Relationship between persistent organic pollutants (POPs) and ranging patterns in common bottlenose dolphins (Tursiops truncatus) from coastal Georgia, USA

Brian C. Balmer,¹,b,⁎ Lori H. Schwacke,¹ Randall S. Wells,¹ R. Clay George,⁵ Jennifer Hogue,⁵ John R. Kucklick,⁶ Suzanne M. Lane,¹ Anthony Martinez,⁵ William A. McLellan⁶, Patricia E. Rosel⁷, Teri K. Rowles,⁸ Kate Sparks,⁴ Todd Speakman,⁶ Eric S. Zolman,⁶ D. Ann Pabst⁶

¹ University of North Carolina Wilmington, Department of Biology and Marine Biology, 601 South College Road, Wilmington, NC 28403, USA
² Chicago Zoological Society, c/o Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA
³ National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, USA
⁴ Georgia Department of Natural Resources, Nongame Wildlife Conservation, One Conservation Way, Brunswick, GA 31520, USA
⁵ National Institute of Standards and Technology, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, USA
⁶ National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, 646 Cajundome Boulevard, Lafayette, LA 70506, USA
⁷ National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, FL 33149, USA
⁸ National Oceanic and Atmospheric Administration, National Marine Fisheries Service, 1315 East-West Highway, Silver Spring, MD 20961, USA

⁎ Corresponding author at: University of North Carolina Wilmington, Department of Biology, 601 South College Road, Wilmington, NC 28403, USA. Tel.: +1 910 617 3238.
E-mail address: bbalmer@mote.org (B.C. Balmer).

Article history:
Received 1 December 2010
Received in revised form 27 January 2011
Accepted 28 January 2011
Available online 26 February 2011

ABSTRACT

Bottlenose dolphins (Tursiops truncatus) are apex predators in coastal southeastern U.S. waters; as such they are indicators of persistent organic pollutants (POPs) in coastal ecosystems. POP concentrations measured in a dolphin’s blubber are influenced by a number of factors, including the animal’s sex and ranging pattern in relation to POP point sources. This study examined POP concentrations measured in bottlenose dolphin blubber samples (n = 102) from the Georgia, USA coast in relation to individual ranging patterns and specifically, distance of sightings from a polychlorinated biphenyl (PCB) point source near Brunswick, Georgia. Dolphin ranging patterns were determined based upon 5 years of photo-identification data from two field sites approximately 40 km apart: (1) the Brunswick field site, which included the Turtle/Brunswick River Estuary (TBRE), and (2) the Sapelo field site, which included the Sapelo Island National Estuarine Research Reserve (SINERR). Dolphins were categorized into one of three ranging patterns from photo-identification data. Individuals with sighting histories exclusively within one of the defined field sites were considered to have either Brunswick or Sapelo ranging patterns. Individuals sighted in both field sites were classified as having a Mixed ranging pattern. Brunswick males had the highest concentrations of PCBs reported for any marine mammal. The pattern of PCB congeners was consistent with Aroclor 1268, a highly chlorinated PCB mixture associated with a Superfund site in Brunswick. PCB levels in Sapelo males were lower than in Brunswick males, but comparable to the highest levels measured in other dolphin populations along the southeastern U.S. Female dolphins had higher Aroclor 1268 proportions than males, suggesting that the highly chlorinated congeners associated with Aroclor 1268 may not be offloaded through parturition and lactation, as easily as less halogenated POPs. Individuals sighted farther from the Superfund point source had lower Aroclor 1268 proportions.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Bottlenose dolphins (Tursiops truncatus) are top-level predators and long-lived residents of bays, estuaries, and tidal marshes along the southeastern United States (reviewed in Shane et al., 1986; Wells and Scott, 1999). Lipophilic persistent organic pollutants (POPs), which are biomagnified in organisms at higher trophic levels, are stored in their lipid-rich blubber, making the bottlenose dolphin a sensitive indicator for POPs in coastal ecosystems (Kucklick et al., in review).

