Comparison of persistent organic pollutants (POPs) between small cetaceans in coastal and estuarine waters of the northern Gulf of Mexico

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A B S T R A C T

Small cetaceans continue to be exposed to elevated levels of persistent organic pollutants (POPs). The goals of this study were to use data from remote biopsy sampling and photographic-identification to compare POP concentrations between small cetacean stocks in the northern Gulf of Mexico. During 2015–2017, 74 remote biopsies were collected in St. Andrew Bay and adjacent coastal waters from two species: common bottlenose dolphin (*Tursiops truncatus*) (N = 28, ♀; N = 42, ♂) and Atlantic spotted dolphin (*Stenella frontalis*) (N = 2, ♀; N= 2, ♂). Common bottlenose dolphin POP concentrations were significantly higher in St. Andrew Bay than coastal waters. Male St. Andrew Bay dolphins had the highest Σ DDT (dichlorodiphenyl-dichloroethane) levels measured in the

southeastern U.S. (67 μg/g, 50–89 μg/g; geometric mean and 95% CI) and showed a significant negative relationship between Σ DDT and sighting distance from a St. Andrew Bay point source.

Introduction

Persistent organic pollutants (POPs) are a group of man-made lipophilic and hydrophobic compounds that were produced for a variety of agricultural and industrial uses. Although there has been significant effort to ban or greatly reduce manufacturing and use of POPs throughout the world (UNEP, 2001), these legacy contaminants continue to be of concern due to their ability to bio-magnify in the marine environment, and to cause acute and chronic health effects at ecosystem, population, and individual levels (reviewed in Murphy et al., 2018). Marine ecosystems are global sinks for POPs, such as polychlorinated biphenyls (PCBs), dichlorodiphenyl-dichloroethanes (DDTs), polybrominated diphenyl ethers (PBDEs), and chlordanes (CHLs). Upper trophic organisms in marine environments have been identified as good bio-indicators of environmental pollution, as these species bio-accumulate contaminants in their tissues over time (reviewed in Green and Larson, 2016).

Small cetaceans are long-lived, high trophic level species that store contaminants, including POPs, in their lipid-rich blubber, and thus, are considered sentinels for assessing marine ecosystem health (Wells et al., 2004). Many small cetacean species are also long-term, year-round residents to nearshore waters, and studying these animals can provide insight into anthropogenic stressors potentially impacting human populations (Hall et al., 2006). POP levels in small cetaceans can vary by species (e.g. Borrell and Aguilar, 2005a; Dirtu et al., 2016), sex (e.g. Aguilar et al., 1999; Borrell and Aguilar, 2005b), age class (e.g. Kuehl and Haebler, 1995; Yordy et al., 2010), geographic region (e.g. Hansen et al., 2004; Kucklick et al., 2011), and temporal scale (e.g. Loganathan and Kannan, 1991; Aguilar et al., 2002).

It is essential for development of effective management strategies to appropriately identify exposure to and effects from these stressors on a given stock. In the U.S., the Marine Mammal Protection Act defines a stock as a group of marine mammals of the same species in a common spatial arrangement that interbreed when mature (MMPA, 16 USC, 1361 et seq.). Stocks of marine mammals are delineated for the purpose of performing stock assessments. However, in some instances, there is spatial overlap of marine mammal stocks and the combination of multiple sampling tools is necessary for differentiation of these stocks. Common bottlenose dolphins (*Tursiops truncatus*) are the primary small cetacean species inhabiting the bays, sounds, and estuaries (BSEs), and coastal (CST) waters of the northern Gulf of Mexico (Mullin et al., 1990). The National Marine Fisheries Service has delineated 31 BSE stocks and three CST stocks, of common bottlenose dolphins in the northern Gulf of Mexico (Hayes et al., 2017). Dolphins that are members of BSE stocks are associated with year-round site fidelity to localized estuarine waters (Hubard et al., 2004; Balmer et al., 2008; Tyson et al., 2011), while dolphins that are members of CST stocks typically have larger ranging patterns, some of which may extend over several hundred kilometers (Balmer et al., 2016). There is currently one stock of

Atlantic spotted dolphins (*Stenella frontalis*) in the northern Gulf of Mexico that primarily inhabits continental shelf (10–200 m) to slope (< 500 m) waters (Fulling et al., 2003), although genetic evidence suggests this stock may more accurately be split into two stocks (Viricel and Rosel, 2014). Limited data have also suggested that this species may have seasonal inshore movements (Caldwell and Caldwell, 1966) which could result in some spatial overlap with CST stocks of common bottlenose dolphins.

In the northern Gulf of Mexico, POP concentrations and profiles have been identified to differ across broad-scale geographic regions. In urbanized sites (e.g. Tampa Bay, Florida), POP profiles in common bottlenose dolphin blubber samples have shown higher levels of PCBs when compared to samples of conspecifics in agricultural sites (e.g. Mississippi Sound and St. Joseph Bay, Florida), where higher levels of organochlorine pesticides (OCPs: e.g. DDT, dieldrin, and mirex) have been observed (Kucklick et al., 2011). Wilson et al. (2012) also identified fine-scale differences in POP concentrations among adjacent northern Gulf of Mexico field sites with common bottlenose dolphins in St. Andrew Bay, Florida, having higher contaminant concentrations across all POP classes than dolphins in St. Joseph Bay, Florida, and St. George Sound, Florida. Currently, POP concentrations for Atlantic spotted dolphins in the northern Gulf of Mexico are not known. However, Méndez-Fernandez et al. (2018) compared POP levels in Atlantic spotted dolphins from four sites in the Atlantic Ocean (Azores, Canary Islands, Caribbean Sea, and São Paulo, Brazil) and reported that POP concentrations and profiles differed depending on agricultural and industrial influences at each site.

