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Baseline

Persistent organic pollutants in the endangered Hawaiian monk seal (*Monachus schauinslandi*) from the main Hawaiian Islands

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ABSTRACT

Little is known about levels or effects of persistent organic pollutants (POPs) in Hawaiian monk seals (HMS) from the main Hawaiian Islands (MHI) subpopulation. This study examined concentrations of a large suite of POPs in blubber and serum of juvenile and adult HMS from the MHI. Adult females have the lowest blubber levels of most POPs, whereas adult males have highest levels. POPs in serum were significantly different in adult males compared with adult females for chlordanes and summed dichlorodiphenyltrichloroethanes (DDTs). Lipid-normalized concentrations of chlordanes, DDTs, polychlorinated biphenyls, and mirex in paired blubber and serum samples were significantly correlated. Contaminant levels from the MHI were at similar or lower levels than those from remote Northwestern Hawaiian Island populations. Determining initial ranges of POPs is an important step towards assessing one of the many potential health threats to this critically endangered species.

The Hawaiian monk seal (*Monachus schauinslandi*, HMS) is a critically endangered pinniped species endemic to the Hawaiian Archipelago with a current estimated population of 1161 individuals (Carretta et al., 2011). Their numbers are declining at a rate of approximately 4.5% in the Northwestern Hawaiian Islands (NWHI) (Carretta et al., 2011). The Hawaiian monk seals' geographic range includes the entire Hawaiian Archipelago, from Kure Atoll in the northwest to the island of Hawaii in the southeast. Across this range, there are two discrete "sub-ranges": the NWHI between Kure Atoll and Nihoa Island, and the main Hawaiian islands (MHI) between Ni'ihau Island and Hawaii Island (Fig. 1). These regions differ in many aspects related to the biology and conservation of the HMS, including population status, threats, level of research, and associated management actions (Baker et al., 2011). The majority of the population (approximately 900 individuals) inhabits the NWHI, where abundance is declining (Carretta et al., 2011). This decline can be attributed to multiple factors, including food limitation, entanglement in marine debris, shark predation, and intraspecific aggression (National Marine Fisheries Service, 2007). A smaller, but growing subpopulation of approximately 150–200 individuals is found in the MHI (Baker et al., 2011). This population has higher survival rates (particularly for juvenile seals) and possibly higher reproductive rates compared with NWHI populations, and the MHI population is estimated to be increasing by approximately 6.5% per year (Baker et al., 2011). Because of the comparative success of this subpopulation, the MHI represents an essential high-quality habitat for HMS recruitment and survivorship.

The disparity of population projections between the MHI and NWHI is counterintuitive because the MHI pose more obvious anthropogenic threats to monk seals than the NWHI, including infectious diseases, fisheries interactions, and human disturbances (Baker and Johanos, 2004; Baker et al., 2011; Littnan et al., 2006; NMFS, 2007). Another possible threat to monk seals in the MHI is exposure to persistent organic pollutants (POPs): industrial byproducts and pesticide chemicals that are frequently associated with urban and agricultural regions. These compounds have been detected in the tissues of HMS from Northwestern Hawaiian Island populations (Willcox et al., 2004; Ylitalo et al., 2008) and have been linked to reproductive effects, immune system dysfunction, and cancer in other pinniped species (Beckmen et al., 2003; de Swart et al., 1995; de Long et al., 1973; Hammond et al., 2005; Kim et al., 2002; Ylitalo et al., 2005). The extent of POP accumulation in the tissues of HMS from the MHI is unknown. Quantifying the incidence and magnitude of POPs in HMS in this population, which is considered to be vital to the recovery of the species is

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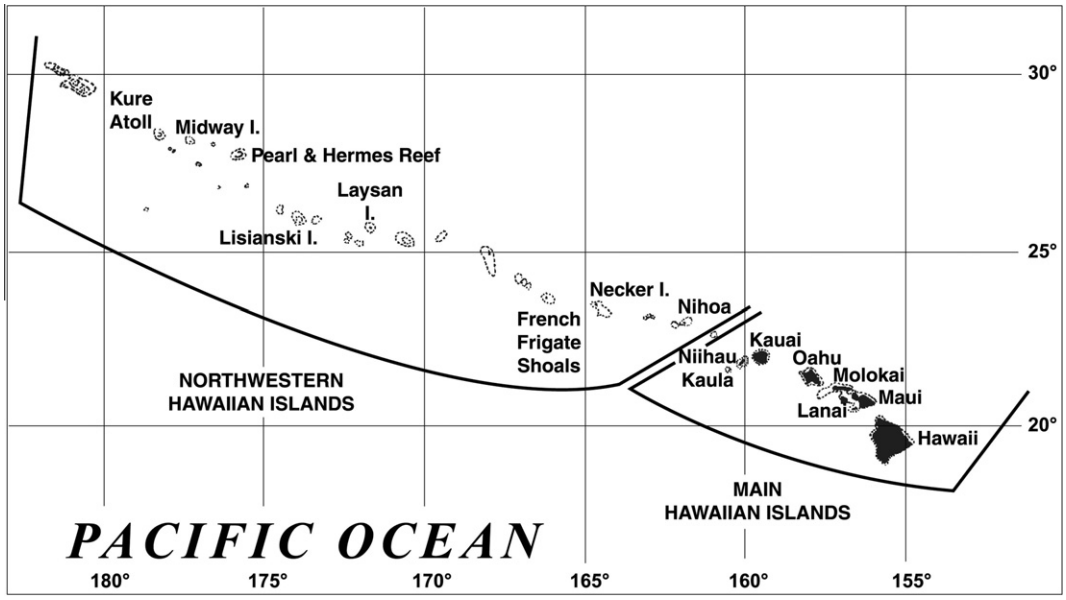

Fig. 1. Map of Hawaiian Archipelago highlighting the 2 sub-ranges of the Hawaiian monk seal: the Northwestern Hawaiian Islands (NWHI) and the main Hawaiian Islands (MHI).

an important conservation priority that was highlighted in the HMS recovery plan (NMFS, 2007).

