Examining the relationships between blubber steroid hormones and persistent organic pollutants in common bottlenose dolphins

Thomas M.Galligan[ab] Brian C.Balmer[c] Lori H.Schwacke[c] Jennie L.Bolton[d] Brian M.Quigley[c] Patricia E.Rosel[e] Gina M.Ylitalo[d] Ashley S.P.Boggs[f]

[a] Medical University of South Carolina, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC, 29412, USA

[b] Virginia Polytechnic Institute and State University, Department of Fish and Wildlife Conservation, 310 West Campus Drive, 101 Cheatham Hall, Blacksburg, VA, 24060, USA

[c] National Marine Mammal Foundation, 3419 Maybank Highway, Site B, Johns Island, SC, 29455, USA

[d] Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA, 98112, USA

[e] National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, 646 Cajundome Boulevard, Lafayette, LA, 70506, USA

[f] National Institute of Standards and Technology, Hollings Marine Laboratory, 331 Fort Johnson Rd, Charleston, SC, 29412, USA

Abstract

Odontocete cetaceans bioaccumulate high concentrations of endocrine disrupting persistent organic pollutants (POPs), including dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyltrichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD) – collectively DDTs – but few studies have explored DDTs-mediated endocrine disruption in cetaceans. Herein, we use remotely collected blubber biopsies from common bottlenose dolphins (Tursiops truncatus) inhabiting a site with high localized DDTs contamination to study the relationships between DDTs exposure and steroid hormone homeostasis in cetaceans. We quantified blubber steroid hormone concentrations by liquid chromatography-tandem mass spectrometry and blubber POP concentrations by gas chromatography-mass spectrometry. We detected six steroid hormones in blubber, including progesterone (P4), 17-hydroxyprogesterone (17OHP4), androstenedione (AE), testosterone (T), cortisol (F), and cortisone (E). Sampled dolphins (n = 62) exhibited exposure to DDT, DDE, DDD, chlordanes (CHLDs), mirex, dieldrin, hexachlorobenzene, polychlorinated biphenyls (PCBs), and brominated diphenyl ethers (BDEs). Using principal components analysis (PCA), we determined that blubber DDTs primarily loaded to the first principal component (PC1) explaining 81.6% of the total variance in POP exposure, while the remaining POPs primarily loaded to the PC2 (10.4% of variance). PC1 scores were negatively correlated with blubber T in males and blubber F in females, suggesting that exposure

to DDTs impacted androgen and corticosteroid homeostasis. These conclusions were further supported by observed negative correlations between T and o,p'-DDE, o,p'-DDD, and p,p'-DDD in males sampled in the fall, and between F and the six individual DDTs and ∑6DDTs in females. Overall, these results suggest that POP-mediated endocrine disruption may have occurred in this stock of dolphins, which could negatively impact their health and fitness. However, this study relied on uncontrolled incidental exposures, making it impossible to establish a causal relationship between DDTs exposure and endocrine effects. Importantly, this study demonstrates that remotely collected blubber biopsies are a useful matrix for studying endocrine disruption in marine mammals.

Keywords

Bottlenose dolphin
Blubber
DDT
Endocrine disruption
Marine mammal
Steroid hormone

1. Introduction

Common bottlenose dolphins (Tursiops truncatus) and other toothed whales (odontocetes) bioaccumulate high concentrations of endocrine disrupting persistent organic pollutants (POPs) due to their high trophic position, long lifespan, and maintenance of extensive lipid reserves in blubber, which puts these species at risk to experience endocrine disruption (Colborn and Smolen, 1996). The organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyltrichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD; collectively referred to as DDTs), a class of POPs, disrupt vertebrate steroid hormone homeostasis by impairing adrenal, gonadal, and placental steroidogenesis (Hart and Straw, 1971; Hart et al., 1971; Jönsson et al., 1993; Lund et al., 1988; Wójtowicz et al., 2007a; Wójtowicz et al., 2007b); hepatic steroid metabolism (Balazs and Kupfer, 1966; Haake et al., 1987; Kupfer et al., 1964; Nowicki and Norman, 1972; Welch et al., 1967; Welch et al., 1971; You et al., 2001); steroid signaling through steroid receptor agonism and antagonism (Clark et al., 1998; Danzo, 1997; Kelce et al., 1995; Nelson et al., 1978; Oien et al., 1997); and steroid transport (Cocco et al., 2004; Nader et al., 2006; van Seters and Moolenaar, 1991). These effects vary by species and individual DDTs. DDTs disrupt both the hypothalamo-pituitary-adrenal (HPA) and HP-gonadal (HPG) axes in vertebrates, and thereby have the capacity to affect homeostasis of all four classes of vertebrate steroid hormones – progestogens, androgens, estrogens, and corticosteroids. Steroid hormones play critical roles in regulating reproductive, stress, metabolic, and developmental physiology and behavior; thus, disruption of steroid hormone homeostasis can have major repercussions for animal health and fitness. Notably, only two studies have examined the impacts of DDTs exposure on cetacean steroid hormone homeostasis. Subramanian et al. (1987) reported that p,p'-DDE and circulating testosterone (T) concentrations were negatively correlated in male Dall's porpoises (Phocoenoides dalli), while aldosterone was not significantly correlated with DDE. Hoydal et al. (2017) found that plasma estrone (E1) was positively correlated with plasma p,p'-DDE in female long-finned pilot whales (Globicephala melas). Because odontocete

cetaceans experience high DDTs exposure (Colborn and Smolen, 1996), it is imperative that we further examine the effects of these exposures on cetacean endocrine function to better conserve these species.

To study POP-mediated endocrine disruption in cetaceans, in which lethal sampling and dosing with contaminants is prohibited in the United States and many other countries, investigators must utilize non-lethally collected sample matrices from animals experiencing incidental POP exposures. That is, access to internal endocrine organs (e.g. gonads, adrenal gland, etc.) is restricted, and investigators cannot control contaminant exposures. Internal tissues could be collected from marine mammals that die from stranding, legal hunting in other countries, or fishery bycatch, but such animals were experiencing acute stress and potentially other health issues immediately preceding death, which confounds physiological findings. Thus, we are largely limited to correlative studies linking systemic measures of POP exposure and endocrine status. Systemic endocrine disruption associated with POP exposure has been detected in several aquatic, long-lived predators, including the two cetaceans discussed above (Dall's porpoise and long-finned pilot whale), polar bears (Ursus maritimus), and American alligators (Alligator mississippiensis). In male polar bears, T, dihydrotestosterone (DHT), and F were negatively correlated with POP burdens (Ciesielski et al., 2017; Oskam et al., 2004; Oskam et al., 2003), while in females, pregnenolone (P5) and androstenedione (AE) were negatively correlated with hydroxylated polychlorinated biphenyls (OH-PCBs) (Gustavson et al., 2015). DDTs exposure was linked to abnormal circulating androgen and estrogen concentrations in male and female American alligators (Guillette Jr. et al., 1999; Guillette Jr. et al., 1995; Guillette Jr. et al., 1994; Guillette Jr. et al., 1996). While it is difficult to establish cause-effect relationships in studies involving incidental exposures – especially without access to internal tissues – these studies are uniquely capable of demonstrating the effects of contaminants under real-world conditions, which cannot be feasibly accomplished in controlled laboratory settings.Due to our specific interest in DDTs, we identified a stock of cetaceans that is likely experiencing elevated incidental DDTs exposure. We selected the common bottlenose dolphins inhabiting St. Andrew Bay (SAB), Florida, USA (Fig. 1). SAB contains a U.S. Environmental Protection Agency National Priorities List (NPL; Superfund) site classified as such due to the presence of high levels of DDTs in sediment. When sampled in 1997, sediment from the NPL site within SAB exhibited total DDT (i.e., sum of the *o,p'* and *p,p'* isomers) concentrations ranging from less than 3.3 μg kg $^{-1}$ to 2800 μg kg $^{-1}$; total DDE ranging from less than 3.3 μg kg $^{-1}$ to 170 μg kg $^{-1}$; and total DDD ranging from less than 10 μg kg $^{-1}$ to 570 μg kg $^{-1}$ (US Department of Health and Human Services, 2000; US Environmental Protection Agency, 1997). Therefore, the bottlenose dolphins in SAB have likely experienced elevated DDTs exposures compared to other stocks in the southeastern United States (Balmer et al., 2015; Kucklick et al., 2011). Im portantly, the only sample matrices currently available from SAB dolphins are remotely collected blubber and skin biopsies, meaning any assessment of endocrine disruption in these animals must be performed with only these two matrices.

