

Investigating the Potential Role of Persistent Organic Pollutants in Hawaiian Green Sea Turtle Fibropapillomatosis

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Supporting Information

ABSTRACT: It has been hypothesized for decades that environmental pollutants may contribute to green sea turtle fibropapillomatosis (FP), possibly through immunosuppression leading to greater susceptibility to the herpesvirus, the putative causative agent of this tumor-forming disease. To address this question, we measured concentrations of 164 persistent organic pollutants (POPs) and halogenated phenols in 53 Hawaiian green turtle (*Chelonia mydas*) plasma samples archived by the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST) project at the National Institute of Standards and Technology Marine Environmental Specimen Bank. Four groups of turtles were examined: free-ranging turtles from Kiholo Bay (0% FP, Hawaii), Kailua Bay (low FP, 8%, Oahu), and Kapoho Bay (moderate FP, 38%, Hawaii) and severely tumored stranded turtles that required euthanasia (high FP, 100%, Main Hawaiian Islands). Four classes of POPs and seven halogenated phenols were detected in at least one of the turtles, and concentrations were low (often <200 pg/g wet mass). The presence of halogenated phenols in sea turtles is a novel discovery; their concentrations were higher than most man-made POPs, suggesting that the source of most of these compounds was likely natural (produced by the algal turtle diet) rather than metabolites of man-made POPs. None of the compounds measured increased in concentration with increasing prevalence of FP across the four groups of turtles, suggesting that these 164 compounds are not likely primary triggers for the onset of FP. However, the stranded, severely tumored, emaciated turtle group ($n = 14$) had the highest concentrations of POPs, which might suggest that mobilization of contaminants with lipids into the blood during late-stage weight loss could contribute to the progression of the disease. Taken together, these data suggest that POPs are not a major cofactor in causing the onset of FP.



INTRODUCTION

Fibropapillomatosis (FP) is a disease that afflicts sea turtles with benign tumors that can grow large enough to inhibit sight, mobility, and foraging.^{1,2} The suspected cause of FP is a herpesvirus because it is often found in the tumored tissues, and the disease has been experimentally transmitted using cell-free extracts of tumor tissue.^{3,4} While the virus is the likely cause of tumor expression, additional environmental stressors may be cofactors in this putative viral-causing disease.^{1,5} Sites with poor environmental quality and intense human land use have higher FP prevalence rate in green sea turtles.^{2,6–8} Investigated stressors include biotoxins, biological vectors of the virus, and chemical pollutants.^{9–17}

Scientists have speculated for decades that environmental pollutants play a role in FP.^{1,2,5,9,16} Chemicals might act either as cocarcinogens or suppress the immune system, which may

allow the virus to grow with less control and promote tumor expression. Green turtles with FP have been shown by several studies to have suppressed immune functions,^{18–22} adding to the suspicion that immunosuppressive pollutants could play a role. Additional evidence that supports this hypothesis can be seen in the simultaneous temporal trends of certain pollutants and the prevalence of FP. The prevalence of FP in green turtles increased in Hawaiian coastal waters and in southern Florida in the 1970s with peaks in the 1990s.^{7,8,23} In Hawaii, prevalence reached 50% of free-ranging turtles at one site (Palauu, Molokai)²³ and approximately 75% of strandings at other

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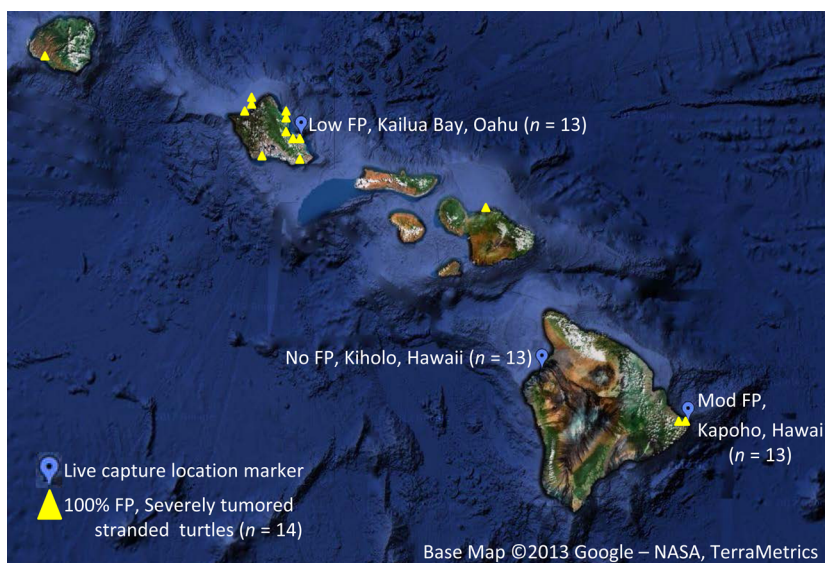


Figure 1. Map of Hawaiian green turtle sampling locations with varying degrees of fibropapillomatosis (FP).

sites (Kaneohe Bay and West Maui).⁸ Most locations have seen a recent decline in FP.^{8,23} This rise and fall in FP is coincident with certain persistent organic pollutant (POP) temporal trends, especially polybrominated diphenyl ethers (PBDEs).²⁴

The Stockholm Convention classifies certain man-made organic pollutants as POPs. To be defined as a POP, the compounds must be greatly persistent in the environment, hydrophobic so they bioaccumulate with age and biomagnify up the food web, globally mobile mainly through atmospheric transport, and toxic.²⁵ POPs include polychlorinated biphenyls (PCBs), organochlorine pesticides, like dichlorodiphenyltrichloroethane (DDT) and chlordanes, and brominated flame retardants, like PBDEs, many of which have been banned or restricted from use in the U.S. and Europe. Most of them have been shown to suppress the immune system.^{26–28} Green turtles accumulate these compounds²⁹ mainly through their diet of algae and sea grasses and also incidental ingestion of sediments, where large concentrations of POPs can reside.

Two studies have attempted to address the question of whether POPs contribute to FP.^{9,16} Both sampled internal tissues of dead Hawaiian green turtles; however, their methods and small sample sizes limited their conclusions. In the first study, limits of detection were so high (20–1000 ng/g) that none of the compounds could be detected in any sample.⁹ The second study¹⁶ showed higher PCBs in the single non-FP turtle, which was a different age class/sex, than two tumored turtles. The objective of the current study was to determine if POPs contribute to FP by analyzing a robust sample size of Hawaiian green turtle plasma samples for POP concentrations using high quality, sensitive analytical methods. Halogenated phenols were also quantified in these samples, which was a novel objective for any sea turtle study.

