

**Title:** 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

1  
2 False killer whales are long-lived, slow to mature, apex predators, and therefore susceptible to  
3 bioaccumulation of persistent organic pollutants (POPs). Hawaiian waters are home to three  
4 distinct populations: pelagic; Northwestern Hawaiian Islands (NWHI) insular; and main  
5 Hawaiian Islands (MHI) insular. Following a precipitous decline over recent decades, the MHI  
6 population was listed as “endangered” under the Endangered Species Act in 2012. This study  
7 assesses the risk of POP exposure to these populations by examining pollutant concentrations  
8 and ratios from blubber samples (n = 56) related to life history characteristics and MHI social  
9 clusters. Samples were analyzed for PCBs, DDTs, PBDEs, and some organochlorine pesticides.  
10 Skin samples (n = 52) were analyzed for stable isotopes  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  to gain insight into MHI  
11 false killer whale foraging ecology. Pollutant levels were similar among populations, although  
12 MHI whales had a significantly higher mean ratio of DDTs/PCBs than NWHI whales. The  
13  $\Sigma\text{PCB}$  concentrations of 28 MHI individuals (68%) sampled were equal to or greater than  
14 suggested thresholds for deleterious health effects in marine mammals. The highest POP values  
15 among our samples were found in four stranded MHI animals. Eight of 24 MHI adult females  
16 have not been documented to have given birth; whether they have yet to reproduce, are  
17 reproductive senescent, or are experiencing reproductive dysfunction related to high POP  
18 exposure is unknown. Juvenile/sub-adults had significantly higher concentrations of certain  
19 contaminants than those measured in adults, and may be at greater risk of negative health effects  
20 during development. Multivariate analyses, POP ratios, and stable isotope ratios indicate varying  
21 risk of POP exposure, foraging locations and potentially prey items among MHI social clusters.  
22 Our findings provide invaluable insight into the ongoing risk POPs pose to the MHI population’s  
23 viability, as well as consideration of risk for the NWHI and pelagic stocks.

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

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## 1. INTRODUCTION

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

41 The MHI population was listed as “endangered” under the U.S. Endangered Species Act (ESA)  
42 in 2012 due to a precipitous population decline between the late 1980s and the early 2000s.  
43 Bradford et al. (2018) estimated only 167 (CV = 0.14) individuals comprise the MHI stock,  
44 which is approximately three times less than an estimate from 1988 (Reeves et al., 2009), and  
45 several other lines of evidence supported a decreasing population trend (Mobley et al. 2000;  
46 Mobley 2004; Baird 2009; Oleson et al. 2010; Silva et al. 2013). Potential causes of population

47 decline in MHI false killer whales include incidental take in commercial and recreational  
48 fisheries (Baird and Gorgone 2005; Baird et al. 2014, 2017), decreased prey biomass and size  
49 (Oleson et al. 2010), reduced genetic diversity (Chivers et al. 2010; Martien et al. 2014), and  
50 susceptibility to adverse health effects associated with exposure to persistent organic pollutants  
51 (POPs) (Ylitalo et al. 2009; Bachman et al. 2014; Foltz et al. 2014). POPs are toxic, man-made  
52 compounds that were used as agricultural pesticides and industrial chemicals. They are  
53 ubiquitous in marine ecosystems due to their widespread use, resistance to degradation,  
54 physicochemical properties, and global range of transport via volatilization and oceanic  
55 circulation (Iwata et al. 1993; Wania and MacKay 1996). False killer whales are particularly  
56 vulnerable to POP exposure as they are apex predators, increasing biomagnification burdens;  
57 long-lived, increasing susceptibility to bioaccumulation; and possess abundant lipid reserves in  
58 blubber, which are ideal repositories for lipophilic POPs (Holden and Marsden 1967; Jones and  
59 de Voogt 1999). Particular contaminants of concern include polychlorinated biphenyls (PCBs),  
60 polybrominated diphenyl ethers (PBDEs), and a number of organochlorine pesticides (OCPs)  
61 (e.g., dichlordiphenyltrichloroethanes or DDTs). Exposure to these pollutants has been correlated  
62 with several negative health effects in marine mammals, including immunosuppression (Ross et  
63 al. 1995; de Swart et al. 1994; Hammond et al. 2005) and disease (Ylitalo et al. 2005; Randhawa  
64 et al. 2015), thyroid disruption (Brouwer et al. 1989, 1998), and reproductive dysfunction  
65 (DeLong et al. 1973; Helle et al. 1976, Subramanian et al. 1987). In addition, thyroid,  
66 reproductive, and cognitive disruption have been observed in laboratory animals exposed to  
67 PBDEs (Eriksson et al. 2002, 2006; de Wit 2002; Talsness 2008).

68 Ylitalo et al. (2009) was the first to report high concentrations of POPs in blubber of MHI false  
69 killer whales and found trends in POP levels among age/sex classes consistent with findings

70 from similar studies on killer whales (*Orcinus orca*), albeit with a small sample size (n = 9)  
71 (Krahn et al. 2007b, 2009; Ross et al. 2000; Ylitalo et al. 2001). Adult males tend to have the  
72 highest POP concentrations as they accumulate POPs throughout their lives, whereas adult  
73 females have the opportunity to offload POPs to their offspring through gestation and, primarily,  
74 through lactation (Ylitalo et al. 2001; Aguilar and Borrell 1994; Ross et al. 2000). In killer  
75 whales, approximately 70-90% of mothers' contaminant burdens have been estimated to be  
76 transferred to offspring during lactation (Mongillo et al. 2012). Consequently, juveniles have  
77 high POP levels with the amount offloaded influenced by birth order (Ylitalo et al. 2001). Once  
78 adult females become reproductively senescent, they continue to accumulate POPs via their diet  
79 (Ross et al. 2000). More recently, Foltz et al. (2014) reported high levels of cytochrome  
80 P4501A1 (CYP1A1) expression, a biomarker of POP exposure, in biopsies sampled from live  
81 MHI false killer whales. Further, they examined total PCB concentrations (n = 33) and found  
82 that 84% of false killer whale biopsies exceeded the suggested 14,700 ng g<sup>-1</sup> threshold for risk of  
83 maternal failure (Schwacke et al. 2002) and 71% exceeded the proposed 17,000 ng g<sup>-1</sup> threshold  
84 for thyroid and immune system disruption in aquatic mammals (Kannan et al. 2000). Differences  
85 in CYP1A1 expression among social clusters were examined but no significant findings were  
86 reported, however knowledge of social clusters at the time of the study was limited (Foltz et al.  
87 2014).

88 While the influence of life history characteristics on POP concentrations in MHI false killer  
89 whales may be generally understood, variance in contaminant concentrations among social  
90 clusters remains unclear. Of particular interest in this study was variance in POP concentrations  
91 between MHI social clusters, while accounting for known drivers of POP levels (i.e., age class,  
92 sex, reproductive status), to investigate inter-group differences in risk of POP exposure. In

93 addition, we were interested in the use of chemical contaminants as indicators of geographic  
94 areas and trophic positions at which MHI social clusters primarily forage. Such findings would  
95 enhance our understanding of these groups' varying spatial use, foraging ecology, and localized  
96 threats to POP exposure. For instance, POP ratios and stable isotopes  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measured in  
97 blubber/epidermis have been used to differentiate cetacean stocks (Krahn et al. 1999; Muir et al.  
98 1996; Witteveen et al. 2009), inform regional sources of contaminants (Calambokidis and  
99 Barlow, 1991; Krahn et al. 1999, 2007a; Muir et al. 1990), and provide insight into foraging  
100 areas differing by pod or social group of upper trophic level odontocetes (Herman et al. 2005;  
101 Krahn et al. 2007a; Schnitzler et al. 2018). Examining POP ratios (e.g.,  $\Sigma\text{DDTs}/\Sigma\text{PCBs}$  and  
102  $\Sigma\text{PBDEs}/\Sigma\text{PCBs}$ ) allows comparison among groups to gain insight into regional differences,  
103 such as urban or agricultural "signatures" (Calambokidis and Barlow, 1991; Krahn et al. 2007a).  
104 Stable isotopes such as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can indicate foraging location (nearshore/offshore) and  
105 trophic position, respectively, as whales accrue these isotopes through their prey (Kelley 2000;  
106 Krahn et al. 2007a).

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

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## **2. MATERIALS AND METHODS**

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### **2.1 Sample collection**

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False killer whale biopsy sampling was conducted around the main Hawaiian Islands from 2008

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through 2012, using a 45 kg pull Barnett RX-150 crossbow and Larsen biopsy tips (25 mm long

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and 8 mm wide), as previously described in Ylitalo et al. (2009). After collection, biopsy

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samples were stored in a cooler with ice packs while in the field and transferred to a -20°C

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freezer for short-term storage before being stored in a -80°C freezer prior to analyses. Biopsies

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from MHI false killer whales reported in Ylitalo et al. (2009) were included in this study (n = 9).