Contamination of the Turtle/Brunswick River Estuary (TBRE) in southern coastal Georgia (Fig. 1) by the highly chlorinated (>5 chlorines) polychlorinated biphenyls (PCBs) mixture Aroclor 1268 has been well documented (Kannan et al., 1997, 1998; Maruya and Lee, 1998; Maruya et al., 2001). The primary PCB congener found in the TBRE are those that comprise Aroclor 1268, a highly chlorinated (>5 chlorines) mixture of PCBs. This mixture was used extensively at a chlor-alkali plant that operated in the TBRE from 1955 to 1994. The site, referred to as LCP Chemicals, was designated a National Priority List (i.e. Superfund) site in 1996 due to extensive environmental
contamination from mercury, lead, PCBs, dioxin, and other organic compounds (EPA, 2007; Kannan et al., 1997). Understanding the long-term impacts of these contaminants requires knowledge of the extent to which they contaminate the adjacent environment and food web. Kannan et al. (1997) measured PCB levels in sediments within the TBRE and determined that sediments sampled from the LCP Chemicals site had PCB concentrations 50 times higher than those measured 500 m from the site. Fish species, including spotted sea trout (Cynoscion nebulosus) and striped mullet (Mugil cephalus), sampled in the TBRE had PCB concentrations that were three times higher than PCB levels measured in fish from the Skidaway River, approximately 100 km north of the TBRE (Maruya and Lee, 1998). High concentrations of PCBs, specifically those with the Aroclor 1268 congener pattern, were also reported from a pilot study which sampled bottlenose dolphins in the TBRE (Pulster et al., 2009). Pulster et al. (2009) compared PCB levels from blubber of live dolphins sampled via remote biopsy in St. Simons Sound and the adjacent Back River in the TBRE with blubber samples from stranded dolphins collected approximately 90 km to the north, near Savannah, Georgia. Even with a small sample size of only four male TBRE dolphins, the study was able to discern a congener pattern indicative of an Aroclor 1268 source and similar to the congener profile documented in prey fish from the area (Pulster et al., 2009, 2005). In addition, Rosel (unpublished NOAA data) reported that mitochondrial DNA control region sequences and microsatellite markers from dolphins remotely biopsied in the TBRE were significantly different from those of dolphins sampled in Savannah, Georgia, and Charleston, South Carolina. Thus, it has been hypothesized that the dolphins in the TBRE and surrounding waters may be long-term residents to this region (Pulster et al., 2009). However, to date, this hypothesis has not been tested and no previous data have been published on ranging patterns of dolphins along this region of the Georgia coast.

This study builds on the previous research of Pulster et al. (2009) by expanding the sampling of dolphins within and outside of the TBRE to examine the relationship between measured POP concentrations and individual dolphin ranging patterns. Biopsy sampling was extended 40 km northeast of the TBRE to the waters in and around the Sapelo Island National Estuarine Research Reserve (SINERR) (Fig. 1). The SINERR is a federal- and state-managed protected area and is the focus of long-term ecological research projects such as water quality monitoring, primary productivity assessment, and fisheries sampling (e.g. Dresser and Kneib, 2007; Hanson and Synder, 1979; Owen and White, 2005). The area surrounding Sapelo Island, including the SINERR, is relatively undeveloped and was chosen with the intent that dolphins in this area could potentially act as a reference group for comparison with dolphins inhabiting the more contaminated TBRE. However, nothing was known about the ranging patterns of bottlenose dolphins within and between the TBRE and SINERR regions. Thus, if dolphins in the SINERR region were found to have elevated POP levels, it would be unclear whether such findings were due to contaminant transport or movement of dolphins between the two regions.

Photo-identification of dorsal fins has proven to be a very effective method of identifying individual dolphins and determining their ranging patterns (e.g. Irvine et al., 1981; Scott et al., 1990; Wells and Scott, 1990). Photo-identification surveys were initiated within the TBRE and SINERR regions to document the presence of individual dolphins and their potential movement between the sites. The goals of this study were to characterize the POP, and specifically PCB, exposure of dolphins in the TBRE and SINERR regions and examine patterns of...
PCB congeners in relation to individual dolphin ranging patterns based upon photo-identification sighting histories.

2. Materials and methods

2.1. Study area

The southern Georgia photo-identification survey area (SGA) included the estuarine waters from Sapelo Sound south to St. Simons Sound, representing approximately 60 km of north–south estuarine shoreline (Fig. 1). The survey area’s eastern boundaries were defined as the mouths of Sapelo, Doboy, Altamaha, and St. Simons Sounds. The western boundaries were defined as 15 km upriver of the Sapelo, Altamaha, and Turtle rivers. The SGA was divided into two field sites based upon the location of major sounds within each site. The Brunswick field site included the TBRE and all estuarine waters from St. Simons Sound north to and including Altamaha Sound. The Sapelo field site excluded Altamaha Sound and covered all estuarine waters north to, and including Sapelo Sound.

2.2. Biopsy sample collection

Biopsy samples from individual bottlenose dolphins were collected during both remote biopsy sampling surveys and a capture-release health assessment. Remote biopsy sampling was conducted in the Brunswick field site in August 2006 and March 2007 and in the Sapelo field site during August 2007, March 2008, and August 2008 utilizing standard techniques demonstrated to be safe and effective in numerous studies of small cetaceans (Kiszka et al., 2010; Sellas et al., 2005; Wells and Scott, 1990). The remote biopsy samples were obtained using a 0.3 m long carbon fiber dart with a 25 mm stainless steel cutterhead, which was propelled by a 0.22 blank charge from a modified 0.22 caliber rifle. The rifle was equipped with a holosight (Bushnell Corporation, Overland Park, KS) to improve sampling accuracy and a digital video camera and/or digital still camera to improve sampling accuracy and a digital video camera and/or digital still camera to identify the sex of the sampled individual using molecular techniques. Samples were utilized to determine POP concentrations in this study. Blubber samples were analyzed for POPs as described previously (Litz et al., 2007). Briefly, approximately 1 g of blubber was minced, dried with sodium sulfate and extracted by pressurized fluid extraction using dichloromethane. Samples were cleaned up by size exclusion chromatography and aluminum solid phase extraction prior to analysis by gas chromatography mass spectrometry. Lipid content was calculated gravimetrically from a weighed portion of the PFE extract. POP concentrations were determined using a gas chromatograph-mass spectrometer (GC/MS; Agilent 6890/5973, Palo Alto, CA).

