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: 13421 ± 7730 $ng.g^{-1}$ lipids, Σ 6 PCBs LFK females: 9683 ± 19007 $ng.g^{-1}$ 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 bottlenose dolphins concentrations are the highest recorded 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.

Capsule:

We observed higher POP levels in dolphins from the Lower Florida Keys (LFK) than from Florida Coastal Everglades (FCE) and an opposite pattern for T-Hg levels (higher in FCE).

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, 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, 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; Poly-ChlorinatedBiphenyls, 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.

MATERIALS AND METHODS

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 (**Figure 1**).

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) (**Figure 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.

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 minutes to remove any impurity of mercury). T-Hg concentrations were determined by atomic absorption spectroscopy (AAS, Direct Mercury Analyzer DMA-80, 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 (1g $Hg.l^{-1}$) 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., 2013, 2012) (**Table 1**).

Persistent Organic Pollutants (POPs).

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 ɣ-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. 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 (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 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 x 0.25 mm (0.25 µm film) DB5ms 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 to 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 70eV 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 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**).

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., 2006; O'Shea et al., 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^{TM}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., 2006, 1998) to calculate a toxic equivalent quantity (TEQ) which is related to the most toxic compound 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD).

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.

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 arose 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 minutes at 94°C was followed by 34 cycles of 45 seconds at 94°C, 45 seconds at 60°C and 60 seconds at 72°C and then final extension for 10 seconds at 72°C. We compared results to controls for a known male and female using gel electrophoresis (1.5% agarose) (Lewis et al., 2013).

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).

RESULTS

In 2008 and 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) (**Figure S1 in supporting information SI**). 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 sub-population. 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)**.

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**).

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**).

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**).

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, $p < 0.05$).

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 (**Figure 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, **Figure 2**).

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 (**Figure 3**).

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 (**Figure 4**). Concentration in β-HCH and HCB are based on the LOD (**Table 2**, **Figure 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 (**Figure 4**).

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, Figure 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, Figure 5**). CB 118 represented

32% 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, Figure S2**) nor PCDF concentrations ($p > 0.05$, **Table 3, Figure 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, Figure S4**). Octa congeners were the greatest proportion of the PCDDs (**Table 3, Figure S2**) and hepta congeners were the largest proportion of the PCDFs in both sample areas (**Table 3, Figure S3**). Penta congeners made up the greatest proportion of the DL-PCBs (**Table 3, Figure S4**).

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 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), $p > 0.05$, **Figure S1**).

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.

POPs. *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., 2006; 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., 2013, 2012; 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 she 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 significant 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., 2015, 2011; Fair et al., 2007; Johnson-Restrepo et al., 2005; Kucklick et al., 2011; Salata et al., 1995) (**Figure 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).

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) (**Tables S1** and **S2**). Unfortunately, no critical threshold exists for PBDE concentrations.

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). 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 (**Tables 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 used of lindane in North America and in Mexico until the end of the 2000s, the volatile, persitent 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).

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 (**Tables S1** and **S2**).

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) (**Figure 7**). To the best of our knowledge, these concentrations are the highest ever recorded in bottlenose dolphins in the world (**Tables 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 a 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 the 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 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.

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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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 (2 sex missing)	11	8	20 (1 sex missing)
PCDD/Fs and **DL-PCBs**	6	0	6	3	2	5
T-Hg	10	0	10	13	9	24 (2 sex missing)

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[1]	13420.5 (11934.3) ±7730 (6044.2-36685.9) n=16	9683.4 (1996.5) ±19006.6 (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[2]	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[3]	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[4]	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[5]	<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

