# Use of chemical profiles in assessing the feeding ecology of eastern North Pacific killer whales

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ABSTRACT

Blubber biopsy samples from adult male North Pacific killer whales (*Orcinus orca*), were analyzed for fatty acids, carbon and nitrogen stable isotopes and organochlorine contaminants. Fatty acid profiles were sufficiently distinct among the three reported ecotypes ("resident," "transient" or "offshore") to correctly classify the whales in this study by ecotype using a previously developed discriminant function model. In addition, a new discriminant function model was developed using data from whales from both the new and the previous studies. PCB profiles in blubber also allowed unambiguous classification of all three killer whale ecotypes (also using both the old and new models). OC concentrations and ratios were used to provide additional insight on the dietary preferences of killer whales biopsied in Alaska, particularly for the offshores about which little dietary information is available. Surprisingly, mean ∑DDT concentrations in the offshores exceeded those of the Alaska transients and were 20 times higher than those of the residents. In addition, mean ∑PCB concentrations of offshores were very similar to those of the transients and were 10 times higher than those of the residents. If the offshores are fish-eaters, concentrations of ∑PCBs and ∑DDTs should be more similar to those in the fish-eating residents, rather than to those of the marine mammal-eating transients—however, the reverse was true. Thus, it appears that offshores feed at a high trophic level or consume species containing high levels of ∑PCB and ∑DDT, perhaps shark or tuna species. Ratios of certain contaminants have been used to define regions from which prey may originate. Offshore contaminant ratios (e.g., ∑DDTs/∑PCBs and p,p'-DDT/∑DDTs) generally fell between those of the West Coast transients and those of the Alaska residents and transients. Because the offshores are known to have a range that extends from Alaska to California, their contaminant ratios and other chemical profiles may represent those from a mix of prey species acquired from California to the Arctic. However, this study demonstrates that offshore killer whales consume prey species that are distinctly different from those of sympatric resident and transient killer whales. To identify the particular species that comprise the diets of offshore killer whales, as well as for the other ecotypes, contaminant profiles and ratios, as well as fatty acid and stable isotope profiles must be measured in many more putative prey species collected from the killer whales' foraging areas.

**KEY WORDS: KILLER WHALE; FEEDING ECOLOGY; FOOD/PREY; PREDATION; BIOPSY SAMPLING; STABLE ISOTOPES; FATTY ACIDS; ORGANOCHLORINES; POLLUTANTS; PACIFIC OCEAN**

## INTRODUCTION

To understand the ecology of marine food webs, assessing the diets and trophic positions of top level marine predators, such as the killer whale (*Orcinus orca*), is essential. For example, predation by killer whales has recently

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been reported to play a role in the population declines of several marine mammal species in Alaskan waters (Barrett-Lennard *et al.*, 1995; Estes *et al.*, 1998; Jefferson *et al.*, 1991; Springer *et al.*, 2003). In order to evaluate this hypothesis, dietary information for Alaskan killer whales is needed. However, traditional methods of diet analysis have known biases and limitations when applied to marine predators. For example, in Alaska, the "field season" generally extends from late spring to early fall, so information is limited about feeding habits of killer whales at other times of the year. In addition, observations are often limited to predation occurring near the ocean surface and little is known about underwater feeding habits of these whales. Stomach content analyses provide data only from relatively recent meals and these analyses are typically biased as a result of differential rates of digestion of hard parts (Tollit *et al.*, 1997; Yonezaki *et al.*, 2003). Finally, stomach contents are available only from stranded animals and these whales may not reflect the eating habits of healthy whales. Thus, to provide data that reflect the long-term diets of killer whales, the use of indirect chemical analysis techniques is necessary.

Fatty acid signature analysis of blubber (Iverson *et al.*, 2004) and stable isotope enrichments of $^{13}C$ and $^{15}N$ in the epidermis (Kelly, 2000) are indirect chemical methods that have been used to assess the dietary preferences and trophic position of marine mammals. In addition, patterns of organochlorine contaminants (OCs) have been shown to differentiate cetacean stocks (Krahn *et al.*, 1999; Muir *et al.*, 1996). Combining the results from two or more of these independent methods allows more confidence in the conclusions than can be obtained from a single technique. For example, fatty acid profiles and stable isotope compositions in biopsy samples were used in combination by Hooker *et al.* (2001) to assess the diet of northern bottlenose whales (*Hyperoodon ampullatus*). All three techniques were combined by Herman *et al.* (in press) to qualitatively examine the dietary specializations of eastern North Pacific killer whale populations.

In North Pacific waters, two "ecotypes" of killer whales have been described ("residents" and "transients") (Bigg, 1982; Ford *et al.*, 2000). These resident and transient whales differ in their genetics (Hoelzel *et al.*, 1998), acoustics (Barrett-Lennard *et al.*, 1996), morphology (Ford *et al.*, 2000) and feeding ecology (Ford *et al.*, 1998; Herman *et al.*, in press). Transients are thought to prey solely on marine mammals and residents are believed to consume fish, principally salmon (Baird and Dill, 1995; Ford *et al.*, 1998; Saulitis *et al.*, 2000). A third ecotype—the "offshore"—has also been proposed (Ford *et al.*, 2000) to describe whales that have been encountered in waters off the coast between California and Alaska (Dahlheim, 2005; Ellis, 2005; Krahn *et al.*, 2004a). Offshore killer whales have been shown to have a different mitochondrial DNA (mtDNA) haplotype from those of resident and transient whales (Barrett-Lennard, 2000; Hoelzel *et al.*, 2002), thus supporting the designation of a third killer whale ecotype. Although few feeding observations have been reported for the offshores, initial data suggest that their diet includes fish (Black, 2005; Dahlheim, 2005; Ford *et al.*, 2000).

In this study, we update and expand the information on the three ecotypes of killer whales using a combination of fatty acid, stable isotope, and organochlorine analyses of biopsy blubber and epidermis to broadly infer the prey preferences of eastern North Pacific killer whales encountered in Alaskan waters. In the previous study (Herman *et al.*, in press), the offshore killer whale sample size was low, so additional biopsy samples have been characterized

using the three indirect chemical methods, thus allowing greater confidence in the results. In addition, the number of biopsy samples analyzed from Alaskan transient killer whales has been significantly expanded in order to provide additional information on these animals.

OC "contaminant ratios," as well as OC profiles, have been used to significantly expand the information available to make comparisons among the killer whale ecotypes and their prey. Characteristic contaminant ratios or "signatures" assist in defining regional sources of OCs (Krahn *et al.*, 1999; Muir *et al.*, 1990). For example, because DDTs were used heavily in California before their ban in the 1970s and also a major spill occurred from a DDT manufacturing plant, the concentrations of ∑DDTs relative to ∑PCBs (i.e., the ∑DDTs/∑PCBs ratio) is typically higher in California marine species than in comparable species from other locations—providing a "California signature" (Brown *et al.*, 1998; Jarman *et al.*, 1996). Another characteristic contaminant signature—the "Asian signature"—has resulted from the continued use of pesticides such as p,p'-DDT and technical HCH long after their ban in much of the rest of the world (de Wit *et al.*, 2004). Due to air and ocean currents, pollutants used in Asia are often found in marine biota from Alaska (de Wit *et al.*, 2004). Finally, p,p'-DDT can be identified as originating in regions of the world (e.g., Central America or Asia) where DDT use as a pesticide has only recently been restricted (de Wit *et al.*, 2004). For example, the ratio of p,p'-DDT to ∑DDTs is low in California biota, indicating an "old" (metabolized) source (Aguilar, 1984), whereas this ratio is higher in Alaskan species due to recent use and transport of p,p'-DDT from Asia. In this study, ratios of contaminants found in killer whales biopsied in Alaska were compared to those in putative prey from Alaska and California and used to provide further insight into the movements and the possible prey preferences of these North Pacific killer whale ecotypes.

