Hylebos Waterways, could independently suppress immune function and increase disease susceptibility in juvenile Chinook salmon (Arkoosh et al. 2001, Arkoosh et al. 1994b).

Most recently, Meador (2014) reported that the cumulative impact of contaminant exposure on juvenile Chinook salmon has affected their marine survival. Meador (2014) reported that juvenile hatchery-produced ocean-type Chinook salmon migrating through contaminated rivers and estuaries had 45% lower marine survival than those from uncontaminated habitats. A parallel analysis of hatchery-produced coho salmon from many of the same hatcheries did not show reduction in marine survival associated with contaminated rivers, indicating that the effects of estuarine contamination depend on species, likely because the Chinook salmon spend more time in estuaries than do coho salmon, which generally move more quickly to offshore marine waters. Meador (2014) concluded that contamination was an important factor affecting the marine survival of Chinook salmon, along with other physical measures of physical habitat degradation that typically accompany contamination of estuarine and nearshore marine habitats.

In summary, although risks to salmon populations in estuarine and nearshore environments have focused largely on alterations to or loss of physical habitat attributes (Bottom et al. 2005, Fresh et al. 2005, Gray et al. 2002), the data presented in this report confirms the findings from other contaminant studies that developed estuarine and nearshore habitats of the Pacific Northwest are also degraded with chemical contaminants that pose a significant risks to salmon populations (Bottom et al. 2005, Fresh et al. 2005, Gray et al. 2002, Johnson et al. 2013, Loge et al. 2005, Meador 2014, Spromberg and Meador 2005). Estuarine and nearshore ecosystems provide a vital role as juvenile rearing habitat for Chinook salmon and can be particularly important in the recovery of species at risk (Feist et al. 2003; Fresh et al. 2005). Furthermore, offshore habitats also contain POP that may impair the health of juvenile salmon, particularly PCBs. To effectively remediate habitat loss and degradation in developed estuarine and nearshore habitats, as well as habitat degradation in offshore habitats, managers must address the factors that impair the structure and function of both physical and chemical attributes of juvenile salmon habitats.

Routes of Contaminant Exposure

Analysis of contaminant body burden (ng/fish) in fish from estuary, nearshore, and offshore habitats revealed that along the migratory pathway salmon accumulated the majority of the mass of POPs in their bodies from offshore habitats, indicating that sources of POPs to fish migrating to the Pacific Ocean are not limited to

contaminant exposure in developed rivers and nearshore habitats. POP contaminant loading from urbanized river system areas and other sources is reaching non-urbanized areas offshore habitats where juvenile Chinook salmon may feed for several months, sometimes accumulating concentrations high enough to potentially impair their health. These findings suggest that that controlling the initial release of contaminants to river system and other sources may be necessary to protect offshore habitats and their associated pelagic species, including Chinook salmon.

For example, the offshore habitat is the predominant habitat along the migratory pathway where juvenile Chinook salmon are exposed to TPCBs, accounting for 85% to 90% of the TPCBs body burden in fish in offshore habitats. Furthermore, 43% of Chinook salmon in offshore habitats accumulated sufficient levels of TPCBs to exceed adverse effects thresholds. Historical input of PCBs into the Puget Sound ecosystem from multiples sources has resulted in the transport of PCBs to offshore habitat such that the a pelagic food web that is highly contaminated with PCBs (O'Neill and West 2007, West et al. 2011a, West et al. 2011b, West et al. 2008). Continued exposure in the offshore waters is a particular concern for Puget Sound Chinook salmon because up to 30% of the population resides in Puget Sound throughout the marine rearing phase rather than migrating to the Pacific Ocean (Chamberlin et al. 2011, O'Neill and West 2009). Indeed, O'Neill and West (2009) estimated that 22% of the adult Puget Chinook salmon had PCB concentrations above the PCB adverse effects threshold (Meador et al. 2002), in large part because of accumulation in the offshore waters of Puget Sound.

Similar to TPCBs, the offshore habitat is the predominant route of exposure for $\sum_{11}$PBDEs and $\sum_{6}$DDTs, accounting for 80 to 88% of the $\sum_{11}$PBDE body burden and 69% to 86% of the $\sum_{6}$DDTs body burden in salmon in offshore habitats. Unlike TPCB, the concentrations of $\sum_{6}$DDTs in salmon in offshore habitats were well below concentrations known to adversely affect salmon health and are not likely to increase in the future, given the very low concentrations detected in estuary and nearshore habitats along their migratory route. Approximately five percent of salmon samples collected in the offshore habitat (one sample from the Central Basin) had a$\sum_{11}$PBDEs concentration high enough to adversely affect fish health, based on known adverse effects concentrations for disease susceptibility and altered thyroid hormones, however the another sample collected had a concentration just below adverse effects concentrations. It is not known if continued PBDE loadings to estuary and nearshore habitats will eventually be transported to offshore habitats at some future time, resulting in a higher percent of salmon with PBDE concentrations above adverse effects concentrations.

Remediation of estuary and nearshore habitats to reduce POP exposure to juvenile Chinook salmon may also be useful to improve the health of juvenile Chinook salmon. Although juvenile Chinook salmon in estuary and nearshore habitats accumulated a lower mass of POPs (i.e., body burden measured as ng POP per fish) than salmon in offshore habitats, salmon in estuary and nearshore habitats of developed river systems often had POPs concentrations (ng POP per g of fish tissue) above adverse effects concentrations. Analysis of contaminant body burden (ng/fish) in fish from estuary and nearshore habitat of individual river systems revealed that the habitat along the migratory pathway where salmon are exposed to POPs (i.e., the route of contaminant exposure) depended on the river system and the contaminant. Thus, management efforts to reduce contaminant exposure in river systems must be prescriptive to the individual river system and contaminant of concern.

For example, the nearshore habitat was the major route of TPCB exposure for fish from the Skagit, Snohomish and Nisqually river systems. However, in the more developed Green/Duwamish River system, most (54 – 61%) of the PCBs were accumulated while fish were migrating through the freshwater and/or estuary portion of their

migratory pathway. From a biological perspective, clean-up efforts should be directed mostly toward the freshwater and estuary habitat, though economic and logistical constraints might also affect any such decision. Moreover, additional assessments are needed to determine if the PCBs are accumulated in the lower estuary or further upriver. However, the Duwamish estuary is a Superfund Site, with highly PCB-contaminated sediments, suggesting that the lower estuary may be a major route of contaminant exposure for juvenile Chinook salmon from this system.

The comparison of PBDE body burdens in Chinook salmon collected from the estuary and the nearshore habitats of the Snohomish River system indicated that the major route of PBDE exposure was the freshwater and/or estuary habitat, rather than the nearshore habitat and thus, efforts to reduce exposure of juvenile salmon to PBDEs should be directed towards freshwater and estuary habitats. Salmon with high PBDE levels from the Snohomish estuary were captured just downstream of the outfall of a waste water treatment facility for the City of Everett; however, it is unknown if that facility was the primary source of PBDEs. A 2009 study of treated wastewater samples from ten POTWs of varying types of treatment process, size, and source of wastewater, distributed around the Puget Sound Basin revealed that PBDE concentration in effluent from another outfall of the Everett POTW that discharges directly into Puget Sound (downstream of where we sampled), was approximately an order of magnitude higher than effluent samples from the other POTWs (WA Dept. of Ecology 2010). It is not known if the Everett outfall nearest the salmon sampling site also had elevated PBDEs. Additional assessments are necessary to identify the specific source of $\sum_{11}$PBDEs that may be contributing to the high levels measured in juvenile Chinook salmon from the Snohomish estuary, including measuring PBDEs in Chinook salmon from higher upstream.

In contrast to the Snohomish system, juvenile Chinook salmon in the Green/Duwamish system accumulated significant portions of their $\sum_{11}$PBDE body burdens from both the freshwater and/or estuary and nearshore habitats. Management efforts to reduce PBDE exposure in salmon from the Green Duwamish system may have to address multiple freshwater and nearshore sources. Similar to the TPCB results, as salmon moved from river systems to offshore habitats their $\sum_{11}$PBDE body burden (ng/g) continued to increase, indicating that they continued to be exposed to $\sum_{11}$PBDEs.

Although the accumulated $\sum_{6}$DDT concentrations were low compared to TPCB and $\sum_{11}$PBDEs, the major route of $\sum_{6}$DDT exposure for salmon from the Skagit and Green/Duwamish river systems was the freshwater and /or estuary habitat. These results indicate that historical use of DDT in agriculture practices within the Skagit River basin, and in urban landscaping practices within the lands surrounding the highly urbanized Green and Duwamish rivers, are continuing to expose juvenile salmon to DDTs, albeit below levels known to cause adverse effects. The major route of $\sum_{6}$DDT exposure for fish migrating through the Snohomish and Nisqually river system was the nearshore habitat.

For these comparisons of POP body burden between estuary and nearshore samples, for each river system, the fish in the nearshore were assumed to have migrated out of the nearest estuary habitat of the same system. Previous studies by Brennan at al. 2004 and Fresh et al. 2005 observed considerable mixing of salmon hatchery populations among fish sampled in the nearshore, including population from outside the basin in which they were captured. However, the CWT information collected from our study indicated that hatchery fish in the nearshore habitats of a particular river system originated from nearby estuary habitats of the same system, possibly because our sampling of the nearshore was in generally more confined to areas closer to the river

mouths and occurred over a narrow time window (i.e., June, approximately a month after the estuary sampling), than those conducted by Brennan et al. (2004) and Fresh et al. (2005).

All calculations to estimate the percent of POPs that were accumulated in freshwater, estuary, nearshore and offshore habitats assume that the Chinook salmon sampled at each of the locations accurately represent the concentration and body burdens of all Chinook salmon at the site. However, our samples sizes at each sample location were small. Composite tissue samples composed of multiple fish were used to dampen the variability associated with small sample size used in this study, however, larger sample sizes would provide more robust estimates.

FUTURE MONITORING AND RESEARCH NEEDS

Chemicals of Emerging Concern

This study of juvenile salmon exposure was limited to contaminants previously documented to be of concern in the Puget Sound ecosystem. Future monitoring efforts should be expanded to include additional chemical of emerging concern (CECs).