Remote blubber biopsy sampling for contaminant analyses in the northern Gulf of Mexico has primarily targeted common bottlenose dolphins in BSE waters, with limited comparisons of POP concentrations between BSE and adjacent CST stocks. For example, Balmer et al. (2015) compared POP concentrations across several field sites in the northern Gulf of Mexico, but also subdivided two of these sites (Chandeleur Sound, Louisiana and Mississippi Sound) in an effort to differentiate POP levels between BSE and CST stocks. This comparison used sampling location as the only parameter for where a sampled animal was grouped (BSE or CST) and in both of these field sites, there is likely some degree of mixing between members of BSE and CST stocks (Hubard et al., 2004; Pitchford et al., 2016; Mullin et al., 2017). Ultimately, no differences in POP concentrations or profiles were identified within the subdivided Chandeleur Sound and Mississippi Sound field sites. To better understand differences in contaminant exposure and subsequent health impacts associated with anthropogenic stressors between BSE and CST stocks, a sampling methodology that specifically targets and appropriately group individuals hypothesized to be members of each stock is necessary.

During 2015–2017, photographic-identification (photo-ID) surveys were conducted in St. Andrew Bay, Florida and adjacent coastal waters to estimate abundance of the St. Andrew Bay BSE Stock, and determine distribution patterns and spatial overlap between this BSE stock and the adjacent Northern Coastal Stock of common bottlenose dolphins (Balmer et al., 2019). Remote biopsy sampling was conducted in addition to photo-ID surveys to provide baseline data on stock structure and contaminant concentrations. The goals for this study were to (1) provide baseline data on POP concentrations in blubber for dolphins in this region and (2) utilize photo-ID and POP data to identify differences in POP concentrations and profiles between dolphins sampled in the BSE and CST waters.

Materials and methods

Study area

The St. Andrew Bay system is a shallow estuarine tidal embayment (Grady, 1981) covering approximately 740,000 acres consisting of four bays (East Bay, North Bay, St. Andrew Bay proper, and West Bay) located along the northeastern shore of the Gulf of Mexico, (i.e. the Florida Panhandle) (SWIM, 2017) (Fig. 1). This embayment is relatively unique among Gulf coast estuaries in that the waters are deep and clear as it receives little freshwater input and sedimentation (Ogren and Brusher, 1977). Mean depth in St. Andrew Bay proper is approximately 5 m, while East, North, and West Bays are generally shallower (2 m) (Ichiye and Jones, 1961). Salinity is approximately 30 parts per thousand (ppt) but can occasionally drop below 10 ppt in locations nearest to freshwater input and farthest from the Gulf (Ichiye and Jones, 1961). St. Andrew Bay is characterized by a diurnal tidal cycle with a mean range of 0.4 m (Salsman et al., 1966). Seagrasses, primarily shoal grass (*Halodule wrightii*) and turtle grass (*Thalassia testudinum*), are found throughout St. Andrew Bay (Grady, 1981; SWIM, 2017). Adjacent coastal waters of St. Andrew Bay are characterized by firm white sandy bottoms with no attached vegetation and clear water with high salinities (~30 ppt) (Naughton and Saloman, 1978). The St. Andrew Bay study area was divided into two subareas (BSE and CST), with the entrance to St. Andrew Bay as the geographic delineation between subareas. The BSE subarea included the four primary bays, while the CST subarea included coastal waters directly adjacent to the estuary and extending approximately 10 km offshore from west of Crooked Island Sound to northwest of Panama City Beach (Fig. 1).

Major sources of both domestic and industrial pollution have been identified in the St. Andrew Bay system including urban stormwater runoff, the WestRock paper mill, and five Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) sites (Defense Fuel Support Point-Lynn Haven, Ethanol Corp, Gulf Oil, Panama City Coastal Systems Station, and Tyndall Air Force Base) (Brim, 1998; EPA, 2007; SWIM, 2017). Ethanol Corp and Gulf Oil are registered as Archived Superfund sites in which the Environmental Protection Agency (EPA) does not require any further cleanup. The Defense Fuel Support Point-Lynn Haven and Panama City Coastal Systems Station sites are not on the National Priority List (NPL), while Tyndall Air Force Base is a NPL site due to high levels of organochlorine pesticides [DDT, DDD (dichlorodiphenyldichloroethane), and DDE (dichlorodiphenyldichloroethylene)] in the sediment of Fred Bayou that empties into East Bay (Fig. 1). Subsequent analyses identified DDTcontaminated fish in Fred Bayou, however, these data suggested that DDT levels were below those of concern and considered to pose no apparent health hazards to the human population in the region (ATSDR, 2000).