## METHODS

### Killer whales sampled

Adult male killer whales were used in this study because reproductive female killer whales can transfer their contaminant burden to their calves, so OC concentrations in females are generally lower than in males and are dependent of the number of times they have given birth (Ross *et al.*, 2000). Biopsies of killer whales were collected in Alaska during the 2003/2004 sampling years. All samples were obtained from live whales using remote biopsy sampling techniques (Barrett-Lennard, 2000; Hoelzel *et al.*, 1998; Ylitalo *et al.*, 2001) and biopsy tips of various lengths (typically 3.0 to 3.5cm). All biopsy samples were stored frozen at -80$^{o}$C until analyzed. In an attempt to standardize sample size, frozen biopsy samples were subjected to two lateral cuts. First, the epidermis was removed by cutting the sample 1-2mm from the inside edge of the epidermis and then a second lateral cut was made 2cm from the inside edge of the epidermis (sample length ~1.8cm). The blubber and epidermis samples acquired from these whales were analyzed for fatty acids, OCs and in a few instances, stable isotope ratios (Table 1). Often, an insufficient quantity of epidermis was available to allow stable isotope analyses to be performed, resulting in data omissions for many samples. Blubber samples from adult male killer whales (n=4) from eastern Tropical Pacific (ETP) waters to the west of Nicaragua were provided by the Southwest Fisheries Science Center (Pitman, 2004). Adult male West Coast (California) transients (n=4) were provided by Nancy Black (Ylitalo *et al.*, in prep). Selected

OC results from both ETP and West Coast transients were used for comparison with those from the Alaskan killer whales.

**Killer whale group structure**

Each whale in this study has been provisionally classified as resident, transient or offshore based on field observations and in some cases, on long-term studies of well-known populations. These classifications have been or will be confirmed by mtDNA haplotype identity (Table 1) (Barrett-Lennard, 2000). All killer whales of each ecotype have been grouped by geographical collection region in this study (Table 1). The offshore killer whales have been combined into a single group, because photo-identification resightings of offshore individuals have been recorded between the Bering Sea, British Columbia and California, indicating that many of these whales move over large areas of the Pacific Ocean and may function as one population (Dahlheim *et al.*, in prep). The "West Coast" adult male transients (n=4) used for comparisons to the Alaska whales are whales are found in the California catalog (Black, 2005).

**Killer whale prey**

OC concentrations in selected putative prey species of killer whales were obtained from a number of sources: whole bodies of California Chinook salmon (O'Neill *et al.*, 2005; Ylitalo *et al.*, 2005a); blubber of gray whales (Krahn *et al.*, 2001); blubber of California sea lions (Ylitalo *et al.*, 2005b); whole bodies of Cook Inlet Chinook (NWFSC, 2005); and blubber of Steller sea lions, harbor seals and northern fur seals (NWFSC, 2005).

**Fatty acid, stable isotope and organochlorine analyses**

Fatty acid concentrations in blubber were determined as reported by Krahn *et al*. (2004b). A standard nomenclature system was used for naming these fatty acids, where 'n' followed by a number refers to the position of the first double bond relative to the alkyl end of the molecule. A full list of all 83 fatty acids measured as part of this study, as well as their abbreviations, systematic and trivial names can be found in Table 1 of Krahn *et al.* (2004b). Abbreviations for groups of fatty acid methyl esters (FAMEs) are: short-chain mono-unsaturated (SCMU), long-chain mono-unsaturated (LCMU) and poly-unsaturated ( PUFA).

Stable isotope analyses of killer whale epidermis samples were conducted as described previously (Herman *et al.*, in press). Stable isotope ratios are expressed in δ notation as per mil (‰) by the following expression:

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

where Z is $^{15}N$ or $^{13}C$ and $R_{sample}$ is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ for the tissue sample. $R_{standard}$ is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ of the corresponding standard (atmospheric air and Pee Dee Belemite limestone respectively).

Blubber samples were analyzed for OC contaminant concentrations using the procedure of Sloan *et al*. (2004). A total of 40 PCB congeners and 24 chlorinated pesticides were determined in these samples. For a list of all OC contaminants measured by this method, refer to Sloan *et al.* (2004). In this manuscript, ΣPCB is the sum of all 40

PCB congeners analyzed; ΣDDTs is the sum of o,p'-DDD, p,p'-DDD, p,p'-DDE, o,p'-DDE, o,p'-DDT and p,p'-DDT; Σchlordanes is the sum of oxychlordane, *gamma*-chlordane, nona-III-chlordane, *alpha*-chlordane, *trans*-nonachlor, and *cis*-nonachlor; and finally the Σhexachlorocyclohexanes (ΣHCHs) is the sum of *alpha*-, *beta*-, and *gamma*-HCH isomers. Total lipids in killer whale biopsy samples, as well as lipid classes. were measured by a TLC-FID method (Ylitalo *et al.*, 2004).

**Statistical analyses**

All multivariate and univariate analyses were conducted on non-transformed data using JMP Statistical Discovery Software (PC profession edition, version 5.01). All FAME concentration data were expressed on a weight percent basis (wt%) by dividing the concentration of each individual FAME by the sum of all FAMEs measured in the sample. Individual PCB congener concentration data were also computed on a wt% basis and expressed as the concentration of each individual PCB relative to the sum of all 40 PCBs measured. Expression of the PCB data on a wt% basis effectively normalized the results in such a way that any differences in measured PCB profiles between two or more samples represented a difference in the pattern of PCBs present and was independent of absolute tissue concentration. In addition, differences in absolute OC contaminant levels were examined by comparing PCB concentrations expressed on a lipid normalized basis (ng/g total lipid).

Linear discriminant function analysis (DFA) of the fatty acid and OC contaminant concentration data was performed on the untransformed wt% results using the interactive forward-stepwise method of variable selection. The mis-classification rates of all optimized DFA models were evaluated using the cross-validation procedure described by Herman *et al.*(in press).

