SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 5, pp. 1079–1087, 2005

Printed in the USA
0730-7268/05 $12.00 + .00

FACTORS INFLUENCING PERSISTENT ORGANIC POLLUTANT CONCENTRATIONS IN THE ATLANTIC WHITE-SIDED DOLPHIN (*LAGENORHYNCHUS ACUTUS*)

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(*Received* 5 *March* 2004; *Accepted* 30 *September* 2004)

Abstract—Assessing the trends of persistent organic pollutants (POPs) in cetaceans is difficult because of age and gender influences on accumulation. Persistent organic pollutants bioaccumulate and are poorly metabolized; hence, concentrations may increase with age in males while females reduce their POP burden through parturition and lactation. Age and gender effects on contaminant concentrations are species specific because of life history and reproductive strategies. These influences must be understood in order to elucidate and assess lifetime POP exposure. The objectives of this study were to determine baseline POP concentrations in blubber samples from the Atlantic white-sided dolphin (*Lagenorhynchus acutus*) and to investigate life history and other influences, such as metabolism, on these concentrations. Forty-seven *L. acutus* blubber samples collected from mass stranding events in Massachusetts, USA (1993–2000), and archived in the National Marine Mammal Tissue Bank at the National Institute of Standards and Technology (Gaithersburg, MD, USA) were analyzed for 55 polychlorinated biphenyl congeners (PCB; 55 congeners), five polybrominated diphenyl ether (PBDE) congeners, and organochlorine pesticides (toxaphene, DDT and metabolites, mirex, dieldrin, chlordanes, hexachlorocyclohexanes, hexachlorobenzene, and endosulfans) by gas chromatography/mass spectrometry (GC-MS). Ages for 19 animals were determined from growth layer groups on decalcified, stained thin tooth sections. Total PCBs (ΣPCB; sum of 55 congeners) were the contaminants present in the highest concentrations in all age classes (0.5–63 μg/g wet mass) followed by sum of DDTs (0.50–43 μg/g wet mass), toxaphene (0.055–31 μg/g wet mass), chlordanes (0.30–24 μg/g wet mass), and PBDEs (0.12–4.0 μg/g wet mass). Body length had a greater statistical influence than age on contaminant burdens in *L. acutus*. Contaminant burdens decreased with length in both male and female *L. acutus*, suggesting metabolic elimination and/or growth dilution in males and off-loading via lactation in females.

Keywords—Dolphin Metabolism Life history Persistent organic pollutant Northwest Atlantic Ocean

INTRODUCTION

Persistent organic pollutants (POPs) have been documented in the marine environment since the 1960s [1]. Subsequently, these contaminants have been measured in many marine mammal species worldwide [2]. Persistent organic pollutants, such as polychlorinated biphenyls (PCBs), brominated flame retardants, and some pesticides (e.g., DDT, chlordane compounds, and toxaphene), bioaccumulate and biomagnify to high concentrations in animals at higher trophic levels, particularly in aquatic food webs [3]. Because of their lipophilic nature, POPs accumulate to highest concentrations in blubber layers of marine mammals. Numerous reports have been published suggesting deleterious effects of organohalogens on mammals including reproductive failure, hormone disruption, and immunosuppression [4–8].

One of the difficulties in assessing the significance of POP contamination in marine mammals, especially delphinids, is the considerable influence of age, gender, and species on the observed contaminant levels. Because of the bioaccumulative nature of the compounds and subsequent slow depuration rates, concentrations tend to increase with age [9–13]. Female marine mammals, however, significantly reduce their contaminant burden through parturition and lactation [9,14,15]. Although cetaceans have the ability to biotransform certain POPs by mixed-function oxidase enzymes [16–18], several studies suggest that metabolic elimination of POPs is fairly limited in cetaceans, thus contributing to bioaccumulation of certain contaminants in marine mammals [19,20]. This is especially true for polychlorinated biphenyl congeners that are unsubstituted with chlorine in the *ortho* and *meta* positions [19,21].

Lagenorhynchus acutus inhabits cold-temperate waters of the North Atlantic, ranging from Cape Cod, Massachusetts, USA, extending up the eastern coast of North America to Greenland, and from Norway to the British Isles [22]. *Lagenorhynchus acutus* is a pelagic, offshore delphinid, preferentially inhabiting waters along the edge of the continental shelf. A relatively large delphinid, *L. acutus* reaches 270 cm in length and may live in excess of 25 years. Females reach sexual maturity at 201 to 210 cm (6–12 years of age) [23]. The calving period lasts approximately 2.5 years (11 months gestation, 18 months lactation) [23]. The diet of *L. acutus* has not been well documented; however, based on limited stomach contents from stranded animals, it consists mainly of fish (herring, silver hake, and smelt), squid, and occasionally shrimp [23,24].

Few data exist on POP contamination in *L. acutus*. This species has been included for comparative purposes in con-

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Certain commercial equipment or instruments are identified in this paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment or instruments are the best available for the purpose.

taminant studies focusing on other marine mammals and appears to have POP concentrations warranting concern. For example, Kuehl et al. [25] examined chemical residues in bottlenose dolphins (*Tursiops truncatus*, $n = 12$), *L. acutus* ($n = 3$), and common dolphins (*Delphinus delphis*, $n = 4$) involved in a mass stranding event and found that *L. acutus* had sum-of-PCB concentrations higher than that of *D. delphis* although lower than *T. truncatus*. Concentrations of hexachlorobenzene (HCB) and chlordane compounds in *L. acutus* were among the highest of all three species examined. Similarly, in Borrel's study [26] of cetaceans from the northeastern North Atlantic, *L. acutus* ($n = 13$) had the second-highest contaminant concentrations for compounds including dichlorodiphenyldichloroethylene (DDE), DDT, and total PCBs (ΣPCBs). These concentrations exceeded those found in nearshore *Phoca phocoena* ($n = 6$). In a study of *L. acutus* ($n = 19$) in Scottish and Irish waters, chlorobiphenyl concentrations were as high as 63 μg/g lipid weight, and ΣDDT concentrations were greater than 20 μg/g lipid weight [27].

