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Concentrations and profiles of organochlorine contaminants in North Pacific resident and transient killer whale (*Orcinus orca*) populations

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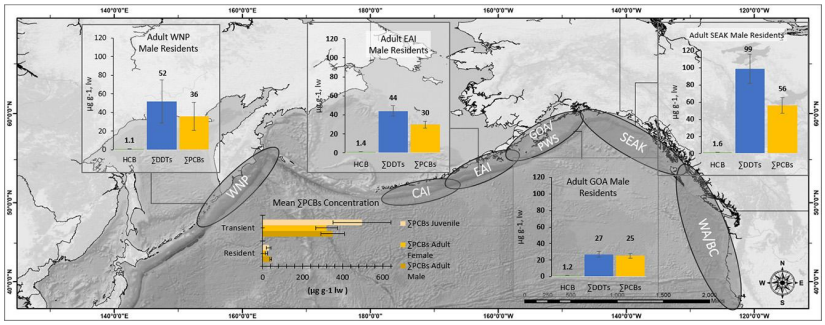
HIGHLIGHTS

- Profiled organochlorines in 98 resident and transient North Pacific killer whales
- Organochlorine profiles used as chemical "fingerprints" to infer foraging habitat
- Transients had higher mean blubber concentrations of OCs than residents.
- Adult females offload organochlorines to calves
- Organochlorine variability in orca largely driven by segregated foraging habitat

GRAPHICAL ABSTRACT



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ABSTRACT

Organochlorine (OC) profiles have been used as chemical "fingerprints" to infer an animal's foraging area. North Pacific killer whale (*Orcinus orca*) populations are exposed to different levels and patterns of OCs based on their prey, distribution, and amount of time spent in a particular area. To characterize concentrations and profiles of OCs found in various populations of North Pacific killer whales, polychlorinated biphenyls (PCBs), including dioxin-like congeners, DDTs, and hexachlorobenzene (HCB), were measured in biopsy blubber samples of photo-identified resident (fish-eating) and transient (mammal-eating) killer whales collected from 1994 through 2002 from Russian Far East waters to the waters of the west coast of the United States, representing 10 populations. We compared blubber OC concentrations based on ecotype (resident vs. transient), sex and reproductive maturity, and geographic area. We also examined OC mixtures to determine if we could detect segregated geographical areas (foraging areas) among the six populations with sufficient sample sizes. Transients had significantly higher OC concentrations than residents and adult male whales had consistently higher OC levels compared to adult females, regardless of ecotype. Our OC profile findings indicate segregated foraging areas

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Chemical fingerprint
Persistent pollutant

for the North Pacific killer whales, consistent with observations of their geographic distributions. Several potential health risks have also been associated with exposure to high levels of contaminants in top-level predators including reproductive impairment, immune suppression, skeletal deformities, and carcinoma. The results of this baseline study provide information on the geographic distribution of OCs found in North Pacific killer whales, results which are crucial for assessing the potential health risks associated with OC exposure in this species.

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1. Introduction

Killer whales (*Orcinus orca*) are widely distributed throughout the world's oceans, but are primarily found at higher latitudes (Mitchell, 1975; Leatherwood and Dahlheim, 1978; Forney and Wade, 2006). In the North Pacific, three different ecotypes of killer whale have been identified and named "residents," "transients," and "offshores." These ecotypes differ genetically (Hoelzel et al., 1998, 2002; Barrett-Lennard, 2000; Parsons et al., 2013; Moura et al., 2015) and in various aspects of their ecology, morphology, and behavior (Ford and Ellis, 1999; Baird, 2000; Barrett-Lennard, 2000; Ford et al., 2000; Dahlheim et al., 2008; Emmons et al., 2018).

Resident killer whales from the North Pacific primarily consume fish with a preference toward salmonids (*Oncorhynchus* spp.), but also consume Pacific herring (*Clupea pallasii*), Pacific halibut (*Hippoglossus stenolepis*), lingcod (*Ophiodon elongates*), and rockfish (*Sebastes* spp.) (Ford et al., 1998; Saulitis et al., 2000; Ford and Ellis, 2006; Hanson et al., 2010; Ford et al., 2016). Because of their preference for salmon, seasonal movements of resident killer whales are strongly tied to Pacific salmon migrations (Ford, 2009). In contrast, the preferred diet of transient killer whales consists of marine mammals with movements linked to the presence of their prey in coastal waters (Baird, 2000; Ford et al., 1998, 2000; Saulitis et al., 2000; Ford, 2009; Dahlheim and White, 2010). Transient killer whales from the North Pacific target a wide variety of prey (e.g., Northern fur seals [*Callorhinus ursinus*], harbor seals [*Phoca vitulina*], Steller sea lions [*Eumetopias jubatus*], gray whales [*Eschrichtius robustus*], minke whales [*Balaenoptera acutorostrata*], Dall's porpoise [*Phocoenoides dalli*], and harbor porpoise [*Phocoena phocoena*]) that likely changes seasonally (Jefferson et al., 1991; Dahlheim and White, 2010). Less information is available on the prey consumed by offshore killer whales. This ecotype has been observed preying on fishes and carcharinid sharks (Ford, 2009; Heise et al., 2003; Herman et al., 2005; Jones, 2006; Krahn et al., 2007a; Dahlheim et al., 2008).

There are several resident killer whale populations in the eastern North Pacific. Two resident killer whale populations, the Southern Resident killer whales and Northern Resident killer whales, inhabit the coastal waters of Washington State, USA and southern British Columbia, Canada (WA/BC). The Southern Resident killer whales are observed as far south as central California and as far north as the southern waters of southeast Alaska (Bigg et al., 1990; Ford et al., 2000; Hanson et al., 2013; Carretta et al., 2017). Northern Resident killer whales primarily inhabit the coastal and inland waters of British Columbia with travels south into Washington State waters and north into southeast Alaska waters (Dahlheim et al., 1997; Ford et al., 2000). There are also several resident killer whale populations found in southeast Alaska (SEAK), throughout the Gulf of Alaska (GOA), and westward to the eastern and central Aleutian Islands (EAI and CAI, respectively) and Bering Sea (Dahlheim and Waite, 1993; Matkin et al., 1999a; Matkin et al., 2007; Muto et al., 2016). Although the ranges of eastern North Pacific resident killer whales overlap, each population appears to have a defined core area, an area the whale populations frequently inhabit (Fig. 1). Within each core area, whether it is offshore or more coastal, available prey species vary. Human development and anthropogenic threats also vary within each core area. For example, the Southern Resident killer whale's core area overlaps with high human population and urban development, whereas killer whales from more remote core areas off Alaska are exposed to less industrial development and other human-related activities.

Similar to eastern North Pacific resident killer whales, several populations of transient killer whales occur in marine waters from California through the GOA, Aleutian Islands, and Bering Sea (Matkin et al., 1999a; Matkin et al., 2012; Muto et al., 2016). Most transient killer whales found in SEAK have been well documented (via photographic matches) to frequently occur throughout the coastal waters of WA/BC (Dahlheim and White, 2010). Conversely, no photographic matches have been found among SEAK transients and whales known from Prince William Sound (Matkin et al., 1999b), or from the GOA, Aleutian Islands, and Bering Sea (Dahlheim, 1997). Recent genotypic and observational data strongly suggest that a subdivision occurs between transients found in the EAI with those that occur in the GOA (Matkin et al., 2007; Durban et al., 2010; Matkin et al., 2012; Parsons et al., 2013). The genotypic data indicate that the eastern point of this subdivision between transients in the EAI and the GOA likely occurs in and near the waters surrounding Kodiak Island. Amchitka Pass represents a division between central and western Aleutian Islands (CAI and WAI) transients (Parsons et al., 2013). Subpopulations of transients were also apparent in the eastern Aleutian Islands and Bering Sea (Parsons et al., 2013).

Organochlorines (OCs), such as DDTs and polychlorinated biphenyls (PCBs), are persistent, widespread environmental contaminants that are resistant to metabolism and have been shown to bioaccumulate through marine food webs (Fisk et al., 2001; Ruus et al., 2002; Hoekstra et al., 2003). However, some large marine mammal species, notably polar bears (*Ursus maritimus*), have an excellent ability to metabolize some OCs (Muir et al., 1988; Letcher et al., 1996, 1998). Accordingly, OCs have been used as intrinsic chemical tracers to infer sources of OCs, foraging areas, and migration patterns of many marine species (Ramos and González-Solís, 2012), including Pacific herring (West et al., 2008), Atlantic salmon (*Salmo salar*) (Svendsen et al., 2008, 2009), bluefish (*Pomatomus saltatrix*) (Deshpande et al., 2016a), bluefin tuna (*Thunnus thynnus*) (Deshpande et al., 2016b), Greenland sharks (*Somniosus microcephalus*) (Fisk et al., 2002), bottlenose dolphins (*Tursiops truncatus*) (Borrell et al., 2006; Fair et al., 2010), humpback whales (*Megaptera novaeangliae*) (Elfes et al., 2010), and killer whales (Herman et al., 2005; Krahn et al., 2007a). Marine environments have distinct OC patterns based on a variety of historical inputs (e.g., industrial discharges, atmospheric deposition, current transport, etc.), and animals that do not readily metabolize these OCs and forage for extended periods of time can accumulate OCs in proportion to their availability in those environments. Species found in more coastal waters near high urban and human development regions are potentially exposed to higher levels of OCs than species found in offshore waters and consequently will have higher body burdens of these pollutants. However, remote, undeveloped offshore areas can receive some input of OCs through various routes, such as long-range atmospheric transport from industrialized areas (Wania and Mackay, 1996).