The MHI have a long history of human activities in comparison to the NWHI, including industrial and agricultural activities resulting in the use and release of POPs into the marine environment (Brasher and Anthony, 2000; Brasher and Wolff, 2004; Oki and Brasher, 2003; Schmitt et al., 1990; Tanita et al., 1976). POPs originate mainly from anthropogenic sources; either from manufactured substances or as chemical byproducts, and are pervasive in the marine environment because they have long half-lives and are resistant to degradation (Bard, 1999). The history of industry and agriculture in Hawaii has involved the use of pesticides such as chlordane, dieldrin, and mirex, and the release of industrial byproducts such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Bevenue et al., 1972; Tanita et al., 1976; Costa and Giordano, 2007). PCBs were used in Hawaii as insulating agents for transformers and were added to manufactured materials such as fuels, paints, adhesives, wood preservatives, and pesticides (Brasher and Wolff, 2004). PBDEs are a class of flame-retardants that have been used in a variety of consumer products. In 2004, two types of PBDE mixtures, the penta-brominateddiphenyl ethers and octa-brominated diphenyl ethers, were banned in Hawaii but are still very pervasive in the environment (Costa and Giordano, 2007). Some POPs can also be transported atmospherically and are likely to have entered the Hawaiian marine environment through remote non-point sources (Bard, 1999; Corsolini et al., 2006; de Wit, 2002; de Wit et al., 2004; Grovhoug, 1992; MacDonald et al., 2000; Montgomery et al., 2009).

Marine mammals acquire POPs through their diet and accumulate them in blubber and other lipid-rich tissues. POP levels can vary across species and individuals by age and sex (Beckmen et al., 1999; Willcox et al., 2004; Ylitalo et al., 2001, 2008), birth order (Beckmen et al., 1999; Ylitalo et al., 2001), or percent lipid content of the tissue (Hall et al., 2008). POPs are known to impact animals in a variety of ways. Exposure to PCBs and organochlorine pesticides (OCPs) in northern fur seals (*Callorhinusursinus*) has been associated with lowered immune response (Beckmen et al., 2003). Captive harbor seals (*Phocavitulina*) fed herring from the heavily polluted Baltic Sea were experimentally determined to have impaired immune function (de Swart et al., 1995). Exposure of California sea lions (*Zalophuscalifornianus*) to OCPs has been correlated with urogenital cancer (Ylitalo et al., 2005) and reproductive impairment (de Long et al., 1973). POP levels have never been identified or quantified in HMS from the MHI, and their effects in this endangered species have not been assessed. Yet, based on studies with other pinniped species, there is the potential for adverse effects of POPs on HMS.

Two previous studies that identified POP levels in the HMS from populations in the NWHI found variability in POP levels associated with age-class and sex (Willcox et al., 2004; Ylitalo et al., 2008). Willcox et al. (2004) assessed POP exposure in seals from the French Frigate Shoals subpopulation and found that adult males had significantly higher PCB levels in blubber samples than reproductive adult females and immature seals of both sexes. Ylitalo et al. (2008) examined contaminants in whole blood and blubber of HMS from 4NWHI populations (French Frigate Shoals, Laysan Island, Pearl and Hermes Reef, and Midway Atoll) and found that adult males and juveniles from Midway Atoll had higher total PCB levels compared with seals of the same age and sex from the 3 other NWHI sites tested. Midway is considered to be the NWHI site with the greatest level of historical human presence and environmental impact. Midway atoll has a long history of human occupation beginning with the Commercial Pacific Cable Company in 1903, and continuing with a large U.S. Navy presence until the site was designated a National Wildlife Refuge managed by the U.S. Fish and Wildlife Service (Speulda-Drews, 2010). It would seem reasonable that seals in the MHI, an area with a longer history of human activity and larger human population than Midway, would have higher contaminant levels than seals from Midway and other NWHI populations.

The objective of the current study was to identify and quantify the levels of a large suite of POPs in blubber and serum samples collected from HMS in the MHI to determine initial contaminant concentration ranges for this critical subpopulation. POP levels were also compared with data from Ylitalo et al. (2008) to assess differences in POP levels from MHI and NWHI subpopulations. This information will facilitate future contaminant monitoring and studies of the effects of these compounds on this critically endangered species.

Blubber and serum samples were collected opportunistically between the years 2000 and 2010 via 1of 2 methods: (1) from live seals during capture for satellite tracking studies, disentanglement, de-hooking or translocation; or (2) during necropsies of seals. For this study, lactating females, nursing pups, molting seals or females that appeared to be pregnant were not handled to avoid potential effects on these sensitive individuals.

Whole blood was collected in a serum separator tube from the extradural vein from live seals or directly from the heart during necropsies (Winchell, 1990). Serum was separated from whole blood via centrifuge, and 1–2 ml of serum was pipetted into a cryovial. Blubber biopsies were collected from the dorsal posterior pelvic area with a 6 mm biopsy punch in both live and necropsied animals. Blubber samples were stored in solvent-rinsed, screw-top teflon vials and serum samples in cryovials and kept on ice until return to the Honolulu, Hawaii laboratory. All samples were stored in a −80 °C freezer prior to analyses.

In the current study, a total of 38 blubber samples and 37 serum samples were used. This study included paired blubber and serum samples (collected simultaneously) of 26 individual seals. Samples were unevenly distributed among 3 age/sex groups: juvenile seals (⩽4 years of age), adult females and adult males (>4 years of age) (Table 1).

Sample preparation, extraction, analysis, quality assurance, and quality control followed standardized methods (Sloan et al., 2004, 2006). Samples were analyzed in batches of 12–14 blubber or serum samples, plus a method blank and a standard reference material (SRM) sample (National Institute for Standards and Technology; blubber SRM 1945; serum SRMs 1957 1589a).

Briefly, each blubber and serum sample was weighed and then mixed with drying agents (sodium and magnesium sulfates). The samples were then transferred to accelerated solvent extraction cells, and surrogate standards (75 μL; PCB 103 and 4,4′-dibromooctafluorobiphenyl) were added to each cell. Using an Accelerated Solvent Extractor (ASE 200, Dionex, Salt Lake City, UT), the POPs and lipids were extracted with 2 cell volumes at 2000 psi and 100 °C using dichloromethane (DCM). The combined extract was collected in 60 ml tubes, a second standard of 75 μL CH HPLC I (tetrachloro-*m*-xylene (TCMX) at 1 ng/μL) was added to each tube and the tube with extract was weighed. Aliquots of 1.5 ml of each sample were transferred to a 2 ml vial and weighed for gravimetric lipid determination. Percent lipids in serum and blubber samples were determined gravimetrically following the method described in Sloan et al. (2004). The remaining extracts were then filtered through a gravity-flow silica/alumina column to remove interfering polar compounds. The pre-cleaned extracts were then concentrated further for additional cleanup using size-exclusion, high-performance liquid chromatography (HPLC) to remove extraneous high molecular weight compounds. The extracts were then concentrated to 2 ml, and a third standard was added (30 ml; GC internal standard for CHs; tetrachloro-*o*-xylene) to the samples to calculate the recovery of the surrogate standard. The samples were then exchanged into isooctane for a final volume of 100 μL to be analyzed by GC/MS (Agilent 5973 MSD, Agilent Technologies, Wilmington, DE).