[1] ∑ 6 NDL-PCBs : CB 28, CB 52, CB 101, CB 138, CB 153, CB 180

[2] ∑ PBDEs : BDE 47, BDE 99

[3] ∑ DDXs : o,p'-DDT, p,p'-DDE, p,p'-DDD

[4] ∑ 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

[5] Data determined on the basis of LOQ

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*	<0.84*	1	0.84	0.84	1.00
1, 2, 3, 7, 8 – PentaCDD	<3.6*	<3.6*	1	3.6	3.6	1.00
1, 2, 3, 4, 7, 8 – HexaCDD	<3.6*	<3.6*	0.1	0.36	0.36	1.00
1, 2, 3, 6, 7, 8 – HexaCDD	<3.6*	<3.6*	0.1	0.36	0.36	1.00
1, 2, 3, 7, 8, 9 - HexaCDD	<3.6*	<3.6*	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*	<3.6*	0.03	0.11	0.11	0.927
2, 3, 4, 7, 8 - PentaCDF	<3.6*	<3.6*	0.3	1.08	1.08	0.927
1, 2, 3, 4, 7, 8 - HexaCDF	2.45	<1.27*	0.1	0.25	0.13	0.715
1, 2, 3, 6, 7, 8 – HexaCDF	<3.6*	<3.6*	0.1	0.36	0.36	0.927
1, 2, 3, 7, 8, 9 - HexaCDF	<3.6*	<3.6*	0.1	0.36	0.36	0.927
2, 3, 4, 6, 7, 8 - HexaCDF	0.85	<0.95*	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*	0.01	0.11	0.04	0.201
OctaCDF (OCDF)	7.67	<9.52*	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	106751	24903	0.00003	3.2	0.75	**0.008**
PCB 114	3685	1624	0.00003	0.11	0.049	0.400
PCB 118	486414	110061	0.00003	14.6	3.3	**0.008**
PCB 123	2668	230	0.00003	0.08	0.007	**0.007**
PCB 156	48916	14076	0.00003	1.47	0.42	**0.008**
PCB 157	11519	5347	0.00003	0.34	0.16	0.055
PCB 167	56668	16512	0.00003	1.7	0.49	**0.008**
PCB 189	18499	8632	0.00003	0.55	0.26	0.055
∑ DL-PCBs (non-ortho PCBs and mono-ortho PCBs)	739001	183035		37	16	**0.008**
∑ PCDD/Fs and DL-PCBs	**739136**	**183168**		**46**	**24.45**	**0.008**

*Data determined on the basis of LOQ

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	n=0	na	10048.3 (9330.5) ± 6637.3 (2221-28760.8) n=13	12313.4 (8511.7) ± 8734.8 (4508.8-29124.6) n=9	0.738

