

**Control of Toxic
Chemicals in Puget Sound Phase 3:
Persistent Bioaccumulative and Toxic
Contaminants in Pelagic Marine
Fish Species from Puget Sound**



Washington Department of
FISH and WILDLIFE

Persistent Bioaccumulative and Toxic Contaminants in Pelagic Marine Fish Species from Puget Sound

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FISH and WILDLIFE

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Acronyms, Abbreviations and Units

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

C	carbon
Ecology	Washington State Department of Ecology
FL	fork length
GLM	General Linear Model (as applied using (SYSTAT, 2007))
GC/MS	gas chromatography/mass spectrometry
HRGC	high resolution gas chromatography
HRMS	high resolution mass spectrometry
IOS	Institute of Ocean Sciences, Sidney, British Columbia
N	nitrogen
NOAA	National Oceanic & Atmospheric Administration
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PBT	persistent, bioaccumulative, and toxic contaminant
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
POP	persistent organic pollutant
PSAMP	Puget Sound Assessment and Monitoring Program
QA/QC	quality assurance/quality control
SRM	standard reference materials
WDFW	Washington Department of Fish and Wildlife

Units of Measurement

gm	gram
mL	milliliters
mm	millimeter
ng/g	nanograms per gram (parts per billion)
‰	permille (parts per thousand)
ppb	part per billion
ppt	part per trillion

Abstract

This study is an assessment of selected persistent bioaccumulative toxic contaminants (PBTs) in a guild of mid-trophic-level pelagic fish predators in Puget Sound. It is part of a consortium of efforts aimed at identifying contaminants of concern in Puget Sound biota, where and how such contaminants enter the food chain, what processes transport toxics to the Sound, and the fate and transport of PBTs once there. We observed patterns of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) in Pacific hake (*Merluccius productus*) and walleye pollock (*Theragra chalcogramma*) that are consistent with other pelagic species such as Pacific herring and Pacific salmon species that have been evaluated in previous studies. PCB, PBDE, and OCPs were all observed in a gradient of concentration, from high in Puget Sound basins that have experienced extensive development, to low in basins where watershed have been less developed. Some pesticides appeared to occur in greater abundance in one Developed Basin (Whidbey Basin) in which extensive development has been in the form of agriculture. PCB congener patterns in hake were consistent with focused, point sources of PCBs that have migrated throughout the ecosystem from urbanized areas over a long period. PBDE congeners and most OCP component patterns were more consistent with ubiquitous, similar terrestrial sources that differed only in magnitude (i.e. congener or compound patterns were similar across regions, but total concentrations were greater in developed regions). Pacific hake and walleye pollock represent a potential source of PBTs to their predators, including other fish predators, marine mammals, birds and humans. Hake exhibited lower PCB concentration than Pacific herring and resident Chinook salmon based on a comparison of contaminant wet weights, however, PCB lipid weights in hake were roughly equivalent to herring, and greater than resident Chinook salmon.

Introduction

Over the past 20 years, researchers from the Puget Sound Assessment and Monitoring Program (PSAMP) at the Washington Department of Fish and Wildlife (WDFW) have monitored and assessed a wide range of toxic contaminants in a number of species from Puget Sound. These efforts have demonstrated the geographic extent of ecosystem contamination, the magnitude of contamination, and temporal trends in these patterns. In addition, monitoring and assessment studies have raised questions regarding the pathways by which contaminants from terrestrial sources enter the Puget Sound food web, and why Puget Sound's pelagic food web carries an unusually high load of some of the persistent and bioaccumulative toxics (PBTs) (West et al. 2008; O'Neill and West 2009). Puget Sound has been identified as a regional source of PBT contamination in harbor seals (*Phoca vitulina*; (Ross et al. 2004; Cullon et al. 2005), and in Southern Resident Killer Whales (*Orcinus orca*; (Ross et al. 2000; Ross 2006 (Krahn et al. 2007), both high level predators that feed primarily in the pelagic food web.

Washington Department of Ecology's (Ecology) Phase 1 and Phase 2 toxics loading and modeling studies reported that surface runoff and aerial deposition represent the primary conveyance mechanisms for polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), organochlorine pesticides (OCPs), and other contaminants from terrestrial sources into Puget Sound. These toxicants represent three important PBT classes to which Puget Sound biota may be exposed in high enough doses to impair their health. Several of these PBTs bioaccumulate through the pelagic food web to high-level predators such as salmon, harbor seals, killer whales, seabirds, and humans. However, the pathways of contaminant flow from their abiotic sources to these species are unclear, making it difficult to prioritize management actions aimed at reducing loading of toxicants, remediating contaminated habitats, or reducing exposure of biota to toxicants. To better protect biota, we have proposed studies to help assess: a) where geographically PBTs enter the pelagic food web (via plankton) from surface runoff and the atmosphere; b) the pathways of toxic contaminants within the pelagic food web from the geographic sources of PBTs to species at the highest trophic levels. The present study is focused on PBT patterns in an intermediate pelagic predator that connects low-trophic-level primary consumers in the pelagic food web with apex predators. This study was designed as a companion to separate, concurrent studies on PBTs in plankton (West et al. 2011) and in harbor seals (Noël et al. 2010).

The current study is focused on a suite of PBTs of concern, and also provides data that supports implementation of the Control of Toxic Chemicals in Puget Sound Initiative (Washington Department of Ecology 2010). A numeric fate/transport/food web model has been developed to evaluate source control scenarios and associated impacts on the Puget Sound ecosystem, to help determine whether existing environmental quality standards are protecting biological resources, and to predict the efficacy of control measures (Pelletier and Mohamedali 2009). Food web contaminant data from the current studies will be used in the Pelletier and Mohamedali (2009) model as input parameters for the pelagic portion of the Puget Sound pelagic food web. These studies cover a wide range of trophic levels in the pelagic food web, representing primary producers (phytoplankton), resident primary consumers (krill), resident secondary consumers (selected pelagic fish species), and resident tertiary consumers (marine mammals).

In this study we collected contaminant data for fish species that occupy an intermediate trophic level in the Puget Sound pelagic food web, and which have been suspected as a primary source of PBTs to apex predators (Cullon et al. 2005). Pacific hake (*Merluccius productus*, family Merlucciidae, hereafter “hake”) and walleye pollock (*Theragra chalcogramma*, family Gadidae, hereafter “pollock”) are important mid-level components of Puget Sound’s pelagic food web. They consume a wide range of zooplankton such as krill, as well as small pelagic forage fishes, with the proportion of fish increasing in the diet as hake grow (Outram and Haegle 1972, Tanasichuk et al. 1991, Robinson 2000). Hake are in turn important prey of harbor seals (Olesiuk et al. 1990, Olesiuk 1993).

The food-web position of hake is similar to Chinook salmon (*Oncorhynchus tshawytscha*), another of Puget Sound’s species of mid-level pelagic fish predator (see review in Quinn 2005); however hake in Puget Sound do not share the long-range migratory behavior exhibited by most Chinook salmon. Although Pacific coastal populations of hake are seasonally highly migratory (Stauffer 1985), hake and pollock living in Puget Sound and the Strait of Georgia are thought to be less migratory, remaining as residents in inland marine waters (Gustafson et al. 2000). O’Neill and West (2009) concluded that residency in Puget Sound was the primary predictor of PBT exposure in Chinook salmon from Puget Sound; however their interpretation was complicated by the wide range in migration behavior of Puget Sound Chinook salmon. Although most Chinook salmon originating from Puget Sound migrate to the Pacific ocean for a significant portion of their life, some remain as residents in Puget Sound year-round, and some exhibit migration movements intermediate to these. Chinook salmon that remain as residents in Puget Sound year-round are often termed “blackmouth”. We chose hake for the present study as representatives of the guild of relatively large-sized midwater pelagic fish predators which live as residents in Puget Sound, including resident Chinook salmon, or blackmouth.

The primary objectives of this study were to (a) compare PBTs concentrations in resident pelagic fish predators across the major regions of Puget Sound, (b) supply tissue residue data for Ecology’s modeling efforts to compare land use patterns (PBTs sources) with PBTs patterns in Puget Sound’s food web, and (c) compare these results with those in other fish species from previous PSAMP studies to provide a clearer understanding of PBTs patterns in fish, relative to potential sources.

Methods

Methods for this study were planned and proposed according to a Quality Assurance Project Plan (QAPP) (West, et al., 2009). These are summarized below, with emphasis on changes that were made to methods anticipated by the QAPP.

Study Design and Sample Collection

We sampled hake or pollock from six hydrologically distinct water bodies and one urbanized embayment, which roughly corresponded to seven of the 14 study areas for which Ecology’s Phase 2 Puget Sound loadings study described land use patterns and contaminant loading (EnviroVision Corporation, et al., 2008). These hake or pollock sampling locations included Strait of Georgia, Strait of Juan de Fuca, Whidbey Basin, Main Basin, South Puget Sound (east), Hood Canal, and Elliott Bay (Figure 1). For simplicity, we combined sample areas from three of

Ecology’s study areas, Hood Canal South, Hood Canal North and Admiralty Inlet into our single “Hood Canal” Basin. Specific sampling locations were selected based on where the fish occurred, targeting well-documented and predictable spawning aggregations, as well as by searching the water column using color fathometers. We conducted sampling in March, May, June, and July 2009. Sampling sites included historical survey stations from WDFW’s Puget Sound Assessment and Monitoring Program (PSAMP) Toxics in Biota efforts^a, locations previously sampled by WDFW staff during Pacific hake abundance surveys, and new locations identified during sampling cruises.

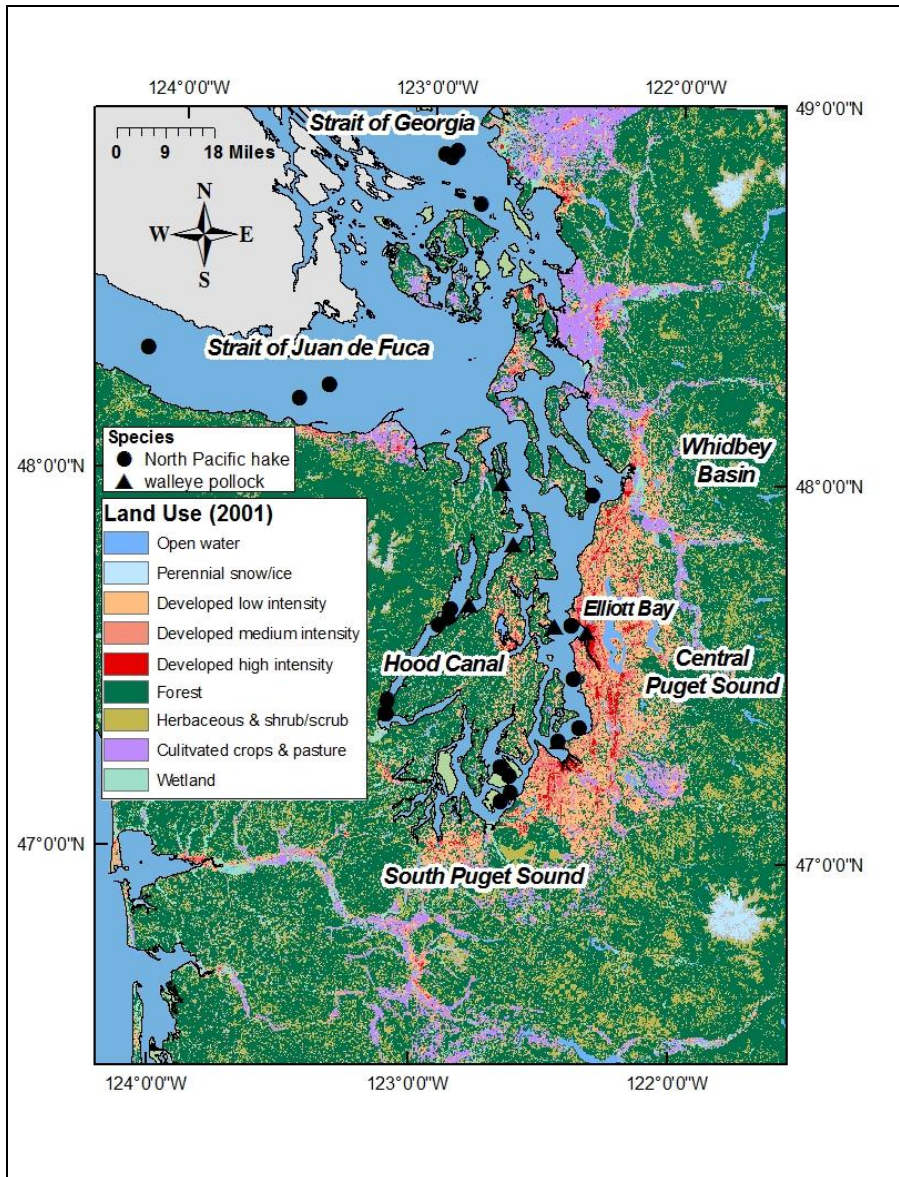


Figure 1. Location of hake and pollock sampling sites in six oceanographic Basins and one urban embayment, relative to degree of land development from a land-use coverage of the Puget Sound Basin. Land use data provided by the NOAA, Coastal Services Center through analysis (Landsat 7 satellite data).

^a http://wdfw.wa.gov/conservation/research/projects/marine_toxics/index.html

Using Ecology's loadings estimate summary from EnviroVision Corporation et al. (2008) we classified our seven sampling areas (hereafter termed Basins) as either "Developed" or "Less Developed". Developed Basins included the Main Basin, South Puget Sound, Elliott Bay, and Whidbey Basin. Puget Sound's Main Basin is clearly the most developed and contains Washington's largest cities and greatest population density. Loadings from two Basins -- Whidbey Basin and Strait of Georgia -- were notable because of their proximity to watersheds with large agriculture coverage. The Strait of Georgia, Hood Canal and the Strait of Juan de Fuca were considered Less Developed Basins, with the last considered an ocean boundary because it represents the area where seaward-flowing Puget Sound waters mix with inland-flowing oceanic waters. These three Less Developed Basins are considered low-exposure "reference" Basins because of their relatively low levels of contaminant loadings (Brandenberger et al. 2010, Washington Department of Ecology and Herrera Environmental Consultants Inc. 2010, EnviroVision Corporation et al. 2008), proximity to undeveloped land coverage (Figure 1), and low contaminant loads in other biota according to other PSAMP's long-term monitoring studies (West et al., 2001).

We tested hypotheses that pelagic fish from Developed Basins would accumulate greater amounts or different patterns of contaminant residues than fish from the Less Developed Basins, and that fish from Developed Basins would differ in contaminant patterns compared to those in fish from Less Developed Basins. Although Elliott Bay is an embayment within the Main Basin, we included it as a discrete sampling location.

We obtained fish for this study using a mid-water rope trawl designed to capture fast moving pelagic fishes, deployed from a 60 ft. limit seiner^a. A few samples were taken opportunistically from other PSAMP or WDFW surveys. Most sampling was conducted at night, when aggregations of midwater species such as Pacific hake are more discrete and easier to discern and target. Once located using a color fathometer, fish aggregations were targeted by depth, with trawl efforts lasting from 10 to 30 minutes. Once retrieved, the contents of the cod end of the net were released onto a large sorting table. Hake and pollock were separated from other taxa and placed together (by species) in labeled Ziploc bags. Bag labels included survey identification number, date, location, species, and total number of fish. Bagged samples were either frozen immediately or held on ice for no longer than two days prior to freezing. Fish were held frozen at -20 °C until they were prepared as samples.

Sample Preparation

Frozen hake and pollock were thawed and measured for fork length (FL, nearest mm) and weighed to the nearest 0.1 gm. Sex was determined from a visual examination of gonads exposed by a small abdominal slit made carefully to minimize loss of fluids or tissue. Otoliths were removed from all fish used in samples to estimate fish age. Fish age was estimated by counting putative annuli from sagittal sections otoliths under magnification. Annuli were exposed by breaking the otolith in half, and the exposed surface was singed with an open flame to enhance visibility of annuli (Chilton and Beamish 1982).

^a The fishing vessel *Chasina* has been regularly contracted for this and related purposes by various WDFW projects since 1993.

We created analytical samples based on size and sex within Basins (Table 1). Analytical samples comprised either composites of 10 to 20 individuals each, or individual fish. Samples were constructed to fill three size or age classes: (a) fish less than 250 mm FL (all as composites), (b) fish between 250 mm and 300 mm FL (all as composites) and (3) fish larger than 300 mm (individuals). We analyzed the largest fish as individuals because of the difficulties involved in capturing a sufficient number of large fish, and the time involved with processing (grinding) large fish bodies.

As outlined in the QAPP for this study, our goal was to obtain six composite samples of hake or pollock per Basin to represent a range of sizes from small, pre-reproductive size classes, to fish that were beginning to mature, and up to six larger (reproductive) individuals per sex per Basin, which would allow Basin-comparison of PBT accumulation by sex and age. However, our samples were dominated by smaller fish, and the largest, reproductive fish processed as individuals were mostly females (Table 1).

