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Geographic specificity of Aroclor 1268 in bottlenose dolphins (*Tursiops truncatus*) frequenting the Turtle/Brunswick River Estuary, Georgia (USA)

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ABSTRACT

Coastal marine resources are at risk from anthropogenic contaminants, including legacy persistent organic pollutants (POPs) with half-lives of decades or more. To determine if polychlorinated biphenyl (PCB) signatures can be used to distinguish among local populations of inshore bottlenose dolphins (*Tursiops truncatus*) along the southeastern U.S. coast, blubber from free-ranging and stranded animals were collected along the Georgia coast in 2004 and analyzed for PCB congeners using gas chromatography with electron capture and negative chemical ionization mass spectrometric detection (GC-ECD and GC-NCI-MS). Mean total PCB concentrations (77±34 μg/g lipid) were more than 10 fold higher and congener distributions were highly enriched in Cl_7–Cl_{10} homologs in free-ranging animals from the Turtle/Brunswick River estuary (TBRE) compared with strandings samples from Savannah area estuaries 90 km to the north. Using principal components analysis (PCA), the Aroclor 1268 signature associated with TBRE animals was distinct from that observed in Savannah area animals, and also from those in animals biopsied in other southeastern U.S estuaries. Moreover, PCB signatures in dolphin blubber closely resembled those in local preferred prey fish species, strengthening the hypothesis that inshore *T. truncatus* populations exhibit long-term fidelity to specific estuaries and making them excellent sentinels for assessing the impact of stressors on coastal ecosystem health.

1. Introduction

The condition and health of coastal ecosystems is a topic of increasing concern as population centers along the coastline continue to grow. Because of their high trophic position and longevity, marine mammals are excellent indicators of stressors impacting coastal systems. Bottlenose dolphins (*Tursiops truncatus*) in particular inhabit estuaries, tidal marshes, and inshore bays of the southeastern U.S. year-round (Shane et al., 1986). Moreover, inshore populations of *T. truncatus* in this region are thought to exhibit long-term site fidelity to specific estuaries (Wells et al., 1980; Caldwell, 2001; Zolman, 2002), making them excellent sentinels for assessment of localized habitat degradation, disturbance and

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exposure to contaminants in the coastal zone (Klinowska, 1991; Gubbins, 2000).

Persistent organic pollutants (POPs) that are known to biomagnify in aquatic ecosystems are a stressor of primary concern to marine mammals. Elevated levels of POPs such as polychlorinated biphenyls (PCBs) are suspected of contributing to immunosuppression and/or reproductive impacts on highly exposed animals, including *T. truncatus* (Kannan et al., 2000; Schwacke et al., 2002). The dominant pathway for bioaccumulation of POPs by marine mammals is dietary ingestion, contributing over 90% of the total intake of POPs (Campbell et al., 1965). For odontocetes such as *T. truncatus*, fish comprise the bulk of the diet (Hamilton and Nishimoto, 1977; Reynolds et al., 2000; Gannon and Waples, 2004). As a result, concentrations of POPs in preferred prey species generally dictate contaminant exposure in dolphin populations, inshore or otherwise.

Whereas POP levels provide little information regarding the location or timing of exposure (DeCaprio et al., 2005), PCB congener patterns in marine mammals have been used to differentiate between local and regional contaminant sources (Westgate and Tolley, 1999; Ross et al., 2000; Hansen et al., 2004) as well as interspecific variations in biotransformation and metabolism (Tanabe et al., 1988). These signatures have been used to infer habitat use, home ranges and in some cases to evaluate population identity and discreteness (Calambokidis, 1986; Cockcroft et al., 1989, Muir et al., 1990; Calambokidis and Barlow, 1991; Storr-Hansen and Spliid, 1993; Pastor et al., 1996; Westgate and Tolley, 1999; Hansen et al., 2004; Herman et al., 2005). In the southeastern U.S., for example, PCB congener profiles were recently reported to be region-specific for inshore *T. truncatus* (Hansen et al., 2004).