Previous studies have reported concentrations of OCs in tissues of killer whales in the North Pacific. Calambokidis et al. (1984) first reported high levels of PCBs and DDTs in blubber of an adult male transient killer whale (PCBs: 250 μg g^{-1}, wet wt.; DDTs: 640 μg g^{-1}, wet wt.) and an adult male Southern Resident killer whale (PCBs: 38 μg g^{-1}, wet wt.; DDTs: 59 μg g^{-1}, wet wt.) that stranded off the coasts of British Columbia and Washington State, respectively, in the

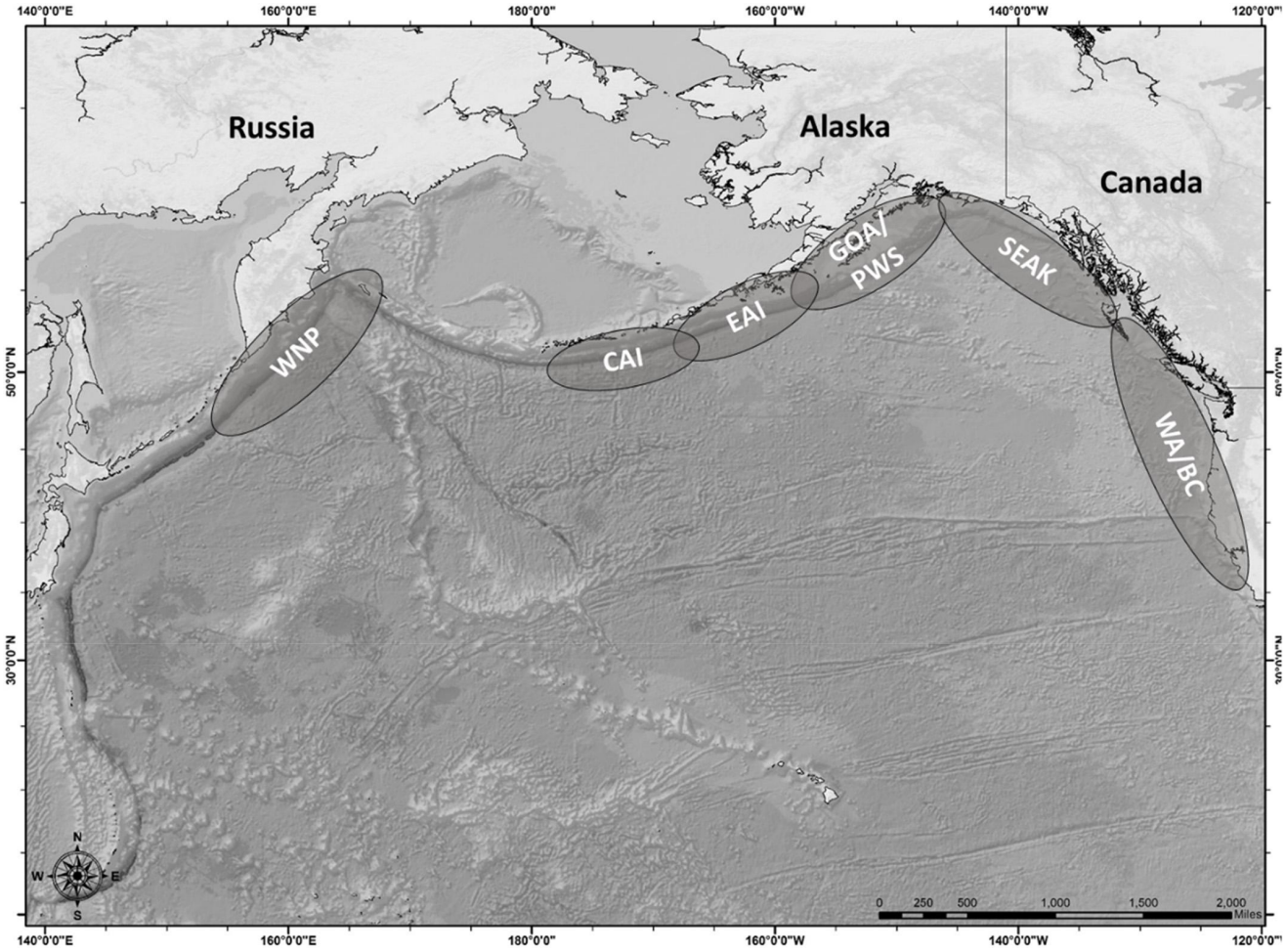

Fig. 1. Map of the North Pacific Ocean detailing core foraging areas inhabited by killer whale populations from which biopsy samples were collected. Ninety eight killer whales from the North Pacific were sampled in the following geographical areas: 1) resident whales from Washington State/British Columbia (WA/BC); 2) resident and transient whales from southeast Alaska (SEAK); 3) resident and transient whales from Gulf of Alaska/Prince William Sound (GOA/PWS); 4) resident and transient whales from eastern Aleutian Islands (EAI); 5) resident whales from Central Aleutian Islands (CAI), and resident whales that occur in the western North Pacific (WNP).

late 1970s. Since then, concentrations of environmental contaminants have been measured in tissues of other stranded or incidentally caught killer whales from the North Pacific Ocean (Ono et al., 1987; Jarman et al., 1996; Hayteas and Duffield, 2000; Krahn et al., 2004; Kajiwara et al., 2006). More recently, concentrations of lipophilic contaminants have been determined in blubber biopsy samples of wild-ranging killer whales (Ross et al., 2000; Ylitalo et al., 2001; Rayne et al., 2004; Herman et al., 2005; Krahn et al., 2007a, 2007b, 2009; McHugh et al., 2007; Wolkers et al., 2007; Noël et al., 2009; Jepson et al., 2016; Atkinson et al., 2019). These studies, as well as others, have revealed large variability in OC levels across regions and within a species.

To increase our knowledge of baseline concentrations and profiles of OCs in various populations of North Pacific killer whales and how these vary geographically over the North Pacific, PCBs (including dioxin-like congeners) and OC pesticides were measured in biopsy blubber samples of 98 wild-ranging resident and transient killer whales sampled from Russian Far East waters to the waters of Washington State, USA. We examined the contribution of dioxin-like PCBs contributing to sum PCB total toxic equivalents ($\sum$PCB TEQ) values as well as the individual DDT and PCB congeners contributing to their respective sum concentrations based on sex and reproductive maturity category. Lastly, we examined the OC mixtures in whale blubber samples to assess whether we could detect segregated foraging areas among six killer whale populations. We hypothesized that populations of North Pacific killer whales would differ in their OC concentrations and profiles based on their ecotype (i.e., resident or transient), foraging area (i.e., geographic distribution and location of core feeding area), and their sex and reproductive maturity category (i.e., adult males, adult females, juveniles or unknown status).

2. Materials and methods

2.1. Field sample collection

From 1994 through 2002, biopsy samples were collected from 98 free-ranging North Pacific killer whales. Remote biopsy sampling techniques were similar to those described in Barrett-Lennard et al. (1996) and Ylitalo et al. (2001). A small core containing skin and blubber (approximately 2.0 to 3.0 cm in length and 0.5 cm in diameter) was obtained from each animal and the blubber portion of the biopsy sample was stored at −20 °C until chemical analysis.

In the current study, killer whales that were biopsy sampled were visually identified using photo-identification catalogues (Dahlheim, 1997; Matkin et al., 1999a). Using the method of Bigg et al. (1986), photographs were taken of the individual killer whales at the time they were biopsy sampled to confirm identification. Sex assignment (i.e., male, female or unknown) was based on direct observations of whales in well-studied populations (e.g., WA/BC, SEAK) or determined

genetically for other populations. Reproductive maturity class (i.e., juvenile, adult or unknown) of each whale was assessed in the field based on long-term sighting data or, if not known, was assigned based on relative body size as described in Herman et al. (2005). We use sex and reproductive maturity class assignments to create four sex/maturity categories (i.e., adult female, adult male, juveniles, and unknowns). Killer whale populations sampled as part of this study are listed in Supplemental Table 1, based on their ecotype (69 residents and 29 transients) and geographic sampling area (i.e., assumed foraging area). Sex and reproductive maturity-category data (e.g., adult males, adult females, and juveniles) are known for the majority of whales sampled and are provided in Supplemental Table 1. The population geographic area assignments follow those described by Herman et al. (2005).

Ninety eight killer whales from the North Pacific were sampled in this study from the following geographical areas: 1) Northern Resident ($n = 1$) and Southern Resident ($n = 3$) killer whales from Washington State/British Columbia (WA/BC); 2) resident ($n = 19$) and transient ($n = 20$) killer whales from southeast Alaska (SEAK); 3) resident ($n = 18$) and transient (n = 2) killer whales from Gulf of Alaska/Prince William Sound (GOA); 4) resident (n = 20) and transient ($n = 7$) killer whales from eastern Aleutian Islands (EAI); and 5) resident killer whales ($n = 3$) from Central Aleutian Islands (CAI). For the purposes of this paper, we also include data on resident killer whales ($n = 5$) that occur in the western North Pacific (WNP) (Fig. 1, Supplemental Table 1).

2.2. OC and lipid analyses

Killer whale biopsy blubber samples were analyzed at the National Marine Fisheries Service's Northwest Fisheries Science Center in Seattle, WA for OCs using a high-performance liquid chromatography/photodiode array detection (HPLC/PDA) method (Krahn et al., 1994). In this method, blubber (0.1 to 0.3 g), sodium sulfate (5 g), hexane/pentane (1:1 v/v), and the surrogate standard (1,2,3,4-tetrachloro-p-dibenzodioxin; 250 ng) were homogenized two times and the extracts combined. The analytes were separated from interfering compounds on a gravity flow cleanup column that contained neutral, basic, and acidic silica gels with hexane/methylene chloride (1:1 v/v). Prior to the cleanup step, a 1-mL aliquot sample extract was removed for lipid quantitation by thin layer chromatography with flame ionization detection (TLC/FID) (Ylitalo et al., 2005a). The remaining sample extract was analyzed for eight dioxin-like PCB congeners (PCBs 77, 105, 118, 126, 156, 157, 169, 189), six other PCB congener groups (PCB 138, PCB 180, PCBs 101/99/149/196, PCBs 128/123, PCBs 153/87, PCBs 170/194), *o,p′*-DDD, *p,p′*-DDD, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT, and hexachlorobenzene (HCB) using a high-performance liquid chromatography with photodiode array detection method (Krahn et al., 1994). The dioxin-like congeners were resolved from other PCBs and OCs (listed above) 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 (collected from 200 to 310 nm) and retention times to those of reference standards in a library. The purity of each analyte was confirmed by comparing spectra within a peak to the apex spectrum. The lower limit of quantitation (LOQ) for PCBs and DDTs ranged from 0.37 to 4.2 ng/ g, wet weight (ww) and 1.2 to 5.6 ng/ g, ww, respectively. The LOQ for HCB ranged from 0.34 to 1.9 ng/ g, ww. The ranges of these LOQ values are typical for the HPLC/PDA and are comparable to or lower than those reported in other studies in which blubber samples were analyzed using the same analytical methods (Ylitalo et al., 2001; Ylitalo et al., 2005a, 2005b; Greig et al., 2007).

Concentrations of summed PCBs ($\sum$PCBs) were calculated using HPLC/PDA analyte concentration data and the following formula: $\sum$PCBs = $\sum$concentrations of 14 PCBs and PCB analyte groups listed above (based on individual response factor) + $\sum$concentrations of "other PCBs" (calculated by summing areas of peaks identified as PCBs and using an average PCB response factor). The response factors of the PCB congeners measured on the HPLC/PDA system are similar (ranging from 0.65 to 0.75) regardless of degree of chlorination or other chemical properties, using an average PCB response factor was warranted for the "other PCBs" measured in the samples. Based on retention times and UV spectral data, the other PCBs included PCBs 31, 66, 70, 110, 182, 190, 200, and 202. Summed DDT ($\sum$DDTs) concentrations were calculated by adding the concentrations of *o,p′*-DDD, *p,p′*-DDD, *p,p′*-DDE, *o,p'*-DDT, and *p,p'*-DDT. Summed PCBs, $\sum$DDTs, and HCB were reported as $\mu g\ g^{-1}$ lipid weight (lw).

