Control of Toxic Chemicals in Puget Sound Phase 3:

Persistent Organic Pollutants in Marine Plankton from Puget Sound

Washington Department of FISH and WILDLIFE

Persistent Organic Pollutants in Marine Plankton from Puget Sound

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Glossary of specialized terms

Bioaccumulation: The buildup of contaminants in an organism's tissues (usually fatty tissue) via ingestion of prey.
Bioconcentration: The increase in contaminants in organisms relative to their aqueous environment
Biomagnification: The increase in contamination levels in predators relative to their prey.
Holoplankton: Organisms which are planktonic for their entire life cycle, such as krill or copepods.
Macrozooplankton: Animals in the water which drift with the currents and are large enough to be visible, usually between 2 to 20 mm in length.
Meroplankton: organisms which are planktonic for only a part of their life cycles, usually the larval stage, such as crab megalopae.
Microzooplankton: Animals in the water which drift with the currents and range in size from 20 to 200 microns (μm).
Nanoplankton: plankton <20 μm (and larger than 2 μm) in size
Plankton: Passively floating animal and plant life in the water that drifts with the currents.
Persistent Organic Pollutant: Organic compounds resistant to degradation that persist in the environment, are capable of long-range transport, and often bioaccumulate in living tissue.
Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.
Particulate Organic Matter: Material of plant or animal origin that is suspended in water.
Toxicant: A toxic agent (chemical compound or mixture) that presents a risk of death, disease, injury, or birth defects in organisms that ingest or absorb it. Toxicants are typically introduced into the environment by human activity.

Acronyms, Abbreviations and Units

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

$\delta^{15}N$	"delta" N (nitrogen), or the ratio of the isotopes ^{15}N to ^{14}N
$\delta^{13}C$	"delta" C (carbon) or the ratio of the isotopes ^{13}C to ^{12}C
C	carbon
DDT	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
FL	fork length
GC/MS	gas chromatography/mass spectrometry
GPS	global positioning system
HPLC	high performance liquid chromatography
HRGC/MS	high resolution gas chromatography/mass spectrometry
IOS	Institute of Ocean Sciences, Sidney, British Columba
N	nitrogen
NOAA	National Oceanic & Atmospheric Administration
OCP	Organo-chlorinated pesticides
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PBT	persistent, bioaccumulative, and toxic chemical
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
POM	particulate organic matter
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

cm	centimeter
m	meter
ft	feet
gm	gram
km	kilometer
kHz	kilohertz
mL	milliliters
mm	millimeters
ng/g	nanograms per gram (parts per billion)
oz	ounces
°C	degrees centigrade
µm	micrometer
$^{0}/_{00}$	permille (parts per thousand)

Abstract

This project was designed to evaluate the extent and magnitude of Persistent Organic Pollutant (POP) exposure in organisms that occupy the lowest trophic levels in the pelagic ecosystem of Puget Sound, and to gain a better understanding of the pathways of contaminants within this food web. To this end zooplanktonic krill, *Euphausia pacifica* and *Thysanoessa spp*, an important food source for pelagic fish in the Puget Sound, and phytoplankton, primary producers at the base of the pelagic food web, were sampled and analyzed for toxic contaminants. Non-migratory pelagic fish species that feed primarily on krill, including Pacific hake (*Merluccius productus*) and walleye pollock (*Theragra chalcogramma*), as well as their predators, harbor seals (*Phoca vitulina*) were assessed in two companion studies. We measured the concentration of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs) in phytoplankton and krill from a broad range of locations representing as wide a range of putative contaminant loadings possible. Because of technical difficulties in isolating phytoplankton from other particulate organic matter, we filtered seawater through 20-μm mesh to select particle sizes that would maximize retention of phytoplankton, but allow smaller particles to pass. The resulting size-selected organic matter was termed Particulate Organic Matter (POM) for this study. POPs, including PCBs, PBDEs, DDTs, and PAHs in both POM and krill exhibited a correlation with urban waters, suggesting urban waters represent areas where POPs enter the pelagic food chain. The Basin-pattern of PBDE accumulation in krill was similar to PCBs; high concentrations in urbanized waters and low concentrations in less developed, more ocean-influenced basins – suggesting a similar mechanism of loading and dispersal in Puget Sound. Overall, PAHs were detected more often and in greater concentration than all other POPs in this study. The greatest concentrations of most POPs were observed in Elliott Bay, one of the two urbanized Basins in this study. This implicates urban waters as an important point of entry for POPs into the pelagic food web. OCPs were observed in low concentration in many krill samples, but were below the limit of detection for most POM samples. Dieldrin was higher in *E. pacifica* from the Whidbey Basin than other Basins. PAHs in some POM appeared to be related to small-scale (sub-basin) shoreline locations relative to nearby land use or activities. Aside from Elliott Bay the next greatest PAH concentrations in POM were observed near to shore, and near to obvious PAH sources e.g., marinas and ferry terminals, even in otherwise relatively undeveloped Basins.

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Introduction

Over the past 20 years researchers from the Washington Department of Fish and Wildlife's (WDFW) Puget Sound Assessment and Monitoring Program (PSAMP) have monitored and assessed a wide range of bioaccumulative and other Persistent Organic Pollutants (POPs) in a number of species representing important ecological guilds in Puget Sound. These efforts have provided a picture of the geographic extent of ecosystem contamination by POPs, the magnitude of contamination, and temporal trends in these patterns. In addition, monitoring and assessment studies have raised questions regarding the pathways by which POPs from terrestrial sources find their way into the Puget Sound food web, and why Puget Sound's pelagic food web exhibits an unusually high exposure to some POPs (West et al. 2008, O'Neill and West 2009). Ross et al. (2004) and Cullon et al. (2005) identified Puget Sound as a regional source of POP contamination in harbor seals, while Ross et al. (2000) and Ross (2006) identified Puget Sound as a regional source of POP contamination in Southern Resident Killer Whales. Moreover, fish in the pelagic food have been identified as the primary source of POPs to these apex predators (Cullon et al. 2005, O'Neill et al. 2006, Krahn et al. 2007, Cullon et al. 2009).

Long-term PSAMP studies support the hypothesis that benthic (bottom-dwelling) species reflect contaminant conditions in sediments. However, assessments of pelagic (open water) species such as Pacific herring (*Clupea pallasi*) suggest the pelagic food web is more directly linked to POPs in Puget Sound's water and pelagic biota rather than sediments. Pacific herring hold unusually high tissue burdens of bioaccumulative POPs (*e.g.*, polychlorinated biphenyls, or PCBs), an observation that is not typically predicted from sediment-as-source food web models. In addition, other research indicates that PCBs and polybrominated diphenyl ethers (PBDEs) have biomagnified in Puget Sound's harbor seals (*Phoca vitulina*) and killer whales (*Orcinus orca*) to levels that have impaired their health (Ross et al. 2000, Ross et al. 2004, Hickie et al. 2007).

Ecology's Phase 2 toxics loading and modeling studies reported surface water runoff and aerial deposition represent the primary conveyance mechanisms for PCBs, PBDEs, organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbon congeners (PAHs) from terrestrial sources into Puget Sound (EnviroVision Corporation et al. 2008). These toxicants represent four important POP classes to which Puget Sound biota are exposed in high enough doses to potentially impair their health. Several of these POPs bioaccumulate through the pelagic food web to predators such as salmon, harbor seals, killer whales, seabirds, and humans. However, the pathways of contaminant flow from their abiotic sources to these predators 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 these biota, we seek to evaluate where (by Basin, defined below) POPs enter the pelagic food web from stormwater and the atmosphere, the pathways of toxic contaminants within the pelagic

food web, and the sources of POPs to species at the highest trophic levels (marine mammals, seabirds, and humans).

Phytoplankton have been identified as an important entry point of POPs to food webs, wherein contaminant molecules are adsorbed or absorbed by water-column biota. Larsson et al. (2000) reviewed the role of phytoplankton in contaminant cycling in the Baltic Sea, and a number of studies have identified such roles in high-latitude marine waters (Chiuchiolo et al. 2004, Fisk et al. 2001, Hoekstra et al. 2003). Hudson et al. (2005) documented the importance of pelagic microbes on the uptake and trophic transfer of POPs in the pelagic food web of Lake Superior. The primary objectives of this study were to (a) evaluate the feasibility of sampling targeted plankton species or guilds for POP analysis, (b) measure POP concentrations in phytoplankton and at least one guild or group of herbivorous zooplankton, (c) supply tissue residue data for Ecology's contaminant modeling efforts and (d) compare POP concentration in phytoplankton and zooplankton across Puget Sound Basins. Results from this study will also be combined with two companion studies on Pacific hake (*Merluccius productus*) (West et al. 2011) and harbor seals (Noël et al. 2011) and with other PSAMP studies to evaluate contaminant transfer across the full range of the pelagic food web.

Methods

Two plankton guilds were targeted in the present study: 1) phytoplankton, representing seasonally abundant, potentially lipid-rich pelagic primary producers to which POPs may sorb directly, and 2) krill species which graze on phytoplankton and serve as the dominant prey for many pelagic fish species. Size selective (20-µm) netting was used to obtain phytoplankton samples. However the nets also retained some micro-heterotrophs (e.g., copepods) as well as inorganic and organic particles that could not be sorted out. Hence, although dominated in mass by phytoplankton, we refer hereafter to these samples as Particulate Organic Matter (POM). *Euphausia pacifica* constituted the majority of krill species, however two other krill species (*Thysanoessa spinifera* and *T. raschii*) were also encountered.

Krill and POM samples were sampled across a wide geographic range of Puget Sound (Figure 1) with a focus on representing the major compartments of the Puget Sound Box Model (Pelletier and Mohamedali 2009). Using Puget Sound basin nomenclature from the 14 study areas defined in the Toxics Loadings surface runoff reports (EnviroVision Corporation et al. 2008) we sampled South Puget Sound, Main Basin, Hood Canal (combined Mid- and North Hood Canal), Admiralty Inlet, Whidbey Basin, Strait of Juan de Fuca, San Juan Archipelago, and the Strait of Georgia. In addition we targeted two urban bays in the Main Basin (Elliott Bay and Commencement Bay). Hereafter these water bodies are all referred to as Basins. Analytes evaluated for all samples include PCBs, PBDEs, PAHs, chlorinated pesticides, percent lipids and percent moisture, and stable isotopes of carbon (C) and nitrogen (N).

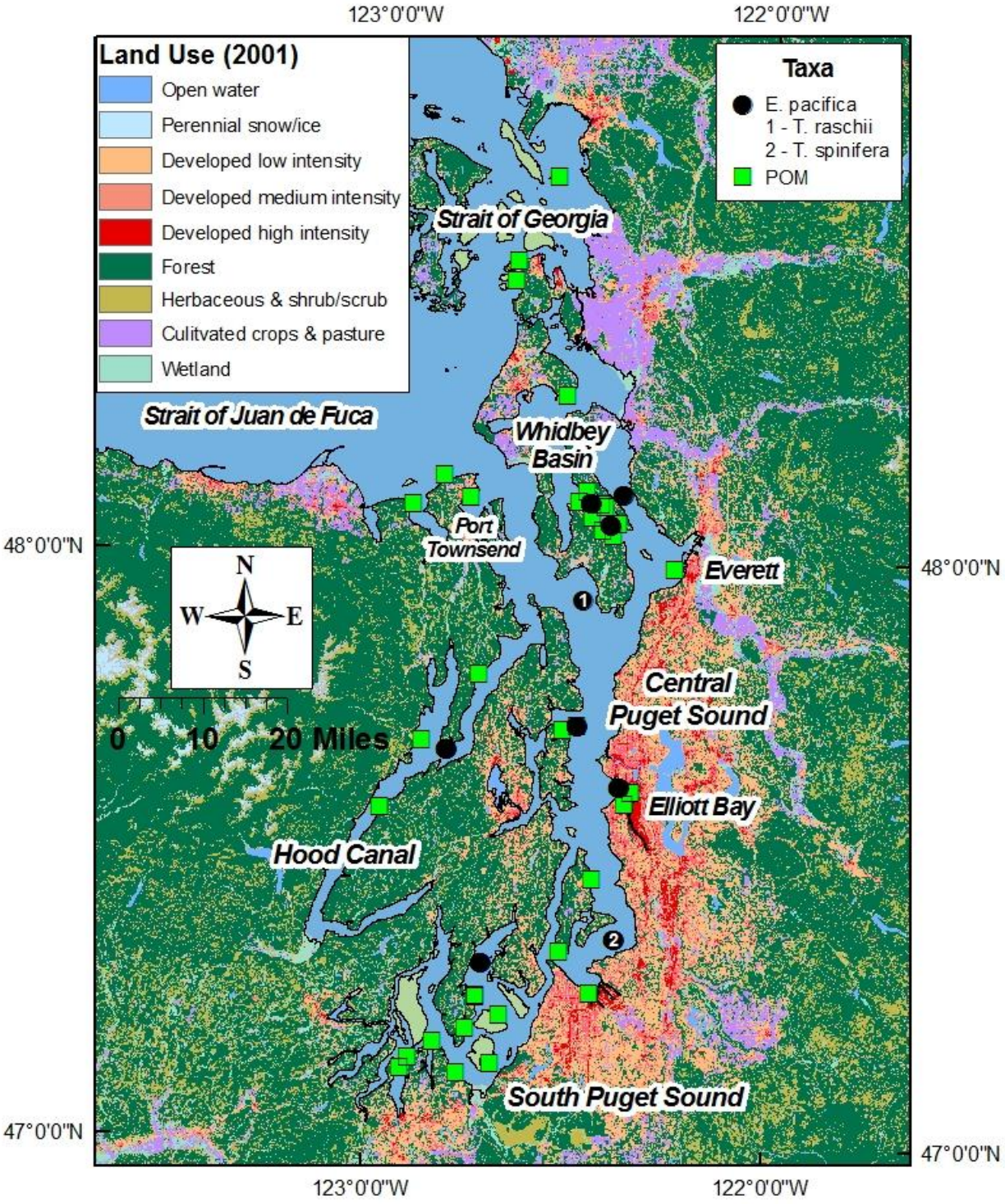

Figure 1. Sampling locations for krill taxa and phytoplankton/particulate organic matter (POM) within eight major oceanographic basins or embayments in Washington State inland waters. Land use data provided by the NOAA, Coastal Services Center.

Study Design and Sample Collection

Twenty-three krill and 35 POM samples were taken altogether, with POM representing all eight Basins; krill were not found in sufficient abundance to collect samples from the San Juan Islands and Strait of Juan de Fuca Basins. Most samples were taken in the summer months of 2009, with a few exceptions (Table 1). All POM were taken from depths shallower than 25 m to the surface and krill were targeted at depths ranging from 53 to 200 m.

Krill

Krill were collected using a modified Kvichak net measuring approximately 3m x 3m at its mouth (Figure 2). The net mesh size was widest at the mouth (10 mm) and became progressively smaller towards the cod end, ending with ~3-mm size at the zippered cod end. The net was suspended between two horizontal beams that opened during deployment by means of floats on the upper beam and 260 lbs of weight on the bottom beam. Sampling consisted of depth-targeted horizontal and oblique tows from a 30-ft research vessel, using pre-marked Kevlar towing line wound on a hydraulic winch.

Figure 2. Modified Kvichak net used to capture krill.

Table 1. Collection information for 58 composite samples of krill (3 species) and Particulate Organic Matter (POM). "nr" indicates data were not recorded.

Basin	Sample Site	Date	SampleID	Species	Latitude	Longitude	Target Depth (m)	Net Max Depth (m)	Bottom Depth (m)
Elliott Bay	Elliott Bay	08/11/2009	09EB-KRW01	*E. pacifica*	47.61384	-122.37329	53-61	67	107
Elliott Bay	Elliott Bay	08/11/2009	09EB-KRW02	*E. pacifica*	47.61384	-122.37329	53-61	67	107
Elliott Bay	Elliott Bay	08/11/2009	09EB-KRW03	*E. pacifica*	47.61384	-122.37329	53-61	67	107
Hood CaNRl	Mid Hood CaNRl	08/05/2009	09HCM-KRW01	*E. pacifica*	47.67442	-122.81394	61-91	108	130
Hood CaNRl	Mid Hood CaNRl	08/05/2009	09HCM-KRW02	*E. pacifica*	47.67442	-122.81394	61-91	108	130
Hood CaNRl	Mid Hood CaNRl	08/05/2009	09HCM-KRW03	*E. pacifica*	47.67442	-122.81394	61-91	108	130
Main Basin	Port Madison	07/22/2009	09PM-KRW01	*E. pacifica*	47.71762	-122.48494	61-91	97	164
Main Basin	Port Madison	07/22/2009	09PM-KRW02	*E. pacifica*	47.71762	-122.48494	61-91	97	164
Main Basin	Port Madison	07/22/2009	09PM-KRW03	*E. pacifica*	47.71762	-122.48494	61-91	97	164
South Puget Sound	Carr Inlet	07/21/2009	09CR-KRW02	*E. pacifica*	47.30116	-122.71477	nr	62	80
South Puget Sound	Carr Inlet	07/21/2009	09CR-KRW03	*E. pacifica*	47.30116	-122.71477	nr	62	80
South Puget Sound	Carr Inlet	07/21/2009	09CR-KRW04	*E. pacifica*	47.30116	-122.71477	nr	62	80
Whidbey Basin	Langley	08/19/2009	09LY-KRW01	*E. pacifica*	48.06140	-122.40846	nr	34	128
Whidbey Basin	Langley	08/19/2009	09LY-KRW02	*E. pacifica*	48.06140	-122.40846	nr	34	128
Whidbey Basin	Langley	08/19/2009	09LY-KRW03	*E. pacifica*	48.06140	-122.40846	nr	34	128
Whidbey Basin	East Point	08/19/2009	09EP-KRW01	*E. pacifica*	48.09807	-122.46296	150-200	69	137
Whidbey Basin	East Point	08/19/2009	09EP-KRW02	*E. pacifica*	48.09807	-122.46296	150-200	69	137
Whidbey Basin	East Point	08/19/2009	09EP-KRW03	*E. pacifica*	48.09807	-122.46296	150-200	69	137
Whidbey Basin	Port Susan	07/01/2009	09PS-KRW01	*E. pacifica*	48.11240	-122.37720	80	79	119
Main Basin	Useless Bay	03/06/2008	08UB-KRW01	*T. raschii*	47.93167	-122.47667	15	15	nr
Main Basin	Vendovi Island	09/26/2009	09VI-KRW01	*T. spinifera*	47.35442	-122.37945	nr	37	51
Main Basin	Vendovi Island	09/26/2009	09VI-KRW02	*T. spinifera*	47.35442	-122.37945	nr	37	51
Main Basin	Vendovi Island	09/26/2009	09VI-KRW03	*T. spinifera*	47.35442	-122.37945	nr	37	51
Str. Juan de Fuca	Port Townsend	08/06/2009	09PT-PPW01	POM	48.10469	-122.76888	nr	nr	55
Commencement Bay	City Waterway	09/22/2009	09CB-PPW01	POM	47.26288	-122.43738	nr	15	18
Elliott Bay	Seattle Waterfront	08/11/2009	09EB-PPW01	POM	47.58580	-122.36087	nr	15	18
Elliott Bay	West Waterway	10/19/2009	09EB-PPW02	POM	47.60488	-122.34522	nr	25	24
Hood CaNRl	Anderson Cove	09/21/2009	09AR-PPW01	POM	47.57183	-122.97812	nr	15	16