Analytes included 6 DDTs (2,4′-DDD, 4′4′-DDD, 2,4′-DDE, 4,4′-DDE, 2,4′-DDT, and 4,4′-DDT), 47 PCB congeners (PCB 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, 159, 170, 171, 177, 180, 182, 183, 187, 190, 191, 194, 195, 199, 205, 206, 208, and 209), 8 chlordane (CHLD) isomers (heptachlor, heptachlor epoxide, oxychlordane, α-chlordane, γ-chlordane, *trans*-nonachlor, *cis*-nonachlor, and nonachlor III), three hexachlorohexanes (HCHs; alpha-, beta-, and gamma-hexachlorohexane), dieldrin, mirex, aldrin, hexachlorobenzene (HCB), and 10 PBDE congeners (PBDE 28, 47, 49, 66, 85, 99, 100, 153, 154, and 183). All concentrations are reported by nanogram (ng) per gram (g) of wet weight then converted to ng/g lipid weight using gravimetrically measured percent lipid in the tissue.

Three age/sex groups compared in the analysis were juveniles, adult males, and adult females. The accumulation of POPs is not expected to differ by sex until sexual maturity is reached and adult females begin to reproduce. This pattern was confirmed for these data using a *t*-test, therefore juvenile males and females were combined in further analysis. Seal ages were known for most individuals from a long-term mark-resight study using rear flipper tags or natural marks for identification (Harting et al., 2004; Henderson and Johanos, 1988). Seals older than 4 years of age were considered adults and seals 4 years of age and younger were considered juveniles following Ylitalo et al., 2008. If the exact age of a seal was not known, a minimum age was used based on first date of identification and size class at that time.

Summed PCBs (ΣPCB) were calculated as the sum of 47 PCB congeners (listed above). Summed DDTs (ΣDDT) is the sum of 6DDT isomers. Sum chlordane (ΣCHLD) is the sum of 8 chlordane isomers. Summed HCHs (ΣHCH) is the sum of 3HCH isomers. Summed PBDEs (ΣPBDE) is the sum of 10 PBDE congeners. Any POPs below the limit of quantification (LOQ) were not included in summed values. For comparison of MHI vs. NWHI contaminant levels (from Ylitalo et al., 2008), ΣPCB_8 included 8 commonly analyzed PCBs (PCBs 101, 118, 128, 138, 153, 156, 170, and 180) and ΣDDT_5 included 5commonly analyzed DDTs (*o,p*-DDD, *p,p′*-DDD, *p,p′*-DDE, *o,p*-DDT, *p,p′*-DDT).

Differences in contaminant levels between MHI age/sex groups and MHI vs. NWHI populations (from Ylitalo et al., 2008) were assessed using analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post hoc test. For ANOVAs, POP concentrations were log-transformed and percent lipid values were arcsine square root transformed to increase normality. Results were examined to determine the existence of outliers after transformation (defined as greater than 3 standard deviations from the mean) for ΣCHLD, ΣDDT, ΣPCB, and ΣPBDE. Two adult male outliers (one high and one low) from the MHI were removed for statistical analysis following these criteria. One additional adult male from Laysan and 1 juvenile from French Frigate Shoals were also removed by this criterion for the MHI vs. NWHI statistical analysis. Simple correlation analysis with log-transformed data was used to compare analyte concentrations of paired serum and blubber samples.

Table 1
Hawaiian monk seal blubber and serum samples collected in the MHI from juveniles (⩽4 yrs, includes weaned pups), and adult (>4 yrs) males and females. Samples are also tallied by origin (necropsy or live biopsy samples).

	Total blubber	Necropsy samples	Live biopsy samples	Total serum	Necropsy samples	Live biopsy samples
Juveniles	**17**	7	10	**16**	2	14
Adult females	**4**	0	4	**8**	0	8
Adult males	**17**	4	13	**13**	1	12
TOTAL	**38**	11	27	**37**	3	34

Principal Component Analysis (PCA) was used to assess patterns of shared variation in POP analytes. PCA has been used in previous studies to determine patterns of contaminants in marine organisms including shellfish (Karouna-Renier et al., 2007), pinnipeds (Bernt et al., 1999; Ross et al., 2004), cetaceans (Krahn et al., 1999), and polar bears (Wouter et al., 2008). The initial sample set for PCA included 71 POPs and 75 samples ($n = 38$ blubber; $n = 37$ for serum). POP analytes that were below the LOQ in more than 75% of the samples were removed from PCA to get closer to the recommended 5:1 sample to variable ratio (McCune and Grace, 2002) to assess patterns among the most common POPs. For the remaining analytes, if concentration for a sample was below the LOQ, a value of one half of the LOQ for that analyte was used in PCA (Karouna-Renier et al., 2007; Ylitalo et al., 2008). Two adult male outliers were removed for PCA analysis (>3 standard deviations). This resulted in a sample set with 21 POPs (*trans*-nonochlor, PCBs 17, 18, 28, 31, 33, 66, 70, 99, 105, 110, 118, 128, 138, 149, 153, 170, 180, 183, 187 and *p,p′*-DDE) and 73 samples. Significant PCA axes were selected based on a p value of <0.05 from randomizations with 999 runs. Differences in PCA axis loading values between age/sex group, sample origin, and tissue type were assessed with ANOVA.

ANOVA and Tukey's HSD were performed using PASW Statistics 18 for Mac (SPSS, Inc., Chicago, IL). PCA and outlier analyses were performed using PC-ORD statistical software version 5 (MjM Software, Gleneden Beach, OR).

Ranges and means for ΣCHLDs, ΣDDTs, ΣPCBs, ΣPBDEs, ΣHCHs, and mirex, by wet and lipid weight, as well as percent lipid, comparing age sex groups are reported in Table 2 for blubber and Table 3 for serum. Adult females had the lowest levels of all POP classes in blubber samples compared with juvenile animals and adult males based on both wet and lipid weight. ΣHCH, HCB and dieldrin were detected in juveniles and adult males but were below the LOQ in blubber samples of adult females. Aldrin was detected in blubber of a single juvenile sample (0.34 ng/g wet; 0.49 ng/g lipid). Endosulfan I was not detected in any blubber samples. No significant differences in blubber concentrations were found among the age/sex groups based on wet weight. However, adult males had significantly higher ΣDDT ($p = 0.024$) by lipid weight than adult females and significantly higher mirex concentrations ($p = 0.020$) by lipid weight than juveniles (Table 2).

