

## ORGANOCHLORINE CONTAMINANTS IN SEA TURTLES: CORRELATIONS BETWEEN WHOLE BLOOD AND FAT

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**Abstract**—Monitoring toxic organochlorine (OC) compounds is an important aspect in wildlife studies, especially in protected species such as sea turtles. The goal of this study was to determine whether blood OC concentrations can predict those in adipose tissue of sea turtles. Blood offers many benefits for monitoring OCs. It can be collected nondestructively from live turtles and can be sampled repeatedly for continuous monitoring. Organochlorine concentrations in blood may better represent the exposure levels of target tissues, but blood concentrations may fluctuate more than those in fatty tissues following recent dietary exposure or lipid mobilization. Paired fat and blood samples were collected from 44 live, juvenile loggerhead sea turtles and 10 juvenile Kemp's ridley sea turtle carcasses. Organochlorines were analyzed using gas chromatography with electron capture detection and mass spectrometry. Lipid-normalized OC concentrations measured in the blood significantly correlated to levels found in the fat samples of both species. This result suggests that sea turtle blood is a suitable alternative to fatty tissues for measuring OCs because blood concentrations reasonably represent those observed in the paired fat samples. However, blood OC concentrations calculated on a wet-mass basis were significantly and inversely correlated to lipid content in the fat samples. Therefore, caution should be used when monitoring spatial or temporal trends, as OC levels may increase in the blood following mobilization of fat stores, such as during long migrations, breeding, or disease events.

**Keywords**—Loggerhead Kemp's ridley Organochlorine Blood Fat

## INTRODUCTION

Organochlorine (OC) compounds, such as pesticides and polychlorinated biphenyls (PCBs), are highly persistent, bioaccumulative, lipophilic contaminants. Most of these compounds were banned from use in the United States and Europe beginning in the 1970s; however, they continue to be detected in both biological and environmental samples worldwide [1]. Chronic exposure to OCs has been shown to affect a variety of biological systems at concentrations much lower than those responsible for acute toxicity. Effects have been demonstrated on the immune, endocrine, developmental, and reproductive systems [2]. The toxicity of OCs poses a considerable hazard for both wildlife and human populations.

The toxicological effects of OC contaminants create special problems for endangered and threatened species such as sea turtles. All seven species of sea turtles are listed nationally or internationally as endangered or threatened [3]. The Kemp's ridley sea turtle (*Lepidochelys kempii*) is considered the most endangered sea turtle species in the world. The number of nesting females of this species plummeted in the 1960s and continued to decline until 1985, when fewer than 1,000 nests were laid on their only known nesting beach at Rancho Nuevo, Mexico [4]. Since then, the population has been increasing, largely because of conservation policies that have reduced poaching and entanglement in fishing gear. Similarly, the log-

gerhead sea turtle (*Caretta caretta*) is listed as threatened on the U.S. Endangered Species Act and has suffered population declines. While the loggerhead subpopulation that nests in southern Florida has been increasing in numbers, the northern subpopulation that ranges from northern Florida to North Carolina (USA) has been decreasing by 2 to 3% per year [4]. The cause of this decline is unknown.

While sea turtles clearly face numerous man-made threats, the effects of environmental contaminants on their health, survival, and reproduction are completely undocumented. The life history of sea turtles creates a challenge for toxicological research, as they are highly migratory and long-lived species. Loggerhead turtles circumnavigate the Atlantic Ocean as pelagic juveniles, spending time in the open ocean and near the Azores, Madeira, and the Canary Islands [5]. Some even enter the Mediterranean Sea during this stage. They return to U.S. estuaries as large juveniles to feed on benthic crustaceans and mollusks. Kemp's ridley sea turtles spend their pelagic juvenile phase in the offshore waters of the Gulf of Mexico or the western North Atlantic Ocean. The age at which these turtles mature is not known but has been estimated to be 21 to 35 years for the loggerhead and 7 to 16 years for the Kemp's ridley [4,6]. Their long migrations facilitate their exposure to diverse contaminant classes arising from a variety of sources, while their long life span allows time for them to accumulate persistent OC contaminants.

Studies reporting contaminant concentrations in sea turtle tissues are limited and widely scattered across contaminant types, geographic locations, species, and tissues (for a review,

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see Pugh and Becker [7]). Furthermore, only one study has reported OC concentrations in sea turtle blood [8]. Since OCs possess a great ability to bioaccumulate into lipid-rich tissues, past studies have primarily analyzed eggs or fatty tissues from dead sea turtles. As shown in snapping turtles, egg concentrations reasonably represent the contaminant burdens of adult nesting females, as OC compounds are maternally transferred into eggs [9]. Fatty tissues, such as fat and liver, can be collected from live animals only using invasive or logistically difficult sampling; therefore, these tissues have traditionally been collected from carcasses stranded on the beach. Samples from dead stranded turtles are solely opportunistic and may be biased toward diseased or partially decomposed animals. As shown with marine mammals, diseased animals may have higher contaminant concentrations than live, healthy organisms [10]. These studies are further complicated because decomposition can result in cross contamination from other tissues or surroundings. The loss or decomposition of lipids may increase the net tissue OC concentrations, as concentrations are typically normalized to lipid content. Organochlorine concentrations in collected tissue samples may decrease because of bacterial degradation of the compounds. An alternative tissue—blood—can be collected from live sea turtles in a relatively noninvasive and nonbiased manner and has recently become a common tissue for measuring OC levels in humans and wildlife [8,11–13].

The use of turtle blood to measure accumulation of OC contaminants may offer many benefits; however, studies in other organisms have shown that the concentrations in blood can fluctuate temporally depending on recent dietary exposure or mobilization of lipid stores. Organochlorine concentrations have been shown to increase in the blood of humans and seals following dramatic weight loss, suggesting that contaminants stored in adipose tissue are released into the blood during lipid mobilization [14,15]. However, a great number of studies examining birds, marine mammals, and humans found that blood OC concentrations were proportional to concentrations present in fatty tissues [11,16–20]. In all these studies, statistically significant, positive correlations were observed between blood and fatty tissue OC contaminant levels. Boon et al. [21] provide data on harbor seals that support a kinetic model in which OC concentrations in the blood are in a dynamic balance with fatty tissues. Overall, these prior studies suggest that blood OC concentrations will fluctuate during lipid mobilization, but in many situations and with many wildlife species blood can be used successfully for monitoring contaminants.

Our study investigated OC concentrations in the blood of sea turtles and compared these to levels found in adipose tissue. Our goal was to determine if blood could be used to monitor OC contaminants in these endangered animals. Temporal changes were also investigated in blood OC concentrations by analyzing and comparing samples taken from loggerhead turtles that were recaptured. Finally, we compared blood OC concentrations to the lipid content in the adipose tissue. This allowed us to determine whether turtles with low adipose lipid content, and possibly those that had mobilized their lipid reserves, had higher levels of OCs in their blood.

## MATERIALS AND METHODS

### *Samples*

During August 2000 and July 2001, 44 free-ranging, juvenile loggerhead sea turtles were captured using the pound-net fishery in Core Sound (NC, USA) and were transported to

the National Marine Fisheries Service in Beaufort (NC, USA). The turtles ranged from 45.7 to 74.1 cm in straight carapace length (SCL; nuchal notch to posterior marginal notch) and from 14.4 to 56.6 kg in weight. Based on these sizes, the turtle ages may fall somewhere between 10 and 30 years of age [6]. Body condition was calculated as weight (kg) divided by the cube of SCL and multiplied by  $10^4$ . The turtles were tagged, and a laparoscopy was performed to determine their sex. Thirty turtles were identified as females and 14 as males. Blood samples (10 ml) were collected from the dorsocervical sinus using Vacutainer double-ended needles inserted directly into Vacutainer blood collection tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Whole blood samples were kept on ice until frozen at  $-20^{\circ}\text{C}$ . Six of the loggerhead turtles were recaptured at a later date, and a second blood sample was drawn.