Over 30,000 chemical substances are in wide commercial use (> 1 ton per year), and the vast majority are not measured in environmental media and have unknown effects on biota (Muir and Howard 2006). For the limited number of environmental studies that have been conducted, endocrine disrupting chemicals, especially estrogenic chemicals (ECs) are of special concern because of their widespread presence in aquatic environments and their potentially far reaching effects on hormone-mediated physiological functions including growth, development, behavior, and reproduction of fish and wildlife. Legacy pollutants like PCBs have long had documented effects on many vertebrate species associated with their estrogenic properties (Bergeron et al. 1994), however, many more ECs are present on the present in aquatic systems. For example, within Puget Sound, da Silva et al. (2013) documented that the likely cause of the vitellogenin (VTG) induction in male English sole from Puget Sound was due to environmental sources of ECs, including, 17β-estradiol (E2), and bisphenol A, which were detected in bile of this species. Pharmaceutical and personal care products are also of emerging concern, as they are detectable in wastewater and stormwater, and may adversely affect aquatic organisms (Kostich et al. 2010, Kostich and Lazorchak 2008, Lubliner et al. 2010, Morace 2012). Pharmaceuticals and personal care products (PPCPs) have been detected in the discharge from waste water treatment plants (Lubliner et al. 2010), and in marine sediments (Long et al. 2013). Throughout the Pacific Northwest, including the Columbia River Basin, some current-use pesticides like pyrethroids are also considered CECs, and can have an adverse impact on the environmental health of anadromous salmonids.

There is ample evidence that juvenile salmon and steelhead in some Puget Sound basin streams are exposed to current use pesticides at levels high enough to cause neurobehavioral toxicity. Low-level exposures to two classes of current-use pesticides, organophosphates and carbamates, directly affect behaviors that are important for salmon survival. Organophosphate and carbamate pesticides inhibit the activity of the acetylcholinesterase (AChE), an enzyme involved with nervous system function. AChE inhibition, may, in turn, disrupt several fish behaviors, including swimming, feeding, predator avoidance, and homing (Sandahl et al. 2005, Scholz et al. 2000). Interference with such basic life activities could clearly have adverse effects on salmon growth, survival, and reproductive success. Additionally, pesticides commonly occur as mixtures, sometimes producing greater-than-additive (i.e. synergistic) effects (Laetz et al. 2009). Baldwin et al. (2009) developed a

model that explicitly linked sublethal AChE inhibition to feeding behavior, food ration, growth, and size at migration, which in turn was then used to estimate size- dependent survival during migration and transition to the sea. Individual survival estimates were then used to calculate population productivity and growth rate. Baldwin et al. (2009) concluded that short-term (i.e., four-day) exposures that are representative of seasonal pesticide use may be sufficient to reduce the growth and size at ocean entry of juvenile Chinook salmon, and, by extension, subsequent size-dependent marine survival. Additionally, some pesticides target aquatic insects that are prey for salmon (reviewed by Macneale et al., 2010).

Limited information is available on the extent to which juvenile salmon are exposed to CECs, including ECs, PPCPs, and current use pesticides, and what effects such exposure might have on long-term survival. There is some evidence that juvenile Chinook salmon are exposed to ECs in estuarine and nearshore waters at levels that can affect their reproductive development. Peck et al. (2011) documented higher plasma levels of estrogen-inducible yolk protein, VTG, in Chinook salmon at sites such as Elliott Bay and the mouth of the Snohomish River than non-exposed hatchery control fish. Juvenile Chinook salmon with elevated VTG during a sensitive early life stage could experience delayed reproductive effects such as those observed in flounder or rainbow trout (Benetau-Pelissero et al. 2001, Hashimoto et al. 2000).

Currently, an independent study is underway in the Skagit and Puyallup systems to characterize Chinook salmon exposure to a wide range of CECs including PPCPs, and industrial compounds believed to be highly relevant to the Puget Sound and to assess the effects of CECs on salmon health (see Yeh et al. 2013). CECs that are detected in this study, especially those that are demonstrated to affect the salmon health, should be considered for long-term monitoring studies of contaminant exposure in juvenile Chinook salmon throughout the Puget Sound region.

Sampling Locations

The geographic scope of this study, although larger than any previous assessment of contaminant exposure for juvenile Chinook salmon from Puget Sound, should be expanded. This study yielded some basic information regarding contaminant exposure of a sensitive life stage of Chinook salmon in Puget Sound, relative to watershed land-use characteristics. However, future monitoring of contaminant exposure should be expanded to more fully assess the additional populations contributing to the production of Puget Sound Chinook salmon. In particular, future monitoring should include populations from Hood Canal, the Nooksack and Stillaguamish river systems.

CONCLUSIONS

A significant proportion of Puget Sound Chinook salmon are at risk for some type of health impairment due to contaminant exposure. Approximately one third of the juvenile Chinook salmon sampled from Puget Sound, regardless of the degree of development, had contaminant concentrations associated with adverse effects. Levels of TPCBs, $\sum_{11}$PBDEs in whole body tissue samples of salmon from the Snohomish, Green/Duwamish and Hylebos/Puyallup river systems, and TPCBs in fish from the offshore habitat of the Whidbey and Central Basins were high enough to potentially cause adverse effects, including reduction in growth, disease resistance, and altered hormone and protein levels. Additionally, $\sum_{42}$PAHs in stomach contents were elevated in salmon from the nearshore habitats of the Snohomish and Green/Duwamish systems, at concentrations high enough to potentially affect growth and alter plasma chemistry and lipid class profiles. Elevated concentrations of copper

and lead were also measured in gills tissue of salmon from developed nearshore marine habitat, however, the potential effects on salmon health are unknown. In contrast, levels of cadmium and nickel in fish gill tissues appear to reflect spatial differences likely associated with naturally occurring levels of these elements in the environment.

Remediation of estuary and nearshore habitats to reduce POP exposure to juvenile Chinook salmon may also be useful to improve the health of juvenile Chinook salmon. However, management efforts to reduce contaminant exposure in river systems must be prescriptive to the individual river system and contaminant of concern. Moreover, sources of POPs to Chinook salmon migrating to the Pacific Ocean are not limited to contaminant exposure in developed rivers and nearshore habitats. POP contaminant loads from urbanized river system areas and other sources are reaching non-urbanized offshore habitats where juvenile Chinook salmon may feed for several months, sometimes accumulating concentrations high enough to potentially impair their health. These findings suggest that controlling the initial release of contaminants to the environment may be necessary to protect offshore habitats and their associated pelagic species, including Chinook salmon.

The results of this study augment previous sampling initiated as early as 1998, and will be used to establish a solid time series of contaminant conditions in juvenile Chinook salmon that can be used to fulfill the *Toxics in Fish Vital Sign* goal of tracking time trends of fish health. Future monitoring of contaminant exposure in juvenile salmon should include CECs in the Puget Sound ecosystem. Additionally, the geographic scope of the monitoring should be expanded to include other river systems that contribute to the production of Puget Sound Chinook salmon, such as salmon populations from Hood Canal, and the Nooksack and Stillaguamish river systems.

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APPENDIX A: Detailed Sample Collection Methods

Fish Collection Efforts – Detailed Descriptions

Skagit Estuary and Nearshore Marine Habitats

On May 30, 2013, with the help of biologists from the Skagit River System Cooperative, 40 juvenile Chinook salmon were collected from three sites within the north fork of the Skagit estuary using a beach seine and two fyke nets (Figure A 1 and Table A 1). The fish were transported on ice back to the Marine Resource Lab (MRL) at the Natural Resources Building (NRB) in Olympia, WA where they were processed for scales, otoliths and stomach contents only. Each fish was frozen and stored individually with their fish ID number. The gills and whole bodies were composited the next day (May 31, 2013). The 40 fish were composited into four samples of each matrix type, with each composite containing 10 fish (Table 2). Due to the small amount of stomach contents collected, the four original composite samples were later combined into one composite containing all 40 fish to guarantee the proper amount was available for chemical analysis (Table 2).

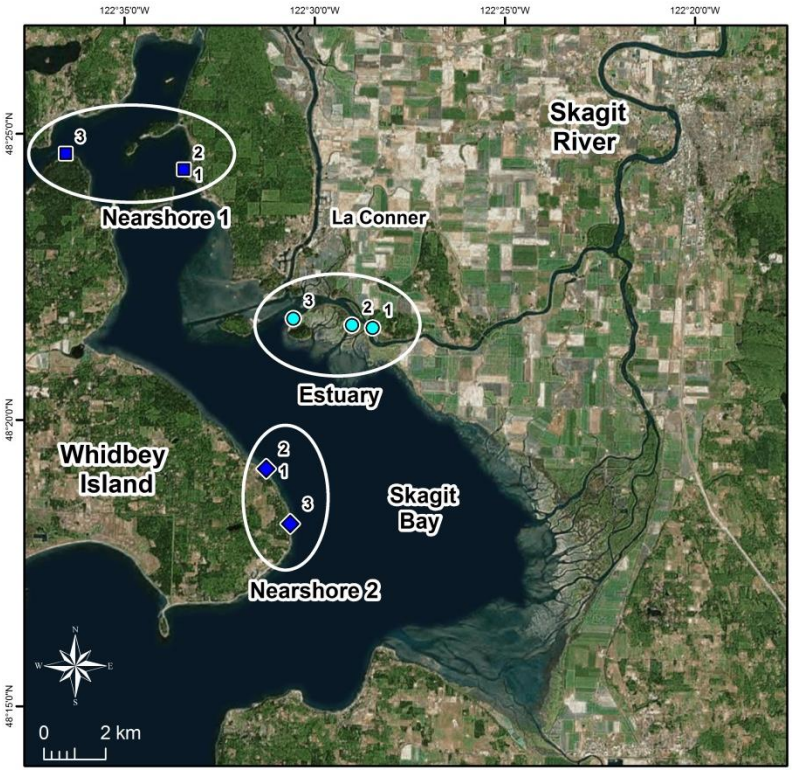

Figure A 1. Juvenile Chinook salmon collection locations in the lower Skagit River (light blue circles), the northern (dark blue squares), and western estuary sites (dark blue diamonds). Note that the symbols for sample locations overlap: the latitude and longitude of the numbered collection sites are provided in Table A 1.