EXPERIMENTAL SECTION

Turtle Capture, Sample Collection, And Sample Processing. In 2011 and 2012, samples were taken from live green turtles to be accessioned into the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST) project. BEMAST is a part of the National Institute of Standard and Technology (NIST) Marine Environmental Specimen Bank's U.S. Pacific Islands Program that archives

marine organism samples for real-time and future retrospective contaminant and health research.³⁰ Thirty-nine free-ranging green turtles were captured along the shoreline by hand, tangle net, or scoop net, and an additional 14 stranded green turtles with severe FP and poor prognosis for survival were sampled. The free-ranging turtles were captured at three sites across the Hawaiian Islands that represent different levels of FP prevalence (Figure 1). Kiholo Bay on the Kona or west coast of Hawaii is an FP-free site; all 13 turtles sampled from this study were free of external tumors. Kailua Bay on the east coast of Oahu is an intermediate FP site; 8% (1 out of 13) of turtles sampled had external tumors. Kapoho Bay on the east coast of Hawaii is a site of relatively high FP prevalence with 38% (5 out of 13) of turtles showing FP tumors. The severely tumored turtles that stranded came from several Main Hawaiian Islands, but mostly from Oahu (Figure 1). Turtles were measured, weighed, tagged, and assessed for severity of FP by assigning a tumor score according to Work and Balazs,²¹ and body condition was calculated as turtle mass (kilograms) divided by the cubed straight carapace length (SCL) (centimeters) multiplied by 100000.^{31,32} Samples of blood and scute were collected and processed using protocols described elsewhere.³⁰ Briefly, blood was drawn using double-ended stainless steel needles into glass sodium heparin Vacutainer blood collection tubes within 15 min of capture for the free-ranging turtles and just prior to administration of euthanasia for stranded turtles. Blood was kept cool, and hematocrit was measured using centrifugation of capillary tubes containing whole blood. Blood was centrifuged within 8 h of collection, and plasma was transferred with hexane-rinsed glass pipettes into solvent-rinsed Teflon vials and stored at $-80\text{ }^{\circ}\text{C}$ or lower. Total extractable organic (TEO) content (a proxy for lipid content³³) was measured in plasma during the POP extraction methods described below. Turtle capture and sampling techniques were approved by National Marine Fisheries Service permit nos. 1581-01 and 15685, Hawaii Department of Land and Natural Resource permit nos. 2011-03, 2012-2, and 2013-32, and IACUC protocols.

POP Extraction and Quantification. Briefly, plasma samples ($\sim 3\text{--}5\text{ mL}$) along with quality control materials were weighed in 35 mL glass focused-microwave extraction

(FME) vessels, spiked gravimetrically with an internal standard solution containing 26 ^{13}C -labeled, deuterated, or fluoro-labeled POPs and 7 ^{13}C -labeled halogenated phenols, and refrigerated overnight. Quality control materials included five field blanks consisting of ~ 5 mL of Millipore water drawn through the same lot of blood collection and processing supplies, six procedural blanks consisting of ~ 5 mL Millipore water, a six-point calibration curve containing a mixture of the targeted POPs and halogenated phenols (Table S1, Supporting Information), eight replicates of NIST Standard Reference Material 1958 Organic Contaminants in Fortified Human Serum, and two replicates of an in-house pooled loggerhead turtle plasma control material (called Cc pool) that has been analyzed in five previously published studies (e.g., ref 34). Compounds were extracted using the FME method described in Keller et al.³⁵ with slight modifications. Briefly, 1.25 mL of hexane-rinsed HCl, 5 mL of hexane-rinsed formic acid, and 5 mL of 25% (volume fraction) dichloromethane (DCM) in hexane were added to the FME vessels. Vessels were microwaved individually using CEM Discover focused microwaves (Matthews, NC) at 90 °C for 3 min with maximum power set at 250 W, maximum pressure at 250 psi, PowerMax on, and stirring at medium speed. Supernatant from centrifuged vessels was pipetted into glass tubes for TEO measurements. Two additional FME rounds were performed with the addition of 5 mL of the 25% DCM in hexane each time to the vessels. Supernatant was combined with the corresponding initial extract. Total extractable organic (TEO, traditionally called lipid content) content was determined gravimetrically from a 10% subsample of the combined extract dried overnight at room temperature and weighed to the nearest 0.0001 mg.

Halogenated phenols were separated from other (parent) POPs by manually shaking separatory funnels containing the extracts plus 5 mL of 1 mol/L KOH in 50% ethanol in water (volume fraction) for 4 min. The bottom KOH layer, containing the phenols or OH fraction, was drained into a glass tube. Two more 5 mL KOH additions were performed; both drained into the same OH fraction tube. The remaining top organic layer was drained into the original glass tube as the parent fraction and was frozen.

To the OH fractions, 9 mL of concentrated HCl and 5 mL of 1:1 DCM/hexane were added. Tubes were rotated for 15 min and centrifuged, and the top DCM/hexane layer was transferred to a new tube. Two more 5 mL portions of 1:1 DCM/hexane were added with rotation, centrifugation, and combined as the organic OH fractions. The OH fractions were evaporated to 0.5 mL and derivatized in the dark with 0.5 mL concentrated diazomethane. Diazomethane was synthesized that day from the reaction of Diazald (Sigma-Aldrich, St. Louis, MO) in ethanol with saturated KOH and captured by bubbling the yellow gas product with a flow of nitrogen into diethyl ether. After derivatization, samples were exchanged to iso-octane and evaporated to 0.5 mL and frozen. The derivatized OH fractions were cleaned using an acidified silica column and readied for gas chromatography single-quadrupole mass spectrometry (GC/MS) by evaporation to 200 μL in glass autosampler vial inserts.

Parent extracts were cleaned up using alumina and acidified silica columns. From a preliminary set of samples ($n = 3$ per turtle group), two fractions were collected from the acidified silica columns as described elsewhere for screening of hexabromocyclododecanes (HBCDDs).³⁵ HBCDDs (as well as pentabromoethylbenzene, hexabromobenzene, 1,2-bis(2,4,6-

tribromophenoxy)ethane, and decabromodiphenylethane) were below detection in all 12 turtle plasma samples (<17 pg/g wet mass), so the second HBCDD fraction was not collected from the remaining green turtle plasma samples, and these brominated flame retardants were not targeted. Parent extracts were evaporated to 200 μL in glass autosampler vial inserts.

Parent extracts were injected (20 μL) first on a GC/MS (Agilent, Palo Alto, CA) for PCBs and pesticides using a programmable temperature vaporization (PTV) inlet operated in the solvent vent mode onto an Agilent 30 m \times 0.18 mm DB-5MS capillary column, 0.18 μm film thickness, with a 5 m \times 0.25 mm Restek Siltek guard column (Bellefonte, PA). The MS source was operated in electron-impact (EI) mode with selected ion monitoring (SIM). Parent extracts were injected (2 μL) a second time on a GC/MS for PBDEs using on-column injection onto an Agilent 10 m \times 0.18 mm DB-5MS capillary column, 0.18 μm film thickness, with the same Restek guard column. The MS source was operated in negative chemical ionization (NCI) mode with methane as the reagent gas with SIM.

OH extracts were injected (20 μL) on a GC/MS using a PTV inlet operated in the solvent vent mode onto an Agilent 30 m \times 0.18 mm DB-5MS capillary column, 0.18 μm film thickness, with the same Restek guard. The MS was operated in EI mode with SIM.

The internal standard approach was used to quantify each compound amount. Amounts of each analyte were calculated using the slope and y -intercept of at least a three-point calibration curve that bracketed the peak area ratios observed in the samples. Concentrations were determined by dividing the calculated analyte mass by the extracted sample mass. Reporting limits (RLs) were determined as described elsewhere.³⁴

Statistical Methods. Statistical tests on health and morphometric data were performed using JMP 11 software (SAS Institute Inc., Cary, NC). Differences between the four groups were tested using analysis of variance (ANOVA) with Tukey multiple comparisons. When data were non-normally distributed or when variances differed, a Kruskal–Wallis test was used to verify the ANOVA. p -values less than 0.05 were considered statistically significant.

Statistical tests on POP data were performed as suggested by Helsel³⁶ using the NADA package for handling data with nondetects (left censored data) in the open-source R software package. Using these tests, nondetects were always included and do not require substitution of values. Statistics of central tendency and variance were calculated using either Kaplan–Meier or regression on order models (“censtats” function). Differences between the four turtle groups were tested using ANOVA in NADA by using either the “cenmle” (a maximum likelihood estimation method) when both normality of data and homogeneity of variance assumptions were met or “cendiff” (a Kruskal–Wallis test) when one or both assumptions were violated. Differences between pairs of turtle groups were tested individually with the cenmle or cendiff functions with a Bonferroni correction to the α value ($p < 0.0083$ was considered significant). Correlations were performed with the “cenken” function, which is a Kendall’s tau correlation for censored data, or “cenreg” function when log-transformed data met the assumptions for this parametric censored linear regression.

