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Spatial variation in the accumulation of POPs and mercury in bottlenose dolphins of the Lower Florida Keys and the coastal Everglades (South Florida)☆

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ABSTRACT

The bottlenose dolphin (*Tursiops truncatus*) is an upper trophic level predator and the most common cetacean species found in nearshore waters of southern Florida, including the Lower Florida Keys (LFK) and the Florida Coastal Everglades (FCE). The objective of this study was to assess contamination levels of total mercury (T-Hg) in skin and persistent organic pollutants (PCBs, PBDEs, DDXs, HCHs, HCB, Σ PCDD/Fs and Σ DL-PCBs) in blubber samples of bottlenose dolphins from LFK (n = 27) and FCE (n = 24). PCBs were the major class of compounds found in bottlenose dolphin blubber and were higher in individuals from LFK (Σ 6 PCBs LFK males: 13,421 ± 7730 ng g^{-1} lipids, Σ 6 PCBs LFK females: 9683 ± 19,007 ng g^{-1} lipids) than from FCE (Σ 6 PCBs FCE males: 5638 ng g^{-1} ± 3627 lipids, Σ 6 PCBs FCE females: 1427 ± 908 ng g^{-1} lipids). These levels were lower than previously published data from the southeastern USA. The Σ DL-PCBs were the most prevalent pollutants of dioxin and dioxin like compounds (Σ DL-PCBs LFK: 739 ng g^{-1} lipids, Σ DL-PCBs FCE: 183 ng g^{-1} lipids) since PCDD/F concentrations were low for both locations (mean 0.1 ng g^{-1} lipids for LFK and FCE dolphins). The toxicity equivalences of PCDD/Fs and DL-PCBs expressed as TEQ in LFK and FCE dolphins is mainly expressed by DL-PCBs (81% LFK - 65% FCE). T-Hg concentrations in skin were significantly higher in FCE (FCE median 9314 ng g^{-1} dw) compared to LFK dolphins (LFK median 2941 ng g^{-1} dw). These concentrations are the highest recorded in bottlenose dolphins in the southeastern USA, and may be explained, at least partially, by the biogeochemistry of the Everglades and mangrove sedimentary habitats that create favourable conditions for the retention of mercury and make it available at high concentrations for aquatic predators.

1. Introduction

Over the last decades, several marine mammals species from various regions around the world have been affected by unusual mortality events, including coastal bottlenose dolphins (*Tursiops truncatus*) along the coasts of the southeastern US. While several possible causative factors have been attributed to these mortalities, a prominent suspect is exposure to toxic contaminants, including persistent organic pollutants (POPs) and some toxic elements (e.g. mercury, Hg), known to affect immune and endocrine systems (Schaefer et al., 2011; Schwacke et al., 2012). In order to better understand the impact of chemical pollution on marine mammal populations, it is critical to understand the drivers of xenobiotic contamination of these marine vertebrates.

The bottlenose dolphin is the most widely distributed small cetacean along temperate and tropical coastlines around the world,

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including along the southeastern coast of the US (Rice, 1998). They are locally abundant in coastal and estuarine habitats, including off South Florida (Barros and Wells, 1998; Mazzoil et al., 2008; Urian et al., 2009). Due to their high trophic position, long lifespan and lipid-rich blubber layer, toothed cetaceans such as bottlenose dolphins generally display high concentrations of xenobiotics in their tissues (Bossart, 2006; Bouquegneau and Joiris, 1988; Vos et al., 2003; Wells and Scott, 2009). Published studies highlight that concentrations are influenced by age, gender, and habitat (Seixas et al., 2008; Stavros et al., 2008; Vos et al., 2003). These characteristics make bottlenose dolphins important sentinels for ecosystems and public health (Reif et al., 2015). However, the potential effects of habitat conditions on the contamination of large marine vertebrates such as coastal dolphins is still poorly understood.

Several studies have found that POPs and T-Hg (total mercury) concentrations in the tissues of dolphins are highly variable in space and time (Balmer et al., 2015; Bryan et al., 2007; Fair et al., 2007; García-Álvarez et al., 2014; Kucklick et al., 2011; Miller et al., 2011; Stavros et al., 2008; Stavros et al., 2007; Woshner et al., 2008; Yordy et al., 2010). For example, the sum of the 6 NDL-PCBs (Non Dioxin Like–PolyChlorinatedBiphenyls) concentration is 5 times higher in male bottlenose dolphins living in the metropolitan area of the Biscayne Bay (near Miami) than those living off the rural coast a few miles south (Litz et al., 2007). There is no available information on pollutant levels on bottlenose dolphins from the waters of the Florida Keys and the coastal Everglades. Quantifying baseline concentrations and patterns of POPs and T-Hg in bottlenose dolphin populations is also critical for risk assessment and monitoring changes in anthropogenic impacts over time.

In the present study, biopsy samples were collected from free-ranging bottlenose dolphins from the Lower Florida Keys (LFK) and the Florida Coastal Everglades (FCE) to assess their POP (PCBs; PolyChlorinatedBiphenyls, PBDEs; PolyBrominatedDiphenylEthers, DDXs; DichloroDiphenyl-Trichloroethanes and metabolites, HCHs; HexaChlorocycloHexanes, HCB; HexaChloroBenzene, DL-PCBs; Dioxin-Like PolyChlorinatedBiphenyls, PCDDs; PolyChlorinatedDibenzoDioxins, PCDFs; PolyChlorinated-DibenzoFurans) and T-Hg concentrations in blubber and skin, respectively. We also aimed to assess the spatial variations of POP and T-Hg concentrations, and the potential role of habitat on their contamination in the coastal waters of South Florida.

2. Materials and methods

2.1. Study sites

The FCE extend from small creeks where freshwater marshes lead to mangrove forests through mangrove-lined channels and inland bays to the coastal oceans of the Gulf of Mexico and Florida Bay. The system is generally oligotrophic and phosphorus-limited with productivity decreasing from the mouths of rivers to upstream marshes (Childers, 2006). There is no human development adjacent to our study areas in FCE. The coastal waters of LFK are dominated by shallow seagrass beds (*Thallasia testudinum* and *Halodule wrightii*) that are subdivided by deeper channels (Lewis et al., 2011). A military base and the city of Key West are adjacent to the LFK study area (Fig. 1).

2.2. Sample collection

Skin and blubber biopsies were collected by using a crossbow (BARNETT Veloci-Speed® Class, 68-kg draw weight) with Finn Larsen (Ceta-Dart, Copenhagen, Denmark) bolts and tips (dart 25-mm long, 5-mm-diameter). The dolphins were hit below the dorsal fin when they were close enough (3–10 m) to the research boat. A total of 51 bottlenose dolphins were sampled in LFK in summer 2008 (National Marine Fisheries Permit No. 779–1633) and in FCE in winter 2013 (National Marine Fisheries Service Permit No. 16314) (Fig. 1, Table 1). Samples were placed in a cooler in the field and subsequently frozen at −20 °C in the laboratory. Skin and blubber tissues were separated before analysis.

2.3. Total mercury (T-Hg) analysis

Approximately 1–13 mg of skin were weighed (0.01 mg precision) and loaded into quartz boats (preheated to 400 °C for 5 min to remove any impurity of mercury). T-Hg concentrations were determined by atomic absorption spectroscopy (AAS, Direct Mercury Analyzer DMA-80, and Milestone) according to the US EPA standard method 7473. This method has been in-house validated for solid samples and quality assurance was assured by measuring blanks (HCl 1%) levels and standardized solution (1 g Hg l^{-1}) before and after every analysis. In addition, Certified Reference Material was analyzed (DORM-2: 4640 μg Hg kg^{-1}) at the beginning and the end of the analysis to monitor the drift of the instrument (Habran et al., 2012, 2013) (Table 1).

2.4. Persistent organic pollutants (POPs)

2.4.1. NDL-PCBs, organochlorine pesticides and PBDEs

The extraction of NDL-PCBs (28, 52, 101, 138, 153 and 180), PBDEs (47 and 99) and organochlorine pesticides (o,p′-DDT, p,p′-DDD, p,p′DDE, HCB, α-HCH, β-HCH and γ-HCH) was performed on about 130 mg of blubber with an Accelerated Solvent Extractor (ASE, Dionex 200, Sunnyvale, USA) using dichloromethane at 80 °C and 0.213 Pa. Before the extraction, 100 μl of a hexanic solution of PCB congener 112 (Dr. Ehrenstorfer®, Augsburg, Germany) was added to the samples as a surrogate internal standard at a concentration of 50 pg μL^{-1}. The fat content was determined gravimetrically by evaporating the solvent at 40 °C until only the fat remains. The extracts were submitted to clean ups with H_2SO_4 and then with Florisil solid phase cartridges (Supelco, Envi-Florisil, Bellefonte, PA) according to the method described by Dyc et al., 2015. Five μL of nonane were used as a keeper and the extract was evaporated under a gentle stream of nitrogen. The extract was reconstituted with 45 μl of *n*-hexane and 50 μl of PCB209 (100 pg μl^{-1} in hexane) as injection volume internal standard (Dr. Erhenstorfer GmbH, Augsburg, Germany). This compound was never detected in the samples during pre-test analysis. The purified extracts were analyzed by high-resolution gas chromatography (Thermo Quest Trace, 2000; Thermo Quest, Milan, Italy) equipped with a ^{63}Ni electron capture detector (ECD). NDL-PCBs, PBDEs and organochlorine pesticides were analyzed on a 60 m × 0.25 mm (0.25 μm film) DB5 ms capillary column (J&W Scientific, USA). Other analytical parameters were described elsewhere (Debier et al., 2003). The quantification was performed by means of the internal standard method. A calibration curve (1.5–250 pg μL^{-1}) was established for each compound of interest. The confirmation of the identity and concentrations of the compounds of interest were periodically performed by high resolution gas chromatograph coupled to an ion trap mass spectrometer (Trace GC Ultra and ITQ 1100 from ThermoQuest). The transfer line temperature was kept at 290 °C and the ion trap temperature was set at 250 °C. The electron ionization (EI) was performed at 70 eV and the ion trap was operating in MS/MS mode. The quality control (QC) was pork fat free of the compounds of interest. The pork fat was spiked with a nominal concentrations of NDL-PCBs and organochlorine pesticides of 5 ng g^{-1} lipid weight forming the QC. The NDL-PCB and the pesticide concentrations in each sample and in the QC were corrected for initial sample weight, and the percentage recovery of the surrogate PCB 112. Recovery rates ranged from 93% ± 22% and