FIGURES : Color for online version only

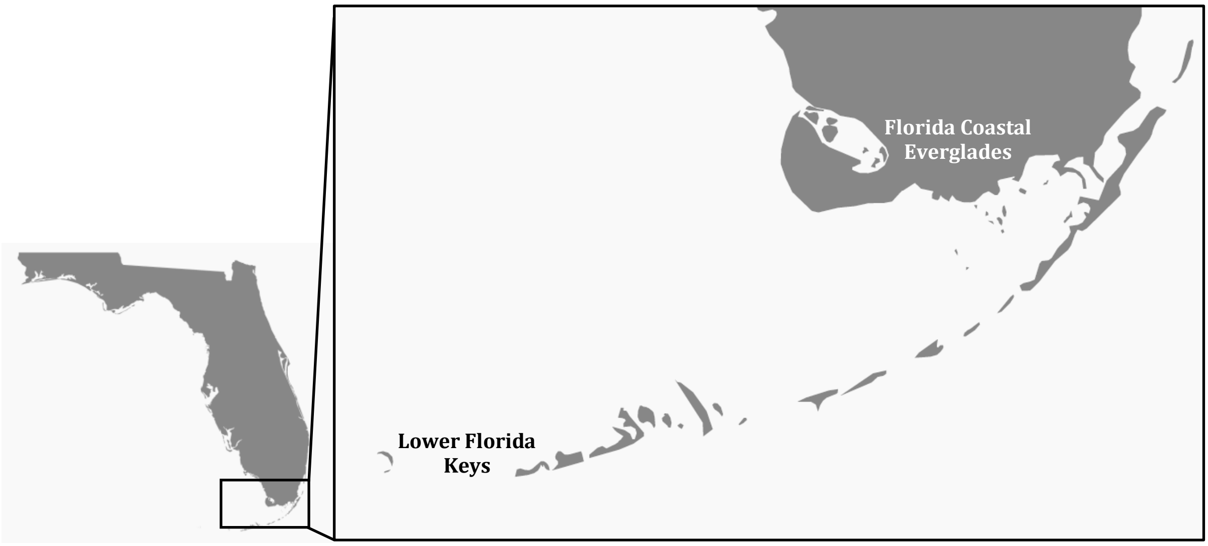
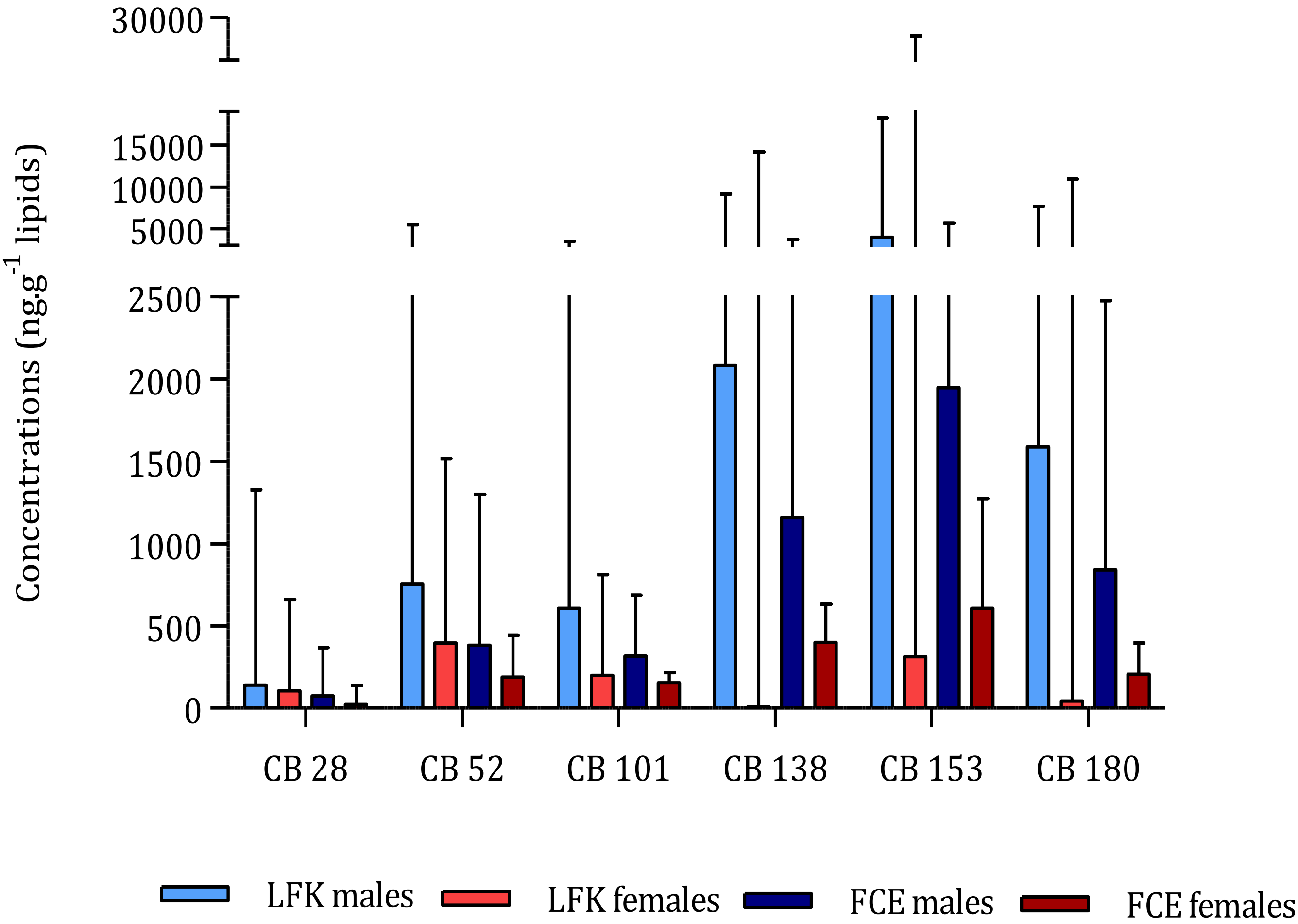