A more recent study of contaminant loads in stranded *L. acutus* from the Gulf of Maine, USA, revealed PCB concentrations similar to those identified in earlier studies [28]. While organohalogen residues were compared between sexes, the small sample size ($n = 6$) and the high variability in concentrations among individuals prohibited a generalization about contaminant concentrations in *L. acutus*. Further, age and reproductive status differences among individuals sampled were not addressed. The objectives of this study were to determine baseline POP concentrations in blubber samples from *L. acutus* inhabiting the northwestern Atlantic as a contribution to long-term biomonitoring and to assess life history and other influences on POP concentrations in this species.

METHODS

Blubber samples for this study were collected according to established National Institute of Standards and Technology (NIST) protocols [29] from 47 *L. acutus* that were found dead or were euthanized after stranding. Necropsy reports indicate that all animals were in good nutritional condition based on blubber thickness; emaciated animals were not included in this study. Morphometric information was collected for each animal, including total length, blubber thickness, gender, and reproductive status (Table 1). Blubber samples were collected from stranding events along the Massachusetts coast, USA, near Cape Cod between the years 1993 and 2000. Approximately 300 g of blubber (skin off, full depth) from each animal was removed just anterior and slightly ventral to the dorsal fin, placed in Teflon® bags or jars on ice, and stored in freezers (−80°C or below) until shipped to NIST, where they were transferred to −150°C liquid nitrogen vapor freezers.

Teeth for 19 of the 47 animals analyzed in this study were available for aging. Briefly, teeth were collected from the left mandible, scraped clean, and decalcified with RDO (Apex Engineering Products, Plainfield, IL, USA), a commercial decalcifying agent (active ingredient HCl). Thin sections were made using a BFS series freezing stage (Physitemp, Clifton, NJ, USA) connected to a SM2000R microtome (Leica, Nussloch, Germany). A hematoxylin solution was used for staining tooth sections. One light and one dark layer typically represent one year of growth [30]. Studies using *T. truncatus* of known age have shown that this pattern is consistent and reliable proxy for determining age [30,31].

Blubber samples were cryogenically homogenized according to established procedures [32]. Sample extraction has been described in detail elsewhere [33]. Briefly, samples were mixed with Na_2SO_4 and added to a pressurized fluid extraction cell along with an internal standard solution containing 4,4′-DDT-d_8, 4,4′-DDE-d_8, 4,4′-dichlorodiphenyldichloroethane (DDD)-d_8, endosulfan I-d_4, PCB 103, and PCB 198 and were extracted with CH_2Cl_2 using pressurized fluid extraction (Dionex, Salt Lake City, UT, USA).

The sample extracts were reduced to between 0.5 and 1 ml by evaporation in a stream of purified N_2 using a Turbovap II (Zymark, Hopkinton, MA, USA). High relative molecular mass compounds were removed by size-exclusion chromatography [33]. The extract was fractionated using a semipreparative aminopropylsilane column (μBondapak NH_2, Waters, Milford, MA, USA) into relatively lower- and higher-polarity fractions (F1 and F2, respectively).

Polychlorinated biphenyl congeners and chlorinated pesticides (excluding toxaphene) were determined using gas chromatography/mass spectrometry (GC-MS; Agilent 6890/5973, Palo Alto, CA, USA) operated in the electron impact mode. Samples were injected (2 μl) by on-column injection onto a 60-m DB-5ms (J&W Scientific, Folsom, CA, USA) capillary column (0.25-mm i.d. × 0.25-μm film thickness). Helium was used as the carrier gas at a constant flow rate of 30 cm/s. The F1 and F2 from the liquid chromatograph column were injected separately using individual selected ion monitoring programs targeting only the analytes in each fraction. For F1, the initial column temperature was 60°C; the temperature was then ramped to 150°C at 25°C/min, then to 200°C at 0.75°C/min; the final ramp brought the temperature to 300°C at 2°C/min with a 10-min hold. For F2, the initial column temperature was 60°C; the temperature was increased to 170°C at 25°C/min, then to 200°C at 1°C/min, then to 240°C at 2°C/min. The final ramp brought the temperature to 300°C at 10°C/min with a 10-min hold. The amount of analyte present was determined using the slope and intercept of the five-point calibration curve prepared from calibration solutions [34]. Standard Reference Material 1945 Organics in Whale Blubber was analyzed with each set of samples as an analytical control material. Measurements for the majority of compounds were in good agreement with certified values (<15% deviation).

Total toxaphene and selected toxaphene congeners were determined using the GC-MS operated in the negative chemical ionization mode. A minimum of three calibration standards were prepared that bracketed the expected toxaphene concentrations in the samples. The toxaphene congeners [35] included toxaphene congener 26, 2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*, 8,8,10,10-octachlorobornane; toxaphene congener 50; 2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*, 8,8,9,10,10-nonachlorobornane; toxaphene congener 62; 2,2,5,5,8,9,9,10,10-nonachlorobornane; and toxaphene congener 32; 2-*endo*, 3-*exo*, 6-*exo*, 8,9,10,10-heptachlorobornane (Promochem, Wesel, Germany). The calibration mixtures were processed the same as the samples. Further details are given in Kucklick et al. [36].

