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Spatial extent, magnitude, and patterns of persistent organochlorine pollutants in Pacific herring (*Clupea pallasii*) populations in the Puget Sound (USA) and Strait of Georgia (Canada)

James E. West^{a,*}, Sandra M. O'Neill^a, Gina M. Ylitalo^b

^aWashington State Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501-1091, USA

^bNorthwest Fisheries Science Center, National Marine Fisheries Service, 2725 Montlake Blvd. E., Seattle, WA 98112, USA

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ABSTRACT

We examined the geographic distribution and magnitude of three persistent organic pollutants (POPs) in Pacific herring, representing three populations from Puget Sound, Washington State, USA and three from the Strait of Georgia (British Columbia, Canada and Washington State). We measured PCBs, DDTs and DDT isomers, and hexachlorobenzene in whole herring using high performance liquid chromatography, which provided a relatively inexpensive estimation of total PCBs, including the most commonly encountered congeners, and DDT isomers. Puget Sound herring were 3 to 9 times more contaminated with polychlorinated biphenyls (PCBs) compared to Strait of Georgia herring and 1.5 to 2.5 times more contaminated with DDTs. Hexachlorobenzene levels were low in all samples, relative to PCBs and DDTs, and one Strait of Georgia population (Cherry Point) had significantly lower HCB levels than the rest. A multidimensional scaling map of the pattern or “fingerprint” of POPs in the six herring populations suggests strong environmental segregation of Puget Sound herring from the Strait of Georgia populations, and isolation of all Strait of Georgia populations from each other. This segregation likely resulted from differential exposure to contaminants, related to where these populations reside and feed, rather than differences in their age, size, trophic level, or lipid content.

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1. Introduction

Much attention has been paid to the accumulation and effects of persistent organic pollutants (POPs) in marine organisms, especially high trophic-level predators, starting with investigations of Baltic Sea biota some 40 years ago (Jensen et al., 1969). In more recent years, biomagnification of POPs resulting from trophic transfer via prey species has been identified as an important pathway for movement of POPs in both marine and freshwater food webs (Thomann, 1989; Bentzen et al., 1996; Fisk et al., 2001; Hoekstra et al., 2003). In addition, the burden

of POPs carried in the bodies of these marine fishes is now being viewed as a significant environmental reservoir of POPs (Mackenzie et al., 2004).

Lipophilic POPs such as polychlorinated biphenyls (PCBs), dichlorodiphenyl-trichloroethane (DDT), chlordanes, polychlorinated dibenzodioxins (PCDDs), and dibenzofurans (PCDFs) have been reported in a food-web context in a number of small, schooling pelagic planktivorous (commonly referred to as forage fish) including Atlantic herring, *Clupea harengus* (Hording et al., 1997; Weisbrod et al., 2001), Baltic herring, *C. harengus* (Strandberg et al., 1998), anchovies, *Engraulis* spp

* Corresponding author. Tel.: +1 360 902 2842; fax: +1 360 902 2944.

E-mail addresses: westjew@dfw.wa.gov (J.E. West), oneilsmo@dfw.wa.gov (S.M. O'Neill), gina.ylitalo@noaa.gov (G.M. Ylitalo).

(Jarman et al., 1996), sprat, *Sprattus sprattus* (Vuorinen et al., 2002), and smelt, *Osmerus mordax* (Hording et al., 1997).

Pacific herring (*Clupea pallasii*), Pacific sandlance (*Ammodytes hexapterus*), osmerid smelts and northern anchovies (*Engraulis mordax*) make up the bulk of the forage fish guild in the Puget Sound and Strait of Georgia food web. Because of their high abundance, high body fat, broad geographic distribution, and central position in the region's food web, they may play an important role in transferring lipophilic POPs to their predators, as well as predators at higher trophic levels. These species are important prey to virtually every large carnivorous fish species, several species of marine mammals, and many piscivorous seabirds in these waters.

Fish predators on this guild include migratory pelagic species such as chinook and coho salmon, *Oncorhynchus tshawytscha* and *O. kisutch* (Brodeur and Percy, 1992; Gearin et al., 1994; Higgs et al., 1995), less migratory pelagics such as Pacific hake, *Merluccius productus* (Outram and Haegle, 1972; Tanasichuk et al., 1991) and spiny dogfish *Squalus acanthias* (Jones and Geen, 1977; Tanasichuk et al., 1991), wide-ranging demersal species such as Pacific cod *Gadus macrocephalus* (Westrheim and Harling, 1983; Walters et al., 1986; Westrheim et al., 1989), reef-resident demersal species such as copper and quillback rockfish, *Sebastes caurinus* and *S. maliger* (Murie, 1995), and benthic

predators such as lingcod, *Ophiodon elongatus* (Cass et al., 1990). In addition, small, schooling, pelagic planktivores are important prey to marine mammals including harbor seals, *Phoca vitulina* (Olesiuk, 1993) and harbor porpoise, *Phocoena phocoena* (Gearin et al., 1994), and they dominate the diet of a number of Puget Sound/Strait of Georgia seabirds, including common murre, *Uria aalge* and rhinoceros auklet, *Cerorhinca monocerata* (Lance and Thompson, 2005).