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Simultaneous to sample collection, individuals were photographed for individual identification

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(see below) and to determine population identity. In addition to biopsies obtained from free-

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ranging individuals, blubber biopsies were included from four stranded (i.e., deceased) whales in

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years 2013, 2015, and 2016.

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### **2.2 Life history and social cluster information**

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Photographs of sampled individuals were compared to the catalog of Baird et al. (2008) to

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determine whether any individuals were sampled on multiple occasions and to assess population

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identity. Age class (i.e., juvenile, subadult, adult) of false killer whales was determined using a

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combination of field assessment, individual sighting histories, and relative size in photographs

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over the entire sighting history of the biopsied individuals. This included body size relative to

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other individuals, presence of calves in close proximity (indicating adulthood), and amount and

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severity of marks which accumulate over time (Baird et al. 2008). Sex of individuals was

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determined genetically at the Southwest Fisheries Science Center using Real-Time PCR

138 (Stratagene) zinc finger gene amplification as described by Morin et al. (2005). For adult  
139 females, determination of reproductive status (i.e., parous, nulliparous, unknown) was based on  
140 field observations and sighting histories (e.g., if seen with calf), and genetic parentage  
141 information if available.

142 MHI false killer whale social cluster assignment was determined through association analyses as  
143 described in Baird et al. (2012, 2019). Analyses were conducted through the program Socprog  
144 2.9 (Whitehead 2009) using individual sighting histories from Cascadia Research Collective's  
145 photo-identification catalog (Baird et al. 2008) from years 2000 to 2018. Eigenvector-based  
146 modularity was used to evaluate association strengths among individuals. Determination of  
147 discrete social clusters within the population was considered when network modularity (Q) was  
148 greater than 0.3 (Newman 2004, 2006). Each individual was assigned to one of five social  
149 clusters based on these analyses (Baird et al. 2019).

### 150 **2.3 Persistent organic pollutant and lipid analyses**

151 Blubber samples were analyzed for a suite of 79 persistent organic pollutants using a gas  
152 chromatography/mass spectrometry (GC/MS) method (Sloan et al. 2014; Ylitalo et al. 2009).  
153 Briefly, blubber was weighed (~ 0.1 to 0.3 g) into a solvent-cleaned glass jar, mixed with sodium  
154 sulfate and magnesium sulfate to remove any water and then the blubber mixture was packed  
155 into an accelerated solvent extractor cell, the surrogate standard was added (PCB103; 75ng) and  
156 analytes of interest were extracted using dichloromethane. Prior to sample cleanup, a 1-mL  
157 portion of extract was removed for percent lipid determination using thin-layer chromatography  
158 with flame ionization detection (TLC-FID) (Ylitalo et al. 2005; Sloan et al. 2014). A high-  
159 performance liquid chromatography (HPLC) internal standard (trichloro-*meta*-xylene; 75 ng)  
160 was added to the remaining extract to calculate the recovery of the surrogate standard. The



161 sample extracts were then cleaned up using a two-step process: removal of highly polar  
162 compounds on a gravity flow glass column containing alumina/silica gel followed by removal of  
163 lipids and other biogenic interferences using HPLC size exclusion chromatography. The extract  
164 volume was concentrated to ~ 100  $\mu$ L and a GC internal standard (tetrachloro-*ortho*-xylene; 30  
165 ng) was added to each sample to calculate the recovery of the HPLC standard. The POPs were  
166 separated on a 60-meter DB-5 GC capillary column and measured on a low-resolution  
167 quadrupole GC/MS system. This system was calibrated using sets of up to ten multi-level  
168 calibration standards of known concentrations.

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

177 All contaminant concentrations are reported in ng/g, lipid weight (ng/g, lipid wt.). Sum PCBs is  
178 the sum concentrations of congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99,  
179 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183,  
180 187/159/182, 191, 194, 195, 199, 205, 206, 208 and 209. Sum DDTs is the sum of *o,p'*-DDD,  
181 *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT. Sum chlordanes (CHLDs) is the  
182 summed levels of *cis*-chlordane, *trans*-chlordane, heptachlor, heptachlor epoxide, *cis*-nonachlor,  
183 *trans*-nonachlor, nona-III-chlordane and oxychlordane. Sum hexachlorocyclohexanes (HCHs) is

184 the summed concentrations of *alpha*-, *beta*-, and *gamma*-HCH isomers, and sum PBDEs is the  
185 summed concentrations of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155 and 183.

186 Concentrations of aldrin, dieldrin, endosulfan I, hexachlorobenzene (HCB) and mirex were also  
187 determined in the biopsy samples.

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

#### 198 **2.4 Stable isotope analyses**

199 False killer whale skin samples were analyzed for stable isotope ratios of carbon and nitrogen  
200 using the method described in Herman et al. (2005). Skin samples were freeze-dried overnight  
201 and subsequently ground to a powder in a micro ball mill. The powdered skin was transferred to  
202 a glass filter paper folded into a cone, folded shut, and the cone was placed into a 33 mL ASE  
203 cell. Lipids were extracted from the powdered skin using two cell volumes of dichloromethane at  
204 25°C and 500 psi. The sample cone was removed from the ASE cell and dried at room  
205 temperature in a fume hood for 10 min. The lipid-free skin samples (0.4 to 0.6 mg dried powder)  
206 were then loaded into tin cups and combusted in a Costech elemental analyzer attached to a

207 Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer. The values were calibrated  
208 against internal laboratory standards (aspartic acid and  $^{15}\text{N}$ -enriched histidine), which were  
209 analyzed after every 10 field samples.

210 Quality assurance measures for stable isotope ratios included the analysis of both continuing  
211 calibration standards and a fish tissue, SRM 1946 (National Institute of Standards and  
212 Technology, Gaithersburg, MD, USA) with each batch of samples (Sloan et al. 2006).

213 Continuing calibration standards were run every 10 field samples, whereas SRM 1946 was run  
214 between every 20 samples. Isotope values for continuing calibration standards and SRM 1946  
215 were within 0.30‰ of the values calibrated against international standards for  $\delta^{15}\text{N}$  and within  
216 0.20‰ for  $\delta^{13}\text{C}$ .

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

230 findings based on whale social clusters or whale age/sex class within a social cluster could be  
231 confidently made.

## 232 **2.5 Statistical analyses**

233 Analysis of variance (ANOVA) followed by the post-hoc Tukey–Kramer honest significant  
234 difference (HSD) test were used to determine if mean POP concentrations, POP ratios, and  $\delta^{13}\text{C}$   
235 and  $\delta^{15}\text{N}$  stable isotopes varied among false killer whale populations, among animals by age and  
236 sex within the MHI stock, or MHI social clusters. All POP concentrations were square root  
237 transformed and the percent lipid data were arcsine square root transformed prior to statistical  
238 analyses to increase homogeneity of variance and achieve normal distribution. Stable isotope  
239 data met assumptions of normal distribution and homogeneity of variance and therefore were not  
240 transformed. As ratios, POP ratios violate the assumption of homogeneity of variance. Therefore,  
241 we place more emphasis on descriptive (qualitative) interpretation of the results over the  
242 statistical outputs for POP ratios. The level of significance used for all statistical tests was  $\alpha \leq$   
243 0.05. All statistical analyses were completed using the program R 3.4.4 (R Development Core  
244 Team 2018).

245 Of particular interest in this study was variation in POP levels among MHI social clusters while  
246 controlling for known life history drivers (i.e., age class, sex, reproductive status) of variance in  
247 POP concentrations. We conducted principal components analysis (PCA) to generate factors that  
248 summarize the majority of variation in the dataset and identify POP classes driving the variance  
249 described by each factor. PCA was performed using the package *psych* (Revelle 2018) by  
250 summed and individual analyte class for MHI false killer whales using a correlation matrix with  
251 varimax rotation. Components with an eigenvalue greater than 1.0 were retained (Cangelosi and  
252 Goriely 2007). Loading weights for retained components on summed and individual analyte

253 classes were evaluated. We then used linear mixed effect models (LMM) to model each retained  
254 principal component factor as a function of fixed covariates age/sex/reproductive class (adult  
255 female parous, adult female nulliparous, adult male, juvenile/subadult) and social cluster  
256 assignment. Whale identity was included as a random effect to account for dependency structure  
257 resulting from repeated sampling of some individuals (i.e., individuals biopsied twice). LMMs  
258 were ran using the package *lme4* (Bates et al. 2015). Cluster 1 was set as the reference level for  
259 the categorical covariate social cluster, such that covariate estimates would be relative to Cluster  
260 1. Cluster 1 had the greatest sample size among social clusters (n = 20) and thus was considered  
261 the most reliable reference group for comparison among other clusters. Similarly, nulliparous  
262 adult females were set as the reference level for age/sex and reproductive class as they generally  
263 are less variable with respect to POP levels, although adult males could have been a suitable  
264 reference as well. Only adult females with known reproductive statuses were included in this  
265 sub-analysis as reproductive status was a covariate of interest for mixed effects models. The final  
266 analytic dataset for this sub-analysis included 36 samples.