Polybrominated diphenyl ethers were determined using an Agilent 5890/5972 GC-MS in the electron-impact mode using selected ion monitoring in each homologue group. The PBDE separation was performed either on a 30- or a 60-m DB-5ms column (0.25-mm i.d. × 0.25-μm film thickness). Calibration mixtures were prepared from individual congener solutions of congeners 47 (2,2′,4,4′-tetra-brominated diphenyl ether [BDE]), 99 (2,2′,4,4′,5-penta-BDE), 100 (2,2′,4,4′,6-penta-BDE), 153 (2,2′,4,4′,5,5′-hexa-BDE), and 154 (2,2′,4,4′,5,6′-

Table 1. National Biomonitoring Specimen Bank (NBSB) identifier and descriptions for white-sided dolphin (*Lagenorhyncus acutus*) samples analyzed in this study[a]

NBSB no.	Sex	*Date stranded	Stranding location (in USA)	Length (cm)	Age (year)	Life history	Blubber thickness (cm)
NM8B254	F	01/29/1998	Wellfleet, MA	147	0.75	Juvenile	1.3
NM8B232	F	01/29/1998	Wellfleet, MA	182		Juvenile	2.0
NM3B038	F	03/23/1993	Barnstable, MA	185		Juvenile	1.8
NM10B104C	F	03/19/1999	Wellfleet, MA	187	2.5	Juvenile	1.8
NM7B132	F	05/28/1997	Duxbury, MA	188		Juvenile	2.2
NM11B210C	F	03/19/1999	Wellfleet, MA	189	2.25	Juvenile	2.2
NM3B036	F	03/23/1993	Barnstable, MA	191		Juvenile	1.8
*NM3B042	F	04/06/1993	Wellfleet, MA	193		Juvenile	1.8
NM11B213C	F	03/19/1999	Wellfleet, MA	194	3.25	Juvenile	1.9
NM8B245	F	01/29/1998	Wellfleet, MA	195		Juvenile	1.7
NM8B235	F	01/29/1998	Eastham, MA	198		Juvenile	1.8
NM8B269	F	01/29/1998	Wellfleet, MA	198		Juvenile	Not available
NM8B241	F	01/29/1998	Eastham, MA	205	5	Juvenile	2.0
NM11B198C	F	04/11/2000	Wellfleet, MA	206	4	Juvenile	1.7
NM8B272	F	01/30/1998	Wellfleet, MA	220		Adult	1.0
NM8B284	F	01/29/1998	Wellfleet, MA	223		Adult	1.3
NM10B046C	F	03/06/1999	Chipman's Cove, MA	224		Adult	2.2
NM8B263	F	01/29/1998	Wellfleet, MA	227		Adult	1.8
NM10B043C	F	03/06/1999	Chipman's Cove, MA	230		Adult	2.0
NM8B257	F	01/29/1998	Eastham, MA	231		Adult	1.8
NM8B275	F	01/30/1998	Wellfleet, MA	235		Adult	Not available
*NM7B135	F	08/12/1997	Great Island, MA	235		Adult	0.80
NM11B186C	F	08/25/2000	Wellfleet, MA	237	13	Adult	1.5
NM10B037C	M	03/19/1999	Wellfleet, MA	150	0.75	Juvenile	1.7
*NM7B096	M	02/02/1997	Brewster, MA	156		Juvenile	1.5
NM7B130	M	05/28/1997	Duxbury, MA	173		Juvenile	2.2
*NM4B054	M	12/31/1994	Eastham, MA	190		Juvenile	2.0
NM10B028C	M	03/19/1999	Wellfleet, MA	193		Juvenile	1.3
NM9B365	M	11/27/1998	Great Island, MA	197		Juvenile	Not available
NM10B101C	M	03/19/1999	Wellfleet, MA	205	2.5	Juvenile	2.0
NM8B244	M	01/30/1998	Wellfleet, MA	208	6	Juvenile	2.0
NM8B251	M	01/29/1998	Wellfleet, MA	211	6	Juvenile	1.8
NM8B260	M	01/29/1998	Wellfleet, MA	220		Adult	Not available
NM8B362	M	11/27/1998	Chipman's Cove, MA	222		Adult	1.5
NM10B107C	M	03/19/1999	Wellfleet, MA	225	9.5	Adult	2.0
NM10B022C	M	03/19/1999	Wellfleet, MA	225		Adult	2.3
NM11B195C	M	04/11/2000	Wellfleet, MA	226	9	Adult	2.3
NM8B248	M	01/30/1998	Wellfleet, MA	230	7	Adult	1.8
NM9B359	M	11/27/1998	Chipman's Cove, MA	230		Adult	1.8
NM8B238	M	01/29/1998	Eastham, MA	237	7.5	Adult	2.4
NM11B138C	M	03/19/1999	Wellfleet, MA	243		Adult	2.3
NM8B278	M	01/31/1998	Wellfleet, MA	244	12	Adult	1.9
NM11B183C	M	08/25/2000	Wellfleet, MA	247	12	Adult	1.5
NM11B189C	M	08/26/2000	Wellfleet, MA	249	7	Adult	1.5
NM11B192C	M	08/25/2000	Wellfleet, MA	257	17	Adult	1.6
NM11B141C	M	03/19/1999	Wellfleet, MA	260		Adult	2.0
NM10B025C	M	03/19/1999	Wellfleet, MA	271		Adult	2.4

[a] Asterisks indicate single stranded animal; all other blubber samples collected from mass strandings.

hexa-BDE) (Cambridge Isotopes, Andover, MA, USA). The PCB 204 or ^{13}C-PBDE 99 was used as the internal standard. The recovery of PCB 198 added at the beginning of the extraction was used to correct for analyte recovery. Standard Reference Material 1945 was analyzed ($n = 5$) with each set of samples. The relative standard deviation of this set of PBDE determinations was less than 10% for each congener [36].

