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Persistent organic pollutants in green sea turtles (*Chelonia mydas*) inhabiting two urbanized Southern California habitats

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ABSTRACT

Within Southern California, east Pacific green sea turtles (*Chelonia mydas*) forage year-round, taking advantage of diverse food resources, including seagrass, marine algae, and invertebrates. Assessing persistent organic pollutants (POP) in green turtle aggregations in the Seal Beach National Wildlife Refuge (SBNWR, n = 17) and San Diego Bay (SDB, n = 25) can help quantify contamination risks for these populations. Blood plasma was analyzed for polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs). PCBs and body size explained much of the separation of turtles by foraging aggregation in a principal component analysis. Turtles from SDB had significantly ($p < 0.001$) higher total PCBs than SBNWR turtles. Most PCBs detected in turtles were non-dioxin-like PCB congeners (153, 138, 99) that are associated with neurotoxicity. Recaptured turtles' POP levels changed significantly over time indicating significant variation in POP levels through time and space, even among adjacent foraging locations.

1. Introduction

Marine species, such as sea turtles, have been negatively impacted by anthropogenic activities, and as a result many sea turtle populations are considered threatened or endangered (Gardner and Oberdorster, 2005; Hamann et al., 2010; Pugh and Becker, 2001). Historically, threats such as overharvesting and habitat loss have severely impacted sea turtle populations, but conservation efforts have initiated recovery for some populations around the world (Hamann et al., 2010; Mazaris et al., 2017; Seminoff et al., 2015). Additional threats, such as pollution, continue to impact marine waterways that sea turtles inhabit (Hamann et al., 2010). Human activities, such as manufacturing, agriculture, and electronic waste produce a variety of anthropogenic contaminants that commonly enter the environment surrounding urban areas that are known to cause cancer, reproductive and immune system impairments across a range of wildlife (Carpenter, 2006; Darnerud et al., 2001; Lohmann et al., 2007; Sindermann, 2005). In particular, large coastal cities can heavily pollute surrounding coastal and estuarine habitats (Sindermann, 2005). Because some sea turtle species, such as green sea turtles (*Chelonia mydas*), demonstrate high site fidelity and extended residency in foraging habitats (Lutz et al., 2002), sea turtles that inhabit these urbanized coastal foraging grounds have higher risk of exposure to anthropogenic pollutants and can accumulate anthropogenic pollutants over time serving as bioindicators for urban habitats (Finlayson et al., 2016; Hamann et al., 2010; Keller, 2013).

Persistent organic pollutants (POPs), one group of anthropogenic contaminants, are made for many industrial and agricultural purposes and are introduced into the environment via urban and agricultural runoff (Flynn and Kleiman, 1997; Gray, 1997; Jones and de Voogt, 1999). POPs include compounds such as polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs). Due to their stability in the environment and accumulation in wildlife, PCBs and OCPs are still found in the

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environment despite being banned in the 1970s (Jones and de Voogt, 1999; Weber et al., 2008). Because of their prevalence and persistence in the environment, there are still numerous questions about the exposure and potential health impacts of POP accumulation in marine species.

Research into the potential effects of PCBs, OCPs, and PBDEs on sea turtle health has increased in recent years (Finlayson et al., 2016; Gardner and Oberdorster, 2005; Keller, 2013; Pugh and Becker, 2001). For example, in vitro DDT exposure has been shown to alter testosterone hormone binding in blood plasma obtained from nesting green sea turtles in Malaysia, suggesting that DDT may disrupt sex steroid-protein binding in green sea turtle blood (Ikonomopoulou et al., 2009). Research on nesting turtles in Malaysia found that hatchlings with higher POP concentrations were smaller in size, which may indicate reduced survival (van de Merwe et al., 2010b). Leatherback sea turtle (*Dermochelys coriacea*) eggs in Costa Rica found that higher POP concentrations were correlated to lower hatching success (De Andrés et al., 2016). Experiments with an immortal sea turtle testis cell line was found to have increased cytochrome 450 aromatase activity following exposure to POPs, indicating sex steroid alterations (Keller and McClellan-Green, 2004). These studies indicate that POPs may competitively inhibit sex hormone binding, affect hatchling survival, and impair immune function through depressed or increased white blood cell count in sea turtles (De Andrés et al., 2016; Keller and McClellan-Green, 2004; Komoroske et al., 2011; Stewart et al., 2011; van de Merwe et al., 2010b). By quantifying POP accumulation in urban green turtle foraging aggregations, wildlife and endangered species managers can assess potential exposure risks and population recovery strategies.

In California, USA, year-round foraging aggregations of green sea turtles are found in several bays and estuaries from Los Angeles to San Diego (Crear et al., 2017, 2016; Eguchi et al., 2010; MacDonald et al., 2012). These locations are highly-urbanized areas known to have POPs present in sediment and wildlife through previous research and sediment analysis (Dodder et al., 2016; Lyons et al., 2014; Lyons and Lowe, 2015; Schiff et al., 2011). Prior research has shown that green sea turtles in San Diego Bay have detectable levels of POPs above the no effect threshold for immunological impairment (e.g., altered lymphocyte production and cytokine gene expression) (Komoroske et al., 2011; Lewison et al., 2011). A growing population of green sea turtles has been identified in the Long Beach/Los Angeles area within the Seal Beach National Wildlife Refuge (SBNWR) and San Gabriel River, which provides heat effluent water allowing year around use of this foraging habitat (Crear et al., 2017, 2016). These SDB and SBNWR green sea turtles provide an opportunity to compare these two subpopulations that inhabit two distinct urban areas.

Considering distance and differences in exposure to pollutants between the two locations, the current study hypothesizes that POPs will differ between green sea turtles from SBNWR and SDB. To assess POP concentration patterns, blood plasma samples will be used to measure POP concentrations in green sea turtles caught in SBNWR and SDB. Previous studies have used lipid-rich tissues, such as liver and adipose, to quantify body burden (Rozman and Klaassen, 2007) of POPs in green sea turtles. However, blood samples have been shown to correlate with POP contamination in lipid-dense organs, albeit at lower concentrations, and thus may provide a non-invasive sampling alternative for measuring POP exposure and bioaccumulation (Finlayson et al., 2016; Gardner and Oberdorster, 2005). This study aims to assess if location-based pollutant signatures are present, how pollutant signatures differ between foraging aggregations, and compare the results with other studies in green turtle POP accumulation.