In order to compare the PCB TEQ concentrations in the current study with those previously reported in killer whales from Kenai Fjords/Prince William Sound, Alaska, we used the mammalian toxic equivalent factors reported in Van den Berg et al. (1998). The PCB TEQs were calculated by multiplying the molar concentration of each dioxin-like congener by the appropriate toxic equivalency factor (TEF) value for that compound. These TEQ concentrations are conservative values as they are calculated solely on measurable concentrations of eight dioxin-like PCBs (i.e., PCBs 77, 105, 118, 126, 156, 157, 169, 189) and did not include polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzo-*p*-furans (PCDFs). In addition, the LOQ values of our PDA are higher than those of low and high resolution gas chromatography/mass spectrometry and thus may also contribute to underestimated TEQ values. In previous studies, PCDDs and PCDFs, as well as dioxin-like PCB congeners, were measured in the blubber of killer whales and TEQs were determined from the data (Kannan et al., 1988; Jarman et al., 1996; Ross et al., 2000; Kajiwara et al., 2006; Noël et al., 2009). Although these studies reported wide ranges of the TEQ values in these animals, dioxin-like PCBs contributed a much larger percentage (>80%) to the TEQs than PCDDs and PCDFs (<20%).

Biopsy blubber samples of the killer whales were analyzed for lipid classes and percent lipid using a Mark 5 Iatroscan TLC/FID (Ylitalo et al., 2005a). Various lipid classes (i.e., sterol esters/wax esters, triglycerides, free fatty acids, cholesterol, polar lipids) were separated on silica-based Chromarods (SIII) and developed in a solvent system containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v). The total percent lipid values were calculated by adding the concentrations of these lipids classes and were reported as percent lipid.

2.3. Quality assurance

Each sample batch contained 8 to 12 field samples, a method blank, and a National Institute of Standards and Technology (NIST) blubber Standard Reference Material (SRM 1945) and were analyzed by HPLC-PDA (Krahn et al., 1994; Ylitalo et al., 2001). The concentrations of ≥70% of individual analytes that were measured in the NIST SRM 1945 were within 35% of either end of the 95% confidence interval range of the published NIST certified or reference OC concentration of that analyte. Method blanks contained no more than four analytes that exceeded four times the LOQ, unless the analyte was not detected in the associated blubber samples in the set. The percent recoveries of the surrogate standard for all field and quality assurance samples ranged from 71% to 99%.

2.4. Statistical analyses

2.4.1. OC concentrations and ratios in North Pacific killer whales

Prior to these analyses, the OC concentrations (not the ratios) were lipid normalized to account for lipid variation among samples (Balmer et al., 2019). These data were then log transformed to meet the criteria for normal distribution and equal variances. We used a generalized linear modelling (GLM) approach to investigate the extent to which variability in contaminant levels (i.e., dependent variables of HCB (ng/g, lw), $\sum$PCBs (ng/g, lw), $\sum$DDTs (ng/g, lw), $\sum$PCB TEQs ($pg\ g^{-1}$, lw) and OC ratios (*p,p′*-DDT/$\sum$DDTs, and $\sum$DDTs/$\sum$PCBs) for the 98 killer

whales was explained by a suite of three independent, fixed effects, variables [i.e., ecotype, foraging area (habitat), sex/maturity category] and one interaction term (ecotype*sex/maturity category). For each dependent variable, we ran all possible model combinations of the four variables (i.e., 15 combinations). Collection year and whale population were not included in our GLM analyses as these data were skewed for certain whale groups (e.g., SEAK killer whales were sampled primarily from 1994 to 1997 whereas the other North Pacific whales were sampled primarily in 2001 and 2002).

All model parameters were estimated by maximizing the likelihood function. To compare models, we calculated four values for each model; Akaike's information criterion (AIC), delta AIC, relative likelihood and AIC weight. Smaller AIC values indicate "better" models, and when comparing two models, we calculated the difference in AIC values (delta AIC; Akaike, 1973; Burnham et al., 2011). A delta AIC of <2 indicates little difference between competing models; a delta AIC of 2–10 indicates moderate support for a difference between the models, and a delta AIC of >10 indicates strong support (Burnham et al., 2011). Relative likelihood represents the likelihood of a model given the data, whereas AIC weight is the discrete probability of each model (Burnham et al., 2011). The best model was defined as having a delta AIC of 0.00, although preference was given to the simplest model if two or more models had a delta AIC of <2. All statistical analyses were conducted in R version 3.5.2 (R Core Team, 2018).

Analysis of variance (ANOVA) and the Tukey–Kramer Honestly Significant Difference (HSD) pairwise post-hoc test were used to compare mean log(∑DDTs lw), log(∑PCBs lw), and log(∑PCB TEQs lw), and untransformed mean OC ratios (∑DDTs/∑PCBs, and *p,p′*-DDE/∑DDTs) between ecotypes, among geographical areas and age/maturity categories, and the interaction (ecotype*sex/maturity category). For mean log(HCB lw), we compared mean values between ecotypes and sex/maturity categories. The Tukey–Kramer HSD test is one of a number of post-hoc methods recommended to use to test differences between pairs of means among groups that contain unequal sample sizes (Zar, 1999).

We also examined differences in mean levels and ratios of the OCs for adult resident males only ($n = 33$) from four geographical areas (WNP, EAI, GOA, and SEAK). Data from adult males were used to avoid any confounding issues associated with differences in OC levels and patterns based on maternal offloading to offspring. Limited samples sizes precluded comparisons among resident adult male whales from other populations or among other sex/maturity categories. The level of significance used for all statistical tests was $p \leq 0.05$. The univariate analysis was completed using JMP Statistical Software (SAS Institute, Inc., Cary, NC).

2.4.2. Assessing segregated diet and foraging areas among killer whale populations

Principal Component Analyses (PCA), as detailed in the software package Primer-E version 6 (Clark and Warwick, 2006; Clark and Gorley, 2006) was used to further evaluate segregation in the relative abundance of OCs in individual whales among resident and transient populations, potentially due to differences in diet and foraging area among populations and/or differences in the composition of sex/maturity categories among populations. The PCA was limited to a comparison among the six populations that had at least five biopsy samples (EAI residents, GOA residents, SEAK residents, WNP residents, EAI transients, and SEAK transients), and included all sex/maturity class samples for each population. Although OC mixtures in marine mammals can differ among sex and reproductive maturity categories because females preferentially offload less hydrophobic contaminants to their offspring via lactation (Ridgway and Reddy, 1995; Pomeroy et al., 1996; Ylitalo et al., 2001; Debier et al., 2003; Desforges et al., 2012), we hypothesized (and tested) that these differences would not be as pronounced as those associated with variation in diet and foraging area among transient and resident populations. Dissimilar contaminant mixtures (i.e., fingerprints) among populations of the same sex/maturity category would suggest inputs of specific OCs associated with different sources (i.e., diet and foraging area of resident and transient populations).

The OC data selected for the PCA analyses excluded three of the 20 OCs (PCB 77, 126, and 169) because they were detected in <30% of the samples. Fifteen of the samples had blank values for at least one analyte because of interference by unidentified analytes preventing quantification. These interference-blank values were randomly distributed among the sample-analyte combinations, except for one sample from a SEAK resident whale, which had five blanks and was excluded from the analyses, resulting in 88 samples. The remaining missing values were replaced with a value calculated by an expectation maximum likelihood algorithm (Primer-E, version 6). For the remaining 17 analytes originally included in the PCA, undetectable values (131 of 1496 sample analyte combinations, 8.8% of the data set) were replaced with the average of the analyte-specific LOQ for this study. Prior to analyzing with PCA, the OC data were pretreated by standardizing (i.e., computing the

Table 1
Results of the generalized linear modelling approach that assessed the independent variables (ecotype, that explained variability in each of the six dependent variables [log(HCB lw), log(∑PCBs lw), log(∑DDTs lw), log(∑PCB TEQs lw), ratio *p,p′*-DDE/∑DDTs, ratio ∑DDTs/∑PCBs]. Relative likelihood (rel.like) is the likelihood of a model given the data, and AIC weight (aic.wt) is the discrete probability of each model. Only models that are indistinguishable (i.e., delta AIC of ≤2.0) are displayed.

Model	AIC	delta.aic	rel.like	aic.wt
Log(HCB)~ecotype*sex/maturity	**63.6**	**0.0**	**1.0**	**0.2**
Log(HCB)~ecotype+ecotype*sex/maturity	63.6	0.0	1.0	0.2
Log(HCB)~ sex/maturity+ecotype*sex/maturity	63.6	0.0	1.0	0.2
Log(HCB)~ecotype+sex/maturity+ecotype*sex/maturity	63.6	0.0	1.0	0.2
Log(∑PCBs)~area + ecotype*sex/maturity	**66.4**	**0.0**	**1.0**	**0.2**
Log(∑PCBs)~ecotype+area + ecotype*sex/maturity	66.4	0.0	1.0	0.2
Log(∑PCBs)~area + sex/maturity+ecotype*sex/maturity	66.4	0.0	1.0	0.2
Log(∑PCBs)~ecotype+area + sex/maturity+ecotype*sex/maturity	66.4	0.0	1.0	0.2
Log(∑PCBs)~ecotype+area + sex/maturity	67.7	1.3	0.5	0.1
Log(∑DDTs)~area + ecotype*sex/maturity	**97.3**	**0.0**	**1.0**	**0.2**
Log(∑DDTs)~ecotype+area + ecotype*sex/maturity	97.3	0.0	1.0	0.2
Log(∑DDTs)~area + sex/maturity+ecotype*sex/maturity	97.3	0.0	1.0	0.2
Log(∑DDTs)~ecotype+area + sex/maturity+ecotype*sex/maturity	97.3	0.0	1.0	0.2
Log(∑DDTs)~ecotype+area + sex/maturity	98.0	0.7	0.7	0.2
Log(∑PCB TEQs)~area + ecotype*sex/maturity	**79.0**	**0.0**	**1.0**	**0.2**
Log(∑PCB TEQs)~ecotype+area + ecotype*sex/maturity	79.0	0.0	1.0	0.2
Log(∑PCB TEQs)~area + sex/maturity+ecotype*sex/maturity	79.0	0.0	1.0	0.2
Log(∑PCB TEQs)~ecotype+area + sex/maturity+ecotype* sex/maturity	79.0	0.0	1.0	0.2
Ratio *p,p′*-DDE/∑DDTs~ecotype + area + sex/maturity	**−303.3**	**0.0**	**1.0**	**0.5**
Ratio ∑DDTs / ∑PCBs ~ ecotype + area + sex/maturity	**22.2**	**0.0**	**1.0**	**0.5**

Best model for each dependent variable shown in bolded text.