As seen in blubber, adult male seals had the highest serum levels of most POP groups whereas adult females the lowest concentrations of these compounds. Dieldrin, HCB, Aldrin and endosulfan I were not detected in any serum samples. Adult males had significantly higher ΣCHLD ($p = 0.041$) by lipid weight in serum than adult females (Table 3).

The predominant PCB congeners measured in the blubber samples were PCB 138, 153, and 180, making up approximately 15%, 28%, and 15% of the ΣPCBs respectively in blubber and 11%, 21%, and 11%, respectively in serum samples (Fig. 2). PCB congener percentages contributing to the summed values were fairly consistent between age/sex groups.

Paired blubber and serum samples for 26 individuals showed significant positive correlations (Pearson correlation, $df = 24$) for ΣPCB ($r^2 = 0.74$, $p < 0.0001$), ΣCHLD ($r^2 = 0.75$, $p < 0.0001$), ΣDDT ($r^2 = 0.62$, $p < 0.0001$), and mirex ($r^2 = 0.56$, $p < 0.003$) by lipid weight (Fig. 3). The concentrations of POPs in blubber were 1–3 orders of magnitude higher than those in serum by wet weight.

Principal component analysis on a subset of the most abundant analytes resulted in one significant axis accounting for 74.89% of the variance. Results indicated shared patterns of variance between PCB congeners with similar chlorination patterns. Lower chlorinated congeners (PCBs 17, 18, 28, 31, 33, and 70) had positive loading values while higher chlorinated congeners (PCBs 153, 170, 180, 183, and 187) had negative loading values for the one significant axis (Table 4).

Sample loading values were compared using ANOVA to assess whether common patterns of measured POP levels were associated with different age/sex groups (juvenile, adult male, adult female), sample origin (live biopsy vs. necropsy), or tissue type (blubber vs. serum). There were no significant differences among age/sex groups using the PCA loading values, indicating that shared variation of POPs did not differ between age/sex group. However, there was a significant difference detected between the 2 sample origins ($p = 0.02$). This result indicates that there was a difference between necropsy and live animal samples in their shared variation patterns of POPs. Likewise, the difference between blubber samples and serum samples was significant ($p = 9.97 \times 10^{-16}$).

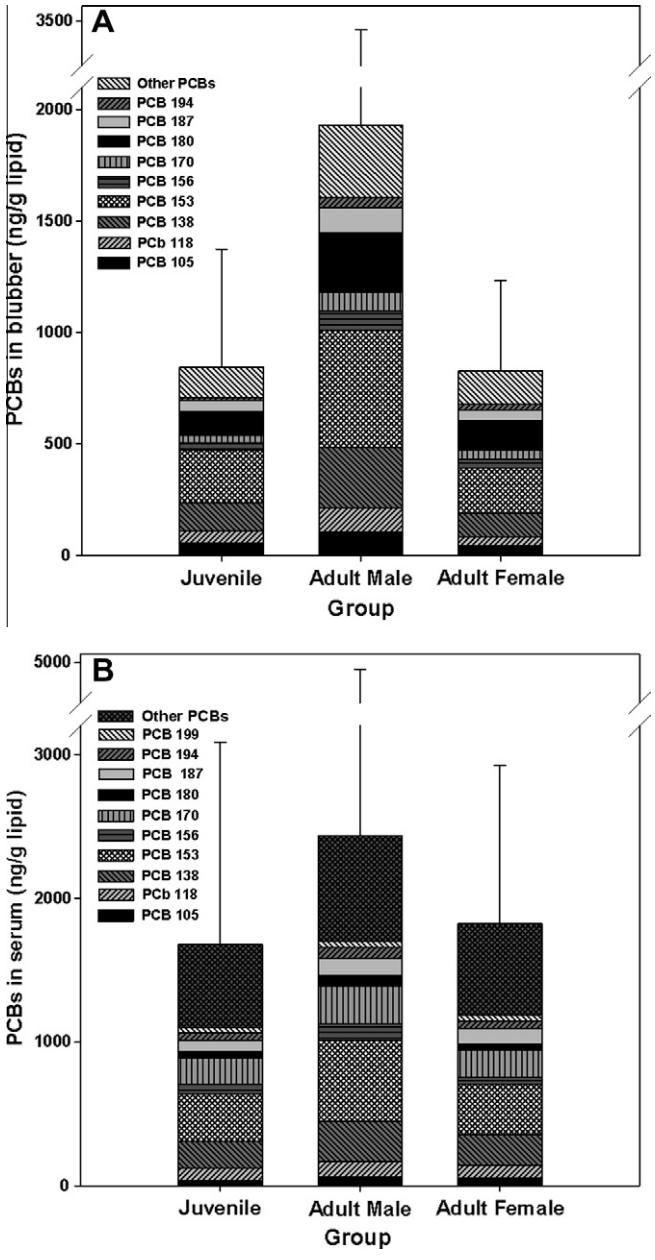

Fig. 2. Mean concentrations (SD 1) of PCBs in (A) blubber and (B) serum of juvenile, adult male, and adult female Hawaiian monk seals. Concentrations of individual congeners that contributed more than 2% to the total ΣPCBs are shown in different patterns. Other PCBs are combined and include PCBs 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 101, 105, 110, 128, 149, 151, 158, 171, 177, 191, 195, 199 (for blubber only), 205, 206, 208, and 209 in blubber and serum.

Table 2
Mean (SE) percent lipid, and ΣHCHs, ΣCHLDs, ΣDDTs, ΣPCBs, ΣPBDEs, HCB, dieldrin, and mirex, in blubber of HMS from the MHI. Significant differences ($p < 0.05$) based on ANOVA between groups for POP classes are shown in bold. Tukey–Kramer HSD, post hoc results indicate groups for which a significant difference ($p < 0.05$) was found. All values are expressed in units of ng/g. Two adult male outliers (>3 SD from mean) were removed from analysis.

