Life history and social structure as drivers of persistent organic pollutant levels and stable isotopes in Hawaiian false killer whales (*Pseudorca crassidens*)

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ABSTRACT

False killer whales are long-lived, slow to mature, apex predators, and therefore susceptible to bioaccumulation of persistent organic pollutants (POPs). Hawaiian waters are home to three distinct populations: pelagic; Northwestern Hawaiian Islands (NWHI) insular; and main Hawaiian Islands (MHI) insular. Following a precipitous decline over recent decades, the MHI population was listed as "endangered" under the Endangered Species Act in 2012. This study assesses the risk of POP exposure to these populations by examining pollutant concentrations and ratios from blubber samples (n = 56) related to life history characteristics and MHI social clusters. Samples were analyzed for PCBs, DDTs, PBDEs, and some organochlorine pesticides. Skin samples (n = 52) were analyzed for stable isotopes $\delta^{13}C$ and $\delta^{15}N$ to gain insight into MHI false killer whale foraging ecology. Pollutant levels were similar among populations, although MHI whales had a significantly higher mean ratio of DDTs/PCBs than NWHI whales. The ∑PCB concentrations of 28 MHI individuals (68%) sampled were equal to or greater than suggested thresholds for deleterious health effects in marine mammals. The highest POP values among our samples were found in four stranded MHI animals. Eight of 24 MHI adult females have not been documented to have given birth; whether they have yet to reproduce, are reproductive senescent, or are experiencing reproductive dysfunction related to high POP exposure is unknown. Juvenile/sub-adults had significantly higher concentrations of certain contaminants than those measured in adults, and may be at greater risk of negative health effects during development. Multivariate analyses, POP ratios, and stable isotope ratios indicate varying risk of POP exposure, foraging locations and potentially prey items among MHI social clusters. Our findings provide invaluable insight into the ongoing risk POPs pose to the MHI population's viability, as well as consideration of risk for the NWHI and pelagic stocks.

KEY WORDS: POPs; Cetaceans; Pacific; Carbon; Nitrogen; Endangered species

1. INTRODUCTION

The false killer whale (*Pseudorca crassidens*) is an upper trophic level marine species that inhabits deep tropical and warm temperate waters around the world, as well as shallower waters near oceanic islands (Baird 2018a). Worldwide, the most studied false killer whales occur in waters around the Hawaiian Islands (Baird 2018a, 2018b), which includes three genetically distinct stocks: pelagic (i.e., offshore), Northwestern Hawaiian Islands (NWHI) insular, and main Hawaiian Islands (MHI) insular (Baird et al. 2008, 2013; Baird 2016; Chivers et al. 2007, 2010; Martien et al. 2014). MHI insular false killer whales have been well-studied for the past twenty years, with detailed life history information on many individuals and documentation of foraging and social behaviors, such as cooperative hunting and food sharing among cohorts (Baird 2016). Social network analyses have identified at least five discrete and enduring social clusters, or groups, within the MHI stock, comprised of highly related and regularly associating individuals (Baird et al. 2012, 2019; Martien et al. 2014, 2019). Previous studies have shown that spatial use (i.e., habitat use) varies by social cluster, identifying geographical "hot spots" throughout the main Hawaiian Islands where these groups spend most of their time (Baird et al. 2012, 2019).

The MHI population was listed as "endangered" under the U.S. Endangered Species Act (ESA) in 2012 due to a precipitous population decline between the late 1980s and the early 2000s. Bradford et al. (2018) estimated only 167 (CV = 0.14) individuals comprise the MHI stock, which is approximately three times less than an estimate from 1988 (Reeves et al., 2009), and several other lines of evidence supported a decreasing population trend (Mobley et al. 2000; Mobley 2004; Baird 2009; Oleson et al. 2010; Silva et al. 2013). Potential causes of population decline in MHI false killer whales include incidental take in commercial and recreational fisheries (Baird and Gorgone 2005; Baird et al. 2014, 2017), decreased prey biomass and size (Oleson et al. 2010), reduced genetic diversity (Chivers et al. 2010; Martien et al. 2014), and susceptibility to adverse health effects associated with exposure to persistent organic pollutants (POPs) (Ylitalo et al. 2009; Bachman et al. 2014; Foltz et al. 2014). POPs are toxic, man-made compounds that were used as agricultural pesticides and industrial chemicals. They are ubiquitous in marine ecosystems due to their widespread use, resistance to degradation, physicochemical properties, and global range of transport via volatilization and oceanic circulation (Iwata et al. 1993; Wania and MacKay 1996). False killer whales are particularly vulnerable to POP exposure as they are apex predators, increasing biomagnification burdens; long-lived, increasing susceptibility to bioaccumulation; and possess abundant lipid reserves in blubber, which are ideal repositories for lipophilic POPs (Holden and Marsden 1967; Jones and de Voogt 1999). Particular contaminants of concern include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and a number of organochlorine pesticides (OCPs) (e.g., dichlordiphenyltrichloroethanes or DDTs). Exposure to these pollutants has been correlated with several negative health effects in marine mammals, including immunosuppression (Ross et al. 1995; de Swart et al. 1994; Hammond et al. 2005) and disease (Ylitalo et al. 2005; Randhawa et al. 2015), thyroid disruption (Brouwer et al. 1989, 1998), and reproductive dysfunction (DeLong et al. 1973; Helle et al. 1976, Subramanian et al. 1987). In addition, thyroid, reproductive, and cognitive disruption have been observed in laboratory animals exposed to PBDEs (Eriksson et al. 2002, 2006; de Wit 2002; Talsness 2008).

Ylitalo et al. (2009) was the first to report high concentrations of POPs in blubber of MHI false killer whales and found trends in POP levels among age/sex classes consistent with findings from similar studies on killer whales (*Orcinus orca*), albeit with a small sample size (n = 9)

(Krahn et al. 2007b, 2009; Ross et al. 2000; Ylitalo et al. 2001). Adult males tend to have the highest POP concentrations as they accumulate POPs throughout their lives, whereas adult females have the opportunity to offload POPs to their offspring through gestation and, primarily, through lactation (Ylitalo et al. 2001; Aguilar and Borrell 1994; Ross et al. 2000). In killer whales, approximately 70-90% of mothers' contaminant burdens have been estimated to be transferred to offspring during lactation (Mongillo et al. 2012). Consequently, juveniles have high POP levels with the amount offloaded influenced by birth order (Ylitalo et al. 2001). Once adult females become reproductively senescent, they continue to accumulate POPs via their diet (Ross et al. 2000). More recently, Foltz et al. (2014) reported high levels of cytochrome P4501A1 (CYP1A1) expression, a biomarker of POP exposure, in biopsies sampled from live MHI false killer whales. Further, they examined total PCB concentrations (n = 33) and found that 84% of false killer whale biopsies exceeded the suggested 14,700 ng g^{-1} threshold for risk of maternal failure (Schwacke et al. 2002) and 71% exceeded the proposed 17,000 ng g^{-1} threshold for thyroid and immune system disruption in aquatic mammals (Kannan et al. 2000). Differences in CYP1A1 expression among social clusters were examined but no significant findings were reported, however knowledge of social clusters at the time of the study was limited (Foltz et al. 2014).

While the influence of life history characteristics on POP concentrations in MHI false killer whales may be generally understood, variance in contaminant concentrations among social clusters remains unclear. Of particular interest in this study was variance in POP concentrations between MHI social clusters, while accounting for known drivers of POP levels (i.e., age class, sex, reproductive status), to investigate inter-group differences in risk of POP exposure. In addition, we were interested in the use of chemical contaminants as indicators of geographic areas and trophic positions at which MHI social clusters primarily forage. Such findings would enhance our understanding of these groups' varying spatial use, foraging ecology, and localized threats to POP exposure. For instance, POP ratios and stable isotopes $\delta^{13}C$ and $\delta^{15}N$ measured in blubber/epidermis have been used to differentiate cetacean stocks (Krahn et al. 1999; Muir et al. 1996; Witteveen et al. 2009), inform regional sources of contaminants (Calambokidis and Barlow, 1991; Krahn et al. 1999, 2007a; Muir et al. 1990), and provide insight into foraging areas differing by pod or social group of upper trophic level odontocetes (Herman et al. 2005; Krahn et al. 2007a; Schnitzler et al. 2018). Examining POP ratios (e.g., ΣDDTs/ΣPCBs and ΣPBDEs/ΣPCBs) allows comparison among groups to gain insight into regional differences, such as urban or agricultural "signatures" (Calambokidis and Barlow, 1991; Krahn et al. 2007a). Stable isotopes such as $\delta^{13}C$ and $\delta^{15}N$ can indicate foraging location (nearshore/offshore) and trophic position, respectively, as whales accrue these isotopes through their prey (Kelley 2000; Krahn et al. 2007a).

Here we examine variance in POP concentrations in Hawaiian false killer whales from all three populations and assess risk of exposure to individuals based on life history factors (i.e., sex, age class, reproductive status) and among MHI social clusters. This study extends the information reported by Ylitalo et al. (2009) and Foltz et al. (2014) by providing a greater sample size (66 more biopsies), updated life history information for biopsied individuals, contemporary social MHI cluster assignments, and biopsies of NWHI and pelagic false killer whales. We examined POP ratios among false killer whale populations and MHI social clusters to gain insight on regionally varying POP exposure, and stable isotopes in MHI whales to infer relative foraging locations and trophic position among social clusters.

2. MATERIALS AND METHODS

2.1 Sample collection

False killer whale biopsy sampling was conducted around the main Hawaiian Islands from 2008 through 2012, using a 45 kg pull Barnett RX-150 crossbow and Larsen biopsy tips (25 mm long and 8 mm wide), as previously described in Ylitalo et al. (2009). After collection, biopsy samples were stored in a cooler with ice packs while in the field and transferred to a -20°C freezer for short-term storage before being stored in a -80°C freezer prior to analyses. Biopsies from MHI false killer whales reported in Ylitalo et al. (2009) were included in this study (n = 9). Simultaneous to sample collection, individuals were photographed for individual identification (see below) and to determine population identity. In addition to biopsies obtained from free-ranging individuals, blubber biopsies were included from four stranded (i.e., deceased) whales in years 2013, 2015, and 2016.

2.2 Life history and social cluster information

Photographs of sampled individuals were compared to the catalog of Baird et al. (2008) to determine whether any individuals were sampled on multiple occasions and to assess population identity. Age class (i.e., juvenile, subadult, adult) of false killer whales was determined using a combination of field assessment, individual sighting histories, and relative size in photographs over the entire sighting history of the biopsied individuals. This included body size relative to other individuals, presence of calves in close proximity (indicating adulthood), and amount and severity of marks which accumulate over time (Baird et al. 2008). Sex of individuals was determined genetically at the Southwest Fisheries Science Center using Real-Time PCR (Stratagene) zinc finger gene amplification as described by Morin et al. (2005). For adult females, determination of reproductive status (i.e., parous, nulliparous, unknown) was based on field observations and sighting histories (e.g., if seen with calf), and genetic parentage information if available.

MHI false killer whale social cluster assignment was determined through association analyses as described in Baird et al. (2012, 2019). Analyses were conducted through the program Socprog 2.9 (Whitehead 2009) using individual sighting histories from Cascadia Research Collective's photo-identification catalog (Baird et al. 2008) from years 2000 to 2018. Eigenvector-based modularity was used to evaluate association strengths among individuals. Determination of discrete social clusters within the population was considered when network modularity (Q) was greater than 0.3 (Newman 2004, 2006). Each individual was assigned to one of five social clusters based on these analyses (Baird et al. 2019).