All fish used for analytical samples were homogenized as whole bodies, with no part removed except otoliths. Analyzing whole bodies minimizes variability in tissue residue concentrations associated with movement of PBTs between the fish's tissues, and better represents the transfer of contaminants to predators that consume whole prey. After an initial thawing to remove otoliths and take measurements, fish were refrozen in preparation for homogenization by grinding. All fish in a composite sample were ground together (still frozen) in a Hobart 4812 meat grinder. To ensure complete homogenization, the ground fish were sent through a ½-inch-bore grinding plate twice and after grinding was complete, each composite was stirred by hand using a pre-cleaned spatula. The fish homogenate was scooped into a pre-cleaned I-Chem® glass jar and held at -20 °C until submission for chemical analysis. Jars were filled to approximately three-quarter volume (~200 gm) to allow a head space for a final mixing prior to removing the sample for analysis.

After each composite was completed, all instruments including all grinder parts that came in contact with fish material were brushed and cleaned in warm tap water mixed with Terg-a-zyme® lab soap, rinsed repeatedly with tap water, and lastly rinsed with isopropyl alcohol (reagent-residue analysis grade). This cleaning technique was repeated between each composite.

Laboratory Analysis

Frozen samples were sent to one of two analytical laboratories via FedEx or by hand. The Institute of Ocean Sciences (IOS), under the management of the Pacific Region of Fisheries and Oceans Canada in Sidney, British Columbia conducted analysis of all analytes. NOAA Fisheries' Northwest Fisheries Science Center, Environmental Conservation Division (ECD) Lab, Seattle, Washington conducted analysis of stable isotopes of carbon (as $\delta^{13}\text{C}$) and nitrogen (as $\delta^{15}\text{N}$). Descriptions of lab methods for these procedures are provided as follows for each lab.

Table 1. Sampling and biological metrics for 60 composite samples of hake and pollock analyzed in this study.

Species	Basin	SampleID	Latitude ^a	Longitude	Date ^b	Comp. (n) ^c	MCL ^d	MCW ^e	MCA ^f	Sex Ratio ^g
hake	Str. Juan de Fuca	09JF-PWW01	48.30886	123.95515	03/17/2009	20	178	30	1.7	3:5:12
hake	Str. Juan de Fuca	09JF-PWW02	48.30702	124.01241	05/13/2009	20	174	28	1.8	2:7:11
hake	Str. Juan de Fuca	09JF-PWW03	48.30702	124.01241	05/13/2009	20	168	26	1.9	4:5:11
hake	Str. Georgia	07SG-PWW01	48.85891	122.95687	05/02/2007	11	144	17	0.9	0:0:11
hake	Str. Georgia	07SG-PWW02	48.85891	122.95687	05/02/2007	11	141	15	0.8	0:0:11
hake	Str. Georgia	09SG-PWW03	48.83866	122.90155	05/19/2009	12	171	28	1.9	0:2:10
hake	Hood Canal	09HC-PWW01	47.53059	123.02650	03/17/2009	20	198	44	1.7	7:11:2
hake	Hood Canal	09HC-PWW02	47.53059	123.02650	03/17/2009	20	198	44	1.5	5:12:3
hake	Hood Canal	09HC-PWW03	47.53059	123.02650	03/17/2009	20	204	49	2.0	9:8:3
hake	Hood Canal	09HC-PWW04	47.41373	123.11033	03/17/2009	10	254	75	3.2	6:3:1
hake	Hood Canal	09HC-PWW05	47.53348	123.02490	03/17/2009	10	294	132	4.3	1:9:0
hake	Hood Canal	09HC-PWW8001	47.63432	122.89490	05/28/2009	1	502	703	8.0	0:1:0
hake	Hood Canal	09HC-PWW8012	47.37764	123.12670	05/27/2009	1	527	859	12.0	0:1:0
hake	Hood Canal	09HC-PWW8043	47.65911	122.88646	03/17/2009	1	340	235	7.0	0:1:0
hake	Hood Canal	09HC-PWW8069	47.37743	123.12430	05/27/2009	1	377	320	6.0	0:1:0
hake	Hood Canal	09HC-PWW8072	47.61520	122.93359	03/18/2009	1	417	423	10.0	0:1:0
pollock	Hood Canal	09HC-WPW01	47.75263	122.72726	03/17/2009	20	154	30	1.1	7:10:3
pollock	Hood Canal	09HC-WPW02	47.75263	122.72726	03/17/2009	20	155	33	1.0	6:11:3
pollock	Hood Canal	09HC-WPW03	47.75263	122.72726	03/17/2009	20	149	27	1.1	7:10:3
hake	Whidbey Basin	09WB-PWW01	48.01640	122.35952	03/16/2009	20	164	42	1.6	7:9:4

^a Best estimate of the centroid of sampling effort for the sample. For single tows the latitude and longitude represent the trawl midpoint. In cases where multiple trawls contributed fish to the composite, centroid is computed as the arithmetic mean of the latitude and longitude for the midpoint of midwater trawls.

^b In cases where fish in a composite were sampled over multiple days, this represent the first day in the trawl effort.

^c Number of individual fish combined in composite

^d Mean Composite Length (mean fork length of all fish in the composite)

^e Mean composite Weight (mean weight in grams of all fish in the composite)

^f Mean Composite Age (mean age of all fish in the composite)

^g male:female:unknown

Table 1. Sampling and biological metrics for 60 composite samples of hake and pollock analyzed in this study.

Species	Basin	SampleID	Latitude ^a	Longitude	Date ^b	Comp. (n) ^c	MCL ^d	MCW ^e	MCA ^f	Sex Ratio ^g
hake	Whidbey Basin	09WB-PWW02	48.02466	122.36307	03/16/2009	20	166	39	1.7	8:8:4
hake	Whidbey Basin	09WB-PWW03	48.02466	122.36307	03/16/2009	20	167	33	1.5	5:10:5
hake	Whidbey Basin	09WB-PWW04	47.96683	122.33826	03/16/2009	10	257	104	2.6	10:0:0
hake	Whidbey Basin	09WB-PWW05	48.02830	122.35950	03/16/2009	9	291	156	3.8	7:2:0
hake	Whidbey Basin	09WB-PWW7711	48.07890	122.42899	03/16/2009	1	332	232	4.0	0:1:0
hake	Whidbey Basin	09WB-PWW7712	48.07890	122.42899	03/16/2009	1	472	670	8.0	0:1:0
hake	Whidbey Basin	09WB-PWW7713	48.07890	122.42899	03/16/2009	1	370	331	5.0	0:1:0
hake	Whidbey Basin	09WB-PWW7715	48.07890	122.42899	03/16/2009	1	325	251	4.0	0:1:0
hake	Whidbey Basin	09WB-PWW7716	48.07890	122.42899	03/16/2009	1	325	221	4.0	1:0:0
hake	Elliott Bay	09EB-PWW01	47.62162	122.40841	03/20/2009	20	152	26	1.6	6:11:3
hake	Elliott Bay	09EB-PWW02	47.62162	122.40841	03/20/2009	20	154	30	1.6	6:12:2
hake	Elliott Bay	09EB-PWW03	47.62162	122.40841	03/20/2009	20	144	23	1.3	6:9:5
hake	Elliott Bay	09EB-PWW04	47.62162	122.40841	03/20/2009	10	262	112	3.4	5:5:0
hake	Elliott Bay	09EB-PWW05	47.62162	122.40841	03/20/2009	10	293	156	3.9	5:5:0
hake	Elliott Bay	09EB-PWW7939	47.62162	122.40841	03/20/2009	1	540	949	10.0	0:1:0
hake	Elliott Bay	09EB-PWW7943	47.62162	122.40841	03/20/2009	1	320	173	3.0	0:1:0
hake	Elliott Bay	09EB-PWW7946	47.62162	122.40841	03/20/2009	1	345	227	4.0	0:1:0
hake	Elliott Bay	09EB-PWW7947	47.62162	122.40841	03/20/2009	1	340	228	5.0	0:1:0
hake	Elliott Bay	09EB-PWW7956	47.62162	122.40841	03/20/2009	1	395	376	5.0	0:1:0
hake	Elliott Bay	09EB-PWW7968	47.62162	122.40841	03/20/2009	1	330	179	4.0	0:1:0
pollock	Elliott Bay	09EB-WPW01	47.60766	122.34849	05/04/2009	9	119	13	1.0	0:1:8
hake	Main Basin	09CB-PWW01	47.31470	122.45172	03/27/2009	20	126	11	1.0	5:8:7
hake	Main Basin	09CB-PWW02	47.31470	122.45172	03/27/2009	20	125	11	1.1	7:6:7
hake	Main Basin	09CB-PWW03	47.31470	122.45172	03/27/2009	20	124	11	1.1	7:8:5
pollock	Main Basin	09CB-WPW01	47.31470	122.45172	03/27/2009	14	127	17	1.1	3:5:6
hake	Main Basin	09CS-PWW01	47.41667	122.38193	07/08/2009	20	187	45	1.1	14:6:0
hake	Main Basin	09CS-PWW02	47.41667	122.38193	07/08/2009	20	186	44	1.3	10:10:0
hake	Main Basin	09CS-PWW03	47.41667	122.38193	07/08/2009	20	179	37	1.3	14:6:0
hake	Main Basin	09CS-PWW04	47.39096	122.37668	07/08/2009	10	259	103	2.8	5:5:0
hake	Main Basin	09CS-PWW05	47.36525	122.37143	07/08/2009	10	294	146	3.7	4:6:0
hake	Main Basin	09CS-PWW7799	47.35240	122.36880	07/08/2009	1	315	200	3.0	0:1:0

Table 1. Sampling and biological metrics for 60 composite samples of hake and pollock analyzed in this study.

Species	Basin	SampleID	Latitude ^a	Longitude	Date ^b	Comp. (n) ^c	MCL ^d	MCW ^e	MCA ^f	Sex Ratio ^g
hake	Main Basin	09CS-PWW7812	47.35240	122.36880	07/08/2009	1	305	168	3.0	0:1:0
hake	Main Basin	09CS-PWW7813	47.48094	122.39505	07/08/2009	1	319	181	5.0	0:1:0
hake	Main Basin	09CS-PWW7819	47.48094	122.39505	07/08/2009	1	367	289	3.0	0:1:0
pollock	Main Basin	09CS-WPW01	47.71151	122.52887	03/19/2009	20	122	14	1.1	4:13:3
pollock	Main Basin	09CS-WPW02	47.71151	122.52887	03/19/2009	20	122	14	1.0	3:13:4
pollock	Main Basin	09CS-WPW03	47.71151	122.52887	03/19/2009	20	125	15	1.2	3:12:5
hake	S. Puget Sound	09SS-PWW01	47.19912	122.63221	03/26/2009	20	150	22	1.0	1:15:4
hake	S. Puget Sound	09SS-PWW02	47.20842	122.63235	03/26/2009	20	152	21	1.0	3:15:2
hake	S. Puget Sound	09SS-PWW03	47.20842	122.63235	03/26/2009	20	147	20	1.0	4:12:4

Chemical Analyses: The IOS laboratory performed ultra-trace analysis of contaminants using high resolution gas chromatography (HRGC) with high resolution mass spectrometry (HRMS) on all 60 samples of hake and pollock. This method was modified in part from USEPA Method 1613, which was originally designed for ultra trace analysis of dioxins and furans. Although the IOS lab is not listed as a Department of Ecology Accredited lab, we submitted to Ecology Form ECY 070-152a “Request to Waive Required Use of Accredited Lab” and received approval to employ this lab.

Chemicals analyzed by IOS included PCB and PBDE congeners, and ten chlorinated pesticides or groups of chlorinated pesticides. A total of 183 individual PCB congeners, 24 coeluting PCB congeners, 52 individual PBDE congeners, 8 coeluters, and 27 individual chlorinated pesticides were quantitated with the HRGC/HRMS.

An abbreviated summary of the HRGC/HRMS procedure is as follows. Approximately 12 grams of fish tissue from each homogenized sample was ground with anhydrous sodium sulphate and then extracted in a glass column using 1:1 (v:v) dichloromethane:hexane. One-third of the extract was removed and spiked with a mixture of ¹³C-labeled organochlorine pesticide internal standards. This pesticide fraction was further processed through florisil using 1:1 (v:v) dichloromethane/hexane, reduced in volume and spiked with surrogate recovery standard.

The two-thirds portion of the extract was spiked with mixtures of ¹³C-labeled PBDE and PCB internal standards. This fraction was processed using alumina and silica columns connected to an automated solvent delivery system (FMS PowerPrep) and eluted sequentially with pentane, 2% dichloromethane in hexane, 1:1 dichloromethane:hexane, and dichloromethane. Fractions from this cleanup were spiked with PBDE and PCB surrogate recovery standards and then analysed by high-resolution gas chromatography / high-resolution mass spectrometry (HRGC/HRMS).

A separate subsample of each tissue homogenate was used to determine percent lipid for each sample. The subsample was ground with anhydrous sodium sulphate and extracted in a glass column using 1:1 (v:v) dichloromethane/hexane. The extracts were evaporated to dryness, cooled, and weighed. Total lipid concentrations were determined gravimetrically.

All samples were processed through the cleanup laboratory as part of extraction batches. Each extraction batch consisted of:

- two procedural blanks; a reagent proof plus a minimal amount of triolein to imitate the behavior of tissue samples during extraction and clean-up,
- 10 tissue samples,
- a replicate of one of the tissue samples, and
- a sample of Certified Reference Material.

All quality control samples (procedural blanks, replicate sample, and Certified Reference Material) were processed through the laboratory in an identical manner to the remainder of the samples in the extraction batch.

The extraction, cleanup, analytical, and quality assurance methods used were adapted from (Ikonomou, et al., 2001). The laboratory reported as N/A (not available) any data point for which recovery of the corresponding internal standard was <20%. The acceptable range for CRM performance versus the laboratory's historical records was ± 2 standard deviations of the historical average. All surrogate standards were obtained from Cambridge Isotope Laboratories (Andover, MA). All results were corrected for internal standard recovery.

Stable Isotopes Analysis

We measured stable isotopes of carbon and nitrogen to calculate the isotopic ratios of ^{13}C to ^{12}C , as $\delta^{13}\text{C}$, and of ^{15}N to ^{14}N , as $\delta^{15}\text{N}$, relative to standardized isotopic ratios. We used $\delta^{15}\text{N}$ as an estimator of trophic level (Hobson, 1999; Post, et al., 2007), and used plankton from West et al. (2011) to evaluate the assumption that this nitrogen ratio was equivalent in the base of the food web across Basins. We used $\delta^{13}\text{C}$ in fish tissues as an independent estimator of the continuum of conditions from estuarine (Puget Sound) to oceanic conditions, with $\delta^{13}\text{C}$ increasing from oceanic to estuarine conditions (Hobson, 1999).

Stable isotope ratios were calculated using carbon and nitrogen isotopes measured from tissue subsamples taken from the same jars used for analysis of chemical contaminants. Wet samples were desiccated in a vacuum freeze dryer. Freeze-dried whole-body subsamples were then powdered in a SPEX 5100 ball mill (Metuchen, N. J) and then weighed into 5x9 mm tin capsules. Stable isotope ratios for the powdered samples were determined using a Costech ECS 4010 elemental analyzer (Valencia, CA) coupled to a Thermo Electron Delta Plus stable isotope ratio mass spectrometer (Bremen, Germany). Stable isotope values were expressed in δ notation as parts-per-thousand (‰) as defined by the following expression:

$$\delta Z = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000,$$

where Z represents ^{15}N or ^{13}C , R_{sample} is the ratio $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ for samples, and R_{standard} is the ratio $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ for the corresponding standards. The lab used two standards each for N and C to define the line used to convert the mass spectrometer signal to sample $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, respectively. Precision for isotope analysis was $\leq \pm 0.3\text{‰}$ for $\delta^{15}\text{N}$ and $\leq \pm 0.2\text{‰}$ for $\delta^{13}\text{C}$. All nitrogen values were referenced to atmospheric nitrogen ($\delta^{15}\text{N}$ for atmospheric N is 0‰ exactly) and carbon values were referenced to Vienna Pee Dee Belemnite, also known as NBS 19 [$\delta^{13}\text{C}$ of NBS 19 $\equiv 1.95\text{‰}$ (Coplen, et al., 2006)].

$\delta^{13}\text{C}$ values were corrected for variable lipid content (rather than pre-extracting lipids from samples) using a correction for aquatic animals proposed by (Post, et al., 2007), presented as “delta delta C” from his Equation 3:

$$\Delta\delta\text{C}^{13} = -3.32 + 0.99 \times \text{C:N},$$

Where C:N is the ratio of carbon to nitrogen by weight in the sample. For simplicity this adjusted $\delta^{13}\text{C}$ is hereafter referred to as $\delta^{13}\text{C}$.

Data Analysis

Of 209 possible PCB congeners, 207 individual or coeluting groups of congeners were reported from the high resolution GC/MS analysis in this study (Table 2). We calculated the sum of PCB congeners (Σ PCBs) as a simple sum of the concentration of all detected values, substituting zero for non-detected congeners. For PBDEs, 59 of 209 possible congeners were reported from the high resolution GC/MS analysis in this study. We estimated total PBDEs by summing all detected values except PBDE-209; significant contamination of blank samples for PBDE-209 indicated its reported values were unreliable. PBDE-209 is considered separately in Results.