A five to seven point calibration curve of compounds was determined from National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) solutions and utilized to quantify all analytes and calibrants. Samples were extracted, cleaned, and analyzed by GC/MS in lots of 30–40 with a minimum of one blank and 1–3 aliquots of NIST SRM 1945 Organics in Whale Blubber (Kucklick et al., 2010). POP concentrations identified within each aliquot of SRM 1945 were within 7.5% ± 3.5% (mean ± standard deviation) of the certified values. The limit of detection (LOD) for each analyte was defined as the greater of (a) the mass of the analyte in the lowest detectable calibration solution divided by the sample mass, or (b) the average mass of the analyte detected in blanks plus three times the standard deviation. The limits of detection ranged from 0.089 ng/g wet mass to 16.9 ng/g wet mass for all measured analytes.

2.3. Biopsy sample analysis

Blubber samples were analyzed for POPs as described previously (Litz et al., 2007). Briefly, approximately 1 g of blubber was minced, dried with sodium sulfate and extracted by pressurized fluid extraction using dichloromethane. Samples were cleaned up by size exclusion chromatography and aluminum solid phase extraction prior to analysis by gas chromatography mass spectrometry. Lipid content was calculated gravimetrically from a weighed portion of the PFE extract. POP concentrations were determined using a gas chromatograph-mass spectrometer (GC/MS; Agilent 6890/5973, Palo Alto, CA).

A five to seven point calibration curve of compounds was determined from National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) solutions and utilized to quantify all analytes and calibrants. Samples were extracted, cleaned, and analyzed by GC/MS in lots of 30–40 with a minimum of one blank and 1–3 aliquots of NIST SRM 1945 Organics in Whale Blubber (Kucklick et al., 2010). POP concentrations identified within each aliquot of SRM 1945 were within 7.5% ± 3.5% (mean ± standard deviation) of the certified values. The limit of detection (LOD) for each analyte was defined as the greater of (a) the mass of the analyte in the lowest detectable calibration solution divided by the sample mass, or (b) the average mass of the analyte detected in blanks plus three times the standard deviation. The limits of detection ranged from 0.089 ng/g wet mass to 16.9 ng/g wet mass for all measured analytes.

2.4. Photo-identification

The photographic records for this study were from three efforts of varying duration and scope, totaling 238 surveys from 2004 to 2009 (Table 1). All efforts were included in this analysis to establish the broadest record possible for each individual dolphin’s sighting history. Dorsal fin images were obtained from remote biopsy sampling surveys conducted in 1–2 week sessions in the TBRE during December 2004, August 2006, and March 2007 and in and around the SINERR during August 2007, March 2008 and August 2008 (Table 1). Contaminant results of biopsy samples from the December 2004 TBRE surveys were previously reported (Pulster et al., 2009) and are not included in this analysis. However, photographic images obtained during the 2004 surveys were included for analysis of individual sighting histories.

Abundance surveys utilizing photo-identification of individuals’ dorsal fins were conducted during every season for 2008 and 2009 in both the Brunswick and Sapelo field sites. During this effort, a 6–7 m, center console vessel with three observers surveyed both field sites to obtain photographs of every individual dolphin’s dorsal fin. Mark-recapture analyses were then performed to determine seasonal abundance (methods reviewed in Balmer et al., 2008) in both the Brunswick and Sapelo field sites.

Radio-tracking was used to identify ranging patterns during summer/fall 2008, following the capture-release health assessment. The two goals of the health assessment were to (1) perform detailed health examinations of bottlenose dolphins from the Brunswick and Sapelo field sites including collection of a surgical wedge biopsy sample for contaminant analysis and (2) attach radio transmitters on bottlenose dolphins to determine short-term ranging patterns. Balmer et al. (2008) have previously described the methodology for radio transmitter attachment and follow-up tracking. Briefly, bottlenose dolphins in both the Brunswick and Sapelo field sites were temporarily captured and restrained utilizing practices similar to those implemented by the Chicago Zoological Society’s Sarasota Dolphin Research
Program (Wells et al., 2004). Radio transmitters were deployed on 28 dolphins (14 male, 14 female) and subsequently tracked by vessel for over 100 days with GPS positions recorded for the visual locations of all tagged individuals.

