Persistent organic pollutants in female humpback whales *Megaptera novaeangliae* from the Gulf of Maine

*Keri A. Baugh[1], Jooke Robbins[2], Irvin R. Schultz[1],
and Gina M. Ylitalo[1]

[1]Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA 98112, USA

[2] Center for Coastal Studies, 5 Holway Avenue, Provincetown, MA 02657, USA

Abstract

Contaminant studies in cetaceans typically focus on males due to confounding effects of reproductive status in females and maternal offloading. However, an improved understanding of contaminant burdens in female cetaceans is needed to better assess potential impacts to populations. In this study, 36 blubber biopsy samples of female humpback whales (*Megaptera novaeangliae*) from the Gulf of Maine were analyzed to examine contaminant loads across females of different ages. Sampled individuals were individually-identified from longitudinal studies and assigned to age class (i.e., adult, subadult, juvenile, calf). Analysis was performed using gas chromatography/mass spectrometry for persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), chlordanes (CHLDs), polybrominated diphenyl ethers (PBDEs), hexachlorocyclohexanes (HCHs), and other organochlorine pesticides (OCPs). The most abundant POPs measured were PCB congeners, with summed values ranging from 380 to 12,300 ng/g, lipid weight, well below the threshold value for adverse health effects. We found significant differences in mean values between adults and juveniles and between adults and subadults, with the exception of the less persistent HCHs for the latter. We also found significant differences in mean levels of ∑HCHs and ∑other OCPs between the juveniles and subadults. Changes over age are consistent with maternal offloading and potentially important for evaluating population health and viability.

Keywords

Humpback whale; POPs; Gulf of Maine; PCBs; DDTs; maternal offloading

1. Introduction

Persistent organic pollutants (POPs) are anthropogenic toxic chemicals that persist in the environment, are resistant to metabolism and degradation, and can be circulated globally via atmospheric transport and ocean currents. Among the different classes of POPs are polychlorinated biphenyls (PCBs), various organochlorine pesticides (OCPs), including dichlorodiphenyltrichloroethanes (DDTs), chlordanes (CHLDs), hexachlorocyclohexanes (HCHs), and the flame retardants, polybrominated diphenyl ethers (PBDEs).

Many POPs continue to be measured in environmental samples despite having been banned for production or open use in the US and many other countries since the 1970s (e.g. Stockholm Convention, (EPA, 2017; Vijgen et al., 2011). Exposure to POPs has been associated with adverse health effects such as immune dysfunction, increased susceptibility to disease, reproductive and endocrine impairment, and neurotoxicity. Such effects have been confirmed in marine mammal species, including: harbor porpoises (*Phocoena phocoena*), harbor seals (*Phoca vitulina*), California sea lions (*Zalophus californianus*), beluga whales (*Delphinapterus leucas*), grey seals (*Halichoerus grypus*) and ringed seals (*Pusa hispida*) (Bergman and Olsson, 1985; Beland et al., 1993; Hammond et al., 2005; Ylitalo et al., 2005a; Murphy et al., 2015). The primary route of POP exposure in marine mammals is through diet and these lipophilic compounds can bioaccumulate to relatively high concentrations in their blubber. As a result, there is a cause for concern, especially for long-lived marine mammals such as cetaceans.

Mysticete cetaceans feed at a lower trophic level than odontocetes and are therefore assumed to be at a lower risk of adverse health effects from POPs, even when residing in the same habitats (Aguilar et al., 1999; Pinzone et al., 2015). However, exposure to lower concentrations may nevertheless be significant in light of the fact that mysticetes are long lived, have large lipid stores, and can offload POPs from mother to calf during gestation and lactation (maternal offloading; Aguilar et al., 1999; Rowe 2008). It is important to also assess the exposure to and impacts of environmental contaminants when evaluating the health of mysticete populations. However, due to the confounding effects of accumulation through diet and maternal offloading, marine mammal studies commonly focus on measuring contaminant concentrations in immature animals or adult males and therefore POPs data is more limited for adult female marine mammals.

The Gulf of Maine, off the east coast of North America, is the site of long-term industrialized activity as well as long-term humpback whale population studies and prior humpback whale toxicological studies (Elfes *et al.* 2010, Ryan *et al.* 2013). The objectives of the current study were to extend that work by characterizing for the first time POP concentrations in blubber of female humpback whales across age classes as well as to better characterize maternal offloading of these compounds to their offspring.