Statistical analysis

Because of the large number of individual compounds measured in each sample, concentrations were summed across similar contaminant classes before statistical analyses. The ΣPCB includes the sum of 55 PCB congeners; ΣDDT includes 2,4′-DDT, 4,4′-DDT, 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, and 4,4′-DDD; Σchlordane includes oxychlordane, *cis*- and *trans*-nonachlor, *cis*- and *trans*-chlordane, and heptachlor epoxide; ΣHCH is the sum of α-, β-, and γ-hexachlorocyclohexanes; ΣPBDE is the sum of five polybrominated diphenyl ethers (PBDE) congeners (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154; BDE = brominated diphenyl ether); and Σtoxaphene was the sum of all 6-Cl, 7-Cl, 8-Cl, and 9-Cl substituted homologues. Concentrations for individual congeners for each organism are described in detail elsewhere [37].

Body length was used to estimate sexual maturity, with females smaller than 210 cm and males smaller than 220 cm classified as juveniles (adapted from Sergeant et al. [23]). To ensure that body condition was not a significant factor affecting contaminant concentration, blubber thickness was tested for normality. To compare concentrations between life history classes, after verifying that no significant differences existed in contaminant concentrations between male and female juveniles, juveniles of both genders were pooled. Contaminant

Table 2. Summary of organohalogen contaminant concentrations (μg/g wet mass) among adult male, adult female, and juvenile *Lagenorhynchus acutus*. Values are mean (±1 standard error) and range. Multiple analysis of variance was performed on log-transformed values to fit assumption of normality. Welch's analysis of variance was used when variances were heteroscedastic. Different letters indicate significant differences among life history classes (multiple analysis of variance results: $l = 0.19$, $F = 3.85$, $p = 0.0001$). Compounds abbreviations are as follows: ΣPCB is the sum of 55 polychlorinated biphenyl congeners; ΣDDT is the sum of 2,4′- and 4,4′- DDD, DDE, and DDT; ΣHCH is the sum of α-, β-, and γ = hexachlorocyclohexanes (HCHs); HCB is hexachlorobenzene, Σtoxaphene is the sum of hexa- through decachlorobornanes; ΣPBDE is the sum of polybrominated diphenyl ethers

Compound	Juvenile ($n = 23$)	Adult female ($n = 9$)	Adult male ($n = 15$)
ΣPCBs	29.4 ± 15.1 A 62.7–13.0	9.41 ± 8.22 B 26.1–0.490	29 ± 9.14 A 49.6–15.5
ΣDDT	15.9 ± 9.65 A 43.3–7.13	4.09 ± 4.22 B 13.2–0.498	14.5 ± 4.09 A 22.6–8.95
Σchlordanes	8.8 ± 5.4 A 23.9–3.25	2.2 ± 2.15 B 6.33–0.285	7.75 ± 2.9 A 13.5–3.96
ΣHCH	0.301 ± 0.17 A 0.821–0.121	0.091 ± 0.049 B 0.149–0.0504	0.196 ± 0.048 A 0.262–0.124
HCB	0.237 ± 0.151 A 0.606–0.0951	0.0506 ± 0.049 B 0.234–0.0127	0.195 ± 0.058 A 0.361–0.114
Dieldrin	1.81 ± 0.835 A 3.94–0.647	0.293 ± 0.321 B 0.639–0.0626	1.39 ± 0.372 A 2.16–0.983
Mirex	0.0593 ± 0.025A 0.112–0.0352	0.0404 ± 0.022 B 0.0643–0.0184	0.0737 ± 0.021 A 0.111–0.0402
Endosulfan I[a]	0.269 ± 0.114 A 0.398–0.0647	0.139 ± 0.062 B 0.147–0.0134	0.0556 ± 0.071 A 0.332–0.0564
ΣToxaphene	13.0 ± 6.67 A 31.1–0.448	3.56 ± 3.97 B 12.4–0.055	10.7 ± 3.98 A 21.6–0.565
ΣPBDEs	2.41 ± 1.16 A 4.03–0.512	0.609 ± 0.517 B 1.35–0.118	1.82 ± 0.764 A 3.25–0.778

[a] Homoscedastic; Welch analysis of variance not used.

concentrations were log transformed to fit the assumption of normality. Multiple analysis of variance was used to compare mean contaminant concentrations among three life history groups: adult males, adult females, and juveniles. The multiple analysis of variance was used as an alternative to repeated analysis of variance, as it minimizes probability of committing a type I error [38]. Individual analyses of variance (Welch's analysis of variance for unequal variances, when applicable) were then used to determine which compounds were significantly different, using contrasts (Tukey–Kramer) to determine among which groups concentrations differed.

Life history effects were further elucidated for males by investigating the relationship between body length or age and contaminant concentration. A backward stepwise regression was used for each contaminant class to determine if length or age was more significant in influencing contaminant concentration. Logarithmic regressions were then used to determine which POPs significantly related to body length or age. Regressions were not applied to females since the onset of female sexual maturity does not occur at a discrete age [23,39] and an age estimate was available for only one adult female.

The biotransformation of individual PCB congeners in marine mammals occurs mainly by cytochrome P450 enzymes [17,21,40]. Boon et al. [41] investigated the biotransformation of PCB congeners in a wide range of marine mammals, including *D. delphis* and *P. phocoena*. Congeners were grouped according to position of vicinal H atoms and number of chlorine substitutions in the *ortho* position. Group I congeners have no vicinal H atoms and ≥2 *ortho*-substituted chlorine atoms. Group II and III congeners have H-atoms in the *ortho* and *meta* positions with ≥2 *ortho*-substituted chlorine atoms and ≤1 *ortho*-substituted chlorine atoms, respectively. Group IV and V congeners have H-atoms in the *meta* and *para* positions with 2 and 3 *ortho*-substituted chlorine atoms, respectively. Congeners were further classified as persistent, biotransformed by the CYP1A and 2B enzyme subfamily (groups II and III), or biotransformed by the CYP2B enzyme subfamily (groups I and IV [41]).