For all three survey efforts, dorsal fin images were graded on both distinctiveness of the dorsal fin, and photographic quality, following the methods of Urian et al. (1999). A catalog of all fins was created with each individual receiving a unique number based on its distinctive markings. Currently, the SGA photo-identification catalog consists of 646 individual bottlenose dolphins. The photo-identification records from the remote biopsy, abundance, and radio-tracking surveys were used to analyze individuals’ sighting histories and classify each biopsy sampled individual into one of three ranging patterns. In this study, a ranging pattern is defined as the photo-identification sighting history for an individual dolphin within the SGA region. If all photo-identification sightings of a biopsy sampled individual were in either the defined Brunswick or Sapelo field site, they were identified as having a “Brunswick” or “Sapelo” ranging pattern, respectively. Biopsy sampled individuals that were sighted in both field sites were identified as having a “Mixed” ranging pattern.

2.5. Data analysis

Blubber samples in this study were analyzed for PCB congeners (IUPAC PCB numbers 18, 28 + 31, 44, 49, 52, 56, 66, 70, 74, 87, 92, 95, 99, 101, 105, 110, 118, 128, 130, 137, 138, 146, 149, 153 + 132, 151, 154, 156, 157, 158, 163, 170, 172, 174, 176, 177, 178, 180, 183, 185, 187, 189, 194, 195, 197, 199, 200, 201, 202, 203 + 196, 206, 207, 208, and 209), polybrominated diphenyl ether (PBDE) congeners (47, 99, 100, 153, and 154), dichlorodiphenyl dichloroethanes (DDTs) (2,4- and 2,5-DDE, and DDT), and 2,4,5-trichlorophenoxyacetic acid, hexachlorobenzene (HCB), dieldrin, and mirex. Σ PCBs was defined as the sum of the 54 PCB congeners. Σ Aroclor 1268 was defined as the sum of the following congeners identified by Maruya and Lee (1998) as indicative of Aroclor 1268 (174, 180, 183, 187, 194, 196, 199, 200, 201, 202, 206, 207, 208, and 209). Aroclor 1268 proportion was calculated as Σ Aroclor 1268/Σ PCBs. To control for lipid content variability between individuals and sampling seasons, POP concentrations for all samples were calculated on a lipid-weight basis and log transformed to meet the assumptions of normality.

Because mothers transfer much of their accumulated lipophilic contaminant loads to their offspring during each pregnancy and associated lactation period (Aguilar et al., 1999; Wells et al., 2005; Yordy et al., 2010), all biopsied individuals were separated based upon sex. Each sampled individual was classified into its respective ranging pattern (Brunswick, Sapelo, or Mixed) based upon its photo-identification sighting history from all survey efforts. If a sampled individual had a non-distinctive fin or had not been sighted pre- or post-biopsy sampling (i.e. its ranging pattern could not be identified), it was excluded from these analyses. The proportion of Aroclor 1268 congeners was arcsine transformed to meet the assumption of normality. A two-way analysis of variance (ANOVA) including sex (male, female) and ranging pattern (Brunswick, Sapelo, Mixed) as factors was performed. When the F-statistic was significant for ranging pattern, pairwise comparisons were made using Tukey’s Honestly Significant Difference (HSD) test.

The location of the LCP Chemicals site (31.189440 N, 81.508330 W) (EPA, 2002), the likely point source for Aroclor 1268 contamination, was used as a reference point and photo-identification sighting histories for each biopsy sampled individual were utilized to calculate the distance of each sighting from this point. Distance for each photo-identification sighting was calculated as the closest on-water distance between the sighting and the reference point using the “Measure” tool in ArcMap 9.2 (ESRI, Redlands, CA). For each individual dolphin, the mean distance to point source was determined from that dolphin’s entire sighting history. Linear regression analysis was performed to examine any relationships between the proportions of Aroclor 1268 congeners, and mean sighting distance from point source. A test for homogeneity of slopes was used to determine interactions between sex and distance from point source.

3. Results

A total of 105 blubber samples were collected via remote biopsy from dolphins in the Brunswick and Sapelo field sites. Of these, 29 remote biopsy samples were excluded because individuals had non-distinctive fins or were not sighted pre- or post-sampling. In addition, 26 samples were collected via surgical biopsy during the capture-release health assessment bringing the total number of samples utilized in this study to 102. Sampled individuals, which were sighted a mean number of 14 ± 12 (± standard deviation) times, were separated by sex and grouped into one of three ranging patterns; Brunswick (♀ = 10, ♂ = 24), Mixed (♀ = 4, ♂ = 18), and Sapelo (♀ = 14, ♂ = 32).