The inland marine and estuarine waters of Washington State and British Columbia comprise an extensive series of relatively deep, fjord-like basins, including the Strait of Georgia and Puget Sound. Over the last 100 years the region has experienced many of the stressors typically endured by nearshore marine ecosystems such as overfishing and habitat loss (West, 1997). Of particular concern is exposure of biota to anthropogenic toxic contaminants, because the region is experiencing rapid population growth. Puget Sound's watershed is more densely populated, and its shoreline more developed with industry and urban centers than the Strait of Georgia, and the Puget Sound is a much smaller body of water than the Strait of Georgia, occupying roughly one-third its surface area and one-sixth its volume. Moreover, the enclosed nature of these inland marine and estuarine waters may impede the dilution of contaminants relative to more

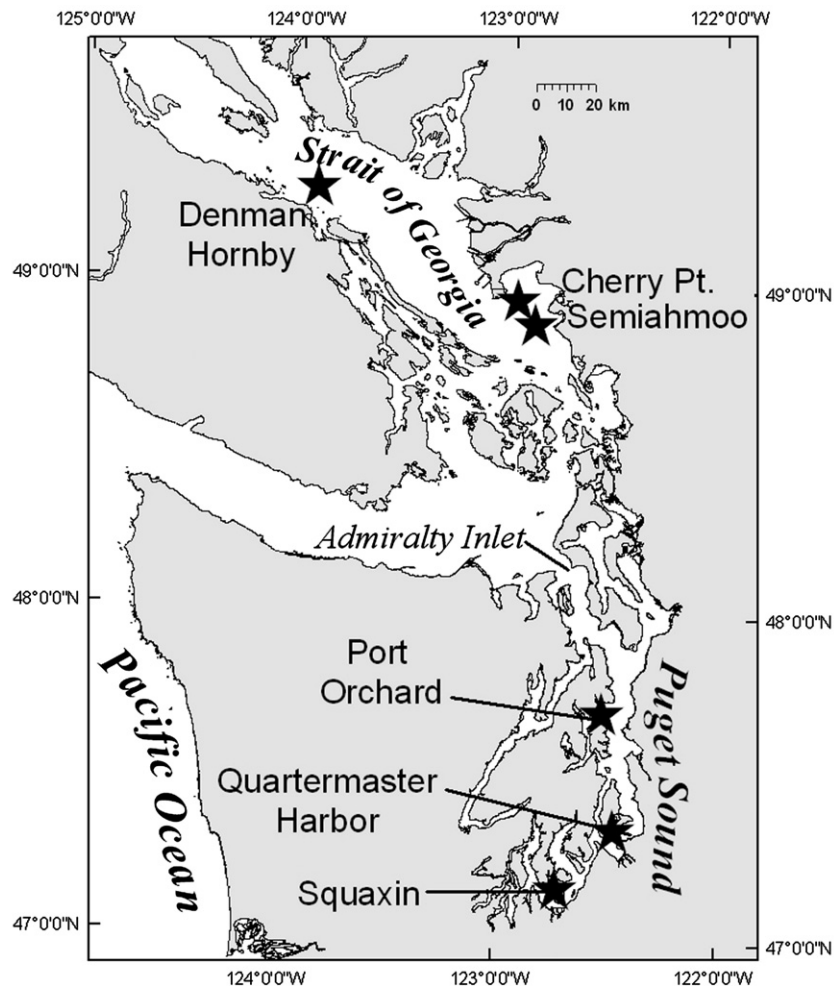


Fig. 1 – Capture location (denoted with black stars) of Pacific herring from six Puget Sound and Strait of Georgia populations.

open coastal ecosystems (Harrison et al., 1994), and in particular, the summer-residence time of water in Puget Sound is roughly double that of the Strait of Georgia (adapted from Thomson, 1994). These characteristics are reflected in greater contamination of Puget Sound's nearshore sediments (Long et al., 2003), and biota such as English sole (*Parophrys vetulus*), a benthic flatfish (West et al., 2001), and blue mussels, a sessile filter-feeding bivalve (Mearns, 2001) with POPs.

We hypothesized that patterns of toxic contaminants might differ between Puget Sound and Strait of Georgia forage fish populations, based on these differences in regional contaminant sources or on population-specific migratory patterns, and that POP patterns in these fish might be distinctive enough to distinguish their populations. Small et al. (2005) identified genetic segregation between Puget Sound and Strait of Georgia herring populations, and we suggest that patterns of toxic contaminants in their bodies can be used to evaluate environmental segregation of marine species like herring.

Using one species, Pacific herring, as a representative of the forage fish guild, our overall goal was to quantify the concentration, distribution, and patterns of POPs in herring from the Puget Sound and Strait of Georgia. We first compared concentrations of PCBs, DDTs and HCB in herring among six populations in this ecosystem to evaluate geographic patterns, while accounting for covariates such as fish age, lipid concentration, and trophic level (estimated using stable isotopes of nitrogen). We then evaluated the patterns of POP similarity/dissimilarity between populations to see whether there existed population "fingerprints", based on contaminant patterns. Finally, we calculated

POP body burdens for herring in the six populations, as a potential estimator of POP dose for their predators.