RESULTS AND DISCUSSION

Historically, PCBs, DDT, chlordanes, HCHs, and chlorobenzenes and their metabolites have been the main POPs quantified in marine mammals [9,20,26]. However, in recent years, other POPs of concern have emerged, including toxaphene and PBDEs. Polychlorinated biphenyls and DDT and associated metabolites tend to dominate POP body burdens in marine mammals [9,20,26]. Relative abundance of other contaminant classes varies according to geographic location, as point sources and atmospheric deposition largely dictate the suite of contaminants in a given area [3,42]. For example, HCHs, toxaphene, and lower-molecular-weight POPs tend to be more abundant in higher latitudes [13]. In some studies, toxaphene has been identified as the dominant POP in marine mammal blubber [13,43]. In the current study of *L. acutus*, PCBs were the most abundant POP class identified in all age/length classes, followed by ΣDDT, Σtoxpahene, Σchlordanes, and ΣPBDEs (Table 2). *Trans*-nonachlor was the most abundant chlordane (59% [7%]; mean [±1 standard deviation]) of Σchlordanes, congener 50 was the most abundant toxaphene congener (7.0% [2%] of Σtoxaphene), and congener 47 (2,2′,4,4′-tetraBDE) was the most abundant PBDE congener (55% [9%] of ΣPBDEs).

An extensive morphometric study of *L. acutus* was conducted by Sergeant in the 1970s, including approximately 70 stranded specimens [23]. The smallest individual was 126 cm in total length, and the largest adult female and male were 243

and 267 cm, respectively. The smallest individual collected in this study was a 147-cm female, and the largest was a 271-cm male (Table 1). The largest adult female was 237 cm in total length. Growth curves (length vs age) have been established for several marine mammal species, with a typical pattern of linear growth in early years, reaching an asymptote as the animal ages. This pattern was established for male and female *L. acutus* by Sergeant et al. [23] and for male *L. acutus* in the current study. For both studies, growth slows as individuals reached the age of sexual maturity. A similar pattern was determined for the striped dolphin, *Stenella coeruleoalba*, which reaches sexual maturity near nine years of age and 190 cm [20]. Sergeant's analysis of reproductive development (testes weight and number of corpora lutea in males and females, respectively) indicates that females reach sexual maturity at 201 to 210 cm (6–12 years of age) and that males reach sexual maturity near 220 cm, or 7 to 12 years of age [23].

A decrease in contaminant concentration in sexually mature adult females has been well documented in marine mammals [9,11,14,15,43]. Adult females pass significant portions of their contaminant concentrations to their offspring through placental transfer and lactation [20,44]. The significance of this mechanism is also related to the length of the lactation period [45]. Marine mammals with long lactation periods have more time to off-load contaminants to their offspring [15,44,46]. Aguilar and Borrel [46] found that 9 to 27% of a mother's total body load is transferred to offspring during a seven-month lactation period in fin whales (*Balaenoptera physalus*). This amount of transfer is low when compared to an estimated 72 to 90% organohalogen transfer in *T. truncatus* and *S. coeruleoalba*. *Tursiops truncatus*, *S. coeruleoalba*, and *L. acutus* which all lactate for approximately 18 months [15,23,44]. Adult female *L. acutus* had significantly lower POP concentrations in all contaminant classes relative to juveniles and adult males (Table 2; $p = 0.0001$). The only exception was endosulfan I, which did not significantly differ between adult males and females or between juveniles and adult males.

Contaminant off-loading from adult females to offspring is illustrated by the significant decrease in contaminant concentrations associated with maturity in female *L. acutus* (Table 2) as well as the relatively high contaminant concentrations seen in the smaller juveniles relative to the larger juveniles. For example, contaminant concentrations in adult females were 12 to 30% of those found in juveniles under 190 cm, suggesting off-loading of 70 to 88%, depending on contaminant class. Additionally, regression analysis of *L. acutus* juveniles (147–211-cm body length) stranded between 1998 and 2000 revealed significant ($p < 0.01$) decreases in contaminant concentrations with length for ΣPCBs ($r^2 = 0.37$), ΣDDT ($r^2 = 0.44$), Σtoxaphene ($r^2 = 0.40$), ΣPBDEs ($r^2 = 0.36$), and ΣHCHs ($r^2 = 0.75$). This suggests that the bulk of the contaminants measured in juveniles is transferred via lactation to newborns, subsequently decreasing through growth dilution and/or metabolism.

Age is an important parameter that is often related to POP concentrations in marine mammals. The age of an animal reveals how long the individual has been exposed to contaminants in its environment. In addition to exposure time, age may help predict reproductive maturity, feeding, and migration behaviors associated with different life history phases. Because of the bioaccumulative nature of the compounds and their slow depuration rates, concentrations tend to increase with age [9–12]. Although this relationship holds true for some male marine mammals [20], another pattern often seen is a slow decrease in POP concentrations after weaning [47], reflecting high growth rates and feeding on less contaminated food [39,45]. Similarly, a pattern noted in males of many species is a steady increase with age after weaning, followed by a plateau [45,48]. This pattern may be related to an induction of mixed-function oxidase response with organohalogen concentrations, thus allowing the individual to biotransform and possibly excrete a portion of the contaminant concentration [48].