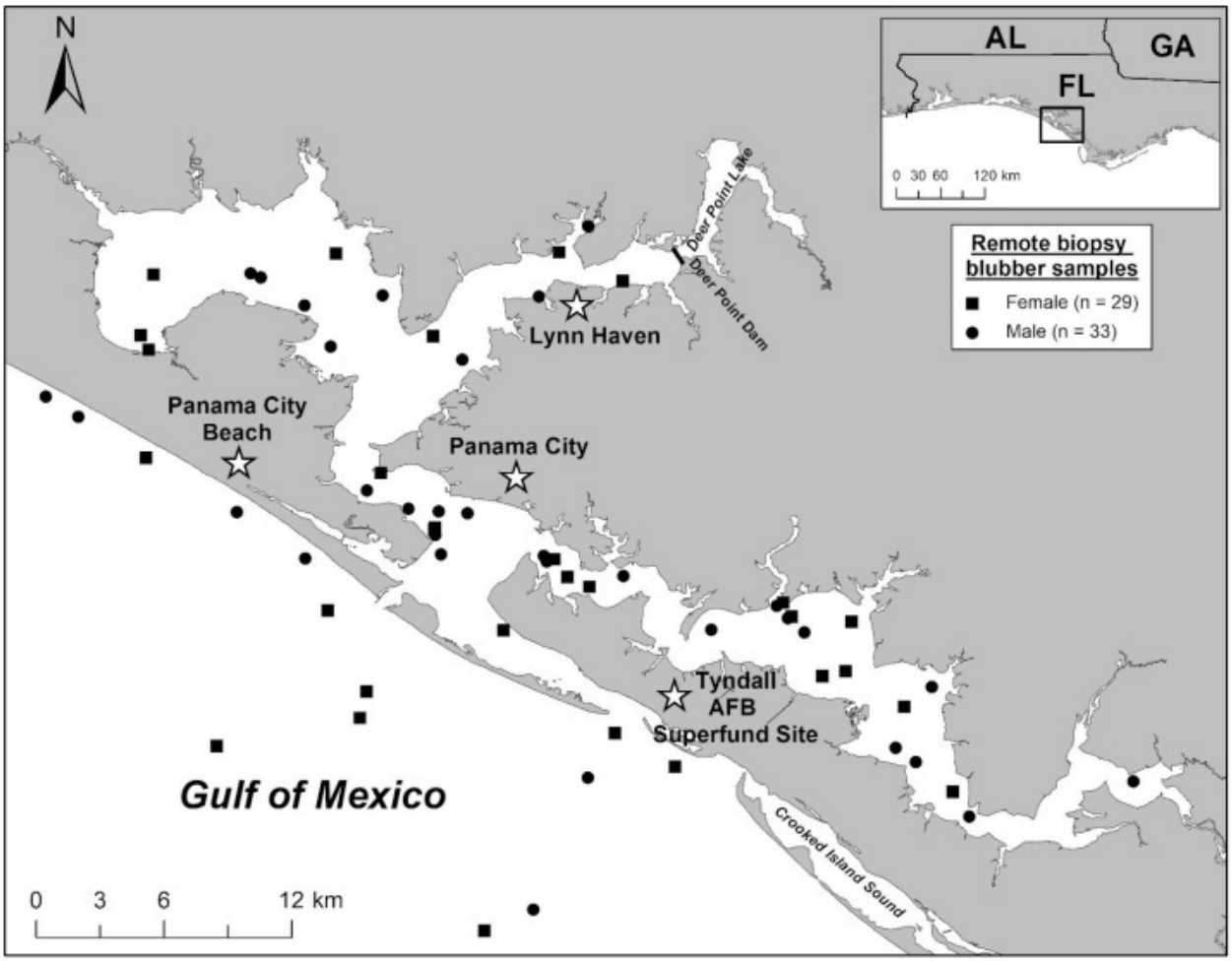

Fig. 1. Sampling distribution in St. Andrew Bay, FL and surrounding coastal waters. AFB = air force base.

Blubber is a hypodermic adipose tissue found in marine mammals. Lipophilic POPs partition into blubber due to its high lipid content, making it a commonly used matrix for POP exposure analysis in marine mammals (Balmer et al., 2015; Kucklick et al., 2011; Pedro et al., 2017; Struntz et al., 2004). Furthermore, cetacean blubber contains a variety of steroid hormones, including progesterone (P4), T, and cortisol (F), and the concentrations of these three hormones in blubber qualitatively reflect systemic physiological state. Specifically, in female cetaceans as in other mammals, progesterone secretion is elevated during pregnancy leading to elevated circulating and blubber progesterone concentrations (Kellar et al., 2006; Kirby and Ridgway, 1984; Mansour et al., 2002; Pérez et al., 2011; Sawyer-Steffan et al., 1983; Trego et al., 2013). In males, circulating T increases at sexual maturity and during breeding season (Harrison and Ridgway, 1971; Schroeder and Keller, 1989), which is reflected in blubber (Kellar et al., 2009). Finally, F concentrations increase in both blood and blubber following exposure to stress stimuli (Beaulieu-McCoy et al., 2017; Champagne et al., 2017; Houser et al., 2011; Kellar et al., 2015; Schroeder and Keller, 1989; Thomson and Geraci, 1986). Through the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS), Boggs et al. (2017) further demonstrated that common bottlenose dolphin blubber contains additional steroid hormones,

including 17-hydroxyprogesterone ($17OHP_4$), 11-deoxycortisol (S), 11-deoxycorticosterone (DOC), corticosterone (B), cortisone (E), and androstenedione (AE). This method also allows for measurement of estrogens, though these were not detected in bottlenose dolphin blubber (Boggs et al., 2017). Skin can be used to determine genotypic sex (Rosel, 2003), which is an important factor to consider because POP burdens and hormone profiles are likely to v ary by sex. Thus, with a single blubber and skin biopsy, investigators can study POP-mediated endocrine disruption in marine mammals.

The purpose of this study is to examine the relationships between POPs and steroid hormones in free-ranging bottlenose dolphins using remotely collected blubber and skin biopsies, with particular emphasis on DDTs. On a broad scale, this study advances our understanding of marine mammal endocrine disruption which will allow for improved mitigation of risks posed to cetaceans by POPs. On a fine-scale, cetaceans in the northern Gulf of Mexico continue to be exposed to cumulative stressors (National Academies of Sciences and Medicine, 2017) and a better understanding of the impacts associated with these stressors is essential for developing restoration strategies for impacted populations.

2. Methods

2.1. Animals and sample collection

Full-depth blubber and skin samples (approximately 1–2 cm in depth) were collected from 62 free-ranging common bottlenose dolphins in St. Andrew Bay, FL, USA and surrounding coastal waters (Fig. 1) by remote dart biopsy using a modified rifle or crossbow, as described in Balmer et al. (2015). Sampling occurred in July 2015 and October 2016. Immediately following sample collection, skin was removed from each biopsy and the blubber (approximately 0.8 g) was halved longitudinally. Skin was stored in 20% dimethyl sulfoxide (DMSO) saturated with sodium chloride at room temperature for genetic analyses, including sex identification. Sex of each individual was determined by molecular genetic analysis. Polymerase chain reaction was used to target X and Y chromosomes in DNA extracted from the skin, as described previously (Rosel, 2003), and was performed at the National Oceanic and Atmospheric Administration (NOAA) Southeast Fisheries Science Center, Lafayette, LA, USA. Blubber subsamples were frozen in a liquid nitrogen dry shipper in the field and stored at −80 °C until subsequent analyses. Blubber subsamples were sent to the National Institute of Standards and Technology (NIST) Environmental Specimen Bank at Hollings Marine Laboratory, Charleston, SC, USA (hormone analyses) and the NOAA Northwest Fisheries Science Center, Seattle, WA, USA (POP analyses).

2.2. Steroid hormone calibration and internal standards

Calibration (cal) and isotopically labelled internal standards (IS) were obtained from various suppliers (Supplemental Table 1). Cal and IS mixture solutions were diluted in methanol, with the concentration of each compound in the final mixtures expressed as a mass fraction (ng compound g−1 mixture).

2.3. Blubber steroid hormone extraction and analysis

Steroids were extracted from bisected remote blubber biopsies (approximately 400 mg of blubber) using a QuEChERS kit (Agilent, Santa Clara, CA, USA), which utilizes a salting out assisted liquid-liquid extraction (SALLE) followed by dispersive solid phase extraction (dSPE), per methods described in Boggs et al. (2017). Briefly, samples were minced in clean borosilicate beakers on dry ice, and then homogenized in water using a bead mill. Prior to homogenization, a known quantity of the IS mixture containing isotopically labelled standards (Supplemental Table 1) was amended to each tube for use in quantification as described below. We then processed homogenates through the SALLE portion of the QuEChERS protocol involving water, acetonitrile, and a kit-provided salt packet (Boggs et al., 2017). Resulting SALLE extracts were further processed through the dSPE portion of the QuECHERS protocol utilizing kit-provided 15 mL C18 dSPE tubes (Boggs et al., 2017). These dSPE extracts were dried, reconstituted in 2 mL water:acetonitrile (80:20, volume fraction), and filtered through a 0.22 μm cellulose acetate filter. Filtrate was dried and then reconstituted in 200 μL methanol. Blanks and cals were extracted identically.

Steroids were then quantified by LC-MS/MS using an Agilent 1200 Series HPLC system with a binary pump and an autosampler linked to an AB Sciex (Framingham, MA, USA) API 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer, per methods described by Galligan et al. (2018). Briefly, progestogens, androgens, and estrogens were separated using a Restek (Bellefonte, PA, USA) Ultra Biphenyl column (250 mm × 4.6 mm, 5 μm particle size) with a gradient of acetonitrile and methanol (both with 0.1% formic acid), while corticosteroids were separated using an Agilent Eclipse Plus C18 column (150 mm × 21 mm, 5.0 μm particle size) and a gradient of methanol and water (both with 0.1% acetic acid). Prior to corticosteroid analysis, an aliquot of extract was solvent exchanged to 50:50 methanol:water (volume fraction) to match gradient starting conditions. Prior to estrogen analysis, an aliquot of extract was dansyl chloride derivatized, per methods described by Galligan et al. (2018), to improve ionization. Two transitions were monitored per compound; the transition with the stronger signal was used for quantification while the other was used to confirm compound identity (Supplemental Table 1).

Sciex Analyst software (version 1.5) was used to integrate chromatographic peaks for quantification. Ratios of target compound peak area to IS peak area were interpolated on standard curve regressions, which were calculated from the extracted cals using the quantitative transition for each analyte (Supplemental Table 2). Each standard curve was comprised of at least three points and fully encompassed the range of measured values (Supplemental Table 2). Reporting limits (RLs) were defined two different ways: observed reporting limits (RLobs) were defined as the lowest calibration standard in the calibration curve; calculated reporting limits (RLcalc) were calculated as three times the standard deviation of the extracted blank measurements plus the mean of these blank measurements (Supplemental Table 1); the larger of these two values was utilized as the censoring threshold, as has been done previously (Alava et al., 2011; Boggs et al., 2016; Boggs et al., 2017; Hoguet et al., 2013; Keller et al., 2012; Ragland et al., 2011; Ragland et al., 2014; Stewart et al., 2011).

2.4. Blubber contaminant extraction and analysis

POP analysis was performed using methods that have been described previously (Balmer et al., 2015; Sloan et al., 2014). Briefly, contaminants were extracted with dichloromethane using

accelerated solvent extraction (ASE) after the blubber was dried with magnesium and sodium sulfate. Prior to sample extract cleanup, a 2 mL fraction was removed for gravimetric total lipid determination as described in Sloan et al. (2014). Polar compounds and lipids were removed from the extracts using a gravity flow column followed by size-exclusion high-performance liquid chromatography. POPs were measured by gas chromatography-mass spectrometry (GC-MS). Included in this analysis were six DDTs (p,p'- and o,p'-DDT, DDE, and DDD), 45 polychlorinated biphenyls (PCBs), 15 brominated diphenyl ethers (BDEs), eight chlordanes (CHLDs), hexachlorobenzene (HCB), dieldrin, and mirex. Individual POPs measured within these classes are the same as those measured in Balmer et al. (2015). Only 11 of the 15 BDE congeners were detected in blubber samples. In the following analyses and discussion, Σ6DDTs, Σ45PCBs, Σ11BDEs, and Σ8CHLDs will refer to the summed concentrations within each of these POP classes. Contaminant values are reported as concentrations (ng of contaminant g−1 of lipid). Measured ranges of POP concentrations are reported in Table 1.

Table 1. Summary of persistent organic pollutant (POP) measurements in St. Andrew Bay common bottlenose dolphin blubber.