2.3 Persistent organic pollutant and lipid analyses

Blubber samples were analyzed for a suite of 79 persistent organic pollutants using a gas chromatography/mass spectrometry (GC/MS) method (Sloan et al. 2014; Ylitalo et al. 2009). Briefly, blubber was weighed (~ 0.1 to 0.3 g) into a solvent-cleaned glass jar, mixed with sodium sulfate and magnesium sulfate to remove any water and then the blubber mixture was packed into an accelerated solvent extractor cell, the surrogate standard was added (PCB103; 75ng) and analytes of interest were extracted using dichloromethane. Prior to sample cleanup, a 1-mL

portion of extract was removed for percent lipid determination using thin-layer chromatography with flame ionization detection (TLC-FID) (Ylitalo et al. 2005; Sloan et al. 2014). A high-performance liquid chromatography (HPLC) internal standard (trichloro-*meta*-xylene; 75 ng) was added to the remaining extract to calculate the recovery of the surrogate standard. The sample extracts were then cleaned up using a two-step process: removal of highly polar compounds on a gravity flow glass column containing alumina/silica gel followed by removal of lipids and other biogenic interferences using HPLC size exclusion chromatography. The extract volume was concentrated to ~ 100 µL and a GC internal standard (tetrachloro-*ortho*-xylene; 30 ng) was added to each sample to calculate the recovery of the HPLC standard. The POPs were separated on a 60-meter DB-5 GC capillary column and measured on a low-resolution quadrupole GC/MS system. This system was calibrated using sets of up to ten multi-level calibration standards of known concentrations.

Percent lipids were determined in the samples using thin-layer chromatography with flame ionization detection (Ylitalo et al., 2005; Sloan et al., 2014). A pre-weighed lipid extract sample was spotted onto a silica Type SIII Chromarod and developed in a chromatography tank containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v) for approximately 25 minutes. Lipid classes were separated based on polarity and measured using flame ionization detection. Percent lipid values were calculated by summing the concentrations of five lipid classes (i.e., sterol esters/wax esters, triglycerides, free fatty acids, cholesterol, phospholipids) for each sample, using the mean of two measurements.

All contaminant concentrations are reported in ng/g, lipid weight (ng/g, lipid wt.). Sum PCBs is the sum concentrations of congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208 and 209. Sum DDTs is the sum of *o,p*'-DDD, *p,p'*-DDD, *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDT and *p,p*'-DDT. Sum chlordanes (CHLDs) is the summed levels of *cis*-chlordane, *trans*-chlordane, heptachlor, heptachlor epoxide, *cis*-nonachlor, *trans*-nonachlor, nona-III-chlordane and oxychlordane. Sum hexachlorocyclohexanes (HCHs) is the summed concentrations of *alpha*-, *beta*-, and *gamma*-HCH isomers, and sum PBDEs is the summed concentrations of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155 and 183. Concentrations of aldrin, dieldrin, endosulfan I, hexachlorobenzene (HCB) and mirex were also determined in the biopsy samples.

A method blank and a National Institute of Standards and Technology (NIST) whale blubber Standard Reference Material (SRM 1945) were analyzed with each sample set as part of a performance-based quality assurance program (Sloan et al. 2019). Concentrations of individual analytes measured in SRM 1945 met the laboratory quality assurance criteria (≥ 70% of individual POPs were within 30% of either end of the 95% confidence interval range of the published NIST certified concentrations) described in Sloan et al. (2019). Method blanks contained no more than five analytes that exceeded two times the lower limit of quantitation (LOQ), unless the analyte was not detected in the associated field samples in the set. Surrogate recoveries for all false killer whale blubber samples ranged from 96 – 120% and met established laboratory criterion (recovery range 60 - 130%).

2.4 Stable isotope analyses

False killer whale skin samples were analyzed for stable isotope ratios of carbon and nitrogen using the method described in Herman et al. (2005). Skin samples were freeze-dried overnight and subsequently ground to a powder in a micro ball mill. The powdered skin was transferred to a glass filter paper folded into a cone, folded shut, and the cone was placed into a 33 mL ASE cell. Lipids were extracted from the powdered skin using two cell volumes of dichloromethane at 25°C and 500 psi. The sample cone was removed from the ASE cell and dried at room temperature in a fume hood for 10 min. The lipid-free skin samples (0.4 to 0.6 mg dried powder) were then loaded into tin cups and combusted in a Costech elemental analyzer attached to a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer. The values were calibrated against internal laboratory standards (aspartic acid and 15N-enriched histidine), which were analyzed after every 10 field samples.

Quality assurance measures for stable isotope ratios included the analysis of both continuing calibration standards and a fish tissue, SRM 1946 (National Institute of Standards and Technology, Gaithersburg, MD, USA) with each batch of samples (Sloan et al. 2006). Continuing calibration standards were run every 10 field samples, whereas SRM 1946 was run between every 20 samples. Isotope values for continuing calibration standards and SRM 1946 were within 0.30‰ of the values calibrated against international standards for $\delta^{15}N$ and within 0.20‰ for $\delta^{13}C$.

Several cetacean studies (Herman et al. 2005; Krahn et al. 2007a,b, 2009; Knoff et al. 2008; Witteveen et al. 2009; Browning et al. 2014) have assessed skin carbon and nitrogen stable isotope ratio values using similar sample preparation protocols (e.g., tissue drying followed by lipid extraction) as described in our study (Herman et al. 2005). However, Ryan et al. (2012) reported that stable isotope ratios of carbon and nitrogen in blubber and skin of three species of baleen whales were significantly different based on lipid vs. non-lipid extraction, noting significant increases in $\delta^{13}C$ values for blubber of all species and significant increases in $\delta^{15}N$ of skin of minke whales only. Based on their findings, the authors recommended that duplicate analyses of lipid-extracted ($\delta^{13}C$ measurements) and non-lipid extracted ($\delta^{15}N$ measurements) tissues of cetaceans be used. In our study, the same sample preparation, isotope analytical protocols and quality assurance criteria were used for all skin samples analyzed. Although lipid-extraction could influence the $\delta^{15}N$ values of false killer whale skin samples reported in our study, these samples were all treated the same and thus, comparisons of our stable isotope findings based on whale social clusters or whale age/sex class within a social cluster could be confidently made.

2.5 Statistical analyses

Analysis of variance (ANOVA) followed by the post-hoc Tukey–Kramer honest significant difference (HSD) test were used to determine if mean POP concentrations, POP ratios, and $\delta^{13}C$ and $\delta^{15}N$ stable isotopes varied among false killer whale populations, among animals by age and sex within the MHI stock, or MHI social clusters. All POP concentrations were square root transformed and the percent lipid data were arcsine square root transformed prior to statistical analyses to increase homogeneity of variance and achieve normal distribution. Stable isotope data met assumptions of normal distribution and homogeneity of variance and therefore were not transformed. As ratios, POP ratios violate the assumption of homogeneity of variance. Therefore, we place more emphasis on descriptive (qualitative) interpretation of the results over the statistical outputs for POP ratios. The level of significance used for all statistical tests was $\alpha \leq$

0.05. All statistical analyses were completed using the program R 3.4.4 (R Development Core Team 2018).

Of particular interest in this study was variation in POP levels among MHI social clusters while controlling for known life history drivers (i.e., age class, sex, reproductive status) of variance in POP concentrations. We conducted principal components analysis (PCA) to generate factors that summarize the majority of variation in the dataset and identify POP classes driving the variance described by each factor. PCA was performed using the package *psych* (Revelle 2018) by summed and individual analyte class for MHI false killer whales using a correlation matrix with varimax rotation. Components with an eigenvalue greater than 1.0 were retained (Cangelosi and Goriely 2007). Loading weights for retained components on summed and individual analyte classes were evaluated. We then used linear mixed effect models (LMM) to model each retained principal component factor as a function of fixed covariates age/sex/reproductive class (adult female parous, adult female nulliparous, adult male, juvenile/subadult) and social cluster assignment. Whale identity was included as a random effect to account for dependency structure resulting from repeated sampling of some individuals (i.e., individuals biopsied twice). LMMs were ran using the package *lme4* (Bates et al. 2015). Cluster 1 was set as the reference level for the categorical covariate social cluster, such that covariate estimates would be relative to Cluster 1. Cluster 1 had the greatest sample size among social clusters (n = 20) and thus was considered the most reliable reference group for comparison among other clusters. Similarly, nulliparous adult females were set as the reference level for age/sex and reproductive class as they generally are less variable with respect to POP levels, although adult males could have been a suitable reference as well. Only adult females with known reproductive statuses were included in this sub-analysis as reproductive status was a covariate of interest for mixed effects models. The final analytic dataset for this sub-analysis included 36 samples.

3. RESULTS

3.1 Sample collection and identity information

Samples (n = 74) were collected from individuals from all three populations: MHI insular (n = 63 samples from 56 individuals); NWHI (n = 8 samples from 8 individuals); pelagic (n = 3 samples from 3 individuals). Of these, POP results were available from 45 samples from the MHI population (41 individuals) and all sampled individuals from the NWHI and pelagic populations. Stable isotope results were available from 51 individuals from the MHI population. Information on demographics, MHI social cluster assignment, and type of analysis completed (POPs and stable isotopes) for each sample is reported in Table 1. Of the stranded individuals, three had sufficient sighting histories for social cluster designation. The fourth individual, HIPc700, had never been sighted previous to its necropsy so social cluster assignment is unknown; therefore, this sample was excluded from any statistical analysis concerning social clusters within the MHI population.

3.2 POPs

Wide ranges of POP concentrations were measured in individual false killer whales from all three populations (Figure 1). For example, concentrations of ΣPCBs and ΣDDTs ranged from 1,000 to 110,000 ng g^{-1}, lipid wt. and 1,100 to 180,000 ng g^{-1}, lipid wt., respectively (Table S1). Levels of ΣPBDEs and organochlorine pesticides (ΣCHLDs, ΣHCHs, HCB, mirex, dieldrin) ranged from < LOQ to 13,000 ng g^{-1}, lipid wt. (Table S1). Concentrations of aldrin and

endosulfan I were < LOQ for all samples analyzed. Proportions of PCB and PBDE congeners by homolog group (i.e., chlorination/bromination level) were generally similar among populations, although NWHI whales had slightly higher proportions of hexabrominated PBDEs (Figure S2). Heavier and recalcitrant (e.g., resistant to metabolism) congeners dominated profiles: hexachlorinated (e.g., PCBs 138, 153) and heptachlorinated (e.g., PCBs 180, 187) PCB congeners accounted for approximately 50% and 27% of ΣPCBs, respectively (Figure S2). Tetrabrominated (e.g., PBDEs 47, 66), pentabrominated (e.g., PBDEs 85, 99), and hexabrominated (e.g., PBDEs 153, 154) PBDEs accounted for 56%, 29%, and 14% of ΣPBDEs, respectively (Figure S2). Trichlorinated (i.e., PCBs 17, 18, 28, 31, 33, 44), tetrachlorinated (e.g., PCBs 49, 52, 66), nonachlorinated (i.e., PCBs 206, 208), and decachlorinated (i.e., PCB 209) PCBs and tribrominated (i.e., PBDE 28) PBDE accounted for less than 2% of congener profiles (Figure S3, S4). Tetrachlorinated PCBs (e.g., PCBs 29, 52, 66) contributed approximately 3% and pentachlorinated PCBs (e.g., PCBs 82, 87, 95, 99) 17% to total PCBs (Figure S2). Mean POP concentrations were not significantly different ($p > 0.05$) among the three whale populations for any of the POP classes measured.

Mean ΣPCB concentrations for all populations exceed both thresholds for adverse health effects proposed by Kannan et al. (2000) and Schwacke et al. (2002), although inferences on NWHI and pelagic populations as a whole are limited due to sample size (Table S1). For the MHI stock, 68% of individuals (28 of 41 individuals; 30 of 45 (67%) samples) exceeded the 17,000 ng g^{-1} ΣPCBs threshold and 71% of individuals (29 of 41 individuals; 31 of 45 (69%) of samples) exceed the 14,700 ng g^{-1} ΣPCBs threshold (Kannan et al. 2000; Schwacke et al. 2002). Of the 4 individuals sampled twice during the study period, two had ΣPCBs levels exceeding health thresholds (HIPc102, adult male; HIPc282, sub-adult female) and two had levels under health thresholds (HIPc116 and HIPc212, adult females) for both pairs of biopsies obtained over the study period (i.e., no changes in exceeding thresholds). Levels of ∑PCBs in 100% of adult males (11 individuals), 55% of adult females (12 of 22 individuals), and 63% of juvenile/subadults (5 of 8 individuals) belonging to the MHI population were greater than or equal to both of those thresholds (Figure 2) (Kannan et al. 2000; Schwacke et al. 2002).