Blubber		Range	Juvenile (SE) $n = 17$	Adult female (SE) $n = 4$	Adult male (SE) $n = 15$	p-value	Tukey–Kramer HSD
	% Lipid	8.4–86	65 (4.3)	44 (13)	39 (4.0)	**0.001**	J/AM
Wet weight	ΣCHLDs	3.0–920	97 (35)	16 (6.0)	99 (64)	0.121	-
	ΣDDTs	23–950	230 (31)	86 (31)	280 (68)	0.102	-
	ΣPCBs	53–3700	510 (110)	370 (170)	800 (250)	0.697	-
	ΣPBDEs	0.49–330	73 (22)	20 (6.5)	52 (20)	0.256	-
	ΣHCHs	0.16–7.3	0.50 (0.10)	ND	1.8 (1.1)	0.126	-
	HCB	0.23–1.9	0.76 (0.12)	ND	0.92 (0.21)	0.502	-
	Dieldrin	0.76–42	10 (7.9)	ND	14 (0)[a]	0.498	-
	Mirex	1.6–95	16 (2.8)	10 (3.7)	26 (6.7)	0.152	-
Lipid weight	ΣCHLDs	13–1500	142 (44)	36 (12)	190 (100)	0.311	-
	ΣDDTs	99–2100	390 (63)	190 (24)	690 (150)	**0.024**	AF/AM
	ΣPCBs	150–6100	800 (150)	770 (220)	1800 (400)	0.102	-
	ΣPBDEs	0.93–400	110 (30)	49 (5.4)	120 (39)	0.756	-
	ΣHCHs	0.23–28	0.82 (0.13)	ND	6.1 (4.5)	0.059	-
	HCB	0.48–4.2	1.1 (0.15)	ND	2.0 (0.50)	0.066	-
	Dieldrin	2.0–56	15 (10)	ND	23 (0)[a]	0.471	-
	Mirex	6.4–210	30 (6.9)	22 (5.5)	65 (15)	**0.020**	J/AM

ND = Not detected.
[a] Dieldrin only found in 1 adult male sample.

Table 3
Mean (SE) percent lipid, and ΣHCHs, ΣCHLDs, ΣDDTs, ΣPCBs, ΣPBDEs, and mirex in serum of HMS from the MHI. Significant differences ($p < 0.05$) based on ANOVA between groups for POP classes are shown in bold. Tukey–Kramer HSD, post hoc results indicate groups for which a significant difference ($p < 0.05$) was found. All values are in units of ng/g.

Serum		Range	Juvenile (SE) $n = 16$	Adult female (SE) $n = 8$	Adult male (SE) $n = 13$	p-value	Tukey–Kramer HSD
	% Lipid	0.12–1.4	0.53 (0.08)	0.47 (0.047)	0.46 (0.04)	0.923	-
Wet weight	ΣCHLDs	0.045–2.6	0.29 (0.17)	0.086 (0.01)	0.82 (0.53)	0.106	-
	ΣDDTs	0.20–6.6	1.4 (0.24)	0.80 (0.26)	2.0 (0.50)	0.082	-
	ΣPCBs	1.4–34	6.7 (2.1)	5.9 (2.8)	8.8 (2.8)	0.816	-
	ΣPBDEs	0.23–11	2.7 (2.1)	0.31 (0.049)	1.2 (0.35)	0.267	-
	ΣHCHs	0.057–0.24	0.12 (0.031)	0.12 (0.062)	0.12 (0)[a]	0.973	-
	Mirex	0.038–0.52	0.14 (0.032)	0.075 (0.015)	0.27 (0.13)	0.120	-
Lipid Weight	ΣCHLDs	11–1200	40 (11)	17 (2.4)	190 (130)	**0.041**	AM/AF
	ΣDDTs	66–1300	320 (64)	150 (35)	410 (95)	0.063	-
	ΣPCBs	230–7600	1400 (350)	1200 (390)	2000 (690)	0.818	-
	ΣPBDEs	49–790	260 (134)	86 (28)	290 (110)	0.362	-
	ΣHCHs	12–86	45 (14)	26 (14)	21 (0)[a]	0.717	-
	Mirex	7.8–86	43 (9.8)	18 (4.5)	46 (12)	0.120	-

[a] HCH only found in 1 adult male sample.

POP concentrations and percent lipid in blubber samples of seals from the MHI in the current study were compared to data reported by Ylitalo et al. (2008) which included seals from 4 sites in the NWHI: French Frigate Shoals (FFS), Laysan Island, Pearl and Hermes Reef (PHR), and Midway Atoll. Of the 5 sites examined between the two studies, Midway had the highest levels of ΣPCB_8 and ΣDDT_5 by lipid and wet weight in most cases (Table 5; Fig. 4). Juveniles from Midway had significantly higher ΣPCB_8 by wet weight compared with MHI ($p = 0.001$) and FFS ($p = 4.5 \times 10^{-6}$) and ΣPCB_8 by lipid weight in juveniles from Midway compared with MHI ($p = 7.8 \times 10^{-5}$) and FFS ($p = 5.2 \times 10^{-5}$). Adult males from Midway had significantly higher ΣPCB_8 by lipid weight compared to animals from all 4 other subpopulations: MHI ($p = 0.002$), FFS ($p = 0.01$), Laysan ($p = 0.001$), and PHR ($p = 0.015$; Table 5; Fig. 4). There were no significant differences found in ΣDDT_5 between sites for any group. Percent lipid was significantly higher in juveniles from the MHI compared with FFS ($p = 2.8 \times 10^{-4}$) and Midway ($p = 0.001$). Percent lipid values in blubber were lowest at Midway for adult males (27%, SE 4.3) and females (27%, SE 2.6) compared to the other sites, although these differences were not significant. Serum POP values from this study were not compared to those from the NWHI because the previous study used whole blood rather than serum.

Differences in POP level were observed between age/sex groups in blubber and serum samples. Adult males had the highest levels of POPs in blubber and adult females had the lowest levels, with juveniles being intermediate. This pattern has been observed in studies of POPs in other pinniped species (Blasius and Goodman-lowe, 2008; Wolkers et al., 2004; Willcox et al., 2004; Ylitalo et al., 2008) and is likely due to the differential accumulation and offloading abilities of adult males and adult females. All seals accumulate POPs throughout their lifetime via the food that they eat. When females give birth and lactate, many of these POPs are offloaded during gestation and to a greater extent by the transference of these lipophilic compounds to the pup via the mother's milk. As a result, it is typical to see POP concentrations increase with age in immature animals of both sexes and adult males but decrease or level off in females once they begin reproducing. This pattern was identified in HMS by both Willcox et al. (2004) and Ylitalo et al. (2008).

