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# Geographic variation of persistent organic pollutants in Hawaiian monk seals *Monachus schauinslandi* in the main Hawaiian Islands

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ABSTRACT: Geographic variation in the levels of persistent organic pollutants (POPs) was assessed in the serum of Hawaiian monk seals *Monachus schauinslandi* from the main Hawaiian Islands. Twenty seals were outfitted with tracking devices to map their home ranges, which were then compared with the POP levels in their serum. Seals with similar ranges were shown to have similar POP levels, and seals with home ranges around the island of O'ahu had significantly higher summed polychlorinated biphenyls and polybrominated diphenyl ethers than seals around the islands of Kaua'i and Moloka'i. This difference was not seen for summed diphenyl-dichlorotriphenylethanes or chlordanes. Non-metric multi-dimensional scaling (NMS) was used to determine if this geographic variation in serum POP levels was associated with specific POPs, watersheds, or state land use districts. The NMS ordination revealed patterns at the island scale, rather than the finer watershed scale. Additionally, there were differences in the land use characteristics adjacent to seals' home ranges between 2 islands: seals with home ranges around O'ahu had a high percentage of area adjacent to urban land use districts, and seals with home ranges around Moloka'i had a high percentage of area adjacent to rural and agricultural land use districts. Integration of serum POP levels and seal home ranges revealed geographic patterns that will help assess the risk of POPs to individual seals. The integrated approach highlighted in this study is applicable to other marine wildlife exposed to local and non-point pollutants.

KEY WORDS: Hawaiian monk seal · *Monachus schauinslandi* · Persistent organic pollutants · Geographic variation · Endangered species · Satellite tracking · Home range analysis · Hawaiian Islands



## INTRODUCTION

Persistent organic pollutants (POPs) are organic molecules, which persist in the environment and accumulate over time in organisms (Bard 1999). POPs have been known to affect the immune function and reproduction of long-lived marine animals, particularly those at high trophic levels (Helle et al. 1976, Addison 1989, De Swart et al. 1995, Beckmen et al. 2003, Hall et al. 2003, Hammond et al. 2005, Mos et al. 2006). Assessing the geographic variation of POPs is critical for understanding the factors affecting the distribution of these pollutants and the risk that they pose to both individual animals and populations. However, studying this geographic variation is inherently difficult due to the large degree of spatial variability in the sources and sinks of these pollutants, because these compounds can have both local (e.g. agricultural runoff, industrial outflows) and remote (e.g. oceanic and atmospheric transport, migrating prey) sources.

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POP levels in the tissues of animals can vary among populations and individuals as a result of differences in behavior, physiology, metabolism, and health (Loughlin et al. 1987, O'Hara & O'Shea 2001, Hall et al. 2008). Marine mammals, including the Hawaiian monk seal *Monachus schauinslandi* (HMS) accumulate POPs through their diet because these compounds bioaccumulate in the body over time and biomagnify through food webs to higher concentrations with each increase of trophic level (Bard 1999). These concentration mechanisms place top-level predators at a particularly high risk for POP exposure (Rowe 2008).

The Hawaiian Islands are susceptible to environmental pollution from both local and remote sources. POPs have a variety of origins, the majority of which are associated with industrial, urban, and agricultural activities. High levels of these compounds occur in urbanized, highly populated areas of the world (Bard 1999). Polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBDEs), and chlorinated pesticides, including aldrin, chlordane, heptachlor, and dichlorodiphenyltrichloroethane (DDT), were used historically in Hawai'i, but most of these compounds are no longer in use (ATSDR 1995, Brasher & Anthony 2000, ATSDR 2002, Costa & Giordano 2007). POPs can also reach the Hawaiian ecosystem from non-local sources. Although most of these compounds have been banned in the United States, many remain ubiquitous in marine ecosystems because they continue to be used throughout the world, do not easily degrade, and are transported long distances by the ocean and the atmosphere (Tanabe et al. 1994, Bard 1999). POPs tend to accumulate in tropical ecosystems where some are still being used, and in Arctic ecosystems due to their global transport patterns (Tanabe et al. 1994). Therefore, Hawaiian fauna can be exposed to POPs from local point sources associated with former agricultural and industrial activities in Hawai'i as well as to those those originating from distant locations that are transported across the Pacific Ocean.

POPs have been linked to a variety of biological effects in marine mammals, including renal lesions (Bergman et al. 2001), impaired immune response (De Swart et al. 1995, Beckmen et al. 2003, Hall et al. 2003, Hammond et al. 2005, Mos et al. 2006), decreased endocrine system function (Beckmen et al. 1999), and impaired reproduction (Helle et al. 1976, Addison 1989).

Several top marine predators in Hawai'i, including the false killer whale *Pseudorca crassidens* (Ylitalo et al. 2009), black-footed albatross *Phoebastria nigripes*, and Laysan albatross *Phoebastria immutabilis* (Finkelstein et al. 2006, 2007), have detectable levels of POPs in their tissues. However, these species exhibit much larger foraging ranges than the HMS, which inhibits the study of potential environmental correlates of these pollutant loads in such far-ranging species. The HMS has a comparatively restricted foraging range, which makes the species ideal for exploring how individual ranges influence exposure to POPs in the main Hawaiian Islands (MHI).

The HMS is a Critically Endangered pinniped species endemic to the Hawaiian Archipelago, with an estimated abundance of 1212 individuals in 2010 (Carretta et al. 2013). The HMS is found across the entire Hawaiian Archipelago, which includes 2 regions: the Northwestern Hawaiian Islands (NWHI) between Kure Atoll and Nihoa Island and the MHI between Ni'ihau Island and Hawai'i Island. The biology and conservation issues for the HMS differ greatly in these 2 regions, including population status, threats, level of research, and associated management actions (Baker et al. 2011). The majority of HMS are found in the NWHI, where abundance is declining at a rate of approximately 4.0% $yr^{-1}$ (Carretta et al. 2013). This decline can be attributed to many factors, including food limitation, entanglement in marine debris, shark predation, and intraspecific aggression (NMFS 2007). The HMS subpopulation in the MHI has higher survival rates, particularly for juvenile seals and higher reproductive rates compared with NWHI subpopulations, and is estimated to be increasing at a rate of approximately 6.5% $yr^{-1}$ (Baker et al. 2011). Because of the comparative success of this subpopulation, the MHI is considered essential high-quality habitat for HMS recruitment and survivorship.

HMS are top marine predators in the Hawaiian Archipelago and forage in a variety of benthic habitats, including shallow reefs, atoll slopes, sand flats, submerged banks, and seamounts (Stewart et al., 2006). The diet of the HMS is extremely varied and consists of nocturnal and diurnal fish, cephalopods, and crustacean taxa, from inshore, offshore, and benthic areas.

