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INFLUENCE OF FORAGING ECOLOGY AND BODY CONDITION ON CONTAMINANT BIOACCUMULATION IN A TOP MARINE PREDATOR

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ECOLOGY AND EVOLUTIONARY BIOLOGY

by

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ABSTRACT

Influence of foraging ecology and body condition on contaminant bioaccumulation in a top marine predator

by

Sarah E. H. Peterson

Environmental contaminants are a continued threat to marine wildlife because they are globally dispersed, bioaccumulate in top predators, and can disrupt physiological pathways, thus leading to adverse health effects. For adult marine mammals, the main source of contaminants is through their diet, therefore foraging behavior, including geographic location, foraging depth, and prey type, may exacerbate or mitigate contaminant exposure. Fluctuating body condition can also significantly affect contaminant concentrations in multiple tissues and thus temporally influence individual toxicological risk.

My dissertation provides an extensive analysis of the assimilation of persistent organic pollutants (POPs) and mercury into the mesopelagic-foraging northern elephant seal. My research integrates foraging behavior (movements, diving, and stable isotopes), physiology (reproductive or molting state and measurements of general body condition), and demographics (age and sex), to understand how these factors influence contaminant dynamics.

Northern elephant seals, *Mirounga angustirostris*, spend the majority of their lives at sea foraging in the mesopelagic (200 – 1000 m depths) North Pacific

Ocean, hundreds to thousands of kilometers from the coastline of North America. Such remoteness creates challenges to understanding the ecosystem where they forage. However, elephant seal life history brings them to shore predictably twice each year for breeding and molting, at which time we can sample their tissues and attach or recover electronic tags to quantify movements and diving behavior.

Every elephant seal I sampled had detectable concentrations of PCBs, PBDEs, organochlorine pesticides and mercury, demonstrating that POPs and mercury are pervasive in the food webs of northern elephant seals. For both POPs and mercury, I showed varying levels of bioaccumulation in elephant seals related to foraging behavior across a large region of the northeast Pacific, indicating that certain regions and depths pose a heightened exposure risk of contaminants to marine predators. However, the relationship between foraging behavior and contaminant bioaccumulation differed among individual compounds. Because predators integrate contaminants from their foraging areas, geographical variability in predator contaminant concentrations may be a useful indicator of the ecotoxicological risk of these contaminants to cryptic or threatened marine predators that live in deep ocean food webs of the North Pacific Ocean.

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The entirety of the research I present in this dissertation took place at the Año Nuevo Colony and it took a literal army of people to help me carry out this research. I am so appreciative to everyone who helped with the elephant seal research program. Specifically, I want to thank the core Año Nuevo field crew, including Chandra Goetsch, Dr. Luis Hückstädt, Liz McHuron, Dr. Patrick Robinson, Mike Tift, and Taiki Adachi (my Japanese PhD student partner in crime who traveled halfway around the world and joined me for every single elephant season). I also want to thank Drs. Melinda Fowler and Cory Champagne

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Many individuals contributed key pieces to this research through USGS, the Toxicological Group at the University of Antwerp, and the Stable Isotope group at UC, Santa Cruz. I am appreciative of Dr. Mark Herzog, Robin Keister, and Trevor Watts for assistance in mercury analysis. Drs. Alin Dirtu and Govindan Malarvannan taught me how to conduct POP analysis in Belgium. I am also appreciative of Caroline Louis for showing me how to run serum lipid analysis. Many thanks to Dyke Andreason, Timothy Lambert, Colin Carney and Rob Franks for their guidance in stable isotope prep and analysis.

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Chapter 3. Peterson SH, Peterson MG, Debier C, et al (*Accepted*) Deep ocean foraging northern elephant seals bioaccumulate persistent organic pollutants. Science of the Total Environment.

INTRODUCTION

Broad context

Environmental contaminants are some of the most insidious threats to human and wildlife health because they can disrupt numerous physiological pathways (Eisler 1987, Ross et al. 1997, Ross et al. 2009). Persistent organic pollutants (POPs), including compounds such as PCBs (polychlorinated biphenyls), DDT (dichlorodiphenyltrichloroethane) and PBDEs (polybrominated diphenyl ethers), and mercury are pervasive in their distribution and detectable in marine ecosystems far from anthropogenic sources (Braune et al. 2005, Campbell et al. 2005, Savinov et al. 2011). Health risks from contaminants such as POPs and mercury may be exacerbated in top predators since POP compounds are resistant to physical and biochemical degradation (Dietz et al. 1996, Muir et al. 1999, Braune et al. 2005), and both of these contaminant groups generally increase in concentration with increasing trophic position (Muir et al. 1999, Bentzen et al. 2008). Negative effects associated with the assimilation of POPs and mercury have been observed in a number of top marine predators (Ross et al. 1997, Muir et al. 1999, Boening 2000).

POPs and mercury accumulate and cycle through the environment from a variety of human-derived sources, including industrial emissions and volatilization from degrading waste-products (Fitzgerald et al. 2007, Yogui & Sericano 2009, Savinov et al. 2011). PBDEs, used as flame retardants in polymeric materials, are increasing in many marine habitats (Yogui & Sericano

2009) and only recently has international regulation been suggested (2009 Stockholm Convention), despite demonstrated health impacts (Ross et al. 2009, Yogui & Sericano 2009). Domestic use of DDT was banned in 1972 and manufacture of PCBs in the United States was banned in 1979, resulting in the leveling off or even decrease in prevalence within some regions. However, while DDTs and PCBs are no longer uniformly increasing in the environment, their presence in marine and terrestrial ecosystems is pervasive and they will continue to pose potential health risks through legacy effects (Loganathan & Kannan 1994, Braune et al. 2005). POP concentrations are decreasing in many geographic regions close to initial pollution sources and increasing in concentration in more remote regions, a pattern attributed to atmospheric transport and oceanic mixing (Aguilar et al. 2002, Lohmann et al. 2007). Mercury is significantly increasing in many remote regions (Monteiro & Furness 1997, Dietz et al. 2009, Dietz et al. 2011) such as the Arctic, where 94.2% of the mercury in biota is estimated to be anthropogenic in origin (Dietz et al. 2009). Anthropogenic atmospheric emissions occur in the form of inorganic mercury, including the gaseous elemental form (Hg^0), which can be oxidized to Hg^{II} and deposited on the ocean, where it subsequently can be converted to organic mercury (methylmercury) and then become integrated into oceanic food webs (Fitzgerald et al. 2007, Blum et al. 2013). Additionally, the deep ocean can serve as a sink for POPs (Nisbet & Sarofim 1972, Lohmann et al. 2007, Takahashi et al. 2010) and mercury

(Sunderland & Mason 2007, Blum et al. 2013), where these compounds then enter deep-ocean food webs.

Exposure risks from environmental contaminants to long-lived animals are difficult to assess; however, negative effects of high concentrations of POPs and heavy metals have been clearly demonstrated across a range of species. Specifically, high concentrations of POPs in wild bottlenose dolphins (*Tursiops truncatus*) strongly correlated with decreased immune system function, increased anemia, and a decrease in thyroid hormones (Schwacke et al. 2012). High DDT concentrations were strongly implicated as a potential cause of premature births in California sea lion (*Zalophus californianus*) females from San Miguel Island, California (DeLong et al. 1973). High doses of mercury fed to captive harp seals (*Pagophilus groenlandicus*) resulted in hepatic dysfunction, renal failure, and eventual death (Ronald et al. 1977). Black-footed albatross (*Phoebastria nigripes*) with relatively high concentrations of mercury showed decreased macrophage phagocytosis, a sign of a compromised immune system (Finkelstein et al. 2007).

Despite the challenges of linking animal health with most environmentally relevant contaminant concentrations, evidence is mounting that persistent exposure to low contaminant levels of POPs and heavy metals may significantly affect the endocrine system and thus reproductive hormones (Reijnders 1986, Subramanian et al. 1987, Bishop et al. 1998), neural or immune system function (de Swart et al. 1996, Basu et al. 2009, Dietz et al. 2011, Schwacke et al. 2012), and even survival (Hall et al. 2009). Relatively low concentrations of mercury in

polar bears (*Ursus maritimus*) negatively altered brain neurochemistry (Basu et al. 2009). Contaminant effects on physiological pathways could have cascading effects and translate into population consequences. For example, compromised immune systems can leave otherwise healthy animal populations vulnerable to pathogens (de Swart et al. 1996, Schwacke et al. 2012). This was demonstrated when increased viral susceptibility caused by contaminants was suspected as a contributing factor in the 1988 deaths of more than 20,000 harbor (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) in Europe from phocine distemper (Das et al. 2003) and the 1990 deaths of more than 700 striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea from a morbillivirus (Aguilar & Borrell 1994a). Quantifying geographic variability in contaminant concentrations and potential exposure is necessary to assess short and long-term health risks of contaminants to individuals and populations.

Prey is considered to be the major source of POPs and heavy metals to adult marine mammals (Das et al. 2003, Campbell et al. 2005); therefore, contaminant exposure for individual predators depends on contaminant levels within their food web. Contaminant levels within food webs can vary geographically, with depth, and with trophic position. Mid- and upper-trophic level epipelagic (0 – 200 m) northeast Pacific predators including albatross (*Phoebastria* spp), humpback whales (*Megaptera novaeangliae*), and Steller sea lions (*Eumetopias jubatus*) displayed geographic variation in total PCBs and mercury (Finkelstein et al. 2006, Elfes et al. 2010, Castellini et al. 2012). Different POP compounds have varying

sources, transport potential, and environmental legacies (Lohmann et al. 2007). In addition to geographic differences in overall contaminant concentrations, ratios of different POPs to each other, such as PCBs:DDE (dichlorodiphenyldichloroethylene: the primary DDT metabolite), or the proportion of different congener "families" (number of chlorine or bromine atoms) making up the total concentration of compounds like ∑PCBs may also vary geographically, based on the distance from contaminant sources. PCBs:DDE varied along a latitudinal gradient in harbor porpoise along the western United States (Calambokidis & Barlow 1991), and the results provided valuable insight into harbor porpoise feeding ecology and proportional levels of coastal contamination by different POPs. DDT was manufactured in southern California and is still detectable in higher concentrations within the southern California Bight (Blasius & Goodmanlowe 2008). More chlorinated or brominated congeners may be less common in food webs farther from a point source than congeners with fewer chlorine or bromine atoms (Ross et al. 2004) due to the increased difficulty of atmospheric or oceanic transport of "heavier" congeners.

Whereas it is clear that foraging behavior influences contaminant exposure to marine predators, many studies have focused on coastal and epipelagic species and less so on mesopelagic (200 – 1000 m) food webs. It is possible that mesopelagic predators may have a greater risk of contaminant exposure than previously considered. POPs, including PCBs and DDTs, were detected in mesopelagic fish sampled in the western Pacific (Takahashi et al. 2010), at

concentrations comparable with those quantified in the blubber of some epipelagic-foraging predators (Barron et al. 2003, Elfes et al. 2010). Mercury profiles in seawater from the Atlantic and Pacific revealed peaks of mercury within the mesopelagic zone (Gill & Fitzgerald 1988, Laurier et al. 2004, Fitzgerald et al. 2007), potentially as a function of particle scavenging that occurs at depth and the conversion of inorganic mercury to bioavailable methylmercury (Fitzgerald et al. 2007, Blum et al. 2013). The highest mercury concentrations in large mesopelagic fish sampled near Hawaii (Choy et al. 2009) were observed in fish sampled from the same depths as the peak mercury concentrations observed in seawater profiles. Mercury in north Atlantic birds foraging on vertically migrating prey that feed within the mesopelagic zone experienced a 4.8 percent/year increase in mercury concentrations over the last 100 years (Monteiro & Furness 1997). Models of global mercury cycling suggest there may be a time lag upwards of several centuries before atmospheric and oceanic equilibration for current levels of anthropogenic mercury emissions in the Pacific (Sunderland & Mason 2007). Together, these studies suggest that mesopelagic predators may have an elevated risk of mercury exposure that will increase if mercury continues to rise in these oceanic habitats.

POPs and mercury assimilate in organisms through a variety of pathways and accumulate within separate storage reservoirs in the body. POPs are lipophilic and accumulate mostly within adipose tissue (Wang et al. 2010, Yordy et al. 2010, Storelli et al. 2012), whereas mercury is protein binding and primarily

accumulates within blood, muscle, keratinous tissues such as hair or nails, and major organs such as the liver and kidney (Wagemann et al. 1998, Woshner et al. 2001, Brookens et al. 2008, Agusa et al. 2011a, Agusa et al. 2011b), and only at low concentrations within blubber (Jones et al. 1976, Agusa et al. 2011b, Monk et al. 2014). POPs associated with lipids are transported through the body in serum and are mainly stored within the adipose tissue (Yordy et al. 2010, Agusa et al. 2011b). During lactation, POPs can move from blubber to serum and subsequently be incorporated into lipid rich milk, which is the main mechanism for offloading POPs from mother to offspring, although a small component of placental transfer also occurs (Debier et al. 2003, Vanden Berghe et al. 2012). Each congener has an octanol-water partitioning coefficient (K_{ow}) value, which is inversely related to the solubility of the congener in water. Congeners with higher K_{ow} values are increasingly non-polar and thus have an affinity to stay bound up in adipose tissue (Vanden Berghe et al. 2012). Congeners with increased chlorination and higher log K_{ow} comprised a greater proportion of PCBs in maternal harbor seals (Wolkers et al. 2004), grey seals (Debier et al. 2003, Sørmo et al. 2003), harbor porpoises (*Phocoena phocoena*) (Weijs et al. 2009), and belugas (*Delphinapterus leucas*) (Desforges et al. 2012) than their offspring, which could be a result of changing congener profiles with age. Older females have likely given birth to more pups (increased parity) and therefore had more opportunities to depurate (offload) congeners with lower log K_{ow} values, while selectively retaining congeners with higher K_{ow} values. In contrast, mercury is

primarily transferred to offspring via the placenta while the fetus is still in the womb, although transfer does continue at low levels through lactation (Habran et al. 2011). Although POPs and mercury are transferred to offspring through different pathways, they are both transferred during crucial periods of offspring growth and development. High doses of POPs and heavy metals negatively affect fetal neural and endocrine development in rats (Gauger et al. 2004), although it is difficult to conclusively determine effects of exposure to lower contaminant dosages. Different transfer routes to offspring (placenta versus milk) and contaminant-specific partitioning into different body tissues makes it crucial to appropriately sample the correct tissues for study of a specific contaminant and determine how body condition and reproductive state affect observed tissue concentrations.

Individuals within a population may experience disproportionate vulnerability to the effects of contaminants because of variability in demographic and physiological parameters; however, these factors are rarely examined concurrently in healthy, free-ranging marine animals. Body composition, quantified as the percent of adipose tissue, has a significant, negative correlation with contaminant concentrations, and fluctuations in adipose tissue as a result of prolonged fasting can increase contaminant concentrations in remaining tissues (Polischuk et al. 1995, Cameron et al. 1996, Debier et al. 2006, Yordy et al. 2010, Debier et al. 2012, Myers & Atkinson 2012, Vanden Berghe et al. 2012). POP concentrations are often higher in males than females because females are able to

pass contaminants to offspring while males are unable to offload any of their accumulated contaminant burden (Addison et al. 1986, Barron et al. 2003, Wang et al. 2007, Storelli et al. 2012). The lack of depuration by males can result in a positive relationship between age and contaminant concentrations, although the same trend does not tend to be observed for female marine mammals. Adult female fin (*Balaenoptera physalus*) and pilot whales (*Globicephala melas*) had stable or declining blubber PCBs with age (Aguilar & Borrell 1994b, Borrell et al. 1995). Non-significant age relationships with POP concentrations were observed for many female pinnipeds, including harbor seals (Wang et al. 2007), Steller sea lions (Barron et al. 2003) and ringed seals (*Pusa hispida*) (Addison et al. 1986), suggesting these species annually offload contaminants to offspring, mostly during lactation, and subsequently ingest more contaminants while feeding over the remainder of the year. Conversely, the relationships between age and mercury concentrations are contradictory in female marine mammals. Some studies, of cetaceans and pinnipeds, observed positive increases in mercury concentration with age (Wagemann et al. 1983, Gaden et al. 2009), whereas other studies of marine mammals found no significant relationships with age (Ikemoto et al. 2004, Woshner et al. 2008, Cardona-Marek et al. 2009, Agusa et al. 2011a).

Northern elephant seals (*Mirounga angustirostris*) are an ideal species to investigate how contaminant bioaccumulation may be influenced by foraging ecology and body condition. Elephant seals, as a species, forage in distinct open-ocean regions, including the remote open-ocean Pacific Subarctic Gyre (Springer

et al. 1999, Le Boeuf et al. 2000, Robinson et al. 2012), as well as near-coastal regions (Le Boeuf et al. 2000, Simmons et al. 2010, Robinson et al. 2012). The northern elephant seal uses four hydrographic ecoregions within the eastern North Pacific, as defined by Longhurst (1998), and is the only pinniped species in these ecoregions that forages in the mesopelagic zone. Individual elephant seals show high geographic fidelity to specific foraging regions within the North Pacific (Simmons 2008), which suggests that individual contaminant bioaccumulation can be attributed to a particular region with increased confidence. Adult elephant seals return to land only two times each year and go through extended fasting periods, one of which is associated with breeding and the other with molting (Le Boeuf et al. 2000, Robinson et al. 2012). Adipose and protein stores are metabolized differently during these two fasts (Costa et al. 1986, Worthy et al. 1992), suggesting that the dynamics of lipophilic POPs and protein binding Hg may be affected differently during the two fasting periods. Additionally, elephant seals have been monitored at the Año Nuevo colony since 1968 and flipper tags are annually attached to several hundred pups, providing a consistent subset of known-age animals within the population, with individual reproductive histories for female seals.

Previous research documented the presence of POPs and mercury in elephant seals from the Año Nuevo colony (Debier et al. 2005, Debier et al. 2006, Habran et al. 2011), thus we know these contaminants bioaccumulate in elephant seals. However, variability in contaminant concentrations among northern elephant

seals has never been quantified relative to foraging behavior, age, multiple fasting states (breeding and molting), or sex of healthy, adult animals. The links between foraging ecology and contaminant bioaccumulation have ecological implications because the foraging range of northern elephant seals overlaps with numerous mesopelagic marine predators, including cetaceans, sharks, and tuna (Block et al. 2011, Pompa et al. 2011). It is significantly more challenging to study many of these overlapping species, due to logistics, difficulty in using non-lethal sampling to obtain life-history parameters, and the fact that many of these species spend the entirety of their lives at sea. Therefore, elephant seals can serve as a sentinel of contaminant presence in these deep-ocean habitats.

Dissertation outline

My dissertation provides an extensive analysis of the assimilation of POPs and mercury into the mesopelagic-foraging northern elephant seal. My research integrates foraging behavior (movements, diving, and stable isotopes), physiology (reproductive or molting state and measurements of general body condition), and demographics (age and sex), to understand how these factors influence contaminant dynamics.

For **Chapter 1**, I examined whether individual seal tissue concentrations and congener profiles were different among the start and end of the breeding and molting fasting periods, and whether age related to total PCBs or the proportion of different congener profiles. By sampling at the start and end of the biannual

foraging trips (four sampling times), I quantified how contaminant concentrations fluctuated in different tissues of a wild marine mammal species across four distinct, annually occurring fasting states. I was able to examine seals at the start and end of one fasting period when females are offloading contaminants to offspring and a second fasting period when females lack the pup depuration and lactation offloading mechanisms. This was an unprecedented study in a wild animal that examined how changes in body condition associated with and without the presence of lactation (for females) affected varying tissue contaminant concentrations. This research also contributed significantly to our understanding of non-lethal contaminant monitoring and tissue concentrations.

In **Chapter 2**, I examined the bioaccumulation of mercury in the blood and muscle of adult female northern elephant seals in relation to foraging behavior, over both the long and the short annual foraging trips. To do so, I integrated satellite tracks, dive records, and stable isotope values (carbon and nitrogen) from the trip with mercury concentrations obtained at the end of the foraging trip. This enabled me to examine which variables describing foraging behavior best explained variability in mercury concentrations. Additionally, I clustered seals into groups of foraging behaviors and tested for broad-scale differences in contaminant bioaccumulation among foraging behavior groups.

For **Chapter 3**, I quantified eight different POP compounds in the blubber and blood (serum) of adult female and male elephant seals, with four main objectives. First, I described the concentrations of all compounds and compared

concentrations between males and females. Second, I examined the correlations between pairs of POP compounds at the end of the ~ seven month long foraging trip, to determine which compounds demonstrate similar patterns of bioaccumulation. Next, using morphometric measurements, I estimated blubber lipid mass and scaled contaminant concentrations to the whole animal to obtain a blubber POP burden (mg POPs) for adult females. Contaminants bioaccumulate while animals forage, and I calculated changes in the blubber burden of female seals to link this directly with foraging behavior. This approach allowed me to quantify POP ingestion while on a foraging trip, which I believe has never been done before in marine mammals. Last, I quantified foraging behavior of elephant seals and examined if POP concentrations differed among clusters of seal behaviors. These observations all provide important insight into the degree of POP contamination for different regions and depths of deep ocean food webs.

Conclusion

The ultimate goal of my dissertation was to quantify tissue concentrations of persistent organic pollutants and mercury in a wide-ranging marine predator. Through this research, I demonstrated that foraging behavior significantly influenced the bioaccumulation of contaminants, although the specific relationships between foraging behavior and bioaccumulation differed among individual compounds. Mercury bioaccumulation was greatest for individuals that foraged further away from the coast at deeper depths and least for individuals that

foraged closer to the coast and in the Subarctic Gyre, whereas bioaccumulation of ∑DDTs and ∑PBDEs was greatest in seals that foraged closest to the coast. Because predators integrate contaminants from their foraging areas, this research contributes valuable information on the potential ecotoxicological risk of POPs and mercury contaminants to marine predators across a large and understudied region of the North Pacific Ocean.

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CHAPTER 1

Effects of age, adipose percent, and reproduction on PCB concentrations and profiles in an extreme fasting North Pacific marine mammal

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1.1. Abstract

Persistent organic pollutants, including polychlorinated biphenyls (PCBs), are widely distributed and detectable far from anthropogenic sources. Northern elephant seals (*Mirounga angustirostris*) biannually travel thousands of kilometers to forage in coastal and open-ocean regions of the northeast Pacific Ocean and then return to land where they fast while breeding and molting. Our study examined potential effects of age, adipose percent, and the difference between the breeding and molting fasts on PCB concentrations and congener profiles in blubber and serum of northern elephant seal females. Between 2005 and 2007, we sampled blubber and blood from 58 seals before and after a foraging trip, which were then analyzed for PCBs. Age did not significantly affect total PCB concentrations; however, the proportion of PCB congeners with different numbers of chlorine atoms was significantly affected by age, especially in the outer blubber. Younger adult females had a significantly greater proportion of low-chlorinated PCBs (tri-, tetra-, and penta-CBs) than older females, with the

opposite trend observed for hepta-CBs, indicating that an age-associated process such as parity (birth) may significantly affect congener profiles. The percent of adipose tissue had a significant relationship with inner blubber PCB concentrations, with the highest mean concentrations observed at the end of the molting fast. These results highlight the importance of sampling across the entire blubber layer when assessing contaminant levels in phocid seals and taking into account the adipose stores and reproductive status of an animal when conducting contaminant research.

1.2. Introduction

Persistent organic pollutants such as polychlorinated biphenyls (PCBs) are harmful to wildlife because they can disrupt endocrine and immune function [1-3]. These effects are especially pronounced in top predators, including marine mammals, because persistent organic pollutants accumulate in adipose tissue and biomagnify with increasing trophic position [4]. The chemical and physical structure of PCBs allows these compounds to persist in the environment long after leaving human-derived sources and, as a result, they are detected in remote regions of the world [5,6]. In general, worldwide, manufacture of PCBs ceased from 1972 – 1984, with the most recent PCB cessation occurring in Russia in 1993 [7]. PCBs are no longer uniformly increasing in the environment [6,8], but their presence in marine and terrestrial ecosystems is widespread.

Demographic and physiological parameters such as age, sex, and adipose stores may affect persistent organic pollutant concentrations. Variability in concentrations within a population may cause certain individuals to be more at risk from the negative effects of persistent organic pollutants. The percent of adipose tissue often has a significant, negative correlation with contaminant concentrations, and fluctuations in adipose tissue as a result of prolonged fasting can increase contaminant concentrations in remaining tissues [9-15]. Persistent organic pollutant concentrations are often higher in males than females because females pass contaminants to offspring while males are unable to offload their contaminant burden [16-18]. For marine mammals, lack of depuration by males results in a positive relationship between age and contaminant concentrations, whereas reproductively active females do not demonstrate the same relationships between age and total contaminant concentrations. Insignificant age trends were observed for many reproductively aged female pinnipeds (seals, sea lions and the walrus), including harbor seals (*Phoca vitulina*) [17], Steller sea lions (*Eumetopias jubatus)* [16] and ringed seals (*Pusa hispida)* [18], suggesting that these species annually offload contaminants to their offspring and subsequently accumulate new contaminants while feeding over the remainder of the year. Conversely, adult female cetaceans, including fin whales (*Balaenoptera physalus*), pilot whales (*Globicephala melas*), Dalls porpoise (*Phocoenoides dalii*), and killer whales (*Orcinus orca*) had declining PCBs in blubber with increasing age [19-22], although that relationship may change after reproductive

senescence [22,23]. However, both pinnipeds and cetaceans do not equally transfer all congeners to their offspring. Offspring are observed with a higher proportion of low-chlorinated congeners than their mothers [14,24-27], which may affect congener profiles in adult females over time.

The major source of contaminants to marine mammals is from their food [4,28]. Therefore, PCB concentrations in wildlife may depend on both foraging location and trophic position. Within similar trophic levels, previous studies showed foraging location to be important. Bottlenose dolphins (*Tursiops truncatus*) foraging adjacent to point-sources of PCBs showed higher concentrations than bottlenose dolphins foraging farther from point sources [29]. Mid-trophic level predators from the northeast Pacific Ocean, including albatrosses (*Phoebastria* spp.), humpback whales (*Megaptera novaeangliae*), killer whales, and harbor seals displayed geographically-associated variability in total PCB concentrations [22,30-33]. The majority of animals from these studies were epipelagic (0-200 m depth) marine predators that also forage within the narrow margin of the continental shelf. Little is known about PCBs in mesopelagic (200-1000 m depth) open-ocean food webs and their effect on predators in these systems.

Assessing contaminants in free-ranging, open-ocean marine predators is complex because demographic parameters, estimates of adipose percent, and indices of foraging behavior are difficult to concurrently obtain from wild and visibly healthy marine predator populations. Northern elephant seals (*Mirounga*

angustirostris) are unique in that all of these variables can be quantified when they arrive on land. They have been monitored at the Año Nuevo colony (California, USA) since 1968, where flipper tags are attached to several hundred pups annually, providing a consistent subset of known-age animals within the population that can be used to investigate relationships between age and contaminant concentrations. Northern elephant seals are long-lived, mesopelagic, high-trophic level predators. Based on diving behavior [34,35], jaw-motion recorders paired with cameras [36], and stomach content analysis [37], northern elephant seals are hypothesized to consume mesopelagic fish and squid. However, little is conclusively known about their diet, since they return from their foraging migrations with nearly all prey completely digested.

Tracking data show that individual elephant seals from the Año Nuevo colony forage in distinct open-ocean regions, including the remote Pacific subarctic gyre [34,35,38], and near-coastal regions [34,35,39]. The northern elephant seal uses four hydrographic ecoregions within the northeastern Pacific, as defined by Longhurst [40], and is the only pinniped species in these ecoregions that consistently forages in the mesopelagic zone. Sampling a large number of individuals could capture the variability in contaminant concentrations between individuals that may utilize varying foraging strategies and obtain an appropriate range of contaminant concentrations for the population.

Elephant seals are also ideal for examining the relationships between adipose percent and contaminant concentrations during the extreme fasting periods they

exhibit on land associated with breeding and molting. Many marine predators go through annual periods of fasting, often associated with long migrations, and the associated mass loss could significantly affect contaminant concentrations in different tissues. Elephant seals return to land from their biannual foraging migrations to breed and molt [34,35]. While on land for breeding/lactation or molting, elephant seals cease feeding and go through extreme reductions in adipose stores, losing up to 40 % of their body mass [41]. Previous studies of PCB concentrations in stranded northern elephant seals along the California coast [42,43] demonstrated the capacity for PCBs in elephant seals to reach concentrations of toxicological concern [44]; however, we still do not know how concentrations fluctuate in healthy, wild animals throughout the year in relation to the naturally occurring extremes of adipose percent. The unique life history strategy of elephant seals, with separate fasting periods for breeding/lactation and molting, can allow us to disentangle the effects of fluctuating adipose percent with the effect of maternal offloading of contaminants. Elephant seal females begin reproducing between the ages of three and six [45]. Females have not been observed to go into reproductive senescence like many cetaceans and average natality is over 80% [35].

Our study builds on the findings of previous research [9,13,46] by incorporating adipose percent estimates for known-age adult females during the breeding and the molting fast. The main objectives of our study were to examine the potential effects of age, adipose percent, and reproductive state (breeding fast

versus molting fast) on PCB concentrations and congener profiles in blubber and serum of northern elephant seal females. By investigating these parameters concurrently we hypothesized that 1) age would not affect total PCB concentrations but would affect congener profiles, resulting in older females with higher chlorinated congeners than younger seals (lower chlorinated congers may be more easily depurated to offspring), 2) total PCB concentrations would increase with decreased adipose percent, and 3) seals would have higher concentrations at the end of molting than at the end of breeding due to the lack of depuration to offspring during the molt.

1.3. Methods

1.3a. Animal sampling

Adult female northern elephant seals (N=58) with no visual health impairments, were selected for tissue sampling at Año Nuevo State Reserve in San Mateo County, California, USA between 2005 and 2007. Most seals (N=54) were known-age individuals between 4 – 17 years old, identified by a uniquely numbered, plastic tag (Dalton jumbo Roto-tags, Oxon, UK) placed in a lobe of their hind flipper. Female elephant seals have two distinct periods of fasting on land (breeding/lactation and molting) between foraging trips (Fig 1.1). The annual breeding fast (January – February) is followed by a short foraging trip (February – April). This is followed by a molting fast (May – June), which precedes a long foraging trip (June – January). These foraging trips are hereafter referred to as the

short and long trips. Individual seals were sampled for the first time at the end of one of the fasting periods (late breeding or late molting) just prior to departure to sea. Those seals that completed the foraging trip and returned to land were then sampled for a second time at the start of the next fasting period (Fig 1.1). Not all seals returned from the foraging trip; therefore pre-and post-foraging trip samples were paired for only some animals (Table 1.1). Seals were handled a maximum of two times during this study. Seals were chemically immobilized using standard protocols while morphometric measurements were taken and tissue samples (blubber and blood) were collected [34,39]. Full thickness blubber cores were collected using sterile, single-use 6 mm biopsy punches (Miltex, Inc., York, Pennsylvania, USA) from the lateral pelvic area, wrapped in aluminum foil marked to identify inner and outer ends of the sample, stored on ice in the field, and frozen at -20 °C until analysis [9]. Blood samples from the extradural vein were collected in 10 mL serum vacutainers and stored on ice in the field. At the laboratory, serum was separated by centrifugation and stored in cryovials at -20 °C until analysis.

All 58 adult females were included in our summary of PCB concentrations (Table 1.1). Only known age females with mass and estimates of adipose percent that reproduced normally were included in our statistical analyses (N = 50 seals). Not every seal had usable samples for all tissue types; therefore, subsets of the 50 seals were used to analyze PCB concentrations in inner blubber, outer blubber, and serum (Table 1.1). Paired late fasting (pre-foraging trip) and early fasting

(post-foraging trip) samples of at least one tissue type (inner blubber, outer blubber or serum) were collected from 27 of the seals.