Excisional biopsies of subcutaneous fat (0.4–4.0 g) were removed from the left inguinal region through a 3-cm transverse incision approximately 3 to 5 cm caudal to the plastron margin. Stainless-steel surgical instruments were hexane-rinsed and sterile, and the surgical site was blocked prior to the procedure by infiltration of a lidocaine local anesthetic (Lidocaine Hydrochloride Injectable-2%, Phoenix Pharmaceutical, St. Joseph, MO, USA). The incisions were closed with 4-O polyglyconate (Maxon, Sherwood-Davis and Geck, Manati, Puerto Rico), in patterns according to surgeon preference, and cyanoacrylate tissue glue. Turtles were observed for a few hours and then released.

Fat samples were stored in hexane-rinsed aluminum foil and were frozen at  $-80^{\circ}\text{C}$  until analyzed for OC contaminants. A portion of a fat biopsy from one turtle was placed in 10% neutral-buffered formalin. Fixed tissue was processed routinely for paraffin sections and stained with hematoxylin and eosin for histological examination.

During a cold stunning event in November 1999 along Cape Cod (MA, USA), blood and fat samples were collected from eight juvenile Kemp's ridley sea turtles that died during rehabilitation. The turtles ranged from 21.9 to 31.1 cm in SCL and were estimated to be 1.25 to 2.25 years of age based on a skeletochronological method that counts the number of annually deposited layers of arrested growth in the humerus [22]. Blood was drained from the heart directly into open Vacutainer tubes containing sodium heparin and was stored at  $-20^{\circ}\text{C}$ . Two types of fat were collected: yellow fat surrounding the internal organs and brown fat along the carapace margin and in the inguinal and shoulder regions. Fat samples were collected using hexane-rinsed stainless-steel instruments and stored in hexane-rinsed foil at  $-80^{\circ}\text{C}$ . Both types of fat, but not blood, were also collected from two Kemp's ridley sea turtles (a female at 45.6 cm SCL, 4.75 years old [6], and a male at 29.7 cm SCL,  $\sim 2$  years old) that drowned in North Carolina in April 1998 and July 2000, respectively.

In addition to the loggerhead and Kemp's ridley sea turtle samples, body fat samples were also collected from one juvenile male green sea turtle (24.2 cm SCL) found dead on August 7, 2000, in Core Sound and one adult female leatherback sea turtle (176.0 cm curved CL) that was euthanized following an injury inflicted by a boat propeller near Long Beach (NC, USA) on June 9, 1999. The blubber layer of the carapace was also sampled from the leatherback turtle along the right margin of the carapace and plastron.

### Organochlorine contaminant analysis

Whole blood and fat samples were analyzed for 55 polychlorinated biphenyl (PCB) congeners and 24 OC pesticides. All analyses were performed using cleaned and hexane-rinsed glassware, stainless-steel instruments, and glass serological and transfer pipettes.

#### Extractions of fat samples

Fat samples and leatherback blubber (0.4–4 g each) were weighed, minced using a hexane-rinsed scalpel, mixed with 40 g anhydrous Na<sub>2</sub>SO<sub>4</sub>, and transferred to 33-ml pressurized fluid extractor cells (Dionex, Salt Lake City, UT, USA). Five calibration solutions were prepared by combining and diluting National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM): 2261 (chlorinated pesticides in hexane), 2275 (chlorinated pesticides in hexane II), 2262 (chlorinated biphenyl congeners in 2,2,4-trimethylpentane), 2274 (PCB congener solution II), and a solution containing 14 additional PCB congeners. The diluted calibration solutions were added to individual pressurized fluid extractor cells resulting in a calibration curve ranging from 0.3 to 400 ng. An internal standard solution was also added containing 70 ng of each of 4,4'-DDT-*d*<sub>8</sub>, 4,4'-dichlorodiphenyldichloroethylene (DDE)-*d*<sub>8</sub>, 4,4'-dichlorodiphenyldichloroethane (DDD)-*d*<sub>8</sub>, Endosulfan I-*d*<sub>4</sub>, PCB 103, and PCB 198. The NIST SRM 1945 (organics in whale blubber) and a blank were also processed with each batch of samples. Samples were extracted with dichloromethane on the pressurized fluid extractor as described elsewhere [23]. Extracts were reduced in volume using purified N<sub>2</sub> (Turbovap II, Zymark, Hopkinton, MA, USA).

#### Extractions of blood samples

While most OCs partition into the plasma of sea turtle blood [8], only whole blood was available for many of the samples in this study. Whole blood samples were extracted using a liquid:liquid extraction method (technique A) described in Keller et al. [8]. Briefly, 3 to 5 g of blood were mixed with 0.2 g of the internal standard solution described previously and allowed to equilibrate at room temperature for 2 h. Samples were treated with formic acid and then extracted three times with 1:1 (v/v) methyl-*tert*-butyl ether:hexane. The organic phases were combined, reduced in volume, and dried with 10 to 20 g anhydrous Na<sub>2</sub>SO<sub>4</sub>. NIST SRM 1589a (PCBs, pesticides, and dioxins/furans in human serum) and blanks were processed with each sample batch.

#### Lipid determination

Lipid content was determined gravimetrically for all fat and blood samples, NIST SRM 1945, and NIST SRM 1589a. Approximately 5 to 10% by weight of each extract was removed and transferred to a tared aluminum weighing boat. The solvent was allowed to evaporate at room temperature for 4 to 12 h, and the dried lipid residue was reweighed to the nearest 0.00001 g for fat and 0.0001 mg for blood.

#### Sample cleanup

High-molecular-mass compounds in the fat samples were removed by gel permeation chromatography [23]. The gel permeation chromatography was used for all the Kemp's ridley blood samples and the loggerhead blood samples from 2000. An alumina column was used for the year 2001 loggerhead blood samples [8].

All sample extracts and the calibration solutions were frac-

tionated into relatively lower- and higher-polarity fractions (F1 and F2, respectively) using a semipreparative aminopropylsilane column (Bondapak NH<sub>2</sub>, Waters, Milford, MA, USA) [23]. Compounds contained in F1 included PCBs, heptachlor, 2,4'-dichlorodiphenyldichloroethylene (DDE), 4,4'-DDE, 2,4'-DDT, hexachlorobenzene (HCB), aldrin, mirex, and oxychlordane. Analytes in F2 included 4,4'-DDT; *cis*- and *trans*-chlordane; *cis*- and *trans*-nonachlor;  $\alpha$ -  $\beta$ -, and  $\gamma$ -hexachlorocyclohexane (HCH); heptachlor epoxide; 2,4'-dichlorodiphenyldichloroethane (DDD); 4,4'-DDD; dieldrin; endrin; endosulfans I and II; and endosulfan sulfate. Each fraction of the fat samples and the corresponding standards and blanks were reduced to approximately 0.5 ml. The blood samples, corresponding blanks, and standards were amended with 5 ng of PCB 14 prior to analysis in order to calculate the recovery of internal standards [8]. The blood extracts were reduced to between 0.05 and 0.1 ml using a stream of purified nitrogen prior to analysis.

#### Contaminant analysis

Both the F1 and the F2 fractions of fat samples were analyzed on a gas chromatograph with dual microelectron capture detectors according to Kucklick et al. [23]. The gas chromatograph–electron capture detector was used to analyze the F1 of the blood samples. For the F2 fraction of the blood samples, a gas chromatograph–mass spectrometer was used operating in the electron-impact mode and using selected ion monitoring using conditions described elsewhere [8]. The calibration standards used for the fat samples were extracted, but standards were not extracted for the blood samples. Thus, the concentrations in the blood were corrected for recovery of the internal standards.

#### Statistics

The contaminant data were not normally distributed even after log transformation; therefore, only nonparametric statistical tests were used (Systat® 8.0 software, SPSS, Chicago, IL, USA). All correlative relationships were tested using the Spearman rank correlation test. The Wilcoxon signed rank test was used to determine a difference in contaminant concentrations between the yellow and brown fat of the Kemp's ridley turtles. The Mann–Whitney test was used to compare mean fat concentrations of OCs in male to female loggerhead turtles. Lipid-normalized OC concentrations (ng/g of lipid in the tissue) were used in all statistical tests, except the correlation analysis between the blood OC concentrations (wet-mass basis) and the percentage lipid in fat biopsies.

## RESULTS

#### Validation of methods

Histological examination was performed on a sample of loggerhead sea turtle fat to validate our fat biopsy sampling method. The tissue floated in formalin, as expected, and histological examination verified our visual tissue identification. The tissue section was composed of sheets of irregular polygonal adipose cells containing large clear vacuoles and small eccentric nuclei, interspersed with sparse fibrovascular stroma, typical of adipose tissue.