It is possible that individual HMS accumulate POPs at varying levels based on individual differences in habitat use and diet. Research in the NWHI (Willcox et al. 2004, Ylitalo et al. 2008) and MHI (Lopez et al. 2012) has documented POPs in HMS blubber, whole blood, and serum. Yet, analysis of geographic patterns of POP levels has been limited to large-scale regional (MHI vs. NWHI) comparisons and differences between sites at which animals were sampled.

However, these comparisons did not take into account geographic habitat use or finer-scale environmental associations (Ylitalo et al. 2008, Lopez et al. 2012).

In the current study, we explored the geographic variation of circulating levels of POPs in HMS serum using data obtained from seal telemetry studies and POP measurements to address 2 specific questions: (1) Do seals that use the same geographic areas have similar POP levels? And, if this pattern is detectable, (2) is the detected geographic variation in HMS serum POP levels associated with any measurable environmental characteristics of these areas? These analyses will help assess the potential threat POPs may pose to the health of individually tracked seals and can be extrapolated to regional populations. This information will, in turn, inform health assessments of individual animals, as well as management decisions regarding priority areas of concentration for POP monitoring efforts in HMS and other marine organisms.

## MATERIALS AND METHODS

### Animal capture and serum collection

Twenty individually known (Harting et al. 2004) and previously marked HMS were fitted with tracking instruments and sampled between 2004 and 2011 on the islands of Kauaʻi, Oʻahu, Molokaʻi, and Hawaiʻi. Seals were captured using a hoop net and physical restraint and were sedated with intravenous diazepam (0.1 to 0.2 mg $kg^{-1}$) in the extradural vein. Upon sedation, seals were measured, biomedically sampled, and equipped with tracking instruments. Whole blood was collected in a serum separator tube from the extradural vein. Serum was separated from whole blood via centrifuge, and 1 to 2 ml of serum was pipetted into a cryovial for POP analysis. Serum was stored at −80°C prior to analyses.

### Instrumentation and tracking

Seals received 1 of 3 different types of tags: Argos-based satellite-linked time-depth recorders (SLTDR) with global positioning technology (MK10; Wildlife Computers; n = 6), Argos SLTDRs without a global positioning system (GPS; ST-16; Wildlife Computers; n = 6), or cell-phone tags with GPS (Sea Mammal Research Unit; n = 8). Tags were attached to the dorsal pelage of the seals using Devcon 10-minute epoxy (ITW Devcon). Location data were recorded until the instruments failed, fell off during molt, or were removed. Nineteen seals were tracked for only 1 time period, and 1 seal (ID# R4DF) was tracked for 2 shorter periods in 2010 and 2011. The locations for this seal in both years showed similar areas of use, so all points for this seal were combined for analysis.

All Argos tag settings and filters followed the methods of Cahoon (2011). A filtering algorithm, SDA-filter (speed−distance−angle-filter; Freitas et al. 2008), was implemented using the software R (R Development Core team 2007) and used to determine invalid points based on unrealistic swim speed, distance traveled, and turning angles. Any point requiring a speed of >2 m $s^{-1}$, turning angles >165° (with a track leading to a point >2500 m away) or 155° (with a track leading to a point >5000 m away) was considered unrealistic due to the unlikelihood that the corresponding location represents a real movement (Freitas et al. 2008). The cell-phone GPS tags use Fastloc GPS technology to obtain locations, with an estimated horizontal error of approximately 55 m. Because these tags transmit data through the cellular phone network and do not rely on satellites, they provide a higher number and more accurate locations than Argos tags, which in the tropics are constrained by the coverage and timing of satellite overpasses.

### POP analysis

Serum POP levels were used for this study because they are presumed to be influenced by the foods consumed most recently (O'Hara & O'Shea 2001) and thus would be indicative of the most recently visited foraging locations.

The serum POP data used in this analysis were a subset of those previously reported by Lopez et al. (2012). HMS serum samples were analyzed for POPs using a gas chromatography/mass spectrometry (GC/MS) method detailed elsewhere (Sloan et al. 2004, 2006, Lopez et al. 2012). Briefly, serum samples were mixed with sodium sulfate and magnesium sulfate and were extracted with dichloromethane using an accelerated solvent extractor. Highly polar compounds were removed from the sample extracts using a gravity flow column containing alumina/silica. The extracts were then further cleaned using high-performance size exclusion liquid chromatography and then analyzed using a low-resolution quadrupole GC/MS system equipped with a 60 m DB-5 GC capillary column and an electron impact mass spectrometer in selected ion monitoring mode. As part of a performance-based quality assurance program (Sloan et al. 2006), a method blank and National Institute of Standards and Technology (NIST) Human Serum Standard Reference Material (SRM 1957 or SRM 1598a) were analyzed with the HMS serum samples. Concentrations of individual analytes measured in SRMs 1957 or 1589a were in good agreement with the reference values published by NIST.

Analytes included 6 DDT metabolites (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), 3 hexachlorocyclohexanes (HCHs; alpha-, beta-, and gamma-hexachlorocyclohexane), dieldrin, mirex, aldrin, hexachlorobenzene (HCB), and 10 PBDE congeners (PBDE 28, 47, 49, 66, 85, 99, 100, 153, 154, and 183). POP concentrations were calculated by both wet weight and lipid weight using gravimetric lipid content determination (Sloan et al. 2004).

### Data exploration

Potential biases in the data were assessed by investigating differences in the size of the home range and core area of individual seals. Linear regression was used to assess the correlation of home range and core area size with 3 continuous variables: (1) minimum estimated seal age (based on identification using rear flipper tags or natural marks), (2) number of tracking locations, and (3) tracking duration. Analysis of variance (ANOVA) was used to assess the influence of 3 categorical variables: tag type (Argos or cell phone), age group (adult or immature), and sex (male or female) on serum POP levels using summed PCB, DDT, PBDE, and CHLD values. Seals older than 4 yr of age were considered adults and those 4 yr of age and younger were considered immature. If significant differences were found between groups or a significant correlation was identified, standardized *z*-scores were used in subsequent analyses to account for the individual deviations from the resulting groups as follows:

$$z = \frac{(\text{individual seal} - \text{group mean})}{(\text{group standard deviation})}$$

### Quantifying home ranges and core areas of tracked seals

Due to differences in both quantity and accuracy of locations generated by Argos and cell phone tags (Costa et al. 2010, Patterson et al. 2010, Witt et al. 2010) we applied analysis techniques that allowed the use and integration of both data types, thus increasing the available sample size and the comparability among the tracking data.