RESULTS AND DISCUSSION

Health indicators, FP prevalence, and FP severity was assessed across the four turtle groups (Table 1). No turtles captured at Kiholo Bay exhibited external tumors. One of the 13 turtles sampled at Kailua Bay had tumors, whereas five of the 13 from Kapoho Bay had tumors, showing the expected increase in prevalence across these three sites. All of the stranded turtles included in this study, regardless of stranding location, had severe tumors and required euthanasia. The average FP score, an indicator of severity of the disease,²¹ increased significantly with the increasing prevalence of FP across the four turtle groups. Hematocrit, an indicator of anemia, was significantly lower in the stranded turtles compared to the free-ranging turtles at the three sites. This trend in hematocrit with FP presence and severity has been documented previously.^{18,21,22}

Turtle length (SCL) increased slightly, but not significantly, through the four groups consistent with previous findings that FP prevalence increases with turtle length from 30 to 70 cm SCL.^{8,37} Turtle mass was consistent across the four groups of turtles. Body condition was lowest in the stranded group, significantly lower than Kiholo and Kapoho turtles ($p < 0.05$), suggesting that this group had experienced weight loss. Plasma TEO (or lipid content) paralleled the body condition finding with the stranded group having approximately half the lipid content in their blood plasma than the free-ranging groups ($p < 0.05$), suggesting that the stranded turtles had not been recently foraging and had utilized much of their lipid stores for survival.

POPs measured in the quality control materials were in agreement with certified values or previous measurements (data not shown). Four classes of POPs and seven halogenated phenols were detected in at least one turtle from this study (Figure 2; Table 2). Total PCBs were the highest of the POP classes, found at 1–2 orders of magnitude greater levels than total chlordanes, total PBDEs, and 4,4'-DDE (Figure 2). The predominant PCB congeners were 153 + 132 > 138 > 99 > 180 + 193 > 118 > 183 > 170 > 105 > 187, which is similar to the typical PCB pattern seen in sea turtles elsewhere.³⁸ Total chlordanes consisted mainly of *trans*-chlordanes > *trans*-nonachlor > *oxy*chlordanes. Total PBDEs consisted of congeners 99 > 153 > 100 > 47 > 154, which is an unusual pattern compared to the typical pattern, in which PBDE 47 is predominant (47 > 99 > 100 > 153 > 154), for most aquatic wildlife tissues.³⁹ Unusual PBDE patterns have been commonly seen in sea turtle and other turtle samples.⁴⁰ For example, adult male loggerhead turtles from Cape Canaveral, FL, that migrated northward after nesting season had a pattern consisting of 154 > 153 > 99 > 100 > 183 > 155 > 47 in plasma,³⁴ and nesting green turtles from Malaysia had a pattern of 153 > 99 > 47 in blood.⁴¹ Possible, yet untested, reasons for this pattern include different biotransformation or elimination rates/pathways in turtles where these patterns are observed. 4,4'-DDE was the only DDT-related compound detected.

The presence of halogenated phenols is a novel discovery for sea turtles. Seven compounds were surprisingly detected with concentrations higher than many POPs (Figure 2). Two hydroxylated PBDEs had concentrations 22–35 times higher than average total PBDEs. These compounds are known to either come from metabolism of manmade PBDEs or naturally produced by marine organisms, including sponges, cyanobacteria, and green, brown, and red algae.^{42,43} Based on their relatively high concentrations compared to PBDEs, the source of these hydroxylated PBDEs in sea turtles is likely derived

Table 1. Morphometric and Health Variables of Hawaiian Green Sea Turtles Measured for Persistent Organic Pollutant Plasma Levels^{a,b}

location/group	Kiholo Bay			Kailua Bay			Kapoho Bay			stranded ^c			
% FP	0%; no			8%; low			38%; moderate			100%; high			
Dates	May 5–6, 2011			Mar 28 and 30, 2011; Jul 10–12, 2012			Nov 18–22, 2011			Jul 31, 2011 to Dec 26, 2012			
health variable	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	range
FP score	13	0 A	0	13	0.15 AB	0.55	13	0.77 B	1.17	14	2.79 C	0.43	2–3
hematocrit (%)	13	32.5 A	5.2	10	32.5 A	6.4	13	33.8 A	3.2	10	17.6 B	5.4	8–26
SCL (cm)	13	53.2 A	5.4	13	57.4 A	10.6	13	58.4 A	11.1	14	60.2 A	13.0	45.4–89.2
mass (kg)	12	20.2 A	5.1	13	25.9 A	14.7	13	32.8 A	23.2	14	29.8 A	22.8	9.3–91
body condition	12	13.9 A	0.8	13	13.1 AB	2.6	13	14.6 A	1.8	14	11.7 B	1.8	8.0–14.0
TEO (%)	13	0.419 A	0.120	13	0.340 A	0.092	13	0.421 A	0.139	14	0.208 B	0.091	0.077–0.390

^aWithin each variable, means that are significantly different across groups have different letters. ^bFP = fibropapillomatosis; SD = standard deviation; SCL = straight carapace length; TEO = total extractable organic content from blood plasma. ^cOne stranded turtle was captured earlier as a free-ranging tumored turtle at Kapoho in Nov 2011.

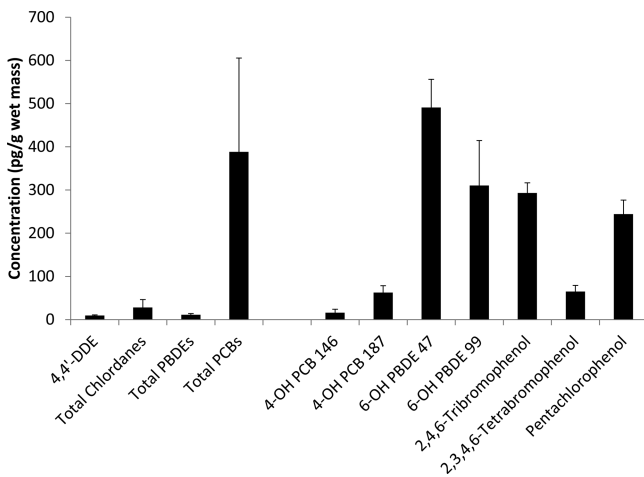


Figure 2. Mean (standard error) plasma levels of persistent organic pollutants and halogenated phenols in 53 Hawaiian green sea turtles regardless of location and fibropapillomatosis state.

naturally from their diet, which is known for this neritic age class to consist mainly of algae.⁴⁴ Two hydroxylated PCBs had concentrations 14–27 times lower than average total PCBs and are likely metabolites of manmade PCBs as hydroxylated PCBs are not known to be natural products.⁴⁵ 2,4,6-Tribromophenol, 2,3,4,6-tetrabromophenol, and pentachlorophenol were the additional halogenated phenols detected at relatively high concentrations compared to POPs. 2,4,6-Tribromophenol is produced by man as a flame retardant, an intermediate in making higher molecular weight flame retardants, and a wood treatment preservative.⁴³ It is also a natural product of marine organisms,⁴³ including acorn worms (*Phoronopsis viridis*)⁴⁶ and several species of green, brown, and red algae.⁴² 2,3,4,6-Tetrabromophenol is used as an intermediate to produce several industrial compounds (for two examples, see US patent 5248432 for damping fluids and lubricants for gyroscopes and US patent 4059655 for plasticizers, pesticides, flame retardants, and textile impregnating agents). Pentachlorophenol has been used since the 1940s and was banned in 1987 as a herbicide but is still used as a fungicidal wood preservative, especially on rounded wooden poles, like utility poles or possibly marine pilings.^{47,48}