2. Methods

2.1. Study sites

San Diego Bay (32° 36′ 54″ N, 117° 6′ 4″ W; SDB) is a natural bay that contains salt marsh, eelgrass bed, mud/tidal flat habitat. SDB's coastline is heavily urbanized with homes, military bases, harbors, and shipyard activity throughout the bay (Fig. 4). Green sea turtles have inhabited these waters for a long period of time feeding on the eelgrass beds within the bay (MacDonald et al., 2012; McDonald et al., 1994). SDB adult greens nest and are from the genetic stock of the Revillagigedo Islands and Michoacan, Mexico (Dutton et al., 2019). The SDB green turtle foraging aggregation has been studied regularly with capture/recapture and monitoring taking place within the bay since 1990 (Eguchi et al., 2010).

The Seal Beach National Wildlife Refuge (33° 44′ 07″ N, 118° 03′ 52″ W; SBNWR) is a wetland area within the Anaheim Bay estuary (Fig. 4). SBNWR estuary contains natural habitat as well as restored habitat that was constructed as part of a restoration project. Within the restored habitat are a series of channels and basins that house eelgrass beds, which green sea turtles are known to forage (Crear et al., 2017, 2016). Green sea turtle capture efforts were conducted within a pond that is fed by a culvert which green sea turtles use to enter the pond and forage. This wetland is adjacent to a naval weapons base with green sea turtles travelling between the ponds in the refuge and the San Gabriel River (Crear et al., 2017, 2016).

The San Gabriel River (33° 45′ 15″ N, 118° 6′ 13″ W; SGR) is a concrete lined river that ends in a 6-km stretch of estuarine habitat (Fig. 4). The river acts as a flood control channel with tributaries throughout the Greater Los Angeles area feeding into the river. There are two power plants 3-km from the river's mouth that use once-through cooling, drawing water from the San Gabriel River to cool steam generators (Crear et al., 2016). As a result, the San Gabriel River's water temperatures are regularly altered via heated water discharge from once-through cooling. Green sea turtles are increasingly found within SGR in recent years and individuals are known to move regularly between the SGR and adjacent SBNWR (Crear et al., 2016).

2.2. Sea turtle capture and sampling

Whole blood samples were collected as described in Barraza et al. (2019). Briefly, green sea turtles were captured, subadults were sexed via testosterone (T) levels in the blood (Allen et al., 2015) and, adult-sized turtles via morphology (Caldwell, 1962), weighed (± 0.1 kg), and measured for curved carapace length (CCL; ± 0.1 cm). Methodology used is from previous research conducted within SDB (32° 36′ 54″ N, 117° 6′ 4″ W) and the SBNWR (33° 44′ 06.8″ N, 118° 03′ 51.9″ W) (Crear et al., 2016; Eguchi et al., 2010). Whole blood (3–10 ml) samples were collected and prepared for POP analysis (see below) following a modified National Institute of Standards and Technology (NIST) protocol to reduce the possibility of sample contamination (Keller et al., 2014b). Changes to NIST protocol include: using kilned glass containers with Teflon lids (Thermo Scientific) instead of Teflon containers, and using kilned aluminum instead of hexane rinse aluminum. Blood was collected with powder free nitrile gloves (Kimtech, Roswell, Georgia) into glass sodium-heparinized tubes (Becton Dickson, San Jose, California) and stored in a cooler with ice packs. At the end of each field day, blood samples were centrifuged at 3000 rpm for 10 min to separate plasma. Plasma was transferred into glass vials. Samples were placed at −20 °C overnight then transferred to −80 °C freezers until POP analysis.

Sea turtles were held for approximately 1 h for morphometric and blood sample collection; afterward, they were released at their location of capture. Additional plasma samples were provided for POP analyses from previous samples collected using NIST protocols (2009–2014) of green sea turtles in SDB (n = 4) and the San Gabriel River (SGR, n = 6), a river within 4 km of SNBWR. These supplementary samples followed collection methods described in Keller et al. (2014b).

2.3. POP analyses

Blood plasma samples were analyzed for 14 PBDEs, 11 OCPs and 54 PCBs (specific analytes listed in Table 2 and Table S4). POPs were

Table 1
Summary persistent organic pollutants (PCB and OCP) detected in blood plasma samples collected from green sea turtles from the Seal Beach National Wildlife Refuge (n = 16) or San Diego Bay (n = 23). Analytes that were not detected in any green sea turtles sampled are not displayed for clarity, including PBDEs. Non-detects are listed as zeros. Mean ± SE and median (range) are ng/g blood plasma. Limit of detection (LOD) for PCB congeners and OCP analytes is 0.27 and 0.18 ng/g, respectively.