Kernel density analysis (KDA) was performed to quantify the geographic areas most frequently utilized by individual seals using the spatial analyst extension of ArcGIS 9.3 (ESRI). A cell size of 100 m and a search radius of 10 km were used based on previous studies (Littnan et al. 2006, Cahoon 2011), and the magnitude of the likely errors in the Argos data was used, based on independent estimates with pinnipeds (Costa et al. 2010). Two polygons were created for each individual: the 95% utilization distribution (UD) contour defined the 'home range', and the 50% UD contour defined the 'core area' (Littnan et al. 2006, Stewart et al. 2006, Curtice et al. 2011).

### Shared space use and POP-level correlation

Shared space use of each pair of seals was calculated using Dice's index, which measures the degree of association between 2 individuals (Dice 1945). Dice's index is defined as $2h/(a + b)$, where $a$ is the area used by Individual A, $b$ is the area used by Individual B, and $h$ is the area shared by A and B. Dice's indices provided spatially explicit distance measures, quantifying the similarity of the home ranges of all pairs of tracked individuals, ranging from 0 (indicating no overlap of the 2 individual seals' home ranges) to 1 (indicating full overlap of the 2 individual seals' home ranges). All pairs of seals were also compared to each other using the Euclidean distance measure to develop to similarity matrices of their POP levels using the lipid weight values and the wet weight values separately. The resulting values ranged from 1 (indicating identical POP levels) to 0 (indicating no overlap in POP levels).

The Mantel test assesses the correlation between 2 similarity (or distance) matrices relating the same sample units. The standardized Mantel statistic ($r$) ranges from 1 to −1 and indicates the strength of the relationship between the 2 matrices. In this case, a positive $r$ value would indicate that seals which share more geographic space have more similar POP levels. Alternatively, a negative $r$ value would indicate that seals which share more geographic space have more dissimilar POP levels (Mantel 1967, McCune & Grace 2002).

The Mantel test was used to determine if seals that utilize similar geographic areas also have similar POP levels. The primary matrix in this analysis was the Dice's index (defined above) of shared space between pairs of seals. The rows and columns of this matrix were the individual seal IDs, with the cell contents being the Dice's index value for the 2 corresponding seals. Two primary matrices contained the Dice's index values for (1) home range and (2) core area. Individual POPs that were not found in any serum samples were removed, which resulted in a secondary matrix with 50 individual standardized POP levels (*x* dimension) in the serum of 20 individual seals (*y* dimension). The primary and secondary matrices were converted to distance matrices during the analysis.

### Island differences in concentrations of POPs

An ANOVA was performed with standardized *z*-score POP concentrations to assess whether variation in serum POP levels (summed PCBs, DDTs, PBDEs, and CHLDs by both wet weight and lipid weight) varied based on island adjacent to core area or home range of seals. Tukey-Kramer honestly significant difference (HSD) post hoc test was used to assess islands between which any observed differences were found.

### Exploring environmental variables associated with POP levels

Attributes of the home ranges and core areas were calculated using ArcGIS 9.3 (ESRI). Geographic information system (GIS) layers for bathymetry, state land use districts, and watersheds were utilized and were obtained from the Hawai'i Mapping Research Group (University of Hawai'i, School of Ocean and Earth Science and Technology: www.soest.hawaii.edu/HMRG/Multibeam/grids.php#50mBathyTopo) and the Hawai'i GIS library (State of Hawai'i Office of Planning, Department of Business, Economic Development, and Tourism: http://hawaii.gov/dbedt/gis/). The average and maximum depths of the home ranges and core areas were determined using the zonal statistics tool in ArcGIS. The percent of the home ranges and core areas adjacent to each of the 8 main Hawaiian Islands were calculated to identify the islands most utilized within the home ranges and core areas.

Four state land use districts, defined by the Hawai'i State Land Use Commission included agricultural, conservation, rural, and urban districts (Fig. S1 in the Supplement at http://www.int-res.com/articles/suppl/n024p249_supp.pdf). The percents of home ranges and core areas adjacent to coastlines with these land use districts that extended at least 1 km inland were calculated. This 1 km buffer was used because, in many areas, the coastline itself is considered conservation district, but the land directly inland has a different designation.

Twenty-nine watershed regions were identified based on the State of Hawai'i Commission on Water Resource Management designations (HCWRM 1990) (Fig. S2 in the Supplement at http://www.int-res.com/articles/suppl/n024p249_supp.pdf). The percents of home ranges and core areas adjacent to these watershed areas were calculated using ArcGIS. Of the 29 watersheds considered, 21 were adjacent to seals' home ranges or core areas. These watersheds are: Kekaha, Koloa, Lihui, Na Pali, Waimea, Ni'ihau (Kaua'i County); Ewa, Honolulu, Kahuku, Kaneohe, Waialua Waianae (O'ahu); Kamalo, Kaunakakai, La'au, North Moloka'i (Moloka'i); Lahaina, Wailuku (Maui); Kohala, Kona, South Hawai'i (Hawai'i Island). Non-metric multi-dimensional scaling (NMS) was used to explore the geographic variation in seal POP concentrations and associations with environmental characteristics. NMS is an ordination technique ideal for characterizing multivariate patterns because it does not impose any assumptions on the shape of the underlying statistical relationships and assesses significance using randomization tests (McCune & Grace 2002). Furthermore, NMS does not assemble discrete groupings of samples, but plots them along multi-dimensional gradients representing combinations of explanatory variables. In our case, those POPs and seals with similar patterns of variability are plotted closer in the resulting multivariate ordination space (Kenkel & Orloci 1986). The reduction in stress, which quantifies the overall fit of the data in ordination space and real space, determines the number of axes that best captures the gradients in the dataset. Based on Clarke's rule of thumb, a stress of 5 to 10 indicates a good ordination with no real risk of drawing false inferences and a stress at the lower end of the 10 to 20 range can still correspond to a usable picture (McCune & Grace 2002). Finally, the test quantifies the correlation of each variable to the empirically selected axes using the Kendal tau statistic (McCune & Grace 2002).

The NMS was performed using 2 distance matrices calculated for all pair-wise combinations of the individual seals considered in this study. The primary matrix included the standardized *z*-scores for lipid weights of individual POP compounds and congeners. The secondary matrix included the average depth, maximum depth, island with the most adjacent monk seal home range or core area, percent of area adjacent to 4 state land use districts (urban, conservation, rural, and agriculture), and percent of area adjacent to 21 watersheds (listed above) for the home range and core area of each individual seal.

A constant (+3) was added to the standardized *z*-scores of POPs to avoid negative numbers for the NMS test, as these pose problems for the use of the Sorensen distance metrics. A Monte Carlo test was performed to assess whether the ordination resulted in stronger axes than would be expected by chance using 250 runs with both the real and randomized data. The number of axes was determined using a minimal reduction of the final stress value by 5 with every additional axis (McCune & Grace 2002).