**RESULTS AND DISCUSSION**

**Classification of ecotypes from blubber fatty acid and PCB profiles**

The fatty acid data from the adult male killer whale blubber samples (n=32; Table 1) were entered into the original DFA model described by Herman *et al.*(in press) and the predicted ecotype classifications of all 32 of these whales were found to agree with the provisional field observation ecotype assignments (Figure 1). Results from these new killer whale samples were combined with the previous fatty acid results (n=53) described by Herman *et al.*(in press) and an updated discriminant function model was derived (Figure 2). This optimized DFA model was based on the proportions of only four individual fatty acids, specifically, C24:1n9, C16:1n5, C14:0 and C16:0. The ability of the new model to successfully predict ecotype based on the wt% data for these four specific fatty acids was tested using the cross-validation procedure and the misclassification rate was determined to be very low (<0.2%).

The mean summed FAME wt% values in each class of blubber fatty acids for each killer whale ecotype (Table 2) were similar to those reported previously by Herman *et al.* (in press). Among the classes of fatty acids listed, SCMU fatty acids were significantly higher in transient whales ($p < 0.001$) compared to the other two ecotypes. Moreover, among all three ecotypes, transient whales had the lowest proportions of LCMU, omega-3 and PUFA fatty acids in their outer blubber layers. However, only LCMU fatty acids were significantly lower in the transient whales ($p <$

0.05). Although the sums of branched-chain fatty acids were similar between residents and transients, the offshore biopsy samples contained consistently lower proportions of branched-chain fatty acids ($p < 0.01$). Furthermore, for resident and transient killer whales, three of the seven characteristic prey fatty acids (Table 2) were significantly different ($p < 0.01$) between these two ecotypes (specifically, C14:1n5, C16:1n7 and C24:1n9) and were qualitatively consistent with the presumed diets of these whales, i.e., predominantly marine fish for residents and marine mammals for transients) (Ford *et al.*, 1998).

The PCB wt% data for the adult male killer whale blubber samples were entered into the original DFA model described in (Herman *et al.*, in press) and the predicted ecotype results were found to agree (no misclassifications) with field observation classifications (Figure 3). An updated DFA model—combining the PCB results (i.e., whales listed in Table 1) with PCB results described in Herman *et al.* (in press)—was based on CB105, CB151 and CB99 (Figure 4). The misclassification rate of the new model was very low and estimated by the cross-validation procedure to be <0.2%.

**Differences in stable isotope ratios and OC concentrations among ecotypes**

Only a few transient and offshore killer whales in this study had sufficient epidermis to analyze for stable isotopes (Table 1). Analyses of blubber from additional resident whales are currently in progress and their stable isotope results will be reported in the future. The results from the completed analyses showed that the single offshore whale had carbon and nitrogen stable isotope enrichment values of $\delta 13C = -16.7$ and $\delta 15N = 16.9$, whereas and the eastern Aleutian Island transient whales (n=6) had mean values of $\delta 13C = -16.2 \pm 0.5$ and $\delta 15N = 18.2 \pm 1.0$, respectively In general, all values were very similar to those reported previously for these ecotypes by Herman *et al.* (in press).

Mean concentrations for ∑PCBs, ∑DDTs, ∑chlordanes and ∑HCHs were measured in the biopsy blubber of the adult male killer whales of the three ecotypes (Table 3). Contaminant concentrations in males increase with age (Ross *et al.*, 2000), so these concentrations generally have large ranges (Table 3). Mean concentrations for all OCs in Alaskan resident and transient killer whales were very similar to those previously reported for the same ecotype by Herman *et al.* (in press). In contrast, the mean ∑PCB, ∑DDT and ∑chlordane concentrations for the four male offshore killer whales (Table 3) were about 2 times the mean values reported for the offshores by Herman *et al.* (in press), possibly because of the previous small sample size (n=2). Surprisingly, ∑DDT concentrations in the offshores exceeded those of the Alaska transients and were 20 times higher than those of the residents. In addition, ∑PCB concentrations of offshores overlapped with the range of PCBs in the Alaska transients and were about 8 times higher than those of the residents. Previously, the male offshores had ∑DDT and ∑PCB levels that were consistently higher than those of the residents and approached, but generally did not exceed, those of the transients (Herman *et al.*, in press). Both in this study and the previous one (Herman *et al.*, in press), ∑chlordanes and ∑HCHs were much higher in the transients than in either the offshores or residents.

**Differences in ratios of OCs for killer whales and putative prey**

In this study, four different ratios of contaminants (∑DDTs/∑PCBs; p,p'-DDT/∑DDTs; ∑chlordanes/∑PCBs; ∑HCHs/∑PCBs; Figures 5-8) were used to evaluate differences among Alaska killer whale populations, as well as to suggest possible prey species for each ecotype. The ratios of eastern ETP killer whales have been included as an illustration of how contaminant ratios can be indicative of unusual patterns of pollutants in a particular region. In the ETP killer whales, the ∑DDTs/∑PCBs ratio (Figure 5), as well as p,p'-DDT/∑DDTs (Figure 6), were much higher than those found for the other killer whales groups, suggesting a recent use of p,p'-DDT that resulted in a high proportion of ∑DDTs in prey. Interestingly, ∑DDTs in ETP whales (1,300,000 ± 520,000 ng/g lipid) were 5 times levels found in the offshores (the group biopsied in Alaska with the highest ∑DDTs), whereas ∑PCBs in ETP whales (22,000 ± 8,300 ng/g lipid) were only about 20% of those in the offshores (Table 3). These results are consistent with both the heavy use of DDTs as a pesticide in areas of Central America (de Wit *et al.*, 2004) and the more limited use of PCBs (de Wit *et al.*, 2004) in a region in which industrialization has been relatively recent. The California transients had the highest concentrations of ∑DDTs (4,000,000 ± 610,000 ng/g lipid) and ∑PCBs (720,000 ± 34,000 ng/g lipid) among all whales in this study (Ylitalo *et al.*, in prep). The OC data for these whales (both lipid normalized concentrations and ∑DDTs/∑PCBs ratios) indicated a definite "California signature." Furthermore, the low p,p'-DDT/∑DDTs ratios in the West Coast transient whales (Figure 6) was also indicative of foraging in California waters, where the source of DDTs is "old", due to the 1970s ban of DDTs in the U.S.

For Alaska residents and transients, p,p'-DDT/∑DDTs and ∑HCHs/∑PCBs ratios were higher than those of the offshores and West Coast transients (Figures 6 and 8), indicating inputs of p,p'-DDT and HCHs (pesticides that have been banned in the U.S.) that are consistent with heavy use of these pesticides in Asia until recently and their transport to Alaskan waters (the "Asian signature") (de Wit *et al.*, 2004). Ratios of ∑chlordanes/∑PCBs (Figure 7) were highest in Alaska resident and transient killer whales, possibly because of chlordane use as a pesticide in Alaska or in Asia (AMAP, 1998; de Wit *et al.*, 2004). As found for fatty acid and contaminant profiles, contaminant ratios for the offshore killer whales were dissimilar to those of the other whale groups. Contaminant ratios in offshore killer whales generally fell between those of the West Coast transients (whales that feed primarily on marine mammals in areas off the California coast) and those of the Alaska residents and transients (Figures 5-8). Because the offshores are known to have a range that extends from Alaska to California (Dahlheim *et al.*, in prep; Matkin *et al.*, 1999), their chemical profiles likely represent a mix of prey species acquired from California to the Arctic. Furthermore, the levels of OCs found in the offshores were similar to those reported for fish-eating southern resident killer whales (Ross *et al.*, 2000), demonstrating that it is possible to attain these high levels of OCs solely by consuming a fish diet.