2. Materials and methods

2.1. Sampling locations

We collected 1055 adult herring using a midwater rope-trawl from pre-spawning aggregations at three locations in Puget Sound, and three in the Strait of Georgia from 1999 through 2004 (Fig. 1). For this study, we defined Puget Sound as the marine and estuarine waters of Central, Southern, and Whidbey basins, and the Strait of Georgia as inland marine and estuarine waters south from Johnstone Strait to the boundary waters of the San Juan Islands. The six fish populations were designated in accordance with stock names used by the regional fisheries agencies that manage the species. Puget Sound populations included herring from Squaxin (1999–2004), Port Orchard (1999–2004), and Quatermaster Harbor (2003). Strait of Georgia populations included Semiahmoo (1999–2004), Cherry Point (1999 and 2001) and Denman/Hornby (1999). We sampled each population as the herring aggregated for spawning, about 1 to 3 weeks prior to the typical spawning period for each population. Squaxin and Quatermaster were always sampled in January, Port Orchard and Semiahmoo in February, Denman/Hornby in March, and Cherry Point in April or May (Table 1). Semiahmoo and Cherry Point populations use similar aggregation and spawning areas but are differentiated by their spawning time.

Table 1 – Summary of life history parameters and contaminant concentrations in composited whole-body samples of six Pacific herring (*Clupea pallasii*) populations from Puget Sound and the Strait of Georgia

	Puget Sound			Strait of Georgia			GLM r^2
	Squaxin Pass	Quatermaster	Port Orchard	Semiahmoo	Denman Hornby	Cherry Point	
Sample size (n)	60	10	56	55	10	20	–
Collection month	Jan	Jan	Feb	Feb	Mar	Apr	–
Mean age	3.1a	2.5bc	2.5bc	2.3c	2.6bc	3.0ab	0.30
Mean std. length (mm)	161a	163a	169b	170b	177c	174c	0.54
Mean wt. (g)	53a	53a,b	59b	59b	67c	71c	0.48
% Lipids	8.0a	8.1a	6.4b	5.1c	5.2c	3.3d	0.62
$\delta^{15}\text{N}$ (‰) [n]	14.0 [20]	–	13.9 [17]	13.0 [13]	–	12.5 [10]	0.93
ng PCBs/g wet	160a	120a	160a	34b	18c	41b	0.88
ng PCBs/fish	9700a	7600a	9200a	2000b	900c	2000d	0.88**
ng PCBs/g lipid	2000a	1500a,b	2500c	690d	370e	1200b	0.76
ng DDTs/g wet	27a	19b	19b	13c	12c,d	11d	0.54*
ng DDTs/fish	1400a	1100a,b	1200b	810c	730c,d	570d	0.54**
ng DDTs/g lipid	330a	240b	300a,b	260b	230b	320a,b	0.26*
ng HCB/g wet	1.5a	1.8a	1.6a	1.5a	1.6a	0.44b	0.71
ng HCB/fish	79a	92a	89a	95a	99a	30b	0.69***
ng HCB/g lipid	19a	22a,b	25b	31c	32c	13d	0.51

All General Linear Models were significant at $p < 0.001$.

An individual sample is a homogenized composite of five whole herring bodies.

PCBs are the sum of 16 identified congeners plus unidentified congeners as described in Materials and methods.

DDTs are the sum of 5 isomers as described in Materials and methods.

Tukey's HSD multiple comparison, lower case letters denote statistical groupings in each row.

* indicates adjustment for fish length.

** indicates adjustment for fish weight.

*** indicates adjustment for length and lipids.

Results for all chemicals presented as geometric means (GLM run on ln-transformed data).

Squared multiple correlation, r^2 , is the proportion of the total variation in the dependent variable explained by the General Linear Model.

2.2. Sample processing

We controlled for variability in POPs associated with age and sex by targeting three-year-old male herring. Males were selected to reduce variability in POP concentration that might occur in females related to maternal transfer of lipophilic POPs to eggs. All males were nearing spawning condition. We used younger herring because they probably migrate shorter distances than adults (e.g., Koistinen et al., 2004) and thus may better reflect local contaminant conditions. In addition, their lower age represented recent exposure, rather than accumulation over many years.

We used standard length measured in the field to pre-select presumed three-year-olds, based on length-at-age data (Lemberg et al., 1997). The ages of individual herring were subsequently estimated by counting annuli from lateral scales taken from under the pectoral fin, and composite groupings were made to best represent three-year-olds. We made 211 composite samples comprising five fish each. All fish were ground whole, including viscera. Stomach contents were not removed, as they appeared to be negligible in sampled specimens. We calculated the mean age, standard length (mm) and weight (g) of fish in each composite and conducted all analyses using these mean composite-values.

POPs were extracted from ground, homogenized whole-body composites using sodium sulfate and pentane/hexane as described in Krahn et al. (1994), or sodium sulfate, magnesium sulfate, and dichloromethane as described by Sloan et al. (2005). All extracts were analyzed by high performance liquid chromatography (HPLC) with photodiode array (Krahn et al., 1994). Briefly, sample extracts were reduced in volume to approximately 1 ml and the POPs were separated from interfering compounds (e.g., lipids and aromatic compounds) on a gravity flow cleanup column that contained neutral, basic and acidic silica gels eluted with hexane/methylene chloride (1:1 v/v). Dioxin-like PCB congeners (PCBs 77, 105, 118, 126, 156, 157, 169, 189) were resolved from other selected PCBs (PCBs 101, 110, 128, 138, 153, 170/194, 180), chlorinated pesticides (*o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT) and hexachlorobenzene (HCB) by HPLC on two Cosmosil PYE analytical columns, connected in series and cooled to 16 °C. The congeners were measured by an ultraviolet (UV) photodiode array detector and were identified by comparing their UV spectra (200–310 nm) and retention times to those of reference standards in a library. The analyte purity was confirmed by comparing spectra within a peak to the apex spectrum. Lipid concentration was measured gravimetrically in all samples, after they were extracted with methylene chloride and homogenized with a tissue grinder (Sloan et al., 2005).