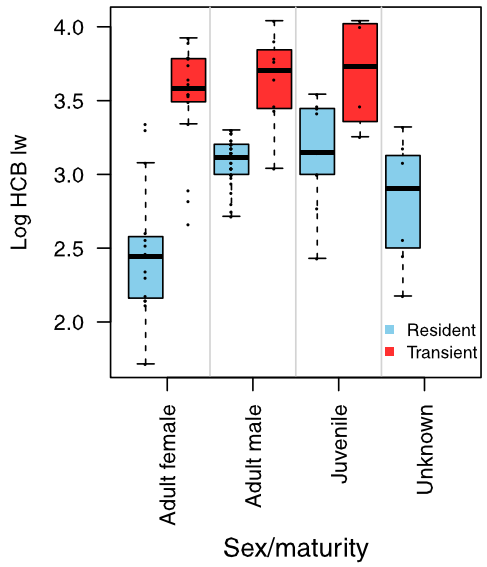

Fig. 2. Plot of log(HCB lw) with respect to sex/maturity class and ecotype. Resident and transient ecotypes are colored blue and red, respectively. Results of analysis of variance (ANOVA) revealed a significant interaction ecotype*sex/maturity class ($F_{2,9} = 4.7$; $p < 0.05$); however, Tukey-Kramer honestly significant difference post-hoc pairwise tests indicated non-significant differences ($p > 0.05$) among sex/maturity classes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

proportional contribution of each OC compound concentration to the total OC concentration in each sample) and then transforming the data by taking the square root to reduce the contribution of dominant compounds. The total number of OCs input to the PCA procedure ($n = 17$) was reduced to five compounds that most efficiently described the OC patterns (HCB, PCB 180, *p,p′*-DDE, and *o,p′*-DDT, and *p,p′*-DDT,) by using the BEST/BVSTEP procedure (Primer-E, version 6) to select OCs that contribute most to explaining the observed OC mixtures. The BEST/BVSTEP identifies the smallest possible subset of a data (i.e., subset of OCs in this case) which, in combination, describes most of the pattern (rho >0.95) of the full data (i.e., all 17 OCs).

To test for significant difference in overall OC mixtures among populations (with all sex/maturity categories included), pairwise comparisons of population mixtures were conducted with ANOSIM, using the R statistic to identify the degree of segregation between-groups as detailed in the software package Primer-E version 6 (Clark and Warwick, 2006; Clark and Gorley, 2006). Values of the ANOSIM R statistic range from 0 (i.e., no segregation, or complete similarity) to 1.0 (i.e., complete segregation, or no similarity) of a population. A *p* value of <0.05 was used as a guide for determining whether the measured segregation between populations (i.e., R statistic) was statistically significant. Additionally, to evaluate difference in OC mixtures among populations due to diet and foraging area (i.e., ecotype and geographic area), independent of the composition of sex/maturity categories among populations, PC1 scores of whales of the same sex/maturity category were analyzed for significant difference among populations using a *t*-test or ANOVA and Holm-Sidak post-hoc tests. The level of significance used for all statistical tests was $p \leq 0.05$.

2.4.3. Toxicological risks of OCs to North Pacific killer whales

Concentrations of ∑PCBs and ∑PCB TEQs (see Supplemental Table 1) measured in individual North Pacific killer whales in the current study were compared to threshold values associated with immune suppression and vitamin A depression in harbor seals (PCBs 17 and TEQs 209 pg g^{-1} lw), (de Swart et al., 1996; Ross et al., 1996; Kannan et al., 2000) and reproductive dysfunction (PCBs 77 μg g^{-1} lw) in ringed seals (Boon et al., 1987).

3. Results

3.1. OC concentrations and ratios in North Pacific killer whales

A wide range of OC concentrations and percent lipid values were determined in the blubber of killer whales from the North Pacific (Supplemental Table 1). Among animals of known sex/maturity category, the lowest concentrations of HCB (0.052 μg g^{-1} lw), ∑DDTs (2.6 μg g^{-1}

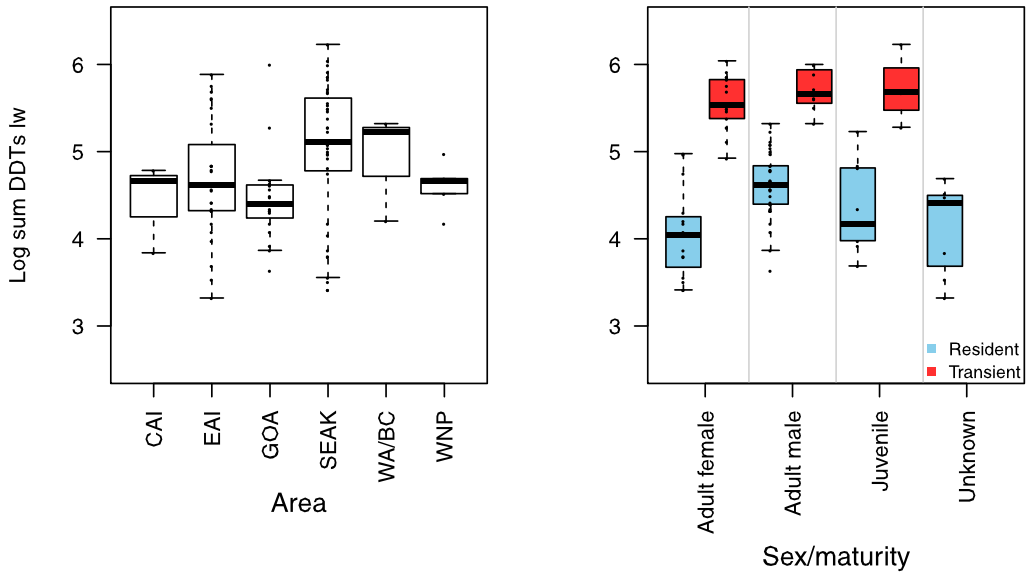

Fig. 3. Plot of log(∑DDTs lw) with respect to a) geographical area, and b) sex/maturity class and ecotype. Resident and transient ecotypes are colored blue and red, respectively. Results of analysis of variance (ANOVA) revealed non-significant differences in log(∑DDTs lw) among geographical areas, as well as non-significant differences in the interaction of ecotype*sex/maturity class. However, significant differences were observed between ecotypes ($F_{1,90} = 182$; $p < 0.001$) and among age and sex classes ($F_{3,95} = 6.9$; $p < 0.01$). Tukey-Kramer honestly significant difference post-hoc pairwise tests indicated non-significant differences ($p > 0.05$) among sex/maturity classes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

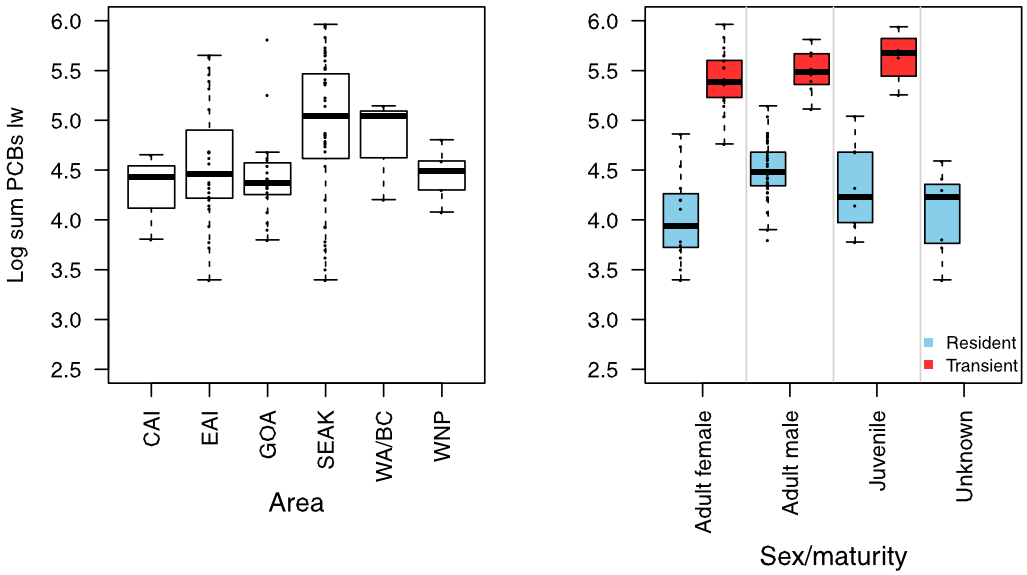

Fig. 4. Plot of log(∑PCBs lw) with respect to a) geographical area, and b) sex/maturity class and ecotype. Resident and transient ecotypes are colored blue and red, respectively. Results of analysis of variance (ANOVA) revealed non-significant differences in log(∑PCBs lw) among geographical areas, as well as non-significant differences in the interaction ecotype*sex/maturity class. Significant differences were observed between ecotypes ($F_{1,90} = 230$; $p < 0.001$) and among sex/maturity classes ($F_{3,95} = 6.9$; $p < 0.01$). Tukey-Kramer honestly significant difference post-hoc pairwise tests indicated non-significant differences ($p > 0.05$) among sex/maturity classes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lw), ∑PCBs (2.5 μg g^{-1} lw), and ∑PCB TEQs (23 pg g^{-1} lw) were measured in the blubber of adult female resident killer whales. The highest concentrations of ∑PCBs and ∑PCB TEQs (920 μg g^{-1} lw and 4700 pg g^{-1} lw, respectively) were found in an adult female transient killer whale. A juvenile transient killer whale had the highest concentration of ∑DDTs (1700 μg g^{-1} lw). The highest concentration of HCB (11 μg g^{-1} lw) was found in the blubber of both a juvenile and an adult male transient whale. We also found that, regardless of ecotype, the rank order of OCs measured was ∑DDTs > ∑PCBs ≫ HCB.