Although mean blubber POP concentrations were lower in adult females than in adult males and juveniles, this difference was not

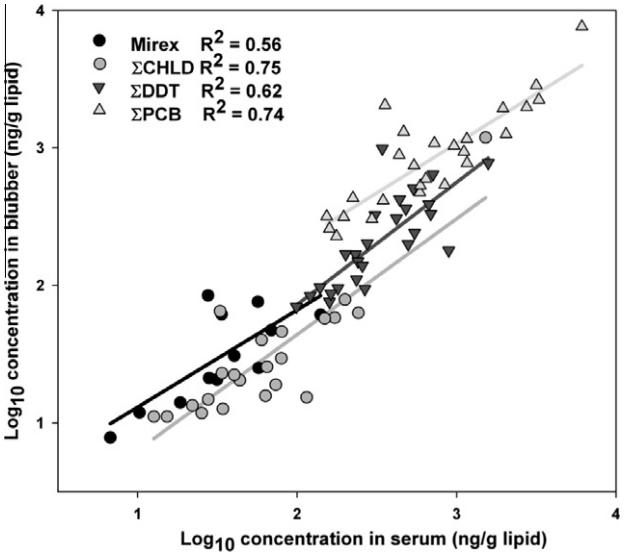

Fig. 3. Correlations between ΣPCBs ($p < 0.0001$), ΣCHLDs ($p < 0.0001$), ΣDDTs ($p < 0.0001$), and mirex ($p < 0.003$) in paired blood and serum samples in individual Hawaiian monk seals.

Table 4
Loading values on one significant PCA axis and Pearson correlations (*r* values) of POPs with PCA Axis 1.

POP	Axis 1	
	loading value	*r* value
Trans-nonachlor	−0.2106	−0.630
pcb 17	0.3206	0.983
pcb 18	0.3247	0.990
pcb 28	0.2897	0.979
pcb 31	0.3295	0.985
pcb 33	0.3433	0.977
pcb 66	0.0398	0.298
pcb 70	0.2288	0.869
pcb 99	−0.1320	−0.852
pcb 105	−0.1059	−0.718
pcb 110	0.2052	0.939
pcb 118	−0.1205	−0.799
pcb 128	−0.1679	−0.924
pcb 138	−0.1713	−0.983
pcb 149	0.0227	0.160
pcb 153	−0.1892	−0.930
pcb 170	−0.1864	−0.825
pcb 180	−0.1974	−0.800
pcb 183	−0.1881	−0.860
pcb 187	−0.2052	−0.814
ppDDE	−0.2299	−0.625

statistically significant ($p > 0.05$) by wet weight for any POP group, and only for ΣDDT by lipid weight. This finding may be caused by the small sample sizes for adult females ($n = 4$) compared with juvenile animals ($n = 17$) and adult males ($n = 16$). Additionally, the exact age and reproductive history of the 4 adult females in the blubber sample set is unknown. All of these animals were first identified as adults and it is unknown if they had given birth before being sampled. If these individuals had not reproduced prior to being sampled, their POP levels would not be expected to differ from those of adult males of similar age.

Differences in POP concentrations in serum may represent variation in recent food consumption. Serum POP levels are believed to reflect what the animal has consumed or metabolized recently and can vary by nutritional condition and season as well as age and sex. Blubber levels are believed to reflect the contaminants that have been stored throughout the lifetime of nonreproductive animals, or since the last reproduction for adult females (O'Hara and O'Shea, 2001), though not all POPs are offloaded during lactation.

PCA showed a significant difference between samples from live seals and from necropsies. Of the individuals whose samples were taken post-mortem, some had known causes of death (e.g. entanglement in fishing net) but many died from unknown causes. Some of the animals were very thin, with relatively low lipid content in their tissues when they died and likely had compromised health. Therefore, POPs may have been further concentrated in the blubber tissue of these individuals prior to death (Hall et al., 2008). No proxies for animal condition (i.e. weight) were collected during sampling, so animal condition could not be taken into account during this analysis.

One individual (ID TK23; adult male) had ΣCHLD, ΣDDT, ΣPCB and ΣPBDE blubber concentrations that were all an order of magnitude greater than the mean for adult males by both wet and lipid weight. TK23 was 23 years old and frequented the south shore of the island of Oahu and had been sighted in and around Pearl Harbor. Pearl Harbor is listed on the National Priorities List of the Environmental Protection Agency as a Superfund site, a site requiring long-term response to clean up hazardous materials or contamination. The contaminants of concern to the EPA in Pearl Harbor include DDTs, dieldrin, and PCBs (Grovhoug, 1992). TK23 died in 2008 and was necropsied within 24 h of death. Sighting records show that he had finished the annual molt approximately 1 month prior to his death and appeared to be in extremely poor condition. Lipid content was 8.73% compared to the mean of 45% (SE 3.5) for other adult males. The post-mortem pathology examination attributed the cause of death to chronic renal disease and mild cardiac disease. It is impossible to determine if the high POP level contributed to this individual's poor condition or death. Yet, based on previous studies of POP effects on other pinnipeds (Beckmen et al., 2003; De Swart et al., 1995; de Long et al., 1973; Hammond et al., 2005; Kim et al., 2002; Ylitalo et al., 2005), it is conceivable that either the high POP content may have impaired this animal's already strained immune system or that high POP levels impaired his immune system, which lead to increased susceptibility to further disease.

OCPs measured in the current study have been used historically to control pests in the main Hawaiian Islands. Although many of these compounds are no longer in use, they are still being found in the environment and organisms of Hawaii because of their resistance to degradation and ability to bioaccumulate in organisms. Previous studies of OCPs have shown high concentrations of chlordane and dieldrin from Hawaii compared with the rest of the nation in water, sediment, and biota (Schultz, 1971; Tanita et al., 1976; Miles et al., 1990; Schmitt et al., 1990; Hunter et al., 1995; Brasher and Anthony, 2000). Aldrin, chlordane, and heptachlor were widely used in Hawaii for termite control until the late 1980s (Brasher and Anthony, 2000). Dieldrin itself is not used as a pesticide but is a metabolite of aldrin. Dieldrin was detected at levels above the LOQ in approximately 16% (6 of 38) of the blubber samples at concentrations between 2.0 ng/g wet and 56 ng/g lipid.

DDT was used in the 1950s in Hawaii as an insecticide (Kartman and Lonergan, 1955). *p,p′*-DDE is a metabolite and degradation product of DDT that is most commonly measured in environmental and biotic samples and is thought to be more potent or harmful than DDT (ATSDR, 2002). Although DDT was banned in the U.S. in 1972, its use has continued elsewhere in the world. The majority of DDT detected in the present study (between 98.7% and 100% of ΣDDT in blubber and 100% of ΣDDT in serum samples by wet weight) was in the form of *p,p′*-DDE. This is consistent with DDTs found in HMS in other studies; Willcox et al., (2004) found no DDT compound other than *p,p′*-DDE and Ylitalo et al., 2008 found

Table 5
Mean (SE) ΣPCB_8 and ΣDDT_5 (ng/g) from the current study (Main Hawaiian Islands; MHI) and Ylitalo et al. (2008) (four NWHI populations: French Frigate Shoals (FFS), Laysan Island, Pearl and Hermes Reef (PHR), and Midway Atoll). Significant differences ($p < 0.05$) based on ANOVA between groups for POP classes are shown in bold. Tukey HSD post hoc results indicate sites for which a significant difference ($p < 0.05$) was found. No significant differences were found for adult females. Two adult males from the MHI, one adult male from Laysan, and one juvenile from FFS were identified as outliers (>3 SD from mean) and removed from analysis.