	Male					Female				
POP	Min. (ng g^{-1} lipid)	Med. (ng g^{-1} lipid)	Max. (ng g^{-1} lipid)	Freq. (%)	% of $\sum_6$DDTs	Min. (ng g^{-1} lipid)	Med. (ng g^{-1} lipid)	Max. (ng g^{-1} lipid)	Freq. (%)	% of $\sum_6$DDTs
p,p'-DDT	4.8	150	1700	100	1.4	<1.8	150	2300	83	3.1
p,p'-DDE	510	13 000	31 000	100	91	8.5	1900	52 000	100	84
p,p'-DDD	3.2	650	3700	100	5.1	61	570	5700	83	9.6
o,p'-DDT	<1.6	81	650	97	0.70	<1.8	55	940	83	1.0
o,p'-DDE	2.4	150	410	100	1.0	<1.8	53	350	83	1.3
o,p'-DDD	<1.4	64	460	94	0.50	<1.8	53	640	83	0.90
$\sum_6$DDTs	520	14 000	34 000	100	NA	8.5	3000	62 000	100	NA
$\sum_8$CHLDs	170	590	2200	100	NA	<2.0	200	1400	97	NA
$\sum_{45}$PCBs	1800	15 000	46 000	100	NA	57	3600	26 000	100	NA
$\sum_{11}$BDEs	62	360	800	100	NA	<2.1	130	710	93	NA
Mirex	13	45	130	100	NA	<2.0	16	94	93	NA
Dieldrin	8.7	34	130	100	NA	<1.2	25	170	79	NA
HCB	2	6.3	25	100	NA	<1.2	7.4	33	76	NA

Min. = minimum, med. = median, max. = maximum, Freq. = detection frequency, NA = not applicable (does not contribute to $\sum_6$DDTs measurement).

2.5. Statistical analyses

IBM (Armonk, NY, USA) SPSS Statistics 24 software was used for all statistical analyses. Principal components analysis (PCA) was used to explore patterns among blubber POP measurements. Values (ng g^{-1} lipid) below RL were assigned a random value between zero and

RL. Data were log10 transformed to minimize potential influence of extreme values before being mean centered at zero and unit scaled. Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy for the whole set and for each individual variable was greater than 0.5; and Bartlett's test of sphericity was significant ($p < 0.05$) (Dziuban and Shirkey, 1974). Factors with eigenvalues greater than 1 were extracted. Varimax rotation was utilized to simplify interpretation. Inclusion of each individual contaminant in the analysis precluded the PCA because many of the contaminants are very highly correlated, leading to a non-positive definite relationship. Thus, included in the PCA were the six individual DDTs, mirex, dieldrin, and HCB, ∑45PCBs, ∑8CHLDs, and ∑11BDEs. Factor loading greater than 0.3 was considered a significant contribution to the variance explained by each PC. Relationships among PC scores and hormones were analyzed by Kendall's tau-b correlations.

We analyzed the relationships between individual hormones and month, pregnancy status, POPs, and PC scores with non-parametric tests on untransformed data in which hormone values below RL were censored to zero, such that all values below RL would be tied in these rank-based tests. These analyses were also stratified by sex because POP exposure and hormone profiles are expected to vary by sex. Samples were collected in two months, July and October. In males, where seasonal variation in hormone profiles is expected, differences in hormone measurements by month were examined by Mann-Whitney U test. In females, animals with blubber P4 values in or exceeding the range for pregnant animals published by Perez et al. were classified as “likely pregnant” (Pérez et al., 2011). F did not differ by pregnancy status but AE did. Thus, analysis of AE in females was stratified by pregnancy status. Kendall's tau-b correlation was used to examine the correlations between hormones and POPs as well as the potentially confounding relationship between blubber F and percent lipid.

3. Results

A total of 29 female (July 2015: n = 17, October 2016: n = 12) and 33 male (July 2015: n = 16, October 2016: n = 17) remote biopsy samples were collected from common bottlenose dolphins inhabiting the bay, sound, and estuary waters of and coastal waters near St. Andrew Bay (Fig. 1). All samples are from unique individuals, as determined by photoidentification and genetics. Due to the remote nature of these sampling efforts, no additional demographic or morphometric data (e.g., age, length, etc.) were collected.

All individuals demonstrated quantifiable concentrations of DDTs (Table 1). Males exhibited quantifiable concentrations of all remaining contaminant classes as well, while fewer than 100% of females had detectable levels of chlordanes, BDEs, mirex, dieldrin, and HCB (Table 1). Two principal components (PCs) were extracted explaining 81.6% and 10.4% of the total variance in POP exposure, respectively (eigenvalues: PC1 = 10.609, PC2 = 1.354). The six DDTs loaded strongly to PC1, and the DDEs also loaded to PC2 (Table 2). The remaining POP variables ($\sum_{45}$PCBs, $\sum_{8}$CHLDs, $\sum_{15}$BDEs, HCB, dieldrin, and mirex) loaded primarily to PC2, although all except mirex also loaded moderately (>0.3) to PC1 (Table 2). PC1 scores were negatively correlated with T in males (Fig. 2A) and F in females (Fig. 2B).Table 2. POP principal components analysis (PCA) variable loading values. Each variable's primary loading is bolded.

Variable	Principal Component 1	Principal Component 2
p,p'-DDT	**0.934**	0.318
p,p'-DDE	**0.683**	0.672
p,p'-DDD	**0.918**	0.348
o,p'-DDT	**0.898**	0.390
o,p'-DDE	**0.791**	0.582
o,p'-DDD	**0.926**	0.299
$\sum_8$CHLDs	0.469	**0.856**
$\sum_{45}$PCBs	0.525	**0.817**
$\sum_{15}$BDEs	0.411	**0.819**
Mirex	0.108	**0.968**
Dieldrin	0.645	**0.671**
HCB	0.473	**0.632**

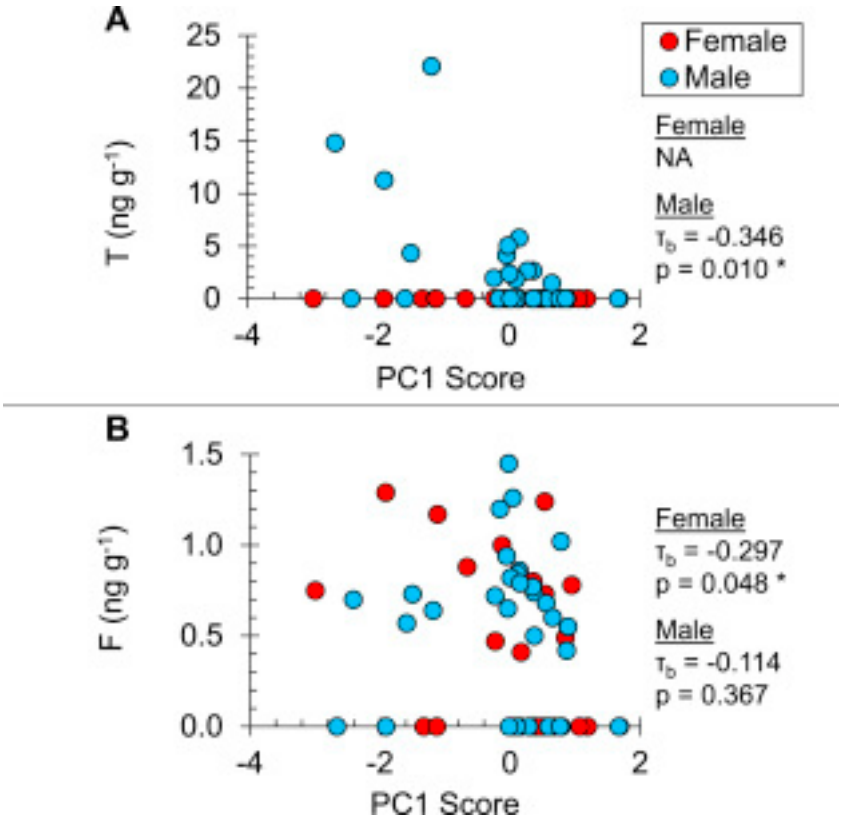

Fig. 2. Kendall's tau-b correlation between principal component 1 (PC1) score and (A) blubber testosterone (T) and (B) blubber cortisol (F) concentration by sex. Hormone values below reporting limit are censored to 0. * indicates significant correlation ($p < 0.05$).

Six steroid hormones were detected in this study, including T, AE, P4, 17OHP4, F, and E, while the remaining five hormones (B, S, DOC, E1, and estradiol [E2]) were not detected in any samples (Table 3). In males, T, AE, and F were most commonly detected (Table 3). T and AE concentrations varied significantly by month in males, with both being higher in October than in

July, while F did not (Table 3). P_4, $17OHP_4$, and E were rarely detected in males (Table 3). In males sampled in October, T exhibited significant negative correlations with *o,p'*-DDD, *o,p'*-DDE, *p,p'*-DDD, and dieldrin (Fig. 3). In males sampled in July, only three out of 16 individuals exhibited quantifiable T (Table 3). AE and F exhibited no significant correlations with individual DDTs, $\sum_6$DDTs, $\sum_{45}$PCBs, $\sum_8$CHLDs, $\sum_{15}$BDEs, HCB, dieldrin, or mirex in males (not shown).

Table 3. Blubber steroid hormone value ranges and detection frequency in St. Andrew Bay common bottlenose dolphins by demographic group. Reported p-values indicate result of Mann-Whitney *U* test comparing hormones in males by month and in females by pregnancy status. * indicates significant difference ($p < 0.05$).