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### 3. RESULTS

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#### 3.1 Sample collection and identity information

270 Samples (n = 74) were collected from individuals from all three populations: MHI insular (n =  
271 63 samples from 56 individuals); NWHI (n = 8 samples from 8 individuals); pelagic (n = 3  
272 samples from 3 individuals). Of these, POP results were available from 45 samples from the  
273 MHI population (41 individuals) and all sampled individuals from the NWHI and pelagic  
274 populations. Stable isotope results were available from 51 individuals from the MHI population.  
275 Information on demographics, MHI social cluster assignment, and type of analysis completed

276 (POPs and stable isotopes) for each sample is reported in Table 1. Of the stranded individuals,  
277 three had sufficient sighting histories for social cluster designation. The fourth individual,  
278 HIPc700, had never been sighted previous to its necropsy so social cluster assignment is  
279 unknown; therefore, this sample was excluded from any statistical analysis concerning social  
280 clusters within the MHI population.

### 281 **3.2 POPs**

282 Wide ranges of POP concentrations were measured in individual false killer whales from all  
283 three populations (Figure 1). For example, concentrations of  $\Sigma$ PCBs and  $\Sigma$ DDTs ranged from  
284 1,000 to 110,000 ng g<sup>-1</sup>, lipid wt. and 1,100 to 180,000 ng g<sup>-1</sup>, lipid wt., respectively (Table S1).  
285 Levels of  $\Sigma$ PBDEs and organochlorine pesticides ( $\Sigma$ CHLDs,  $\Sigma$ HCHs, HCB, mirex, dieldrin)  
286 ranged from < LOQ to 13,000 ng g<sup>-1</sup>, lipid wt. (Table S1). Concentrations of aldrin and  
287 endosulfan I were < LOQ for all samples analyzed. Proportions of PCB and PBDE congeners by  
288 homolog group (i.e., chlorination/bromination level) were generally similar among populations,  
289 although NWHI whales had slightly higher proportions of hexabrominated PBDEs (Figure S2).  
290 Heavier and recalcitrant (e.g., resistant to metabolism) congeners dominated profiles:  
291 hexachlorinated (e.g., PCBs 138, 153) and heptachlorinated (e.g., PCBs 180, 187) PCB  
292 congeners accounted for approximately 50% and 27% of  $\Sigma$ PCBs, respectively (Figure S2).  
293 Tetrabrominated (e.g., PBDEs 47, 66), pentabrominated (e.g., PBDEs 85, 99), and  
294 hexabrominated (e.g., PBDEs 153, 154) PBDEs accounted for 56%, 29%, and 14% of  $\Sigma$ PBDEs,  
295 respectively (Figure S2). Trichlorinated (i.e., PCBs 17, 18, 28, 31, 33, 44), tetrachlorinated (e.g.,  
296 PCBs 49, 52, 66), nonachlorinated (i.e., PCBs 206, 208), and decachlorinated (i.e., PCB 209)  
297 PCBs and tribrominated (i.e., PBDE 28) PBDE accounted for less than 2% of congener profiles  
298 (Figure S3, S4). Tetrachlorinated PCBs (e.g., PCBs 29, 52, 66) contributed approximately 3%

299 and pentachlorinated PCBs (e.g., PCBs 82, 87, 95, 99) 17% to total PCBs (Figure S2). Mean  
300 POP concentrations were not significantly different ( $p > 0.05$ ) among the three whale  
301 populations for any of the POP classes measured.

302 Mean  $\Sigma$ PCB concentrations for all populations exceed both thresholds for adverse health effects  
303 proposed by Kannan et al. (2000) and Schwacke et al. (2002), although inferences on NWHI and  
304 pelagic populations as a whole are limited due to sample size (Table S1). For the MHI stock,  
305 68% of individuals (28 of 41 individuals; 30 of 45 (67%) samples) exceeded the 17,000 ng g<sup>-1</sup>  
306  $\Sigma$ PCBs threshold and 71% of individuals (29 of 41 individuals; 31 of 45 (69%) of samples)  
307 exceed the 14,700 ng g<sup>-1</sup>  $\Sigma$ PCBs threshold (Kannan et al. 2000; Schwacke et al. 2002). Of the 4  
308 individuals sampled twice during the study period, two had  $\Sigma$ PCBs levels exceeding health  
309 thresholds (HIPc102, adult male; HIPc282, sub-adult female) and two had levels under health  
310 thresholds (HIPc116 and HIPc212, adult females) for both pairs of biopsies obtained over the  
311 study period (i.e., no changes in exceeding thresholds). Levels of  $\Sigma$ PCBs in 100% of adult males  
312 (11 individuals), 55% of adult females (12 of 22 individuals), and 63% of juvenile/subadults (5  
313 of 8 individuals) belonging to the MHI population were greater than or equal to both of those  
314 thresholds (Figure 2) (Kannan et al. 2000; Schwacke et al. 2002).

315 The influence of sex and age class on POP concentrations for false killer whales from the MHI  
316 population was examined as it was the only population that had sufficient numbers of whales  
317 represented by the three age/sex categories (i.e., adult male, adult female, juvenile/subadult).

318 Contaminant data for both male and female juvenile/subadult whales were combined as no  
319 significant differences in mean concentrations of POPs were found between sexes ( $\Sigma$ PCBs,  $p =$   
320 0.8286;  $\Sigma$ DDTs,  $p = 0.7524$ ;  $\Sigma$ CHLDs,  $p = 0.7523$ ;  $\Sigma$ PBDEs,  $p = 0.4842$ ;  $\Sigma$ HCHs,  $p = 0.4657$ ;  
321 HCB,  $p = 0.8169$ ; dieldrin,  $p = 0.8514$ ; mirex,  $p = 0.9883$ ). Significant differences in mean

322 concentrations of  $\Sigma$ PCBs ( $p = 0.0111$ ),  $\Sigma$ DDTs ( $p = 0.0032$ ), and  $\Sigma$ CHLDs ( $p = 0.0131$ ) were  
323 found between adult males and adult females, with males having higher concentrations for all  
324 three (Figure 3). Mean concentrations of HCB ( $p = 0.0485$ ),  $\Sigma$ PBDEs ( $p = 0.0063$ ),  $\Sigma$ HCHs ( $p =$   
325  $0.0249$ ), and dieldrin ( $p = 0.0334$ ) were significantly different between juvenile/subadult whales  
326 and adult females, with higher levels in juveniles/subadults for all three (Figure 3). A significant  
327 difference in mean levels of sum DDTs ( $p = 0.0391$ ) was found between juvenile/subadult  
328 whales and adult males, with adult males having higher levels. Although the mean  
329 concentrations of  $\Sigma$ PBDEs were elevated in juvenile/subadult whales compared to adult male  
330 false killer whales (Figure 3), this difference was not significant ( $p = 0.1781$ ). No other  
331 significant differences in mean percent lipid or POP concentrations were found among the  
332 age/sex classes. Proportions of PCB and PBDE congeners by homolog group were similar  
333 among age/sex/reproductive classes and mirror profiles for all false killer whale populations  
334 (Figure S2, S3, S4). For instance, the predominant congeners among these groups were hexa-  
335 and heptachlorinated PCBs and tetra- and pentabrominated PBDEs (Figure S2). However, parous  
336 adult females had slightly higher proportions of octachlorinated PCBs and hexabrominated  
337 PBDEs (Figure S2).

338 Taking reproductive status (nulliparous, parous, unknown) of adult females from the MHI  
339 population into account, those known to have had at least one calf (i.e., parous) had significantly  
340 (all  $p$ 's  $< 0.05$ ) lower levels of all POP classes than adult females that had never been observed  
341 with a calf (i.e., nulliparous) (Figure 4). There were several adult females in our dataset that we  
342 were unable to confidently determine reproductive status due to limited sighting histories. A  
343 number of these individuals had concentrations of  $\Sigma$ PCBs and  $\Sigma$ DDTs that were comparable to  
344 nulliparous adult females (Figure 4). Additionally, there were four mother/offspring pairs in our



345 dataset allowing us to examine maternal offloading relationships (Figure S1). As expected,  
346 mothers had much lower levels of most contaminants, including  $\Sigma$ PCBs,  $\Sigma$ DDTs,  $\Sigma$ CHLDs,  
347  $\Sigma$ PBDEs, and mirex, than their offspring (Figure S1).