To begin to understand PCB metabolism in green turtles, the ratio of total hydroxylated PCBs to total PCBs was calculated for the six turtles that had detectable levels. The ratios ranged from 0.037 to 16.6 with an average \pm standard deviation of 3.0 ± 6.7 . Since the maximum ratio was an outlier, the median is a better description of central tendency at 0.38. Compared to blood samples from a variety of other species, with a focus on organisms that forage in the sea, the green turtle ratio falls in line with other reptile and bird ratios (Figure 3). This comparison suggests that the green turtle's ability to hydroxylate PCBs is higher than cartilaginous and bony fish but much lower than the efficiently metabolizing polar bear (Figure 3). The hydroxylating cytochrome P450 enzyme, CYP1A, can be induced in reptiles by chemicals that activate the aryl–hydrocarbon receptor, but the ability of this enzyme in reptiles to metabolize two substrates (7-ethoxyresorufin and PCB 77) appears to be lower than birds and mammals.⁴⁹ Limited CYP1A activity was detected in green sea turtle liver tissue.⁵⁰ Taken together, these studies suggest that green turtles

Table 2. Mass Fractions (pg/g wet mass) of Detectable Persistent Organic Pollutants and Halogenated Phenols in Blood Plasma of Hawaiian Green Sea Turtles with ANOVA *p*-Values Indicating Differences among Groups with Varying Prevalence of Fibropapillomatosis (FP) (See Figure 4 for multiple Comparison Test Results)^a

	no FP, Kiholo (n = 13)			low FP, Kailua (n = 13)			moderate FP, Kapoho (n = 13)			100% FP, stranded (n = 14)			ANOVA <i>p</i> -value	
	median	mean	SD	n > RL	median	mean	SD	n > RL	median	mean	SD	n > RL		
4,4'-DDE	<RL			0 ^b	10.3	13.7	8.36	3	<RL	8.11	17.9	16.2	5	0.0246
total chlordanes	<RL			0 ^b	11.7	13.9	4.86	5	<RL	5.99	94.9	251	4	0.0220
total PBDEs	<RL			0	<RL			1	<RL	17.9	33.3	35.3	6	0.0007
total PCBs	2.84	25.1	51.9	4 ^b	91.7	144	165	9	<RL	170	1260	2890	14	<0.0001
2,4,6-tribromophenol	244	347	242	12	362	364	122	13	210	265	125	124	12	0.0076
2,3,4,6-tetrabromophenol	45.8	75.6	81.6	8	<RL			1	79.3	174	133	42.3	3	0.0001
pentachlorophenol	<RL			2	337	367	150	11	130	174	112	359	11	0.0001
4-OH PCB 146	<RL			1	<RL			2	<RL	0.423	29.6	84.1	3	0.4440
4-OH PCB 187	32.2	104	180	9	54.6	64.0	47.7	10	<RL	5.25	73.6	133	6	0.0208
6-OH PCB 47	955	1040	515	13	271	416	320	12	243	377	283	165	7	<0.0001
6-OH PCB 99	602	1130	1190	13	<RL			0	<RL	60.5	210	210	1 ^c	<0.0001

^aDDE = dichlorodiphenylchloroethylene; PBDE = polybrominated diphenyl ether; PCB = polychlorinated biphenyl; SD = standard deviation; n > RL = number of samples with concentration greater than reporting limit. ^bOut of 12, one sample was lost. ^cOut of 13, one sample was lost.

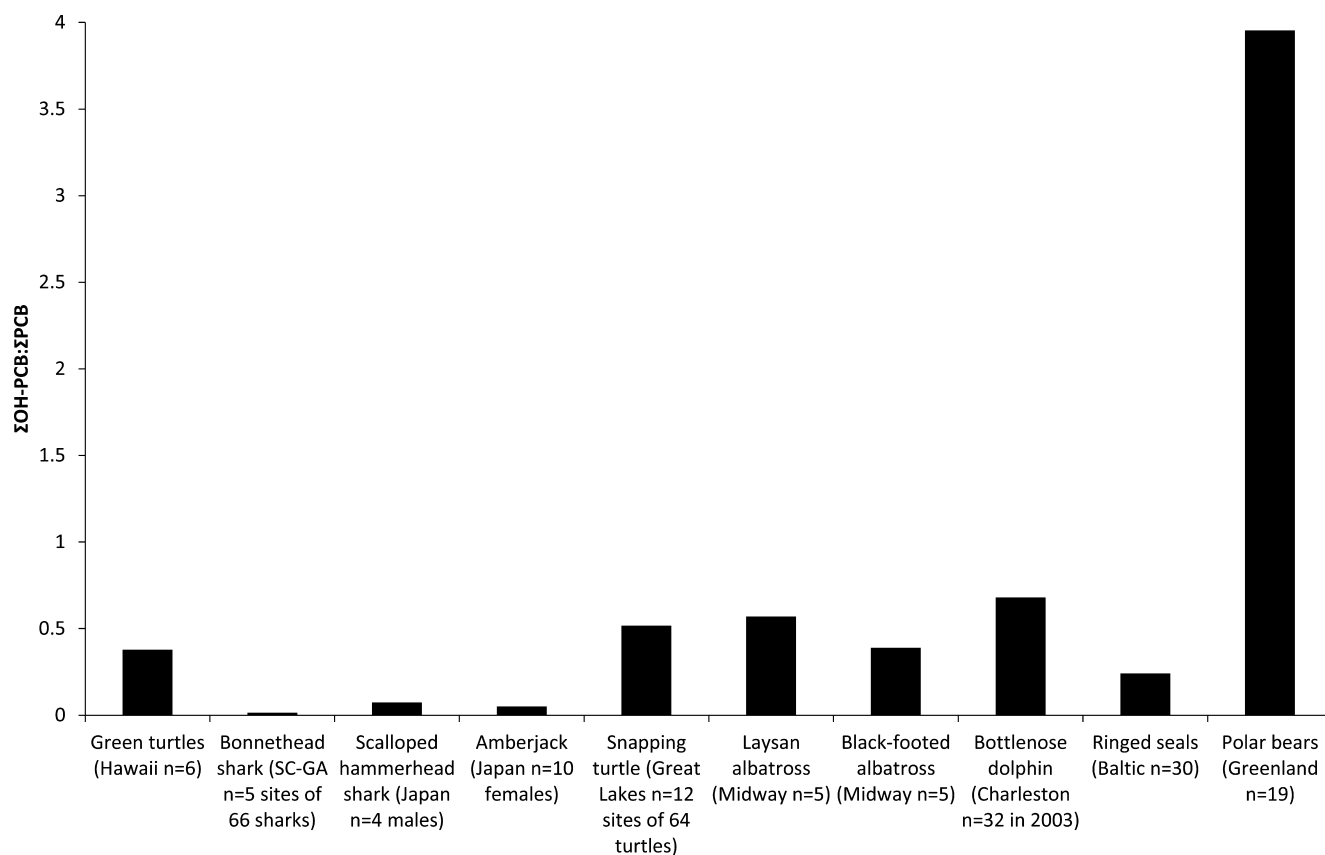


Figure 3. Comparison of the ratio of Σ OH-PCBs to Σ PCBs in a variety of species. Median or means taken from other studies^{53,56–61} were used to calculate these ratios.

may use enzymes other than CYP1A in biotransformation of PCBs.