Male dolphins had significantly higher mean concentrations for all POP classes than did females (Table 2). Mean percent lipid was significantly higher in female dolphins than male dolphins (P = 0.0022). Σ PCB and Σ Aroclor 1268 differed significantly across all ranging patterns. There were no significant differences in mean

<table>
<thead>
<tr>
<th>Date</th>
<th>Field site</th>
<th>Survey type</th>
<th># of individuals sighted</th>
<th># of remote biopsy samples obtained</th>
<th># of surgical biopsy samples obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>14–17 Dec. 2004</td>
<td>Brunswick</td>
<td>Remote biopsy</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21–30 Aug. 2006</td>
<td>Brunswick</td>
<td>Remote biopsy</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12–23 Mar. 2007</td>
<td>Brunswick</td>
<td>Remote biopsy</td>
<td>114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–31 Aug. 2007</td>
<td>Sapelo</td>
<td>Remote biopsy</td>
<td>169</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04–16 Feb. 2008</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17–27 Mar. 2008</td>
<td>Sapelo</td>
<td>Remote biopsy</td>
<td>77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>01–11 Apr. 2008</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 Jul.–9 Aug. 2008</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–28 Aug. 2008</td>
<td>Sapelo</td>
<td>Remote biopsy</td>
<td>106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06–16 Oct. 2008</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 Jan.–9 Feb. 2009</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 Mar.–11 Apr. 2009</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06–16 Jul. 2009</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>196</td>
<td></td>
<td></td>
</tr>
<tr>
<td>03–14 Aug. 2009</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Aug.–9 Oct. 2009</td>
<td>Brunswick and Sapelo</td>
<td>Radio tracking</td>
<td>224</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–24 Oct. 2009</td>
<td>Brunswick and Sapelo</td>
<td>Abundance</td>
<td>179</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 Oct.–20 Nov. 2009</td>
<td>Brunswick and Sapelo</td>
<td>Radio tracking</td>
<td>69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
percent lipid and all other POP classes, across male ranging patterns. The highest Σ PCB concentrations in male dolphins were 2870 µg/g (Brunswick), 756 µg/g (Mixed), and 333 µg/g (Sapelo). Brunswick males had significantly higher mean Σ PCB and Σ Aroclor 1268 concentrations than did Sapelo males (P<0.0001 and P<0.0001, respectively). Mean Σ PCB and Σ Aroclor 1268 concentrations for Mixed males were significantly lower than Brunswick males (P=0.0036 and P=0.0024, respectively) and significantly higher than Sapelo males (P=0.0028 and P=0.0090, respectively). The highest Σ PCB concentrations measured in female dolphins were 339 µg/g (Brunswick), 154 µg/g (Mixed), and 279 µg/g (Sapelo). There were no significant differences in mean percent lipid, Σ PCB, Σ Aroclor 1268, and all other POP classes between females across ranging patterns. However, the low sample size (n=4) for Mixed females limits interpretation of contaminant data associated with this ranging pattern in comparison to the other female ranging patterns.

Aroclor 1268 proportion in male dolphins differed significantly between all three ranging patterns (P<0.0001 for all pairwise comparisons), with Brunswick males having the highest proportion followed by Mixed, and Sapelo males (Table 2). Brunswick and Mixed females had a significantly higher proportion of Aroclor 1268 (P<0.0001 and P=0.0009, respectively) than did Sapelo females. Aroclor 1268 proportion did not differ significantly between Brunswick and Mixed females (P=0.9288).

Linear regression analysis was performed to identify relationships between Aroclor 1268 proportion and mean sighting distance from the point source for each biopsy sampled individual (Fig. 2). For both male and female dolphins, there was a negative relationship between the proportion of Aroclor 1268 congeners and mean sighting distance from the point source (males: R²=0.6842, P<0.0001; females: R²=0.7137, P<0.0001). The slopes of the regression lines did not differ between males and females (P=0.4020).

Table 2

<table>
<thead>
<tr>
<th>POP class</th>
<th>Σ PCB (µg/g)</th>
<th>Σ Aroclor 1268 proportion</th>
<th>Σ PBDE (µg/g)</th>
<th>Σ DDT (µg/g)</th>
<th>Σ CHL (µg/g)</th>
<th>HCB (µg/g)</th>
<th>Dieldrin (µg/g)</th>
<th>Mirex (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brunswick</td>
<td>25.12</td>
<td>509.56A</td>
<td>407.78A</td>
<td>0.77B</td>
<td>3.85</td>
<td>36.77</td>
<td>6.30</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>(13.17–37.07)</td>
<td>(369.04–703.59)</td>
<td>(290.30–572.78)</td>
<td>(0.74–0.80)</td>
<td>(2.79–5.32)</td>
<td>(21.93–61.65)</td>
<td>(4.31–0.22)</td>
<td>(0.03–0.60)</td>
</tr>
<tr>
<td>Mixed</td>
<td>27.90</td>
<td>253.57</td>
<td>170.71</td>
<td>0.68B</td>
<td>5.12</td>
<td>28.55</td>
<td>5.75</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(17.02–38.77)</td>
<td>(177.89–361.45)</td>
<td>(119.14–244.61)</td>
<td>(0.65–0.71)</td>
<td>(3.78–6.95)</td>
<td>(16.87–48.32)</td>
<td>(3.68–0.01)</td>
<td>(0.04–0.07)</td>
</tr>
<tr>
<td>Sapelo</td>
<td>23.57</td>
<td>115.73F</td>
<td>69.10F</td>
<td>0.60F</td>
<td>2.48</td>
<td>20.49</td>
<td>3.83</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>(14.39–32.74)</td>
<td>(91.66–146.13)</td>
<td>(54.97–86.86)</td>
<td>(0.58–0.62)</td>
<td>(1.95–3.17)</td>
<td>(14.03–29.93)</td>
<td>(2.76–5.34)</td>
<td>(0.03–0.04)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brunswick</td>
<td>32.80</td>
<td>116.47A</td>
<td>94.87A</td>
<td>0.85A</td>
<td>0.63</td>
<td>15.68</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>(12.71–52.90)</td>
<td>(78.14–173.60)</td>
<td>(64.41–139.72)</td>
<td>(0.79–0.84)</td>
<td>(0.22–1.82)</td>
<td>(2.79–48.10)</td>
<td>(0.24–1.63)</td>
<td>(0.01–0.04)</td>
</tr>
<tr>
<td>Mixed</td>
<td>28.61</td>
<td>45.94A</td>
<td>35.15A</td>
<td>0.78A</td>
<td>0.38</td>
<td>1.59</td>
<td>0.49</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>(17.98–40.03)</td>
<td>(28.75–101.72)</td>
<td>(19.43–63.60)</td>
<td>(0.55–1.00)</td>
<td>(0.05–2.57)</td>
<td>(0.23–10.99)</td>
<td>(0.08–3.05)</td>
<td>(0.00–0.03)</td>
</tr>
<tr>
<td>Sapelo</td>
<td>36.44</td>
<td>48.27B</td>
<td>30.60B</td>
<td>0.63B</td>
<td>1.27</td>
<td>10.03</td>
<td>1.31</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>(19.94–53.84)</td>
<td>(27.25–85.50)</td>
<td>(17.72–52.86)</td>
<td>(0.59–0.67)</td>
<td>(0.06–2.55)</td>
<td>(3.98–25.32)</td>
<td>(0.37–4.74)</td>
<td>(0.02–0.04)</td>
</tr>
</tbody>
</table>