The influence of sex and age class on POP concentrations for false killer whales from the MHI population was examined as it was the only population that had sufficient numbers of whales represented by the three age/sex categories (i.e., adult male, adult female, juvenile/subadult). Contaminant data for both male and female juvenile/subadult whales were combined as no significant differences in mean concentrations of POPs were found between sexes (ΣPCBs, $p = 0.8286$; ΣDDTs, $p = 0.7524$; ΣCHLDs, $p = 0.7523$; ΣPBDEs, $p = 0.4842$; ΣHCHs, $p = 0.4657$; HCB, $p = 0.8169$; dieldrin, $p = 0.8514$; mirex, $p = 0.9883$). Significant differences in mean concentrations of ΣPCBs ($p = 0.0111$), ΣDDTs ($p = 0.0032$), and ΣCHLDs ($p = 0.0131$) were found between adult males and adult females, with males having higher concentrations for all three (Figure 3). Mean concentrations of HCB ($p = 0.0485$), ΣPBDEs ($p = 0.0063$), ΣHCHs ($p = 0.0249$), and dieldrin ($p = 0.0334$) were significantly different between juvenile/subadult whales and adult females, with higher levels in juveniles/subadults for all three (Figure 3). A significant difference in mean levels of sum DDTs ($p = 0.0391$) was found between juvenile/subadult whales and adult males, with adult males having higher levels. Although the mean concentrations of ΣPBDEs were elevated in juvenile/subadult whales compared to adult male false killer whales (Figure 3), this difference was not significant ($p = 0.1781$). No other significant differences in mean percent lipid or POP concentrations were found among the

age/sex classes. Proportions of PCB and PBDE congeners by homolog group were similar among age/sex/reproductive classes and mirror profiles for all false killer whale populations (Figure S2, S3, S4). For instance, the predominant congeners among these groups were hexa- and heptachlorinated PCBs and tetra- and pentabrominated PBDEs (Figure S2). However, parous adult females had slightly higher proportions of octachlorinated PCBs and hexabrominated PBDEs (Figure S2).

Taking reproductive status (nulliparous, parous, unknown) of adult females from the MHI population into account, those known to have had at least one calf (i.e., parous) had significantly (all p's < 0.05) lower levels of all POP classes than adult females that had never been observed with a calf (i.e., nulliparous) (Figure 4). There were several adult females in our dataset that we were unable to confidently determine reproductive status due to limited sighting histories. A number of these individuals had concentrations of ΣPCBs and ΣDDTs that were comparable to nulliparous adult females (Figure 4). Additionally, there were four mother/offspring pairs in our dataset allowing us to examine maternal offloading relationships (Figure S1). As expected, mothers had much lower levels of most contaminants, including ΣPCBs, ΣDDTs, ΣCHLDs, ΣPBDEs, and mirex, than their offspring (Figure S1).

Of highlighted concern were the alarmingly high POP levels measured in blubber samples of stranded false killer whales. Among the four stranded individuals, the lowest ΣPCB concentration measured was 43,000 ng g^{-1} (lipid wt.), more than twice the highest suggested health threshold for PCBs (Kannan et al. 2000), and highest was 110,000 ng g^{-1} (lipid wt.). ΣDDTs concentrations ranged from 58,000 ng g^{-1} to 180,000 ng g^{-1} (lipid wt.). Bachman et al. (2014) also reported POP concentrations from a stranded Hawaiian false killer whale however its levels were lower than those reported in the current study (Table 2). Lailson-Brito et al. (2012) reported POP concentrations for a single stranded false killer whale from the southeastern Brazilian coast that had levels similar to our findings (ΣPCBs: 63,700 ng g^{-1}; ΣDDTs: 17,900 ng g^{-1}). POP concentrations in these stranded false killer whales were among the highest compared to what has been previously reported on other odontocetes found in Hawaiian waters and regions throughout the Eastern North Pacific (Table 2). Although the influence of POP exposure on these individuals' deaths cannot be confidently resolved, these whales had the highest POP levels among all individuals in our dataset suggesting that some associated negative health effects may have been at play.

3.3 Multivariate analyses

Principal components analysis generated three factors that summarized the most variance in the dataset (Table 3). Factor 1 explained 42% of the variance and had high loadings for ΣPCBs, ΣDDTs, ΣCHLDs, and mirex. Mixed effects model outputs (Figure 5; Table S2) showed a statistical increase in factor 1 estimates for adult males (estimate, 0.92; p = 0.008) and animals within Cluster 3 (estimate, 0.94; p = 0.030), and a statistical decrease for parous adult females (estimate, -0.83; p = 0.048). The second factor explained 33% of the variance, with high loadings for ΣHCHs, ΣHCB, and dieldrin. The mixed model for this factor showed an increase in estimates for animals within Cluster 4, albeit not significantly (estimate, 0.91; p = 0.052). Factor 3 accounted for 18% of the variance and was highly loaded for ΣPBDEs. The mixed model for this factor showed a statistical decrease in factor 3 estimates for parous adult females (estimate, -1.25; p = 0.019), adult males (estimate, -1.02; p = 0.018), and for animals within Cluster 2 (estimate, -0.87; p = 0.033). Recall estimates for age/sex/reproductive status class were relative

to nulliparous adult females and estimates for social clusters were relative to Cluster 1.

3.4 POP ratios

Ratios of ΣDDTs/ΣPCBs and ΣPBDEs/ΣPCBs among false killer whale populations and MHI social clusters are shown in Figure (6). The MHI population had a statistically significant greater mean ΣDDTs/ΣPCBs (1.20 ± 0.42) ratio than NWHI false killer whales (0.83 ± 0.16; $p = 0.0254$). We found no apparent or statistically significant difference in average ratios of ΣDDTs/ΣPCBs between MHI and pelagic populations, although the small number of whales sampled from the pelagic population may have reduced our ability to find significant differences in POP ratios. The NWHI stock had a lower mean ratio of ΣDDTs/ΣPCBs (0.83 ± 0.16) compared to the pelagic stock (1.10 ± 0.17), albeit not significantly. No significant differences were found in mean ratios of ΣPBDEs/ΣPCBs between populations, although the MHI stock had greater variation among individuals (Figure 6B). No statistically significant differences were found in mean ratios of ΣDDTs/ΣPCBs or ΣPBDEs/ΣPCBs among MHI social clusters, although ratios appear to vary within clusters to some extent (Figure 6C, D).

3.5 Stable isotopes

Mean carbon and nitrogen values among MHI social clusters were generally similar, ranging from -16.9 to -15.4 and 11.1 to 13.1, respectively (Figure 7). As previously noted, stable isotope values were not available for individuals within Cluster 3 (Table 1). Cluster 1 had significantly lower $\delta^{13}C$ values than Cluster 2 ($p = 0.02081$), although it should be noted the scale of difference is small (Figure 7). No significant differences were found in $\delta^{15}N$ levels among all social clusters although Clusters 1 and 2 had slightly higher values for that isotope (Figure 7). Provided the variation in isotope values within social clusters (Figure 7), we further investigated differences between age/sex classes. This sub-analysis was restricted to individuals within Cluster 1 as it had the largest sample size and the most representatives from each age/sex class. Carbon and nitrogen stable isotopes were generally similar among age/sex classes within Cluster 1, although there was some variation among those groups (Table S3).

4. DISCUSSION

The precipitous population decline observed in the endangered MHI false killer whale stock over recent decades has been linked to several potential causes, including exposure to POPs (Ylitalo et al. 2009; Baird and Gorgone 2005; Baird 2009). Extensive study of this population has provided a unique dataset allowing us to investigate how contaminant profiles and exposure risk vary among individuals. Notably, we provide the first comprehensive study examining and identifying drivers of variance in POP concentrations, ratios, and stable isotopes among MHI false killer whale social clusters. We enhanced our understanding of the risk POP exposure poses to the endangered MHI population with a greater sample size and contemporary life history information for biopsied individuals. This is also the first study to report POP concentrations for NWHI and pelagic false killer whale populations.

4.1 POPs

4.1.1 Influence of life history characteristics on POPs

The trends in POP concentrations among age/sex class for MHI false killer whales in this study

follow what was speculated in Ylitalo et al. (2009) and are comparable to those previously published on killer whales (Herman et al. 2005; Krahn et al. 2009; Ylitalo et al. 2001). As seen in Figure (3), there is quite a bit of variation in POP concentrations within age/sex classes. Variability in POP levels among adult males in our study may be caused by several factors, including age and birth order (Ross et al. 2000; Ylitalo et al. 2001). For instance, we would expect older adult males to have higher levels than their younger counterparts, and first-born male offspring to have particularly high levels. Future studies that refine individual age estimates (e.g., through epigenetic aging) may aid in understanding this variation.

Variation in POPs measured in adult females is likely driven by reproductive status (Figure 4). MHI adult females known to have at least one calf prior to biopsy collection had among the lowest POP levels among all biopsies analyzed in this study as a result of maternal offloading. Consequently, POP levels measured in offspring surpassed or were close to both suggested ΣPCBs thresholds for negative health effects (Figure S1) (Kannan et al. 2000; Schwacke et al. 2002). Of the 24 adult females (MHI), eight whales have never been reported to give birth (Cascadia Research Collective 2019). A majority of these nulliparous females are characterized by extremely high POP concentrations; whether these individuals are reproductively impaired due to POP exposure or have simply have yet to reproduce is unknown. We were unable to determine the reproductive status of nine adult females from the MHI stock due to sparse sighting histories (Cascadia Research Collective 2019). As mentioned previously, future studies using estimated age may help determine if these females are younger and reproductively active or older and reproductively senescent.

Interestingly, juvenile and sub-adult false killer whales from the MHI population had lower levels of most contaminants compared to adults, but elevated levels of ΣPBDEs, dieldrin, and HCB (Figure 3). Similar findings were reported on two sub-adult whales in Ylitalo et al. (2009) that were included in the current study, so results from additional biopsies confirm this trend for these particular POP classes. The high concentrations of ΣPBDEs in younger whales is concerning as exposure to PBDEs has been linked to neurotoxic effects during neonatal brain development in mice (Eriksson et al. 2002, 2006; Viberg et al. 2003). As juveniles/sub-adults undergo rapid development of their physiological systems, they may metabolize lipids which could redistribute POPs to their bloodstream or other organs where damage could occur (Hickie et al. 1999, 2007). For example, immune system dysfunction has been observed in male common bottlenose dolphins (*Tursiops truncatus*) with increased contaminant concentrations in blood (Lahvis et al. 1995).

4.1.2 POPs among MHI social clusters

Results from PCA followed by LMMs indicate that, while controlling for life history drivers of POP variance (i.e., age, sex, reproductive status), POP classes and concentrations vary by MHI social cluster. The LMM for PC factor 1 (Table S2, Figure 5) showed that relative to Cluster 1, Cluster 3 had a significantly positive correlation with contaminants highly loaded on factor 1 (ΣPCBs, ΣDDTs, ΣCHLDs, mirex; Table 3). This could reflect regional differences in POP exposure or contamination in prey items, although we might have expected Clusters 1 and 3 to have comparable POP concentrations as they have similar high-density areas (Baird et al. 2019). In addition, only four blubber samples were obtained from whales in Cluster 3, in which three were from stranded whales (Table 1). In the current study, stranded whales had the highest POP concentrations measured among all individuals analyzed. Thus, inferences on this statistical

finding are limited due to sample size; better sample representation for Cluster 3 (i.e., analysis of additional biopsies) would aid understanding of risk of POP exposure for this social cluster. Parous adult females were negatively correlated while adult males were positively correlated with loadings for factor 1 (Figure 5) relative to nulliparous adult females; however, this finding was expected provided known patterns in POPs among age/sex/reproductive class groups.

The LMM for factor 2 showed that Cluster 4 was positively correlated with contaminants highly loaded on this factor (ΣHCHs, HCB, dieldrin; Table 3) relative to Cluster 1, albeit not significantly ($p = 0.052$; Figure 5). For example, Cluster 4 false killer whales generally had higher levels of ΣHCHs (140 ng g^{-1} lipid wt.), HCB (290 ng g^{-1} lipid wt.), and dieldrin (120 ng g^{-1} lipid wt.) relative to Cluster 1 (72, 120, and 55 ng g^{-1} lipid wt., respectively). Previous studies suggest high density areas for Cluster 4 are off eastern Oʻahu, Molokaʻi, Lānaʻi, Kahoʻolawe, and western Maui (Baird et al. 2019). Animal husbandry, sugarcane, and pineapple plantations are the primary land uses in these regions, which could be associated with high levels of agricultural pesticides measured in Cluster 4 false killer whales (State of Hawaiʻi 2015).