Ten organochlorine pesticides or groups are reported in this study (Table 3). Some pesticide results were presented and analyzed as summations, based on groupings of related compounds. Dichloro-diphenyl-trichloroethanes (DDTs) were presented as the sum of six DDT-related chemicals (Σ DDTs). Σ Chlordanes were presented as the sum of two chlordane isomers, two nonachlor isomers, and heptachlor, Σ Chlorobenzenes (Σ CBZs) were presented as the sum of hexachlorobenzene, 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene, and pentachlorobenzene. Σ Hexachlorocyclohexanes (Σ HCHs) were presented as the sum of alpha-, beta-, delta-, and gamma-hexachlorocyclohexane. Σ Endosulfan were presented as the sum of endosulfan sulfate, endosulfan II, and endosulfan sulfate. Six compounds, heptachlor, octachlorostyrene, aldrin, dieldrin, mirex, and methoxychlor, were presented as single compounds (Table 3).

If necessary, PBT data were log-transformed to meet the requirements of normality and constant variance for parametric analysis, or to linearize curvilinear relationships for regression analysis. Data are presented on a wet weight basis throughout, except for specific cases as noted wherein PBTs are presented on a lipid basis.

A General Linear Model (GLM) as implemented in SYSTAT (2007) was used to measure the statistical significance of differences in PBT concentrations in fish among the six Basins and one embayment (hereafter referred to as Basins). We considered $\delta^{15}\text{N}$, tissue lipid content, fish size (fork length), age and sex as covariates in this comparison. These covariates were evaluated prior to GLM analyses using visual examination of scatterplots and with linear regressions to determine whether we could eliminate the effects of a covariate *a priori* to performing GLM analyses on data subsets, and to ensure that autocorrelated covariates were not included together in GLM runs. For example, fish length, age and $\delta^{15}\text{N}$ were all strongly correlated with each other, so we selected only one of those covariates (fish length) as a covariate in some of the GLM analyses.

Multiple comparisons testing (Tukey's Honestly-Significant-Difference Test, SYSTAT v. 12) was used to conduct pairwise comparisons of Basins for GLM analyses where the Basin effect was significant. We considered test results statistically significant at probability (p) levels of ≤ 0.05 (α threshold=0.05).

Table 2. List of contaminant types analyzed in Pacific hake and walleye pollock

Contaminant or Group	No. of composites	No. of Individual Compounds	Units	Basis	Laboratory^b	Dates Analyzed
PCB congeners	60	183 ^a	ng/g	wet	IOS	Nov.-Dec. 2009
PBDE congeners	60	52 ^c	ng/g	wet	IOS	Nov.-Dec. 2009
Organochlorine Pesticides	60	27	ng/g	wet	IOS	Nov.-Dec. 2009
DDTs	60	6	ng/g	wet	IOS	Nov.-Dec. 2009
Chlordanes	60	5	ng/g	wet	IOS	Nov.-Dec. 2009
Dieldrin	60	1	ng/g	wet	IOS	Nov.-Dec. 2009
Hexachlorocyclohexanes	60	4	ng/g	wet	IOS	Nov.-Dec. 2009
Chlorobenzenes	60	4	ng/g	wet	IOS	Nov.-Dec. 2009
Aldrin	60	1	ng/g	wet	IOS	Nov.-Dec. 2009
Octachlorostyrene	60	1	ng/g	wet	IOS	Nov.-Dec. 2009
Mirex	60	1	ng/g	wet	IOS	Nov.-Dec. 2009
Endosulfans	60	3	ng/g	wet	IOS	Nov.-Dec. 2009
Methoxychlor	60	1	ng/g	wet	IOS	Nov.-Dec. 2009
Total Lipids	60	-	%	wet	IOS	Nov.-Dec. 2009
Total Solids	60	-	%	wet	IOS	Nov.-Dec. 2009
δ^{15} Nitrogen (ppt)	60	-	‰	dry	NOAA	Dec. 2009
δ^{13} Carbon (ppt)	60	-	‰	dry	NOAA	Dec. 2009

Table 3. Summary of pesticide detection frequencies and limits by major pesticide groups.

Pesticide	CAS registry number	No. Samples Analyzed	Mean Detection Limit (pg/g)	No. of Detected Values	Frequency of Detected Values	
<u>DDTs</u>	o,p'-DDE	3424-82-6	57	4.9	55	96%
	p,p'-DDE	72-55-9	60	5.0	60	100%
	o,p'-DDD	53-19-0	58	4.6	58	100%
	p,p'-DDD	72-54-8	60	5.1	60	100%
	o,p'-DDT	789-02-6	58	5.2	56	97%
	p,p'-DDT	50-29-3	60	6.4	59	98%
<u>Chlordanes</u>	trans-chlordane	5103-74-2	60	2.3	60	100%
	cis-chlordane	5103-73-1	60	2.5	60	100%
	trans-nonachlor	39765-80-5	60	2.7	60	100%
	cis-nonachlor	39765-80-5	60	8.0	60	100%
	heptachlor	76-44-8	58	1.7	24	41%
<u>Chlorobenzenes</u>						
1,2,3,5+1,2,4,5- tetrachlorobenzene*	see footnote	52	0.59	52	100%	
1,2,3,4- tetrachlorobenzene	634-66-2	52	0.59	52	100%	
pentachlorobenzene	608-93-5	59	0.44	59	100%	
hexachlorobenzene	118-74-1	59	0.25	59	100%	
<u>Hexachlorocyclohexanes</u>						
alpha-hexachlorocyclohexane	319-84-6	60	1.6	60	100%	
beta- hexachlorocyclohexane	319-85-7	60	1.8	60	100%	
delta- hexachlorocyclohexane	319-86-8	60	1.8	15	25%	
gamma- hexachlorocyclohexane	58-89-9	58	1.8	58	100%	
<u>Endosulfans</u>						
endosulfan	115-29-7	60	19	9	15%	
endosulfan sulfate	1031-07-8	56	3.9	53	95%	
endosulfan II	33213-65-9	59	27	18	31%	
<u>Individual Compounds</u>						
dieldrin	60-57-1	60	5.5	60	100%	
octachlorostyrene	29082-74-4	55	0.29	55	100%	
aldrin	309-00-2	60	2.9	6	10%	
mirex	2385-85-5	60	0.54	60	100%	
methoxychlor	72-43-5	60	5.8	1	2%	

*combination of 1,2,3,5-tetrachlorobenzene (CAS# 634-90-2) and 1,2,4,5-tetrachlorobenzene (CAS# 95-94-3)

Results

Biological Metrics

Overall, trawl catches were dominated numerically by hake ($n=556$), followed by pollock ($n=143$). Pacific cod (*Gadus macrocephalus*) and Pacific tomcod (*Microgadus tomcod*) were caught infrequently and were not analyzed. We created 60 analytical samples (composites or individuals, hereafter referred to as samples), of which 52 were hake and 8 were pollock, over all Basins (Table 1). We analyzed at least three samples of hake from each of the seven Basins, and four pollock samples from the Main Basin, three from Hood Canal, and one from Elliott Bay (Table 4). Average size and age of hake in composite samples varied widely across Basins, ranging from 150 to 294 mm FL, and a modal FL of 140 mm (Figure 2). Mean age of fish in composites ranged from <1 year to 12 years (Table 1). All pollock were small (<170 mm FL) and young (mean <2 years of age in composites).

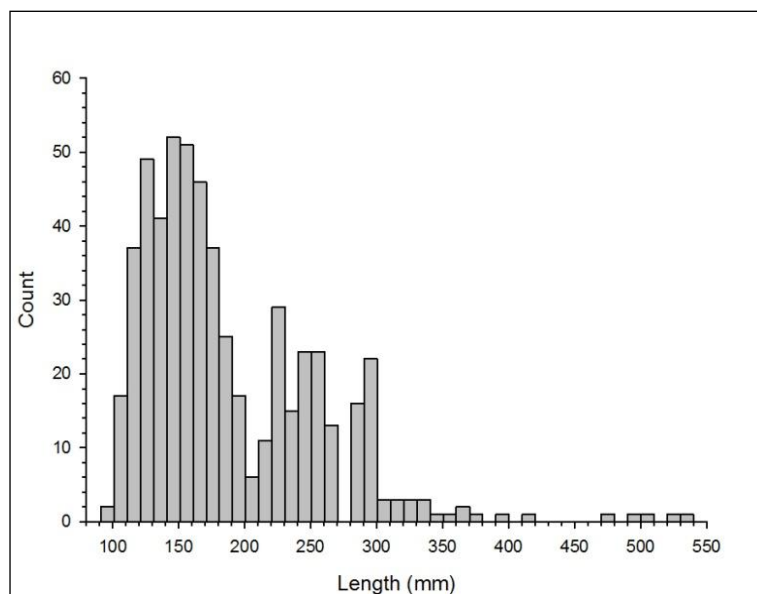


Figure 2. Size frequency distribution of all hake used in samples for analytical chemistry.

Mixing fish together by size in composite samples was meant to maximize the number of fish represented across a wide range of sizes, covering the full range of pre-reproductive condition to fully reproductive. However there were insufficient numbers of large (reproductive) hake to create same-sex composite samples in sufficient numbers to compare across Basins. Hence, using the range of sizes and

sexes available to us we created three types of samples: (a) composites of up to 20 hake or 20 pollock^a each of a size (<250 mm FL) to be clearly pre-reproductive and of unknown sex, (b) composites of up to 10 hake of an intermediate size (250 to 300 mm FL) wherein some fish were likely beginning to reproduce, and (c) large, undoubtedly reproductive hake (>300 mm FL) analyzed as individuals (Table 1). For unknown reasons, large male hake were rare in this study; 23 of 24 of the largest individual hake were females, and only nine of those 23 were of a size (>370 mm) that we had a high certainty were old enough to have been fully reproductive.

^a All pollock analyzed in this study were small/pre-reproductive

Table 4. Biological data (means) for samples of all Pacific hake and walleye pollock, summarized by species and Basin.

	Strait of Georgia	Str. Juan de Fuca	Hood Canal	Whidbey Basin	Main Basin	S. Puget Sound	Elliott Bay	Total
<i>All Pacific hake (Merluccius productus)</i>								
Number of composites	3	3	10	10	12	3	11	52
Weight(g)	20	28	288	208	104	21	225	171
Length (mm)	152	174	331	287	232	150	298	263
Age (years)	1.2	1.8	5.6	3.6	2.4	1.0	3.9	3.4
Tissue Lipids (%)	0.9	1.4	1.2	2.5	1.2	2.0	1.8	1.6
δN^{15} (‰)	12.0	12.3	14.0	13.7	13.6	13.5	13.9	13.6
δC^{13} (‰)	-18.2	-18.9	-18.3	-17.4	-16.4	-16.8	-16.7	-17.3
<i>All Walleye pollock (Theragra chalcogramma)</i>								
Number of composites	--	--	3	--	4	--	1	8
Weight(g)	--	--	30	--	15	--	13	20
Length (mm)	--	--	153	--	124	--	119	134
Age (years)	--	--	1.0	--	1.1	--	1.0	1.0
Tissue Lipids (%)	--	--	0.8	--	0.9	--	1.0	0.9
δN^{15} (‰)	--	--	13.2	--	13.1	--	13.8	13.2
δC^{13} (‰)	--	--	-17.7	--	-16.4	--	-17.7	-17.1
<i>Pacific hake (Merluccius productus) < 250 mm fork length</i>								
Number of composites	3	3	3	3	6	3	3	24
Weight(g)	19.9	27.8	45.8	37.8	26.2	20.9	26.5	28.9
Fork Length (mm)	152	174	200	166	155	150	150	163
Age (years)	1.2	1.8	1.7	1.6	1.1	1.0	1.5	1.4
Tissue Lipids (%)	0.94	1.4	0.88	2.4	1.5	2.0	2.4	1.6
δN^{15} (‰)	12.0	12.3	12.9	13.2	12.8	13.5	13.2	12.8
δC^{13} (‰)	-18.2	-18.9	-18.6	-17.7	-16.7	-16.8	-17.3	-17.6

The metric $\delta^{13}\text{C}$ from fish tissue provided an independent estimate of the distribution of the fish populations along a gradient of environmental conditions from ocean-influenced to estuary-influenced (Figure 3). We selected our sampling locations to represent a range of conditions across this continuum, with the simple assumption that fish living in Basins with a nearer and more direct connection to oceanic waters would exhibit lower contamination and perhaps different patterns of contamination than those from more developed (nearshore/estuarine) Basins. We observed a significant decrease in $\delta^{13}\text{C}$ in fish across Basins, with $\delta^{13}\text{C}$ declining as distance from estuarine waters increased and proximity to oceanic waters decreased (Figure 3), suggesting that our gradient assumption was valid. This gradient is used later in this report as a continuous variable in testing the geographic distribution of PBTs.

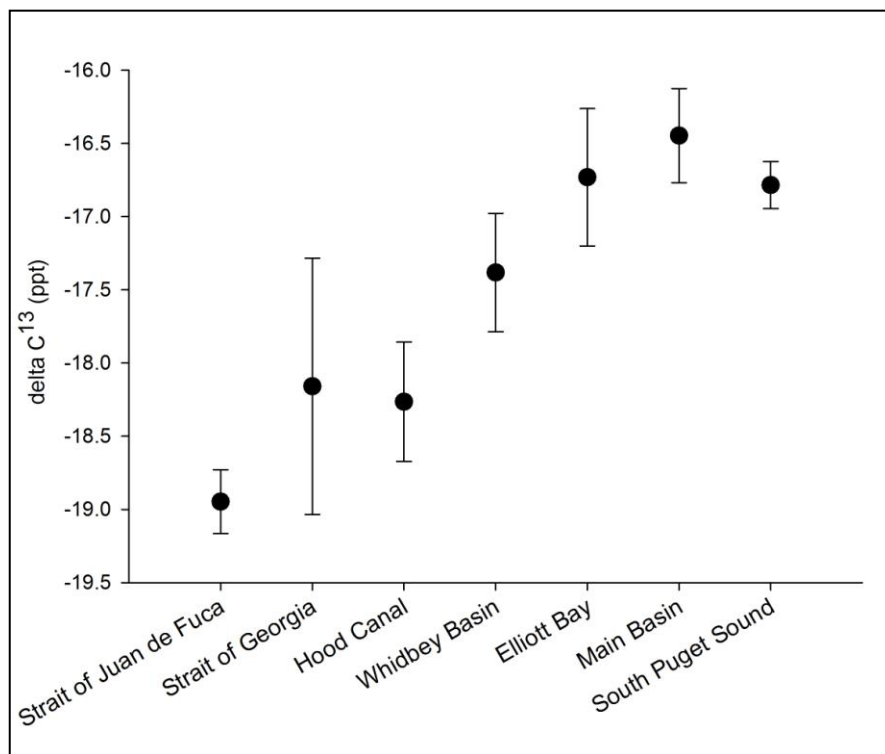


Figure 3. Distribution of $\delta^{13}\text{C}$ in hake across seven sampling Basins in Puget Sound. Mean ratio \pm 95% confidence interval.

PBT Overview

Specific geographic comparisons of PBTs are made below, accounting for covariate effects among Basins. Of the 27 individual pesticide compounds reported here, 21 occurred in at least 95% of the samples. Aldrin, methoxychlor and Endosulfan occurred rarely (<20% of samples) (Table 3).

PCBs dominated the chemical classes by concentration, with sample measurements ranging from 2.7 to 118 ng/g wet wt. for both species (Table 5). PBDE concentrations were roughly one-fourth of PCBs by Basin, with concentrations ranging from 0.7 to 28 ng/g wet wt. Of the eleven organochlorine pesticides (OCP) or groups we analyzed, Σ DDTs and Σ Chlordanes exhibited the greatest concentrations, ranging from 0.5 to 9.5 ng/g wet wt. (Table 5). Σ Chlorobenzenes, dieldrin and Σ HCHs were almost always detected (Table 2), however with low concentrations (median values from 0.1 to 0.7 ng/g wet wt. – Table 5). Octachlorostyrene, mirex, and Σ Endosulfans were detected in all Basins at low concentrations (maximum of 0.15 ng/g wet wt. in hake from Elliott Bay (Table 5). Octachlorostyrene, mirex, endosulfans, aldrin and methoxychlor are not analyzed beyond their entries in Tables 2 and 5.

Table 5 Summary of concentration data (ng/g wet wt.) for 11 PBTs in hake and pollock from seven Puget Sound Basins. “nc” indicates statistics that were not calculated because there were fewer than three detected values. In cases where no analyte was detected, the minimum (min) and maximum (max) values are presented as “<” the average limit of quantitation for that analyte.