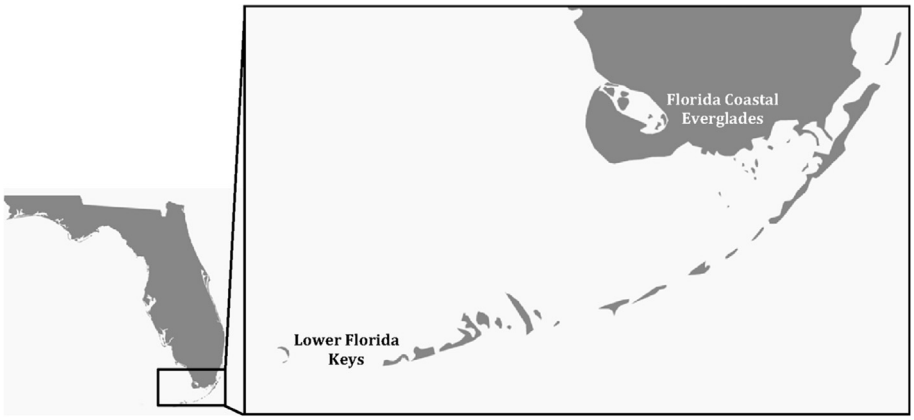

Fig. 1. The study was conducted in the Lower Florida Keys (Key West coastal waters) and in the Florida Coastal Everglades (southwest of the Everglades National Park) https://freevectormaps.com.

Table 1
Number of samples analyzed in blubber (POPs, PCDD/Fs and DL-PCBs) and in skin (T-Hg) of bottlenose dolphins from the Lower Florida Keys and the Florida Coastal Everglades.

	Lower Florida Keys			Florida Coastal Everglades		
	M	F	All samples	M	F	All samples
POPs	16	8	26	11	8	20
PCDD/Fs and DL-PCBs	6	0	6	3	2	5
T-Hg	10	0	10	13	9	24

103% ± 23% for QC and surrogate internal standard respectively and were in good agreement with requirements of SANCO (SANCO, 2014). The limit of detection (LOD) was 0.02 ng g^{-1} lipid weight and the measured limit of quantification (LOQ) determined with PCB spiked lard was established at 0.7 ng g^{-1} lipid weight (Table 1).

2.5. DL-PCBs and PCDD/Fs

Eleven male samples were selected for the determination of 17 WHO PCDD/Fs, and the 12 WHO dioxin-like PCBs (Table 1). Because of their potential low concentrations in marine mammal tissues, PCDD/F and DL-PCB data are difficult to interpret in reproductively active females. In fact, females may exhibit variations in pollutant concentrations because of their own reproduction history (Thron et al., 2004) and because of the placental and lactation transfer of toxic compounds to their offspring (Hall et al., 2006a,b; O'Shea and Tanabe, 2003; Schwacke et al., 2002). To remove this known variability, only the data from males were used for geographic comparisons (Dorneles et al., 2016).

This analytical process also allows the determination of the 6 NDL-PCBs and some PBDEs. A home made spiking solution containing the $^{13}C_{12}$ labelled version of the analytes of interest at known concentrations (mix of individual solutions from Cambridge Isotope Laboratories, USA and Wellington Laboratories, Canada) was used as internal standard and mix to the sample prior the ASE extraction. Clean-up stages were performed by low pressure preparative liquid chromatography with an automated purification Power Prep™System (FMS, Waltham, USA) including acidic silica, basic alumina and carbon columns. The process has been previously described (Focant et al., 2001). The final extracts were concentrated to few μL and the two recovery standards $^{13}C_{12}$ PCB 80, 200 pg μl^{-1} (10 μl) and $^{13}C_{12}$ PBDEs 78 and 138, 200 pg μl^{-1} (10 μl) were added to the mono-*ortho* fraction, whilst the mix $^{13}C_6$ 1,2,3,4 TCDD 1.25 pg μl^{-1} and $^{13}C_{12}$ 1,2,3,4,7,8,9 HPCDF, 3.125 pg μl^{-1} (5 μl) was added to the PCDD/Fs. The analyses were performed by GC-HRMS using Autospec Ultima High Res Mass Spectrometer (Waters, Manchester, UK) coupled with an Agilent 6890 GC (GMI, Minnesota, USA). The process was operated via electron ionization mode using a selected ion monitoring (SIM) in splitless mode (Pinzone et al., 2015).

To assess the danger of the dioxin-like compound concentrations found in dolphin blubber samples, concentrations of every PCDD/F and DL-PCB congener were multiplied by their related toxic equivalency factor (TEF) (Van Den Berg et al., 1998, 2006) to calculate a toxic equivalent quantity (TEQ) which is related to the most toxic compound 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD).

2.6. Data presentation

The Σ 6 NDL-PCBs congeners (28, 52, 101, 138, 153 and 180) were chosen as priority compounds for POP analysis by the Scientific Panel on Contaminants in the Food Chain of EFSA (CONTAM Panel) (European Food Safety Authority (EFSA), 2010). The ΣPCBs is the total sum of the 18 PCB congeners that were analyzed; the ΣPBDEs is the sum of the BDE 47 and BDE 99; the ΣHCH is the sum of the α-HCH, β-HCH and γ-HCH; the ΣDDXs is the sum of o,p′-DDT, p,p′-DDE and p,p′-DDD. We also calculated the following ratios: Σ 6 PCBs/ΣPCBs and p,p′-DDE/ΣDDXs.

2.7. Gender identification

Genomic DNA of FCE dolphins was extracted from skin tissues using the DNeasy-blood and tissue kit (QIAGEN). The gender of each individual was determined by PCR amplification of the SRY and ZFX/ZFY fragments followed by agarose gel electrophoresis (2.5%) following a previously described protocol (Rosel, 2003). In LFK, gender identification has been conducted over the course of a longitudinal study conducted by the Tropical Dolphin Research Foundation. Gender was determined for all individuals sampled using a multiplex reaction (Lewis et al., 2013). Polymerase Chain reaction (PCR) mixture of 1 ml of template was used, 0.3 ml of each primer (ZFX forward and reverse, and SRY forward and reverse), 0.2 ml dNTPs, 0.25 ml $MgCl_2$, 2.0 ml buffer, 0.05 ml Taq polymerase, and 15.3 ml ddH_2O for a final volume of 20 ml. Initial denaturization for 4 min at 94 °C was followed by 34 cycles of 45 s at 94 °C, 45 s at 60 °C and 60 s at 72 °C and then final extension for 10 s at 72 °C. We compared results to controls for a known male and female using gel electrophoresis (1.5% agarose) (Lewis et al., 2013).

Table 2
Lipids percentages and organic pollutant concentrations (ng g^{-1} lipids) in blubber of bottlenose dolphins from the Lower Florida Keys and the Florida Coastal Everglades. Data are showed as mean (median) ± standard deviation (min-max) n = number of samples and p-value for the comparison between male and female dolphins from the Lower Florida Keys and the Florida Coastal Everglades (Mann-Whitney, $p < 0.05$). Significant p-value are in bold.