Girth and length measurements were taken at eight locations along the seal, including six locations where blubber thickness was measured dorsally, laterally and ventrally using a handheld ultrasound backfat meter (Scanoprobe, Ithaca, New York, USA) [35]. Body mass was determined by rolling the seal onto a canvas sling and using a hand-winch to suspend them from a 1000 ± 1.0 kg Dyna-Link digital scale attached to a metal tripod [35]. The percent of adipose tissue was estimated using the morphometric measurements and a truncated cones technique [47,48].

1.3b. Chemical analyses

Blubber biopsies were cut into three equal parts. Inner (closest to the muscle) and outer (closer to the skin) blubber layers were analyzed separately in 2008 at the University of Liege. Using a Thermo Quest Trace 2000 gas chromatograph coupled with a ^{63}Ni ECD (Thermo Quest, Trace 2000, Milan, Italy), serum and each section of blubber biopsy were analyzed separately for 22 PCB congeners (IUPAC 28, 44, 70, 87, 95, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194, 195, 206, and 209). Details of sample preparation, clean-up, and analysis, including quality assurance, are provided in Debier et al. [49]. The limit of detection (LOD) was fixed at a level three times the background noise of the chromatogram. The limit of detection of PCB congeners was therefore 0.006 ng g^{-}

^{1} (ppb) of serum fresh weight, 2 ng g^{-1} (ppb) of serum lipid weight and 0.7 ng g^{-1} (ppb) of lipid weight of adipose tissue in our analytical conditions. The limit of quantification (LOQ) of PCB congeners was determined by means of spiked bovine serum and bovine fat samples used as quality control (QC) and was the lowest concentration which could be quantified and which showed a recovery range between 70 % and 130 % [50]. This concentration also corresponds to at least 10 times the background noise of the chromatogram. In these conditions, the LOQ for each PCB congener was 0.03 ng g^{-1} (ppb) of serum fresh weight, 10 ng g^{-1} of serum lipid weight and 2.5 ng g^{-1} of lipid weight in adipose tissue. Congeners falling below the LOQ for elephant seal samples were recorded as measured concentrations. When a congener concentration was recorded as zero, this means that the congener was not detected at all in the sample. Blubber concentrations were quantified as ng g^{-1} lipid, while serum samples were quantified both by unit of serum lipid (ng g^{-1} lipid) and unit of whole serum (ug L^{-1}). Lipids in serum were quantified as described in Vanden Berghe et al. [14]. In summary, different lipid classes (total cholesterol, phospholipids, triacylglycerols and nonesterified fatty acids) were quantified using enzymatic kits from Diasys (Diasys Diagnostics System, Holzheim, Germany) and Wako (Wako Chemicals USA Inc., Richmond, Virginia, USA). Concentrations of each lipid class were calculated using kit-specific recommendations, on the basis of standard equivalents. All lipid classes were added together to obtain serum total lipid concentrations.

1.3c. Statistical analyses

Total PCB concentrations: Linear mixed effects (LME) models were run using the nlme package [51] in the statistical software R (version 2.15.2 [52]), to examine effects of age and percent adipose tissue (fixed effects) on total contaminant concentrations in inner blubber, outer blubber and serum. Models were run separately for each tissue type. Blubber composition is stratified and differences in fatty acid profiles have been observed between the blubber layers [53,54]; therefore, our analysis focused on the inner and outer blubber layers. Models were run for both lipid-normalized serum ∑PCBs as well as ∑PCBs in serum measured per unit of wet weight. When models on serum ∑PCBs yielded the same trends, we only report lipid-normalized results. LME models included ∑PCB concentrations from samples collected at all time periods (Fig 1.1). Residuals of each model were plotted against fixed effects to examine deviations from homogeneous variance, and data were log-transformed or appropriate variance structures were incorporated when necessary [55]. We treated individual as a random effect to account for repeated measurements for some individuals and the model was fit using restricted maximum likelihood (REML). For tests without repeated measures from individuals, general linear models were used to compare tissue concentrations at the end of the breeding and molting fasts, to examine potential influence of life history (i.e. fasting while lactating versus fasting with

no offloading mechanism) on mean contaminant concentrations, while accounting for adipose percent.

The subset of individuals with paired pre- and post-foraging trip samples were additionally analyzed using paired t-tests to examine if ∑PCB concentrations in tissues (inner blubber, outer blubber, and serum) changed between the start and end of the short or the long foraging trip. T-tests were also used to examine whether the magnitude and direction of change in tissue concentrations were the same for both foraging trips (short versus long). The level of statistical significance for all analyses was set at $p \leq 0.05$.

PCB congener profiles: PCB concentrations were further examined at the level of specific congeners and congener families, based on the degree of chlorination. Low-chlorinated biphenyls included all congeners with three or four chlorines (tri- and tetra-CBs). The percentage of the total contaminant concentration made up by each congener family was calculated for every sample (inner blubber, outer blubber, and serum). Octa-, nona-, and deca-CBs were below the detection limit for the majority of samples, therefore statistical analyses were focused on tri/tetra-CBs, penta-CBs, hexa-CBs, and hepta-CBs. LME models were run with the percentage of each congener family as the response variable, after an arcsin square root transformation was applied [56], to examine the proportional contribution of each congener family to the total PCB concentration. Age and adipose percent were fixed effects and individual was included as a random effect.

1.4. Results

PCBs were detected in all samples collected from 58 adult female northern elephant seals, ranging from 4 – 17 years of age (Table 1.1). ∑PCB concentrations ranged from 356 – 2722 ng g^{-1} lipid in blubber and 512 – 2,837 ng g^{-1} lipid in serum (Table 1.1; Fig 1.2).

The majority of inner and outer blubber PCB profiles in female seals were comprised of eight congeners: PCB-101, -110, -118 (penta-CBs), -138, -153 (hexa-CBs), -180, -183, and -187 (hepta-CBs), all of which had a mean percent contribution of the total burden greater than 5% (Fig 1.3). These eight congeners comprised 84.9 ± 4.5 % of the total PCB concentration in inner blubber and 90.2 ± 3.6 % in outer blubber. PCB-153 was the most common congener, comprising 15.8 – 28.5 % and 14.1 – 38.8 % of the ∑PCB burden in inner and outer blubber, respectively. Hexa-CBs were the dominant congeners in blubber for all sampling periods, on average comprising >40 % of ∑PCBs. Octa-, nona-, and deca-chlorobiphenyls combined comprised less than 1.7 % of the total blubber PCB burden, and octa-CBs were the most prevalent of these high-chlorinated congener groups.

Serum PCB profiles had seven congeners that contributed a mean percent of more than 5 % of the total PCB concentration: PCB-101, -110, -118, -138, -153, -180, and -183. These seven congeners comprised 78.8 ± 5.9 % of the total serum

concentrations. PCB-153 comprised between 14.0 – 26.4 % of the total serum PCB concentrations.

1.4a. Influence of age

There was no detectable relationship between age and total PCB concentrations in inner blubber, outer blubber or serum ($p \geq 0.230$). However, age had a significant effect on congener groups (Fig 1.4; Table 1.2). There was a significant, negative relationship between age and the percent of penta-CBs ($F_{1,44} = 12.8$, $p<0.001$) and a significant, positive relationship between age and the percent of hepta-CBs ($F_{1,44} = 12.4$, $p=0.001$) in inner blubber. There was no detectable relationship between age and the percent of either tri/tetra-CBs or hexa-CBs in the inner blubber. In the outer blubber, there was also a significant, negative relationship between age and the percent of penta-CBs ($F_{1,47} = 30.1$, $p<0.001$) and a significant positive relationship between age and the percent of hepta-CBs ($F_{1,47} = 35.7$, $p<0.001$). Additionally, in the outer blubber, the percent of tri/tetra-CBs had a significant, negative relationship with age ($F_{1,47} = 15.1$, $p<0.001$) and the percent of hexa-CBs had a significant, positive relationship with age ($F_{1,47} = 15.8$, $p<0.001$). The same significant, negative relationship observed for both blubber layers between age and the percent of penta-CBs was also detectable for lipid-normalized serum ($F_{1,47} = 14.0$, $p<0.001$) and serum concentrations using wet-weight. The percent of tri/tetra-CBs also had a significant, positive relationship with age in the serum ($F_{1,47} = 7.2$, $p=0.010$),

which was the opposite trend as observed in the outer blubber layer. The percent of hexa-CBs and hepta-CBs in serum did not change with age.

1.4b. Influence of adipose percent and fasting state

Elephant seals were fattest early in the breeding season fast (mean ± SD: 34.7 ± 2.3 %; range: 30.9 – 39.9 %) and leanest late in the breeding fast (mean ± SD: 29.3 ± 1.7 %; range: 26.0 – 32.0 %). Seals sampled early in the molting fast had a mean adipose percent of 30.8 ± 2.7 % (range: 23.4 – 33.8 %) compared to 30.9 ± 2.4 % (range: 26.1 – 36.1 %) late in the molting fast, indicating that adipose and lean tissue were lost in similar proportions over the course of the molt.

The percent of adipose tissue had a significant relationship with total PCB concentrations, although this relationship was only observed for the inner blubber layer and serum measured by unit of wet weight and not for the outer blubber or lipid-normalized serum concentrations (Fig 1.5). Overall, inner blubber ∑PCB concentrations were higher for seals that were leaner ($F_{1,20} = 19.7$, $p<0.001$). Direct comparisons of late breeding and late molting, while accounting for adipose percent, revealed that seals had higher mean inner blubber ∑PCB concentrations at the end of the molt ($F_{1,26} = 13.1$, $p=0.001$) (Fig 1.6). Conversely, outer blubber ∑PCB concentrations did not have a significant relationship with adipose percent ($F_{1,21} = 2.4$, $p=0.136$), and there was no detectable difference between ∑PCB concentrations in late breeding and late molting outer blubber samples ($F_{1,28} = 1.1$, $p=0.310$; Fig 1.6).

The relationship between the percent of adipose tissue and total serum ∑PCB concentrations was dependent on whether the concentrations were analyzed using lipid-normalized or wet weight concentrations. Serum ∑PCBs measured per unit lipid initially appeared to have a significant relationship with adipose percent ($F_{1,21} = 6.1$, p=0.023); however, when the single highest serum concentration was removed from analysis (2837 ng g^{-1} lipid measured during the early molting fast) this relationship became non-significant ($F_{1,20} = 2.2$, p=0.157). Serum ∑PCB concentrations measured per unit of wet weight had a significant relationship with adipose percent even with the highest concentration removed ($F_{1,20} = 10.1$, p=0.005). There was a significant difference between mean late breeding and late molting serum ∑PCB concentrations per unit lipid ($F_{1,31} = 11.9$, p=0.002) but there was no significant difference between the late breeding and late molting serum ∑PCB concentrations per unit of wet weight ($F_{1,31} = 1.6$, p=0.213).

Adipose percent had significant relationships with some congener groups in blubber and serum (Table 1.2). Fatter seals had a significantly greater percent of tri/tetra-CBs in their inner blubber than seals with lower adipose percentages ($F_{1,19} = 13.6$, p=0.002), while the opposite was true for adipose percent and the proportion of penta-CBs in the inner blubber ($F_{1,19} = 8.3$, p=0.010). There was no detectable relationship between adipose percent and the proportion of hexa-CBs or hepta-CBs in the inner blubber. In the outer blubber, adipose percent also had a significant, positive relationship with the proportion of tri/tetra-CBs ($F_{1,21} = 12.9$, p=0.002) and a significant, negative relationship with the proportion of penta-CBs

($F_{1,21}$ = 12.6, p=0.002). Additionally, adipose percent had a significant, negative relationship with the proportion of hepta-CBs in the outer blubber ($F_{1,21}$ = 18.8, p<0.001). There was no detectable relationship between adipose percent and the proportion of hexa-CBs in the outer blubber. In serum, there was a significant, positive relationship between adipose percent and the proportion of penta-CBs ($F_{1,21}$ = 6.4, p=0.020), the opposite trend to that observed in blubber. There were no significant relationships detected between adipose percent and the proportion of tri/tetra-CBs, hexa-CBs or hepta-CBs in serum.

1.4c. Paired tissue samples

Paired tissue samples from the same individuals pre- and post-foraging revealed that changes in ∑PCB concentrations within individuals differed by tissue type and whether the change was measured across the short or long foraging trip (Fig 1.7). The concentration of ∑PCBs in inner blubber decreased across the long (t = 6.2, df = 7, p<0.001) and short trips (t = 3.3, df = 12, p=0.007); however, the magnitude of decrease was greater over the course of the long trip (t = 4.5, df = 19, p<0.001). The outer blubber layer changed in concentration across both the short (t = -2.8, df = 13, p=0.015) and long trips (t = 2.3, df = 7, p=0.05) but the direction of the change differed between the trips (t = 3.4, df = 20, p=0.003). ∑PCBs increased in outer blubber over the course of the short trip but decreased over the course of the long trip. Lipid-normalized serum ∑PCB concentrations demonstrated the same differences between foraging trips

as observed in outer blubber ($t = 4.6$, $df = 20$, $p<0.001$). Lipid-normalized serum ∑PCB concentrations increased over the course of the short trip ($t = -3.1$, $df = 13$, $p=0.008$) but decreased over the course of the long trip ($t = 3.7$, $df = 7$, p-value=0.008). Changes in ∑PCB concentrations in serum measured by unit wet weight did not significantly change across the short trip ($t = -1.9$, $df = 13$, $p=0.081$) but significantly decreased across the long trip ($t = 3.5$, $df = 7$, $p = 0.010$), and changes in ∑PCB concentrations between the trips were significantly different ($t = 4.2$, $df = 20$, $p<0.001$).

1.5. Discussion

Our findings highlight that toxic contaminants are detectable in seals that forage in remote depths of the Pacific Ocean, thousands of kilometers from anthropogenic sources. Mesopelagic and open-ocean foraging behaviors do not leave northern elephant seals immune to the accumulation of PCBs, as PCBs were detected in blubber and blood of all 58 sampled northern elephant seal females. PCB concentrations in elephant seal serum and blubber sampled during early and late breeding in the present study are similar to those previously observed in elephant seal females [13] and weaned pups [9,46] from the same breeding colony (Año Nuevo). However, the mean PCB concentration in samples collected at the end of the molting fast in our study are higher than the mean PCB concentration in samples previously collected at the end of the breeding fast [13]. Our study is the first to quantify PCB concentrations in free-ranging, female northern elephant

seals across the full range of naturally occurring body conditions and annual life history phases. Our results demonstrate the importance of accounting for both adipose percent and reproductive state when interpreting contaminant concentrations.

Quantification of ∑PCBs in marine mammals varies somewhat based on the number of congeners that are quantified in each study, which makes direct comparison of ∑PCBs or the percent of individual congeners difficult between studies. Additionally, the reporting of individual congeners varies between study, making one to one comparisons difficult. Nevertheless, some broad comparisons can be made between our observations and other published studies, realizing that the absolute values may differ somewhat based on study design. Female northern elephant seals have substantially lower PCB concentrations than stranded adult California sea lions (*Zalophus californianus*) [57], but adipose percentages are lower in most stranded animals than elephant seals from the present study. Blubber PCB concentrations in elephant seals from our study are higher than those found in free-ranging, coastally-foraging adult female harbor seals from Kodiak Island, the southern Alaska Peninsula, and Prince William Sound [17], although these harbor seals forage further north than elephant seals from the present study and there are no comparable studies for free-ranging adult harbor seals foraging along the west coast of the United States of America. Given that all seals from our study harbor PCBs, greater sampling of animals across this region is warranted, especially because of the paucity of studies reporting PCB

concentrations for free-ranging adult pinnipeds from the eastern North Pacific. This would provide more complete documentation of baseline contaminant ranges within multiple species and the ability to appropriately compare species that utilize more similar geographic ranges.

The most common PCB congener quantified in elephant seal blubber for our study is CB-153, which contributes the greatest proportion to ∑PCBs in a broad range of marine mammal species [14,17,26,58,59]. The mean contribution of CB-138 and CB-180 in the present study placed those congeners among the top five congeners contributing to the ∑PCB concentration in northern elephant seals. These same congeners are among the top five most important congeners for other pinnipeds, including adult fur seals [58], harbor seals [17] and Hawaiian monk seals [59] from the Pacific, as well as for gray seals [14] and harbor seals [26] from the Atlantic.

1.5a. Influence of age

The absence of a significant age relationship with ∑PCB concentrations indicates that elephant seals likely offload contaminants during lactation and replenish contaminants while foraging. Age does not affect ∑PCB concentrations in the blubber of other female pinnipeds, including harbor seals [17], Steller sea lions [16], and ringed seals [18]. ∑PCB concentrations in female odontocete and mysticete blubber decrease with increasing age [19,20], although this relationship reverses in post-reproductive females after reproduction senescence and the

associated cessation of lactation [22,23]. These observations indicate that there are fundamental differences in how contaminants accumulate in cetaceans and pinnipeds, possibly driven by life history strategies and reproductive senescence.

Based on previous research on elephant seals from Año Nuevo quantifying average ∑PCB concentrations in milk [13] and average milk production in female elephant seals [41], females may annually offload approximately 23.9 mg of PCBs during an average 26.5 day [41] lactation period. This estimated transfer for northern elephant seals is similar to the calculated transfer of PCBs (27.0 ± 11.2 mg) from grey seal female to their pups [60]. Concentrations of ∑PCBs in adult female elephant seal blubber, serum and milk tissues (present study; [13]) are lower than the concentrations observed in some highly contaminated marine mammal species [22,29,31]. However, transfer of contaminants to the pup is during a critical period of pup development for the immune, endocrine, and nervous systems [24] and maternal transfer of PCBs can affect fetal brain development, as observed in rats [61]. It is unlikely that PCBs are the only anthropogenic contaminant acquired by an individual [13]; therefore, each seal could have additional contaminants within blubber or other tissue compartments. It is possible that contaminants may interact, potentially in a synergistic way, but this has yet to be fully examined.

Age significantly affects the proportion of PCB congener families in female elephant seal blubber and serum. This suggests a positive relationship between an age-associated process, such as the number of times a female has given birth, and

the proportion of higher chlorinated congeners in elephant seal tissues. Congeners with increased chlorination are more lipophilic and have a higher octanol-water partitioning coefficient (K_{ow}). This decreases the transfer efficiency from maternal blubber to offspring through milk, potentially due to differences in of mobilization efficiency from blubber [14] as well as diffusion across the boundary of the mammary gland [14,25,62]. Congeners with higher log K_{ow} comprised a greater proportion of PCBs in maternal harbor seals [24], grey seals [14,25], harbor porpoises [26], and belugas [27] than their offspring, which may occur in northern elephant seals and cause the relationships observed between age and specific congener families. Older female seals have increased parity and greater adipose mass loss and milk production during lactation, which leads to more opportunities to depurate congeners with lower K_{ow} values, while selectively retaining congeners with higher K_{ow} values. Another potential mechanism that can explain the age relationship observed in our study is the preferential metabolism of lower chlorinated congeners. It is possible that both selective excretion through milk and preferential metabolism could play a role in the age and congener trends observed in female northern elephant seals.

1.5b. Influence of adipose percent and fasting state

Total PCB concentrations in inner blubber and serum (wet weight) have significant, negative relationships with the percent of adipose tissue. However, this trend is not observed in outer blubber or serum measured per unit lipid. This

suggests that varying physiological and temporal mechanisms mediate fluctuations within the main reservoir (blubber) and the transport system (serum) of PCBs in northern elephant seal females. Asynchronous fluctuations between inner blubber, outer blubber, and serum are likely due to differential use of these tissue components during fasting and lactation, and the potential for transfer of contaminants to offspring. Trends observed here are supported by previous studies. In general, inner blubber ∑PCB concentrations may be more dynamic than outer blubber ∑PCB concentrations because inner blubber is the main energy reserve mobilized during fasting [63]. Fatty acid profiles for the inner blubber layer of adult female elephant seals change significantly over the lactation-associated fasting period, whereas the proportion of different classes of fatty acids in outer blubber remains stable through lactation [53]. Fatty acid profiles of inner blubber are similar to those observed in milk, which supports inner blubber as the main contributor of fatty acids to milk synthesis [53]. Additionally, outer blubber has a high proportion of medium chain length monounsaturated fatty acids, which are theorized to serve in a thermoregulatory capacity [53]. Thus, changes in PCB concentrations correspond most strongly with changes in maternal lipid content, especially in the inner blubber layer.

When animals are at their fattest, outer blubber concentrations may be higher than inner blubber concentrations [13,49]. PCB concentrations may increase in the inner blubber while fasting, because an animal utilizes the blubber reserve from the inside first and concentrates contaminants in the remaining tissue. This

may cause the inner blubber PCB concentrations to become higher than the outer blubber PCB concentrations by the end of fasting [13,49,53]. In the present study, we observe the same trend occurring during the molting fast, although the overall concentrations in inner blubber are highest at the end of the molting fast. This may be attributed to the life history phase, with seals operating more as a closed system during the molting fast because lactation does not occur during the molt and there is no offloading of contaminants to offspring (Table 1.1).

Biannual fasting periods of seals on land are associated with breeding or molting and result in significant reductions in adipose tissue and body mass [41,64]. Female seals are on land for different life history stages (breeding/lactation and molting), which likely causes the differences in how energy stores are used during the two fasting periods. Female seals during the breeding season lose approximately 58% of their fat mass and lose significantly more fat than lean tissue [41]. Female seals during the molt lose an average of 38% of their fat mass and similar proportions of fat and lean tissue [64]. It is important to note that seals are at their fattest at the beginning of the breeding season and adipose reserves are not as great when seals arrive for the start of the molt, as seals have approximately three times longer to forage at sea prior to the breeding season than in between the breeding and molting seasons (Fig 1.1) [35]. Accounting for fat stores, we observed that the highest mean PCB concentrations in inner blubber occurred at the end of the molting fast, when seals were both relatively thin and unable to depurate contaminants to their offspring, rather than

at the end of the breeding fast when seals were at their leanest. This suggests that both the fat content of the animal and its reproductive condition affect contaminant concentrations. Elephant seals only transfer contaminants to offspring during the long foraging trip (gestation) and the subsequent fasting period on land (lactation) and not during the molting fast; therefore, reproductive transfer to offspring, through the placenta and highly lipid-rich milk, may be responsible for this observed difference.

While concentrations of PCBs in blubber and serum in our study are below the concentration threshold that corresponds with immune system suppression in harbor seals [44], concentrations in elephant seals fluctuate significantly relative to body condition. Elevated PCB concentrations in inner blubber and the mobilization of PCBs to serum at the end of a fasting period, as observed in elephant seals [13] and grey seals [14,49], could increase the vulnerability of individuals to cumulative effects from additional contaminants or physiological stressors that reduce their adipose tissue reserves and concentrate contaminants in remaining tissues. Our observations of increased ∑PCB concentrations at lower adipose percentages are consistent with observations in other marine mammals, including humpback whales and polar bears (*Ursus maritimus*), that undergo significant fasting periods [65,66].

Animals may be especially vulnerable to the effects of contaminants if normal fasting causes increased tissue concentrations and is then followed by decreased post-fasting foraging opportunities. This was the possible explanation for elevated

PCB concentrations observed in a female from the present study. This female lost adipose tissue over the course of a short, post-breeding foraging trip, which corresponded to an increase in blubber and serum PCB concentrations of 92% and 213%, respectively, over her late fasting concentrations. Her tissue concentrations, measured before and after the foraging trip, are among the highest observed during any time period and, based on our observations of other seals in this study, it is likely that her PCB tissue concentrations would have been even higher at the end of the molting fast. Similar trends are observed in polar bears, where fluctuations in seasonal food availability outside of the hibernation period can influence body condition and thus affect PCB concentrations [66]. These observations have implications for other fasting animals, especially marine predators that fast while migrating or provisioning offspring.

1.5c. Paired samples

Examining paired tissue samples from the same individuals before and after a foraging trip provides insight into certain dynamics that are not observable in a larger population level analysis. These results are unique because tissue samples were paired between the start and end of a foraging period and not between the start and end of a fasting period, as is common for studies on the PCB dynamics in the blubber and blood of marine mammals. Inner blubber is the most metabolically active layer in northern elephant seals [53] and ∑PCB concentrations in our study vary with adipose percent both at population-level and

individual-level analyses. Individuals show a decrease in ∑PCB concentrations within the inner blubber layer over the course of a foraging trip; however, the magnitude of the decrease is greater over the long trip than the short trip, which may partially be explained by differences in adipose tissue gained over these trips [35] due to differences in trip length (Fig 1.1). The ∑PCBs in outer blubber and serum at the individual level present a more complicated story, increasing in concentration across the short trip but decreasing in concentration across the long trip, which suggests that PCB dynamics in outer blubber may be more complex than previously thought.

1.5d. Conclusion

Overall, our findings demonstrate that PCBs accumulate in female northern elephant seals that forage across the mesopelagic north Pacific. While total accumulation of PCBs does not change with age, our findings reveal that more chlorinated PCB congeners comprise a higher proportion of the total PCB burden of older females while younger seals have a higher proportion of less-chlorinated PCBs. At this time, the health consequences of these observations remain unknown.

Northern elephant seals exhibit a set of life history strategies that depend on two seasonal fasting periods. Our findings suggest that female northern elephant seals are most vulnerable to high PCB concentrations at low adipose percentages, which occur at the end of their two seasonal fasts. Depuration to pups may

mitigate high blubber PCB concentrations in adult female tissues during the breeding fast and prevent overall PCB concentrations from increasing with increasing age. However, no mitigating mechanism is known to occur during the molting fast, and therefore, our results suggest the female elephant seals are most vulnerable to increased concentrations of PCBs during their molting fast. Free ranging adult male elephant seals may accumulate significantly more PCBs than females since they have no ability to mitigate their contaminant load through reproductive transfer. More research is needed on males to verify this hypothesis.

In addition, the inverse relationship between body condition and total PCB concentration in the inner blubber has implications for the development of bio-monitoring strategies. First, the presence of a significant, negative relationship between adipose percent and PCB concentration in the inner blubber, but not in the outer blubber, indicates the complexity of blubber as a biological indicator. For elephant seals, our results suggest that PCB concentrations of inner blubber represent recent trends in contaminant fluctuation, while PCB concentrations of outer blubber, a tissue of slower turnover, may represent longer-term accumulation. Bio-monitoring of more elusive marine predators, where biopsy darting is the only option, may provide misleading results if only the outer-most tissue is sampled. It is imperative that adipose percent and the life history of an animal be fully considered for both the design of contaminant monitoring programs and appropriate interpretation of the results.

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Table 1.1. ∑PCB concentrations in northern elephant seals (N=58). ∑PCB concentrations are lipid-normalized (ng g^{-1} lipid) for inner blubber, outer blubber and serum. Serum ∑PCB concentrations are also reported by wet weight (μg L^{-1}). Samples were collected at four different times of year (see Fig 1.1) from 2005 – 2007.

Tissue		Late breeding fast (Sample 1A)	Early molting fast (Sample 1B)	Paired samples	Late molting fast (Sample 2A)	Early breeding fast (Sample 2B)	Paired samples
Inner	Sample size	N=18 (17)	N=13 (13)	13	N=16 (12)	N=31 (25)	8
	Mean ± sd	1241 ± 545	884 ± 179		1647 ± 574	690 ± 160	
	(range)	(495 – 2502)	(557– 1204)		(708– 2722)	(473 – 1120)	
Outer	Sample size	N=19 (18)	N=16 (15)	14	N=18 (13)	N=32 (25)	8
	Mean ± sd	912 ± 262	1170 ± 314		1003 ± 336	900 ± 221	
	(range)	(497 – 1461)	(777– 2024)		(356– 1597)	(504 – 1417)	
Serum	Sample size	N=20 (19)	N=16 (15)	14	N=20 (15)	N=29 (23)	8
	Mean ± sd	818 ± 210	1207 ± 517		1061 ± 343	838 ± 255	
	(range)	(542– 1362)	(770 – 2837)		(723– 1772)	(511 – 1495)	
Serum	Mean ± sd	7.8 ± 1.8	9.0 ± 3.1		8.1 ± 2.6	6.2 ± 1.4	
(wet weight)	(range)	(4.6 – 10.9)	(6.0 – 17.9)		(4.9 – 14.3)	(4.3 – 9.4)	

Note. A subset of animals with known ages and body composition measurements were used for statistical analyses (sample sizes in parentheses). Late breeding (1A) – early molt (1B) samples were taken before and after the short foraging trip and late molt (2A) – early breeding (2B) samples were taken before and after the long trip. Numbers of paired samples (same seal) are given for each tissue type because not all seals were repeatedly sampled. Some seals were sampled twice while other seals were only sampled once.

Table 1.2. Relationship between the percent of the ΣPCB concentrations in inner blubber, outer blubber and serum made up by each congener group and A) adipose percent or B) age. Relationship indicated by statistically significant and positive relationship (+), significant and negative relationship (–), or no significant relationship (n.s.).

Statistical test	Chlorination	Inner blubber	Outer blubber	Serum
Age	Tri/Tetra-CBs (3-4)	n.s.	–	+
	Penta-CBs (5)	–	–	–
	Hexa-CBs (6)	n.s.	+	n.s.
	Hepta-CBs (7)	+	+	n.s.
Adipose percent	Tri/Tetra-CBs (3-4)	+	+	n.s.
	Penta-CBs (5)	–	–	+
	Hexa-CBs (6)	n.s.	n.s.	n.s.
	Hepta-CBs (7)	n.s.	–	n.s.

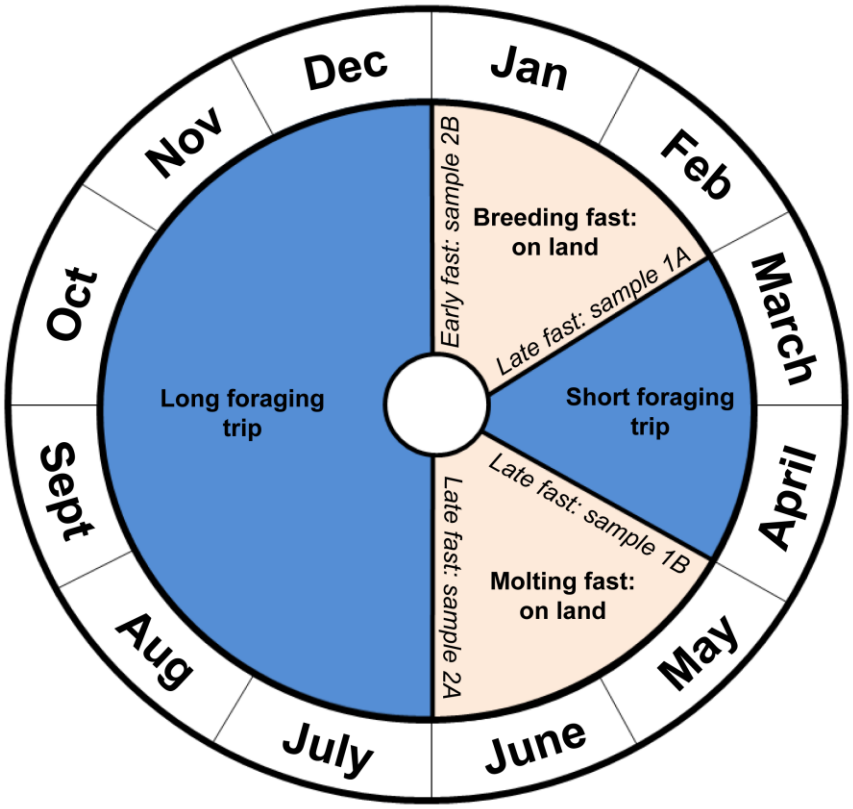

Figure 1.1. One year in the life of a female northern elephant seal. Tissue samples from the same animals (i.e. 1A/1B and 2A/2B) were collected just prior to the start of a foraging trip (late fasting) and just after the end of that foraging trip (early fasting).