On June 19, 2013, with the help of five biologists from the Skagit River System Cooperative, 42 juvenile Chinook salmon were collected from four sites within North Skagit Bay (Skagit Nearshore 1; Figure A 1 and Table A 1) using a beach seine. Two, four minute sets were made at three of the four total locations. At the last location, two Chinook with CWTs were caught and subsequently released because additional fish were not needed for sample collection. The fish were transported on ice to the National Oceanic and Atmospheric Association's (NOAA) laboratory in Mukilteo where three biologists from NOAA's Northwest Fisheries Science Center (NWFSC) assisted the Puget Sound Ecosystem Monitoring Program (PSEMP) group with processing the fish. Thirty fish were processed as described and stomach contents, gills and whole bodies were composited into five samples of each matrix type, with each composite containing six fish (Table 2). Due to the small amount of stomach

Table A 1. Juvenile Chinook collection information for all estuary and nearshore sites

Map #	Study Location	Collection Site	Sample Date	Latitude	Longitude	Gear
1	Skagit	Estuary	5/30/2013	48.3628	-122.4712	beach seine
2			5/30/2013	48.3635	-122.4803	fyke net
3			5/30/2013	48.3649	-122.5060	fyke net
1		Nearshore 1	6/19/2013	48.4076	-122.5557	beach seine
2			6/19/2013	48.4076	-122.5557	beach seine
3			6/19/2013	48.4113	-122.6076	beach seine
1		Nearshore 2	6/20/2013	48.3210	-122.5163	beach seine
2			6/20/2013	48.3210	-122.5163	beach seine
3			6/20/2013	48.3051	-122.5052	beach seine
1	Snohomish	Estuary	5/28/2013	48.0068	-122.1782	beach seine
2			5/28/2013	48.0017	-122.1778	beach seine
1		Nearshore 1	6/26/2013	48.0304	-122.2366	beach seine
2			6/26/2013	48.0352	-122.2511	beach seine
1		Nearshore 2	6/26/2013	47.9633	-122.2469	beach seine
2			6/26/2013	47.9588	-122.2588	beach seine
3			6/26/2013	47.9542	-122.2904	beach seine
4			7/11/2013	47.9591	-122.2704	beach seine
5			7/11/2013	47.9632	-122.2471	beach seine
6			7/11/2013	47.9591	-122.2705	beach seine
1	Green/	Estuary	5/22/2013	47.5562	-122.3454	beach seine
2	Duwamish		5/22/2013	47.5561	-122.3459	beach seine
3			5/22/2013	47.5560	-122.3465	beach seine
4			5/22/2013	47.5561	-122.3473	beach seine
1		Nearshore 1	6/24/2013	47.5875	-122.3775	beach seine
2			6/24/2013	47.5842	-122.3696	beach seine
3			6/24/2013	47.5837	-122.3699	beach seine
4			6/24/2013	47.5875	-122.3775	beach seine
5			6/24/2013	47.5903	-122.3810	beach seine
6			6/24/2013	47.5965	-122.3839	beach seine
1		Nearshore 2	6/25/2013	47.6170	-122.3584	beach seine
2			6/25/2013	47.6185	-122.3610	beach seine
3			6/25/2013	47.6202	-122.3632	beach seine
1	Hylebos/	Estuary	6/13/2013	47.2795	-122.3955	beach seine
2	Puyallup		6/13/2013	47.2726	-122.3803	beach seine
3			6/13/2013	47.2722	-122.3797	beach seine
1		Nearshore 1	6/12/2013	47.2925	-122.4121	beach seine
2			6/12/2013	47.2930	-122.4126	beach seine
3			6/12/2013	47.2930	-122.4125	beach seine
4			6/12/2013	47.2922	-122.4116	beach seine
5			6/12/2013	47.2973	-122.4287	beach seine
6			6/12/2013	47.2972	-122.4294	beach seine

Continued.

Table A 1 continued. Juvenile Chinook collection information for all estuary and nearshore sites

Map #	Study Location	Collection Site	Sample Date	Latitude	Longitude	Gear
7	Hylebos/	Nearshore 1	6/12/2013	47.2969	-122.4305	beach seine
8	Puyallup		6/12/2013	47.2975	-122.4281	beach seine
1	(continued)	Nearshore 2	6/13/2013	47.2691	-122.4486	beach seine
2			6/13/2013	47.2689	-122.4483	beach seine
3			6/13/2013	47.2757	-122.4631	beach seine
4			6/13/2013	47.2758	-122.4632	beach seine
5			6/13/2013	47.2689	-122.4484	beach seine
6			6/13/2013	47.2685	-122.4479	beach seine
1	Nisqually	Estuary	5/20/2013	47.0978	-122.6987	beach seine
2			5/20/2013	47.0700	-122.7027	beach seine
3			5/20/2013	47.0774	-122.7080	beach seine
1		Nearshore 1	6/18/2013	47.1491	-122.6361	lampara seine
2			6/18/2013	47.1419	-122.6961	lampara seine
3			6/18/2013	47.1043	-122.6919	lampara seine
4			6/18/2013	47.1109	-122.6888	lampara seine
5			6/18/2013	47.1095	-122.6733	lampara seine
6			6/18/2013	47.1302	-122.6540	lampara seine
7			6/18/2013	47.1388	-122.6331	lampara seine
1		Nearshore 2	6/18/2013	47.1271	-122.7057	lampara seine
2			6/18/2013	47.1131	-122.7424	lampara seine
3			6/18/2013	47.1159	-122.7367	lampara seine
4			6/18/2013	47.1041	-122.7258	lampara seine
5			6/18/2013	47.0968	-122.7216	lampara seine
6			6/18/2013	47.1056	-122.7166	lampara seine

contents collected, the four original composite samples were later combined into one composite containing all 30 fish to guarantee the proper amount was available for chemical analysis (Table 2). In addition, the 12 fish not used for sample collection were stored in a Ziploc bag in a -20° C freezer for possible future analysis.

The second nearshore site was sampled on June 20, 2013, with the same help from the Skagit River System Cooperative. Fifty-five juvenile Chinook salmon were collected from three beach seine sets at two locations within the western part of Skagit Bay (Skagit Nearshore 2; Figure A 1 and Table A 1). Forty of these fish were transported on ice to NOAA's laboratory in Mukilteo where two biologists from NOAA's NWFSC assisted the PSEMP group with processing the fish. Thirty of the 40 fish collected for tissue chemistry were then processed as described above and stomach contents, gills and whole bodies were composited into five samples of each matrix type, with each composite containing six fish (Table 2). After further consideration, the five original stomach contents were later combined into one composite containing all 30 fish to guarantee the proper amount was available for chemical analysis (Table 2).

Snohomish Estuary and Nearshore Marine Habitats

On May 28, 2013, 39 juvenile Chinook salmon were collected from two different locations in the Snohomish estuary by staff from the Tulalip Tribe and NOAA biologists (Table A 1and Figure A 2) using a beach seine. The

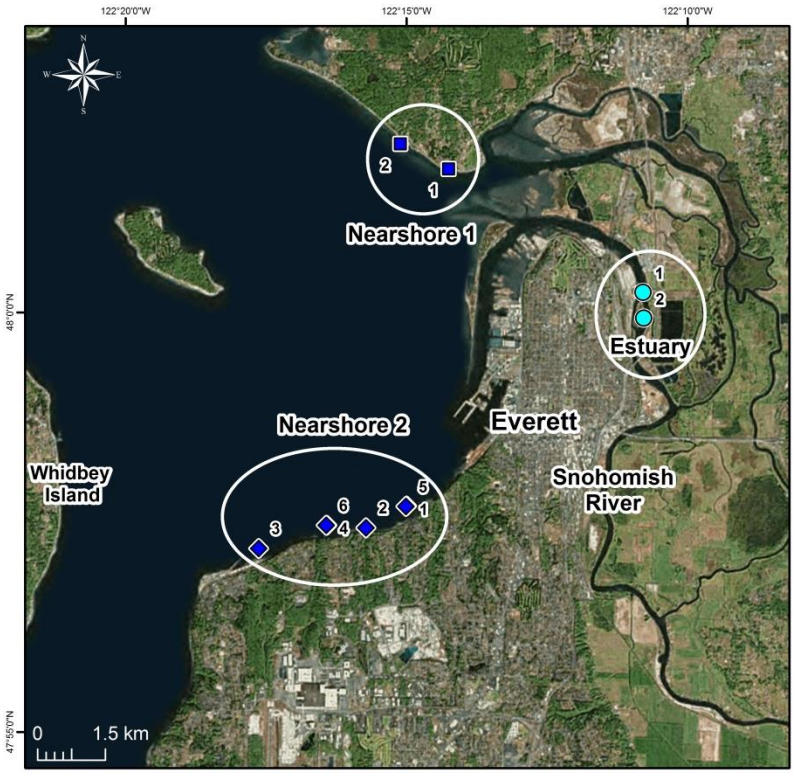

Figure A 2. Juvenile Chinook salmon collection locations in the Snohomish River (light blue circles), the northern (dark blue squares), and southern estuary sites (dark blue diamonds). Note that the symbols for sample locations overlap: the latitude and longitude of the numbered collection sites are provided in Table A 1.

fish were transported on ice back to the MRL where they were processed for scales, otoliths and stomach contents only. Each fish was frozen and stored individually with their fish ID number and the gills and whole bodies were composited the next day (May 29, 2013). The stomach contents, gills and whole bodies were composited into four samples of each matrix type, with three composites containing 10 fish each and one composite with nine fish (Table 2). After further consideration, the four original stomach contents composites were later combined into one composite containing all 39 fish to guarantee the proper amount was available for chemical analysis (Table 2).

On June 26, 2013, 55 juvenile Chinook salmon were collected by staff from the Tulalip Tribe and NOAA biologists from two locations in the northern part of the Snohomish River nearshore marine shoreline (Snohomish Nearshore 1; Table A 1 and Figure A 2) using a beach seine. Thirty fish were processed for tissue chemistry samples. The stomach contents, gills, and whole bodies were then composited into five samples of each matrix type with each composite containing six fish (Table 2). Because of the small amount of stomach contents collected from the 30 fish used for tissue chemistry, another 10 fish were processed for stomach contents alone and combined into one composite (Table 2), as a supplemental sample. After further consideration, the five original stomach contents were later combined into one composite containing all 30 fish to guarantee the proper amount was available for chemistry analysis (Table 2). Lastly, five juvenile Chinook salmon that were not used for sample collection are stored in a -20° C freezer in the MRL for possible future analysis.

Sampling for juvenile Chinook salmon in the southern nearshore marine habit of the Snohomish system (Snohomish Nearshore 2; Table A 1 and Figure A 2) took place over the course of two days due to low catch numbers during the first collection attempt. On June 26, 2013, five juvenile Chinook were collected from three

sites in the nearshore marine shoreline and on July 11, 2013, an additional 23 fish were collected from three sites using a beach seine. A total of five composites of each matrix type were created from the 28 total fish collected. Stomach contents, gills and whole bodies from fish collected on June 26, 2013 were combined into one composite per matrix. The three matrix types from the remaining fish collected in July were combined into three composites of six fish each and one composite contained five fish (Table 2).

Green/Duwamish Estuary and Nearshore Marine Habitats

On May 22, 2013, with the help of biologists from NOAA NWFSC, 42 juvenile Chinook salmon were collected from four sites near Kellogg Island within the lower Duwamish estuary (Table A 1 and Figure A 3) using a beach seine. The fish were transported to the NRB's MRL on ice and processed the day of collection. Forty fish were processed as described and stomach contents, gills and whole bodies were composited into four samples of each matrix type with each composite containing 10 fish (Table 2). After further consideration, the four stomach contents composites were combined into one composite containing all 40 fish to guarantee the proper amount was available for chemical analysis. The two remaining fish from this site were stored for potential future analysis.