A method blank and a National Institute of Standards and Technology (NIST) blue mussel standard reference material (SRM 1974a or SRM 1974b) were analyzed with each sample set containing 12–14 field samples. Concentrations of $\geq 70\%$ of individual analytes that were measured in the NIST SRM were within 35% of either end of the 95% confidence interval range of the published NIST certified concentrations (Schantz et al., 1997). Duplicate analyses were done for 10% of the tissue samples, with relative standard deviations $\leq 30\%$ for more than 80% of analytes detected in the samples. Method blanks contained no more than four analytes that exceeded

four times the limit of quantitation (LOQ), unless the analyte was not detected in the associated tissue samples in the set. The percent recovery of the surrogate standard (1,2,3,4-tetrachloro-*p*-dibenzodioxin; 250 ng) ranged from 62 to 107%.

Throughout this paper PCBs are presented as summed PCB congeners, which were calculated using the following formula: PCBs = the sum of the concentrations of the 16 PCBs listed above (based on individual response factors) plus the sum of the concentrations of other unidentified PCBs (calculated by summing areas of peaks identified as PCBs and using an average PCB response factor). DDTs are presented as the sum of the five DDT isomers noted above. If a DDT isomer was not detected, a concentration of one half the detection limit (typically <0.5 ng/g) was used for that analyte in the DDT sum.

We measured $\delta^{15}\text{N}$ isotope concentration in a subset of 60 samples from four of our six populations (Cherry Point, Semiahmoo, Port Orchard, and Squaxin) in 2001 and 2002 to estimate relative trophic position of herring (e.g., Post 2002). We assumed equivalent isotopic baselines across the populations, and that the sampled years represented samples taken prior to 2001 and 2002 (i.e. that $\delta^{15}\text{N}$ was relatively stable within populations, across the years we sampled). Stable isotope analyses of whole-body composites of herring were conducted on lipid-extracted tissues (Herman et al., 2005). Precision for $\delta^{15}\text{N}$ analysis was $\leq \pm 0.3\%$. A standard reference material (NIST SRM 1946) was processed with every 20 analyses to monitor analytical accuracy (Sloan et al., 2006). The $\delta^{15}\text{N}$ values were reported as deviations in parts per thousand (‰) from atmospheric nitrogen.

The significance of population differences in concentration of POPs was tested using a General Linear Model (GLM from SPSS Inc., 2000), with population as the primary factor, and age, length or weight, lipids, and $\delta^{15}\text{N}$ (when available) as covariates. We avoided mixing fish age, length or weight in any GLM because they are strongly correlated with each other. We used a stepwise GLM, with the goal of deriving a predictive regression model that reduced effects of covariates (by adjusting POP concentrations from all populations to the grand mean of the covariate), maximized the amount of variation explained by the model (r^2), and contained as few covariates as possible (favoring simplicity). Hence, some significant covariates, especially those defined in complex interactions, were excluded from the final models, if they explained little of the total variation.

We used multidimensional scaling (MDS from Clarke and Warwick, 2001) to assess POP patterns in herring bodies, constructing a two-dimensional unitless configuration or “map” of points that describe groupings of contaminants based on the similarity of the relative contribution of each class of POPs to the total POPs in a sample (see Clarke and Gorley, 2001). In brief, the algorithm attempts to satisfy conditions prescribed by a contaminant-similarity matrix to place similar samples together, and dissimilar samples apart in low-dimensional space with the least amount of stress. Data were standardized by computing the proportional contribution of each POP concentration to the total POP concentration in each sample, and then transformed by taking square root, to reduce the contribution of the dominant POP class (i.e. PCBs). Bray–Curtis similarity data were plotted in two-dimensions, on unitless axes. Pairwise comparisons of MDS populations were conducted

with Analysis of Similarities (ANOSIM), using the R statistic to identify the main between-population differences (Clarke and Warwick, 2001). Values of R range from zero (no separation, or complete similarity) to 1.0 (complete separation, or no similarity) of populations.

3. Results

3.1. PCBs

Whole bodies of herring from the three Puget Sound populations contained significantly greater PCB levels (3 to 9 times) than those measured in the Strait of Georgia populations (Table 1, Fig. 2a) and were consistent over time. Temporal variability in PCB concentration within populations was either an insignificant or a negligible contributor to the total variation in PCB concentration, leading us to pool samples across years. For the three populations we sampled over the full 1999–2004 range, two exhibited no significant temporal trend in PCBs (linear regression of ln-transformed PCB concentration by year, $p=0.59$ for Squaxin and 0.80 for Port Orchard). The third, Semiahmoo, exhibited a significant negative trend ($p=0.011$), however, time explained only a small amount (9.8%) of variation in PCB concentration for that population, and the trend was strongly affected by three high-leverage points in 1999. Hence, we ignored temporal variation in our spatial comparisons of PCBs for the six populations.