	Lower Florida Keys			Florida Coastal Everglades		
	M	F	p-value	M	F	p-value
Lipids %	18.1 (16.3) ± 12 (2–41.7) n = 16	27.6 (22.9) ±11.4 (18.8–46.4) n = 8	0.081	7.4 (5.8) ± 5.2 (3–21.9) n = 11	6.8 (6.8) ± 2.8 (310.7) n = 8	0.836
Σ 6 NDL-PCBs[a]	13,420.5 (11,934.3) ±7730 (6044.2–36685.9) n = 16	9683.4 (1996.5) ±19,006.6 (390.5–56100.08) n = 8	**0.022**	5637.9 (4082.5) ± 3627.0 (1826.4–13349.6) n = 11	1426.9 (1619.6) ± 907.6 (396.3–2978.4) n = 8	**0.001**
ΣPBDEs[b]	453.2 (408.3) ± 289.8 (47.8–1048.5) n = 16	210.8 (36.6) ± 368.4 (6.4–1083.9) n = 8	**0.018**	151.2 (115.7) ±136.2 (40.7–471.3) n = 11	79.8 (45.6) ± 78.6 (9.6–215.5) n = 8	0.127
ΣDDXs[c]	3889.3 (1786.3) ± 5287 (673.4–20918.3) n = 16	3777.5 (220.8) ± 8564.5 (19.7–24820.5) n = 8	0.134	3236.5 (2071.8) ± 3255.7 (1079.1–12462.0) n = 11	1065.8 (1010.7) ± 1019.5 (28.7–3242.6) n = 8	**0.015**
ΣHCHs[d]	149.8 (77.1) ± 198.7 (8.2–680.0) n = 16	135.6 (100.1) ± 125.1 (35.4–422.8) n = 8	0.520	85.0 (44.8) ±103 (14.2–348.0) n = 11	29.6 (23.6) ±25.5 (10.3–90.9) n = 8	0.148
HCB[e]	<11.1 (<7.3) ± <12.1 (<2.1–<51.7) n = 16	<12.1 (<6.8) ± <10.6 (<3.4–<32) n = 8	0.975	<6.6 (<7.2) ±<3.0 (<1.5–<10.3) n = 11	<5.9 (<6.6) ±<3.2 (<1.5–<9.9) n = 8	0.650

[a] Σ 6 NDL-PCBs : CB 28, CB 52, CB 101, CB 138, CB 153, CB 180.
[b] Σ PBDEs : BDE 47, BDE 99.
[c] ∑ DDXs: o,p′-DDT, p,p′-DDE, p,p′-DDD.
[d] ∑ HCH: α-HCH (Data of 48 out of 51 samples determined on the basis of LOQ), β-HCH (Data determined on the basis of LOQ), γ-HCH.
[e] Data determined on the basis of LOQ.

2.8. Data analysis

The normality of data was assessed using a Shapiro test (Shapiro et al., 1968). Because the majority of data deviated from a normal distribution, the non-parametric Mann-Whitney *U* test (Whitney, 1951) was used for spatial and sex comparisons of all the chemical tracers, ΣPCBs, Σ 6 NDL-PCBs, ΣPBDEs, and ΣDDXs, ΣHCHs and HCB, PCDD/Fs and DL-PCBs. Statistical analyses were conducted with *Statistica* (version 10).

3. Results

In 2008, 2013, 51 skin and blubber biopsies were collected from dolphins in LFK (n = 27) and FCE (n = 24), respectively. In FCE, dolphins were sampled from 5 different areas: Florida Bay (n = 5), Whitewater Bay (n = 12), Joe River (n = 5), Shark River (n = 1) and Oyster Bay (n = 1) (Fig. S1 in supporting information). Concentrations of 6 NDL-PCBs, PBDEs, DDXs, HCHs and HCB were determined in all individuals in order to quantify POP contamination. Out of these 51 individuals, 11 males were selected for further analysis including the study of dioxins, furans and dioxin-like PCBs (Table 1) to construct a detailed contamination profile of this subpopulation. In order to express our results on a lipid weight basis, samples with a lipid percentage lower than 2% were excluded from analysis (n = 1 sample from LFK and n = 4 samples from the Whitewater Bay in FCE) (EU 589/2014), resulting in a total of 46 samples being analyzed. In addition, T-Hg concentrations were measured in skin samples from 34 individuals, including 10 from LFK and 24 from FCE (**Table 1**).

3.1. Lipids

Blubber lipid percentage were significantly higher dolphins from LFK for both sexes (males: U = 32, p = 0.006; females: U = 0, p < 0.001, Table 2).

3.2. POPs

All the determined compounds were detected at quantifiable levels (>LOQ) in our samples except for HCB (below the LOQ), α-HCH (detected in 3 out of 51 samples) and β-HCH (below the LOQ) (Table 2).

3.3. Gender differences

POP data were obtained for 16 males and 8 females from LFK and for 11 males and 8 females from FCE. Male bottlenose dolphins from LFK displayed significantly higher Σ 6 NDL-PCBs and ∑PBDEs concentrations than females (U = 26, p = 0.022; U = 25, p = 0.018 respectively) and males from FCE displayed significantly higher Σ 6 NDL-PCBs and ∑DDXs concentrations than females (U = 5, p = 0.001; U = 14, p = 0.015 respectively, Table 2).

3.4. Spatial variation

The concentrations of Σ 6 NDL-PCBs and ΣPBDEs were significantly higher in males from LFK than in males from FCE (U = 26, p = 0.002; U = 25, p = 0.002, respectively). For females, ΣHCH concentrations were significantly higher in LFK individuals than those from FCE (U = 4, p = 0.004, Table 2). There was no significant spatial variation among the main sampled sub-areas of FCE (Florida Bay, n = 5; Whitewater Bay, n = 12; and Joe River, n = 5), with the exception of ΣHCH concentrations which were significantly higher in males from Florida Bay (n = 4) than those from Whitewater Bay (n = 5) (Mann-Whitney, U = 0, p < 0.05).

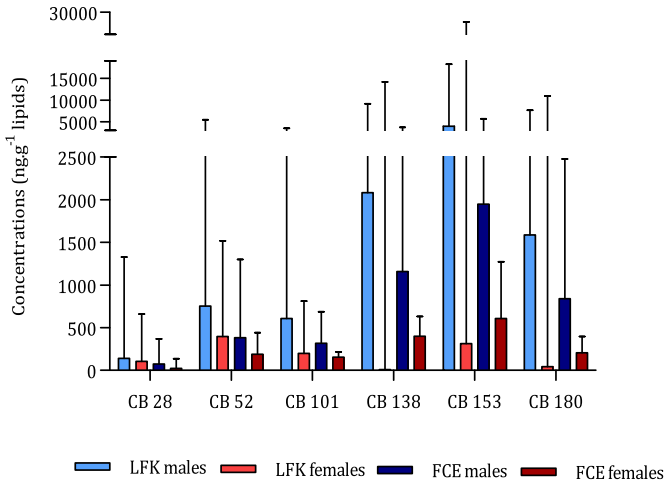

Fig. 2. Median and maximum concentrations of CB 28, 52, 101, 153, 138 and 180 (ng g^{-1} lipids) in bottlenose dolphins from the Lower Florida Keys (LFK) and the Florida coastal Everglades (FCE).

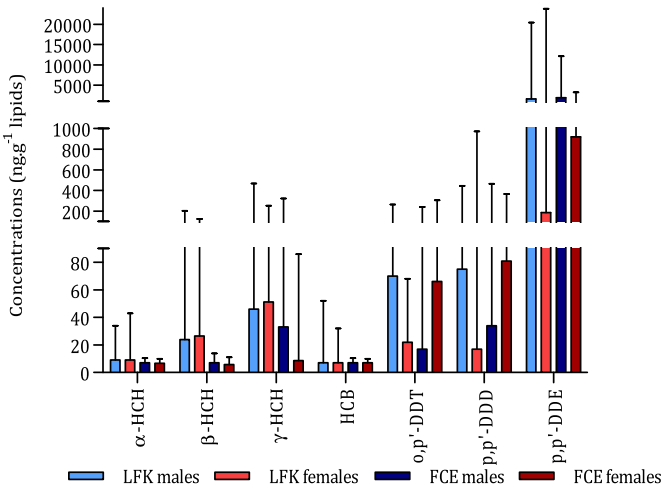

Fig. 4. Median and maximum concentrations of α-HCH, β-HCH, γ-HCH, HCB, o,p′-DDT, p,p′-DDD and p,p′-DDE (ng g^{-1} lipids) in bottlenose dolphins from the Lower Florida Keys (LKF) and the Florida coastal Everglades (FCE).

3.5. NDL-PCBs

The Σ 6 NDL-PCBs displayed the highest concentrations among the studied contaminants with a contribution of all organic pollutants of 75% and 62% for males, and 70% and 54% for females in dolphins from LFK and FCE, respectively (Table 2). CB 153 was the most common congener present for all sampled individuals, representing 43% and 40% for males and 48% and 34% for females of all PCBs in dolphins from LFK and FCE, respectively (Fig. 2). Concentrations for each congener of the 6 NDL-PCBs were significantly higher in males from LFK compared to those from FCE (CB 52: U = 43, p = 0.028; CB 101: U = 39, p = 0.017; CB 138: U = 46, p = 0.041; CB 153: U = 36, p = 0.011; CB 180: U = 41, p = 0.022) with the exception of CB 28, which was similar across sampled regions (U = 53, p > 0.05). Concentrations for CB 28 and CB 52 were significantly higher in females sampled in LFK compared to females sampled in FCE (U = 5, p = 0.005; U = 11, p = 0.03, respectively, Fig. 2).

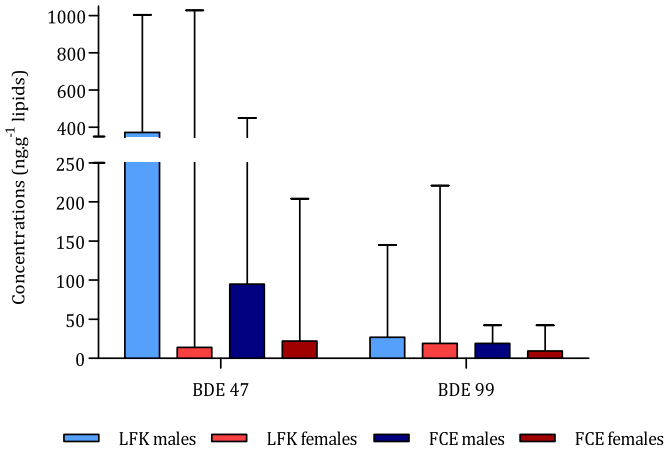

Fig. 3. Median and maximum concentrations of BDE 47 and BDE 99 (ng g^{-1} lipids) in bottlenose dolphins from the Lower Florida Keys (LFK) and the Florida coastal Everglades (FCE).