	Male: July (n = 16)				Male: October (n = 17)				
Hormone (Abbreviation)	Min. (ng g^{-1})	Med. (ng g^{-1})	Max. (ng g^{-1})	Freq. (%)	Min. (ng g^{-1})	Med. (ng g^{-1})	Max. (ng g^{-1})	Freq. (%)	p-value
Androstenedione (AE)	< RL	1.2	3.89	75	< RL	3.0	9.78	94	0.014 *
Corticosterone (B)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
Cortisol (F)	< RL	0.75	1.5	69	< RL	0.55	0.82	65	0.063
Cortisone (E)	< RL	< RL	0.50	31	< RL	< RL	< RL	0	0.127
11-Deoxycorticosterone (DOC)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
11-Deoxycortisol (S)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
Estradiol (E_2)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
Estrone (E_1)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
17-Hydroxyprogesterone ($17OHP_4$)	< RL	< RL	< RL	0	< RL	< RL	4.64	12	0.581
Progesterone (P_4)	< RL	< RL	340	6.3	< RL	< RL	< RL	0	0.763
Testosterone (T)	< RL	< RL	4.1	19	< RL	1.8	22	59	0.037 *
	Female: Non-pregnant (n = 20)				Female: Pregnant (n = 9)				
Hormone (Abbreviation)	Min. (ng g^{-1})	Med. (ng g^{-1})	Max. (ng g^{-1})	Freq. (%)	Min. (ng g^{-1})	Med. (ng g^{-1})	Max. (ng g^{-1})	Freq. (%)	p-value
Androstenedione (AE)	< RL	< RL	0.65	5	< RL	2.2	19	67	0.004 *
Corticosterone (B)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
Cortisol (F)	< RL	0.61	1.3	65	< RL	< RL	1.1	22	0.077
Cortisone (E)	< RL	< RL	0.39	10	< RL	< RL	< RL	0	0.694
11-Deoxycorticosterone (DOC)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
11-Deoxycortisol (S)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND

Hormone (Abbreviation)	Female: Non-pregnant (n = 20)				Female: Pregnant (n = 9)				p-value
	Min. (ng g^{-1})	Med. (ng g^{-1})	Max. (ng g^{-1})	Freq. (%)	Min. (ng g^{-1})	Med. (ng g^{-1})	Max. (ng g^{-1})	Freq. (%)	
Estradiol (E_2)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
Estrone (E_1)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
17-Hydroxyprogesterone ($17OHP_4$)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND
Progesterone (P_4)	< RL	< RL	< RL	0	44	220	600	100	NV
Testosterone (T)	< RL	< RL	< RL	0	< RL	< RL	< RL	0	ND

Min. = minimum measured value; Med. = median measured value; Max. = maximum measured value; Freq. = detection frequency; < RL = value below reporting limit; ND = not detected; NV = comparison not valid among females because progesterone was used to assign pregnancy status.

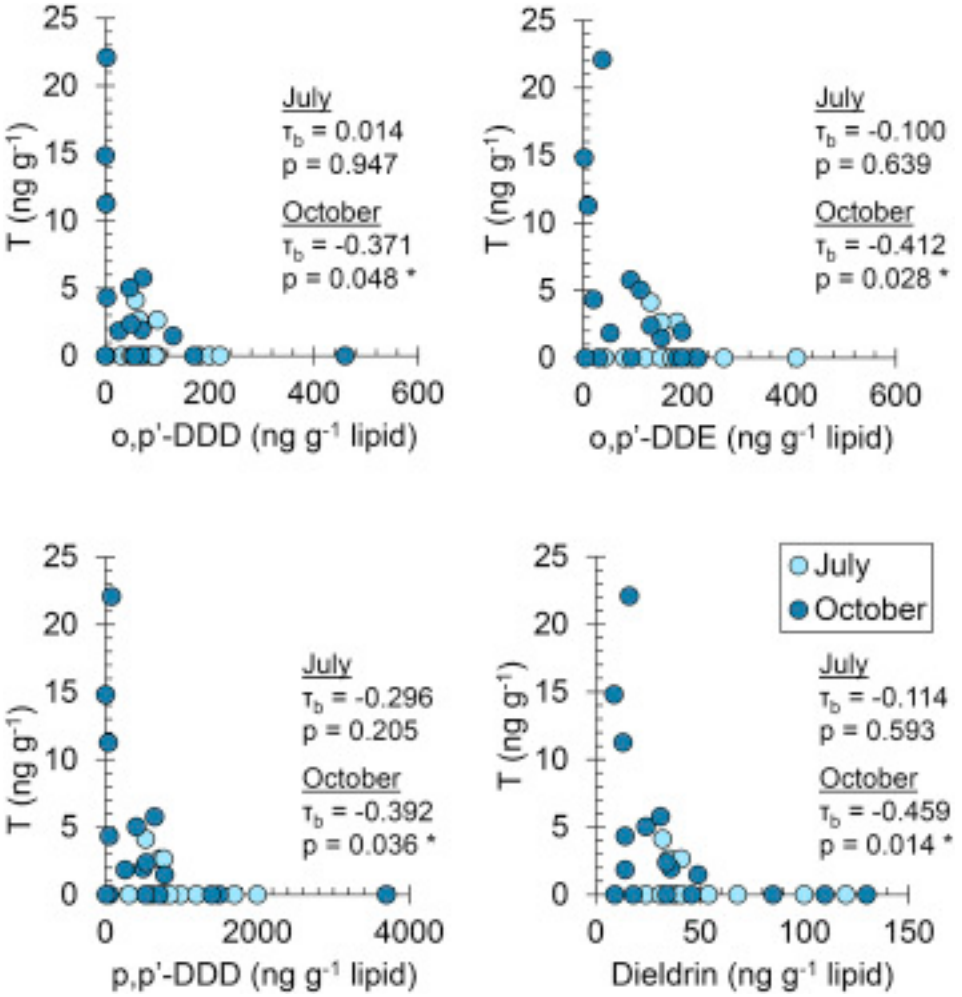

Fig. 3. Kendall's tau-b correlations between blubber testosterone (T) and o,p'-DDD, o,p'-DDE, p,p'-DDD, and dieldrin in males by month. Values below reporting limit are censored to 0. * indicates significant correlation ($p < 0.05$).

Blubber P4 values reported for pregnant females in Pérez et al. (2011) were used as a reference to identify possible pregnant animals in the current dataset. Females with blubber P4 values exceeding the threshold for pregnant animals (>32 ng g−1) were classified as pregnant (n = 9 [31%]). Only P4, F, and AE were commonly detected in females; the other hormones were rarely detected (Table 3). F in females did not vary significantly by pregnancy status (Table 3). F was negatively correlated with each of the individual DDTs (Fig. 4) as well as ∑45PCBs, ∑8CHLDs, ∑15BDEs, HCB, dieldrin, and mirex (Fig. 5, Fig. 6). AE did vary by pregnancy status, with only pregnant animals commonly exhibiting detectable AE (Table 3), but AE was not significantly

correlated with any POPs when stratified by pregnancy status (not shown).

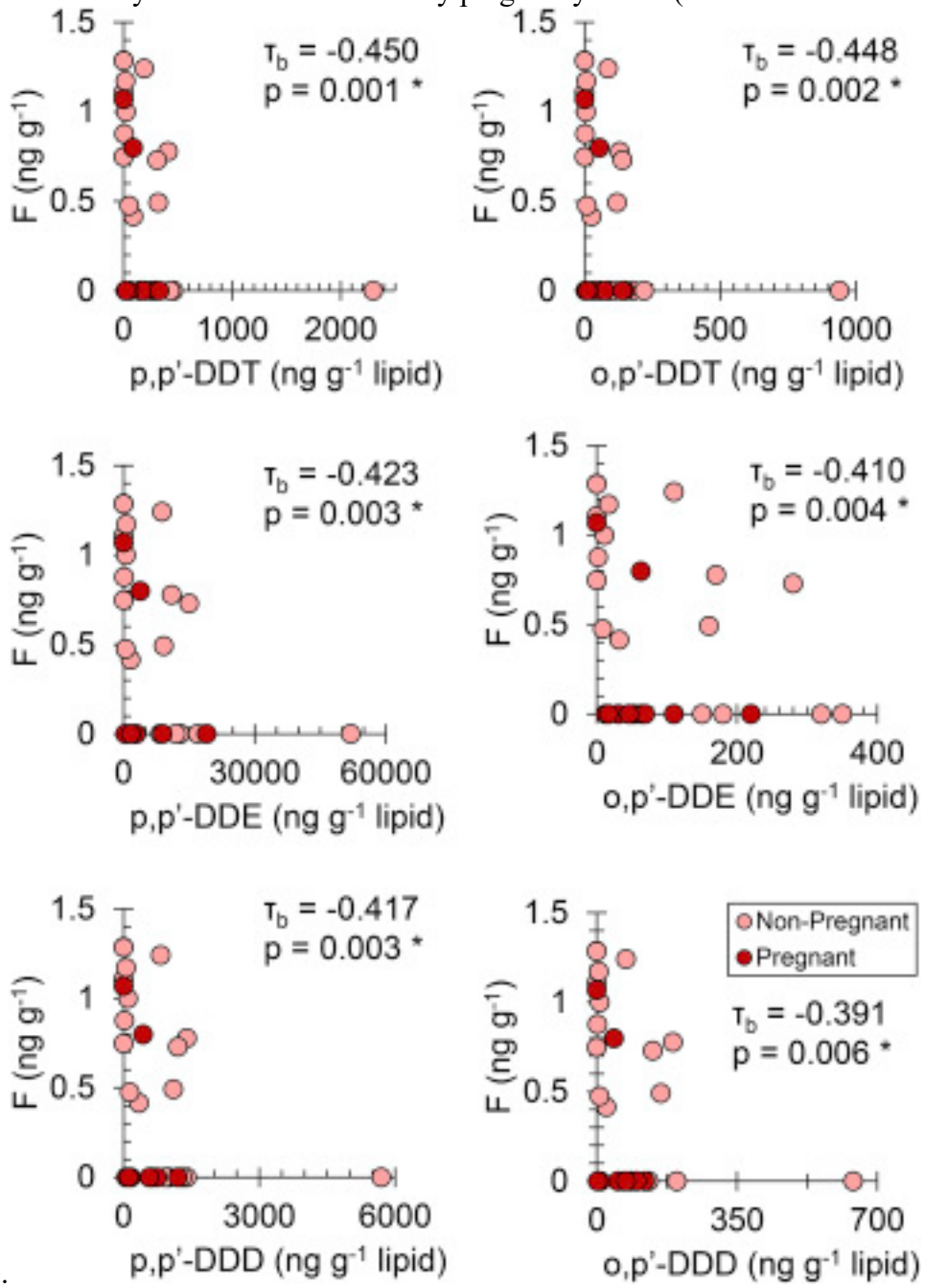

Fig. 4. Kendall's tau-b correlations between blubber cortisol (F) and the individual DDTs in females. Values below reporting limit are censored to 0. * indicates significant correlation ($p < 0.05$).