PC factor 3 was highly loaded for ΣPBDEs (Table 3) and the LMM for this factor showed parous adult females, adult males, and Cluster 2 false killer whales were negatively correlated with ΣPBDEs relative to nulliparous adult females and Cluster 1, respectively. We expected to find parous adult females and adult males with lower levels of this POP class, as previous findings showed juveniles and nulliparous adult females generally have higher concentrations of ΣPBDEs (Figure 3). It was interesting to find lower ΣPBDE levels in Cluster 2 false killer whales, as their high density area overlaps part of those of Clusters 1 and 3 (mostly limited to northwestern Hawaiʻi and southeast Maui) (Baird et al. 2019). PBDEs are used for industrial purposes, such as flame retardants and used in manufacturing furniture and plastics (EPA 2017), such that we would expect to see higher PBDE exposure near more urbanized regions. While there are urbanized areas near Cluster 2's range, these regions are generally less urbanized compared to other counties throughout the state (State of Hawaiʻi 2013). Our results may reflect the more remote habitat use or foraging locations of Cluster 2 relative to other social clusters.

Our results suggest that MHI social groups likely forage in different areas around the Hawaiian Islands- reinforcing findings from satellite tag studies (Baird et al. 2012, 2019)- and may be subject to varying degrees of exposure to certain POP classes. These findings could also reflect variability in the types of prey these groups primarily consume, however stable isotope results would provide a more distinct indication of this inference as they more directly reflect what is consumed.

4.2 POP ratios

We compared POP ratios ΣDDTs/ ΣPCBs and ΣPBDEs/ ΣPCBs among false killer whale populations and MHI social clusters that may be characteristic of agricultural or urban "signatures" associated with foraging locations. An unexpected finding was the significant difference in mean ΣDDTs/ ΣPCBs ratios between MHI and NWHI false killer whales, with the former having an elevated mean ratio (Figure 6). Because the Northwestern Hawaiian Islands are relatively remote compared to the main Hawaiian Islands, we would expect MHI false killer whales to have greater ΣPCBs concentrations relative to ΣDDTs compared to NWHI false killer whales, as PCBs are more often associated with urban environments. A study on endangered Hawaiian monk seals (*Monachus schauinslandi*) also found elevated levels of ΣPCBs in seals from Midway (located in Northwestern Hawaiian region) compared to those found on the main

Hawaiian Islands (Lopez et al. 2012). It was speculated that PCBs became pervasive in the NWHI region as a result of over 50 years of military occupation, where cleanup efforts following military activities were not sufficient (Forney, 2010; Lopez et al. 2012). This finding highlights the persistent aspect of POPs and that even populations occurring in remote regions are subject to their exposure and associated negative health effects. The lack of significant findings in mean ΣPBDEs/ ΣPCBs ratios among the three false killer whale populations suggests that all stocks have relatively similar exposures to these industrial-associated contaminants, although MHI false killer whales have greater range in ratio values (Figure 6). In addition, only three biopsies from the pelagic population were available for this study; thus, additional biopsies from this stock may be useful in making inferences on the entire population.

Neither mean ratios of ΣDDTs/ ΣPCBs nor ΣPBDEs/ ΣPCBs differed significantly among MHI social clusters. However, as seen in Figure (6), there is quite a bit of variation in ratios within and among groups. This could indicate that a broader agricultural or urban signature (i.e., ΣDDTs/ ΣPCBs, ΣPBDEs/ ΣPCBs) may not be apparent among MHI social groups that share an overall foraging range of the main Hawaiian Islands, but rather more refined regional differences in contaminated prey items as suggested by the multivariate findings. In addition, inter-individual differences (i.e., sex, age class) in POP ratios could be a plausible driver of variation in mean ratios among MHI social clusters and false killer whale populations. For example, variability in maternal offloading burdens to calves may cause greater variation in mean ratios when analyzing an entire social group/population.

4.3 Stable isotopes

As noted previously, stable isotope analysis of epidermis can be used to provide insight into trophic positions and geographic locations where marine mammals forage (Kelley, 2000). Cluster 1 had significantly lower mean levels of $\delta^{13}C$ than Cluster 2, suggesting that Cluster 1 whales may forage farther offshore than Cluster 2 whales. While there were no statistically significant findings in mean $\delta^{13}C$ values among the other clusters, Figure (7) suggests that Clusters 4 and 5 may feed in more inshore regions compared to Cluster 1, although the difference was small. Average stable isotope $\delta^{15}N$ levels were not significantly different among MHI social groups, so it appears these groups consume prey at similar trophic positions. Main Hawaiian Islands insular false killer whales are known to eat a variety of pelagic and reef-associated game fish (Baird 2016), such as yellowfin tuna (*Thunnus albacares*), mahimahi, (*Coryphaena hippurus*), several species of jack (*Caranx sp.*), and some squid species have been documented in the stomach contents of stranded individuals (West et al. unpublished data). Variation in diet by age class or sex has not been assessed based on observational studies, although whales frequently share prey (Baird 2016), so such variation may be difficult to assess. Compared to stable isotope findings from false killer whales from the southwestern Atlantic (Riccialdelli et al. 2010; Bisi et al. 2013; Botta et al. 2012), our stable isotope values were generally lower in both $\delta^{13}C$ and $\delta^{15}N$. These differences in stable isotope values among false killer whale studies may be due to variations in diets of the whales from different regions, and/or differences in isotopic baseline values for various oceanic basins that these whales inhabit (Graham et al. 2010). Other factors such as different tissues analyzed or tissue preparation protocols (lipid extracted versus non-lipid extracted sample preparation, Ryan et al. 2012) may also contribute to differences in these stable isotope ratios. Some of these studies used bone/dentition samples from stranded false killer whales that may be less susceptible to variation associated with time or environmental conditions compared to blubber/skin biopsies (Walker and

Macko, 1999; Kelley, 2000). For instance, complete turnover of skin cells was estimated to be around 73 days for a similar species (Hicks et al. 1985), suggesting isotope values reflect their diet during 1-2 months prior to sampling. In addition, the scale of measurements at which comparisons in the current study were made is small (Figure 7), such that identifying trophic discrimination would be challenging; it is very possible that statistical findings are an artifact of inherent variability in stable isotope values. Therefore, we exercise caution in our biological interpretation of these statistical findings and consider these differences as an initial exploration of the possibility for seasonal variation in prey items or foraging locations among social groups.

5. CONCLUSIONS

Concentrations of POPs measured in false killer whales from all three populations found in Hawaiian waters rank among the highest documented in Eastern North Pacific odontocetes (Table 2). NWHI and pelagic false killer whales are not as well-studied as the MHI stock, however, high levels of POPs measured in these whales raise concern regarding the conservation status of these populations. Importantly, this study provides evidence that POPs continue to be a relevant and pressing risk to the endangered MHI false killer whales, with juveniles/sub-adults at greater risk of adverse health effects linked to their exposure. Monitoring MHI false killer whales' health (e.g., respiratory microbiome, body condition) is essential in elucidating the threat of POP exposure in these animals. For example, respiratory microbiome analysis of breath samples from free-ranging cetaceans has been a recent advancement in monitoring health and disease for several species, including humpback whales (*Megaptera novaeangliae*) (Apprill et al. 2017; Acevedo-Whitehouse, Rocha-Gosselin, and Gendron, 2009), killer whales (Raverty et al. 2017), Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Nelson et al. 2019), and a number of Hawaiian odontocetes, including 2 false killer whales (Lerma et al. 2019). In addition, a number of studies have assessed individual body condition to gain insight into population health, using photographs (Bradford et al. 2012) and, more recently, photogrammetry (Durban et al. 2016; Fearnbach et al. 2018). Periods of nutritional stress may accelerate deleterious health effects associated with POPs (e.g., compromised immune system, reproductive failure) as has been speculated of the critically endangered Southern Resident killer whales in Washington State/British Colombia (Lundin et al. 2016; Wasser et al. 2017).

Chemical profiles (i.e., POPs and stable isotopes) of MHI social clusters presented here do not completely describe their foraging ecology and regionally varying POP exposure but have informed what we know of these groups in several ways. Our findings indicate that some social clusters have higher concentrations of certain POP classes than others and likely feed in different regions and potentially on different prey types, which we suspect is associated with their varying spatial use (Baird et al. 2012, 2019). This indicates that some social clusters may be more vulnerable to POP exposure than others; although as additional biopsies are obtained for POP and stable isotope analyses and information on MHI social structure increases, our understanding of these relationships will become more clear.

Given the longevity of this species (i.e., slow growth and reproduction rates) (Kasuya 1986), we recommend incorporating the risk of POPs and potential adverse health effects associated with them into management of all three false killer whale populations when considering their long-term viability.

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Figure 1. Distribution of total POP concentrations (ng g^{-1} lipid wt.) ΣDDTs and ΣPCBs (A), ΣCHLDs, mirex and ΣPBDEs (B) and dieldrin, HCB and ΣHCHs (C) measured in blubber of false killer whales from main Hawaiian Islands (MHI), Northwestern Hawaiian Islands (NWHI), and pelagic (PEL) populations. Black dots represent outliers.

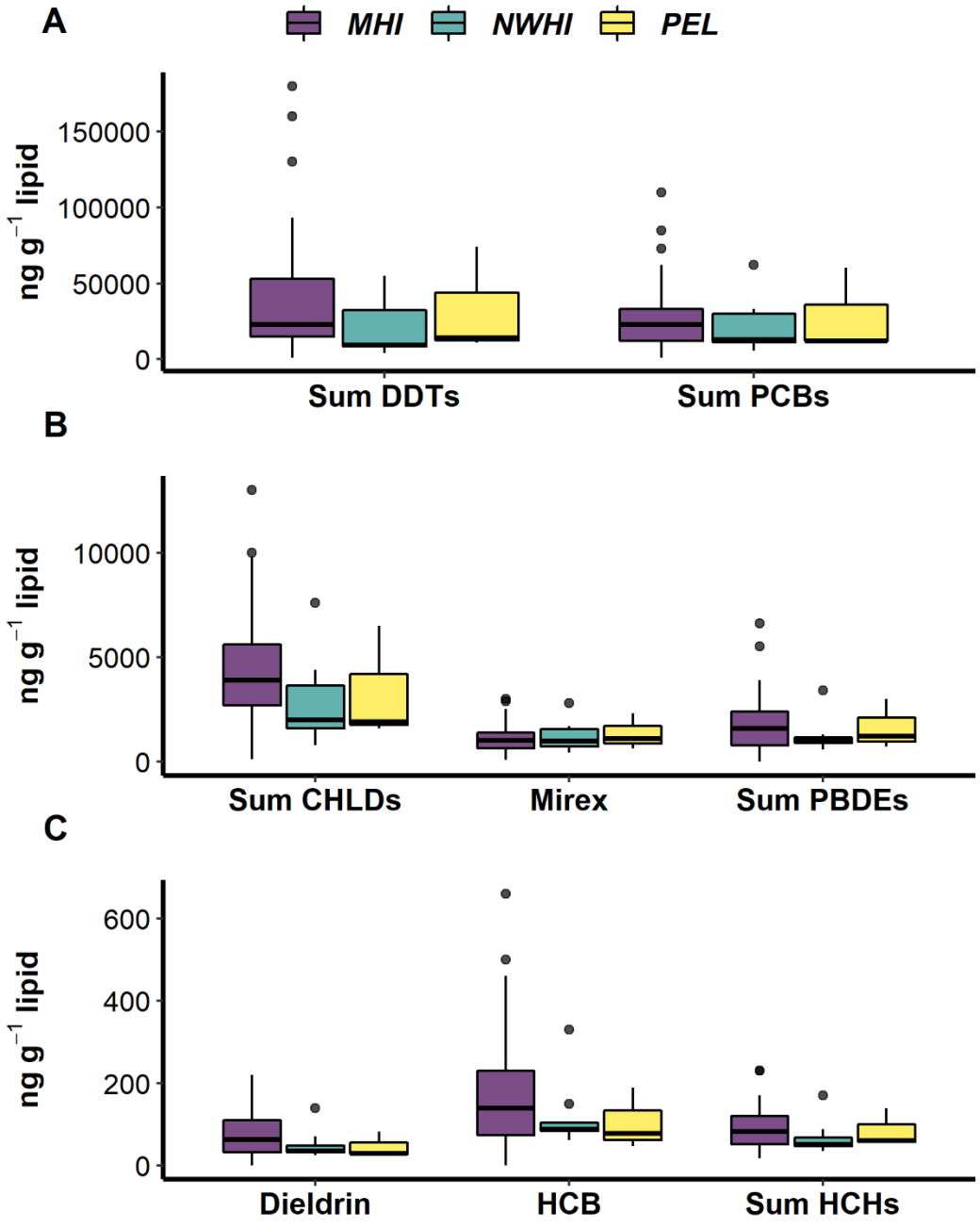

Figure 2. Sum PCBs concentrations (ng g^{-1} lipid wt.) measured in main Hawaiian Islands false killer whales by age/sex class. Solid horizontal line represents the threshold for thyroid and immune system dysfunction in aquatic mammals suggested by Kannan et al. (2000) and dashed line represents the threshold for maternal failure in bottlenose dolphins proposed by Schwacke et al. (2002). Black dots represent outliers. *Male and female juveniles/sub-adults were combined as no significant differences in concentrations for any POP class were found.