Fig. 1 – The study was conducted in the Lower Florida Keys (Key West coastal waters) and in the Florida Coastal Everglades (southwest of the Everglades National Park southwest area) https://freevectormaps.com.



764
765
766 Fig. 2 – Median and maximum concentrations of CB28, 52, 101, 153, 138 and 180 (ng.g^{-1} lipids) in bottlenose
767 dolphins from the Lower Florida Keys (LFK) and the Florida coastal Everglades (FCE).

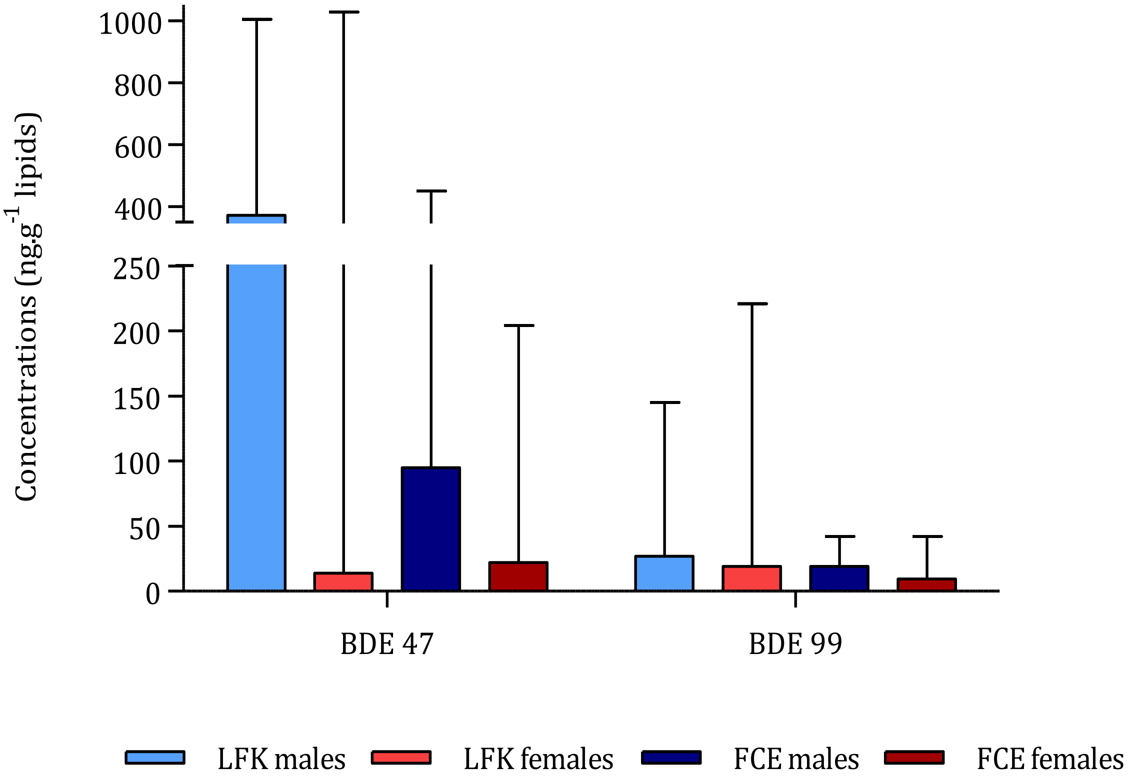


Fig. 3 – Median and maximum concentrations of BDE 47 and BDE 99 (ng.g^{-1} lipids) in bottlenose dolphins from the Lower Florida Keys (LFK) and the Florida coastal Everglades (FCE).