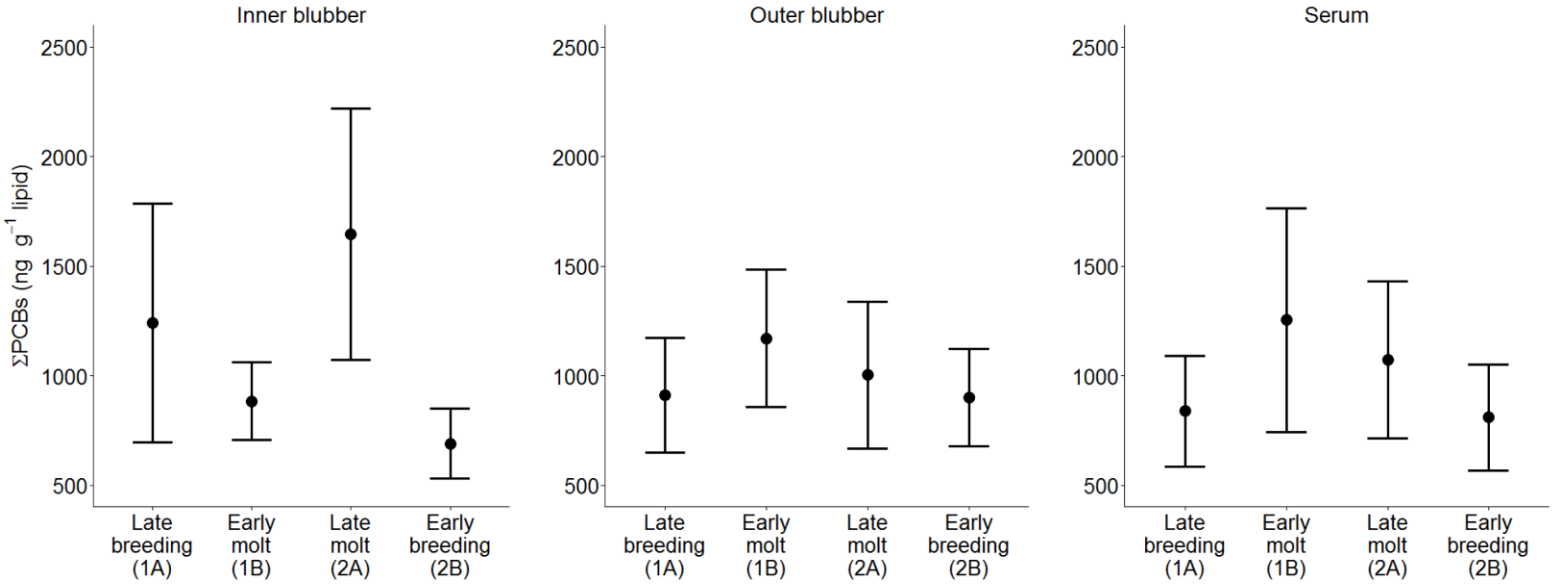

Figure 1.2. ∑PCB concentrations (mean ± SD) for inner blubber, outer blubber, and serum samples measured in elephant seals during four different sampling periods (refer to Fig 1.1 for timing of samples).

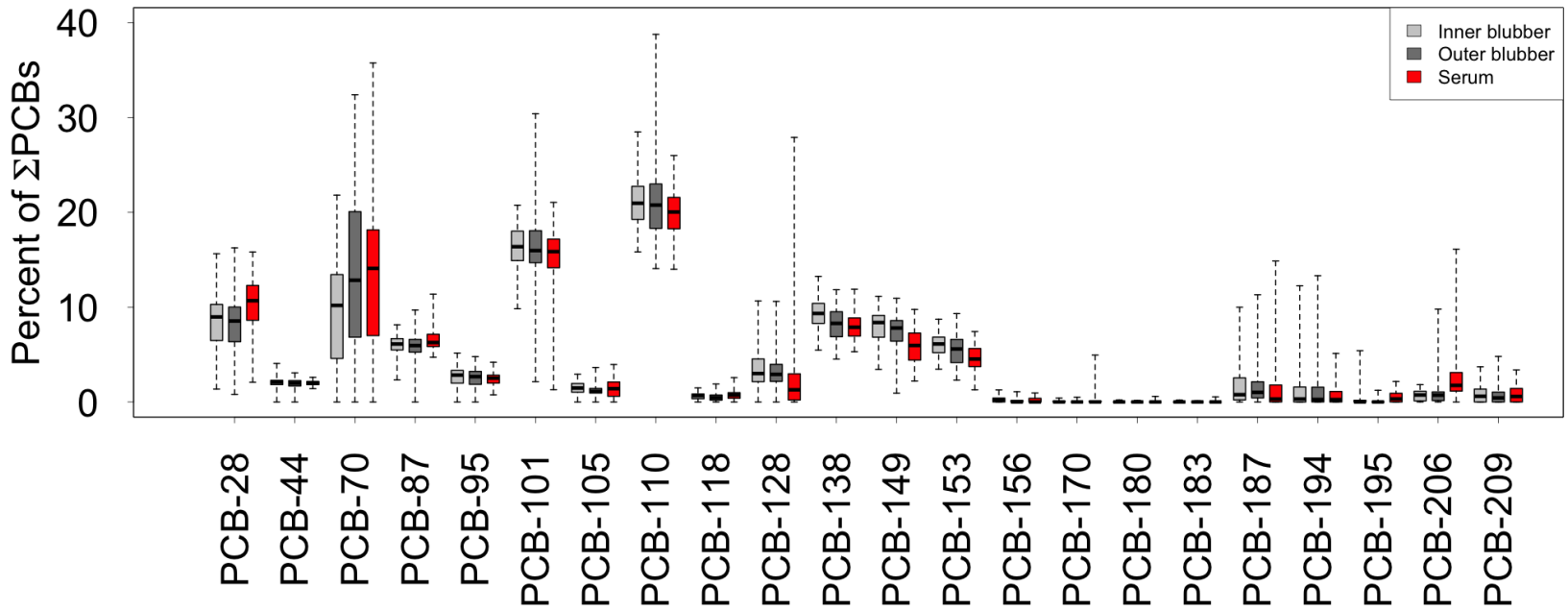

Figure 1.3. Proportions of each congener within the inner blubber, outer blubber, and serum of northern elephant seal females. Whiskers encompass the full range of the data. All sampling periods are combined, which means that some females contributed two samples while other females only are represented by one sample.

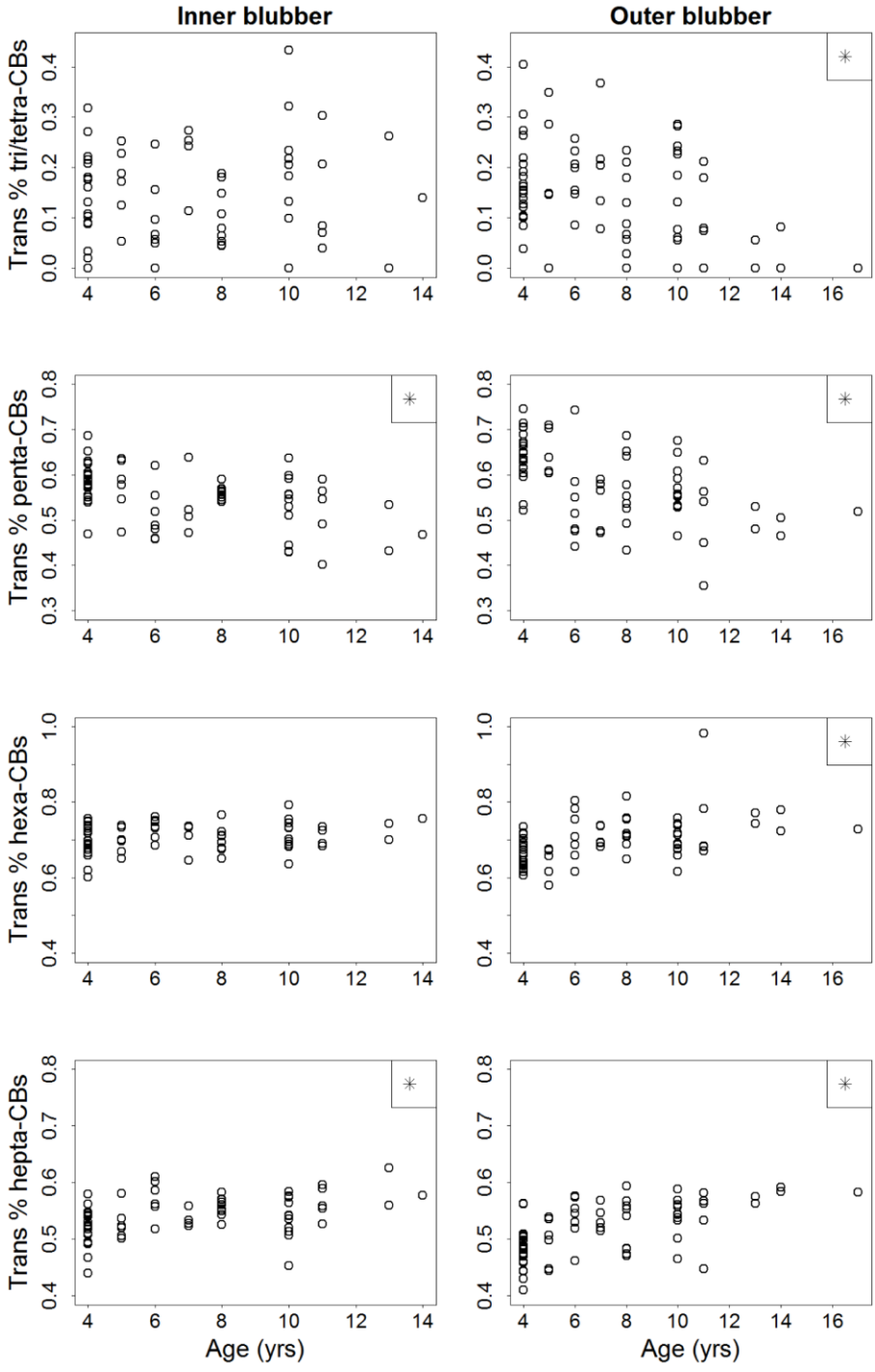

Figure 1.4. Inner and outer blubber congener profiles by age for all sampling periods combined. Statistics were run on transformed percentages (arc-sin square root). Asterisks indicate a statistically significant relationship with age.

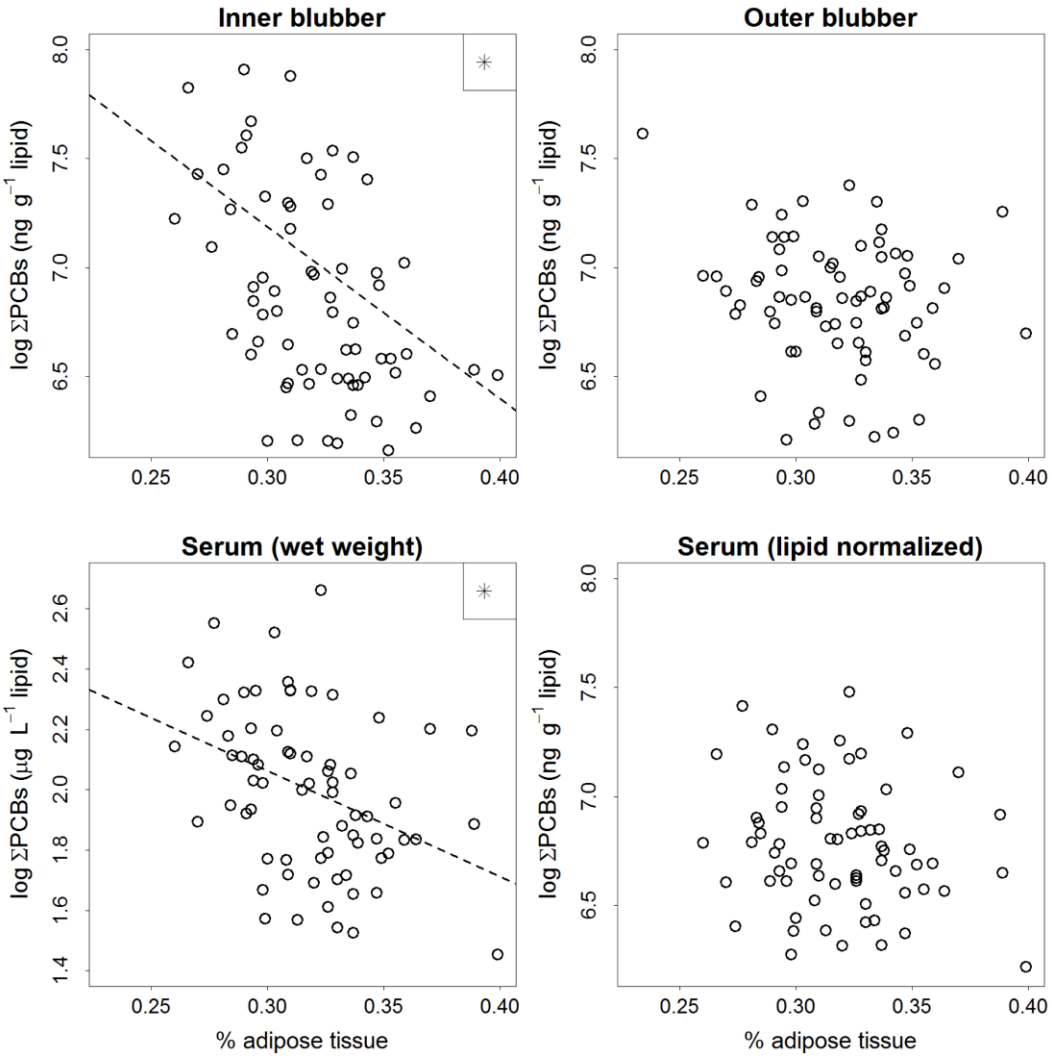

Figure 1.5. Inner blubber, outer blubber and serum (wet weight and lipid-normalized) ∑PCB concentrations relative to body condition (percent of adipose tissue). Statistical analyses were run using log transformed ∑PCB concentrations. Samples are from all sampling periods combined. Asterisks indicate statistically significant relationships.

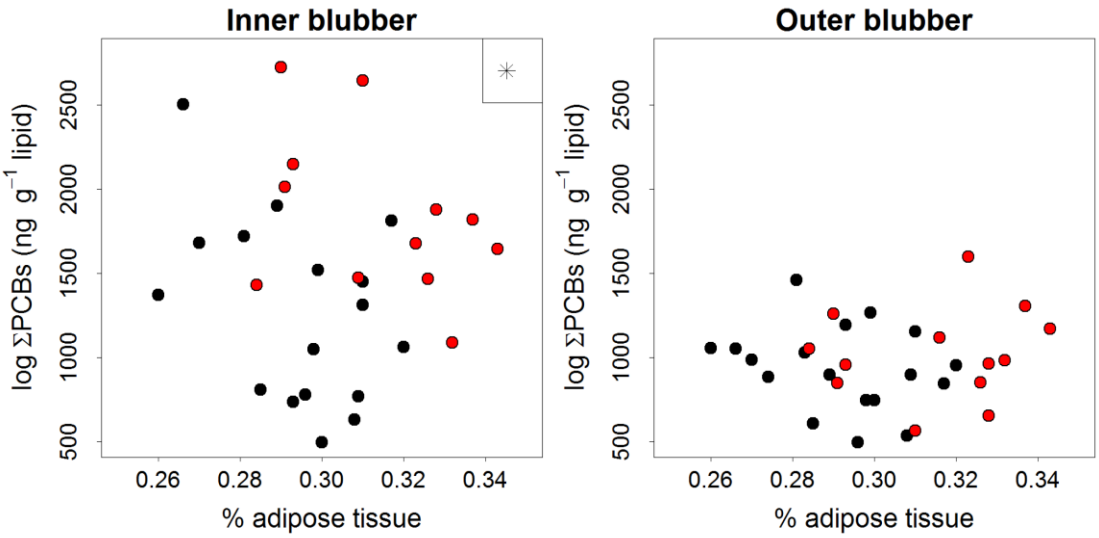

Figure 1.6. ∑PCB concentrations in individual blubber samples at the end of the breeding/lactation fast (black) and the molting fast (red), relative to body condition (percent of adipose tissue). Asterisk indicates a statistically significant difference between groups.

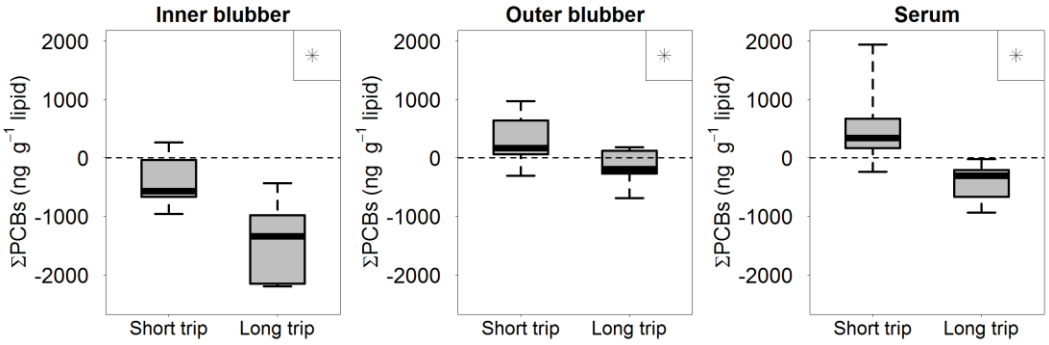

Figure 1.7. Changes in paired ∑PCB concentrations over the course of a foraging trip (same individuals pre- and post-foraging) for inner blubber, outer blubber and serum. Asterisks indicate statistically significant differences between the two trips. Refer to Fig 1.1 for annual life history phases and differences between the short and long trip.

CHAPTER 2

Marine foraging ecology influences mercury bioaccumulation in deep-diving northern elephant seals

SH Peterson, J Ackerman, DP Costa

2.1. Abstract

Mercury contamination of oceans is prevalent worldwide and methylmercury concentrations in the mesopelagic zone (200 - 1000 m) are increasing more rapidly than in surface waters. Yet, mercury bioaccumulation in mesopelagic predators has been understudied. Northern elephant seals (*Mirounga angustirostris*) biannually travel thousands of kilometres to forage within coastal and open-ocean regions of the northeast Pacific Ocean. We coupled satellite telemetry, diving behaviour, and stable isotopes (carbon and nitrogen) from 77 adult females, and showed that variability among individuals in foraging location, diving depth, and δ^{13}C values were correlated with mercury concentrations in blood and muscle. We identified three clusters of foraging strategies, and these resulted in substantially different mercury concentrations: 1) deeper-diving and offshore foraging seals had the greatest mercury concentrations, 2) shallower-diving and offshore foraging seals had intermediate levels, and 3) coastal and more northerly foraging seals had the lowest mercury concentrations. Additionally, mercury concentrations were lower at the end of the seven-month

long foraging trip (n = 31) than after the two-month long post-breeding trip (n = 46). Our results indicate that foraging behaviour influences mercury exposure and mesopelagic predators foraging in the northeast Pacific Ocean may be at high risk for mercury bioaccumulation.

2.2. Introduction

Mercury, a non-essential trace element toxic to humans and wildlife [1, 2], is widespread and increasing in the marine environment [3-6]. Although sources are both natural and anthropogenic, the overwhelming majority of mercury in the biota of remote marine regions, such as the Arctic, originates from human activities [5, 7]. Anthropogenic atmospheric emissions occur in the form of inorganic mercury, including the gaseous elemental form (Hg^0), which can be oxidized to Hg^{II} and deposited on the ocean, where it subsequently can be converted to organic mercury (methylmercury) [8, 9]. At this point, methylmercury can enter and biomagnify in oceanic food webs [5, 10]. Models of global mercury cycling suggest a time lag of decades to centuries before current levels of anthropogenic emissions equilibrate between the atmosphere and ocean [11, 12]. As a result, mercury concentrations in the world's oceans are expected to continue increasing [11, 12].

Specific zones within the marine water column, including the epipelagic (0 - 200 m) and the mesopelagic (200 - 1000 m), differ with respect to biogeochemical cycling of mercury [9], which may have significant implications for

bioaccumulation in marine predators. Increasing evidence indicates that the mesopelagic is a critical zone for entry of methylmercury into oceanic food webs, although specific mechanisms leading to oceanic mercury methylation and the subsequent integration into deep-ocean food webs are not yet fully understood [8]. The mesopelagic zone contains higher total mercury and methylmercury concentrations than either the epipelagic (0 - 200 m) or the zones below 1000 m [4, 9, 13, 14], and over the past century mercury concentrations have increased more rapidly in the mesopelagic zone than in the other oceanic zones. For example, methylmercury concentrations in water collected from the mesopelagic zone were higher in 2006 at all locations in the North Pacific than in previous studies [4]. Additionally, mercury in North Atlantic birds foraging on prey of mesopelagic origins experienced a 3.5 - 4.8 % yr^{-1} increase in mercury concentrations over the last 100 years, a much faster rate of increase than that observed in shallower, epipelagic foraging seabirds (1.1 - 1.9 % yr^{-1}) [3]. Mercury concentrations in large predatory fish sampled near Hawaii were highest in fish species that foraged within the mesopelagic and lowest in fish foraging in the epipelagic [14]. Despite the accumulating evidence that the mesopelagic has higher levels of mercury contamination, mercury exposure in top mesopelagic predators has been little studied. This could be problematic because mercury can adversely affect reproduction, development, behaviour, and nervous system function in many organisms, and may be toxic even at low levels [15].

We followed known-age adult female northern elephant seals (*Mirounga angustirostris*) to quantify how variability in foraging behaviour, including geography, diving depth, and stable isotopes (carbon and nitrogen) can explain mercury bioaccumulation in a mesopelagic predator. Specifically, we used variables to describe foraging locations and diving behaviour, in addition to stable isotopes, to determine what best explained the variability observed in mercury concentrations in blood and muscle. Additionally, we used the same variables to identify clusters of seals with similar foraging behaviours, and examined if the clusters corresponded with overall differences in mercury concentrations. We studied the northern elephant seal, because it is the only pinniped species (seals, sea lions, and walrus) in the northeast Pacific Ocean that forages almost entirely on fish and squid in the mesopelagic [16-19], and their foraging range overlaps with more cryptic, mesopelagic marine predators that may also be vulnerable to methylmercury bioaccumulation, including cetaceans, sharks, and tuna [20, 21]. Importantly, adult females vary substantially both in diving behaviour (i.e., median foraging dive depth during the day can differ among individuals by nearly 300 m) and geographic location [16, 17, 22, 23]. Annually, adult females undergo two foraging trips, ranging upwards of 10,000 km over seven months (pre-breeding, gestational; hereafter long foraging trip) or 5,000 km over two months (post-breeding; hereafter short foraging trip). Females come to shore after the long foraging trip to give birth and come to shore after the short foraging trip to moult (Fig 2.1).

2.3. Methods

In order to relate mercury concentrations in adult female elephant seals to foraging behaviour at sea, we deployed satellite-transmitters and time-depth recorders at the start of either the short or long foraging trip [17] and non-lethally collected whole blood (hereafter referred to as blood) and muscle biopsies from all animals at the end of their foraging trip (Fig 2.1). From 2011 - 2013, we sampled blood and muscle, to represent mercury bioaccumulation over potentially different time scales, once each from 77 known-age (4 - 13 yr) adult females. We sampled seals on average nine days after arrival to the Año Nuevo colony (Año Nuevo State Reserve, San Mateo County, California, USA) for breeding (5 - 6 days post-parturition; n = 31) and two days after arriving to the colony for the annual moult (n = 46). We used standard protocols to immobilize seals in order to attach or remove instruments, collect morphometrics, and sample tissues [16, 17, 22].

We used published protocols to prepare tissue samples for mercury and stable isotope analysis [24-26]. We analysed whole blood and muscle samples for total mercury (Hg_T) because the mercury in these tissues from marine mammals is almost entirely methylmercury [27-29]. Tissue samples were analysed at the U.S. Geological Survey Dixon Field Station Mercury Lab using a Milestone DMA-80 Direct Mercury Analyzer (Milestone, Shelton, CT, USA). Quality assurance measures during each batch included reference materials certified by the National

Research Council of Canada, Ottawa, Canada (DORM-3, DOLT-3 or DOLT-4, and TORT-3), continuing calibration verifications, system and method blanks, and duplicate samples. Recoveries (mean ± SE) were 101.9 ± 0.7 % (n = 46) for certified reference materials and 101.4 ± 0.9 % (n = 67) for calibration verifications. Absolute relative percent difference for duplicates averaged 4.1 ± 0.8 % (n = 53). Mercury concentrations were generated using wet weight (ww) for whole blood samples and dry weight (dw) for muscle samples. We analysed red blood cells for δ^{13}C and δ^{15}N, using a Carlo Erba Elemental Analyzer interfaced with a ThermoFinnigan Delta Plus XP mass spectrometer (Light Stable Isotope Lab, UC Santa Cruz), because they represent integrated diet over a period of weeks to months leading up to sampling [30, 31]. The average experimental precision for isotope samples, calculated by averaging the standard deviation for the sets of in-house standards (Pugel) among all isotope runs, was 0.10 ‰ for δ^{13}C and 0.08 ‰ for δ^{15}N.

We used time-depth recorders to determine if Hg_T concentrations varied with foraging depth or proportional use of the water column, and used satellite locations in order to determine if the geographic location of the foraging trip influenced Hg_T concentrations. Tracking and diving data were processed using standard filtering techniques and protocols [17]. All dive locations were georeferenced using the entire satellite track. We modified previously published dive type classification [17] and combined all active-bottom dives and v-shaped dives greater than 400 m as putative foraging dives, because jaw accelerometer

tags deployed on elephant seals capture jaw motion events in 70 - 90 % of all dives below 450 m [18] and an even higher percentage of v-shaped dives below 400 m (Y. Naito, personal communication). Because elephant seals demonstrate a diel diving pattern [17], we assigned dives to day or night based on the solar zenith angle associated with each dive and quantified median and 90^{th} percentile depths for each individual separately for day and night. We quantified the percent of the total dives that were benthic, to identify seals that spend a greater proportion of time foraging along the bottom. Additionally, we calculated an overall dimensionless dive index for each seal to quantify proportional use of the water column (0 = at the surface, 1 = on the seafloor), by dividing the maximum depth of each dive by bathymetry (using the ETOPO1 1 Arc-Minute Global Relief Model [32]) and then averaging all dives. We calculated all geographic variables using a satellite track that was linearly interpolated to one location every eight hours (three locations per day). Every location was assigned to a hydrographic ecoregion [23], including the California Current Upwelling Region, the Coastal Alaska Downwelling Region, the Subarctic Gyre, and the North Pacific Polar Front (NPPF). The most common ecoregions were the California Current and the NPPF, but all seals must travel through the California Current to reach the NPPF. Because the proportions of time spent in these two ecoregions were negatively correlated, we only quantified the proportion of locations over the course of the foraging trip that occurred in the California Current to use as a geographic variable in the statistical analyses.

We set up two identical sets of candidate models to explain the variability in blood (n = 77 seals) and muscle (n = 70 seals) Hg_T concentrations, respectively, using general linear models. The variables in the full model set included the foraging trip (short trip or long trip), seal age, median depth of foraging dives during the day (m), median depth of night foraging dives (m), 90th percentile depth of day foraging dives (m), 90th percentile of night foraging dives (m), maximum latitude obtained during the trip (°N), median distance of all seal locations during a trip to the continental shelf (km), median dive index (dive depth/ocean depth), proportion of time spent in the California Current ecoregion, the δ^{13}C value (‰), and the δ^{15}N value (‰). In order to determine if blood or muscle Hg_T concentrations were better explained using a more recent time scale of behaviour, we calculated all of the same diving and geographic variables using the 60 most recent days of data prior to sample collection for each foraging trip (on average, the short trips are 75 days and long trips are 220 days [17]).

We ran all possible combinations of the variables, except for a specified subset described below, in the statistical program R, version 3.0.2 [33]. Specifically, we did not allow models to contain both δ^{13}C and δ^{15}N because these variables were highly correlated. We also did not allow variables calculated at different time scales (the entire trip duration vs. the most recent 60 days) to appear in the same model. We used Akaike Information Criterion corrected for small sample sizes (AIC_c) to rank candidate models, and considered candidate models for biological importance when $\Delta AIC_c \leq 2.0$ [34]. We calculated evidence

ratios for each variable included in the top model, by dividing the Akaike weight of the top model by the Akaike weight of the same model without the variable of interest, which allows for comparison of the relative weight of support between models [34]. We also calculated the relative variable importance, which is the sum of the Akaike weights for all models containing the variable of interest, to compare the relative weight of support for different variables [35]. Foraging behaviour variables calculated over the final 60 days of a foraging trip did not fall within the top 95 % of cumulative Akaike weights for blood and muscle analyses, clearly indicating that the quantification of foraging behaviour over the entire foraging trip better explained the variability in mercury concentrations than just quantifying the most recent behaviour. Thus, we report only results for models including variables calculated over the full foraging trip length.

Next, to identify unique clusters of seals based on foraging behaviour, including seals from both foraging trips, we used a combination of principal components analysis (PCA) and hierarchical cluster analysis using the FactoMineR package in R [36, 37]. We used the same variables as previously described, except for the percentage of time in the California Current, and added the percentage of benthic dives and both δ^{13}C and δ^{15}N values. The PCA produced unrotated factors and we used the eigenvalue threshold of 1.0 to retain principal components. We used PCA factor scores as input variables to the cluster analysis, which was run using hierarchical clustering on principal components using Euclidean distance and the average linking method [36]. Clusters were

identified based on intra-cluster inertia [37]. We then separately tested blood and muscle Hg_T concentrations for broad-scale differences between the identified cluster groups, using AIC_c. We used AIC_c to compare five models; different only in how the seals were grouped using the three identified clusters (Table 2.1).

2.4. Results

We detected mercury in all blood and muscle samples collected from adult female elephant seals at the Año Nuevo colony from 2011 - 2013 (Table 2.2). Upon return to land from a foraging trip, seals ranged in Hg_T from 0.18 - 0.65 μg g^{-1} ww in whole blood (n = 77), and from 1.90 - 10.15 μg g^{-1} dw in muscle (n = 70). The mean $\delta^{13}C$ value was -19.5 ± 0.3 ‰ (range -20.1 to -18.7 ‰) and the mean $\delta^{15}N$ value was 14.7 ± 0.9 ‰ (range 13.2 to 16.8 ‰). We assessed tissue concentrations for the assumptions of normality and homogeneous variance and did not transform concentrations of mercury in blood or muscle.

The geographic location of the Año Nuevo colony causes all seals to spend at least a portion of the start and end of a foraging trip in the California Current as they transit to and from their at-sea foraging locations; however, the time spent in the California Current varied widely from ≤ 20 % (n = 19) to ≥ 80 % (n = 8; Fig 2.1). Median depth of foraging dives ranged from 475 - 760 m during the day and 440 - 613 m at night, while the 90th percentile of foraging dives ranged from 641 - 1061 m during the day and 574 - 965 m at night (Table 1).