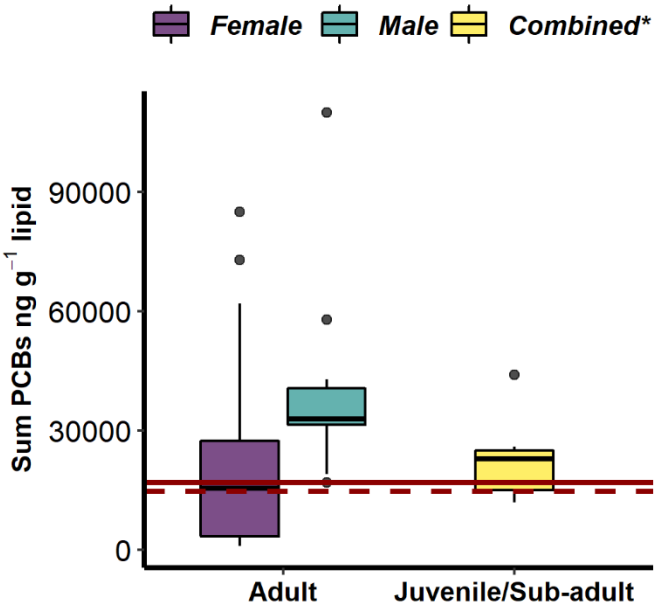

Figure 3. Distribution of total POP concentrations (ng g^{-1} lipid wt.) ΣDDTs and ΣPCBs (A), ΣCHLDs, mirex and ΣPBDEs (B) and dieldrin, HCB and ΣHCHs (C) by age/sex class in main Hawaiian Islands false killer whales. Black dots represent outliers. Male and female juveniles/sub-adults were combined as no significant differences in concentrations for any POP class were found.

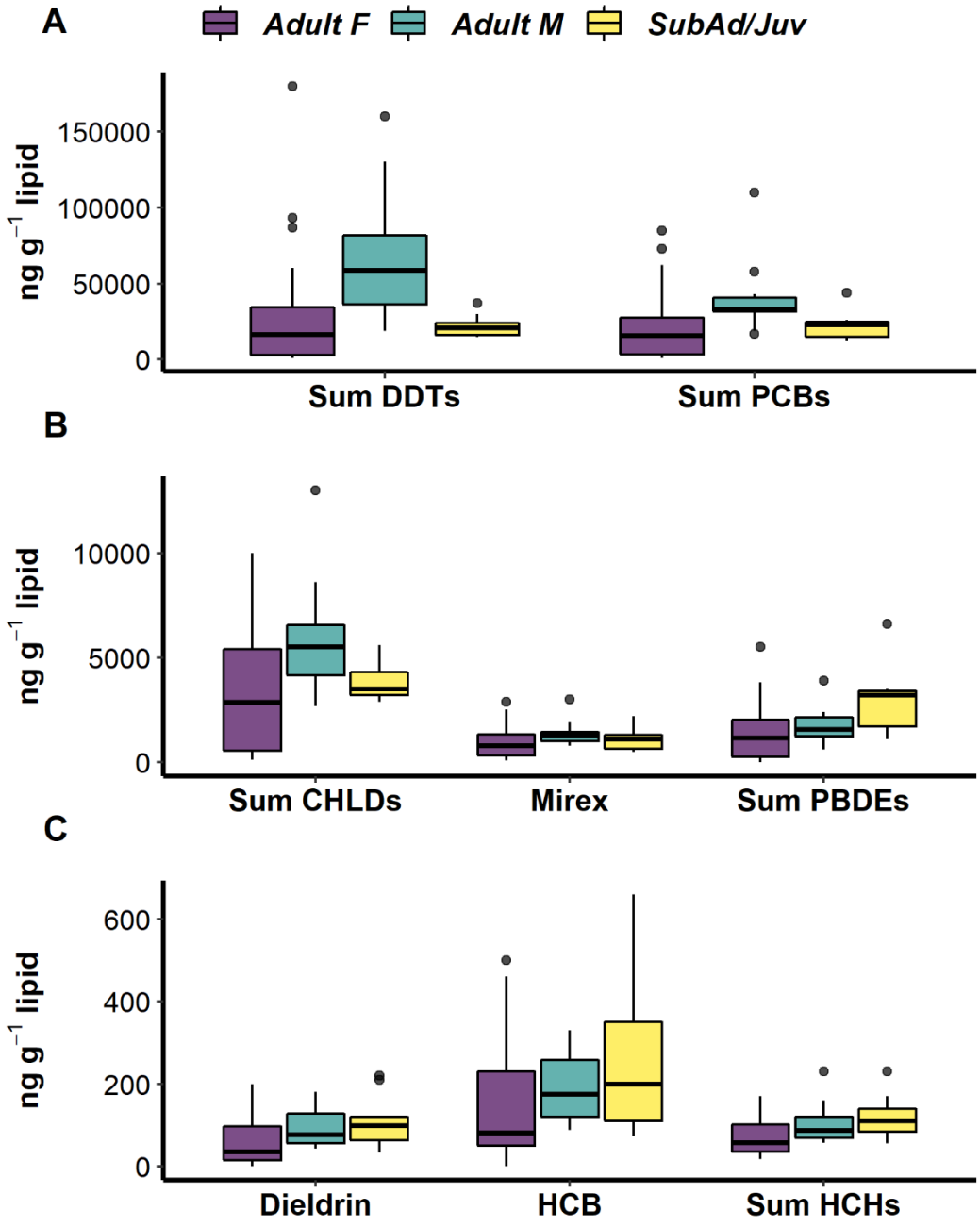

Figure 4. Distribution of concentrations (ng g^{-1} lipid wt.) of sum PCBs (A), DDTs (B) and PBDEs (C) measured in main Hawaiian Islands adult females by reproductive status (nulliparous = has never given birth; parous = given birth to at least one calf; unknown = insufficient sighting history). Black dots represent outliers.

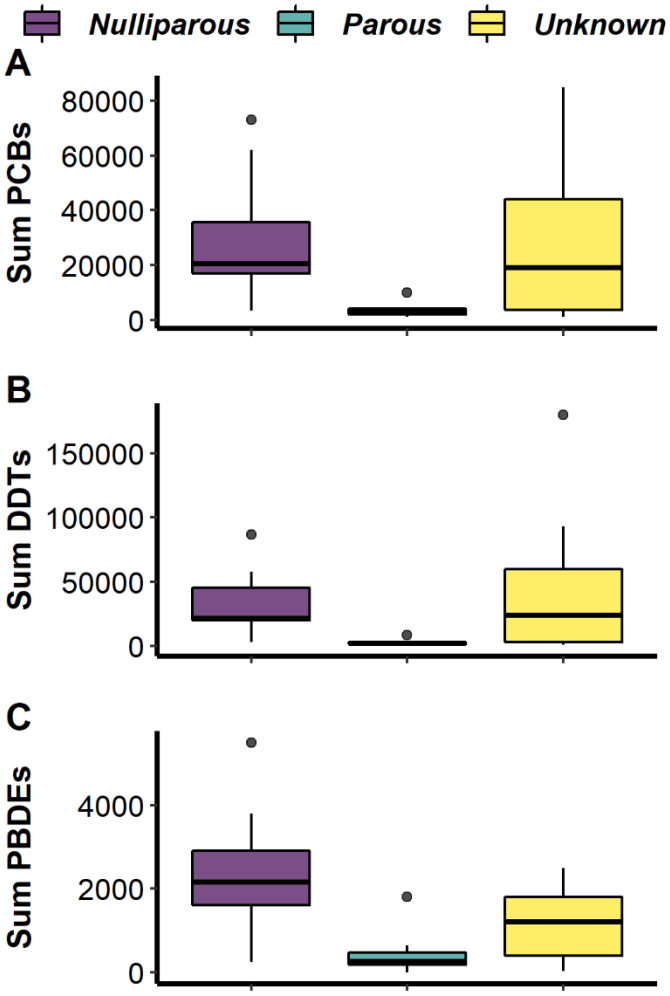

Figure 5. Linear mixed model estimates (coefficients) for each retained principal component (PC) factor generated from principal components analysis on main Hawaiian Islands false killer whales. Model estimates for social cluster are relative to Cluster 1 and estimates for age/sex/reproductive class are relative to nulliparous adult females.

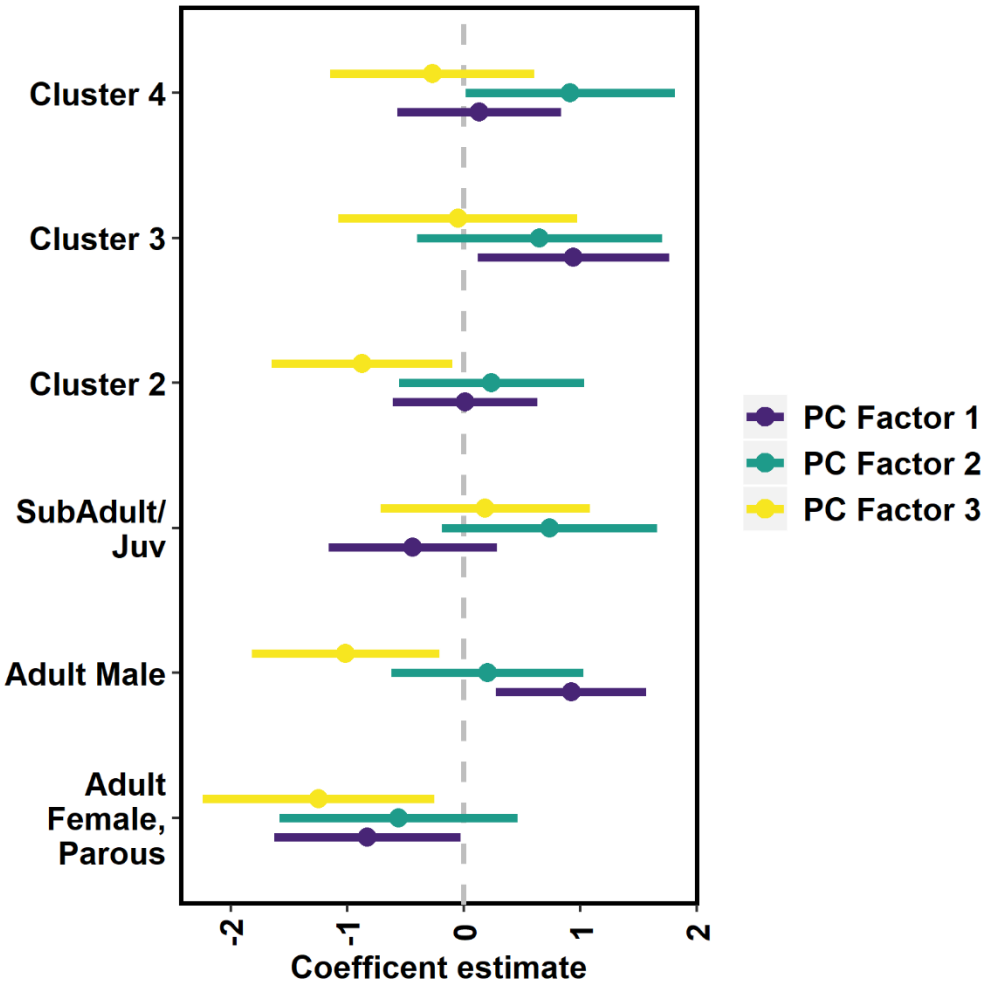

Figure 6. Ratios of sum DDTs/PCBs and sum PBDEs/PCBs by false killer whale population (A, B) and main Hawaiian Islands (MHI) social cluster (C, D). Black dots represent outliers. NWHI = Northwestern Hawaiian Islands.