The NIST SRMs representing tissue matrices similar to adipose and blood were analyzed for OCs alongside each batch of turtle samples to validate the chemical analysis. Organochlorine concentrations were measured in four samples of NIST SRM 1945 (whale blubber) and five bottles of NIST

Table 1. Lipid-normalized organochlorine contaminant concentrations (ng/g lipid) in fat biopsies and blood from 44 juvenile loggerhead sea turtles<sup>a</sup>

	Fat biopsies (ng/g lipid)			Whole blood (ng/g lipid)		
	Mean (SD) <sup>b</sup>	<i>n</i> > LOD <sup>c</sup>	Median (quartiles)	Mean (SD)	<i>n</i> > LOD	Median (quartiles)
Total PCBs	2,010 (2,960)	44	1,010 (619–2,360)	2,490 (3,700)	44	2,030 (1,020–2,810)
α-HCH	0.270 (1.46)	2	<LOD <sup>d</sup>	<LOD	0	<LOD
β-HCH	0.358 (1.03)	5	<LOD	<LOD	0	<LOD
γ-HCH	3.27 (10.6)	7	<LOD	<LOD	0	<LOD
HCB	2.57 (6.40)	12	<LOD (<LOD–1.85)	<LOD	0	<LOD
Mirex	43.7 (62.1)	39	18.8 (9.05–42.7)	21.2 (39.2)	27	7.58 (<LOD–20.5)
Dieldrin	35.3 (87.2)	38	18.8 (7.00–30.3)	20.1 (25.8)	26	15.1 (<LOD–27.4)
Heptachlor epoxide	10.4 (12.2)	29	8.85 (<LOD–15.1)	8.02 (15.9)	16	<LOD (<LOD–10.6)
<i>trans</i> -Chlordane	<LOD	0	<LOD	10.1 (15.7)	30	7.72 (<LOD–12.8)
<i>cis</i> -Chlordane	<LOD	0	<LOD	0.406 (1.56)	3	<LOD
<i>trans</i> -Nonachlor	107 (147)	42	70.5 (43.6–103)	56.7 (99.4)	43	34.6 (22.4–61.0)
<i>cis</i> -Nonachlor	5.17 (6.58)	25	3.72 (<LOD–8.34)	9.05 (6.95)	37	8.61 (4.98–11.5)
Oxychlordane	134 (277)	40	37.5 (26.8–163)	25.8 (35.7)	35	14.9 (8.21–37.1)
Total chlordanes	246 (412)	43	125 (83.2–262)	102 (151)	43	67.8 (42.3–124)
4,4'-DDD	<LOD	0	<LOD	4.09 (6.45)	20	<LOD (<LOD–5.78)
4,4'-DDE	445 (643)	41	250 (144–476)	300 (578)	41	172 (99.8–333)
2,4'-DDT	7.03 (13.1)	11	<LOD (<LOD–4.14)	0.286 (1.90)	1	<LOD
Total DDTs	452 (643)	41	254 (144–477)	305 (577)	42	192 (101–337)
Percentage lipid	26.3 (20.6)		26.1 (4.68–42.6)	0.262 (0.0804)		0.257 (0.209–0.306)

<sup>a</sup> PCB = polychlorinated biphenyl; HCH = hexachlorohexane; HCB = hexachlorobenzene; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene.

<sup>b</sup> SD = standard deviation.

<sup>c</sup> Number of samples out of 44 that were above the limit of detection (1 ng/g wet mass for fat; 10 pg/g wet mass for blood).

<sup>d</sup> <LOD = below the limit of detection.

SRM 1589a (human serum). The values obtained from these NIST SRMs were reported and discussed elsewhere [8,24]. The values we obtained for the majority of the OCs differed from the certified values by less than 30%.

#### Contaminant concentrations and lipid content

Organochlorine contaminant concentrations and lipid content in the tissues of loggerhead and Kemp's ridley sea turtles are listed in Tables 1 and 2, respectively. The patterns of PCB congeners in the blood and fat of both species are shown in Figure 1. Lipid content in loggerhead adipose tissue was extremely variable among individuals (range = 0.255–64.7%), emphasizing the importance of normalizing the OC concentrations to lipid content for better comparisons of OC concentrations between individual turtles and between different tissues. The lipid content in the adipose tissues of the Kemp's ridley sea turtle varied only slightly, with the exception of one turtle. This turtle was extremely emaciated and had 0.521% lipid in its brown fat and no apparent yellow fat. The blood lipid content (range = 0.209–0.306% in loggerheads) varied less than the fat lipid content for both species.

Some OC compounds were detectable in one tissue and not in another (Tables 1 and 2). No blood sample from either loggerhead or Kemp's ridley turtles had detectable concentrations of HCHs, while low concentrations of HCHs were found in at least five of the fat samples. *Trans*-chlordane was not found in any loggerhead fat biopsy, but low concentrations were measurable in 30 of the blood samples. These discrepancies are likely due to the difficulty in measuring these compounds at their low concentrations and the differing limits of detection between the tissues. Limits of detection were 1 ng/g wet mass for the fat and 10 pg/g wet mass for the blood samples.

Overall, the mean lipid-normalized blood OC concentra-

tions were similar to the fat concentrations for both species. Likewise, the concentrations of all the major OC compounds in the Kemp's ridley yellow fat were not significantly different than those in the brown fat samples (Wilcoxon,  $p > 0.05$ ; Table 2). The OC concentrations in the yellow fat were significantly correlated to those in the brown fat for all the major PCB congeners, total PCBs, and all the major pesticides, including HCHs, mirex, dieldrin, total chlordanes, and total DDTs (Spearman rank correlation,  $p < 0.05$ ; Table 3).

#### OC concentrations in fat versus blood

The OC concentrations in fat significantly correlated to blood concentrations for both species and for all the major PCB congeners, total PCBs, and all the major pesticides, including mirex, total chlordanes, and total DDTs (Spearman rank correlation,  $p < 0.05$ ; Table 3 and Fig. 2). Even when the loggerhead turtle with the highest levels of OCs was removed from the comparisons, the correlations between blood and fat concentrations remained statistically significant.

#### Blood OC concentrations versus lipid content in fat biopsies

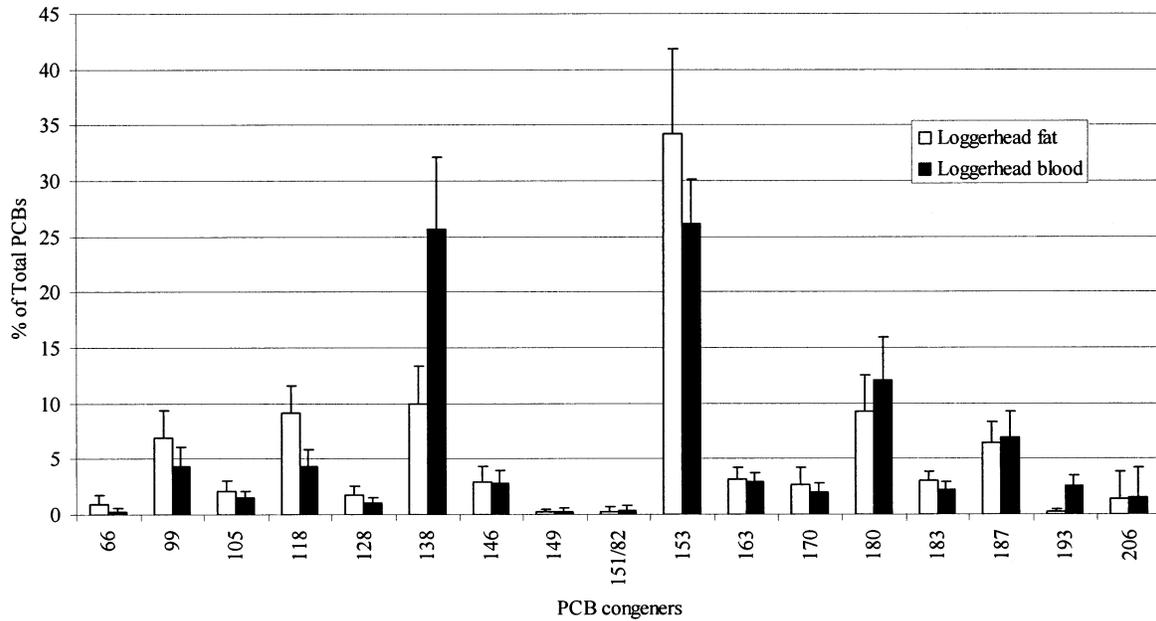
Lipid content in the fat of the loggerhead sea turtles was not significantly correlated with body condition (Fig. 3A). This result suggested that emaciated turtles did not have lower lipid content in their fat. On the other hand, the percentage lipid in the fat was inversely correlated to concentrations of blood OCs on a wet-mass basis (Fig. 3B and C;  $r_s = -0.403$  for total PCBs,  $-0.431$  for heptachlor epoxide,  $-0.671$  for total chlordanes,  $-0.551$  for mirex, and  $-0.361$  for total DDTs; all  $p$  values  $< 0.02$ ). This suggested that turtles that mobilized their lipid stores had higher concentrations of OCs circulating in their blood.