Biopsy sample collection

Remote biopsy samples were collected using a Barnett Panzer V crossbow (Barnett Outdoors, LLC, Tarpon Springs, Florida, USA). The methodology for sample collection and in-field processing has been described in detail in Sinclair et al. (2015). Briefly, samples were collected from individual dolphins at a distance of 2–10 m, targeting the flank of the animal below the dorsal fin and above the midline (Gorgone et al., 2008). Remote biopsy samples consisted of skin and a full-depth section of blubber approximately 0.7–0.8 g in weight. Subsamples were processed and prepared for storage immediately after the sample was collected. The skin sample for genetic analyses was stored at room temperature in 20% dimethyl sulfoxide (DMSO) saturated with sodium chloride (NaCl). DNA was extracted from each skin sample with a Qiagen DNeasy Tissue and Blood extraction kit (Hilden, Germany), and used to determine sex via polymerase chain reaction (PCR) methods described by Rosel (2003). The blubber sample used for contaminant analyses was stored in a pre-cleaned Teflon vial (Savillex, Eden Prairie, Minnesota, USA), frozen in a liquid N_2 dry shipper in the field, and stored at −80 °C in the lab prior to sample analysis. Digital photographs were obtained to identify sampled individuals using dorsal fin identification (Urian et al., 1999). The remote biopsy sampling was conducted under National Oceanic and Atmospheric Administration (NOAA) Scientific Research Permit Number 14450 and NOAA IACUC approval (Atlantic IACUC-2017-001) issued to Keith D. Mullin, NOAA Southeast Fisheries Science Center.

Biopsy sample analysis

Full-depth blubber samples were extracted and analyzed using gas chromatography/mass spectrometry (GC/MS) for POPs as described previously (Schwacke et al., 2014; Sloan et al., 2014). Briefly, approximately 0.4 g of minced blubber was dried with sodium and magnesium sulfate and extracted with dichloromethane on an accelerated solvent extractor. The extract was cleaned on a gravity flow column containing alumina/silica to remove polar compounds. The pre-cleaned sample extract was further cleaned using size-exclusion chromatography to remove lipids prior to GC/MS analysis. Percent lipid was determined gravimetrically as described in Sloan et al. (2014) and lipid classes (i.e. sterol esters/wax esters, triglycerides, free fatty acids, cholesterol, phospholipids) were determined in a 1 mL subsample of pre-cleaned sample extract using thin-layer chromatography with flame ionization detection (TLC-FID) (Ylitalo et al., 2001, Sloan et al., 2014). Duplicate TLC-FID analyses were conducted for each sample extract and the percent contribution of each lipid class to the sum of the five classes was determined for each sample run. Then, for each lipid class, the average percent contribution between the two sample runs was reported.

Concentrations of POPs are reported as μg/g (lipid weight). In total, 77 compounds were analyzed including 45 PCB congeners (Σ PCB) (IUPAC PCB numbers 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 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, 200, 201, 202, 205, 206, 207, 208, and 209), 15 PBDE congeners (Σ PBDE) (28, 47, 49, 66, 85, 99, 100, 153, 154, 155, 183, two unidentified pentabrominated diphenyl ethers, one unidentified hexabrominated diphenyl ether, and one unidentified heptabrominated diphenyl ether), 6 DDT isomers (Σ DDT) (o,p′-DDD, DDE, and DDT; and p,p′-DDD, DDE, and DDT), 8 CHLs (Σ CHL) (alpha chlordane, cis-nonachlor, beta chlordane, heptachlor, heptachlor epoxide, nonachlor III, oxychlordane, and trans-nonachlor), hexachlorobenzene (HCB), dieldrin, and mirex. Summed POPs (Σ POPs) included all 77 analyzed compounds and total organochlorine pesticides (Σ OCPs) included the sum of Σ DDTs, Σ CHLs, HCB, dieldrin, and mirex.

Samples were extracted, cleaned, and analyzed by GC/MS in sample batches of 10–13 field samples, as well as one solvent (dichloromethane) method blank and a National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM) 1945 Organics in Whale Blubber. Individual analyte concentrations measured in NIST SRM 1945 were in excellent agreement with reference values published by NIST. The concentration of each analyte measured in the NIST SRM 1945 was, on average, within 16% of the published NIST certified value. The lower limit of quantitation (LOQ) for each analyte was defined as the greater of either the analyte mass in the lowest detectable calibration solution divided by the sample mass, or the analyte's average mass detected in blanks plus three times the standard deviation (Sloan et al., 2014). The LOQ values for PCB congeners ranged from < 0.00067 μg/g (wet weight) to < 0.0080 μg/g (wet weight). For chlorinated pesticides and PBDEs, the LOQ values ranged from < 0.00066 μg/g, wet weight to < 0.0084 μg/g, wet weight and < 0.00068 μg/g, wet weight to < 0.0084 μg/g, wet weight, respectively.