3.6. PBDEs

Concentrations of BDE 47 and BDE 99 were significantly higher in LFK male dolphins than in FCE male dolphins (U = 26, p = 0.002; U = 45, p = 0.034, respectively). There was no detectable difference in BDE 47 and BDE 99 concentrations in females from the two sampling regions (U = 27, p > 0.05; U = 31, p > 0.05, respectively). BDE 47 represented 88% and 77% for males and 56% and 64% for females of all PBDEs in dolphins from LFK and FCE, respectively (Fig. 3).

3.7. Pesticides

The predominant isomer of HCH family is γ-HCH. Concentrations of γ-HCH were significantly higher in LFK than in FCE for female dolphins (U = 7, p = 0.01), but were not statistically distinguisable between sampled regions for males. The γ-HCH represented 60% of all PBDEs for males in both location and 52% and 45% of all PBDEs in females from LFK and FCE, respectively (Fig. 4). Concentration in β-HCH and HCB are based on the LOD (Table 2, Fig. 4). ΣDDXs concentrations were not significantly different between the two regions (males: U = 74, p > 0.05; females: U = 30, p = 0.875). In both sampled regions, the p,p'-DDE was the predominant compound, representing 89% and 93% for males and 68% and 73% for females of the ΣDDXs in LFK and FCE individuals, respectively (Fig. 4).

3.8. DL-PCBs and PCDD/Fs

Unlike many PCDD/Fs, all dioxin-like PCBs (DL-PCBs) were detected at quantifiable levels (>LOQ) (Table 3). In both regions, Σ 6 NDL-PCBs represented 93% of the Σ PCBs in male dolphins. But, Σ DL-PCBs was the major contributor to the sum of dioxin and dioxin like compounds. The concentrations of Σ DL-PCBs and Σ PCDD/Fs were significantly higher in LFK than in FCE dolphins (p = 0.008 and p = 0.008, respectively). PCDD/Fs expressed as TEQ represented 19% and 35% of Σ PCDD/Fs and Σ DL-PCBs of LFK and FCE dolphins, respectively (Table 3, Fig. 5). CB 126 and CB 118 expressed as TEQ were the predominant congeners in the dioxin and dioxin-like compound contamination pattern in male dolphins. CB 126 represented 25% and 35% of Σ PCDD/Fs and Σ DL-PCBs of LFK and FCE dolphins, respectively (Table 3, Fig. 5). CB 118 represented 32%

Table 3
PCDD/Fs and DL-PCBs concentrations (pg.g^{-1} lipids) of bottlenose dolphins from the Lower Florida Keys (LFK, n = 6) and the Florida Coastal Everglades (FCE, n = 5). Data are showed as mean concentrations, TEF (toxic equivalency factor) and TEQ (toxicity equivalent) (pg.g^{-1} lipids) and p-value ($p < 0.05$). Significant differences for TEQ values are shown in bold.

Congener	Mean concentrations		TEF	TEQ		p-value
	LFK	*FCE*		*LFK*	*FCE*	
PCDDs						
2, 3, 7, 8 -TetraCDD	<0.84[a]	<0.84[a]	1	0.84	0.84	1.00
1, 2, 3, 7, 8-PentaCDD	<3.6[a]	<3.6[a]	1	3.6	3.6	1.00
1, 2, 3, 4, 7, 8-HexaCDD	<3.6[a]	<3.6[a]	0.1	0.36	0.36	1.00
1, 2, 3, 6, 7, 8-HexaCDD	<3.6[a]	<3.6[a]	0.1	0.36	0.36	1.00
1, 2, 3, 7, 8, 9-HexaCDD	<3.6[a]	<3.6[a]	0.1	0.36	0.36	1.00
1, 2, 3, 4, 6, 7, 8-HeptaCDD	21	17	0.01	0.21	0.17	0.522
OctaCDD (OCDD)	40	53	0.0003	0.012	0.016	0.522
PCDFs						
2, 3, 7, 8 -TetraCDF	4	4.28	0.1	0.4	0.43	0.411
1, 2, 3, 7, 8—PentaCDF	<3.6[a]	<3.6[a]	0.03	0.11	0.11	0.927
2, 3, 4, 7, 8-PentaCDF	<3.6[a]	<3.6[a]	0.3	1.08	1.08	0.927
1, 2, 3, 4, 7, 8-HexaCDF	2.45	<1.27[a]	0.1	0.25	0.13	0.715
1, 2, 3, 6, 7, 8-HexaCDF	<3.6[a]	<3.6[a]	0.1	0.36	0.36	0.927
1, 2, 3, 7, 8, 9-HexaCDF	<3.6[a]	<3.6[a]	0.1	0.36	0.36	0.927
2, 3, 4, 6, 7, 8 - HexaCDF	0.85	<0.95[a]	0.1	0.08	0.09	0.715
1, 2, 3, 4, 6, 7, 8-HeptaCDF	18	14	0.01	0.18	0.14	0.411
1, 2, 3, 4, 7, 8, 9-HeptaCDF	11	<3.6[a]	0.01	0.11	0.04	0.201
OctaCDF (OCDF)	7.67	<9.52[a]	0.0003	0.002	0.003	0.411
∑ PCDD/Fs	135	133		8.7	8.45	1.00
Non-ortho PCBs						
PCB 77	3426	1274	0.0001	0.34	0.13	0.714
PCB 81	251	243	0.0003	0.07	0.07	0.927
PCB 126	114	86	0.1	11.4	8.6	0.411
PCB 169	92	45	0.03	2.76	1.35	0.120
Mono-ortho PCBs						
PCB 105	106,751	24,903	0.00003	3.2	0.75	**0.008**
PCB 114	3685	1624	0.00003	0.11	0.049	0.400
PCB 118	486,414	110,061	0.00003	14.6	3.3	**0.008**
PCB 123	2668	230	0.00003	0.08	0.007	**0.007**
PCB 156	48,916	14,076	0.00003	1.47	0.42	**0.008**
PCB 157	11,519	5347	0.00003	0.34	0.16	0.055
PCB 167	56,668	16,512	0.00003	1.7	0.49	**0.008**
PCB 189	18,499	8632	0.00003	0.55	0.26	0.055
∑ DL-PCBs (non-ortho PCBs and mono-ortho PCBs)	739,001	183,035		37	16	**0.008**
∑ PCDD/Fs and DL-PCBs	**739,136**	**183,168**		**46**	**24.45**	**0.008**

[a] Data determined on the basis of LOQ.

and 13% of Σ PCDD/Fs and Σ DL-PCBs of LFK and FCE dolphins, respectively. The concentrations of CB 105, CB 118, CB 123, CB 156 and CB 167 were all significantly higher in LFK dolphins than in FCE ones (all comparisons, $p < 0.01$).

The contamination profiles of LFK and FCE dolphins were also analyzed according to the different congener proportions of PCDDs, PCDFs and DL-PCBs (calculated from the data in pg. g^{-1} lipids) grouped by chlorination degree. Neither PCDD concentrations ($p > 0.05$, Table 3, Fig. S2) nor PCDF concentrations ($p > 0.05$, Table 3, Fig. S3) were significanty different between dolphins from LFK and FCE. DL-PCBs concentrations were higher in LFK than in FCE dolphins ($p < 0.05$, Table 3, Fig. S4). Octa congeners were the greatest proportion of the PCDDs (Table 3, Fig. S2) and hepta congeners were the largest proportion of the PCDFs in both sample areas (Table 3, Fig. S3). Penta congeners made up the greatest proportion of the DL-PCBs (Table 3, Fig. S4).

3.9. T-Hg

T-Hg was detected at quantifiable levels (>LOQ) in all individuals (Table 4). Due to the fact that no sample of LFK females has been analyzed, we only conducted gender comparisons on FCE

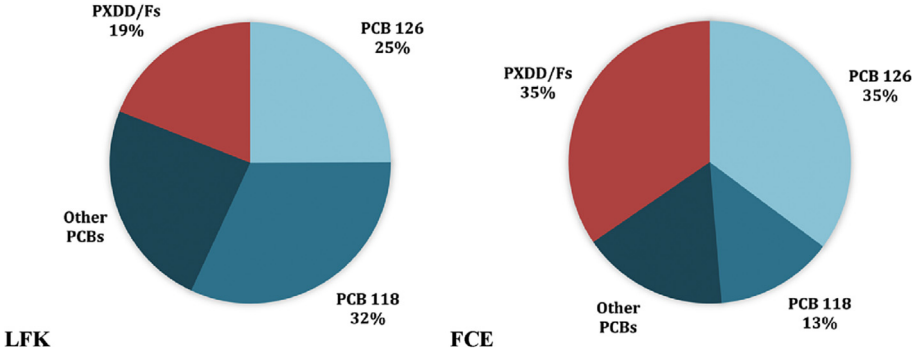

Fig. 5. Proportions of PCDD/Fs and DL-PCBs (TEQ) in bottlenose dolphins from the Lower Florida Keys (LKF) and the Florida coastal Everglades (FCE).

Table 4
Total mercury concentrations (T-Hg, ng g^{-1} dry weight) in skin of male bottlenose dolphins from the Lower Florida Keys and the Florida Coastal Everglades. Data are showed as mean (median) ± standard deviation (min-max) n = number of samples and p-value for the comparison between LFK and FCE dolphins (Mann-Whitney, $p < 0.05$).