Table 2. Lipid-normalized organochlorine contaminant concentrations (ng/g lipid) in fat and blood samples from juvenile Kemp's ridley sea turtles<sup>a</sup>

	Yellow fat (ng/g lipid)			Brown fat (ng/g lipid)			Whole blood (ng/g lipid)		
	Mean (SD)	n > LOD <sup>b</sup>	Median (quartiles)	Mean (SD)	n > LOD <sup>c</sup>	Median (quartiles)	Mean (SD)	n > LOD <sup>d</sup>	Median (quartiles)
Total PCBs	1,050 (1,220)	9	726 (206–1,270)	1,110 (1,030)	10	887 (240–1,540)	985 (1,250)	8	404 (176–1,240)
α-HCH	6.09 (4.59)	7	5.31 (3.54–8.17)	10.2 (15.8)	7	5.06 (0.638–11.3)	<LOD <sup>e</sup>	0	<LOD
β-HCH	50.8 (100)	5	3.90 (<LOD–21)	41.4 (68.6)	8	9.45 (3.99–25.6)	<LOD	0	<LOD
γ-HCH	21.7 (35.1)	5	1.86 (<LOD–28.6)	30.6 (46.2)	6	15.0 (<LOD–43.5)	<LOD	0	<LOD
HCB	20.3 (14.7)	8	20.1 (10.7–32.4)	15.1 (13.2)	9	10.4 (3.94–26.1)	1.33 (2.55)	2	<LOD (<LOD–1.02)
Mirex	5.26 (6.82)	6	2.88 (<LOD–7.02)	5.34 (6.13)	7	3.53 (0.783–6.62)	7.12 (13.5)	4	1.73 (<LOD–6.06)
Dieldrin	33.8 (22.3)	9	43.5 (13.6–43.7)	51.7 (66.2)	10	32.5 (14.9–53.3)	17.9 (16.3)	6	14.4 (8.49–25.1)
Heptachlor epoxide	24.2 (30.5)	9	10.9 (8.31–26.5)	31.3 (38.0)	8	18.9 (3.27–37.5)	13.3 (10.6)	7	10.4 (6.09–20.9)
trans-Chlordane	0.844 (2.53)	1	<LOD	2.80 (3.95)	5	0.720 (<LOD–5.22)	2.74 (5.12)	3	<LOD (<LOD–3.14)
cis-Chlordane	1.37 (1.81)	4	<LOD (<LOD–2.40)	1.12 (1.95)	3	<LOD (<LOD–1.79)	0.816 (1.52)	2	<LOD (<LOD–0.743)
trans-Nonachlor	54.7 (46.9)	9	35.9 (23.7–79.1)	109 (173)	10	53.3 (24.4–102)	41.2 (50.2)	8	27.4 (18.7–36.8)
cis-Nonachlor	10.4 (8.62)	8	6.64 (3.86–17.7)	9.77 (9.29)	8	5.64 (2.37–19.3)	11.8 (6.06)	8	10.3 (7.07–14.2)
Oxychlordane	90.8 (98.9)	9	43.8 (16.7–123)	117 (162)	10	66.6 (27.6–135)	20.6 (24.3)	6	10.4 (5.49–29.0)
Total chlordanes	158 (138)	9	80.3 (49.6–234)	240 (331)	10	144 (60.7–266)	77.2 (81.6)	8	56.9 (36.3–73.5)
4,4'-DDD	2.76 (4.58)	4	<LOD (<LOD–2.18)	2.96 (5.11)	5	0.795 (<LOD–1.98)	5.97 (1.79)	8	5.74 (4.84–6.57)
4,4'-DDE	154 (111)	9	103 (54.8–256)	254 (332)	10	169 (69.9–276)	166 (147)	8	119 (76.7–178)
Total DDTs	156 (113)	9	103 (54.8–257)	257 (332)	10	175 (71.5–287)	172 (147)	8	24 (83.4–185)
Percentage lipid	65.8 (10.6)		70.7 (57.7–74.8)	62.0 (23.9)		72.8 (57.6–74.3)	0.461 (0.313)		0.347 (0.289–0.463)

<sup>a</sup> Refer to Table 1 for explanation of abbreviations.<sup>b</sup> Number of samples out of nine that were above the limit of detection (LOD).<sup>c</sup> Number of samples out of 10 that were above the LOD.<sup>d</sup> Number of samples out of eight that were above the LOD.<sup>e</sup> <LOD = below LOD (1 ng/g wet mass for fat; 10 pg/g wet mass for blood).

A)



B)

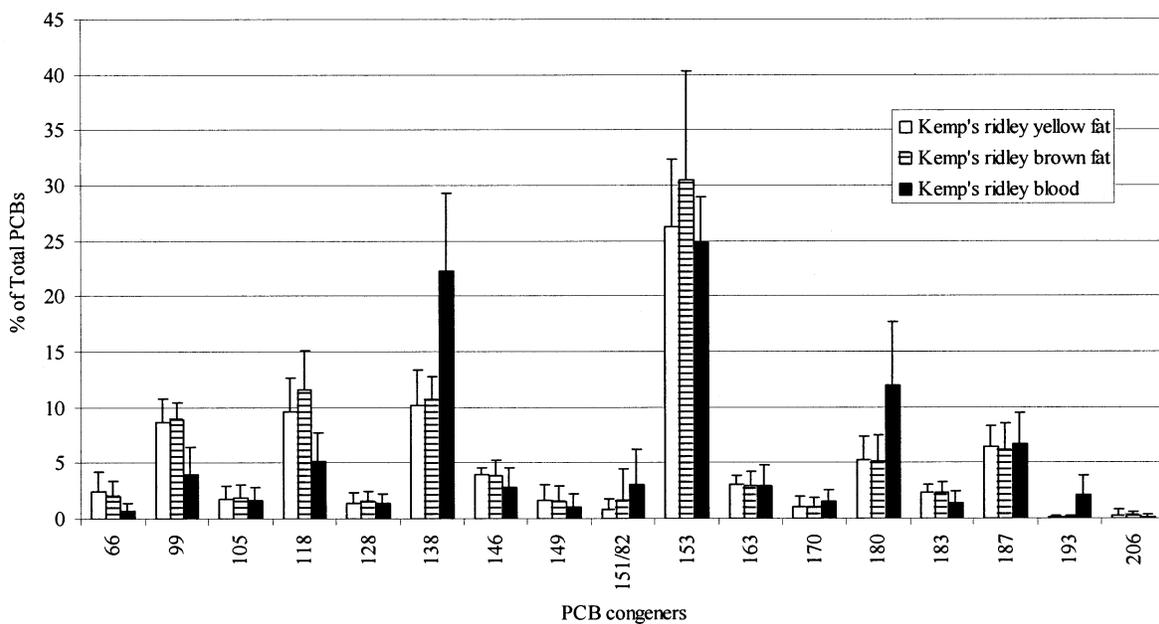


Fig. 1. Pattern of polychlorinated biphenyl (PCB) congeners (wet-mass basis) in blood and fat of (A) loggerhead and (B) Kemp's ridley sea turtles (mean and standard deviation). Only congeners with greater than 2% of the total PCB concentration in at least one of the tissues are included. Sample sizes are 44 for loggerhead blood and fat, 9 for yellow fat, 10 for brown fat, and 8 for blood of Kemp's ridley sea turtles.