348 Of highlighted concern were the alarmingly high POP levels measured in blubber samples of  
349 stranded false killer whales. Among the four stranded individuals, the lowest  $\Sigma$ PCB  
350 concentration measured was 43,000 ng g<sup>-1</sup> (lipid wt.), more than twice the highest suggested  
351 health threshold for PCBs (Kannan et al. 2000), and highest was 110,000 ng g<sup>-1</sup> (lipid wt.).  
352  $\Sigma$ DDTs concentrations ranged from 58,000 ng g<sup>-1</sup> to 180,000 ng g<sup>-1</sup> (lipid wt.). Bachman et al.  
353 (2014) also reported POP concentrations from a stranded Hawaiian false killer whale however its  
354 levels were lower than those reported in the current study (Table 2). Lailson-Brito et al. (2012)  
355 reported POP concentrations for a single stranded false killer whale from the southeastern  
356 Brazilian coast that had levels similar to our findings ( $\Sigma$ PCBs: 63,700 ng g<sup>-1</sup>;  $\Sigma$ DDTs: 17,900 ng  
357 g<sup>-1</sup>). POP concentrations in these stranded false killer whales were among the highest compared  
358 to what has been previously reported on other odontocetes found in Hawaiian waters and regions  
359 throughout the Eastern North Pacific (Table 2). Although the influence of POP exposure on these  
360 individuals' deaths cannot be confidently resolved, these whales had the highest POP levels  
361 among all individuals in our dataset suggesting that some associated negative health effects may  
362 have been at play.

### 363 **3.3 Multivariate analyses**

364 Principal components analysis generated three factors that summarized the most variance in the  
365 dataset (Table 3). Factor 1 explained 42% of the variance and had high loadings for  $\Sigma$ PCBs,  
366  $\Sigma$ DDTs,  $\Sigma$ CHLDs, and mirex. Mixed effects model outputs (Figure 5; Table S2) showed a  
367 statistical increase in factor 1 estimates for adult males (estimate, 0.92; p = 0.008) and animals

368 within Cluster 3 (estimate, 0.94;  $p = 0.030$ ), and a statistical decrease for parous adult females  
369 (estimate, -0.83;  $p = 0.048$ ). The second factor explained 33% of the variance, with high loadings  
370 for  $\Sigma$ HCHs,  $\Sigma$ HCB, and dieldrin. The mixed model for this factor showed an increase in  
371 estimates for animals within Cluster 4, albeit not significantly (estimate, 0.91;  $p = 0.052$ ). Factor  
372 3 accounted for 18% of the variance and was highly loaded for  $\Sigma$ PBDEs. The mixed model for  
373 this factor showed a statistical decrease in factor 3 estimates for parous adult females (estimate, -  
374 1.25;  $p = 0.019$ ), adult males (estimate, -1.02;  $p = 0.018$ ), and for animals within Cluster 2  
375 (estimate, -0.87;  $p = 0.033$ ). Recall estimates for age/sex/reproductive status class were relative  
376 to nulliparous adult females and estimates for social clusters were relative to Cluster 1.

### 377 **3.4 POP ratios**

378 Ratios of  $\Sigma$ DDTs/ $\Sigma$ PCBs and  $\Sigma$ PBDEs/ $\Sigma$ PCBs among false killer whale populations and MHI  
379 social clusters are shown in Figure (6). The MHI population had a statistically significant greater  
380 mean  $\Sigma$ DDTs/ $\Sigma$ PCBs ( $1.20 \pm 0.42$ ) ratio than NWHI false killer whales ( $0.83 \pm 0.16$ ;  $p =$   
381  $0.0254$ ). We found no apparent or statistically significant difference in average ratios of  
382  $\Sigma$ DDTs/ $\Sigma$ PCBs between MHI and pelagic populations, although the small number of whales  
383 sampled from the pelagic population may have reduced our ability to find significant differences  
384 in POP ratios. The NWHI stock had a lower mean ratio of  $\Sigma$ DDTs/ $\Sigma$ PCBs ( $0.83 \pm 0.16$ )  
385 compared to the pelagic stock ( $1.10 \pm 0.17$ ), albeit not significantly. No significant differences  
386 were found in mean ratios of  $\Sigma$ PBDEs/ $\Sigma$ PCBs between populations, although the MHI stock had  
387 greater variation among individuals (Figure 6B). No statistically significant differences were  
388 found in mean ratios of  $\Sigma$ DDTs/ $\Sigma$ PCBs or  $\Sigma$ PBDEs/ $\Sigma$ PCBs among MHI social clusters, although  
389 ratios appear to vary within clusters to some extent (Figure 6C, D).

### 390 **3.5 Stable isotopes**

391 Mean carbon and nitrogen values among MHI social clusters were generally similar, ranging  
392 from -16.9 to -15.4 and 11.1 to 13.1, respectively (Figure 7). As previously noted, stable isotope  
393 values were not available for individuals within Cluster 3 (Table 1). Cluster 1 had significantly  
394 lower  $\delta^{13}\text{C}$  values than Cluster 2 ( $p = 0.02081$ ), although it should be noted the scale of  
395 difference is small (Figure 7). No significant differences were found in  $\delta^{15}\text{N}$  levels among all  
396 social clusters although Clusters 1 and 2 had slightly higher values for that isotope (Figure 7).  
397 Provided the variation in isotope values within social clusters (Figure 7), we further investigated  
398 differences between age/sex classes. This sub-analysis was restricted to individuals within  
399 Cluster 1 as it had the largest sample size and the most representatives from each age/sex class.  
400 Carbon and nitrogen stable isotopes were generally similar among age/sex classes within Cluster  
401 1, although there was some variation among those groups (Table S3).

402

403

#### 4. DISCUSSION

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

414

## 4.1 POPs

415

### *4.1.1 Influence of life history characteristics on POPs*

416 The trends in POP concentrations among age/sex class for MHI false killer whales in this study  
417 follow what was speculated in Ylitalo et al. (2009) and are comparable to those previously  
418 published on killer whales (Herman et al. 2005; Krahn et al. 2009; Ylitalo et al. 2001). As seen in  
419 Figure (3), there is quite a bit of variation in POP concentrations within age/sex classes.

420 Variability in POP levels among adult males in our study may be caused by several factors,  
421 including age and birth order (Ross et al. 2000; Ylitalo et al. 2001). For instance, we would  
422 expect older adult males to have higher levels than their younger counterparts, and first-born  
423 male offspring to have particularly high levels. Future studies that refine individual age estimates  
424 (e.g., through epigenetic aging) may aid in understanding this variation.

425 Variation in POPs measured in adult females is likely driven by reproductive status (Figure 4).  
426 MHI adult females known to have at least one calf prior to biopsy collection had among the  
427 lowest POP levels among all biopsies analyzed in this study as a result of maternal offloading.  
428 Consequently, POP levels measured in offspring surpassed or were close to both suggested  
429  $\Sigma$ PCBs thresholds for negative health effects (Figure S1) (Kannan et al. 2000; Schwacke et al.  
430 2002). Of the 24 adult females (MHI), eight whales have never been reported to give birth  
431 (Cascadia Research Collective 2019). A majority of these nulliparous females are characterized  
432 by extremely high POP concentrations; whether these individuals are reproductively impaired  
433 due to POP exposure or have simply have yet to reproduce is unknown. We were unable to  
434 determine the reproductive status of nine adult females from the MHI stock due to sparse  
435 sighting histories (Cascadia Research Collective 2019). As mentioned previously, future studies  
436 using estimated age may help determine if these females are younger and reproductively active

437 or older and reproductively senescent.

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

#### 450 *4.1.2 POPs among MHI social clusters*

451 Results from PCA followed by LMMs indicate that, while controlling for life history drivers of  
452 POP variance (i.e., age, sex, reproductive status), POP classes and concentrations vary by MHI  
453 social cluster. The LMM for PC factor 1 (Table S2, Figure 5) showed that relative to Cluster 1,  
454 Cluster 3 had a significantly positive correlation with contaminants highly loaded on factor 1  
455 ( $\Sigma$ PCBs,  $\Sigma$ DDTs,  $\Sigma$ CHLDs, mirex; Table 3). This could reflect regional differences in POP  
456 exposure or contamination in prey items, although we might have expected Clusters 1 and 3 to  
457 have comparable POP concentrations as they have similar high-density areas (Baird et al. 2019).  
458 In addition, only four blubber samples were obtained from whales in Cluster 3, in which three  
459 were from stranded whales (Table 1). In the current study, stranded whales had the highest POP

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

466 The LMM for factor 2 showed that Cluster 4 was positively correlated with contaminants highly  
467 loaded on this factor ( $\Sigma$ HCHs, HCB, dieldrin; Table 3) relative to Cluster 1, albeit not  
468 significantly ( $p = 0.052$ ; Figure 5). For example, Cluster 4 false killer whales generally had  
469 higher levels of  $\Sigma$ HCHs ( $140 \text{ ng g}^{-1}$  lipid wt.), HCB ( $290 \text{ ng g}^{-1}$  lipid wt.), and dieldrin ( $120 \text{ ng g}^{-1}$   
470 lipid wt.) relative to Cluster 1 ( $72, 120, \text{ and } 55 \text{ ng g}^{-1}$  lipid wt., respectively). Previous studies  
471 suggest high density areas for Cluster 4 are off eastern O‘ahu, Moloka‘i, Lāna‘i, Kaho‘olawe,  
472 and western Maui (Baird et al. 2019). Animal husbandry, sugarcane, and pineapple plantations  
473 are the primary land uses in these regions, which could be associated with high levels of  
474 agricultural pesticides measured in Cluster 4 false killer whales (State of Hawai‘i 2015).