The GLM results for the North Pacific killer whales indicated that most of the variability in the lipid-normalized concentrations of HCB was best explained by ecotype*sex/maturity category interaction (Table 1; Fig. 2). Although the results of ANOVA revealed a significant interaction ($p < 0.001$) ecotype*sex/maturity category for the log(HCB lw), these differences could not be distinguished by Tukey-Kramer HSD post-hoc pairwise tests. For ∑DDTs, ∑PCBs, and ∑PCB TEQs, the variability was best explained by the ecotype*sex/maturity category interaction followed by geographical area (Table 1; Figs. 3 through 5). ANOVA results for the log(∑DDTs lw) and log(∑PCBs lw) showed significant differences between ecotypes ($p < 0.001$) and among age/maturity categories ($p < 0.01$) but Tukey-Kramer HSD did not find differences among the sex/maturity categories (Figs. 3 and 4). Non-significant

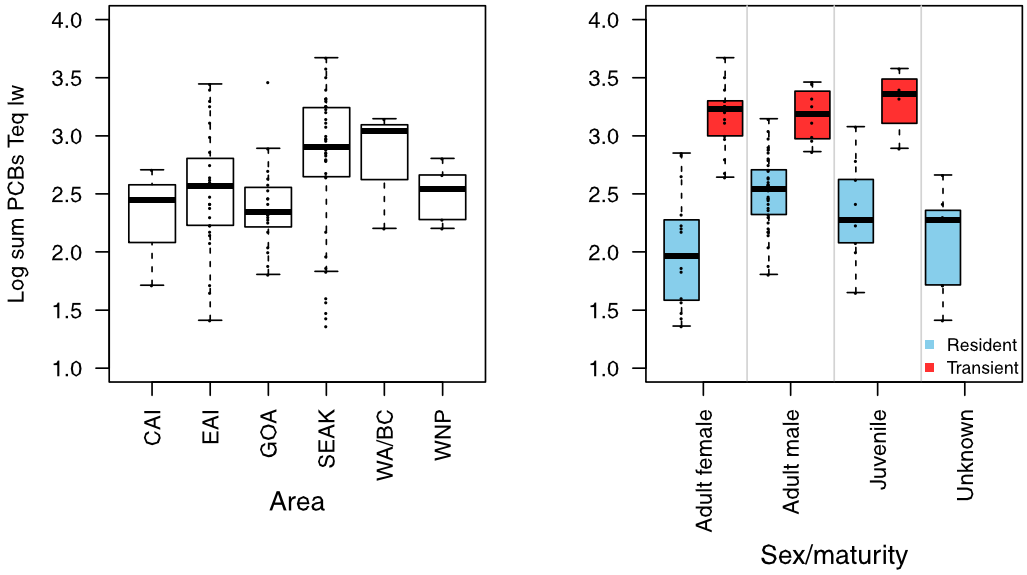

Fig. 5. Plot of log(∑PCB TEQs lw) with respect to a) geographical area and b) sex/maturity class and ecotype. Resident and transient ecotypes are colored blue and red, respectively. Results of analysis of variance (ANOVA) revealed non-significant differences in log(∑PCBs TEQs lw) among geographical areas, yet a significant interaction ecotype*sex/maturity class was observed ($F_{2,90} = 3.2$; $p < 0.05$). Tukey-Kramer honestly significant difference post-hoc pairwise tests indicated non-significant differences ($p > 0.05$) for the interaction ecotype*sex/maturity class. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

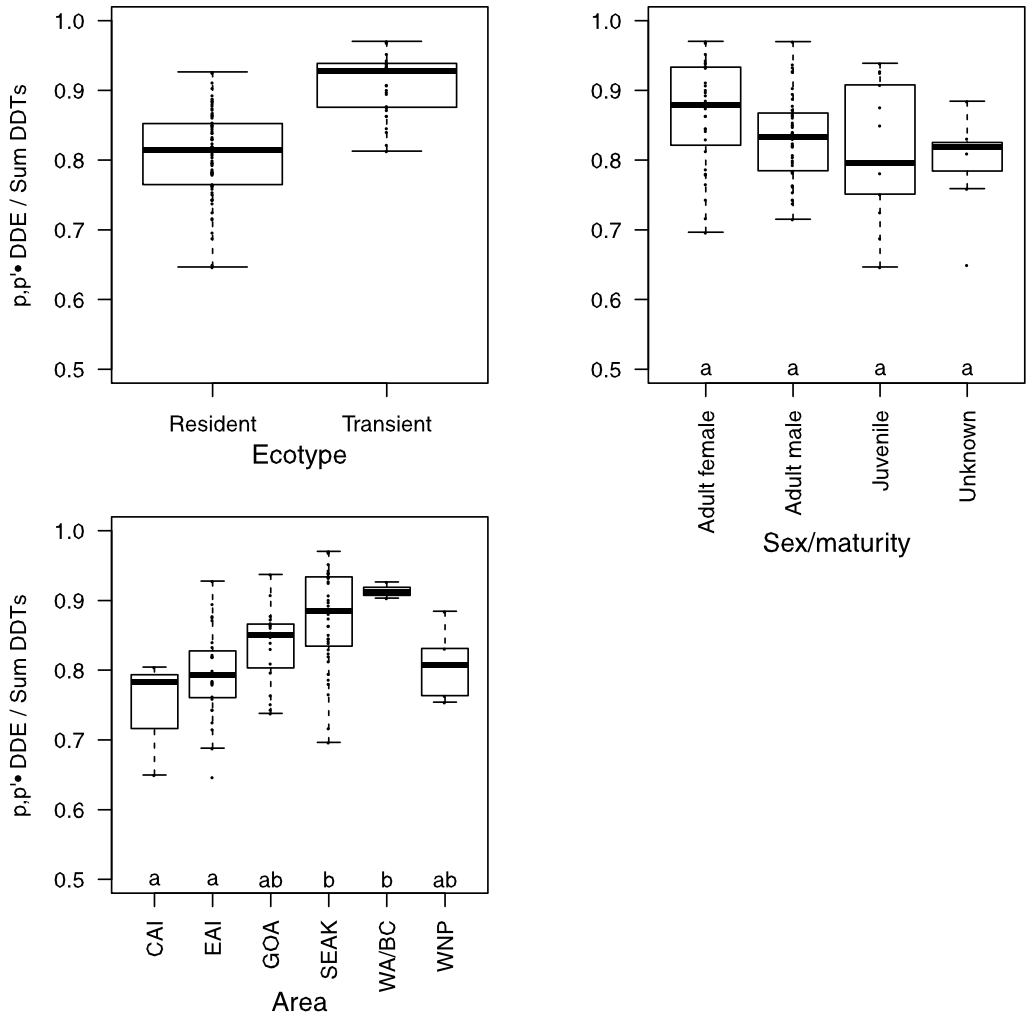

Fig. 6. The ratio of *p,p′*-DDE/∑DDTs with respect to a) ecotype, b) sex/maturity class, and c) geographical area. ANOVA tests revealed significant differences in the ratio of *p,p′*-DDE/∑DDTs between ecotypes ($F_{1,95} = 59$; $p < 0.001$), among sex/maturity classes ($F_{3,93} = 2.9$; $p < 0.05$), and geographical areas ($F_{5,91} = 7.3$; $p < 0.001$). Tukey-Kramer honestly significant difference post-hoc pairwise tests indicated significant differences ($p < 0.05$) between geographical areas, which are denoted by different letters, but did not detect differences among sex/maturity classes ($p > 0.05$).

differences in log(∑DDTs lw) and log(∑PCBs lw) were found among geographical areas or in the ecotype*sex/maturity category interaction. ANOVA tests revealed non-significant differences in log(∑PCB TEQs lw) among geographical areas but found significant interaction of ecotype*sex/maturity category ($p < 0.05$) (Fig. 5). However, differences among the categories were not detected using the Tukey-Kramer post-hoc tests.

For the other two dependent variables, ratios of *p,p′*-DDE/∑DDTs and ∑DDTs/∑PCBs, the variability was best explained by ecotype, sex/maturity category, and geographical area (Table 1; Figs. 6–7). We found significant differences in mean *p,p′*-DDE/∑DDTs ratios between ecotypes ($p < 0.001$), with transients having a higher mean ratio than the resident mean ratio. Significant differences for this ratio were also found among geographical areas ($p < 0.001$), with the CAI and EAI whales having lower mean ratios compared to the ratios determined in SEAK and SRKW whales. Significant differences in mean ratios of *p,p′*-DDE/∑DDTs were found among sex/maturity categories ($p < 0.05$) but the post-hoc pairwise tests did not denote differences among the categories. Significant differences in the ratio of ∑DDTs/∑PCBs were found between ecotypes ($p < 0.01$) and geographical area ($p < 0.0001$), with transients having higher mean ratios than residents and whales from EAI and SEAK having higher mean ∑DDTs/∑PCBs ratios compared to the mean value determined for the GOA whales. Among sex/maturity categories, no significant differences were found in either of these ratios.

To assess if we could detect any additional patterns within a similar sex/maturity category, ANOVA and Tukey's HSD were used to compare mean concentrations of OCs and mean OC ratios among WNP, EAI, GOA, and SEAK resident adult male killer whales. The mean (±SE) OCs (HCB, ∑DDTs, ∑PCBs), ∑PCB TEQs and percent lipid values varied among adult male residents from four populations (WNP, EAI, GOA, and SEAK; Table 2). The percent lipid values in the blubber biopsy samples from adult male WNP, EAI, GOA, and SEAK resident killer whales ranged from 5.0% to 38% (Table 2). Adult male WNP resident killer whales had statistically significant lower lipid percent in blubber biopsy values compared to the EAI ($p = 0.0489$) and GOA ($p = 0.0455$) adult male residents. SEAK adult male residents had significantly higher mean concentrations of ∑DDTs ($p = 0.0120$), ∑PCBs ($p = 0.0200$), and ∑PCB TEQs ($p = 0.0143$) compared to EAI adult male residents. SEAK adult male residents also had significantly higher mean concentrations of ∑DDTs ($p < 0.0001$), ∑PCBs ($p = 0.0014$), and ∑PCB TEQs ($p = 0.0003$) compared to GOA adult male residents. The mean HCB concentration in the SEAK adult male residents was significantly higher than