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Basin	Sample Site	Date	SampleID	Species	Latitude	Longitude	Target Depth (m)	Net Max Depth (m)	Bottom Depth (m)
Hood CaNRl	Dosewallips	09/21/2009	09DW-PPW01	POM	47.68900	-122.87803	nr	12	12
Hood CaNRl	Thorndyke Bay	09/21/2009	09TB-PPW01	POM	47.80225	-122.73605	nr	15	17
Main Basin	Point Dalco	09/22/2009	09DO-PPW01	POM	47.33300	-122.51600	nr	15	15
Main Basin	Point Beals	09/22/2009	09BL-PPW01	POM	47.45758	-122.43785	nr	15	16
Main Basin	Pt. Monroe	09/22/2009	09PMN-PPW01	POM	47.71033	-122.52080	nr	15	20
Main Basin	Port Madison	07/22/2009	09PM-PPW01	POM	nr	nr	nr	nr	nr
San Juan Islands	Burrows Bay	08/19/2009	09AB-PPW01	POM	48.47430	-122.66599	nr	20	25
San Juan Islands	Guemes Channel	08/19/2009	09SH-PPW01	POM	48.50920	-122.66136	nr	10	9
San Juan Islands	Eliza Island	08/18/2009	09EI-PPW01	POM	48.65229	-122.56337	nr	20	40
South Puget Sound	Tolmie State Park	08/14/2009	09TL-PPW01	POM	47.12213	-122.76788	nr	15	15
South Puget Sound	Budd Inlet	07/28/2009	09BI-PPW01	POM	47.12882	-122.90828	nr	20	90
South Puget Sound	Cole Pt.	09/18/2009	09CE-PPW01	POM	47.13823	-122.68285	nr	15	17
South Puget Sound	Dover point	09/18/2009	09DV-PPW01	POM	47.14722	-122.88965	nr	17	18
South Puget Sound	Johnson Point	09/18/2009	09JP-PPW01	POM	47.17405	-122.82732	nr	15	14
South Puget Sound	Filucy Bay	08/14/2009	09FL-PPW01	POM	47.19918	-122.74665	nr	8	8
South Puget Sound	Gertrude Island	09/18/2009	09GI-PPW01	POM	47.22187	-122.66155	nr	20	22
South Puget Sound	Carr Inlet	08/14/2009	09SD-PPW01	POM	47.25145	-122.72453	nr	8	8
South Puget Sound	Carr Inlet	09/18/2009	09SD-PPW02	POM	47.25542	-122.72390	nr	15	16
Str. Juan de Fuca	Diamond Pt	08/06/2009	09JF-PPW02	POM	48.08975	-122.91362	nr	25	218
Str. Juan de Fuca	McCurdy Point	08/06/2009	09JF-PPW01	POM	48.14008	-122.83575	nr	15	49
Whidbey Basin	Port Gardner	09/24/2009	09PG-PPW01	POM	47.98664	-122.24384	nr	12	23
Whidbey Basin	Langley	08/19/2009	09LY-PPW01	POM	48.04237	-122.40348	nr	25	45
Whidbey Basin	North of Langley	09/24/2009	09LYN-PPW01	POM	48.05155	-122.42920	nr	15	20
Whidbey Basin	Pebble Beach	09/24/2009	09PBL-PPW01	POM	48.06270	-122.39035	nr	15	99
Whidbey Basin	Saratoga City	09/24/2009	09SAR-PPW01	POM	48.07380	-122.45828	nr	15	23
Whidbey Basin	MabaNR	09/24/2009	09MB-PPW02	POM	48.09146	-122.42503	nr	15	82
Whidbey Basin	MabaNR	08/19/2009	09MB-PPW01	POM	48.09739	-122.43560	nr	25	55
Whidbey Basin	East Point	09/24/2009	09EP-PPW01	POM	48.10023	-122.49295	nr	15	19

Table 1. Collection information for 58 composite samples of krill (3 species) and Particulate Organic Matter (POM). "nr" indicates data were not recorded.

Basin	Sample Site	Date	SampleID	Species	Latitude	Longitude	Target Depth (m)	Net Max Depth (m)	Bottom Depth (m)
Whidbey Basin	Elger Bay	09/24/2009	09ELG-PPW01	POM	48.11722	-122.47295	nr	15	94
Whidbey Basin	Polnell Point	08/19/2009	09PN-PPW01	POM	48.27933	-122.52855	nr	20	22

A sonar depth sounder (Furuno or SIMRAD) split-set at 50 and 200 kHz was used to locate diffuse sound-scattering layers (which often indicate the presence of zooplankton). Once such a layer was identified on the depth sounder, a target sampling depth was determined and length of trawl line calculated. A ratio of approximately 2:1 (line:net depth) was used to reach the target depth. The net was fished at approximately 2 knots (boat speed) for 5 to 30 minutes, depending on the strength of the signal observed. A ReefNet Inc. Sensus Ultra™ dive data logging device, attached to the Kvichak's upper net beam, was used to record the actual depth sampled. Recorded depth information was downloaded and viewed immediately after each tow to verify sampling depth and make any needed corrections for subsequent tows.

Once on deck, the contents of the cod end were released into a pre-cleaned sorting basin. The collected organisms were held in seawater obtained on site and immediately size-sorted, using pre-cleaned stainless steel sieves (>3-mm mesh) to remove debris and large, unwanted organisms. Once larger, unwanted organisms (e.g., fishes, algae, and jellies) and debris were removed, the remaining krill and other smaller taxa were concentrated by passing the sample through pre-cleaned, stainless steel sieves varying in mesh size from 500µm to 3,000 µm. This filtrate was then transported to a clean working table where the krill taxa were manually isolated individually using pre-cleaned forceps and/or stainless steel spatulas and placed into 2-oz, pre-cleaned I-Chem™ brand sample jars. Multiple sample jars were collected per sample site. Sample jars were labeled, placed on ice immediately, and frozen to at least -20 °C within 72 hours of collection. Such composite samples remained frozen until analyzed in the laboratory.

A random subsample of krill from each sample was preserved in a 5% buffered formalin solution, as a voucher for species verification and for estimating the size class frequency of each sample. All sampling gear was washed in the lab using soap and fresh water between sampling efforts and stored in covered containers.

Particulate Organic Matter (POM)

We targeted phytoplankton using conical phytoplankton lift-nets designed specifically to retain phytoplankton with as little damage to the cells as possible (Figure 3). The two phytoplankton nets used in this study measured: a) 25-cm mouth diameter by 60 cm length, and b) 30-cm mouth diameter by 100 cm length. Both nets were attached to a stainless steel ring, had 20-µm square Nitex mesh, and were equipped with closed-cod-end jars. Each net ring was attached to a 3-point bridle secured to a 30-m nylon line.

Phytoplankton were sampled from surface waters to a depth of 25 m with vertical net lifts. All phytoplankton sampling was conducted between the months of July and September except one sample which was obtained in mid-October (Table 1). Lifts were made from a drifting boat to

minimize the effects of currents on net performance. The boat engine was switched off prior to sampling to avoid contamination of gear and samples by exhaust fumes.

Because of its small mesh size (20-µm) a net-mouth "bow wave" (water pushed upward in advance of the net mouth opening) was produced at the top of the sampling net as it was towed upwards through the water column. This bow wave was observed as a flurry of turbulent water that thrust aside any surface-layer particles just in advance of the net mouth breaching the surface. We took advantage of this to reduce the likelihood of introducing contaminants that may have been associated with the sea surface micro-layer. Although it occurred in all tows, the bow wave was especially pronounced when the net appeared more clogged with POM, i.e. when phytoplankton density was high.

Figure 3. Conical plankton net and sieve (20 µm mesh pore size each) used to sample particulate organic matter (POM) and concentrate it into a paste.

Once on deck, the phytoplankton net was suspended until most of the water had drained and all visible POM was concentrated into the cod end. The cod end was then detached and the slurry gently poured into a 20-µm mesh sieve to further drain and concentrate the sample. Any large, unwanted organisms (e.g., macro-algal debris, comb jellies, and zooplankton) were removed using pre-clean tweezers and/or stainless steel spatulas. The resulting green "paste" of POM was then gently scooped out of the 20-µm sieve and placed into a 2-oz, pre-cleaned I-Chem™ brand sample jar using a pre-cleaned stainless steel spatula. Multiple lifts and filtering (up to 25 times on occasion) were needed to obtain sufficient volume of POM for a single sample jar. POM samples were labeled, immediately placed on ice, and frozen to at least -20 °C within 72 hours of collection. Composites remained frozen until analyzed in the laboratory.

A voucher sample for phytoplankton identification was collected at each site by gently pouring some of the concentrated POM from the cod-end jar into either a 125ml glass jar or a 20 mL scintillation vial. Buffered formalin solution was added to make a ~0.8 - 1% formaldehyde solution for preservation.

Krill Species Identification and Body Length Measurement

We estimated the size frequency distribution of krill from each sample by measuring the total length of all preserved krill individuals in related voucher subsamples. Total body length was measured as a proxy for age, which is an estimate of total possible contaminant exposure time. The mean size of krill from each subsample was applied to and included as a covariate for each sample, and mean POP concentration adjusted as appropriate in analysis of covariance (described later).

Krill were identified to species and measured for total body length by WDFW staff, Olympia (Figure 4). Total body length (mm) was measured from the beginning of the carapace (starting between the eyes, but not including the rostrum) to the end of telson, including spines. Images of krill bodies were captured using a stereo-viewing microscope, using 63x magnification, mounted with a Leica DFC295 digital camera. The resulting images were imported into the Image-Pro 6.0 software package where the line measurement tool, adjusted with a calibration slide, was used to measure lengths. Due to the curved nature of preserved specimens, each krill was measured in five contiguous sections (1 for the carapace; 4 for the curve of the abdominal segments; 1 for the telson and spines). The five sections were then added together to estimate total body length (mm).

POM Species Identification and Semi-quantitative Measurement

Identification and semi-quantitative assessment of the dominant phytoplankton taxa in POM samples was performed under contract by staff at the King County Environmental Laboratory (KCEL), Seattle. An aliquot of each formalin preserved voucher sample was placed on a Palmer-Maloney-type counting chamber (0.059 ml or 0.066 ml) and covered with a cover slip. Observations were made on a Nikon 80i microscope, with DIC and phase contrast at 100x – 600x magnification. Images were captured with an attached digital camera.

Each slide was examined in its entirety and all phytoplankton were identified to the lowest practical taxon. A semi-quantitative measure of relative abundance was determined by examining nine fields along two perpendicular axes at 100x and noting the dominant taxon, on a cell number basis, in each field. Taxon dominance was generally recorded at the genus level unless, a) only one species is known to occur in Puget Sound, or b) one species was conspicuously dominant. The nine fields were then tallied and taxon dominance assigned as *dominant* (taxon dominant in > 50% of fields), *subdominant* (taxon dominant in > 25% of fields), or *present* (all other taxa). We note that this protocol tended to favor species with smaller cells, and that species that appeared abundant, but not dominant, often did not meet criteria to be

Figure 4. Example of measurements made on a single krill.

called subdominant. Additionally, we assumed nanoplankton were not retained by our sampling net; individuals identified here were typically larger than 20-μ diameter.

Similarly, a subjective measure of relative abundance based on observation was also used to note species/genera that appeared *abundant* or *very abundant*. This evaluation was based on a combination of cell number and cell volume and its purpose was to note species abundances that may not have been captured with the semi-quantitative method described above, as may be the case with blooms of species with "medium-large" cells.

Identification and development of sample analysis protocols were aided with the use of the Horner (2002 and Tomas (1997) taxonomic and identification guides for phytoplankton.

Laboratory Analysis

All chemical analyses for krill and phytoplankton were conducted at NOAA's Northwest Fisheries Science Center in Seattle, Washington. In this study we termed all contaminants POPs, rather than Persistent Bioaccumulative Toxics (PBTs) because PAH compounds tend not to accumulate in many organisms. The POPs we measured in krill and POM comprised three major halogenated groups: (a) polychlorinated biphenyls (46 congeners of PCB), (b) polybrominated diphenyl ethers (15 congeners of PBDEs), and (c) 23 chlorinated pesticides (Table 2). We also measured 38 polycyclic aromatic hydrocarbons (PAHs, or simply aromatic hydrocarbons) and PAH alkylated homologs. In addition to contaminant analyses we measured stable isotopes of carbon and nitrogen, total extractible lipids, and the percentage of water in each sample. Brief descriptions for each analysis method follow.

Chlorinated and Aromatic Hydrocarbons

Composite krill and POM samples were homogenized using a hand-held electric mixer, and then extracted and analyzed for POPs using accelerated solvent extraction and gas chromatography/mass spectrometry according to Sloan et al. (2004) and Sloan et al. (2005). In brief, this method involves: (1) extraction of tissue using methylene chloride in an accelerated solvent extraction procedure, (2) clean-up of the methylene chloride extract on a single stacked silica gel/alumina column, (3) separation of chlorinated hydrocarbons and aromatic hydrocarbons from the bulk lipid and other biogenic material by high-performance size exclusion liquid chromatography, and (4) analysis on a low resolution quadrupole GC/MS system equipped with a 60-meter DB-5 GC capillary column. The instrument was calibrated using sets of up to ten multi-level calibration standards of known concentrations.

Five modifications to this procedure were employed to achieve greater sensitivity (lower detection limits) and to ensure adequate analysis for plankton samples that had low mass. 1) a small aliquot (250 µL) of extract was used for gravimetric lipid analyses instead of using approximately one third of the extract (~20 mL). 2) The total remaining extract after silica/alumina cleanup proceeded to HPLC cleanup instead of applying half of the extract to HPLC cleanup and holding half of the extract in reserve. 3) The final volume of concentrated, cleaned up extract for GC/MS analyses was reduced to 50 µL instead of 100 µL. 4) The samples were quantified using additional, lower concentration GC/MS calibration standards for chlorinated hydrocarbons and PBDEs. 5) The amount of internal standards added to the samples was reduced to 20 µL instead of 75 µL to be appropriate for the more concentrated sample analyzed by GC/MS.

Total extractible lipids was measured gravimetrically using a separate 250 µL aliquot of extract This 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.

Table 2. Analytes measured for composites of krill and POM samples.

Parameter	No. of composites	Congeners/Types	Units	Dates Analyzed
PCB congeners	60	40	ng/g	March 2010
PBDE congeners	60	15	ng/g	March 2010
Organochlorine Pesticides	60	23	ng/g	March 2010
PAHs		2	ng/g	
Total Lipids	60	-	%	March 2010
Total Solids	60	-	%	March 2010
δ^{15}Nitrogen (ppt)	60	-	‰	March 2010
δ^{13}Carbon (ppt)	60	-	‰	March 2010

All contaminant concentrations were reported in ng/g (parts per billion), wet weight. Analytes reported were as follows:

$\sum_{46}$PCBs is the sum of detected values of congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209.

$\sum_{10}$PBDEs is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, and 183.

$\sum_{6}$DDTs is the sum of detected values of *o,p*'-DDD, *p,p'*-DDD, *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDT and *p,p*'-DDT.

$\sum_{8}$Chlordanes is the sum of detected values of oxychlordane, *gamma*-chlordane, nona-III-chlordane, *alpha*-chlordane, *trans*-nonachlor and *cis*-nonachlor, heptachlor, and heptachlor epoxide

$\sum_{3}$HCHs (hexachlorocyclohexanes) is the sum of detected values of *alpha*-, *beta*-, and *gamma*-HCH isomers.

∑LMWPAH (low molecular weight PAHs) is the sum of detected values of acenaphthylene, acenaphthene, fluorene, C_1- through C_3-fluorenes, dibenzothiophene, C_1-through C_4-dibenzothiophenes, phenanthrene, anthracene C_1- through C_4-phenanthrene/anthracenes, and retene.

∑HMWPAH (high molecular weight PAHs) is the sum of detected values of fluoranthene, pyrene, C_1-through C_4-fluoranthene/pyrenes, benz[*a*]anthracene, chrysene/triphenylene, C_1-through C_4-chrysenes, benzo[*b*]fluoranthene, benzo[*j*]fluoranthenes/benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno-pyrene, dibenzoanthracene, and benzo[z]pyrene.

∑PAHs is the sum of LMWPAHs and HMWPAHs.

As part of performance-based laboratory quality assurance (Sloan, et al. 2004), quality control samples [a method blank, replicate and Standard Reference Materials (SRMs, e.g., NIST 1974b and 1947)] were analyzed with each sample set. Results obtained for SRMs 1974b and 1947 were in excellent agreement with certified and reference values published for these materials by the National Institute of Standards and Technology. In addition, the other quality control samples met established laboratory criteria.

Stable Isotopes Analysis

We measured stable isotopes of carbon and nitrogen to calculate the isotopic ratios of ^{13}C to ^{12}C as $\delta^{13}C$, and of ^{15}N to ^{14}N, as $\delta\ ^{15}N$, relative to standardized isotopic ratios. We used $\delta^{15}N$ as an estimator of trophic level, *sensu* Hobson (1999) and Post et al. (2007). We used $\delta^{13}C$ in plankton tissues as an independent estimator of the continuum of conditions from estuarine (Puget Sound) to oceanic conditions, with $\delta^{13}C$ increasing from oceanic to estuarine conditions (Hobson 1999, West et al. 2011).

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:

$$\delta Z = [(R_{sample}/R_{standard})\text{-}1]x1000,$$

where Z represents ^{15}N or ^{13}C, R_{sample} is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ for samples, and $R_{standard}$ is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ 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 $\delta^{15}N$ and $\delta^{13}C$ values, respectively. Precision for isotope analysis was $\leq \pm 0.3$‰ for $\delta^{15}N$ and $\leq \pm 0.2$‰ for $\delta^{13}C$. All nitrogen values were referenced to atmospheric nitrogen ($\delta^{15}N$ for atmospheric N is 0‰ exactly) and carbon values were referenced to Vienna Pee Dee Belemnite, also known as NBS 19 [($\delta^{13}C$ of NBS 19 ≡ 1.95‰ (Coplen, et al., 2006).

$\delta^{13}C$ 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:

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

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

Data Analysis

Summary statistics for analytes presented in tables were calculated as arithmetic means, medians, and 10^{th} and 90^{th} percentiles. All data and analyses were conducted on wet weight POP concentrations.

Comparison of tissue POP concentration between basins was performed with parametric analysis of variance using a General Linear Model (GLM, Systat 2007) on natural log-transformed analyte concentration, with Basin as the classification variable. Percent lipids and $\delta^{15}N$ were included as covariates. In no case was either covariate a significant contributor to explaining variability of POP in any model containing any combination of covariates at the α=0.05 level, and so neither %Lipids nor $\delta^{15}N$ included in final comparisons. Geometric mean POP concentrations and 95^{th}% confidence intervals were back-calculated from least squares means generated by the GLM, and plotted in Figures. Tukey's Honestly Significant Difference (THSD) test was used *post hoc* for pairwise Basin comparisons of least squares means generated by the GLMs.