2.4a. Tissue mercury concentrations

Blood mercury. Variability in blood Hg_T concentrations of seals could be explained by variability in foraging behaviour, age, and whether the animal was sampled after the short or the long foraging trip. The most important variables to explain blood Hg_T included the 90th percentile of foraging dive depths during the night, the $\delta^{13}C$ value, the percent of time in the California Current, and the foraging trip (Fig 2.2; Table 2.3). The top three models (within a ΔAIC_c of 2) all contained these four variables, with individual variable weights > 0.97, indicating their overriding importance. Removing one variable at a time from the top model and comparing this reduced model to the top model indicated that the top model was > 6800 times more likely than similar models but without each one of these four variables. In contrast, the maximum latitude was in the top three models but the top model was only 3.4 times more likely than the same model without maximum latitude. Additionally, the top model (adj r^2 = 0.65; Fig 2.2; Table 2.3) included age; however, age only had a variable weight of 0.51 and was only 1.1 times more likely than the same model without age. The median dive depth of foraging dives during the day was in the fifth ranked model (ΔAIC_c = 2.26), and was considered an uninformative parameter with a variable weight of 0.31. There was no support for the remaining variables. The top model was a significantly better fit than the null model, with a ΔAIC_c of 74.

Accounting for the other variables in the top model, blood Hg_T concentrations were 0.11 ± 0.02 µg g^{-1} ww higher in blood after the short compared with the long

foraging trip. For every 100 m increase in the 90th percentile of foraging dives at night, Hg_T concentrations increased 0.06 ± 0.01 µg g^{-1} ww. Concentrations of Hg_T also increased by 0.15 ± 0.03 µg g^{-1} per mil increase in $\delta^{13}C$ values (Fig 2.2). Concentrations of Hg_T decreased by 0.04 ± 0.03 µg g^{-1} ww with each 10˚ increase in the maximum latitude reached during the foraging trip and decreased by 0.02 ± 0.01 µg g^{-1} ww for every 10 % increase in the proportion of time spent in the California Current (Fig 2.2). Lastly, Hg_T concentrations in blood increased with age at a rate of approximately 0.01 ± 0.01 µg g^{-1} ww per yr.

Muscle mercury. Muscle samples were obtained from only 70 of the seals; therefore muscle analysis was restricted to a smaller sample size. Results for muscle analysis were similar to those observed in blood but had lower explanatory power than blood (lower adj-r^2 for the top model). Seven models explaining the variability in muscle Hg_T concentrations of adult female elephant seals were within a ΔAIC_c of 2, and, similar to the best models for blood Hg_T concentrations, each model included the foraging trip, the 90th percentile of foraging dives during the night, and the $\delta^{13}C$ value (Fig 2.3). The combined Akaike weight for the models containing all three of these variables was 0.52, and their individual variable weights were 0.77, 0.68, and 0.87, respectively. The median distance to the continental shelf was also included in the most parsimonious model (adj r^2 = 0.40; Fig 2.3; Table 2.4), with a cumulative Akaike weight of 0.47. The relative variable weights for the other variables included in the other models within a ΔAIC_c of 2 were 0.32 for age, 0.52 for the median dive

depth of foraging dives during the day, 0.41 for the median dive depth of foraging dives during the night, 0.36 for the dive index, and 0.04 for the δ^{15}N value (Table 2.4). Unlike for Hg_T concentrations in blood, the maximum latitude reached during the foraging trip did not appear in the top models explaining Hg_T concentrations in muscle.

Accounting for the other variables in the top model, muscle Hg_T concentrations were 0.98 ± 0.44 µg g^{-1} dw higher after the short foraging trip than after the long foraging trip. Muscle Hg_T concentrations increased 1.63 ± 0.62 µg g^{-1} dw with each per mil increase in δ^{13}C value and increased by 0.59 ± 0.29 µg g^{-1} dw with each 100 m increase in depth for the deep foraging dives during the night (the 90th percentile of foraging dives during the night). Additionally, muscle Hg_T concentrations increased by 0.14 ± 0.08 µg g^{-1} dw with each 100 km increase of the median distance from the continental shelf (Fig 2.3). Evidence ratios indicated that the top model (adj r^2 = 0.40; Table 2.4) was 188 times more likely than the model without the δ^{13}C value, 123 times more likely than the model without trip, 105 times more likely than the model without the median distance to the continental shelf, and 11 times more likely than the model without the 90th percentile of foraging dive depths during the night. The top model was a better fit than the null model, with a ΔAIC of 30.

2.4b. Cluster analysis for foraging strategies

Adult female elephant seals were grouped into three clusters based on foraging behaviour, using hierarchical clustering of factor scores from the three retained principal components (Fig 2.4; Table 2.1). Seals in the first cluster (n = 30; hereafter northerly seals) had foraging trips closer to the continental shelf, were more northerly in maximum latitude, contained a higher proportion of benthic dives, had average dives that used a greater proportion of the water column, foraged shallower during the day, and had shallower 90^{th} percentiles of foraging dives during day and night than the overall mean values for all seals (Fig 2.4; Table 2.2). The northerly cluster was not distinguished by isotope values. Seals in the second cluster (n = 37; hereafter shallower, offshore seals) and the third cluster (n = 10; hereafter deeper, offshore seals) foraged much further offshore than the northerly seals but differed between each other in terms of diving depth and isotope values. Mean δ^{13}C and δ^{15}N values were lower for the shallower, offshore seals when compared with the deeper, offshore seals (Table 2.2). All three clusters included seals from both foraging trips (Table 2.2).

We observed substantial differences in mercury concentrations between the three clusters of seals. The most parsimonious model for Hg_T concentrations in both blood and muscle included all three identified clusters as separate groups and had substantial weight (Akaike weight ≥ 0.88), whereas the next best model contained less than three separate clusters and had little weight (Akaike weight ≤ 0.08) and a $\Delta AIC_c > 6$ (Table 2.1). The more northerly foraging seals had the lowest median blood and muscle Hg_T concentrations of the three clusters (Fig

2.4). Median Hg_T concentrations in blood and muscle concentrations were 19 % and 35 % higher in the shallower, offshore foraging seals than in the more northerly foraging seals. In turn, the deeper, offshore foraging seals had median Hg_T concentrations in blood and muscle that were 40 % and 26 % higher, respectively, than in the shallower, offshore foraging seals and 67 % and 66 % higher, respectively, than in the more northerly foraging seals (Fig 2.4).

2.5. Discussion

We linked individual foraging behaviour with mercury concentrations of a top marine predator foraging in the mesopelagic. Individual foraging behaviour of adult female northern elephant seals substantially influenced mercury bioaccumulation, and seals could be broadly clustered into separate groups based on foraging behaviour. This indicates that individuals are not at equal risk to mercury exposure. We found that elephant seals that foraged offshore in the deepest parts of the mesopelagic had mercury concentrations in blood that were 40 % higher than seals foraging offshore but at shallower depths, and 67 % higher than seals foraging closer to the continental shelf, more northerly in latitude, and at shallower depths. Elephant seals foraged across a wide section of the northeast Pacific, overlapping with several sites that have been sampled for mercury within the water column. The lowest blood mercury concentrations from our study were from females that foraged further north, near the Subarctic Gyre, and the highest blood mercury concentrations in our study were from females that foraged further

south, within the Transition Zone along the North Pacific Polar Front (Figs 2.1 and 2.4). The negative relationship we observed between blood mercury concentrations and maximum latitude in elephant seals (Fig 2.2) corresponds with regional variability in mercury concentrations within water profiles [4, 13]. The greatest mercury concentrations measured in water at sites to the north were between 200 - 400 m in depth, whereas the peaks of mercury measured at sites further south were between 500 - 800 m in depth, indicating that mercury concentrations at similar depths in the water column change substantially from north to south in the northeast Pacific [4, 13]. Elephant seals typically forage deeper than 400 m, which may mean that seals in the Subarctic Gyre foraged deeper than the depths in the water column where the highest mercury concentrations occur, but seals further south foraged at the depths with the highest mercury concentrations. The high geographic fidelity of individual elephant seals to foraging areas [38, 39] makes it highly probable that the same individuals would consistently accumulate more mercury while foraging.

In addition to geographic variability in water column mercury concentrations, individual diving behaviour strongly influenced mercury bioaccumulation. The majority of seals forage deeper during the day than at night, based on the vertical movement of prey within the water column [17]. However, some seals continued to dive to deeper depths during the night, and it was specifically these individuals that had the highest mercury concentrations. Because mercury distributions vary within the water column, seals that spent more time deeper in the water column

may have foraged on a higher proportion of non-vertically migrating prey or prey migrating up in the water column at night from even deeper depths. These species could either contain greater concentrations of mercury because of their position within the water column or they could represent a higher trophic level.

Elephant seal carbon isotopes, but not nitrogen isotopes, were important to explain some of the variability in mercury concentrations. This suggests that the positive relationship between δ^{13}C values and mercury may be influenced more by a combination of oceanographic processes and latitude and less by trophic position. The region in the northeast Pacific where elephant seals foraged encompasses wide variability in δ^{13}C and δ^{15}N values at the base of the food web [40-42], which makes it difficult to directly compare isotope values to infer trophic position. Within the northeast Pacific, near shore in the California Current tends to be more enriched in ^{13}C and becomes depleted in ^{13}C moving offshore [43, 44]; however, offshore ecoregions can vary significantly in δ^{13}C values as a result of both latitude and depth [40, 45]. Elephant seals from all three clusters spent substantial periods of time outside of the California Current ecoregion. Elephant seals foraging outside of the California Current ecoregion that were enriched in ^{13}C could have been foraging on prey from a deeper food web because oceanographic and biological processes associated with increasing depth can cause deeper food webs to become enriched in ^{13}C [40, 42, 46], although it is also possible that these animals could have been foraging on prey from a higher trophic level.

The seasonality of life history events may help explain the higher mercury concentrations we observed in blood and muscle after the short foraging trip (mean duration was 73 days) when compared with the long foraging trip (mean duration was 223 days). Mercury can be removed from circulation if it binds to tissue like hair that grows and subsequently become inert [47], and female elephant seals undergo an annual moult after the short foraging trip (Fig 2.1). Mercury is detectable in elephant seal hair (S. Peterson 2014, unpublished data), with a mean concentration comparable with the highest mean hair concentrations documented for females of other free-ranging pinniped species [48, 49], indicating that elephant seals annually offload substantial amounts of mercury into hair. Following the annual moult, female elephant seals return to the ocean for the long foraging trip, at which time gestation occurs. Maternal offloading of methylmercury in marine mammals occurs mostly via the placenta during gestation and to a much lesser extent during lactation [50, 51]. For elephant seals arriving from the short foraging trip, there has been no recent maternal transfer of methylmercury and the greatest amount of time has elapsed since the prior moult (Fig 2.1). Lastly, the increased mass and body condition (i.e., growth dilution effect due to increase in body mass) of seals at the end of the long foraging trip, in preparation for the extended lactation and fasting period associated with breeding, likely reduces mercury concentrations in internal tissues because changes in body condition can influence mercury concentrations in vertebrates [51, 52]. Thus, the higher mercury concentrations we observed in seal blood and muscle after the

short foraging trip (about 150 days shorter than the long foraging trip) were likely caused by decreased body mass (mass approximately 16 % less) and the lack of ability to depurate mercury into developing offspring or through moult.

To our knowledge, the mean mercury concentration in the blood of female elephant seals (n = 77, 0.40 ± 0.11 μg g^{-1} ww) was the highest measured for any free-ranging pinniped species. Northern elephant seals bioaccumulated more mercury than their marine mammal counterparts that forage closer to the coast and within the neritic zone of the northeast Pacific. For comparison, mercury concentrations in the blood of adult females were 0.24 ± 0.21 μg g^{-1} ww (mean ± sd) for harbour seals (*Phoca vitulina*, n = 27, California, USA; [49]), were < 0.30 μg g^{-1} ww for California sea lions (*Zalophus californianus*, n = 19, California, USA; S. Peterson 2014, unpublished data), and < 0.36 μg g^{-1} ww, with one exception, for Steller sea lions (*Eumetopias jubatus*, n = 30, Alaska, USA; L. Rea 2014, personal communication). Additionally, mercury concentrations in the blood of female elephant seals were also substantially higher than in other marine mammals, such as female polar bears (*Ursus maritimus*) from the Alaskan Arctic (≤ 0.21 μg g^{-1} ww, n = 17) [53] and sea otters (*Enhydra lutris*) from the northeast Pacific (≤ 0.13 μg g^{-1} ww, n = 20) [54]. While fasting, lipid and muscle tissues are catabolized to fuel the energy demands of an animal, at which point mercury can move from muscle tissue into the blood stream and increase blood mercury concentrations, as observed previously in northern elephant seal females [51]. Since all of the blood samples in our study were from seals at the start of a fasting

period, mercury concentrations would likely have been even greater during late fasting, which would only increase blood mercury concentrations in elephant seals, making them even higher than other free-ranging northeast Pacific pinnipeds.

We observed consistently high mercury concentrations across a range of ages, despite annual offloading of a portion of the mercury burden through reproduction and moult. This indicates that the mesopelagic is a significant and consistent source of mercury into these predators. Although blood mercury concentrations increased with age, the magnitude of the effect was small but suggests that female seals may not depurate or demethylate mercury at the same rate of ingestion. However, because females can reproduce every year until death [55], there is no post-reproductive period without maternal transfer of mercury during which time females would be even more vulnerable to bioaccumulation. Although mercury toxicity benchmarks for marine mammals are difficult to develop, 99 % of elephant seals exceeded the prominently used clinical neurotoxicity threshold of 0.21 μg g^{-1} whole blood for marine mammals [56, 57], based on thresholds developed for humans [58].

Altogether, the high mercury concentrations we observed in elephant seals indicate that the mesopelagic zone in the northeast Pacific Ocean is an important source of mercury exposure to marine predators. Further, our study demonstrated that variability in individual foraging behaviours can significantly influence bioaccumulation of mercury, even within a single species. Mercury

concentrations in the world's oceans are projected to increase even if anthropogenic mercury emissions are halted [11, 12], thus furthering the risk of mercury exposure to predators foraging within the mesopelagic zone.

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Table 2.1. In order to test for broad scale differences in mercury concentrations in blood and muscle of adult female northern elephant seals, we used principal components analysis followed by hierarchical clustering, which resulted in three identified foraging clusters. To determine how well the different clusters explained the variability in mercury concentrations in blood and muscle, we created different combinations of clusters to generate a set of models that we compared using AIC_c. Cluster 1 is the northerly cluster, cluster 2 is the shallower diving and offshore cluster, and cluster 3 is the deeper diving and offshore cluster.

tissue	**model: separation of clusters**	adj r^2	k	log lik	AIC_c	ΔAIC_c	akaike weight
blood	cluster 1, cluster 2, cluster 3	0.32	3	76.69	-147.04	0.00	0.982
	clusters 1 & 2 combined, cluster 3	0.25	3	72.61	-138.90	8.14	0.017
	clusters 2 & 3 combined, cluster 1	0.19	3	69.60	-132.86	14.18	0.001
	intercept only (no clusters)	0.00	2	61.10	-118.04	29.00	0.000
	clusters 1 & 3 combined, cluster 2	0.00	3	61.43	-116.54	30.50	0.000
muscle	cluster 1, cluster 2, cluster 3	0.18	3	-126.45	259.25	0.00	0.884
	clusters 1 & 2 combined, cluster 3	0.10	3	-129.79	265.94	6.69	0.083
	clusters 2 & 3 combined, cluster 1	0.13	3	-128.81	263.99	4.74	0.031
	intercept only (no clusters)	0.00	2	-134.08	272.33	13.08	0.001
	clusters 1 & 3 combined, cluster 2	0.01	3	-133.28	272.92	13.67	0.001

Table 2.2. Total Hg (mean ± sd) in whole blood (µg g^{-1} ww) and muscle (µg g^{-1} dw) of adult female northern elephant seals (*Mirounga angustirostris*) sampled at the Año Nuevo colony (California USA), shown separately for the two foraging trips (short and long; Fig 2.1). Females were clustered into three groups based on diving variables, geographic variables and stable isotope ratios (asterisks indicate variables important in distinguishing clusters). The clusters are referred to as northerly (1), shallower diving, offshore (2), and deeper diving, offshore (3).

variable	cluster 1 (northerly)	cluster 2 (shallower, offshore)	cluster 3 (deeper, offshore)	overall
blood Hg_T short foraging trip	0.36 ± 0.10 (n = 19)	0.45 ± 0.07 (n = 19)	0.56 ± 0.10 (n = 8)	0.43 ± 0.11 (n = 46)
blood Hg_T long foraging trip	0.30 ± 0.08 (n = 11)	0.36 ± 0.08 (n = 18)	0.45 ± 0.10 (n = 2)	0.35 ± 0.09 (n = 31)
muscle Hg_T short foraging trip	5.00 ± 1.52 (n = 17)	5.95 ± 1.41 (n = 18)	7.02 ± 1.94 (n = 7)	5.75 ± 1.67 (n = 42)
muscle Hg_T long foraging trip	3.86 ± 1.16 (n = 11)	5.12 ± 1.39 (n = 16)	5.77 (n = 1)	4.65 ± 1.41 (n = 28)
maximum latitude (°N)	51.5 ± 4.6*	46.0 ± 2.3*	42.9 ± 3.4*	47.8 ± 4.8
median distance to continental shelf (km)	278 ± 188*	962 ± 206*	936 ± 139*	687 ± 383
median day dive depth (m)	592 ± 38*	662 ± 28*	702 ± 30*	639 ± 52
90^{th} percentile day dive depth (m)	710 ± 71*	818 ± 64*	829 ± 49*	777 ± 85
% benthic dives (%)	6.0 ± 4.1*	3.2 ± 1.4*	3.3 ± 2.2	4.3 ± 3.2
90^{th} percentile night dive depth (m)	631 ± 41*	667 ± 59	722 ± 98*	660 ± 65
mean dive index (%)	22.2 ± 7.7*	13.7 ± 2.5*	15.4 ± 1.3	17.3 ± 6.4
$\delta^{13}C$ (‰)	-19.4 ± 0.4	-19.6 ± 0.2*	-19.1 ± 0.2*	-19.5 ± 0.3
$\delta^{15}N$ (‰)	14.6 ± 0.9	14.3 ± 0.5*	15.9 ± 0.9*	14.7 ± 0.9
median night dive depth (m)	516 ± 34	516 ± 42	571 ± 38*	523 ± 42

Table 2.3. Model selection to explain blood Hg_T concentrations in adult female northern elephant seals sampled at the Año Nuevo colony (California, USA) from 2011 - 2013. Top models with ΔAIC_c < four and the null model (intercept only: bottom of table) are shown below. Variables included in each model are indicated by "x." Age is the age of the seal (yr) at deployment, 90 % day and 90 % night are the 90th percentiles of day and night foraging dive depths (m), med day and med night are the median day and night foraging dive depths (m), max lat is the maximum latitude reached during the trip, cont shelf is the median distance from the continental shelf (200 m isobaths) during the foraging trip, Dive index is the proportional use of the water column, % CA is the percent of the trip spent in the California current ecoregion, and the trip is whether the trip was the short or long foraging trip. More details of the variables are provided in the main text.

age	$\delta^{13}C$	90% day	med day	90% night	med night	max lat	cont shelf	dive index	% CA	trip	adj r^2	k	loglik	AIC_c	ΔAIC_c	akaike weight
x	x			x		x			x	x	0.65	8	105.05	-191.99	0.00	0.0797
	x			x		x			x	x	0.65	7	103.72	-191.81	0.18	0.0728
x	x			x		x	x		x	x	0.65	9	105.42	-190.16	1.83	0.0319
	x			x		x	x		x	x	0.64	8	103.98	-189.84	2.15	0.0272
x	x		x	x		x			x	x	0.65	9	105.21	-189.73	2.26	0.0257
x	x		x	x					x	x	0.64	8	103.87	-189.63	2.36	0.0245
x	x			x	x	x			x	x	0.65	9	105.15	-189.61	2.37	0.0243
	x		x	x		x			x	x	0.64	8	103.84	-189.56	2.43	0.0236
x	x			x					x	x	0.64	7	102.59	-189.55	2.43	0.0236
	x			x	x	x			x	x	0.64	8	103.82	-189.53	2.46	0.0233
	x			x					x	x	0.63	6	101.34	-189.47	2.51	0.0227
x	x			x		x		x	x	x	0.65	9	105.06	-189.43	2.55	0.0222
x	x	x		x		x			x	x	0.65	9	105.06	-189.42	2.56	0.0221
	x		x	x					x	x	0.63	7	102.51	-189.40	2.59	0.0218
	x			x		x		x	x	x	0.64	8	103.74	-189.35	2.63	0.0214
	x	x		x		x			x	x	0.64	8	103.73	-189.35	2.64	0.0213
	x			x			x		x	x	0.63	7	102.30	-188.98	3.01	0.0177
	x			x				x	x	x	0.63	7	102.27	-188.92	3.07	0.0172
x	x			x			x		x	x	0.64	8	103.50	-188.88	3.11	0.0168
x	x			x				x	x	x	0.64	8	103.47	-188.83	3.16	0.0164
x	x		x	x				x	x	x	0.64	9	104.38	-188.08	3.91	0.0113
	x		x	x				x	x	x	0.64	8	103.07	-188.03	3.96	0.0110
x	x		x	x		x	x		x	x	0.65	10	105.67	-188.00	3.99	0.0108
											0.00	2	61.10	-118.04	7.94	6.99E-18

Table 2.4. Model selection to explain muscle Hg_T concentrations in adult female northern elephant seals sampled at the Año Nuevo colony (California, USA) from 2011 - 2013. Top models with ΔAIC_c < four (of 6132 possible models) and the null model (intercept only: bottom of table) are shown below. Variables included in each model are indicated by "x." Age is the age of the seal (yr) at deployment, 90 % day and 90 % night are the 90th percentiles of day and night foraging dive depths (m), med day and med night are the median day and night foraging dive depths (m), max lat is the maximum latitude reached during the trip, cont shelf is the median distance from the continental shelf (200 m isobaths) during the foraging trip, Dive index is the proportional use of the water column, % CA is the percent of the trip spent in the California current ecoregion, and the trip is whether the trip was the short or long foraging trip. More details of the variables are provided in the main text.

age	$\delta^{13}C$	90% day	med day	90% night	med night	max lat	cont shelf	dive index	% CA	trip	adj r^2	k	log lik	AIC_c	ΔAIC_c	akaike weight
	x			x			x			x	0.40	6	-114.31	241.96	0.00	0.0299
	x		x	x				x		x	0.40	7	-113.38	242.56	0.60	0.0221
	x		x	x			x			x	0.40	7	-113.53	242.86	0.90	0.0191
	x		x	x						x	0.38	6	-115.20	243.73	1.77	0.0123
x	x			x			x			x	0.39	7	-114.00	243.80	1.84	0.0119
	x			x	x		x			x	0.39	7	-114.02	243.85	1.89	0.0116
	x			x			x	x		x	0.39	7	-114.05	243.91	1.95	0.0113
x	x		x	x				x		x	0.40	8	-112.82	244.00	2.04	0.0107
	x			x			x		x	x	0.39	7	-114.29	244.38	2.43	0.0089
	x			x		x	x			x	0.39	7	-114.30	244.40	2.44	0.0088
	x	x		x			x			x	0.39	7	-114.31	244.42	2.46	0.0087
	x		x	x			x	x		x	0.40	8	-113.12	244.59	2.64	0.0080
	x		x	x					x	x	0.39	7	-114.40	244.61	2.65	0.0079
	x			x				x		x	0.37	6	-115.64	244.62	2.66	0.0079
x	x		x	x			x			x	0.40	8	-113.14	244.64	2.68	0.0078
	x		x	x				x	x	x	0.40	8	-113.14	244.65	2.69	0.0078
	x	x	x	x				x		x	0.40	8	-113.22	244.81	2.85	0.0072
	x		x	x	x			x		x	0.40	8	-113.26	244.88	2.92	0.0069
	x			x	x		x				0.37	6	-115.86	245.04	3.09	0.0064
	x		x	x		x		x		x	0.39	8	-113.36	245.08	3.12	0.0063
	x	x	x	x			x			x	0.39	8	-113.40	245.15	3.19	0.0061
x	x		x	x						x	0.38	7	-114.68	245.16	3.20	0.0060
	x		x	x	x		x			x	0.39	8	-113.40	245.16	3.20	0.0060
	x			x				x	x	x	0.38	7	-114.69	245.19	3.23	0.0059
	x		x	x		x	x			x	0.39	8	-113.46	245.29	3.33	0.0057
	x		x	x			x		x	x	0.39	8	-113.51	245.38	3.42	0.0054
	x		x	x		x				x	0.38	7	-114.80	245.41	3.45	0.0053

	x		x				x			x	0.36	6	-116.11	245.56	3.60	0.0049
x	x			x			x	x		x	0.39	8	-113.68	245.72	3.76	0.0045
x	x			x	x		x			x	0.39	8	-113.69	245.74	3.78	0.0045
	x			x	x		x	x		x	0.39	8	-113.74	245.84	3.88	0.0043
	x				x		x				0.35	5	-117.50	245.93	3.97	0.0041
											0.00	2	-134.08	272.33	30.37	7.58E-09

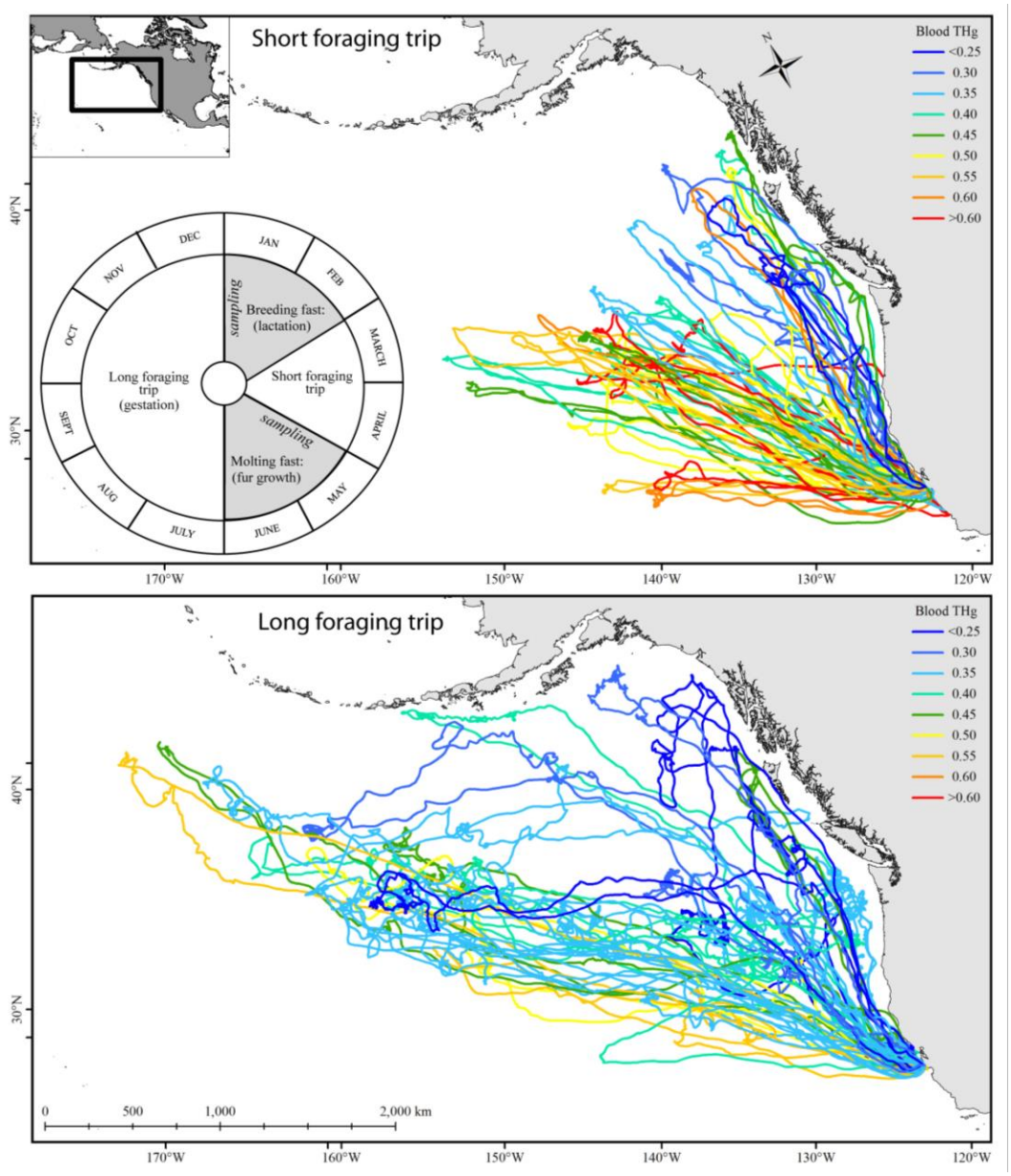

Figure 2.1. Blood Hg_T (µg g^{-1} ww) in relation to foraging location and behaviour of adult female northern elephant seals (*Mirounga angustirostris*) from the Año Nuevo colony, California USA. Seals were satellite tracked during the short, post-breeding foraging trip (top panel; n = 46) and the long, pre-breeding foraging trip (bottom panel; n = 31). The inset shows one year in the life of adult females and the timing of sample collection. Gestation occurs during the long foraging trip. Note that we show periods of time that the majority of animals are ashore, although individual seals are ashore for less than the full period shown because seals arrive over a several week period. These seals were at sea for a mean duration of 73 and 223 days for the short and long foraging trips, respectively.