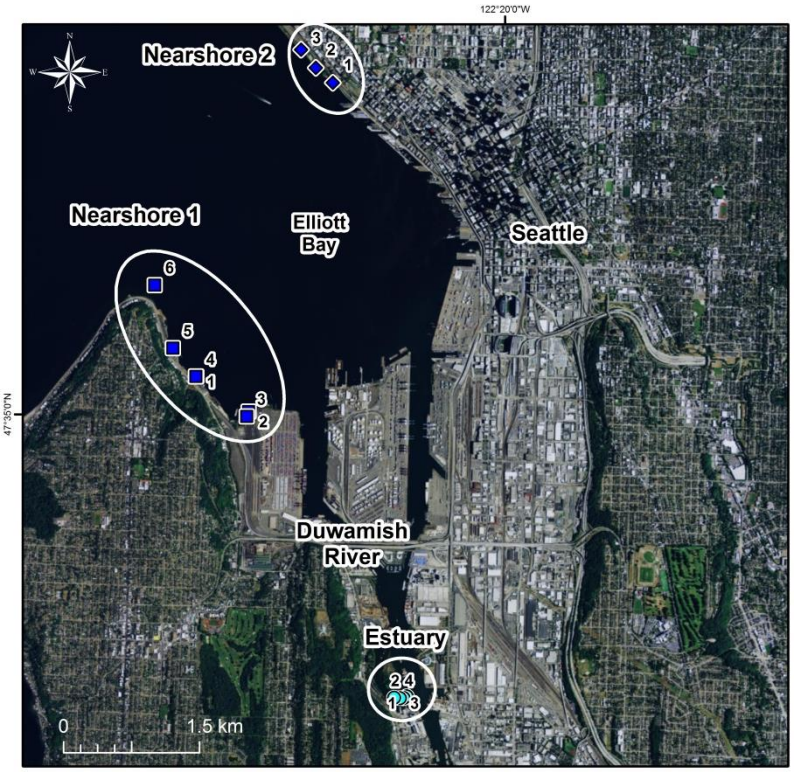

Figure A 3. Juvenile chinook collection locations in the lower Green/Duwamish River (light blue circles) and the northern (dark blue diamonds) and southern (dark blue squares) sides of the estuary.Note that the symbols for sample locations overlap: the latitude and longitude of the collections sites are provided in Table A 1.

On June 24, 2013, with the help of biologists from NOAA NWFSC, 65 juvenile Chinook salmon were collected from six sites in the western portion of Elliott Bay (Green/Duwamish Nearshore 1) using a beach seine (Table A 1 and Figure A 3). The fish were transported to NOAA NWFSC in Seattle on ice and processed the day of collection. Thirty-one fish were then processed as described and stomach contents, gills and whole bodies were combined into five samples of each matrix type, with four samples containing six fish each and one sample containing seven fish. Later, after further consideration, the five samples of stomach contents were combined

into one composite containing the contents from 31 fish to guarantee the proper amount was available for chemical analysis (Table 2). The 25 remaining fish not used for sample collection were stored in a -20° C freezer in the MRL for possible future analysis.

The following day, on June 25, 2013, with the help of the NOAA NWFSC biologists, 64 juvenile Chinook salmon were collected from three sites in the northern portion of Elliott Bay (Green Nearshore 2; Table A 1 and Figure A 3) using a beach seine. Fifty-four fish were transported to the NOAA NWFSC in Seattle on ice and 10 were kept alive and transported to the lab in aerated coolers. All fish were processed the day of collection. Thirty fish transported on ice were then processed as described and stomach contents, gills and whole bodies were combined into five samples of each matrix type, with each composite containing tissue from six fish (Table 2). Prior to chemical analysis, the five composite samples of stomach contents were combined into one composite containing stomach contents from 30 fish to guarantee the proper amount was available for chemical analysis (Table 2). The remaining 14 fish were stored in a Ziploc bag in a -20° C freezer at the MRL for possible future analysis.

Hylebos Waterway and Puyallup Nearshore Marine Habitats

On June 13, 2013, WDFW and NOAA NWFSC biologists collected five juvenile Chinook salmon from three sites in the Hylebos Waterway in Tacoma (Table A 1 and Figure A 4) using a beach seine. The fish were transported to the MRL on ice and processed as described on the day of collection. The stomach contents, gills and whole bodies were combined into one composite sample for each matrix type (Table 2).

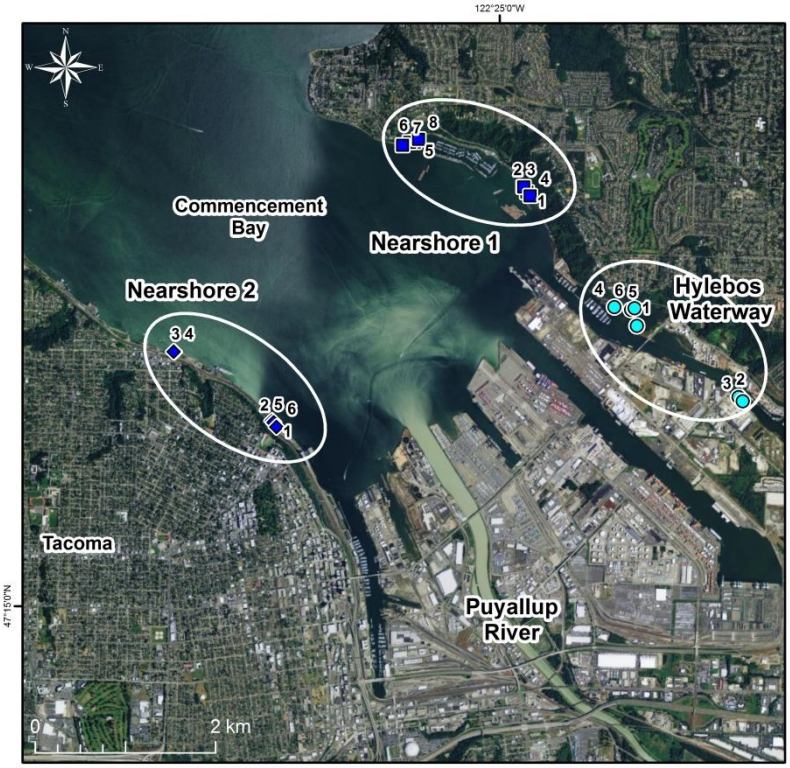

Figure A 4. Juvenile Chinook salmon collection locations in the Hylebos Waterway (light blue circles), and the eastern (dark blue diamonds) and western (dark blue diamonds) sides of the Puyallup estuary. Note that the symbols for sample locations overlap: the latitude and longitude of the collection sites are provided in Table A 1.

On June 12, 2013, WDFW and NOAA NWFSC biologists collected 57 juvenile Chinook salmon from eight sites in the northern portion of Commencement Bay (Hylebos/Puyallup Nearshore 1) using a beach seine (Table A 1 and Figure A 4). The remaining fish were transported on ice to the NRB in Olympia where they were processed as described. Thirty fish were processed for stomach contents, gills and whole bodies into five samples of each matrix type with each composite containing six fish (Table 2). Prior to chemical analysis, the five stomach contents composite samples were combined into one composite containing stomach contents from 30 fish (Table 2). Finally, 15 fish not used for sample collection were stored in a Ziploc bag in a -20° C freezer in the MRL for possible future analysis.

On June 13, 2013, WDFW and NOAA NWFSC biologists collected 37 juvenile Chinook salmon from six sites in the southern portion of Commencement Bay (Hylebos/Puyallup Nearshore 2; Table A 1 and Figure A 4) using a beach seine. The fish were transported to the NRB in Olympia where they were processed as described on the day of collection. The 37 fish were processed for stomach contents, gills and whole bodies into five samples of each matrix type with three composites containing tissue from seven fish and two composites containing tissue from eight fish (Table 2).

Nisqually Estuary and Nearshore Marine Habitats

On May 20, 2013, with the help of two biologists from the Nisqually River Foundation, 40 juvenile Chinook were collected from three different sites in the Nisqually estuary using a beach seine (Table A 1 and Figure A 5). The fish were transported to the NRB's MRL on ice and stored in the freezer until processing approximately two weeks later on June 5, 2013. The 40 fish were processed as described and stomach contents, gills and whole bodies were combined into four samples of each matrix type, with each composite containing 10 fish (Table 2).

Sampling of both nearshore marine habitats sites took place on June 18, 2013, approximately one month after the estuary sampling. A lampara seining method, in which two boats deploy a purse seine in the nearshore, was used to collect the salmon. In addition to the two biologists from the Nisqually River Foundation, two biologists from the United States Geographic Service (USGS) helped collect the juvenile Chinook salmon. Chinook salmon were collected from two distinct areas of the nearshore, the east side, Nearshore 1, and the west side, Nearshore 2, with collection beginning on the east side, moving to the west side and then back to the east side at the end of the day. A total of 43 juvenile Chinook salmon were collected from seven different sites in Nearshore 1 (east side; Table A 1 and Figure A 5) and a total of 45 were collected from six different sites in Nearshore 2 (west side; Table A 1 and Figure A 5). All the fish were transported on ice and all biological samples were resected and stored accordingly on the day of collection. For each nearshore location, 35 juvenile chinook were processed as described and their stomach contents, gills and whole bodies were composited into fives samples of each matrix type, with each composite containing seven fish (Table 2). In addition, 10 fish collected in Nearshore 2 were not used for sample collection and are stored in a Ziploc bag in a -20° C freezer for possible future analysis.

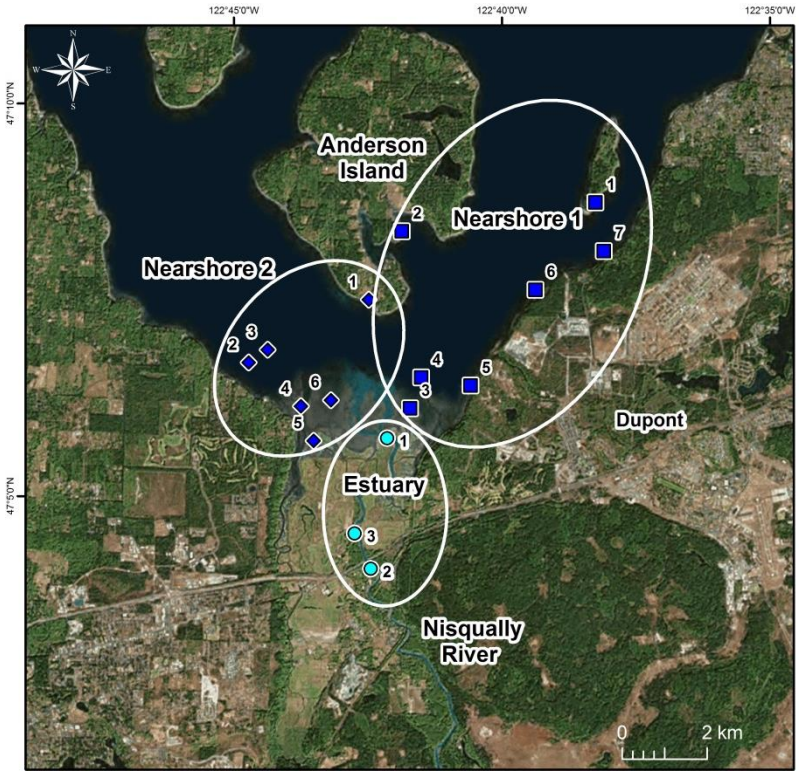

Figure A 5. Juvenile Chinook salmon collection locations in the Nisqually River (light blue circles), the eastern (dark blue squares) and western (dark blue diamonds) sides of the estuary. Note that the symbols for sample locations overlap: the latitude and longitude of the numbered collection sites are provided in Table A 1.