2. Materials and Methods

Sample collection

Blubber samples were collected from 36 free-ranging female humpback whales in the Gulf of Maine (western North Atlantic Ocean) between 2004 and 2012 (Figure 1). Samples were collected from the lateral flank by biopsy sampling techniques (Palsbøll et al. 1991). Samples were kept on ice in the field and then frozen at -80 degrees Celsius until analysis. A long-term photo-identification catalog of individual Gulf of Maine humpback whales (Center for Coastal Studies, Provincetown, MA) was used to determine age class of individuals at the time of sampling. Calves were dependent offspring within the first year of birth, and juveniles when independent but no more than four years of age. Subadult females had reached the minimum documented age at first calving in this population (age 5, Clapham 1992; Robbins 2007) but had not yet observed with a calf themselves. Females were categorized as adults when known to have given birth to at least one calf. For three individuals, samples were available from two different ages.

Chemical analyses

POPs were extracted from samples and analyzed for POPs using gas chromatography/mass spectrometry (GC/MS) as described in Sloan et al., 2014. Prior to extraction of POPs with dichloromethane using an automated pressurized solvent extractor, blubber samples (0.1–0.3 g) were mixed with drying agents (sodium sulfate and magnesium sulfate) and spiked with a surrogate standard (PCB 103; 75 ng). A single stacked gravity flow silica gel/alumina column was used to remove any highly polar compounds from the sample. POPs were separated from the bulk lipid and other biogenic material present in each sample using high performance size exclusion liquid chromatography. After separation of analytes using a 60 m Agilent DB-5 GC capillary column, they were determined on an Agilent 5973 MS that was operated in selected ion monitoring and electron impact mode. Specificsof GC operating conditions and monitored ions can be found in Sloan et al., 2014. The instrument was calibrated using ten levels of standards of known concentrations. All blubber contaminant concentrations were reported in ng/g, lipid weight. The concentrations of PCB congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, and 209 were used to calculate summed PCBs (ΣPCBs).. The concentrations of *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, and *p,p'*-DDT were used to calculate summed DDTs (ΣDDTs). The concentrations of heptachlor, oxychlordane, *β*-chlordane, nona-III-chlordane, α-chlordane, *trans*-nonachlor, and *cis*-nonachlor were used to calculate summed chlordanes (ΣCHLDs). The concentrations of PBDE congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183 were used to calculate summed PBDEs (ΣPBDEs). The concentrations of α-hexachlorocyclohexane, *β*- hexachlorocyclohexane, and *γ*-hexachlorocyclohexane (lindane) were used to calculate summed hexachlorocyclohexanes (ΣHCHs). The concentrations of hexachlorobenzene (HCB), aldrin, mirex, and endosulfan I were used to calculate summed other OC pesticides (ΣOCPs).

Lipid content

A 1.5 mL extract subsample was aliquoted for determination of percent lipid gravimetric, as well as to measure lipid classes using thin-layer chromatography with flame ionization detection (TLC/FID) (Ylitalo et al., 2005b; Sloan et al. 2014). Lipophilic POP concentrations were normalized using percent lipid of each blubber sample. Lipid class profiles (i.e., sterol esters/wax esters, triglycerides, free fatty acids, cholesterol, phospholipids/polar lipids) were evaluated because the accumulation of lipophilic POPs may be influenced by the proportion of neutral lipids (e.g., triglycerides) in the blubber (Krahn et al., 2004).

Quality assurance

A solvent (dichloromethane) method blank and a National Institute of Standards and Technology (NIST) whale blubber Standard Reference Material (SRM 1945) were analyzed with each humpback whale blubber sample set. No more than five analytes exceeded 2 times the lower limit of quantitation (LOQ) for each method blank. For each sample set (10-12 blubber samples), concentrations of ≥ 70% of individual analytes that were measured in the NIST SRM 1945 were within 30% of the upper and lower ends of the 95% confidence interval range of the published NIST certified concentrations. The LOQs ranged from < 0.37 to < 10 ng/g wet weight for the PCBs, < 0.37 to < 9.9 ng/g wet weight for the organochlorine pesticides (DDTs, CHLDs, HCB, aldrin, mirex, and endosulfan I), and < 0.36 to < 2.6 ng/g wet weight for the PBDEs. The percent recoveries of the surrogate standard in the field and associated quality assurance samples ranged from 89% to 109%. Other quality control elements met established laboratory criteria (Sloan et al., 2019).