Congeners	San Diego Bay			Seal Beach National Wildlife Refuge		
	n > LOD	Median (Range)	Mean ± SE	n > LOD	Median (Range)	Mean ± SE
PCB066	1	0 (0–0.27)	0.01 ± 0.05	0	0 (0)	0
PCB087	1	0 (0–0.31)	0.01 ± 0.06	0	0 (0)	0
PCB095	11	0 (0–0.22)	0.05 ± 0.06	1	0 (0–0.11)	0.01 ± 0.007
PCB099	21	0.73 (0–3.31)	0.86 ± 0.68	1	0 (0–0.66)	0.05 ± 0.04
PCB101	1	0 (0–0.13)	0.006 ± 0.02	1	0 (0–0.14)	0.008 ± 0.008
PCB105	1	0 (0–0.22)	0.01 ± 0.04	0	0 (0)	0
PCB110	0	0 (0)	0	1	0 (0–0.08)	0.005 ± 0.004
PCB114	1	0 (0–0.44)	0.02 ± 0.08	0	0 (0)	0
PCB118	18	0.175 (0–0.94)	0.21 ± 0.19	0	0 (0–0.17)	0.01 ± 0.01
PCB128	11	0 (0–0.70)	0.12 ± 0.15	0	0 (0–0.12)	0.007 ± 0.007
PCB138	23	2.07 (0.13–11.82)	2.92 ± 2.61	16	0.15 (0.07–2.06)	0.32 ± 0.12
PCB141	0	0 (0)	0	1	0 (0–0.13)	0.008 ± 0.008
PCB149	22	0.095 (0–0.33)	0.12 ± 0.06	16	0.05 (0.02–0.28)	0.07 ± 0.02
PCB151	17	0.03 (0–0.11)	0.03 ± 0.03	6	0 (0–0.09)	0.01 ± 0.006
PCB153	22	1.345 (0–11.92)	2.79 ± 3.01	16	0.08 (0.03–1.32)	0.18 ± 0.08
PCB156	3	0 (0–0.10)	0.01 ± 0.02	0	0 (0)	0
PCB157	3	0 (0–0.09)	0.01 ± 0.02	0	0 (0)	0
PCB158	1	0 (0–0.20)	0.01 ± 0.04	0	0 (0)	0
PCB167	9	0 (0–0.22)	0.06 ± 0.07	0	0 (0)	0
PCB168 + 132	1	0 (0–0.12)	0.01 ± 0.02	1	0 (0–0.13)	0.008 ± 0.008
PCB170	12	0.015 (0–0.31)	0.07 ± 0.08	1	0 (0–0.07)	0.007 ± 0.005
PCB174	9	0 (0–0.10)	0.02 ± 0.02	2	0 (0–0.14)	0.01 ± 0.008
PCB177	9	0 (0–0.10)	0.02 ± 0.03	1	0 (0–0.08)	0.007 ± 0.005
PCB180	23	0.21 (0.05–2.02)	0.38 ± 0.41	11	0.04 (0–0.21)	0.05 ± 0.01
PCB183	20	0.075 (0–0.57)	0.15 ± 0.14	1	0 (0–0.10)	0.01 ± 0.006
PCB187	23	0.26 (0.05–0.66)	0.30 ± 0.15	13	0.03 (0–0.35)	0.06 ± 0.02
PCB189	1	0 (0–0.02)	0.001 ± 0.003	0	0 (0)	0
PCB194	5	0 (0–0.15)	0.02 ± 0.04	0	0 (0)	0
PCB195	1	0 (0–0.04)	0.002 ± 0.007	0	0 (0)	0
PCB201	9	0 (0–0.38)	0.08 ± 0.10	0	0 (0–0.08)	0.005 ± 0.005
PCB206	4	0 (0–0.09)	0.01 ± 0.02	0	0 (0)	0
PCB209	1	0 (0–0.04)	0.002 ± 0.007	0	0 (0)	0
Σ PCB	**23**	**5.07 (0.36–30.11)**	**8.31 ± 7.25**	**16**	**0.32 (0.12–5.46)**	**0.85 ± 0.33**
Chlordane-gamma	19	0.095 (0–0.19)	0.09 ± 0.05	14	0.1 (0–0.2)	0.10 ± 0.01
Chlordane-alpha	1	0 (0–0.09)	0.004 ± 0.02	1	0 (0–0.08)	0.005 ± 0.005
Trans-Nonachlor	19	0.09 (0–0.51)	0.12 ± 0.11	11	0.05 (0–0.21)	0.05 ± 0.01
4,4′-DDE	1	0 (0–0.40)	0.02 ± 0.07	0	0 (0)	0
2,4′-DDD	0	0 (0)	0	1	0 (0–0.89)	0.05 ± 0.05
Cis-Nonachlor	3	0 (0–0.14)	0.01 ± 0.03	0	0 (0)	0
Σ OCP	**21**	**0.23 (0–0.75)**	**0.25 ± 0.16**	**14**	**0.15 (0–0.95)**	**0.20 ± 0.05**
Σ POP	**23**	**5.275 (0.36–30.79)**	**8.57 ± 7.36**	**16**	**0.44 (0.25–5.61)**	**1.05 ± 0.34**
% Lipid	**23**	**0.5 (0.13–2.61)**	**0.68 ± 0.10**	**16**	**0.41 (0.16–1.06)**	**0.47 ± 0.05**

extracted using a soxhlet extraction with dichloromethane solvent. Blood plasma was placed directly into sodium sulfate in a thimble to dry and placed through a soxhlet extraction overnight. Extracts were column cleaned with an Alumina-B/Silica column to reduce lipid interference and concentrated with a vacuum-sealed rotary-evaporator followed by nitrogen evaporation. Extracts were analyzed using an Agilent Gas Chromatograph Mass Spectrometer (7890A/5975C) equipped with a J&W 60 m, 0.25 mm ID, 0.25 μm film thickness DB-5 column via a splitless injection at a temperature of 285 °C. The oven temperature profile was programmed from 45 °C to 150 °C at 20 °C/min, and then to 300 °C at 2.5 °C/min. PCBs and OCPs were analyzed using a mass selective detector (MSD) in Electron Ionization (EI) selected ion monitoring (SIM) mode to scan for PCB and OCP specific ions at 1.67 times/s. PBDEs were analyzed using the MSD in Negative Ion Chemical Ionization (NCI) mode using methane. A standard curve for all pollutants was based on a 6-point linear regression calibration curve with an R^2 value of 0.99. All calibration standards were NIST traceable standards (AccuStandard©). For quality assurance, each sample batch was analyzed with a standard reference material (SRM 1957, NIST), two laboratory blank spikes, and a laboratory blank. Limits of detection in sea turtle plasma were determined using spiked sea turtle plasma with a 6-point linear regression; the standard deviation of spikes was divided by the slope of the regression and multiplied by 3.3. For a conservative approach, the congener with the highest limit of detection for each POP type was used as the limit of detection for all congeners. Recovery surrogates (TCMX, PCB 30, PCB 112, and PCB 198) were added to each sample prior to extraction, and all samples were quantified using the internal standards dibromobiphenyl and tetrabromobiphenyl. Blank spikes, SRM values, and recovery surrogates were analyzed for percent recovery to assess the accuracy and precision of the selected methods (supplementary Tables S2, S3, and S4). To account for lipid content difference, percent lipid was determined via weighing a 25% lipid split of extract and dividing by 25% volume of blood plasma used in the extract.

2.4. Statistical analyses

Statistical analyses were done using R (version 3.3.3; R Core Team, 2018), with a significance threshold of alpha = 0.05. To assess current differences in POP levels in SDB and SBNWR turtles, samples collected before 2015 were not included in summary statistics of POPs. Samples collected before 2015 were used to provide data for key habitat

locations for which little other data exist, and to provide data for future comparisons. Four blood plasma samples from SDB green sea turtles and six blood plasma samples from individuals in the SGR collected during another study were processes and analyzed separately. Since no PBDEs were detected below or above LOD in any samples, they were not included in the current study's analyses.