Photographic-identification and ranging pattern classification

Photo-ID sighting records were compiled for surveys conducted in 2015–2017 in the St. Andrew Bay study area (Table 1). The photo-ID sighting records included those collected during capture-recapture surveys (Balmer et al., 2019) as well as remote biopsy sampling surveys. Capture-recapture surveys utilized photo-ID of individuals' dorsal fins to estimate abundance during a given sampling period. During this effort, a 6.3 m, center-console vessel was used at a survey speed of approximately 30 km/h to search for dolphins along pre-defined transects in the BSE and CST waters of the study area (transect lines and survey methodology detailed in Balmer et al., 2019). At least three observers, including the operator, were required, with each observer covering 60° of the 180° forward of the vessel. A sighting was recorded any time a dolphin was encountered. Sighting data were recorded onto a data sheet and included: time, location (GPS coordinates), total number of dolphins, group behavior(s), and various observational and environmental parameters (reviewed in Melancon et al., 2011). A Canon EOS-1DX (Canon USA Inc., Melville, New York, USA) with a 100–400 mm telephoto lens (or comparable digital camera and lens) was used to capture dorsal fin images of each individual in the group. Effort was made to photograph all dolphins within a sighting (full photo coverage) regardless of distinctiveness.

Remote biopsy survey effort followed a similar methodology to that of capture-recapture with a few modifications. The primary goal of remote sampling was to efficiently and safely collect samples from free-ranging dolphins in the study area. With these considerations in mind, remote biopsy sightings were conducted opportunistically (i.e. not following defined transect lines), and targeted individual dolphins or groups in which age class (e.g. no neonates in group and/or calves that consistently remained in echelon position) and dolphin behavior (e.g. directional travel with multiple surfacing events) maximized the likelihood of safely collecting a sample. Dorsal fin images were obtained of every remote biopsy sampled individual and compared to the St. Andrew Bay photo-ID catalog, consisting of 558 distinctive individuals (Balmer et al., 2019).

For both types of survey effort, all digital photographs were downloaded and sorted using protocols discussed in Speakman et al. (2010). A standardized approach was used to grade photographic quality and dorsal fin distinctiveness (Urian et al., 2014). Photographic quality of the best left and/or right side dorsal fin image was graded based upon the focus, contrast, angle, dorsal fin visibility, and proportion of the dorsal fin within the image frame. Digital dorsal fin images with a Q-1 (excellent) or Q-2 (good) quality grade were included in data analyses; images with a Q-3 (poor) grade were excluded. A distinctiveness rating (D1-very distinctive, D2-moderately distinctive, D3-not distinctive) was given to each sighting of all identified individuals, as agreed upon by two experienced researchers (BQ and TS). The same two experienced researchers matched and verified all individual dorsal fins. Photographs and associated sighting data were entered into FinBase (Adams et al., 2006), a customized Microsoft Access (Microsoft Corporation, Redmond, Washington, USA) database.

Photo-ID records from the capture-recapture and remote biopsy survey efforts were used to analyze individuals' sighting histories and classify each sampled individual into one of three ranging patterns. In this study, a ranging pattern is defined as the geographic photo-ID sighting history for an individual dolphin within the study area. If all photo-ID sightings of a biopsy sampled individual were in either the BSE or CST subarea, they were identified as having a “BSE” or “CST” ranging pattern, respectively. Biopsy sampled individuals sighted in both subareas were identified as having a “BOTH” ranging pattern.

Statistical analyses

Adult female cetaceans transfer the majority of their body burden of lipophilic contaminants (approximately 80%) to their calves through lactation (Cockcroft et al., 1989; Yordy et al., 2010). In contrast, male cetaceans have no substantive mechanism for offloading contaminants, thus body burdens remain relatively stable or increase over a male's lifespan (Wells et al., 2005; Yordy et al., 2010). Accordingly, POP concentrations were reported separately for males and females.

Concentrations of POPs from all remote blubber samples were lipid normalized to reduce variation related to lipid content (Struntz et al., 2004), and then log transformed to meet the statistical assumptions of equal variance and normality.

Prior to statistical analyses, concentration values below the LOQ were replaced with ½ of the LOQ value and analytes with a detection rate of $< 75\%$ were removed (Kucklick et al., 2011). POP data were separated by species, and for common bottlenose dolphins, a multivariate analysis of variance (MANOVA; JMP 14.1.0, SAS Institute, Cary, North Carolina, USA) with all contaminant classes (Σ PCB, Σ DDT, Σ CHL, Σ PBDE, HCB, dieldrin, mirex; dependent variables), and sex (female, male) and ranging pattern (BSE, CST, BOTH) (independent variables) was conducted. When the MANOVA indicated a significant multi-variate effect, a two-way ANOVA including sex and ranging pattern as factors was performed for each contaminant class to provide insight into the differences identified by the MANOVA. When the F-statistic was significant for ranging pattern in the ANOVApairwise comparisons were made using Tukey's Honestly Significant Difference (HSD) test.

The location of the Tyndall Air Force Base CERCLA site in Fred Bayou (30.0875 N, −85.5866 W) (Fig. 1) has been identified as a point source for DDT contamination (ATSDR, 2000), and was used as a reference point to compare photo-ID sighting histories and DDT levels for each remotely sampled common bottlenose dolphin in the study area. The closest on-water distance between each sighting and the reference point was determined using the "Measure" tool in ArcMap 10.6 (ESRI, Redlands, California, USA). For each individual biopsied dolphin, the mean distance to the point source was determined from that dolphin's photo-ID sighting history. Linear regression analysis was performed separately for males and females to examine the relationship between Σ DDT and mean sighting distance from the point source.