475 PC factor 3 was highly loaded for  $\Sigma$ PBDEs (Table 3) and the LMM for this factor showed parous  
476 adult females, adult males, and Cluster 2 false killer whales were negatively correlated with  
477  $\Sigma$ PBDEs relative to nulliparous adult females and Cluster 1, respectively. We expected to find  
478 parous adult females and adult males with lower levels of this POP class, as previous findings  
479 showed juveniles and nulliparous adult females generally have higher concentrations of  $\Sigma$ PBDEs  
480 (Figure 3). It was interesting to find lower  $\Sigma$ PBDE levels in Cluster 2 false killer whales, as their  
481 high density area overlaps part of those of Clusters 1 and 3 (mostly limited to northwestern  
482 Hawai‘i and southeast Maui) (Baird et al. 2019). PBDEs are used for industrial purposes, such as

483 flame retardants and used in manufacturing furniture and plastics (EPA 2017), such that we  
484 would expect to see higher PBDE exposure near more urbanized regions. While there are  
485 urbanized areas near Cluster 2's range, these regions are generally less urbanized compared to  
486 other counties throughout the state (State of Hawai'i 2013). Our results may reflect the more  
487 remote habitat use or foraging locations of Cluster 2 relative to other social clusters.

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

#### 494 **4.2 POP ratios**

495 We compared POP ratios  $\Sigma$ DDTs/ $\Sigma$ PCBs and  $\Sigma$ PBDEs/ $\Sigma$ PCBs among false killer whale  
496 populations and MHI social clusters that may be characteristic of agricultural or urban  
497 "signatures" associated with foraging locations. An unexpected finding was the significant  
498 difference in mean  $\Sigma$ DDTs/ $\Sigma$ PCBs ratios between MHI and NWHI false killer whales, with the  
499 former having an elevated mean ratio (Figure 6). Because the Northwestern Hawaiian Islands are  
500 relatively remote compared to the main Hawaiian Islands, we would expect MHI false killer  
501 whales to have greater  $\Sigma$ PCBs concentrations relative to  $\Sigma$ DDTs compared to NWHI false killer  
502 whales, as PCBs are more often associated with urban environments. A study on endangered  
503 Hawaiian monk seals (*Monachus schauinslandi*) also found elevated levels of  $\Sigma$ PCBs in seals  
504 from Midway (located in Northwestern Hawaiian region) compared to those found on the main  
505 Hawaiian Islands (Lopez et al. 2012). It was speculated that PCBs became pervasive in the

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

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

### 525 **4.3 Stable isotopes**

526 As noted previously, stable isotope analysis of epidermis can be used to provide insight into  
527 trophic positions and geographic locations where marine mammals forage (Kelley, 2000).

528 Cluster 1 had significantly lower mean levels of  $\delta^{13}\text{C}$  than Cluster 2, suggesting that Cluster 1



529 whales may forage farther offshore than Cluster 2 whales. While there were no statistically  
530 significant findings in mean  $\delta^{13}\text{C}$  values among the other clusters, Figure (7) suggests that  
531 Clusters 4 and 5 may feed in more inshore regions compared to Cluster 1, although the  
532 difference was small. Average stable isotope  $\delta^{15}\text{N}$  levels were not significantly different among  
533 MHI social groups, so it appears these groups consume prey at similar trophic positions. Main  
534 Hawaiian Islands insular false killer whales are known to eat a variety of pelagic and reef-  
535 associated game fish (Baird 2016), such as yellowfin tuna (*Thunnus albacares*), mahimahi,  
536 (*Coryphaena hippurus*), several species of jack (*Caranx sp.*), and some squid species have been  
537 documented in the stomach contents of stranded individuals (West et al. unpublished data).  
538 Variation in diet by age class or sex has not been assessed based on observational studies,  
539 although whales frequently share prey (Baird 2016), so such variation may be difficult to assess.  
540 Compared to stable isotope findings from false killer whales from the southwestern Atlantic  
541 (Riccialdelli et al. 2010; Bisi et al. 2013; Botta et al. 2012), our stable isotope values were  
542 generally lower in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . These differences in stable isotope values among false  
543 killer whale studies may be due to variations in diets of the whales from different regions, and/or  
544 differences in isotopic baseline values for various oceanic basins that these whales inhabit  
545 (Graham et al. 2010). Other factors such as different tissues analyzed or tissue preparation  
546 protocols (lipid extracted versus non-lipid extracted sample preparation, Ryan et al. 2012) may  
547 also contribute to differences in these stable isotope ratios. Some of these studies used  
548 bone/dentition samples from stranded false killer whales that may be less susceptible to variation  
549 associated with time or environmental conditions compared to blubber/skin biopsies (Walker and  
550 Macko, 1999; Kelley, 2000). For instance, complete turnover of skin cells was estimated to be  
551 around 73 days for a similar species (Hicks et al. 1985), suggesting isotope values reflect their

552 diet during 1-2 months prior to sampling. In addition, the scale of measurements at which  
553 comparisons in the current study were made is small (Figure 7), such that identifying trophic  
554 discrimination would be challenging; it is very possible that statistical findings are an artifact of  
555 inherent variability in stable isotope values. Therefore, we exercise caution in our biological  
556 interpretation of these statistical findings and consider these differences as an initial exploration  
557 of the possibility for seasonal variation in prey items or foraging locations among social groups.

558

559

## 5. CONCLUSIONS

560 Concentrations of POPs measured in false killer whales from all three populations found in  
561 Hawaiian waters rank among the highest documented in Eastern North Pacific odontocetes  
562 (Table 2). NWHI and pelagic false killer whales are not as well-studied as the MHI stock,  
563 however, high levels of POPs measured in these whales raise concern regarding the conservation  
564 status of these populations. Importantly, this study provides evidence that POPs continue to be a  
565 relevant and pressing risk to the endangered MHI false killer whales, with juveniles/sub-adults at  
566 greater risk of adverse health effects linked to their exposure. Monitoring MHI false killer  
567 whales' health (e.g., respiratory microbiome, body condition) is essential in elucidating the threat  
568 of POP exposure in these animals. For example, respiratory microbiome analysis of breath  
569 samples from free-ranging cetaceans has been a recent advancement in monitoring health and  
570 disease for several species, including humpback whales (*Megaptera novaeangliae*) (Apprill et al.  
571 2017; Acevedo-Whitehouse, Rocha-Gosselin, and Gendron, 2009), killer whales (Raverty et al.  
572 2017), Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Nelson et al. 2019), and a number  
573 of Hawaiian odontocetes, including 2 false killer whales (Lerma et al. 2019). In addition, a  
574 number of studies have assessed individual body condition to gain insight into population health,

575 using photographs (Bradford et al. 2012) and, more recently, photogrammetry (Durban et al.  
576 2016; Fearnbach et al. 2018). Periods of nutritional stress may accelerate deleterious health  
577 effects associated with POPs (e.g., compromised immune system, reproductive failure) as has  
578 been speculated of the critically endangered Southern Resident killer whales in Washington  
579 State/British Columbia (Lundin et al. 2016; Wasser et al. 2017).

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

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

593

594

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1069

#### FIGURE CAPTIONS

1070 **Figure 1.** Distribution of total POP concentrations (ng g<sup>-1</sup> lipid wt.) ΣDDTs and ΣPCBs (A), ΣCHLDs,  
1071 mirex and ΣPBDEs (B) and dieldrin, HCB and ΣHCHs (C) measured in blubber of false killer whales  
1072 from main Hawaiian Islands (MHI), Northwestern Hawaiian Islands (NWHI), and pelagic (PEL)  
1073 populations. Black dots represent outliers.