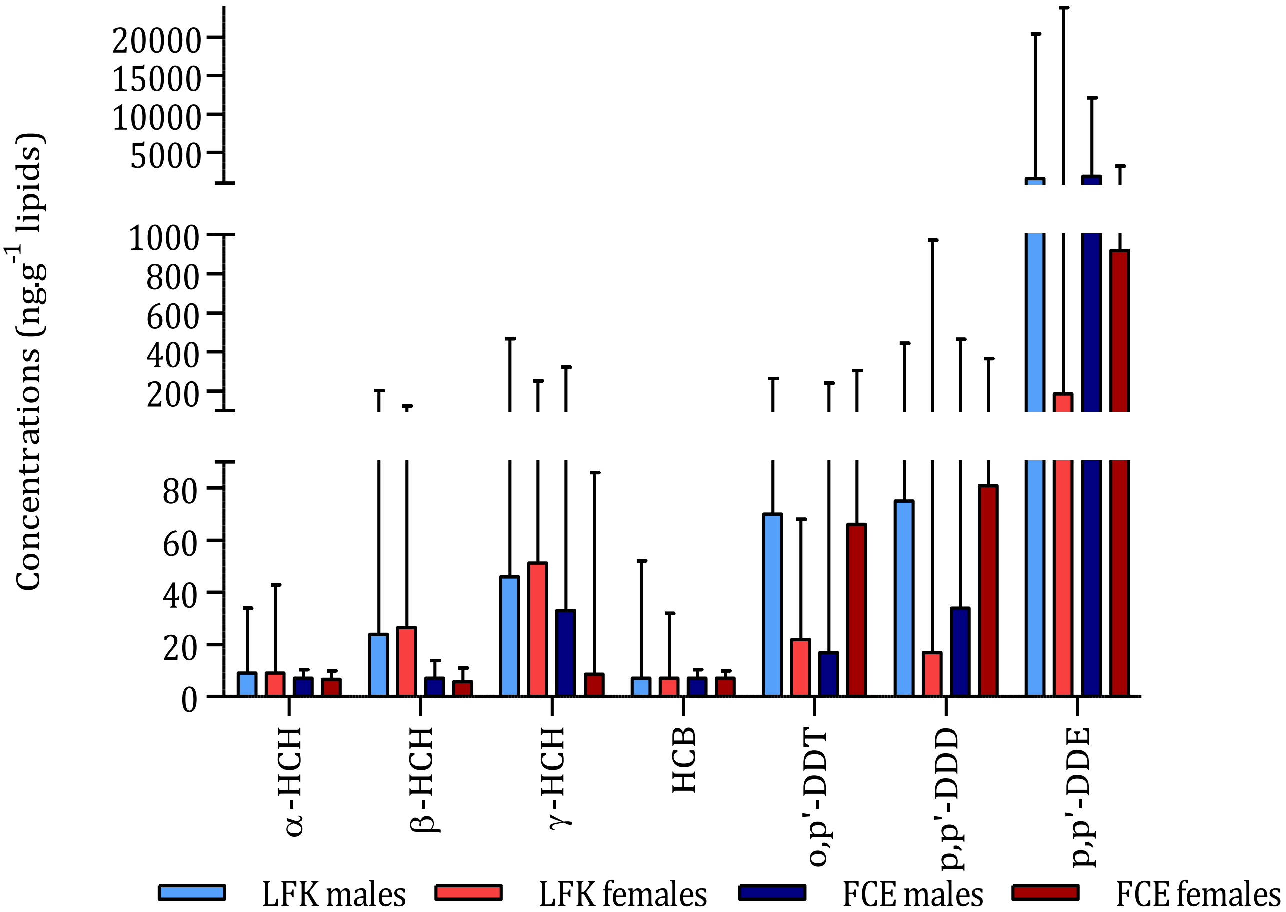


Fig. 4 – Median and maximum concentrations of α-HCH, β-HCH, γ-HCH, HCB, o,p'-DDT, p,p'-DDD and p,p'-DDE (ng.g^{-1} lipids) in bottlenose dolphins from the Lower Florida Keys (LKF) and the Florida coastal Everglades (FCE).