		Pacific Hake							Walleye Pollock		
		Str. Juan de Fuca	Strait of Georgia	Hood Canal	Whidbey Basin	Elliott Bay	Main Basin	South Sound	Hood Canal	Elliott Bay	Main Basin
ΣPCBs	n ^a	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	2.7	3.4	9.0	29	29	23	32	6.9	120	11
	max	6.6	6.6	38	67	72	68	34	8.4	120	26
	mean	4.2	5.3	15	44	45	33	33	7.6	nc	15
	10th pctl.	2.7	3.4	9.0	30	30	24	32	6.9	nc	11
	median	3.3	5.7	10	41	44	28	33	7.4	nc	12
	90th pctl.	6.6	6.6	33	65	67	52	34	8.4	nc	26
ΣPBDEs	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	0.74	1.3	1.4	7.3	8.0	6.5	7.3	1.6	5.0	3.8
	max	1.9	3.6	8.2	21	28	19	7.9	2.0	5.0	7.8
	mean	1.1	2.8	2.6	13	14	9.4	7.6	1.7	nc	4.9
	10th pctl.	0.74	1.3	1.5	8.0	8.4	6.8	7.3	1.6	nc	3.8
	median	0.80	3.6	1.7	13	13	7.8	7.6	1.7	nc	4.0
	90th pctl.	1.9	3.6	6.1	20	23	16	7.9	2.0	nc	7.8
ΣDDTs	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	2.8	2.5	1.1	4.1	2.1	2.1	3.5	1.5	4.4	2.1
	max	3.2	2.9	5.9	9.5	6.7	7.3	3.6	2.1	4.4	4.0
	mean	3.0	2.7	2.6	6.0	4.6	4.1	3.6	1.8	nc	2.7
	10th pctl.	2.8	2.5	1.2	4.5	2.3	2.2	3.5	1.5	nc	2.1
	median	3.0	2.8	1.9	5.8	4.5	3.9	3.6	1.7	nc	2.4
	90th pctl.	3.2	2.9	5.9	8.3	6.7	6.2	3.6	2.1	nc	4.0
cont'd...											

^a Number of samples with detected values/number of samples analyzed

Table 5 Summary of concentration data (ng/g wet wt.) for 11 PBTs in hake and pollock from seven Puget Sound Basins. “nc” indicates statistics that were not calculated because there were fewer than three detected values. In cases where no analyte was detected, the minimum (min) and maximum (max) values are presented as “<” the average limit of quantitation for that analyte.

		Pacific Hake							Walleye Pollock		
		Str. Juan de Fuca	Strait of Georgia	Hood Canal	Whidbey Basin	Elliott Bay	Main Basin	South Sound	Hood Canal	Elliott Bay	Main Basin
ΣChlordanes	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	0.48	0.46	0.57	1.7	1.2	0.78	1.2	0.42	1.3	0.56
	max	0.57	0.60	2.3	2.7	2.8	1.7	1.3	0.51	1.3	1.2
	mean	0.53	0.53	1.0	2.0	1.8	1.2	1.2	0.46	nc	0.76
	10th pctl.	0.48	0.46	0.60	1.7	1.2	0.85	1.2	0.42	nc	0.56
	median	0.55	0.53	0.78	1.9	1.6	1.1	1.2	0.46	nc	0.63
	90th pctl.	0.57	0.60	2.2	2.4	2.4	1.6	1.3	0.51	nc	1.2
ΣChlorobenzenes	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	0.21	0.17	0.16	0.58	0.26	0.11	0.42	0.22	0.14	<0.004
	max	0.31	0.22	1.1	0.84	1.0	0.35	0.54	0.25	0.14	0.31
	mean	0.26	0.19	0.38	0.71	0.56	0.25	0.47	0.24	nc	0.19
	10th pctl.	0.21	0.17	0.17	0.59	0.27	0.13	0.42	0.22	nc	0.004
	median	0.25	0.18	0.30	0.71	0.59	0.27	0.44	0.24	nc	0.23
	90th pctl.	0.31	0.22	0.79	0.83	0.84	0.33	0.54	0.25	nc	0.31
Dieldrin	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	0.07	0.07	0.07	0.34	0.14	0.05	0.25	0.07	0.10	0.09
	max	0.09	0.08	0.68	0.46	0.63	0.19	0.25	0.09	0.10	0.16
	mean	0.08	0.08	0.19	0.39	0.31	0.13	0.25	0.08	nc	0.12
	10th pctl.	0.07	0.07	0.07	0.35	0.14	0.06	0.25	0.07	nc	0.09
	median	0.08	0.08	0.12	0.40	0.32	0.13	0.25	0.08	nc	0.11
	90th pctl.	0.09	0.08	0.46	0.45	0.47	0.18	0.25	0.09	nc	0.16

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Table 5 Summary of concentration data (ng/g wet wt.) for 11 PBTs in hake and pollock from seven Puget Sound Basins. “nc” indicates statistics that were not calculated because there were fewer than three detected values. In cases where no analyte was detected, the minimum (min) and maximum (max) values are presented as “<” the average limit of quantitation for that analyte.

		Pacific Hake							Walleye Pollock		
		Str. Juan de Fuca	Strait of Georgia	Hood Canal	Whidbey Basin	Elliott Bay	Main Basin	South Sound	Hood Canal	Elliott Bay	Main Basin
ΣHexachloro- cyclohexanes	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	0.22	0.13	0.08	0.29	0.09	0.05	0.31	0.12	0.09	0.09
	max	0.32	0.16	0.77	0.70	0.65	0.41	0.37	0.13	0.09	0.16
	mean	0.26	0.14	0.22	0.46	0.33	0.18	0.33	0.13	nc	0.12
	10th pctl.	0.22	0.13	0.09	0.32	0.11	0.09	0.31	0.12	nc	0.09
	median	0.23	0.14	0.15	0.43	0.32	0.18	0.31	0.13	nc	0.11
	90th pctl.	0.32	0.16	0.54	0.66	0.54	0.29	0.37	0.13	nc	0.16
Octachlorostyrene	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	0.009	0.005	0.003	0.012	0.014	0.003	0.021	0.004	0.007	0.005
	max	0.009	0.008	0.015	0.031	0.039	0.042	0.028	0.005	0.007	0.022
	mean	0.009	0.006	0.006	0.022	0.022	0.017	0.024	0.004	nc	0.010
	10th pctl.	0.009	0.005	0.003	0.013	0.014	0.008	0.021	0.004	nc	0.005
	median	0.009	0.006	0.005	0.022	0.019	0.016	0.023	0.005	nc	0.006
	90th pctl.	0.009	0.008	0.011	0.030	0.033	0.029	0.028	0.005	nc	0.022
Mirex	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	0.009	0.006	0.003	0.010	0.007	0.006	0.013	0.007	0.022	0.007
	max	0.010	0.009	0.017	0.019	0.026	0.024	0.014	0.008	0.022	0.018
	mean	0.010	0.008	0.007	0.013	0.013	0.011	0.013	0.008	nc	0.011
	10th pctl.	0.009	0.006	0.003	0.010	0.008	0.007	0.013	0.007	nc	0.007
	median	0.010	0.007	0.005	0.013	0.011	0.010	0.013	0.007	nc	0.009
	90th pctl.	0.010	0.009	0.014	0.017	0.023	0.019	0.014	0.008	nc	0.018
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Table 5 Summary of concentration data (ng/g wet wt.) for 11 PBTs in hake and pollock from seven Puget Sound Basins. “nc” indicates statistics that were not calculated because there were fewer than three detected values. In cases where no analyte was detected, the minimum (min) and maximum (max) values are presented as “<” the average limit of quantitation for that analyte.

		Pacific Hake							Walleye Pollock		
		Str. Juan de Fuca	Strait of Georgia	Hood Canal	Whidbey Basin	Elliott Bay	Main Basin	South Sound	Hood Canal	Elliott Bay	Main Basin
ΣEndosulfans	n	3/3	3/3	10/10	10/10	11/11	12/12	3/3	3/3	1/1	4/4
	min	<0.004	0.012	<0.004	0.025	0.026	0.009	0.015	<0.004	0.050	0.011
	max	0.013	0.037	0.100	0.090	0.146	0.086	0.053	0.011	0.050	0.032
	mean	0.006	0.029	0.024	0.057	0.067	0.033	0.028	0.007	nc	0.018
	10th pctl.	0.000	0.012	0.003	0.030	0.031	0.015	0.015	0.003	nc	0.011
	median	0.005	0.037	0.018	0.062	0.057	0.024	0.017	0.009	nc	0.013
	90th pctl.	0.013	0.037	0.066	0.080	0.116	0.071	0.053	0.011	nc	0.032
Aldrin	n	0/3	0/3	0/10	2/9	2/9	1/12	0/3	0/3	0/1	0/4
	min	<0.0023	<0.0040	<0.0027	0.0017	0.0017	0.0028	<0.0050	<0.0022	<0.0013	<0.0030
	max	<0.0023	<0.0040	<0.0027	0.0017	0.0023	0.0028	<0.0050	<0.0022	<0.0013	<0.0030
	mean	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
	10th pctl.	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
	median	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
	90th pctl.	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
Methoxychlor	n	0/3	0/3	1/10	0/9	0/9	0/12	0/3	0/3	0/1	0/4
	min	<0.0033	<0.0042	0.0412	<0.0075	<0.0063	<0.0043	<0.0104	<0.0027	<0.0036	<0.0043
	max	<0.0033	<0.0042	0.0412	<0.0075	<0.0063	<0.0043	<0.0104	<0.0027	<0.0036	<0.0043
	mean	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
	10th pctl.	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
	median	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
	90th pctl.	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc

PBTs-Basin Comparison Using Small (pre-reproductive) Hake

In most cases PBTs concentrations were correlated with one or more of the biological metrics: fish size, age, trophic level ($\delta^{15}\text{N}$), or lipids. A complete set of these biological metrics was not available for all fish samples in all Basins. To control for covariates among Basins for which a comparable set of biological metric data were available, a select subset of hake data were analyzed. Hence, among-Basin comparisons for most PBTs were made for the most common size classes, which were the youngest (<2 year-old) hake, of 110 to 250 mm fork length. Hake < 2 years of age were all sexually immature, and so there was likely no variability in PBTs associated with gender differences expected at reproductive ages. Average $\delta^{15}\text{N}$ varied over a range of less than 2 ‰ in these hake samples (Table 4), and only 1 ‰ in their primary prey, *Euphausia pacifica* (Table 4, West et al. 2011) indicating that these fish were likely feeding within a similar trophic level. To be more certain however, we included $\delta^{15}\text{N}$ and tissue lipid concentration as covariates in the General Linear Model (GLM) analyses for Basin-effects. In no case was trophic level or lipids a significant contributor (either $p>0.05$ in GLM runs, or significant factors explained only a trivial amount of model variability). These covariates were dropped from further analyses, and for brevity their results are not shown.

Of the seven organohalogen compounds or groups we included in the Basin comparison for small hake, ΣPCBs exhibited the greatest concentrations overall, with geometric mean tissue residues ranging from 26 to 36 ng/g wet wt. in fish from the four Developed Basins (Figure 4a). ΣPCBs in hake from these four Developed Basins were statistically different from those from Less Developed Basins, but were indistinguishable from each other ($F_{6,17}=61.9$, $p<0.0001$ for full model of \ln -transformed ΣPCBs by Basin, Tukey's Honestly Significant Difference pairwise comparison, $p>0.66$ between hake from Developed Basins). ΣPCBs in hake from the three Less Developed Basins were significantly lower than those in the four Developed Basins, with concentrations roughly one-third of the range observed in Developed Basins' samples (THSD, $p<0.0001$ for each pairwise comparison of Less Developed with Developed Basin samples). Although Strait of Georgia and Strait of Juan de Fuca hake had significantly lower ΣPCBs than Hood Canal hake, ΣPCBs in hake from these three Basins were all low (< 10 ng/g wet wt).

ΣPBDE concentrations in small hake exhibited a similar Basin pattern as ΣPCBs , however overall their concentrations were lower -- roughly one-fourth of ΣPCBs by Basin. Geometric mean ΣPBDEs ranged from 7.3 to 8.7 ng/g wet wt. in the four Developed Basins and less than 3 ng/g wet wt. in the three Less Developed Basins (Figure 4b). Similar to PCBs, ΣPBDE concentrations were statistically indistinguishable among Developed Basins, higher in Developed Basins than Less Developed Basins, and indistinguishable for two of the three pairings of Less Developed Basins: Multiple Linear Regression, $F_{6,17}=32.1$, $p<0.0001$ for full model of \ln -transformed ΣPBDEs by Basin, Tukey's Honestly Significant Difference pairwise comparison, $p>0.96$ between hake from Developed Basins, $p<0.0001$ for each comparison of Developed Basin with Less Developed Basin, $p>0.44$ for Strait of Georgia versus Hood Canal and Strait of Juan de Fuca, and $p=0.017$ for the Strait of Georgia-Hood Canal comparison.

ΣDDTs exhibited the greatest concentration of all organochlorine pesticides in small hake, with geometric means ranging from 2 to 5 ng/g wet wt (Figure 4c). All other pesticides exhibited concentrations less than 2 ng/g wet wt. In general, organochlorine pesticides exhibited a slightly

different spatial distribution pattern than PCBs and PBDEs. Whereas \sum PCBs and \sum PBDEs in hake from the four Developed Basins were statistically indistinguishable, the concentration of four pesticides, \sum DDTs, \sum Chlordanes, \sum Chlorobenzenes and dieldrin in small hake from the agricultural (Developed) Whidbey Basin was greater than two other Developed Basins (Main Basin and South Puget Sound) as well as from the three Less Developed Basins (Figure 4c-f; GLM of ln-transformed PBT concentration Basin, with $p < 0.0001$ for the full model for each pesticide, $\alpha = 0.05$ for Tukey Honestly Significant Difference pairwise comparisons). The concentration of these pesticides in small hake from Elliott Bay was intermediate between those in Whidbey Basin hake and hake from the Main Basin and South Puget Sound. \sum HCHs appeared broadly distributed among Basins at low levels (less than 0.6 ng/g wet wt) although small hake from Whidbey Basin had greater concentrations than small hake from the Main Basin and the three Less Developed Basins (Figure 4g).

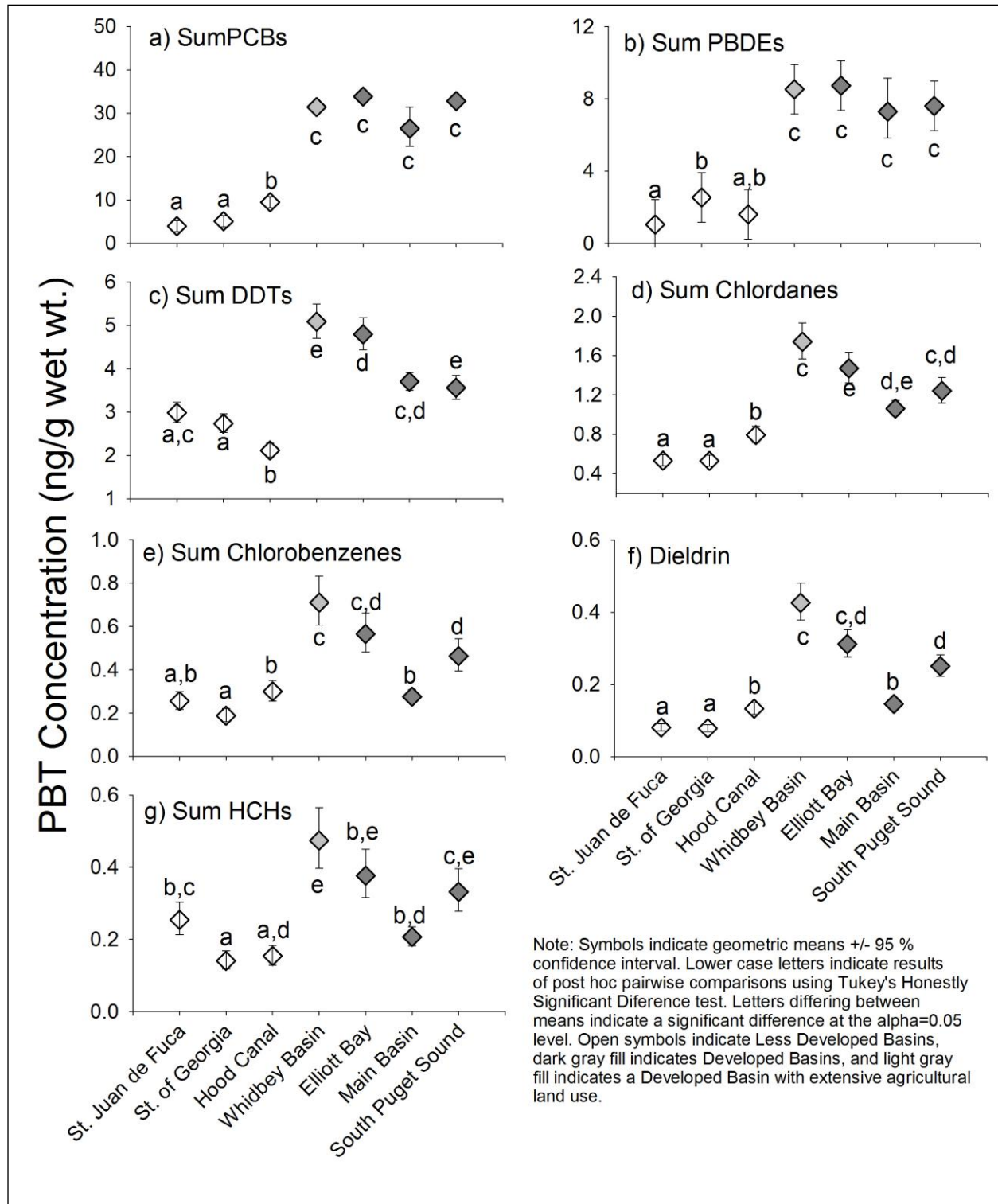


Figure 4. Summary of seven persistent bioaccumulative toxic chemicals in pre-reproductive Pacific hake from seven sampling Basins in PugetSound.