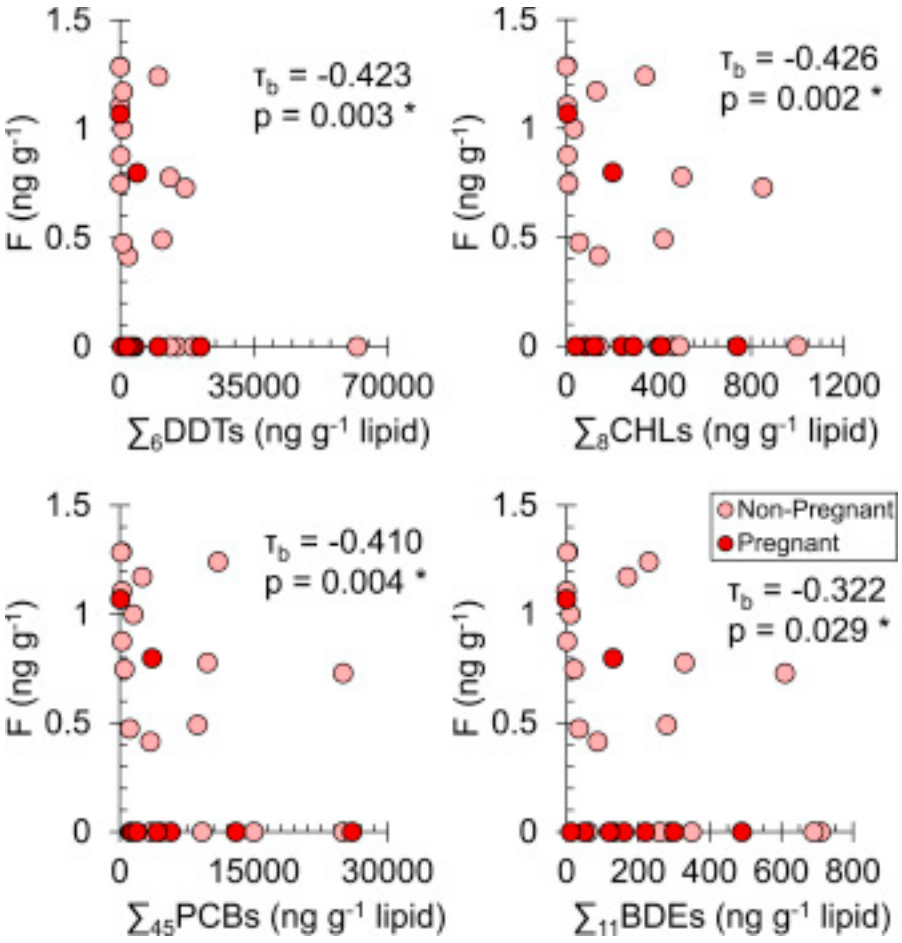

Fig. 5. Kendall's tau-b correlations between blubber cortisol (F) and summations of contaminants by class in females. Values below reporting limit are censored to 0. * indicates significant correlation ($p < 0.05$).

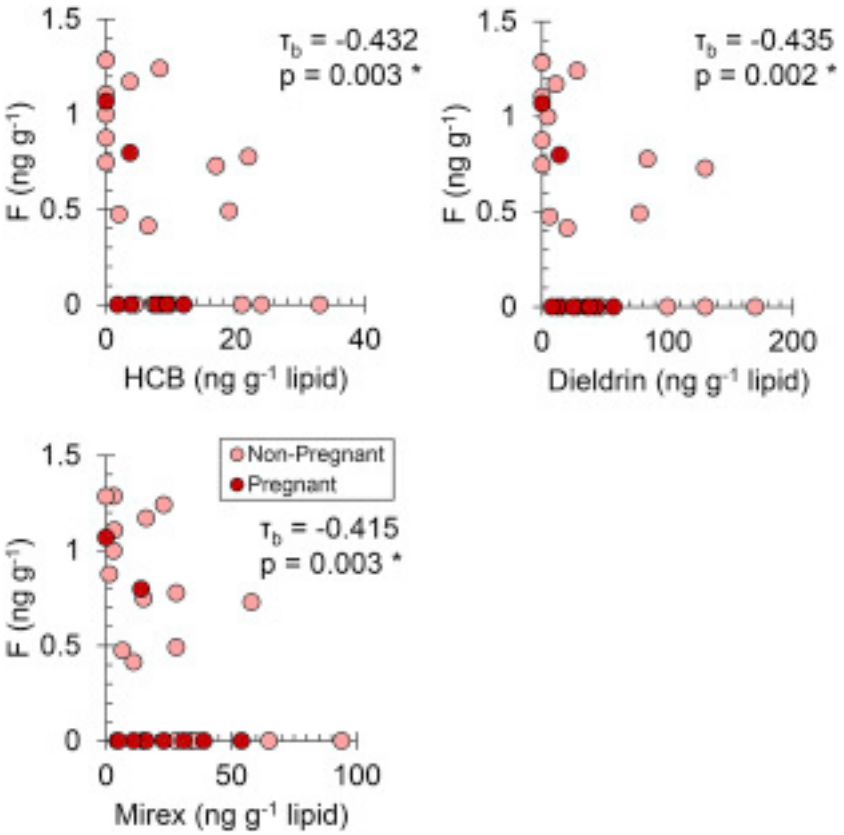

Fig. 6. Kendall's tau-b correlations between blubber cortisol (F) and HCB, dieldrin, and mirex in females. Values below reporting limit are censored to 0. * indicates significant correlation ($p < 0.05$).

Blubber F concentration and lipid percent were negatively correlated in both males and females (Fig. 7). Blubber percent lipid was not significantly associated with PC1 scores (not shown).

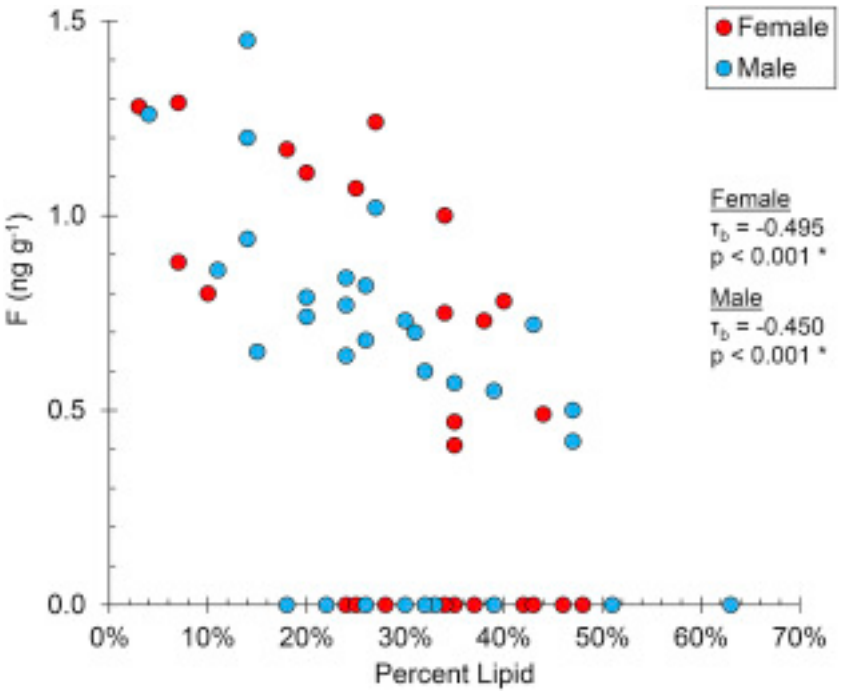

Fig. 7. Kendall's tau-b correlations between blubber cortisol (F) and blubber percent lipid by sex. Values below reporting limit are censored to zero. * indicates significant correlation ($p < 0.05$).

4. Discussion

To our knowledge, this is the first study of DDTs-mediated endocrine disruption in bottlenose dolphins. We examined the relationships between steroid hormones and POPs in the blubber of common bottlenose dolphins inhabiting a US EPA Superfund site. We first explored the relationships among the measured POPs by PCA, and found that the six DDTs measured in blubber all loaded to the first principal component (PC1), while the remaining POP classes (PCBs, PBDEs, CHLDs, mirex, HCB, and dieldrin) loaded to PC2. This suggests that variation in DDTs exposure contributes the majority of the variance in total POP exposure measured in this study, which confirms that SAB is a suitable stock for assessing DDTs exposure specifically. However, these relationships elucidated by PCA are not absolute, meaning it is impossible to consider the effects of DDTs exposure independently of the remaining POPs. Nonetheless, since the DDTs contributed most strongly to PC1, we used PC1 scores to initially examine of the relationships between collective DDTs exposures and blubber steroid hormone values, while we use PC2 scores to assess relationships with non-DDTs POPs. T (in males) and F (in females) are negatively correlated with PC1 scores but not PC2 scores, which suggests that androgen and corticosteroid homeostasis were affected by DDTs exposure specifically. We stratified this and the remaining analysis by sex because POP exposure and endocrine profiles vary by sex in common bottlenose dolphins. Male and female common bottlenose dolphins exhibit differential POP exposure profiles because females offload significant portions of their POP burden during lactation when they mobilize lipid reserves – and thus, the POPs stored within – to support milk production, while males have no such life history trait which would provide the opportunity to offload POPs (Aguilar et al., 1999; Wells et al., 2005). Therefore, POP measures in males can be considered an estimate of their lifetime exposure to POPs, whereas in post-parturient females

they cannot (Yordy et al., 2010). Without detailed life history data, such as age, parity, and number of lactations, it is impossible to estimate lifetime exposure in females. As such, studying the impacts of POP exposure in this species requires sex-specific interpretation.

We further explored the relationship between T and POPs by assessing bivariate correlations between T and the individual DDTs, ∑6DDTs, and the other POP classes in males. Based on the relationships observed in Dall's porpoise and other marine predators, we predicted that T and DDE would be negatively correlated in male common bottlenose dolphins (Ciesielski et al., 2017; Oskam et al., 2004; Oskam et al., 2003; Subramanian et al., 1987). Notably, only 19% of males sampled in July had quantifiable T whereas 59% sampled in October had quantifiable blubber T. Since circulating and blubber T values are known to increase during breeding season in another cetacean species (Kellar et al., 2009), this variation by month may suggest that males in this study were more reproductively active during fall than summer. In other Gulf of Mexico stocks, breeding season occurs in spring, not fall, but other stocks in the southeastern U.S. exhibit bimodal breeding patterns, with peaks in spring and summer or fall (McFee et al., 2014; Urian et al., 1996). Our findings suggest that the SAB stock may have a fall breeding season in addition to spring. The three animals with the highest T concentrations, which seem to be driving these relationships, were all sampled in coastal waters in close proximity to SAB. This may indicate that the Northern Coastal Stock, which range includes the coastal waters near SAB, has a different breeding pattern than the bay, sound, and estuary stocks in this region. Testing these hypotheses would require additional remote sampling of the SAB and Northern Coastal stocks and surveying of these regions throughout the year. It is important to note that July and October samples were collected in different years, meaning that observed differences by month may actually be differences by year. Alternatively, it is possible that a disproportionately high number of sexually immature individuals – who would be expected to have low T values – were sampled in July 2016 while more sexually mature individuals were sampled in October 2015. Without age information we cannot make this determination. In future studies, age could potentially be estimated by examining changes in blubber fatty acid composition (Herman et al., 2008; Herman et al., 2009; Marcoux et al., 2015) or changes in DNA methylation of remote skin samples (Polanowski et al., 2014), as performed in other cetaceans, or through using laser photogrammetry to measure dolphin length (Webster et al., 2010) in parallel with remote biopsy sampling. Nonetheless, there were significant differences in T in males by month, which prompted us to stratify analyses in males by month.