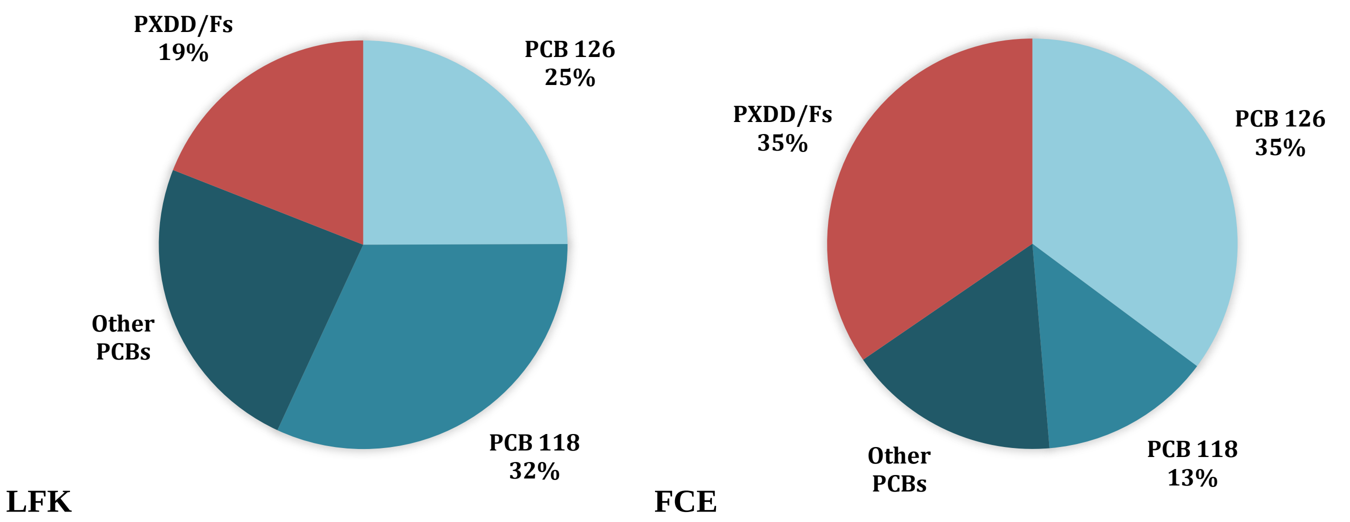


Fig. 5 - Proportions of PCDD/Fs and DL-PCBs (TEQ) in bottlenose dolphins from the Lower Florida Keys (LKF) and the Florida coastal Everglades (FCE).