Results

Remote biopsy samples were collected from 74 small cetaceans in the St. Andrew Bay study area: common bottlenose dolphin (N = 28, ♀; N = 42, ♂) and Atlantic spotted dolphin (N = 2, ♀; N = 2, ♂) (Table 1, Fig. 1). Sampled individuals were sighted a mean ± S.D. of 4 ± 2 times with a range of 1–11 sightings. Of the 70 common bottlenose dolphin biopsy samples, 42 (N = 24, ♀; N = 18, ♂) were sighted exclusively in the BSE subarea, 24 were sighted exclusively in the CST subarea (N = 15, ♀;N = 9, ♂), and four were sighted in BOTH subareas (N = 3, ♀; N = 1, ♂). Of the four Atlantic spotted dolphin biopsy samples, all were sighted exclusively in the CST subarea.

POP class concentrations followed a similar general trend across species, sex, and ranging pattern with Σ PCB being the highest, followed by Σ DDT, Σ CHL, and Σ PBDE, with mirex, dieldrin, and HCB all at lower levels (Table 2). BSE male common bottlenose dolphins had the highest mean concentrations across all POP classes with Σ PCB closely followed by Σ DDT as the individual POP classes with highest concentrations. For common bottlenose dolphins, the MANOVA revealed a highly significant effect of sex [$F (9, 58) = 9.9272$, $P < 0.0001$], and ranging pattern [$F (18, 116) = 9.9112$, $P < 0.0001$, Wilk's $\Lambda = 0.0155$] on contaminant concentrations. The follow-up two-way ANOVA yielded significant differences across sex and ranging patterns on contaminant concentrations. Male common bottlenose dolphins had significantly higher mean concentrations across all POP classes than concentrations measured in females ($P < 0.0001$). For both male and female common bottlenose dolphins, Σ POP (♂, $P < 0.0001$; ♀, $P = 0.0038$), Σ OCP (♂, $P < 0.0001$; ♀, $P = 0.0004$), Σ PCB (♂, $P = 0.0034$; ♀, $P = 0.0133$), and Σ DDT (♂, $P < 0.0001$; ♀, $P = 0.0002$) were significantly higher in the BSE ranging pattern than the CST. Dieldrin was also significantly higher in the male BSE ranging pattern than the CST, but not in females (♂, $P = 0.0127$; ♀, $P = 0.1288$). There was no significant difference in ranging pattern and Σ PBDE (♂, $P = 0.2906$; ♀, $P = 0.4440$), mirex (♂, $P = 0.3614$; ♀, $P = 0.8124$), and HCB (♂, $P = 0.5159$; ♀, $P = 0.3684$) for both male and female common bottlenose dolphins. For males, the relationship between Σ DDT and mean sighting distance from the Tyndall Air Force Base CERCLA site was negative ($R^2 = 0.2449$; $P = 0.0012$) but there was no relationship for females ($R^2 = 0.0085$; $P = 0.6415$) (Fig. 2).

Discussion

Small cetacean populations throughout the world continue to be exposed to, and impacted by elevated levels of POPs (e.g. Jepson et al., 2016; Gui et al., 2017; Murphy et al., 2018). Differences in POP concentrations and profiles have been identified on both broad (e.g. Northern and Southern hemispheres: Aguilar et al., 2002) and fine (e.g. adjacent bays and coastal waters: Westgate and Tolley, 1999) spatial scales. Photo-ID is increasingly being utilized as a tool to better understand fine-scale impacts associated with anthropogenic stressors (e.g. Litz et al., 2007; Adams et al., 2008; Balmer et al., 2011; Titcomb et al., 2017; Genov et al., 2018).

This study utilized photo-ID in combination with chemical contaminant data obtained from remote biopsy samples to demonstrate differences in mean POP concentrations between northern Gulf of Mexico common bottlenose dolphins in BSE and adjacent CST waters. Based upon photo-ID sighting histories from remote biopsied individuals, BSE dolphins are likely members of the St. Andrew Bay Stock while CST dolphins are presumably members of the Northern Coastal Stock. The site fidelity and movement patterns of these two different stocks might factor in the identified differences in POP levels. The dolphins identified as members of the St. Andrew Bay Stock show high site fidelity (sighted across seasons and years) to the localized estuarine waters with limited movements outside of the St. Andrew Bay study area (Balmer et al., 2019). In contrast, the members of the Northern Coastal Stock show lower site fidelity to the St. Andrew Bay study area with extended movements along the coast (Balmer et al., 2016; Balmer et al., 2019). The physical features of St. Andrew Bay, including shallow depth, high salinity as a result of reduced freshwater inflow, minimal tidal flushing, and sediment composition, make this system vulnerable to accumulating higher levels of pollution (Brim and Handley, 2002). Thus, the higher contaminant concentrations measured in BSE dolphins are likely a result of prolonged, direct exposure to point (e.g. CERCLA Sites) and non-point (e.g. agricultural and urban runoff) source pollutants associated with development in the region and run-off into estuarine waters. The lower contaminant levels measured in CST dolphins are likely attributed to individuals residing in a more open water habitat that is farther from these anthropogenic contaminant sources.