The ANOVA and linear regression analyses were performed using SPSS 19.0 for Mac (IBM). Mantel tests and NMS ordinations were performed using PC-ORD Version 5 (MjM Software).

## RESULTS

### Tag deployment

The 20 HMS tagged between 2004 and 2011 were tracked for between 44 and 288 d (Table 1). There was no significant difference in tag deployment duration between Argos and cell phone tags ($F$ = 0.030; df = 1,18; p = 0.865. No significant differences (p > 0.05) were found in the size of the home range or core area between tag types, age classes, or sex (Table 2). Regression analysis revealed no significant relationship between the size of the home range or core area and the minimum estimated seal age, the number of locations, or the number of days tracked (Table 2).

### Home range and core area assessment

Home range sizes ranged from 268.4 to 2078.3 km$^2$ (mean: 923.2 km$^2$; SD: 444.7). Core area sizes ranged from 66.7 to 282.6 km$^2$ (mean: 130.7 km$^2$; SD: 65.3) (Table 3). One seal's core area was around the north side of the island of Ni'ihau, but the majority of its home range was adjacent to the coastline of Kaua'i.

Table 1. Summary of tracked Hawaiian monk seals, showing the individual seal ID, the tag type (Argos or Cell), tag number, age group (adult or immature), minimum age (yr), sex (M: male, F: female), start and end date of track, deployment duration (d), and number of geographic locations recorded

| Seal ID | Tag type | Tag no. | Age group | Minimum age at sampling (yr) | Sex | Start track (mo/d/yr) | End track (mo/d/yr) | Tag deployment duration (d) | No. of locations |
|---|---|---|---|---|---|---|---|---|---|
| R012 | Cell | 11393 | Adult | 11 | M | 3/1/2010 | 8/20/2010 | 172 | 7091 |
| R018 | Cell | 11478 | Adult | 9 | M | 6/9/2010 | 12/8/2010 | 182 | 6416 |
| R4DI | Cell | 11337 | Adult | 5 | M | 2/9/2010 and 6/15/2011 | 2/23/2010 and 7/26/2011 | 55 | 1967 |
| RE70 | Cell | 11420 | Adult | 7.5 | M | 3/27/2010 | 5/10/2010 | 44 | 1844 |
| RI11 | Cell | 11419 | Adult | 6 | M | 3/27/2010 | 10/9/2010 | 196 | 6019 |
| RR70 | Cell | 11396 | Adult | 7 | M | 6/29/2010 | 8/31/2010 | 63 | 2881 |
| RK35 | Cell | 10603 | Adult | 6 | F | 10/3/2007 | 2/22/2008 | 142 | 7283 |
| R4DF | Cell | 11476 | Adult | 5 | F | 7/9/2010 | 11/25/2010 | 139 | 6344 |
| RI19 | Argos | 79749 | Immature | 3.5 | M | 6/11/2004 | 9/6/2004 | 87 | 216 |
| RM38 | Argos | 42687 | Immature | 2.5 | M | 2/20/2004 | 7/19/2004 | 150 | 392 |
| RO36 | Argos | 73370 | Immature | 1 | M | 8/10/2007 | 10/21/2007 | 72 | 244 |
| RR64 | Argos | 73367 | Immature | 4 | M | 8/9/2007 | 11/22/2007 | 105 | 79 |
| RK03 | Argos | 42681 | Adult | 7 | M | 5/5/2004 | 8/17/2004 | 104 | 107 |
| T34M | Argos | 42684 | Adult | 20.5 | M | 2/24/2004 | 4/26/2004 | 62 | 74 |
| TT40 | Argos | 42680 | Adult | 19.5 | M | 2/19/2004 | 7/3/2004 | 135 | 337 |
| RO42 | Argos | 74954 | Immature | 1 | F | 8/24/2007 | 12/12/2007 | 110 | 304 |
| RV11 | Argos | 73362 | Immature | 2 | F | 8/20/2007 | 10/25/2007 | 66 | 75 |
| R4DP | Argos | 79750 | Adult | 5 | F | 6/14/2008 | 3/29/2009 | 288 | 745 |
| R5AU | Argos | 34877 | Adult | 5 | F | 4/13/2004 | 1/8/2005 | 270 | 350 |
| R6AJ | Argos | 73364 | Adult | 5 | F | 8/9/2007 | 11/5/2007 | 88 | 442 |

Table 2. Results of tracking metadata analysis. ANOVA was performed for categorical variables: tag type, age class, and sex for Hawaiian monk seals *Monachus schauinslandi*. Regression was performed for continuous variables: minimum estimated age, number of locations, and number of days tracked. Separate analyses were performed for core area and home range. No tests yielded significant ($p < 0.05$) results

| | Core area | | Home range | |
|---|---|---|---|---|
| | ANOVA | | | |
| **Variable (sample size)** | *F* | p | *F* | p |
| Tag type (Argos: n = 12, Cell: n = 8) | 1.383 | 0.255 | 0.318 | 0.580 |
| Age class (adult: n = 14, immature: n = 6) | 1.165 | 0.295 | 0.054 | 0.819 |
| Sex (male: n = 13, female: n = 7) | 0.232 | 0.636 | 0.414 | 0.528 |
| | Regression | | | |
| **Variable (range)** | $r^2$ | p | $r^2$ | p |
| Minimum estimated age (1.1–20.75 yr) | 0.204 | 0.111 | 0.063 | 0.373 |
| Number of locations (74–23 421) | 0.073 | 0.856 | 0.047 | 0.475 |
| Number of days tracked (44–288) | 0.073 | 0.874 | 0.019 | 0.862 |

Table 3. Summary of tracked Hawaiian monk seal (*Monachus schauinslandi*) space use for individual seals, showing the size, average and maximum depths, and the location most used by seals within their core area (50% utilization distribution [UD]) and home range (95% UD) from kernel density analysis. PB: Penguin Banks