779

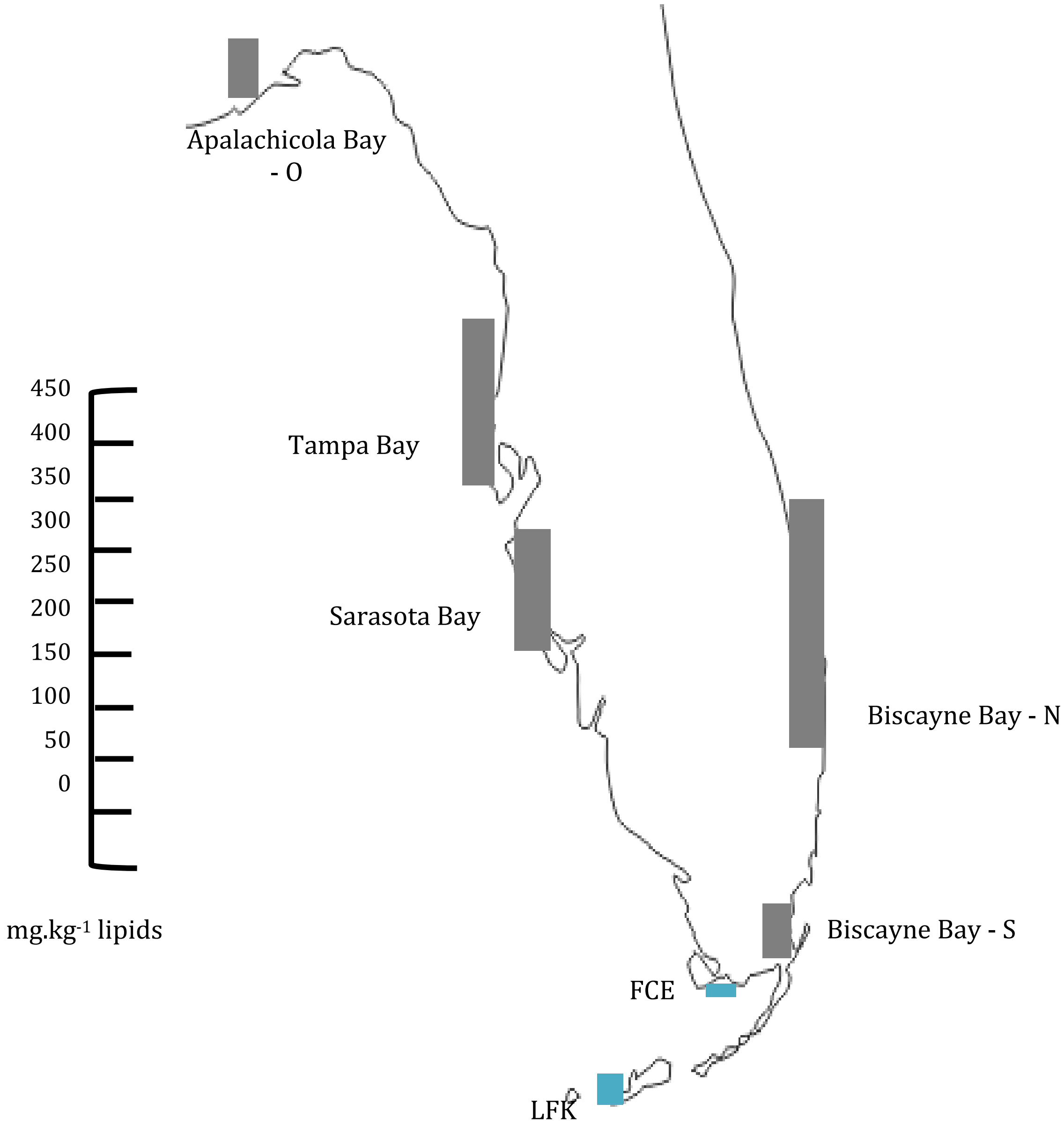


Fig. 6 – Review of mean PCB concentrations ($mg.kg^{-1}$ lw) in blubber of bottlenose dolphins in the south-eastern US from the literature (Kucklick et al. 2011, Pulster et al. 2009, Adams et al. 2014, Balmer et al. 2015) and the present study (blue lines). Adapted from Kucklick et al. 2011.