Two methods were used to summarize POP data. First, all detected PCB and OCP analytes were summed to calculate total POP (ΣPOPs) concentration detected per turtle and summed by pollutant group to calculate total PCB or total OCPs (ΣPCB or ΣOCP) per turtle. Second, a non-parametric Kaplan-Meier model (as described in Helsel, 2012) was used to account for detections below the limit of detection (LOD) and to provide an average value for each PCB and OCP analyte. For a conservative approach to avoid overestimation, and because of resource limitations, the highest congener LOD for PCBs and OCPs, respectively, was used as the cut-off for categorizing values below LOD in the Kaplan-Meier models (Table S1). ΣPOPs/PCBs/OCPs for individuals were not calculated using the Kaplan-Meier model because the data were over 70% censored, which previous research has shown to not provide a good estimate of concentrations (Antweiler and Taylor, 2008). As a result, to avoid problems added by substitution (Helsel, 2012) or statistical treatment (Antweiler and Taylor, 2008), ΣPCB and ΣOCP included all detected values (whether above or below LOD), and non-detected analytes (analytes not found at all in scans) were treated as zero. To normalize data and account for individual lipid differences, detected POPs were measured in ng/g blood plasma, converted to ng/g lipid and natural log transformed for all statistical analyses. POPs that were not detected in any plasma samples were omitted from summary tables (Tables 1 and 2) for clarity.

One SBNWR turtle and three SDB turtles were recaptured and had repeat blood samples taken. Mean POP concentrations of recaptured turtles were used for analyses; no green turtle was captured more than twice. Using the R package *vegan* (Oksanen et al., 2015), principal component analyses (PCA) were conducted to assess location-based pollutant patterns and included CCL, ΣPCB, and ΣOCP of each individual. Using the R package *cluster* (Maechler et al., 2016), k-means cluster analyses of the PCA were conducted to assess how individuals clustered by location. Regression analyses were used to find relationships between POP types and CCL. A multivariate analysis of variance (MANOVA) was used to compare ΣPCB and ΣOCP between locations and among size classes. A second MANOVA was conducted that only included turtles of similar size for comparison (Table 2; between 60 and 85 cm CCL), and therefore included similarly aged turtles that are considered sub-adults (Eguchi et al., 2012; Figueroa et al., 1992; Juarez-Ceron et al., 2003).

3. Results

From August 2015 to May 2017, whole blood samples were collected from 23 green sea turtles from SDB and 16 green sea turtles from SBNWR. Green sea turtles captured in SDB were significantly larger than individuals from SBNWR ($p < 0.001$; Fig. 1). Of the 79 POPs assessed (14 PBDE congeners, 54 PCB congeners, and 11 OCP analytes), only 32 PCB congeners (LOD = 0.27 ng/g) and 6 OCP analytes (LOD = 0.18 ng/g) were detected at concentrations above the LOD (see Table 1). PCB congeners not detected in any samples included: PCB 3, 8, 18, 28, 31, 33, 37, 44, 49, 52, 56 (60), 70, 74, 77, 81, 97, 119, 123, 126, 169, and 199 (200). OCP analytes not detected in any samples included: 4,4′-DDMU, 2,4′-DDE, 4,4′-DDD, 2,4′-DDT, and 4,4′-DDT. No PBDE analytes (PBDE 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190, and 209) were detected (LOD = 0.86 ng/g) in any green turtle blood plasma sample.

PCA k-means cluster analysis grouped green sea turtles from the same location together with some overlap between the different locations (Fig. 2). From the PCA, PC1 (ΣPCBs) accounted for 58% of the variance, and PC2 (CCL) accounted for 30% of the variance. There were more PCB congeners and OCP analytes detected (Table 1) in SDB turtles (30 PCB congeners; 5 OCPs) than SBNWR turtles (19 PCB congeners; 4 OCPs). Several congeners that displayed differences in the frequency of detection between populations included PCB 180, 187, 149, and 153 (see Table 1 and Table 2). PCB 180 and 187 were detected in all SDB turtles, PCB 99 was only detected in one SBNWR turtle but detected in nearly all SDB turtles, PCB 149 and 153 were detected in all SBNWR turtles (and all but one SDB turtle), and PCB 138 was detected in every turtle captured in the present study. Overall, the concentration of PCBs strongly varied between SDB and SBNWR green turtle plasma samples; in particular, SDB turtles had much higher concentrations of PCB 153, PCB 138, and PCB 99 in their blood plasma. With the exception of moderate levels of PCB 118 in SDB green sea turtles, there were low to non-detectable levels of dioxin-like PCBs (Table 1), such as PCB 77, 126, 169, and 105 in the plasma of all green sea turtles from the current study. The ΣPCBs detected in all turtles made up 95.1% by ng/g lipid of all POPs detected, and ΣOCPs made up 4.9% ng/g lipid of POPs detected. There were no significant differences in ΣPCB and ΣOCP concentrations between male ($n = 6$) and female green sea turtles ($n = 11$) regardless of capture location (data not shown). Not all turtles in the current study had sex determined by testosterone measurements; all sex undetermined turtles were juveniles. Two MANOVAs of PCB and OCP concentrations, one including all samples and another including only sub-adults, both showed that location, not CCL or the interaction of CCL and location, was a significant ($p < 0.001$) factor for PCB concentrations (Table 3).

The MANOVA including all samples indicated that SDB green sea turtles had significantly more ΣPCBs (7.16, 6.93 ± 0.22 ln ng/g lipid; median, mean ± SE) in their blood plasma than SBNWR (4.33, 4.49 ± 0.22 ln ng/g lipid) green sea turtles (Fig. 3A). MANOVA comparing similarly sized turtles (see methods) demonstrated that sub-adults from SDB (6.45, 6.26 ± 0.29 ln ng/g lipid) had significantly more ΣPCBs (4.34, 4.36 ± 0.21 ln ng/g lipid) than SBNWR sub-adult turtles (Fig. 3B).