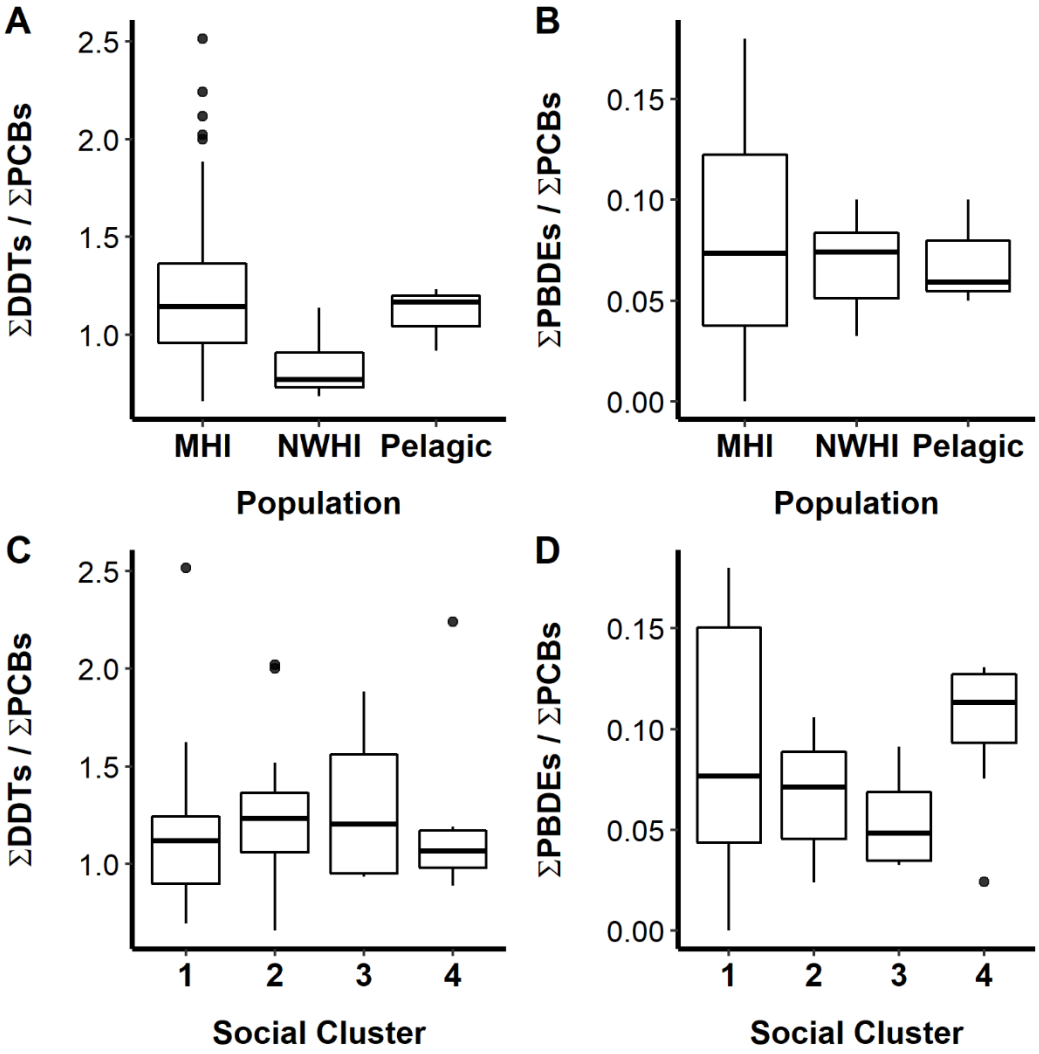

Figure 7. Mean ± standard error values for carbon and nitrogen stable isotopes by main Hawaiian Islands (MHI) false killer whale social cluster.

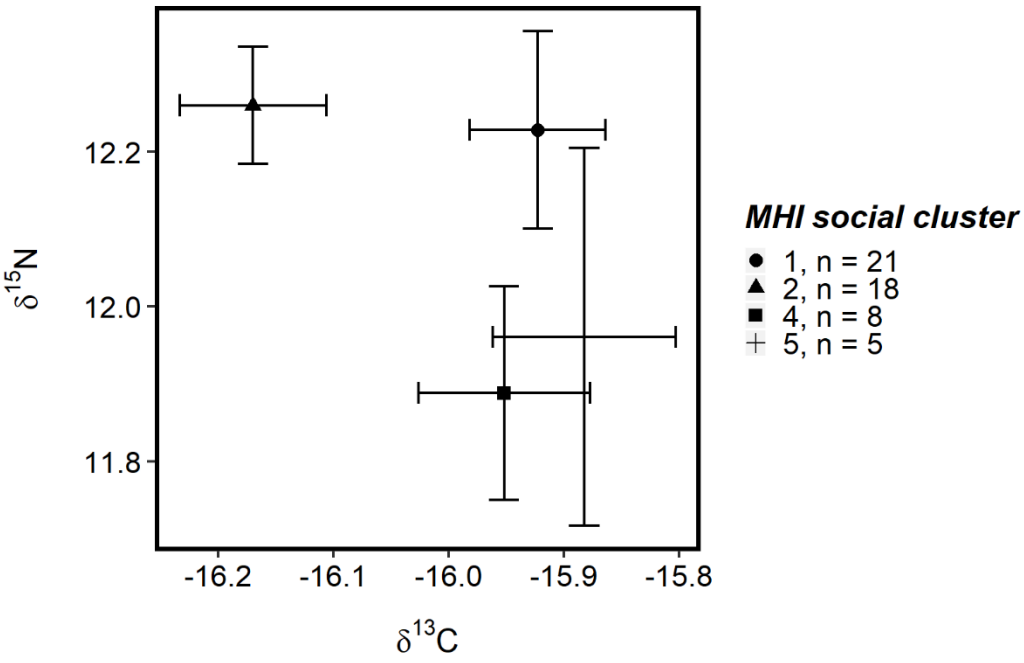

Table 1.
False killer whale life history information, main Hawaiian Islands (MHI) social cluster assignment, and analysis type by biopsy sample.

Animal ID	Sample collection date	Population	Age class	Sex	Social cluster	POPs	Stable isotopes
HIPc101	12/19/2009	MHI	Adult	Female[2]	4	x	x
HIPc102	7/16/2008	MHI	Adult	Male	1	x	
HIPc102	8/11/2010	MHI	Adult	Male	1	x	x
HIPc106	8/11/2010	MHI	Adult	Female[1]	1	x	x
HIPc114	8/25/2011	MHI	Adult	Male	1	x	x
HIPc115	8/11/2010	MHI	Adult	Male	1	x	x
HIPc116	7/16/2008	MHI	Adult	Female[2]	1	x	x
HIPc116	8/25/2011	MHI	Adult	Female[3]	1	x	x
HIPc117	7/16/2008	MHI	Adult	Female[2]	1	x	x
HIPc120	7/16/2008	MHI	Adult	Female[3,a]	1	x	
HIPc120	8/25/2011	MHI	Adult	Female[4]	1		x
HIPc133	8/11/2010	MHI	Adult	Male	1		x
*HIPc162	10/6/2013	MHI	Adult	Male	3	x	
*HIPc164	9/28/2016	MHI	Adult	Male	3	x	
HIPc179	7/16/2008	MHI	Adult	Male	1	x	
HIPc179	12/18/2009	MHI	Adult	Male	1		x
HIPc181	8/25/2011	MHI	Adult	Male	1	x	x
HIPc184	12/19/2009	MHI	Adult	Male	4	x	x
HIPc186	12/10/2009	MHI	Adult	Female[1]	3	x	
*HIPc198	11/7/2015	MHI	Adult	Female[1]	3	x	
HIPc203	8/11/2010	MHI	Adult	Female[1]	1	x	
HIPc204	8/11/2010	MHI	Adult	Male	5		x
HIPc207	8/14/2010	MHI	Sub-adult	Male	2		x
HIPc210	12/18/2009	MHI	Adult	Male	1		x
HIPc211	8/20/2011	MHI	Adult	Male	2	x	x
HIPc212	7/26/2008	MHI	Adult	Female[3,b]	1	x	x
HIPc212	7/28/2010	MHI	Adult	Female[3,b]	1	x	x
HIPc214	7/26/2008	MHI	Sub-adult	Female	1		x
HIPc216	7/28/2010	MHI	Adult	Female	1		x
HIPc220	8/14/2010	MHI	Adult	Female[4]	2		x
HIPc230	8/20/2011	MHI	Adult	Female[2,c]	2	x	x
HIPc233	8/20/2011	MHI	Adult	Female[4]	2		x
HIPc266	12/19/2009	MHI	Adult	Female[1]	4	x	x
HIPc282	7/26/2008	MHI	Sub-adult	Female[b]	1	x	
HIPc282	7/28/2010	MHI	Sub-adult	Female[b]	1	x	x
HIPc310	8/11/2010	MHI	Adult	Female[4]	1		x
HIPc312	7/26/2008	MHI	Juvenile	Female[a]	1	x	
HIPc313	7/16/2008	MHI	Adult	Female[3]	1	x	
HIPc316	8/25/2011	MHI	Adult	Female[3]	1	x	x
HIPc320	12/18/2009	MHI	Sub-adult	Male	1	x	x
HIPc338	8/20/2011	MHI	Adult	Female[4]	2		x

HIPc339	8/20/2011	MHI	Adult	Female[1]	2	x	x
HIPc351	12/19/2009	MHI	Sub-adult	Female	4	x	x
HIPc352	12/18/2009	MHI	Juvenile	Male	4		x
HIPc352	12/19/2009	MHI	Juvenile	Male	4	x	x
HIPc365	12/10/2009	MHI	Adult	Female[4]	5		x
HIPc366	12/10/2009	MHI	Adult	Male	5		x
HIPc367	12/10/2009	MHI	Juvenile	Female[4]	5		x
HIPc369	12/10/2009	MHI	Juvenile	Male	5		x
HIPc372	12/19/2009	MHI	Adult	Male	4	x	x
HIPc375	12/19/2009	MHI	Juvenile	Female	4	x	x
HIPc379	8/20/2011	MHI	Adult	Female[4]	2		x
HIPc381	8/20/2011	MHI	Adult	Female[2]	2	x	x
HIPc382	8/20/2011	MHI	Adult	Female[3,c]	2	x	x
HIPc383	8/20/2011	MHI	Adult	Female[2]	2	x	x
HIPc384	8/14/2010	MHI	Sub-adult	Male	2	x	x
HIPc387	8/20/2011	MHI	Adult	Male	2	x	x
HIPc391	8/14/2010	MHI	Adult	Female[1]	2	x	x
HIPc392	8/14/2010	MHI	Sub-adult	Male	2	x	x
HIPc396	8/14/2010	MHI	Adult	Female[2]	2	x	x
HIPc397	8/20/2011	MHI	Adult	Female[2]	2	x	x
HIPc398	8/20/2011	MHI	Adult	Female[1]	2	x	x
*HIPc700	11/21/2016	MHI	Adult	Female[2]	UK	x	
HIPc525	6/14/2012	NWHI	Adult	Male	NA	x	
HIPc529	6/13/2012	NWHI	Juvenile	Female	NA	x	
HIPc532	6/14/2012	NWHI	Sub-adult	Male	NA	x	
HIPc533	6/14/2012	NWHI	Adult	Female[2]	NA	x	
HIPc538	6/14/2012	NWHI	Adult	Male	NA	x	
HIPc542	6/14/2012	NWHI	Adult	Male	NA	x	
HIPc543	6/14/2012	NWHI	Juvenile	Male	NA	x	
HIPc544	6/14/2012	NWHI	Adult	Female[2]	NA	x	
HIPc291	4/21/2008	PEL	Adult	Female[2]	NA	x	
HIPc292	4/21/2008	PEL	Adult	Female[2]	NA	x	
HIPc850	4/21/2008	PEL	Adult	Male	NA	x	

[1] Nulliparous (never observed with a calf)

[2] Infrequent sighting data; association with a calf is unknown

[3] Parous (observed with a calf pre-biopsy)

[4] Stable isotope analyses only; reproductive status not considered

*Stranded

[a,b,c] Mother/offspring pair

Population abbreviations: MHI = main Hawaiian Islands; NWHI = Northwest Hawaiian Islands; PEL = pelagic

NA = not applicable

Table 2.