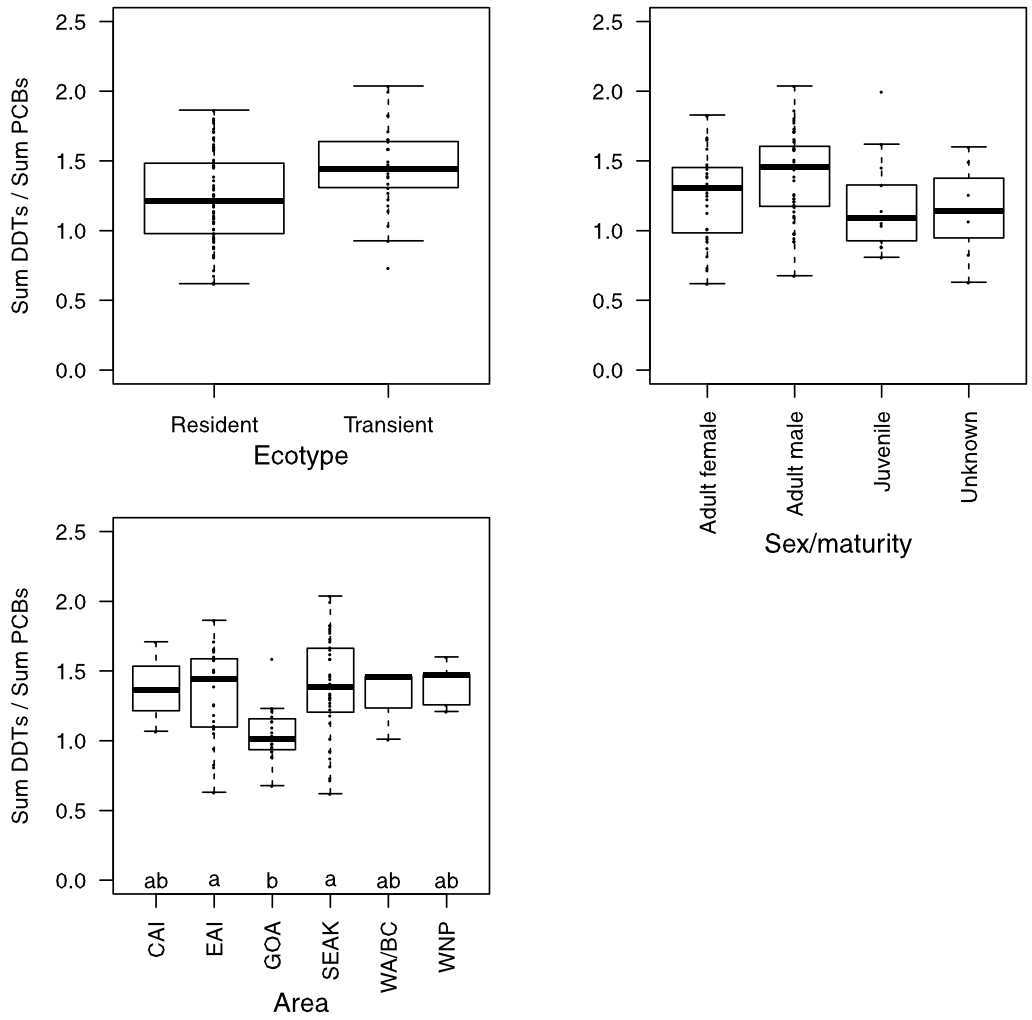

Fig. 7. The ratio of ∑DDTs/∑PCBs with respect to a) ecotype, b) sex/maturity class, and c) geographical area. ANOVA tests revealed significant differences in the ratio of ∑DDTs/∑PCBs between ecotypes ($F_{1,95} = 9.8$; $p < 0.01$) and among areas ($F_{5,91} = 7.3$; $p < 0.001$), but not among sex/maturity classes. Tukey-Kramer honestly significant difference post-hoc pairwise tests indicated significant differences ($p < 0.05$) between areas (denoted by different letters).

those determined in the GOA ($p = 0.0286$) or WNP ($p = 0.0430$), but no significant difference in mean HCB levels was found between EAI and SEAK adult males ($p = 0.3643$). Significant differences in the mean ∑DDTs / ∑PCBs were found among all populations (SEAK and GOA adult males: $p < 0.0001$; EAI and GOA adult males: $p < 0.0001$; SEAK and WNP adult males: $p = 0.0310$; WNP and GOA adult males $p = 0.0227$; SEAK and EAI adult males: $p = 0.0150$) except between WNP and EAI resident killer whales ($p = 0.9285$; Table 2). The EAI resident killer whales had a significantly different mean *p,p'*-DDE / ∑DDTs ratio than adult males from GOA ($p = 0.0023$) and SEAK ($p = 0.0128$).

Table 2
Mean (±SE) and concentration range of organochlorines (μg g^{-1}, lipid weight, lw, for HCB, ∑DDTs, ∑PCBs and pg g^{-1} lw for ∑PCB TEQs), percent lipid, and mean organochlorine ratios in blubber biopsy samples from adult male western North Pacific (WNP), eastern Aleutian Island (EAI), Gulf of Alaska (GOA), and southeast Alaska (SEAK) resident killer whales.

Population	*n*	Percent lipid	μg g^{-1}, lw			pg g^{-1}, lw	OC Ratios (±SE)	
			HCB	∑DDTs	∑PCBs	∑PCB TEQs	∑DDTs/∑PCBs	*p,p'*-DDE/∑DDTs
WNP resident	3	6.3 ± 1.1	1.1 ± 0.30	52 ± 23	36 ± 15	380 ± 140	1.385	0.775
(range)		(5.0–8.6)	(0.56–1.6)	(15–94)	(12–64)	(160–640)	±0.087	±0.016
Significance[1]		B	B	A, B	A, B	A, B	B	A, B
EAI resident	11	19 ± 2.2	1.4 ± 0.11	44 ± 5.5	30 ± 3.6	330 ± 38	1.455	0.778
(range)		(6.7–31)	(0.87–2.0)	(21–69)	(16–49)	(140–550)	±0.070	±0.011
Significance[1]		A	A, B	B	B	B	B	B
GOA resident	14	19 ± 2.2	1.2 ± 0.09	27 ± 3.7	25 ± 3.3	250 ± 35	1.040	0.838
(range)		(6.0–38)	(0.52–1.7)	(4.3–47)	(6.3–48)	(64–500)	±0.041	±0.011
Significance[1]		A	B	B	B	B	C	A
SEAK resident	5	9.5 ± 1.2	1.8 ± 0.09	120 ± 5.2	67 ± 2.8	770 ± 54	1.763	0.845
(range)		(7.0–13)	(1.6–2.0)	(100−130)	(60–71)	(620–950)	±0.018	±0.012
Significance[1]		A, B	A	A	A	A	A	A

[1]Unlike letters indicate significant differences, Tukey-Kramer Honestly Significant Difference (HSD) test, $p < 0.05$.

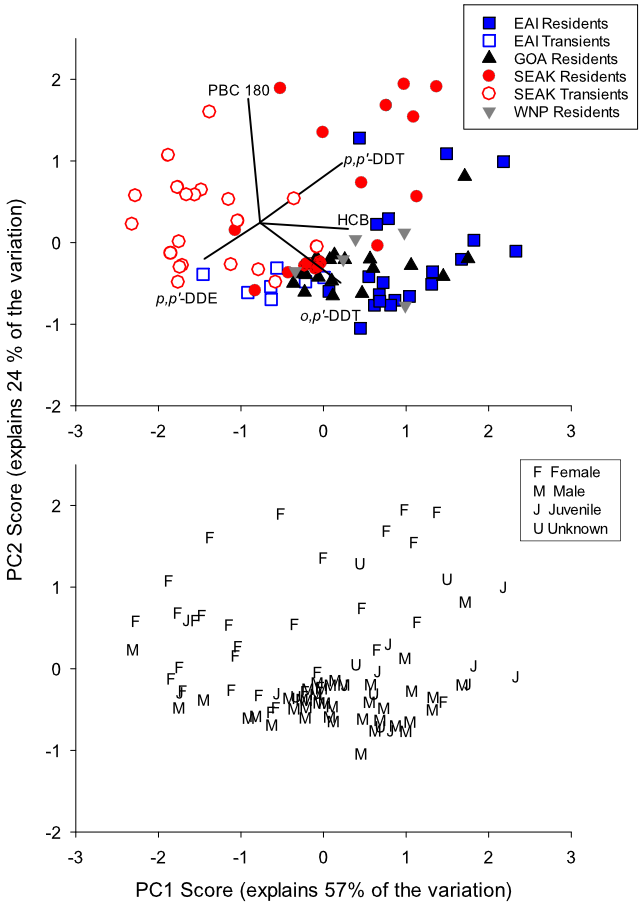

Fig. 8. Plot of the first two principal components based on the Princinpal Component Analysis (PCA) of proportions of 5 OCs (HCB, PCB 180, *p,p′*-DDE *p,p′*-DDT, and *o,p′*-DDT) measured in blubber samples of killer whales populations from the North Pacific Ocean revealing the degree of segregation on OC mixtures among populations (upper panel) and among sex/maturity classes (lower pane). Collectively, both PCAs explain 81.5% of the variation, with PC1 accounting for 57.4%, showing higher proportions of HCB, *p,p′*-DDT, and *o,p′*-DDT in resident whale populations higher proportions of *p,p′*-DDE in transient populations. PC2 accounts for 24% of the variation, with higher proportions of PCB180 in adults females and higher proportion of *p,p′*-DDE in adult males (upper panel).EAI = eastern Aleutian Islands; GOA = Gulf of Alaska; SEAK = southeast Alaska; and WNP = western North Pacific.

3.2. Assessing segregated diet and foraging areas among killer whale populations

A comparison of OC mixtures in killer whales indicated significant segregations in the six populations analyzed: EAI residents, GOA residents, SEAK residents, WNP residents, EAI transients, and SEAK transients (Fig. 8, upper panel; Table 3 $R = 0.451$, $p = 0.001$), which included all adult males, adult females, juveniles and unknowns in each population. Overall, PC1 accounted for 57.4% of the variation and illustrated larger difference in OC mixtures of the transient vs. resident populations but also smaller difference among populations of the same ecotype. Positive loadings for PC1 were highest for HCB, *p,p′*-DDT, and *o,p′*-DDT and were more dominant in resident populations whereas negative loading were highest for *p,p′*-DDE and more dominant in transient populations revealing higher accumulation of *p,p′*-DDE in the transient populations that feed at a higher trophic level. In contrast, PC2 accounted for 24% of the variation and better illustrated differences in OC mixtures among sex/maturity class. Positive loadings for PC2 were highest for highly lipophilic OCs PCB180 followed by *p,p′*-DDT, a mixture observed more in adult females, whereas negative loadings of PC2 were highest *o,p′*-DDT, followed by *p,p′*-DDE, mixture observed more in adults males, suggesting females are retaining these more lipophilic OCs.

Table 3
ANOSIM statistical results for pair-wise comparison of organochlorine (OC) patterns among killer whale populations among. R varies between 0 and 1, although small negative values close to zero are possible. R values closer to 1 signify a higher degree of separation. Statistically significant differences are noted with an *. SEAK = Southeast Alaska, EAI = Eastern Aleutian Island, GOA = Gulf of Alaska, WNP = Western North Pacific.