There was no significant difference in ΣOCPs between turtles captured in SDB (3.65, 2.90 ± 0.66 ln ng/g lipid) and SBNWR (3.80, 2.48 ± 0.94 ln ng/g lipid); and there was no relationship between ΣOCPs and turtle CCL ($R^2 = 0.006$, $p = 0.52$). The most commonly-occurring OCPs in green turtle plasma from both locations were chlordane-gamma and trans-nonachlor. Most of the DDT metabolites were not detected or were only present in blood plasma from one turtle at each location (Table 1).

Only four green sea turtles were recaptured for the duration of the study, three from SDB and one from SBNWR. Three of the four turtles showed increased (+70.22%, +248.30%, +367.55% ng/g lipid) ΣPOP concentrations from their initial capture (Table 4). One turtle had decreased (−23.01%) ΣPOPs from its initial capture (Table 4). Changes in ΣPOP concentrations were mainly driven by increases (+74.29%, +240.35%, +383.71%) or decreases (−24.41%) in ΣPCB concentrations.

Samples from previous SDB green turtle blood collections had significantly ($p = 0.002$) higher ΣPCBs (8.95, 8.75 ± 0.30 ln ng/g lipid) and significantly ($p = 0.001$) higher ΣOCPs (5.66, 5.63 ± 0.18 ln ng/g lipid) than SDB green sea turtles measured in the current study. Green sea turtles from SGR had significantly ($p = 0.004$) higher ΣPCBs (7.99, 7.52 ± 0.64 ln ng/g lipid) but similar ΣOCPs (5.09, 5.18 ± 0.49 ln ng/g lipid) than green sea turtles from SBNWR. Results from additional samples are provided the supplementary section for future comparisons (Table S4).

Percent recovery of SRM, plasma, blank, and blank spikes TCMX recovery surrogate accuracy was 67.71–99.95% and precision was ± 1.79–6.88% (see Table S2). In all samples, accuracy of PCB 30 was 77.23–100.80%, and precision was 1.38–6.75%. Detection of PCB 112 had an accuracy of 92.17–105.65% and a precision of 1.75–6.38%. Accuracy of PCB 198 was 70.42–100.56% in all samples, and precision of PCB 198 in all samples was ± 0.49–8.16%. Detection of FPBDE had an accuracy of 76.55–88.01% and precision of 3.44–8.77%. Detection of DFPBDE had an accuracy of 52.35–74.75% and precision of 2.26–6.70%. There was no outside contamination detected in any blanks analyzed.

Table 2
Summary persistent organic pollutants (PCB and OCP) estimated in blood plasma samples collected from green sea turtles from the Seal Beach National Wildlife Refuge (n = 16) or San Diego Bay (n = 23) using Kalpan-Meier model estimates. NAs are present where the model could not provide an estimate. Mean ± SEM and 95% confidence interval (Conf. Int.) are ng/g blood plasma.

Congener	San Diego Bay		Seal Beach National Wildlife Refuge	
	Mean ± SEM	95% Conf. Int.	Mean ± SEM	95% Conf. Int.
PCB 3	0	0	0	0
PCB 8	0	0	0	0
PCB 18	0	0	0	0
PCB 28	0	0	0	0
PCB 31	0	0	0	0
PCB 33	0	0	0	0
PCB 37	0	0	0	0
PCB 44	0	0	0	0
PCB 49	0	0	0	0
PCB 52	0	0	0	0
PCB 56 + 60	0	0	0	0
PCB 66	0.27 ± NA	NA	0	0
PCB 70	0	0	0	0
PCB 74	0	0	0	0
PCB 77	0	0	0	0
PCB 81	0	0	0	0
PCB 87	0.31 ± NA	NA	0	0
PCB 95	0	0	0	0
PCB 97	0	0	0	0
PCB 99	0.88 ± 0.16	0.57–1.19	0	0
PCB 101	0	0	0	0
PCB 105	0	0	0	0
PCB 110	0	0	0	0
PCB 114	0.44 ± NA	NA	0	0
PCB 118	0.33 ± 0.035	0.26–0.40	0	0
PCB 119	0	0	0	0
PCB 123	0	0	0	0
PCB 126	0	0	0	0
PCB 128	0.29 ± 0.02	0.25–0.34	0	0
PCB 138	2.91 ± 0.62	1.69–4.12	0.33 ± 0.02	0.28–0.40
PCB 141	0	0	0	0
PCB 149	0.33 ± NA	NA	0.28 ± NA	NA
PCB 151	0	0	0	0
PCB 153	2.75 ± 0.72	1.35–4.16	0.54 ± NA	NA
PCB 156	0	0	0	0
PCB 157	0	0	0	0
PCB 158	0	0	0	0
PCB 167	0	0	0	0
PCB 168 + 132	0	0	0	0
PCB 169	0	0	0	0
PCB 170	0.28 ± 0.002	0.28–0.28	0	0
PCB 174	0	0	0	0
PCB 177	0	0	0	0
PCB 180	0.49 ± 0.09	0.31–0.66	0	0
PCB 183	0.32 ± 0.02	0.28–0.35	0	0
PCB 187	0.35 ± 0.03	0.29–0.40	0	0
PCB 189	0	0	0	0
PCB 194	0	0	0	0
PCB 195	0	0	0	0
PCB 199 + 200	0	0	0	0
PCB 201	0.34 ± 0.002	0.34–0.35	0	0
PCB 206	0	0	0	0
PCB 209	0	0	0	0
4,4′-DDMU	0	0	0	0
Chlordane-gamma	0	0	0	0
2,4′-DDE	0	0	0	0
Chlordane-alpha	0	0	0	0
trans-Nonachlor	0.43 ± 0.005	0.42–0.44	0	0
4,4′-DDE	0.40 ± NA	NA	0	0
2,4′-DDD	0	0	0.89 ± NA	NA
4,4′-DDD	0	0	0	0
2,4′-DDT	0	0	0	0
cis-Nonachlor	0	0	0	0
4,4′-DDT	0	0	0	0

