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Persistent Organic Pollutants (POPs) in Blood and Blubber of Common Bottlenose Dolphins (*Tursiops truncatus*) at three northern Gulf of Mexico sites following the *Deepwater Horizon* Oil Spill

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Abstract.
Common bottlenose dolphins (*Tursiops truncatus*), including those impacted by the 2010 *Deepwater Horizon* (DWH) oil spill, inhabit the coastal and estuarine waters of the northern Gulf of Mexico (GoM). In response to the spill, dolphin health assessments conducted in Barataria Bay, Louisiana – a site that experienced heavy and prolonged oiling – uncovered a high prevalence of health abnormalities and individuals in poor body condition. Although the health effects observed were suggestive of petroleum toxicity, a lack of pre-spill information regarding dolphin health raises the possibility that other environmental factors may have contributed to the adverse health of dolphins in this oil-impacted area. To assess how exposure to other environmental pollutants may affect the health of northern GoM dolphin populations impacted by the DWH oil spill, a suite of 68 persistent organic pollutants (POPs), including PCBs, PBDEs and organochlorine pesticides, was determined in blood and a subset of blubber samples collected during health assessments of 145 bottlenose dolphins at three GoM sites: two oil impacted sites -Barataria Bay, LA (BB), and Mississippi Sound, MS (MS) and an unimpacted reference site - Sarasota Bay, FL (SB). Overall, levels of POPs at all three sites appeared comparable or lower than concentrations previously reported for coastal bottlenose dolphin populations outside of the northern GoM. POP levels measured in BB dolphins were also comparable or lower than those measured at the unimpacted reference site (SB) within the northern GoM. Additionally, the relationship between blubber and blood contaminant levels in a smaller subset of BB and SB suggests that BB animals were not experiencing elevated blood-contaminant concentrations as a result of their poor body condition. Cumulatively, these results suggest that background levels of POPs measured are unlikely to produce the health abnormalities previously reported for BB dolphins.

Keywords: persistent organic pollutants, contaminants, marine mammals, dolphins, Gulf of Mexico, Deepwater Horizon

1. Introduction

Common bottlenose dolphins (*Tursiops truncatus*) inhabit the bays, sounds, estuaries, and coastal waters of the northern Gulf of Mexico (GoM) where they are exposed to a variety of anthropogenic threats, including the residual effects from the 2010 *Deepwater Horizon* (DWH) oil spill (Vollmer and Rosel, 2013). The explosion and subsequent sinking of the DWH drilling rig, located approximately 60 km off the coast of southeastern Louisiana, resulted in the release of an estimated 3.19 million barrels (134 million gallons) of crude oil and 1.84 million gallons of dispersants into the northern GoM over a three-month period (DWH NRDA Trustees, 2016). The spill led to the direct oiling of more than 1600 km of wetlands, shoreline, and barrier islands from western Louisiana to the Florida Panhandle and unknown impacts to deep sea waters and benthic ecosystems (Kujawinski et al., 2011; McNutt et al., 2012; Michel et al., 2013). Several years later, remnants of the spill and its effects were still evident {Michel et al., 2013; Hsing et al., 2013; Smith et al., 2017).

In 2011, health assessments of bottlenose dolphins were conducted at two locations within the northern GoM - Barataria Bay, LA (BB), an area that received heavy and prolonged oiling, and an unimpacted (unoiled) reference site, Sarasota Bay, FL (SB) where oil was not observed (Schwacke et al., 2014). Veterinary examinations of BB dolphins uncovered a high prevalence of severe lung disease, as well as evidence of other health abnormalities, such as poor body condition, abnormally low levels of adrenal hormones, and elevated markers of inflammation that were non-existent or less prevalent in dolphins of SB and other western Atlantic locations unaffected by oil (Schwacke et al., 2014). Subsequent health assessments of bottlenose dolphins in Barataria Bay in 2013 and 2014 found that lung disease and impaired stress response persisted for at least 4 years post-spill (Smith et al., 2017). Furthermore, follow-up monitoring showed that BB dolphins also experienced reduced reproductive success and higher mortality rates relative to dolphins in unimpacted locations in the years following the spill (Lane et al., 2015).

The uncommon and severe health effects observed in BB dolphins are strongly suggestive of petroleum toxicity in other species and strongly suggestive of impacts caused by exposure to oil. However, without knowledge of the health of BB dolphins prior to the spill, the possibility that pre-existing and/or co-existing environmental stressors have influenced the severity of the observed health effects must be considered.

As inhabitants of inshore waters, bottlenose dolphins are particularly vulnerable to exposure to anthropogenic contaminants such as persistent organic pollutants (POPs) (Houde et al., 2005). POPs are lipophilic chemicals that bioaccumulate readily in tissues, such as blubber, and blood of bottlenose dolphins (Yordy et al., 2010a; Yordy et al., 2010b), potentially predisposing them to adverse reproductive, immunological and endocrine-related effects (Schwacke et al., 2002; Schwacke et al., 2012). The degree of POP exposure can vary both within sympatric populations (Litz et al., 2007; Wells et al., 2005; Yordy et al., 2010c) and between allopatric populations (Balmer et al., 2011; Balmer et al., 2015; Hansen et al., 2004; Kucklick et al., 2011) as a result of differences in environmental concentrations, life histories, feeding ecologies, and individual movements, making POPs an important factor to consider when assessing environmental impacts on dolphin health.

Blubber serves as the primary repository for POPs in cetaceans, holding more than 90% of an individual's total body burden of contaminants (Yordy et al., 2010b). A previous study was undertaken to examine POP concentrations in blubber of bottlenose dolphins from several northern GoM sites following the DWH oil spill (Balmer et al., 2015). However, it has been established that for individuals experiencing a decline in body condition – such as those observed following the spill – changes in lipid can result in a redistribution of contaminants, leading to a concomitant increase of blood contaminant levels (Yordy et al., 2010a), and a heightened risk of systemic toxic effects (Kim et al., 2010; Lassiter and Hallam, 1990). Therefore, knowledge of both blubber and blood contaminant levels is important for assessing potential health risks in stressed populations.

To better understand how exposure to environmental pollutants affects the health of northern GoM dolphin populations impacted by the DWH oil spill, POP levels were determined in blood samples collected from capture-release health assessments of bottlenose dolphins during 2011 health assessments in BB and SB (Schwacke et al., 2014) as well as from follow-up health assessments conducted in 2013 and 2014 at each location, and a third site in 2013, Mississippi Sound, MS (MS), which received moderate shoreline oiling relative to BB (Michel et al., 2013). Our objectives were to evaluate differences in background POP exposure levels between the dolphins from the three northern GoM locations and to compare the relationship between contaminant concentrations in a subset of matching blubber and blood samples collected during the 2011 health assessments to determine whether the BB dolphins experienced an increase in bioavailable POP concentrations in blood as a result of their poor body condition.