Results

Sample Composition and Morphometry

Particulate Organic Matter

Relative counts of phytoplankton taxa indicated that centric diatoms or dinoflagellates were either dominant or subdominant in all POM samples (Table 3). Pennate diatom and silicoflagellate phytoplankton were also present in most samples, and diversity ranged from a low of 21 (Main Basin, Outer Commencement Bay) to a high of 216 (Whidbey Basin) identified phytoplankton taxa.

Although we targeted phytoplankton blooms in this sampling, it was difficult to collect enough mass of POM for a sample. We typically needed to make 5 to 20 net-lifts to concentrate enough POM for analysis. POM scooped from the net mesh into the jar had the consistency of pudding, with a brown to green color (Figure 3). We avoided centrifuging or vacuum-filtering samples to minimize the risk of cells rupturing, however as a result we probably retained more extracellular water. %Moisture in POM samples ranged from 92 to 96% (Table 4).

Although the presence of small zooplankton (e.g., copepod nauplii, ciliates and rotifers) was noted in some samples, their numbers were low compared to phytoplankton. This is probably

Table 3. Relative abundance of phytoplankton taxa from eight Puget Sound Basins. Taxa were termed "Dominant" if cell counts were >50% of nine fields examined (100x) and as "Subdominant" if cell counts dominated >25% of nine fields. "Dia"= centric diatom.

Basin/Bay	Location	Collection Date in 2009	Dominant Taxon	Subdominant Taxa
Strait of Georgia/San Juan Islands	Eliza Island	18-Aug	*Skeletonema costatum* (Dia)	
	Alexander Beach	19-Aug	*Skeletonema costatum* (Dia)	
	Ship Harbor	19-Aug	*Skeletonema costatum* (Dia)	
Strait of Juan de Fuca	Discovery Bay	6-Aug		*Ceratium fusus* (Dino)
	McCurdy Point	6-Aug	*Thalassiosira rotula* (Dia)	
	Port Townsend	6-Aug	*Thalassiosira rotula* (Dia)	
Whidbey Basin	Langley	19-Aug	*Thalassiosira* sp. (Dia)	
	Mabana	19-Aug	*Thalassiosira* sp. (Dia)	
	East Point	24-Sep	*Chaetoceros* sp., Hyalochaete (Dia)	
	Elger Bay	24-Sep	*Chaetoceros* sp., Hyalochaete (Dia)	
	Mabana	24-Sep	*Chaetoceros* sp., Hyalochaete (Dia)	
	N. Langley	24-Sep	*Chaetoceros* sp., Hyalochaete (Dia)	
	Pebble Beach	24-Sep	*Chaetoceros* sp., Hyalochaete (Dia)	
	Saratoga City	24-Sep	*Chaetoceros* sp., Hyalochaete (Dia)	
	Port Gardner	24-Sep	*Rhizosolenia setigera* (Dia)	
Main Basin	Port Madison	22-Jul	*Coscinodiscus wailesii* (Dia)	*Ceratium fusus* (Dino)
	Point Beals	22-Sep	*Skeletonema costatum* (Dia)	
	Point Dalco	22-Sep	*Skeletonema costatum* (Dia)	
	Point Monroe	22-Sep		*Chaetoceros* sp., Hyalochaete and *Skeletonema costatum* (Dia)
Comm. Bay	Comm. Bay	22-Sep	*Skeletonema costatum* (Dia)	
Elliott Bay	Elliott Bay	11-Aug	*Ceratium fusus* (Dino)	
	Elliott Bay	19-Oct	*Thalassiosira* sp. (Dia)	

continued….

Table 3. Relative abundance of phytoplankton taxa from eight Puget Sound Basins. Taxa were termed "Dominant" if cell counts were >50% of nine fields examined (100x) and as "Subdominant" if cell counts dominated >25% of nine fields. "Dia"= centric diatom.

Basin/Bay	Location	Collection Date in 2009	Dominant Taxon	Subdominant Taxa
South Puget Sound	Budd Inlet	28-Jul	*Ceratium fusus* (Dino)	
	Filucy Bay	14-Aug	*Chaetoceros* sp., Hyalochaete (Dia)	
	South Head	14-Aug		*Chaetoceros* sp., Hyalochaete and *Rhizosolenia setigera* (Dia)
	Tolmie State Park	14-Aug	*Akashiwo sanguinea* (Dino)	
	Cole Point	18-Sep	*Skeletonema costatum* (Dia)	
	Dover Point	18-Sep	*Skeletonema costatum* (Dia)	
	Gertrude Island	18-Sep	*Rhizosolenia setigera* (Dia)	*Skeletonema costatum* (Dia)
	Johnson Point	18-Sep	*Skeletonema costatum* (Dia)	
Hood Canal	Anderson Cove	21-Sep	*Chaetoceros concavicornis* (Dia)	
	Dosewallips	21-Sep	*Chaetoceros concavicornis* (Dia)	
	Thorndyke Bay	21-Sep	*Chaetoceros concavicornis* (Dia)	

because the rate at which the phytoplankton net was lifted through the water was slow enough for zooplankters to easily avoid capture.

Krill

We encountered krill species generally in the same areas of Puget Sound that had been described in some detail by Cooney (1971) forty years ago. Overall we observed what appears to have been low abundance of krill, relative to Cooney's narrative descriptions and his echograms of sound-scattering layers. Cooney used a narrow-beam echosounder operating at 100 kHz. We used a dual-beam echosounder, sending both wide and narrow beams, at 50 and 200 kHz, and so bracketed Cooney's equipment. Cooney routinely observed a well-defined diffuse sound scattering layers on most of his transects, of up to 25 m in thickness, during the summer months we sampled. We never observed such strong scattering layers, even though we regularly scanned across a wide range of signal strengths for each beam. In areas where we reliably observed a sound scattering layer from which we sampled krill, the layer was typically at or near the bottom, difficult to discern (faint image on the screen) and less than a few meters in thickness. On more than several occasions we observed this layer moving quickly upward just as the sun set. After the sun set the sound-scattering layer typically disappeared, and krill became difficult to locate. Our most successful tows were made just at dusk, with the net wire set at a depth to sample the observed layer, typically a few meters off the seafloor.

Cooney used 80 cm-diameter bongo nets (total area approximately 1 m^2) and we used a single net with square opening approximately 3m x 3m (9 m^2). Although we made systematic searches across long tracks throughout Puget Sound with our acoustic equipment and also with the net, we generally found krill only in a few specific areas where we ultimately collected them (Figure 1). These locations also generally correlated with locations where some of their primary fish-predators aggregate. These predators, Pacific hake (*Merluccius productus*) and walleye pollock (*Theragra chalcogramma*) are the subject of a companion study (West et al 2011) to this report.

Of the krill taxa sampled, *Euphausia pacifica* was the most frequently encountered and was the dominant krill species in terms of numbers and biomass, in samples from all five basins and embayments. *Thysanoessa spinifera*, a comparatively larger and more raptorial krill species, was the dominant species at one site in the Main Basin (Vashon Island, Figure 5). *T. spinifera* were also present in Port Madison (Main Basin) and Port Susan (Whidbey Basin), where they were noted as an incidental species among the numerically dominant *E. pacifica*. *T. raschii* dominated one sample from the southern end of Admiralty Inlet in the Main Basin (Useless Bay, Figure 5). We also note that this collection was made in March of 2008 from a separate pilot survey of plankton in Puget Sound, a year prior to sample collection for the other two species.

Body length of individual *E. pacifica* ranged widely within Basins (7.3 to 25.7 mm) however their mean length varied only slightly between Basins. *E. pacifica* from the Main Basin (mean 19.3 mm) were slightly larger than those from the South Puget Sound (17.4 mm), Hood Canal

(16.6 mm), Elliott Bay (15.4 mm), and Whidbey Basin (15.3 mm) Whidbey Basin *E. pacifica* were also smaller than those from the South Puget Sound and Hood Canal. This range of these mean sizes (14.8 to 19.3 mm) is consistent with the range of sizes reported by Cooney (1971) for *E. pacifica* in the late summer/fall months of their first year of life in Puget Sound. These data suggest krill were all hatched the previous spring, with the sizes we reported representing approximately four months of growth after metamorphosis from their furcilia stage.

T. spinifera were larger (mean length 24.6 mm), than *E. pacifica* (19.3 mm) and T. raschii (16.6 mm). These differences may be related to sample timing, (September, July, March respectively), differences in species, age, diet or some other factors. It appears that *T. spinifera* may simply be a larger species -- a comparison of our *T.spinifera* sizes (median 24 mm TL) with growth trajectories reported by Tanasichuk (1998) suggest that our *T. spinifera* were the same age (approximately 4 months) as our *E. pacifica.*

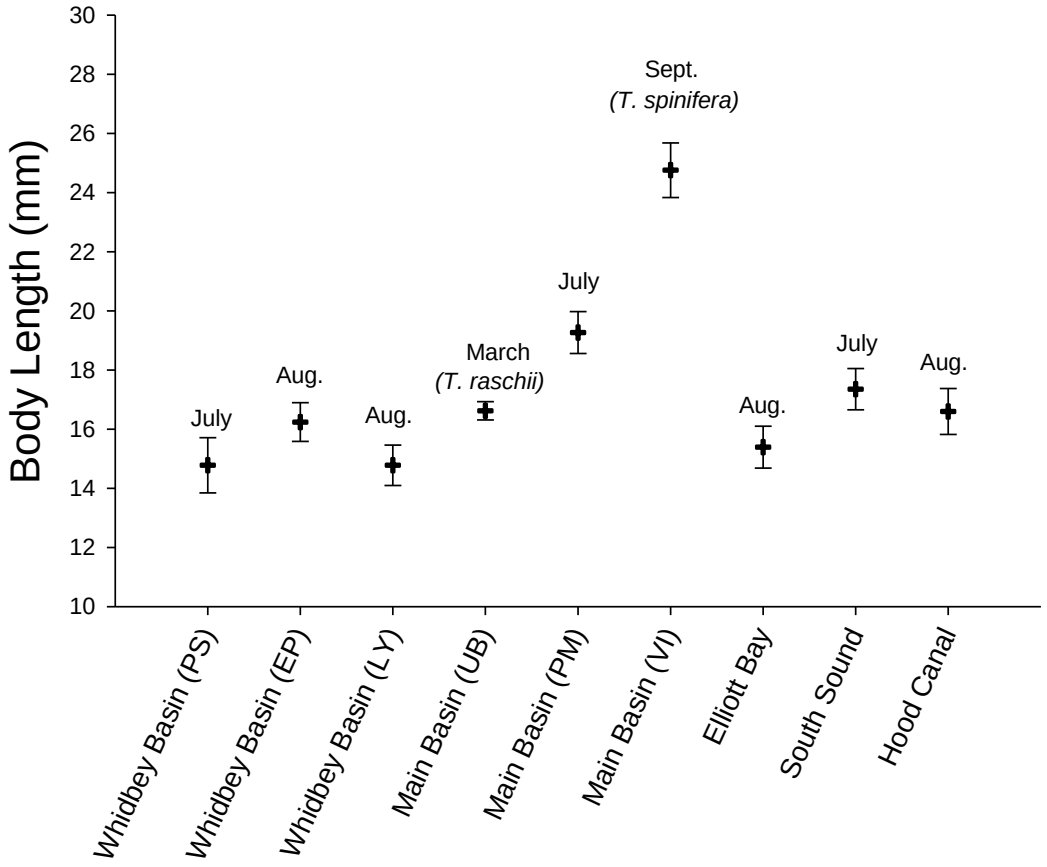

Figure 5. Mean body length (mm, ±99th % confidence interval) of krill from selected locations representing five basins in the Puget Sound. Sampling month is indicated above each symbol. All data represent *Euphausia pacifica*, unless otherwise noted.

Stable Isotopes

$\delta^{13}C$ values in POM were characterized primarily by a relatively small range across seven of eight Basins (18.2 to 21.7‰) but with high variability within Basins (Table 4). $\delta^{13}C$ was almost 10 ‰ lower in Hood Canal than the next lowest mean value from the other Basins, and $\delta^{13}C$ samples within Hood Canal ranged 6 ‰ across its three samples. $\delta^{13}C$ in POM from Hood Canal (30.4‰) was significantly lower than POM from the four other Basins with a minimum sample size of three (GLM of adjusted $\delta^{13}C$ by Basin, $F_{(4,23)}=28.7$, $p<0.0001$; THSD pairwise comparison, $p<0.0001$ for each Basin pairwise comparison with Hood Canal). Mean $\delta^{13}C$ ranged from -21.7 to -18.2 ‰ in San Juan Islands, Whidbey Basin, South Sound, and the Main Basin (Table 4). Of these four, $\delta^{13}C$ in POM from the Main Basin was significantly greater than Whidbey Basin (THSD pairwise comparison, $p<0.0001$, whereas all others were statistically indistinguishable (THSD pairwise comparison, $p>0.05$).

Table 4. Comparison of biometric data (mean values) and trophic status (mean and standard deviations, sd) for three species of krill (*Euphausia pacifica, Thysanoessa spinifera, T. raschii*,) and POM from eight Basins.

Species/type	Basin	n	Moisture (%)	Total Extractible Lipids (%)	Total Length (mm)	$\delta^{13}C$[a] (‰)	sd	$\delta^{15}N$ (‰)	sd
E. pacifica	Hood Canal	3	83.8	2.5	16.6	-17.7	0.06	9.0	0.063
E. pacifica	Whidbey Basin	7	84.9	2.7	15.4	-16.4	0.27	9.1	0.125
E. pacifica	Main Basin	3	81.5	2.0	19.3	-14.9	0.06	8.6	0.033
E. pacifica	Elliott Bay	3	82.9	1.9	15.4	-14.6	0.04	8.8	0.063
E. pacifica	S. Puget Sound	3	85.6	0.49	17.4	-16.1	nc	9.7	nc
T. raschii	Main Basin	1	78.5	1.8	16.6	-18.6	nc	9.0	nc
T.spinifera	Main Basin	3	76.5	9.3	24.8	-16.0	0.07	11.5	0.023
POM	Str. Juan de Fuca	3	92.2	0.37	--	-18.7	2.11	6.2	0.299
POM	San Juan Islands	3	94.1	0.18	--	-20.5	0.58	6.3	0.060
POM	Hood Canal	3	96.0	0.13	--	-30.4	3.27	7.1	1.365
POM	Whidbey Basin	10	95.6	0.08	--	-21.3	1.22	7.8	0.280
POM	Main Basin	4	94.1	0.37	--	-18.2	2.23	7.0	0.892
POM	Elliott Bay	2	93.1	0.47	--	-21.7	nc	9.4	nc
POM	Commencement Bay	1	95.0	0.35	--	-20.0	nc	7.9	nc
POM	S. Puget Sound	9	94.6	0.26	--	-21.0	1.03	9.0	0.763

[a]adjusted for lipid content – see Methods

nc = not calculated

$\delta^{13}C$ in krill, most notably *E. pacifica*, generally increased in Basins moving from oceanic (Hood Canal) to more estuarine (Table 4). *E. pacifica* from Hood Canal exhibited significantly lower $\delta^{13}C$ than *E. pacifica* from the four other Basins with a sample size of at least three; Whidbey Basin, Main Basin, South Sound, and Elliott Bay (GLM of $\delta^{13}C$ by Basin, $F_{(4,14)}=148$, $p<0.0001$; THSD pairwise comparison, $p<0.0001$ for each pairwise comparison with Hood Canal). In addition, *E. pacifica* from the Main Basin and Elliott Bay exhibited significantly greater $\delta^{13}C$

than all other Basins, and $\delta^{13}C$ in Whidbey Basin and South Sound *E. pacifica* was intermediate between Hood Canal and the Whidbey and Main Basins (THSD, pairwise comparisons, $p<0.05$). Although sample size for the two *Thysanoessa* species was too small to make conclusions about their $\delta^{13}C$ signal, they generally followed the pattern exhibited by *E. pacifica*.

POM exhibited relatively wide variation in $\delta^{15}N$ across Basins, with values ranging from 6.2‰ in the Strait of Juan de Fuca to 9.4 ‰ from Elliott Bay (Table 4). For the five Basins with 3 or more samples of $\delta^{15}N$, South Puget Sound exhibited the greatest mean $\delta^{15}N$ (9.0 ‰), which was significantly greater than POM from the Whidbey Basin, Main Basin, Hood Canal, and San Juan Islands GLM of $\delta^{15}N$ by Basin, $F_{(4,24)}=12.0$, $p<0.0001$, THSD pairwise comparison of South Sound with four other Basins, p value ranging from 0.005 to <0.0001). Among the four other Basins, $\delta^{15}N$ in San Juan Islands POM was significantly lower than Whidbey Basin (THSD pairwise comparison, $p=0.022$), while POM from Main Basin, Hood Canal, and Whidbey Basin were indistinguishable (THSD pairwise comparison, $p>0.05$).

$\delta^{15}N$ results for *E. pacifica* suggest that the trophic level of populations we sampled were equivalent across basins for that species. Although $\delta^{15}N$ varied significantly among the five Basins with three or more $\delta^{15}N$ samples (GLM of $\delta^{15}N$ by Basin, $F_{(4,14)}=51.5$, $p<0.0001$), the widest range in $\delta^{15}N$ values among the locations sampled was less than half (1.18‰) the trophic fractionation estimate proposed by Post (2002) as the degree of $\delta^{15}N$ enrichment separating trophic levels in aquatic food webs (3.4‰) (Table 4). Among our three krill species, *T. spinifera* (from the Main Basin exhibited a greater $\delta^{15}N$ (11.5‰) than the other two species from that basin (range 8.6 to 8.9 for *E. pacifica*, from Elliott Bay and other Main Basin locations – Table 4). This difference, combined with *T. spinifera's* larger size and raptorial feeding appendages suggest that this species occupies a higher trophic level than either *E. pacifica* or *T. raschii*. Although $\delta^{15}N$ results suggest that *T. raschii* occupy a similar trophic level to *E. pacifica*, its greater lipid content and dry weight suggest some other differences in feeding ecology. For these reasons we treated *T. spinifera* and *T. raschii* separately from the dominant species, *E. pacifica* in the following POP analyses.

Contaminant Analysis Overview

This pilot survey of POPs in krill and phytoplankton presented unique difficulties related to capture, sorting and processing of the organisms, and in chemical analysis of some compounds. Although we were successful at targeting and sorting krill according to species, phytoplankton were targeted by simply filtering POM by size.

Lipid concentrations in POM were uniformly low, ranging from less than 0.1% to 0.5%, wet weight (Table 4). Such a range is near to the limit of reliable quantitation for gravimetric lipid analyses. Because such low lipid values have a large effect when computing PCB concentrations on a lipid basis using the commonly employed ratio method, small inaccuracies in quantitation in

this range can contribute to spurious conclusions. For these reasons we do not present lipid-normalized data in this report, and only include analysis of lipids when feasible.

Overall, $\sum_{46}$PCBs, $\sum_{10}$PBDEs, $\sum_{6}$DDTs, $\sum_{3}$HCHs and hexachlorobenzene were the most abundant contaminants or groups measured in this study. In POM, $\sum_{46}$PCBs were reported from all samples, $\sum_{10}$PBDEs from 19/35 samples, $\sum_{6}$DDTs from 23/35 samples, $\sum_{3}$HCHs from 24/25 samples, and hexachlorobenzene from 10/35 samples (Table 5). This means that at least one congener or contributing compound was detected within each summed group. The remaining five contaminants, all organochlorine pesticides (OCPs), were rarely detected in POM; 5/35 samples for mirex, 3/35 for $\sum_{8}$Chlordanes, and 0/35 for dieldrin, aldrin, and endosulfan sulfate. In krill, all contaminants except dieldrin, aldrin, mirex, and endosulfan were detected in all samples. The average limit of quantitation (LOQ) for all POPs ranged from 0.14 to 0.78 ng/g wet wt. in POM and 0.012 to 0.081 ng/g wet wt. in krill (Table 6).