The apparent variation by month, regardless of whether it is a function of physiology or sampling bias, further complicates our analysis. In males sampled in October, T is negatively correlated with o,p'-DDE as well as both isomers of DDD and dieldrin, but similar patterns do not emerge in July, likely because only three males in July had quantifiable T. These results in October further suggest that high concentrations of DDD and/or o,p'-DDE negatively impact T homeostasis in male common bottlenose dolphins. If DDTs exposure had no impact on T homeostasis, one would expect a positive correlation between T and DDTs in males because T is linked to sexual maturity – i.e., males with higher T would likely be older (Galligan et al. 2018, in prep.), and should therefore exhibit higher DDTs measurements compared to males with lower T. Therefore, the negative correlations here may indicate that males with higher o,p'-DDE and DDD exposure experience impaired T secretion and/or elevated T metabolism compared to males with lower exposure. However, it is possible that dieldrin, other POPs, or a mixture of

POPs is responsible for disrupting T homeostasis, and these DDTs are only positively correlated with the causative contaminants. Similarly, perhaps DDTs and T are only correlated because they are each associated with an alternative causative agent that was not considered in this study, such as exposure to contaminants of emerging concern, additional stressors, or differences in foraging behavior, habitat use, or prey availability. This conclusion also assumes that the dose response curve is linear, which may not be the case for POP-mediated endocrine disruption. Without performing controlled dosing studies we cannot conclusively determine whether DDTs exposure impacts T homeostasis in male common bottlenose dolphins, but these results argue that further investigation is warranted. Because T plays an important role in regulating male reproductive biology, resource managers should consider examining reproductive endpoints in male dolphins from SAB, particularly in relation to other dolphin stocks with lower DDTs exposure.

We expected to observe a positive correlation between p,p'-DDE and E1 in females, because this relationship was observed in female long-finned pilot whale plasma (Hoydal et al., 2017). However, E1 was not detected in any samples in this study, which could be due to the limitations of our analytical method or differences in estrogen physiology between these two species.

We further explored the relationship between F and POPs by assessing bivariate correlations between F and the individual DDTs, ∑6DDTs, and the other POP classes in females. We observed significant negative correlations between F and the individual DDTs in females. Similar relationships have been observed in male polar bears, and several DDTs (specifically p,p'-DDT, p,p'-DDE, p,p'-DDD, and o,p'-DDD) have been shown to disrupt adrenal function in other mammals, including grey seals (Jönsson et al., 1992; Jönsson et al., 1993; Lund, 1994; Lund et al., 1988; Oskam et al., 2004; Young et al., 1973). Furthermore, adrenal abnormalities were observed in beluga whales from the St. Lawrence Estuary – a population which is characterized by high DDTs exposure – and Hudson Bay, but it remains unclear whether this phenomenon is causally linked to contaminant exposure (Lair et al., 1997; Martineau et al., 1987). As in males, these results are difficult to interpret because exposures are incidental and complex, and F is similarly correlated with the other POPs measured, making it impossible to consider DDTs independently of the other POPs. Interpretation is furthered hindered by the occurrence of lactational POP offloading in females and our lack of life history data. Additionally, it is important to note that F is likely involved in blubber lipolysis, as suggested by the negative correlation between blubber F and percent lipid reported herein, and by the relationships between F, expression of lipolytic genes, and fasting in phocids reported by Kershaw and Hall (2016) and Khudyakov et al. (2017). This may play a role in producing the observed negative correlation between blubber F and POPs.

We propose two mechanisms linking blubber POPs, F, and lipids. First, individuals with higher circulating F concentrations should exhibit higher blubber F concentrations than individuals with low circulating F. High circulating F would also lead to increased blubber lipolysis and, thereby, increased mobilization of POPs stored in blubber. This would produce a negative correlation between blubber F and POPs, which would not be indicative of POP-mediated endocrine disruption, but rather of the role F plays in regulating lipid homeostasis. Alternatively, the negative relationship between POPs and F may be directly caused by POP-mediated adrenal disruption. Animals (particularly males) with high total lifetime POP exposure would exhibit

high blubber POP concentrations and, therefore, may be more likely to experience adrenal disruption than those with lower blubber POPs. This in turn would lead to diminished F secretion and ultimately lower blubber F concurrent with high blubber POPs. It is important to note that PC1 and blubber percent lipid are not significantly correlated, suggesting that the relationship between PC1 and F is not driven by differences in lipid content. While not conclusive, these results should prompt further investigation of adrenal health in this stock, as well as the interactions between blubber lipid content, F, and POPs. Considering the important roles adrenal hormones play in vertebrate stress physiology, nutrient homeostasis, immune function, and development, POP-mediated adrenal disruption could have serious implications for bottlenose dolphin health and fitness.

Future studies should consider employing capture-release sampling methods as a means to more directly assess common bottlenose dolphin health (i.e., reproductive, stress, immunological, and developmental endpoints) and the impacts of anthropogenic stressors on common bottlenose dolphins in SAB and other regions in the southeastern U.S. In SAB, researchers should seek to integrate hormone and POP measurements with demographics, direct measures of reproductive physiology and success, stress response, and immune physiology to improve our understanding of DDTs-mediated endocrine disruption and its potential downstream effects on the health and fitness of cetaceans. Additionally, future work could assess other stocks with differential POP exposure profiles to better elucidate effects associated with DDTs versus other POPs.

5. Conclusions

The evidence presented herein suggests that DDTs are negatively impacting steroid hormone homeostasis in common bottlenose dolphins inhabiting SAB, though we are limited in our ability to establish causality due to the legal and logistical difficulties associated with performing toxicological studies in marine mammals. This study provides a critical basis for future studies to further assess the impacts of DDTs and other POPs on common bottlenose dolphin health.

This study demonstrates the utility of remotely collected blubber and skin biopsies for the assessment of POP-mediated endocrine disruption in cetaceans. Compared to capture-release studies, remote biopsy-based studies can provide a logistically feasible and economical methodology to identify cetacean populations experiencing endocrine disruption, which will greatly enhance our ability to assess the impacts of anthropogenic contaminants on marine mammal populations.

6. Compliance with ethical standards

St. Andrew Bay remote biopsy sampling was conducted under NMFS permit no. 14450-04 with protocols reviewed and approved by the NOAA National Marine Fisheries Service Atlantic IACUC.

Conflicts of interest

The authors declare that they have no conflict of interest in the publication of this manuscript. Commercial equipment, instruments, or materials are identified to specify adequately the

experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor the National Oceanographic and Atmospheric Administration, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Acknowledgements

Funding (including indirectly through provision of necessary equipment, personnel, and/or supplies): This work was supported through a multi-agency collaboration with the Navy Sea Systems Command, Naval Undersea Warfare Center, Newport Division (contract number: N00178-12-D-6974-3); NOAA National Ocean Service National Centers for Coastal Ocean Science; National Marine Mammal Foundation; NOAA NMFS Southeast Fisheries Science Center; NOAA NMFS Southeast Regional Office; NOAA NMFS Northwest Fisheries Science Center; National Institute of Standards and Technology; Medical University of South Carolina; Florida Department of Environmental Protection; Dolphin Relief and Research; Gulfarium Marine Adventure Park; and Gulf World Marine Park. We thank Dr. Teri Rowles of the NOAA Fisheries Office of Protected Resources for her financial support under the Marine Mammal Health and Stranding Response Program. We would like to acknowledge staff at NIST Charleston and the Environmental Specimen Bank at Hollings Marine Laboratory for archival of the specimens analyzed in this manuscript. We would like to thank Kevin Huncik (NIST) for assistance with instrument maintenance, support, and troubleshooting. We would also like to thank Kevin Barry, John Brusher, Penn Clarke, Larry Fulford, Keith Mullin, Carrie Sinclair, Todd Speakman, and Eric Zolman for their assistance with logistics, sampling methodology, and sample collection for the field component of this project. We thank Bernadita Anulacion, Daryle Boyd, Jonelle Herman, and Catherine Sloan from NOAA Northwest Fisheries Science Center for contaminant and data analyses.

References

Aguilar et al., 1999

A. Aguilar, A. Borrell, T. Pastor

Biological factors affecting variability of persistent pollutant levels in cetaceans

J. Cetacean Res. Manag., 1 (1999), pp. 83-116

Alava et al., 2011

J.J. Alava, J.M. Keller, J. Wyneken, L. Crowder, G. Scott, J.R. Kucklick

Geographical variation of persistent organic pollutants in eggs of threatened loggerhead sea turtles (Caretta caretta) from southeastern United States

Environ. Toxicol. Chem., 30 (2011), pp. 1677-1688

Balazs and Kupfer, 1966

T. Balazs, D. Kupfer

Effect of DDT on the metabolism and production rate of cortisol in the Guinea pig

Toxicol. Appl. Pharmacol., 9 (1966), pp. 40-43

Balmer et al., 2015

B.C. Balmer, G.M. Ylitalo, L.E. McGeorge, K.A. Baugh, D. Boyd, K.D. Mullin, P.E. Rosel, C. Sinclair, R.S. Wells, E.S. Zolman, L.H. Schwacke

Persistent organic pollutants (POPs) in blubber of common bottlenose dolphins (Tursiops truncatus) along the northern Gulf of Mexico coast, USA

Sci. Total Environ., 527–528 (2015), pp. 306-312

Beaulieu-McCoy et al., 2017

N.E. Beaulieu-McCoy, K.K. Sherman, M.L. Trego, D.E. Crocker, N.M. Kellar

Initial validation of blubber cortisol and progesterone as indicators of stress response and maturity in an otariid; the California sea lion (Zalophus californianus)

Gen. Comp. Endocrinol., 252 (2017), pp. 1-11

Boggs et al., 2016

A.S.P. Boggs, J.A. Bowden, T.M. Galligan, L.J. Guillette, J.R. Kucklick

Development of a multi-class steroid hormone screening method using Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS)

Anal. Bioanal. Chem., 408 (2016), pp. 4179-4190

Boggs et al., 2017

A.S.P. Boggs, T.B. Schock, L.H. Schwacke, T.M. Galligan, J.S. Morey, W.E. McFee, J.R. Kucklick

Rapid and reliable steroid hormone profiling in Tursiops truncatus blubber using liquid chromatography tandem mass spectrometry (LC-MS/MS)

Anal. Bioanal. Chem., 409 (21) (2017), pp. 5019-5029

Champagne et al., 2017

C.D. Champagne, N.M. Kellar, D.E. Crocker, S.K. Wasser, R.K. Booth, M.L. Trego, D.S. Houser

Blubber cortisol qualitatively reflects circulating cortisol concentrations in bottlenose dolphins