POP and halogenated phenol concentrations measured in the four turtle groups with varying prevalence of FP are shown in Table 2. Even though many samples were below the reporting limit for many compounds, analysis of variance could be performed using the NADA package in R, which takes into account the reporting limit for samples below detection. Of the 11 compounds detected in at least one turtle, all but one (4-OH PCB 146) significantly differed among the four turtle groups ($p < 0.05$, Table 2). The differences are visually displayed for eight of the compounds in Figure 4. If POPs play a role in triggering tumor formation, one might expect POP levels to increase stepwise through the four groups of turtles as FP prevalence increased. However, this was not seen for any of the compounds measured (Figure 4), leading to the conclusion that POPs do not trigger FP. For the four POP classes (4,4'-DDE, total chlordanes, total PBDEs, and total PCBs), the highest concentrations were observed in the low FP site and the stranded turtles (Table 2; Figure 4a,b). The higher levels at Kailua Bay (the low FP site) may be explained by the fact that this bay has the most densely populated and developed watershed of the three sites. The higher levels in the stranded turtles are likely due to emaciation and recent mobilization of lipids and contaminants from fatty stores into the blood. These explanations are supported by previous studies that have shown that human population density and emaciation contribute to higher POP levels in sea turtle blood.^{51,52}

For the halogenated phenols, a variety of patterns were observed among the groups (Table 2; Figure 4c–h). The

lowest concentrations of 4-OH PCB 187 were observed at Kapoho Bay, the moderate FP site, which also had the lowest total PCB concentrations (Figure 4c). This congruency lends additional support that the hydroxylated PCBs detected in the turtles were metabolites of parent manmade PCBs. The concentrations of the hydroxylated PBDEs (Figure 4d,f), on the other hand, were in opposition of the concentrations of total PBDEs (Figure 4b). All turtles from Kiholo Bay, the FP-free site, had detectable levels of 6-OH PBDE 47 and 6-OH PBDE 99, and this site had the highest concentrations, significantly different from the other three groups (Figure 4d,f). However, this site had nondetectable levels of parent total PBDEs (Table 2; Figure 4b). The inconsistency between the parent PBDE compounds and their possible hydroxylated metabolites suggests that the hydroxylated PBDEs are likely natural chemicals produced by the algae the turtles are eating. The preferred diet items at the three sites of free-ranging turtles are known and differ dramatically among the sites.⁴⁴ Only one previous study has analyzed the same algal genera found in the turtle diets for concentrations of OH-PBDEs and 2,4,6-tribromophenol.⁴² This allowed a preliminary comparison; however, it should be noted that these three algal samples were collected from the Philippines, very distant from the Hawaiian turtle foraging grounds of the current study. Kiholo turtles are unique in their selection of *Cladophora* species, which had nondetectable 6-OH PBDE 47 in the Philippines. *Acanthophora spicifera* is the dominant algal prey of Kailua turtles, is not eaten by turtles from the other two sites, and has low but detectable concentrations of 6-OH PBDE 47 in the Philippines. Neither of these comparisons help explain why Kiholo turtles had the

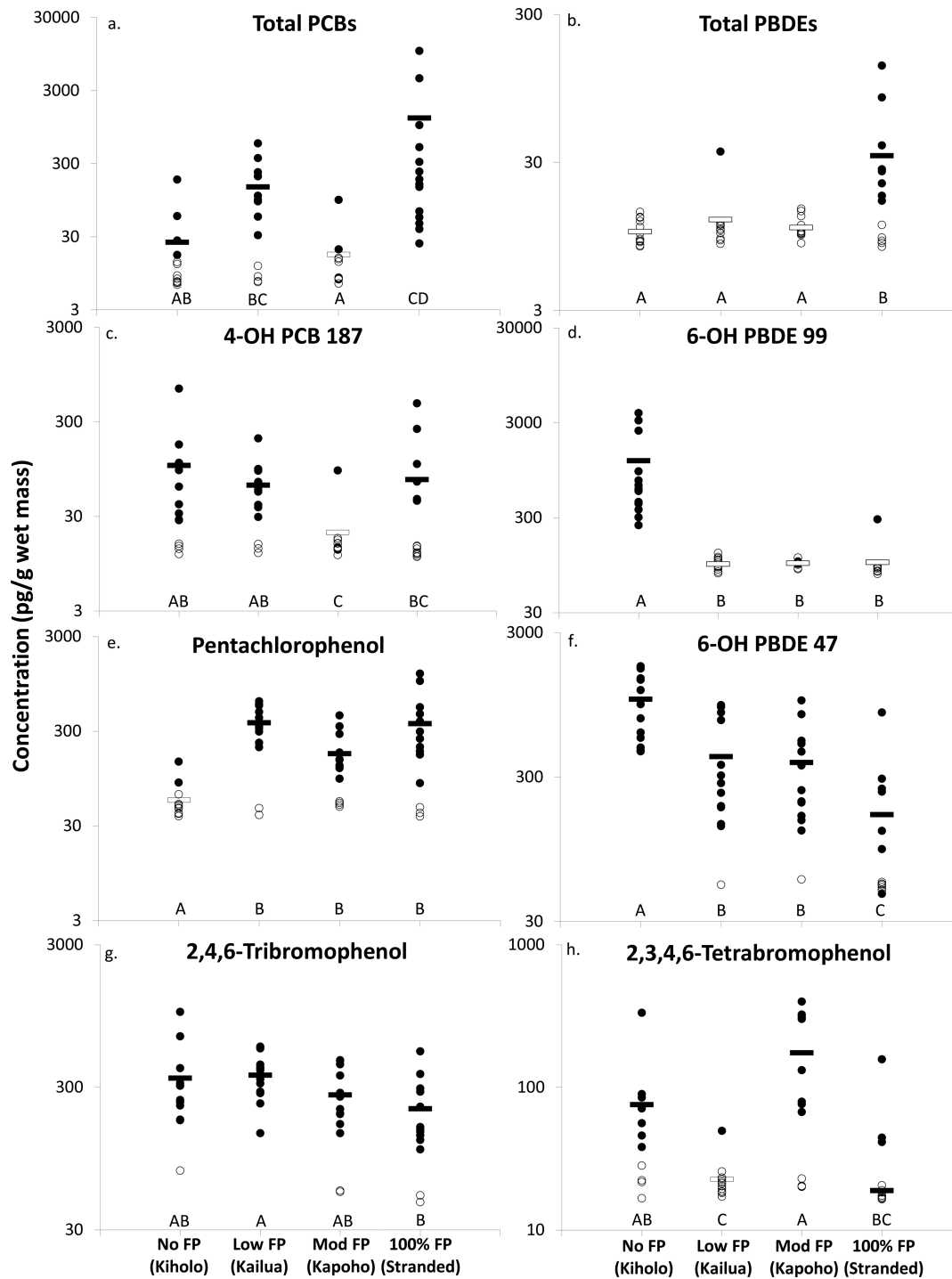


Figure 4. Comparison of plasma levels of persistent organic pollutants and halogenated phenols in Hawaiian green turtles with varying degrees of fibropapillomatosis (FP). Dots are individual turtle samples (black >RL; white = 1/2 RL). Bars are means (black from NADA; white calculated with nondetects substituted as 1/2 RL). Different capital letters above the x-axis indicate significant differences among groups.

highest concentration of this compound. Future studies should simultaneously collect turtle tissues and their algal prey from the same site because plant production of these compounds likely varies geographically and quickly with environmental changes.