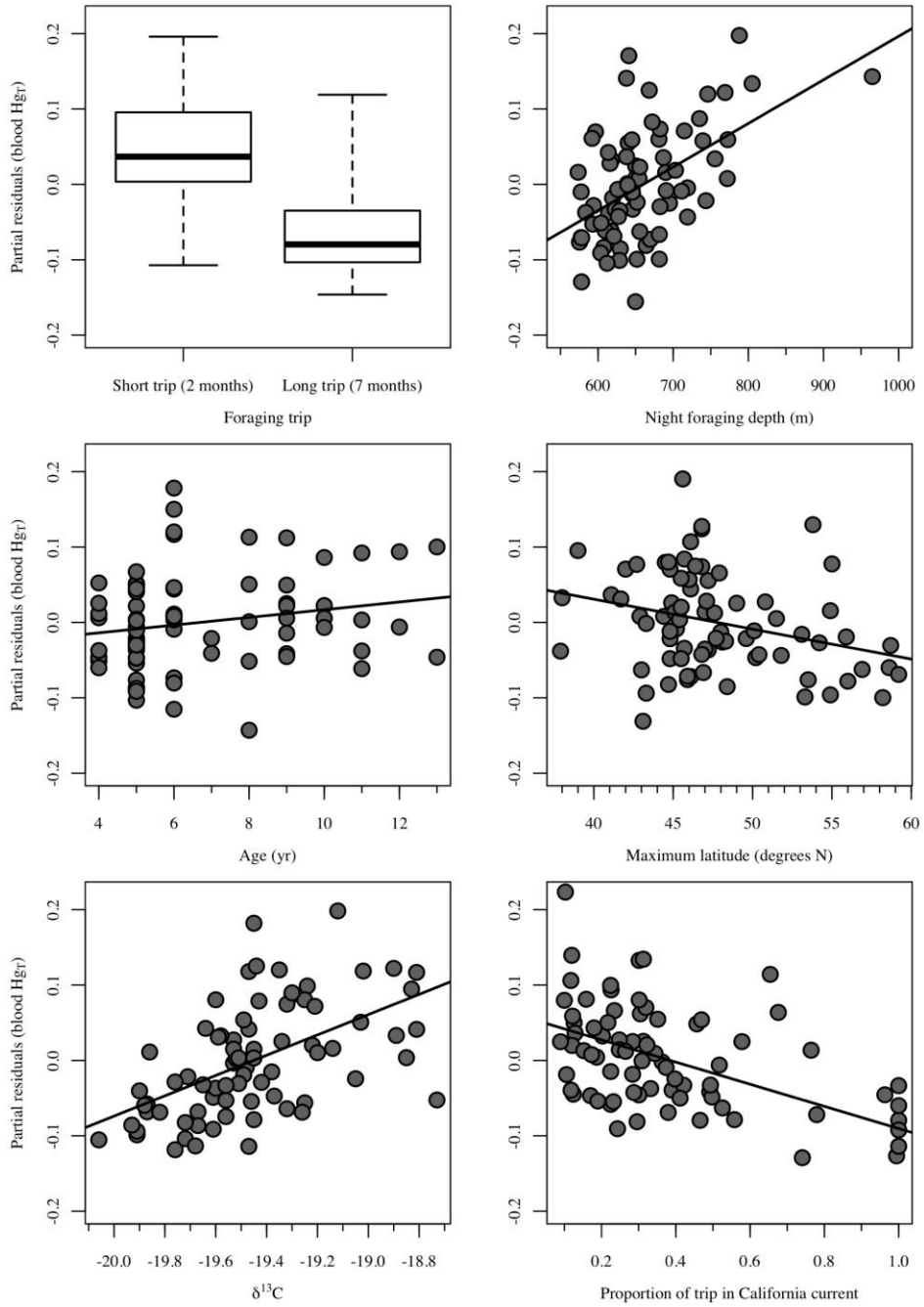

Figure 2.2. Panels show partial residual plots for each of the variables in the top model (each panel shows the relationship between blood Hg_T and an independent variable while accounting for all other independent variables in the top model) to explain blood Hg_T concentrations in adult female northern elephant seals (*Mirounga angustirostris*) sampled at the Año Nuevo colony. The short foraging trip was post-breeding, whereas the long foraging trip was pre-breeding, when gestation occurs (see Fig 2.1). Night foraging depth was the 90th percentile of night foraging dive depths, maximum latitude was the furthest north location during the trip, $\delta^{13}C$ values were from red blood cells, and proportion of trip in the California Current was the proportion of time spent in that ecoregion.

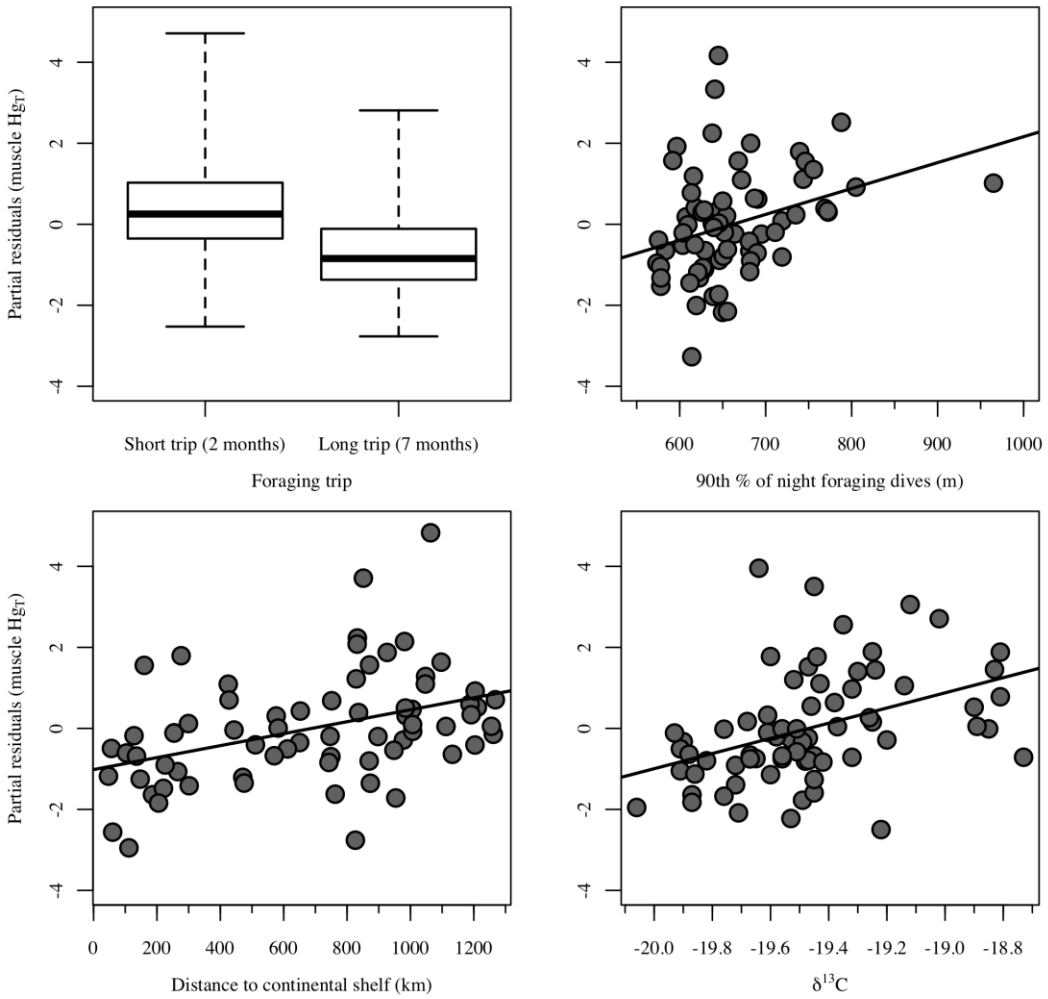

Figure 2.3. Panels show partial residual plots for each of the variables in the top model (each panel shows the relationship between muscle Hg_T and an independent variable while accounting for all other independent variables in the model) to explain muscle Hg_T concentrations in adult female northern elephant seals (*Mirounga angustirostris*) sampled at the Año Nuevo colony (California, USA). The short foraging trip was post-breeding, whereas the long trip was post-moulting, when gestation occurs. Night foraging depth was the 90th percentile of foraging dive depths at night, distance to the continental shelf was the median distance from the 200 m isobath during the trip, and $\delta^{13}C$ was the isotope value from red blood cells.

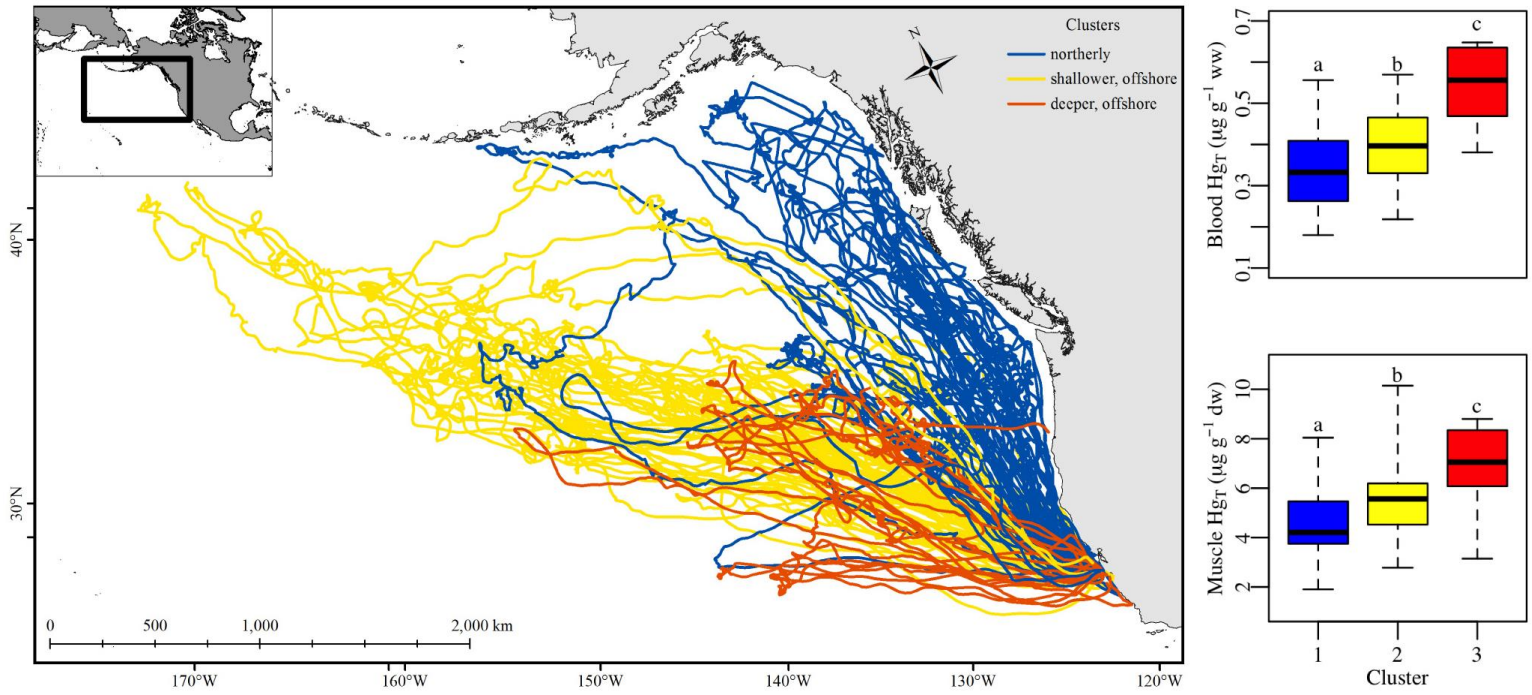

Figure 2.4. Hierarchical analysis on principal components, using geography, diving behaviour, and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to describe foraging behaviour of satellite-tracked adult female northern elephant seals (*Mirounga angustirostris*; n = 77) from the Año Nuevo colony, resulted in three clusters: blue (northerly; n = 30), yellow (shallower diving, offshore; n = 37), and red (deeper diving, offshore; n = 10). All three clusters included seals sampled after both the short and long foraging trips (see Fig 2.1) and had substantially different blood and muscle Hg_T concentrations

.

CHAPTER 3

Deep-ocean foraging northern elephant seals bioaccumulate persistent organic pollutants

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3.1 Abstract

As top predators in the North Pacific Ocean, northern elephant seals (*Mirounga angustirostris*) are vulnerable to bioaccumulation of persistent organic pollutants (POPs). Our study examined a suite of POPs in blubber (inner and outer) and blood (serum) of free-ranging northern elephant seals. For adult females (N = 24), we satellite tracked and sampled the same known-age seals before and after their approximately seven month long foraging trip. For males, we sampled different adult and sub-adults before (N = 14) and after (N = 15) the same foraging trip. For females, we calculated blubber burdens for all compounds. The highest POP concentrations in males and females were found for ∑DDTs and ∑PCBs. In both blubber and serum, males had greater concentrations than females for almost all compounds. For males and females, ∑DDT and ∑PBDEs were highly correlated in blubber and serum. While ∑PCBs were highly correlated with ∑DDTs and ∑PBDEs in blubber and serum for males, ∑PCBs showed weaker correlations with both compounds in females. As females gained mass during their foraging trip, concentrations of nearly all POPs in inner and

outer blubber decreased; however, the absolute burden in blubber increased for each compound, indicating ingestion of contaminants while foraging. Additionally, clusters of seal foraging behavior, based on geography, diving behavior, and stable carbon and nitrogen isotopes, corresponded with differences in ∑DDTs, ∑PBDEs, MeO-BDE 47, as well as the ratio of ∑DDTs to ∑PCBs, indicating the potential for behavior to heighten or mitigate contaminant exposure. Bioaccumulation of POPs by northern elephant seals supports the mesopelagic zone as a sink for POPs and highlights the potential of elephant seals as a sentinel of POP contamination in deep ocean food webs.

3.2. Introduction

Persistent organic pollutants (POPs) are a continued threat to wildlife because they are widely dispersed, bioaccumulate in top predators, and can disrupt physiological pathways, thus leading to adverse health effects (Tartu et al., 2015; Jenssen, 2006). Despite international regulation (2009 Stockholm Convention) and bans of some POPs by individual countries, the presence of polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its metabolites, polybrominated diphenyl ethers (PBDEs), chlordanes (CHLs), and other POPs in marine and terrestrial ecosystems remains pervasive (Braune et al., 2005; Farrington and Takada, 2014). While POPs have multiple sources and mechanisms of transport, the deep ocean can serve as a sink (Farrington and Takada, 2014), where POPs can enter marine food webs and can magnify with

trophic level (Weijs et al., 2009). POPs are resistant to biological degradation and associate mainly with lipids in biological organisms (Muir et al., 1999). The legacy of POPs is important for marine mammals because persistent exposure to even low levels of POPs can influence mammalian endocrine systems (Tanabe, 2002), neural function (Haijima et al., 2010; Winneke, 2011) and immune systems (Ross et al., 1996; Schwacke et al., 2012). Bioaccumulation of POPs in marine mammals may have population-level consequences through the combination of weakened immune function and infectious disease (Hall et al., 2006). In addition, POPs in adult female marine mammals are concerning because POPs can transfer from mother to offspring via placental transfer and lactation (Greig et al., 2007; Vanden Berghe et al., 2012; Wolkers et al., 2004), meaning that young animals are exposed to a suite of contaminants during critical periods of development.

The main source of POPs to marine mammals is through food (Muir et al., 1999), therefore specific foraging behaviors, including location or diet, may exacerbate or mitigate POP exposure. For example, proximity to industrialization and point sources of contaminant entry into the environment can result in higher POP bioaccumulation in some marine mammals than others (Frouin et al., 2011; Lopez et al., 2014; Ross et al., 2004; Schwacke et al., 2012). Differences in diet, within and among species, can also influence bioaccumulation of POPs (Bentzen et al., 2008; Ross et al., 2000). Concentrations of POPs are often higher in males than females because females can transfer contaminants to offspring while males

are unable to offload any of their accumulated contaminant burden (Barron et al., 2003; Storelli et al., 2012; Wang et al., 2007). Marine mammals are vulnerable to bioaccumulation of POPs because they often have relatively long life spans over which to accumulate POPs, and many species are more susceptible to biomagnification due to their high trophic position.

Studies of contaminant accumulation in mammals are challenging because factors such as animal age and body condition can confound analyses. Contaminants are primarily reported in concentrations, either by wet weight or lipid weight; however, concentrations of POPs are significantly influenced by body condition and physiological state in both stranded and free-ranging animals (Debier et al., 2012; Hall et al., 2008; Myers and Atkinson, 2012; Peterson et al., 2014). Varying contaminant concentrations have been observed for a significant number of marine mammal species, although many studies have focused on quantification of contaminants in stranded animals and less so in free-ranging animals. Contaminant concentrations in stranded and deceased animals may not represent healthy animals due to the complications of disease, dehydration, or starvation; therefore, sampling free-ranging animals provides a more complete quantification of the range of bioaccumulation experienced by a population as a whole. Additionally, quantification of the blubber burden of contaminants (mg contaminant) and the subsequent change in burden over time makes it possible to track changes in contaminants contained within the blubber layer, regardless of physiological changes. Indeed, variations of blubber POP concentrations, which

are directly related to physiological state and blubber mass, do not necessarily reflect variations of POP burden. For example, increased POP concentrations in blubber of northern elephant seals at the end of the lactation-associated fast, compared with the beginning of the fast, may actually correspond to a decreased body burden as a result of POP excretion through milk (Debier et al., 2012). In contrast, lower POP concentrations in elephant seal blubber after a foraging trip (Peterson et al., 2014) may correspond to a greater body burden as a result of foraging. The challenges associated with repeatedly sampling the same free-ranging animals have limited the number of studies that directly link individual foraging behavior with contaminant bioaccumulation or changes in contaminant burden.

As relatively long-lived, high trophic level predators foraging in the mesopelagic (200-1000 m) northeast Pacific Ocean, northern elephant seals (*Mirounga angustirostris*) can serve as biomonitors of remote ocean habitats that are difficult to sample. Elephant seals undergo biannual foraging trips, ranging upwards of 5,000 or 10,000 km depending on the season, within several open-ocean and near-coastal hydrographic ecoregions, including the Subarctic Gyre, the North Pacific Polar Front, and the California Current (Le Boeuf et al., 2000; Robinson et al., 2012; Springer et al., 1999). The northern elephant seal is the only pinniped species in the North Pacific that forages almost entirely on fish and squid in the mesopelagic zone (Antonelis et al., 1987; Le Boeuf et al., 2000; Naito et al., 2013). Biannual foraging trips (Fig 3.1) are interspersed with extensive

fasting periods on land, at which time individuals lose up to 40% of their body mass (Costa et al., 1986; Worthy et al., 1992).

Our study is one of few to repeatedly sample individuals at the start and end of a long foraging trip and to calculate variations in the contaminant mass in blubber. For this study, our objectives were to use free-ranging northern elephant seals to: 1) Measure a suite of POP compounds and compare the concentrations between adult females and males before and after the foraging trip, 2) Determine how well correlated different POP compounds are in females and males upon arrival to the colony at the end of the foraging trip, 3) Quantify changes in concentrations (females – paired seals, males – unpaired seals) and blubber burdens (females) from the start to the end of the foraging trip, and 4) Determine if contaminant concentrations and blubber burdens in females vary with clusters of foraging behavior.

3.3. Methods

3.3a. Animal sampling

We collected paired blubber and blood samples from adult northern elephant seals at the Año Nuevo State Reserve (37.11° N, 122.33° W) in 2012 and 2013. Known-age females (N = 24), ranging in age from four to twelve years, were sampled before (late in the molting fast) and after (early in the breeding fast) the approximately seven month foraging trip (Fig 3.1). Blubber cores and blood samples were also collected from 29 unique male northern elephant seals at two

points in their life history: 14 seals were sampled at the end of the molting fast and 15 seals were sampled at the start of the early breeding fast (Fig 3.1). Due to the challenges associated with repeatedly sampling males, we were unable to compare paired male samples across the foraging trip. However, we were able to collect blubber and serum samples from four paired males early and late in the breeding fast (48 – 53 days between samples), to examine if changes in POP concentrations were associated with changes in body condition.

To attach satellite tags, obtain tissue samples, and to collect morphometric measurements, we followed standard procedures for chemical immobilization of elephant seals (Le Boeuf et al. 2000, Robinson et al. 2012). We collected blood samples from the extradural vein, stored samples on ice for return to the lab on the same day, centrifuged blood, and stored serum samples in glass vials. A full-thickness blubber core was collected from the lateral pelvic area of each seal using a sterile 6 mm biopsy punch (Miltex, Inc., York, Pennsylvania, USA) and stored in aluminum foil. Red blood cells were collected for stable carbon and nitrogen analysis and were analyzed following previously published methods (Hückstädt et al., 2011; Peterson et al., *Accepted*). Blubber cores and blood samples were stored at -20°C until analysis.

We collected tissue samples and morphometric measurements each time an animal was handled. Measurements included girth and length taken at eight locations along the seal, including six locations where blubber thickness was measured dorsally, laterally, and ventrally using either a handheld ultrasound

backfat meter (Scanoprobe, Ithaca, New York, USA) or a Signos handheld portable ultrasound (Signostics Ltd, Clovelly Park, Australia) (Robinson et al., 2012; Schwarz et al., *Accepted*). Body mass (females only) was determined by rolling each seal into a canvas sling and using a hand-winch to suspend them from a 1000 ± 1.0 kg Dyna-Link digital scale attached to a metal tripod (Robinson et al. 2012).

3.3b. Laboratory analysis

In all samples, 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209), seven PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183), three DDTs (*p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT), five chlordanes (CHLs: OxC (oxychlordane), CC (*cis*-chlordane), TC (*trans*-chlordane), TN (*trans*-nonachlor), CN (*cis*-nonachlor)), hexachlorobenzene (HCB), hexachlorocyclohexane (α-, β-, and γ-HCH), and two naturally-produced methoxylated PBDEs (2′-MeO-BDE 68 and 6-MeO-BDE 47) were targeted. Extraction, clean-up, and concentration measurement methods followed protocols described in Vanden Berghe et al. (2012).

In brief, the skin layer and fur (1.3 cm deep on average, Schwarz et al., *Accepted*), was removed from the outer portion of the biopsy core, and the remaining blubber was cut into inner and outer segments of approximately equal mass. Each blubber segment was dried with Na_2SO_4, placed in a thimble, and

spiked with internal standards (BDE 77, BDE 128, ε-HCH, and CB 143). Lipids and target contaminants were extracted using hot Soxhlet extraction with hexane/acetone (3:1, v:v) for 2 h. An aliquot of the extract (typically $1/8^{th}$) was used for gravimetric determination of lipids (105 °C, 1 h). The rest of the extract was cleaned on approximately eight g acid silica (44% H_2SO_4, w/w) and eluted with 20 ml hexane, followed by 15 ml dichloromethane. The cleaned extract was evaporated to dryness and reconstituted in 150 µl iso-octane.

Serum samples were split for separate determination of target contaminants and lipids. For contaminant determination, serum samples were spiked with internal standards (CB 143, ε-HCH, and BDE 77) diluted with Milli-Q water, mixed with formic acid for protein denaturation, and sonicated for 20 min. Samples were extracted on solid phase extraction cartridges (Oasis HLB, 60 mg/3 ml). Extracts were eluted with dichloromethane (7.5 ml), concentrated, and further reconstituted with hexane (0.5 ml). Extracts were cleaned on 1 g acid silica (44% H_2SO_4, w/w) topped with 100 mg Na_2SO_4, eluted with 10 ml hexane:dichloromethane (1:1, *v:v*), evaporated to near-dryness, and resolubilized in 100 µl iso-octane. Four lipid classes in serum (total cholesterol, phospholipids, triacylglycerides, and non-esterified fatty acids) were determined with enzyme kits from Diasys Diagnostic Systems (Holzheim, Germany) and Wako Chemicals (Neuss, Germany), with the concentrations of each lipid class calculated on the basis of standard equivalents. Total lipid concentrations were calculated as the sum of the four lipid classes (Debier et al. 2006, Vanden Berghe et al. 2012).

PBDEs, MeO-PBDEs, CHLs, HCB, and HCHs were measured by gas chromatography-electron capture negative ion/mass spectrometry (GC-ECNI/MS) on a 30 m × 0.25 mm × 0.25 µm DB-5 column (J&W Scientific, Folsom, CA, USA) by monitoring two ions *m/z* = 79 and 81 (for PBDEs and MeO-PBDEs) and two specific ions for each pesticide. DDTs and PCBs were measured by gas chromatography-electron ionization/mass spectrometry (GC-EI/MS) on a 25 m × 0.22 mm × 0.25 µm HT-8 column (SGE, Zulte, Belgium) by monitoring 2 ions for each homologue group.

We scaled lipid-normalized POP concentrations up to a blubber burden for each seal using a multiple step process. We used the relative contribution of the inner and outer blubber layers to the full thickness blubber core, based on mass, to obtain an overall lipid-normalized POP concentration in blubber and an overall percent lipid of the blubber. Using the percent lipid for full thickness blubber, and both morphometric and ultrasound measurements, we estimated blubber lipid mass for each seal using the steps described in Schwarz et al., (*Accepted*). The overall lipid-normalized concentration of each POP compound in blubber was then multiplied by the lipid mass of each seal to obtain a pre- and post-foraging trip blubber burden (mg POP compound in blubber per seal). In order to compare POP bioaccumulation among clusters of seals with varying departure mass and mass gained while foraging, we calculated a burden of POP compound gained per kg of blubber lipid gained over the foraging trip.

3.3c. Quality control

For quality control (QC), we randomly analyzed procedural blanks, solvent blanks, and standards throughout the extraction process. Recoveries for individual PCB and PBDE congeners ranged between 75 and 104% (RSD < 12%). For each analyte, the mean procedural blank value was used for subtraction to determine final analyte concentrations. After blank subtraction, the limit of quantification (LOQ) was set at 3 x SD of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio S/N (signal to noise) equal to 10. A standard reference material SRM 1945 (PCBs, OCPs and PBDEs in whale blubber) was used to test the accuracy of the method. Measured values did not deviate more than 15% from the certified values.

3.3d. Animal movement and behavior

To determine if contaminant bioaccumulation varied with foraging behavior, including diving behavior, geography, and stable isotopes (Objective 4), we used a combination of satellite-transmitters and time-depth recorders on adult female seals. We used five-minute epoxy to attach satellite-transmitters (Wildlife Computers, Bellevue WA: MK-10 AF, SPOT4 and SPOT5) and time-depth recorders (Wildlife Computers, Bellevue WA: MK9 or MK10) to seals at the end of the fasting period on land prior to departure. We recovered instruments when seals returned to land at the end of a foraging trip. One time-depth recorder failed to record accurate information; therefore at-sea foraging behavior in relation to

contaminants was restricted to 23 females. We used standard filtering techniques and protocols (Robinson et al., 2012), georeferenced all dives, and interpolated the satellite track to three locations per day (one location every eight hours). From the georeferenced dives and the interpolated satellite tracks, we quantified a set of eight diving and geographic variables: median day foraging dive depth, median night foraging dive depth, 90^{th} percentile depth of day foraging dives, 90^{th} percentile depth of night foraging dives, percent of benthic dives, the average of a dimensionless dive index to quantify proportional use of the water column (dividing the maximum depth of each dive by the bathymetry, obtained from the ETOPO1 1 Arc-Minute Global Relief Model (Amante and Eakins, 2009)), maximum latitude reached during the trip, and median distance to the continental shelf.

We used the eight quantified geographic and diving variables in conjunction with $\delta^{13}C$ and $\delta^{15}N$ from elephant seal red blood cells to identify clusters of seal behavior, using a combination of principal components analysis (PCA) followed by hierarchical cluster analysis with the FactoMineR package in R (Husson et al., 2014). This method produces unrotated factors and we used the eigenvalue threshold of 1.0 to retain principal components, which were then input as variables into the cluster analysis. Clusters were identified based on intra-cluster inertia (using Euclidean distance), although we set the minimum number of clusters at three to achieve finer resolution of groups, based on previous research with a larger number of individuals (Peterson et al., *Accepted*).

3.3e. Statistical analysis

For Objective 1, we used linear mixed effects models to test for differences in concentrations of POPs (natural log transformed to meet the assumptions of normality and homogenous variance) in inner blubber, outer blubber, and serum between males and females. We ran mixed effects models separately for all POP compounds at each of two sampling periods (pre-foraging/late molting and post-foraging/early breeding). In each model, we included sex, tissue type, and an interaction between sex and tissue type as fixed effects, with individual seal as a random effect nested within sex. If the interaction was significant, we conducted three contrasts to compare concentrations between males and females in inner blubber, outer blubber, and serum. For Objective 2, we examined correlations among contaminant concentrations at the end of the foraging trip using Pearson's product moment correlation. For correlation analysis, when pairs of concentrations violated the assumption of bivariate normality, both concentrations were natural log-transformed. If this transformation was insufficient to obtain bivariate normality, we used a non-parametric Spearman's rank correlation. We examined the relationship between contaminants separately for each sex because exploratory regression analysis revealed numerous cases where there was either a significant difference in slope or intercept between the sexes. We calculated R^2 to describe the amount of variation statistically explained by each association of compounds. For Objective 3, we used paired t-tests to compare pre-foraging to

post-foraging contaminant concentrations (for serum, outer blubber, and inner blubber) and blubber burdens in females. For males, we used unpaired t-tests to compare pre-foraging concentrations to post-foraging concentrations. In addition, we used paired t-tests to examine differences in contaminant concentrations (inner blubber, outer blubber, and serum) of four males across their breeding-season fast. Finally, for Objective 4, we used ANOVA to test for differences among the identified clusters of seals for 1) blubber burden gain scaled by lipid gain (mg contaminant gained per kg lipid gained), 2) contaminant concentrations (inner and outer blubber), and 3) the ratio of the concentrations (inner and outer blubber) of the two most prevalent compounds, ΣPCBs and ΣDDTs. We initially used Analysis of Covariance (ANCOVA) with the percent of adipose tissue as a covariate (main effect) for the analysis of concentrations in inner blubber; however, the inclusion of the percent of adipose tissue for early breeding samples was not significant for any contaminant compound ($p > 0.279$) and was therefore removed from the final analyses. Data were transformed (natural log or reciprocal (1/x) transformation) if necessary to meet the assumptions of normality and homogeneous variance. If clusters were significantly different, we conducted post-hoc pairwise tests with a Holm correction for repeated analysis. All statistical analyses were conducted either in the statistical package R, version 3.1.0 (R Development Core Team, 2012) or JMP Pro 11, and statistical significance was set at $\alpha = 0.05$.

3.4. Results

3.4 Objective 1. Persistent organic pollutant concentrations

All elephant seals had detectable lipid-normalized concentrations of ΣPCBs, ΣDDTs, ΣCHLs and ΣPBDEs in blubber and serum samples before and after their long foraging trip (Table 3.1). Additionally, HCB, α-HCH, β-HCH, and 6-MeO-BDE 47 were detected in all blubber samples (Table 3.1), whereas γ-HCH was only detected in 6% of both inner and outer blubber samples and 2-MeO-BDE 68 was only detected in 22% of inner blubber, but not detected in outer blubber (both compounds only detected during early breeding). Fewer compounds had 100% detectability in serum samples (Table 3.1). The specific compounds with the highest mean concentrations in serum and blubber of northern elephant seal males and females were ΣDDTs, of which > 98% was the DDT metabolite, *p,p'*-DDE (Table 3.1). The second and third most prevalent compounds were ΣPCBs and ΣCHLs, respectively (Table 3.1).

Overall, for the majority of POP compounds pre- and post-foraging trip, male elephant seals had significantly greater concentrations in blubber (inner and outer) and serum than females (Table 3.2). The naturally produced 6-MeO-BDE 47 was the only compound with greater concentrations in both the blubber and serum of females than in males, which was observed at both time periods ($p \leq 0.002$). Pre- and post-foraging trip, concentrations of ΣDDTs, ΣPCBs, ΣCHLs, and β-HCH were greater in males than females in blubber and serum ($p \leq 0.026$). Pre-foraging trip, there was a marginally non-significant difference in the mean concentration

of ΣPBDEs in males and females ($F_{1,36}$ = 4.0, p = 0.054), and post-foraging trip ΣPBDEs were significantly greater in the blubber and serum of males than females ($p \leq 0.008$). Pre- and post-foraging trip concentrations of α-HCH were not significantly different between males and females in blubber ($p \geq 0.088$). In contrast, both pre- and post-foraging trip concentrations of α-HCH in serum were greater in males than females ($p \leq 0.001$). Pre-foraging trip concentrations of HCB in blubber and serum were significantly different between males and females ($p \leq 0.020$). However, in contrast with other compounds, post-foraging trip concentrations of HCB were greater for females in serum ($p < 0.001$), while concentrations were significantly greater in males in inner blubber (t = -2.0, p = 0.050) and nearly significant in outer blubber (t = -1.8, p = 0.068).