Concentrations of summed DDTs, PCBs and PBDEs measured in blubber of Eastern North Pacific odontocetes.

Species	Ecotype/population	Collection region	Collection year(s)	n	ng g^{-1}, lipid weight ∑DDTs	∑PCBs	∑PBDEs
Killer whale[1]	Southern Resident	Puget Sound/BC	2004-2013	78	53,000 ± 50,000	36,000 ± 30,000	4700 ± 3400
Killer whale (AM)[2]	Resident, Transient, Offshore	Alaska	2001-2003	14	25,000 ± 2800	15,000 ± 1700	NA
Killer whale (JM)[3]*	UK	Hawai‘i	2008	1	171,000	93,200	938
Melon-headed whale[3]*	UK	Hawai‘i	2010-2011	4	31,000 ± 45,000	15,600 ± 23,300	409 ± 432
Striped dolphin[3]*	UK	Hawai‘i	1997-2010	6	20,000 ± 5,510	13,800 ± 7,260	258 ± 139
Bottlenose dolphin[3]*	UK	Hawai‘i	2009, 2011	3	15,000 ± 7,700	11,800 ± 7,340	1070 ± 172
False killer whale (AM)[4]*	UK	British Columbia	1987, 1989	2	990,000 ± 1,300,000	40,000 ± 8500	NA
False killer whale[3]*	MHI	Hawai‘i	2010	1	28,200	26,200	1,650
False killer whale[6]*	MHI	Hawai‘i	2013-2016	4	120,000 ± 59,000	75,000 ± 29,000	2,900 ± 1,200
False killer whale[5,6]	MHI, NWHI, PEL	Hawai‘i	2008-2012	52	28,000 ± 28,000	22,000 ± 17,000	1600 ± 1300

NA = not analyzed

Population abbreviations: MHI = main Hawaiian Islands; NWHI = Northwestern Hawaiian Islands; PEL = pelagic

[1]data from Krahn et al. 2007, 2009; unpublished NWFSC data

[2]data from Herman et al. 2005

[3]data from Bachman et al. 2014

[4]data from Jarman et al. 1996

[5]data from Ylitalo et al. 2009

[6]data from current study

*stranded animal

Table 3.
Summary of retained varimax-rotated PCA factors and loading weights for POP classes in main Hawaiian Islands (MHI) false killer whales (n = 36, restricted data set). Loadings greater than 0.50 are bolded.

	Factor 1	***Factor 2***	***Factor 3***
% variance explained	0.42	0.33	0.18
eigenvalue	3.33	2.63	1.4
ΣPCBs	**0.87**	0.34	0.33
ΣDDTs	**0.94**	0.28	0.10
ΣCHLDs	**0.80**	0.43	0.32
ΣPBDEs	0.32	0.43	**0.83**
ΣHCHs	0.31	**0.74**	0.26
HCB	0.30	**0.91**	0.26
Dieldrin	0.45	**0.79**	0.36
Mirex	**0.75**	0.26	0.47

Figure S1. Persistent organic pollutant (POP) concentrations (ng g^{-1} lipid wt.) sum DDTs and sum PCBs (A), sum CHLDs, mirex, and sum PBDEs (B), and dieldrin, HCB, and sum HCHs (C) measured in main Hawaiian Islands false killer whale mother offspring pairs (from left to right, pairs: HIPc120 (mother) / HIPc312 (offspring); HIPc212 (mother) / HIPc282 (offspring); second biopsy for latter pair; HIPc382 (mother) / HIPc230 (offspring)).

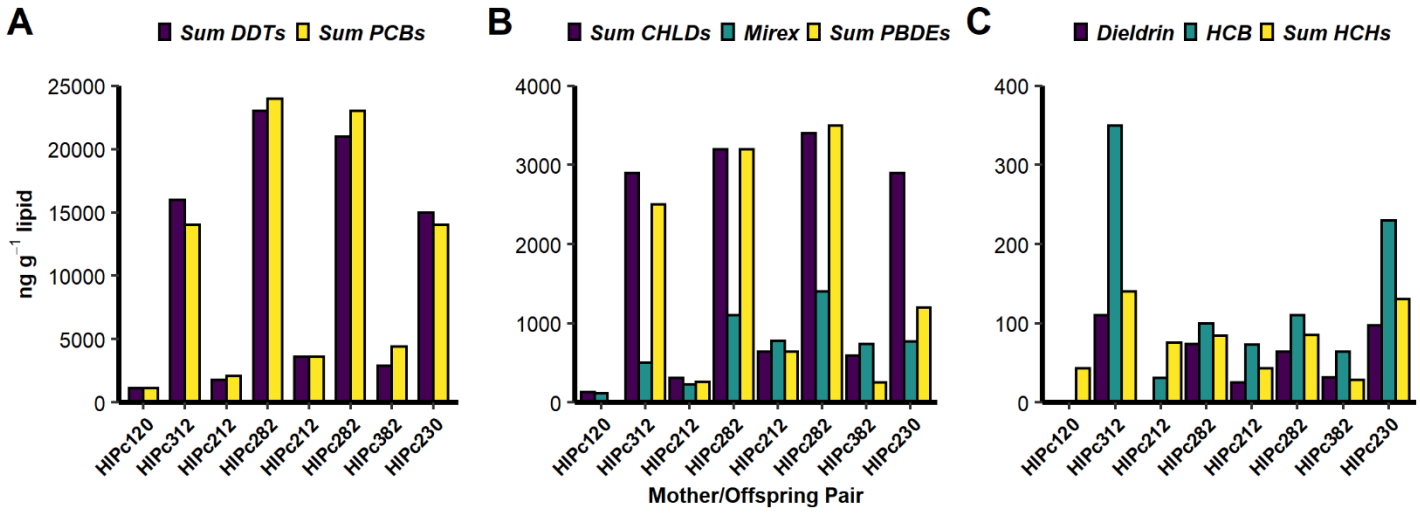

Figure S2. Composition of PCB (A, C) and PBDE (B,D) congeners by homolog group for false killer whale populations (A, B) and among age/sex/reproductive class groups within the main Hawaiian Islands (MHI) population (C, D). Deca-, nona-, and trichlorinated PCBs and tri- and heptabrominated PBDEs contributed less than 2% to total sums and therefore are not shown. For plots A, B: NWHI = Northwestern Hawaiian Islands; PEL = pelagic. For plots C, D: Ad. = Adult; Null. = Nulliparous; UK = Unknown reproductive status.

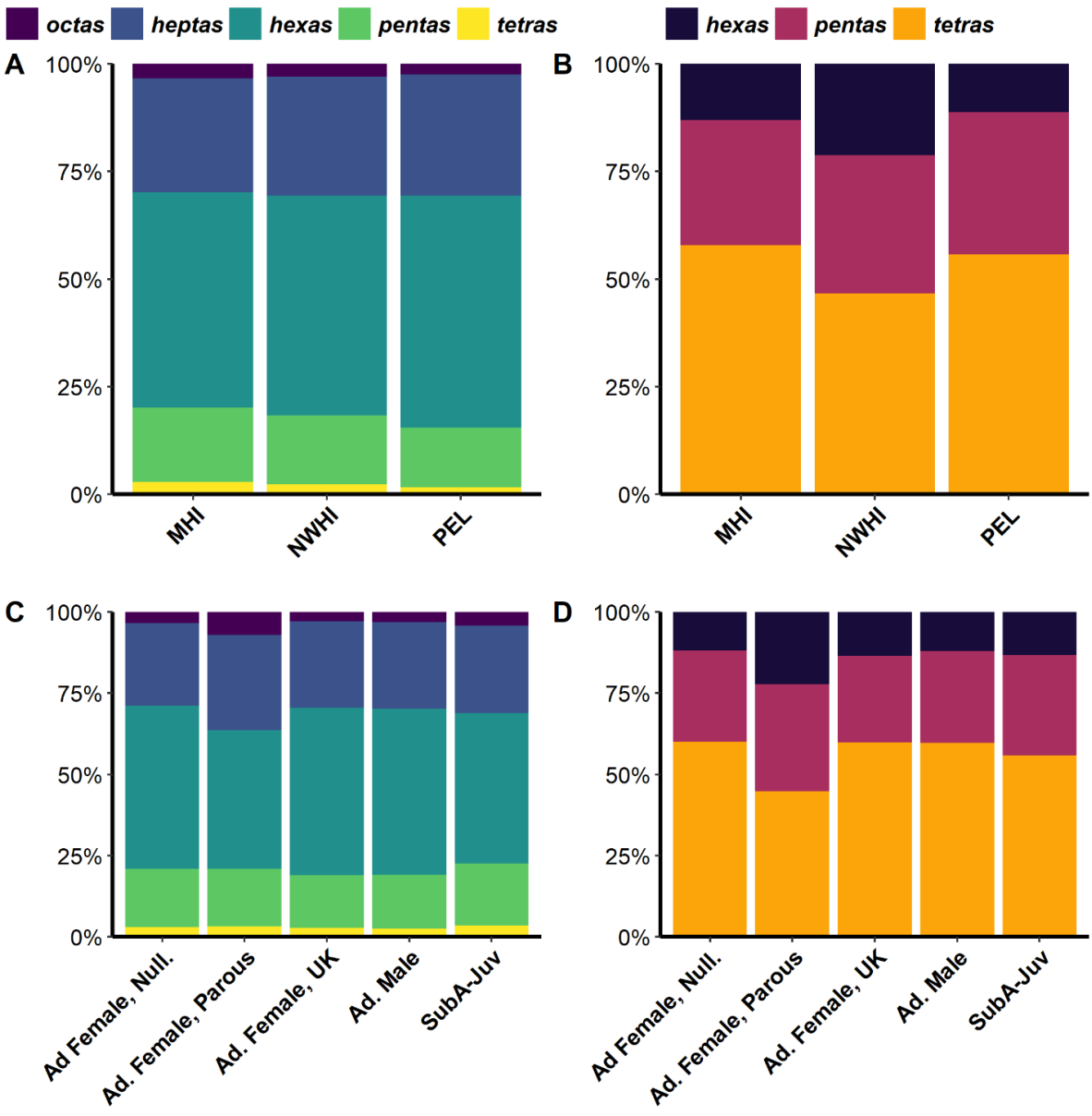

Figure S3. Mean relative contribution (%) of PCB congeners to the total sum of PCBs measured in false killer whale populations from Hawaiian waters. MHI = main Hawaiian Islands; NWHI = Northwestern Hawaiian Islands; PEL = pelagic population.