	Lower Florida Keys			Florida Coastal Everglades		
	M	F	p-value	M	F	p-value
T-Hg	2936.0 (3634.8)± 2082.7 (293.9–5713.3) n = 9	na	n = 0	10,048.3 (9330.5) ± 6637.3 (2221–28760.8) n = 13	12,313.4 (8511.7) ± 8734.8 (4508.8–29124.6) n = 9	0.738

individuals (n = 13 males, n = 9 females; Table 1). However, no difference was detected (U = 53, $p > 0.05$). Regarding geographical differences, T-Hg concentrations were significantly higher in males from FCE than in males from LFK (U = 14, p = 0.003). We did not detect any spatial variation in concentrations within FCE (i.e. among Florida Bay (n = 5), Whitewater Bay (n = 12) and Joe River (n = 5), $p > 0.05$, Fig. S1).

4. Discussion

Lipid percentage, POP concentrations in blubber as well as T-Hg in skin differed strikingly between bottlenose dolphins from LFK and FCE. The reason for the blubber lipid percentage of males being more than twice higher in LFK (median 16.3%) than in FCE (median 5.8%) remains unclear. Dolphins from LFK were all sampled during summer (June–August) while most dolphins from FCE were sampled during winter (February–March). One would expect then a higher lipid percentage of dolphins sampled in winter because of the well described seasonnal blubber variation associated to water temperature (Kucklick et al., 2011; Samuel and Worthy, 2004). Other hypotheses to explain such discrepancy include nutritionnal status and prey preferences between the two locations whereas biases related to sampling cannot be totally excluded.

4.1. POPs

4.1.1. NDL-PCBs

PCBs constitute the POP class generally present at higher concentrations in marine mammals even though imports, manufacturing, and commissioning of new materials containing PCBs were banned in 1979 in the US (EPA, 1979). Biological effects, such immunosuppression and reproductive problems, can be observed at concentrations of 17,000 ng of PCBs per gram of lipids (Hall et al., 2006a,b; Jepson et al., 2005; Kannan et al., 2000). The health of bottlenose dolphins is impacted in populations with the highest POP exposure (max: 761,000 ng g^{-1} lipids) (Schwacke et al., 2012). Indeed, dolphins from Sapelo and Brunswick (Georgia), total PCBs were significantly correlated with circulating thyroid homones (free T4) and functional immune response (Schwacke et al., 2012). Most of bottlenose dolphins from LFK and FCE were below this threshold (Table 2). However, five males from LFK had concentrations higher than 17,000 ng g^{-1} lipids. One female from LFK also presented concentrations higher than this threshold and more than 10 times higher than the second most contaminated female from our sampling. Male bottlenose dolphins sampled in LFK displayed higher Σ 6 NDL-PCBs concentrations than males sampled in FCE. This is not surprising given the remote nature of FCE relative to the more urbanized Florida Keys where inputs of POPs into the environment have been higher (EPA, 1996; Finkl and Charlier, 2003). Σ 6 NDL-PCBs concentrations in female bottlenose dolphins did not differ between the two sampled regions, but those levels were significantly lower than concentrations observed in males. This gender-related difference is most likely related to maternal transfer of PCBs to offspring (Aguilar et al., 1999). Indeed, pregancy or lactation may cause excretion and redistribution of pollutants across various tissues of the female leading to POP transfer from the mother to the offspring (Habran et al., 2012, 2013; Honda et al., 1987; Leonel et al., 2012; Sager and Girard, 1994; Wagemann et al., 1988). The reason why a LFK female presented Σ 6 NDL-PCBs concentrations more than 10 times higher than the second most contaminated female, remains unclear. We can hypothesize that there was no transfer to the offspring or that this female came from another highly contaminated population. One interesting result was the higher concentrations in CB 28 and CB 52 in LFK females than in FCE females. These congeners are the less chlorinated molecules among the measured PCBs and consequently the less hydrophobic ones. Several studies showed a reduced efficiency in maternal transfer for the higher halogenated compounds (Dorneles et al., 2010; Ikonomou and Addison, 2008). FCE females could have had a significantly higher number of pregnancies during their lives than LFK females. Moreover, this geographical difference in CB 28 (the less hydrophobic among the PCBs measured) concentrations was verified for females, but not for males. Low levels of pollutants in female dolphins does not preclude the possibility of harmful effects on their health, or that of their calves. Levels of Σ 6 NDL-PCBs in LFK male dolphins were below the concentrations previously described in dolphins from other locations in Florida and in the Gulf of Mexico (Balmer et al., 2011, 2015; Fair et al., 2007; Johnson-Restrepo et al., 2005; Kucklick et al., 2011; Salata et al., 1995) (Fig. 6). The highest PCB concentrations have been described in bottlenose dolphins from Brunswick, GA where Aroclor 1268-a highly chlorinated PCB mixture-was released into the aquatic environment during decades of local industrial activities (Balmer et al., 2011; Kucklick et al., 2011; Wirth et al., 2014). In our study region, the Σ 6 NDL-PCBs concentrations were low compared to other locations in the southeastern US. Highly dynamic marine currents in South Florida may serve to dilute pollutants and help explain the low concentrations observed. In addition, the remediation efforts implemented in 2000 to protect the LFK and FCE environments may also contribute (Finkl and Charlier, 2003).

4.2. PBDEs

PBDEs concentrations of males were significantly higher in LFK than in FCE, but below the range of other dolphin populations along the southeastern US (Kucklick et al., 2011) (Table S1 and S2). Unfortunately, no critical threshold exists for PBDE concentrations.

4.3. Pesticides

In Florida, DDT was banned in 1972 (Fishel, 2013). However, p,p′-DDE is still the most commonly detected metabolite of DDXs (DDT and metabolites) found in bottlenose dolphin tissues. This may be explained by the fact that p,p′-DDE is the most accumulative molecule among DDXs (Fishel, 2013; McKinney et al., 2012). Our results confirm that ΣDDXs concentrations varied considerably with adjacent watersheds and land use (Adams et al., 2014). Aguilar (1984) suggested that a ratio p,p'-DDE/ΣDDXs greater than 0.6 suggests the absence of recent contamination by ΣDDXs because of the persistence of the p,p′-DDE in the environment (Aguilar, 1984).

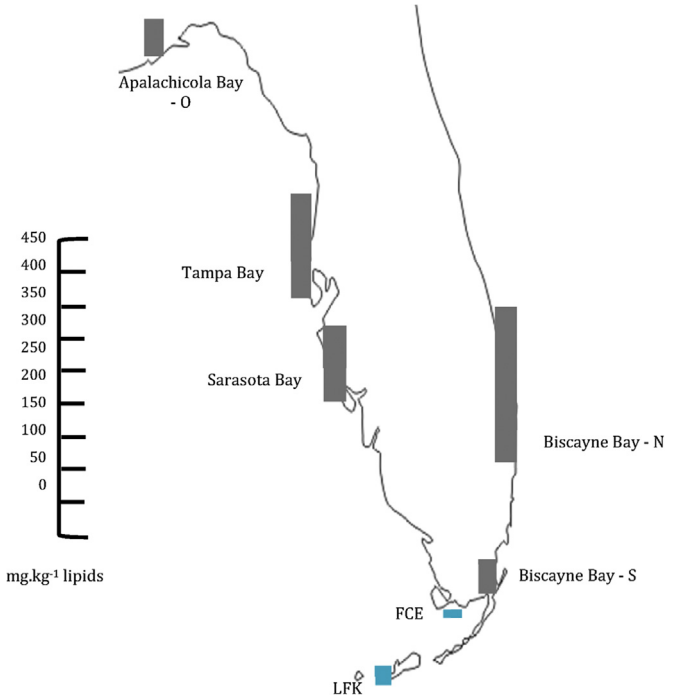

Fig. 6. Review of mean PCB concentrations (mg kg^{-1} lw) in blubber of bottlenose dolphins in the south-eastern US from the literature (Kucklick et al., 2011; Pulster et al., 2009; Adams et al., 2014; Balmer et al., 2015) and the present study (blue lines). Adapted from Kucklick et al., 2011. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The ratios in our study were 0.83 (LFK) and 0.85 (FCE), confirming the absence of recent DDT use in the area.

Low concentrations also were observed for ΣHCH (Fair et al., 2010; Hansen et al., 2004; Salata et al., 1995) and HCB (Fair et al., 2010; Kucklick et al., 2011) in dolphins from LFK, FCE, and other locations in the southeastern US (Table S1 and S2). The use of all isomers of HCH was banned in the US in 1978 (Fishel, 2013) (except for the γ-HCH), which likely explains the low concentrations observed. Lindane (γ-HCH) represented 64% and 79% of the ΣHCH contamination in LFK dolphins and in FCE dolphins, respectively. However, a recent study has shown that β-HCH was the isomer of higher importance in marine mammals from the Northern Hemisphere (Dorneles et al., 2015). The lidane importance of the present study could be related with the important veterinary, agricultural and pharmaceutical use of lindane in North America and in Mexico until the end of the 2000s, the volatile, persistent and bioaccumulative proprieties of lindane, and with the late ban. Actually, unlike other isomers of HCH, lindane was banned in the US in 2002 - explaining its prevalence relative to other HCH isomers (Fishel, 2013).