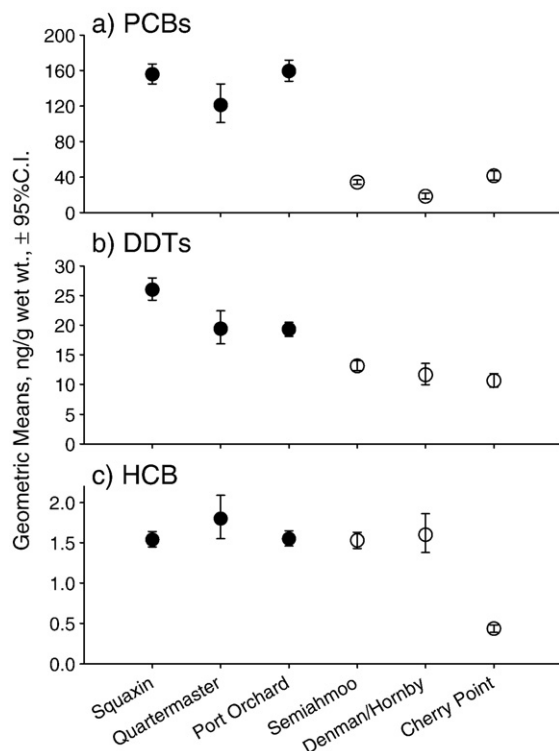


Fig. 2 – (a–c). Concentration of a) PCBs, b) DDTs, and c) hexachlorobenzene (HCB) in six herring populations from the Puget Sound (filled circles) and Strait of Georgia (open circles). Symbols indicate geometric means, and vertical bars define 95% confidence intervals.

Trophic status was not a significant covariate of spatial variation in PCB concentrations across the subset of four populations for which we measured $\delta^{15}\text{N}$. Although $\delta^{15}\text{N}$ was significantly greater in the two sampled Puget Sound populations (Squaxin, at 14.0‰ and Port Orchard at 13.9‰) than the two Strait of Georgia populations (Semiahmoo at 13.0‰, and Cherry Point at 12.5‰; ANOVA of $\delta^{15}\text{N}$ by population, $p<0.0001$, Tukey Multiple Range Test), $\delta^{15}\text{N}$ did not correlate with PCBs for these four populations. This analysis, albeit on a subset of our POP data, suggests that trophic differences among our populations were not strong predictors of PCBs. Hence, our remaining analyses on spatial variation in PCB concentrations were made without the $\delta^{15}\text{N}$ covariate.

Whole-body lipid concentration was not a significant covariate in the stepwise GLM for PCB concentration (ng PCB/g fish, wet wt.), and computing the GLM on lipid-normalized data (ng PCB/g lipid) did not affect the model to an appreciable degree. Fish weight, length, and age (run in separate GLMs) explained a negligible amount of variability in the PCB concentration (approximately 1% each), and correlations between PCB concentration and these covariates were significant (and only weakly so) for only two of the six populations. Hence, none of the biological covariates we tested contributed appreciably to explaining the observed spatial variation in PCB levels, and were omitted from the stepwise GLM for PCBs.

The final model predicted wet weight PCB concentration in the six populations using location alone, and explained 88% of PCB variation, with geometric mean concentrations ranging from 120 to 160 ng/g whole body, wet weight, in the Puget Sound populations and 18 to 41 ng/g in the Strait of Georgia populations ($p<0.001$ — Table 1). Using mean composite weight to compute total body burden, a predator consuming whole Puget Sound herring would receive on average roughly 7600 to 9700 ng of PCB per fish, whereas predators feeding on Strait of Georgia herring would receive 900 to 2000 ng PCBs per fish (Table 1).

3.2. DDTs

DDTs were measured in all herring composites at low to moderate levels (compared to PCBs), and Puget Sound samples were more contaminated than those from the Strait of Georgia, regardless of covariate effects (Table 1, Fig. 2). In addition, unexplained variability in the General Linear Model for DDTs was much greater (46%) than with PCBs (12%). As with PCBs, lipid levels explained an insignificant amount of variability in DDT concentration, and were therefore omitted from the model. For the three populations sampled across all years, Semiahmoo herring exhibited a weak ($r^2=0.092$) although significant ($p=0.014$) negative temporal trend in DDTs, whereas Squaxin and Port Orchard herring showed no temporal trend in DDTs ($p=0.089$ and 0.70). These results suggested that temporal trends were relatively unimportant in our analyses of variance, and so time was omitted from subsequent analyses (i.e., samples were pooled across years). Trophic level ($\delta^{15}\text{N}$) was not a significant covariate (for the subset of four populations).

Fish size (standard length) accounted for 14% of the total explained by the final GLM, with population accounting for the remaining 40% of the total explained. Therefore we reported fish length-adjusted DDT concentrations with geometric

means ranging from 19 to 26 ng/g wet wt. in the Puget Sound, and 11 to 13 ng/g wet wt. in the Strait of Georgia herring (Fig. 2b, Table 1).

We also observed significant spatial trends in the relative distribution of DDT isomers. Firstly, *p,p'*-DDE accounted for most (73 to 100%) of DDTs and was detected in all samples. Virtually no *o,p'*-substituted DDTs were detected (although *o,p'*-DDE was not quantified by our method) in any samples. *p,p'*-DDD was detected equally across populations, in 95 to 100% of samples. The unmetabolized parent compound *p,p'*-DDT was detected in greater frequency in Puget Sound than Strait of Georgia — 80%, 40% and 82% of samples in Squaxin, Quatermaster, and Port Orchard, respectively, and 60% 10% and 0% of samples in Semiahmoo, Cherry Point, and Denman/Hornby, respectively. The ratio of *p,p'*-DDT:DDTs was significantly lower in two Puget Sound populations (Squaxin and Port Orchard) than Strait of Georgia's Semiahmoo population (Fig. 3, ANOVA, $p < 0.001$, Tukey's multiple range test). We omitted Cherry Point and Denman/Hornby populations from this ANOVA because *p,p'*-DDT was rarely detected in these populations, and Quatermaster was omitted because its *p,p'*-DDT exhibited unusually high variability that we suspect was related to its small sample size ($n = 10$).