The following Basin comparisons are based on GLM tests of natural log-transformed POP concentrations versus Basin in separate tests of POM and *E. pacifica*, using the THSD test to make *post hoc* pairwise Basin comparisons. Only Basins with three or more samples were included in the statistical comparisons. Geometric means and confidence intervals are presented in the following figures, and for reference individual sample concentrations for Basins with fewer than 3 samples for the statistical test are included as separate symbols.

Table 5. Summary of POP concentrations data in Particulate Organic Matter (POM) and three species of krill from eight sampling Basins. Number of samples analyzed is indicated in parentheses next to each Basin name. See Table 6 for a summary of limits of quantitation (LOQ).

		Particulate Organic Matter (POM, n=35)								*Euphausia pacifica (n=19)*					*T. raschii*	*T. spinifera*
		Juan de Fuca (3)	San Juan Islands (3)	Hood Canal (3)	Whidbey Basin (10)	Main Basin (4)	Elliott Bay (2)	Comm. Bay (1)	South Sound (9)	Hood Canal (3)	Whidbey Basin (7)	Main Basin (3)	Elliott Bay (3)	South Sound (3)	Main Basin (1)	Main Basin (3)
Σ_{46} PCBs	n detects	3	3	3	10	4	2	1	9	3	7	3	3	3	1	3
	minimum	2.1	2.7	2.1	2.1	2.6	4.9	2.8	2.2	2.9	4.2	3.8	11.0	3.4	6.6	17.2
	maximum	3.4	7.8	2.4	4.3	3.5	10.3	2.8	3.6	3.4	5.1	4.0	12.5	4.7	6.6	18.6
	mean	2.9	4.8	2.3	2.5	3.0	nc	nc	2.7	3.1	4.6	3.9	11.7	4.1	nc	17.6
	10^{th} pctle.	2.1	2.7	2.1	2.1	2.6	nc	nc	2.2	2.9	4.2	3.8	11.0	3.4	nc	17.2
	median	3.3	4.0	2.3	2.3	3.0	nc	nc	2.7	3.0	4.7	3.9	11.7	4.1	nc	17.2
	90^{th} pctle.	3.4	7.8	2.4	3.6	3.5	nc	nc	3.4	3.4	5.0	4.0	12.5	4.7	nc	18.6
Σ_{10}PBDEs	n detects	2	3	0	2	3	2	1	6	3	7	3	3	3	1	3
	minimum	0.13	0.15	<LOQ	0.11	0.11	0.44	0.27	<LOQ	0.14	0.44	0.52	0.98	0.22	1.50	7.61
	maximum	0.42	0.28	--	0.19	0.18	1.45	0.27	0.86	0.19	0.78	0.57	1.92	0.36	1.50	8.28
	mean	nc	0.19	--	nc	0.15	nc	nc	0.29	0.17	0.59	0.55	1.42	0.28	nc	7.89
	10^{th} pctle.	nc	0.15	--	nc	0.11	nc	nc	0.01	0.14	0.44	0.52	0.98	0.22	nc	7.61
	median	nc	0.16	--	nc	0.17	nc	nc	0.20	0.17	0.56	0.57	1.36	0.26	nc	7.78
	90^{th} pctle.	nc	0.28	--	nc	0.18	nc	nc	0.81	0.19	0.76	0.57	1.92	0.36	nc	8.28
Σ_6DDTs	n detects	2	3	1	3	3	2	1	8	3	7	3	3	3	1	3
	minimum	0.028	0.038	0.021	0.012	0.022	0.471	0.093	0.023	0.204	0.297	0.236	0.540	0.120	2.40	2.30
	maximum	0.193	0.089	0.021	0.035	0.100	0.857	0.093	0.150	0.254	0.378	0.265	0.745	0.200	2.40	2.42
	mean	nc	0.059	nc	0.024	0.052	nc	nc	0.061	0.225	0.333	0.255	0.634	0.160	nc	2.38
	10^{th} pctle.	nc	0.038	nc	0.012	0.022	nc	nc	0.023	0.204	0.298	0.236	0.540	0.120	nc	2.30
	median	nc	0.050	nc	0.025	0.034	nc	nc	0.039	0.218	0.326	0.263	0.617	0.160	nc	2.41
	90^{th} pctle.	nc	0.089	nc	0.035	0.100	nc	nc	0.145	0.254	0.375	0.265	0.745	0.200	nc	2.42
Σ_3HCHs	n detects	2	3	0	5	3	2	1	8	3	7	3	3	3	1	3
	minimum	0.029	0.032	<LOQ	0.011	0.020	0.104	0.089	0.016	0.141	0.080	0.163	0.142	0.065	0.385	0.750
	maximum	0.250	0.173	--	0.040	0.048	1.451	0.089	0.077	0.153	0.182	0.168	0.196	0.120	0.385	0.830
	mean	nc	0.098	--	0.024	0.031	nc	nc	0.051	0.146	0.153	0.166	0.164	0.101	nc	0.794
	10^{th} pctle.	nc	0.032	--	0.011	0.020	nc	nc	0.018	0.141	0.095	0.163	0.142	0.065	nc	0.750
	median	nc	0.089	--	0.020	0.026	nc	nc	0.058	0.144	0.162	0.166	0.154	0.119	nc	0.801
	90^{th} pctle.	nc	0.173	--	0.040	0.048	nc	nc	0.076	0.153	0.179	0.168	0.196	0.120	nc	0.830

continued.

Table 5. Summary of POP concentrations data in Particulate Organic Matter (POM) and three species of krill from eight sampling Basins. Number of samples analyzed is indicated in parentheses next to each Basin name. See Table 6 for a summary of limits of quantitation (LOQ).

		Particulate Organic Matter (POM, n=35)								*Euphausia pacifica (n=19)*					*T. raschii*	*T. spinifera*
		Juan de Fuca (3)	San Juan Islands (3)	Hood Canal (3)	Whidbey Basin (10)	Main Basin (4)	Elliott Bay (2)	Comm. Bay (1)	South Sound (9)	Hood Canal (3)	Whidbey Basin (7)	Main Basin (3)	Elliott Bay (3)	South Sound (3)	Main Basin (1)	Main Basin (3)
Σ_8CHLD	n detects	0	0	0	0	1	1	0	1	3	7	3	3	3	1	3
	minimum	<LOQ	<LOQ	<LOQ	<LOQ	0.020	0.099	<LOQ	0.028	0.101	0.131	0.143	0.228	0.077	0.771	1.055
	maximum	--	--	--	--	0.020	0.099	--	0.028	0.135	0.239	0.164	0.347	0.110	0.771	1.289
	mean	--	--	--	--	nc	nc	--	nc	0.115	0.171	0.154	0.283	0.094	nc	1.187
	10th pctle.	--	--	--	--	nc	nc	--	nc	0.101	0.133	0.143	0.228	0.077	nc	1.055
	median	--	--	--	--	nc	nc	--	nc	0.109	0.173	0.154	0.273	0.094	nc	1.216
	90th pctle.	--	--	--	--	nc	nc	--	nc	0.135	0.229	0.164	0.347	0.110	nc	1.289
hexachlorobenzene	n detects	1	0	0	1	1	1	0	6	3	7	3	3	3	1	3
	minimum	0.027	<LOQ	<LOQ	0.018	0.027	0.530	<LOQ	0.013	0.086	0.067	0.075	0.083	0.032	0.210	0.340
	maximum	0.027	--	--	0.018	0.027	0.530	--	0.029	0.093	0.085	0.084	0.092	0.044	0.210	0.380
	mean	nc	--	--	nc	nc	nc	--	0.017	0.088	0.076	0.079	0.088	0.039	nc	0.357
	10th pctle.	nc	--	--	nc	nc	nc	--	0.013	0.086	0.067	0.075	0.083	0.032	nc	0.340
	median	nc	--	--	nc	nc	nc	--	0.014	0.086	0.076	0.078	0.090	0.041	nc	0.350
	90th pctle.	nc	--	--	nc	nc	nc	--	0.028	0.093	0.084	0.084	0.092	0.044	nc	0.380
dieldrin	n detects	0	0	0	0	0	0	0	0	3	6	3	3	0	1	3
	minimum	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.041	0.067	0.045	0.045	<LOQ	0.200	0.220
	maximum	--	--	--	--	--	--	--	--	0.062	0.110	0.062	0.070	--	0.200	0.320
	mean	--	--	--	--	--	--	--	--	0.051	0.085	0.052	0.060	--	nc	0.267
	10th pctle.	--	--	--	--	--	--	--	--	0.041	0.068	0.045	0.045	--	nc	0.220
	median	--	--	--	--	--	--	--	--	0.050	0.082	0.050	0.066	--	nc	0.260
	90th pctle.	--	--	--	--	--	--	--	--	0.062	0.108	0.062	0.070	--	nc	0.320
aldrin	n detects	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0
	minimum	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.017	<LOQ	0.033	<LOQ	<LOQ	<LOQ
	maximum	--	--	--	--	--	--	--	--	--	0.019		0.033	--	--	--
	mean	--	--	--	--	--	--	--	--	--	nc		nc	--	--	--
	10th pctle.	--	--	--	--	--	--	--	--	--	nc		nc	--	--	--
	median	--	--	--	--	--	--	--	--	--	nc		nc	--	--	--
	90th pctle.	--	--	--	--	--	--	--	--	--	nc		nc	--	--	--

continued.

Table 5. Summary of POP concentrations data in Particulate Organic Matter (POM) and three species of krill from eight sampling Basins. Number of samples analyzed is indicated in parentheses next to each Basin name. See Table 6 for a summary of limits of quantitation (LOQ).

		Particulate Organic Matter (POM, n=35)								*Euphausia pacifica (n=19)*					*T. raschii*	*T. spinifera*
		Juan de Fuca (3)	San Juan Islands (3)	Hood Canal (3)	Whidbey Basin (10)	Main Basin (4)	Elliott Bay (2)	Comm. Bay (1)	South Sound (9)	Hood Canal (3)	Whidbey Basin (7)	Main Basin (3)	Elliott Bay (3)	South Sound (3)	Main Basin (1)	Main Basin (3)
mirex	n detects	1	0	0	0	1	1	1	1	0	0	0	2	3	1	3
	minimum	0.034	<LOQ	<LOQ	<LOQ	0.018	0.510	0.024	0.530	<LOQ	<LOQ		0.027	0.440	0.530	0.021
	maximum	0.034	--	--	--	0.018	0.510	0.024	0.530	--	--		0.520	0.790	0.530	0.030
	mean	nc	--	--	--	nc	nc	nc	nc	--	--		nc	0.597	nc	0.024
	10^{th} pctle.	nc	--	--	--	nc	nc	nc	nc	--	--		nc	0.440	nc	0.021
	median	nc	--	--	--	nc	nc	nc	nc	--	--		nc	0.560	nc	0.022
	90^{th} pctle.	nc	--	--	--	nc	nc	nc	nc	--	--		nc	0.790	nc	0.030
α-endosulfan	n detects	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	minimum	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	maximum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	mean	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	10^{th} pctle.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	median	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	90^{th} pctle.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
TPAH	n detects	3	3	3	10	4	2	1	9	3	7	3	3	2	1	3
	minimum	2.3	27.5	1.7	1.8	6.3	149.3	54.9	5.4	5.6	7.2	5.6	16.8	7.6	33.5	32.5
	maximum	109.0	61.5	5.2	16.4	13.1	2020.6	54.9	35.4	6.4	10.9	18.0	18.1	7.9	33.5	37.3
	mean	42.7	42.6	2.9	5.1	8.9	1084.9	nc	17.0	5.9	8.1	9.8	17.7	7.8	nc	34.7
	10^{th} pctle.	2.3	27.5	1.7	2.0	6.3	149.3	nc	5.9	5.6	7.2	5.6	16.8	7.6	nc	32.5
	median	16.9	38.7	1.9	2.8	8.2	1084.9	nc	16.1	5.8	7.6	5.7	18.1	7.8	nc	34.3
	90^{th} pctle.	109.0	61.5	5.2	15.2	13.1	2020.6	nc	33.0	6.4	10.4	18.0	18.1	7.9	nc	37.3

Table 6. Average limit of quantitation (LOQ) for 24 analytes or congener groups (ng/g wet wt.)

Analyte	POM	Krill
PCB congeners	0.016	0.015
PBDE congeners	0.078	0.051
p,p'-DDD	0.017	--
p,p'-DDE	0.014	--
p,p'-DDT	0.014	0.014
o,p'-DDD	0.014	0.016
o,p'-DDE	0.014	0.016
o,p'-DDT	0.014	0.013
α-hexachlorocyclohexane	0.015	--
β-hexachlorocyclohexane	0.025	0.076
γ-hexachlorocyclohexane	0.015	0.020
α-chlordane	0.014	--
γ-chlordane	0.015	0.022
trans-nonachlor	0.014	--
cis-nonachlor	0.015	0.020
heptachlor epoxide	0.014	0.016
oxychlordane	0.050	0.048
nonachlor III	0.014	0.012
heptachlor	0.014	0.025
hexachlorobenzene	0.015	--
dieldrin	0.050	0.059
aldrin	0.017	0.014
mirex	0.014	0.013
α-endosulfan	0.054	0.048
PAHs	0.064	0.081

$\sum_{46}$PCBs

Of the 46 PCB congeners tested in this study, 36 were measured as individual congeners, and four groups were measured as coeluters (Table 7). For 30 congeners in the di- through hexa-chlorinated biphenyls, all were detected in > 75% of POM and krill samples; only two (PCB 180 and the coeluting group PCB187(159,182)) were detected in most POM samples. The 11 other hepta- through deca-chlorinated biphenyls were detected in 0 to 40% of POM samples. This pattern was similar for krill except that two hepta-chlorinated biphenyls that were rare in POM (PCB177 and PCB183) were detected in all krill samples.

Table 7. Frequency of detection for the 46 congeners detected in 35 POM and 23 krill samples from this study. Numbers in parentheses indicate coeluting congeners.

PCB Congener	Homolog Group	POM	Krill (3 spp)
PCB17	Tri	100.0%	100.0%
PCB18	Tri	100.0%	100.0%
PCB28	Tri	100.0%	100.0%
PCB31	Tri	100.0%	100.0%
PCB33	Tri	100.0%	100.0%
PCB44	Tetra	100.0%	100.0%
PCB49	Tetra	100.0%	100.0%
PCB52	Tetra	100.0%	100.0%
PCB66	Tetra	100.0%	100.0%
PCB70	Tetra	100.0%	100.0%
PCB74	Tetra	100.0%	100.0%
PCB82	Penta	97.1%	100.0%
PCB87	Penta	100.0%	100.0%
PCB95	Penta	100.0%	100.0%
PCB99	Penta	100.0%	100.0%
PCB101(90)	Penta	100.0%	100.0%
PCB105	Penta	100.0%	100.0%
PCB110	Penta	100.0%	100.0%
PCB118	Penta	100.0%	100.0%
PCB128	Hexa	100.0%	100.0%
PCB138(163,164)	Hexa	100.0%	100.0%
PCB149	Hexa	100.0%	100.0%
PCB151	Hexa	91.4%	100.0%
PCB153(132)	Hexa	100.0%	100.0%
PCB156	Hexa	80.0%	100.0%
PCB158	Hexa	85.7%	100.0%
PCB170	Hepta	37.1%	95.7%
PCB171	Hepta	8.6%	34.8%
PCB177	Hepta	31.4%	100.0%
PCB180	Hepta	94.3%	100.0%
PCB183	Hepta	40.0%	100.0%
PCB187(159,182)	Hepta	97.1%	100.0%
PCB191	Hepta	0.0%	17.4%
PCB194	Octa	5.7%	56.5%
PCB195	Octa	5.7%	34.8%
PCB199	Octa	5.7%	60.9%
PCB205	Octa	20.0%	47.8%
PCB206	Nona	5.7%	30.4%
PCB208	Nona	2.9%	21.7%
PCB209	Deca	2.9%	26.1%

$\sum_{46}$PCB was calculated for all POM and *E. pacifica* samples, and a statistical comparison of Basins was possible for six Basins for POM and all five Basins for *E. pacifica* (solid bars, Figure 6). Overall, there was high variability within Basins for POM, with a relatively uniform mean concentration of PCBs in POM between the Basins. Only one of the six testable Basins exhibited a significant difference: San Juan Islands POM had a higher $\sum_{46}$PCB concentration (4.8 ng/g wet wt.) than three other Basins, Hood Canal, Whidbey Basin and South Sound (GLM of ln-transformed $\sum_{46}$PCB, $F_{(5,26)}$ =3.37, p=0.018, THSD pairwise comparisons , p<0.05 for significant differences). All others were statistically indistinguishable from each other with THSD pairwise comparison, p values >0.05. The two individual POM samples from Elliott Bay exhibited a wide range of concentrations, 4.9 and 10.3 ng/g wet wt., both of which were higher than the maximum $\sum_{46}$PCB from all the other Basins except San Juan Islands (Table 5).

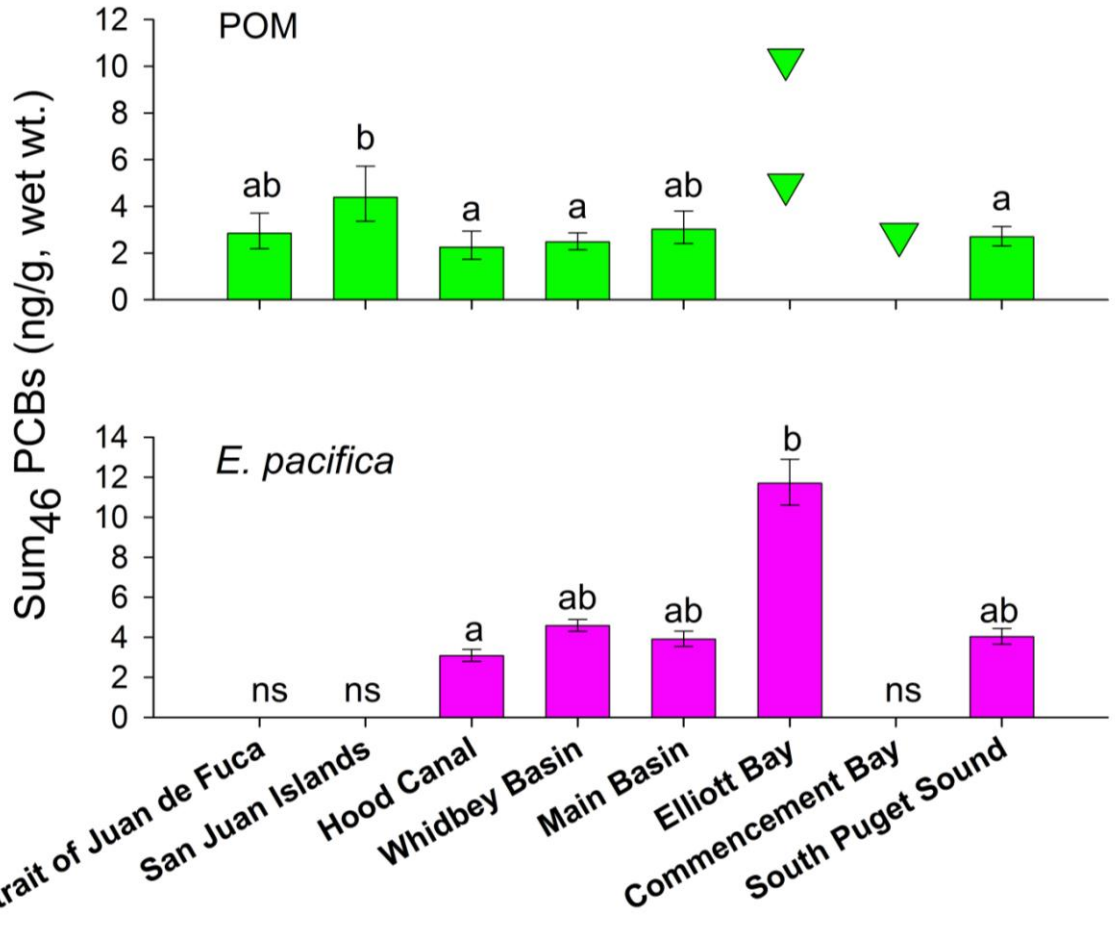

Figure 6. Geometric mean ±95% confidence intervals for $\sum_{46}$PCB in POM (green bars, upper plot) and *E. pacifica* (pink bars, lower plot). Individual sample concentrations are presented as triangles where n<3 for a Basin. Not sampled denoted as "ns". Bars with different lower case letters indicate THSD significant difference at α=0.05.