| Seal ID | Tagging location | Core area (50% UD) | | | | Home range (95% UD) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Area ($km^2$) | Average depth (m) | Maximum depth (m) | Location most utilized | Area ($km^2$) | Average depth (m) | Maximum depth (m) | Location most utilized |
| R018 | Kauaʻi | 71.80 | 219.04 | 522.15 | Niʻihau | 1359.48 | 515.59 | 2145.51 | Kauaʻi |
| R4DI | Kauaʻi | 113.25 | 189.26 | 763.14 | Kauaʻi | 833.53 | 597.83 | 1944.26 | Kauaʻi |
| RI19 | Kauaʻi | 133.20 | 298.82 | 1286.21 | Kauaʻi | 1153.03 | 468.16 | 2100.52 | Kauaʻi |
| RK03 | Kauaʻi | 272.35 | 371.11 | 1552.54 | Kauaʻi | 2078.31 | 964.34 | 3712.78 | Kauaʻi |
| RK35 | Kauaʻi | 214.58 | 357.15 | 1433.01 | Kauaʻi | 575.37 | 966.10 | 1966.24 | Kauaʻi |
| RM38 | Kauaʻi | 282.55 | 334.62 | 1492.99 | Kauaʻi | 1648.33 | 1040.92 | 3022.13 | Kauaʻi |
| TT40 | Kauaʻi | 87.96 | 504.08 | 1480.12 | Kauaʻi | 395.55 | 849.19 | 1981.22 | Kauaʻi |
| R4DP | Oʻahu | 77.21 | 235.72 | 1120.02 | Kauaʻi | 268.40 | 817.04 | 1811.86 | Kauaʻi |
| R012 | Oʻahu | 79.32 | 116.62 | 460.96 | Oʻahu | 827.34 | 332.49 | 1411.27 | Oʻahu |
| R4DF | Oʻahu | 82.06 | 173.04 | 569.91 | Oʻahu | 894.86 | 363.54 | 1502.08 | Oʻahu |
| RR70 | Oʻahu | 86.15 | 111.65 | 450.45 | Oʻahu | 979.74 | 221.24 | 1012.07 | Oʻahu |
| T34M | Oʻahu | 66.65 | 464.28 | 1012.07 | Oʻahu | 677.52 | 410.91 | 2005.17 | Oʻahu |
| R5AU | Molokaʻi | 164.40 | 48.72 | 66.95 | PB | 1255.77 | 143.2 | 1101.86 | Molokaʻi |
| R6AJ | Molokaʻi | 143.73 | 48.03 | 63.23 | PB | 1139.26 | 160.28 | 1101.85 | Molokaʻi |
| RE70 | Molokaʻi | 116.60 | 69.1 | 196.9 | Molokaʻi | 398.69 | 120.22 | 435.5 | Molokaʻi |
| RI11 | Molokaʻi | 97.62 | 41.96 | 127.95 | Molokaʻi | 1092.05 | 149.38 | 1022.54 | Molokaʻi |
| RO36 | Molokaʻi | 172.49 | 56.70 | 122.19 | Molokaʻi | 726.68 | 124.19 | 769.14 | Molokaʻi |
| RR64 | Molokaʻi | 74.71 | 87.74 | 179.64 | Molokaʻi | 489.52 | 93.18 | 435.5 | Molokaʻi |
| RV11 | Molokaʻi | 96.08 | 106.9 | 285.52 | Molokaʻi | 691.64 | 470.13 | 1966.3 | Molokaʻi |
| RO42 | Hawaiʻi | 180.74 | 504.55 | 1651.09 | Hawaiʻi | 979.49 | 1079.68 | 2582.88 | Hawaiʻi |
| Minimum | | 66.65 | 41.96 | 63.23 | | 268.40 | 93.18 | 435.50 | |
| Maximum | | 282.55 | 504.55 | 1651.09 | | 2078.31 | 1079.68 | 3712.78 | |
| Mean | | 130.67 | 216.95 | 741.85 | | 923.23 | 494.38 | 1701.53 | |
| SD | | 65.25 | 158.66 | 576.90 | | 444.70 | 343.66 | 832.20 | |

Two individuals had core areas offshore over Penguin Banks and home ranges around the island of Molokaʻi. All other seals had their core areas and home ranges adjacent to the same island. Seven individuals had their core areas and home ranges adjacent to the island of Kauaʻi. Four individuals' core areas were adjacent to Oʻahu. Five individuals had core areas and home ranges adjacent to Molokaʻi. One seal's core area and home range was adjacent to the Island of Hawaiʻi (Fig. 1, Table 3).