Populations	R	p	
SEAK transient vs. EAI resident	0.857	0.001	*
SEAK transient vs. WNP resident	0.737	0.001	*
SEAK transient vs. GOA resident	0.687	0.001	*
SEAK transient vs. SEAK resident	0.486	0.001	*
EAI transient vs. WNP resident	0.563	0.004	*
EAI transient vs. EAI resident	0.470	0.002	*
EAI transient vs. GOA resident	0.341	0.013	*
EAI transient vs. SEAK resident	0.100	0.154	
EAI transient vs. SEAK transient	0.285	0.004	*
GOA resident vs. WNP resident	0.436	0.016	*
EAI resident vs. GOA resident	0.374	0.001	*
SEAK resident vs. GOA resident	0.372	0.001	*
EAI resident vs. SEAK resident	0.289	0.001	*
EAI resident vs. WNP resident	0.037	0.350	
SEAK RESIDENT VS. WNP RESIDENT	−0.062	0.631	

Based on an examination of all individuals in each population (i.e., inclusion of all sex/maturity classes), the greatest degree of segregation occurred between SEAK and EAI transients with each of the resident ecotypes (Table 3, R ranges from 0.857 to 0.341 for all but one comparison). Transients (Fig. 9, open symbols) generally distributed further to the left on the PC1 axis (i.e., higher proportion *p,p′*-DDE and lower proportion of HCB, *p,p′*-DDT, and *o,p′*-DDT), than residents (Fig. 9, solid symbols). All comparisons of OC profiles between transients and resident populations were significant ($p \leq 0.01$) except for the comparison between EAI transients and SEAK residents ($p = 0.15$), which also had considerably less segregation ($R = 0.100$) than all other comparisons of transients and residents ($R \geq 0.341$). For SEAK and EAI transients, the OC profiles were also significantly different from each other, with SEAK transients having higher proportions of *p,p′*-DDE, but the degree of segregation was less (Table 3, $R = 0.285$, $p = 0.004$). Overall, less segregation in OC mixtures was observed among resident populations, however, the EAI, GOA, and SEAK populations are all significantly different from each other (Table 3 ($p < 0.001$ for all comparisons), with the greatest segregation between EAI and GOA ($R = 0.374$, followed by SEAK and GOA ($R = 00.372$), and least between EAI and SEAK ($R = 0.289$). The WNP residents were significantly segregated from the GOA resident killer whale population (Table 3, $R = 0.436$, $p = 0.016$) but not the SEAK and EAI resident populations (Table 3, ($p \geq 0.35$); however, there were only five individuals within WNP population, potentially limiting our ability to discriminate true differences that may exist.

Examinantion of a subset of PCA scores among populations of individuals of the same sex/maturity demonstate that variation in OC patterns among populations was primarily determined by ecotype, followed by geographic feeding area (Figs. 9 and 10) rather than the sex/maturity class of the whales. For example, the PCA scores for female transient and resident killer whales from SEAK (Fig. 9, upper panel) illustrate that transient females have a much lower average PC1 score than the resident females (−1.1298 vs. 0.355, *t*-test, $p < 0.001$) Likewise, the PCA scores for male transient and resident killer whales from EAI (Fig. 9, lower panel) illustrate that transient females have a much lower average PC1 score than resident females (−0.646 vs. 0.844, t-test, $p < 0.001$). Furthermore, examination of the PCA scores among resident males (Fig. 10), illustrate significant segregation among populations (ANOVA, $p < 0.001$) with average PC1 score for EAI males (0.844) significantly higher than those from GOA males (0.214) and SEAK males (−0.316), which were not significantly different from each other (Holm Sidak post-hoc test). The WNP resident males also

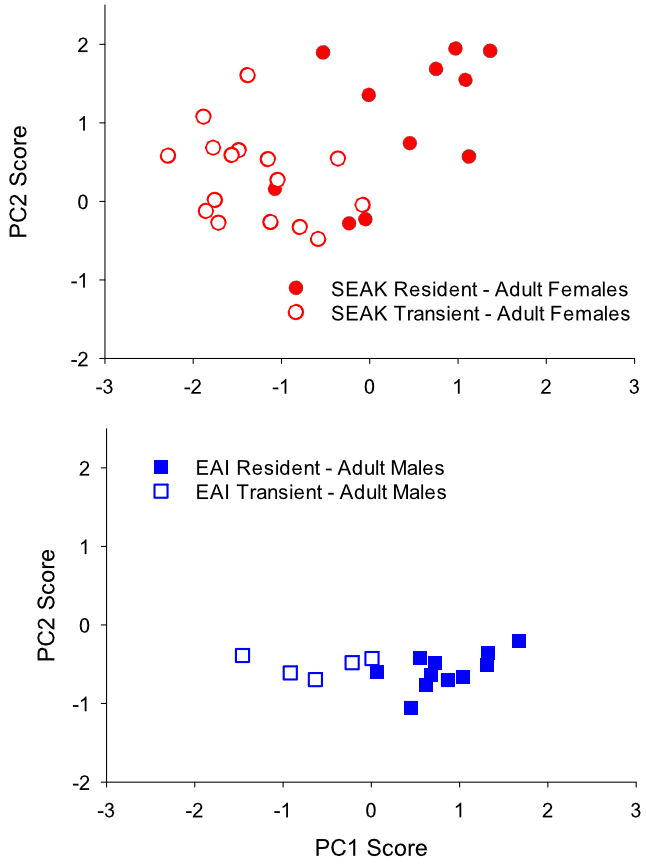

Fig. 9. Plot of the PC1 and PC2 scores for resident (solid symbols) and trantient (open symbols) populations of SEAK adult females (upper plot) and EAI adult males (lower plot), revealing the segregation in PC1 scores for OC mixtures between resident and transient populations within a geographical (foraging) area is not influenced by sex/maturity of the whales. Plotted scores are of subset scores from the PCA of proportions of 5 OCs measured in blubber samples of killer whale populations from the North Pacific Ocean plotted in Fig. 8). SEAK = southeast Alaska EAI = eastern Aleutian Islands, SEAK = southeast Alaska.

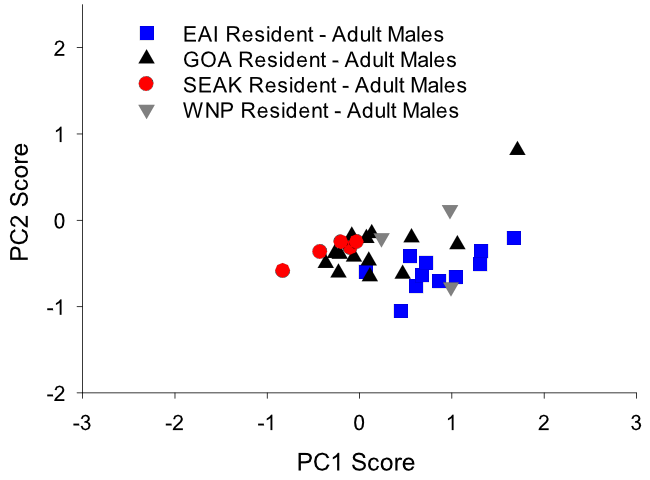

Fig. 10. Plot of the PC1 and PC2 scores for adult males from SEAK (circles symbols), GOA (upward triangles symbols), WNP (downward trianglsymbols), and EAI (square symbols) resident populations, suggesting that segregation in PC1 scores for organochlorine (OC) mixtures among resident population is primarily determined by diet and geographical (foraging) area rather than sex/maturity of whales. Plotted scores are a subset of scores from the PCA of proportions of 5 OCs measured in blubber samples of killer whale populations from the North Pacific Ocean plotted in Fig. 8. EAI = eastern Aleutian Island; SEAK = southeast Alaska, GOA = Gulf of Alaska, and WPN = Western North Pacific.

had a significantly higher average PC1 score (0.736) than the SEAK resident males.

3.3. Toxicological risks of OCs to North Pacific killer whales

Of the 69 resident killer whales analyzed in the current study (Supplemental Table 1), 62% (43/69) of these animals had blubber ∑PCB levels that exceeded the threshold for immune suppression and vitamin A depression whereas only 4% (3/69) of these whales (2 adult males from SRKW population and 1 juvenile from SEAK) had blubber PCB concentrations above the ringed seal reproductive dysfunction threshold. All of the transient whales except one adult female from SEAK (∑PCBs: 58 μg g^{-1} lw) had blubber ∑PCB levels that exceeded both of these threshold values. With regard to blubber PCB TEQ concentrations, all transient (29/29) and 57% (39/69) of the resident whales had values that exceeded the 209 pg g^{-1} lw threshold value.

4. Discussion

4.1. OC concentrations and ratios in North Pacific killer whales

Variability in OC concentrations measured in the blubber of marine mammals is largely due to several factors including diet, age, sex or reproductive history, birth order, body composition, and nutritive condition (Borrell, 1993; de Swart et al., 1994; Aguilar et al., 1999; Ross et al., 2000; Ylitalo et al., 2001; Krahn et al., 2007a; Krahn et al., 2009; Mongillo et al., 2016). In the current study, baseline concentrations and profiles of OCs in various populations of North Pacific killer whales were characterized from biopsy blubber samples of 98 wild-ranging resident and transient killer whales sampled from across the North Pacific, ranging from the Russian Far East to Washington State, USA. We hypothesized that populations of North Pacific killer whales would differ in their OC concentrations and profiles based on their ecotype, geographical (foraging) area, and by sex/maturity category. In addition, our study provides information on the levels of ∑PCB TEQs for killer whale populations across this region.

Transient killer whales across the expanse of the North Pacific had significantly higher mean blubber concentrations of OCs than those measured in residents in the same sex/maturity categories (See Figs. 2–5). These findings were expected because previous feeding ecology and observational field studies have shown that North Pacific transient killer whales feed primarily at a higher trophic level (i.e., marine mammals) than residents, whose diet is composed primarily of fish (Ford et al., 1998, 2000; Ford and Ellis, 2006; Saulitis et al., 2000; Herman et al., 2005; Krahn et al., 2007a; Matkin et al., 2007; Dahlheim and White, 2010). Because OCs are generally resistant to metabolism and environmental degradation, and the majority of OC accumulation in adult killer whales is from their diet, we would expect killer whales that feed at higher trophic levels to have a higher exposure to these pollutants. Moreover, our results corroborate findings reported in previous studies in which OC levels in transient killer whales were substantially higher than in resident killer whales (e.g., Ross et al., 2000; Ylitalo et al., 2001; Herman et al., 2005; Krahn et al., 2007a).

In both transient and resident killer whales, adult females had lower OC concentrations than that observed in juvenile or adult male killer whales (See Figs. 2–5; Supplemental Table 1). Numerous studies have found significant differences in OC concentrations between adult male and adult female killer whales (Ross et al., 2000; Ylitalo et al., 2001; Krahn et al., 2009). This is because reproductive females are able to transfer a substantial amount of their OC body burdens to their calves, especially through lactation (Fukushima and Kawai, 1981; Tanabe et al., 1981; Tanabe et al., 1982; Borrell et al., 1995). Unlike adult whales that receive the majority of OCs from their diet, calves receive the majority of their pollutant load from their mothers through transplacental

transfer and nursing (Tanabe et al., 1982; Aguilar and Borrell, 1994; Borrell et al., 1995; Ridgway and Reddy, 1995; Desforges et al., 2012; Cadieux et al., 2016).