Accumulation of PBTs using All Hake

We evaluated the potential for accumulation of PBTs in hake by testing correlations of PBT concentration with hake age, across the 11 year age span represented by our samples. This analysis was hampered by the lack of older specimens, notably males. Hence, accumulation patterns we observed here were attributable primarily to female hake. In addition, relative trophic level of hake ($\delta^{15}\text{N}$) correlated strongly with both fish age (Figure 5a) and fish size (Figure 5b), indicating an ontogenetic shift in diet during their life span. As a result, increases in PBT concentration with fish age may be attributed to both bioaccumulation (increase with exposure time), and from exposure as adults to greater concentration in higher-trophic-level prey.

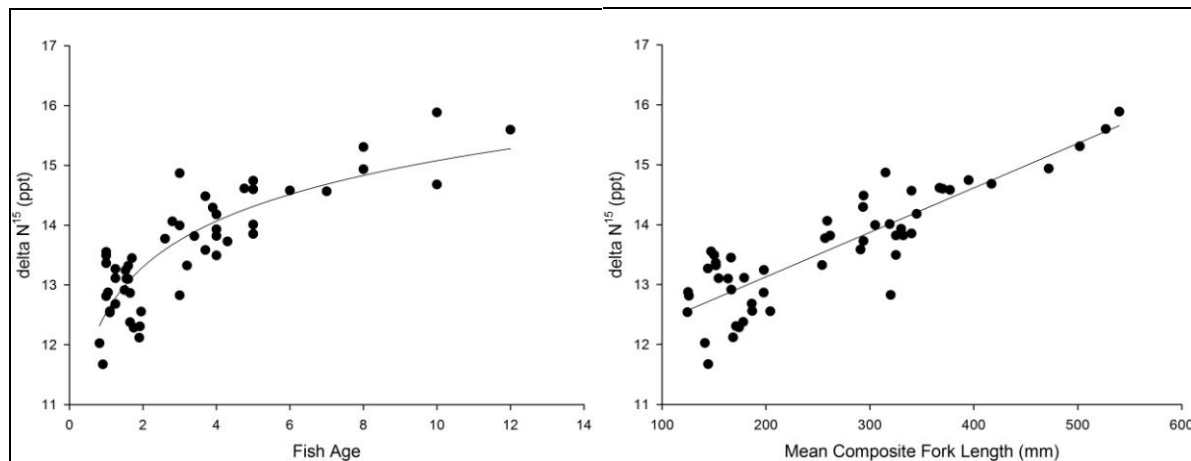


Figure 5a-b. Increase in estimated trophic level ($\delta^{15}\text{N}$) with estimated hake age (5a) and fish length (5b).

Applying multiple linear regression analysis of ln-transformed PBT concentrations by fish size (as a proxy for age and trophic level), tissue lipid content and $\delta^{13}\text{C}$ (as an indicator of location) we observed four basic patterns in PBT accumulation in hake: 1) $\sum\text{PCBs}$ and $\sum\text{Chlordanes}$ increased with fish size, increasing with proximity to estuarine/urbanized waters, and with lipid content; 2) $\sum\text{PBDEs}$ and $\sum\text{DDTs}$ did not increase with fish size, but increased with proximity to estuarine/urbanized waters, and with lipid content; and c) Dieldrin, $\sum\text{Chlorobenzenes}$ and $\sum\text{Hexachlorocyclohexanes}$ were predicted best with lipids alone, without regard to sampling location or fish size (Table 6). In all cases lipids did not correlate with fish size, supporting the inclusion of both factors separately in the multiple regression models. In addition, analogous models substituting age or trophic level ($\delta^{15}\text{N}$) for fish size yielded the same results (not shown for brevity).

These three factors, $\delta^{13}\text{C}$, fork length and lipids, or subsets of factors explained much of the variability in PBTs, (adjusted r^2 ranging from 0.63 to 0.89; Table 6). Additionally, analysis of PBT concentrations expressed on a lipid basis (i.e., as ng PBT/ g lipids) reduced the predictive capacity of these models, suggesting that including lipids as a covariate (or factor) provided a better explanation of the relationship between lipids and PBTs (*sensu* (Hebert and Keenleyside 1995) rather than evaluating a simple ratio of lipids:PBTs.

Table 6. Model parameters from backwards stepwise multiple linear regression of PBTs by Basin, with three covariates. Coefficients reported from analysis of ln-transformed contaminants.

Contaminant	Adj. r ²	covariate	coefficient	SE	p	Std.coeff
ΣPCBs	0.78	constant	12	1.05	<0.001	--
		Lipids	0.63	0.064	<0.001	0.65
		Fork length	0.001	0.001	0.026	0.15
		δC ¹³	0.61	0.059	<0.001	0.70
ΣChlordanes	0.71	Constant	3.1	0.71	<0.001	--
		Lipids	0.43	0.044	<0.001	0.76
		Fork length	0.001	<0.001	0.006	0.22
		δC ¹³	0.23	0.044	<0.001	0.44
ΣPBDEs	0.80	Constant	12.9	1.1	<0.001	--
		Lipids	0.69	0.068	<0.001	0.66
		Fork length	--	--	0.28	--
		δC ¹³	0.71	0.063	<0.001	0.74
ΣDDTs	0.62	Constant	3.3	0.80	<0.001	--
		Lipids	0.45	0.049	<0.001	0.81
		Fork length	--	--	0.11	--
		δC ¹³	0.15	0.045	0.002	0.30
Dieldrin	0.72	constant	--	--	0.36	--
		Lipids	0.69	0.026	<0.001	0.75
		Fork length	--	--	0.45	--
		δC ¹³	0.104	0.055	0.067	0.15
ΣChlorobenzenes	0.69	constant	-1.74	0.84	0.045	--
		Lipids	0.54	0.052	<0.001	0.84
		Fork length	--	--	0.31	--
		δC ¹³	--	--	0.79	--
ΣHCHexanes	0.89	constant	-3.2	0.57	<0.001	--
		Lipids	0.67	0.035	<0.001	0.9
		Fork length	--	--	0.41	--
		δC ¹³	--	--	0.23	--

Although using δ¹³C as an indicator of location has advantages for such statistical analyses, its primary disadvantage is that it makes it difficult to see patterns graphically. To remedy this we looked at accumulation patterns of PBTs in hake by comparing fish samples using Basin as a categorical variable, pooling samples from the four Developed Basins together in one class (“Developed”), and the three Less Developed Basins together in a second class (“Less Developed”). In this way we could plot the relationship between PBTs and fish size and lipid content separately for the two Basin classes, with a fairly large sample size in three dimensions.

The two PBT classes that exhibited both size- and lipid-accumulation, ΣPCBs and ΣChlordanes are shown in Figure 6a-b. In each case the ln-transformed PBT concentration was predicted well with a combination of fish size and tissue lipid concentration, with the predicted linear regression plane showing greater length- and lipid-specific accumulation in fish from Developed Basins (see Table 6 for regression parameters).

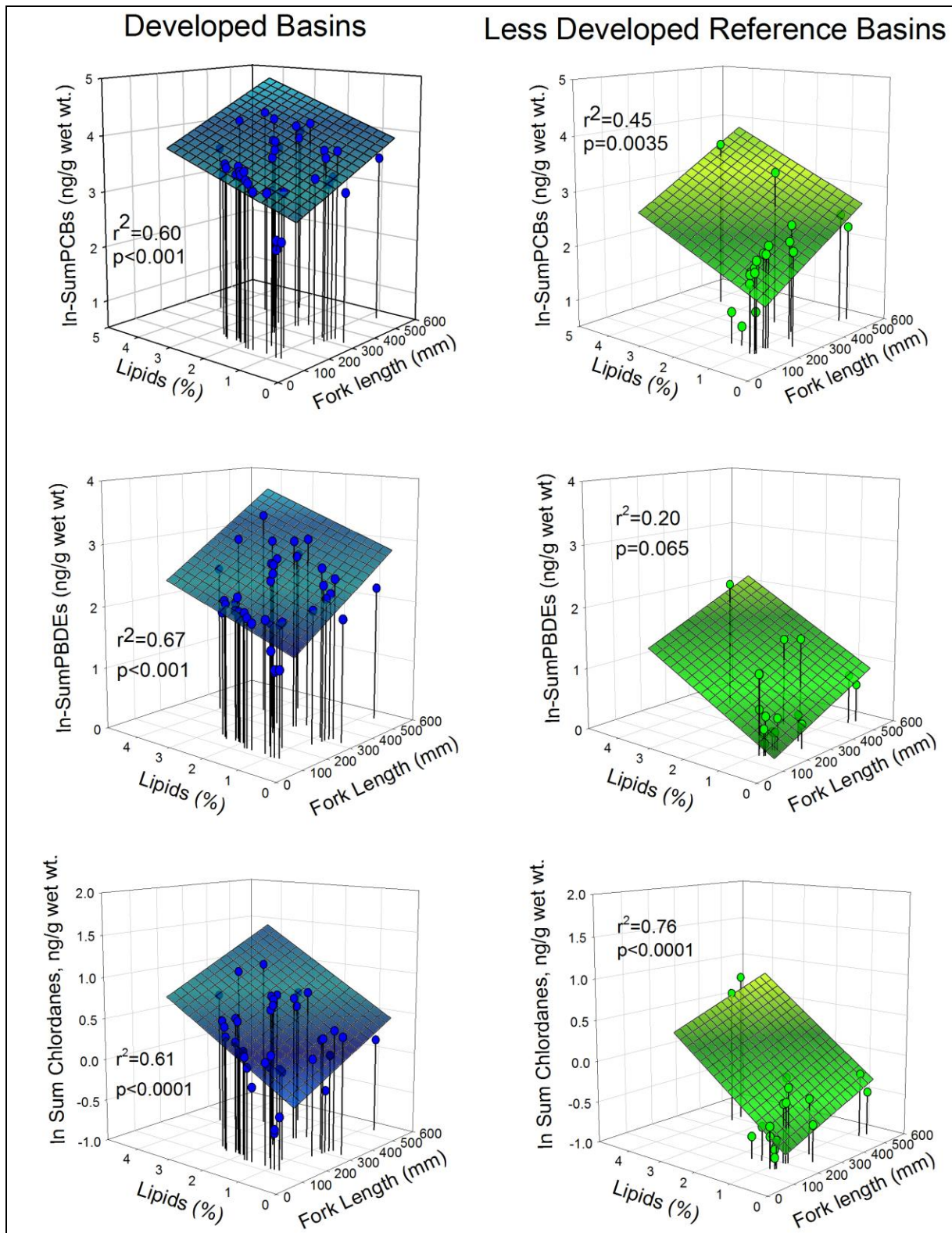


Figure 6a-c. Accumulation of PCBs (a, top row), Chlordanes (b, middle row) and PBDEs (c, bottom row) with fish size and tissue lipid concentration for gadoids from Developed Basins (left side) and Less Developed Reference Basins (right side). Scatterplots with predictive response plane generated using multiple linear regression.

Three dimensional graphical analysis also revealed evidence for accumulation of PBDEs in hake, even though fish length was not a significant factor in the regression analysis using δC^{13} above (Table 6). When separated and plotted by Basin type, PBDEs showed a significant increase in concentration with both fish length and lipids in the Developed Basins but not the Less Developed Basins (Figure 6-c).

Patterns of Individual Congeners or Compounds

PCB Patterns

One hundred ninety three of the 207 PCB congeners analyzed by the high resolution GC/MS were detected at least once in a sample; 14 were never detected. Twenty four congeners were identified in coeluting groups of two to three congeners each. Fifty-two congeners or coeluting groups were measured in concentrations >0.1 ng/g, which accounted for 92% of the total mean concentrations. All PCB homolog groups were analyzed except monochlorobiphenyls. Penta- and hexa-chlorobiphenyls accounted for roughly 75 % of the totals on average for each Basin, followed by hepta- and tetra- (roughly 15% and 8%). Each of the di-, tri-, octa-, nona-, and deca-chlorobiphenyl groups accounted for less than 5% of the total (Figure 7).

The congener pattern in hake varied among Basins, with fish from Developed Basins not only containing greater Σ PCB residues as described above, but also trending towards a dominance of congeners with a greater molecular weight. Low-molecular-weight congeners such as tetra-chlorobiphenyls as a % of total PCBs declined across Basins from oceanic to estuarine, while high molecular-weight congeners such as hepta-chlorobiphenyls increased (Figure 7). This trend can be seen more clearly by comparing the sum of low molecular weight (LMW) PCBs (di- through penta-) with the sum of high molecular weight (HMW; hexa- through deca-) homolog groups across Basins. The HMW:LMW ratio was lowest in Less Developed Basins, and increased with proximity to Developed Basins, especially Elliott Bay (Figure 8). This trend was consistent for both pre-reproductive hake and older hake, however the HMW:LMW ratio was consistently greater in the older fish.

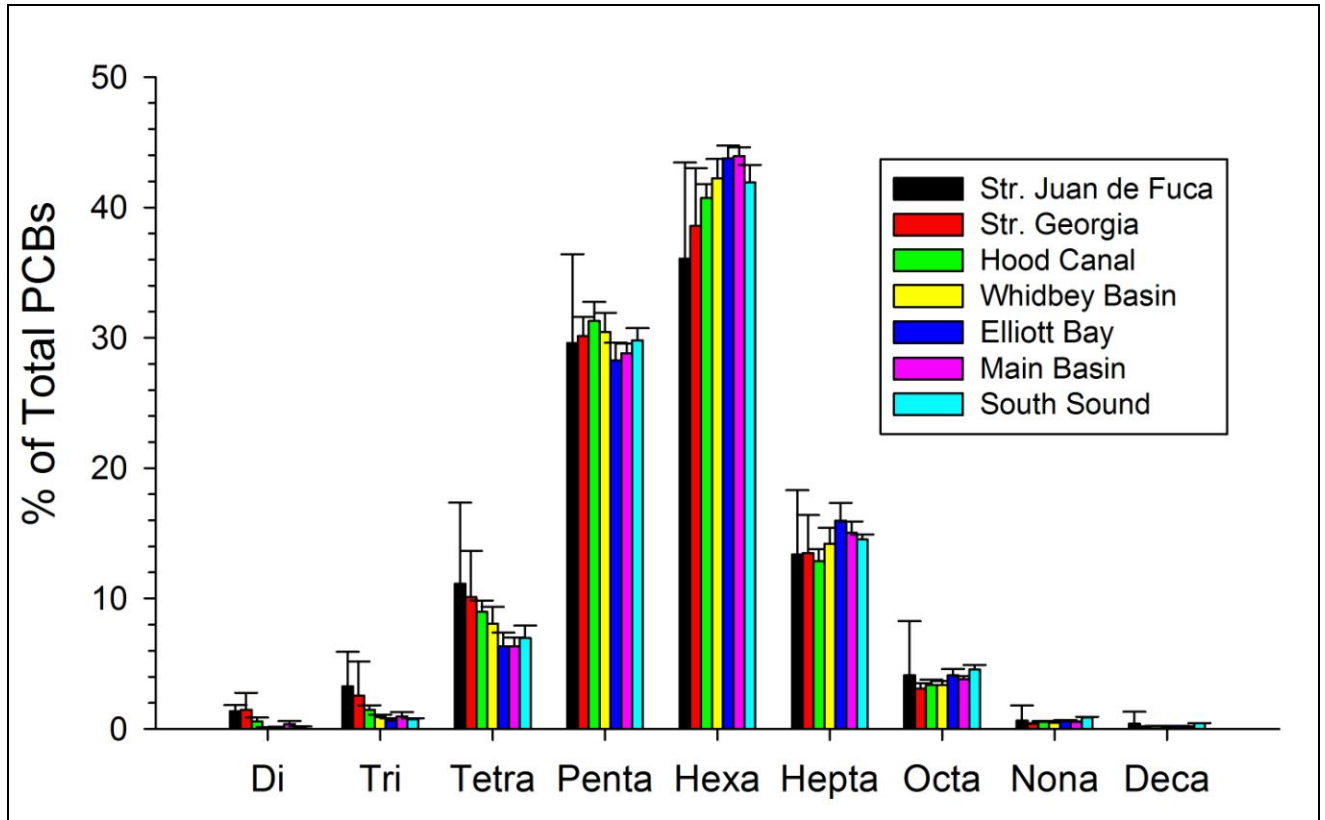


Figure 7. Relative abundance (as percent of total PCBs) of PCB homolog groups in hake across the seven sampling Basins.