2. Materials and Methods

2.1 Study dates and locations

In 2011, 2013, and 2014, health assessments of bottlenose dolphins (*Tursiops truncatus*) were conducted at three locations within the northern GoM (Smith et al., 2017; Schwacke et al., 2014). The three locations targeted included Barataria Bay, Louisiana (BB), an area that received prolonged and heavy oiling (Michel et al., 2013); Mississippi Sound, Mississippi (MS), which received a lesser degree of oiling (Michel et al., 2013); and Sarasota Bay, Florida (SB), where no oil was observed following the DWH spill (Figure 1). Sampling was conducted in the summers of 2011 (1 year post-spill; BB and SB only), 2013 (three years post-spill; BB, SB and MS) and 2014 (four years post-spill; BB only). Blood was collected from a total of 145 dolphins over three years, including a limited number of dolphins (n = 16) repeatedly sampled in multiple years. Although matching blubber samples were collected from each individual captured, only a subset of those data (n = 22), collected from SB and BB in 2011 and previously used by Schwacke et al. (2014) to assess potential health impacts of the DWH spill, is presented here for comparison with concentrations in blood. Details regarding the timing of each health assessment and a breakdown of the age and sex of dolphins sampled at each location are provided in Supporting Information (Table S1).

2.2 Life history data

Capture methods have been described in detail elsewhere (Schwacke et al., 2014) and followed those described for prior dolphin capture–release health assessments (Wells et al., 2004). From each captured individual a suite of samples was collected for health assessment, contaminant analysis, and life history determination. Sex was confirmed through direct examination while age was determined by examination of growth layer groups in a tooth (Hohn et al., 1989) or from knowledge of birth year from observational studies. Each individual was assigned to a life history class (subadult, adult male, adult female) based on age and sex using previously determined criteria (Schwacke et al., 2009; Schwacke et al., 2010). If age was unavailable, total body length was used to categorize individuals.

2.3 Sample collection

Blubber and blood samples were obtained and stored as described previously (Wells et al., 2005; Yordy et al., 2010a). Briefly, after application of anesthetic consisting of 2% lidocaine with epinephrine and rinsing of the site with chlorhexiderm and methanol, a full-depth blubber biopsy was surgically removed from the dolphin's left side, approximately 10 cm below and 10 cm caudal to the posterior insertion of the dorsal fin. Blood was drawn from the tail fluke into glass sodium heparin blood collection tubes (BD Vacutainer, Franklin Lakes, NJ). Immediately following collection, blood was centrifuged to isolate the plasma fraction and blubber was sub-sampled using solvent-rinsed instruments. Both plasma and blubber were placed in Teflon jars and frozen in a liquid nitrogen vapor shipper. After transport to the analytical laboratory, samples were stored at -80 ^{o}C until analysis.

2.4 Contaminant analysis

Plasma and blubber samples were extracted and analyzed for POPs using gas chromatography/mass spectrometry (GC/MS) as described previously (Sloan et al., 2014). This method involves: (1) extraction of tissues using dichloromethane in an accelerated solvent extraction procedure, (2) clean-up of the dichloromethane sample extract on a single stacked silica gel/alumina column, (3) separation of POPs from lipid and other biogenic materials by high-performance size exclusion liquid chromatography, and (4) analysis on a low resolution quadrupole GC/MS system equipped with a 60-meter DB-5 GC capillary column. The GC/MS system was calibrated using sets of up to ten multi-level calibration standards of known concentrations. Percent lipid was determined gravimetrically.

A method blank and a standard reference material (SRM) - either National Institute of Standards and Technology (NIST) SRM 1945 Organics in Whale Blubber or NIST SRM 1958 Organic Contaminants in Fortified Human Serum - were analyzed with each dolphin sample set as part of a performance-based quality assurance program (Kucklick et al., 2010; Sloan et al., 2006). The procedural (method) blank consisted of a solvent (methylene chloride) run through the extraction, cleanup and gas chromatography/mass spectrometry procedures along with field samples. Concentrations of individual analytes measured in NIST SRM 1945 were in excellent agreement with the certified or reference values published by NIST. For NIST SRM 1958, 59% to 86% of the analytes were within 30% of either end of the 95% confidence interval of the NIST reference values. All other quality control samples met established laboratory criteria except where noted. The limit of quantification (LOQ) for each analyte was defined as the mass of the analyte in the lowest detectable calibration divided by the sample mass.

2.5 Statistics

All statistics were performed using Statistica (Statsoft, 2006). A total of 184 compounds were targeted during analysis. Prior to statistical analyses, analytes with a rate of detection <80% were removed (PCBs 82, 200, 205; PBDEs 28, 49, 66, 85 and 183; and pesticides alpha-, beta- and gamma-hexachlorohexane; *trans*-chlordane, aldrin; heptachlor, and endosulfan I) The remaining compounds that were below the limit of detection were replaced with a value equal to ½ the LOQ. The resulting dataset included concentration data for 69 compounds: 48 polychlorinated biphenyl (PCB) congeners (including some congeners that coelute) (17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 196, 199, 201, 202, 206, 207, 208, 209), six polybrominated diphenyl ether (PBDE) congeners (47, 99, 100, 153, 154, 155), six DDTs (2,4'- and 4,4'- DDT, DDD and DDE), six chlordanes (CHLs; *cis*-chlordane, *cis*-nonachlor, *trans*-nonachlor, nona-III-chlordane, heptachlor epoxide, oxychlordane), hexachlorobenzene

(HCB), dieldrin and mirex. Concentration data were lipid-normalized and log-transformed prior to statistical analyses to meet assumptions of normality and equal variance.

POP concentrations have been shown to significantly decline following parturition and lactation (Wells et al., 2005; Yordy et al., 2010c) and are therefore difficult to interpret in reproductively mature female dolphins. Thus, differences in contaminant concentrations between adult females and adult males/subadults were initially assessed using a multivariate analysis of variance (MANOVA) followed by Tukey's Honestly Significant Difference test (HSD) before excluding adult female dolphins from subsequent statistical comparisons. However, a summary of contaminant concentrations for adult females is provided in Supporting Information (Table S2) for reference.

For the two sites that were sampled on multiple occasions (SB and BB), a factorial ANOVA including sampling year and location as covariates was used to determine whether lipid-normalized concentrations of major contaminant groups varied between collection years before making the decision to combine plasma data from multiple years for further analysis. Data from 2014 were excluded from this analysis due to a lack of corresponding SB samples for this year. Results of this analysis are provided as Supporting Information (Table S3).

To assess differences in contaminant concentrations between locations, the geometric mean (GM) and 95% confidence interval (CI) were calculated for POP concentrations expressed on a lipid-weight basis (ng/g lipid) in adult males and subadults of both sexes (Table 1). For dolphins sampled in repeat years only one time point was selected at random for inclusion in summary statistics. Concentrations of POPs were compared between the three sites using multivariate analysis of covariance (MANCOVA) including length (a proxy for age) as a covariate as described previously (Schwacke et al., 2014).

The relationship between blubber and blood concentrations was assessed using matched blubber and plasma data from a subset of male dolphins from SB and BB in 2011. Blubber/blood partition coefficients were calculated for each individual as the ratio of lipid-normalized concentrations in blubber and matching plasma sample as described previously (Yordy et al., 2010a). Site-specific differences in blubber/blood partition coefficients for major contaminant classes were assessed by multivariate analysis of variance (MANOVA).