#### Blood OC concentrations in recaptured turtles

To investigate the temporal variability of blood OC concentrations, we collected and analyzed a second blood sample on recapturing six loggerhead turtles. The duration between sampling ranged from 17 to 403 d, and all turtles grew over this time (Table 4). The blood lipid content changed on average between the first and second sampling times by 27.7% (standard deviation = 48.7%) (Table 4). The blood OC concentra-

tions varied considerably (Fig. 4). Organochlorine concentrations increased in four of the six turtles, while a decreasing trend was observed in the other two turtles. Turtle identifications (IDs) 5 and 6 were recaptured more than one year after the initial capture date. Their blood PCB concentrations nearly doubled, as did the concentrations in turtle ID 1, which was recaptured after only 17 d. The average percentage difference between the first and second sampling times (standard deviation) was an increase of 39.7% (69.2%) for total PCBs, 89.5%

Table 3. Spearman rank correlation coefficients ( $p$  values) of lipid-normalized organochlorine contaminant concentrations between various tissues of sea turtles<sup>a</sup>

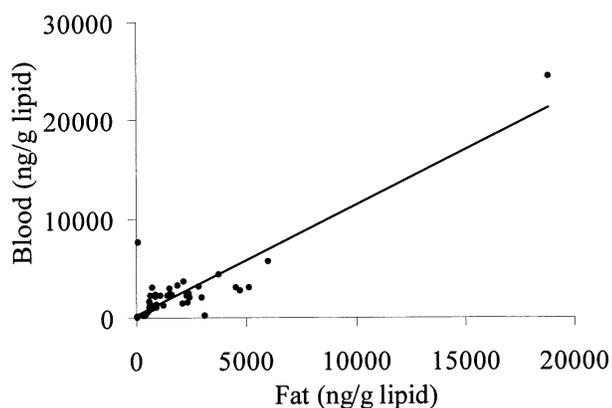
	Loggerhead fat biopsies vs whole blood	Kemp's ridley brown fat vs whole blood	Kemp's ridley yellow fat vs whole blood	Kemp's ridley yellow fat vs brown fat
Total PCBs	0.634 (<0.001)	0.976 (0.001)	0.893 (0.02)	0.929 (<0.002)
Mirex	0.634 (<0.001)	0.762 (<0.05)	0.786 (0.05)	0.933 (0.001)
Dieldrin	0.582 (<0.001)	0.786 (<0.05)	NS <sup>b</sup>	0.904 (<0.005)
Heptachlor epoxide	0.436 (<0.005)	NS	NS	NS
<i>cis</i> -Chlordane	ND <sup>c</sup>	NS	NS	0.750 (<0.05)
<i>trans</i> -Nonachlor	0.752 (<0.001)	1.000 (<0.001)	0.964 (0.005)	0.967 (<0.001)
<i>cis</i> -Nonachlor	NS	0.905 (0.005)	0.857 (<0.05)	0.883 (<0.005)
Oxychlordane	0.697 (<0.001)	0.857 (<0.02)	0.964 (0.005)	0.767 (<0.05)
Total chlordanes	0.722 (<0.001)	0.905 (0.005)	0.929 (0.01)	0.867 (0.005)
4,4'-DDD	ND	NS	NS	0.833 (0.01)
4,4'-DDE	0.657 (<0.001)	0.857 (<0.02)	0.786 (0.05)	0.950 (<0.001)
Total DDTs	0.665 (<0.001)	0.905 (0.005)	0.857 (<0.05)	0.917 (0.002)

<sup>a</sup> Refer to Table 1 for explanation of abbreviations.

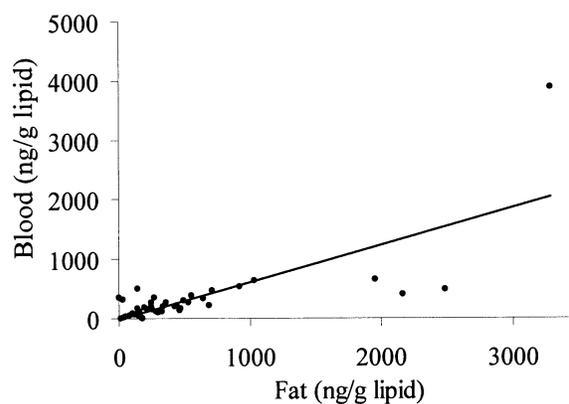
<sup>b</sup> NS = not significant,  $p > 0.05$ .

<sup>c</sup> ND = not determined because these compounds were below the limit of detection in at least one tissue.

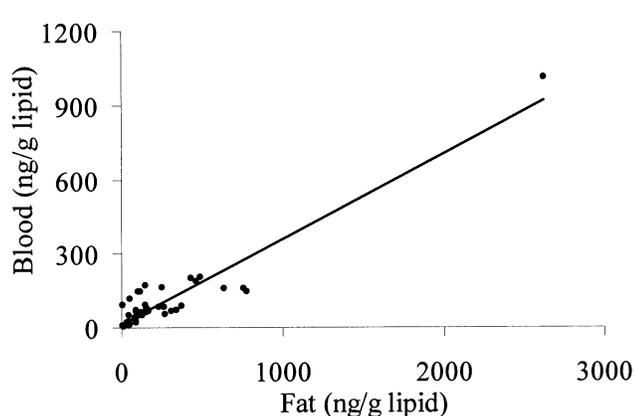
A) Total PCBs



B) Total DDTs



C) Total Chlordanes



D) Mirex

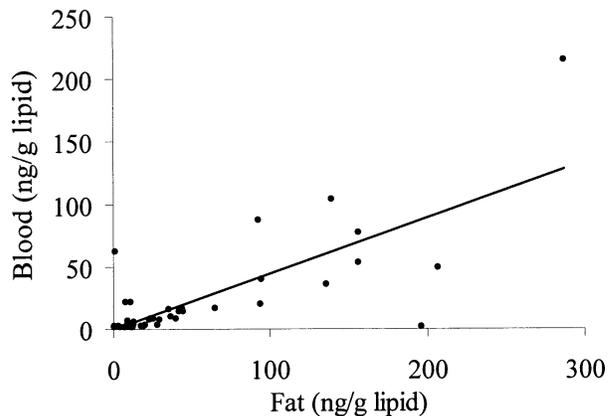


Fig. 2. Relationships between fat biopsy and whole blood concentrations of organochlorine contaminants in loggerhead sea turtles. Organochlorine concentrations are lipid normalized. Linear regression and Spearman rank correlation statistics for total polychlorinated biphenyls (PCBs) (A) were  $y = 1.12x + 239$ ,  $r^2 = 0.798$ ,  $r_s = 0.634$ ,  $p < 0.001$ ; for total DDTs (B),  $y = 0.628x - 10.8$ ,  $r^2 = 0.557$ ,  $r_s = 0.665$ ,  $p < 0.001$ ; for total chlordanes (C),  $y = 0.345x + 16.0$ ,  $r^2 = 0.885$ ,  $r_s = 0.722$ ,  $p < 0.001$ ; and for mirex (D),  $y = 0.446x - 0.294$ ,  $r^2 = 0.577$ ,  $r_s = 0.634$ ,  $p < 0.001$ .

(239%) for mirex, 14.2% (59.2%) for total chlordanes, and 4.6% (57.3%) for total DDTs. Changes in blood OC concentrations did not significantly correlate to duration between sampling events for any compound.

#### Are contaminant concentrations dependent on sex and size?

The effect of sex and size (as a proxy for age) on contaminant concentrations was investigated in the loggerhead sea turtles. Male and female loggerhead sea turtles did not significantly differ in OC concentrations measured in their fat on a lipid-normalized or wet-mass basis (data shown in Keller [24]). Since no differences were seen, the OC concentrations in both males and females were combined in Table 1. The only contaminants that showed significant but weak correlations to turtle length were fat concentrations of mirex and total chlordanes on a wet-mass basis ( $r_s = -0.370$  and  $-0.422$ , respectively;  $p < 0.05$ ), which were inversely related to turtle length. Total chlordanes also showed a weak but significant correlation to turtle weight ( $r_s = -0.299$ ;  $p < 0.05$ ). Interestingly, these correlations were negative, indicating that larger turtles had lower concentrations of these compounds than smaller turtles.