Statistical analyses

Prior to statistical analyses, percent lipid results were arcsine square transformed and summed concentrations of POPs were log transformed to obtain a more normal distribution and equal variances. Analysis of variance (ANOVA) and the Tukey-Kramer honestly significance difference test (HSD) were used to determine if the mean concentrations of the various classes of POPs were significantly different among the four age classes with a level of significance at $P \leq 0.05$. Statistical analyses were completed using Statistical analyses were completed using R (version R-4.0.4).

3. Results and Discussion

Concentrations of summed POPs based on whale age class are reported in Table 1. All POP classes were detected in every individual whale, with concentrations ranging from 6.2 (∑HCHs) to 12,300 ng/g, lipid weight (∑PCBs). The rank order of POP classes determined in the whale blubber were PCBs > DDTs > PBDEs ≅ CHLDs > other OCPs > HCHs. The mean blubber POP levels (ng/g lipid weight) for the four age classes of the female humpback whales ranged from 1,700 to 5,900 for ∑PCBs, 400 to 2,100 for ∑DDTs, 210 to 980 for ∑CHLDs, 190 to 980 for ∑PBDEs, 34 to 170 for ∑other OCPs, and 20 to 64 for ∑HCHs (Table 1). The predominant analytes contributing to each of the corresponding summed values for each age class were as follows: PCB 153 (16 to 19%) and PCB 138 (13 to 15%) to ∑PCBs, *p,p'*-DDE (67 to 72%) to ∑DDTs, *trans*-nonachlor (58 to 60%) to ∑CHLDs, PBDE 47 (66 to 75%) to ∑PBDEs, α-hexachlorocyclohexane (46 to 61%) and *β* -hexachlorocyclohexane (29 to 42%) to ∑HCHs, and HCB (88 to 97%) to ∑other OCPs. Mean blubber levels reported previously in adult males from this humpback whale population (Elfes et al., 2010) were up to 2 to 13 times higher than the mean values of females in the current study.

The lipid content of the blubber samples decreased with animal age class, being highest in calves (46.4%) and lowest in adults (32.6%, Table 1). The lipids measured in the biopsy blubber samples consisted primarily of neutral triglycerides (85 to 100% of total lipid). In addition to triglycerides, blubber samples from a juvenile and nursing adult contained 7.4% and 14.6% phospholipids respectively. The near homogenous lipid class profiles of the female humpback whale blubber samples across all age classes indicate that POP concentrations were not biased due to lipid composition.

Association between POPs and lipids with age class

Mean summed POP values differed significantly ($p < 0.05$) among the four age classes of female whales, with juveniles having significantly higher levels than the mean values in adults (for all 5 POP classes and other OCPs) and subadults (for HCHs and other OCPs). In addition, subadult females had significantly higher (p < 0.04) mean summed POP values than adult females for all POP classes except HCHs. No other significant differences ($p > 0.05$) in mean POP values were found among the age classes. The changes in the concentrations of POPs from one age class to the next are likely due to a combination of factors. Additive factors can be attributed to accumulation via diet and maternal transfer of contaminants (Aguilar et al. 1999). Previous cetacean studies have shown that lipophilic compounds are transferred from mother to calf primarily during lactation and, to a lesser extent during gestation (Cockcroft et al., 1989, Krahn et al., 2009). Birth order can also influence concentrations of lipophilic contaminants such as PCBs and DDTs, with first born animals having higher levels than non-first born (Cockcroft et al., 1989; Ylitalo et al., 2001; Wells et al., 2005). In addition to maternal offloading, other factors that can decrease POP concentrations include biotransformation as well as dilution related to growth or changes in blubber lipid content (Aguilar et al., 1999).

Comparison of the measured POP values between adults and calves indicated that there was a 1.1 to 2.8 times greater concentration in calves. The initial summed POP levels of the calves are assumed to be primarily from lactational and to a lesser extent, gestational transfer from their mothers. The decrease in summed POP concentrations between the juvenile and the subadult age classes was unexpected. There is a possibility that some individuals classified as subadults were actually adults that were missed in reproductive years or experienced calf mortality prior to being seen. Such individuals could therefore have already undergone some contaminant offloading. Although both adult and subadults likely accumulate POPs through their diet, the decrease in summed POP levels between these age classes suggests maternal offloading may be the predominate factor that determines POP levels in adult females. In the current study, the youngest sampled parous female was at least 7 years and the oldest sampled subadult was 12 years. The summed concentrations of the five POP classes generally decreased with age after age 4 and became relatively constant (Figure 2).