Strong and highly significant correlations between contaminant concentrations in blubber and blood have been previously demonstrated for bottlenose dolphins in SB (Yordy et al., 2010a). However, the strength and nature of this correlation has not been assessed for dolphins elsewhere, and could be subject to environmental or physiological differences that alter the relationship between tissues. Therefore, correlations between blubber and blood contaminant concentrations were assessed for SB and BB both individually and jointly. To determine whether the relationship between blubber and blood contaminant concentrations differed between the two sites, the singular and interactive effects of location and blubber contaminant levels on plasma contaminant levels were assessed using ANCOVAs. Regression parameters relating lipid-normalized concentrations of major contaminant classes in blubber and plasma were then calculated for SB and BB dolphins combined using major axis (Model II type) regression to provide unbiased estimates for use in future calculations (Yordy et al., 2010a).

3. Results and Discussion

Plasma was collected from a total of 145 bottlenose dolphins at three northern GoM sites between 2011 and 2014. Thirty dolphins were sampled in SB (13 ♀, 17 ♂) in 2011 ($N = 15$) and 2013 ($N = 15$), ninety-five dolphins were sampled in BB (60 ♀, 35 ♂) in 2011 ($N = 32$), 2013 (*N* = 31), and 2014 (*N* = 32) and twenty dolphins were sampled in MS (9 ♀, 11 ♂) in 2013 only (Table S1).

Age, sex, and reproductive status can have a significant influence on POP concentrations in bottlenose dolphins (Wells et al., 2005; Yordy et al., 2010c). Contaminant levels in mature females are particularly influenced by age and reproductive history as a result of the offloading of contaminants concomitant with parturition and, predominantly, lactation (Wells et al., 2005; Yordy et al., 2010c). Female dolphins typically offload 80% of their organic contaminant burden to their first calf through lactation, whereafter, her tissue concentrations tend to remain low, but may increase between subsequent calves (Wells et al., 2005; Yordy et al., 2010c). Consistent with previous observations in bottlenose dolphins, total contaminant concentrations decreased with age (proxied by length) in GoM female bottlenose dolphins (Figure S1) and were lower in adult females than in adult male and subadult dolphins (Tables 1 and S2). Plasma concentrations of total POPs (∑POP) for adult female dolphins in SB (*N* = 7; geometric mean = 7,180 ng/g lw; 95% CI 3,100 – 16,700 ng/g lw) and BB (*N* = 38; geometric mean = 9,340 ng/g lw; 95% CI 7,010 – 12,500 ng/g lw) were significantly lower relative to adult males and subadults from their respective locales (95% CIs of 41,500 – 59,800 ng/g lw and 32,000 – 40,800 ng/g lw, respectively; Tables 1 and S2; MANOVA, $p < 0.05$). In contrast, contaminant levels in adult females from MS were closer to concentrations measured in adult males and subadults from the same locale; ∑POP concentrations in plasma of MS adult females (*N* = 7, geometric mean = 33700 ng/g lw, 95% CI 13,000 – 87,600 ng/g lw) were at the lower end but overlapped with the range of concentrations measured in the plasma of adult male and subadult dolphins (95% CI 68,100 – 107,000 ng/g lw; Table 1, MANOVA, $p > 0.05$), emphasizing the importance of knowing the reproductive history of female cetaceans before interpreting associated contaminant data. To avoid the bias that can originate from the use of contaminant data from reproductively mature female dolphins of unknown history, only samples from adult males and (non-pregnant) subadults were used in following comparisons.

Plasma contaminant concentrations did not vary significantly between sampling years in SB and BB (males and subadults, lipid-normalized concentrations, ANOVA, $p > 0.05$), with the exception of mirex ($p = 0.02$) and ∑PBDEs ($p = 0.016$), which were significantly higher in BB dolphins sampled in 2011 compared to those sampled in 2013 (Table S3). Given the minimal influence of sampling year on contaminant concentrations, the time points for each location were merged for subsequent analyses (Table 1).

3.1. Comparison with populations outside the northern Gulf of Mexico. Few published contaminant values for bottlenose dolphin plasma are available to compare the concentrations measured in the current study; however, those available suggest that concentrations of POPs in northern GoM dolphin populations are either lower than, or comparable, to those of dolphin populations from the western North Atlantic (Houde et al., 2006). Total plasma PCBs for all adult males and subadults sampled in the current study (geometric mean, 30,400 ng/g lw; 95% CI 26,800 -34,500 ng/g lw) are lower than those previously reported for plasma of bottlenose dolphins from the east coast of Florida, South Carolina, New Jersey, and Bermuda in 2003-2004 (Houde et al., 2006). Direct comparisons between the two studies must be interpreted cautiously, given the near ten-year elapse in time and variations in the number and selection of PCBs included in the analysis (i.e., this study, 42 congeners; Houde et al. 2006, 121 congeners). However, other analyses comparing blubber contaminant levels in bottlenose dolphins across southeastern U.S. sites yielded similar observations. A spatial analysis of blubber contaminant levels in bottlenose dolphins across multiple GoM and Atlantic locations, including two of the three sites in the current study (MS and SB), found GoM dolphin populations exhibited POP levels at the lower end of the range reported for populations found in the inshore waters of the eastern US for the majority of contaminant classes measured (Kucklick et al., 2011). Furthermore, a more recent analysis of remotely collected bottlenose dolphin blubber biopsies from northern GoM sites, including BB and MS, in 2010 and 2011 also concluded that POP levels were within the lower half of concentrations reported for other southeastern U.S. sites (Balmer et al., 2015).

3.2. Site specific differences within the northern Gulf of Mexico. Significant differences were also detected between plasma contaminant levels of SB, BB and MS dolphins (Table 1, Figure 2). Plasma lipid content, which can influence contaminant levels in blood, did not vary significantly between sites (Table 1, ANOVA, $p > 0.05$); therefore, contaminant concentrations were expressed on a lipid-normalized basis to facilitate geographic comparisons.

Located along the eastern shoreline of the GoM, SB received no visible oiling following the DWH spill, and is also the site of a resident dolphin population for which numerous years of health and contaminant exposure data exist (Scott et al., 1990; Wells, 2014). The plasma POP concentrations for SB dolphins reported here (Tables 1 and S4, Figure 2) fell within the range of those previously reported for juvenile and adult male dolphins sampled at this location from 2002 to 2005 (Yordy et al., 2010a) suggesting that the levels of legacy POPs have not changed significantly within the resident dolphin population over the past decade. Also consistent with previous reports, SB dolphins tended to have elevated levels of chlordanes relative to other locations, both within and outside of the GoM (Kucklick et al., 2011).

In comparison to the other two GoM sites, MS dolphins exhibited the highest overall plasma contaminant levels (∑POPs; MANOVA, $p<0.005$), which were on average, nearly one and one half times higher than concentrations detected in dolphins from the SB reference site (Tables 1 and S4, Figure 2). This difference was predominantly driven by higher concentrations of ∑PCBs, ∑DDTs, and ∑PBDEs in MS dolphins (MANOVA, $p \leq 0.005$). The elevated levels of POPs measured in MS animals is explicable given the area's longstanding involvement in the shipbuilding trade, an industry which has been associated with historical use of these chemicals elsewhere (Maruyama et al., 1983; Xin et al., 2011).

In stark contrast to the high contaminant levels measured in the plasma of MS dolphins, concentrations measured in the dolphins of BB were the lowest of all three sites (Tables 1 and S4, Figure 2). Concentrations of all measured contaminant groups were either comparable (∑PCBs, ∑PBDEs) or significantly lower (∑DDTs, ∑CHLs, HCB, mirex, dieldrin) than concentrations measured at the SB reference site. These results are consistent with the statistical differences detected by Schwacke et al. (2014) in a smaller set of blubber samples collected from male bottlenose dolphins in SB in 2010/2011 and BB in 2011. Cumulatively, these results add to growing evidence that the background levels of POPs are insufficient to produce the health abnormalities previously reported for BB dolphins (Schwacke et al., 2014).

