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# The distribution and stratification of persistent organic pollutants and fatty acids in bottlenose dolphin (*Tursiops truncatus*) blubber

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## HIGHLIGHTS

- We examined pollutants (POPs) and fatty acids (FA) in dolphin blubber to assess stratification.
- Dietary and biosynthesized FAs stratified reflecting recent feeding or storage.
- Total POPs were stratified but the stratification direction varied among individuals.
- Profiles of individual POPs were consistent among blubber layers and body locations.
- POP assessment in partial depth biopsies can be used however variability will be greater.

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## ABSTRACT

Blubber has been used for decades to monitor exposure of marine mammals to persistent organic pollutants (POPs). However, little is known about POP variability as a function of blubber depth and across the body of the animal. Remote blubber biopsy sampling (e.g, projectile biopsy) is the most common technique used to acquire samples from free-swimming animals, yet such techniques may result in variable sampling. It is important to understand whether blubber stratification or body location affects POP concentration or the concentration of other important blubber constituents such as fatty acids (FA). To investigate the influence of sampling depth and location on POP concentration, full depth blubber samples were taken from one stranded bottlenose dolphin (*Tursiops truncatus*) at six different body sites to assess variation in FA distribution and contaminant storage with body location. Three of the samples from different body locations were separated into histologically distinct layers to examine the effect of blubber depth and body location on POPs and FAs. In this individual, both POPs and FAs were heterogeneous with blubber depth and body location. POP concentrations were significantly greater in ventral (average ΣPBDEs 1350 ng/g lipid) and anterior (average ΣPCBs 28700 ng/g lipid) body locations and greater in the superficial blubber layer (average ΣPCBs 35500 ng/g lipid) when compared to the deep (8390 ng/g lipid) and middle (23,700 ng/g lipid) layers. Proportionally more dietary FAs were found in dorsal blubber and in middle and deep layers relative to other locations while the reverse was true for biosynthesized FAs. Stratification was further examined in blubber from the same body location in five additional stranded bottlenose dolphins. Although FAs were stratified with blubber depth, lipid-normalized POPs were not significantly different with depth, indicating that POP concentrations can vary in an individual with blubber depth though the direction of POP stratification is not consistent among individuals.

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## 1. Introduction

The marine environment is a global sink for lipophilic persistent organic pollutants (POPs) that are legacies of prior use (e.g, organochlorine pesticides, polychlorinated biphenyls) or current domestic use (e.g, brominated flame retardants) (Dachs et al., 2002). Due to their resistance to degradation and lipophilic properties, POPs accumulate in the lipid-rich tissues of animals, such as the blubber of cetaceans, where they can have significant negative effects on animal health and reproductive ability (Beland et al., 1993; Schwacke et al., 2011; Wells et al., 2005).

Samples from odontocetes (toothed whales) have been used to gauge POP contamination in the marine environment due to the high trophic positions of these animals and often close association

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with coastal areas where sampling is easier to conduct (Kucklick et al., 2011). Non-lethal remote biopsy sampling of blubber has become a common method for sampling odontocetes, rather than sampling blubber from stranded or fisheries bycatch animals. POP concentrations in blubber samples obtained from free ranging animals are preferable to those obtained from stranded animals as the habitat use history and health status of stranded animals are generally difficult to determine. Conversely, results obtained from living animals can sometimes be coupled to known habitat preference or association with contaminated sites (Balmer et al., 2011; Fair et al., 2007; Kucklick et al., 2011; Litz et al., 2007; Pulster et al., 2009).

While samples from remotely collected biopsies provide important information on POP exposure in odontocetes, it is recognized that blubber tissue is not homogeneous with depth or across the body (Evans et al., 2004; Montie et al., 2008; Struntz et al., 2004). Further, the influence of this variability on POP distribution in small odontocetes (i.e., the bottlenose dolphin (*Tursiops truncatus*)) that are most commonly sampled is largely unknown. This uncertainty can lead to difficulty in interpreting POP and/or fatty acid (FA) data when the biopsy sample does not penetrate the entire blubber layer during collection. Sampling the same location on different free-swimming animals using a projectile biopsy is also difficult.

In the bottlenose dolphin, adipocyte dimensions, vascularization, and distribution of structural collagen fibers in blubber has been shown to vary with depth (McClelland et al., 2012; Pond, 1978; Struntz et al., 2004) with superficial, middle and deep layers being identified. Within the depth of the bottlenose dolphin blubber, the middle and deep layers, those closest to the muscle interface, appear to be the most metabolically dynamic serving as the main location for accumulation and release of nutritional lipids (Struntz et al., 2004). Likewise, blubber composition varies with body location. For example, in bottlenose dolphins the tailstock blubber is thought to serve a mainly locomotor function and is not a physiologically active site for sequestration or release of energy (Pabst, 1996), while thoracic blubber is the main region associated with energy storage and release. Specific changes in blubber composition have also been observed with age, reproductive status, season, and geographic location (Dunkin et al., 2005; Samuel and Worthy, 2004; Struntz et al., 2004).