1074

1075 **Figure 2.** Sum PCBs concentrations ( $\text{ng g}^{-1}$  lipid wt.) measured in main Hawaiian Islands false killer  
1076 whales by age/sex class. Solid horizontal line represents the threshold for thyroid and immune system  
1077 dysfunction in aquatic mammals suggested by Kannan et al. (2000) and dashed line represents the  
1078 threshold for maternal failure in bottlenose dolphins proposed by Schwacke et al. (2002). Black dots  
1079 represent outliers. \*Male and female juveniles/sub-adults were combined as no significant differences in  
1080 concentrations for any POP class were found.

1081

1082 **Figure 3.** Distribution of total POP concentrations ( $\text{ng g}^{-1}$  lipid wt.)  $\Sigma$ DDTs and  $\Sigma$ PCBs (A),  $\Sigma$ CHLDS,  
1083 mirex and  $\Sigma$ PBDEs (B) and dieldrin, HCB and  $\Sigma$ HCHs (C) by age/sex class in main Hawaiian Islands  
1084 false killer whales. Black dots represent outliers. Male and female juveniles/sub-adults were combined as  
1085 no significant differences in concentrations for any POP class were found.

1086

1087 **Figure 4.** Distribution of concentrations ( $\text{ng g}^{-1}$  lipid wt.) of sum PCBs (A), DDTs (B) and PBDEs (C)  
1088 measured in main Hawaiian Islands adult females by reproductive status (nulliparous = has never given  
1089 birth; parous = given birth to at least one calf; unknown = insufficient sighting history). Black dots  
1090 represent outliers.

1091

1092 **Figure 5.** Linear mixed model estimates (coefficients) for each retained principal component (PC) factor  
1093 generated from principal components analysis on main Hawaiian Islands false killer whales. Model  
1094 estimates for social cluster are relative to Cluster 1 and estimates for age/sex/reproductive class are  
1095 relative to nulliparous adult females.

1096

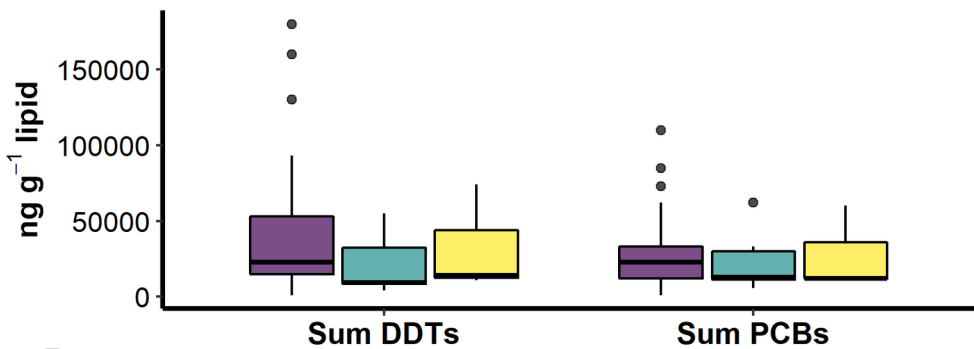
1097 **Figure 6.** Ratios of sum DDTs/PCBs and sum PBDEs/PCBs by false killer whale population (A, B) and  
1098 main Hawaiian Islands (MHI) social cluster (C, D). Black dots represent outliers. NWHI = Northwestern  
1099 Hawaiian Islands.

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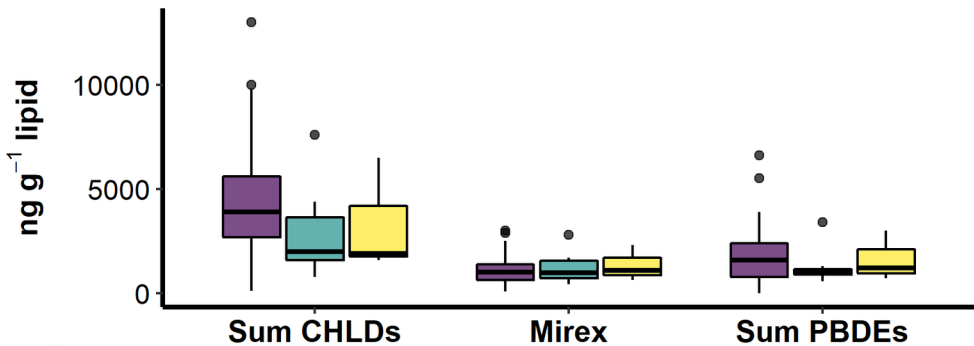
1101 **Figure 7.** Mean  $\pm$  standard error values for carbon and nitrogen stable isotopes by main Hawaiian Islands  
1102 (MHI) false killer whale social cluster.