3.4 Objective 2. Correlations between contaminants

We found significant, positive relationships between concentrations of most, but not all, compounds in male and female elephant seals in blubber and serum after the foraging trip, upon arrival to the colony at the start of the breeding season (Table 3.3a-c). In males and females, ∑DDTs, and ∑PBDEs had significant, positive correlations ($p < 0.05$, Table 3.3a-c) in inner blubber (males: R^2 = 0.83; females: R^2 = 0.85), outer blubber (males: R^2 = 0.77, females: R^2 = 0.88) and serum (males: R^2 = 0.77; females: R^2 = 0.59). Similarly, correlations between ∑DDTs and ∑PCBs were strong for males in all tissue types ($R^2 \geq 0.83$, $p < 0.001$). However, in females, less variation was statistically explained by the

association of these compounds in inner blubber (R^2 = 0.37, p = 0.001) and serum (R^2 = 0.46, p < 0.001), and there was no significant relationship in outer blubber (R^2 = 0.11, p = 0.115; Table 3.3a-c). Additionally, in both males and females, ∑CHLs had significant, positive correlations with ∑DDTs and ∑PCBs in inner blubber ($R^2 \geq 0.44$), outer blubber ($R^2 \geq 0.40$), and serum ($R^2 \geq 0.42$). In females, ∑CHLs were significantly and positively correlated with ∑PBDEs in inner blubber, outer blubber, and serum ($R^2 \geq 0.17$, $p \leq 0.044$), whereas these two compounds in males were only significantly correlated in the inner blubber (*rho* = 0.53, p = 0.047) and not in the outer blubber or serum. In males, ∑PCBs were significantly correlated with ∑PBDEs in inner blubber (R^2 = 0.61, p < 0.001), outer blubber (R^2 = 0.56, p = 0.001) and serum (R^2 = 0.49, p = 0.004), but in females were only significantly correlated in inner blubber (R^2 = 0.18, p = 0.036) and serum (R^2 = 0.18, p = 0.041), and not in outer blubber (R^2 = 0.04, p = 0.345; Table 3.3a-c). For males and females, α-HCH and HCB were positively correlated in all tissue types ($R^2 \geq 0.30$, p < 0.021). In females but not males, β-HCH was positively correlated with ∑CHLs in blubber and serum ($R^2 \geq 0.18$, $p \leq 0.037$; Table 3.3a-c)

3.4 Objective 3. Change in contaminant concentrations and blubber burdens

Female concentrations: Using paired pre- and post-foraging trip tissue samples from adult females, we detected significant changes in almost all contaminant concentrations in blubber (inner and outer) and serum across the

foraging trip (Fig 3.2; Table 3.1). Additionally, the percent of lipid in inner blubber and serum significantly increased across the foraging trip ($t = 4.67$, $df = 23$, $p < 0.001$; $t = 2.18$, $df = 23$, $p = 0.040$, respectively; Table 3.1), but the percent of lipid in outer blubber did not significantly change between those time periods ($t = 0.32$, $df = 23$, $p = 0.753$; Table 3.1). The majority of POP concentrations in elephant seal females decreased across the foraging trip, with 6-MeO-BDE 47 the only contaminant to increase in concentration within the blubber while seals were foraging (Table 3.4).

Concentrations of ∑DDTs, ∑PCBs, ∑CHLs, ∑PBDEs, and β-HCH significantly decreased across the long foraging trip in inner blubber, outer blubber, and serum ($p < 0.044$; Table 3.1; Table 3.4). Concentrations of HCB and α-HCH decreased in inner blubber and outer blubber ($p < 0.023$) but not in serum ($p > 0.160$; Table 3.4). Concentrations of 6-MeO-BDE 47 increased within inner and outer blubber ($p < 0.001$) but serum concentrations did not significantly change (Table 3.4). Due to the low detectability of γ-HCH and 2-MeO-BDE 68, we did not test for changes in concentration of these compounds across the foraging trip.

Male concentrations: Using unpaired samples for adult males, we detected very few changes in POP concentrations across the foraging trip and we did not detect any overall differences in lipid percent; however, the variability in male contaminant concentrations was substantially higher than that observed in females (Table 3.1; Table 3.5). In male serum, only concentrations of ∑CHLs, HCB, and

α-HCH decreased across the foraging trip ($p < 0.034$; Table 3.5). In blubber, changes were only observed between pre-and post-trip concentrations of HCB and α-HCH in inner blubber ($p < 0.015$; Table 3.1; Table 3.5). The only contaminant observed in males to increase in concentration across the foraging trip was 6-MeO-BDE47 measured in serum ($t = -3.10$, $df = 15.6$, $p = 0.007$; Table 3.1). Conversely, when paired tissue samples from four males were examined across the breeding fast (48-53 days of fasting between samples; Table 3.6), concentrations of ∑DDTs, ∑PCBs, ∑CHLs, HCB, and β-HCH significantly increased in inner blubber, outer blubber, and serum ($p < 0.042$; Tables 3.6-3.7). Concentrations of ∑PBDEs increased in serum across the breeding fast ($t = 5.0$, $p = 0.016$), but did not change in blubber ($p \geq 0.087$; Table 3.6). Similarly, concentrations of α-HCH increased in serum across the breeding fast ($t = 5.7$, $p = 0.011$) but did not change in blubber. Concentrations of 6-MeO-BDE 47 did not significantly change across the breeding fast in blubber or serum (Table 3.6).

Female blubber burdens: In adult females, blubber burdens of ∑PCBs, ∑DDTs, ∑CHLs, ∑PBDEs, HCB, α-HCH, β-HCH, and 6-MeO-BDE 47 significantly increased across the foraging trip, based on paired pre- and post-foraging trip blubber samples ($p < 0.001$; Fig 3.2; Table 3.8). Elephant seal females gained a mean ± SD (range) of 25.1 ± 13.4 mg (4.1 - 56.3 mg) ∑PCBs, 40.1 ± 18.4 mg (13.9 - 82.9 mg) ∑DDTs, 7.8 ± 4.0 mg (2.0 - 15.9 mg) ∑CHLs, and 0.7 ± 0.4 mg (0.2 - 1.5 mg) ∑PBDEs between sampling periods, over the long foraging trip (Table 3.8). These increases resulted in female elephant seals

arriving to the colony with approximately 60.5 ± 16.5 mg ∑PCBs, 103.2 ± 31.0 mg ∑DDTs, 19.8 ± 4.8 mg ∑CHLs, and 1.8 ± 0.7 mg ∑PBDEs in their adipose tissue (Table 3.8).

3.4 Objective 4. Geography and foraging behavior

Adult female northern elephant seals (N = 23) were at sea for a mean ± SD (range) of 224 ± 4 days (217 – 233 days), with the distal location of the trip located 3,364 ± 1,062 km (range 1,126 – 5,206 km) from the Año Nuevo colony (Fig 3.3). The median distance of individual seals from the continental shelf ranged from 47 – 1204 km, with maximum distances from the continental shelf of 188 – 1646 km. The maximum latitude reached by individuals ranged from 42.0 – 59.2 °N. Stable carbon and nitrogen isotope values quantified in red blood cells were -19.5 ± 0.3‰ (-20.1 to -18.8‰) for $\delta^{13}C$ and 14.4 ± 0.9‰ (13.4 to 16.5‰) for $\delta^{15}N$. The median depth of foraging dives for individual seals was 633 ± 51 m (539 – 760 m) during the day and 486 ± 22 m (440 – 532 m) during the night. The 90^{th} percentile of foraging dives for individual seals was 776 ± 93 m (641 – 938 m) during the day and 653 ± 43 (578 – 740) during the night. Individual seals only had 4.8 ± 3.5% (10.7 – 35.5%) of total dives identified as benthic with an overall mean dive index (quantifying proportional use of the water column) of 15.5 ± 6.3% (10.7 – 35.5%).

Adult female elephant seals were clustered into three groups, the preset minimum number of groups, based on their foraging behavior, including

geographic location variables, diving behavior variables, and both carbon and nitrogen isotopes (Fig 3.3). The three clusters could be broadly classified into a northerly cluster (N = 4), a cluster enriched in ^{13}C and ^{15}N isotopes (hereafter the isotopes cluster) (N = 3), and an offshore cluster (N = 16). Based on the variables important in cluster creation, seals in the northerly cluster were characterized by a more northerly maximum latitude (57.9 ± 1.7 °N), used a greater proportion of the water column (dive index = 24.8 ± 8.1%), had shallower median day and night foraging dive depths (556 ± 22 m and 459 ± 13 m, respectively), had shallower 90^{th} percentile of dive depths during the day (687 ± 31 m), and had a smaller median distance to the continental shelf (197 ± 96 km) than the overall mean value for all seals. Seals in the isotopes cluster were more enriched in ^{13}C (-18.85 ± 0.04‰), more enriched in ^{15}N (16.36 ± 0.04‰), and had a deeper median depth of foraging dives at night than the overall mean for all seals. The seals in the offshore cluster had an average maximum latitude of 47.8 ± 3.7 °N, used a smaller proportion of the water column (dive index = 12.2 ± 1.2%), had a smaller proportion of benthic dives (3.6 ± 1.6%), had a greater median distance to the continental shelf (880 ± 225 km), and had a deeper median and 90^{th} percentile of foraging dive depths during the day (652 ± 34 m and 812 ± 81 m, respectively) than the overall mean for all seals.

Both the burden of contaminant (mg) gained per kg lipid gained over the foraging trip and contaminant concentration (inner and outer blubber) differed among clusters for some compounds (Fig 3.3). The mg POP gained per kg lipid

gained over the foraging trip differed among clusters for ∑DDTs, ∑PBDEs, and 6-MeO-BDE 47 ($F_{2,20}$ = 7.14, p = 0.005; $F_{2,20}$ = 6.47, p = 0.007; $F_{2,20}$ = 21.35, p < 0.001, respectively; Fig 3.3). Pairwise tests revealed that the isotope cluster had greater ∑DDTs and ∑PBDEs gained per kg lipid gained than the offshore cluster (p = 0.004 and p = 0.008), but was not significantly different from the northerly cluster (p = 0.088 and p = 0.221), and the northerly cluster was not significantly different than the offshore cluster (p = 0.222 and p = 0.221; Fig 3.3). The mg gain of 6-MeO-BDE 47 per kg lipid gained was lower for the northerly cluster than the isotope or offshore clusters (p < 0.001), which were not different from each other (p = 0.476).

Concentrations of ∑DDTs, ∑PBDEs, and 6-MeO-BDE 47 differed among clusters in both inner blubber ($F_{2,20}$ = 7.77, p = 0.003; $F_{2,20}$ = 8.4, p = 0.002; $F_{2,20}$ = 15.23, p < 0.001, respectively; Fig 3.3) and outer blubber ($F_{2,20}$ = 10.67, p < 0.001; $F_{2,20}$ = 13.60, p < 0.001; $F_{2,20}$ = 5.03, p = 0.017; $F_{2,20}$ = 10.35, p < 0.001, respectively; Fig 3.3), the same pattern demonstrated using the burden of these four contaminants gained per kg lipid gained. There was no detectable difference in the mg contaminant gained per kg lipid gained over the foraging trip for ∑PCBs, ∑CHLs, HCB, α-HCH, or β-HCH among the clusters of elephant seals.

Pairwise tests revealed the same differences in POP concentrations between clusters of northern elephant seals for inner and outer blubber as those observed for the mg of contaminant gained per kg lipid gained while foraging. Concentrations of ∑DDTs and ∑PBDEs were higher in inner and outer blubber of

seals from the isotope cluster than the offshore cluster (inner: $p = 0.003$ and $p = 0.002$; outer: $p < 0.001$ and $p < 0.001$) or the northerly cluster (inner: $p = 0.007$ and $p = 0.013$; outer: $p = 0.001$ and $p = 0.001$), which were not different from each other ($p > 0.586$). Concentrations of 6-MeO-BDE 47 were higher in the offshore cluster than the northerly cluster in inner blubber ($p < 0.001$) and outer blubber ($p = 0.001$), and concentrations were higher in the offshore cluster than the isotope cluster in inner blubber ($p = 0.032$) and outer blubber ($p = 0.048$). There was no difference between concentrations of 6-MeO-BDE 47 between the northerly and isotope cluster in either inner or outer blubber ($p > 0.111$). There was no detectable difference in the concentration of ∑PCBs, ∑CHLs, HCB, β-HCH, or α-HCH among the clusters of elephant seals.

The ratio between the two most prevalent contaminant compounds, ∑DDTs and ∑PCBs, was significantly different between clusters of elephant seal females for inner and outer blubber ($F_{2,20} = 11.6$, $p < 0.001$ and $F_{2,20} = 12.0$, $p < 0.001$, respectively). Ratios of ∑DDTs to ∑PCBs ranged from 1.0 – 3.4 in inner blubber and 1.2 – 3.8 in outer blubber. For both inner blubber and outer blubber, the ratios were significantly greater in the isotopes cluster than the northerly cluster ($p < 0.026$) and the offshore cluster ($p < 0.001$), which were not significantly different from each other ($p < 0.216$) (Fig 3.4). While we could not assign male elephant seals to geographic foraging regions because they were not satellite tracked, males had $\delta^{13}C$ values ranging from -19.2 to -16.5 and had ratios of ∑DDTs to ∑PCBs

ranging from 1.1 – 3.2 in inner blubber and 1.3 – 3.8 in outer blubber, overlapping with the ratios observed in females (Fig 3.4).

3.5. Discussion

Adult northern elephant seals revealed variation in bioaccumulation of POPs with sex and foraging behavior. All females and males had measurable concentrations of DDTs, PCBs, CHLs, and PBDEs in blubber and blood, demonstrating the legacy of these contaminants across an extensive expanse of the mesopelagic northeast Pacific Ocean.

For adult female and male elephant seals, the predominant POP compounds in blubber and serum were ∑DDTs, followed by ∑PCBs, which is in contrast with free-ranging adults from several other northeastern Pacific marine mammal species. Although some individual elephant seals had ratios of ∑DDTs to ∑PCBs close to 1, no elephant seals had greater concentrations of ∑PCBs than ∑DDTs (Fig 3.4). In contrast, concentrations of ∑PCBs were greater than ∑DDTs in the blubber of Steller sea lions (*Eumetopias jubatus*) from Alaska and monk seals (*Neomonachus schauinslandi*) from the Hawaiian Islands, (Lion et al., 1996; Lopez et al., 2012; Willcox et al., 2004). Additionally, female resident killer whales (*Orcinus orca*) from the Kenai Fjords in Alaska (Ylitalo et al., 2001) and belugas (*Delphinapterus leucas*) from Cook Inlet, Alaska (Hoguet et al., 2013) also had greater concentrations of ∑PCBs than ∑DDTs in blubber. In blubber of male harbor seals from the Gulf of Alaska, concentrations of ∑PCBs were greater

than ∑DDTs; however, the relationship between these two compounds was less clear for females (Wang et al., 2007). In contrast to these species but similar to northern elephant seals from the present and previous studies (Debier et al., 2012), concentrations of ∑DDTs were greater than ∑PCBs in the blubber of bottlenose dolphins (*Tursiops truncatus*) in San Diego Bay (Reddy et al., 2001) and California sea lions (*Zalophus californianus*) found along the California coast (Blasius and Goodmanlowe, 2008; Kannan et al., 2004; Ylitalo et al., 2005), although the only published concentrations of these compounds in adult California sea lion blubber are for stranded animals.

Geographic variability in the relative distribution of ∑DDTs to ∑PCBs in the northeastern Pacific may partially explain why elephant seals differ from more northerly- and offshore- distributed species. Sea otters (*Enhydra lutris*) sampled in Alaska had greater ∑PCBs than ∑DDTs in serum, whereas otters sampled in California had greater ∑DDTs than ∑PCBs (Jessup et al., 2010). Similarly, harbor porpoises (*Phocoena phocoena*) and humpback whales (*Megaptera novaeangliae*) sampled along the west coast of North America demonstrated increasing concentrations of ∑PCBs relative to ∑DDTs in blubber with increasing latitude (Calambokidis and Barlow, 1991; Elfes et al., 2010). Synthesis of these studies provides strong evidence for past and current distributional gradients of ∑DDTs relative to ∑PCBs in marine predators across the northeastern Pacific Ocean. In the present study, we observed a lower ratio of ∑DDTs to ∑PCBs for the northerly and offshore seals than the seals foraging closer to the coast and further

south (Fig 3.4). However, despite female elephant seals foraging a significant distance from shore across a large latitudinal range of the northeastern Pacific, the relationship of ∑DDTs to ∑PCBs for all seals appeared less similar to their northerly or highly offshore (i.e., monk seals from Hawaii) pinniped counterparts and more similar to marine mammals from southerly latitudes in the North Pacific.

POPs have not previously been quantified in free-ranging adult male northern elephant seals, and the two most prevalent POP compounds were ∑DDTs and ∑PCBs. Mean concentrations of ∑DDTs in males from the present study were greater than ∑DDTs in free ranging adult harbor seals (*Phoca vitulina*) from the Gulf of Alaska (Wang et al., 2007) and adult male Hawaiian monk seals from multiple colonies (Lopez et al., 2012; Willcox et al., 2004). In contrast, the mean concentrations of ∑PCBs in male Steller sea lions captured in British Columbia (Alava et al., 2012) and male Steller sea lions from the Bering Sea and Prince William Sound (Wang et al., 2011) were greater than the mean concentrations of ∑PCBs we observed in elephant seals. It should be noted, however, that direct comparison of ∑PCBs is difficult due to the variability in congeners quantified in each study. Mean ∑PCBs in elephant seal males were similar to male monk seals from the main Hawaiian Islands (Lopez et al., 2012), although other colonies of monk seals (Midway Island and French Frigate Shoals) demonstrated increased localized contamination of PCBs (Willcox et al., 2004; Ylitalo et al., 2008).

We observed greater concentrations of most POP compounds in males versus females, similar to previous studies on other pinniped species (Barron et al., 2003; Lopez et al., 2012; Wang et al., 2007). This is not surprising, given that females offload a large proportion of their contaminant burden to their offspring, with some transfer occurring during gestation but the primary transfer occurring through lactation (Debier et al., 2012; Frouin et al., 2012; Vanden Berghe et al., 2010). For northern elephant seals, most females give birth to a pup every year and offload a significant proportion of their contaminant burden to their pup (Debier et al., 2012; Peterson et al., 2014). In addition to reproductive explanations for differences in contaminant concentrations between the females and males, male elephant seals also differ from the majority of female elephant seals in foraging location and diving behavior, as well as potential differences in prey selection (Le Boeuf et al., 2000). The only compound found in greater concentrations in the blubber of females was the naturally produced 6-MeO-BDE 47, which may be attributed to the offshore nature of female foraging in contrast with the coastal nature of male foraging (Le Boeuf et al., 2000; Simmons et al., 2007). This hypothesis was additionally supported within females, with the greatest concentrations of 6-MeO-BDE 47 observed in the cluster of females identified as foraging furthest away from the continental shelf (Fig 3.3). Limited information exists regarding sources or accumulation patterns of MeO-BDEs in the northeastern Pacific (Alonso et al., 2014). It is unlikely that differences in age between males and female elephant seals caused the differences we observed, as

reproductive aged males likely overlapped in age with the older females we sampled (Condit et al., 2014).

The difference in correlations between females and males among compounds may indicate either differential geographic distributions or varying rates of metabolism or reproductive transfer among compounds. For example, correlations ∑DDTs and ∑PCBs in blubber and serum were weaker, especially for females, than the strong correlations observed for both females and males between ∑DDTs and ∑PBDEs. In addition, ∑CHLs were well correlated with ∑PCBs and less so with ∑DDTs or ∑PBDEs. While there is evidence for selective maternal transfer of some POP compounds over others (Debier et al., 2003; Vanden Berghe et al., 2012) and the potential exists for differential metabolism of certain POP compounds over others, these physiological processes would likely be consistent among elephant seals (Louis et al., 2015). Thus, variability in geographic distribution of POPs or differences in foraging behavior associated with geography are more likely explanations for the patterns we observed. Long range transport potential varies among POP compounds, with PCBs (specifically, the less chlorinated congeners) more likely to reach regions farther from their initial source than either DDTs or PBDEs (Lohmann et al., 2007; Wania and Dugani, 2003). This was supported by female elephant seals from our study because they returned from a wide range of foraging areas with similar bioaccumulation of ∑PCBs, indicating ubiquity in distribution, whereas bioaccumulation of ∑DDTs and ∑PBDEs was highest in animals that foraged closer to the continental shelf

(Fig 3.3). In male seals, all three compounds were highly correlated, which may be attributed to the more uniform, coastal foraging behavior of males. Additionally, the timeline of use for these compounds may contribute to their environmental distribution. Whereas worldwide manufacture of PCBs ceased in the early 1990s (Breivik et al., 2002), the use of PBDEs is more recent in some regions and thus these compounds may not have yet reached more remote locations, and the use of DDT for insect control in parts of the world (including Mexico and some regions of Asia) continues or was recently phased out (Lohmann et al., 2007).

Foraging behavior influenced bioaccumulation of some POP compounds in mesopelagic foraging adult female northern elephant seals. In previous research on epipelagic foraging marine predators from the North Pacific Ocean, intraspecific variability in POP concentrations was observed in shearwaters (*Calonectris leucomelas*) (Ito et al., 2013), skipjack tuna (*Katsuwonus pelamis*) (Ueno et al., 2003), humpback whales (Elfes et al., 2010), and Hawaiian monk seals (Lopez et al., 2012), and attributed to differences in geographic location. However, the influence of foraging behavior on POP concentrations may depend on foraging characteristics beyond geography. Our cluster analysis of female seals, characterized using geography, stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$), and diving behavior, showed that $\sum$DDTs and $\sum$PBDEs were greatest in the seals that foraged further south, closer to the continental shelf, and were more enriched in $\delta^{13}C$ and $\delta^{15}N$ (Fig 3.3). Of note, one female in this cluster foraged in a

geographic region overlapping with females from the offshore cluster; however, her stable isotope ratios and her ∑DDTs and ∑PBDEs were different from the offshore females, providing evidence for an interplay between the multiple components of foraging behavior that influence contaminant bioaccumulation. The seals that foraged more northerly and at shallower depths, near the Subarctic Gyre, had the lowest concentrations of 6-MeO-BDE 47, suggesting that regions in the north Pacific may vary in the production of this naturally-produced brominated compound.

Our study showed that elephant seals ingest a suite of POPs while foraging in the mesopelagic North Pacific. The influence of body condition on blubber concentrations of POPs (Debier et al., 2012; Hall et al., 2008; Myers and Atkinson, 2012) makes it challenging to use concentrations in blubber to track temporal changes in POPs in marine predators that fluctuate markedly in body condition as a consequence of annual life history events. Concentrations of POPs in previous studies on elephant seals decreased in response to improved body condition and mass gain, which often occurs during foraging (Peterson et al., 2014), and increased while fasting (Debier et al., 2012). For our study, contaminant concentrations in female elephant seals decreased over the foraging trip, but blubber burdens of all compounds increased, indicating ingestion of POPs during the foraging trip at a higher rate than they were being metabolically processed or transferred to a developing fetus.

We provided an important comparison between POP tissue concentrations and blubber burdens in a free ranging marine predator across the duration of an approximately seven month foraging trip. Concentrations of POP compounds in blubber (inner and outer) decreased while animals gained mass during their foraging trip, with the exception of 6-MeOBDE 47, whereas the absolute burden of all POP compounds in blubber increased across the foraging trip. Our results demonstrate that regardless of any differences in foraging characteristics, all female elephant seals gained POPs while foraging. To account for varying animal size and foraging success, we did not directly compare blubber burden with clusters of seal foraging behavior, but instead used the ratio of POP burden gained to the lipid mass gained while foraging. Importantly, the differences among clusters of elephant seal foraging behavior for POPs were consistent regardless of whether we examined concentrations in blubber (inner or outer) at the end of the foraging trip or the burden of contaminant gained per kg of lipid gained. These observations suggest that, in the case of elephant seals sampled at the end of a foraging trip, contaminant concentrations alone can be used to indicate the influence of recent foraging behavior on POP exposure.

As mesopelagic predators, northern elephant seals utilize a large portion of the northeastern Pacific Ocean, and all individuals demonstrated POP bioaccumulation. The inability of males to offload POPs to offspring possibly coupled with differences in foraging behavior from females resulted in greater bioaccumulation of POPs in the males, as seen in other marine mammal species.

Tissue correlations and individual burdens suggest uneven distribution of major POP compounds in marine food webs across the foraging range of northern elephant seals. Our calculation of blubber burdens for adult females allowed us to distinguish ingestion of POPs over the course of an extensive foraging trip, despite mass dilution effects on tissue concentrations. We showed that the deep-ocean foraging strategy of elephant seals does not eliminate their risk for POP exposure and further supports that the mesopelagic ocean is a sink for legacy POPs.

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Table 3.1. The same adult female (F) elephant seals (N = 24) were sampled during late molting and early breeding for POPs in inner blubber, outer blubber and serum. Unpaired males (M) were sampled during late molting (N = 14) and early breeding (N = 15). POP concentrations were quantified in all three tissue types for every animal. Blubber percent lipid is reported as percent lipid (w/w) and serum is reported as g lipid dL^{-1} serum (mean ± sd) . Lipid-normalized concentrations (ng g^{-1} lipid) are reported as mean ± sd (*number of samples above the detection limit*[a]: range of concentrations). Italics indicate what percent of the total (i.e., ΣPCBs or ΣPBDEs) can be attributed to a specific group of congeners (i.e., Σtri-CBs) or a specific congener (i.e., BDE 47).

		Late molting			Early breeding		
Type	Sex	Inner	Outer	Serum	Inner	Outer	Serum
Percent lipid	F	82.1 ± 6.0 (65.7 – 91.7)	82.4 ± 3.2 (75.9 – 87.2)	0.942 ± 0.120 (0.710 – 1.232)	87.4 ± 2.4 (81.9 – 91.1)	82.7 ± 4.5 (75.8 – 94.3)	0.998 ± 0.063 (0.873 – 1.093)
	M	80.4 ± 5.2 (67.9 – 88.5)	71.2 ± 5.9 (60.0 – 83.1)	1.108 ± 0.147 (0.740 – 1.321)	82.6 ± 5.5 (72.3 – 89.0)	72.9 ± 5.9 (60.6 – 83.5)	1.039 ± 0.215 (0.796 – 1.633)
ΣDDTs[b]	F	1736.0 ± 603.2 (902.9 – 3354.8)	1579.1 ± 487.6 (865.1 – 2677.2)	1273.9 ± 387.2 (623.7 – 2360.6)	1180.5 ± 536.5 (598.1 – 3086.8)	1473.4 ± 485.7 (875.3 – 2803.7)	684.8 ± 339.0 (365.3 – 1912.0)
	M	3161.3 ± 2189.2 (1355.3 – 8730.2)	2848.1 ± 1774.6 (1054.6 – 7107.3)	2066.3 ± 869.0 (823.4 – 3778.1)	3030.7 ± 2952.9 (705.7 – 12689.5)	3458.8 ± 3450.0 (770.8 – 15061.3)	1896.5 ± 1699.8 (390.1 – 7532.6)
% p,p'-DDE	*F*	*99.2 ± 0.3 (98.3 – 99.7)*	*99.2 ± 0.3 (98.5 – 99.7)*	*99.0 ± 0.2 (98.0 – 99.0)*	*98.6 ± 0.5 (97.4 – 99.7)*	*99.0 ± 0.3 (98.3 – 99.7)*	*98.3 ± 0.4 (98.0 – 99.0)*
	M	*99.5 ± 0.3 (98.9 – 99.8)*	*99.5 ± 0.3 (99.0 – 99.9)*	*99.1 ± 0.5 (98.0 – 100.0)*	*99.2 ± 0.6 (97.5 – 99.9)*	*99.5 ± 0.3 (98.9 – 99.9)*	*99.1 ± 0.7 (98.0 – 100.0)*
ΣPCBs[c]	F	1111.4 ± 278.3 (580.7 – 1806.2)	800.5 ± 142.1 (501.3 – 1053.2)	673.9 ± 169.0 (445.4 – 997.1)	722.6 ± 169.9 (422.1 – 1029.8)	781.1 ± 135.5 (539.6 – 1046.8)	322.0 ± 73.7 (194.7 – 470.2)
	M	1684.2 ± 997.6 (733.5 – 4610.6)	1559.6 ± 1013.2 (584.4 – 4396.7)	994.4 ± 372.7 (437.1 – 1679.7)	1557.0 ± 838.1 (448.8 – 3942.7)	1516.1 ± 815.9 (517.0 – 3975.1)	922.3 ± 494.6 (236.6 – 2317.7)
% Σtri-CBs	F	*<0.1 ± <0.1 (<0.1 – <0.1)*	*0.0 ± 0.0 (0.0 – 0.0)*	*<0.1 ± <0.1 (<0.1 – <0.1)*	*<0.1 ± <0.1 (<0.1 – 0.3)*	*0.0 ± 0.0 (0.0 – 0.0)*	*0.1 ± 0.4 (<0.1 – 1.9)*
	M	*<0.1 ± <0.1 (<0.1 - <0.1)*	*0.0 ± 0.0 (0.0 – 0.0)*	*<0.1 ± <0.1 (<0.1 - <0.1)*	*<0.1 ± <0.1 (<0.1 - <0.1)*	*0.0 ± 0.0 (0.0 – 0.0)*	*<0.1 ± <0.1 (<0.1 - <0.1)*
% Σtetra-CBs	F	*2.8 ± 0.7*	*3.7 ± 0.8*	*2.1 ± 0.8*	*3.4 ± 0.9*	*3.7 ± 0.8*	*2.5 ± 0.9*