In the current study, concentrations of all contaminant groups measured in BB dolphins were found to be significantly lower than those measured in dolphins from MS (Tables 1 and S4, Figure 2), which is in contrast to findings from a previous study examining POP concentrations in blubber of bottlenose dolphins from northern GoM sites that found no significant differences in exposure between the two sites (Balmer et al., 2015). It is unclear why the two studies have conflicting observations, however, despite superficial similarities between the analyses, notable differences in their methodologies exist, including tissue types used for comparisons (this study - plasma; previous study - blubber), and time periods covered (this study - 2011-2014; previous study - 2010-2011). Future analysis of stored blubber samples collected from 2013 and 2014 health assessments in BB and MS should help to resolve the reason for these differences.

3.3. Relationship of blubber and plasma concentrations. The relationship between blubber and plasma POP concentrations has been previously examined in SB dolphins and has been shown to be significantly influenced by changes in blubber lipid content (Yordy et al., 2010a). As blubber lipid stores are reduced, lipophilic contaminants, such as POPs, may redistribute from blubber into blood (Yordy et al., 2010a), potentially increasing the risk of experiencing health effects. Health assessments of dolphins following the DWH spill observed a higher proportion of underweight individuals in BB (25%) as compared to SB (4%), prompting concern that BB dolphins may have undergone an increase in bioavailable POPs as a result of their poor body condition (Schwacke et al., 2014).

Blubber/plasma partition coefficients, which provide an indication of the distribution of POPs between the two tissue compartments, averaged 0.939 for BB dolphins (95% CI 0.844 – 1.03) and 0.867 for SB dolphins (range, 0.798 – 0.935; Table S5). At both sites, blubber/blood partition coefficients for $\sum$DDTs and $\sum$CHLs averaged ~1, indicating these contaminant groups were equally distributed between blubber and blood. The remainder of contaminants analyzed, including PCBs and PBDEs, exhibited partition coefficients < 1, indicating these groups were generally more concentrated in plasma than blubber (Table S5).

Despite a higher prevalence of individuals in poor body condition, BB dolphins did not exhibit reduced blubber lipid content (%) relative to SB dolphins (Student's t-test, $p > 0.05$). Additionally, blubber/blood partition coefficients were not significantly different for the majority of contaminants measured in SB and BB dolphins (MANOVA, $p > 0.05$), suggesting that the relative tissue distribution of these chemicals did not differ between dolphins from the two sites at the time of sampling. The two exceptions for which significant differences between SB and BB dolphins were observed - PCBs and mirex - were lower for SB animals, indicating that dolphins from this location were experiencing elevated blood levels in relation to blubber for these select classes of contaminants (Table S5).

POP concentrations in blubber and plasma were significantly and positively correlated for all contaminant groups measured at both sites individually and together. (Tables 2 and S6, Figure 3). The R^2 values ranged from 0.789 to 0.932 for BB ($p<0.001$) and from 0.794 to 0.957 for SB ($p <0.001$) (Table S6). The regression parameters relating blubber and blood for SB dolphins as mentioned in Table 2 agree strongly with those previously reported for the area (R^2 values range: 0.824 to 0.970, $p<0.001$; Yordy et al., 2010a), supporting the use of these parameters for reliably estimating tissue concentrations in this population. Furthermore, location did not have a significant effect on the slope of the regression equation relating blubber and blood contaminant levels, indicating that the nature of this relationship did not significantly vary between sites (ANCOVA, $p>0.05$). The similar blubber-blood relationship observed for the two locations provides further support that at the time of sampling, BB dolphins were not experiencing elevated blood POP levels stemming from their observed poor body condition(s).

4. Conclusions

Similar to other marine mammal populations around the world, bottlenose dolphins inhabiting BB have measurable exposure to POPs. However, several findings from the current study suggest that the level of background exposure to POPs in BB dolphins is unlikely to significantly contribute to the health abnormalities observed in this population (Schwacke et al., 2014). First, concentrations of POPs in BB dolphins are either lower, or comparable, to those of presumably healthy dolphin populations found within and beyond the northern Gulf of Mexico. Secondly, BB dolphins do not appear to be experiencing elevated blood contaminant levels; consequently, observed health effects are unlikely to be related to the mobilization of POPs as a result of a decline in body condition. Cumulatively, these results add to the weight of evidence suggesting health impacts observed in BB dolphins are the result of exposure to oil (Schwacke et al, 2014; Smith et al., 2017; Lane et al., 2015).

Additionally, the strength and the nature of the relationship between contaminant levels in blubber and blood reported here strongly agrees with that previously reported for SB dolphins (Yordy et al., 2010a) and does not vary significantly between SB and BB dolphin populations, providing further support for the use of these regression parameters for reliably estimating contaminant concentrations in one tissue given the other. The ability to use blubber for predicting blood-level exposures, and vice-versa, expands the capacity to make comparisons and assess risks to wild dolphin populations subject to sampling constraints.

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Supporting Information Available: Additional summary tables and statistical details of results.

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Table 1. Plasma POP concentrations (ng/g lipid) and percent lipid content in adult male and subadult bottlenose dolphins from three sites in the northern Gulf of Mexico. Values are geometric means (95% confidence intervals) computed at a mean body length of 229.8 cm.

	Sarasota Bay, FL	**Barataria Bay, LA**	**Mississippi Sound, MS**
Sampling years	2011, 2013	2011, 2013, 2014	2013
Sample[1]	20 (2)	46 (9)	13
Age range	2 - 30	3 - 27	6 – 30
Lipid (%)	0.29 (0.26 – 0.32)[a]	0.26 (0.25 – 0.28)[a]	0.26 (0.23 – 0.29)[a]
ΣPCBs[2]	27000 (22300 - 32500)[a]	26900 (23900 - 30400)[a]	55500 (44300 - 69500)[b]
ΣDDTs[3]	11000 (8930 - 13500)[a]	5600 (4890 - 6420)[b]	19600 (15200 - 25300)[c]
ΣCHLs[4]	8000 (6640 -9700)[a]	1540 (1360 - 1750)[b]	4210 (3330 - 5330)[c]
ΣPBDEs[5]	1490 (1220 - 1810)[a]	1390 (1220 - 1580)[a]	3800 (2980 - 4860)[b]
HCB[6]	97.7 (83.8 – 114)[a]	68.6 (62.0 – 75.9)[b]	162 (134 - 196)[c]
Mirex	794 (624 - 1000)[a]	74.4 (63.4 – 87.1)[b]	553 (410 - 745)[a]
Dieldrin	805 (668 – 969)[a]	413 (365 – 467)[b]	919 (730 - 1158)[a]
ΣOCPs[7]	20900 (17200 – 25400)[a]	7770 (6840 - 8840)[b]	25900 (20300 - 32900)[a]
∑POPs[8]	49800 (41500 - 59800)[a]	36100 (32000 - 40800)[b]	85500 (68100 – 107000)[c]

Note: Statistical differences were evaluated using MANCOVA using length (a proxy for age) as a covariate followed post hoc by Tukey's HSD. Groups sharing the same letter are not statistically different.