1103

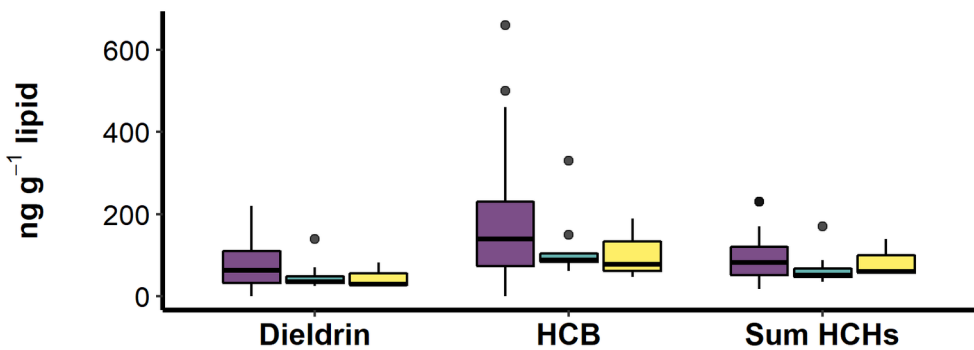
**A**  *MHI*  *NWHI*  *PEL*



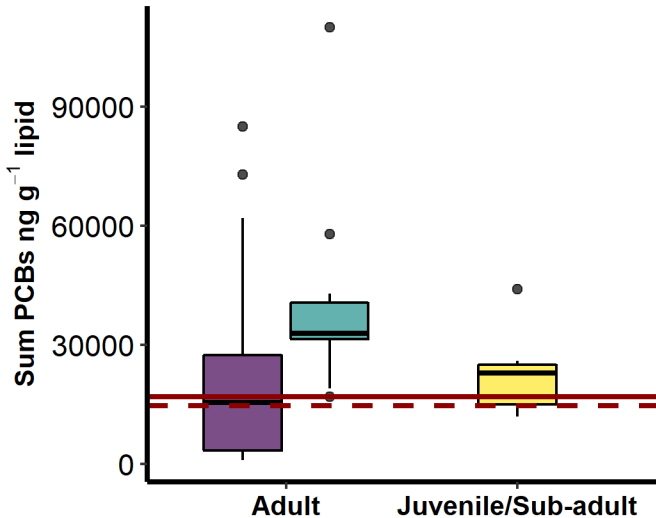
**B**



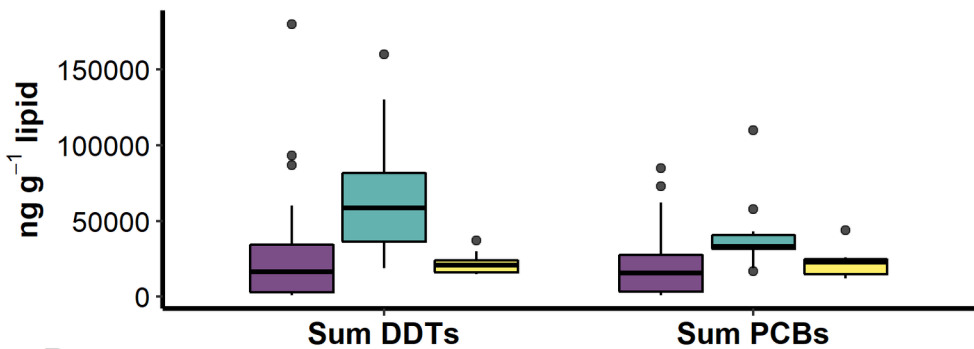
**C**



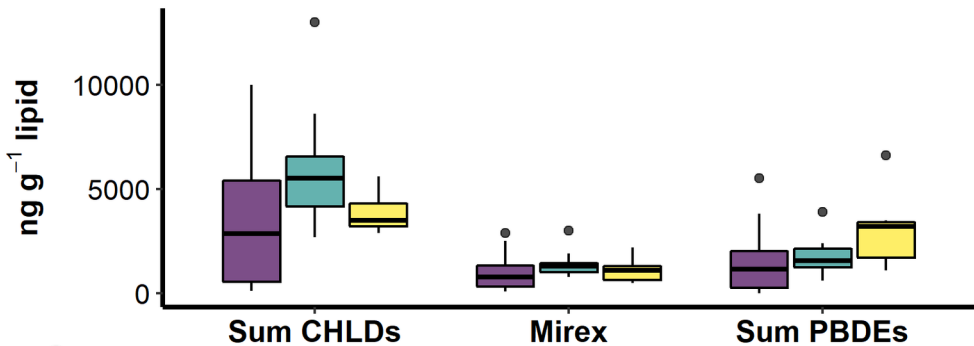
Female Male Combined\*



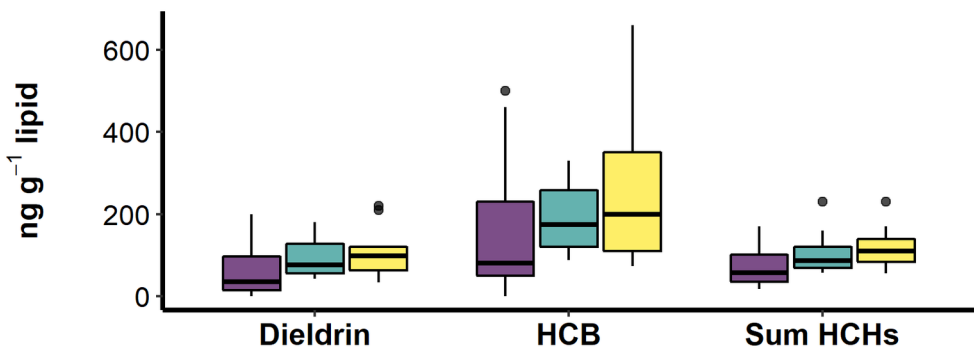
**A** ■ *Adult F* ■ *Adult M* ■ *SubAd/Juv*



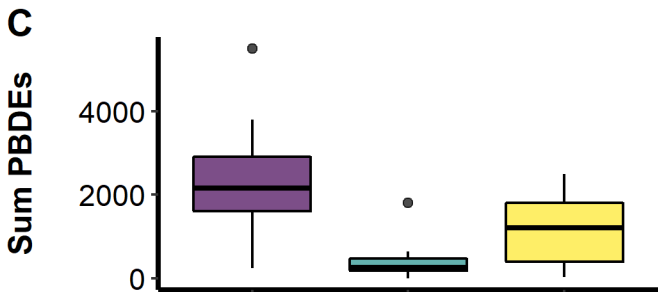
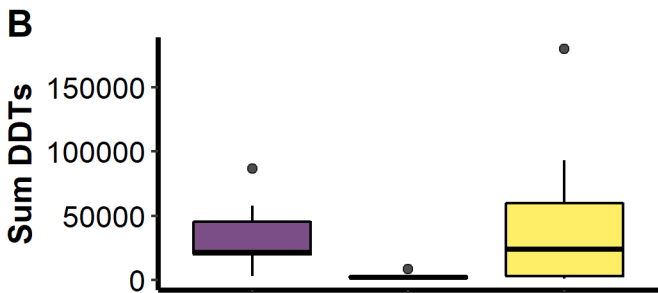
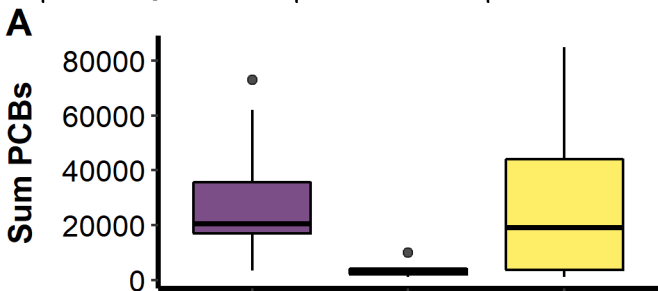
**B**



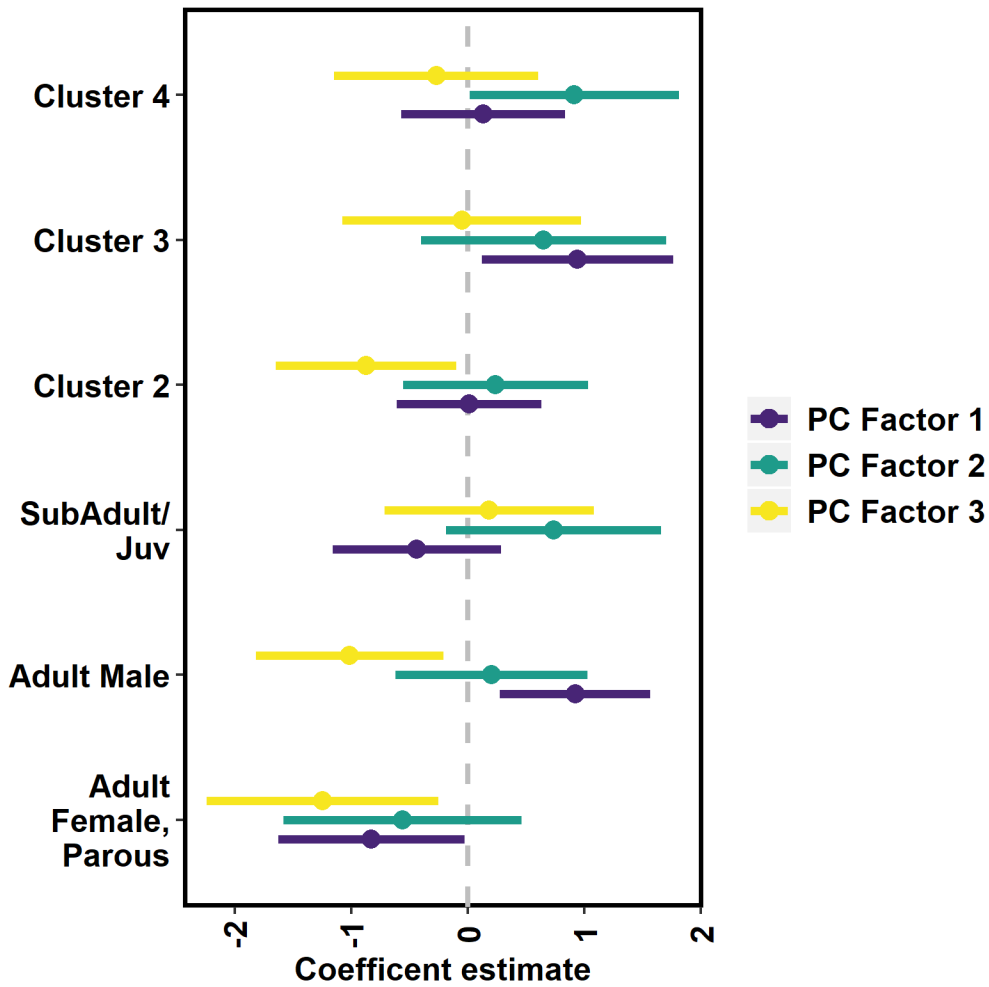
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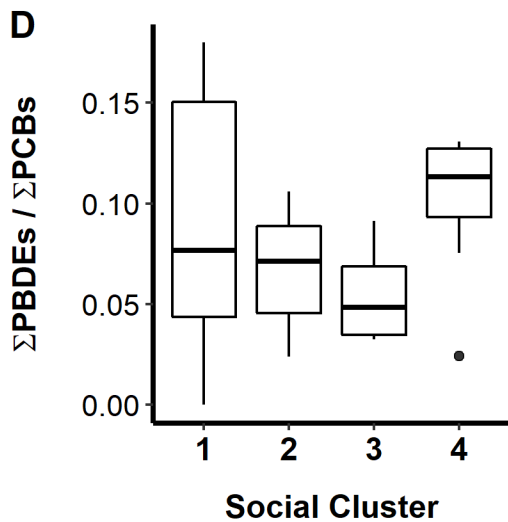
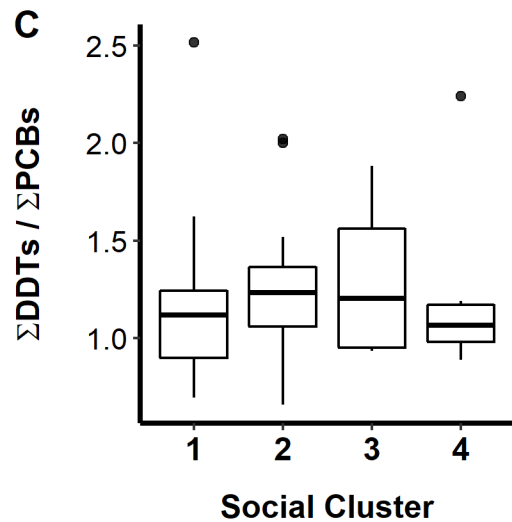
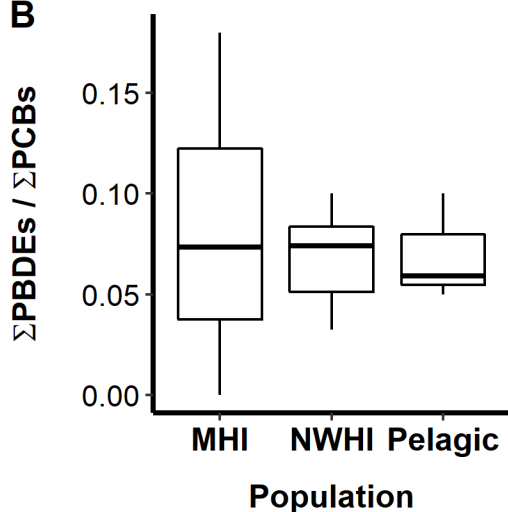
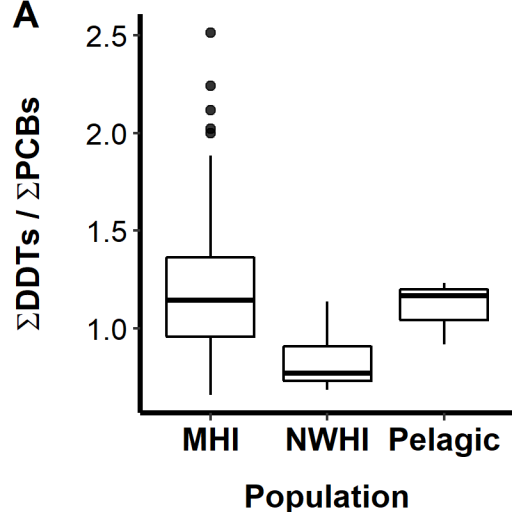