## DISCUSSION

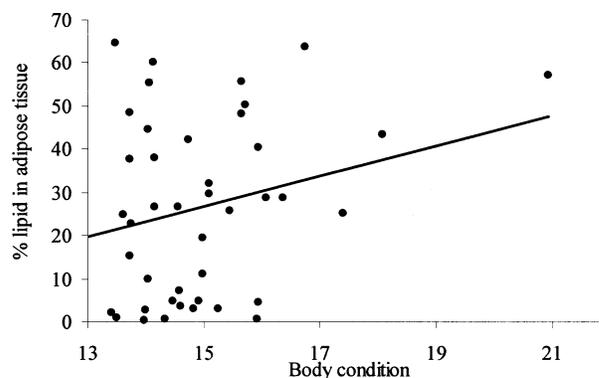
### Sea turtle OC concentrations: Comparison to past studies

Some studies have been published on OC concentrations in the fat of sea turtles (Table 5). Large variation has been seen previously in OC concentrations among individual turtles. In the current study, the variation in the concentrations of most of the contaminants could not be explained by sex or size of the turtles.

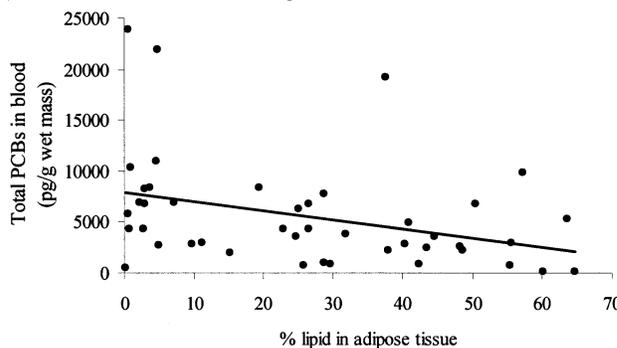
Kucklick et al. [25] found that polycyclic aromatic hydrocarbon concentrations in urban estuarine sediments were highly variable even among sites that were close in proximity. Hyland et al. [26] showed that total PCB and total DDT concentrations in the sediment of North Carolina estuaries range over two orders of magnitude depending on the sampling location. Therefore, spatial habitat choice may explain some of the variance observed in sea turtle tissue concentrations of OCs. Many juvenile loggerhead turtles captured at this study site (Core Sound) show strong site fidelity [27]. They return to a particular area in successive years and remain in this area for extended parts of a season. This localized feeding may contribute to the variation seen in their contaminant levels. It is possible that one turtle may continually feed in a highly contaminated site, while another may feed in a less contaminated area. Additionally, these juvenile turtles are thought to use offshore habitats for their winter foraging grounds or follow a more coastal migratory route to southern, warmer waters [28]. Turtles that take the offshore route may accumulate lesser amounts of contaminants than those closer to the coast. Evidence of this comes from a study in which loggerhead sea turtles that were captured closest to the mouths of rivers that drain large watersheds with local point sources of mercury had higher recent mercury exposure [29].

The total PCB and total DDT concentrations in loggerhead turtles from the current study were roughly half those measured in the fat from dead loggerhead turtles stranded in Virginia and North Carolina in the early 1990s [30] and a quarter of the concentration measured in loggerhead turtles stranded in the Mediterranean Sea in 1994 and 1995 [31]. These observed differences in OC concentration may be the result of a number

### A) Body condition vs. % lipid in fat



### B) Blood PCB concentrations vs. % lipid in fat



### C) Blood OC pesticide concentrations vs. % lipid in fat

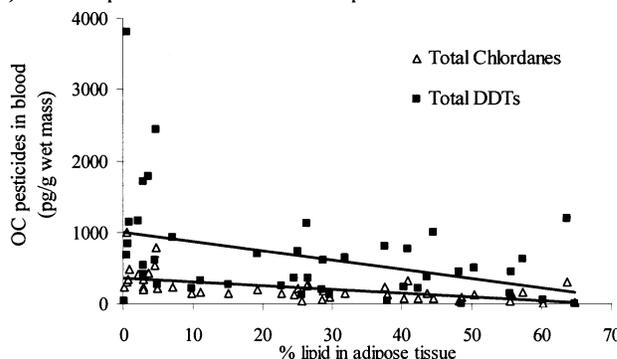


Fig. 3. Relationships of body condition and blood organochlorine concentrations (pg/g wet mass) to percentage lipid content in the adipose tissue of loggerhead sea turtles. (A) Percentage lipid did not correlate to body condition (see *Materials and Methods* for calculation). Linear regression and Spearman rank correlation statistics were  $y = 3.51x - 25.8$ ,  $r^2 = 0.0711$ ,  $r_s = 0.232$ ,  $p > 0.10$ . (B) Polychlorinated biphenyl (PCB) and (C) organochlorine (OC) pesticide concentrations in the blood (pg/g wet mass) were correlated to percentage lipid in the fat biopsies. Linear regression and Spearman rank correlation statistics for total PCBs (B) were  $y = -89.2x + 7,900$ ,  $r^2 = 0.122$ ,  $r_s = -0.403$ ,  $p < 0.02$ ; for total DDTs (C),  $y = -13.1x + 1,010$ ,  $r^2 = 0.148$ ,  $r_s = -0.361$ ,  $p < 0.02$ ; and for total chlordanes (C),  $y = -5.33x + 363$ ,  $r^2 = 0.330$ ,  $r_s = -0.671$ ,  $p < 0.02$ .

of factors, including spatial or temporal differences in OC environmental concentrations. Turtles inhabiting coastal waters of the more urban Mediterranean Sea or Chesapeake Bay may be exposed to higher levels of contaminants than turtles in the less urban Core Sound. In addition, PCB concentrations along the U.S. coast have decreased over the past nine years,

Table 4. Information on six recaptured loggerhead sea turtles

Turtle ID	Date		Days between sampling	Size <sup>a</sup>		Sex <sup>a</sup>	% Lipid in blood	
	1st capture	2nd capture		1st capture	2nd capture		1st capture	2nd capture
1	6/29/2001	7/16/2001	17	59.0	59.2	F	0.195	0.315
2	6/15/2001	7/16/2001	31	59.3	59.8	F	0.170	0.226
3	6/2/2000	8/11/2000	70	54.3	55.7	F	0.165	0.304
4	7/17/2001	10/8/2001	83	64.9	66.2	F	0.208	0.191
5	6/16/2000	7/18/2001	397	73.3	73.6	M	0.266	0.385
6	6/5/2000	7/13/2001	403	73.5	75.4	F	0.199	0.101

<sup>a</sup> Size = straight carapace length (cm) from nuchal notch to posterior marginal notch. Sex based on plasma testosterone concentrations.

as demonstrated by decreasing concentrations measured in bivalves through the National Oceanic and Atmospheric Administration Mussel Watch Program [32].

Another potential cause for the observed difference between the loggerhead sea turtle OC concentrations in this study and previous studies may be sampling bias. All the past studies utilized tissues from carcasses of stranded sea turtles, thereby biasing their sampling toward diseased or injured turtles. Animals dead from disease may contain higher concentrations of OCs than the live, apparently healthy turtles analyzed in our study. This relationship has been seen in other marine wildlife. For example, striped dolphins that died from a morbillivirus epizootic in the Mediterranean Sea had nearly threefold higher concentrations than healthy dolphins in the same area [10]. Therefore, using tissues from dead animals could complicate interpretation of contaminant data, and future studies should examine more closely the prospect that diseased turtles have higher levels of contaminants than healthy ones.