Offshore Basins

During July and October 2013, a WDFW PSEMP biologist took part in two Canadian Department of Fisheries and Oceans (DFO) mid-water trawl surveys within four major basins of Puget Sound onboard the *CCGS W.E. Ricker*, a 190 foot research vessel. A total of total of 30 juvenile Chinook salmon were collected from 6 tows in Central Basin from July 9-10, 2013 (Table A 2). An additional 73 juvenile Chinook salmon were collected from 5 tows in Central Basin (n = 25), five tows in South Basin (n = 28), two tows in Whidbey Basin (n = 10) and two tows in Admiralty Inlet (n = 10) from October 3-6, 2013 (Table A 2 and Figure 1)

In brief the collection process went as follows; 1) after the net was emptied, the catch was divided by species, 2) a maximum of 10 juvenile Chinook salmon were immediately randomly chosen, 3) length and weight measurements were recorded, 4) fish type (i.e., adipose intact, CWTs present, adipose clipped) was noted and recorded, 5) the fish were returned to the DFO crew for further processing if necessary (i.e., fins snips, stomach content analysis, scale collection) and finally, 6) all whole body samples were saved in a Ziploc bag and stored in a -20° C freezer onboard the boat. All samples collected on board the *CCGS W.E. Ricker* were then transported to the MRL and stored in a -20° C freezer.

Table A 2. Juvenile Chinook collection information for offshore sites

Offshore Location	Station ID	Sample Date	Latitude	Longitude	Gear
Admiralty Inlet	AI02	10/6/2013	47.9932	-122.6594	Midwater trawl
	AI03	10/6/2013	48.0592	-122.6223	Midwater trawl
Whidbey Basin	WB10	10/5/2013	48.0122	-122.3426	Midwater trawl
	WB11	10/5/2013	47.9383	-122.3457	Midwater trawl
Central Basin	CPS02	7/9/2013	47.4929	-122.3997	Midwater trawl
(July)	CPS03	7/9/2013	47.4228	-122.3635	Midwater trawl
	CPS05	7/9/2013	47.3610	-122.4198	Midwater trawl
	CPS06	7/9/2013	47.3611	-122.5389	Midwater trawl
	CPS07	7/9/2013	47.4136	-122.5309	Midwater trawl
	CPS11	7/10/2013	47.7146	-122.4252	Midwater trawl
Central Basin	CPS16	10/3/2013	47.5536	-122.4210	Midwater trawl
(October)	CPS23	10/3/2013	47.4195	-122.5292	Midwater trawl
	CPS26	10/6/2013	47.7001	-122.4380	Midwater trawl
	CPS28	10/6/2013	47.8647	-122.4877	Midwater trawl
	CPS29	10/6/2013	47.9436	-122.5059	Midwater trawl
South Basin	SPS02	10/4/2013	47.2242	-122.8317	Midwater trawl
	SPS04	10/4/2013	47.1663	-122.7464	Midwater trawl
	SPS04	10/4/2013	47.1663	-122.7464	Midwater trawl
	SPS05	10/4/2013	47.1464	-122.6653	Midwater trawl
	SPS06	10/4/2013	47.3310	-122.6998	Midwater trawl
	SPS07	10/4/2013	47.2406	-122.6692	Midwater trawl

APPENDIX B: Data Quality Control Check (POPs, PAHs, and trace metals)

The data quality control checks for chemical analyses met the criteria outlined in the QAPP for this project (O'Neill et al. 2013) except for minor deviations (discussed below) that did not compromise the usability of the results.

POPs Analyses - Continuing calibration verification standards for RSD were met for all analytes in all analytical sample sets except for three POP analytes (aldrin, dieldrin, and beta-chlordane) in one set of whole body tissue samples (set PS2956); the relative standard deviation (RSD) of aldrin, dieldrin, and beta-chlordane responses relative to the surrogate standard were 16.0, 17.7 and 16.0, respectively, just outside the ≤ 15% quality control criteria. These slight violations of the QC criteria we not considered to affect the reported values, especially because these analytes were seldom detected in other samples sets.

Replicate Standard Deviations (RSD) control criteria for sample replicates (RSDs are to be ≤ 15% for ≥ 90% of the analytes that have concentrations ≥ 1 ng/g) were meet for all samples sets except one sample in set PS2977 and one sample in set PS3088. One of the three replicate samples in set PS2977 had levels of certain PCB congeners (e.g., CB 99, CB101/90, CB118, CB138/153/164) that were approximately 30 – 60% higher than the levels reported for the other two replicate samples. An examination of the ultraviolet peak patterns from the size-exclusion clean up step indicated that this sample had a different pattern from the other two triplicate samples, suggesting that this sample was not analytically homogenous to the other two replicate samples. In set PS3088, two analytes, BDE47 and BDE99, the RSD values were 16.9% and 73.6%, respectively. In some instances, the concentrations of analytes were so low that they were detected in one sample or two samples but were below the LOQ in the other sample(s) — in these cases the RSD may be >50%, but this is an artifact of the LOQ. For all replicates, we reported the original values rather than the replicates.

Overall, the limit of quantitation (LOQ) for most organic contaminants (Table B 1 and Table B 2) fell below the expected ranges specified in the QAPP for this project (O'Neill et al. 2013).

PAHs Analyses -Continuing calibration verification standards for PAHs samples were met for all analytes except for IDP – indeno[1,2,3-*cd*]pyrene, however, this did not affect the reported values because IDP comprises < 1% of the summed total PAHs concentration. In addition, the method blank and surrogate recovery quality control samples all met established laboratory criteria. Sample replicates were not performed due to insufficient sample volume.

Concentrations of individual analytes measured in SRM 1974c were generally in excellent agreement with the certified and reference values published by NIST with the exception of a few analytes that were just outside the acceptable confidence interval for each analyte, and thus did not substantively affect our reporting results. The quality control criteria for SRMs that 70% of the individual analytes are to be within the 30% of either end of the 95% confidence interval of the certified SRM value were met for all but three sets of samples, PS2979, PS2980, and PS3091. In each of these sets, two analytes (1MP – 1-methyphenanthrene (1MP) and benzo[*j*]fluoranthene +benzo[*k]*fluoranthene) were just outside the acceptable confidence interval for each analyte, and did not substantively affect our reporting results. For set PS2979, three additional analytes (3MP – 3-methylphenanthrene and 9MP – 9-methylphenanthrene; and BeP – benzo[*e*]pyrene) were also just outside the acceptable confidence control limits for certified reference values (i.e., 4.2 vs. 4.1 n/g g for IMP, 5.9 vs. 5.4. for 3MP and 9.8 vs 9.6 for BeP).

Table B 1. Average limit of quantitation (LOQ) for 25 analytes or congener groups (ng/g wet weight) reported in juvenile Chinook whole bodies (less gills and stomach contents).

Analyte	Average LOQ
Hexachlorobenzene	0.16
α-hexachlorocyclohexane	0.15
β-hexachlorocyclohexane	0.15
γ-hexachlorocyclohexane (lindane)	0.15
α-chlordane	0.15
cis-nonachlor	0.15
β-chlordane	0.15
Heptachlor	0.15
heptachlor epoxide	0.15
nonachlor III	0.15
Oxychlordane	0.15
trans-nonachlor	0.16
Aldrin	0.15
Dieldrin	0.15
Mirex	0.15
α-endosulfan	0.15
o,p'-DDD	0.15
o,p'-DDE	0.15
o,p'-DDT	0.15
p,p'-DDD	0.15
p,p'-DDE	-
p,p'-DDT	0.15
$\sum_{11}$PBDEs	0.15
TPCBs	0.15

Table B 2. Limit of quantitation (LOQ) ranges for analytes or analyte groups (see Table 2 for groupings) analyzed in this study. LOQs for groups are the range of values for individual analytes within the group. Original LOQs reported in wet weight.

Analyte or Group	Range of LOQs (ng/g)
$\sum_{42}$PAHs	0.12 – 7.6
TPCBs	0.09 – 0.29
$\sum_{11}$PBDEs	0.089 – 0.29
$\sum_{6}$DDTs	0.089 – 0.29
$\sum_{8}$Chlordanes	0.089 – 0.29
$\sum_{3}$HCHs	0.088 – 0.29
Aldrin	0.090 – 0.28
Dieldrin	0.090 – 0.28
HCB	0.11 – 0.29
Mirex	0.090 – 0.29
Endosulfan 1	0.090 – 0.29

The SRM performance for 2,6-dimethylnaphthalene (DMN), one of the C2-naphthalenes (C2-NPH) indicated a high bias for that analyte. In addition, DMN values in our field samples were 28 to 70 times higher than the NIST reference value, indicating a high bias. As a result, we subtracted the concentration of DMN reported in each field sample from the concentration reported for C2-NPH in that field sample. This recommended change in concentrations of C2-NPHs also affected the reported values for summed LMW and $\sum_{42}$PAHs. After subtracting out the DMN values any sample set where the associated method blank had a value greater than LOQ (i.e., naphthalenes) all measured values that were less than 5X the method blank were set to 0. The method blanks concentrations were then subtracted from all remaining non-zero values in the sample set. The summed values (LMW, HMW, $\sum_{42}$PAHs) were then recalculated using only the detected values, with zeroes substituted for non-detected (< LOQ) analytes, within each group.

The reported alkylated homologue for some analytes were designated with an "i" qualifier by the analytical laboratory because, one (or more) significant peak(s) within the elution range of the homolog group had a retention time that did not match those in a known PAH pattern, which means the alkyl group may have contained peaks that were not part of a recognized oil pattern (Table B 3). Although this qualifier was noted, these data were used "as is" (i.e. not censored or modified) for all summations and analyses in this study. For the EIM database, they are flagged as estimated values (NJ).