3.3. Hexachlorobenzene

Concentrations of HCB were low compared to PCBs and DDTs in all six populations sampled, ranging from the lower limit of quantitation (mean of 0.065 ng/g) to 2.3 ng/g, wet weight. The HCB population-pattern was substantially different than that exhibited by PCBs or DDTs (Fig. 2c). The spring spawning population (Cherry Point) exhibited a much lower HCB concentration (0.44 ng/g) than herring from the other five locations, whose HCB ranged narrowly, from 1.5 to 1.8 ng/g (Table 1). Unlike PCBs and DDTs, HCB correlated with lipids

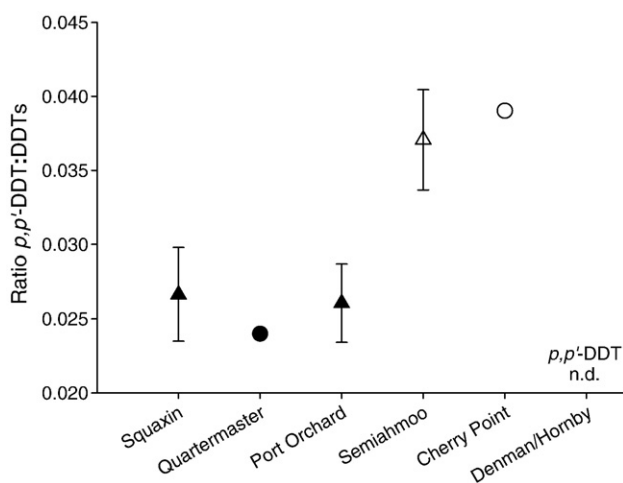


Fig. 3—DDT ratios in six populations of Puget Sound (filled symbols) and Strait of Georgia (open symbols) herring. Mean ratios were computed as the quotient of parent compound (*p,p'*-DDT) to DDTs. Circles indicate sample sizes too low to be included in ANOVA. *p,p'*-DDT was not detected in any Denman/Hornby samples. Vertical bars define 95% confidence intervals.

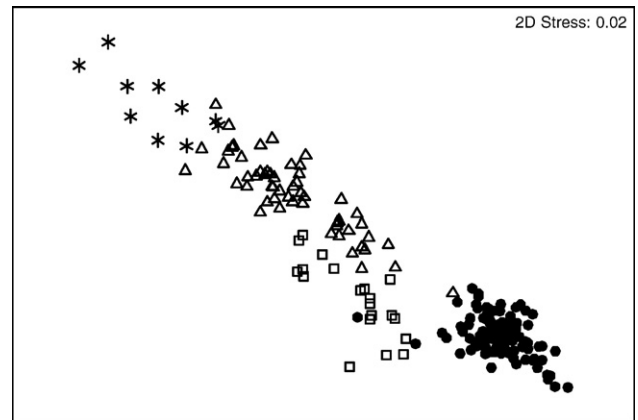


Fig. 4—Multidimensional scaling map of PCBs, DDTs, and HCB for six populations of Pacific herring. Three populations from the Puget Sound were all highly similar and so are represented with a single symbol, ●. Populations from the Strait of Georgia are denoted with * (Denman/Hornby), Δ (Semiahmoo), and □ (Cherry Point). Stress < 1.0 indicates a high probability that the groupings shown were not made by chance (Clarke and Warwick 2001).

(at least weakly so) in three of the six populations (Denman/Hornby, Semiahmoo, and Port Orchard; $r^2 = 0.62, 0.41, \text{ and } 0.14$, respectively). The population factor alone explained 62.3% of the variation in HCB, while lipids and the station \times lipid interaction explained 6.8%, combined. Neither fish weight nor $\delta^{15}\text{N}$ contributed significantly to explaining HCB variation. Both the full GLM (including lipids as a covariate) and the simpler model (excluding lipids) showed that Cherry Point herring had significantly lower HCB levels than herring from any other population.

3.4. Environmental segregation

Collectively, the multidimensional scaling (MDS) pattern of the three POPs measured in our six herring populations showed clear segregation between the three Puget Sound and three Strait of Georgia populations (Fig. 4, ANOSIM, R from 0.85 to 1 for the nine possible pairwise comparisons). In addition, the distribution of points in the plot generally mimicked the north-to-south geographic distribution of collection locations for the populations, even though the plot itself contains unitless axes. The three Puget Sound populations in the lower (“southern”) portion of the MDS plot were indistinguishable or barely separable from each other (ANOSIM, R from 0.12 to 0.38), and above, or further “northward” in the Strait of Georgia, we observed increasing dissimilarity with (or segregation from) Puget Sound, as R values increased from 0.85 (Quatermaster vs Cherry Point) to 1.0 (Denman/Hornby vs all three Puget Sound populations) in the northerly direction. Within the Strait of Georgia, Cherry Point herring were moderately dissimilar to Semiahmoo (ANOSIM, $R = 0.50$), and highly dissimilar to Denman/Hornby (ANOSIM, $R = 0.97$), while Semiahmoo herring were moderately dissimilar to Denman/Hornby (ANOSIM, $R = 0.66$).