[1] Summary statistics include only one sample from each individual. For individuals sampled repeatedly, only one timepoint selected at random is included. The number of replicate samples omitted are indicated in parentheses.

[2] ∑PCBs includes 48 PCB congeners (including congeners that coelute). See Materials and Methods for full list.

[3] ∑DDTs includes 2,4'- and 4,4'-DDT, DDE and DDD.

[4] ∑CHLs includes *alpha*-chlordane, *cis*-nonachlor, *trans*-nonachlor, nonachlor III, heptachlor epoxide, and oxychlordane.

[5] ∑PBDEs includes congeners 47, 99, 100, 153, 154, and 155.

[6] Hexachlorobenzene

[7] ∑OCPs includes ∑DDTs, ∑CHLs, HCB, mirex and dieldrin.

[8] ∑POPs is sum of all measured analytes.

Table 2. Model II regression parameters relating POP concentrations (log, ng/g lipid) in blubber and plasma of bottlenose dolphins from Sarasota Bay, FL and Barataria Bay, LA[1].

	R^2	slope (b1)	intercept (b0)	p-value
ΣPCBs	0.875	1.07	- 0.217	<0.001
ΣDDTs	0.947	1.11	- 0.492	<0.001
ΣCHLs	0.970	1.09	- 0.341	<0.001
ΣPBDEs	0.847	1.03	0.005	<0.001
HCB	0.824	0.926	0.338	<0.001
mirex[2]	0.959	1.07	- 0.009	<0.001
dieldrin	0.919	1.11	- 0.160	<0.001
∑POPs	0.919	1.10	- 0.431	<0.001

[1]Concentrations were log-transformed for regression analyses to improve normality. Regression parameters relate to the specific function: log $\text{concentration}_{plasma}$ = b0+ b1*log $\text{concentration}_{blubber}$.

[2]Model II regression could not converge on a solution therefore results of Model I regression are shown.

Figure Captions.

Figure 1. Map of study sites within the northern Gulf of Mexico.

Figure 2. Persistent organic pollutant concentrations measured in plasma of adult male and subadult bottlenose dolphins from Sarasota Bay, FL (n = 20), Barataria Bay, LA (n = 46) and Mississippi Sound, MS (n = 13). Bars represent geometric mean concentration computed at the mean body length (229.8 cm) and whiskers represent 95% CI around the mean. Asterisks represent locations having concentrations significantly different than the reference site, Sarasota Bay, FL (*ANOVA, $p < 0.001$).

Figure 3. Relationship of blubber and plasma contaminant levels in dolphins from Sarasota Bay, FL and Barataria Bay, LA. Model II regression and 95% confidence region describing matched blubber and blood concentrations of **(A)** ∑PCBs, $R^2 = 0.875$, $p < 0.001$; **(B)** ∑DDTs, $R^2 = 0.947$, $p < 0.001$; **(C)** ∑CHLs, $R^2 = 0.970$, $p < 0.001$; and **(D)** ∑PBDEs, $R^2 = 0.847$, $p < 0.001$.

Figure 1.