Of the four testable Basins for *E. pacifica*, $\sum_{46}$PCB was statistically greatest in Elliott Bay, with a mean concentration (11.7 ng/g wet wt.) more than double the next lower concentration, (4.6 ng/g, wet wt., Whidbey Basin (Figure 6; GLM of ln-transformed $\sum_{46}$PCB, $F_{(4,14)}=106$, $p<0.0001$, all THSD pairwise comparisons with Elliott Bay, $p\leq 0.001$). Hood Canal exhibited the lowest concentration (3.1 ng/g wet wt.), which was significantly lower than all other Basins (all THSD pairwise comparisons with Hood Canal, $p<0.035$). Main Basin, Whidbey Basin, and South Sound were all statistically indistinguishable from each other (THSD pairwise comparisons, $p>0.10$)

$\sum_{46}$PCB in *T. spinifera* from the Main Basin were the greatest of any sample in the study, with a mean concentration of 17.6 ng/g wet wt., and one Main Basin sample of *T. raschii* exhibited a concentration of 6.6 ng/g wet wt. (Table 5). *T. spinifera* were also the largest species, and they exhibited the greatest lipid content, and the greatest δ^{15}N (Table 5).

$\sum_{10}$PBDEs:

Of the ten congeners quantitated by the method used in this study, seven were detected in POM, and eight in krill. Two congeners (BDE-85 and BDE-183) were never detected in krill and three (BDE-28, BDE-85 and BDE-183) were never detected in POM. Three congeners were dominant by frequency of occurrence (BDE-47, -99, and -100), occurring in 53 to 100% of krill and 9 to 54% of POM samples (Table 8).

Table 8. Frequency of detection (%) for ten PBDE congeners in 52 samples of POM and *E. pacifica* analyzed in this study.

	POM	*E. pacifica*
N	35	17
BDE28	0	12
BDE47	45.7	100
BDE49	0	12
BDE66	2.9	11.8
BDE85	2.9	0.0
BDE99	54.3	100
BDE100	8.6	52.9
BDE153	5.7	5.9
BDE154	2.9	5.9
BDE183	0	0

Detected concentrations of $\sum_{10}$PBDE in POM ranged from a maximum of 1.5 ng/g wet wt. in Elliott Bay to <0.3 ng/g wet wt. in all other Basins -- Table 5). The large number of non-detected values, wide variability, and low concentrations resulted in no ability to discern patterns with confidence in POM (Figure 7). There was no significant difference in $\sum_{10}$PBDE between the three Basins that had three or more samples with detected values (San Juan Islands, Main Basin, and South Sound; GLM of ln-transformed $\sum_{10}$PBDE by Basin, $F_{(2,8)}=1.1$, $p=0.38$), and no PBDE congeners were detected in at least one POM sample from five of eight Basins. $\sum_{10}$PBDE concentrations in POM from the two Elliott Bay samples were both three to five times greater than the maximum $\sum_{10}$PBDE from any other Basin.

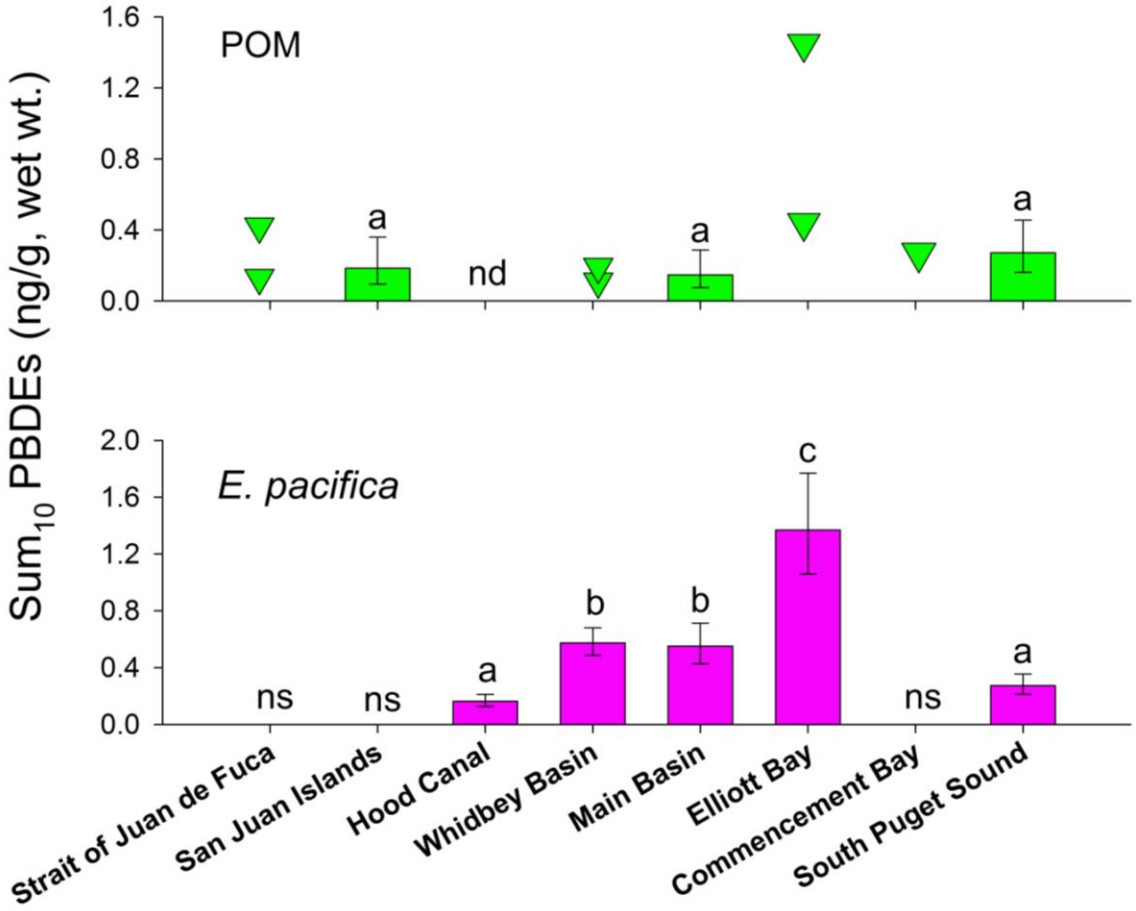

Figure 7. Geometric mean ± 95% confidence intervals for Σ_{10}PBDEs in POM (green bars, upper plot), and *E. pacifica* (pink bars, lower plot). Individual sample concentrations are presented as triangles where n<3 for a Basin). Not sampled denoted as "ns". Not detected denoted as "nd". Bars with different lower case letters indicate THSD significant difference at α=0.05.

$\sum_{10}$PBDEs in *E. pacifica* samples from Elliott Bay were significantly greater (mean 1.4 ng/g wet wt.) than any other Basin (GLM of ln-transformed $\sum_{10}$PBDE by Basin, $F_{(4,14)}=38.7$, $p<0.0001$,

THSD pairwise comparison of Elliott Bay with all other Basins, $p<0.005$). $\sum_{10}$PBDE concentrations in Whidbey and Main Basin were roughly four-fifths of Elliott Bay samples, and *E. pacifica* from Hood Canal and South Sound were in turn, roughly one-half the concentration of those from Whidbey Basin and Main Basin (Table 5).

Similar to PCBs, $\sum_{10}$PBDE in *T. spinifera* (from the Main Basin) were much greater that *E. pacifica* from any other Basin (roughly four times greater than *E. pacifica* from Elliott Bay). The concentration of $\sum_{10}$PBDE in *T. raschii* from the Main Basin was similar to *E. pacifica* from Elliott Bay (Table 5).

$\sum_6$DDTs

Five of six possible DDT isomers or metabolites were detected in POM, and six in *E. pacifica* , with *p,p'*-DDD and *p,p'*-DDE dominant in both groups (Table 9). $\sum_6$DDTs in POM ranged from 0.012 ng/g wet wt. in a sample from Whidbey Basin to 0.86 ng/g wet wt. in one sample from Elliott Bay (Table 5). For the four Basins with at least 3 POM samples with detected DDTs, (San Juan Islands, Whidbey Basin, Main Basin, and South Sound) there was no significant difference in $\sum_6$DDTs between them (GLM of ln-transformed $\sum_6$DDTs by basin, $F_{(3,13)}=1.0$, $p=0.40$; Figure 8).

$\sum_6$DDTs in *E. pacifica* were significantly greater in Elliott Bay (0.63 ng/g wet wt.) than any of the other four tested basins, followed by Whidbey Basin, which was greater than the Main Basin and Hood Canal, which were in turn, greater than South Sound (Table 5 -- GLM of ln-transformed $\sum_6$DDTs by Basin, $F_{(4,14)}=43.4$, $p<0.0001$, with THSD pairwise comparisons, $\alpha=0.05$).

Table 9. Frequency of detection (%) for six DDT isomers analyzed in 52 samples of POM and *E. pacifica* in this study.

	POM	*E. pacifica*
N	35	17
o,p'-DDD	17.1	17.6
o,p'-DDE	0	65
o,p'-DDT	11.4	23.5
p,p'-DDD	40	100
p,p'-DDE	65.7	100
p,p'-DDT	8.6	52.9

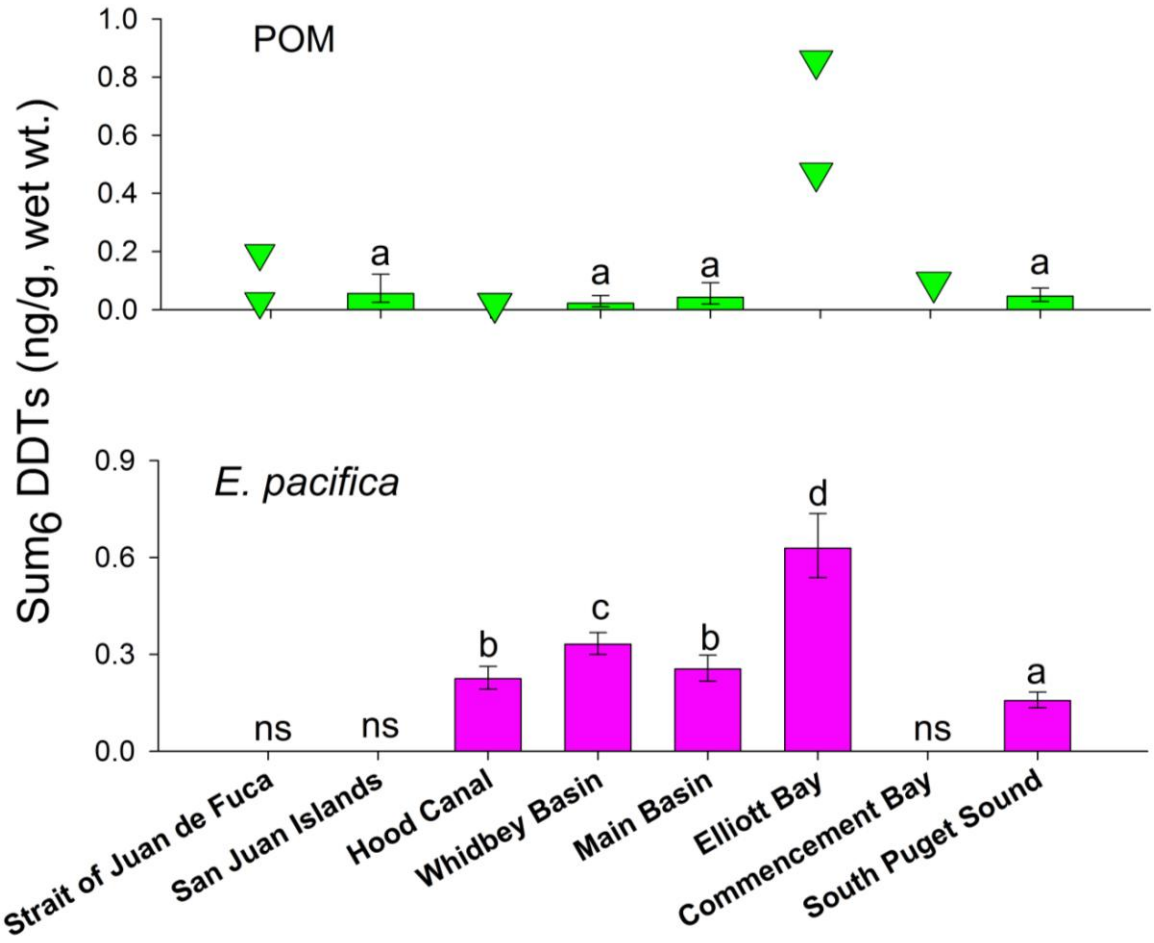

Figure 8. Geometric mean ± 95% confidence intervals for $\sum_6$DDTs in POM (green bars, upper plot) and *E. pacifica* (pink bars, lower plot). Individual sample concentrations are presented as triangles where n<3 for a Basin. Not sampled denoted as "ns". Bars with different lower case letters indicate THSD significant difference at α=0.05.

$\sum_3$HCHs

Three hexachlorocyclohexane compounds were detected in POM and krill, α-HCH, β-HCH, and γ-HCH (also called lindane; Table 10). $\sum_3$HCH concentration in POM was similar in magnitude and distribution to $\sum_6$DDTs. Concentrations were below 0.10 ng/g wet wt. in all samples except for one sample from Elliott Bay, which exhibited a concentration ten times greater than any other (1.5 ng/g wet wt).

Unlike $\sum_6$DDTs, the pattern of $\sum_3$HCH concentration in *E. pacifica* was more uniform across the five Basins that had 3 or more samples (Figure 9-- GLM of ln-transformed ∑HCH by Basin for South Sound, Hood Canal, Whidbey Basin, Elliott Bay, and Main Basin, $F_{(4,14)}$=2.6, p=0.86).

The concentration of ∑HCH from individual samples ranged from 0.08 to 0.20 ng/g wet wt. across all Basins (Table 5).

Table 10. Frequency of detection (%) for HCH isomers analyzed in this study.

	POM	*E. pacifica*
N	35	17
α-HCH	57.1	100
β-HCH	14.3	88.2
γ-HCH	57.1	88.2

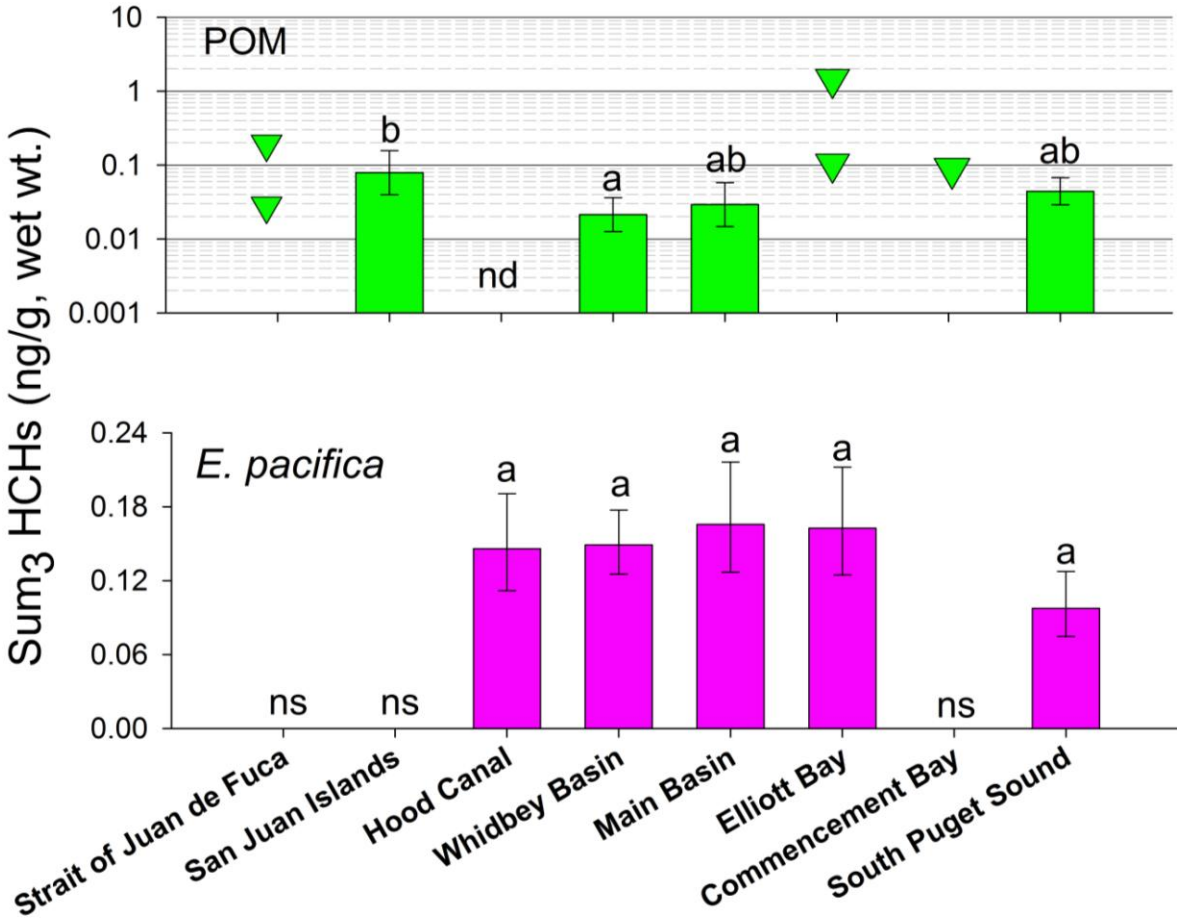

Figure 9. Geometric mean ± 95% confidence intervals for Sum HCHs in POM (green bars, upper plot – note log scale to accommodate high individual POM value from Elliott Bay) and *E. pacifica* (pink bars, lower plot). Individual sample concentrations are presented as triangles where n<3 for a Basin. Not sampled denoted as "ns". Not detected denoted as "nd". Bars with different lower case letters indicate THSD significant difference at α=0.05.