P-value (ranging pattern): P<0.0001

P-value (sex): P<0.0001

Fig. 2. Relationship between the proportions of Aroclor 1268 congeners found in the blubber of each biopsy sampled individual and its calculated mean sighting distance from LCP Chemicals.
4. Discussion

This study confirms that dolphins utilizing the TBRE are exposed to extraordinarily high levels of PCBs. The maximum PCB concentration measured in a Brunswick male was over 1.5 times greater than the maximum PCB level measured in transient, male Pacific killer whales (Orcinus Orca), which were previously reported to have the highest PCB levels of any cetacean (Krahm et al., 2007; Ross et al., 2000). Biomagnification of contaminant concentrations has been extensively documented in marine mammal species (reviewed in Houde et al., 2005). Transient killer whales, at the top of the northeastern Pacific marine food web, primarily feed on other marine mammal species (Ford et al., 1998), therefore high contaminant levels would be expected in these individuals through biomagnification. Bottlenose dolphins along the southeastern U.S. are also considered top-level, marine predators (reviewed in Wells et al., 2005). However, bottlenose dolphin prey is primarily based on lower trophic levels such as pinfish (Lagodon rhomboides), mullet (Mugil spp.), and a variety of soniferous fish species (Barros and Odell, 1990; Barros and Wells, 1998; Berens McCabe et al., 2010; Gannon and Waples, 2004). Thus, based solely on trophic level differences, it would be expected that bottlenose dolphin contaminant concentrations should typically be lower than those of transient killer whales. The higher levels of PCBs measured in Brunswick male dolphins compared to male transient killer whales is related to the proximity of this population to a major PCB point source and the exposure to these contaminants within their localized environment due to their ranging patterns.

Σ PCB concentrations measured in male dolphins that were only sighted in the Sapelo field site were lower than in Brunswick males, but were comparable to those measured for male bottlenose dolphins in northern Biscayne Bay, Florida (Litz et al., 2007). These males were previously reported to have the highest PCB concentrations for bottlenose dolphins in the southeastern U.S. The Sapelo Island National Estuarine Research Reserve (SINERR) has been identified in numerous studies as a “pristine” reference site based upon the minimal amount of urbanization in the region (e.g. Alberts et al., 1990; Chalmers et al., 1985; Plumley et al., 1980). The elevated levels of PCBs and high Aroclor 1268 proportion in Sapelo male dolphins suggest otherwise. Although there are limited industrial influences surrounding the SINERR, dolphins that have been sighted exclusively in this region have elevated PCB levels associated with a point source located 40 km southwest of their observed ranging pattern. Future research is necessary to identify the pathways leading to Aroclor 1268 contamination in Sapelo dolphins, such as determining contaminant levels and movement patterns of key bottlenose dolphin prey fish species.

Contaminated prey or sediments are the most likely routes leading to dolphin exposure as the Aroclor 1268 mixture is extremely hydrophobic (mean log Kow = 7.9 L/kg) (Maruya and Lee, 1998) and water transport is unlikely.