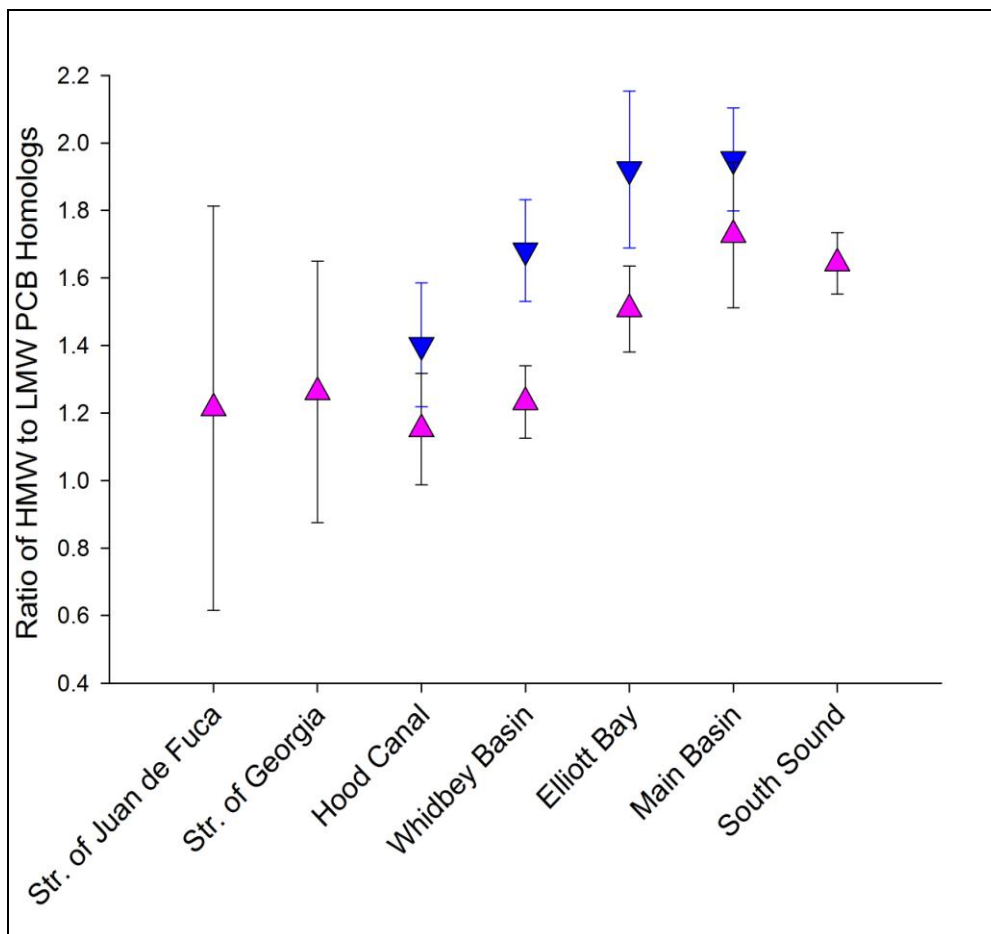


Figure 8. Ratio of high molecular weight homolog groups (sum of hexa-through deca-chlorinated biphenyls) to low molecular weight groups (sum of di-through penta-chlorinated biphenyls) for pre reproductive Pacific hake (upward triangles) and adult hake (downward triangles).

PBDEs

BDE-209

The decabrominated diphenylether BDE-209 was detected in all our hake and pollock samples, however it was also detected in all blank (solvent) control samples, often at concentrations equivalent to or exceeding those in our tissue samples. Because of its ubiquity in laboratory and other settings it is difficult to protect samples from external sources during processing. Of the 59^a samples analyzed for PBDE-209, 54 values were qualified as not detected because their concentration was less than three- times the average concentration of PBDE-209 in the two blank solvents from their corresponding batches. Five values exceeded three-times the average solvent blank concentration for hake: two samples from the Main Basin (1.004 and 0.69 ng/g wet wt.), two from Elliott Bay (0.70 and 0.81), and one from Hood Canal (2.7 ng/g wet wt.). Average solvent blank concentrations for the two batches in which these samples were analyzed were

^a One of 60 samples failed analysis for PBDE-209 and was not re-run

0.1833 and 0.1830 ng/g wet wt. These five samples were each composed of individual fish, all were females, and all were greater than 300 mm in length (see Table 1, SampleIDs; 09CS-PWW06, 09CS-PWW08, 09EB-PWW7946, 09EB-PWW7947, and 09HC-PWW8043).

Congener Patterns

Although we detected 14 PBDE congeners in virtually all samples, Σ PBDE concentration (not including BDE-209) was dominated by four congeners (BDE-47, -49, -99, and -100); these accounted for an average 91% of the Σ PBDE across all Basins (Figure 9). Because only 14 congeners were regularly detected in this analysis, we were unable to complete a comprehensive homolog profile as was done with PCBs. However abundance of the four dominant PBDE congeners in fish tissues suggest a relatively uniform pattern across all Basins. BDE-47 accounted for over 50% of Σ PBDE on average across all Basins, followed by BDE-49 and -100, which ranged from 10 to 15%, and BDE-99, which ranged from 2 to 7% of Σ PBDE.

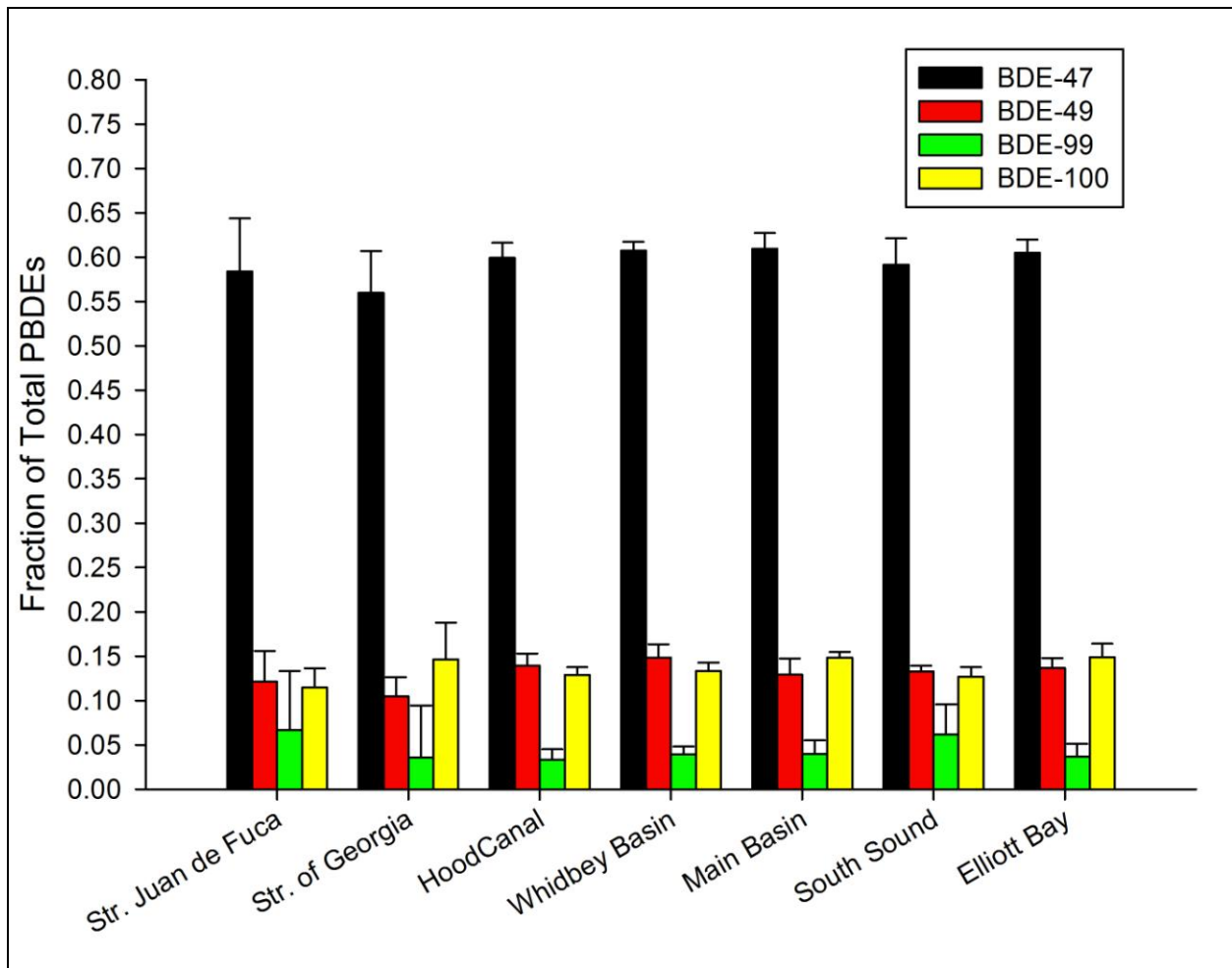


Figure 9. Relative abundance (as fraction of total PBDEs) of the four dominant PBDE congeners in hake from all Basins.

Comparison of PCBs and PBDEs

Σ PBDE concentration occurred in hake with a remarkable consistency relative to PCBs, across species, fish sizes, and locations. Σ PBDE concentration was roughly one-fourth the concentration of Σ PCBs at the low-concentration end of the scale, decreasing to one-third in samples at the higher end (Figure 10). This relationship is described with the following quadratic regression model:

$$\Sigma\text{PBDE} = a * \Sigma\text{PCB} + b * \Sigma\text{PCB}^2,$$

Wherein:

$$\begin{aligned} a &= 0.20, p < 0.0001; \\ b &= 0.0019, p = 0.0001; \\ y_0 &\text{ not significant, } p = 0.30; \\ r^2 &= 0.94, \text{ and } p < 0.0001 \text{ overall.} \end{aligned}$$

DDTs and Related Compounds

For the six DDT compounds measured here, we observed a consistent pattern in hake across all Basins, with a strong dominance of the DDT metabolite *p,p'*-DDE (accounting for an average of 68 to 81% of Σ DDTs), followed by *p,p'*-DDD and *p,p'*-DDT (Figure 11). The ratio of parent-to-total, calculated as (*o,p'*-DDT + *p,p'*-DDT)/ Σ DDTs was low in hake from all Basins (between 0.04 and 0.10, on average; Figure 12). Hake from Elliott Bay, Main Basin, South Puget Sound and Whidbey Basin each exhibited a significantly greater ratio than Hood Canal fish (ANOVA of the ratio transformed by arcsine of the square root, by sampling Basin, $p < 0.0001$, Tukey's multiple range test by Basin; $p < 0.0001$ for Elliott Bay:Hood Canal; $p = 0.051$ for Hood Canal:South Puget Sound; $p = 0.0045$ for Hood Canal:Whidbey Basin; $p = 0.074$ for Hood Canal:Main Basin; all other Basin pairwise comparisons $p > 0.10$).

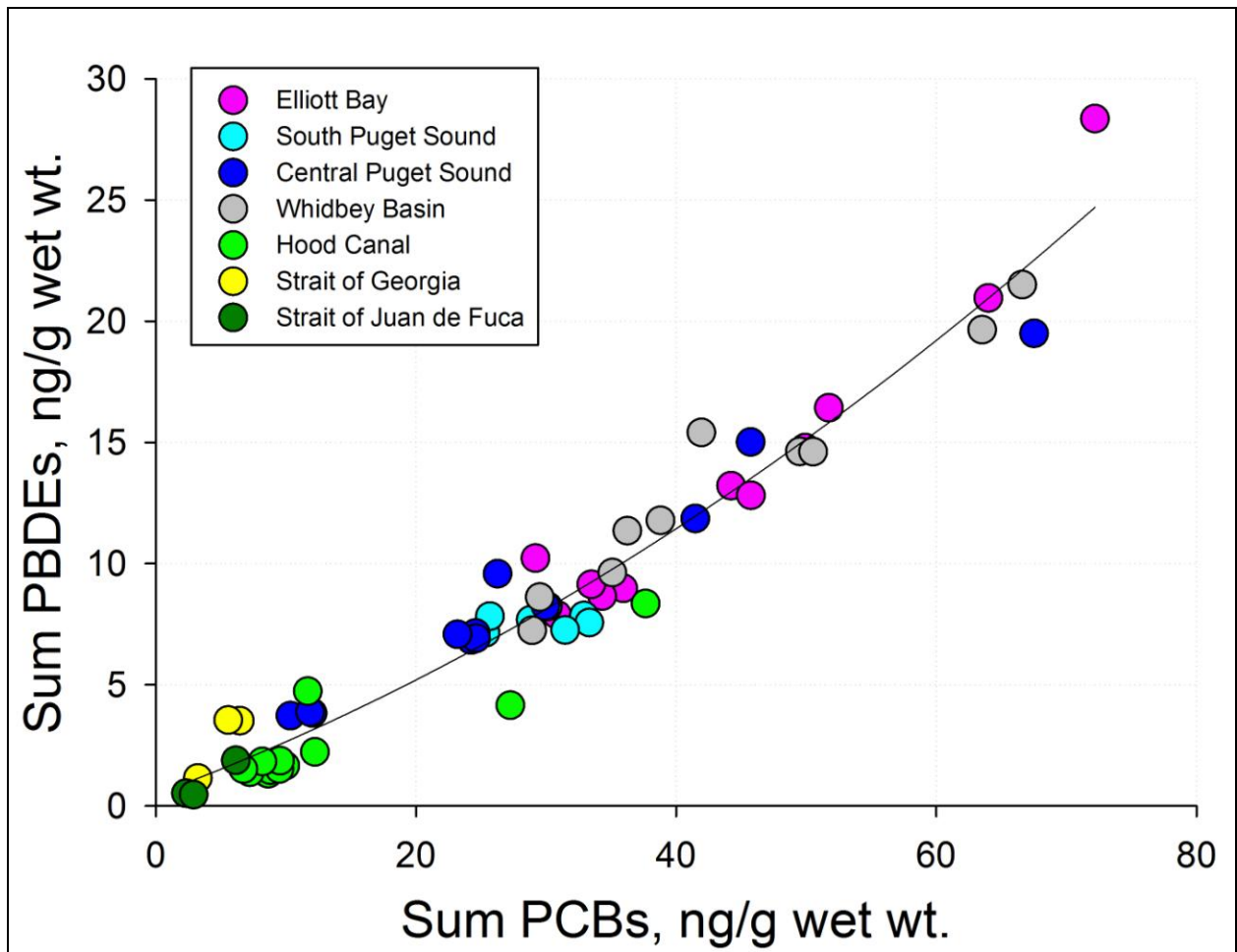


Figure 10. Comparison of PBDEs and PCBs in all tissue samples.

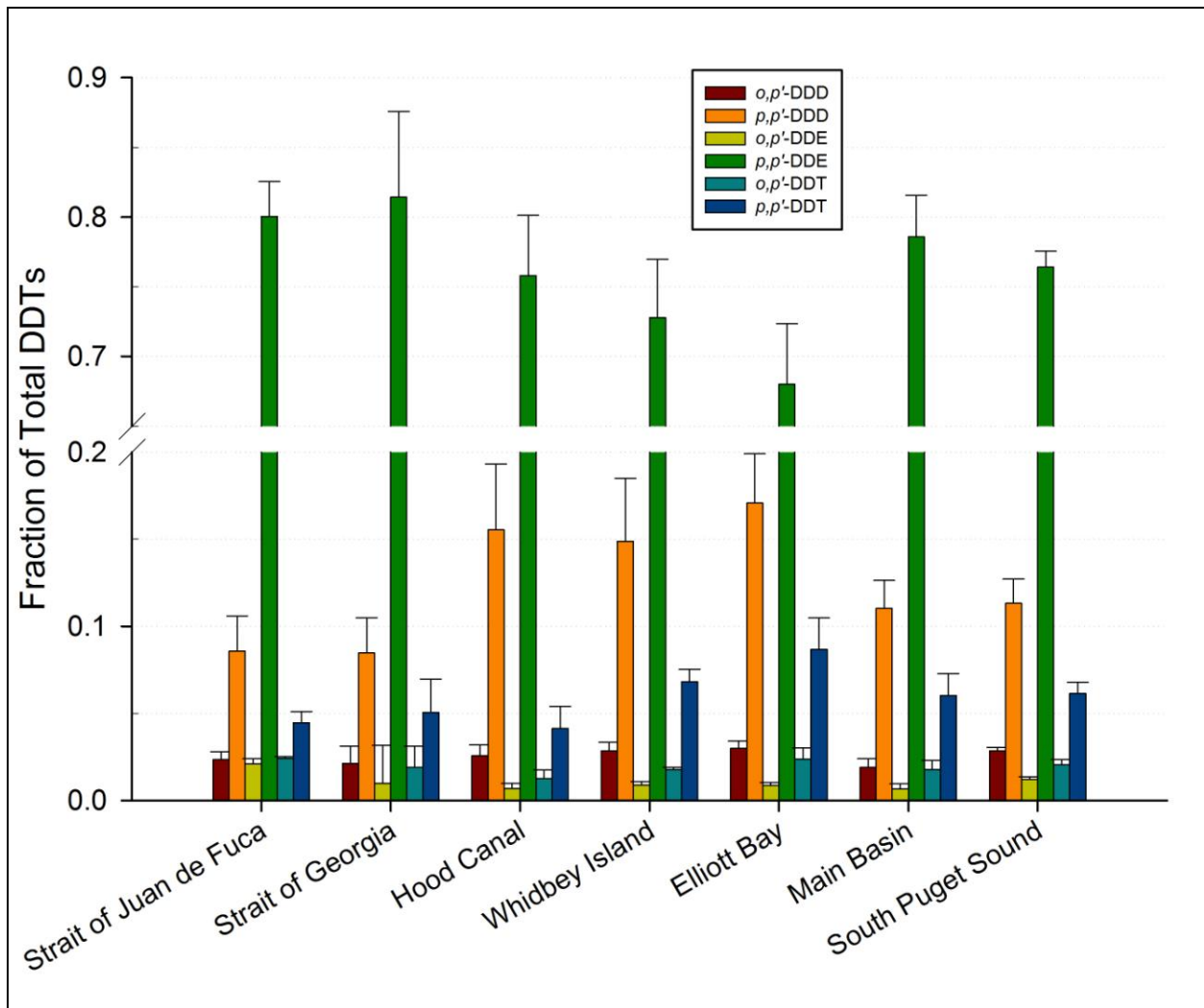


Figure 11. Relative abundance of DDT isomers (as a fraction of total DDTs) in hake from all Basins.