4. Discussion

The present study assesses persistent organic pollutant (POP) exposures and patterns in an abundant, small, schooling, pelagic planktivore in the Puget Sound and Strait of Georgia ecosystem. The Pacific herring we sampled in Puget Sound were 3 to 9 times more contaminated with PCBs and 1.5 to 2.5 times more contaminated with DDTs than those in the Strait of Georgia. Variation in biological traits such as trophic status, lipid content, and fish age, length and weight did not account for the observed spatial variation in PCB concentrations, primarily because we controlled for covariates by study design. Mean fish age ranged only from 2.3 to 3.1 years, which effectively factored out this covariate. Lipids ranged more widely among populations, from 3.3 to 8%, however PCBs did not correlate well with lipids, precluding their use as a predictor. Hexachlorobenzene was remarkably consistent among populations, except for Cherry Point herring, whose exposure to hexachlorobenzene was exceptionally low.

It is interesting to note that even though the two Strait of Georgia populations exhibited significantly greater $\delta^{15}\text{N}$ than the two Puget Sound populations, this covariate also failed as a predictor of PCBs, DDTs, and HCB. As with fish age, this is probably related to the relatively small range of the covariate; mean $\delta^{15}\text{N}$ ranged only 1.5‰, which is less than half the concentration typically thought to represent a full trophic level difference (Post, 2002). Hence, it is unlikely that trophic level differences were associated with the patterns we observed in POPs.

The multidimensional scaling (MDS) map of PCBs, DDTs, and HCB suggests differential contaminant exposures that illustrate environmental segregation of the Puget Sound herring from Strait of Georgia populations. The Puget Sound populations clustered as a single group, exhibiting the highest levels of all three contaminants. We interpret this as evidence that they forage primarily within Puget Sound, where they are likely exposed to higher regional sources of these POPs. The Puget Sound has 9.5 times more people per km² of its drainage area¹, covers only one-third the surface area and occupies one-sixth the volume of the Strait of Georgia. In addition, Puget Sound's narrow connection to oceanic waters and shallow sills at Admiralty Inlet tend to isolate its waters from relatively cleaner oceanic waters (Harrison et al., 1994), and its summer flushing time is roughly twice that of the Georgia Basin (Thomson, 1994). These characteristics increase the likelihood that loadings of POPs are greater, and that POPs are retained longer in the Puget Sound system.

All three Strait of Georgia populations were strongly isolated from the Puget Sound populations in MDS. In addition, the Strait of Georgia populations were moderately to strongly isolated from each other. The separation between Cherry Point and Semiahmoo populations is especially interesting because these populations aggregate and spawn in essentially the same area, but at different times of the year. Fishery managers have long recognized the asynchronous

spawn timing of Semiahmoo (winter), and Cherry Point (spring) herring as an important factor separating these populations (Stout et al., 2001), perhaps even genetically (Small et al., 2005). The unique contaminant profile of the Cherry Point population, which resulted primarily from its relatively low HCB concentration and relatively high PCB concentration, is evidence that this population is also environmentally segregated from other Puget Sound and Strait of Georgia herring populations.

Environmental segregation of herring populations may occur as a result of differential migration behavior, which can affect the amount of time populations spend in contaminated habitats, resulting in different contaminant fingerprints. Two "types" of herring have been described in Puget Sound (Penttila, 1986), the Strait of Georgia (Taylor, 1964), and Southeast Alaska (Carlson, 1980). Migratory herring forage in oceanic habitats widely separated from inland spawning habitats, and so make regular migrations between the two. In contrast, local, or resident populations remain in inland waters year-round, feeding and spawning in the same region. The Puget Sound populations we sampled are generally considered resident in Puget Sound (Penttila, 1986), and Strait of Georgia populations are considered more migratory (Hay et al., 2001; Stout et al., 2001). Puget Sound herring, because of their residency, would have year-round proximity to urbanized waters, whereas migrants such as the Strait of Georgia populations probably feed in oceanic-coastal waters with much lower land-based POP inputs.

The ratio of *p,p'*-DDT to DDTs was greater in Strait of Georgia herring than those we sampled from Puget Sound, which further supports our hypothesis that herring in these two regions are environmentally segregated. Possible explanations for the greater Strait of Georgia ratios could be related to differential historic use patterns, or differences in current sources. Bailey et al. (2000) documented atmospheric transport of DDTs from east Asia to the western Canadian arctic, which may represent a current source of DDT with a relatively high *p,p'*-DDT:DDT ratio. If Strait of Georgia POP sources are dominated by such far-field atmospheric inputs (as suggested by Cullon et al., 2005), one might infer the atmospheric transport of POPs described by Bailey et al. (2000) as a possible explanation for the higher *p,p'*-DDT to DDTs ratio we observed in the Strait of Georgia herring. Moreover, if DDTs in Puget Sound biota are dominated by historic (more metabolized) local sources, the higher local concentrations may mask a far-field atmospheric signal.