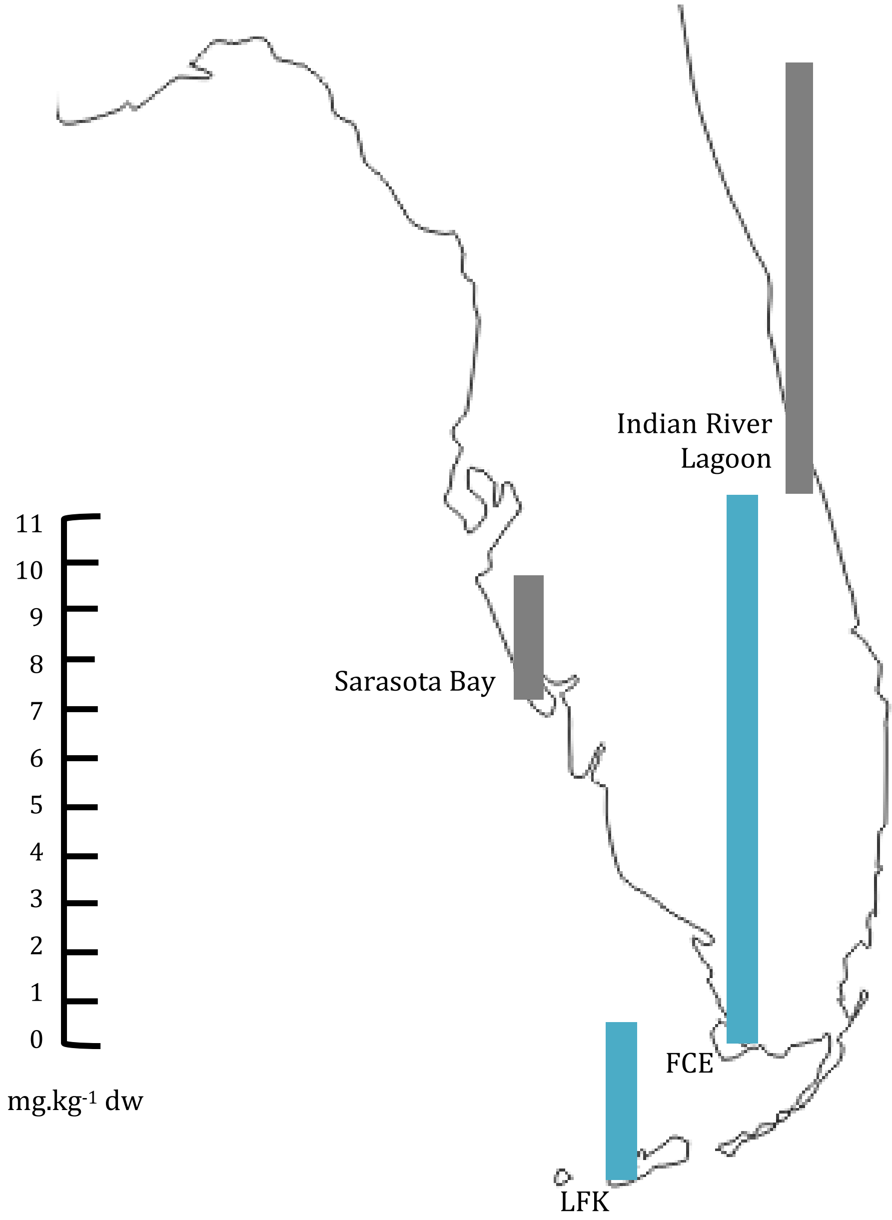
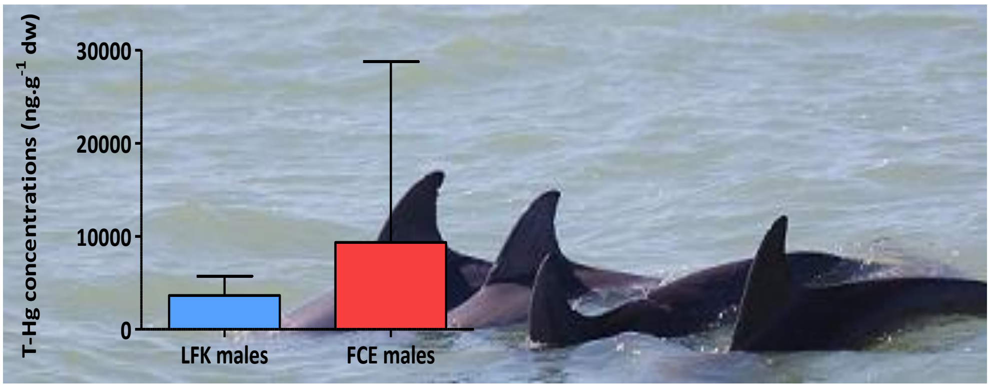


Fig. 7 – Review of mean T-Hg concentrations (mg.kg^{-1} dw) in skin of bottlenose dolphins from Florida from the literature (Woshner et al. 2008; Stavros et al. 2011) and the present study (blue lines).


T-Hg concentrations (ng.g-1 dw)
30000
20000
10000
0
LFK males
FCE males