FA stratification has been observed in bottlenose dolphin blubber. Higher concentrations of biosynthesized and branched chain FAs are generally more abundant in the superficial layer, higher concentrations of dietary FAs are found in deep blubber, and intermediate layers have characteristics of both superficial and deep layers (Samuel and Worthy, 2004), suggesting that lipid mobilization and deposition is most active in the deep blubber (Koopman et al., 1996). Although FA composition does vary with blubber depth, it has not been shown to vary at different body sites in the same dolphin (Samuel and Worthy, 2004).

Struntz et al. (2004) hypothesized that the deep adipocytes of juvenile bottlenose dolphins may be preferentially filled with lipid from dietary sources, suggesting that contaminants in association with dietary lipids would also be deposited in the deep blubber layers. It is not known, however, if contaminants remain in the deep blubber layer with the possibility of re-entering the bloodstream during lipid catabolism or if they are permanently retained in adipocytes in the superficial blubber layer. Recent data suggest the former and that the expression of CYP1A, an enzyme involved in xenobiotic metabolism, is heterogeneously distributed in the blubber, with higher expression in the deep layer compared to superficial layers, implying POP metabolism occurs to a greater extent in this deep layer (Montie et al., 2008). Few studies have assessed POP stratification with blubber depth in small odontocetes; however, several studies have been conducted using larger whales, though POPs were found to be homogenously distributed (Evans et al., 2004). It is likely that POPs are in a dynamic equilibrium with adipocytes and other tissues and that this equilibrium is a function of lipid content (Yordy et al., 2010a).

The aim of this study was to examine the relationship of POP and FA distributions with body location and blubber depth in order to better understand how POPs are arranged in bottlenose dolphin blubber and provide insight on bottlenose dolphin exposure to labile fat-soluble contaminants. In addition, this study aims to provide a better understanding of how to interpret POP data from blubber samples obtained from different body locations and blubber depths.

## 2. Materials and Methods

### 2.1. Sample Collection

Blubber samples were collected from six subadult male bottlenose dolphins that were either stranded or were incidentally killed in fishing operations near the North Carolina and Virginia coasts (Table 1). Subadult (i.e. reproductively immature) animals were classified based on total body length and testicular measurements. Only animals that were code 1 or code 2 (euthanized or freshly dead, respectively) were used (Hofman, 1991). Only subadult males were chosen in order to minimize variation between individuals due to life-history characteristics and state of decomposition.

Blubber samples from ESG001 were used in the body location portion of this study. Duplicate full depth blubber samples were collected from all six sites shown in Fig. 1. These samples were wrapped in aluminum foil, placed in Teflon bags and stored at −80 °C. Full depth blubber was sampled from the mid-thoracic region just below the caudal insertion of the dorsal fin (Fig. 1, site 3) of the remaining five individuals in Table 1 for the blubber layer analysis. After initial collection, these samples were placed in freezer bags and stored at −20 °C until subsampling. Samples were partially thawed and subsampled, with subsamples stored at −80 °C or below until analysis.

### 2.2. Histological Preparation and Analysis

Samples from sites 3, 4 and 5 (Fig. 1) from ESG001 and samples from site 3 of the remaining five animals (Table 1) were used to determine depth-dependency of POPs and FAs. Prior to sectioning, a portion of each sample was histologically prepared, embedded in paraffin, stained with hematoxylin and eosin (H&E) stain and mounted according to Schmacher et al. (1993). The slides were placed on a light box and digital photographs were taken using a VideoLabs Student Cam™ (Golden Valley, MN, USA) ocular camera. Images were then imported into an image editing program, analySIS® (Soft Imaging System GmbH,

**Table 1**
Level A data from six subadult male bottlenose dolphins that either stranded or were incidentally killed on the coasts of North Carolina and Virginia between 1999 and 2006. Animal body masses that could not be obtained are listed as "NE". SI (Smithsonian Institute) Code indicates the degree of decomposition of the carcass. Code 2 is a fresh dead animal with no skin peeling and organs intact.

| Animal ID | Length (cm) | Mass (kg) | Body condition | Reproductive status | Sample collection date | Strand location | SI code |
|---|---|---|---|---|---|---|---|
| EMM 006 | 197 | NE | Robust | Immature | 6, May 1999 | Surf City/North Topsail, NC | 2 |
| PTM 117 | 189 | 73.6 | Not emaciated | Immature | 15, March 2000 | Emerald Isle, NC | 2 |
| SAE 003 | 173 | NE | Robust | Immature | 20, April 2002 | Wrightsville Beach, NC | 2 |
| VMSM 2000-1049 | 207 | 114.0 | Robust | Immature | 29, September 2000 | Norfolk, VA | 2 |
| WAM 572 | 196 | 93.0 | Robust | Immature | 10, November 2002 | Sunset Beach, NC | 2 |
| ESG 001 | 203 | 114.4 | Not emaciated | Immature | 31, October 2006 | Atlantic Beach, NC | 2 |