4.4. DL-PCBs and PCDD/Fs

The most important contribution for the toxicity equivalences of PCDD/Fs and DL-PCBs expressed as TEQ in LFK and FCE dolphins are mainly provided by DL-PCBs (81% LFK - 65% FCE), similar to Guiana dolphins (*Sotalia guianensis*) from Guanabara Bay Brazil where DL-PCBs represented 98.8% of the total TEQ (Dorneles et al., 2013). The past use of PCBs (NDL-PCBs and DL-PCBs) in industry drives the observed pattern, while PCDD/Fs are produced mostly released by combustion reactions. These compounds preferentially bind to poorly soluble water particles (WHO, 2010) which contributes to their low presence in the marine environment, including in bottlenose dolphins sampled for our study (Table S1 and S2).

4.5. T-Hg

T-Hg concentrations measured in skin sampled from FCE males were significantly higher than in male dolphins from LFK and other regions in Florida including Sarasota Bay and the Indian River Lagoon (Stavros et al., 2011; Woshner et al., 2008) (Fig. 7). To the best of our knowledge, these concentrations are the highest ever recorded in bottlenose dolphins in the world (Table S1 and S2). A national monitoring study (*Mussel Watch*) was conducted from 1990 to 1998 using mollusc species (*Chama sinuosa* and *Crassostrea virginica*) as bioindicators of water pollution from the US coasts. T-Hg concentrations in molluscs from LFK (*Chama sinuosa*) were below the critical threshold of 0.23 μg g^{-1} dry weight, above which the water is considered heavily contaminated (O'Connor, 2002). Unlike LFK, molluscs taken from Joe River (*Crassostrea virginica*) in FCE had concentrations above this limit (O'Connor, 2002). These high T-Hg concentrations can be explained by the presence of mangroves in FCE. Mangrove ecosystems have an important influence on the biogeochemical cycle of Hg (Bergamaschi et al., 2012; Silva et al., 2003). These ecosystems have a very high organic content, promoting the development of anaerobic bacteria in the sediment, enabling the methylation of mercury. Furthermore, mangrove mud is naturally acidic (pH 3–4), which facilitates mercury availability for anaerobic bacteria. Indeed, when elemental mercury (Hg^{2+}) binds to the DOC (dissolved organic carbon) it is rarely methylated because DOC molecules are too large to pass

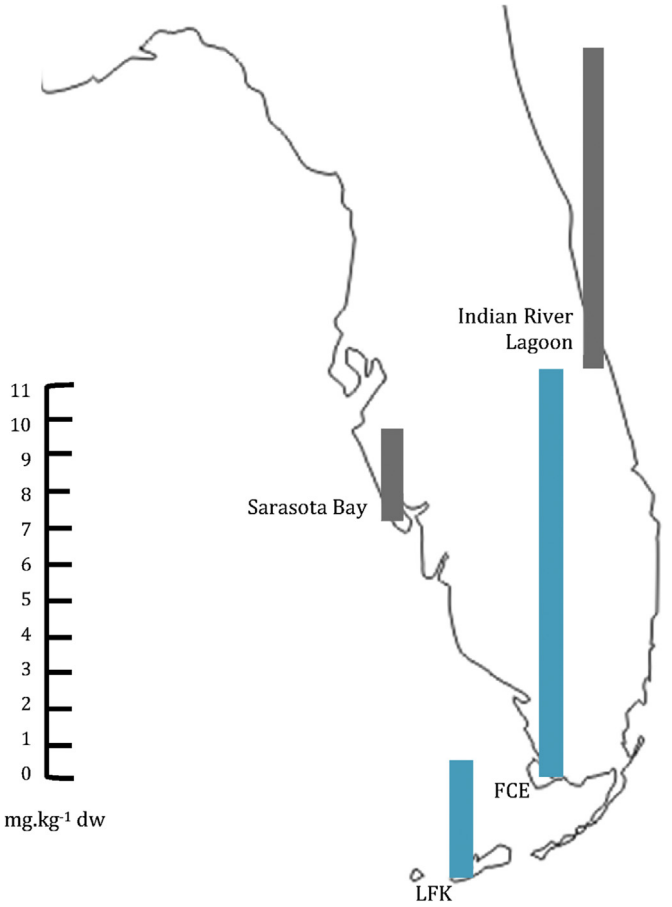

Fig. 7. Review of mean T-Hg concentrations (mg kg^{-1} dw) in skin of bottlenose dolphins from Florida from the literature (Woshner et al., 2008; Stavros et al., 2011) and the present study (blue lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

through the bacterial cell membranes. The acidic pH of the mangroves decreases the affinity of DOC for mercury and therefore allows Hg^{2+} to pass the bacterial cell membrane and to be methylated (Barkay et al., 1997; Bergamaschi et al., 2012; Miskimmin et al., 1992). Methylmercury is then released into the aquatic environment after contact with water sediments (Silva et al., 2003). It may then attach to the DOC that promotes solubility (Liu et al., 2008), transport by complexing to the DOM (dissolved organic material) and therefore be available in the water column (Ravichandran, 2004). Moreover, it has been shown that in addition to the DOC contributions, tidally driven export from mangroves represents a significant potential source of Hg and MeHg to nearby coastal waters of South Florida (Bergamaschi et al., 2012). MeHg is known to biomagnify along the food chain up top-predators including bottlenose dolphins (Evans and Crumley, 2005; Schaefer et al., 2014). These high T-Hg concentrations in dolphins suggest that fish from FCE have also higher T-Hg concentrations than fish from LFK (Schaefer et al., 2015). Further studies are needed to determine the predominant form of mercury in dolphin tissues from this region.

5. Conclusion

This study showed that LFK and FCE dolphins exhibit low concentrations of organic persistent pollutants compared to other populations. However, mercury does not follow the same trend as POPs, which raises concerns about its impact on the health of bottlenose dolphins from FCE. To the best of our knowledge, these concentrations are the highest ever recorded in bottlenose dolphins. Further studies are needed to determine the mercury concentrations at different trophic levels in this region. This would suggest that the high mercury concentrations constitute consequence of the proximity to mangrove ecosystems that increase mercury bioavailability.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.10.005.

References

Adams, J., Speakman, T., Zolman, E., Mitchum, G., Wirth, E., Bossart, G.D., Fair, P. a., 2014. The relationship between land use and emerging and legacy contaminants in an Apex predator, the bottlenose dolphin (*Tursiops truncatus*), from two adjacent estuarine watersheds. Environ. Res. 135, 346–353. http://dx.doi.org/10.1016/j.envres.2014.08.037.

Aguilar, A., 1984. Relationship of DDE/ΣDDT in marine mammals to the chronology of DDT input into the ecosystem. Can. J. Fish. Aquat. Sci. 41, 840–844.

Aguilar, A., Borrell, A., Pastor, T., 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. J. Cetacean Res. Manag. 83–116.

Balmer, B.C., Schwacke, L.H., Wells, R.S., George, R.C., Hoguet, J., Kucklick, J.R., Lane, S.M., Martinez, A., McLellan, W.A., Rosel, P.E., Rowles, T.K., Sparks, K., Speakman, T., Zolman, E.S., Pabst, D.A., 2011. Relationship between persistent organic pollutants (POPs) and ranging patterns in common bottlenose dolphins (*Tursiops truncatus*) from coastal Georgia, USA. Sci. Total Environ. 409, 2094–2101. http://dx.doi.org/10.1016/j.scitotenv.2011.01.052.

Balmer, B.C., Ylitalo, G.M., McGeorge, L.E., Baugh, K.L., Boyd, D., Mullin, K.D., Rosel, P.E., Sinclair, C., Wells, R.S., Zolman, E.S., Schwacke, L.H., 2015. Persistent organic pollutants (POPs) in blubber of common bottlenose dolphins (*Tursiops truncatus*) along the northern Gulf of Mexico coast, USA. Sci. Total Environ. 527–528, 306–312. http://dx.doi.org/10.1016/j.scitotenv.2015.05.016.

Barkay, T., Gillman, M., Turner, R.R., 1997. Effects of dissolved organic carbon and salinity on bioavailability of mercury. Appl. Environ. Microbiol. 63, 4267–4271.

Barros, N.B., Wells, R.S., 1998. Prey and feeding patterns of resident bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. J. Mammal. 79, 1045–1059.

Bergamaschi, B.A., Krabbenhoft, D.P., Aiken, G.R., Patino, E., Rumbold, D.G., Orem, W.H., 2012. Tidally driven export of dissolved organic carbon, total mercury, and methylmercury from a mangrove-dominated estuary. Environ. Sci. Technol. 46, 1371–1378.

Bossart, G., 2006. Marine mammals as sentinel species for oceans and human health. Oceanography 19, 134–137.

Bouquegneau, J.M., Joiris, C., 1988. The fate of stable pollutants–heavy metals and organochlorines in marine organisms. Adv. Comp. Environ. Physiol. 2, 219–247.

Bryan, C.E., Christopher, S.J., Balmer, B.C., Wells, R.S., 2007. Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: implications for non-invasive monitoring. Sci. Total Environ. 388, 325–342.

Childers, D., 2006. A synthesis of long-term research by the Florida Coastal Everglades LTER Program. Hydrobiologia 569, 531–544.

Debier, C., Pomeroy, P.P., Dupont, C., Joiris, C., Comblin, V., Le Boulengé, E., Larondelle, Y., Thomé, J.P., 2003. Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*. Mar. Ecol. Prog. Ser. 247, 237–248. http://dx.doi.org/10.3354/meps247249.