	N	Percent lipid (SE)	Wet weight (ng/g sample)		Lipid weight (ng/g lipid)	
			ΣPCB_8 (SE)	ΣDDT_5 (SE)	ΣPCB_8 (SE)	ΣDDT_5 (SE)
Juvenile						
MHI	17	65 (4.3)	370 (80)	230 (31)	590 (110)	390 (63)
FFS	27	44 (3.3	300 (73)	250 (59)	880 (200)	700 (160)
Laysan	3	47 (1.5)	280 (69)	260 (89)	610 (150)	560 (190)
Midway	13	42 (3.4)	1100 (170)	410 (95)	2800 (440)	2000 (190)
p-Value		**1.38×10^{-4}**	**1.4×10^{-5}**	0.154	**3.21×10^{-5}**	0.160
Tukey–Kramer HSD results		MHI, FFS; MHI, Midway;	MHI, Midway; FFS, Midway		MHI, Midway; FFS, Midway	
Adult male						
MHI	15	39 (4.0)	590 (190)	280 (68)	1300 (300)	690 (150)
FFS	7	38 (5.4)	350 (91)	160 (38)	1000 (210)	440 (78)
Laysan	8	48 (4.4)	310 (74)	280 (94)	680 (160)	590 (170)
PHR	6	40 (5.2)	730 (420)	570 (390)	1600 (910)	1200 (840)
Midway	5	27 (4.3)	2100 (1000)	410 (110)	7400 (3300)	1500 (240)
p-Value		0.125	0.071	0.600	**0.001**	0.109
Tukey–Kramer HSD results					MHI, Midway; FFS, Midway; Laysan, Midway; PHR, Midway	
Adult female						
MHI	4	44 (13)	270 (120)	88 (31)	550 (160)	190 (24)
Laysan	2	57 (7.5)	190 (140)	190 (140)	320 (200)	300 (210)
Midway	6	27 (2.6)	260 (67)	76 (25)	960 (230)	270 (77)
p-Value		0.095	0.866	0.172	0.591	0.936

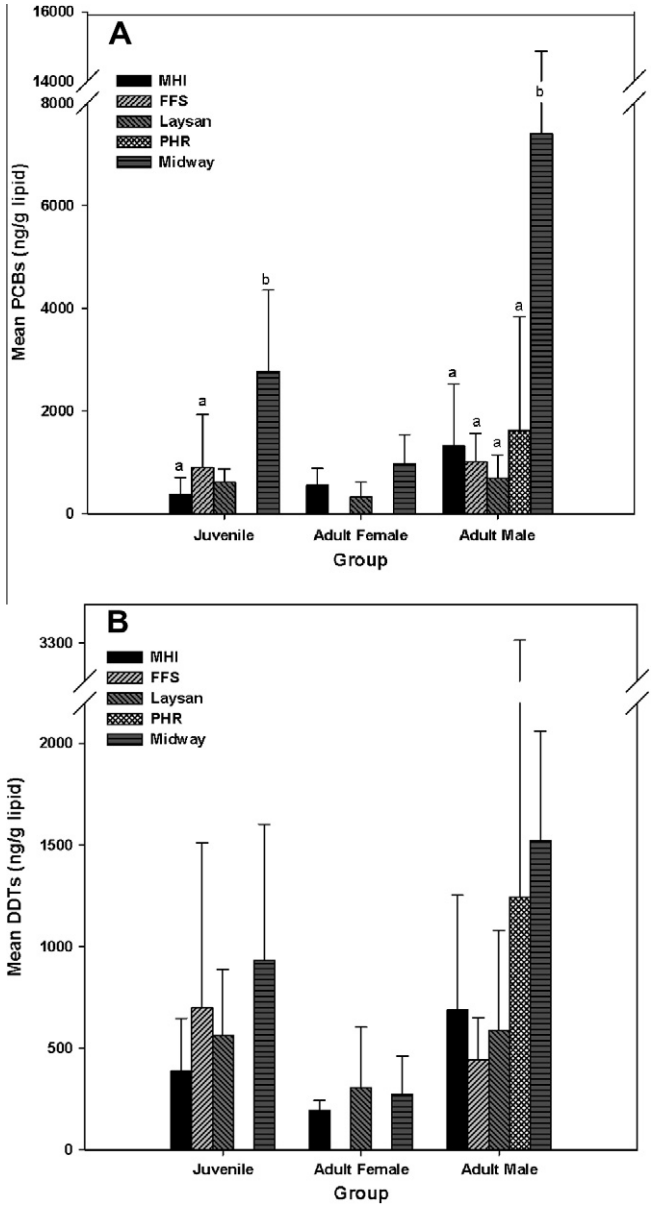

Fig. 4. Mean concentrations (ng/g lipid) of (A) 8 commonly analyzed PCB congeners (PCB 101, 118, 128, 138, 153, 156, 170, and 180) and (B) 5 commonly analyzed DDTs (*o,p*-DDD, *p,p′*-DDD, *p,p′*-DDE, *o,p*-DDT and *p,p′*-DDT) in blubber of Hawaiian monk seals, comparing 5 populations: main Hawaiian Islands (MHI; present study), French Frigate Shoals (FFS), Laysan Island, Pearl and Hermes Reef (PHR), and Midway Atoll (Ylitalo et al., 2008). Bars with unlike letters differ significantly.

only *p,p′*-DDE except for two seals from Laysan in which a small amount of *p,p′*-DDT was found. However, although the ΣDDT in blubber samples from the current study contained a majority of *p,p′*-DDE, all 6 metabolites were detected in at least one blubber sample and represented between 0.003% and 0.67% of the ΣDDT in each sample. The half-life of DDT is estimated to be between 1.5 and 3 days in the atmosphere as vapor but degrades more slowly in an ecosystem. It is estimated that in tropical climates all DDT will degrade to DDD or DDE within a year and in temperate regions half of the DDT will degrade within 5 years but may remain for up to 30 years (ATSDR, 2002). Finding even a small amount of DDT in samples of animals at this high trophic level is rare and may indicate a more recent source in the environment than was previously known.