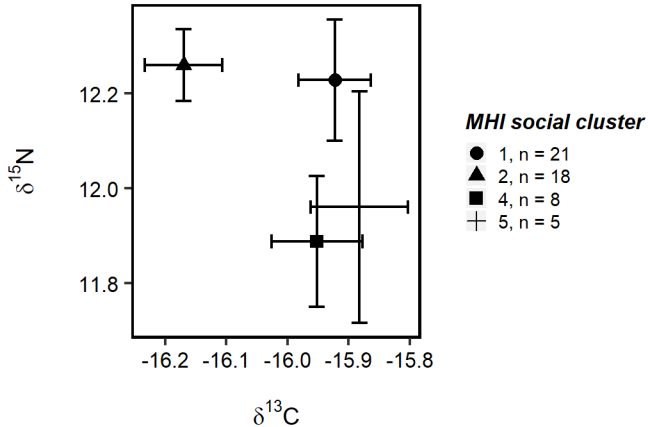
Nulliparous Parous Unknown











**Table 1.**

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

<b>Animal ID</b>	<b>Sample collection date</b>	<b>Population</b>	<b>Age class</b>	<b>Sex</b>	<b>Social cluster</b>	<b>POPs</b>	<b>Stable isotopes</b>
HIPc101	12/19/2009	MHI	Adult	Female <sup>2</sup>	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 <sup>1</sup>	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 <sup>2</sup>	1	x	x
HIPc116	8/25/2011	MHI	Adult	Female <sup>3</sup>	1	x	x
HIPc117	7/16/2008	MHI	Adult	Female <sup>2</sup>	1	x	x
HIPc120	7/16/2008	MHI	Adult	Female <sup>3,a</sup>	1	x	
HIPc120	8/25/2011	MHI	Adult	Female <sup>4</sup>	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 <sup>1</sup>	3	x	
*HIPc198	11/7/2015	MHI	Adult	Female <sup>1</sup>	3	x	
HIPc203	8/11/2010	MHI	Adult	Female <sup>1</sup>	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 <sup>3,b</sup>	1	x	x

HIPc212	7/28/2010	MHI	Adult	Female <sup>3,b</sup>	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 <sup>4</sup>	2		x
HIPc230	8/20/2011	MHI	Adult	Female <sup>2,c</sup>	2	x	x
HIPc233	8/20/2011	MHI	Adult	Female <sup>4</sup>	2		x
HIPc266	12/19/2009	MHI	Adult	Female <sup>1</sup>	4	x	x
HIPc282	7/26/2008	MHI	Sub-adult	Female <sup>b</sup>	1	x	
HIPc282	7/28/2010	MHI	Sub-adult	Female <sup>b</sup>	1	x	x
HIPc310	8/11/2010	MHI	Adult	Female <sup>4</sup>	1		x
HIPc312	7/26/2008	MHI	Juvenile	Female <sup>a</sup>	1	x	
HIPc313	7/16/2008	MHI	Adult	Female <sup>3</sup>	1	x	
HIPc316	8/25/2011	MHI	Adult	Female <sup>3</sup>	1	x	x
HIPc320	12/18/2009	MHI	Sub-adult	Male	1	x	x
HIPc338	8/20/2011	MHI	Adult	Female <sup>4</sup>	2		x
HIPc339	8/20/2011	MHI	Adult	Female <sup>1</sup>	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 <sup>4</sup>	5		x
HIPc366	12/10/2009	MHI	Adult	Male	5		x
HIPc367	12/10/2009	MHI	Juvenile	Female <sup>4</sup>	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 <sup>4</sup>	2		x
HIPc381	8/20/2011	MHI	Adult	Female <sup>2</sup>	2	x	x
HIPc382	8/20/2011	MHI	Adult	Female <sup>3,c</sup>	2	x	x
HIPc383	8/20/2011	MHI	Adult	Female <sup>2</sup>	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 <sup>1</sup>	2	x	x
HIPc392	8/14/2010	MHI	Sub-adult	Male	2	x	x
HIPc396	8/14/2010	MHI	Adult	Female <sup>2</sup>	2	x	x
HIPc397	8/20/2011	MHI	Adult	Female <sup>2</sup>	2	x	x
HIPc398	8/20/2011	MHI	Adult	Female <sup>1</sup>	2	x	x
*HIPc700	11/21/2016	MHI	Adult	Female <sup>2</sup>	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 <sup>2</sup>	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 <sup>2</sup>	NA	x	
HIPc291	4/21/2008	PEL	Adult	Female <sup>2</sup>	NA	x	
HIPc292	4/21/2008	PEL	Adult	Female <sup>2</sup>	NA	x	
HIPc850	4/21/2008	PEL	Adult	Male	NA	x	

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<sup>1</sup> Nulliparous (never observed with a calf)

<sup>2</sup> Infrequent sighting data; association with a calf is unknown

<sup>3</sup> Parous (observed with a calf pre-biopsy)

<sup>4</sup> Stable isotope analyses only; reproductive status not considered

\*Stranded

<sup>a,b,c</sup> 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 <sup>-1</sup> , lipid weight		
					∑DDTs	∑PCBs	∑PBDEs
Killer whale <sup>1</sup>	Southern Resident	Puget Sound/BC	2004-2013	78	53,000 ± 50,000	36,000 ± 30,000	4700 ± 3400
Killer whale (AM) <sup>2</sup>	Resident, Transient, Offshore	Alaska	2001-2003	14	25,000 ± 2800	15,000 ± 1700	NA
Killer whale (JM) <sup>3*</sup>	UK	Hawai'i	2008	1	171,000	93,200	938
Melon-headed whale <sup>3*</sup>	UK	Hawai'i	2010-2011	4	31,000 ± 45,000	15,600 ± 23,300	409 ± 432
Striped dolphin <sup>3*</sup>	UK	Hawai'i	1997-2010	6	20,000 ± 5,510	13,800 ± 7,260	258 ± 139
Bottlenose dolphin <sup>3*</sup>	UK	Hawai'i	2009, 2011	3	15,000 ± 7,700	11,800 ± 7,340	1070 ± 172
		British					
False killer whale (AM) <sup>4*</sup>	UK	Columbia	1987, 1989	2	990,000 ± 1,300,000	40,000 ± 8500	NA
False killer whale <sup>3*</sup>	MHI	Hawai'i	2010	1	28,200	26,200	1,650
False killer whale <sup>6*</sup>	MHI	Hawai'i	2013-2016	4	120,000 ± 59,000	75,000 ± 29,000	2,900 ± 1,200

False killer whale <sup>5,6</sup>	MHI, NWHI, PEL	Hawai'i	2008-2012	52	28,000 ± 28,000	22,000 ± 17,000	1600 ± 1300
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NA = not analyzed

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Population abbreviations:

MHI = main Hawaiian

Islands; NWHI =

Northwestern Hawaiian

Islands; PEL = pelagic

<sup>1</sup>data from Krahn et al. 2007, 2009; unpublished NWFSC

data

<sup>2</sup>data from Herman et al. 2005

<sup>3</sup>data from Bachman et al. 2014

<sup>4</sup>data from Jarman et al. 1996

<sup>5</sup>data from Ylitalo et al.

2009

<sup>6</sup>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.

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