		(1.8 – 5.0)	*(2.6 – 5.6)*	*(0.8 – 4.5)*	*(1.9 – 5.4)*	*(2.3 – 5.1)*	*(1.6 – 4.7)*
	M	*3.3 ± 0.8*	*3.6 ± 1.1*	*3.3 ± 0.8*	*3.0 ± 1.4*	*3.5 ± 1.5*	*2.3 ± 1.1*
		(1.8 – 4.6)	*(1.4 – 6.0)*	*(1.6 – 5.1)*	*(1.4 – 6.5)*	*(1.5 – 6.3)*	*(0.8 – 5.0)*
% Σpenta-CBs	F	*17.0 ± 2.2*	*19.4 ± 2.6*	*25.5 ± 3.4*	*17.8 ± 2.5*	*18.6 ± 2.6*	*32.4 ± 3.1*
		(13.4 – 22.3)	*(15.5 – 24.0)*	*(19.5 – 31.9)*	*(13.7 – 22.5)*	*(15.0 – 22.9)*	*(27.5 – 38.5)*
	M	*21.8 ± 1.5*	*22.0 ± 1.6*	*32.9 ± 2.8*	*20.2 ± 2.6*	*21.4 ± 2.9*	*29.2 ± 4.3*
		(19.0 – 24.4)	*(19.4 – 24.5)*	*(28.1 – 37.7)*	*(16.2 – 23.8)*	*(17.0 – 26.7)*	*(21.4 – 34.9)*
% Σhexa-CBs	F	*51.9 ± 1.5*	*52.8 ± 1.4*	*52.5 ± 1.9*	*52.2 ± 1.6*	*52.5 ± 1.4*	*48.9 ± 2.6*
		(48.6 – 54.4)	*(49.6 – 55.5)*	*(46.8 – 54.9)*	*(49.3 – 55.1)*	*(50.0 – 54.6)*	*(43.0 – 52.6)*
	M	*54.0 ± 1.4*	*54.4 ± 1.6*	*49.6 ± 2.3*	*54.1 ± 2.0*	*54.2 ± 2.4*	*51.7 ± 2.4*
		(51.9 – 57.3)	*(51.1 – 57.3)*	*(44.1 – 53.2)*	*(49.5 – 57.1)*	*(49.0 – 57.6)*	*(47.0 – 55.1)*
% Σhepta-CBs	F	*22.3 ± 2.7*	*19.2 ± 2.7*	*17.4 ± 2.6*	*21.0 ± 2.5*	*20.1 ± 2.6*	*14.4 ± 1.3*
		(16.0 – 27.2)	*(15.5 – 24.4)*	*(13.5 – 22.3)*	*(16.9 – 24.9)*	*(15.7 – 24.4)*	*(11.8 – 16.2)*
	M	*16.8 ± 1.3*	*16.2 ± 1.1*	*12.5 ± 1.3*	*18.3 ± 2.3*	*16.8 ± 2.1*	*14.8 ± 2.8*
		(14.7 – 18.5)	*(14.8 – 18.2)*	*(10.8 – 15.1)*	*(15.1 – 22.7)*	*(13.9 – 21.2)*	*(10.7 – 21.1)*
% Σocta-CBs	F	*3.9 ± 0.9*	*2.7 ± 0.7*	*1.4 ± 0.3*	*3.5 ± 0.8*	*2.9 ± 0.7*	*1.1 ± 0.1*
		(2.2 – 5.4)	*(1.8 – 4.0)*	*(0.9 – 2.2)*	*(2.2 – 4.8)*	*(1.9 – 3.9)*	*(0.8 – 1.4)*
	M	*2.7 ± 0.5*	*2.4 ± 0.4*	*1.0 ± 0.2*	*3.2 ± 0.7*	*2.7 ± 0.6*	*1.3 ± 0.4*
		(2.0 – 3.9)	*(2.0 – 3.5)*	*(0.8 – 1.3)*	*(2.0 – 4.0)*	*(1.7 – 3.9)*	*(0.7 – 2.2)*
% Σnona-CBs	F	*0.2 ± <0.1*	*0.1 ± <0.1*	*<0.1 ± <0.1*	*0.2 ± <0.1*	*0.1 ± <0.1*	*<0.1 ± <0.1*
		(0.1 – 0.4)	*(<0.1 – 0.2)*	*(<0.1 – 0.1)*	*(<0.1 – 0.3)*	*(<0.1 – 0.2)*	*(<0.1 – 0.1)*
	M	*0.1 ± <0.1*	*0.1 ± <0.1*	*<0.1 ± <0.1*	*0.2 ± <0.1*	*0.1 ± <0.1*	*<0.1 ± <0.1*
		(<0.1 – 0.3)	*(<0.1 – 0.2)*	*(<0.1 – 0.1)*	*(<0.1 – 0.3)*	*(<0.1 – 0.2)*	*(<0.1 – 0.2)*
% Σdeca-CBs	F	*0.1 ± <0.1*	*<0.1 ± <0.1*	*0.0 ± 0.0*	*0.1 ± <0.1*	*<0.1 ± <0.1*	*0.0 ± 0.0*
		(<0.1 – 0.3)	*(<0.1 – 0.1)*	*(0.0 – 0.0)*	*(<0.1 – 0.2)*	*(<0.1 – 0.1)*	*(0.0 – 0.0)*
	M	*<0.1 ± <0.1*	*<0.1 ± <0.1*	*0.0 ± 0.0*	*<0.1 ± <0.1*	*<0.1 ± <0.1*	*0.0 ± 0.0*
		(<0.1 – 0.1)	*(<0.1 – <0.1)*	*(0.0 – 0.0)*	*(<0.1 – 0.1)*	*(<0.1 – <0.1)*	*(0.0 – 0.0)*
ΣCHLs[d]	F	361.7 ± 85.1	277.9 ± 41.0	196.5 ± 38.6	234.6 ± 56.2	257.6 ± 32.9	112.0 ± 24.8
		(242.8 – 532.3)	(211.2 – 352.6)	(120.5 – 271.3)	(158.1 – 373.2)	(207.2 – 327.9)	(74.9 – 175.1)
	M	578.7 ± 265.4	498.4 ± 247.9	322.2 ± 93.6	484.0 ± 126.9	492.0 ± 130.3	251.8 ± 74.3
		(320.2 – 1262.2)	(247.2 – 1061.3)	(187.9 – 468.7)	(237.1 – 684.5)	(233.6 – 729.2)	(111.2 – 350.7)
% OxC	*F*	*16.3 ± 2.4*	*17.5 ± 2.7*	*22.0 ± 2.9*	*15.8 ± 2.3*	*17.0 ± 2.4*	*23.1 ± 3.0*
		(13.3 – 21.9)	*(13.5 – 22.4)*	*(18.0 – 28.4)*	*(12.7 – 21.8)*	*(13.9 – 21.8)*	*(18.8 – 28.5)*
	M	*20.0 ± 2.4*	*20.8 ± 2.2*	*28.2 ± 2.3*	*18.6 ± 2.0*	*19.6 ± 1.9*	*24.0 ± 2.4*
		(17.0 – 26.2)	*(17.8 – 26.1)*	*(24.9 – 33.5)*	*(14.8 – 21.3)*	*(15.9 – 23.1)*	*(20.2 – 28.3)*

% TC	*F*	*1.1 ± 0.2 (0.9 – 1.6)*	*1.3 ± 0.2 (1.0 – 1.7)*	*1.0 ± 0.2 (0.7 – 1.4)*	*1.2 ± 0.1 (0.9 – 1.6)*	*1.2 ± 0.1 (1.1 – 1.6)*	*1.3 ± 0.4 (1.0 – 3.2)*
	M	*1.0 ± 0.5 (0.4 – 1.9)*	*1.0 ± 0.5 (0.4 – 1.9)*	*0.9 ± 0.4 (0.4 – 1.6)*	*0.9 ± 0.3 (0.5 – 1.3)*	*1.0 ± 0.3 (0.5 – 1.5)*	*0.8 ± 0.3 (0.4 – 1.3)*
% CC	*F*	*2.9 ± 0.5 (1.8 – 3.8)*	*3.1 ± 0.4 (2.3 – 3.8)*	*3.5 ± 0.7 (2.1 – 4.7)*	*4.1 ± 1.0 (2.6 – 6.3)*	*3.2 ± 0.5 (2.3 – 4.3)*	*6.4 ± 1.7 (0.9 – 8.5)*
	M	*2.1 ± 0.8 (0.7 – 3.3)*	*2.0 ± 0.8 (0.8 – 3.4)*	*2.8 ± 1.1 (1.4 – 4.8)*	*2.3 ± 0.9 (1.1 – 4.4)*	*2.0 ± 0.7 (1.1 – 3.7)*	*3.1 ± 1.3 (1.2 – 5.5)*
% TN	*F*	*67.5 ± 2.5 (62.0 – 71.2)*	*65.9 ± 2.4 (61.0 – 69.2)*	*58.6 ± 2.5 (53.5 – 63.2)*	*66.4 ± 2.0 (62.0 – 70.6)*	*66.0 ± 1.9 (62.2 – 68.6)*	*54.7 ± 2.2 (51.0 – 60.2)*
	M	*63.2 ± 1.9 (59.7 – 66.8)*	*62.6 ± 2.1 (57.9 – 66.0)*	*52.7 ± 1.7 (50.3 – 56.8)*	*64.7 ± 2.2 (61.5 – 70.0)*	*64.1 ± 2.4 (59.5 – 69.4)*	*55.1 ± 2.3 (51.0 – 60.0)*
% CN	*F*	*12.2 ± 0.5 (11.2 – 13.3)*	*12.2 ± 0.4 (11.5 – 13.5)*	*14.9 ± 0.7 (13.2 – 16.5)*	*12.5 ± 0.5 (11.2 – 13.3)*	*12.6 ± 0.5 (11.7 – 13.7)*	*14.5 ± 0.8 (13.2 – 15.9)*
	M	*13.6 ± 1.0 (11.9 – 15.5)*	*13.6 ± 1.0 (12.2 – 16.3)*	*15.4 ± 1.2 (13.8 – 18.5)*	*13.4 ± 0.9 (12.1 – 15.3)*	*13.4 ± 1.2 (11.8 – 15.8)*	*16.9 ± 1.4 (15.5 – 19.9)*
α-HCH	F	7.0 ± 2.0 (3.1 – 11.7)	5.6 ± 1.2 (2.8 – 8.2)	2.7 ± 0.7 (1.0 – 4.0)	4.8 ± 1.3 (2.6 – 7.5)	4.7 ± 1.0 (2.3 – 7.4)	2.4 ± 0.8 (1.0 – 4.1)
	M	7.0 ± 1.5 (4.0 – 9.3)	6.6 ± 1.6 (3.1 – 9.4)	3.8 ± 1.0 (2.2 – 5.3)	5.6 ± 1.4 (4.0 – 7.7)	5.6 ± 1.6 (2.9 – 8.8)	1.7 ± 0.7 (*13:* 1.0 – 3.8)
β-HCH	F	36.2 ± 10.8 (18.1 – 60.1)	35.5 ± 9.5 (19.0 – 54.1)	19.2 ± 6.5 (7.7 – 29.8)	26.0 ± 9.6 (18.8 – 55.8)	30.5 ± 9.3 (20.0 – 57.2)	14.6 ± 4.1 (7.1 – 25.5)
	M	61.6 ± 19.5 (37.6 – 119.0)	61.1 ± 20.8 (37.4 – 123.9)	33.6 ± 11.6 (20.2 – 69.7)	50.4 ± 10.4 (34.6 – 70.3)	56.0 ± 9.3 (41.4 – 72.1)	27.8 ± 5.8 (17.0 – 35.0)
HCB	F	21.7 ± 4.0 (10.0 – 26.3)	22.5 ± 2.5 (16.9 – 27.1)	36.0 ± 13.1 (14.1 – 61.4)	15.4 ± 3.7 (8.6 – 21.7)	19.3 ± 6.0 (10.2 – 30.4)	31.7 ± 10.4 (7.2 – 47.6)
	M	31.2 ± 6.1 (15.9 – 43.2)	29.7 ± 8.1 (14.9 – 42.0)	61.3 ± 23.1 (15.6 – 86.7)	21.7 ± 7.9 (7.5 – 36.3)	25.9 ± 9.0 (12.1 – 42.9)	12.5 ± 8.4 (*12*: 2.0 – 30.8)
ΣPBDEs[e]	F	32.1 ± 14.1 (14. – 76.0)	27.0 ± 11.3 (12.2 – 52.0)	18.2 ± 7.8 (7.9 – 43.5)	21.2 ± 11.6 (10.3 – 61.9)	24.8 ± 10.6 (13.2 – 55.6)	9.1 ± 6.1 (4.4 – 27.4)
	M	44.9 ± 31.6 (18.4 – 118.7)	43.6 ± 30.7 (13.2 – 112.8)	26.1 ± 18.8 (11.7 – 68.7)	59.9 ± 68.2 (13.3 – 264.6)	63.0 ± 76.4 (14.8 – 284.7)	28.7 ± 33.8 5.4 – 135.5)
% BDE-47	*F*	*66.3 ± 5.8 (55.3 – 79.2)*	*68.6 ± 7.6 (47.1 – 79.3)*	*76.6 ± 7.4 (58.9 – 88.1)*	*64.3 ± 7.1 (47.2 – 78.6)*	*66.9 ± 6.3 (56.3 – 78.1)*	*73.8 ± 11.4 (38.8 – 87.4)*

	M	*76.2 ± 4.4*	*74.5 ± 7.1*	*84.3 ± 5.3*	*70.7 ± 5.5*	*72.4 ± 3.5*	*83.3 ± 4.3*
		(69.9 – 85.4)	*(52.9 – 84.0)*	*(74.9 – 90.3)*	*(60.6 – 78.3*	*(65.6 – 77.1)*	*(73.7 – 90.1)*
% BDE-99	*F*	*8.7 ± 1.6*	*9.9 ± 5.7*	*6.4 ± 5.7*	*9.7 ± 4.7*	*8.9 ± 1.6*	*8.5 ± 9.4*
		(4.2 – 13.1)	*(5.0 – 28.6)*	*(0.5 – 25.8)*	*(4.2 – 29.8)*	*(5.0 – 12.9)*	*(1.2 – 39.1)*
	M	*4.6 ± 1.6*	*5.8 ± 5.1*	*3.4 ± 3.2*	*7.9 ± 4.9*	*7.4 ± 2.6*	*2.5 ± 2.8*
		(1.8 – 7.0)	*(2.2 – 23.0)*	*(0.3 – 10.3)*	*(3.1 – 21.2)*	*(4.0 – 13.4)*	*(0.5 – 9.8)*
% BDE-100	*F*	*10.3 ± 1.0*	*10.1 ± 1.2*	*7.3 ± 1.5*	*9.8 ± 0.9*	*10.2 ± 1.2*	*7.1 ± 2.0*
		(8.5 – 12.9)	*(7.7 – 13.1)*	*(4.3 – 9.9)*	*(7.5 – 11.6)*	*(8.6 – 13.1)*	*(3.7 – 11.8)*
	M	*10.3 ± 1.9*	*10.5 ± 2.0*	*6.3 ± 1.1*	*11.1 ± 3.2*	*10.8 ± 2.9*	*7.7 ± 2.2*
		(7.9 – 14.8)	*(7.8 – 14.8)*	*(4.0 – 8.4)*	*(7.1 – 18.9)*	*(7.5 – 18.2)*	*(3.7 – 11.4)*
% BDE-154	*F*	*10.1 ± 3.9*	*7.4 ± 3.2*	*6.7 ± 2.9*	*10.8 ± 4.2*	*9.1 ± 3.8*	*6.3 ± 3.3*
		(3.9 – 18.5)	*(2.6 – 15.0)*	*(2.2 – 11.9)*	*(3.7 – 19.7)*	*(3.0 – 16.9)*	*(1.9 – 13.0)*
	M	*4.7 ± 1.9*	*5.2 ± 1.6*	*2.7 ± 1.4*	*5.5 ± 1.9*	*5.1 ± 1.8*	*3.6 ± 2.1*
		(1.4 – 8.5)	*(2.3 – 7.9)*	*(1.0 – 6.0)*	*(2.2 – 9.3)*	*(1.9 – 7.9)*	*(1.2 – 10.0)*
6-MeO-BDE 47	F	2.6 ± 0.6	1.9 ± 0.4	0.8 ± 0.7	3.1 ± 0.8	2.4 ± 0.7	1.0 ± 0.5
		(1.3 – 4.0)	(1.2 – 2.9)	(*23*: 0.2 – 2.2)	(1.5 – 4.5)	(1.4 – 4.4)	(*23*: 0.2 – 1.9)
	M	1.7 ± 0.9	1.5 ± 0.6	0.2 ± 0.7	2.2 ± 1.4	1.5 ± 0.8	0.1 ± 0.6
		(0.6 – 4.0)	(0.4 – 2.8)	(*7*: 0.2 – 0.8)	(0.6 – 5.3)	(0.4 – 3.4)	(*14*: 0.2 – 1.9)

[a]The number of samples above the detection limit for a given compound is only presented if the compound was detected in less than 100% of samples.

[b]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[c]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[d]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[e]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

Table 3.2a. Statistical output for linear mixed effects models comparing POP concentrations between male and female northern elephant seal serum and blubber (inner and outer) samples before the foraging trip (at the end of the molting), with individual included as a random effect. If the interaction between sex and tissue type was significant, post-hoc contrasts were run to just compare each tissue type between males and females. If there was no interaction, then the main effects (sex and tissue type) were examined. Significant differences ($p < 0.05$) are bolded.

Pre-foraging

POP compound	fixed effects	test statistics	post-hoc contrasts
ΣDDTs[a]	**sex**	**$F_{1,36} = 13.2$, $p < 0.001$**	-----
	tissue type	**$F_{2,72} = 63.1$, $p < 0.001$**	-----
	interaction	$F_{2,72} = 0.329$, $p = 0.721$	-----
ΣPCBs[b]	sex	$F_{1,36} = 13.7$, $p < 0.001$	**serum: $t = -3.0$, $p = 0.004$**
	tissue type	$F_{2,72} = 98.6$, $p < 0.001$	**inner: $t = -2.8$, $p = 0.007$**
	interaction	**$F_{2,72} = 5.0$, $p = 0.009$**	**outer: $t = -4.6$, $p < 0.001$**
ΣCHLs[c]	**sex**	**$F_{1,36} = 26.4$, $p < 0.001$**	-----
	tissue type	**$F_{2,72} = 221.7$, $p < 0.001$**	-----
	interaction	$F_{2,72} = 1.1$, $p = 0.325$	-----
α-HCH	sex	$F_{1,36} = 5.3$, $p = 0.027$	**serum: $t = -3.7$, $p < 0.001$**
	tissue type	$F_{2,72} = 155.3$, $p < 0.001$	inner: $t = -0.2$, $p = 0.867$
	interaction	**$F_{2,72} = 6.4$, $p = 0.003$**	outer: $t = -1.7$, $p = 0.088$
β-HCH	**sex**	**$F_{1,36} = 30.9$, $p < 0.001$**	-----
	tissue type	**$F_{2,72} = 483.2$, $p < 0.001$**	-----
	interaction	$F_{2,72} = 0.5$, $p = 0.592$	-----
HCB	sex	$F_{1,36} = 20.1$, $p < 0.001$	**serum: $t = -4.9$, $p < 0.001$**
	tissue type	$F_{2,72} = 68.0$, $p < 0.001$	**inner: $t = -3.5$, $p < 0.001$**
	interaction	**$F_{2,72} = 3.1$, $p = 0.050$**	**outer: $t = -2.4$, $p = 0.020$**
ΣPBDEs[d]	sex	$F_{1,36} = 4.0$, $p = 0.054$	-----
	tissue type	**$F_{2,72} = 86.9$, $p < 0.001$**	-----
	interaction	$F_{2,72} = 1.2$, $p = 0.302$	-----
6-MeO-BDE 47	**sex**	**$F_{1,36} = 19.7$, $p < 0.001$**	-----
	tissue type	**$F_{2,72} = 142.0$, $p < 0.001$**	-----
	interaction	$F_{2,72} = 2.8$, $p = 0.066$	-----

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

Table 3.2b. Statistical output for linear mixed effects models comparing POP concentrations between male and female northern elephant seal serum and blubber (inner and outer) samples at the end of the foraging trip (at the beginning of the breeding season), with individual included as a random effect. If the interaction between sex and tissue type was significant, post-hoc contrasts were run to just compare each tissue type between males and females. If there was no interaction, then the main effects (sex and tissue type) were examined. Significant differences ($p < 0.05$) are bolded.

Post-foraging			
POP compound	fixed effects	test statistics	post-hoc contrasts
ΣDDTs[a]	sex	$F_{1,37} = 20.7$, $p < 0.001$	**serum: $t = -5.1$, $p < 0.001$**
	tissue type	$F_{2,74} = 238.3$, $p < 0.001$	**inner: $t = -4.4$, $p < 0.001$**
	interaction	**$F_{2,74} = 6.3$, $p = 0.003$**	**outer: $t = -3.8$, $p < 0.001$**
ΣPCBs[b]	sex	$F_{1,37} = 40.8$, $p < 0.001$	**serum: $t = -8.0$, $p < 0.001$**
	tissue type	$F_{2,74} = 356.6$, $p < 0.001$	**inner: $t = -5.6$, $p < 0.001$**
	interaction	**$F_{2,74} = 22.7$, $p < 0.001$**	**outer: $t = -4.7$, $p < 0.001$**
ΣCHLs[c]	sex	$F_{1,36} = 95.9$, $p < 0.001$	**serum: $t = -9.9$, $p < 0.001$**
	tissue type	$F_{2,74} = 426.1$, $p < 0.001$	**inner: $t = -9.0$, $p < 0.001$**
	interaction	**$F_{2,74} = 4.2$, $p = 0.019$**	**outer: $t = -7.8$, $p < 0.001$**
α-HCH	sex	$F_{1,37} = 0.0$, $p = 0.900$	**serum: $t = 3.7$, $p < 0.001$**
	tissue type	$F_{2,74} = 224.4$, $p < 0.001$	inner: $t = -1.7$, $p = 0.101$
	interaction	**$F_{2,74} = 17.7$, $p < 0.001$**	outer: $t = -1.7$, $p = 0.092$
β-HCH	sex	**$F_{1,37} = 68.7$, $p < 0.001$**	-----
	tissue type	**$F_{2,74} = 549.4$, $p < 0.001$**	-----
	interaction	$F_{2,74} = 0.7$, $p = 0.489$	-----
HCB	sex	$F_{1,37} = 3.8$, $p = 0.058$	**serum: $t = 7.5$, $p < 0.001$**
	tissue type	$F_{2,74} = 3.2$, $p = 0.046$	**inner: $t = -2.0$, $p = 0.050$**
	interaction	**$F_{2,74} = 33.2$, $p < 0.001$**	outer: $t = -1.8$, $p = 0.068$
ΣPBDEs[d]	sex	$F_{1,37} = 13.4$, $p < 0.001$	**serum: $t = -4.2$, $p < 0.001$**
	tissue type	$F_{2,74} = 241.0$, $p < 0.001$	**inner: $t = -3.6$, $p < 0.001$**
	interaction	**$F_{2,74} = 5.1$, $p = 0.008$**	**outer: $t = -2.8$, $p = 0.008$**
6-MeO-BDE 47	**sex**	**$F_{1,37} = 11.2$, $p = 0.002$**	-----
	tissue type	**$F_{2,74} = 160.5$, $p < 0.001$**	-----
	interaction	$F_{2,74} = 0.1$, $p = 0.883$	-----

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

*****Negative correlation.

Table 3.3a. Squared correlation coefficients (R^2) in italics and *p*-values (in bold if ≤ 0.05) between lipid normalized contaminant concentrations in inner blubber (analyzed using natural log transformed concentrations, except where noted^{+}, when untransformed data were analyzed) at the end of a foraging trip for adult female (df = 22) and male (df = 13) northern elephant seals (*Mirounga angustirostris*). All correlations were done using Pearson's product moment correlation, except where noted^{++}, when Spearman's rank correlations were conducted (rho is reported). Negative relationships between compounds are indicated with an *.

Compounds	Sex	∑DDTs[a]	∑PCBs[b]	ΣCHLs[c]	β-HCH	ΣPBDE[d]	HCB	α-HCH
∑PCBs	female	***0.37***						
		0.001						
	male	***0.83***						
		<0.001						
ΣCHLs	female	***0.44***	***0.69***$^{+}$					
		<0.001	***<0.001***					
	male	***0.45***	***0.77***					
		0.006	**<0.001**					
β-HCH	female	***0.46***	*0.04*	***0.41***				
		<0.001	0.316	**<0.001**				
	male	*0.06*	*0.02*	0.01^{+}				
		0.395	0.582	0.706				
ΣPBDEs	female	***0.85***	***0.18***	***0.24***	***0.36***			
		<0.001	**0.036**	**0.015**	**0.002**			
	male	***0.83***	***0.61***	***0.53***$^{++}$	*0.18*			
		<0.001	**<0.001**	**0.047**	0.119			
HCB	female	*0.07*	*0.00*$^{+}$	*0.02*	*0.06*	*0.11*		
		0.195	0.818	0.510	0.257	0.116		
	male	****0.55***	****0.40***	*******0.08*$^{+}$	****0.27***$^{+}$	****0.56***		
		0.001	**0.013**	0.317	**0.045**	**0.001**		
α-HCH	female	*0.00*	****0.19***$^{+}$	*******0.03*	*0.06*	*0.03*	***0.52***	
		0.950	**0.032**	0.416	0.237	0.399	**<0.001**	
	male	**0.25*	****0.27***	*******0.12*$^{+}$	**0.05*$^{+}$	**0.19*	***0.44***$^{+}$	
		0.056	**0.048**	0.215	0.403	0.102	**0.008**	
6-MeO-BDE 47	female	**0.03*	*0.02*$^{+}$	*0.00*	**0.09*	**0.10*	*0.01*$^{+}$	**0.04*$^{+}$
		0.401	0.473	0.848	0.161	0.130	0.598	0.379
	male	**0.12*	**0.09*	**0.04*$^{+}$	****0.50***$^{+}$	**0.18*	***0.35***	*0.05*
		0.197	0.271	0.451	**0.003**	0.115	**0.020**	0.406

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

*****Negative correlation.

Table 3.3b. Squared correlation coefficients (R^2) in italics and *p*-values (in bold if ≤ 0.05) between lipid normalized contaminant concentrations in outer blubber (analyzed using natural log transformed concentrations, except where noted[+], when untransformed data were analyzed) at the end of a foraging trip for adult female (df = 22) and male (df = 13) northern elephant seals (*Mirounga angustirostris*). All correlations were done using Pearson's product moment correlation, except where noted[++], when Spearman's rank correlations were conducted (rho is reported). Negative relationships between compounds are indicated with an *.

Compounds	Sex	∑DDTs[a]	∑PCBs[b]	∑CHLs[c]	β-HCH	ΣPBDEs[d]	HCB	α-HCH
∑PCBs	female	*0.11*						
		0.115						
	male	***0.86***						
		<0.001						
∑CHLs	female	***0.31***	***0.40***					
		0.005	***<0.001***					
	male	***0.40***	***0.65***					
		<0.011	**<0.001**					
β-HCH	female	***0.29***	**0.09*	***0.18***				
		0.007	0.149	**0.037**				
	male	**0.02*	**0.03*	*0.01[+]				
		0.629	0.567	0.787				
ΣPBDEs	female	***0.88***	*0.04*	***0.17***	***0.29***			
		<0.001	0.345	**0.043**	**0.006**			
	male	***0.77***	***0.56***	*0.45[++]*	*0.04*			
		<0.001	**0.001**	0.092	0.488			
HCB	female	**0.10*	**0.02[+]*	**0.04*	**0.03*	**0.08*		
		0.131	0.493	0.326	0.426	0.190		
	male	****0.52***	****0.36***	*0.00[+]*	*0.00[+]*	****0.45***		
		0.002	**0.017**	0.884	0.877	**0.006**		
α-HCH	female	**0.03*	****0.31***[+]	**0.03[+]*	*0.12*	*0.00*	***0.30***[+]	
		0.465	**0.005**	0.409	0.102	0.94	**0.005**	
	male	****0.60***[++]	**0.22*	*0.04[+]*	*0.05[+]*	**0.11*	***0.35***[+]	
		0.021	0.074	0.453	0.411	0.236	**0.021**	
6-MeO-BDE 47	female	**0.01*	***0.29***	*0.08*	****0.20***	**0.04*	*0.02*	**0.06*
		0.587	**0.006**	0.184	**0.026**	0.383	0.548	0.251
	male	**0.07*	**0.05*	*0.00[+]*	**0.24*	**0.14*	***0.28***	*0.01*
		0.354	0.434	0.918	0.067	0.166	**0.042**	0.787

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

*****Negative correlation.

Table 3.3c. Squared correlation coefficients (R^2) in italics and *p*-values (in bold if ≤ 0.05) between lipid normalized contaminant concentrations in serum (analyzed using natural log transformed concentrations, except where noted[+], when untransformed data were analyzed) at the end of a foraging trip for adult female (df = 22) and male (df = 13) northern elephant seals (*Mirounga angustirostris*). All correlations were done using Pearson's product moment correlation, except where noted[++], when Spearman's rank correlations were conducted (rho is reported). Negative relationships between compounds are indicated with an *.

Compounds		∑DDTs[a]	∑PCBs[b]	∑CHLs[c]	β-HCH	ΣPBDEs[c]	HCB	α-HCH
∑PCBs	female	***0.46*** **<0.001**						
	male	***0.85*** **<0.001**						
∑CHLs	female	***0.56*** **<0.001**	***0.77***[+] **<0.001**					
	male	***0.42*** **0.009**	**0.69** **<0.001**					
β-HCH	female	***0.38*** **0.001**	***0.23***[+] **0.017**	***0.50*** **<0.001**				
	male	*0.07* 0.334	*0.06* 0.379	0.14[+] 0.171				
ΣPBDEs	female	***0.59*** **<0.001**	***0.18*** **0.041**	***0.17*** **0.044**	*0.08* 0.179			
	male	***0.77*** **<0.001**	***0.49*** **0.004**	*0.17* 0.126	*0.16* 0.137			
HCB	female	*0.08* 0.193	**0.01*[+] 0.693	*0.03*[+] 0.416	*0.12*[+] 0.092	*0.12* 0.104		
	male	*0.00* 0.936	*0.01* 0.755	*0.12* 0.205	*0.13*[+] 0.188	**0.01* 0.765		
α-HCH	female	*0.08* 0.173	*-0.05* 0.270	*0.01* 0.704	***0.29*** **0.006**	*0.05* 0.312	***0.46*** **<0.001**	
	male	*0.00* 0.885	*0.00* 0.833	*0.00* 0.813	***0.53***[++] **0.044**	*0.01* 0.789	***0.79***[++] **<0.001**	
6-MeO-BDE 47	female	*0.05* 0.303	***0.32*** **0.003**	*0.06*[+] 0.246	**0.02*[+] 0.529	*0.01* 0.644	**0.11*[+] 0.115	**0.08* 0.168
	male	**0.46*[++] 0.082	**0.47*[++] 0.074	**0.05*[+] 0.410	**0.23*[+] 0.069	**0.19* 0.097	*0.00*[+] 0.902	*0.00* 0.964

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

Table 3.4. Statistical output for paired t-tests comparing POP concentrations between female northern elephant seal serum and blubber samples pre-and post-foraging trip.

Contaminant type	Tissue type	DF	*t*-value	*p*-value
ΣDDTs[a]	**Serum**	**23**	**9.59**	**< 0.001**
	Inner	**23**	**6.13**	**< 0.001**
	Outer	**23**	**3.55**	**0.002**
ΣPCBs[b]	**Serum**	**23**	**9.86**	**< 0.001**
	Inner	**23**	**6.59**	**< 0.001**
	Outer	**23**	**2.13**	**0.044**
ΣCHLs[c]	**Serum**	**23**	**9.40**	**< 0.001**
	Inner	**23**	**6.70**	**< 0.001**
	Outer	**23**	**4.80**	**< 0.001**
α-HCH	Serum	23	1.45	0.160
	Inner	**23**	**4.55**	**< 0.001**
	Outer	**23**	**4.54**	**< 0.001**
β-HCH	**Serum**	**23**	**4.34**	**< 0.001**
	Inner	**23**	**6.73**	**< 0.001**
	Outer	**23**	**5.39**	**< 0.001**
HCB	Serum	23	1.20	0.241
	Inner	**23**	**6.02**	**< 0.001**
	Outer	**23**	**2.44**	**0.023**
ΣPBDEs[d]	**Serum**	**23**	**6.77**	**< 0.001**
	Inner	**23**	**5.44**	**< 0.001**
	Outer	**23**	**2.26**	**0.034**
6-MeO-BDE 47	Serum	23	-1.16	0.257
	Inner	**23**	**-4.98**	**< 0.001**
	Outer	**23**	**-4.89**	**< 0.001**

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and183).

Table 3.5. Statistical output for unpaired t-tests (Welch's) comparing POP concentrations between male northern elephant seal serum and blubber samples collected pre-and post-foraging trip.

Contaminant type	Tissue type	DF	*t*-value	*p*-value
ΣDDTs[a]	Serum	21.2	0.34	0.736
	Inner	25.7	0.14	0.893
	Outer	21.2	-0.61	0.552
ΣPCBs[b]	Serum	25.9	0.45	0.660
	Inner	25.5	0.37	0.714
	Outer	25.0	0.13	0.900
ΣCHLs[c]	**Serum**	**24.8**	**2.23**	**0.035**
	Inner	18.4	1.21	0.241
	Outer	19.4	0.09	0.933
α-HCH	**Serum**	**23.7**	**6.44**	**< 0.001**
	Inner	**24.4**	**2.59**	**0.015**
	Outer	26.9	1.59	0.123
β-HCH	Serum	18.7	1.68	0.109
	Inner	**19.5**	**1.93**	**0.069**
	Outer	17.7	0.85	0.406
HCB	**Serum**	**16.2**	**7.44**	**< 0.001**
	Inner	**26.2**	**3.63**	**0.001**
	Outer	27.0	1.19	0.244
ΣPBDEs[d]	Serum	22.17	-0.25	0.803
	Inner	20.1	-0.77	0.452
	Outer	18.7	-0.91	0.374
6-MeO-BDE 47	**Serum**	**15.6**	**-3.10**	**0.007**
	Inner	23.9	-1.01	0.324
	Outer	24.8	-3.19	0.752

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

Table 3.6. The same adult male elephant seals (N = 4) were sampled during early breeding and late breeding for POPs in inner blubber, outer blubber and serum. Blubber is reported as percent lipid and serum is reported as g lipid dL^{-1} serum. Lipid-normalized POP concentrations (ng g^{-1} lipid) are reported as mean ± sd (range). Italics indicate what percent of the total (i.e. ΣPBDEs) can be attributed to a specific congener (i.e. BDE 47).