Mirex was used extensively in Hawaii as a pesticide to control mealy bugs in pineapples. It has also been used in the U.S. mainland, specifically in the Great Lakes region and the southeastern U.S., as both a fire retardant additive and pesticide. When mirex was banned in the rest of the U.S. in 1978, its use was allowed to continue in Hawaii until on-hand supplies were exhausted (ATSDR, 1995). Mirex has been detected in terrestrial and marine organisms in Hawaii at higher levels than those found in mainland organisms (Johnson et al., 1976; Lee et al., 1975; Ylitalo et al. 2009). Mirex was present in >92% (35 of 38) of blubber samples at concentrations ranging from 6.4 ng/g lipid to 211 ng/g lipid and approximately 41% (15 of 37) of serum samples at concentrations between 7.8 ng/g lipid and 86 ng/g lipid.

An unexpected finding of the current study was that MHI monk seal contaminant levels were comparable to or lower than those previously reported in animals from the NWHI (Willcox et al., 2004; Ylitalo et al., 2008). Mean ΣPCB_8 were significantly higher by both wet and lipid weight in juveniles from Midway compared with MHI and FFS, and by lipid weight in adult males from Midway compared with all other sites (Table 5).

When the military base on Midway was closed in 1996, substantial cleanup activities occurred to remove chemical contaminants, including DDT and PCBs that had accumulated after more than 50 years of military occupation (Forney, 2010). However, these compounds are so persistent in the environment that the cleanup activities were likely not sufficient to completely eliminate POPs.

While this helps to explain the higher POP concentrations at Midway compared with other NWHI sites measured, it does not account for why these levels are higher than in the MHI. It is possible that the environment and lower trophic levels directly adjacent to Midway are more contaminated or the contamination is more concentrated than in the areas in which the MHI seals feed. However, even when Midway samples are removed from statistical analysis, MHI samples are still not the highest in many groups of POPs. There is not much information regarding POP levels in lower trophic organisms in any area in Hawaii and none comparing MHI to NWHI. Many of these lipophilic persistent compounds can be transported atmospherically. PCBs have been measured in air and seawater samples in areas adjacent to the Hawaiian Archipelago (Iwata et al., 1993, 1994), and some have been measured in rainwater samples in Hawaii (Bevenue et al., 1972). Although in many aspects, the NWHI and MHI are considered separate ecosystems, their proximity may subject them to similar levels of non-point source POPs.

Another important consideration when comparing these data is that the seals included are not always exclusive to the sites in which they were sampled. Although seals at the different sites are referred to as subpopulations, there is significant inter-island movement of HMS throughout their range. Seals from the three western-most atolls in particular (Pearl and Hermes Reef, Midway Atoll, and Kure Atoll) have a high rate of inter-island migration and similar foraging areas (Schultz et al., 2010). Other studies have showed differences in POP levels in marine mammals based on geographic location and/or foraging area (Borrell et al., 2007; Muir et al., 2000; Ross et al., 2004; Del Toro et al., 2006). In addition to the geographic differences between the two subpopulations, there may be variation in HMS foraging behavior and diet between the MHI and NWHI sites, which could influence the POPs consumed by seals from these different areas and thus, the measured POP concentrations in this study. This has been shown in populations of killer whales showing differences in POPs based on different diets or ecotypes (Ylitalo et al., 2001). Based on a recent unpublished study, the MHI HMS diet is similar to that of seals in the NWHI with some differences in variety and prevalence of prey

species (Goodman-Lowe, 1998; Cahoon, 2011). However, the diet study in the NWHI (Goodman-Lowe, 1998), which is being compared to the more recent study (Cahoon, 2011), does not include samples from Midway, so cannot serve to explain all of the differences observed in the current study. It is also possible that there could have been shifts in the prey base or diet in these locations between the time periods of the two studies.

The findings of this study suggest that the risk of POP exposure for HMS from the MHI is likely not higher than for HMS from the NWHI, contrary to what was speculated prior to the current study. Given the relative success of HMS from the MHI subpopulation, it does not appear that POPs are limiting growth at a population level, but may still be a health risk for individual animals.

HMS in the current study had intermediate levels of POPs compared with pinnipeds from previous studies. Mirex levels were lower in HMS from the current study than those measured in harbor seals from the northwestern Atlantic coast (Shaw et al., 2005), but higher than those from California sea lions, harbor seals, and northern elephant seals from California (Blasius and Goodmanlowe, 2008). Mean ΣPCBs found in the blubber of HMS in this study were overall lower than those reported previously in California sea lions, harbor seals, and northern elephant seals (*Mirounga angustirostris*) from California (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001) but are higher than those determined in grey seals (*Halichoerus grypus*) from Canada (Sørmo et al., 2003), southern elephant seals (*Mirounga leonine*) from Antarctica(Miranda-Filho et al., 2007), and harbor seals from Norway (Wolkers et al., 2004). ΣDDT concentrations in blubber in the current study were lower than California sea lions, harbor seals and northern elephant seals from California (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001), and higher than southern elephant seals from Antarctica (Miranda-Filho et al., 2007). These differences are likely due to differential PCB and DDT exposure of pinnipeds in different areas, differing diets, differing body conditions, or varying extraction and quantification techniques used among the studies.

POPs have been linked to various effects in other pinniped species (Beckmen et al., 2003; De Swart et al., 1995; Ylitalo et al., 2005; de Long et al., 1973) and similar effects may also occur in HMS. However, there may be differential susceptibility to effects of these compounds in HMS as seen when comparing other pinniped species (Kim et al., 2002, De Swart et al., 1995, Hammond et al., 2005) as a result of differences in absorption, storage, metabolism, or molecular binding. The current study quantifies the amount of POPs in HMS from a very important population. To date, no study has been conducted to determine the effects of POPs on this highly endangered species. To fully assess the risk of exposure to POPs, the molecular and immune response of the HMS to these contaminants should be assessed.

Although MHI seals sampled at different islands were combined in this study, different habitat use can occur among individuals (Cahoon, 2011), thereby resulting in varying contaminant exposures. The MHI has a wide array of habitats, and some of these habitats may pose more of a threat as a result of pesticides, industrial chemicals, and other POPs. For example, some islands (i.e. Molokai) have almost no history of engaging in industrial activities, but have historically high agricultural use. Likewise, certain areas of Oahu are highly industrialized and would be expected to have higher background levels of certain POPs in the environment. Although mean levels of POPs in the MHI were not higher than those found in the NWHI, some individual seals from the MHI had very high contaminant levels. This could be a result of differential habitat use or foraging behavior. Additional research is needed to assess pollutant levels at specific locations within the MHI in which POPs may be more of a threat to indivudal HMS that frequent those areas. Information on these geographic patterns of POP concentrations in the MHI may help scientists make informed decisions regarding future conservation and management efforts for HMS such translocations and health assessments.

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