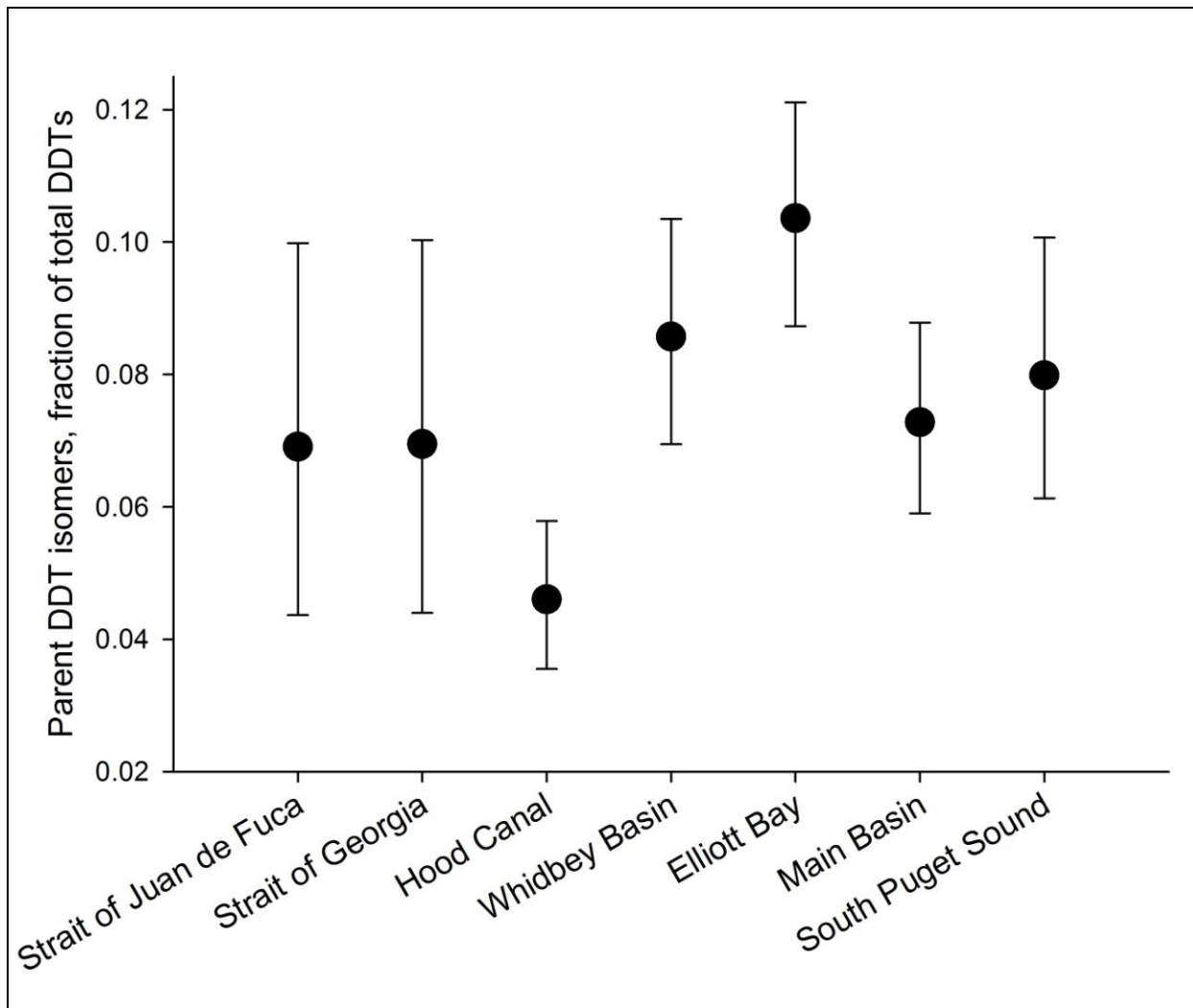


Figure 12. Comparison of the ratio of parent DDT isomers to total DDTs ratios in hake from all Basins. Lower case letters indicate statistical groupings from GLM analysis.

Σ Chlordanes:

Of the five chlordane-related compounds measured, four (*trans*-nonachlor, *cis*-nonachlor, *trans*-chlordane and *cis*-chlordane) occurred in all samples. These four accounted for an average of 42%, 19%, 11%, and 28%, respectively, of the Σ Chlordanes in all samples from all Basins (Figure 13). Heptachlor was only detected in 40% of samples and accounted for <2% of the Σ Chlordanes when it was present. The relative abundance of these compounds was consistent among Basins.

Σ Chlorobenzenes:

Of the four chlorobenzenes identified in hake, hexachlorobenzene was dominant, accounting for an average of 78 to 90% of Σ Chlorobenzenes (Figure 14). Pentachlorobenzene and two forms

of tetrachlorobenzene accounted for the remainder, in decreasing order of importance. The relative abundance of these compounds was nearly identical in hake across Basins.

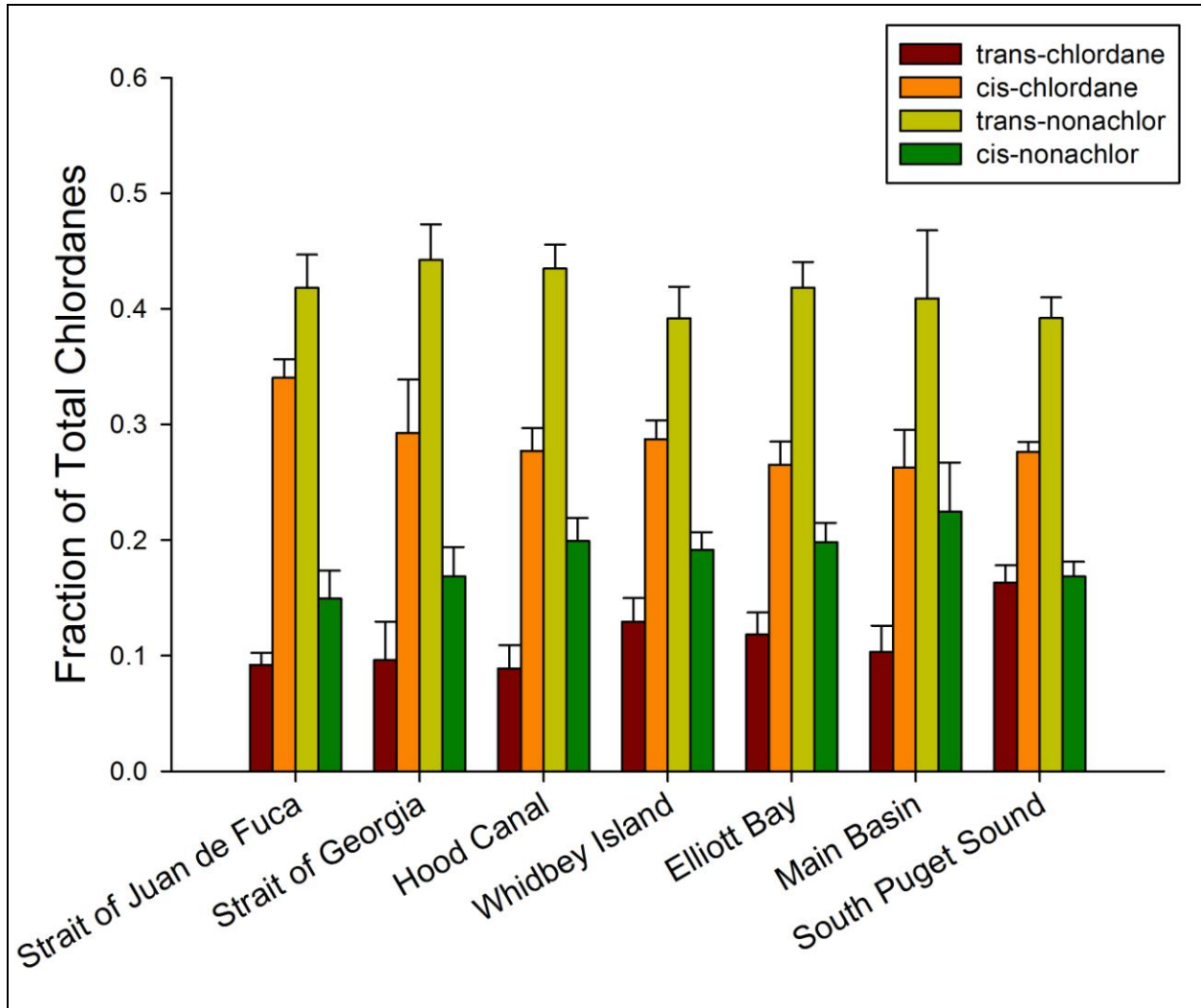


Figure 13. Relative abundance of chlordane-related compounds, as a fraction of total chlordanes in hake from all Basins.

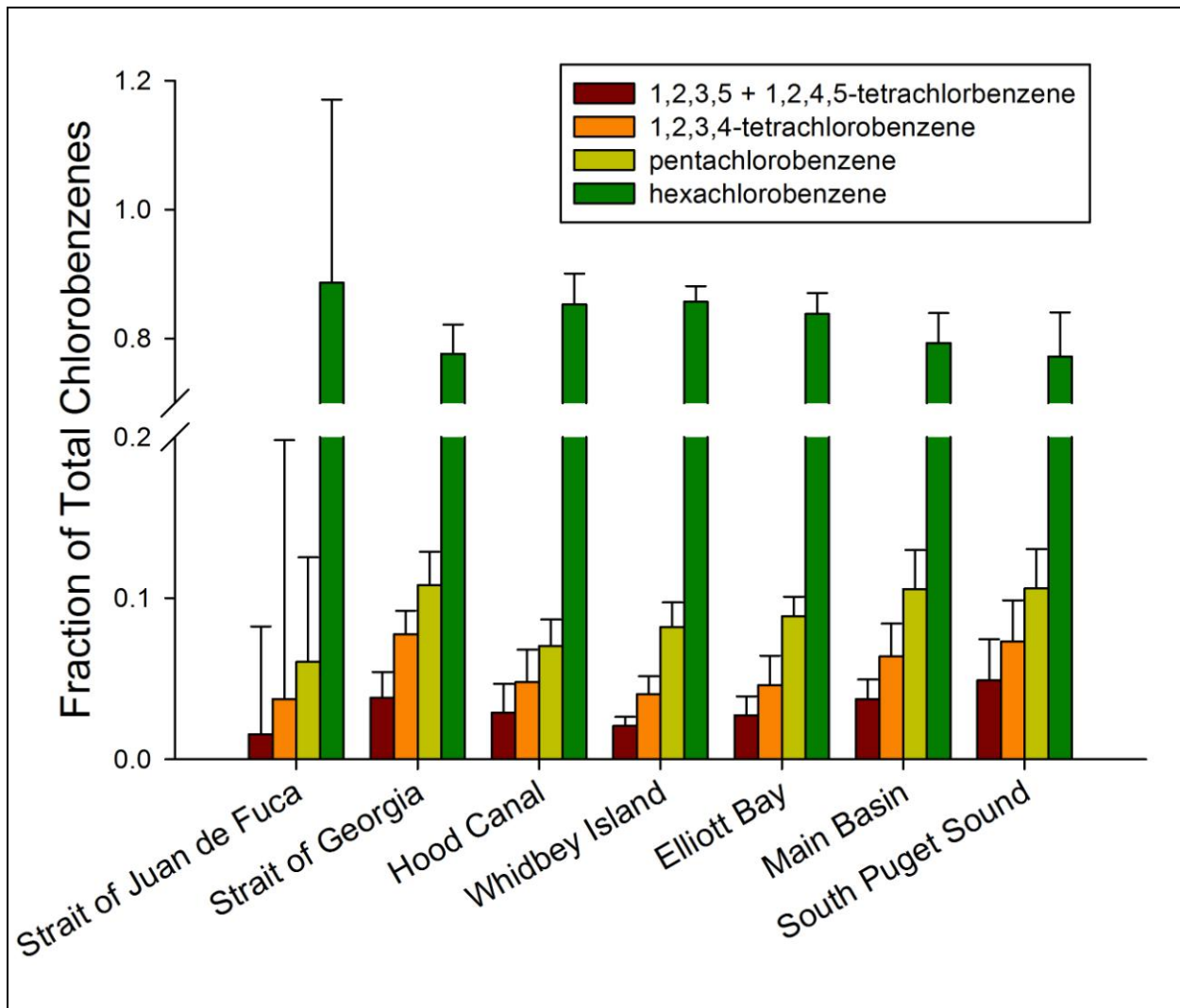


Figure 14. Relative abundance of chlorobenzene compounds (as a fraction of total chlorobenzenes) in hake from all Basins.

Σ Hexachlorocyclohexanes (HCHs):

α -HCH and β -HCH accounted for most (78 to 92%) of the Σ HCHs. Lindane, the gamma isomer of hexachlorocyclohexane (γ -HCH) was detected in all samples but accounted for only 8 to 22% of the Σ HCHs (Figure 15). The relative abundance of these three compounds appeared to be consistent across Basins.

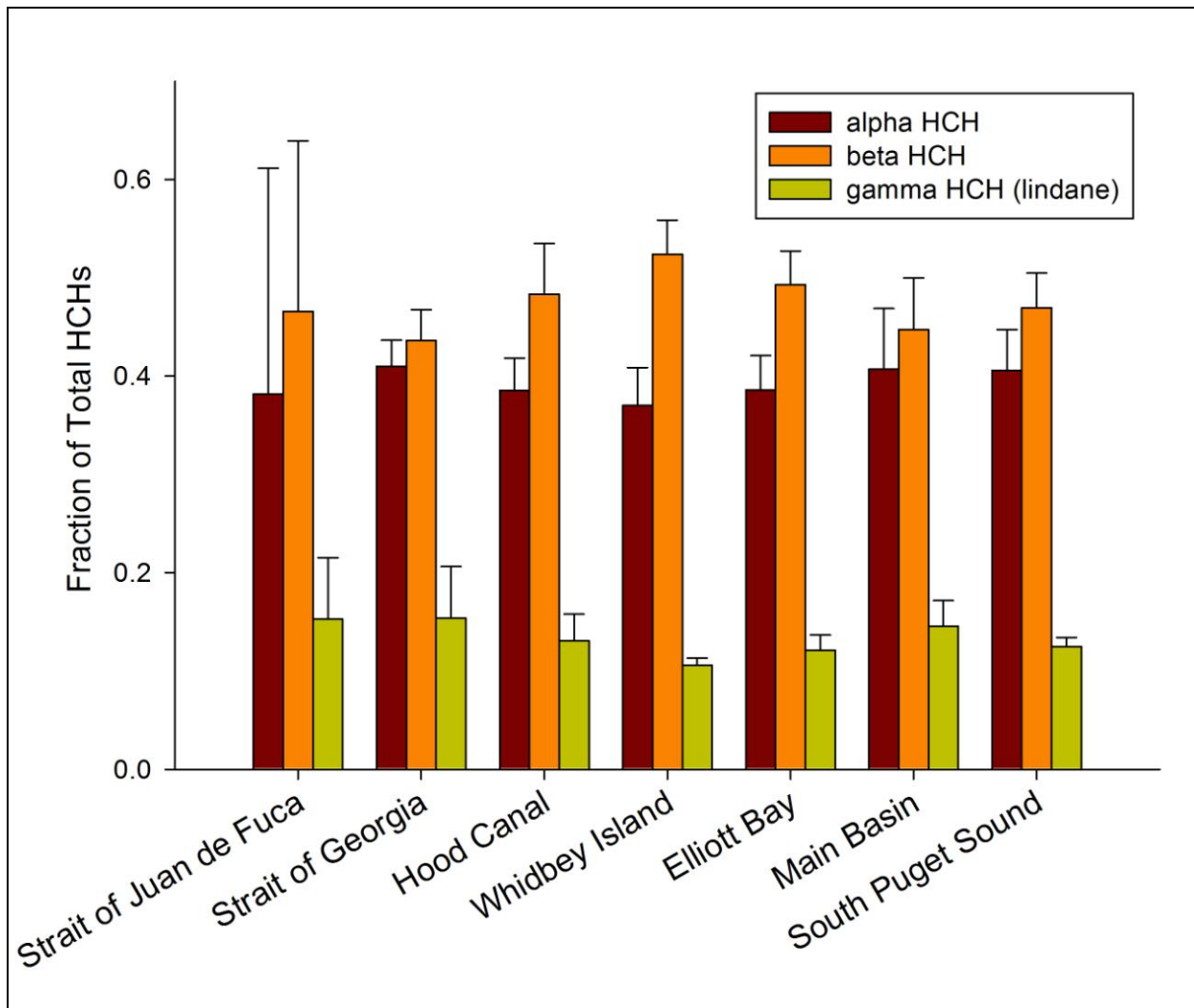


Figure 15. Relative abundance of hexachlorocyclohexane compounds (as a fraction of total hexachlorocyclohexanes) in hake from all Basins.