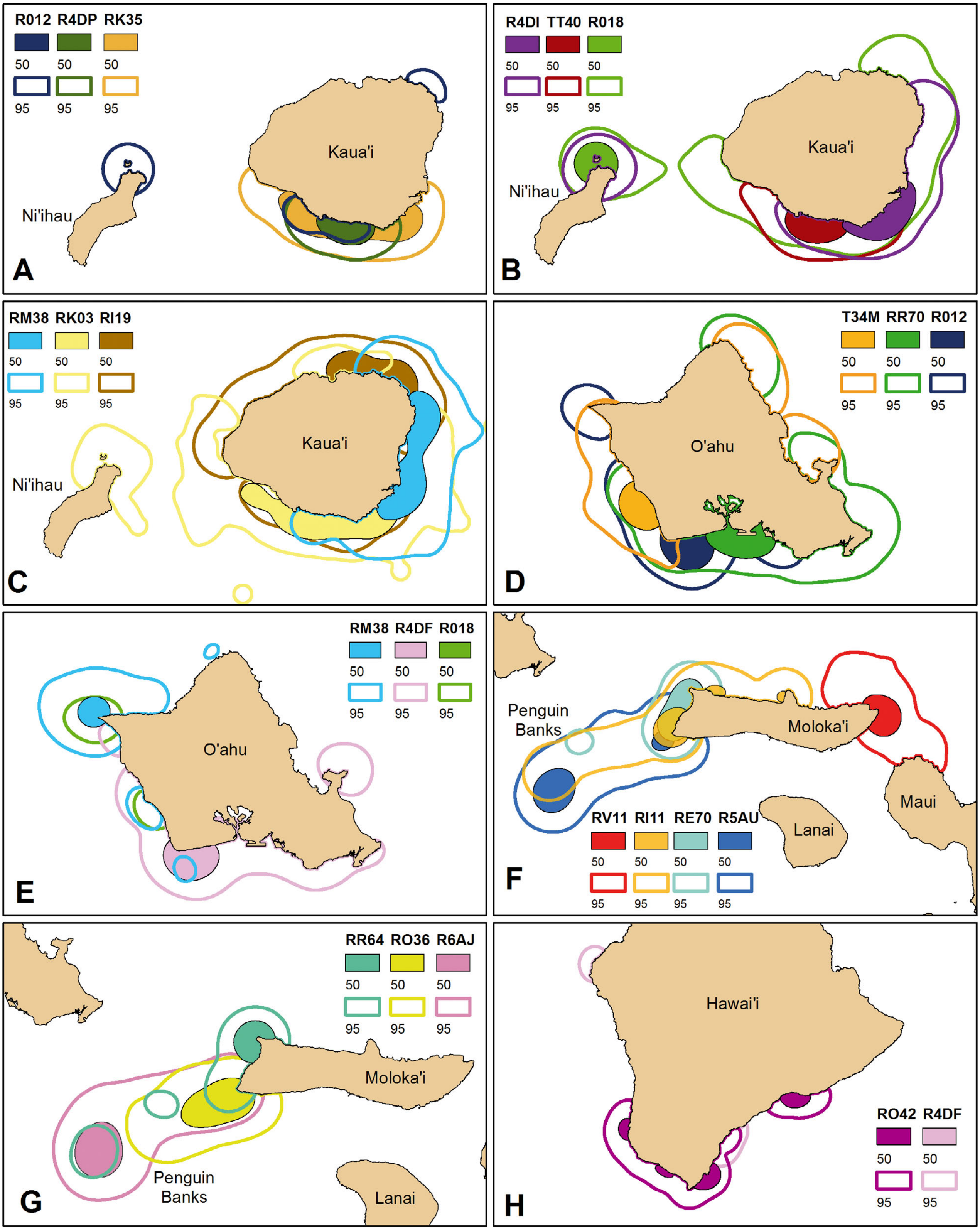


Fig. 1. Core areas (solid) and home ranges (outline) for 20 Hawaiian monk seals *Monachus schauinslandi* in (A,B,C) Kaua'i county; (D,E) O'ahu; (F,G) Maui county; and (H) on the island of Hawai'i. Seal IDs as in Table 1; 50 and 95 refer to 50 and 95% utilization distribution for core areas and home ranges, respectively

## POP levels in serum

The levels of summed DDTs differed significantly by sex. Therefore, the concentrations of individual POPs were converted to *z*-scores, standardized for gender as described in the 'Materials and methods' section. Other POP groups showed differences by sex that were not statistically significant. Mean concentrations of summed chlordanes, PCBs, DDTs, and PBDEs in male and female HMS serum are reported in Table 4.

## Shared space use and POP correlation: Mantel test results

Significant positive relationships were found between the spatial overlap of seals' home ranges and core areas (Dice's index) and seals' serum POP levels (by lipid weight) based on 50 individual compounds and congeners ($r = 0.145$, $p = 0.035$ for core area vs. POP; $r = 0.242$, $p = 0.005$ for home range vs. POP). This finding indicated that seals that shared more of their core areas and home ranges had more similar POP levels.

## Island differences in POP level

ANOVA results showed significant differences between islands ($p < 0.05$) for mean concentrations of ΣPCB and ΣPBDE based on wet weight and lipid weight. After standardizing the data for any effect of sex, seals that spent most of their time around Oʻahu were found to have significantly higher concentrations of ΣPCBs and ΣPBDEs than those around Molokaʻi and Kauaʻi (Table 5).

Table 4. Mean (±SD) concentrations of summed chlordanes (ΣCHLDs), summed polychlorinated biphenyls (ΣPCB), summed DDTs (ΣDDT), and summed polybrominated diphenyl ethers (ΣPBDE) determined in the serum of Hawaiian monk seals *Monachus schauinslandi* based on sex. The p-values are based on ANOVAs with log-transformed data. **Bold** text indicates a significant ($p < 0.05$) difference

| | Male mean (SD, n = 13) | Female mean (SD, n = 7) | *F* | p |
|---|---|---|---|---|
| **Wet weight (ng g$^{-1}$)** | | | | |
| ΣCHLD | 0.62 (1.3) | 0.09 (0.05) | 2.054 | 0.169 |
| ΣPCB | 10 (9.5) | 3.5 (1.4) | 4.358 | 0.051 |
| ΣDDT | 2.2 (1.7) | 0.91 (0.70) | 6.581 | **0.019** |
| ΣPBDE | 0.47 (0.64) | 0.08 (0.14) | 1.863 | 0.189 |
| **Lipid weight (ng g$^{-1}$)** | | | | |
| ΣCHLD | 140 (320) | 20 (10) | 0.156 | 0.697 |
| ΣPCB | 1600 (1900) | 760 (300) | 0.076 | 0.786 |
| ΣDDT | 440 (330) | 190 (150) | 5.082 | **0.037** |
| ΣPBDE | 17 (33) | 10 (27) | 0.204 | 0.657 |

## Environmental variables associated with POP level: NMS results

The NMS test resulted in 2 axes, based on the stress reduction criterion (McCune & Grace 2002), with a final stress of the 2-dimensional solution of 10.73. This result yielded a significantly higher reduction in stress than would be expected by chance based on a randomization test with 1000 iter-

Table 5. Mean (±SE) concentrations of summed chlordanes (ΣCHLDs), summed DDTs (ΣDDTs), summed polychlorinated biphenyls (ΣPCBs), and summed polybrominated diphenyl ethers (ΣPBDEs) measured in the serum of Hawaiian monk seals *Monachus schauinslandi* from the main Hawaiian Islands. Significant differences ($p < 0.05$) based on ANOVA between home range islands for standardized POP levels are shown in **bold**. ANOVAs did not include the island of Hawaiʻi due to the small sample size (n = 1). Tukey-Kramer honestly significant difference (HSD), post hoc results indicate islands between which a significant difference was found. ND: not detected

| | KauaʻI (n = 8) | Oʻahu (n = 4) | Molokaʻi (n = 7) | Hawaiʻi (n = 1) | p-value | Tukey-Kramer HSD |
|---|---|---|---|---|---|---|
| **Wet weight** | | | | | | |
| ΣCHLDs | 0.22 (0.07) | 1.6 (1.2) | 0.10 (0.03) | 0.06 | 0.107 | |
| ΣDDTs | 2.2 (0.68) | 2.3 (0.9) | 1.0 (0.25) | 0.70 | 0.798 | |
| ΣPCBs | 7.3 (1.8) | 17 (6.8) | 3.6 (0.69) | 2.1 | **0.035** | Oʻahu/Molokaʻi |
| ΣPBDEs | 0.18 (0.1) | 1.4 (0.51) | 0.23 (0.18) | ND | **0.004** | Oʻahu/Molokaʻi, Oʻahu/Kauaʻi |
| **Lipid weight** | | | | | | |
| ΣCHLDs | 39.0 (8.4) | 390 (270) | 18 (5.0) | 15.39 | 0.086 | |
| ΣDDTs | 390 (82) | 610 (250) | 200 (55) | 179.49 | 0.379 | |
| ΣPCBs | 1300 (200) | 4400 (1700) | 650 (110) | 538.46 | **0.012** | Oʻahu/Kauaʻi, Oʻahu/Molokaʻi |
| ΣPBDEs | 33 (17) | 380 (150) | 40 (30) | ND | **0.002** | Oʻahu/Kauaʻi, Oʻahu/Molokaʻi |

ations (p = 0.004). The proportion of the observed variance captured by the first 2 dimensions ($r^2$) was 1.9 and 60.4 %, respectively, for a cumulative value of 62.3 % (Fig. 2). The orthogonality (independence) of the 2 axes was 97.8 %.