Pentachlorophenol was higher at Kailua, Kapoho, and in the stranded turtles than at Kiholo (Figure 4e). Its primary use is in wood treatment, and to our knowledge it is not a natural product. The reason for lower concentrations on the Kona coast of Hawaii is unknown, but possible explanations may lie

in the number of wood treatment facilities or amount of treated wood in the watersheds. Alternatively, Kiholo Bay may receive less runoff than the other locations because it is the only site on the eastern lee side of an island. 2,4,6-Tribromophenol was relatively similar across the four turtle groups with only Kailua and the stranded turtles significantly differing from each other (Figure 4g). This compound is produced by *Cladophora* (algae eaten by Kiholo turtles), *Gracilaria* (eaten by Kailua and Kapoho turtles), *A. spicifera* (eaten by Kailua turtles), and other algal species growing in the Philippines.^{42,44} Since this

compound is commonly produced by several algal species, it is likely to be consumed by turtles from all three sites. Kapoho Bay turtles had the highest levels of 2,3,4,6-tetrabromophenol with Kailua Bay and stranded turtles having significantly lower levels (Figure 4h). The reason for the differences in this compound is unknown, and few studies have analyzed environmental samples for this compound.^{45,53} It was not detected in sharks from South Carolina and Georgia,⁵³ but a tetrabromophenol compound, possibly with this structure, was detected in plasma of Swedish men.⁴⁵

Concentrations of certain compounds were significantly correlated to each other (Table S2, Supporting Information). Possible explanations for positive correlations we observed may include a common source (6-OH PBDE 47 vs 6-OH PBDE 99), a metabolite of a parent compound (4-OH PCB 187 vs total PCBs), or simply covarying in the environment (total PCBs and 4,4'-DDE). The lack of correlations between total PBDEs and the hydroxylated PBDEs provides more evidence that the hydroxylated PBDEs are from a natural source. With all turtles included, we observed some significant correlations between compound concentrations and morphometric/health indicators (Table S3, Supporting Information). Most notably, as FP score increased and body condition decreased, total PCBs increased and the food-derived hydroxylated PBDEs decreased. As plasma lipids (TEO) and hematocrit decreased, total PCBs, total PBDEs, and 4,4'-DDE increased and food-derived hydroxylated PBDEs decreased. All of these significant correlations can be explained by weight loss, lipid mobilization, and reduced food intake in the stranded turtle group. When this group is excluded from the analyses, three interesting correlations were observed (Table S4, Supporting Information). Total PCBs decreased with increasing turtle length, which can be explained by the ontogenetic shift that these turtles underwent from foraging omnivorously during an earlier pelagic juvenile stage to a lower trophic level of algae during this neritic juvenile stage.⁴⁴ As FP score increased and body condition decreased in the free-ranging turtles 6-OH PBDE 47 concentrations decreased. Again, this could be explained by reduced food intake by tumored turtles; thus, they are accumulating lower levels of algae-derived natural products.

For a global comparison, 4,4'-DDE and PCB levels in Hawaiian green turtles were approximately 1 order of magnitude lower than green turtle blood examined elsewhere (Texas,⁵⁴ San Diego,⁵⁵ Malaysia⁴¹). PBDEs levels were similar to those measured in green turtle blood from Malaysia.⁴¹ The 4,4'-DDE and PCB levels in Hawaiian green turtles were approximately 2 orders of magnitude lower than loggerhead turtle blood from the southeastern U.S. coast.³⁸ It is not possible to compare the green turtle POP levels to other Hawaiian wildlife because no other study from this region has analyzed blood plasma and comparing different tissues is not appropriate.

In conclusion, many lines of evidence indicate that these 164 POPs and halogenated phenols are not likely the triggers for onset of FP. First, the concentrations of POPs and halogenated phenols were very low (less than a few hundred pg/g) in the Hawaiian green turtle plasma. These levels are lower than concentrations measured in other sea turtles. Second, the levels did not follow a pattern of increasing concentrations with increasing prevalence of FP. The hypothesis that POPs cause immunosuppression, which may lead to the herpesvirus causing FP, is not supported by the documented time order of events. Immunosuppression is only observed in sea turtles after tumors

have formed and severity has reached higher FP scores.^{21,22} With these new data on contaminant concentrations, we conclude that none of these 164 compounds is a primary contributor to FP in green turtles, but the rise of POP concentrations in late-stage, severely tumored, emaciated turtles might contribute to progression of the disease through immunosuppression or toxic effects to other organs or systems. Other environmental contaminants should not be discounted as possible contributors before they are investigated. The presence of halogenated phenols is a novel finding for sea turtles, suggesting that sea turtles are exposed to more compounds that remain to be discovered in their tissues. The sources of these compounds and their effects on sea turtles should be investigated.

■ DISCLAIMER

Commercial equipment or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by NIST or the U.S. government, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

■ ASSOCIATED CONTENT

📄 Supporting Information

Table S1 lists compounds measured with abbreviations. Tables S2–S4 show correlative results. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Aguirre, A. A.; Lutz, P. L. Marine turtles as sentinels of ecosystem health: Is fibropapillomatosis an indicator? *EcoHealth* **2004**, *1*, 275–283.
- (2) Herbst, L. H. Fibropapillomatosis of marine turtles. *Annu. Rev. Fish Dis.* **1994**, *4*, 389–425.
- (3) Herbst, L. H.; Jacobson, E. R.; Klein, P. A.; Balazs, G. H.; Moretti, R.; Brown, T.; Sundberg, J. P. Comparative pathology and patho-

genesis of spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). *Vet. Pathol.* **1999**, *36*, 551–564.

(4) Herbst, L. H.; Jacobson, E. R.; Moretti, R.; Brown, T.; Sundberg, J. P.; Klein, P. A. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. *Dis. Aquat. Organ.* **1995**, *22* (1), 1–12.

(5) Herbst, L. H.; Klein, P. A. Green turtle fibropapillomatosis: Challenges to assessing the role of environmental cofactors. *Environ. Health Perspect.* **1995**, *103* (Suppl 4), 27–30.

(6) dos Santos, R. G.; Martins, A. S.; Torezani, E.; Baptistotte, C.; Farias, J. D.; Horta, P. A.; Work, T. M.; Balazs, G. H. Relationship between fibropapillomatosis and environmental quality: a case study with *Chelonia mydas* off Brazil. *Dis. Aquat. Organ.* **2010**, *89* (1), 87–95.

(7) Foley, A. M.; Schroeder, B. A.; Redlow, A. E.; Fick-Child, K. J.; Teas, W. G. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the eastern United States (1980–98): Trends and associations with environmental factors. *J. Wildlife Dis.* **2005**, *41* (1), 29–41.

(8) Van Houtan, K. S.; Hargrove, S. K.; Balazs, G. H. Land use, macroalgae, and a tumor-forming disease in marine turtles. *PLoS One* **2010**, *5* (9), No. e12900, DOI: 10.1371/journal.pone.0012900.

(9) Aguirre, A. A.; Balazs, G. H.; Zimmerman, B.; Galey, F. D. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. *Mar. Pollut. Bull.* **1994**, *28* (2), 109–114.

(10) Aguirre, A. A.; Spraker, T. R.; Balazs, G. H.; Zimmerman, B. Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. *J. Wildlife Dis.* **1998**, *34* (1), 91–98.

(11) Anderson, Y.; Landsburg, J.; Balazs, G. H.; Carthy, R. The relationship between the potential tumor-promoting dinoflagellates *Prorocentrum* spp. and green turtle fibropapillomatosis: preliminary results of a comparison between Hawaii and Florida. In *Proceedings of the Twenty-First Annual Symposium on Sea Turtle Biology and Conservation*; Coyne, M. S., Clark, R. D., compilers; NOAA Technical Memorandum NMFS-SEFSC-528, **2005**, p 95; <http://www.nmfs.noaa.gov/pr/pdfs/species/turtlesymposium2001.pdf> (accessed Jul 1, 2014).