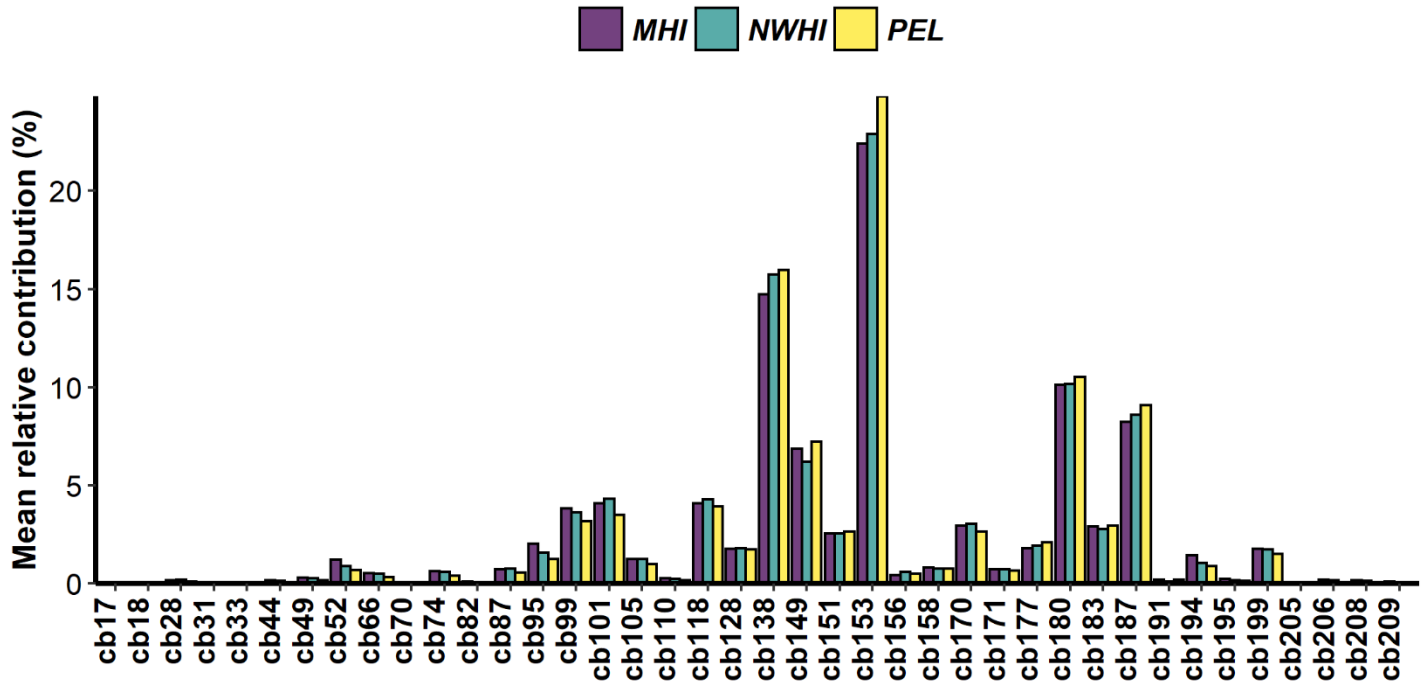

Figure S4. Mean relative contribution (%) of PBDE congeners to the total sum of PBDE measured in false killer whale populations from Hawaiian waters. MHI = main Hawaiian Islands; NWHI = Northwestern Hawaiian Islands; PEL = pelagic population.

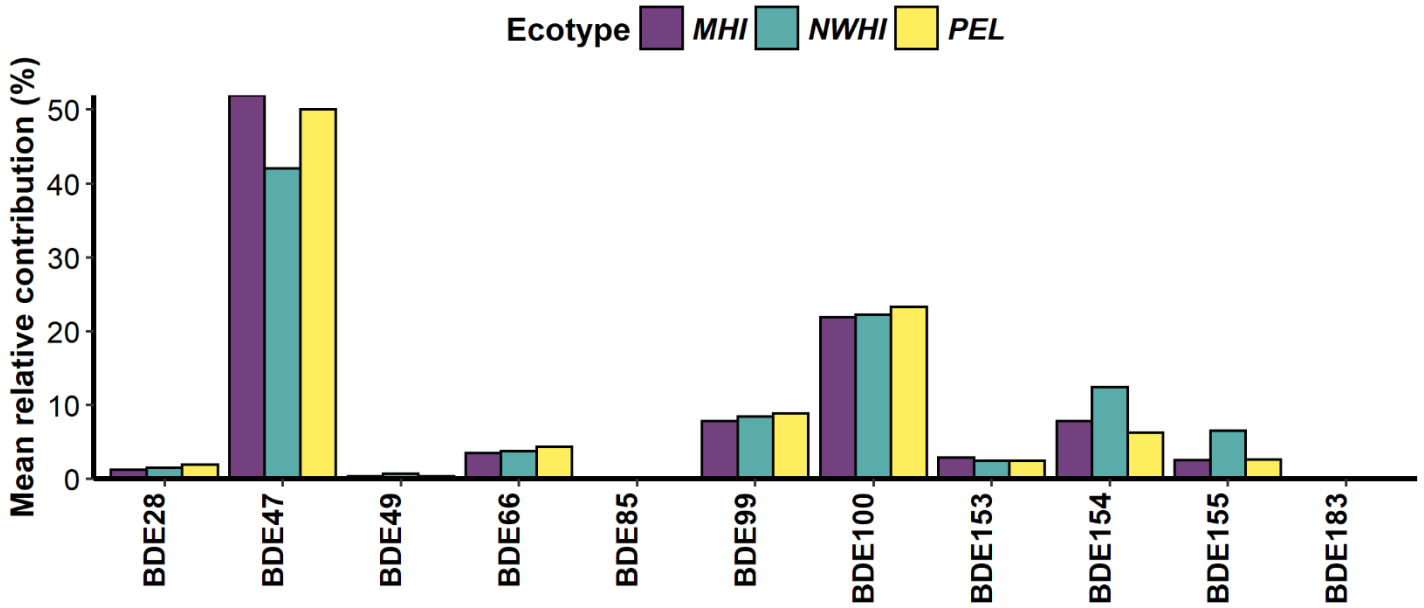

Table S1.

Percent lipid and persistent organic pollutant concentrations measured in blubber biopsies from all three Hawaiian false killer whale populations.

		ng g^{-1} (parts per billion)							
False killer whale population	**% lipid**	**Sum HCHs[1]**	**Sum CHLDs[2]**	**Sum PCBs[3]**	**Sum DDTs[4]**	**Sum PBDEs[5]**	**dieldrin**	**HCB[6]**	**mirex**
MHI[7] (n = 45)									
Mean ± SD (wet wt.)	21 ± 11	18 ± 15	900 ± 960	6,100 ± 7,900	8,900 ± 14,000	410 ± 440	18 ± 17	39 ± 37	240 ± 240
Concentration range	5.2 - 58	1.7 - 66	16 - 4,200	140 - 35,000	140 - 55,000	< LOQ - 1,900	< 1.8 - 56	< 3.3 - 150	13 - 1,000
Mean ± SD (lipid wt.)	—	90 ± 51	4,100 ± 3,000	27,000 ± 23,000	38,000 ± 41,000	1,800 ± 1,400	83 ± 57	180 ± 140	1,100 ± 700
Concentration range	—	18 - 230	130 - 13,000	1,000 - 110,000	1,100 - 180,000	< LOQ - 6,600	< LOQ - 220	< LOQ - 660	87 - 3,000
NWHI[7] (n = 8)									
Mean ± SD (wet wt.)	19 ± 7.0	11 ± 3.5	460 ± 240	3,500 ± 2,300	3,200 ± 2,700	200 ± 74	8.1 ± 2.8	19 ± 6.5	200 ± 100
Concentration range	9.2 - 27	6.3 - 16	180 - 910	1,300 - 7,800	910 - 8,900	130 - 310	4.3 - 13	8.0 - 30	95 - 400
Mean ± SD (lipid wt.)	—	69 ± 44	2,900 ± 2,200	22,000 ± 19,000	20,000 ± 18,000	1,300 ± 900	52 ± 38	120 ± 88	1,200 ± 760
Concentration range	—	36 - 170	790 - 7,600	5,700 - 62,000	4,000 - 55,000	570 - 3,400	25 - 140	62 - 330	440 - 2,800
PEL[7] (n = 3)									
Mean ± SD (wet wt.)	20 ± 6.2	17 ± 11	680 ± 630	5,900 ± 6,200	6,900 ± 7,900	350 ± 280	9.4 ± 7.5	21 ± 17	280 ± 210
Concentration range	13 - 25	7.8 - 30	250 - 1,400	1,600 - 13,000	1,800 - 16,000	92 - 650	3.8 - 18	10 - 41	83 - 500
Mean ± SD (lipid wt.)	—	85 ± 47	3,300 ± 2,700	28,000 ± 28,000	33,000 ± 36,000	1,600 ± 1,200	46 ± 32	110 ± 75	1,300 ± 860
Concentration range	—	55 - 140	1,600 - 6,500	12,000 - 60,000	11,000 - 74,000	710 - 3,000	26 - 83	47 - 190	640 - 2,300

[1] Sum HCHs = summed concentrations of alpha-, beta-, and gamma-hexachlorocyclohexane (HCH) isomers

[2] Sum CHLDs = summed concentrations of *cis*-chlordane, *trans*-chlordane, heptachlor, heptachlor epoxide, *cis*-nonachlor, *trans*-nonachlor, nona-III-chlordane and oxychlordane

[3] Sum PCBs = summed concentrations of congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208 and 209

[4] Sum DDTs = summed concentrations of *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT

[5] Sum PBDEs = summed concentrations of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155 and 183

[6] HCB = hexachlorobenzene

[7] Population abbreviations: MHI = main Hawaiian Islands; NWHI = Northwest Hawaiian Islands; PEL = pelagic

< LOQ = less than the lower limit of quantitation

Table S2.

Linear mixed-effects model results for retained PCA factors 1-3 for persistent organic pollutant classes in main Hawaiian Islands (MHI) false killer whales (n = 36, restricted dataset). Covariates included age/sex class (adult male/female, sub-adult/juvenile) paired with reproductive status (nulliparous/parous) and social cluster assignment (1-4). Statistically significant covariates ($p < 0.05$) are bolded.

	Factor 1			***Factor 2***			***Factor 3***		
Model	**Estimate**	**SE**	***p*-value**	**Estimate**	**SE**	***p*-value**	**Estimate**	**SE**	***p*-value**
Intercept	-0.17	0.31	0.601	-0.38	0.40	0.348	0.75	0.39	0.065
Age/sex class, relative to nulliparous adult females									
Adult female, parous	-0.83	0.40	**0.048**	-0.56	0.51	0.283	-1.25	0.50	**0.019**
Adult male	0.92	0.32	**0.008**	0.20	0.41	0.627	-1.02	0.40	**0.018**
Sub-adult/juvenile	-0.44	0.36	0.236	0.74	0.46	0.122	0.18	0.45	0.686
Social cluster, relative to cluster 1									
Cluster 2	-0.01	0.31	0.973	0.24	0.40	0.553	-0.87	0.39	**0.033**
Cluster 3	0.94	0.41	**0.030**	0.65	0.53	0.228	-0.05	0.51	0.923
Cluster 4	0.13	0.35	0.711	0.91	0.45	0.052	-0.27	0.44	0.540

Table S3.
Summary of stable isotope $\delta^{13}C$ and $\delta^{15}N$ values from main Hawaiian Islands (MHI) Cluster 1 false killer whales by age/sex class.

Adult female (n = 11)		
Mean ± SD	-16.0 ± 0.08	12.3 ± 0.21
Range of ratios	-16.4 – -15.5	11.1 – 13.1
Adult male (n = 7)		
Mean ± SD	-15.9 ± 0.10	12.1 ± 0.18
Range of ratios	-16.5 – -15.6	11.6 – 12.7
Subadult female (n = 2)		
Mean ± SD	-15.7 ± 0.05	12.3 ± 0.30
Range of ratios	-15.8 – -15.6	12.0 – 12.6
Subadult male (n = 1)		
Mean	-15.7	11.8
All Cluster 1 individuals (n = 21)		
Mean ± SD	-15.9 ± 0.27	12.2 ± 0.59
Range of ratios	-16.5 – -15.5	11.1 – 13.1
All MHI individuals (n = 52)		
Mean ± SD	-16.0 ± 0.28	12.2 ± 0.48
Range of ratios	-16.6 – -15.4	11.1 – 13.1