For both male and female common bottlenose dolphins, the majority of POP class concentrations were generally similar to, or lower than, those measured in other southeastern U.S. field sites (Kucklick et al., 2011; Balmer et al., 2015). However, BSE male dolphins were identified as having the highest Σ DDT levels (67 μg/g, 50–89 μg/g; geometric mean and 95% CI) in the southeastern U.S. (Kucklick et al., 2011; Balmer et al., 2015) and some of the highest levels globally (reviewed in Gui et al., 2018) with one sampled individual's Σ DDT concentration measured as 164 μg/g. There was a significant negative relationship ($P = 0.0012$) between Σ DDT and mean sighting distance from the Tyndall Air Force Base CERCLA site for male common bottlenose dolphins. However, the strength of the association ($R^2 = 0.2449$) for this relationship was weak. Although these results should be taken cautiously, there is an indication that DDT exposure may be, at some level, associated with the proximity to this site. A similar, stronger relationship was observed with common bottlenose dolphins near a Superfund Site, associated with extremely high levels of PCBs, in Brunswick, Georgia, in which dolphins with ranging patterns closer to this site had higher levels of site-specific PCB contaminants (Balmer et al., 2011). There was no relationship between Σ DDT and mean sighting distance to the Tyndall Air Force Base CERCLA site for female common bottlenose dolphins. This is likely due to adult female cetaceans offloading contaminants through lactation (Cockcroft et al., 1989; Ylitalo et al., 2001; Yordy et al., 2010), thereby preventing an assessment of the exposure of contaminants in females' blubber over time.

While only four Atlantic spotted dolphins were sampled, these represent the first published POP data for this species in the northern Gulf of Mexico. Based upon color phase, all four appeared to be juvenile (speckled) to young adult (mottled) (reviewed in Herzing, 1997). Méndez-Fernandez et al. (2018) reported POP concentrations for Atlantic spotted dolphins in four field sites throughout the Atlantic Ocean. The POP levels measured from the four individuals in the northern Gulf of Mexico are on the lower end of the range from this more robust study. Although admittedly a small sample size, the low POP levels identified could potentially be due to the age class for these sampled individuals, with the two females not reaching sexual maturity and thus, not had the opportunity to offload contaminants through lactation. Future research to collect additional remote biopsy samples from Atlantic spotted dolphins in the northern Gulf of Mexico would enhance our knowledge of POP exposure in this stock/species.

Remote biopsy sample analyses and photo-ID data are increasingly being used in parallel to provide further insights into small cetacean biology and management. For example, Wilson et al. (2017) utilized stable isotope values from remote biopsy skin samples and photo-ID sighting histories of common bottlenose dolphins in St. George Sound (~150 km east of St. Andrew Bay) to identify two different groups of dolphins in the region: high site fidelity dolphins preying primarily upon pinfish (*Lagodon rhomboides*), pigfish (*Orthopristis chrysoptera*), and mojarra (*Eucinostomus gula*), and low site fidelity dolphins preying primarily upon silver perch (*Bairdiella chrysoura*). These differences in prey selection may be indicative of different stocks (BSE = high site fidelity; CST = low site fidelity) overlapping in St. George Sound. Similarly, Rosel et al. (2017) utilized genetic assignment tests from skin samples collected from remote biopsies and strandings of common bottlenose dolphins to differentiate between the Barataria Bay Stock and adjacent Western Coastal Stock, near Grand Isle, Louisiana. In addition, Thomas et al. (2017) developed an integrated framework incorporating these genetic results with stable isotope ratios to differentiate CST versus BSE source stocks across the northern Gulf of Mexico. In addition to the skin and blubber samples used for sexing and POP concentrations, respectively, in this study, each remote biopsy sample was subsampled further for retrospective projects including stable isotopes and fine-scale genetic analyses. Future research incorporating these analyses with the photo-ID and POP data would provide additional insight into differences between BSE and CST dolphins in the northern Gulf of Mexico.

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Author contributions

The authors are a mix of field and laboratory researchers that at a minimum did two of the following: 1) photo-ID survey and remote biopsy sampling design [Brian Balmer (BB), Stephanie Watwood (SW), Brian Quigley (BQ), Keith Mullin (KM), Patricia Rosel (PR), Teri Rowles (TR), Todd Speakman (TS), Eric Zolman (EZ), and Lori Schwacke (LS)], 2) collection of photo-ID data and remote biopsy samples (BB, BQ, TS, and EZ), 3) genetics and POP laboratory analyses [Gina Ylitalo (GY), Jennie Bolton (JB), Lynsey Wilcox (LW) and PR], 4) photo-ID analyses (BQ and TS), 5) photo-ID and POP statistical analyses (BB), and 6) drafted and/or edited sections of the manuscript (BB, GY, SW, BQ, JB, KM, PR, TR, TS, LW, EZ, and LS).

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Table 1 Photographic-identification (photo-ID) sighting and remote biopsy sampling effort from 2015 to 2017 in the St. Andrew Bay study area.