Other Organochlorine Pesticides (OCPs)

None of the other OCPs (∑Chlordanes, hexachlorobenzene, dieldrin, aldrin, mirex, or endosulfan) occurred in more than negligible frequency or concentration in POM (Table 5) and so no Basin comparisons were made for POM. In *E. pacifica*, chlordanes, hexachlorobenzene and dieldrin were commonly detected (see Basin comparisons following). No Basin comparisons are presented here for aldrin, mirex, and endosulfan I because they were rarely detected in *E. pacifica*.

$\sum_8$Chlordanes

$\sum_8$Chlordanes used in the following analysis were dominated by five abundant chlordane-compounds; α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, and heptachlor epoxide. Three other chlordane-compounds were never or rarely detected (Table 11).

At least one chlordane compound was detected in all *E. pacifica* samples. The greatest concentration of $\sum_8$Chlordanes was observed in Elliott Bay (0.35 ng/g wet wt. from one sample), which was significantly greater than all other Basins (GLM of ln-transformed $\sum_8$Chlordanes by Basin, $F_{(4,14)}=16.7$, $p<0.0001$; THSD, $p<0.001$ for pairwise comparisons of Elliott Bay with Whidbey Basin, Main Basins, Hood Canal and South Sound).

Table 11. Frequency of detection (%) for eight chlordane and chlordane-related isomers analyzed in this study. * indicates isomers included in ∑chlordanes for statistical analysis of between-Basin differences in *E. pacifica* and POM samples.

	POM	*E. pacifica*
N	35	17
*α-chlordane	2.9	100
*γ-chlordane	8.6	94.1
*trans-nonachlor	5.7	100
*cis-nonachlor	0	82.4
*heptachlor epoxide	0	52.9
oxychlordane	0	17.6
nonachlor III	0	0
heptachlor	0	0

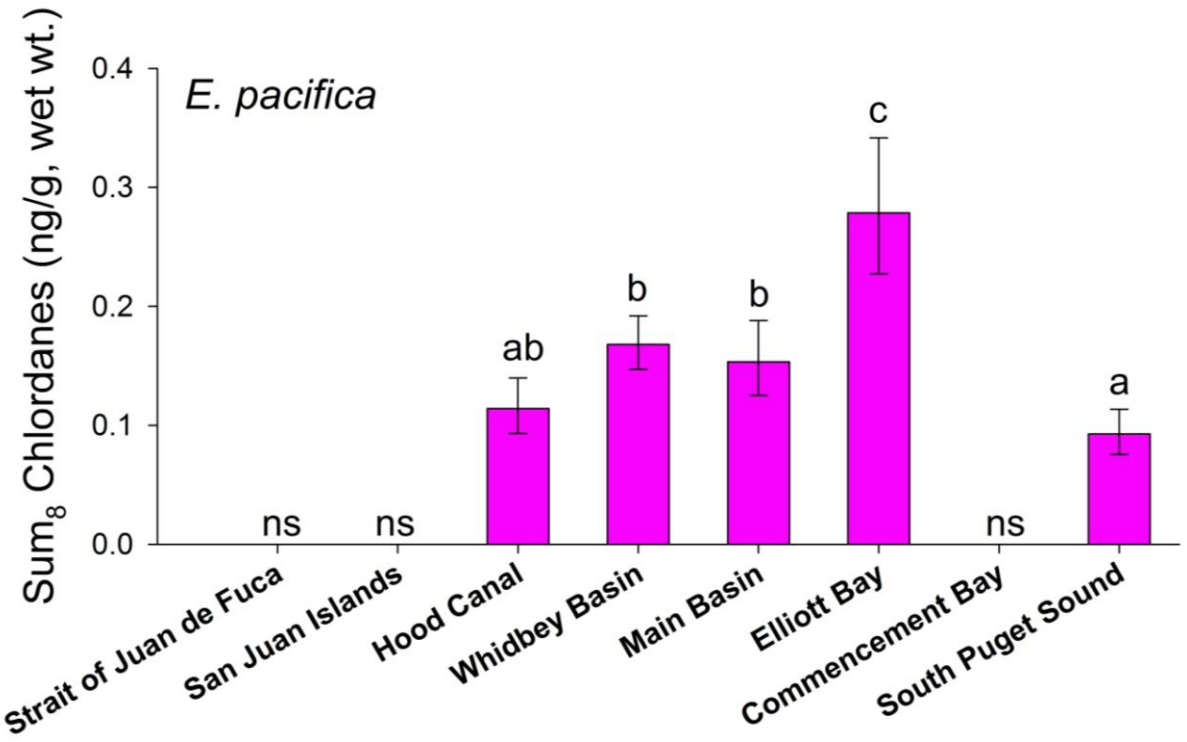

Figure 10. Geometric mean ± 95% confidence intervals for $\sum_8$Chlordanes in *E. pacifica*. Not sampled denoted as "ns". Bars with different lower case letters indicate THSD significant difference at α=0.05.

Hexachlorobenzene

Hexachlorobenzene was detected in all *E. pacifica* samples, with the lowest concentration from South Sound, and uniform concentrations across the other four tested Basins (Table 5 -- GLM of ln-transformed HCB by Basin, $F_{(4,14)}$=43.0, p<0.0001; THSD, p<0.001 for pairwise comparisons of South Sound with Elliott Bay, Main Basin, Whidbey Basin, and Hood Canal; Figure 11). All pairwise comparisons among other Basins indicated no significant differences (THSD, p>0.05).

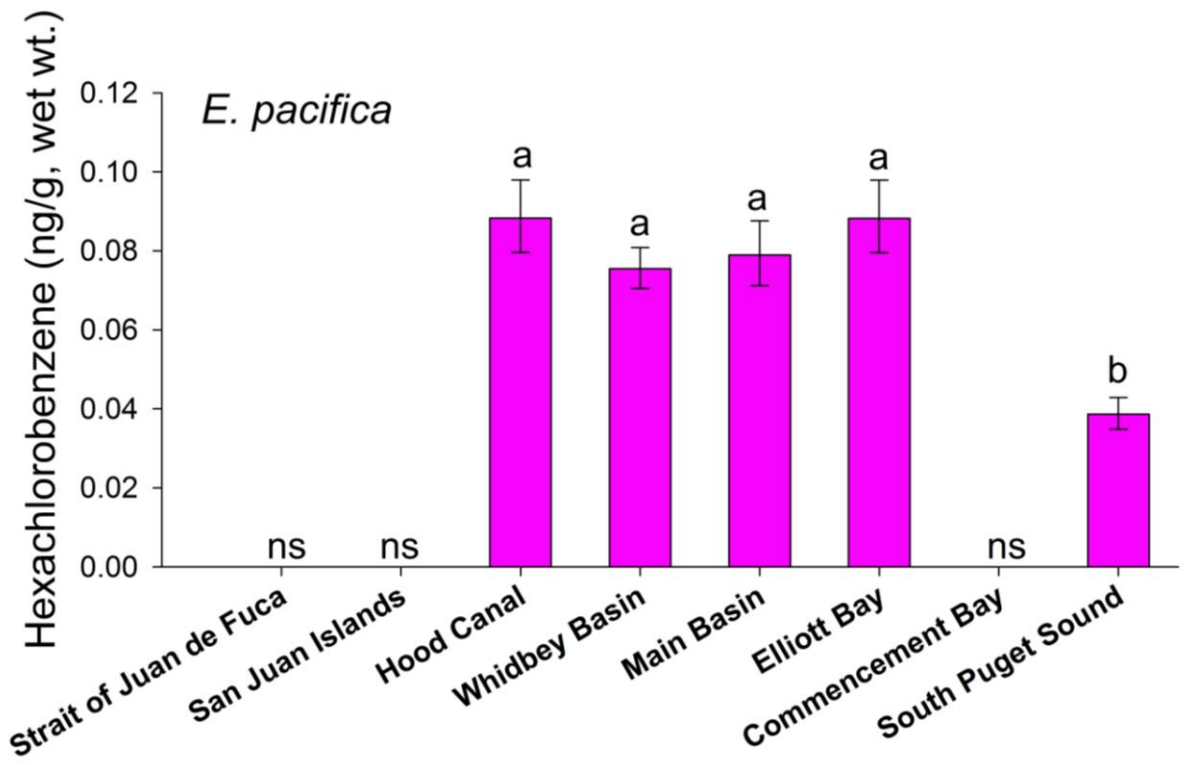

Figure 11. Geometric mean ± 95% confidence intervals for hexachlorobenzene in POM (green bars) and *E. pacifica* (pink bars). Individual sample concentrations are presented as triangles where n<3 for a Basin. Not sampled denoted as "ns". Bars with different lower case letters indicate THSD significant difference at α=0.05.

Dieldrin

Dieldrin was detected at low concentrations in all *E. pacifica* samples except for non-detects in one of seven from Whidbey Basin and all three from South Puget Sound (Table 5). Of the four testable Basins, the concentration of dieldrin in *E. pacifica* from Whidbey Island was significantly greater than Hood Canal (GLM of ln-transformed dieldrin by Basin, $F_{(3,11)}=6.5$, p=0.009; THSD p=0.017 for the Whidbey Basin comparisons with Hood Canal and p>0.05 for all other pairwise comparisons – Figure 12).

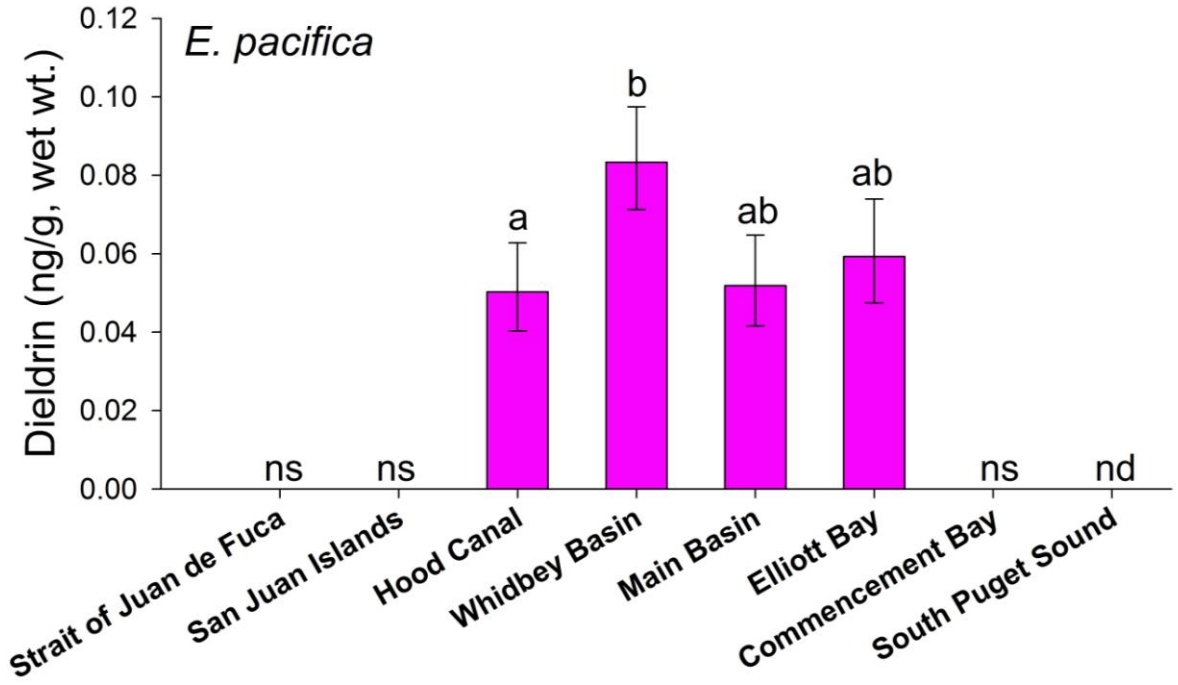

Figure 12. Geometric mean ± 95% confidence intervals for dieldrin in POM (green bars) and E. pacifica (pink bars). Individual sample concentrations are presented as triangles where n<3 for a Basin. Not sampled denoted as "ns". Not detected denoted as "nd". Bars with different lower case letters indicate THSD significant difference at α=0.05.

∑PAHs

PAH compounds were detected in all POM and krill samples (Table 5). Mean concentration of ∑PAH ranged from 3 to 1,084 ng/g wet wt. in POM and 6 to 35 ng/g wet wt. in the three krill species. Low-molecular-weight (LMW) PAH compounds were slightly more abundant than high-molecular-weight (HMW) accounting for 55% and 45% respectively of ∑PAH s (Table 12). The phenanthrene/anthracene group, comprising the parent compound, its four (C_1-C_4) alkylated homologs, and 1-methyl-7-isopropyl phenanthrene (retene) were the most abundant of PAH classes, accounting for 35.4% of TPAH, on average. Fluoranthenes (16.1%), chrysenes (11.5%), fluorenes (11.1%), and the single compound pyrene (8%) accounted for an additional 47.2%. All remaining compounds contributed less than 5% to the total, on average.

Overall we observed some of the greatest ∑PAH s in our urban Basins; for both POM and *E. pacifica* from Elliott Bay and in POM from Commencement Bay. In POM, ∑PAHs in individual samples from our two urban Basins ranged from 55 ng/g wet wt. in Commencement Bay to 149 to 2,020 ng/g wet wt. in Elliott Bay (compared to median concentrations ranging from 3 to 43

Table 12. Frequency of occurrence of Low Molecular Weight (LMW) and High Molecular Weight (HMW) polycyclic aromatic hydrocarbon compounds in POM and krill.

LMW Compounds	Mean % of Total	HMW Compounds	Mean % of Total
acenaphthylene (ACY)	0.9%	fluoranthene (FLA)	10.2%
acenaphthene (ACE)	2.4%	pyrene (PYR)	8.4%
fluorene (FLU)	2.8%	C_1-F/P	3.2%
C_1-Fluorene	2.2%	C_2-F/P	1.7%
C_2-Fluorene	2.6%	C_3-F/P	0.7%
C_3-Fluorene	3.5%	C_4-F/P	0.3%
dibenzothiophene (DBT)	0.5%	benzo[*a*]anthracene (BAA)	1.0%
C_1-dibenzothiophene	0.6%	chrysene† (CHR)	7.2%
C_2-dibenzothiophene	1.3%	C_1-chrysene	1.3%
C_3-dibenzothiophene	1.0%	C_2-chrysene	1.1%
C_4-dibenzothiophene	0.6%	C_3-chrysene	0.6%
anthracene (ANT)	0.7%	C_4-chrysene	1.4%
phenanthrene (PHN)	10.4%	benzo[*b*]fluoranthene (BBF)	1.8%
C_1-P/A	8.2%	benzo[*k*]fluoranthene†† BKF)	1.2%
C_2-P/A	8.2%	benzo[*e*]perylene (BEP)	1.2%
C_3-P/A	6.1%	benzo[*a*]pyrene (BAP)	0.8%
C_4-P/A	1.1%	perylene (PER)	1.2%
retene*	1.3%	indeno-pyrene (IDP)	0.9%
Total	54.6%	dibenzoanthracene (DBA)	0.1%
		benzo[*z*]pyrene (BZP)	1.2%
		Total	45.4%

*1-methyl-7-isopropyl phenanthrene

†coeluted with triphenylene

††coeluted with benzo[*j*]fluoranthene

ng/g wet wt. from the other Basins; Table 5). Of particular note here is the large disparity in the two Elliott Bay POM concentrations, of which one was taken in August, 2009 at the Seattle Waterfront, and the other taken in October, 2009 at the mouth of Duwamish River's west waterway. In both sampling efforts we targeted observable phytoplankton blooms, however in addition to the obvious seasonal and spatial differences between the samples, several other factors could account for the observed disparity; dominant phytoplankton species in the two Elliott Bay samples were different – dinoflagellates (dominated by *Ceratium fusus*) in August, and diatoms (dominated by *Thalassiosira* sp) in October. Carbon isotope and lipid content of

these samples were also different, resulting in disparate $\delta^{13}C$ values (-22.5 in the October sample *vs* -20.9 in the August sample).

The next greatest mean ∑PAH concentrations in POM were observed from the San Juan Islands and Strait of Juan de Fuca (mean of 43 ng/g wet wt.), with Strait of Juan de Fuca POM exhibiting a range of 107 ng/g, and San Juan Islands POM only 34 ng/g. These two were indistinguishable from South Sound and Main Basin POM (GLM of ln-transformed ∑PAH by Basin, $F_{(5,26)}=6.53$, $p=0.001$; THSD pairwise comparisons, $p>0.05$, while these four exhibited ∑PAH that were significantly greater than Whidbey Basin and Hood Canal (THSP, $p<0.02$).

∑PAH in *E. pacifica* was greatest from Elliott Bay (17 ng/g wet wt.), which was significantly greater than the other three Basins that had sufficient sample size to allow testing (GLM of ln-transformed ∑PAH by Basin, $F_{(3,12)}=7.71$, $p=0.008$; THSD, $p=0.029$, 0.042 and 0.007 for Elliott Bay comparisons with Main Basin, Whidbey Basin, and Hood Canal, respectively).

∑PAHs were generally greater in POM than krill for Elliott Bay and South Sound (visual comparison of means for Basins with sample size >2 with individual concentrations, Figure 13). This disparity was especially apparent in samples from Elliott Bay, where ∑PAH in POM was 23 to 270x greater than *E. pacifica* from that location. For the three Basins where statistical comparisons were possible ($n\geq3$ for both POM and *E. pacifica*), there was no significant difference in ∑PAHs between POM and *E. pacifica* for the Main Basin and Hood Canal (ANOVA of ln-transformed ∑PAHs between POM and *E. pacifica*, $F_{(1,5)}=0.003$, $p=0.958$ for the Main Basin, and $F_{(1,4)}=5.4$, $p=0.082$ for Hood Canal). *E. pacifica* exhibited significantly greater ∑PAHs than POM in Whidbey Basin (ANOVA of ln-transformed ∑PAHs between POM and *E. pacifica*, $F_{(1,15)}=0.6.9$, $p=0.019$).