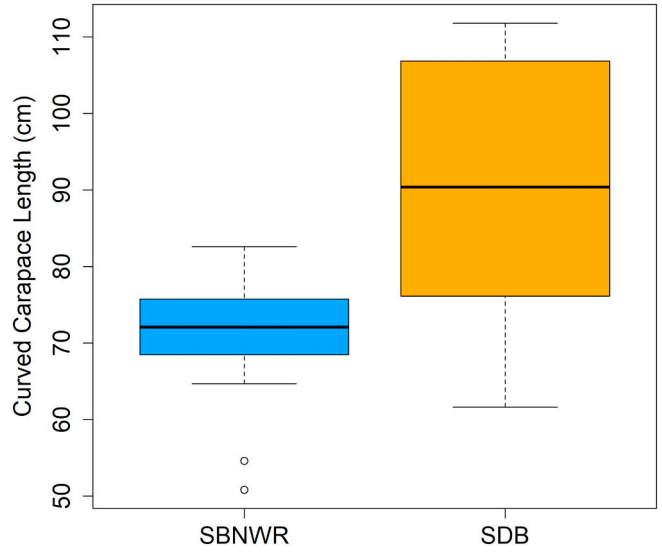

Fig. 1. Boxplot of curved carapace length (CCL; cm) of green sea turtles captured from Seal Beach National Wildlife Refuge (SBNWR, n = 16 turtles) and San Diego Bay (SDB, n = 23 turtles). Boxes are the middle 50% quartile with the line representing the median, whiskers are top and bottom 25% quartile. X-axis represents CCL and y-axis capture location of green sea turtles. SDB turtles are significantly ($p \leq 0.001$) larger than SBNWR.

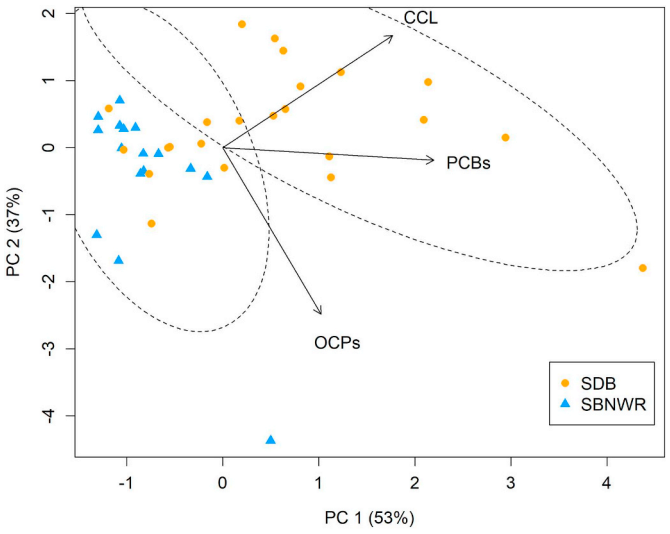

Fig. 2. Principal component analysis of ΣPCBs and ΣOCPs in blood plasma lipid and curved carapace length (CCL) of green sea turtles from the Seal Beach National Wildlife Refuge (SBNWR; n = 16 green sea turtles) and San Diego Bay (SDB; n = 23 green sea turtles). Vectors indicate the direction that each factor affects principal component scores for each turtle (point). PC1 (x-axis) refers to ΣPCBs in ng/g blood plasma lipid, and PC2 refers to CCL (cm).

4. Discussion

4.1. Location pollutant signatures

As expected, there were unique location-specific pollutant signatures in green sea turtles inhabiting SBNWR and SDB. Green sea turtles from SBNWR had similar PBDE and OCP concentrations to SDB green sea turtles; however, the majority of POPs detected in green turtle blood plasma were PCBs. As a result, PCBs constituted the highest proportion of green turtle POP loads by ng/g lipid and were the strongest factor in the separation of green sea turtles by location in the PCA loading plot (Fig. 2). These patterns are reflected in the higher concentration and variety of PCBs in SDB green turtle blood plasma relative to SBNWR green sea turtles, which suggests that SDB green sea turtles experience higher PCB exposure than SBNWR green sea turtles (Table 1; Table 2).

Table 3
Multivariate Analysis of Variance (MANOVA) results of persistent organic pollutant concentrations in green sea turtles inhabiting the Seal Beach National Wildlife Refuge (n = 16) or San Diego Bay (n = 23). "PCBs" are polychlorinated biphenyls, "OCPs" are organochlorinated pesticides, and "CCL" refers to curved carapace length. "All" refers to comparisons that include all turtles captured and analyzed in the current study (n = 39); and "Sub-Adult" refers to comparisons which only include similar sized subadult turtles (n = 23). "Comparison" refers to the dependent and independent variables in the MANOVA. Significant differences have p values in bold below.

Comparison	Mean squares	F Value	P Value
All PCBs: Location	55.61	60.77	**p < 0.001**
All PCBs: CCL	2.02	2.21	p = 0.14
All PCBs: CCL*Location	2.32	2.53	p = 0.12
All OCPs: Location	0.23	0.12	p = 0.73
All OCPs: CCL	0.39	0.2	p = 0.65
All OCPs: CCL*Location	0.90	0.48	p = 0.50
Sub-Adult PCBs: Location	19.66	29.93	**p < 0.001**
Sub-Adult PCBs: CCL	1.32	2.01	p = 0.17
Sub-Adult PCBs: CCL*Location	0.01	0.02	p = 0.89
Sub-Adult OCPs: Location	0.10	0.04	p = 0.84
Sub-Adult OCPs: CCL	0.01	0.004	p = 0.94
Sub-Adult OCPs: CCL*Location	0.24	0.10	p = 0.75

4.2. Differences between sample locations

The current study measured PCB concentrations green sea turtles from two locations, approximately 160 km apart. The two green sea turtle aggregations have some biological factors (e.g., size and sex) that could influence their PCB accumulation patterns. Previous research has shown that SDB green sea turtles' POPs correlate with CCL (Komoroske et al., 2011); therefore, it could be expected that larger, older individuals would have higher PCB concentrations as seen in other sea turtle species (Finlayson et al., 2016; Pugh and Becker, 2001). SDB green sea turtles were larger than green sea turtles caught in SBNWR (Fig. 1), and PCB differences could be due to longer exposure times. Although bioaccumulation with age/size has been found in turtles from other locations (Finlayson et al., 2016; Gardner and Oberdorster, 2005) and the data often trended with CCL (Fig. 2, 3C), the current study did not find significant relationships between size (CCL) and PCB levels (Table 3). However, the current study captured only a small size range of SBNWR green sea turtles (50.8–82.6 cm CCL); thus, future capture of an increased range in size of individuals from SBNWR would help determine if there is a relationship between size and POP levels. It is possible that SBNWR currently represents a relatively new year-round expansion of green turtle foraging habitat, utilized primarily by smaller juvenile individuals that will take some time to grow into adults. It is also possible that adult turtles could not fit into the culverts that leads into the capture location within SBNWR as there were adult turtles caught within the SGR in previous sampling years. However, one adult turtle was seen in SBNWR, indicating some adults could access the ponds inside SBNWR.