In some instances, length appears to be more influential than age in determining contaminant patterns. Reproductive maturity may be more dependent on the size than age of the individual [45,48]. The relationship between POP concentration and age in male *L. acutus* was further examined by logarithmic regressions of pesticide concentrations on length. Ages were available for 12 male *L. acutus*. To determine whether age or length had more influence on pesticide concentration, backward stepwise multiple linear regressions were carried out for each class of pesticide to determine which parameter had a significant effect. For *L. acutus*, length was a better indicator than age in determining concentrations of most contaminants, having greater r^2 and significant p values (Fig. 1). This may be due to a small number of known ages or an incomplete cross section of individuals, particularly older males. For females, age estimates may be less important, as contaminant concentrations are highly dependent on reproductive maturity and reproductive success (e.g., number of offspring), which may not be dependent on age or length alone. All contaminant classes decreased significantly with length in males with the exception of ΣPCBs, ΣDDT, and Σchlordanes.

A surprising result of this study is that contaminant burdens do not appear to increase in adult male *L. acutus* with either length or age. Numerous reports have shown that when comparing life history classes, adult males have increasing POP burdens with age leading to significantly higher POP concentrations compared to females, which can off-load POPs to young [9–12]. Hoekstra et al. [13], for example, found that toxaphene, ΣPCBs, ΣDDT, and Σchlordanes all significantly increased with age in male bowhead whales (*Balaena mysticetus*). However, in this study, mean concentrations did not significantly differ between juvenile and adult male *L. acutus* (Table 2). Furthermore, logarithmic regression analyses revealed that concentrations of several POPs, including toxaphene, actually decreased with length in males (Fig. 1).

Several factors may account for these observations. One explanation for lower concentrations in adult male *L. acutus* is so-called growth dilution. In this scenario, the juvenile male receives a large initial contaminant load through nursing. Once the juvenile is weaned, it experiences several years of linear growth in conjunction with a switch in food source from milk to fish, squid, and other prey items that have a lower contaminant concentration [32,36,45,49]. The result is a decline in concentration after weaning. This theory is supported by the relationship between length of both male and female juvenile *L. acutus* and contaminant concentration. When comparing total POP burden (ΣPOP) with body length, concentrations significantly decrease with length ($r^2 = 0.21$, $p = 0.03$; figure not shown). Because only prereproductive individuals were analyzed, off-loading via parturition and lactation is not a possible explanation, leaving only the possibility of dilution through growth or loss through biotransformation.

Another possible explanation for lower POP concentrations in adult males relative to juvenile animals is biotransformation. The biotransformation of PCBs and most likely PBDEs as well