Date	Survey type	Total # of field days	Total # of dolphins sighted	Total # of remote biopsy samples obtained
13–21 Jul 2015	Photo-ID	9	241	
22–25, 27–29 Jul 2015	Remote biopsy	7	148	27
12–18 Oct 2015	Photo-ID	7	396	
19–23 Oct 2015	Remote biopsy	5	66	12
18–21, 23–27 Apr 2016	Photo-ID	9	461	
13–20 Oct 2016	Photo-ID	8	432	
21–25 Oct 2016	Remote biopsy	5	124	15
11–12, 14–15, 19 Jul 2017	Photo-ID	5	121	
17–18, 20–22 Jul 2017	Remote biopsy	5	85	20

Table 2

Persistent organic pollutant (POP) concentrations (μg/g lipid; geometric mean, 95% CI) and percent lipid content (geometric mean, 95% CI) measured in remote biopsy samples from 70 common bottlenose dolphins (N = 42, ♂; N = 28, ♀) and four Atlantic spotted dolphins (N = 2, ♂; N = 2, ♀). Both species were grouped by sex and common bottlenose dolphins were also grouped by ranging pattern (exclusively BSE, exclusively CST, or both BSE and CST). Statistically significant values from the two-way ANOVA are in bold and statistically homogeneous groups determined using Tukey's Honestly Significant Difference (HSD) test are indicated by shared letter superscripts.1

	Lipid	Σ POP[1]	Σ OCP[2]	Σ PCB[3]	Σ DDT[4]	Σ CHL[5]	Σ PBDE[6]	Mirex	Dieldrin	HCB[7]
T. truncatus males BSE (N = 24)	0.2^{A} (0.2–0.3)	150^{A} (110–190)	70^{A} (53–93)	70^{A} (53–93)	67^{A} (50–89)	2.6^{A} (2.0–3.5)	1.5^{A} (1.2–1.9)	0.2^{A} (0.1–0.3)	0.2^{A} (0.1–0.2)	0.0^{A} (0.0–0.0)
CST (N = 15)	0.2^{A} (0.1–0.4)	42^{B} (25–69)	13^{B} (7.6–22)	28^{B} (17–45)	11^{B} (6.1–18)	1.7^{A} (1.2–2.6)	1.1^{A} (0.7–1.8)	0.3^{A} (0.2–0.4)	0.1^{B} (0.1–0.2)	0.0^{A} (0.0–0.0)
BOTH (N = 3)	0.4^{A} (0.2–0.6)	120^{A,B} (100–140)	49^{A} (40–61)	68^{A,B} (57–82)	44^{A} (35–59)	3.8^{A} (3.0–4.8)	2.2^{A} (1.2–3.9)	0.3^{A} (0.2–0.5)	0.3^{A} (0.2–0.5)	0.0^{A} (0.0–0.1)
P-value (ranging pattern)	$P = 0.4197$	$P < 0.0001$	$P < 0.0001$	$P = 0.0034$	$P < 0.0001$	$P = 0.1136$	$P = 0.2906$	$P = 0.3614$	$P = 0.0127$	$P = 0.5159$
T. truncatus females BSE (N = 18)	0.3^{A} (0.2–0.4)	24^{A} (14–43)	11^{A} (6.1–21)	12^{A} (7.2–20)	11^{A} (5.7–20)	0.5^{A} (0.3–0.8)	0.3^{A} (0.1–0.5)	0.0^{A} (0.0–0.1)	0.0^{A} (0.0–0.1)	0.0^{A} (0.0–0.0)
CST (N = 9)	0.3^{A} (0.2–0.3)	3.5^{B} (1.2–9.6)	0.6^{B} (0.2–2.2)	2.5^{B} (0.9–6.9)	0.4^{B} (0.1–1.6)	0.1^{A} (0.0–0.3)	0.2^{A} (0.1–0.4)	0.0^{A} (0.0–0.1)	0.0^{A} (0.0–0.1)	0.0^{A} (0.0–0.0)
BOTH (N = 1)	0.3^{A}	5.8^{A,B}	2.2^{B}	3.5^{A,B}	1.9^{B}	0.2^{A}	0.1^{A}	0.0^{A}	0.0^{A}	0.0^{A}
P-value (ranging pattern)	$P = 0.9294$	$P = 0.0038$	$P = 0.0004$	$P = 0.0133$	$P = 0.0002$	$P = 0.0354$	$P = 0.4440$	$P = 0.8124$	$P = 0.1288$	$P = 0.3684$
S. frontalis										
Male (N = 2)	0.4 (0.3–0.4)	47 (18–37)	6.1 (4.0–9.1)	18 (12–26)	4.7 (2.9–7.6)	1.1 (0.9–1.2)	1.7 (1.6–1.8)	0.1 (0.1–0.2)	0.1 (0.1–0.1)	0.0 (0.0–0.0)
Female (N = 2)	0.2 (0.2–0.3)	3.6 (1.4–9.1)	0.9 (0.0–24)	4.0 (0.4–45)	0.6 (0.0–26)	0.1 (0.0–3.9)	0.2 (0.0–4.9)	0.0 (0.0–0.2)	0.1 (0.0–0.1)	0.0 (0.0–0.0)

[1] Σ POP is the sum of all measured persistent organic pollutant compounds.

[2] Σ OCP includes the sum concentration of all ΣDDTs, ΣCHLs, HCB, mirex and dieldrin.