The range and magnitude of *p,p'*-DDT:DDTs ratios we observed in both Puget Sound and Strait of Georgia herring was relatively small (0.024 to 0.039), compared to the original technical DDT product, which contained approximately 0.74 *p,p'*-DDT:DDTs. Bignert et al. (1998) reported *p,p'*-DDT:DDTs in Baltic Sea herring of approximately 0.5 in the 1970s, when DDT was still being used in that region, and a decline to near 0.1 in the 1990s, after two decades of usage-bans. The lower ratios we observed in Puget Sound and the Strait of Georgia at the turn of the century seem consistent with that decline, relative to the 1990s Baltic herring observations.

There are several possible pathways for POPs to become entrained in the populations of Pacific herring we studied, but it is generally thought that diet is the dominant pathway in adult fish (Borgmann and Whittle, 1992). The diet of Pacific

¹ Calculated from data presented in: Georgia Basin–Puget Sound Ecosystem Indicators Report [online]. Available from <http://www.env.gov.bc.ca/spd/gbpse/population/index.html>.

herring in Puget Sound and Strait of Georgia is poorly documented. However, adult Pacific herring along the Pacific coast from Washington to Alaska seem to rely heavily on phytoplanktivorous krill (especially *Euphausia pacifica*), calanoid copepods, and larval invertebrates and fishes (Wailles, 1936; Robinson, 2000; Iverson et al., 2002). These planktonic prey have no obvious, direct trophic connections to sediment sources of POPs. However, biota in the pelagic food web may take up hydrophobic contaminants directly from the water column (e.g., Del Vento and Dachs, 2002) before incoming contaminants settle, or after contaminants are resuspended into the pelagic habitat from disturbed sediments. Gustafsson et al. (1999) showed that a sessile, filter-feeding bivalve, blue mussel (*Mytilus edulis*) can rapidly concentrate PCBs from their food (phytoplankton) or directly from the water. Mearns (2001) documented blue mussels from Puget Sound with PCB concentrations two to 13 times higher than mussels from the Strait of Georgia, suggesting that PCBs in Puget Sound's phytoplankton or water column mimic the regional PCB gradient we observed in herring.

PCBs could also move from contaminated benthic biota to the pelagic food web via their reproductive products. Wailles (1936) indicated that "ova" was an important diet item in juvenile herring, and pelagic larvae of benthic invertebrates like crabs *Cancer* spp are common in the adult herring diet. We hypothesize that maternal transfer of lipophilic POPs (see Niimi, 1983; Miller, 1993) like PCBs from benthic invertebrates and fishes could be a significant pathway of sediment-PCBs to the pelagic habitat. In addition, maternal transfer of POPs to eggs and larvae among pelagic species themselves may recycle POPs within the pelagic food web (wherein pelagic planktivores consume each others' eggs and larvae), isolating the pelagic POP burden from the benthos. Such biological recycling of POPs may result in POPs being retained by herring, especially for populations that reside in a relatively isolated system like Puget Sound.

The regional differences in PCBs we observed among Pacific herring populations are also reflected in three of their important predators, chinook and coho salmon, and harbor seals. O'Neill et al. (1998) observed higher PCBs in coho salmon originating from Southern and Central Puget Sound (equivalent to our definition of "Puget Sound" in this paper) than those originating from Northern Puget Sound (equivalent to our definition of southern Strait of Georgia). O'Neill et al. (2006) also reported higher PCBs in chinook salmon that reside in Puget Sound, compared to Puget Sound chinook salmon that migrate to the Pacific Ocean, and compared to chinook that originate from the Strait of Georgia. Ross et al. (2004) and Cullon et al. (2005) reported that Puget Sound harbor seals and their prey (analyzed as a food-basket composite) were seven times more contaminated with PCBs than those from the Strait of Georgia. Harbor seal preys were dominated by pelagic species like Pacific herring (79% in Strait of Georgia and 65% in Puget Sound). Based on PCB-homolog and congener analyses, Ross et al. (2004) and Cullon et al. (2005) concluded that PCB patterns in seals and their prey in the Strait of Georgia were consistent with atmospheric transport and deposition (dominated by lighter congeners), whereas Puget Sound PCBs (dominated by heavier congeners) probably resulted from direct, regional inputs from urbanization and industrialization of the Puget Sound basin.

The many species of herring predators comprise a wide range of feeding ecologies, and so herring in Puget Sound may serve as an important nexus of POP distribution in that ecosystem. Sedentary benthic predators like rockfishes and lingcod may draw POPs from the pelagic food web to the benthos, by feeding on herring that have accumulated POPs over a wider geographic range. Other herring predators such as Pacific salmon and piscivorous seabirds are wide-ranging or highly migratory, and so Puget Sound herring may represent a source of POPs that are biotransported to more distant North Pacific or North American habitats. Biotransport of POPs over long distances has already been documented in sockeye salmon (*Oncorhynchus nerka*, Ewald et al., 1998) from the northern Pacific Ocean to Alaskan lakes, and in northern fulmars (*Fulmarus glacialis*) from the North Atlantic Ocean to high arctic ponds (Blais et al., 2005).

In summary, Pacific herring in Puget Sound are significantly contaminated with PCBs, and to a lesser degree, DDTs and HCB, and represent a notable source of bioavailable, albeit mobile, POPs in the Puget Sound food web. The magnitude of this herring-POP-source relative to other pelagic species is unknown as yet, and so highlights the need for broader food-web based toxics research. The POP patterns we observed substantiate the hypothesis that Puget Sound herring are environmentally isolated from populations outside the Sound, and illustrate the utility of contaminant "fingerprinting" in elucidating geographic range and other important life history characteristics.

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