For each ranging pattern within the southern Georgia survey area (SGA), female dolphins had significantly lower mean Σ PCB and Σ Aroclor 1268 concentrations, but significantly higher proportions of Aroclor 1268 than males. Female cetaceans, upon reaching sexually maturity, offshore the majority of their contaminants to their first born offspring, primarily through lactation (reviewed in Aguilera et al., 1999). For example, PCB concentrations measured in adult female bottlenose dolphins from Sarasota are much lower than those of juvenile females from the same community (Wells et al., 2005; Yordy et al., 2010). Yordy et al. (2010) identified significant changes in POP profiles of female bottlenose dolphins at sexual maturity, where the smallest, least lipophilic contaminants were offloaded through lactation to their first offspring. The predominant Aroclor 1268 congeners are highly chlorinated and therefore may not partition to the milk during lactation, making them resistant to offloading (Kannan et al., 1997, 1998; Yordy et al., 2010). Thus, the proportion of Aroclor 1268 in female dolphins would be expected to be higher than in males, as females offload the less lipophilic contaminants and retain the most lipophilic contaminants. The results of this study suggest that SGA female bottlenose dolphins either continue to be exposed to PCBs, or are not offloading contaminants at the same rate as dolphins in other regions, or some combination of these two processes.

Schwacke et al. (2002) suggested that risk of reproductive failure, such as neonate mortality, would be highest for primiparous female bottlenose dolphins, but that following a successful birth and lactation, the risk of reproductive failure would be reduced with a lower contaminant load. The high PCB levels in SGA females, maintained over the course of a reproductive lifetime, may also maintain the high risk for reproductive failure, even for subsequent reproductive events. Photo-identification data from the 2008 survey effort identified six neonates within the SGA, only one of which survived until the following year (B. Balmer, unpublished data), yielding an annual neonate survival rate of 0.167. For comparison, Speakman et al. (2010) calculated an annual neonatal survival rate of 0.754 (95% CI = 0.647–0.878) for bottlenose dolphins in the Charleston Estuarine Stock. In Sarasota Bay, Florida, the average annual overall neonatal survival is approximately 80%, with about 50% of first-born calves surviving the first year (Wells and Scott, 1990; Wells et al., 2005). Although our SGA estimate is only for a single year, and survival rates often vary greatly across years, these results suggest that dolphin reproductive potential in the SGA may be limited in comparison to other estuarine areas. Knowledge of life history parameters from standing data is necessary to improve the accuracy of neonatal survivorship estimates. However, collection of high quality stranded carcasses in the SGA has been hampered by geographic remoteness, high tidal flux, and other logistical constraints in the region. Enhanced stranding response, stranding reporting and continuation of photo-identification surveys in the SGA are all needed in order for survival estimates to be calculated and compared with other dolphin populations.

The PCB congeners that comprise Aroclor 1268 have been identified as a point source pollutant from the LCP Chemicals Superfund site (Kannan et al., 1997; Kucklick et al., in review; Maruya and Lee, 1998; Pulster and Maruya, 2008). There was a significant negative relationship between the proportion of Aroclor 1268 congeners and mean sighting distance from the LCP Superfund site, indicating that the exposure of a SGA dolphin is directly associated to its proximity to this site. Although PCBs are ubiquitous contaminants and there is potentially some background exposure resultant from long-range environmental transport, the high levels and proportion of Aroclor 1268 congeners indicate that PCB exposure of the sampled dolphins was predominantly from this single point source. Other studies along the southeastern U.S. have reported elevated levels of highly chlorinated PCB congeners in bottlenose dolphins (Hansen et al., 2004; Houde et al., 2006; Kucklick et al., in review; Maruya and Lee, 1998; Pulster and Maruya, 2008). Watanabe et al. (2000) determined that over 60% of the PCB profile measured in liver samples from stranded bottlenose dolphins consisted of six (hexa) and seven (hepta) chlorobiphenyls. Similarly, in blood plasma samples from bottlenose dolphins obtained during capture-release health assessments, the predominant PCB homolog groups measured were those that contained between five (penta) and seven (hepta) chlorobiphenyls (Yordy et al., 2010). However, the specific PCB profile of the highly chlorinated congeners associated with Aroclor 1268 have only been identified along the southern coast of Georgia (Kucklick et al., in review). Although our study has identified SGA dolphins with localized ranging patterns exclusively within the Brunswick and Sapelo field sites, future research is necessary to determine if other groups of dolphins are entering the SGA as well as prey species’ movements into and out of the region.

Kucklick et al. (in review) utilized POP concentrations measured in bottlenose dolphins at 14 locations along the southeastern U.S. and Gulf of Mexico coasts, to identify geographic differences in POPs. The
contaminant levels measured in the Brunswick and Sapelo field sites for this study were two of the locations included in this analysis. Kucklick et al. (in review) confirmed the results of this study, which identified that Brunswick dolphins had the highest Σ PCB concentrations measured along the southeastern U.S. and Gulf of Mexico coasts. Σ PBDE concentrations in SGA dolphins were comparable to dolphins sampled in Charleston, SC, and Mississippi Sound, and higher than dolphins sampled in all other sampling locations. Mirex concentrations in SGA dolphins were comparable to dolphins sampled in Sarasota Bay, FL, Tampa Bay, FL, and Mississippi Sound, and higher than all other sampling locations. Σ DDT, Σ CHL, HCB, and dieldrin concentrations were intermediate in SGA dolphins, in comparison to all other sampling locations. The geographic differences in POP concentrations provide an additional tool to identify bottlenose dolphin stock delineations.