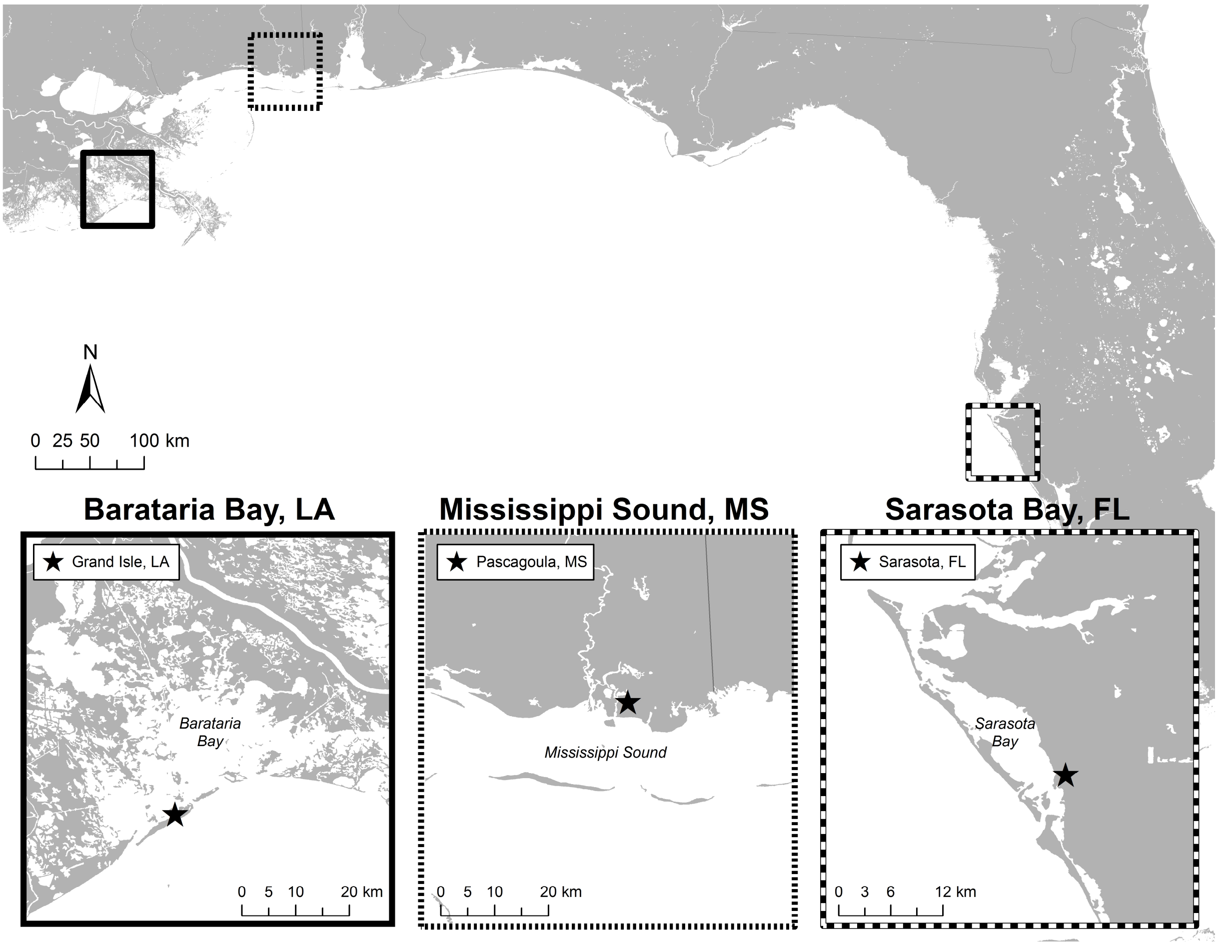
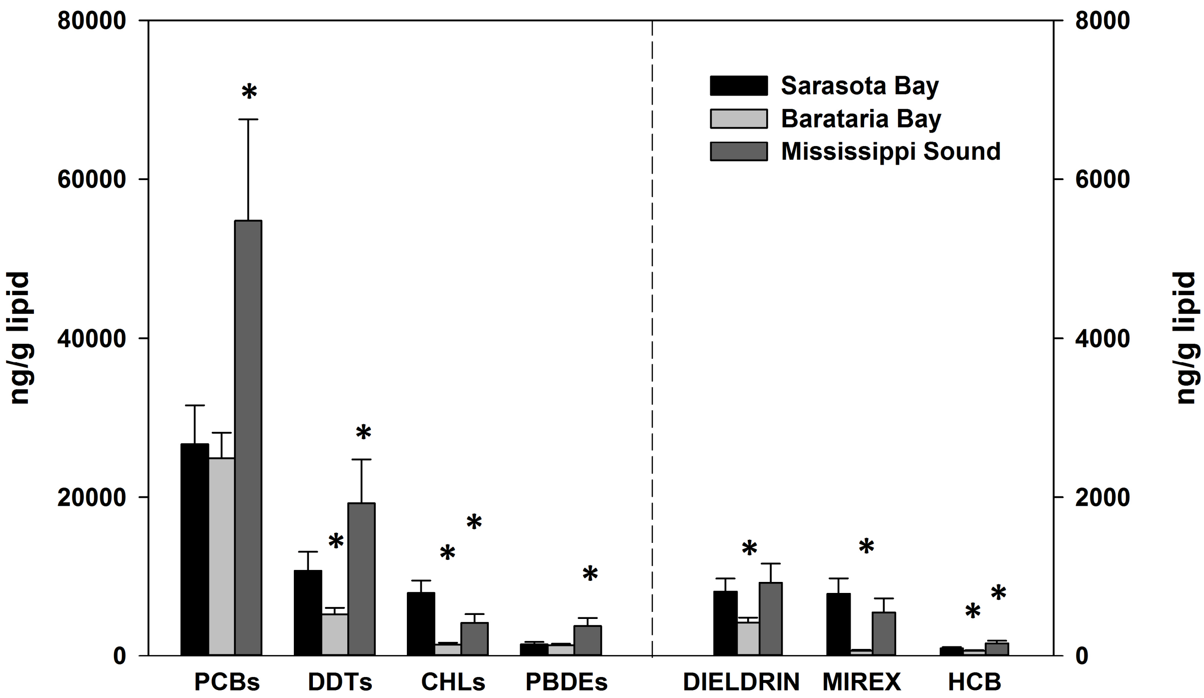
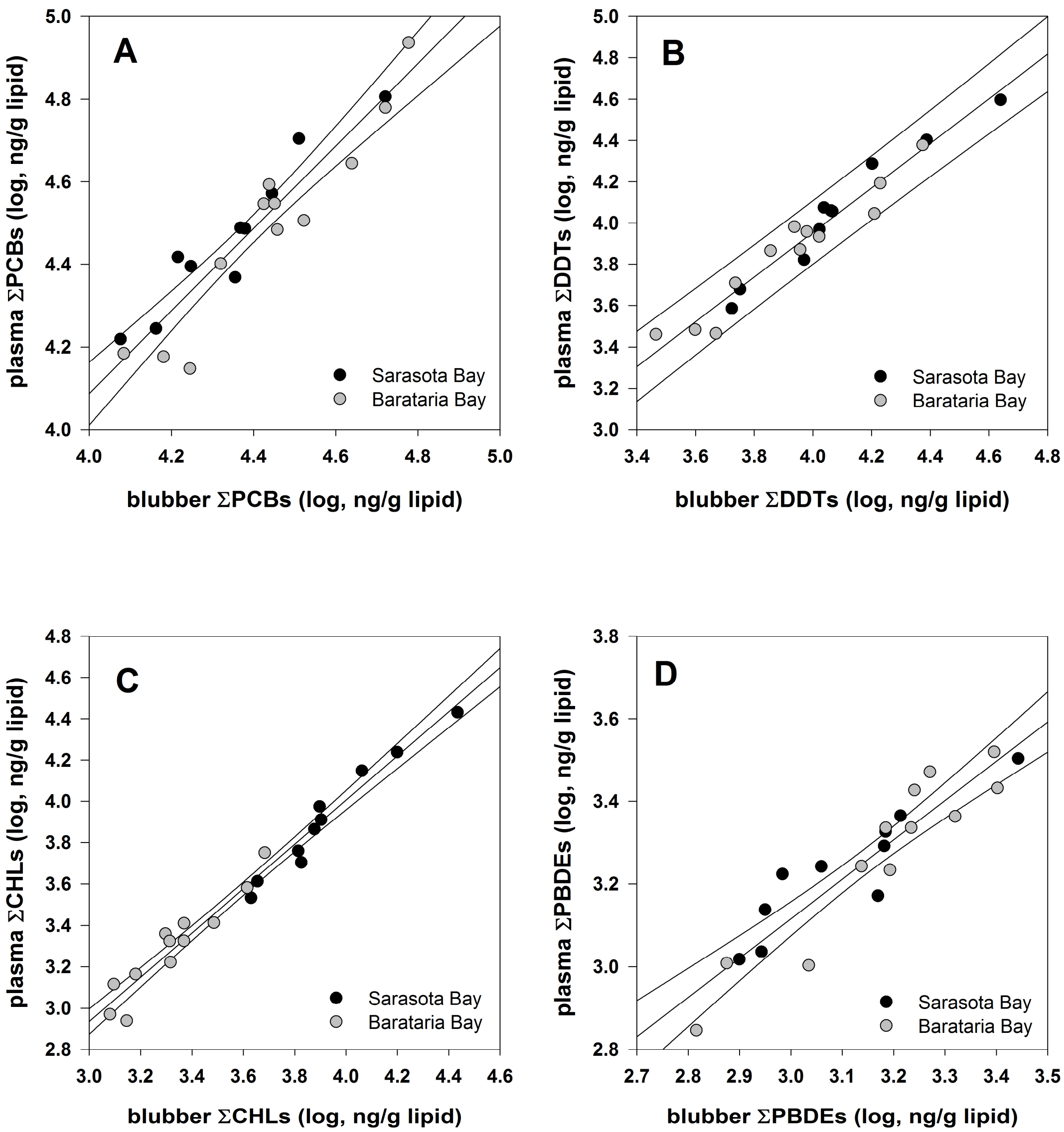

N
0 25 50 100 km
Barataria Bay, LA
Grand Isle, LA
Barataria Bay
0 5 10 20 km
Mississippi Sound, MS
Pascagoula, MS
Mississippi Sound
0 5 10 20 km
Sarasota Bay, FL
Sarasota, FL
Sarasota Bay
0 3 6 12 km

Figure 2.



80000
60000
40000
20000
0
ng/g lipid
8000
6000
4000
2000
0
ng/g lipid
Sarasota Bay
Barataria Bay
Mississippi Sound
PCBs
DDTs
CHLs
PBDEs
DIELDRIN
MIREX
HCB

Figure 3.


A
plasma ΣPCBs (log, ng/g lipid)
blubber ΣPCBs (log, ng/g lipid)
Sarasota Bay
Barataria Bay
B
plasma ΣDDTs (log, ng/g lipid)
blubber ΣDDTs (log, ng/g lipid)
Sarasota Bay
Barataria Bay
C
plasma ΣCHLs (log, ng/g lipid)
blubber ΣCHLs (log, ng/g lipid)
Sarasota Bay
Barataria Bay
D
plasma ΣPBDEs (log, ng/g lipid)
blubber ΣPBDEs (log, ng/g lipid)
Sarasota Bay
Barataria Bay

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Supporting Information for

Persistent Organic Pollutants (POPs) in Blood and Blubber of Common Bottlenose Dolphins (*Tursiops truncatus*) at three northern Gulf of Mexico sites following the *Deepwater Horizon* Oil Spill

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Table S1. Breakdown of sampled dolphins by age and sex class.