Table B 3. Percent of low molecular weight PAH analyte values censored with an "i' qualifier or treated as a non-detect because it had less than five times the concentration of the method blank.

Analyte	"i"	< 5 x Blank
Naphthalene (NPH)		79
C1-naphthalenes (C1NPH)		76
C2-naphthalenes (C2NPH)		70
C3-naphthalenes (C3NPH)		73
C4-naphthalenes (C4NPH)	64	
C2-fluorenes (C2FLU)	21	
C3-fluorenes (C3FLU)	30	
C3-dibenzothiophenes (C3DBT)	3	
Phenanthrene (PHN)		39
C1-phenanthrenes/anthracenes (C1PHN)		45
C2-phenanthrenes/anthracenes (C2PHN)	42	
C3-phenanthrenes/anthracenes (C3PHN)	21	

Overall, the range of limit of quantitation (LOQ) for PAHs (Table B 2) fell within the expected ranges specified in the QAPP for this project (O'Neill et al. (2013). However, the LOQs for some PAHs were higher than anticipated and all of these high LOQs (< 7.3 - < 7.6 ng/g wet weight) came from the same sample analysis: Snohomish Nearshore 2 – composite #3.

Metals Analyses – All of the methods blank were in acceptable limits except for the method blank associated with copper for batch B14C057 and for the methods blank associated with lead for batch B14C071. All samples for both batches with sufficient sample volume were extracted and all of the samples associated with these methods blanks were reported without qualification. Five of the samples from batch B14C057, (all form the

Skagit system), had insufficient tissue volume for re-extraction so as a result, the method blank was subtracted from those five estimates and that blank corrected value was used for statistical analyses.

Of the five metals analyzed, only cadmium and lead had samples measured below method detection limits (nine and seven out of 67, respectively).

APPENDIX C: Summary Statistics of Persistent Organic Pollutants Measured in Juvenile Chinook Salmon Whole Body Tissue

Table C 1. Summary of estimated total PCB (TPCB) concentration (ng/g ww) data measured in juvenile Chinook salmon whole body (less gill and stomach content) composite samples. NC = not calculated

TPCBs		# Samples analyzed	Lipid mean (%)	n detects	Minimum	Maximum	Geometric mean	25th Percentile	Median	75th Percentile
Skagit	System	14	1.1	14	5.3	9.3	7.3	6.4	7.7	8.6
	Estuary	4	1.1	4	7.8	8.9	8.2	7.9	8.1	8.4
	Nearshore 1	5	1.1	5	5.3	9.3	7.8	7.5	8.7	9.0
	Nearshore 2	5	1.0	5	5.4	7.4	6.3	5.5	6.2	7.0
Snohomish	System	14	1.5	14	8.4	32	16	10	15	24
	Estuary	4	1.1	4	22	32	27	24	28	32
	Nearshore 1	5	2.0	5	8.4	19	11	9.5	10	10
	Nearshore 2	5	1.4	5	10	27	15	12	14	15
Green/ Duwamish	System	14	1.6	14	20	90	46	33	54	63
	Estuary	4	2.1	4	20	66	32	25	29	40
	Nearshore 1	5	1.4	5	22	81	48	47	54	54
	Nearshore 2	5	1.4	5	37	90	59	57	57	65
Hylebos/ Puyallup	System	11	2.2	11	16	46	24	22	23	26
	Estuary	1	2.1	1	46	46	NC	NC	NC	NC
	Nearshore 1	5	3.0	5	22	33	25	23	25	25
	Nearshore 2	5	1.5	5	16	26	21	19	21	22
Nisqually	System	14	1.0	14	8.6	20	13	12	13	14
	Estuary	4	1.2	4	11	13	12	12	12	12
	Nearshore 1	5	0.89	5	8.6	20	13	12	13	13
	Nearshore 2	5	0.97	5	11	19	14	12	14	17
Offshore (basins)	Admiralty Inlet	2	0.76	2	8.3	9.3	8.8	NC	NC	NC
	Whidbey	2	1.1	2	21	23	22	NC	NC	NC
	Central - Jul	6	0.65	6	12	30	19	17	18	25
	Central - Oct	5	0.94	5	13	37	23	19	27	28
	South	6	1.3	6	16	33	25	23	24	30

Table C 2. Summary of $\sum_{11}$PBDE concentration (ng/g ww) data measured in juvenile Chinook salmon whole body (less gill and stomach content) composite sample. NC = not calculated

$\sum_{11}$PBDEs		# Samples analyzed	Lipid mean (%)	n detects	Minimum	Maximum	Geometric mean	25th Percentile	Median	75th Percentile
Skagit	System	14	1.1	14	1.3	6.0	2.4	1.6	2.3	2.9
	Estuary	4	1.1	4	1.3	2.6	1.8	1.4	1.7	2.2
	Nearshore 1	5	1.1	5	1.4	2.5	1.9	1.5	2.2	2.3
	Nearshore 2	5	1.0	5	2.0	6.0	3.8	3.0	4.6	4.6
Snohomish	System	14	1.5	14	3.1	40	8.2	3.9	5	19
	Estuary	4	1.1	4	17	40	29	28	33	36
	Nearshore 1	5	2.0	5	3.1	4.4	3.8	3.6	3.8	4.0
	Nearshore 2	5	1.4	5	3.4	19	6.5	4.3	5.6	7.6
Green/ Duwamish	System	14	1.6	14	1.1	20	4.2	2.5	4.3	6.6
	Estuary	4	2.1	4	1.1	6.6	2.9	1.3	4.0	6.6
	Nearshore 1	5	1.4	5	1.8	20	4.6	3.5	4.0	4.2
	Nearshore 2	5	1.4	5	2.2	7.3	5.0	4.3	6.5	7.0
Hylebos/ Puyallup	System	11	2.2	11	2.9	35	7.0	4.5	5.3	11
	Estuary	1	2.1	1	13	13	NC	NC	NC	NC
	Nearshore 1	5	3.0	5	2.9	35	8.8	3.8	8.7	16
	Nearshore 2	5	1.5	5	3.3	6.2	4.9	5.1	5.2	5.3
Nisqually	System	14	1.0	14	0.94	4.8	2.0	1.4	1.8	3.5
	Estuary	4	1.2	4	4.0	4.8	4.2	4.0	4.1	4.3
	Nearshore 1	5	0.89	5	0.94	1.9	1.6	1.6	1.7	1.9
	Nearshore 2	5	0.97	5	1.1	1.8	1.4	1.2	1.3	1.5
Offshore (basins)	Admiralty Inlet	2	0.76	2	1.2	1.3	1.3	NC	NC	NC
	Whidbey	2	1.1	2	3.4	5.0	4.1	NC	NC	NC
	Central - Jul	6	0.65	6	1.7	3.8	2.6	2.2	2.5	3.2
	Central - Oct	5	0.94	5	1.7	4.3	2.8	2.4	2.5	3.6
	South	6	1.3	6	1.8	3.8	2.5	2.0	2.4	3.2

Table C 3. Summary of $\sum_6$DDT concentration (ng/g ww) data measured in juvenile Chinook salmon whole body (less gill and stomach content) composite samples. NC = not calculated

$\sum_6$DDTs		# Samples analyzed	Lipid mean (%)	n detects	Minimum	Maximum	Geometric mean	25th Percentile	Median	75th Percentile
Skagit	System	14	1.1	14	1.0	2.3	1.5	1.3	1.5	1.9
	Estuary	4	1.1	4	1.3	2.3	1.9	1.8	2.0	2.1
	Nearshore 1	5	1.1	5	1.0	1.6	1.2	1.1	1.2	1.3
	Nearshore 2	5	1.0	5	1.3	2.0	1.6	1.4	1.5	1.7
Snohomish	System	14	1.5	14	1.4	4.6	2.3	1.9	2.3	2.7
	Estuary	4	1.1	4	2.7	4.6	3.5	2.9	3.6	4.3
	Nearshore 1	5	2.0	5	1.8	2.4	2.0	1.9	1.9	2.0
	Nearshore 2	5	1.4	5	1.4	2.6	2.0	1.6	2.2	2.3
Green/ Duwamish	System	14	1.6	14	2.5	6.9	3.9	3.3	4.1	4.4
	Estuary	4	2.1	4	4.0	4.5	4.3	4.2	4.3	4.4
	Nearshore 1	5	1.4	5	2.5	6.9	4.0	3.1	4.3	4.4
	Nearshore 2	5	1.4	5	3.0	4.8	3.7	3.1	3.7	3.9
Hylebos/ Puyallup	System	11	2.2	11	2.6	5.8	4.2	3.6	4.5	5.3
	Estuary	1	2.1	1	5.5	5.5	NC	NC	NC	NC
	Nearshore 1	5	3.0	5	2.6	5.8	4.6	4.8	5.2	5.3
	Nearshore 2	5	1.5	5	2.7	4.5	3.6	3.2	3.9	4.0
Nisqually	System	14	1.0	14	1.1	2.3	1.8	1.6	1.8	2.0
	Estuary	4	1.2	4	1.6	2.0	1.7	1.7	1.7	1.8
	Nearshore 1	5	0.89	5	1.1	2.3	1.7	1.6	1.7	2.0
	Nearshore 2	5	0.97	5	1.5	2.1	1.9	1.9	1.9	2.1
Offshore (basins)	Admiralty Inlet	2	0.76	2	0.80	0.99	0.89	NC	NC	NC
	Whidbey	2	1.1	2	1.1	2.5	1.7	NC	NC	NC
	Central - Jul	6	0.65	6	1.2	2.8	1.6	1.3	1.5	1.8
	Central - Oct	5	0.94	5	1.4	2.6	1.8	1.5	1.6	1.8
	South	6	1.3	6	0.63	2.3	1.1	0.84	0.99	1.3

Table C 4. Summary of $\sum_8$chlordane concentration (ng/g ww) data measured in juvenile Chinook salmon whole body (less gill and stomach content) composite samples. All $\sum_8$chlordane concentrations were included in the summary statistics (i.e., detects and non-detects). Samples that were measured below the limit of quantitation are labeled with LOQ after the value. NC = not calculated