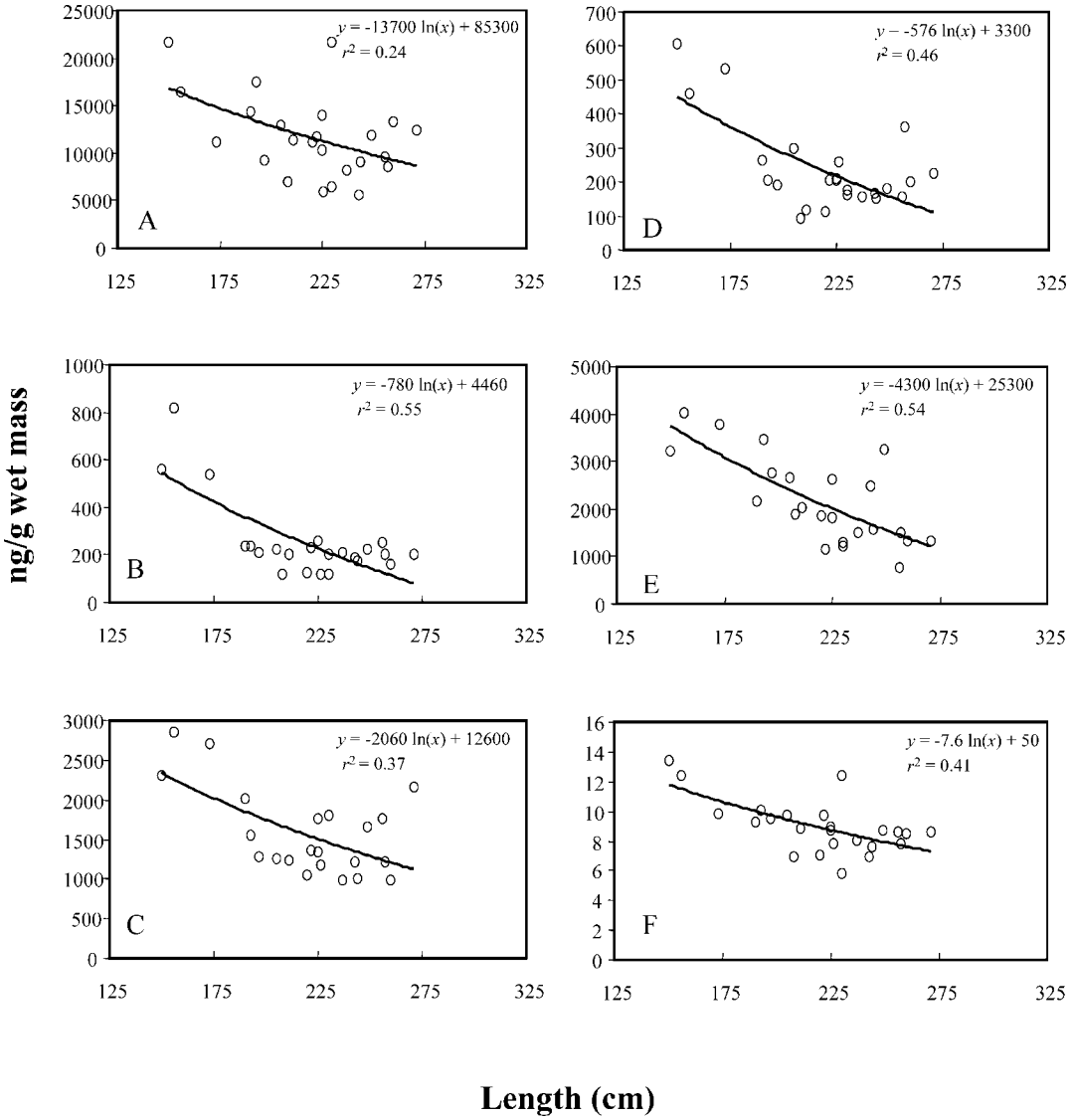

Fig. 1. Relationship between length and (**A**) Σtoxaphene (sum of hexa- through deca-chlorobornanes, (**B**) ΣHCH (sum of α-, γ-, and β-hexachlorocyclohexanes), (**C**) dieldrin, (**D**) HCB (hexachlorobenzene), (**E**) ΣPBDEs (sum of bromodiphenylether congeners 47, 99, 100, 153, and 154), and (**F**) endosulfan I in male *Lagenorhynchus acutus*.

involves the oxidation of parent compounds [7,21,50]. There are three main enzyme types that function in PCB biotransformation; phenobarbital-type (PB-type), 3-methylcholanthrene-type (3-MC-type), and mixed type [7,21]. The 3-MC pathway involves the CYP1A subfamily of the cytochrome P450 enzymes, hydroxylating congeners with H-atoms in *ortho* and *meta* positions with at least one non–*ortho*-substituted chlorine atom [18,21,41]. With CYP1A metabolism, the oxygen atom is not conformationally hindered, and the intermediate may conjugate with other molecules in phase II biotransformation reactions [7,21,40,50].

Phenobarbital-induced metabolism functions with the CYP2B subfamily of P450 enzymes, inserting oxygen into conformationally hindered sites of planar molecules [7,21,41]. Hydroxylation occurs on congeners with H-atoms in the *para* and *meta* positions and multiple *ortho*-substituted chlorine atoms [8,41]. The conformational hindrance of the oxygenated molecule thus inhibits conjugation with other molecules, preventing the intermediate from continuing to phase II metabolism and resulting in phenolic metabolites, some of which have been shown to have hormone-like activity [7,40]. Relevant literature provides conflicting evidence for the existence of CYP2B in cetaceans [18,21,51]. The CYP3A is present in delphinids and is thought to be induced by the xenobiotics that induce CYP2B [52]. For example, recent work by Li et al. [52] showed that CYP2B was minimal to absent in *Lagenorhynchus albirostris* microsomes treated with PCBs, whereas CYP1A and CYP3A were induced.

To look for evidence of CYP1A and CYP2B biotransformation in *L. acutus*, the ratio of PCB congeners 180 (group I); 128, 138, and 170 (group II); 105, 118, and 156 (group III); and 52, 101 (group IV), and 149 (group V) relative to PCB 153 were calculated (PCB_x/PCB_{153}) as done by Boon et al. [41]. Ratio values from male *L. acutus* were then regressed against their length (Fig. 2). Significant negative relationships were observed for congeners with vicinal H atoms in the *ortho*, *meta* positions with one or two *ortho*-substituted chlorines (groups II and III) as well as PCB congeners with vicinal H atoms in the *meta*, *para* positions and two to three *ortho*-chlorines (group IV and V) suggesting *L. acutus* possesses