Sex is another biological factor that may influence pollutant load (Keller, 2013). Green sea turtles from SDB are mostly female (> 75%) and of adult-size (> 85 cm CCL), whereas turtles collected from SBNWR are also mostly female, but smaller than adult-size (< 85 cm CCL) (Allen et al., 2016, 2015). Female green sea turtles can maternally offload POPs by metabolizing fat reserves that transfer lipids, and therefore POPs, to their eggs (Munoz and Vermeiren, 2019; van de Merwe et al., 2010b). As a result, there is potential for adult female green sea turtles to exhibit lower blood plasma POPs due to maternal offloading, yet, the data did not support that conclusion as female and male adult turtles in this study had similar POP concentrations. The current study had only six adult males, and all but one was from SDB, which is insufficient to observed sex-based POP differences.

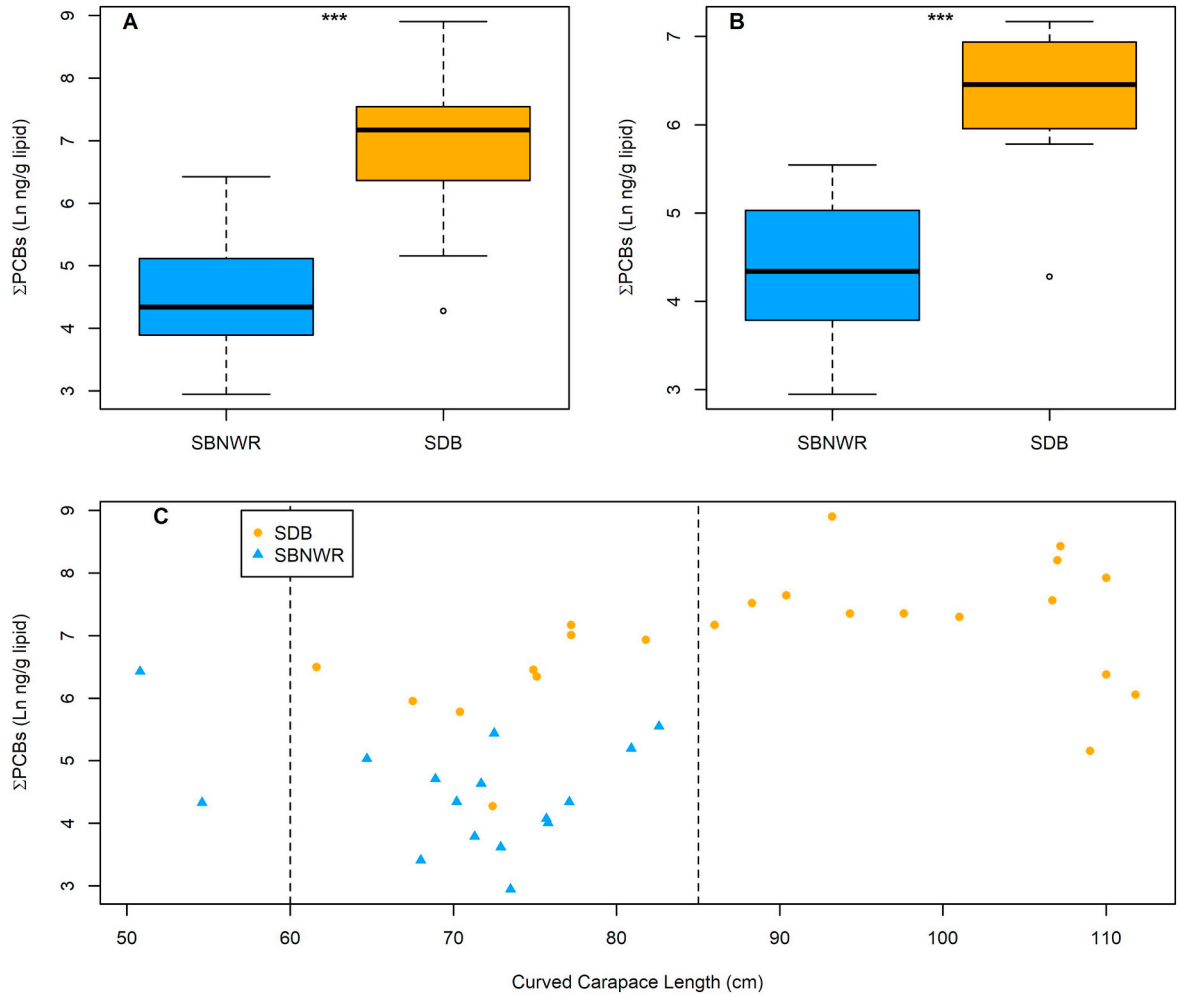

Fig. 3. (A) Natural log transformed ΣPCBs in blood plasma lipid of green sea turtles from Seal Beach National Wildlife Refuge (SBNWR; n = 16 turtles) and San Diego Bay (SDB; n = 23 turtles). (B) Natural log transformed ΣPCBs in blood plasma lipid of sub-adult (between 60 and 85 cm CCL) green sea turtles from Seal Beach National Wildlife Refuge (n = 14 turtles) and San Diego Bay (n = 9 turtles). Y-axis represents ng/g lipid ΣPCBs natural log transformed in green turtle plasma samples (corrected for lipid content). X-axis represents location of sea turtles captured. Asterisks indicate significant differences via one-way ANOVA (*** ≤ 0.001). (C) Relationship of natural log ΣPCBs in blood plasma lipid and curved carapace length of green sea turtles from Seal Beach National Wildlife Refuge (n = 16 turtles) and San Diego Bay (n = 20 turtles). X-axis represents curved carapace length in centimeters of sea turtles captured. Y-axis represents natural log transformed ng/g ΣPCBs in sea turtle blood plasma lipid.