Table S2. Geometric means and 95% CI of plasma POP concentrations in adult female bottlenose dolphins from three sites within the Gulf of Mexico.

Table S3. Statistical significance results (p-values) from factorial ANOVA examining effect of year and location covariates on contaminant concentrations measured in plasma of subadult and adult male dolphins from Sarasota Bay, FL and Barataria Bay, LA.

Table S4. Statistical significance results (p-values) from MANCOVA and subsequent Tukey's HSD test examining concentrations of contaminants measured in plasma of subadult and male dolphins from Sarasota Bay, Barataria Bay and Mississippi Sound with length as a covariate.

Table S5. Tissue partition coefficients (arithmetic mean, 95% CI) for blubber and plasma of bottlenose dolphins and p-values of MANOVA comparing differences between sites.

Table S6. Model II regression parameters (slope, intercept, R^2 values) relating blubber and blood concentrations separately for Sarasota Bay and Barataria Bay dolphins and statistical significance results (p-values) from ANCOVA examining the singular and interactive effects of location and blubber contaminant concentrations on plasma contaminant concentrations.

Figure S1. Total plasma persistent organic pollutants (ΣPOPs) versus length in female dolphins from Barataria Bay, LA.

Table S1. Number of plasma and (matching blubber) samples from Sarasota Bay, Barataria Bay, and Mississippi Sound dolphins grouped by life history class. Criteria for assigning age/sex class are the same used by Schwacke et al. 2014 and were adopted from Schwacke et al. 2009 and 2010.

Age/Sex class	**Criteria[1]**	**Sarasota Bay**		**Barataria Bay**			**Mississippi Sound**
		May 2011	May 2013	June 2011	June 2013	June 2014	July 2013
Subadult	≥ 2 years and < 10 years;						
Male	≥ 200 cm and < 240 cm	7 (7)	5	3 (3)	10	3	6
Female		1	4	7	7	6	2
Adult	≥ 10 years; ≥ 240 cm						
Male		3 (3)	2	9 (9)	5	5	5
Female		4	4	13	9	18	7
Total		**15 (10)**	**15**	**32 (12)**	**31**	**32**	**20**

[1]Age was used for assigning class; if age was not available, assignment was based on length. Pregnant females were always classified as adults, regardless of age or length.

Table S2. Plasma POP concentrations (ng/g lipid) and percent lipid content (geometric mean, 95% CI) in adult female bottlenose dolphins from three sites in the northern Gulf of Mexico.

	Sarasota Bay, FL	Barataria Bay, LA	Mississippi Sound, MS
Sampling years	2011, 2013	2011, 2013, 2014	2013
Sample size[1]	7 (1)	38 (2)	7
Age range[2]	NA - 43	NA - 42	NA
Lipid (%)	0.27 (0.22 – 0.32)	0.25 (0.22 – 0.28)	0.27 (0.23 – 0.31)
ΣPCBs[3]	4590 (1960 - 10700)	7238 (5470 - 9570)	22600 (8970 - 57000)
ΣDDTs[4]	1050 (417 - 2660)	1150 (820 - 1630)	6970 (2500 - 19400)
ΣCHLs[5]	899 (400 -2020)	350 (257 - 476)	1430 (473 - 4330)
ΣPBDEs[6]	241 (110 - 531)	353 (258 - 482)	1600 (530 - 4850)
HCB[7]	24.9 (14.8 – 41.8)	28.6 (23.0 – 35.5)	81 (36.6 -179)
Mirex	162 (67.3 – 387)	26.7 (20.9 – 34.0)	298 (131 - 674)
Dieldrin	120 (49.5 – 290)	145 (113 – 185)	366 (147 - 912)
ΣOCPs[8]	2320 (992 – 5420)	1730 (1260 - 2380)	9310 (3380 - 25700)
∑POPs[9]	7180 (3100 - 16700)	9340 (7010 - 12500)	33700 (13000 – 87600)

Note: Statistical differences between locations were not evaluated given the potential bias originating from unknown reproductive history on contaminant concentrations.

[1] Summary statistics include only one sample from each individual. For individuals sampled in 2011 and 2013, only one timepoint selected at random is included. The number of replicate samples omitted are indicated in parentheses.

[2] Ages for some individuals were not available (NA).

[3] ∑PCBs includes 48 PCB congeners. See Materials and methods for full list.

[4] ∑DDTs includes 2,4’- and 4,4’-DDT, DDE AND DDD.

[5] ∑CHLs includes alpha-chlordane, cis-nonachlor, trans-nonachlor, nonachlor III, heptachlor epoxide and oxychlordane.

[6] ∑PBDEs includes 47, 99, 100, 153, 154, and 155.

[7] Hexachlorobenzene

[8] ∑OCPs includes ∑DDTs, ∑CHLs, HCB, mirex and dieldrin.

[9] ∑POPs is sum of all measured analytes.

Table S3. Statistical significance results (p-values) from factorial ANOVA examining effect of year and location covariates on contaminant concentrations measured in plasma of subadult and adult male dolphins from Sarasota Bay, FL and Barataria Bay, LA collected in 2011 and 2013. Plasma contaminant concentrations (ng/g lipid) were log-transformed prior to analysis. Red text (*) indicates significant p-value at the α=0.05 level.

	Year	Location	Year*Location
Σ PCBs	0.204	0.627	0.522
Σ DDTs	0.288	<0.001*	0.330
Σ CHLs	0.140	<0.001*	0.342
Σ PBDEs	0.016*	0.589	0.259
HCB	0.523	<0.001*	0.797
Mirex	0.022*	<0.001*	0.164
Dieldrin	0.102	<0.001*	0.280
Σ OCPs	0.206	<0.001*	0.383
Σ POPs	0.192	0.012*	0.477

Table S4. Statistical significance results (p-values) from MANCOVA and subsequent Tukey's HSD test examining concentrations of contaminants measured in plasma of subadult and male dolphins from Sarasota Bay (SB), Barataria Bay (BB) and Mississippi Sound (MS) with length as a covariate. Red text indicates significant p-value at the α=0.05 level.

Contaminant Class	**MANCOVA p-values**		**Tukey's HSD Pairwise Comparisons p-values**		
	Length	Location	SB / BB	SB / MS	BB / MS
Σ PCBs	<0.001*	<0.001*	0.994	<0.001*	<0.001*
Σ DDTs	<0.001*	<0.001*	<0.001*	0.005*	<0.001*
Σ CHLs	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Σ PBDEs	<0.001*	<0.001*	0.887	<0.001*	<0.001*
HCB	0.258	<0.001*	<0.001*	<0.001*	<0.001*
Mirex	<0.001*	<0.001*	<0.001*	0.092	<0.001*
Dieldrin	0.989	<0.001*	<0.001*	0.644	<0.001*
Σ OCPs	<0.001*	<0.001*	<0.001*	0.539	<0.001*
∑ POPs	<0.001*	<0.001*	0.02*	0.003*	<0.001*

Table S5. Tissue partition coefficients (arithmetic mean, 95% CI) for blubber and plasma of bottlenose dolphins and p-values of MANOVA comparing differences between sites. Red text (*) indicates significant p-value at the α=0.05 level.