The PCB concentrations measured in dead Kemp's ridley turtles from Massachusetts (USA) in this study were extraordinarily similar to the findings of two previous studies on this species with only one exception (Table 5). Polychlorinated biphenyl concentrations in Kemp's ridley turtles that stranded in New York (USA) in 1985 were roughly double those measured in the turtles analyzed in this study [33]. Lake et al. [33] also analyzed tissues from turtles that stranded four years later in 1989. The PCB concentrations in 1989 were half those measured in 1985 and similar to the levels measured in the

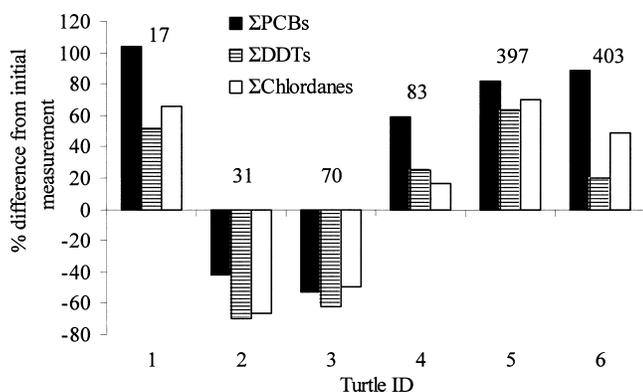


Fig. 4. Percentage difference of polychlorinated biphenyls (PCBs) and organochlorine pesticide concentrations (ng/g lipid) measured in whole blood samples collected from six loggerhead sea turtles that were captured twice. Percentage difference was calculated as (concentration at time 2 - concentration at time 1)/(concentration at time 1) × 100. Numbers above bars indicate the number of days between capture events and blood sampling. Contaminants were determined in only a single sample per turtle per sampling event.

current study. The concentrations of DDE in this study were half those previously reported for Kemp's ridley sea turtles [30,33]. These differences could be due to temporal or spatial trends in environmental OC concentrations or variations in analytical techniques.

Compared to other sea turtle species, the OC concentrations were higher in the fat of Kemp's ridley and loggerhead sea turtles than in green and leatherback sea turtles (Table 5). These species differences could be explained by differing trophic feeding levels. The crab- and mollusk-eating loggerhead and Kemp's ridley sea turtles feed higher on the food chain than the herbivorous green sea turtles, which consume a variety of sea grasses and algae. The leatherback sea turtle feeds primarily on jellyfish.

An interesting finding highlighted during this study is the apparent difference between OC concentrations in the adipose tissue and the blubber of a single leatherback sea turtle. The adipose concentrations were similar to previous reports for leatherback adipose tissue (near 100 ng/g wet mass total PCBs), but the blubber concentrations were remarkably higher (2,330 ng/g wet mass total PCBs) than reported in any fatty tissues of any sea turtle species. Davenport et al. [34] reported 1,200 ng/g lipid total PCBs in a fatty tissue of a single leatherback turtle, but it was unclear whether the tissue was adipose or blubber. This previously anomalous value was more comparable to the concentrations measured in the blubber of the leatherback turtle from our current study (9,040 ng/g lipid total PCBs). Recent analysis of a larger sample set ( $n = 6$  turtles), however, indicates little difference between adipose and blubber concentrations (J. Keller, unpublished data).

The shell of the leatherback sea turtle differs from that of all the other sea turtle species. The bony structures of the shell are greatly reduced, and the majority of this structure consists of a thick lipid layer, similar to the blubber layer of marine mammals. This insulating blubber layer is thought to help the leatherback turtle maintain its body temperature above the ambient water temperature and facilitate deep diving [35].

#### Pattern of PCB congeners

The patterns of PCB congeners in the blood and fat of loggerhead sea turtles were similar to those in the Kemp's ridley sea turtles (Fig. 1). The sea turtle congener profiles were also similar to previously reported patterns for these tissues in other species. As expected, the dominant congeners (PCBs 138, 153, and 180) found in the loggerhead and Kemp's ridley sea turtles in our study were the dominant congeners in adipose tissue of green sea turtles from Hawaii (USA) [36] and in tissues of animals inhabiting the Great Lakes. Notably, these specific congeners were prevalent in plasma of Lake Erie (USA) water snakes and snapping turtles and in eggs of herring

Table 5. Mean (standard deviation) organochlorine contaminant concentrations (ng/g wet mass unless stated otherwise) in fat of sea turtles<sup>a</sup>

Species	Age class/sex <sup>b</sup>	Status	Tissue	Year	Location <sup>bc</sup>	PCB 153	Total PCBs	4,4'-DDE	Total DDTs	n	Reference
Loggerhead	J	Live	Adipose	2000 and 2001	NC	80.9 (86.4)	256 (269)	64.4 (64.8)	67.0 (68.7)	44	This study
Loggerhead	J and A	Fresh dead to decomposed	Adipose	1991 and 1992	VA and NC	146 (120)	551 (473)	195 (266)	206 (268)	20	[30]
Loggerhead	J and A	Dead	Adipose	1994 and 1995	Med. Sea	241 (17.7)	840 (60.0)	509 (173)	528 (185)	3	[31]
Loggerhead	J	Dead	Adipose	1993	Med. Sea	87.9 (NA)	334 (179)			4	[42]
Loggerhead	NA	Dead	Adipose	1968	NA		647	300		1	[33]
Kemp's ridley	J	Fresh dead	Yellow fat	1998-2000	MA and NC	161 (173)	701 (893)	99.6 (76.4)	101 (77.9)	9	This study
Kemp's ridley	J	Fresh dead	Brown fat	1998-2000	MA and NC	135 (127)	525 (545)	90.9 (71.9)	92.8 (73.6)	10	This study
Kemp's ridley	J	Decomposed	Adipose	1991	VA and NC	189 (96.4)	660 (333)	194 (98.2)	223 (106)	3	[30]
Kemp's ridley	J	Dead	Adipose	1989	NY	161 (95.6)	476 (273)	232 (157)	261 (176)	6	[33]
Kemp's ridley	J	Dead	Adipose	1985	NY	384 (289)	1,250 (985)	386 (250)	454 (298)	7	[33]
Leatherback	AF	Euthanized	Adipose	1999	NC	41.0	129	13.2	13.2	1	This study
Leatherback	AF	Euthanized	Blubber	1999	NC	664	2,330	288	292	1	This study
Leatherback	AM	Dead	Adipose	1993 and 1995	Scotland	26.9 <sup>d</sup>	113 <sup>d</sup>	33.5 <sup>d</sup>	36 <sup>d</sup>	2	[31]
Leatherback	AM	Drowned in fishing gear	Adipose	1993-1996	Wales and Scotland	42.3 (32.7)	152 (94.3)	45.0 (30.8)		3	[43]
Leatherback Green	AM	Dead	Blubber?	1988	Wales		1,200 <sup>e</sup>			1	[34]
Green	JM	Moderately decomposed	Adipose	2000	NC	28.7	81.1	15.0	15.0	1	This study
Green	J	Dead	Adipose	1995	Med. Sea	15.3 (16.0)	136 (113)	9.13 (8.73)	12.4 (9.93)	3	[31]
Green	M	Dead	Adipose	1998	NE Australia	70 <sup>e</sup>	171 <sup>e</sup>	45 <sup>e</sup>	53 <sup>e</sup>	1	[44]
Green	JM, AF, AM	Fresh dead	Adipose	1992 and 1993	Hawaii		285 (330) <sup>f</sup>			3	[36]
Green	J	Dead	Adipose	NA	Hawaii		22.9 (21.3)	4.97 (8.82)		5	[45]
Green	J	Dead or euthanized	Adipose	NA	Hawaii		<1,000 <sup>g</sup>	<100 <sup>g</sup>		12	[46]

<sup>a</sup> Refer to Table 1 for explanation of abbreviations.<sup>b</sup> J = juvenile; A = adult; F = female; M = male; NA = not available.<sup>c</sup> NC = North Carolina; VA = Virginia; Med. = Mediterranean; MA = Massachusetts; NY = New York.<sup>d</sup> Standard deviation was not calculated on sample size of 2.<sup>e</sup> Values were reported as ng/g lipid.<sup>f</sup> Values were reported as ng/g dry weight.<sup>g</sup> Analytical limit of detection for total PCBs was 1,000 ng/g and for 4,4'-DDE was 100 ng/g.

gulls and mud puppies [12]. These congeners also dominated the PCB profile in fatty tissues of Alaskan (USA) marine mammals [23].