NOAA has defined five coastal and nine estuarine North Western Atlantic (NWA) bottlenose dolphin stocks, based upon photo-identification, telemetry, and genetic studies at multiple locations along the southeastern U.S. coast (reviewed in Waring et al., 2009). Numerous NWA bottlenose dolphin stocks overlap with each other and the precise delineations of these stocks, and movements of individuals between these stocks, are currently not well understood. On a broad-scale, Hansen et al. (2004) identified differences in POP concentrations between individual dolphins biopsy sampled in multiple states along the southeastern U.S. Similarly, Litz et al. (2007) identified significant differences in POP exposure of different bottlenose dolphin communities in the localized estuary of Biscayne Bay, Florida. The results of this study suggest that the elevated POP levels and patterns may provide insight into Georgia bottlenose dolphin population structure. The two NOAA defined stocks in this region are the South Carolina/Georgia Coastal Stock (SCGCS) and the Southern Georgia Estuarine Stock (SGES) (Waring et al., 2009). The SCGCS includes all of the coastal waters of South Carolina and Georgia out to 25 m in depth. The SGES includes all of the estuarine waters from Altamaha Sound south to the Cumberland Sound (Georgia/Florida border). The spatial extent, ranging patterns, and overlap between these two stocks are not well understood. Dolphins that live in the estuarine waters to the north of the SGES, including Sapelo Island and the SINERR, are not classified into any stock at this time. The results from the photo-identification data and measured contaminant concentrations from this study suggest that Brunswick and Sapelo bottlenose dolphins may be part of separate estuarine stocks; SGES and a previously undefined stock beginning at the Altamaha Sound and extending northward, respectively. Recent studies determining seasonal abundance estimates, as well as ranging and movement patterns of bottlenose dolphins within the Brunswick and Sapelo field sites will augment this study and enhance these proposed changes in current SGA stock delineations.

The results of this study suggest that POP, and specifically Aroclor 1268, contamination extends farther outside of the TBRE than previously documented. Elevated levels of POPs, such as PCBs, have been identified as potential stressors to marine mammals (reviewed in Houde et al., 2005). Numerous studies have linked high tissue levels of PCBs to deleterious effects on reproduction and immune function (Aguilar and Borrell, 1998; DeLong et al., 1973; Helle et al., 1976; Martineau et al., 1987). However, identifying POPs as a causative factor of reproductive failure and immune suppression has proven difficult due to the logistical, political, and ethical constraints involved with marine mammals (reviewed in Schwacke et al., 2002). SGA bottlenose dolphins have extremely high levels of PCBs, specifically the highly chlorinated congeners associated with Aroclor 1268, which have been suggested to be resistant to offloading. Individual dolphins within the SGA have relatively localized distribution patterns facilitating routine follow up monitoring. Thus, the bottlenose dolphins within the SGA provide a unique opportunity to identify possible deleterious effects associated with chronic PCB exposure.

Disclaimer

This publication does not constitute an endorsement of any commercial product or intend to be an opinion beyond scientific or other results obtained by the National Institute of Standards and Technology (NIST) and National Oceanic and Atmospheric Administration (NOAA). No reference shall be made to NOAA, or this publication furnished by NOAA, to any advertising or sales promotion which would indicate or imply that NOAA recommends or endorses any proprietary product mentioned herein, or which has as its purpose an interest to cause the advertised product to be used or purchased because of this publication.

Acknowledgments

This research was funded by NOAA’s Ocean and Human Health Initiative and NOAA’s Marine Mammal Health and Stranding Response Program, and conducted under Scientific Research Permit Number 932-1905/MA-009526 issued by NOAA Fisheries and IACUC permit numbers HQ-2009-001 and UNCW 2007-016. Additional support was also provided by the Chicago Zoological Society, University of North Carolina Wilmington, and Georgia Department of Natural Resources. We would like to thank Jennifer Yordy and Aurore Guichard for assistance with contaminant analyses; John Schwacke and Andrew Westgate for logistical assistance and support during the telemetry portion of this study; Jeff Adams for database creation and maintenance; Penn Clarke and Barbara Danielson for logistical and field efforts; and Dr. Forrest Townsend, Larry Fulford, Jesse Wicker, and all of the participants in the NOAA sponsored health assessment. We also thank the Sapelo Island National Estuarine Research Reserve, and particularly Dorset Hurley, for support of this research and logistical assistance.

References

Dresser BK, Kneib RT. Site fidelity and movement patterns of wild subsadult red drum, Sciaenops ocellatus (Linnaeus), within a salt marsh-dominated estuarine landscape. Fish Manag Ecol 2007;14:183–90.