Dorneles, P.R., Lailson-Brito, J., Bisi, T.L., Domit, C., Barbosa, L.A., Meirelles, A.C.O., Carvalho, V.L., Malm, O., Azevedo, A.F., Brose, F., Das, K., Scippo, M.-L., 2016. Guiana dolphins (*Sotalia guianensis*) and DR-CALUX for screening coastal Brazilian environments for dioxins and related compounds. Arch. Environ. Contam. Toxicol. http://dx.doi.org/10.1007/s00244-016-0299-z.

Dorneles, P.R., Lailson-Brito, J., Dirtu, A.C., Weijs, L., Azevedo, A.F., Torres, J.P.M., Malm, O., Neels, H., Blust, R., Das, K., Covaci, A., 2010. Anthropogenic and naturally-produced organobrominated compounds in marine mammals from Brazil. Environ. Int. 1, 36–60. http://dx.doi.org/10.1016/j.envint.2009.10.001.

Dorneles, P.R., Lailson-Brito, J., Secchi, E.R., Dirtu, A.C., Weijs, L., Dalla Rosa, L., Bassoi, M., Cunha, H.A., Azevedo, A.F., Covaci, A., 2015. Levels and profiles of chlorinated and brominated contaminants in Southern Hemisphere humpback whales, *Megaptera novaeangliae*. Environ. Res. 138, 49–57. http://dx.doi.org/10.1016/j.envres.2015.02.007.

Dorneles, P.R., Sanz, P., Eppe, G., Azevedo, A.F., Bertozzi, C.P., Martínez, M.A., Secchi, E.R., Barbosa, L.A., Cremer,.M, Alonso, M.B., Torres, J.P.M., Lailson-Brito, J., Malm, O., Eljarrat, E., Barceló, D., Das, K., 2013. High accumulation of PCDD, PCDF, and PCB congeners in marine mammals from Brazil: a serious PCB problem. Sci. Total Environ. 463–464, 309–318.

Dyc, C., Covaci, A., Debier, C., Leroy, C., Delcroix, E., Thomé, J.P., Das, K., 2015. Pollutant exposure in green and hawksbill marine turtles from the Caribbean region. Reg. Stud. Mar. Sci. 2, 158–170. http://dx.doi.org/10.1007/s00244-003-2110-1.

EPA, U.S.E.P.A, 1996. Water quality protection program for the Florida Keys national marine sanctuary. First Bienn. Rep. Congr.

EPA, U.S.E.P.A, 1979. Polychlorinated biphenyls (PCBs): manufacturing, processing, distribution in commerce, and use prohibitions. Fed. Regist. 44.

European Food Safety Authority (EFSA), 2010. Results of the monitoring of non dioxin-like PCBs in food and feed. EFSA J. 8 (7), 35. http://dx.doi.org/10.2903/j.efsa.2010.1701.

Evans, D.W., Crumley, P.H., 2005. Mercury in Florida Bay fish: spatial distribution of elevated concentrations and possible linkages to Everglades restoration. Bull. Mar. Sci. 77, 321–345.

Fair, P.A., Adams, J., Mitchum, G., Hulsey, T.C., Reif, J.S., Houde, M., Muir, D., Wirth, E., Wetzel, D., Zolman, E., McFee, W., Bossart, G.D., 2010. Contaminant blubber burdens in Atlantic bottlenose dolphins (*Tursiops truncatus*) from two southeastern US estuarine areas: concentrations and patterns of PCBs, pesticides, PBDEs, PFCs, and PAHs. Sci. Total Environ. 408, 1577–1597.

Fair, P.A., Mitchum, G., Hulsey, T.C., Adams, J., Zolman, E., McFee, W., Wirth, E., Bossart, G.D., 2007. Polybrominated diphenyl ethers (PBDEs) in blubber of free-ranging bottlenose dolphins (*Tursiops truncatus*) from two southeast Atlantic estuarine areas. Arch. Environ. Contam. Toxicol. 53, 483–494.

Finkl, C.W., Charlier, R.H., 2003. Sustainability of subtropical coastal zones in southeastern Florida: challenges for urbanized coastal environments threatened by development, pollution, water supply, and storm hazards. J. Coast. Res. 19, 934–943.

Fishel, F.M., 2013. Pesticides and Prior Informed Consent. Univ. Florida IFAS Ext, pp. 1–5.

Focant, J.F.J.-F., Eppe, G., Pirard, C., De Pauw, E., 2001. Fast clean-up for

polychlorinated dibenzo-p-dioxins, dibenzofurans and coplanar polychlorinated biphenyls analysis of high-fat-content biological samples. J. Chromatogr. A 925, 207–221. http://dx.doi.org/10.1016/S0021-9673(01)01047-0.

García-Álvarez, N., Boada, L.D., Fernández, A., Zumbado, M., Arbelo, M., Sierra, E., Xuriach, A., Almunia, J., Camacho, M., Luzardo, O.P., 2014. Assessment of the levels of polycyclic aromatic hydrocarbons and organochlorine contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean. Mar. Environ. Res. 100, 48–56. http://dx.doi.org/10.1016/j.marenvres.2014.03.010.

Habran, S., Crocker, D.E., Debier, C., Das, K., 2012. How are trace elements mobilized during the postweaning fast in Northern elephant seals? Environ. Toxicol. Chem. 31, 2354–2365.

Habran, S., Pomeroy, P.P., Debier, C., Das, K., 2013. Changes in trace elements during lactation in a marine top predator, the grey seal. Aquat. Toxicol. 126, 455–466. http://dx.doi.org/10.1016/j.aquatox.2012.08.011.

Hall, A.J., Hugunin, K., Deaville, R., Law, R.J., Allchin, C.R., Jepson, P.D., 2006. The risk of infection from polychlorinated biphenyl exposure in the harbor porpoise (*Phocoena phocoena*): a case-control approach. Environ. Health Perspect. 114, 704–711.

Hall, A.J., McConnell, B.J., Rowles, T.K., Aguilar, A., Borrell, A., Schwacke, L., Reijnders, P.J.H., Wells, R.S., 2006. Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. Environ. Health Perspect. 114, 60–64.

Hansen, L.J., Schwacke, L.H., Mitchum, G.B., Hohn, A.A., Wells, R.S., Zolman, E.S., Fair, P.A., 2004. Geographic variation in polychorinated biphenyl and organochlorine pesticide concentrations in the blubber of bottlenose dolphins from the US Atlantic coast. Sci. Total Environ. 319, 147–172.

Honda, K., Yamamoto, Y., Kato, H., Tatsukawa, R., Tatsukawa, R., 1987. Heavy metal accumulations and their recent changes in southern minke whales *Balaenoptera acuturostrata*. Arch. Environ. Contam. Toxicol. 16, 209–216.

Ikonomou, M.G., Addison, R.F., 2008. Polybrominated diphenyl ethers (PBDEs) in seal populations from eastern and western Canada: an assessment of the processes and factors controlling PBDE distribution in seals. Mar. Environ. Res. 66, 225–230. http://dx.doi.org/10.1016/j.marenvres.2008.02.004.

Jepson, P.D., Bennett, P.M., Deaville, R., Allchin, C.R., Baker, J.R., Law, R.J., 2005. Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. Environ. Toxicol. Chem. 24, 238–248.

Johnson-Restrepo, B., Kannan, K., Addink, R., Adams, D.H., 2005. Polybrominated diphenyl ethers and polychlorinated biphenyls in a marine foodweb of coastal Florida. Environ. Sci. Technol. 39, 8243–8250.

Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Hum. Ecol. Risk Assess. 6, 181–201.

Kucklick, J., Schwacke, L., Wells, R., Hohn, A., Guichard, A., Yordy, J., Hansens, L., Zolman, E., Wilson, R., Litz, J., Nowacek, D., Rowles, T., Pugh, R., Balmer, B., Sinclair, C., Rosel, P., 2011. Bottlenose dolphins as indicators of persistent organic pollutants in the western North atlantic ocean and northern Gulf of Mexico. Environ. Sci. Technol. 45, 4270–4277.

Leonel, J., Taniguchi, S., Sasaki, D.K., Cascaes, M.J., Dias, P.S., Botta, S., Marcos, M.C., Montone, R.C., 2012. Contamination by chlorinated pesticides, PCBs and PBDEs in Atlantic spotted dolphin (*Stenella frontalis*) in western South Atlantic. Chemosphere 86, 741–746. http://dx.doi.org/10.1016/j.chemosphere.2011.10.049.

Lewis, J.S., Wartzok, D., Heithaus, M., Krützen, M., 2013. Could relatedness help explain why individuals lead in bottlenose dolphin groups? PLoS One 8. http://dx.doi.org/10.1371/journal.pone.0058162.

Lewis, J.S., Wartzok, D., Heithaus, M.R., 2011. Highly dynamic fission-fusion species can exhibit leadership when traveling. Behav. Ecol. Sociobiol. 65, 1061–1069.

Litz, J.A., Garrison, L.P., Fieber, L.A., Martinez, A., Contillo, J.P., Kucklick, J.R., 2007. Fine-scale spatial variation of persistent organic pollutants in bottlenose dolphins (*Tursiops truncatus*) in Biscayne Bay, Florida. Environ. Sci. Technol. 41, 7222–7228.

Liu, G., Cai, Y., Philippi, T., Kalla, P., Scheidt, D., Richards, J., Scinto, L., Appleby, C., 2008. Distribution of total and methylmercury in different ecosystem compartments in the Everglades: implications for mercury bioaccumulation. Environ. Pollut. 153, 257–265. http://dx.doi.org/10.1016/j.envpol.2007.08.030.