Contaminant ratios in selected prey species (Figures 5-8, bottom) generally showed the same regional distinctions found for killer whales. For example, ∑DDTs/∑PCBs ratios were highest (Figure 5) and p,p'-DDT/∑DDTs ratios were lowest (Figure 6) in the California prey (Chinook salmon and California sea lions)—again indicative of the California signature. In contrast, the ∑HCHs/∑PCBs ratios (Figure 8), indicative of the "Asian signature" were high in Alaskan prey (Chinook, Steller sea lions and northern fur seals). Unexpectedly, this ∑HCHs/∑PCBs ratio was

also high in California Chinook. Because California Chinook are a migratory species, HCHs may have been acquired from eating prey in other regions of the North Pacific. As expected, California sea lions had a lower relative ∑HCHs/∑PCBs ratio.

Each contaminant (or group of summed contaminants) is biomagnified to a different extent, so contaminant ratios change as contaminants from prey are assimilated by the predator (Fisk *et al.*, 2001; Hoekstra *et al.*, 2003). However, biomagnification factors (BMFs) appear to be species-specific (Fisk *et al.*, 2001; Hoekstra *et al.*, 2003) and no BMFs have been reported for killer whales. Consequently, OC contaminant ratios cannot be directly compared among killer whales and their likely prey until these BMF values are known. However, qualitative comparisons can be made. For example, West Coast transient killer whales and one of their known prey species, California sea lions (Black, 2005), have similar contaminant signatures (high ∑DDTs/∑PCBs, as well as low p,p'-DDT/∑DDTs, ∑chlordanes/∑PCBs and ∑HCHs/∑PCBs). Furthermore, Steller sea lion pups cannot be ruled out as prey of Alaska transients, because the "Asian signature" (low ∑DDTs/∑PCBs, as well as high ∑chlordanes/∑PCBs and ∑HCHs/∑PCBs) was identified in both prey and predator. Even though Steller sea lion pups could be possible prey for transient killer whales, other marine mammals from the Gulf of Alaska/Aleutian Islands area have ratios that also show an Asian signature (e.g., Figures 5-8; northern fur seals) and thus, could also be potential prey. In fact, transient killer whales may very well prey on a number of different marine mammal species, altering their target prey by availability of favored species. Alternatively, two or more marine mammal prey may have "complimentary" ratios, where a high ratio in one species is balanced by a low ratio in others, allowing the species together to be considered as possible prey. Contaminant ratios are only a single indicator and are intended to be used in combination with the other chemical profiles to provide an accurate assessment of most likely prey for killer whales.

In the previous study (Herman *et al.*, in press), residents and transients were shown to have distinct fatty acid and OC patterns, as well as fatty acid, OC and stable isotope ratio values, that were consistent with those of their putative prey. In contrast, offshore whales were found to have contradictory results—fatty acid profiles were consistent with a fish diet, whereas both OC and stable isotope results suggested that these whales might be feeding at a high trophic level (Herman *et al.*, in press). The current results for the offshores add to the complexity of determining their prey. Concentrations of ∑PCBs and ∑DDTs in offshores approached or exceeded those of the Alaska transients, although levels were lower than those found in West Coast transients (Table 3). Moreover, contaminant ratios found for offshores fell between those found for the West Coast transients and the Alaska whales. One explanation would be that significant proportions of the long-term diets for offshore killer whales may be acquired during their movements to more southern latitudes. In particular, offshores frequent waters off California in winter (Black, 2005), where higher levels of ∑PCB and ∑DDT are typically found in marine fish compared to equivalent fish species found in Alaska waters (Brown *et al.*, 1998; Jarman *et al.*, 1996). Alternatively, the preferential prey of offshore whales may be very different from those of residents and comprise larger, longer-lived marine fish species that are themselves consuming at a much higher trophic level and bioaccumulating high levels of OC contaminants. For example, rather than eating fish species presumed to be the traditional prey of their resident

killer whale counterparts (e.g., salmon), offshores may consume significant quantities of higher trophic level species, such as shark or tuna (Black, 2005). Finally, a mixed, "opportunistic" diet for offshores, comprising both marine fish and marine mammals (e.g., northern fur seal), cannot be entirely ruled out from current fatty acid, stable isotope, and OC contaminant results. To better define the prey of offshores, samples of other possible prey species (e.g., blue and thresher shark, albacore tuna, squid) are currently being collected for analysis.

**Recommendations**

As a result of the current study, additional areas of essential research have been identified. First, contaminant profiles and ratios, as well as fatty acid profiles and stable isotope enrichment values must be measured in many more putative killer whale prey species. These prey species, as often as possible, should be collected from known killer whale foraging areas. Second, mathematical models are needed to correlate the chemical signatures in biopsy blubber of these top-level predators with corresponding chemical signatures in their likely prey.

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**Table 1. Location, ecotype and genetic information about adult male killer whale biopsy samples analyzed for fatty acids[#], stable isotopes[%] and organochlorines[X]**

| Sample# | FA | SI | OCs | Animal Identification | Collection date | Ecotype | Location | Region/category | Haplotype |
|---|---|---|---|---|---|---|---|---|---|
| 1 | # | % | x | 9-16-04 ENC 8 #2 | 9/16/2004 | offshore | Southeast Alaska | AK/OFF | |
| 2 | # | | x | AKW-04-006 | 7/8/2004 | offshore | Kenai Fjords | AK/OFF | |
| 3 | # | | x | AKW-04-007 | 7/8/2004 | offshore | Kenai Fjords | AK/OFF | |
| 4 | # | | x | EA-03-08 | 7/10/2003 | offshore | Eastern Aleutian Islands | AK/OFF | OFF |
| 5 | # | | x | AE040728-01 | 7/28/2004 | resident | Eastern Aleutian Islands | EAI/R | |
| 6 | # | | x | AE040728-02 | 7/28/2004 | resident | Eastern Aleutian Islands | EAI/R | |
| 7 | # | | x | AE040728-04 | 7/28/2004 | resident | Eastern Aleutian Islands | EAI/R | |
| 8 | # | | x | AE040728-05 | 7/28/2004 | resident | Eastern Aleutian Islands | EAI/R | |
| 9 | # | | x | AE040728-06 | 7/28/2004 | resident | Eastern Aleutian Islands | EAI/R | |
| 10 | # | | x | AE040728-07 | 7/28/2004 | resident | Eastern Aleutian Islands | EAI/R | |
| 11 | # | | x | AE040818-02 | 8/18/2004 | resident | Eastern Aleutian Islands | EAI/R | |
| 12 | # | | x | AE040819-01 | 8/19/2004 | resident | Central Aleutian Islands | CAI/R | |
| 13 | # | | x | AKW-04-012 | 8/14/2004 | resident | Kenai Fjords ? | GOA/R | |
| 14 | # | | x | AKW-04-013 | 8/15/2004 | resident | Kenai Fjords ? | GOA/R | |
| 15 | # | | x | EA-03-05 | 7/7/2003 | resident | Eastern Aleutian Islands | EAI/R | NR |
| 16 | # | | x | EA-03-11 | 8/1/2003 | resident | Eastern Aleutian Islands | EAI/R | NR |
| 17 | # | % | x | AE040804-01 | 8/4/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |
| 18 | # | % | x | AE040815-01 | 8/15/2004 | transient | Alaska Peninsula North | EAI/T [04] | |
| 19 | # | % | x | AE040817-01 | 8/17/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |
| 20 | # | | x | CA040711-01 | 7/11/2004 | transient | Southeast Alaska | SEA/T | |
| 21 | # | | x | EA-03-10 | 7/30/2003 | transient | Eastern Aleutian Islands | EAI/T [03] | GAT1 |
| 22 | # | | x | FP-03-03 | 5/20/2003 | transient | Eastern Aleutian Islands | EAI/T [03] | GAT1 |
| 23 | # | | x | FP-03-06 | 5/22/2003 | transient | Eastern Aleutian Islands | EAI/T [03] | GAT1 |
| 24 | # | | x | FP-03-07 | 5/30/2003 | transient | Eastern Aleutian Islands | EAI/T [03] | GAT1 |
| 25 | # | % | x | FP-03-08 | 5/30/2003 | transient | Eastern Aleutian Islands | EAI/T [03] | GAT1 |
| 26 | # | % | x | FP-03-09 | 5/31/2003 | transient | Eastern Aleutian Islands | EAI/T [03] | GAT1 |
| 27 | # | | x | FP-04-01 | 5/4/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |
| 28 | # | | x | FP-04-02 | 5/4/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |
| 29 | # | | x | FP-04-08 | 5/5/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |
| 30 | # | % | x | FP-04-14 | 5/8/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |
| 31 | # | | x | FP-04-25 | 5/30/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |
| 32 | # | | x | UNAK-04-06 | 7/30/2004 | transient | Eastern Aleutian Islands | EAI/T [04] | |