We also examined the percent contribution of PCB homologs to ∑PCBs to determine if there were qualitative differences among the four age classes of whales (Figure 3). PCB congeners were grouped according to the number of chlorine atoms [e.g., pentachlorinated congeners (5 Cl atoms), hexachlorinated congeners (6 Cl atoms)]. We found that the composition of the different PCB homolog groups varied slightly among the four age classes, with a tendency for the higher chlorinated homologs (i.e., heptachlorobiphenyls, octachlorobiphenyls, nonachlorobiphenyls) to be proportionately more abundant in adults and less in the calves. In contrast, hexachlorobiphenyls, the homolog group with the most detected congeners, were proportionately more abundant in calves and progressively decreased between the age classes (Figure 3). Previous cetacean studies have shown that higher chlorinated PCBs are not as readily offloaded from mother to offspring. (Aguilar et al., 1999; Yordy et al., 2010). The age associated decrease in pentachlorobiphenyls may be a consequence of growth dilution and/or elimination such as through biotransformation.

Changes in POPs determined from repeat samples and other studies

Age-related changes in ∑POPs concentrations were further evaluated based on three female humpback whales sampled twice, each more than a year apart (Figure 4). These results suggest that POP levels initially increase with age, peaking around age 4-5 and then continually decrease with age. These results highlight how age and other life history factors at time of sampling are important variables to consider when comparing POP levels in female humpback whales with prior studies.

Blubber POP concentrations reported in the current study were compared with values reported previously in humpback whales from the western North Atlantic, eastern North Atlantic, southwestern Indian Ocean, and the western Antarctic waters (Metcalf et al. 2004; Elfes et al., 2010; Ryan et al., 2013; Dorneles et al., 2015; Das et al., 2017, Table 2). Mean ∑PCB levels in adult females from the current study were approximately 1.9 to 630 times higher than levels in adult females from other populations, with the lowest levels reported in whales from the South Western Indian Ocean. Mean ∑PCBs levels determined in two calves in the current study were 2.9 times higher than levels measured in calves from the Gulf of St. Lawrence (Metcalfe et al., 2004). Mean ∑PCBs levels in adult females were higher than levels in adult males from the other studies, with the exception of those collected from the same sampling region (Elfes et al., 2010).

The highest observed PCB concentrations in the present study are below the most widely used threshold of 17,000 ng/g, lipid weight for onset of adverse effects in aquatic mammals (Kannan et al. 2000). However, a more recent study has suggested the threshold may be lower at 9,000 ng/g, lipid weight (Jepson et al. 2016). This lower threshold value is within the range of observed values in juvenile whales (Table 1). An added concern is the increased sensitivity of neonatal and juvenile life stages towards perturbations in thyroid hormone action (Zoeller sand Rovett 2004), which is one of the known effects of PCB exposure in marine mammals (Brouwer et al. 1989). Estimates of toxicity threshold values integrate results from many studies and generally are not specific for juvenile life stages nor fully consider potential synergistic interactions with other POPs present in the whales. Thus, toxicity thresholds for the actual mixture of POPs may be much lower, especially for juveniles that are potentially more sensitive and yet have the highest levels of contaminants in humpback whales.

4. Conclusion

In summary, POPs varied with age class in blubber samples from female humpback whales from the Gulf of Maine. Among the five POP classes, mean summed POP levels were highest in juveniles and subadults and lowest in adults. Among the four age classes, POP mean concentrations followed the order ∑PCB > ∑DDTs > ∑CHLDs > ∑PBDEs > other OCPs and

HCHs. Due to the confounding effects of maternal offloading, POPs data collected from female marine mammals is less common than that of males, but due to adverse health effects such as increased susceptibility to disease, immune and reproductive dysfunction and endocrine impairment, is important in assessing the health of a population. In addition, POP concentration data, such as those reported in the current study, can be used to develop models that can help determine the potential impacts of these toxic compounds on population growth of cetaceans (Hall et al., 2018).

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Certain commercial equipment or instruments are identified in the paper in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by NOAA Fisheries, nor does it imply the equipment is the best available for the purpose.

CRdiT authorship contribution statement

Keri A. Baugh: Investigation, formal analysis, data curation, visualization, writing - original draft. **Jooke Robbins**: Investigation, conceptualization, formal analysis, data curation, Writing - original draft, review, editing, and funding acquisition. **Irvin R. Schultz**: Conceptualization, formal analysis, visualization, Writing - original draft, review and editing. **Gina M. Ylitalo**: Investigation, conceptualization, formal analysis, data curation, visualization, Writing - original draft, review and editing, project administration, funding acquisition.

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