PCB contamination in the Turtle/Brunswick River estuary (TBRE) near Brunswick, Georgia (USA) is dominated by highly chlorinated homologs (Cl_7–Cl_{10}) associated with Aroclor 1268 (Kannan et al., 1997; Maruya and Lee, 1998a). Because of the large inventory, extreme persistence and hydrophobicity of this relatively simple technical mixture, PCB concentrations and congener distributions in sediment, aquatic invertebrates and fish have not changed substantially over the past decade (Maruya and Lee, 1998b; Pulster et al., 2005). The uniqueness of Aroclor 1268 has more recently been used to distinguish between fish inhabiting the TBRE from those in nearby coastal estuaries (Pulster et al., 2005). However, no information on PCB congener levels and distributions in marine mammals utilizing this estuary have been published.

The objective of this study was to determine if PCB congener patterns, including that associated with Aroclor 1268, could be used to (1) link inshore dolphins with contaminated prey fish and (2) distinguish among populations of inshore *T. truncatus* frequenting southeastern U.S. Atlantic estuaries. To address this, blubber from stranded and free-ranging animals were collected along the Georgia coast and inside the TBRE, and analyzed for a comprehensive suite of PCB congeners. These data were compared with previously published results on preferred *T. truncatus* prey species to determine the geospecificity of PCB congener patterns of animals frequenting the TBRE.

2. Experimental section

2.1. Materials

All sample glassware was exhaustively hand-washed and rinsed with tap water, kiln-fired and rinsed with acetone and hexane prior to use. Solvents (Optima grade) and reagents (Na_2SO_4 and Florisil) were purchased from Fisher Scientific. High purity PCB standards were purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA), Ultra Scientific (North Kingstown, RI, USA), or AccuStandard (New Haven, CT, USA).

2.2. Sample collection

The collection of fish species preferred by *T. truncatus* was previously reported in Pulster et al. (2005). Targeted species were silver perch (*Bairdiella chrysoura*), spot (*Leiostomus xanthurus*), spotted seatrout (*Cynoscion nebulosus*), and striped mullet (*Mugil cephalus*). Collected during the spring and fall of 2003 from Wassaw Sound near Savannah, Georgia (SAV) and St. Simons Sound near Brunswick, Georgia (BRN) (Fig. 1), whole fish were sorted by species, size range, site and season and homogenized into composite samples. The number of individuals/composite samples for SAV and BRN were 243/18 and 328/31, respectively.

Blubber biopsies from seven free-ranging *T. truncatus* were collected in December 2004 by projectile dart following the methods of Hansen et al. (2004) under Marine Mammal Protection Act (MMPA) Permit No. 779-1633-00. These animals were biopsied in a relatively small area (~50 km^2) well within the confines of the TBRE (Fig. 1). Briefly, blubber and skin plugs were obtained using an air rifle and pre-cleaned stainless steel hollow darts from the dorsal area of each animal. Retrieved samples were handled using stainless steel implements and stored in glass containers, placed on ice immediately after collection, and subsequently frozen at −80 °C within 12 h.

Blubber samples from *T. truncatus* stranded in Wassaw, Ossabaw and St. Catherines Sounds in the general vicinity of Savannah (Fig. 1) were collected in March–June 2004 by Georgia Department of Natural Resources personnel in accordance with NOAA's MMPA. Full depth blubber samples were excised using solvent rinsed stainless implements from the left side of the dorsal region of each animal, placed in clean, solvent-rinsed I-Chem jars, stored in ice-filled coolers and subsequently frozen at −80 °C until analysis. Age class was designated in accordance with previously published guidelines for coastal *T. truncatus* forms (Geraci and Lounsbury, 2005; Ridgway and Harrison, 1999). Additional details for blubber sampling and determination of age/sex of biopsied animals are documented elsewhere (Pulster et al., submitted).

2.3. Extraction and PCB analysis

After thawing at room temperature, each blubber (0.5–1 g) and fish tissue (5 g) sample was homogenized with kiln-fired (~500 °C for 13 h) Na_2SO_4, packed into a 33-ml stainless steel cell and extracted with CH_2Cl_2 for three sequential cycles at 100 °C and 2000 psi using a Dionex ASE 200 system (Salt Lake City, UT, USA). Ten percent by volume of each extract was