There were some general patterns in ordination scores on the 2 axes based on the island adjacent to the seals' core area. However, there was a high degree of individual variability leading to exceptions to every pattern. Seals with core areas adjacent to O'ahu had negative ordination scores for Axis 1 and positive ordination scores for Axis 2 (Fig. 2). Seals with core areas adjacent to Kaua'i had negative ordination scores for Axis 1 (except for individual TT40) and negative ordination scores for Axis 2 (except for individual R4DP; Fig. 2). Seals with core areas adjacent to Moloka'i or Penguin Bank had positive ordination scores for Axis 1 (except for individual R5AU) and negative ordination scores for Axis 2 (except for individual RV11; Fig. 2).

The variables of core areas and home ranges around O'ahu watersheds (Honolulu, Ewa, Kahuku, Kaneohe, and Waianae) and core areas and home ranges around urban land use districts had negative ordination scores for Axis 1 and positive ordination scores for Axis 2. The variables of core areas and home ranges adjacent to Moloka'i watersheds (Kaunakakai and La'au) and core areas and home ranges around rural land use districts had positive ordination scores for Axis 1 and negative ordination scores for Axis 2. All environmental variable (size, depth, land use, and watershed) correlations to the NMS axes are reported in Table S1 in the Supplement at http://www.int-res.com/articles/suppl/n024p249_supp.pdf.

POPs showed a narrow range in ordination space compared with seals and environmental variables (Fig. 3). All POPs were positively correlated with Axis 2 and most were negatively correlated with Axis 1. Trichlorinated PCBs had lower ordination scores, while all other PCBs had higher ordination scores for Axis 2 (Fig. 3). The four PBDEs (47, 99, 100, and 153) present in the samples of these seals had negative ordination scores on Axis 1. All POP level correlations to the 2 NMS axes are reported in Table S2 in the Supplement at http://www.int-res.com/articles/suppl/n024p249_supp.pdf.

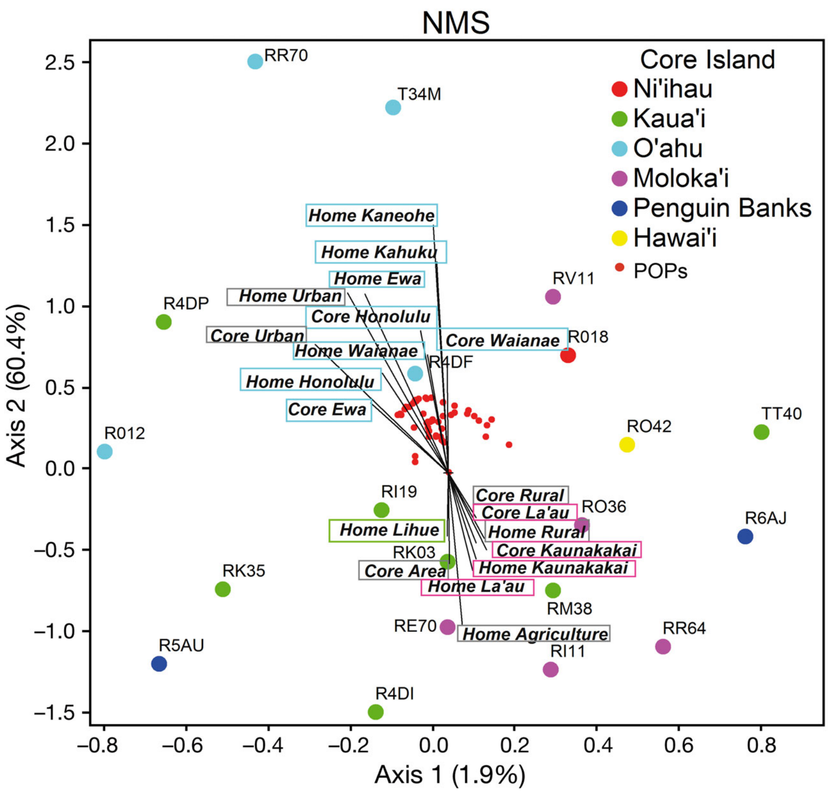


Fig. 2. Non-metric multidimensional scaling (NMS) ordination plot of Hawaiian monk seals *Monachus schauinslandi* (colored dots), persistent organic pollutants (POPs; small red dots), home range, and core area variables with $r^2 > 0.10$ (vectors). Colored outlines represent the island associated with each variable

## DISCUSSION

### Shared space use and POP level

Core area and home range size are not necessarily representative of one another and can vary among individuals. The Mantel's tests showed that both of these range metrics had a significant positive relationship with POP level in serum, indicating that HMS that share the same geographic range have similar serum POP levels. A limitation of the Mantel test is that it only gives a significant or non-significant result based on all POP compounds. Consequently, although all POPs were included in the test, it is unclear which might be responsible for the results. Despite this limitation, the Mantel test was useful in identifying an overall pattern. Further information was gained in exploring the data using other methods.

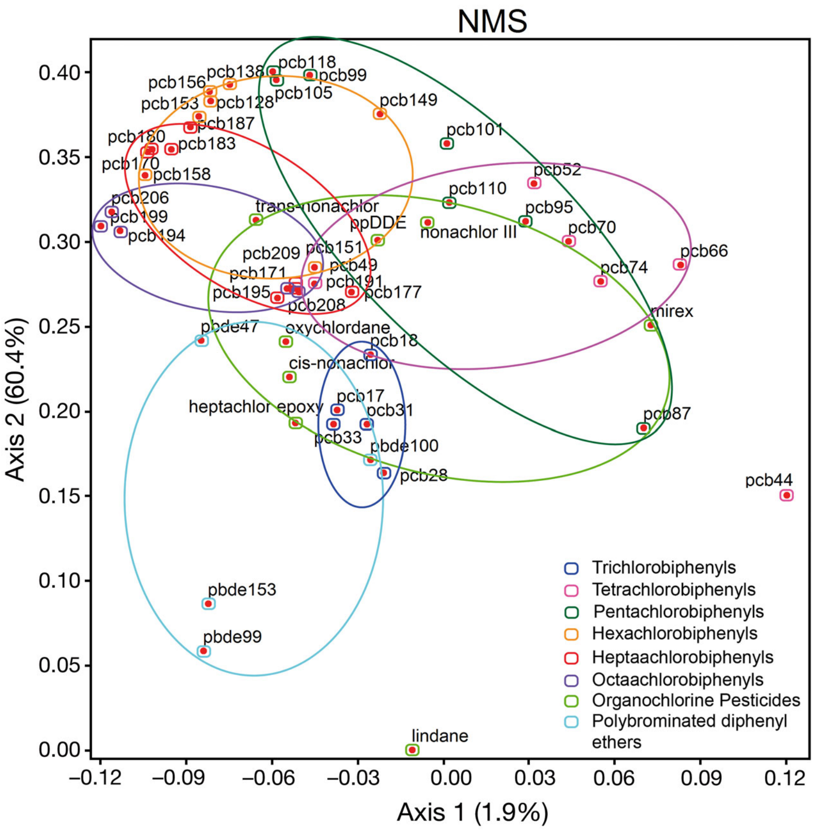


Fig. 3. Ordination plot for all POPs, color-coded by type and chlorination pattern (for polychlorinated biphenyls). The ellipses indicate groups of POP type