	Sarasota Bay (N = 10)	**Barataria Bay (N= 12)**	**p-value**
Σ PCBs	0.760 (0.700 – 0.822)	0.889 (0.797 – 0.982)	0.041*
Σ DDTs	1.09 (0.975 – 1.21)	1.15 (1.04 – 1.28)	0.463
Σ CHLs	1.04 (0.937 – 1.14)	1.09 (0.969 – 1.21)	0.539
Σ PBDEs	0.751 (0.676 – 0.826)	0.817 (0.741 – 0.893)	0.244
HCB	0.653 (0.586 – 0.721)	0.596 (0.543 – 0.648)	0.196
Mirex	0.615 (0.543 – 0.689)	0.830 (0.741 – 0.920)	0.002*
Dieldrin	0.735 (0.650 – 0.820)	0.777 (0.692 – 0.862)	0.503
Σ POPs	0.867 (0.798 – 0.935)	0.939 (0.844 – 1.03)	0.258

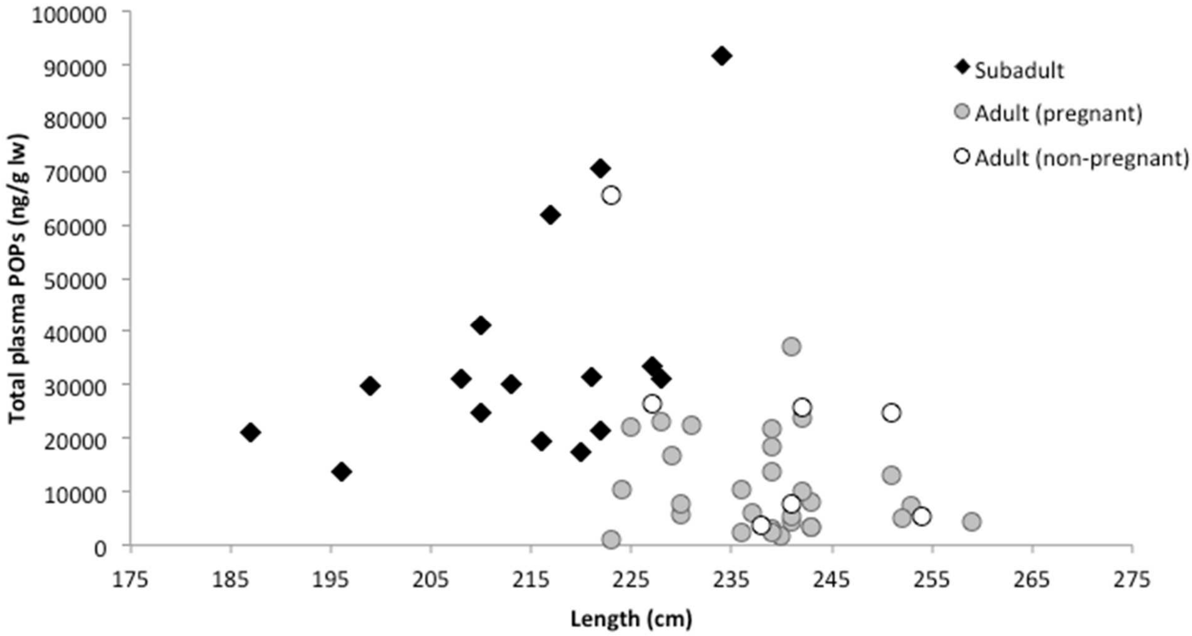
Table S6. Model II regression parameters (slope, intercept, R^2 values) relating blubber and blood concentrations separately for Sarasota Bay and Barataria Bay dolphins and statistical significance results (p-values) from ANCOVA examining the singular and interactive effects of location and blubber contaminant concentrations on plasma contaminant concentrations. Red text (*) indicates significant p-value at the α=0.05 level.

		Regression Parameters[a]		ANCOVA		
		Sarasota Bay (N = 10)	Barataria Bay (N= 12)	Location	Blubber concentration	Location*Blubber concentration
Σ PCBs	slope (b1)	0.999	1.16	0.351	<0.001*	0.400
	intercept (b0)	0.128	-0.634			
	R^2	0.911	0.902			
Σ DDTs	slope (b1)	1.15	1.07	0.465	<0.001*	0.450
	intercept (b0)	-0.660	-0.324			
	R^2	0.956	0.932			
Σ CHLs	slope (b1)	1.17	1.24	0.978	<0.001*	0.917
	intercept (b0)	-0.696	-0.820			
	R^2	0.957	0.896			
Σ PBDEs	slope (b1)	0.884	1.15	0.144	<0.001*	0.159
	intercept (b0)	0.490	-0.379			
	R^2	0.828	0.888			
HCB	slope (b1)	0.770	1.31	0.061	<0.001*	0.057
	intercept (b0)	0.607	-0.270			
	R^2	0.794	0.789			
Mirex	slope (b1)	0.771	0.817[b]	0.113	<0.001*	0.565
	intercept (b0)	0.841	0.440[b]			
	R^2	0.931	0.910[b]			
Dieldrin	slope (b1)	1.02	1.34	0.184	<0.001*	0.189
	intercept (b0)	0.073	-0.738			
	R^2	0.886	0.868			
Σ POPs	slope (b1)	1.05	1.13	0.659	<0.001*	0.690
	intercept (b0)	-0.146	-0.552			
	R^2	0.939	0.909			

[a]Concentrations were log-transformed for regression analyses to improve normality. Regression parameters relate to the specific function: log $\text{concentration}_{plasma} = b0 + b1 * \log \text{concentration}_{blubber}$, where concentrations are in units of ng/g lipid. All regressions were statistically significant ($p < 0.05$) at the α=0.05 level.

[b]Model II regression could not converge on a solution therefore results of Model I regression are shown.

Figure S1. Total plasma persistent organic pollutants (ΣPOPs) versus length in female dolphins from Barataria Bay, LA.



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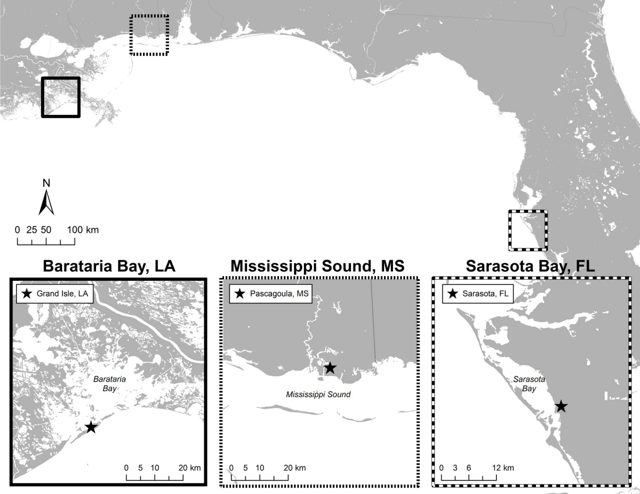
N
0 25 50 100 km
Barataria Bay, LA
Grand Isle, LA
Barataria Bay
0 5 10 20 km
Mississippi Sound, MS
Pascagoula, MS
Mississippi Sound
0 5 10 20 km
Sarasota Bay, FL
Sarasota, FL
Sarasota Bay
0 3 6 12 km