Abbreviations: R=resident; T=transient; OFF=offshore; SEA = Southeast Alaska; EAI = Eastern Aleutian Islands; GOA = Gulf of Alaska; AK = Alaska.

**Table 2. Mean ± SD FAME compositions† in blubber biopsy samples from adult male offshore, resident and transient killer whale**

| | Offshores (n=4) | Residents (n=12) | Transients (n=16) |
|---|---|---|---|
| Σ n-6 | 2.22 ± 0.26 | 2.19 ± 0.36 | 2.15 ± 0.37 |
| Σ n-3 | 3.73 ± 1.14 | 4.41 ± 1.97 | 3.51 ± 1.09 |
| Σ saturated | 16.75 ± 1.57 | 13.99 ± 2.21 | 15.10 ± 2.44 |
| Σ SCMU‡ | 20.69 ± 2.59 | 31.05 ± 3.52 | 39.36 ± 5.48 |
| Σ LCMU¥ | 47.15 ± 2.62 | 46.86 ± 4.22 | 38.33 ± 4.95 |
| Σ PUFA§ | 7.41 ± 1.52 | 8.09 ± 2.50 | 7.22 ± 1.48 |
| Σ branched | 1.33 ± 0.10 | 1.76 ± 0.32 | 1.63 ± 0.34 |
| *Individual fatty acids having relatively high concentrations in pinnipeds/cetaceans* | | | |
| C14:1n5 | 1.38 ± 0.31 | 3.45 ± 0.66 | 4.17 ± 0.58 |
| C16:1n7 | 19.59 ± 1.46 | 21.38 ± 2.48 | 27.06 ± 5.03 |
| C18:1n9 | 34.72 ± 1.20 | 27.49 ± 1.93 | 25.68 ± 3.02 |
| *Individual fatty acids having relatively high concentrations in fish* | | | |
| C16:0 | 8.71 ± 0.84 | 5.23 ± 1.08 | 5.64 ± 0.93 |
| C18:0 | 1.34 ± 0.07 | 0.92 ± 0.14 | 0.93 ± 0.16 |
| C22:6n3 | 0.92 ± 0.44 | 0.93 ± 0.72 | 0.57 ± 0.31 |
| C24:1n9 | 0.25 ± 0.05 | 0.22 ± 0.04 | 0.07 ± 0.02 |
| %FA | 19.8 ± 3.6 | 21.2 ± 11.5 | 23.0 ± 6.7 |

† compositions expressed in units of percentage of total fatty acids by mass (wt%)
‡ sum of all short-chain mono-unsaturated fatty acid methyl esters (C ≤ 16)
¥ sum of all long-chain mono-unsaturated fatty acid methyl esters (C >16)
§ sum of all poly-unsaturated fatty acid methyl esters

**Table 3. Mean ± SD and (range) of organochlorine contaminants (ng/g lipid) and percent lipid in blubber biopsy samples from adult male offshore, resident and transient killer whales from Alaska***

| | Offshores (n = 4) | Residents (n = 12) | Transients (n = 16) |
|---|---|---|---|
| Σ PCBs | 110,000 ± 22,000 (79,000 – 130,000) | 14,000 ± 4,400 (9,400 – 25,000) | 140,000 ± 68,000 (58,000 – 310,000) |
| Σ DDTs | 420,000 ± 100,000 (290,000 – 510,000) | 21,000 ± 11,000 (12,000 – 46,000) | 240,000 ± 140,000 (76,000 – 550,000) |
| Σ Chlordanes | 16,000 ± 2,300 (13,000 – 18,000) | 6,900 ± 1,500 (4,800 – 9,600) | 76,000 ± 34,000 (40,000 – 170,000) |
| Σ HCHs | 500 ± 93 (440 – 620) | 620 ± 220 (210 – 910) | 10,000 ± 3,900 (3,200 – 16,000) |
| %lipid | 17.9 ± 3.7 | 21.7 ± 13.8 | 21.5 ± 7.4 |

*For comparison, concentrations (ng/g lipid) in adult male West Coast (California) transients (n=4) were: Σ PCBs = 720,000 ± 34,000; Σ DDTs = 4,000,000 ± 610,000; Σ Chlordanes = 50,000 ± 4,100; and Σ HCHs = 3,800 ± 1,100 (Ylitalo *et al.*, in prep).