Examination of OC ratios between ecotypes revealed that transient killer whales had significantly higher ∑DDTs/∑PCBs ratios than resident killer whales (Fig. 7). Krahn et al. (2007a) also found higher ∑DDTs/∑PCBs ratios in adult male West Coast transients compared to those determined in adult male residents. Borrell (1993) examined the variations in ∑DDTs/∑PCBs ratios in long-finned pilot whales (*Globicephala melas*), Atlantic white-sided dolphins (*Lagenorhynchus acutus*), harbor porpoises, fin whales (*Balaenoptera physalus*), sei whales (*B. borealis*), and sperm whales (*Physeter macrocephalus*) from the northeastern North Atlantic and found an increasing ratio with body size and a decreasing ratio with trophic level. In contrast, Pinzone et al. (2015) found that long-finned pilot whales, fin whales, and sperm whales from the Mediterranean Sea that feed at different trophic levels did not have significant differences in ∑DDTs/∑PCBs, further suggesting that other factors may also influence this ratio in cetaceans.

It has also been previously suggested that geographical foraging areas influence the ∑DDTs/∑PCBs ratio. For example, among the three Southern Resident killer whale pods (J, K, and L pods), the ∑DDTs/∑PCBs ratios were significantly higher in K and L pods than the ratios determined for the J pod (Krahn et al., 2007b, 2009). Relatively high levels of DDTs are found in the marine environment off California, creating what has been called a "California signature" where ∑DDTs are high relative to ∑PCBs (Calambokidis and Barlow, 1991; Jarman et al., 1996; Krahn et al., 2007b). Consequently, salmon stocks that originate from the more urban southern waters generally have higher DDTs than stocks that originate from British Columbia or Alaska (Mongillo et al., 2016), as this OC was primarily used as an insecticide in agricultural regions (EPA, 1972; Eganhouse et al., 2000; Blasius and Goodmanlowe, 2008). Supported by field observations of K and L pods off California waters and no observations of J pod, the authors suggested this difference in the chemical fingerprint was likely due to the pods feeding in spatially distinct areas during certain times of the year. Krahn et al. (2007a) also reported that GOA resident killer whales had statistically lower ∑DDTs/∑PCBs ratios and statistically higher ∑PBDEs / ∑PCBs than the EAI or CAI whales. Although the primary driver for the variations in this ratio through the food web is not well known, it is likely that the geographic proximity to regional OC sources is an important driver.

Some OC ratios can be used to estimate how recently the pollutant loading into a local ecosystem occurred. For example, *p,p′*-DDE, a stable and persistent isomer that originates from the breakdown of DDT, is more abundant over time and through the food web (Aguilar, 1984; Borrell, 1993) than the parent *p,p′*-DDT. In pinnipeds and odontocetes, it has been estimated that the conversion of DDT to DDE can take years and continue after the DDT exposure has ceased (Aguilar, 1984). A relatively higher ratio of *p,p′*-DDE/∑DDTs may suggest that DDT has been in the environment for a long time, whereas a relatively smaller ratio may indicate exposure to a 'fresher' source of this OC pesticide. This ratio can also be influenced by the concentration of ∑DDT in the individual (higher body burdens of ∑DDT can result in higher rates of dehydrochlorination and result in higher levels of DDE) (Borrell and Aguilar, 1987; Martineau et al., 1987). In the current study, transient killer whales in all sex/maturity categories had significantly higher *p,p′*-DDE/∑DDTs than resident killer whales in the corresponding class, reflecting its persistence through the food web and may also reflect the intensification of dehydrochlorination given their relatively higher body burdens than resident whales. The mean *p,p′*-DDE/∑DDTs values measured in resident and transient North Pacific killer whales was above 0.8 and 0.9, respectively (Fig. 6). Similarly, McHugh et al. (2007) sampled blubber from killer whales from British and Irish waters and reported that the *p,p′*-DDE/∑DDTs ratio ranged from 0.7 to 0.96, suggesting a more historical contamination source of DDTs.

4.2. Assessing segregated diet and foraging areas among killer whale populations

Distinct chemical fingerprints, suggesting distinct foraging areas and/or diets, were measured among the four resident and two transient populations (Fig. 8), based on the PCA analyses of the relative abundance of five OC analytes: HCB, non-dioxin-like PCB 180, and DDT metabolites *p,p′*-DDE, *p,p′*-DDT, and *o,p′*-DDT. The greatest distinction in fingerprints occurred between transient populations and resident populations, consistent with the differences in OC concentrations and ratios observed for these ecotypes. As discussed above, transient killer whales in all sex/maturity categories had significantly higher mean *p,p′*-DDE/∑DDTs ratios than resident killer whales in the corresponding sex/majority category, likely associated with persistence of *p,p′*-DDE through the food web. However, we also observed differences in fingerprints of EAI and SEAK transients, likely associated with their foraging areas. The SEAK transients have been well documented (via photographic matches) to frequently occur throughout the coastal waters of British Columbia and Washington State (Dahlheim and White, 2010). Conversely, SEAK transients have not been observed in Prince William Sound (Matkin et al., 1999b), or the Gulf of Alaska, Aleutian Islands, and Bering Sea (Dahlheim, 1997).

We also observed significant differences in chemical fingerprints among resident populations, consistent with differences in *p,p′*-DDE/∑DDTs among adult males from resident populations. The EAI resident killer whales had a significantly different mean *p,p′*-DDE/∑DDTs ratio than adult males from GOA and SEAK. SEAK residents, which are known to inhabit waters off southeast Alaska, had significantly different profiles than EAI and GOA residents (Table 2). It is likely that SEAK resident killer whales consume salmon with a more southern range for part of the year including those from British Columbia, Washington, Oregon and California given these populations are known to consume Chinook salmon; the Chinook salmon in SEAK are dominated by southern stocks; and strong synchronized demographic rates were found between SEAK resident killer whales and SRKWs, which suggests a common environmental driver (Ward et al., 2016). These southern stocks of adult Chinook salmon (e.g. stocks from British Columbia, Washington, Oregon, and California) tend to have higher OC concentrations than Chinook populations from Alaska (Mongillo et al., 2016). Supporting this hypothesis, we found significantly higher OC concentrations in blubber of adult male SEAK resident killer whales than in adult males from the WNP, EAI, and GOA that likely consume different salmon stocks and other fish species. We also found significant differences in mean blubber percent lipid values among these adult resident males based on geographic areas, but the reasons for these differences are not known. It should be noted that the SEAK adult males were sampled approximately four to seven years prior to adult males from the three other geographical areas. The effects of different sampling years on the killer whale blubber OC concentrations, however, are likely to be negligible as the declines in concentrations of PCBs, DDTs, and HCB in marine biota have been estimated to range from 2 to 8% per year since their attaining maximum concentrations in the 1980s (AMAP, 2015; West et al., 2017; Bolton et al., 2020). It is more likely that the differences in OC profiles among resident adult male killer whales are largely driven by their segregated feeding locations. Future effort should include additional collection of biopsy samples of these populations to compare and assess temporal trends to this baseline study to confirm if concentrations have continued to decline since the early 2000s.

4.3. Toxicological risks of OCs to North Pacific killer whales

A number of potential health risks have been associated with exposure to high levels of contaminants in top-level predators such as killer whales. For example, Hall et al. (2006a) reported that the risk of infection in harbor porpoise from the United Kingdom doubled when blubber PCB concentrations exceeded 45 μg g^{-1} lipid. Thus, OC exposure

may indirectly affect populations of marine mammals by increasing susceptibility to opportunistic pathogens at lower exposure levels than are necessary to observe direct toxicity or death. In addition, high levels of OCs in other marine mammal species have been linked to reproductive impairment, immune suppression, anemia, endocrine disruption, skeletal deformities, and carcinoma (Reijnders, 1986; Subramanian et al., 1987; de Swart et al., 1995; Ross et al., 1996; Beckmen et al., 2003; Schwacke et al., 2012; Ylitalo et al., 2005b; Desforges et al., 2016).

Although the health effects from mixture interactions is largely unknown, neglecting to consider potential interactive effects may underestimate risk to individual killer whales or their population (Mongillo et al., 2016). For example, certain mixtures of pollutants can interact additively or synergistically and enhance toxicity (e.g. Eriksson et al., 2006; Gao et al., 2009), whereas some may have antagonistic interactions and reduce toxicity (e.g. Yordy et al., 2010). Desforges et al. (2017) used contaminant mixtures from the blubber of polar bears and killer whales for in-vitro experiments with immune cells of multiple species and observed lower effect levels from the mixtures relative to the single compounds. Pellacani et al. (2014) investigated the cytotoxic effects of several combinations of persistent pollutants and observed antagonistic, additive, and synergistic interactions from one mixture. Because real world exposures of mixtures can contain a range of interactions, it makes predicting effects extremely difficult.

There have been several attempts to model relative impacts of OCs on marine mammal populations. Hall et al. (2006b) estimated the effects of PCB accumulation rates on potential population growth rates in a bottlenose dolphin population and found that the current PCB accumulation rates may be depressing the population growth rate. More recently, Desforges et al. (2018) modeled relative PCB effects on killer whale reproduction and immune function to assess the potential risk on long-term viability and population size. The model predicted the high risk of population crashes for many killer whale populations (e.g., those found in more industrialized urban areas and those that eat at higher trophic levels). Their predictions have since been the center of an active and ongoing debate in the online (eLetter) forum of the journal Science about how PCBs could cause population declines.

In the current study, transients appear to be at higher risk of health effects compared to residents as their blubber PCBs and PCB TEQs (Supplemental Table 1) exceeded threshold levels established for marine mammals. As previously noted, we found that 97% of transient North Pacific killer whales had blubber $\sum$PCBs that exceeded the reproductive dysfunction threshold value established for ringed seals (Boon et al., 1987) while only 4% of the resident whales had blubber concentrations that exceeded this value. Regardless of geographical area, adult males comprised the largest faction of animals that had blubber PCB and PCB TEQ levels that exceeded immune suppression and vitamin A thresholds for marine mammals, also placing them at higher risk of deleterious effects. Therefore, examining differences in OC concentrations based on ecotype, as well as population and sex/maturity class is important when assessing the relative risk of potential health impacts from OC exposure in North Pacific killer whales.

4.4. Conclusion

North Pacific killer whales are long-lived, high trophic level predators that are exposed to multiple stressors. Some of these populations are in close proximity to human activities and development and thus exposed to relatively high levels of OCs, making them particularly vulnerable to adverse health effects. Our study results indicate high variability in OC concentrations in North Pacific killer whales and that segregation in foraging areas appears to be a primary driver of variability in OC profiles we observed among the populations within the ecotypes. Current concentrations and profiles of organochlorine contaminants in North Pacific resident and transient killer have likely reduced over the 20-year period since samples were collected. The results of this baseline study are crucial for examining time trend changes in the region and assessing the potential health risks associated with OC exposure in this species.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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