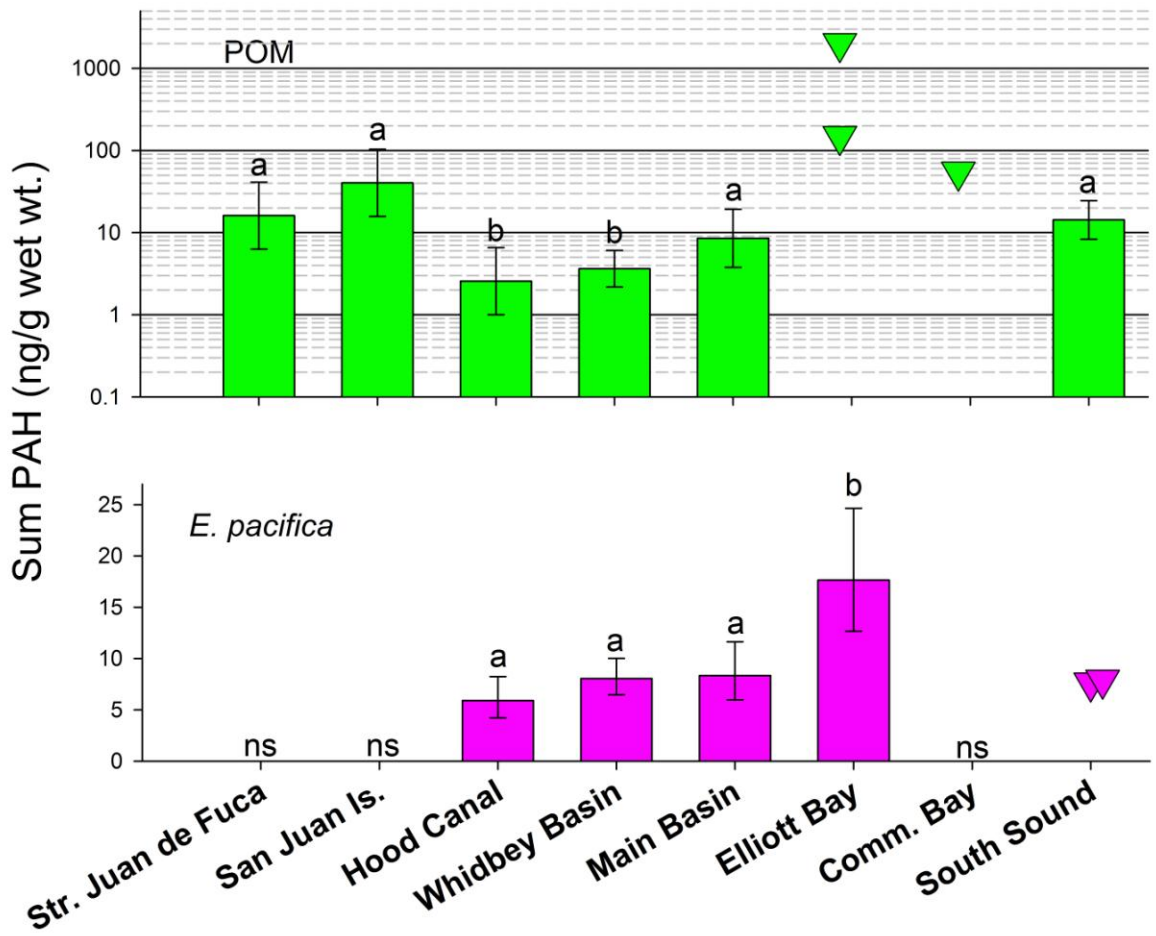

Figure 13. Geometric mean ± 95% confidence intervals for ∑PAHs in POM (green bars, upper plot -- note log scale to accommodate high Elliott Bay values) and E. pacifica (pink bars, lower plot). Individual sample concentrations are presented as triangles where n<3 for a Basin. Not sampled denoted as "ns". Bars with different lower case letters indicate THSD significant difference at α=0.05.

POPs in *T. spinifera* and *T. raschii*

Overall, all POPs were substantially greater in the three *T. spinifera* composites (three samples taken from one location in the Main Basin near Vashon Island; Figure 1) than the other krill species and in POM in all Basins (Table 5). This species had POP concentrations ranging from 1.5 to 5.5 times greater than *E. pacifica* from Elliott Bay, the Basin that consistently exhibited the greatest POP concentrations in *E. pacifica*. On average *T. spinifera* were 25% to 67% larger than *E. pacifica* (Figure 4), they exhibited a higher lipid concentration (9.3%, which was over 3 times greater than any *E. pacifica* samples) and a relatively high mean $\delta^{15}N$ (11.5‰, which was 1.8 to 2.9 times greater than *E. pacifica* --Table 3).

One *T. raschii* composite, taken near Useless Bay, Whidbey Island (Figure 1) exhibited concentrations of ∑PCBs and ∑PBDEs similar to other *E. pacifica* from Elliott Bay or other Basins. This species showed two to three times-greater concentration of most organochlorine pesticides and PAHs than *E. pacifica* from Elliott Bay. *T. raschii* were similar in size (Figure 4), lipid content, and $\delta^{15}N$ to *E. pacifica*.

Pattern analysis of PCBs

Analysis of homolog and congener distribution in POM and krill was hampered by the limited number of congeners (n=40 congeners or coeluting groups) available from the analysis used in this study. Overall we observed greater abundance of higher molecular weight PCBs in a) POM from Elliott Bay compared to POM from other Basins (Figure 14), and b) in *E. pacifica* from Elliott Bay and in both *Thysanoessa* species from the Main Basin compared to *E. pacifica* from other Basins (Figure 15. This pattern was more pronounced in the krill species, as noted by the relatively high proportion of penta- (blue bars) and hexachlorinated (yellow bars) congeners.

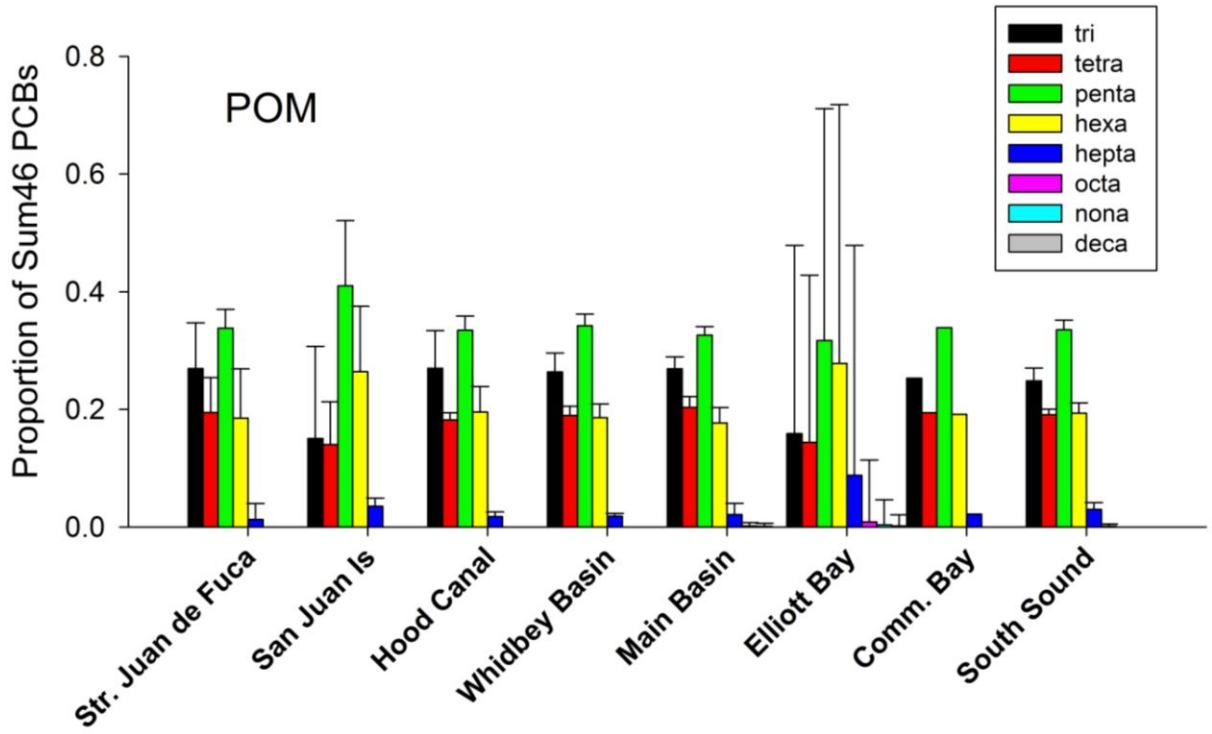

Figure 14. Relative abundance of eight PCB homolog groups in POM, each expressed as a mean fraction of the Sum46PCBs ± 95% confidence interval.

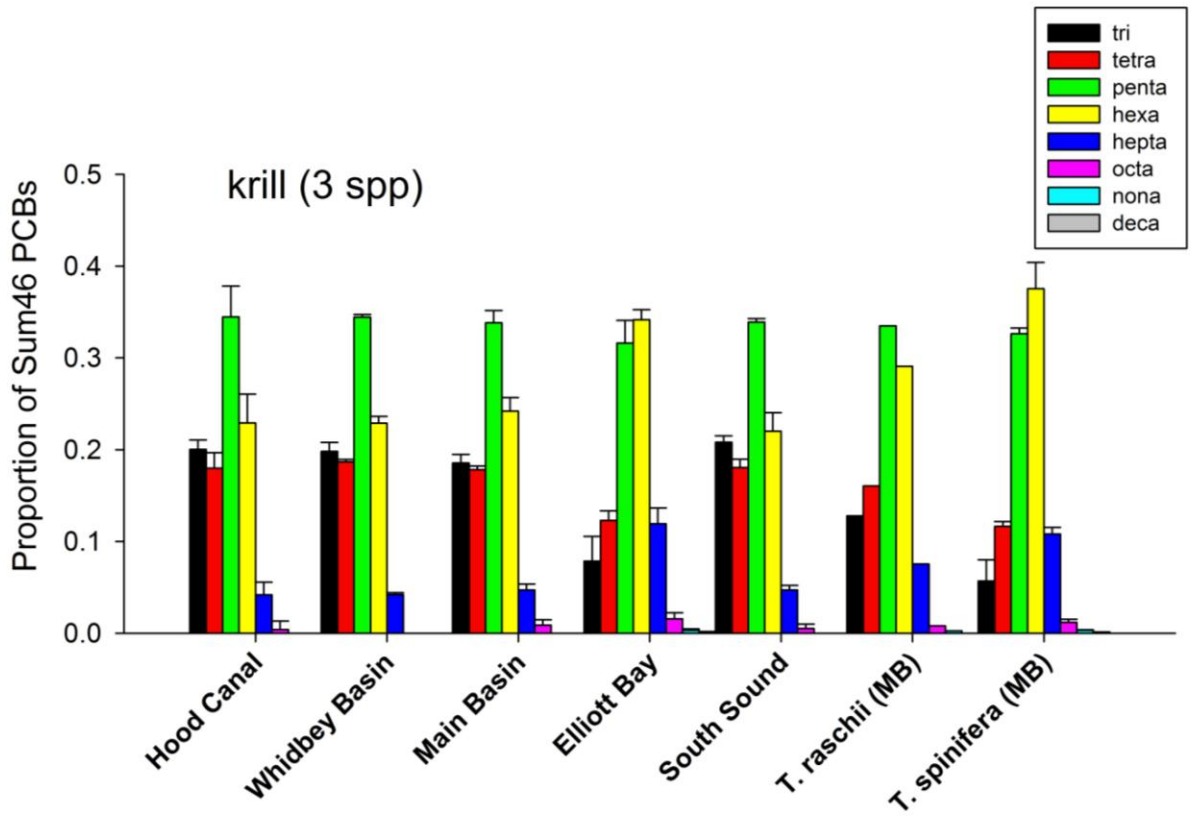

Figure 15. Relative abundance of eight PCB homolog groups in krill, each expressed as a mean fraction of the Sum46PCBs ± 95% confidence interval. All krill are *E. pacifica* except for *Thysanoessa* spp as noted from the Main Basin (MB).

Pattern analysis of PAHs

Overall the PAH pattern (expressed as proportion of each of 38 compounds to ∑PAHs) in POM was distinguished from krill by consistently greater abundance of high molecular weight (mass 252, 276 and 278 groups), 5-ring compounds such as benzo[*k*]fluoranthene (BKF), benzo[*e*]pyrene (BEP), and benzo[*a*]pyrene (BAP) (Figure 16, red bars). Many of these compounds were not detected in krill (Figure 17). In addition, POM appeared to exhibit greater proportions of four-ring compounds, notably benzo[*a*]anthracene (BAA), and the alkylated homologs of chrysene (CHR) and fluoranthene/pyrene ($C_{1\text{-}4}$-F/A). Other notable species differences include the relative lack of acenaphthene (ACE) and acenaphthylene (ACY) in POM, and alkylated homologs of phenanthrene/anthracene and fluoranthene/pyrene in krill.

In most Basins the parent compounds PHN/ANT, FLA/PYR, and CHR exhibited greater concentration than their alkylated homologs for both POM and krill. One exception to this pattern was POM from San Juan Islands, where C_1 homologs of these groups were equivalent or greater in proportion than their parent compounds.

In comparing the urban Basins to less Developed Basins, POM from Elliott Bay and Commencement Bay exhibited a dominance of high molecular weight, four-ring compounds, whereas 3-ring fluorene, phenanthrene and anthracene compounds were more abundant in POM from other Basins. This pattern was generally true, although less pronounced, in *E. pacifica* from Elliott Bay than the other Basins.

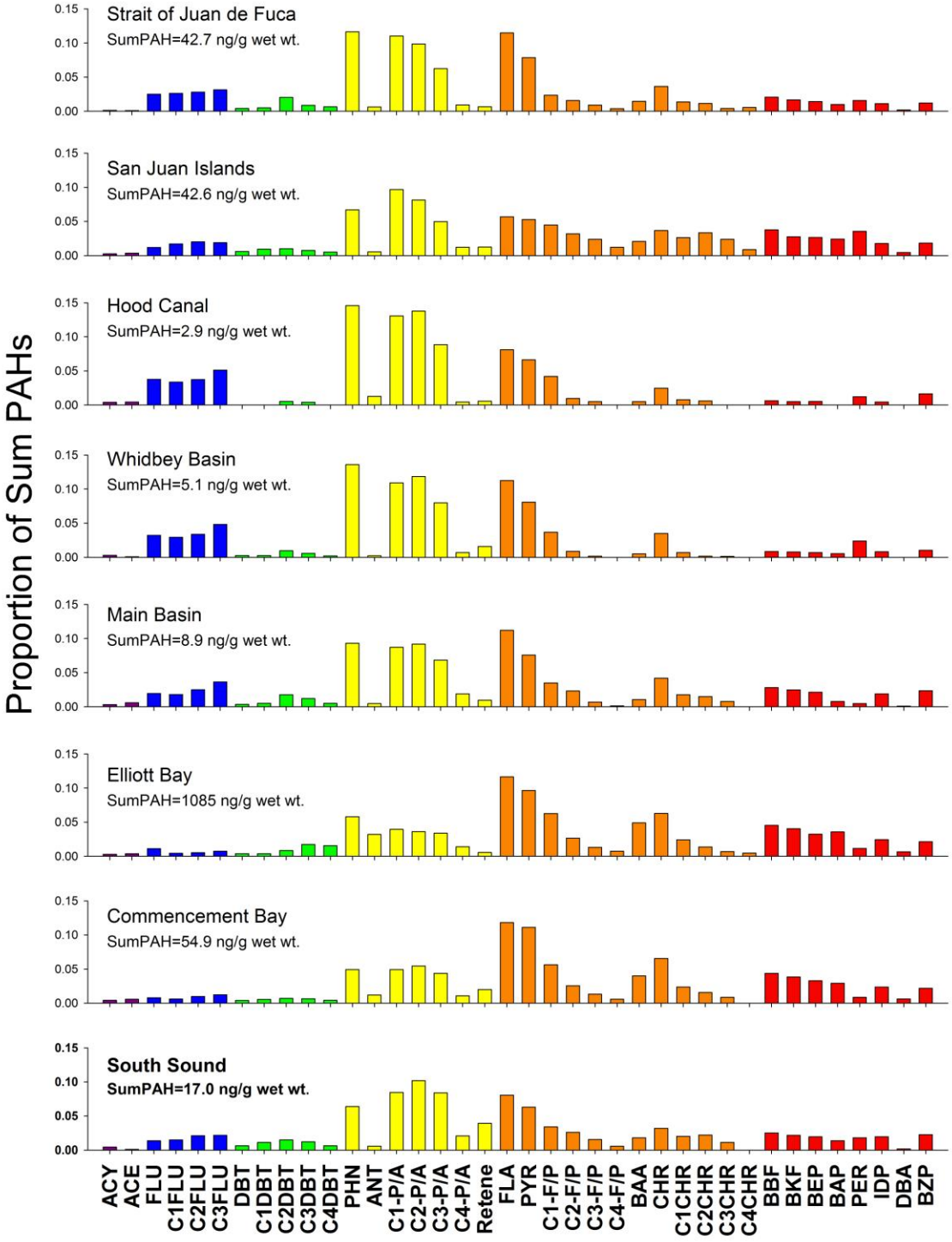

Figure 16. Relative abundance of 38 PAH compounds in POM from eight Puget Sound Basins. Each compound expressed as an average proportion of ∑PAH. PAHs arranged by increasing molecular weight and number of rings from left-to-right. See Table 12 for PAH abbreviations.

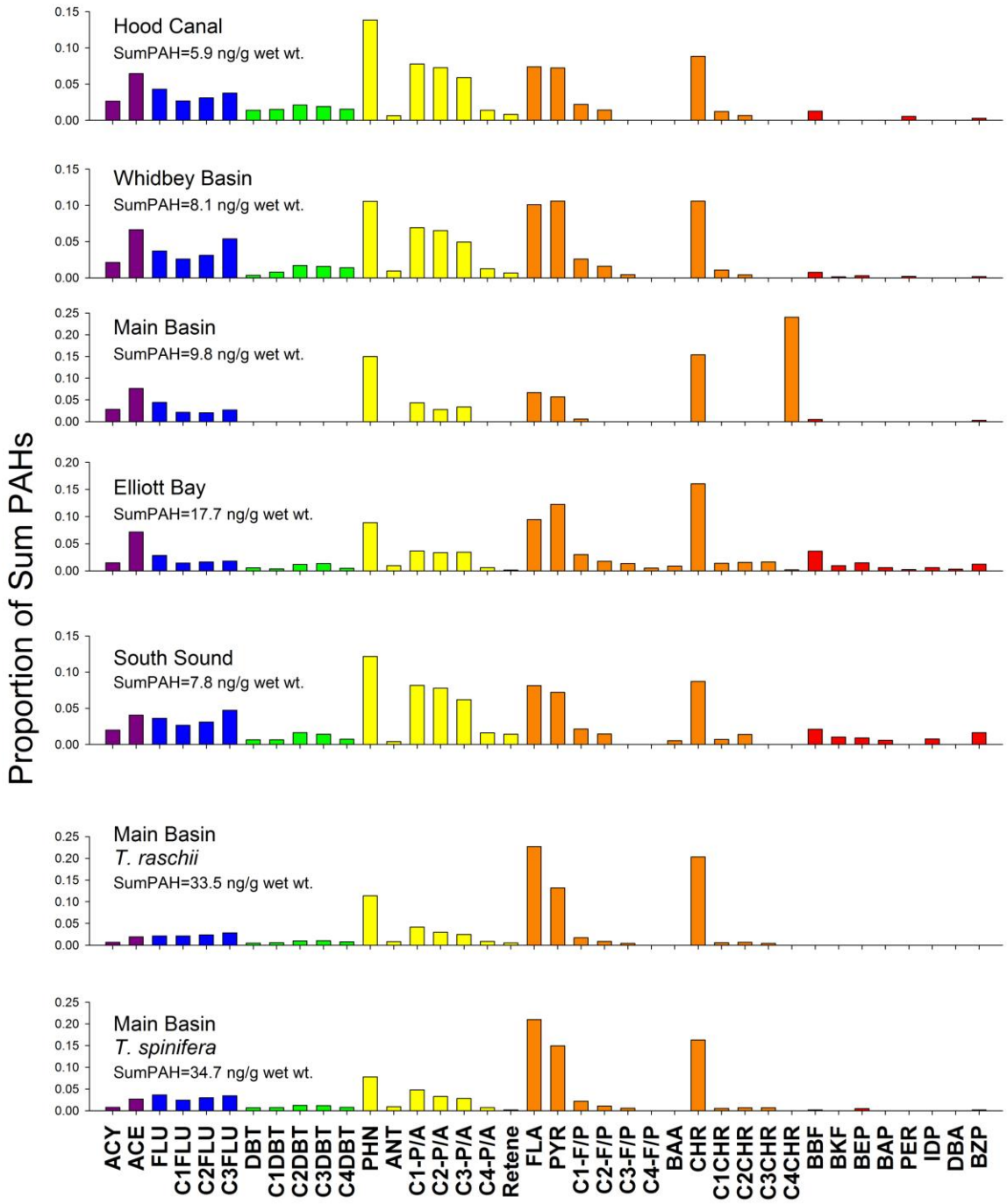

Figure 17. Relative abundance of 38 PAH compounds in three species of krill from five Puget Sound Basins. Krill are *E. pacifica* unless noted otherwise noted. Each compound expressed as an average proportion of ∑PAH. PAHs arranged by increasing molecular weight and number of rings from left-to-right. See Table 12 for PAH abbreviations.