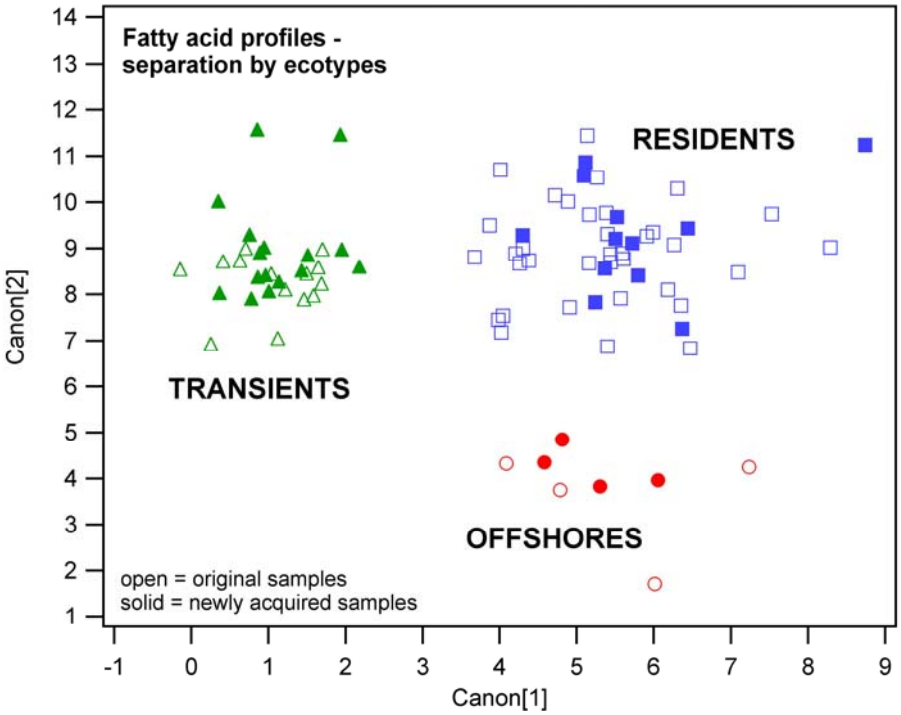


Figure 1. Discriminant function showing successful classification of adult male killer whale ecotypes based on the fatty acid profiles of their blubber biopsies. Points labeled with solid shapes are the canonical scores computed for newly acquired samples using the original discriminant model described in Herman *et al*. (in press).

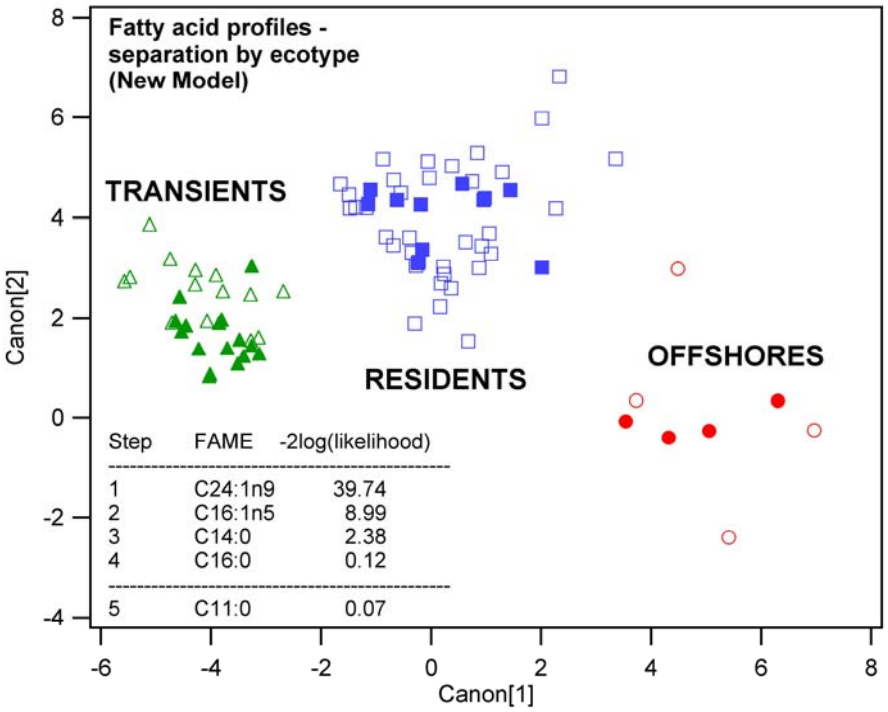


Figure 2. Revised discriminant function model showing successful classifications of adult male killer whale ecotypes based on the FAME profiles of their blubber biopsies. The updated model includes all killer whale samples from the original model (Herman *et al.*, in press) plus all adult male killer whales listed in Table 1.

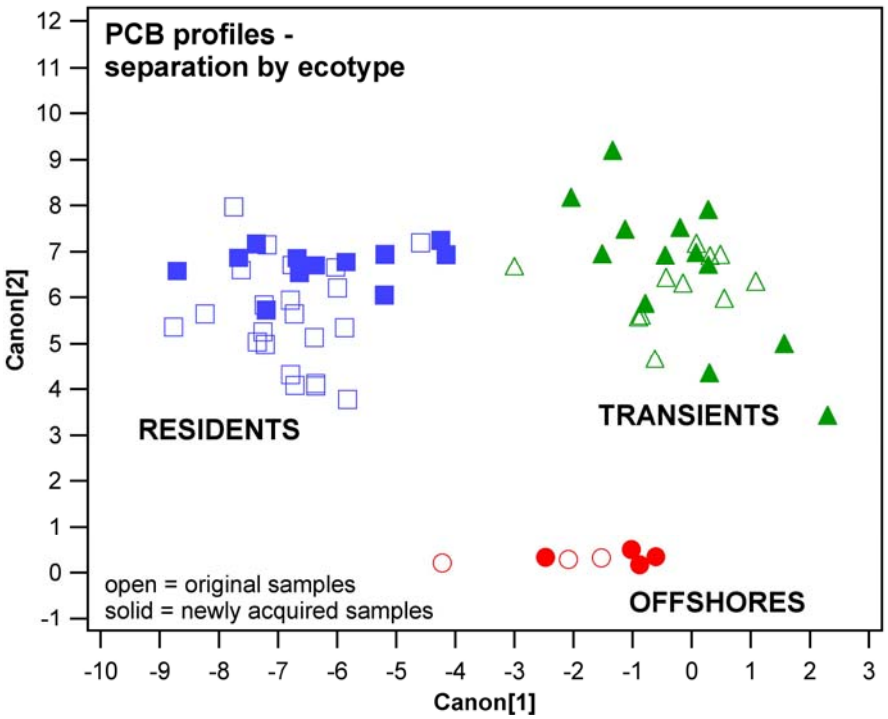


Figure 3. Discriminant function showing successful classification of adult killer whale ecotypes based on the PCB patterns of their blubber biopsies. Points labeled with solid shapes are the canonical scores computed for newly acquired samples using the original discriminant model described in Herman *et al*. (in press).