PBTs in Walleye Pollock

We analyzed PBTs in eight composites of small, pre-reproductive pollock. Three composites were from Hood Canal, four from the Main Basin, and one from Elliott Bay. Trophic level of pollock and hake were indistinguishable within Basins, with average $\delta^{15}\text{N}$ varying over a range of less than 0.7‰ (Table 4). In the only Basin where the average fish size in composites was similar enough to allow comparison of like-sized samples (3 composites of the smallest sized fish each from the Main Basin), $\delta^{15}\text{N}$ was statistically indistinguishable between the species (GLM, $p=0.326$, $F_{(1,5)}=1.19$, for a comparison of $\delta^{15}\text{N}$ between hake and pollock for average fish size <140 mm FL).

Although trophic level was similar for same-size fish between species in one Basin, lipid concentration in pollock from the Main Basin (0.93%) was significantly lower -- only roughly one-half of hake (1.7%) from the same Basin (GLM, $p=0.001$, $F_{(1,5)}=40.5$). Because of this disparity and the overall limited size distribution of pollock, we analyzed them for PBTs

separately. We also limited the PBT analysis to the subset of composites in the Main Basin as above, wherein average fish size in composites was similar (<140 mm FL) to provide us information about potential species differences, rather than any elucidation of Basin PBT patterns.

Of the seven PBTs analyzed in small hake, hake exhibited significantly greater concentration of Σ PCBs and Σ HCHs than pollock (geometric means from GLM analysis, 28.1 vs 14.1 ng/g wet wt. for Σ PCBs, and 5.2 vs 2.5 ng/g wet wt. for Σ HCHs respectively; GLM of ln-transformed PBT variable by Species, $p=0.036$, $F_{(5,1)}=8.08$ for Σ PCBs and $p=0.001$, $F_{(1,5)}=25.2$ for Σ HCHs). All other comparisons exceeded the $\alpha=0.05$ GLM comparison criterion.

The single composite of pollock sampled from Elliott Bay was unique in two ways: it contained the smallest fish of any composite in the study, yet the greatest Σ PCB concentration, 118 ng/g wet wt., of any composite (Sample ID 09EB-WPW01, Table 1). This concentration was over 60% greater than hake from that Basin and almost double that of any other hake sample from any Basin. These fish were taken near to the Seattle waterfront, unlike hake, which were taken in tows further away from the waterfront, in deeper waters to the north.

Discussion

The primary objectives of this study were to (a) compare PBTs concentrations in resident pelagic fish predators across the major regions of Puget Sound, (b) supply tissue residue data for Ecology's modeling efforts to compare land use patterns (PBTs sources) with PBTs patterns in Puget Sound's food web, and (c) compare these results with those in other fish species from previous PSAMP studies to provide a clearer understanding of PBTs patterns in fish, relative to potential sources.

Basin Comparison

Overall, the PBT results in hake from this report support similar findings on Chinook salmon (O'Neill and West 2009) and on Pacific herring (West et al. 2008) regarding the importance of proximity to developed watersheds for predicting PBT tissue residues in pelagic species. Here, we observed greater size- and lipid-specific accumulation of PCBs, PBDEs, and chlordanes, and lipid-associated accumulation of dieldrin and DDTs in a resident pelagic fish predator, Pacific hake, from Developed Basins compared to Less Developed Basins. This is consistent with watershed-scale sources of pollutants related to land use described in EnviroVision (2008), including ongoing point sources, surface runoff, and atmospheric transport. The Basin distribution of PBTs reported here are also consistent with the distribution of legacy PBTs in existing abiotic and biotic reservoirs.

Chlorobenzenes and hexachlorocyclohexanes occurred in hake in low concentrations equally across Basins, with variability explained primarily by tissue lipid content; from this we infer a more uniform distribution of these PBTs in the region. Such a uniform, low level of exposure may be consistent with atmospheric transport of these pollutants from diffuse sources.

Of particular note is the significantly greater tissue residues of pesticides in Whidbey Basin and Elliott Bay, compared with the other Developed Basins. All pesticides or groups showed elevated levels in one of these Basins compared with either the Main Basin or South Puget Sound. Although many of these compounds have restricted use it is possible that eroding watershed soils release chronic, low-levels of these contaminants, which enter receiving waters via stormwater runoff (e.g., Johnson et al. 1988).

Results from the multiple linear regression models and 3 dimensional plots highlight the importance of accounting for life history characteristics when evaluating spatial and temporal variability of PBTs in biota. Our results support the hypothesis that residency in Puget Sound, or nearness to developed watersheds, is one of the primary factors associated with exposure to these PBTs for pelagic fish species (O'Neill and West 2009, West et al., 2008). Even so, all eight PBTs were measured in all samples, indicating some exposure to these contaminants even in strongly ocean-influenced waters from Less Developed Basins (e.g., Strait of Juan de Fuca and Hood Canal) in Basins that receive much lower contaminant loads than Developed Basins.

The inclusion of highly explanatory life history factors can help untangle complicated variability in PBT patterns. We observed low \sum PCBs and \sum PBDEs in some of our oldest fish, two from a Less Developed Basin (Hood Canal) and one each from Elliott Bay and Whidbey Basin, in which we otherwise might have expected to see high exposure because of their greater age. In fact these individuals were all females, and each exhibited low tissue lipid content, probably

resulting from recent spawning activity. It is likely that the relatively low PBT concentrations in these older female individuals resulted from transfer of lipophilic PBTs (depuration) to eggs from spawning activity over their lifetime. Although we were unable to sample older male hake, and therefore failed to document their tissue loading patterns, our analytical models were generally consistent with and accommodated expected accumulation of PBTs in females and subsequent loss via reproduction (*sensu* Larsson 1996), by including both size and lipids in the regressions.

Data Transfer and Use in Subsequent Modeling

The data generated in this report will be transferred to Washington Department of Ecology staff for inclusion in ongoing contaminant fate and transport modeling efforts. These data include explanatory cofactors such as $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, tissue lipid concentration, fish age, and sex. $\delta^{13}\text{C}$ was particularly useful in this context because it helped explain the distribution of our samples across the ocean-to-estuary gradient. Others have reported similar C^{13} depletion in marine organisms sampled across similar gradients (Das, et al. 2003, Hobson 1999), and in future it may be useful to verify similar patterns in other biota.

In addition, $\delta^{15}\text{N}$ correlated strongly with hake size, supporting the assumption that trophic level of this species increases as the fish grows. This substantiates observations of a shift in prey from zooplankton (e.g., krill and copepods) to fishes and squid as hake grow larger (Stauffer 1985).

Understanding the feeding ecology and prey selection of predators may help to fine tune estimates of PBT transfer from their prey. Female hake grow larger than males (Stauffer 1985), and may lose PBTs as they grow older and reproduce many times during their lifetime. Based on our hake data, predators such as harbor seals that target the largest prey in a population may be exposed to lower PBT loads from their prey than those targeting smaller-sized individuals; smaller-sized hake prey may have a greater proportion of males.

Comparison of Hake with Other Species

The Basin-pattern of contaminant loads we observed for hake were consistent with those previously reported for two other pelagic fishes; Pacific herring (*Clupea pallasii*) (West et al. 2008) and Chinook salmon (*Oncorhynchus tshawytscha*) (O'Neill and West 2009). Herring exhibited a three-to five-fold greater PCB concentration in populations from the Main Basin and Southern Puget Sound (Developed Basins), compared to same-age herring from the southern Strait of Georgia (a Less Developed Basin). Puget Sound-resident Chinook salmon (often termed "blackmouth") sampled from the Main Basin carried PCB concentrations three times greater than older conspecific individuals that had spent the majority of their saltwater life in the ocean.

We also compared PBT concentration between these pelagic species (using PCBs as a model) to help understand their relative importance as potential sources in the trophic transfer of PBTs to their predators. Based upon comparison of wet weight PCBs, Pacific herring appear to pose a greater potential for PBT transfer than hake or resident Chinook salmon. Herring from Southern Puget Sound, the Main Basin and Strait of Georgia exhibited a five- to seven-fold greater concentration of $\sum\text{PCBs}$ on a wet weight basis (160, 160 and 34 ng/g, respectively) than our

subset of young hake^a from these same Basins (30, 30 and 5.0 ng/ g wet wt. respectively) (Table 7). Resident Chinook salmon reported by O’Neill and West (2009) from the Main Basin exhibited PCB concentration (74 ng/g wet wt.) intermediate to herring and hake. Based on PCB accumulation plots in O’Neill and West (2009), smaller resident Chinook salmon (closer in size to our largest hake) would be predicted to exhibit a lower concentration (below 50 ng/g wet weight.), which is roughly equivalent to the maximum reported here for any hake from the Main Basin (Table 5).

Table 7. Comparison of PCB concentration in Pacific hake^a from the present study with Pacific herring in similar areas reported by West et al. (2008), Puget Sound Assessment and Monitoring Program (PSAMP).

	S. Puget Sound		Main Basin		Str. of Georgia	
	Herring	Hake	Herring	Hake	Herring	Hake
sample size	60	3	56	6	55	3
mean age	3.1	1.0	2.5	1.1	2.3	1.2
mean length (mm)	161	150	169	155	170	152
mean wt. (g)	53	21	59	26	59	20
% lipids	8.0	2.0	6.4	1.5	5.1	0.94
$\delta^{15}\text{N}$ (‰)	14.0	13.5	13.9	12.8	13.0	12.0
ngPCBs/g wet	160	32.6	160	26.3	34	5.1
ngPCBs/g lipid	2,000	1500	2,500	3300	690	560

^asubset of small hake for comparison with herring

If predators target lipid content of their prey to satisfy metabolic needs, herring and hake appear to present a more equivalent PCB transfer potential. Hake exhibited lower lipid content ($\leq 2\%$) than herring (5 to 8%) so if predators consume more hake than herring to acquire equivalent dietary lipids, the potential PCB trophic transfer disparity between herring and hake is lessened. This is seen in a comparison of PCBs on a tissue lipid basis; West et al. (2008) reported 2,000 ng PCBs/g lipid for herring from Southern Puget Sound, 2,500 ng PCBs/g lipid from the Main Basin and 690 ng PCBs/g lipid from Strait of Georgia herring, and we found 1,500, 3,300 and 560 ng PCBs/g lipid, respectively, for hake in these three Basins.

Resident Chinook salmon from O’Neill et al. (2009) exhibited over five times the lipid content of hake; re-computation of Chinook summary results to lipid weight based on data in their Table 3 indicates Main Basin resident Chinook likely exhibited a PCB concentration of 1,100 ng/g lipid wt., roughly one-third the lipid-weight concentration of hake and one-half that of herring from the same Basin.

^a We used our subset of young hake for this comparison because they best matched the herring from West et al. (2008) in size and age (Table 7).

PCB and PBDE Patterns

High resolution, ultra-trace (part-per-trillion) quantitation facilitated PCB pattern analysis because it resulted in a greater number of congeners (203 for PCBs, 52 for PBDEs) than is typically identified in monitoring programs.

The PCB pattern in hake wherein the proportion of higher-molecular-weight PCB congeners decreased with distance from Developed Basins is consistent with that reported for harbor seals by Ross et al. (2004), Cullon et al. (2005), and Noël et al. (2011). These results agree with the hypothesis that heavier, more chlorinated congeners tend to move more slowly through the environment than lighter fractions, primarily related to molecular weight or size, particle-affinity and lipophilicity. Lighter congeners are more volatile and likely to move greater distances from their source before becoming entrained in biota or sediments. Such differential movement in the environment (often termed “distillation”) has been demonstrated for PCBs in the atmosphere on a global scale (Simonich and Hites, 1995). Stronger patterns may occur with greater time, and so may be easier to observe in legacy contaminants such as PCBs that have been in the environment for a long time.

Our observation of uniformity in BDE ratios in hake across Basins suggests three possible causes: 1) there are too few PBDE congeners in the environment or quantitated by the method to identify existing patterns, 2) PBDE congeners are more uniformly distributed because they have had less time than PCBs to “distill” in the environment, and 3) the source type of PBDEs is more uniform across the landscape, albeit with more volume in Basins that are adjacent to developed watersheds. In order to more fully evaluate these patterns and develop hypotheses about the proximate sources of PBDEs in biota, a more thorough comparison of PBDE patterns in fish (and other biota) samples is needed, along with a comparison of PBDE patterns in sediments, stormwater, the atmosphere, and other conveyance mechanisms.

PBDE 209

The analytical issues we encountered for PBDE-209 allow only qualified conclusions regarding this congener in hake. Part-per-trillion analysis of this congener clearly creates difficulties in controlling external procedural contamination samples during processing. Other studies have shown that BDE-209 is abundant in Puget Sound sediments -- this congener was the third most abundant PBDE congener detected in surface sediment samples from 210 stations throughout Puget Sound, accounting for 26% of the total PBDE load in surface sediments (Dutch and Weakland 2009). However BDE-209 is not expected to enter and pass through the food chain easily to higher trophic levels for a number of reasons including its strong affinity to particles and large molecule size, which impedes its ability to pass across cell membranes (see review in Ross et al. 2009). We observed five instances where PBDE-209 concentration exceeded three-times the mean PBDE value in corresponding solvent blanks. Each of these fish was a female, and they were all medium-size fish (between 315 and 346 mm FL).

Pesticides

The relative abundance of pesticides in hake followed a pattern similar to that found in harbor seals, i.e., $\sum\text{DDTs} > \sum\text{Chlordanes} > \text{all others}$, and with *p,p'*-DDE the dominant DDT isomer (Noël et al. 2011). The original commercial DDT product comprised approximately 93% *p,p'*- and *o,p'*-DDT, with the four other compounds accounting for the rest. This pattern was

essentially reversed in our fish samples: DDT parent isomers accounted for only an average of 7.8% of Σ DDTs in hake, probably resulting from metabolism and environmental degradation of parent compounds to DDE and DDD metabolites, an observation made by others in a number of systems. This ratio, although low overall, was significantly greater in hake from the four Developed Basins. A possible reason for this is chronic, low-level release of historic, terrestrial DDT sources from eroding watershed soils (e.g., (Johnson, et al., 1988), or urban point sources of new product (Olson et al. 2008).

Summary and Conclusions

The purpose of this study was to investigate the spatial distribution of persistent, bioaccumulative and toxic (PBT) chemicals in pelagic fish in the Puget Sound ecosystem. Motivation for this study was threefold: 1) to evaluate the association between the degree of land development in the Puget Sound Basin as a factor associated with high PBT exposure in pelagic fishes, 2) to compare PBT patterns in pelagic fishes among Puget Sound Basins that have experienced a wide range of watershed development, 3) to evaluate the presence of PBTs in the prey base for harbor seals, a species of interest that is the focus of a companion study; and 4) to provide data for a PBT trophic transfer model used by Ecology to predict ecosystem response to management activities designed to reduce toxics in the ecosystem .

PBT results from this study for hake and pollock sampled across the inland marine and estuarine waters of Washington support several major conclusions:

1. Eleven PBTs or PBT classes were broadly distributed throughout Puget Sound's pelagic fish in at least trace (part-per-trillion) concentrations. Three were rarely detected (methoxychlor, aldrin and endosulfan).
2. Three contaminants or classes (PCBs, PBDEs, and Chlordanes) exhibited greater bioaccumulation with increasing age, which elevates the risk of exposure to trophically higher predators in their food web via biomagnification
3. Differing congener patterns between PCBs and PBDEs suggest a fundamental difference in contaminant pathways and fate and transport in Puget Sound's pelagic food web.
 - PCB patterns in fish were consistent with congeners spreading out from well-defined, urban sources, with individual congeners being transported through the ecosystem at different rates, resulting in a distillation of heavier versus lighter congeners across Basins in the Puget Sound ecosystem
 - PBDE congener patterns were consistent across Basins suggesting similar broad-geographic-scale inputs that load the ecosystem with consistent congener ratios, with differences between Basins evident as total amount (concentration) of PBDEs.
 - The difference in PCB and PBDE patterns between Basins may also be related to:
 - the amount of time these PBTs have been present in the ecosystem – the PCB pattern is the result of approximately 70 years of inputs and distillation within the environment, compared to a few decades for PBDEs

- differential susceptibility to metabolism of the two contaminant types
4. PCB concentration was consistently 25-30% greater than PBDEs
 5. Pesticide exposures in hake seemed to reflect land use patterns: concentrations were greatest in Whidbey Basin and Elliott Bay fish, two Basins that receive surface runoff from watersheds that are either agriculturalized or otherwise have exhibited high pesticide use.
 6. Hake from Developed Basins received a DDT loading pattern that contained a greater proportion of the original DDT product (i.e., was less environmentally weathered) than the pattern observed in Hood Canal fish, suggesting newer sources of DDTs exist in Developed Basins
 7. Other than DDTs, the patterns of individual pesticide constituents within their groupings were remarkably uniform across Basins, suggesting a similar conveyance mechanism and fate/transport of these compounds.
 8. The relative abundance and Basin-distribution of PBTs in Pacific hake was consistent with Pacific herring and Puget Sound-resident Chinook salmon from other studies.
 9. The potential for trophic transfer of PBTs to harbor seals and other apex predators appeared to be greatest from Pacific herring, followed by Pacific hake, and Chinook salmon. The wide variability in lipid content of these prey needs to be considered when modeling such potential.

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