	Early breeding			Late breeding		
Type	Inner	Outer	Serum	Inner	Outer	Serum
Lipids	83.2 ± 7.5 (72.3 – 89.0)	70.2 ± 9.7 (60.6 – 83.5)	0.980 ± 0.161 (0.862 – 1.209)	76.3 ± 5.4 (70.2 – 83.2)	81.9 ± 6.4 (75.0 – 88.9)	0.770 ± 0.177 (0.602 – 0.995)
ΣDDTs[a]	2748.7 ± 1378.1 (1646.1 – 4733.3)	3135.3 ± 1180.3 (1993.4 – 4724.3)	1688.9 ± 363.0 (1226.1 – 2109.8)	5963.1 ± 1842.0 (3557.5 – 7469.9)	3634.3 ± 1382.8 (2405.6 – 5563.9)	3512.5 ± 761.3 (2444.5– 4146.5)
% p,p'-DDE	*99.4 ± 0.4 (98.9 – 99.8)*	*99.5 ± 0.4 (99.0 – 99.8)*	*99.2 ± 0.4 (98.7 – 99.5)*	*99.7 ± 0.2 (99.5 – 99.9)*	*99.6 ± 0.2 (99.4 – 99.9)*	*99.3 ± 0.4 (98.9 – 99.6)*
ΣPCBs[b]	1513.7 ± 332.9 (1160.2 – 1954.6)	1563.4 ± 371.1 (1208.6 – 2085.2)	848.2 ± 159.9 (701.9 – 1072.4)	3336.1 ± 1271.1 (2285.1 – 5091.7)	1818.1 ± 421.6 (1333.6 – 2340.0)	1712.9 ± 645.1 (1028.4 – 2577.7)
% Σtri-CBs	*<0.1 ± <0.1 (<0.1 – <0.1)*	*0.0 ± 0.0 (0.0 – 0.0)*	*<0.1 ± <0.1 (<0.1 – <0.1)*	*<0.1 ± <0.1 (<0.1 – <0.1)*	*0.0 ± 0.0 (0.0 – 0.0)*	*<0.1 ± <0.1 (<0.1 – <0.1)*
% Σtetra-CBs	*2.3 ±1.2 (1.6 – 4.0)*	*2.9 ± 1.5 (1.8 – 5.0)*	*1.7 ± 0.8 (0.8 – 2.8)*	*2.1 ± 0.9 (1.4 – 3.4)*	*2.7 ± 1.7 (1.5 – 5.1)*	*1.8 ± 0.6 (1.3 – 2.6)*
% Σpenta-CBs	*18.8 ± 3.2 (16.4 – 23.5)*	*19.6 ± 3.1 (17.0 – 23.9)*	*27.1 ± 5.3 (21.4 – 34.0)*	*18.5 ± 2.9 (16.6 – 22.9)*	*20.6 ± 4.7 (15.8 – 27.0)*	*27.7 ± 2.1 (25.4 – 30.4)*
% Σhexa-CBs	*54.8 ± 1.2 (53.3 – 55.9)*	*55.4 ± 2.1 (52.5 – 57.3)*	*52.8 ± 2.7 (48.9 – 55.1)*	*54.3 ± 1.5 (52.7 – 55.8)*	*55.1 ± 2.9 (51.2 – 58.0)*	*53.4 ± 2.3 (50.8 – 56.3)*
% Σhepta-CBs	*19.5 ±2.8 (15.7 – 22.2)*	*18.0 ± 2.6 (15.0 – 21.2)*	*16.4 ± 3.5 (12.7 – 21.1)*	*20.0 ± 2.3 (16.9 – 22.2*	*17.6 ± 3.8 (13.5 – 22.6)*	*15.3 ± 1.1 (14.3 – 16.9)*
% Σocta-CBs	*3.4 ± 0.9 (2.1 – 4.0)*	*3.0 ± 0.8 (2.1 – 3.9)*	*1.5 ± 0.6 (0.8 – 2.2)*	*3.8 ± 0.9 (2.6 – 4.7)*	*2.9 ± 1.2 (1.6 – 4.5)*	*1.3 ± 0.2 (1.1 – 1.5)*
% Σnona-CBs	*0.2 ± < 0.1 (<0.1 – 0.2)*	*0.2 ± < 0.1 (<0.1 – 0.2)*	*0.1 ± <0.1 (< 0.1 – 0.2)*	*0.3 ± 0.1 (0.1 – 0.4)*	*0.2 ± 0.1 (<0.1 – 0.3)*	*<0.1 ± <0.1 (<0.1 – 0.1)*
% Σdeca-CBs	*<0.1 ± <0.1 (< 0.1 – 0.1)*	*<0.1 ± <0.1 (<0.1 – <0.1)*	*0.0 ± 0.0 (0.0 – 0.0)*	*0.1 ± <0.1 (<0.1 – 0.2)*	*<0.1 ± <0.1 (<0.1 – 1.0)*	*0.0 ± 0.0 (0.0 – 0.0)*
ΣCHLs[c]	537.8 ± 69.1 (440.4 – 593.0)	575.1 ± 92.6 (445.2 – 645.9)	471.7 ± 194.8 (183.0 – 597.2)	1097.5 ± 276.1 (718.4 – 1366.9)	646.8 ± 68.6 (554.1 – 717.0)	1025.3 ± 300.0 (621.0 – 1254.1)

% TN	*63.9 ± 1.0*	*64.0 ± 1.2*	*36.3 ± 14.4*	*62.8 ± 0.5*	*62.2 ± 1.2*	*29.6 ± 6.5*
	(63.2 – 65.3)	*(63.0 – 65.8)*	*(23.3 – 56.4)*	*(62.1 – 63.4)*	*(61.1 – 63.8)*	*(25.5 – 39.2)*
% OxC	*19.9 ± 1.5*	*20.1 ± 0.8*	*16.6 ± 5.8*	*21.3 ± 1.0*	*21.8 ± 1.1*	*15.0 ± 3.3*
	(17.8 – 21.3)	*(19.3 – 21.2)*	*(10.1 – 24.2)*	*(20.5 – 22.7)*	*(20.7 – 23.1)*	*(11.2 – 18.4)*
% CN	*13.4 ± 1.3*	*13.4 ± 1.7*	*10.9 ± 3.4*	*13.9 ± 1.7*	*13.6 ± 1.6*	*8.6 ± 1.5*
	(12.5 – 15.3)	*(11.8 – 15.8)*	*(8.5 – 15.9)*	*(12.5 – 16.3)*	*(12.6 – 15.9)*	*(7.3 – 10.7)*
% CC	*1.9 ± 0.7*	*1.6 ± 0.4*	*1.7 ± 1.0*	*1.2 ± 0.4*	*1.5 ± 0.5*	*0.7 ± 0.3*
	(1.2 – 2.8)	*(1.1 – 2.1)*	*(0.5 – 2.7)*	*(0.6 – 1.6)*	*(1.0 – 2.1)*	*(0.3 – 0.9)*
% TC	*0.8 ± 0.2*	*0.9 ± 0.3*	*0.5 ± 0.2*	*0.8 ± 0.3*	*0.9 ± 0.2*	*0.4 ± 0.2*
	(0.5 – 1.0)	*(0.5 – 1.0)*	*(0.2 – 0.8)*	*(0.5 – 1.1)*	*(0.5 – 1.0)*	*(0.2 – 0.5)*
α-HCH	5.6 ± 1.5	5.1 ± 1.1	1.6 ± 0.4	6.6 ± 0.9	6.2 ± 1.2	3.2 ± 0.9
	(4.2 – 7.4)	(3.9 – 6.4)	(1.3 – 2.1)	(5.3 – 7.4)	(4.6 – 7.3)	(2.2 – 4.3)
β-HCH	52.7 ± 14.4	57.7 ± 13.4	27.5 ± 6.9	99.2 ± 27.6	75.5 ± 20.7	54.7 ± 9.2
	(39.3 – 70.3)	(42.7 – 72.1)	(19.2 – 33.4)	(72.1 – 124.6)	(55.7 – 99.3)	(43.6 – 65.1)
HCB	25.5 ± 7.8	31.1 ± 8.8	14.4 ± 10.7	47.3 ± 14.0	36.5 ± 9.2	69.2 ± 21.9
	(17.8 – 36.3)	(23.1 – 42.9)	(1.8 – 26.4)	(28.0 – 61.0)	(26.7 – 45.9)	(45.2 – 93.3)
ΣPBDEs[d]	67.6 ± 69.4	77.7 ± 84.7	31.4 ± 29.1	125.1 ± 110.3	84.7 ± 89.5	63.9 ± 39.0
	(19.8 – 169.1)	(20.9 – 202.5)	(9.4 – 72.4)	(31.0 – 281.1)	(21.4 – 215.6)	(31.1 – 120.3)
% BDE-47	*70.7 ± 5.2*	*69.7 ± 2.9*	*82.1 ± 3.7*	*72.8 ± 4.4*	*75.4 ± 2.2*	*76.5 ± 9.9*
	(67.2 – 78.3)	*(65.6 – 71.8)*	*(77.9 – 85.7)*	*(68.2 – 78.0)*	*(73.2 – 78.4)*	*(62.7 – 86.4)*
% BDE-99	*8.0 ± 3.8*	*9.9 ± 2.5*	*3.1 ± 3.5*	*5.5 ± 1.7*	*5.1 ± 1.5*	*10.0 ± 9.6*
	(3.1 – 12.3)	*(7.6 – 13.3)*	*(0.5 – 8.4)*	*(3.6 – 7.0)*	*(2.9 – 6.2)*	*(3.6 – 24.3)*
% BDE-100	*10.9 ± 3.3*	*10.6 ± 2.8*	*7.7 ± 2.3*	*11.1 ± 2.9*	*10.7 ± 3.2*	*7.2 ± 0.9*
	(7.1 – 13.7	*(7.5 – 13.0)*	*(5.2 – 10.4)*	*(7.7 – 14.0)*	*(6.9 – 13.7)*	*(6.4 – 8.5)*
% BDE-154	*5.5 ± 3.0*	*5.5 ± 2.5*	*4.6 ± 3.8*	*6.4 ± 3.4*	*4.9 ± 2.0*	*4.0 ± 2.4*
	(2.6 – 9.3)	*(2.6 – 7.9)*	*(1.9 – 10.0)*	*(2.9 – 10.6)*	*(2.9 – 6.7)*	*(1.4 – 7.2)*
6-MeO-BDE 47	2.7 ± 1.4	1.9 ± 1.1	0.7 ± 0.5	2.6 ± 1.1	2.2 ± 0.9	0.8 ± 0.5
	(1.4 – 4.5)	(1.0 – 3.4)	(0.4 – 1.4)	(1.7 – 4.1)	(1.4 – 3.4)	(0.5 – 1.5)

[a]ΣDDTs are the sum of *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

Table 3.7. Statistical output for paired t-tests comparing POP concentrations between male northern elephant seal serum and blubber samples early and late in the breeding fast.

Contaminant type	Tissue type	DF	*t*-value	*p*-value
ΣDDTs[a]	**Serum**	**3**	**7.6**	**0.005**
	Inner	**3**	**5.3**	**0.013**
	Outer	**3**	**4.4**	**0.022**
ΣPCBs[b]	**Serum**	**3**	**2.6**	**0.038**
	Inner	**3**	**3.7**	**0.035**
	Outer	**3**	**3.5**	**0.039**
ΣCHLs[c]	**Serum**	**3**	**8.6**	**0.003**
	Inner	**3**	**5.2**	**0.014**
	Outer	**3**	**4.1**	**0.026**
α-HCH	**Serum**	**3**	**5.7**	**0.011**
	Inner	3	1.2	0.309
	Outer	3	2.7	0.076
β-HCH	**Serum**	**3**	**15.0**	**<0.001**
	Inner	**3**	**5.9**	**0.010**
	Outer	**3**	**4.6**	**0.019**
HCB	**Serum**	**3**	**8.0**	**0.004**
	Inner	**3**	**3.9**	**0.030**
	Outer	**3**	**3.4**	**0.042**
ΣPBDEs[d]	**Serum**	**3**	**5.0**	**0.016**
	Inner	3	2.7	0.074
	Outer	3	2.5	0.087
6-MeO-BDE 47	Serum	3	2.8	0.069
	Inner	3	-0.4	0.725
	Outer	3	1.5	0.224

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

Table 3.8. Mean ± SD (range) of the estimated blubber burden (mg) pre- and post-foraging trip in adult female northern elephant seals (*Mirounga angustirostris*), the mean burden of contaminant gained over the approximately seven month long, post-molting foraging trip, and the percent increase in the blubber burden over the foraging trip. Statistical results are for paired t-tests comparing the change in the burden of POPs in the blubber between pre-and post-foraging trip (N = 24).

Contaminant type	Pre-foraging burden (mg)	Post-foraging burden (mg)	Gain (mg)	% Increase in burden	DF	*t*-value	*p*-value
ΣDDTs[a]	63.1 ± 25.7 (33.7 – 146.4)	103.2 ± 31.0 (59.3 – 179.4)	40.1 ± 18.4 (13.9 – 82.9)	71.2 ± 37.2 (17.4 – 162.2)	23	-10.67	< 0.001
ΣPCBs[b]	35.3 ± 7.6 (21.9 – 51.0)	60.5 ± 16.5 (40.2 – 106.1)	25.1 ± 13.4 (4.1 – 56.3)	73.3 ± 37.3 (9.2 – 173.4)	23	-9.22	< 0.001
ΣCHLs[c]	12.0 ± 2.8 (8.0 – 17.0)	19.8 ± 4.8 (12.1 – 32.9)	7.8 ± 4.0 (2.0 – 15.9)	69.1 ± 39.1 (12.2 – 185.3)	23	-9.63	< 0.001
α-HCH	0.2 ± 0.1 (0.1 – 0.4)	0.4 ± 0.1 (0.2 – 0.6)	0.1 ± 0.1 (-0.1 – 0.4)	72.1 ± 65.4 (-21.2 – 214.7)	23	-6.48	< 0.001
β-HCH	1.4 ± 0.6 (0.6 – 3.0)	2.2 ± 0.8 (1.4 – 4.6)	0.8 ± 0.4 (0.3 – 1.6)	68.4 ± 38.7 (16.7 – 190.9)	23	-10.40	< 0.001
HCB	0.9 ± 0.2 (0.5 – 1.3)	1.4 ± 0.4 (0.6 – 2.4)	0.5 ± 0.4 (-0.2 – 1.5)	72.1 ± 67.6 (-21.3 – 288.3)	23	-6.33	< 0.001
ΣPBDEs[d]	1.1 ± 0.6 (0.5 – 3.1)	1.8 ± 0.7 (0.9 – 3.4)	0.7 ± 0.4 (0.2 – 1.5)	69.8 ± 46.5 (12.4 – 182.0)	23	-8.81	< 0.001
6-MeO-BDE 47	0.1 ± < 0.1 (<0.1 – 0.1)	0.3 ± 0.1 (0.1 – 0.4)	0.2 ± 0.1 (<0.1 – 0.3)	214 ± 108.4 (40.5 – 525.0)	23	-11.52	< 0.001

[a]ΣDDTs are the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

[b]ΣPCBs are the sum of 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209).

[c]ΣCHLs are the sum of OxC, TC, CC, TN, and CN.

[d]ΣPBDEs are the sum of 7 PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183).

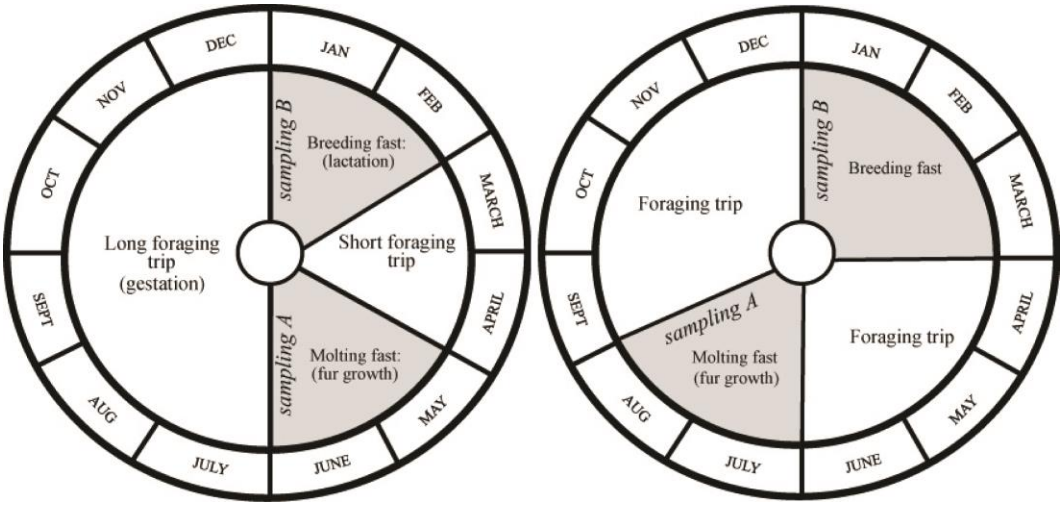

Figure 3.1. One year in the life of a northern elephant seal. A year in the life of an adult female (left) or adult male (right) northern elephant seal (*Mirounga angustirostris*). Individual females are on land for approximately four-five weeks during the breeding fast and five-six weeks during the molting fast. Males are on land for longer during the breeding fast and a similar length of time during the molting fast. Note that individual animals are onshore for less time than the full periods shown above because seals do not all arrive to the colony at the same time.

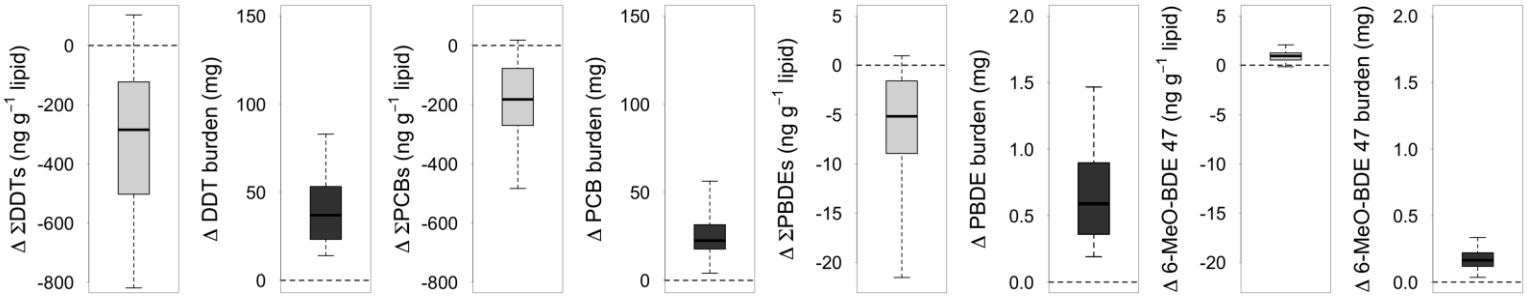

Figure 3.2. Change in POP concentrations and burdens over a foraging trip. Paired pre and post-foraging trip samples for 24 adult female elephant seals show the change in concentration (full blubber cores) and the change in blubber burden (mg) of three anthropogenic and one naturally produced (6-MeO-BDE 47) POPs across an approximately seven month long foraging trip. All eight compound groups measured in this study significantly decreased in concentration across the foraging trip, except for 6-MeO-BDE 47. Burden, however, increased for all compounds. Note that the axes labels are not the same for all compounds. All eight compounds are shown in Fig 3.5.

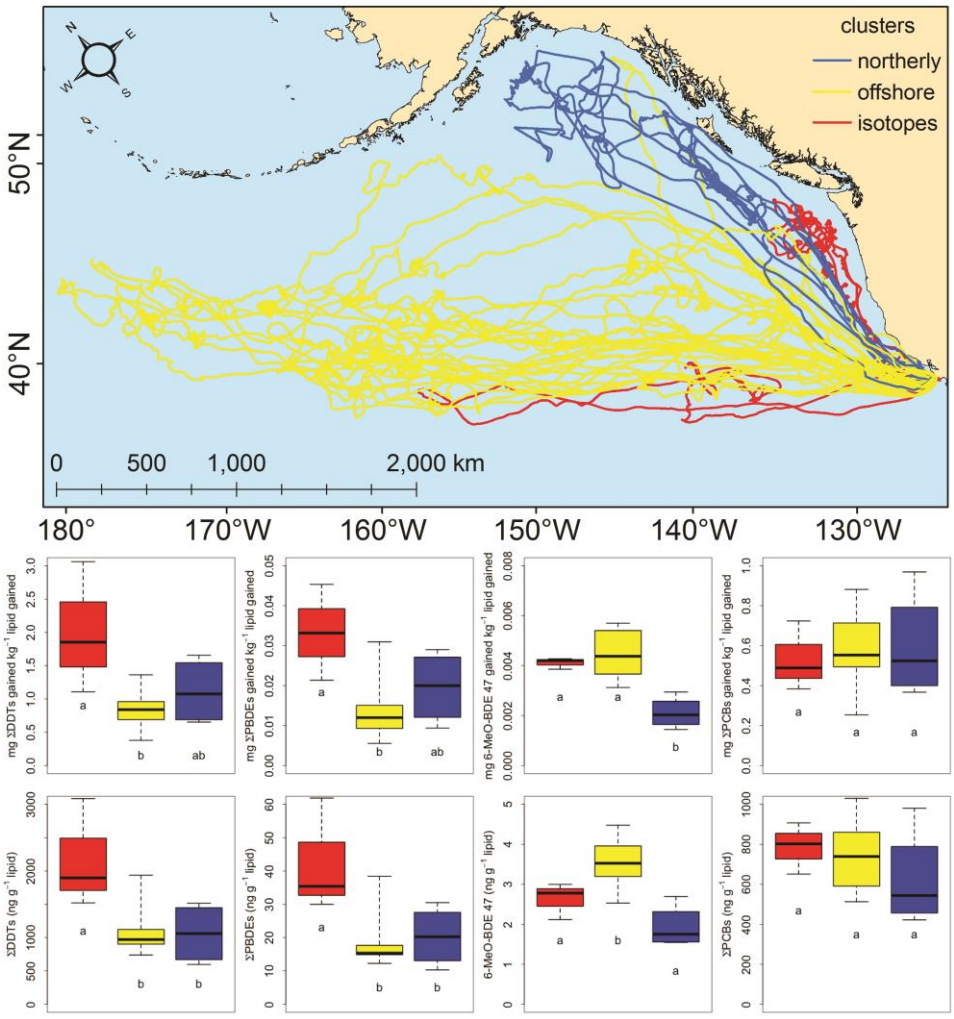

Figure 3.3. Clusters of seal behavior along with corresponding POP concentrations and blubber burdens. Northern elephant seals (*Mirounga angustirostris*) were clustered into three groups based on geographic variables, diving variables and stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$). The clusters in the map and the boxplots have corresponding colors. The top row of boxplots shows clusters by the burden of contaminant (mg) gained for each kg of lipid gained between sampling periods, pre- and post-foraging trip. The bottom row shows lipid normalized contaminant concentrations in inner blubber. Letters indicate significant differences between clusters. ∑PCBs were not significantly different between clusters, but were included as a reference. Untransformed data are shown here, although, when necessary, concentrations were transformed to meet assumptions of normality and homogenous variance.

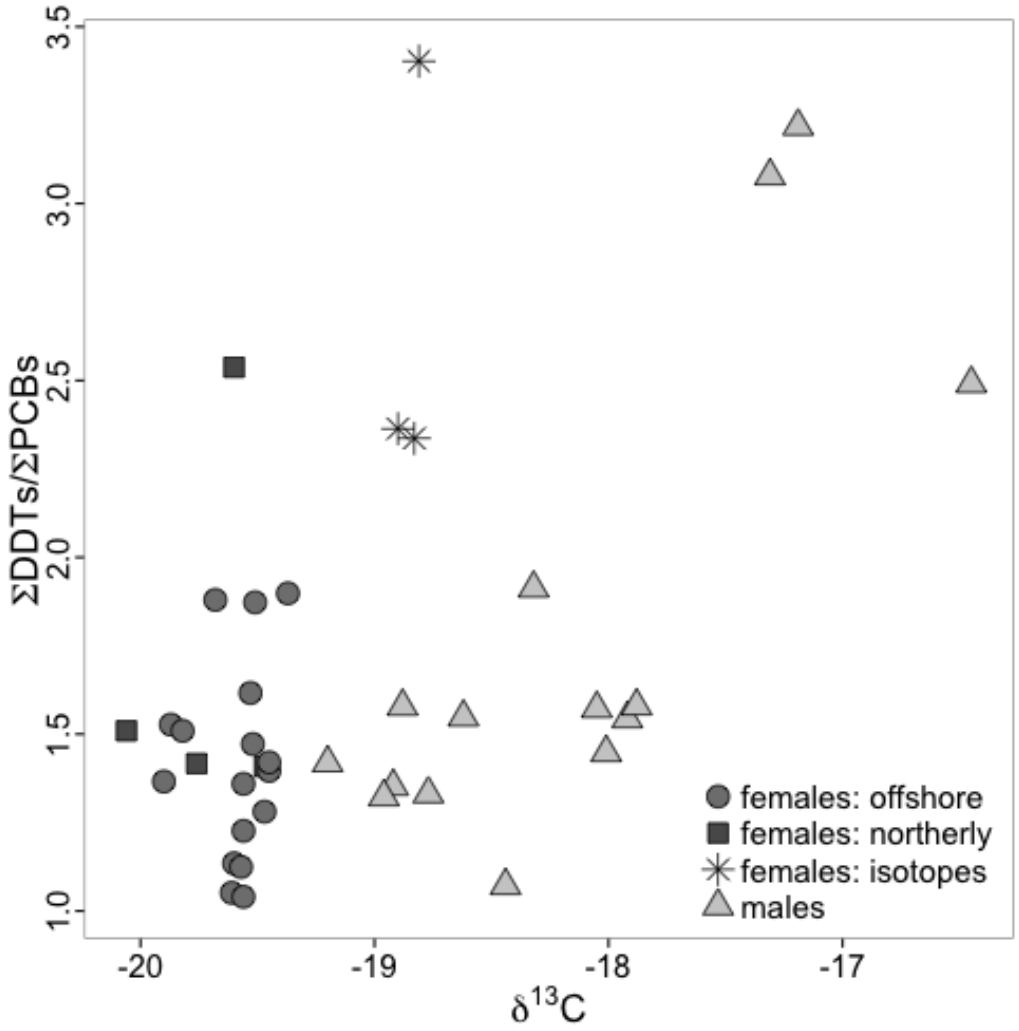

Figure 3.4. Ratios of ∑DDTs to ∑PCBs in elephant seals. Ratios of ∑DDTs to ∑PCBs in relation to $\delta^{13}C$ values from adult female and male (sub-adult 4 and adult age classes) northern elephant seals (*Mirounga angustirostris*) sampled at the Año Nuevo colony upon returning from a foraging trip. Females are grouped based on a cluster analysis, using diving behavior, geography and stable C and N isotopes. Males were not satellite tracked.

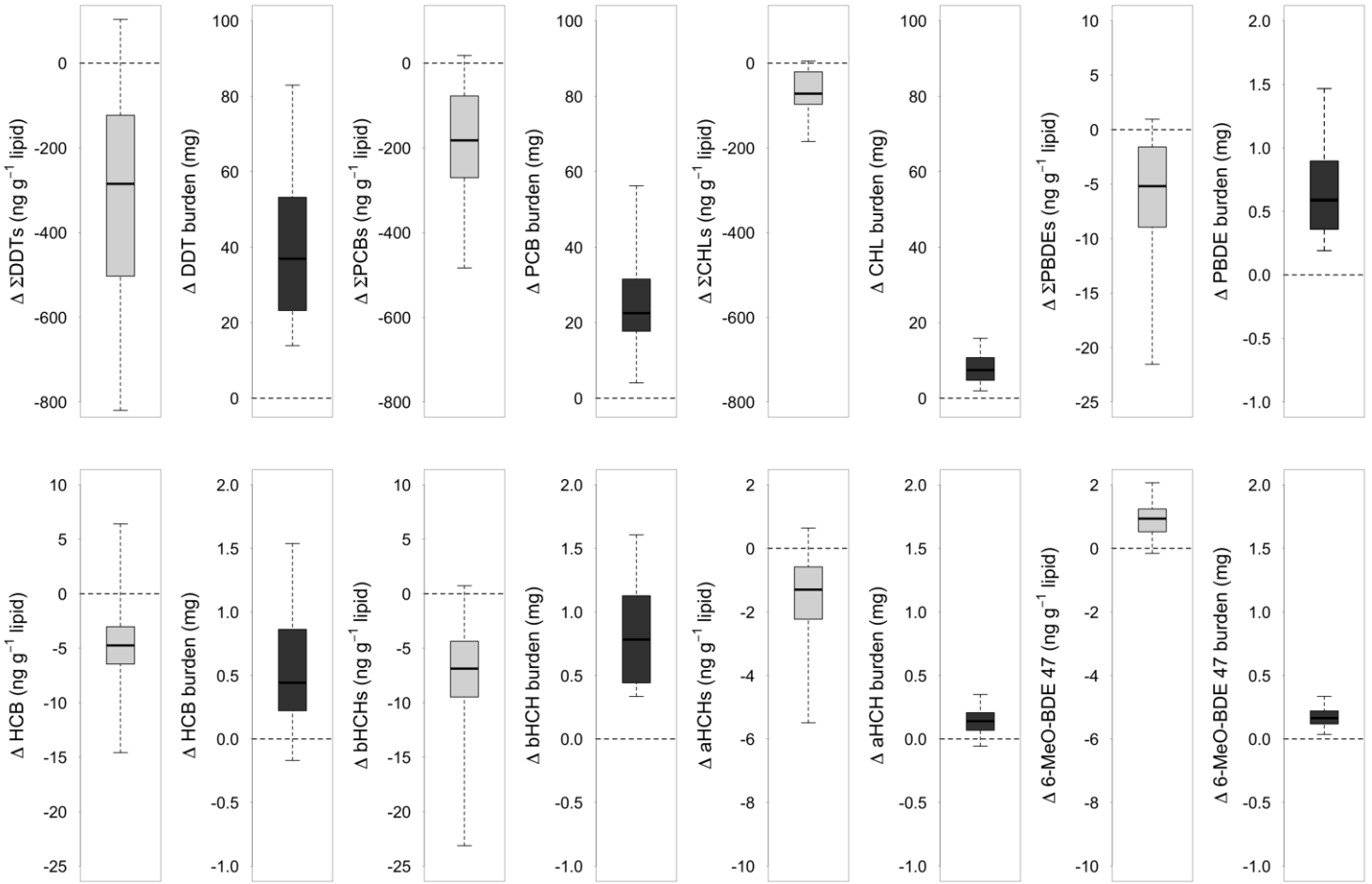

Figure 3.5. Paired pre and post-foraging trip samples for 24 adult female northern elephant seals (*Mirounga angustirostris*) showing the change in concentration (full blubber cores) and the change in blubber burden (mg) of all eight POP compounds. Note that the axes labels are not the same for all compounds.