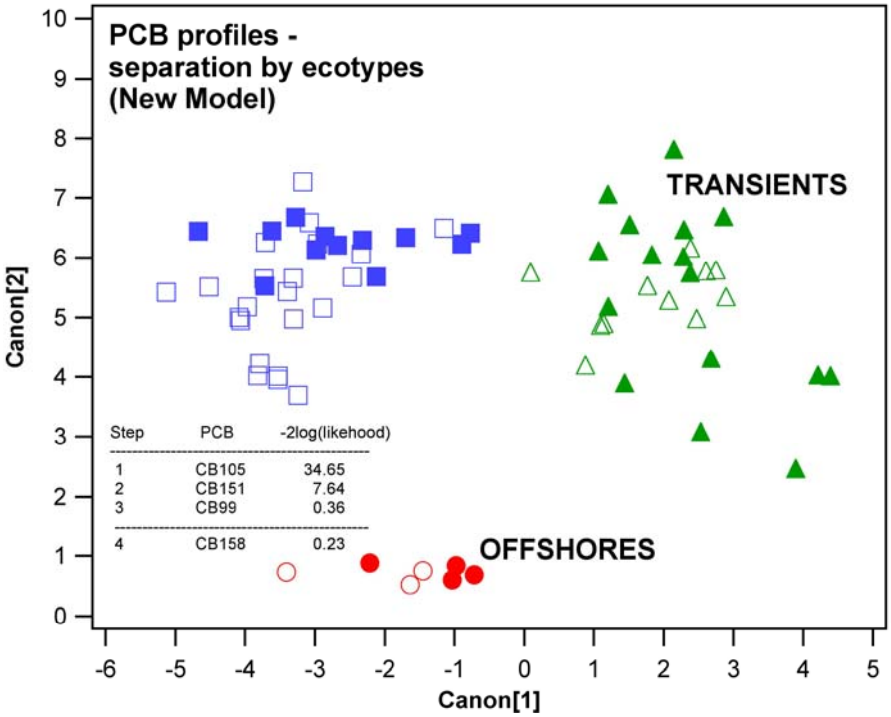


Figure 4. Revised discriminant function model showing successful classifications of adult killer whale ecotypes based on the PCB patterns of their blubber biopsies. All samples analyzed to date are included in the new model.

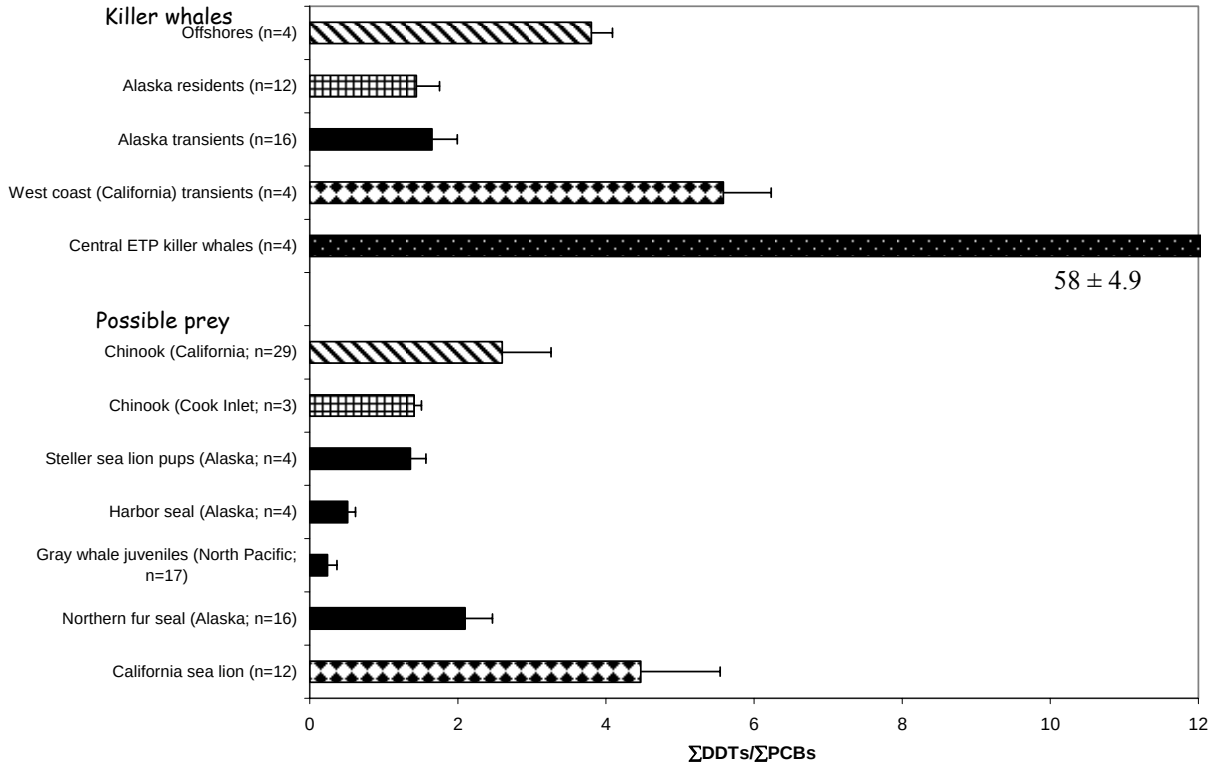


Figure 5. The ratios Σ DDTs /Σ PCBs in adult male killer whale populations and selected putative prey species (matching patterns).

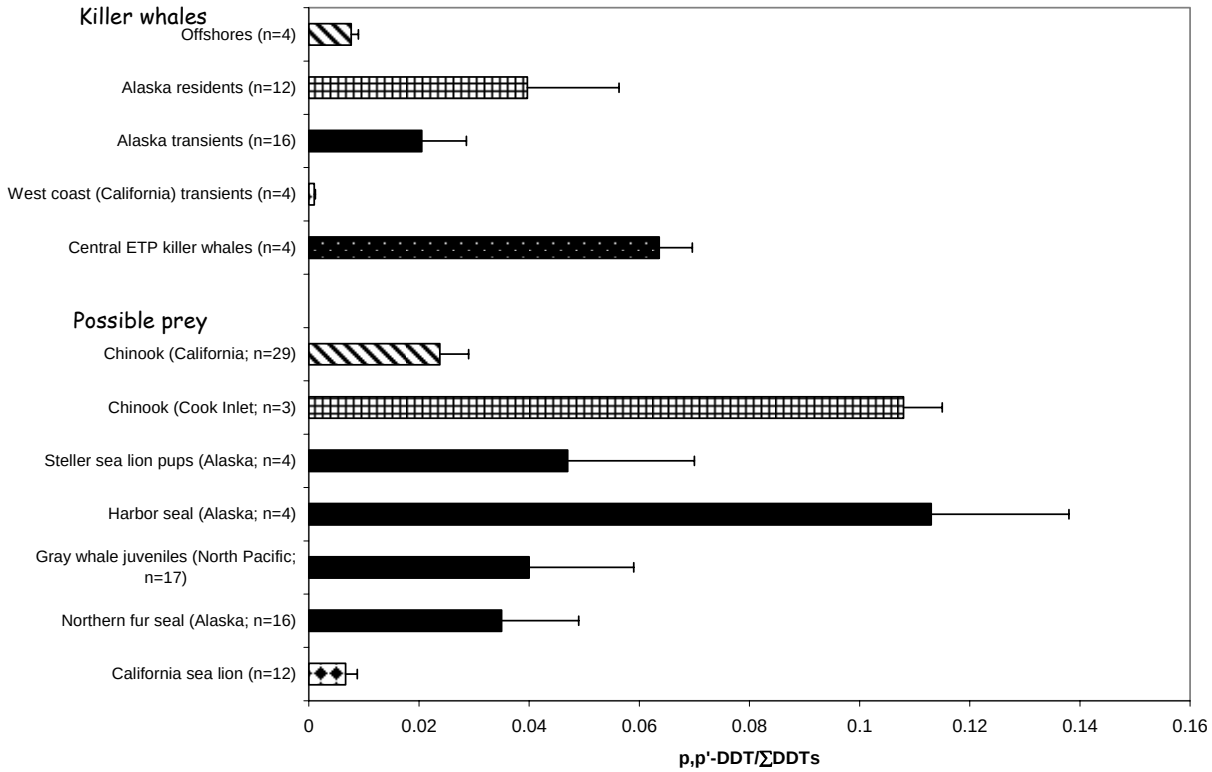


Figure 6. The ratios p,p'-DDT/Σ DDTs in adult male killer whale populations and selected putative prey species (in matching patterns).