Interestingly, a difference was observed between the congener composition of blood and fat of the sea turtles. As shown in Figure 1, PCB 138 made up a greater percentage of the total PCBs in the blood than in the fat. This congener has only ortho-meta vicinal hydrogens and is metabolized by the methylcholanthrene-type enzyme (cytochrome P4501A) subfamily in mammals [37]. The relatively high concentrations of this compound, along with the other PCB congeners that are metabolized by this enzyme subfamily (PCBs 99, 118, 128, and 170), suggest that sea turtles may have weak P4501A activity. The lack of congeners that are typically metabolized by phenobarbital-type enzymes (cytochrome P4502B), including PCBs 52, 95, 101, 151, 149, 185, and 174, suggested that this enzyme activity may dominate the phase I metabolism of contaminants in sea turtles, as has been suggested in polar bears [23]. It should be noted that these enzymes have been well characterized in mammals, but little is known about their occurrence in reptiles; therefore, these conclusions should be viewed with caution. Four cytochrome P450 proteins have been partially isolated from the liver of a Kemp's ridley sea turtle, and although the identity of these isozymes has not been fully characterized, low levels of P4501A activity were evident [38].

#### *Contaminants versus age and sex*

In other wildlife, it has been shown that contaminant concentrations are dependent on age, sex, and nutritional status. Adult males typically contain higher concentrations of organochlorines than females because females can release portions of their contaminant burden through their eggs, milk, or tissues of their offspring [9,39]. In addition, it has been shown that older individuals possess higher concentrations of OCs through extended accumulation of the compounds [39]. Organochlorine concentrations did not differ between male and female loggerhead turtles analyzed in the current study. A difference was not expected because the juvenile females sampled in this study were not of breeding age and therefore had not yet transferred contaminants to their eggs.

Few correlations were seen between OC concentrations and loggerhead size. Larger turtles unexpectedly had lower concentrations of chlordanes and mirex. A possible explanation for this finding may be growth dilution. Loggerhead sea turtles may accumulate these compounds at a higher rate during an earlier life stage. Lower exposure to the compounds and/or faster growth during the benthic juvenile stage would serve to dilute their tissue contaminant levels. During an earlier life stage, the pelagic juveniles inhabit not only open ocean habitat but also coastal areas of Madeira, the Azores and Canary Islands, Africa, Portugal, and the waters of the Mediterranean Sea. When the turtles return to the U.S. coast, their growth during the benthic juvenile stage may dilute their previous accumulation of these compounds. However, without knowing the migratory history and previous contaminant exposure of these animals, we can offer this as only a possible explanation.

At this time, it is difficult to interpret age-dependent changes in contaminant concentrations for sea turtles. Noninvasive methods are not available to age live sea turtles. Size is the only available approximation of their age, and the accuracy of this method is questionable, as their growth rates can be plastic [6]. Loggerhead sea turtle hatchlings begin life at 4 cm

in straight carapace length and grow to more than 100 cm as reproducing adults. The single size class (45.7–74.1 cm) represented in this study, the benthic juvenile stage, is a mere portion of their life history. Future research should incorporate different age/size classes into these analyses, including eggs, hatchlings, pelagic juveniles, benthic juveniles, and adults. An alternative but more difficult approach to this question would entail repeated measurements of OC contaminants on individual turtles over time. Interestingly, the two turtles (IDs 5 and 6) that were recaptured after one year showed evidence of accumulation of OCs in their blood (Fig. 4). The second blood sample from both turtles had higher levels of all OCs than the first sample.

#### *Yellow versus brown fat in Kemp's ridley sea turtle*

Two types of fat, yellow and brown, were observed and analyzed for OCs in the Kemp's ridley sea turtles. The yellow fat surrounded the viscera, while the brown fat was located in the inguinal regions and along the margins of the plastron. To our knowledge, neither the lipid composition nor the functions of these two fat types has been described in sea turtles. Though brown in color, the brown fat of sea turtles differs from brown fat of mammals, which contains adipocytes with multiple small vacuoles and serves a thermoregulatory function, particularly in hibernating animals [40]. Histology on the brown fat from the loggerhead sea turtle showed that it more closely resembled mammalian white fat, which contains adipocytes with a single or few large lipid vacuoles. Histological examination was not performed on the Kemp's ridley brown or yellow fat.

The yellow and brown fat collected from the Kemp's ridley turtles in this study contained similar levels of lipid and all OC contaminants. These similarities suggest that the deposition, mobilization, and possibly the function of these two fat stores are similar. Additionally, this indicates that either fat type may be used to monitor OC concentrations.

Previous sea turtle studies have shown significant correlations between OC concentrations in different tissue compartments. Organochlorine concentrations (total PCBs, 4,4'-DDE, and *trans*-nonachlor) in adipose tissue of Kemp's ridley sea turtles significantly correlated to concentrations in their liver [33]. In eggs, the concentrations of total PCBs were correlated to concentrations in the chorioallantoic membrane, which is left behind after the turtle emerges [41]. Chorioallantoic membranes can therefore be sampled noninvasively to measure contaminants at the embryonic life stage, whereas the current study investigated the use of blood as a noninvasive sample to monitor OCs in later life stages.

#### *Fluctuations in blood OC concentrations*

In addition to size and sex, nutritional status may also influence OC concentrations, especially concentrations observed in the blood. Large changes in blood OC concentrations were observed in the six recaptured loggerhead turtles. These changes did not correlate to the length of time between sampling events. Turtles that were recaptured after an entire year and after only 17 d exhibited nearly 100% increases in OC concentrations. These changes were much larger than analytical error expected from repeated sample analysis [8]. For example, the relative standard deviation from five separate analyses of NIST SRM 1589a (PCBs, pesticides, and dioxins/furans in human serum) was only 4% for total PCBs and 2% for total DDTs [8].

It is known that blood OC levels may fluctuate depending

on recent diet, hydration status, short-term changes in metabolism, or mobilization of lipid stores. Lipid mobilization is likely to occur in turtles during long migrations or when they are yolking eggs, fasting, or diseased or when food is scarce. Lydersen et al. [14] found that mean blood OC concentrations in harp seals increased by nearly 100% after 28 d of fasting in the laboratory. Even larger increases in blood OC concentrations were found in wild harp seals after dramatic weight loss during lactation and molting. Similarly, loggerhead turtles in this study with lower fat lipid stores had higher levels of blood OCs, suggesting that these compounds were mobilized into the blood along with lipid stores. Conclusions based on this small sample size are limited; however, it is evident that blood OC concentrations can change dramatically over short intervals of time. Unfortunately, fat biopsies were not collected on recapture, so the changes in adipose OC concentrations are unknown. This fluctuation in blood concentrations, though, should be considered when designing an OC monitoring study relying solely on blood measurements.

#### Blood versus fat OC concentrations

The primary goal of this study was to determine if the high variability seen in blood OC concentrations of sea turtles could be explained by the accumulated concentrations found in the adipose tissue. Our data suggest that this is the case. Concentrations of all the major OC compounds in the blood correlated to those in the fat for both loggerhead and Kemp's ridley sea turtles. Similar relationships have also been noted in humans, marine mammals, and birds [11,16–21]. Reddy et al. [11] observed a significant relationship between blubber and red blood cell concentrations of HCB, total DDTs, *trans*-nonachlor, and the dominant PCB congeners in captive bottlenose dolphins. Correlations were also seen between subcutaneous fat and plasma for chlordanes, DDE, HCB, HCHs, and total PCBs in polar bears [17]. Likewise, Henny and Meeker [16] have shown that blood plasma concentrations of 4,4'-DDE were significantly related to brain concentrations in American kestrels. It is important to note that correlations of blood OCs to fat OCs observed in the current study were stronger than the correlations of blood OCs to fat lipid content. These results and previously documented correlations in other species indicate that OC contaminants in blood can reasonably predict OC concentrations in fatty tissues.

In conclusion, our data suggest that in most situations blood can be used to measure OC burden in sea turtles. This conclusion is based on findings of previous studies with other organisms and our finding that blood OC concentrations in two sea turtle species were correlated to concentrations present in the fatty tissues. Since blood carries contaminants to sensitive tissues and organs, blood OC concentrations may be the most relevant to toxicological studies. However, we caution the use of blood in studies that plan to monitor spatial or temporal changes in OC concentrations. Turtles undergoing nutritional changes, seasonal migrations, or disease events may be mobilizing their lipid stores that could cause drastic fluctuations in blood OC concentrations. Future studies may clarify contaminant concentrations in sea turtles that are undergoing these changes and provide insight as to how these events truly affect blood OC concentrations.

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