(12) Arthur, K.; Limpus, C.; Balazs, G.; Capper, A.; Udy, J.; Shaw, G.; Keuperbennett, U.; Bennett, P. The exposure of green turtles (*Chelonia mydas*) to tumour promoting compounds produced by the cyanobacterium *Lyngbya majuscula* and their potential role in the aetiology of fibropapillomatosis. *Harmful Algae* **2008**, *7* (1), 114–125.

(13) Herbst, L. H.; Greiner, E. C.; Ehrhart, L. M.; Bagley, D. A.; Klein, P. K. Serological association between spirorchidiasis, herpesvirus infection, and fibropapillomatosis in green turtles from Florida. *J. Wildlife Dis.* **1998**, *34* (3), 496–507.

(14) Landsberg, J. H.; Balazs, G. H.; Steidinger, K. A.; Baden, d. G.; Work, T. M.; Russell, D. J. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. *J. Aquat. Anim. Health* **1999**, *11*, 199–210.

(15) Lu, Y.; Yu, Q.; Zamzow, J. P.; Wang, Y.; Losey, G. S.; Balazs, G. H. Detection of green turtle herpesviral sequence in saddleback wrasse *Thalassoma duperrey*: a possible mode of transmission of green turtle fibropapilloma. *J. Aquat. Anim. Health* **2000**, *12*, 58–63.

(16) Miao, X.-S.; Balazs, G. H.; Murakawa, S. K. K.; Li, Q. X. Congener-specific profile and toxicity assessment of PCBs in green turtles (*Chelonia mydas*) from the Hawaiian Islands. *Sci. Total Environ.* **2001**, *281*, 247–253.

(17) Greenblatt, R. J.; Work, T. M.; Balazs, G. H.; Sutton, C. A.; Casey, R. N.; Casey, J. W. The Ozobranchus leech is a candidate mechanical vector for the fibropapilloma-associated turtle herpesvirus found latently infecting skin tumors on Hawaiian green turtles (*Chelonia mydas*). *Virology* **2004**, *321* (1), 101–110.

(18) Aguirre, A. A.; Balazs, G. H.; Spraker, T. R.; Gross, T. S. Adrenal and hematological responses to stress in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas. *Physiol. Zool.* **1995**, *68* (5), 831–854.

(19) Cray, C.; Varella, R.; Bossart, G. D.; Lutz, P. Altered *in vitro* immune responses in green turtles (*Chelonia mydas*) with fibropapillomatosis. *J. Zoo Wildlife Med.* **2001**, *32* (4), 436–440.

(20) Lutz, P. L.; Cray, C.; Sposato, P. L. *Studies of the association between immunosuppression and fibropapillomatosis within three habitats of Chelonia mydas*; Southwest Fisheries Science Center Administrative Report H-01–01C, National Marine Fisheries Service: Honolulu, HI, 2001; http://www.pifsc.noaa.gov/library/pubs/admin/SWFC_Admin_Report_01-01C.pdf (accessed Jul 1, 2014).

(21) Work, T. M.; Balazs, G. H. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. *J. Wildlife Dis.* **1999**, *35* (4), 804–807.

(22) Work, T. M.; Rameyer, R. A.; Balazs, G. H.; Cray, C.; Chang, S. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. *J. Wildlife Dis.* **2001**, *37* (3), 574–581.

(23) Chaloupka, M.; Balazs, G. H.; Work, T. M. Rise and fall over 26 years of a marine epizootic in Hawaiian green sea turtles. *J. Wildlife Dis.* **2009**, *45* (4), 1138–1142.

(24) Law, R.; Allchin, C.; Deboer, J.; Covaci, A.; Herzke, D.; Lepom, P.; Morris, S.; Tronczynski, J.; Dewit, C. Levels and trends of brominated flame retardants in the European environment. *Chemosphere* **2006**, *64* (2), 187–208.

(25) Lammel, G.; Lohmann, R. Identifying the research needs in the global assessment of toxic compounds 10 years after the signature of the Stockholm Convention. *Environ. Sci. Pollut. Res.* **2012**, *19* (6), 1873–1874.

(26) Kohler, H. R.; Triebkorn, R. Wildlife ecotoxicology of pesticides: Can we track effects to the population level and beyond? *Science* **2013**, *341* (6147), 759–765.

(27) Ross, P. S.; De Swart, R. L.; Addison, R.; Van Loveren, H.; Vos, J. G.; Osterhaus, A. D. Contaminant-induced immunotoxicity in harbour seals: Wildlife at risk? *Toxicology* **1996**, *112*, 157–169.

(28) Fernie, K. J.; Mayne, G.; Shutt, J. L.; Pekarik, C.; Grasman, K. A.; Letcher, R. J.; Drouillard, K. Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. *Environ. Pollut.* **2005**, *138* (3), 485–493.

(29) Hermanussen, S.; Matthews, V.; Pöpke, O.; Limpus, C. J.; Gaus, C. Flame retardants (PBDEs) in marine turtles, dugongs and seafood from Queensland, Australia. *Mar. Pollut. Bull.* **2008**, *57* (6–12), 409–418.

(30) Keller, J. M.; Pugh, R.; Becker, P. R. *Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST): Rationale, protocols, and initial collections of banked sea turtle tissues*, National Institute of Standards and Technology Internal Report 7996, Gaithersburg, MD, 2014.

(31) Bjørndal, K. A.; Bolten, A. B.; Chaloupka, M. Y. Green turtle somatic growth model: evidence for density dependence. *Ecol. Applic.* **2000**, *10* (1), 269–282.

(32) Keller, J. M.; Kucklick, J. R.; Stamper, M. A.; Harms, C. A.; McClellan-Green, P. D. Associations between organochlorine contaminant concentrations and clinical health parameters in loggerhead sea turtles from North Carolina, USA. *Environ. Health Perspect.* **2004**, *112* (10), 1074–1079.

(33) Grimvall, E.; Rylander, L.; Nilsson-Ehle, P.; Nilsson, U.; Stromberg, U.; Hagmar, L.; Ostman, C. Monitoring of polychlorinated biphenyls in human blood plasma: Methodological developments and influence of age, lactation, and fish consumption. *Arch. Environ. Contam. Toxicol.* **1997**, *32* (3), 329–336.

(34) Ragland, J. M.; Arendt, M. D.; Kucklick, J. R.; Keller, J. M. Persistent organic pollutants in blood plasma of satellite-tracked adult male loggerhead sea turtles (*Caretta caretta*). *Environ. Toxicol. Chem.* **2011**, *30* (7), 1549–1556.

(35) Keller, J. M.; Swarthout, R. F.; Carlson, B. K.; Yordy, J.; Guichard, A.; Schantz, M. M.; Kucklick, J. R. Comparison of five extraction methods for measuring PCBs, PBDEs, organochlorine pesticides, and lipid content in serum. *Anal. Bioanal. Chem.* **2009**, *393* (2), 747–760.

(36) Helsel, D. R. *Nondetects and Data Analysis: Statistics for Censored Environmental Data*; John Wiley & Sons: Hoboken, 2005.