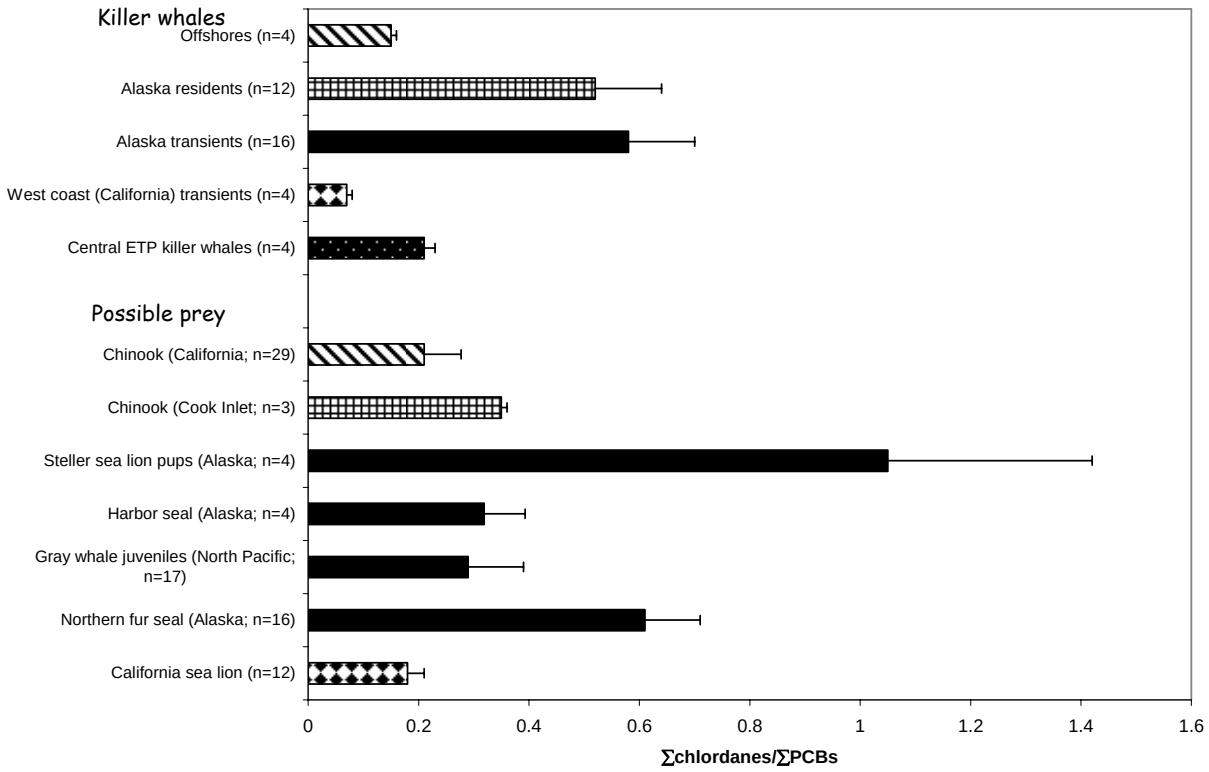


Figure 7. The ratios Σ chlordanes /Σ PCBs in adult male killer whale populations and selected putative prey species (matching patterns).

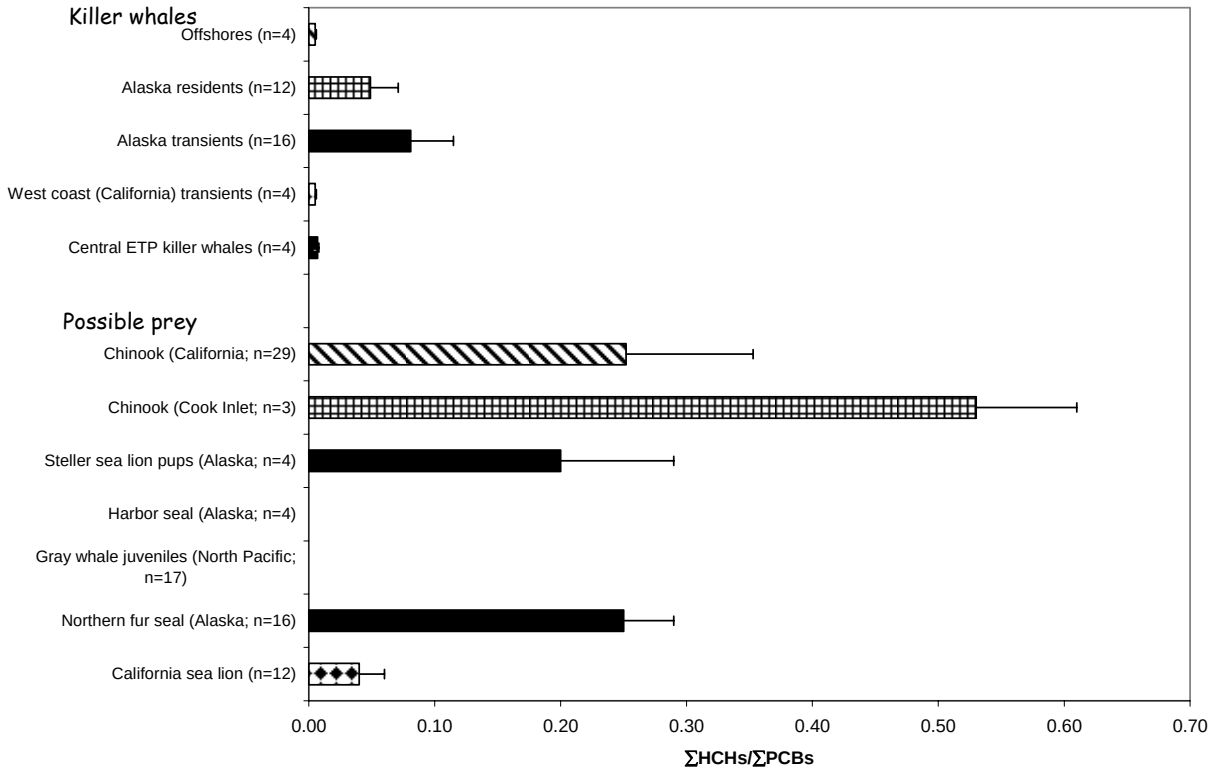


Figure 8. The ratios Σ HCHs /Σ PCBs in adult male killer whale populations and selected putative prey species (matching patterns).