Mar. Mamm. Sci., 33 (2017), pp. 134-153

Ciesielski et al., 2017

T.M. Ciesielski, I.T. Hansen, J. Bytingsvik, M. Hansen, E. Lie, J. Aars, B.M. Jenssen, B. Styrishave

Relationships between POPs, biometrics and circulating steroids in male polar bears (Ursus maritimus) from Svalbard

Environ. Pollut., 230 (2017), pp. 598-608

Clark et al., 1998

E.J. Clark, D.O. Norris, R.E. Jones

Interactions of gonadal steroids and pesticides (DDT, DDE) on gonaduct growth in larval tiger salamanders, Ambystoma tigrinum

Gen. Comp. Endocrinol., 109 (1998), pp. 94-105

Cocco et al., 2004

P. Cocco, A. Loviselli, D. Fadda, A. Ibba, M. Melis, A. Oppo, S. Serra, A. Taberlet, M. Tocco, C. Flore

Serum sex hormones in men occupationally exposed to dichloro-diphenyl-trichloro ethane (DDT) as young adults

J. Endocrinol., 182 (2004), pp. 391-397

Colborn and Smolen, 1996

T. Colborn, M. Smolen

Epidemiological Analysis of Persistent Organochlorine Contaminants in Cetaceans, Reviews of Environmental Contamination and Toxicology

Springer (1996), pp. 91-172

Danzo, 1997

B.J. Danzo

Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins

Environ. Health Perspect., 105 (1997), p. 294

Dziuban and Shirkey, 1974

C.D. Dziuban, E.C. Shirkey

When is a correlation matrix appropriate for factor analysis? Some decision rules

Psychol. Bull., 81 (1974), pp. 358-361

Galligan et al., 2018

T.M. Galligan, L.H. Schwacke, D.S. Houser, R.S. Wells, T. Rowles, A.S.J.G. Boggs, c. endocrinology

Characterization of circulating steroid hormone profiles in the bottlenose dolphin (Tursiops truncatus) by liquid chromatography–tandem mass spectrometry (LC–MS/MS), 263 (2018), pp. 80-91

Guillette et al., 1999

L.J. Guillette Jr., J.W. Brock, A.A. Rooney, A.R. Woodward

Serum concentrations of various environmental contaminants and their relationship to sex steroid concentrations and phallus size in juvenile American alligators

Arch. Environ. Contam. Toxicol., 36 (1999), pp. 447-455

Guillette et al., 1995

L.J. Guillette Jr., T.S. Gross, D.A. Gross, A.A. Rooney, H.F. Percival

Gonadal steroidogenesis in vitro from juvenile alligators obtained from contaminated or control lakes

Environ. Health Perspect., 103 (1995), pp. 31-36

Guillette et al., 1994

L.J. Guillette Jr., T.S. Gross, G.R. Masson, J.M. Matter, H.F. Percival, A.R. Woodward

Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida

Environ. Health Perspect., 102 (1994), pp. 680-688

Guillette et al., 1996

L.J. Guillette Jr., D.B. Pickford, D.A. Crain, A.A. Rooney, H.F. Percival

Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment

Gen. Comp. Endocrinol., 101 (1996), pp. 32-42

Gustavson et al., 2015

L. Gustavson, T.M. Ciesielski, J. Bytingsvik, B. Styrishave, M. Hansen, E. Lie, J. Aars, B.M. Jenssen

Hydroxylated polychlorinated biphenyls decrease circulating steroids in female polar bears (Ursus maritimus)

Environ. Res., 138 (2015), pp. 191-201

Haake et al., 1987

J. Haake, M. Kelley, B. Keys, S. Safe

The effects of organochlorine pesticides as inducers of testosterone and benzo [a] pyrene hydroxylases

Gen. Pharmacol. Vasc. Syst., 18 (1987), pp. 165-169

Harrison and Ridgway, 1971

R.J. Harrison, S.H. Ridgway

Gonadal activity in some Bottlenose dolphins (Tursiops truncatus)

J. Zool., 165 (1971), pp. 355-366

Hart and Straw, 1971

M.M. Hart, J.A. Straw

Studies on the site of action of o,p'-DDD in the dog adrenal cortex. I. Inhibition of ACTH-mediated pregnenolone synthesis

Steroids, 17 (1971), p. 559

Hart et al., 1971

M.M. Hart, E.S. Swackhamer, J.A. Straw

Studies on the site of action of o,p'-DDD in the dog adrenal cortex. II. TPNH- and corticosteroid precursor-stimulation of o,p'-DDD inhibited steroidogenesis

Steroids, 17 (1971), p. 575

Herman et al., 2008

D.P. Herman, C.O. Matkin, M. Ylitalo, G, J.W. Durban, M.B. Hanson, M.E. Dahlheim, J.M. Straley, P.R. Wade, K.L. Tilbury, R.H. Boyer

Assessing age distributions of killer whale Orcinus orca populations from the composition of endogenous fatty acids in their outer blubber layers

Mar. Ecol. Prog. Ser., 372 (2008), pp. 289-302

Herman et al., 2009

D.P. Herman, G.M. Ylitalo, J. Robbins, J.M. Straley, C.M. Gabriele, P.J. Clapham, R.H. Boyer, K.L. Tilbury, R.W. Pearce, M.M. Krahn

Age determination of humpback whales Megaptera novaeangliae through blubber fatty acid compositions of biopsy samples

Mar. Ecol. Prog. Ser., 392 (2009), pp. 277-293

Hoguet et al., 2013

J. Hoguet, J.M. Keller, J.L. Reiner, J.R. Kucklick, C.E. Bryan, A.J. Moors, R.S. Pugh, P.R. Becker

Spatial and temporal trends of persistent organic pollutants and mercury in beluga whales (Delphinapterus leucas) from Alaska

Sci. Total Environ., 449 (2013), pp. 285-294

Houser et al., 2011

D.S. Houser, L.C. Yeates, D.E. Crocker

Cold stress induces an adrenocortical response in bottlenose dolphins (Tursiops truncatus)

J. Zoo Wildl. Med., 42 (2011), pp. 565-571

Hoydal et al., 2017

K.S. Hoydal, B. Styrishave, T.M. Ciesielski, R.J. Letcher, M. Dam, B.M. Jenssen

Steroid hormones and persistent organic pollutants in plasma from North-eastern Atlantic pilot whales

Environ. Res., 159 (2017), pp. 613-621

Jönsson et al., 1992

C.J. Jönsson, B.-O. Lund, Å. Bergman, I. Brandt

Adrenocortical toxicity of 3-methylsulphonyl-DDE; 3: studies in fetal and suckling mice

Reprod. Toxicol., 6 (1992), pp. 233-240

Jönsson et al., 1993

C.J. Jönsson, B.O. Lund, I. Brandt

Adrenocorticolytic DDT-metabolites: studies in mink, Mustela vison and otter, Lutra lutra

Ecotoxicology (London), 2 (1993), pp. 41-53

Kelce et al., 1995

W.R. Kelce, C.R. Stone, S.C. Laws, L.E. Gray, J.A. Kemppainen, E.M. Wilson

Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist

Nature, 375 (1995), pp. 581-585

Kellar et al., 2015

N.M. Kellar, K.N. Catelani, M.N. Robbins, M.L. Trego, C.D. Allen, K. Danil, S.J. Chivers

Blubber cortisol: a potential tool for assessing stress response in free-ranging dolphins without effects due to sampling

PLoS One, 10 (2015), p. e0115257

Kellar et al., 2009

N.M. Kellar, M.L. Trego, C.I. Marks, S.J. Chivers, K. Danil, F.I. Archer

Blubber testosterone: a potential marker of male reproductive status in short-beaked common dolphins

Mar. Mamm. Sci., 25 (2009), pp. 507-522

Kellar et al., 2006

N.M. Kellar, M.L. Trego, C.I. Marks, A.E. Dizon

Determining pregnancy from blubber in three species of delphinids

Mar. Mamm. Sci., 22 (2006), pp. 1-16

Keller et al., 2012

J.M. Keller, L. Ngai, J.B. McNeill, L.D. Wood, K.R. Stewart, S.G. O'Connell, J.R. Kucklick

Perfluoroalkyl contaminants in plasma of five sea turtle species: comparisons in concentration and potential health risks

Environ. Toxicol. Chem., 31 (2012), pp. 1223-1230

Kershaw and Hall, 2016

J.L. Kershaw, A.J. Hall

Seasonal variation in harbour seal (Phoca vitulina) blubber cortisol - a novel indicator of physiological state?

Sci. Rep., 6 (2016), p. 21889

Khudyakov et al., 2017

J.I. Khudyakov, C.D. Champagne, L.M. Meneghetti, D.E. Crocker

Blubber transcriptome response to acute stress axis activation involves transient changes in adipogenesis and lipolysis in a fasting-adapted marine mammal

Sci. Rep., 7 (2017), p. 42110

Kirby and Ridgway, 1984

V. Kirby, S. Ridgway

Hormonal evidence of spontaneous ovulation in captive dolphins, Tursiops truncatus and Delphinus delphis

Rep. Int. Whal. Comm., 6 (1984), pp. 459-464

Kucklick et al., 2011

J. Kucklick, L. Schwacke, R. Wells, A. Hohn, A. Guichard, J. Yordy, L. Hansen, E. Zolman, R. Wilson, J. Litz, D. Nowacek, T. Rowles, R. Pugh, B. Balmer, C. Sinclair, P. Rosel

Bottlenose dolphins as indicators of persistent organic pollutants in the Western North atlantic ocean and northern Gulf of Mexico

Environ. Sci. Technol., 45 (2011), pp. 4270-4277

Kupfer et al., 1964

D. Kupfer, T. Balazs, D.A. Buyske

Stimulation by o,p′-DDD of cortisol metabolism in the Guinea pig

Life Sci., 3 (1964), pp. 959-964

Lair et al., 1997

S. Lair, P. Beland, S. De Guise, D. Martineau

Adrenal hyperplastic and degenerative changes in beluga whales

J. Wildl. Dis., 33 (1997), pp. 430-437

Lund, 1994

B.-O. Lund

In vitro adrenal bio activation and effects on steroid metabolism of DDT, PCBs and their metabolites in the gray seal (Halichoerus grypus)

Environ. Toxicol. Chem., 13 (1994), pp. 911-917

Lund et al., 1988

B.-O. Lund, Å. Bergman, I. Brandt

Metabolic activation and toxicity of a DDT-metabolite, 3-methylsulphonyl-DDE, in the adrenal Zona fasciculata in mice

Chem. Biol. Interact., 65 (1988), pp. 25-40

Mansour et al., 2002

A.A. Mansour, D.W. Mkay, J. Lien, J.C. Orr, J.H. Banoub, N. ØIen, G. Stenson

Determination of pregnancy status from blubber samples in minke whales (Balaenoptera acutorostrata)