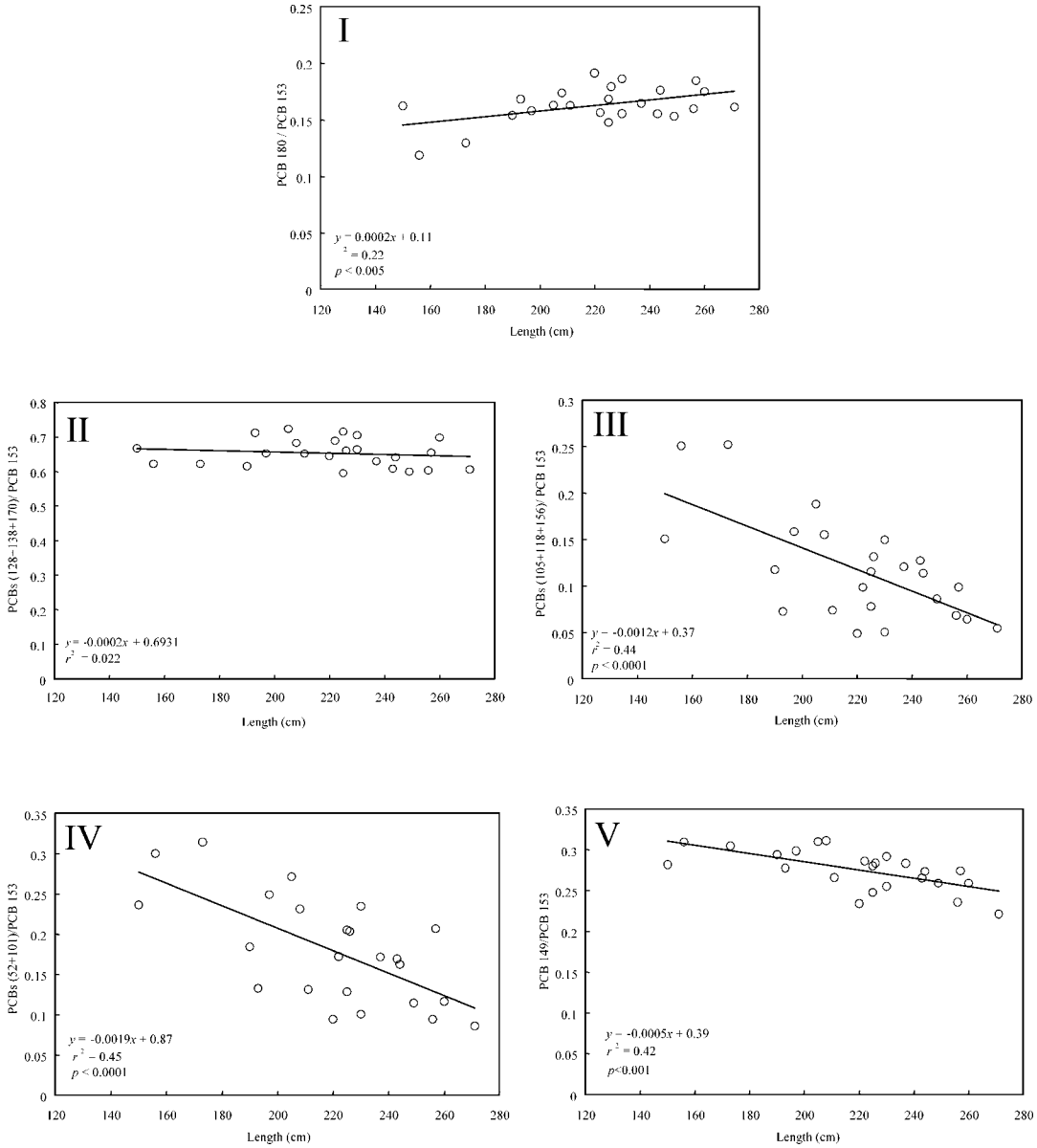

Fig. 2. Ratios of individual polychlorinated biphenyl (PCB) congeners to PCB 153 versus length in male *Lagenorhynchus acutus*. Groups I to V are those specified by Boone et al. [41].

both CYP1A and CYP2B-like biotransformation capabilities (Fig. 2). The congener included in group I, PCB 180, increased slightly with length relative to PCB 153, indicating that this compound has a greater bioaccumulation potential than PCB 153. Within the group II compounds, the behavior of PCB congener 128 differed from PCB congeners 138 and 170. While the slopes for PCB congeners 138 and 170 were not significantly different from zero, suggesting poor biotransformation of these compounds, the slope of PCB congener 128 versus length was significantly negative. This compound possesses two unsubstituted *ortho-* and *meta-* position hydrogen atoms, unlike PCB congeners 138 and 170, which possess only one vicinal hydrogen group, and is more similar in structure to PCB 105 and thus may be biotransformed by a similar mechanism. Within other congener groups, individual congeners behaved similarly to other congeners in the group.

Several observations can be made from the relationships shown in Figure 2. The slopes of the relationships do not appear to change with length, implying constant biotransformation (or elimination) with age (length is proxy for age), as has been observed in humans [53]. Using such data, it may be possible to calculate biotransformation rates relative to poorly biotransformed congeners such as PCB congeners 153 or 180 if the initial ratios in food are stable over the animal's life span. One of the highest negative slopes was observed for the group III compounds, which are thought to be biotransformed mainly by CYP1A. Since odontocetes are thought to have low CYP2B, which is the major biotranformation route for group IV compounds [41], other unidentified enzymes may be responsible for the change in the ratio, such as CYP3A, which has been identified in *L. albirostris* and has a similar function as CYP2B in other mammals [52]. Despite low CYP2B activity in cetaceans, in vitro studies with selected PCB congeners have demonstrated the production of hydroxylated metabolites in beluga whale and pilot whale livers [18]. The resulting mixture of PCB congeners in *L. acutus* becomes increasingly depleted in the dioxin-like group III congeners with length relative to the group I and II congeners. Congeners

in group I and II have been shown in rodent models to have greater immunosuppression activity relative to the group III and IV congeners [54]. An alternative explanation to the reduction of certain PCB congeners relative to PCB 153 in *L. acutus* with length is a change in food from milk, as an animal is weaned, to a fish and squid diet. This is probably not the case, as fish and squid should be more enriched in metabolically labile congeners relative to PCB 153 when compared to milk.

CONCLUSION

While POP concentrations and life history patterns have been well documented for several marine mammal species, the toxicological significance of POPs remains in question. Previous studies drawing from laboratory and semifield experiments have suggested that the 10% effects concentration, or EC10, value for PCBs in marine mammal blubber is approximately 15 μg/g wet mass [8,55]. Approximately 80% of the animals in this study had ΣPCB concentrations greater than this value, and all but two juvenile animals had concentrations exceeding the median effective concentration, or EC50, value of 33 μg/g wet mass proposed by Schwacke et al. [8]. These elevated POP concentrations may affect recruitment of first-borns to the *L. acutus* population, as recent studies have shown that 60 to 80% of primaparous *T. truncatus* are considered at risk for reproductive failure in the form of stillborns and/or high neonatal mortality [8].

Further research needs to be conducted in cetaceans to help determine how body burdens and not simply concentrations within blubber change with age. Currently, it is difficult to perform a mass balance of contaminants on cetaceans, as little is known about tissue distributions, and morphometric relationships with organ/tissue mass and volume with age are limited. Additional research should also be conducted describing concentrations of POPs in prey items to better elucidate the exposure to POPs from food sources. This is important both for describing contaminant intake and for examining the biotransformation of POPs.

Acknowledgement—The authors thank the Cape Cod Stranding Network and the New England Aquarium for help in procuring and storing samples and H. Stapleton and J. Baker for initial aid with PBDE analysis.

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