$\sum_8$Chlordanes		# Samples analyzed	Lipid mean (%)	n detects	Minimum	Maximum	Geometric mean	25th Percentile	Median	75th Percentile
Skagit	System	14	1.1	8	0.10 (LOQ)	0.24	0.16	0.13	0.15	0.20
	Estuary	4	1.1	4	0.13	0.23	0.15	0.13	0.13	0.16
	Nearshore 1	5	1.1	2	0.10 (LOQ)	0.24	0.16	0.13	0.13	0.22
	Nearshore 2	5	1.0	2	0.15 (LOQ)	0.21	0.16	0.15	0.15	0.17
Snohomish	System	14	1.5	14	0.21	0.84	0.52	0.46	0.54	0.65
	Estuary	4	1.1	4	0.55	0.78	0.64	0.60	0.63	0.68
	Nearshore 1	5	2.0	5	0.34	0.63	0.49	0.47	0.51	0.53
	Nearshore 2	5	1.4	5	0.21	0.84	0.46	0.35	0.46	0.70
Green/ Duwamish	System	14	1.6	14	0.21	0.84	0.52	0.46	0.54	0.65
	Estuary	4	2.1	4	0.55	0.78	0.64	0.60	0.63	0.68
	Nearshore 1	5	1.4	5	0.34	0.63	0.49	0.47	0.51	0.53
	Nearshore 2	5	1.4	5	0.21	0.84	0.46	0.35	0.46	0.70
Hylebos/ Puyallup	System	11	2.2	11	0.68	3.6	2.0	1.6	2.0	2.9
	Estuary	1	2.1	1	1.5	1.5	NC	NC	NC	NC
	Nearshore 1	5	3.0	5	0.68	3.6	2.1	2.0	2.5	3.3
	Nearshore 2	5	1.5	5	1.6	3.2	2.0	1.6	1.7	2.4
Nisqually	System	14	1.0	10	0.16	0.67	0.25	0.18	0.21	0.32
	Estuary	4	1.2	1	0.18(LOQ)	0.29 (LOQ)	0.22	0.20	0.21	0.24
	Nearshore 1	5	0.89	4	0.16	0.67	0.27	0.18	0.23	0.35
	Nearshore 2	5	0.97	5	0.16	0.40	0.24	0.19	0.20	0.33
Offshore (basins)	Admiralty Inlet	2	0.76	0	0.14 (LOQ)	0.15 (LOQ)	0.15	NC	NC	NC
	Whidbey	2	1.1	1	0.16 (LOQ)	0.62	0.32	NC	NC	NC
	Central – Jul	6	0.65	4	0.15 (LOQ)	0.47	0.21	0.16	0.18	0.21
	Central – Oct	5	0.94	5	0.22	0.53	0.30	0.24	0.27	0.33
	South	6	1.3	6	0.20	0.55	0.35	0.27	0.35	0.48

Table C 5. Summary of HCB concentration (ng/g ww) data measured in juvenile Chinook salmon whole body (less gill and stomach content) composite samples. All HCB concentrations were included in the summary statistics (i.e., detects and non-detects). Samples that were measured below the limit of quantitation are labeled with LOQ after the value. NC = not calculated

HCB		# Samples analyzed	Lipid mean (%)	n detects	Minimum	Maximum	Geometric mean	25th Percentile	Median	75th Percentile
Skagit	System	14	1.1	8	0.11 (LOQ)	0.19	0.19	0.13	0.15	0.17
	Estuary	4	1.1	3	0.11	0.17	0.13	0.12	0.13	0.14
	Nearshore 1	5	1.1	3	0.13 (LOQ)	0.19	0.15	0.13	0.14	0.18
	Nearshore 2	5	1.0	2	0.15 (LOQ)	0.18	0.16	0.15	0.15	0.17
Snohomish	System	14	1.5	12	0.14	11	0.31	0.18	0.28	0.32
	Estuary	4	1.1	3	0.14	0.18	0.17	0.16	0.18	0.18
	Nearshore 1	5	2.0	5	0.27	0.36	0.30	0.29	0.30	0.30
	Nearshore 2	5	1.4	4	0.14	11	0.53	0.26	0.32	0.34
Green/ Duwamish	System	14	1.6	10	0.12	0.35	0.20	0.16	0.22	0.25
	Estuary	4	2.1	4	0.20	0.35	0.28	0.24	0.29	0.34
	Nearshore 1	5	1.4	3	0.14	0.28	0.19	0.15	0.17	0.23
	Nearshore 2	5	1.4	3	0.12	0.23	0.17	0.14	0.18	0.23
Hylebos/ Puyallup	System	11	2.2	11	0.14	0.37	0.24	0.18	0.27	0.33
	Estuary	1	2.1	1	0.37	0.37	NC	NC	NC	NC
	Nearshore 1	5	3.0	5	0.26	0.34	0.30	0.27	0.33	0.33
	Nearshore 2	5	1.5	5	0.14	0.30	0.18	0.14	0.14	0.21
Nisqually	System	14	1.0	3	0.11	0.29	0.18	0.16	0.18	0.20
	Estuary	4	1.2	0	0.18 (LOQ)	0.29 (LOQ)	0.21	0.18	0.20	0.24
	Nearshore 1	5	0.89	1	0.16	0.20	0.18	0.16	0.18	0.20
	Nearshore 2	5	0.97	2	0.11	0.18	0.15	0.14	0.16	0.17
Offshore (basins)	Admiralty Inlet	2	0.76	0	0.14 (LOQ)	0.15 (LOQ)	0.15	NC	NC	NC
	Whidbey	2	1.1	1	0.16 (LOQ)	0.18	0.17	NC	NC	NC
	Central – Jul	6	0.65	0	0.13 (LOQ)	0.22 (LOQ)	0.15	0.14	0.15	0.16
	Central – Oct	5	0.94	4	0.14	0.23	0.17	0.15	0.15	0.17
	South	6	1.3	5	0.15 (LOQ)	0.35	0.22	0.18	0.21	0.25

Table C 6. Summary of dieldrin concentration (ng/g ww) data measured in juvenile Chinook salmon whole body (less gill and stomach content) composite samples. All dieldrin concentrations were included in the summary statistics (i.e., detects and non-detects). Samples that were measured below the limit of quantitation are labeled with LOQ after the value. NC = not calculated

Dieldrin		# Samples analyzed	Lipid mean (%)	n detects	Minimum	Maximum	Geometric mean	25th Percentile	Median	75th Percentile
Skagit	System	14	1.1	1	0.09 (LOQ)	0.18	0.13	0.12	0.13	0.15
	Estuary	4	1.1	0	0.09 (LOQ)	0.12 (LOQ)	0.11	0.11	0.12	0.12
	Nearshore 1	5	1.1	1	0.10 (LOQ)	0.18 (LOQ)	0.14	0.13	0.13	0.15
	Nearshore 2	5	1.0	0	0.13 (LOQ)	0.15 (LOQ)	0.15	0.15	0.15	0.15
Snohomish	System	14	1.5	4	0.097 (LOQ)	0.25	0.15	0.13	0.14	0.16
	Estuary	4	1.1	1	0.12 (LOQ)	0.16	0.14	0.14	0.15	0.15
	Nearshore 1	5	2.0	2	0.13 (LOQ)	0.23	0.16	0.14	0.16	0.17
	Nearshore 2	5	1.4	1	0.097 (LOQ)	0.25	0.14	0.11	0.14	0.14
Green/ Duwamish	System	14	1.6	12	0.12	0.66	0.29	0.20	0.27	0.46
	Estuary	4	2.1	4	0.3	0.48	0.37	0.31	0.36	0.43
	Nearshore 1	5	1.4	4	0.17 (LOQ)	0.66	0.37	0.23	0.52	0.53
	Nearshore 2	5	1.4	4	0.12	0.23	0.19	0.18	0.19	0.23
Hylebos/ Puyallup	System	11	2.2	10	0.15 (LOQ)	1.9	0.36	0.18	0.37	0.53
	Estuary	1	2.1	1	0.37	0.37	NC	NC	NC	NC
	Nearshore 1	5	3.0	4	0.15 (LOQ)	1.9	0.40	0.17	0.38	0.58
	Nearshore 2	5	1.5	5	0.15	0.64	0.31	0.19	0.32	0.48
Nisqually	System	14	1.0	0	0.11 (LOQ)	0.28 (LOQ)	0.17	0.15	0.18	0.20
	Estuary	4	1.2	0	0.18 (LOQ)	0.28 (LOQ)	0.21	0.18	0.20	0.24
	Nearshore 1	5	0.89	0	0.12 (LOQ)	0.20 (LOQ)	0.17	0.16	0.18	0.20
	Nearshore 2	5	0.97	0	0.11 (LOQ)	0.18 (LOQ)	0.14	0.13	0.14	0.16
Offshore (basins)	Admiralty Inlet	2	0.76	0	0.14 (LOQ)	0.15 (LOQ)	0.15	NC	NC	NC
	Whidbey	2	1.1	1	0.12	0.16 (LOQ)	0.14	NC	NC	NC
	Central - Jul	6	0.65	0	0.13 (LOQ)	0.22 (LOQ)	0.15	0.14	0.15	0.16
	Central - Oct	5	0.94	0	0.10 (LOQ)	0.15 (LOQ)	0.13	0.13	0.14	0.14
	South	6	1.3	0	0.14 (LOQ)	0.17 (LOQ)	0.15	0.14	0.16	0.17

APPENDIX D: Summary Statistics of Polycyclic Aromatic Hydrocarbons Measured in Juvenile Chinook Salmon Stomach Contents

Table D 1. Summary of summed PAHs (ng/g ww) measured in juvenile Chinook salmon stomach contents composite samples. NC = not calculated

$\sum_{42}$PAHs		# Samples analyzed	n detects	Minimum	Maximum	Arithmetic mean	25th Percentile	Median	75th Percentile
Skagit	System	3	3	27	44	35	31	35	40
	Estuary	1	1	NC	NC	35	NC	NC	NC
	Nearshore 1	1	1	NC	NC	44	NC	NC	NC
	Nearshore 2	1	1	NC	NC	27	NC	NC	NC
Snohomish	System	6	6	80	32,000	5,800	200	360	1,100
	Estuary	1	1	NC	NC	460	NC	NC	NC
	Nearshore 1	1	1	NC	NC	1,300	NC	NC	NC
	Nearshore 2	4	4	80	32,000	8,200	150	210	8,300
Green/ Duwamish	System	3	3	490	11,000	4,300	860	1,200	6,200
	Estuary	1	1	NC	NC	490	NC	NC	NC
	Nearshore 1	1	1	NC	NC	1,200	NC	NC	NC
	Nearshore 2	1	1	NC	NC	11,00	NC	NC	NC
Hylebos/ Puyallup	System	7	7	130	1,700	440	140	170	420
	Estuary	1	1	NC	NC	590	NC	NC	NC
	Nearshore 1	1	1	NC	NC	1,700	NC	NC	NC
	Nearshore 2	5	5	130	250	170	140	140	170
Nisqually	System	14	14	2.1	42	17	5.1	12	25
	Estuary	4	4	10	40	19	13	19	28
	Nearshore 1	5	5	5.1	42	21	5.3	17	37
	Nearshore 2	5	5	2.1	26	8.1	2.8	4.7	5.2
Offshore (basins)	Admiralty Inlet	2	2	2.1	4.3	3.2	NC	NC	NC
	Whidbey	2	2	4.7	230	120	NC	NC	NC
	Central - Jul	5	5	3.1	16	6.4	3.6	4.4	5.0
	Central - Oct	5	5	2.9	23	8.5	2.9	4.0	9.5
	South	6	6	2.0	12	4.8	3.0	3.6	5.0