Mar. Mamm. Sci., 18 (2002), pp. 112-120

Marcoux et al., 2015

M. Marcoux, V. Lesage, G.W. Thiemann, S.J. Iverson, S.H. Ferguson

Age estimation of belugas, Delphinapterus leucas, using fatty acid composition: a promising method

Mar. Mamm. Sci., 31 (2015), pp. 944-962

Martineau et al., 1987

D. Martineau, P. Béland, C. Desjardins, A. Lagacé

Levels of organochlorine chemicals in tissues of beluga whales (Delphinapterus leucas) from the St. Lawrence Estuary, Québec, Canada

Arch. Environ. Contam. Toxicol., 16 (1987), pp. 137-147

McFee et al., 2014

W.E. McFee, T.R. Speakman, L. Balthis, J.D. Adams, E.S. Zolman

Reproductive seasonality of a recently designated bottlenose dolphin stock near Charleston, South Carolina, U.S.A

Mar. Mamm. Sci., 30 (2014), pp. 528-543

Nader et al., 2006

N. Nader, G.r. Raverot, A.s. Emptoz-Bonneton, H. Déchaud, M. Bonnay, E. Baudin, M. Pugeat

Mitotane Has an Estrogenic Effect on Sex Hormone-Binding Globulin and Corticosteroid-Binding Globulin in Humans

J. Clin. Endocrinol. Metab., 91 (2006), pp. 2165-2170

National Academies of SciencesE.Medicine, 2017

National Academies of Sciences, E., Medicine

Approaches to Understanding the Cumulative Effects of Stressors on Marine Mammals

National Academies Press (2017)

Nelson et al., 1978

J.A. Nelson, R.F. Struck, R. James

Estrogenic activities of chlorinated hydrocarbons

J. Toxicol. Environ. Health, 4 (1978), pp. 325-339

Nowicki and Norman, 1972

H.G. Nowicki, A.W. Norman

Enhanced hepatic metabolism of testosterone, 4-androstene-3, 17-dione, and estradiol-17β in chickens pretreated with DDT or PCB

Steroids, 19 (1972), pp. 85-99

Oien et al., 1997

C.W. Oien, C. Hurd, D.P. Vorojeikina, S.F. Amold, A.C. Notides

Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells

Biochem. Pharmacol., 53 (1997), pp. 1161-1172

Oskam et al., 2004

I. Oskam, E. Ropstad, E. Lie, A. Derocher, Ø. Wiig, E. Dahl, S. Larsen, J.U. Skaare

Organochlorines affect the steroid hormone cortisol in free-ranging polar bears (Ursus maritimus) at Svalbard, Norway

J. Toxicol. Environ. Health, 67 (2004), pp. 959-977

Oskam et al., 2003

I.C. Oskam, E. Ropstad, E. Dahl, E. Lie, A.E. Derocher, y. Wiig, S. Larsen, R. Wiger, J.U. Skaare

Organochlorines Affect the Major Androgenic Hormone, Testosterone, in Male Polar Bears (Ursus Maritimus) at Svalbard

J. Toxicol. Environ. Health, Part A, 66 (2003), pp. 2119-2139

Pedro et al., 2017

S. Pedro, C. Boba, R. Dietz, C. Sonne, A. Rosing-Asvid, M. Hansen, A. Provatas, M.A. McKinney

Blubber-depth distribution and bioaccumulation of PCBs and organochlorine pesticides in Arctic-invading killer whales

Sci. Total Environ., 601–602 (2017), pp. 237-246

Pérez et al., 2011

S. Pérez, Á. García-López, R. De Stephanis, J. Giménez, S. García-Tiscar, P. Verborgh, J. Mancera, G. Martínez-Rodriguez

Use of blubber levels of progesterone to determine pregnancy in free-ranging live cetaceans

Mar. Biol., 158 (2011), pp. 1677-1680

Polanowski et al., 2014

A.M. Polanowski, J. Robbins, D. Chandler, S.N. Jarman

Epigenetic estimation of age in humpback whales

Mol. Ecol. Resour., 14 (2014), pp. 976-987

Ragland et al., 2011

J.M. Ragland, M.D. Arendt, J.R. Kucklick, J.M. Keller

Persistent organic pollutants in blood plasma of satellite-tracked adult male loggerhead sea turtles (Caretta caretta)

Environ. Toxicol. Chem., 30 (2011), pp. 1549-1556

Ragland et al., 2014

J.M. Ragland, D. Liebert, E. Wirth

Using Procedural Blanks to Generate Analyte-Specific Limits of Detection for Persistent Organic Pollutants Based on GC-MS Analysis

Anal. Chem., 86 (2014), pp. 7696-7704

Rosel, 2003

P.E. Rosel

PCR-based sex determination in Odontocete cetaceans

Conserv. Genet., 4 (2003), pp. 647-649

Sawyer-Steffan et al., 1983

J.E. Sawyer-Steffan, V.L. Kirby, W.G. Gilmartin

Progesterone and estrogens in the pregnant and nonpregnant dolphin, Tursiops truncatus, and the effects of induced ovulation

Biol. Reprod., 28 (1983), pp. 897-901

Schroeder and Keller, 1989

J.P. Schroeder, K.V. Keller

Seasonality of serum testosterone levels and sperm density in Tursiops truncatus

J. Exp. Zool., 249 (1989), pp. 316-321

Sloan et al., 2014

C.A. Sloan, B.F. Anulacion, K.A. Baugh, J.L. Bolton, D. Boyd, R.H. Boyer, D.G. Burrows, D.P. Herman, R. Pearce, G.M. Ylitalo

Northwest Fisheries Science Center's analyses of tissue, sediment, and water samples for organic contaminants by gas chromatography/mass spectrometry and analyses of tissue for lipid classes by thin layer chromatography/flame ionization detection

(2014)

Stewart et al., 2011

K.R. Stewart, J.M. Keller, R. Templeton, J.R. Kucklick, C. Johnson

Monitoring persistent organic pollutants in leatherback turtles (Dermochelys coriacea) confirms maternal transfer

Mar. Pollut. Bull., 62 (2011), pp. 1396-1409

Struntz et al., 2004

D. Struntz, W. McLellan, R. Dillaman, J. Blum, J. Kucklick, D. Pabst

Blubber development in bottlenose dolphins (Tursiops truncatus)

J. Morphol., 259 (2004), pp. 7-20

Subramanian et al., 1987

A. Subramanian, S. Tanabe, R. Tatsukawa, S. Saito, N. Miyazaki

Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of northwestern North Pacific

Mar. Pollut. Bull., 18 (1987), pp. 643-646

Thomson and Geraci, 1986

C. Thomson, J. Geraci

Cortisol, aldosterone, and leucocytes in the stress response of bottlenose dolphins, Tursiops truncatus

Can. J. Fish. Aquat. Sci., 43 (1986), pp. 1010-1016

Trego et al., 2013

M.L. Trego, N.M. Kellar, K. Danil

Validation of blubber progesterone concentrations for pregnancy determination in three dolphin species and a porpoise

PLoS One, 8 (2013), p. e69709

Urian et al., 1996

K. Urian, D. Duffield, A. Read, R. Wells, E. Shell

Seasonality of reproduction in bottlenose dolphins, Tursiops truncatus

J. Mammal., 77 (1996), pp. 394-403

US Department of Health and Human ServicesU.D. and Services, 2000

US Department of Health and Human Services, U.D.

U.D.o.H.a.H. Services (Ed.), Public Health Assessment for Tyndall Air Force Base, Panama City, Bay County, FL, CERCLIS No. FLI570024124 (2000)

US Environmental Protection AgencyU.E and AuthorAnonymous, 1997

US Environmental Protection Agency, U.E

NPL Site Narrative for Tyndall Air Force Base

US Environmental Protection Agency, U.E. (1997)

van Seters and Moolenaar, 1991

A.P. van Seters, A.J. Moolenaar

Mitotane increases the blood levels of hormone-binding proteins

Acta Endocrinol., 124 (1991), pp. 526-533

Webster et al., 2010

T. Webster, S. Dawson, E. Slooten

A simple laser photogrammetry technique for measuring Hector's dolphins (Cephalorhynchus hectori) in the field

Mar. Mamm. Sci., 26 (2010), pp. 296-308

Welch et al., 1967

R.M. Welch, W. Levin, A.H. Conney

Insecticide Inhibition and Stimulation of Steroid Hydroxylases in Rat Liver

J. Pharmacol. Exp. Ther., 155 (1967), pp. 167-173

Welch et al., 1971

R.M. Welch, W. Levin, R. Kuntzman, M. Jacobson, A.H. Conney

Effect of halogenated hydrocarbon insecticides on the metabolism and uterotropic action of estrogens in rats and mice

Toxicol. Appl. Pharmacol., 19 (1971), pp. 234-246

Wells et al., 2005

R.S. Wells, V. Tornero, A. Borrell, A. Aguilar, T.K. Rowles, H.L. Rhinehart, S. Hofmann, W.M. Jarman, A.A. Hohn, J.C. Sweeney

Integrating life-history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (Tursiops truncatus) in Sarasota Bay, Florida

Sci. Total Environ., 349 (2005), pp. 106-119

Wójtowicz et al., 2007a

A.K. Wójtowicz, M. Kajta, E. Gregoraszczuk

DDT- and DDE-induced disruption of ovarian steroidogenesis in prepubertal porcine ovarian follicles: a possible interaction with the main steroidogenic enzymes and estrogen receptor beta

J. Physiol. Pharmacol., 58 (2007), pp. 873-885

Wójtowicz et al., 2007b

A.K. Wójtowicz, T. Milewicz, E.Ł. Gregoraszczuk

DDT and its metabolite DDE alter steroid hormone secretion in human term placental explants by regulation of aromatase activity

Toxicol. Lett., 173 (2007), pp. 24-30

Yordy et al., 2010

J.E. Yordy, R.S. Wells, B.C. Balmer, L.H. Schwacke, T.K. Rowles, J.R. Kucklick

Life history as a source of variation for persistent organic pollutant (POP) patterns in a community of common bottlenose dolphins (Tursiops truncatus) resident to Sarasota Bay, FL

Sci. Total Environ., 408 (2010), pp. 2163-2172

You et al., 2001

L. You, M. Sar, E. Bartolucci, S. Ploch, M. Whitt

Induction of hepatic aromatase by p,p′-DDE in adult male rats

Mol. Cell. Endocrinol., 178 (2001), pp. 207-214

Young et al., 1973

R.B. Young, M.J. Bryson, M.L. Sweat, J.C. Street

Complexing of DDT and o,p'DDD with adrenal cytochrome P-450 hydroxylating systems

J. Steroid Biochem., 4 (1973), pp. 585-591