Bioaccumulation and Biomagnification of POPs

Although we did not measure POP concentrations in water in this study and so cannot calculate bioaccumulation factors directly for POM, the concentration of most POP classes in POM appeared to reflect their Basin conditions – urban POM exhibited higher concentrations than POM from Less Developed Basins. This simple observation, combined with the relatively high concentration of some POPs in POM suggests that these organisms were exposed to, and either adsorbed or absorbed contaminants from the water.

Our sampling for POM and krill occurred over large areas and across several months, making it difficult to match krill samples with POM upon which they could have grazed. Krill and POM samples were taken only where significant populations aggregated, and we never encountered enough krill to sample from the San Juan Islands, Eastern Strait of Juan de Fuca, or Commencement Bay.

POM and krill from Hood Canal and South Sound were either sampled too far apart in space or time to be considered synoptic. The closest time- and location-matches between krill and POM were in Elliott Bay, Whidbey Basin, and selected Main Basin sampling sites. A comparison of PCB concentration between POM and krill from data subset of these three Basins generally showed a greater concentration in krill than POM, shown as three points above the 1:1 ratio line in Figure 18. For PAHs, two locations (Whidbey and Main Basins) exhibited greater concentration in *E. pacifica* than POM (Figure 19). POM from Elliott Bay exhibited an eight-fold greater concentration of ∑PAHs than *E. pacifica* taken from that location. This latter difference was driven by the exceptionally high ∑PAH concentration in one of the two Elliott Bay POM samples.

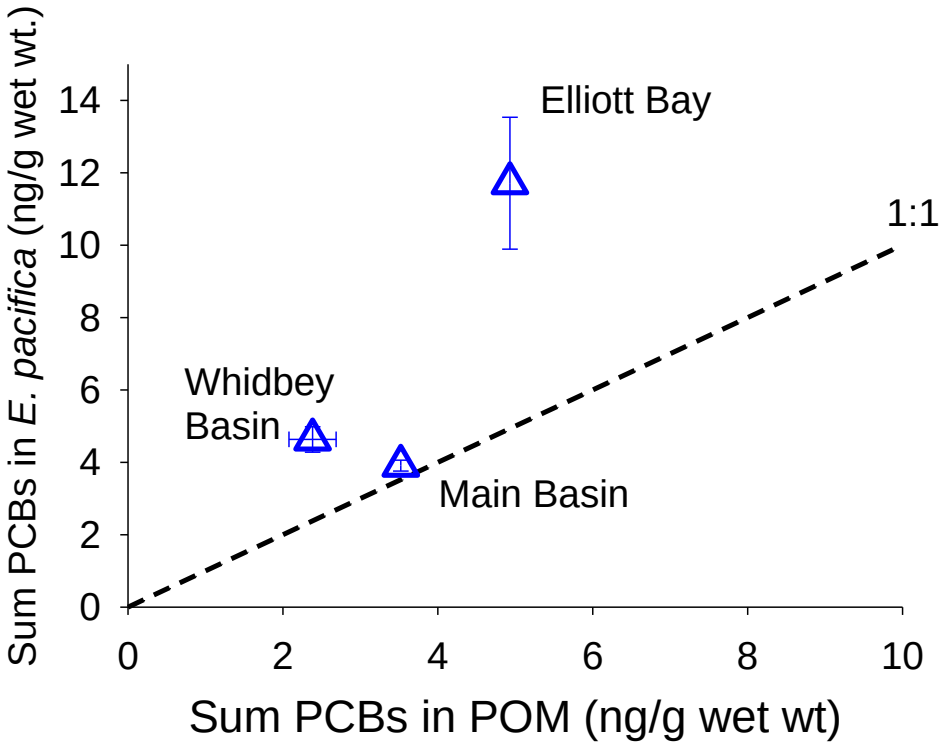

Figure 18. Comparison of PCBs in krill and POM for locations where samples were collected synoptically.

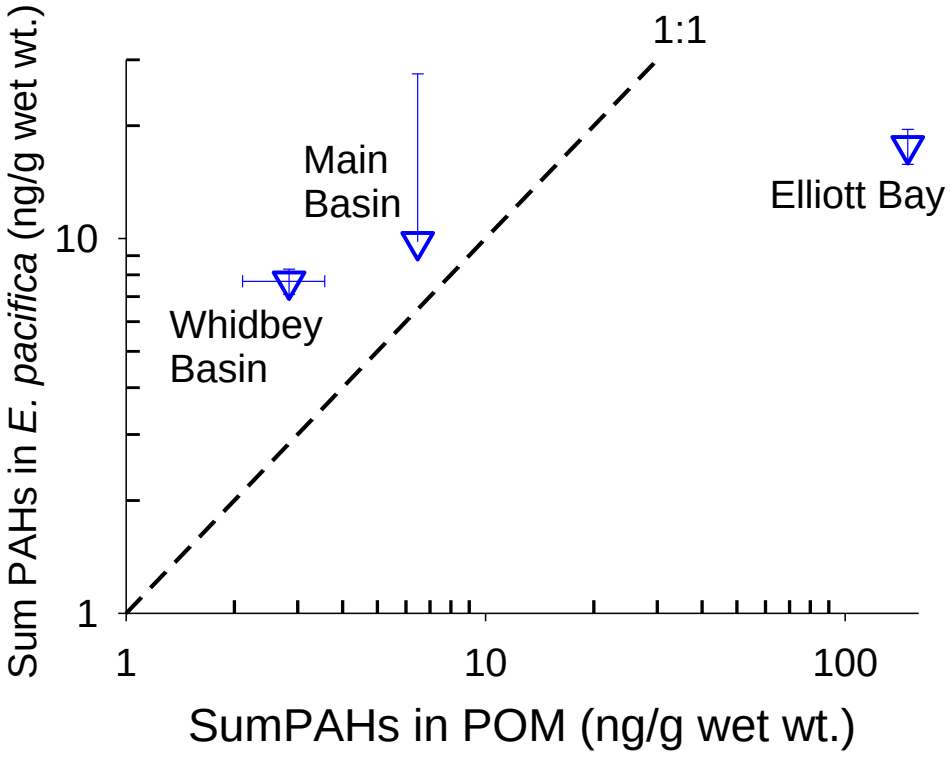

Figure 19. Comparison of PAHs in krill and POM for locations where samples were collected synoptically. Note logarithmic scale.

Discussion

The primary objectives of this study were to evaluate the extent and magnitude of selected persistent organic pollutants (POPs) in Puget Sound's plankton, with a focus on primary producers (phytoplankton) and a representative primary consumer (krill). Data reported in this study represent the first broad-geographic-scale evaluation of POPs in the base of Puget Sound's pelagic food chain, and complement two other companion studies focusing on predators of krill (West et al. 2011), and apex predators (harbor seals, Noël et al. 2011). Results from these three studies will be combined with ongoing POP monitoring studies covering a wide range of fishes to further evaluate pathways of POPs in Puget Sound's pelagic food web. In addition this study provides input data for Department of Ecology models and efforts on fate and transport of POPs in Puget Sound.

Basin Comparison

As observed with Pacific hake (*Merluccius productus*) in a companion study (West et a., 2011), Elliott Bay stood out as the Basin wherein krill exhibited the greatest body residues of three persistent bioaccumulative toxics, PCBs, PBDEs and DDTs, and Hood Canal as the Basin with the least concentrations. The other Basins exhibited intermediate concentrations, supporting the hypothesis that urban areas in Puget Sound represent a focus of these contaminants entering the pelagic food web at its lowest trophic levels. Because *E. pacifica* were not found in Strait of Juan de Fuca Basins we are unable to use this species to compare conditions from inland waters with ocean-boundary waters.

Basin patterns of these three persistent bioaccumulative contaminants were less easily interpreted in POM because we only took three samples from urban bays -- two from Elliott Bay (both widely separated in time and space), and one from Commencement Bay, and variability in POM samples was great within Basins. In any case, PCBs, PBDEs and DDTs in the two individual POM samples were greatest from Elliott Bay as well, but not from the single Commencement Bay POM sample.

PAHs, although not considered bioaccumulative in many organisms, were the most ubiquitous of the persistent organic pollutants (POPs) that we measured, and occurred in the greatest concentration of all POPs measured in this study, in both krill and POM. Many PAH compounds are considered persistent in the environment, however many can also be metabolized by organisms (Varanasi, 1989), and as such may not accumulate in their bodies. Warshawsky et al. (1990) observed evidence of PAH metabolism by freshwater green algae, and Rust et al. (2004) reported a wide range in ability among invertebrates to metabolize PAHs. Our measurements of PAHs in both POM and krill provide evidence that these contaminants can accumulate in the species we measured.

This evidence is especially pertinent to questions regarding the fate of PAHs in the pelagic food web and transfer of these contaminants to predators. Pacific herring, a primary predator of krill in Puget Sound, exhibited significant exposure to PAHs, as shown by measurements of PAH-metabolites in their bile (Puget Sound Action Team 2002). However, lacking accumulation of PAH residues in herring tissues there is little evidence that PAHs are trophically transferred to predators that consume herring, suggesting PAHs tend to accumulate only in the lower trophic levels in Puget Sound's pelagic food web.

PAHs presented a somewhat different Basin pattern than PCBs, PBDEs and DDTs. Although POM from Elliott Bay clearly showed the greatest PAH concentrations, individual samples from other Basins, (San Juan Islands, Strait of Juan de Fuca, and South Sound) showed relatively high concentrations. We also observed that POM exhibiting these highest PAH concentrations were taken near to marinas, ferry terminals, or shoreline roadways. Although this study was not designed to test exposure patterns on such a small scale, this coincidence is notable. Moreover, it suggests the importance of understanding shoreline or shore-based development as sources of PAH to the pelagic food web, even in non-urbanized or less developed Basins.

The Basin pattern of non-DDT related organochlorine pesticides was impossible to evaluate using POM, because of the preponderance of non-detects. *E. pacifica* from South Sound were particularly low in chlordanes, hexachlorobenzene and dieldrin, and *E. pacifica* from Whidbey Basin were comparatively high in dieldrin. This latter observation mirrors results from Pacific hake (West et al. 2011), and may be related to land use patterns in that Basin.

PCB homolog patterns support an hypothesis of urban areas as source of PCBs to POM and krill. We observed a greater proportion of higher-molecular-weight PCB homolog groups (hexa- and hepta-chlorinated biphenyls) in both POM and *E. pacifica* from Elliott Bay, and an increase in relative abundance of lighter homolog groups with increasing distance from Elliott Bay. A full analysis of PCB patterns was not possible in krill and POM in this study because the analytical method only reported 40 PCB congeners or coeluting groups. However West et al. (2011) observed a similar and more obvious pattern in Pacific hake using 203 congeners, as did Ross et al. (2004), Cullon et al. (2005), and Noel et al. (2011) in harbor seals (*Phoca vitulina*). These results agree with the hypothesis that heavier, more chlorinated congeners tend to move more slowly from their source through the environment than lighter fractions, primarily related to molecular weight or size, particle-affinity and lipophilicity.

Congener-patterns were not evaluated in PBDEs because so few congeners were detected (seven in POM, and eight in krill). Moreover, detection limits in this study for PBDEs congeners were in the 0.05 to 0.07 ng/g wet wt. range, compared to the detection limits at roughly one-hundredth that range for individual PBDE congeners in the high-resolution GC/MS method used with Pacific hake in West et al. (2011). Overall however, the dominant PBDE congeners in POM and krill were congruent with those from a number of their primary predators including Pacific hake

(West et al., 2011), and other species monitored by WDFW/PSAMP including Chinook salmon, coho salmon, and Pacific herring (WDFW unpublished data, Puget Sound Assessment and Monitoring Program).

Forensic analysis of PAH patterns in abiotic media is sometimes used to infer terrestrial sources (e.g., Yunker, et al. 2002), however difficulties arise in applying those principles to living organisms. Differential affinity, uptake and metabolism of individual PAH chemicals from the environmental mix to which organisms are exposed may mask patterns that could otherwise distinguish sources. Given these issues, the ubiquitous presence of many of the 38 PAH compounds we measured in POM and krill invite some inference of potential sources. Perhaps the most obvious pattern we observed in the relative abundance of PAHs was the greater representation in POM of four- and five-ring, high molecular weight compounds, compared to krill. Such a pattern is consistent with exposure to combustion sources ((Yunker, et al., 1999). The presence of dibenzothiophenes in POM and krill from most Basins indicates some exposure to petroleum products, however, the relatively low concentration of that parent compound to its alkylated homologs suggests a petroleum source that had been weathered (i.e. was not fresh).

Trophic Transfer of POPs

Follow-up studies are planned to compare POP results in POM and krill from this study within the context of the full Puget Sound food web, including secondary (e.g., Pacific hake and Pacific herring) and tertiary consumers (e.g., harbor seals) in Puget Sound. We observed some evidence for trophic transfer of POPs between the lowest trophic levels in Puget Sound -- primary producers to primary consumers, however conclusions vary depending on whether analyses are conducted on a wet- dry-, or lipid-basis. Our wet-weight comparison of POPs between POM and krill was consistent with magnification of concentration from the primary producers to consumers. However, this pattern was not consistent when analyzed on a dry weight basis (not shown in this report, for brevity), wherein POP concentrations in POM generally exceeded krill.

As mentioned above, isolating phytoplankton and removing ambient, extracellular water from POM samples without damaging cells (and losing cell contents) is particularly problematic. Methods used by others include centrifuging (Chiuchiolo, Dickhut et al. 2004), vacuum-filtering (e.g., (Hobson, Fisk et al. 2002), freeze-drying gravity-filtered samples (Taylor et al. 1991) and freezing gravity-filtered samples. In order to preserve the integrity of cells and minimize the risk of losing cell contents via rupturing or volatilization, we chose the last method. Whereas %Moisture in krill was roughly 85%, and consistent with other organisms we have analyzed, %Moisture in POM was closer to 95%, and because of this, POP concentrations in POM may have been underestimated using wet weight. Analysis of dry weights may alleviate this issue however it introduces error associated with the %Moisture method.

Sampling Considerations

Sampling sufficient particulate matter from surface waters using phytoplankton lift nets was relatively straightforward; however isolating phytoplankton from field samples was unfeasible. We were confident that the bulk of our POM samples comprised phytoplankton but we cannot rule out the possibility that inorganic matter, including anthropogenic substances such as microplastics contributed to the contaminant loads we measured.

Isolating and sorting primary consumer-plankton was tedious but feasible, especially for large-bodied species such as euphausiid krill. Krill appeared to adequately reflect local contaminant conditions on a spatial scale that was useful to evaluate Basin-loading questions where they were present. Although locally abundant, we had difficulty in finding krill in many areas of Puget Sound. It is possible that targeting krill during their spawning season (spring) may make it easier to locate populations that may otherwise have eluded us in this study. We commonly observed several other large-bodied, vertically migrating zooplankton in our samples, including glass shrimp (*Pasiphaea* spp), arrow worms (Phylum Chaetognatha), copepods, and amphipods, however krill were the most consistently abundant group.

Krill Species Comparison

Of the three krill species we sampled, *T. spinifera* was distinguished from the others in their greater size, trophic level, tissue lipids, and concentration of virtually all POPs. Such wide disparity in POP tissue residues between otherwise similar (taxonomically closely related) species may be related to these life history characteristics. This necessitates careful selection of species and sorting to avoid mixing species in zooplankton samples. Some of the variability in our data may have been related to incomplete sorting, however we observed *T. spinifera* from only one location in the Main Basin, and those samples appeared to have been taken from mono-specific krill swarms.

T. spinifera also appeared to exhibit a shift in PCB homolog towards heavier congeners, compared with *E. pacifica* and *T. raschii* from the same Basin. Because this species was taken from the same Basin (same putative PCB source) as other species that showed the "lighter" PCB signature, it seems likely a species-specific difference in feeding ecology or metabolic capacity can influence residue patterns at this low level in the food chain.

Summary & Conclusions

This report represents the first efforts at identifying patterns of toxic contaminants in primary producers and primary consumers in Puget Sound's pelagic food web. This work is part of a larger effort aimed at a) monitoring status and trends of toxic contaminants in Puget Sound, b) identifying where, when and how contaminants enter the food chain, c) understanding the fate and transport of contaminants in the food web, and d) providing guidance for determining how best to spend limited resources in reducing the exposure of Puget Sound's biota to contaminants.

The most important conclusions from this effort are:

- Overall, PAHs were the most frequently detected of the POPs, and they occurred in greatest concentrations across all Basins in both POM and krill, compared to the bioaccumulative POPs
- POPs, including PCBs, PBDEs, DDTs, and PAHs in both POM and krill exhibited a correlation with urban waters, suggesting urban waters represent areas where POPs enter the pelagic food chain.
- Relatively high PAHs were also found in POM from some areas of less developed Basins, but near to shoreline developments such as marinas or roadways, suggesting these developments as sources of PAHs in the pelagic food web.
- PAHs probably accumulate and are transferred up the food chain from phytoplankton to krill, to their fish-predators, and are subsequently metabolized by fish-predators
- PAH patterns in POM appeared to be dominated by pyrogenic compounds, although they also contained some petrogenic constituents in a pattern that suggested weathering of oil.
- although POP concentrations in krill suggest bioaccumulation from consuming contaminated food, evidence for bioaccumulation of POPs from POM from this study is equivocal
- the Basin-pattern of PBDE accumulation in krill was similar to PCBs; high concentrations in urbanized waters and low concentrations in less developed, more ocean-influenced basins – suggesting a similar mechanism of loading and dispersal in Puget Sound
- variability in population abundance, timing, and spatial distribution, as well as difficulties in isolating organisms for analysis make phytoplankton a difficult indicator of POP exposure
- short lived and resident primary consumers such as krill integrate contaminant and trophic conditions and may be suitable for measuring POPs low in the pelagic food chain
- the organochlorine pesticide diledrin, appeared to be higher in waters nearer to agricultural land use, albeit in extremely low concentrations overall.

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