Mazzoil, M., Reif, J., Youngbluth, M., Murdoch, M., Bechdel, S., Howells, E., McCulloch, S., Hansen, L., Bossart, G., 2008. Home ranges of bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida: environmental correlates and implications for management strategies. Ecohealth 5, 278–288.

McKinney, M.L., Schoch, R.M., Yonavjak, L., 2012. In: Jones, Ba (Ed.), Environmental Science: Systems and Solutions.

Miller, D.L., Woshner, V., Styer, E.L., Ferguson, S., Knott, K.K., Gray, M.J., Wells, R.S., O'Hara, T.M., 2011. Histologic findings in free-ranging Sarasota bay bottlenose dolphin (*Tursiops truncatus*) skin: mercury, Selenium, and seasonal factors. J. Wildl. Dis. 47, 1012–1018.

Miskimmin, B.M., Rudd, J.W.M., Kelly, C.A., 1992. Influence of dissolved organic carbon, pH, and microbial respiration rates on mercury methylation and demethylation in lake water. J. Can. des Sci. halieutiques Aquat 49, 17–22.

O'Connor, T.P., 2002. National distribution of chemical concentrations in mussels and oysters in the USA. Mar. Environ. Res. 53, 117–143.

O'Shea, T.J., Tanabe, S., 2003. Persistent ocean contaminants and marine mammals: a retrospective overview. In: Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J. (Eds.), Toxicology of Marine Mammals. Taylor and Francis, London, New-York, pp. 99–134.

Pinzone, M., Budzinski, H., Tasciotti, A., Ody, D., Lepoint, G., Schnitzler, J., Scholl, G., Thomé, J.P., Tapie, N., Eppe, G., Das, K., 2015. POPs in free-ranging pilot whales, sperm whales and fin whales from the Mediterranean Sea: influence of biological and ecological factors. Environ. Res. 142, 185–196. http://dx.doi.org/10.1016/j.envres.2015.06.021.

Ravichandran, M., 2004. Interactions between mercury and dissolved organic matter–A review. Chemosphere 55, 319–331.

589/2014, 2014. Commission Regulation (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2012 Text with EEA relevance. Off. J. Eur. Union 164, 18–40.

Reif, J., Schaefer, A., Bossart, G., 2015. Atlantic bottlenose dolphins (*Tursiops truncatus*) as A Sentinel for exposure to mercury in humans: closing the loop. Vet. Sci. 2, 407–422. http://dx.doi.org/10.3390/vetsci2040407.

Rice, D.W., 1998. Marine mammals of the world. Systematic and distribution. Spec. Publ. Soc. Mar. Mammal. 1–231.

Rosel, P.E., 2003. PCR-based sex determination in Odontocete cetaceans. Conserv. Genet. 4, 647–649. http://dx.doi.org/10.1023/A:1025666212967.

Sager, D.B., Girard, D.M., 1994. Long-term effects on reproductive parameters in female rats after translactational exposure to PCBs. Environ. Res. 66, 52–76.

Salata, G.G., Wade, T.L., Sericano, J.L., Davis, J.W., Brooks, J.M., 1995. Analysis of Gulf of Mexico bottlenose dolphins for organochlorine pesticides and PCBs. Environ. Pollut. 88, 167–175.

Samuel, A.M., Worthy, G. a., 2004. Blubber as a function of body site, season, and reproductive state. Can. J. Zool. 82, 1933–1942. http://dx.doi.org/10.1139/z05-001.

SANCO, 2014. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANCO/12571/2013 Supersedes SANCO/12495/2011. Off. J. Eur. Communities 2002, 8–36, 48.

Schaefer, A.M., Jensen, E.L., Bossart, G.D., Reif, J.S., 2014. Hair mercury concentrations and fish consumption patterns in Florida residents. Int. J. Environ. Res. Public Health 11, 6709–6726. http://dx.doi.org/10.3390/ijerph110706709.

Schaefer, A.M., Stavros, H.-C.W.C.W., Bossart, G.D., Fair, P. a, Goldstein, J.D., Reif, J.S., 2011. Associations between mercury and hepatic, renal, endocrine, and hematological parameters in atlantic bottlenose dolphins (*Tursiops truncatus*) along the eastern coast of Florida and South Carolina. Arch. Environ. Contam. Toxicol. 61, 688–695. http://dx.doi.org/10.1007/s00244-011-9651-5.

Schaefer, A.M., Titcomb, E.M., Fair, P.A., Stavros, H.-C.W., Mazzoil, M., Bossart, G.D., Reif, J.S., 2015. Mercury concentrations in Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting the Indian River Lagoon, Florida: patterns of spatial and temporal distribution. Mar. Pollut. Bull. 97, 544–547. http://dx.doi.org/10.1016/j.marpolbul.2015.05.007.

Schwacke, L.H., Voit, E.O., Hansen, L.J., Wells, R.S., Mitchum, G.B., Hohn, A.A., Fair, P.A., 2002. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the southeast United States coast. Environ. Toxicol. Chem. 21, 2752–2764.

Schwacke, L.H., Zolman, E.S., Balmer, B.C., De Guise, S., George, R.C., Hoguet, J., Hohn, A.A., Kucklick, J.R., Lamb, S., Levin, M., Litz, J.A., McFee, W.E., Place, N.J., Townsend, F.I., Wells, R.S., Rowles, T.K., 2012. Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (*Tursiops truncatus*). Proc. Biol. Sci. 279, 48–57. http://dx.doi.org/10.1098/rspb.2011.0665.

Seixas, T.G.T.G., Kehrig, H.D. a, Costa, M., Fillmann, G., Di Beneditto, A.P.M., Secchi, E.R., Souza, C.M.M., Malm, O., Moreira, I., 2008. Total mercury, organic mercury and selenium in liver and kidney of a South American coastal dolphin. Environ. Pollut. 154, 98–106. http://dx.doi.org/10.1016/j.envpol.2008.01.030.

Shapiro, S.S., Wilk, M.B., Chen, H.J., 1968. A comparative study of various tests of normality. J. Am. Stat. Assoc. 1343–1372.

Silva, L.F.F., Machado, W., Lisboa Filho, S.D., Lacerda, L.D., 2003. Mercury accumulation in sediments of a mangrove ecosystem in SE Brazil. Water. Air. Soil Pollut. 145, 67–77.

Stavros, H.C.W., Bossart, G.D., Hulsey, T.C., Fair, P.A., 2008. Trace element concentrations in blood of free-ranging bottlenose dolphins (*Tursiops truncatus*): influence of age, sex and location. Mar. Pollut. Bull. 56, 371–379.

Stavros, H.C.W., Bossart, G.D., Hulsey, T.C., Fair, P.A., 2007. Trace element concentrations in skin of free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeast Atlantic coast. Sci. Total Environ. 388, 300–315.

Stavros, H.C.W., Stolen, M., Durden, W.N., McFee, W., Bossart, G.D., Fair, P.A., 2011. Correlation and toxicological inference of trace elements in tissues from stranded and free-ranging bottlenose dolphins (*Tursiops truncatus*). Chemosphere 82, 1649–1661.

Thron, K.U., Bruhn, R., Mclachlan, M., 2004. The influence of age, sex, body-condition, and region on the levels of PBDEs and toxaphene in harbour porpoises from European waters. Fresenius Environ. Bull. 13, 146–155.

Urian, K.W., Hofmann, S., Wells, R.S., Read, A.J., 2009. Fine-scale population structure of bottlenose dolphins (*Tursiops truncatus*) in Tampa Bay, Florida. Mar. Mammal. Sci. 25, 619–638.

Van Den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., Van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Wærn, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

Van Den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M.,

Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241. http://dx.doi.org/10.1093/toxsci/kfl055.

Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J., 2003. In: Taylor, F. (Ed.), Toxicology of Marine Mammals.

Wagemann, R., Stewart, R.E.A., Lockhart, W.L., Stewart, B.E., Poveldo, M., 1988. Trace metals and methylmercury: associations and transfer in harp seals (*Phoca groenlandica*) mothers and their pups. Mar. Mamm. Sci. 4, 339–355.

Wells, R.S., Scott, M.D., 2009. Common bottlenose dolphin *Tursiops truncatus*. In: Perrin, W., Wursig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals, p. 1352.

Whitney, D.R., 1951. A bivariate extension of the U statistic. Ann. Math. Stat. 274–282.

WHO, 2010. Exposure to dioxins and dioxin-like substances: a major public health concern. Prev. Dis. through Heal. Environ.

Wirth, E.F., Pennington, P.L., Cooksey, C., Schwacke, L., Balthis, L., Hyland, J., Fulton, M.H., 2014. Distribution and sources of PCBs (Aroclor 1268) in the Sapelo island national estuarine research reserve. Environ. Monit. Assess. 186, 8717–8726. http://dx.doi.org/10.1007/s10661-014-4039-4.

Woshner, V., Knott, K., Wells, R., Willetto, C., Swor, R., O'Hara, T., 2008. Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus*) from sarasota bay, FL: interaction and relevance to life history and hematologic parameters. Ecohealth 5, 360–370.

Yordy, J.E., Pabst, D.A., McLellan, W.A., Wells, R.S., Rowles, T.K., Kucklick, J.R., 2010. Tissue-specific distribution and whole-body burden estimates of persistent organic pollutants in the bottlenose dolphin (*Tursiops truncatus*). Environ. Toxicol. Chem. 29, 1263–1273.