APPENDIX E: Summary Statistics of Trace Metals Measured in Juvenile Chinook Salmon Gill Tissue

Table E 1. Summary of cadmium concentration (mg/kg ww) data measured in juvenile Chinook salmon, gill tissue composite samples. All cadmium concentrations were included in the summary statistics (i.e., detects and non-detects). Samples that were measured below the method detection limit are labeled with MDL after the value. NC = not calculated

Cd		# Samples analyzed	n detects	Minimum	Maximum	Arithmetic mean	25th Percentile	Median	75th Percentile
Skagit	System	14	14	0.022	0.052	0.037	0.034	0.036	0.045
	Estuary	4	4	0.036	0.048	0.042	0.037	0.042	0.048
	Nearshore 1	5	5	0.022	0.036	0.030	0.023	0.032	0.035
	Nearshore 2	5	6	0.033	0.052	0.042	0.036	0.040	0.047
Snohomish	System	14	14	0.041	0.10	0.069	0.051	0.060	0.094
	Estuary	4	4	0.043	0.058	0.053	0.051	0.056	0.057
	Nearshore 1	5	5	0.091	0.10	0.098	0.095	0.099	0.10
	Nearshore 2	5	5	0.041	0.067	0.053	0.044	0.050	0.062
Green/ Duwamish	System	14	10	0.010 (MDL)	0.026	0.016	0.011	0.014	0.020
	Estuary	4	1	0.010 (MDL)	0.013	0.011	0.010	0.010	0.011
	Nearshore 1	5	5	0.012	0.024	0.018	0.013	0.020	0.020
	Nearshore 2	5	4	0.010 (MDL)	0.026	0.018	0.015	0.017	0.022
Hylebos/ Puyallup	System	11	11	0.012	0.026	0.021	0.019	0.022	0.024
	Estuary	1	1	0.022	0.022	NC	NC	NC	NC
	Nearshore 1	5	5	0.018	0.026	0.022	0.019	0.023	0.025
	Nearshore 2	5	5	0.012	0.025	0.019	0.016	0.021	0.023
Nisqually	System	14	9	0.010 (MDL)	0.032	0.016	0.010	0.015	0.018
	Estuary	4	0	0.010 (MDL)	0.010 (MDL)	0.010	0.010	0.010	0.010
	Nearshore 1	5	4	0.010 (MDL)	0.032	0.019	0.015	0.017	0.022
	Nearshore 2	5	5	0.013	0.024	0.017	0.015	0.015	0.018

Table E 2. Summary of copper concentration (mg/kg ww) data measured in juvenile Chinook salmon, gill tissue composite samples. NC = not calculated

Cu		# Samples analyzed	n detects	Minimum	Maximum	Arithmetic mean	25th Percentile	Median	75th Percentile
Skagit	System	14	14	0.37	0.79	0.56	0.47	0.57	0.65
	Estuary	4	4	0.47	0.79	0.64	0.56	0.65	0.73
	Nearshore 1	5	5	0.37	0.66	0.48	0.39	0.50	0.50
	Nearshore 2	5	7	0.43	0.69	0.58	0.57	0.58	0.64
Snohomish	System	14	14	0.45	0.61	0.51	0.48	0.50	0.55
	Estuary	4	4	0.47	0.61	0.54	0.53	0.55	0.57
	Nearshore 1	5	5	0.46	0.56	0.49	0.47	0.48	0.49
	Nearshore 2	5	5	0.45	0.59	0.51	0.50	0.50	0.52
Green/ Duwamish	System	14	14	0.54	0.76	0.62	0.58	0.59	0.67
	Estuary	4	4	0.54	0.59	0.57	0.55	0.57	0.58
	Nearshore 1	5	5	0.58	0.76	0.65	0.59	0.62	0.68
	Nearshore 2	5	5	0.56	0.75	0.64	0.59	0.62	0.68
Hylebos/ Puyallup	System	11	11	0.59	0.85	0.71	0.64	0.72	0.76
	Estuary	1	1	0.59	0.59	NC	NC	NC	NC
	Nearshore 1	5	5	0.60	0.73	0.67	0.60	0.70	0.72
	Nearshore 2	5	5	0.68	0.85	0.77	0.73	0.79	0.79
Nisqually	System	14	14	0.46	0.76	0.58	0.48	0.57	0.65
	Estuary	4	4	0.47	0.55	0.51	0.47	0.50	0.54
	Nearshore 1	5	5	0.59	0.73	0.65	0.63	0.66	0.67
	Nearshore 2	5	5	0.46	0.76	0.56	0.48	0.49	0.59

Table E 3. Summary of lead concentration (mg/kg ww) data measured in juvenile Chinook salmon, gill tissue composite samples. All lead concentrations were included in the summary statistics (i.e., detects and non-detects). Samples that were measured below the method detection limit are labeled after the value. NC = not calculated

Pb		# Samples analyzed	n detects	Minimum	Maximum	Arithmetic mean	25th Percentile	Median	75th Percentile
Skagit	System	14	14	0.022	0.13	0.060	0.027	0.046	0.084
	Estuary	4	4	0.048	0.13	0.093	0.061	0.097	0.13
	Nearshore 1	5	5	0.042	0.12	0.070	0.044	0.050	0.090
	Nearshore 2	5	8	0.022	0.031	0.025	0.023	0.025	0.025
Snohomish	System	14	9	0.020	0.36	0.060	0.020	0.024	0.039
	Estuary	4	3	0.037 (MDL)	0.36	0.15	0.039	0.091	0.20
	Nearshore 1	5	1	0.020 (MDL)	0.039	0.024	0.020	0.020	0.020
	Nearshore 2	5	5	0.021	0.052	0.029	0.023	0.023	0.024
Green/ Duwamish	System	14	14	0.025	0.35	0.10	0.071	0.086	0.11
	Estuary	4	4	0.030	0.10	0.069	0.039	0.071	0.10
	Nearshore 1	5	5	0.025	0.18	0.087	0.070	0.075	0.082
	Nearshore 2	5	5	0.076	0.35	0.15	0.089	0.11	0.12
Hylebos/ Puyallup	System	11	11	0.049	0.48	0.12	0.062	0.078	0.10
	Estuary	1	1	0.48	0.48	NC	NC	NC	NC
	Nearshore 1	5	5	0.049	0.067	0.060	0.059	0.061	0.062
	Nearshore 2	5	5	0.078	0.19	0.11	0.091	0.093	0.11
Nisqually	System	14	12	0.019	0.057	0.036	0.024	0.037	0.044
	Estuary	4	4	0.034	0.057	0.041	0.035	0.037	0.044
	Nearshore 1	5	4	0.020 (MDL)	0.045	0.033	0.021	0.038	0.039
	Nearshore 2	5	4	0.019	0.051	0.034	0.020	0.032	0.049

Table E 4. Summary of nickel concentration (mg/kg ww) data measured in juvenile Chinook salmon, gill tissue composite samples. NC = not calculated

Ni		# Samples analyzed	n detects	Minimum	Maximum	Arithmetic mean	25th Percentile	Median	75th Percentile
Skagit	System	14	14	0.031	0.23	0.10	0.056	0.069	0.15
	Estuary	4	4	0.18	0.23	0.20	0.19	0.21	0.22
	Nearshore 1	5	5	0.054	0.084	0.072	0.067	0.071	0.082
	Nearshore 2	5	9	0.031	0.059	0.051	0.054	0.055	0.057
Snohomish	System	14	14	0.028	0.073	0.051	0.040	0.049	0.062
	Estuary	4	4	0.061	0.073	0.067	0.062	0.067	0.072
	Nearshore 1	5	5	0.028	0.050	0.037	0.031	0.037	0.041
	Nearshore 2	5	5	0.040	0.073	0.052	0.044	0.048	0.054
Green/ Duwamish	System	14	14	0.040	0.083	0.058	0.048	0.058	0.062
	Estuary	4	4	0.053	0.083	0.064	0.058	0.061	0.067
	Nearshore 1	5	5	0.051	0.081	0.064	0.056	0.059	0.071
	Nearshore 2	5	5	0.040	0.061	0.047	0.042	0.045	0.047
Hylebos/ Puyallup	System	11	11	0.028	0.11	0.059	0.039	0.058	0.070
	Estuary	1	1	0.059	0.059	NC	NC	NC	NC
	Nearshore 1	5	5	0.028	0.057	0.039	0.033	0.035	0.042
	Nearshore 2	5	5	0.058	0.11	0.079	0.060	0.080	0.088
Nisqually	System	14	14	0.032	0.091	0.052	0.041	0.047	0.059
	Estuary	4	4	0.045	0.091	0.059	0.047	0.049	0.061
	Nearshore 1	5	5	0.032	0.062	0.041	0.034	0.036	0.040
	Nearshore 2	5	5	0.045	0.083	0.057	0.047	0.047	0.061

Table E 5. Summary of zinc concentration (mg/kg ww) data measured in juvenile Chinook salmon, gill tissue composite samples. NC = not calculated

Zn		# Samples analyzed	n detects	Minimum	Maximum	Arithmetic mean	25th Percentile	Median	75th Percentile
Skagit	System	14	14	30	39	323	31	32	35
	River	4	4	30	31	30	30	30	31
	Estuary 1	5	5	30	39	35	34	35	37
	Estuary 2	5	5	30	36	32	31	32	33
Snohomish	System	14	14	28	36	32	30	32	34
	Estuary	4	4	29	32	30	29	29	30
	Nearshore 1	5	5	28	34	32	32	32	33
	Nearshore 2	5	5	32	36	34	32	35	36
Green/ Duwamish	System	14	14	25	32	27	25	28	29
	Estuary	4	4	28	29	28	28	28	28
	Nearshore 1	5	5	25	29	27	25	27	27
	Nearshore 2	5	5	25	32	27	25	26	29
Hylebos/ Puyallup	System	11	11	32	39	36	34	36	38
	Estuary	1	1	33	33	NC	NC	NC	NC
	Nearshore 1	5	5	32	39	36	35	36	38
	Nearshore 2	5	5	33	39	36	35	36	38
Nisqually	System	14	14	22	38	31	28	32	34
	Estuary	4	4	22	29	25	24	24	26
	Nearshore 1	5	5	28	38	34	34	35	37
	Nearshore 2	5	5	29	34	32	31	33	33