### Geographic variation in POP level

Concentrations of summed POPs compared between islands showed that those seals with home ranges adjacent to Oʻahu had significantly higher levels of ΣPCBs and ΣPBDEs than those seals with home ranges adjacent to Molokaʻi and Kauaʻi. This may indicate that the prey that compose the monk seal diet are more highly contaminated with PCBs and PBDEs around the more urbanized island of Oʻahu compared with the less industrialized islands of Kauaʻi and Molokaʻi. The differences between islands were not significant for pesticide POP groups (ΣDDT and ΣCHLD).

The differences in serum PCB and PBDE levels observed for Hawaiian monk seals with different ranges in the current study are likely the result of the variation in POP levels in the prey species between the different islands rather than a variation in the species composition of the diet or metabolic factors. A difference in pesticide POP groups (DDT and CHLD) was likely not seen because most of the main Hawaiian Islands have a history of agriculture and pesticide use (ATSDR 1995, Brasher & Anthony 2000, ATSDR 2002, Costa & Giordano 2007). Additionally, these compounds are highly ubiquitous, not only throughout the Hawaiian Islands, but in other areas of the tropics. They can be found in measurable quantities in areas that have only remote sources since they can be transported globally (Tanabe et al. 1994, Bard 1999).

Seals that spent most of their time around Oʻahu had higher levels of PCBs and PBDEs in their serum, and, historically, the island of Oʻahu has had more industry and known use of many more of the POP compounds included in this study compared with other Hawaiian Islands. Oʻahu is currently the only Hawaiian Island which has Superfund sites (locations identified by the Environmental Protection Agency as areas that require long-term response to clean hazardous materials or contamination). These sites include the Pearl Harbor Naval Complex and multiple naval communication sites where there has been a history of military activities that included PCB transformer sites and disposal of organic pollutants and metals (US EPA 2013).

Variation in diet has been assessed between the NWHI and the MHI, but not within MHI locations (Cahoon et al. 2013). Likewise, no stable isotope data for HMS have been published to determine if seals from different locations are feeding on different trophic level species, which could influence the POP levels they are being exposed to through diet. However, foraging behaviors (i.e. depth, trip duration, habitat use) are similar within MHI seals, and no large difference was found between the diets of MHI and NWHI seals (Goodman-Lowe 1998, Cahoon et al. 2013), indicating that it is unlikely that seals on Oʻahu are consuming different prey than those from other MHI locations.

Geographic variation in POP levels due to varying overlap with anthropogenic activities in different regions has been previously documented in other pinniped species. For instance, Ross et al. (2004) found differences in PCB, polychlorinated dibenzodioxin (PCDD), and polychlorinated dibenzofuran (PCDF) levels in harbor seal blubber in the Pacific

Northwest region of North America based on the types of industrial activities in different regions. Loughlin et al. (2002) detected similarities in PCB levels based on foraging range of northern fur seals *Callorhinus ursinus* in the Pribilof Islands. Geographic variation was also found for California sea lions *Zalophus californianus* along the coast of northern Baja California and California. A decreasing north–south gradient was seen in chlorinated hydrocarbons, likely reflecting the same gradient in California sea lion prey species (Del Toro et al. 2006).

### Variables associated with POP level

It is unclear why significant differences were found in the level of DDT between males and females, but were not found in other POPs. Other POP groups showed a difference, with males having higher levels of chlordanes, PCBs, and PBDEs than females, but the difference was not found to be statistically significant at an α-level of 0.05. Males are expected to have higher levels than females, particularly after reaching reproductive age, because females have the ability to offload POPs through lactation.

In contrast to the initial analysis of POP levels in seals of different age classes and sex, individual compounds and congeners were used in the NMS analysis to investigate shared gradients and geographic patterns associated with different islands and watershed characteristics. The bulk of the geographic variability captured by the NMS was related to the island. The core areas and home ranges with high proportions of area adjacent to urban land-use districts were grouped with O‘ahu watersheds because this island is characterized by the highest proportion of coastal urban land-use districts. Conversely, those core areas and home ranges with high proportions of area adjacent to rural and agricultural land-use districts were grouped with the watersheds of Moloka‘i (Fig. 2).

There was little geographic variation in POP levels at the watershed scale since the characteristics of the watersheds adjacent to the seal habitats were fairly similar within each island. Thus, there were few differences in the Kendall correlations with the ordination axes amongst watersheds from the same islands. This finding reinforces the notion that large-scale atmospheric transport may be primarily responsible for some of the observed POP levels rather than localized point sources.

Although this portion of the study was mainly exploratory in nature due to limitations in available data, it provided an initial assessment of potential sources of POPs to monk seal home ranges and core areas. We considered including additional variables in this analysis such as water quality parameters and sewage outflow. These were not, however, included due to lack of availability or poor spatial coverage, resulting in incomplete overlap with the home ranges and core areas. To further investigate the influence of watersheds, independent of the corresponding island, future studies could examine specific watershed attributes such as rainfall, stream flow, human population, land use within each watershed, presence of livestock, and water quality.

The current study integrated the data from spatial habitat use patterns and serum POP levels of 20 HMS tracked within the MHI. The knowledge gained from this study can be beneficial in a number of ways. First, these results represent an important baseline for HMS health monitoring in the future as this important population continues to grow. It is believed that POPs do not presently represent a direct or immediate threat to the HMS population in the MHI (Lopez et al. 2012). However, this study has identified methods that will allow managers to utilize this fine-scale geographical information to assess the POP levels and health status of seals in the future, should indicators arise of increasing POP levels in the ecosystem. These findings may be able to inform current and future management actions by helping to identify locations and environmental conditions associated with high and low pollutant levels in the HMS within the MHI in combination with other geographically variable threats. This information can help to identify the seals most at risk of POP contamination and its associated effects, based on the seals' typical geographic home range. For instance, if an individual seal is found to have health issues, it can be determined whether POPs may be playing a role as a result of that individual's geographic home range. Finally, this study has identified methods that may be used in the assessment of the risk of POPs to other species in the Hawaiian Islands and other locations at a variety of geographic scales.

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