- (37) Chaloupka, M.; Work, T. M.; Balazs, G. H.; Murakawa, S. K. K.; Morris, R. Cause-specific temporal and spatial trends in green sea turtle strandings in the Hawaiian Archipelago (1982–2003). *Mar. Biol.* **2008**, *154* (5), 887–898.
- (38) Keller, J. M.; Kucklick, J. R.; Harms, C. A.; McClellan-Green, P. D. Organochlorine contaminants in sea turtles: Correlations between whole blood and fat. *Environ. Toxicol. Chem.* **2004**, *23* (3), 726–738.
- (39) Hites, R. A. Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. *Environ. Sci. Technol.* **2004**, *38* (4), 945–956.
- (40) Keller, J. M., Exposure to and effects of persistent organic pollutants. In *The Biology of Sea Turtles Vol. III*; Wyneken, J., Lohmann, K. J., Musick, J. A., Eds.; CRC Press: Boca Raton, 2013; pp 285–328.
- (41) van de Merwe, J. P.; Hodge, M.; Whittier, J. M.; Ibrahim, K.; Lee, S. Y. Persistent organic pollutants in the green sea turtle *Chelonia mydas*: Nesting population variation, maternal transfer, and effects on development. *Mar. Ecol.: Prog. Ser.* **2010**, *403*, 269–278.
- (42) Haraguchi, K.; Kotaki, Y.; Relox, J. R.; Romero, M. L. J.; Terada, R. Monitoring of naturally produced brominated phenoxyphenols and phenoxyanisoles in aquatic plants from the Philippines. *J. Agric. Food. Chem.* **2010**, *58* (23), 12385–12391.
- (43) Vetter, W. Marine halogenated natural products of environmental relevance. *Rev. Environ. Contam. Toxicol.* **2006**, *188*, 1–57.
- (44) Arthur, K. E.; Balazs, G. H. A comparison of immature green turtle (*Chelonia mydas*) diets among seven sites in the main Hawaiian Islands. *Pac. Sci.* **2008**, *62* (4), 205–217.
- (45) Hovander, L.; Malmberg, T.; Athanasiadou, M.; Athanasiadis, I.; Rahm, S.; Bergman, A.; Wehler, E. K. Identification of hydroxylated PCB metabolites and other phenolic halogenated pollutants in human blood plasma. *Arch. Environ. Contam. Toxicol.* **2002**, *42*, 105–117.
- (46) Gribble, G. W. The diversity of naturally occurring organobromine compounds. *Chem. Soc. Rev.* **1999**, *28* (5), 335–346.
- (47) U.S. EPA. *Basic Information about Pentachlorophenol in Drinking Water*. <http://water.epa.gov/drink/contaminants/basicinformation/pentachlorophenol.cfm#two> (Jan 6, 2014).
- (48) Bolin, C. A.; Smith, S. T. Life cycle assessment of pentachlorophenol-treated wooden utility poles with comparisons to steel and concrete utility poles. *Renew. Sustain. Energy Rev.* **2011**, *15* (5), 2475–2486.
- (49) Schlezinger, J. J.; Keller, J.; Verbrugge, L. A.; Stegeman, J. J. 3,3',4,4'-tetrachlorobiphenyl oxidation in fish, bird and reptile species: relationship to cytochrome P450 1A inactivation and reactive oxygen production. *Comp. Biochem. Physiol. Part C* **2000**, *125*, 273–286.
- (50) Richardson, K. L.; Lopez Castro, M.; Gardner, S. C.; Schlenk, D. Polychlorinated biphenyls and biotransformation enzymes in three species of sea turtles from the Baja California Peninsula of Mexico. *Arch. Environ. Contam. Toxicol.* **2010**, *58* (1), 183–193.
- (51) Keller, J. M.; Thorvalson, K.; Sheridan, T.; Harms, C. A.; Segars, A. L.; Beasley, J.; Day, R. D.; Peden-Adams, M. M.; Kucklick, J. R.; Norton, T. Organic contaminant concentrations change in debilitated loggerhead turtle plasma during recovery in rehabilitation. In *Proceedings of the Twenty-Seventh Annual Symposium on Sea Turtle Biology and Conservation*; Rees, A. F., Frick, M., Panagopoulou, A., William, K., Compilers; NOAA Technical Memorandum NMFS-SEFSC-569, **2008**; pp 20–21; www.nmfs.noaa.gov/pr/pdfs/species/turtlesymposium2007.pdf (accessed Jul 1, 2014).
- (52) O'Connell, S. G.; Arendt, M.; Segars, A.; Kimmel, T.; Braun-McNeill, J.; Avens, L.; Schroeder, B.; Ngai, L.; Kucklick, J. R.; Keller, J. M. Temporal and spatial trends of perfluorinated compounds in juvenile loggerhead sea turtles (*Caretta caretta*) along the east coast of the United States. *Environ. Sci. Technol.* **2010**, *44* (13), 5202–5209.
- (53) Bazan, K. L. Persistent organic pollutants in shark blood plasma from estuaries along the southeast U.S. coast. Master's Thesis, College of Charleston, Charleston, SC, 2011.
- (54) Swarthout, R. F.; Keller, J. M.; Peden-Adams, M.; Landry, A. M.; Fair, P. A.; Kucklick, J. R. Organohalogen contaminants in blood of Kemp's ridley (*Lepidochelys kempii*) and green sea turtles (*Chelonia mydas*) from the Gulf of Mexico. *Chemosphere* **2010**, *78* (6), 731–741.
- (55) Komoroske, L. M.; Lewison, R. L.; Seminoff, J. A.; Deheyn, D. D.; Dutton, P. H. Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. *Chemosphere* **2011**, *84* (5), 544–552.
- (56) Houde, M.; Pacepavicius, G.; Wells, R. S.; Fair, P. A.; Letcher, R. J.; Alaea, M.; Bossart, G. D.; Hohn, A. A.; Sweeney, J.; Solomon, K. R.; Muir, D. C. Polychlorinated biphenyls and hydroxylated polychlorinated biphenyls in plasma of bottlenose dolphins (*Tursiops truncatus*) from the Western Atlantic and the Gulf of Mexico. *Environ. Sci. Technol.* **2006**, *40* (19), 5860–5866.
- (57) Klasson-Wehler, E.; Bergman, A.; Athanasiadou, M.; Ludwig, J. P.; Auman, H. J.; Kannan, K.; Van Den Berg, M.; Murk, A. J.; Feyk, L. A.; Giesy, J. P. Hydroxylated and methylsulfonyl polychlorinated biphenyl metabolites in albatrosses from Midway Atoll, North Pacific Ocean. *Environ. Toxicol. Chem.* **1998**, *17* (8), 1620–1625.
- (58) Letcher, R. J.; Fernie, K. J.; Shahmiri, S.; Chu, S.; De Solla, S. R.; Sandau, C. D. Snapping turtles (*Chelydra serpentina*) from Canadian Great Lakes Areas of Concern (AOCs): Metabolic formation versus bioaccumulation of hydroxylated PCBs and PBDEs. In *SETAC North American Meeting Abstract Book, Montreal*; SETAC Pensacola Office: Pensacola, FL, 2006; pp 368–369; <http://www.setac.org/store/ViewProduct.aspx?id=1248711>.
- (59) Nomiya, K.; Uchiyama, Y.; Horiuchi, S.; Eguchi, A.; Mizukawa, H.; Hirata, S. H.; Shinohara, R.; Tanabe, S. Organohalogen compounds and their metabolites in the blood of Japanese amberjack (*Seriola quinqueradiata*) and scalloped hammerhead shark (*Sphyrna lewini*) from Japanese coastal waters. *Chemosphere* **2011**, *85* (3), 315–321.
- (60) Routti, H.; Letcher, R. J.; Arukwe, A.; van Bavel, B.; Yoccoz, N. G.; Chu, S. G.; Gabrielsen, G. W. Biotransformation of PCBs in relation to phase I and II xenobiotic-metabolizing enzyme activities in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. *Environ. Sci. Technol.* **2008**, *42* (23), 8952–8958.
- (61) Sandala, G. M.; Sonne-Hansen, C.; Dietz, R.; Muir, D. C. G.; Valters, K.; Bennett, E. R.; Born, E. W.; Letcher, R. J. Hydroxylated and methyl sulfone PCB metabolites in adipose and whole blood of polar bear (*Ursus maritimus*) from East Greenland. *Sci. Total Environ.* **2004**, *331* (1–3), 125–141.