[3] Σ PCB includes the sum concentrations of 45 PCB congeners. See Balmer et al. (2015) for a complete list.

[4] Σ DDT includes the sum concentrations of *o,p'*-DDD, DDE, and DDT; and *p,p'*-DDD, DDE, and DDT.

[5] Σ CHL includes the sum concentrations of alpha chlordane, *cis*-nonachlor, beta chlordane, heptachlor, heptachlor epoxide, nonachlor III, oxychlordane, and *trans*-nonachlor.

[6] Σ PBDE includes the sum concentrations of PBDEs 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, 183, Br5DE04, Br5DE05, Br6DE01, and Br7DE01 (unidentified congeners with 5, 6, or 7 bromines).

[7] Hexachlorobenzene

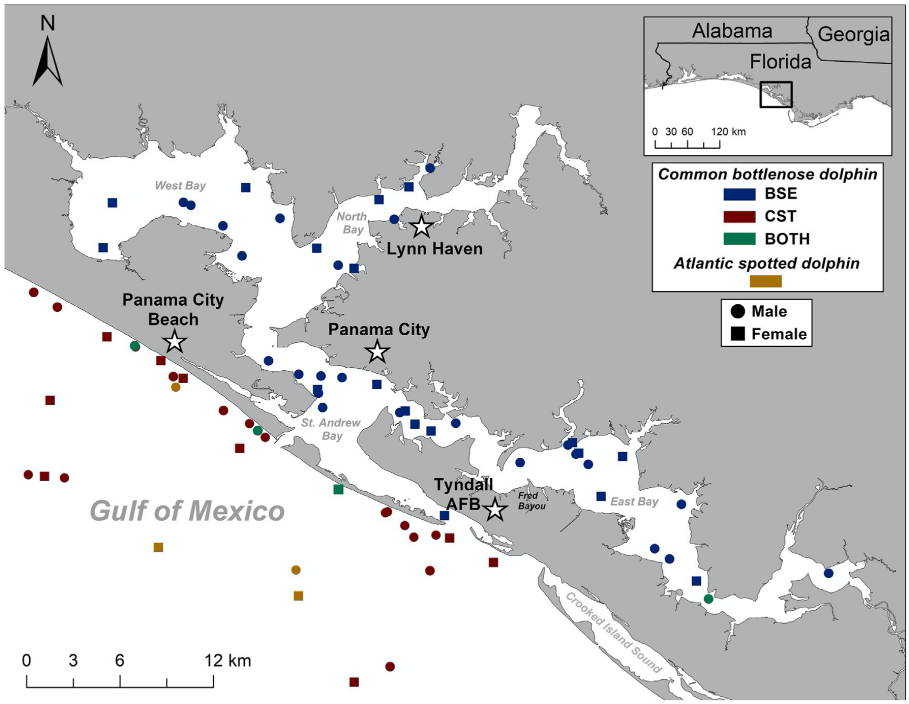

Fig. 1. St. Andrew Bay study area remote biopsy sampling locations during 2015–2017, grouped by species, sex, and ranging pattern.

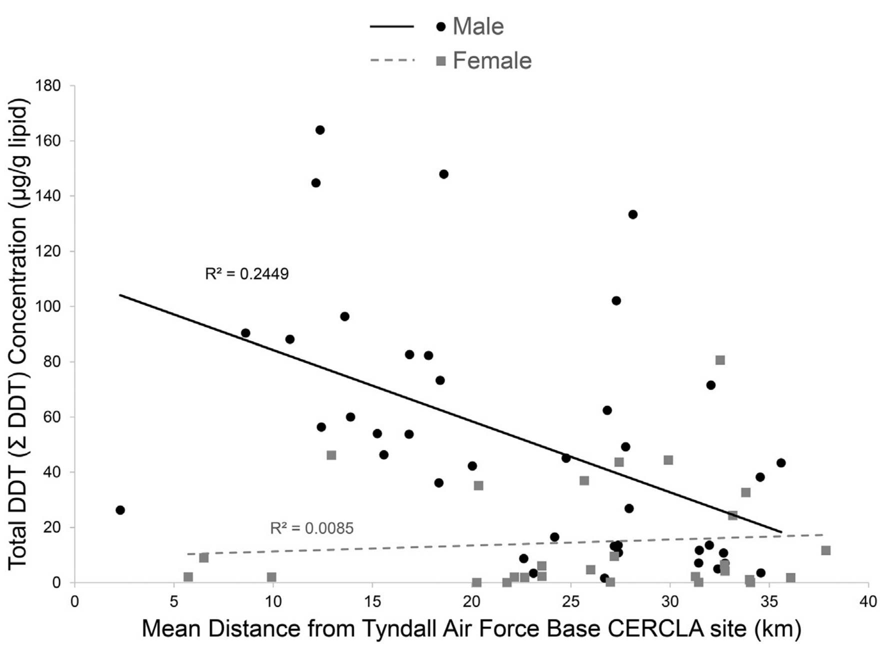

Fig. 2. Relationship between Σ DDT found in blubber from all biopsy sampled common bottlenose dolphins (N = 70), grouped by sex, and mean sighting distance from Tyndall Air Force Base Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) site.