Green sea turtles in SBNWR have access to anthropogenically-warmed waters from power plants within SGR (Crear et al., 2017, 2016). In contrast, SDB green sea turtles in the present study live under more natural conditions—although it should be noted that data from Komoroske et al. (2011) in SDB were from a period when waters were warmed from the south San Diego Bay power plant. Anthropogenically warmed waters could explain why SDB green sea turtles had lower PCB levels when the power plant was active (since monitoring began) than after power plant closure in December 2010. When SDB green sea turtles had access to anthropogenically warmed waters, their growth rate was similar to green sea turtles that inhabit tropical waters (Eguchi et al., 2012). While not confirmed, it is postulated that SBNWR/SGR green sea turtles have an accelerated growth rate due to their access to anthropogenically-warmed waters. Depending on pollutant availability, accelerated growth rates could be diluting SBNWR green sea turtles' PCB concentrations through increased mass from growth and the opposite for SDB green sea turtles currently inhabiting non-warmed waters. While it can be argued that warmer waters can increase the rate at which green sea turtles feed on PCB contaminated food, thereby increasing their potential PCB exposure, results indicate that overall, SBNWR turtles have lower PCB concentrations than SDB green sea turtles.

4.3. Comparison to previous research

Previous research by Komoroske et al. (2011) examined POPs in SDB green sea turtles and captured a size range of green sea turtles similar to those captured in the current study. In the current study, green sea turtles from SDB and SBNWR had lower chlordane gamma and chlordane alpha than previously assessed in SDB, possibly indicating a reduction in bioavailable pesticides or exposure (Komoroske et al., 2011; Lewison et al., 2011). It is also important to note that Komoroske et al. (2011) had different LODs for each of the POPs analyzed than the current study, with LODs lower in the current study for PCBs and OCPs but higher for PBDEs. Unlike Komoroske et al. (2011), the current study did not find any PBDEs in blood plasma samples. These findings could indicate a decrease in PBDE contamination at both sites samples were last taken, especially since PBDEs concentrations are decreasing throughout the Southern California Bight (Dodder et al., 2016, 2012). The current study had a higher LOD and recovery surrogates were relatively low compared to other analytes, but SRM measurements were accurate and precise. There is a possibility that the higher LOD would miss low PBDE concentrations in green turtle blood plasma; however, there was no indication of any PBDE concentrations at or below the LOD in gas chromatography mass spectrometry scans.

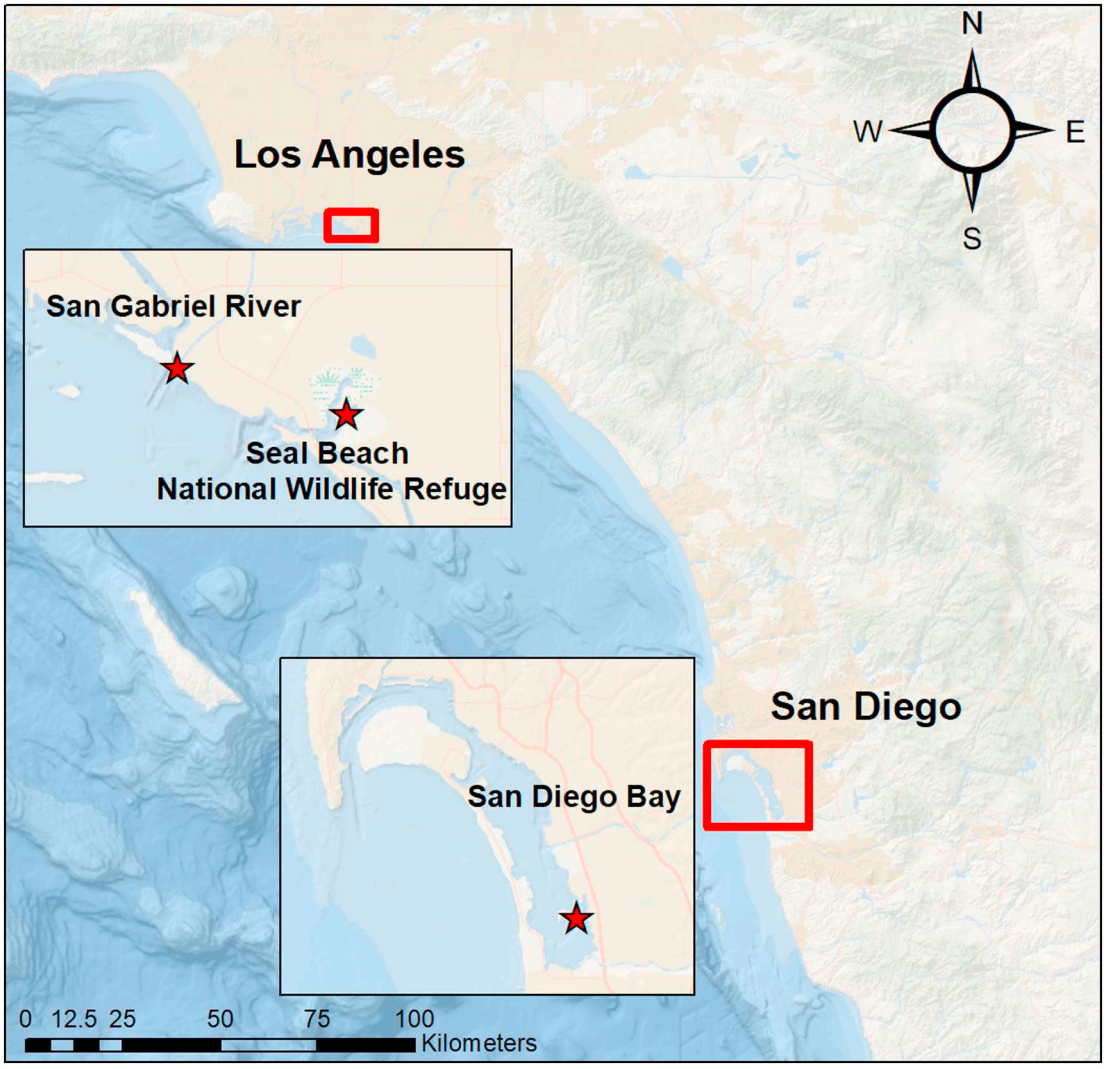

Fig. 4. Capture locations (red stars) for green sea turtles inhabiting the Los Angeles area (top square) and the San Diego area (bottom square) within Southern California, USA. Top square shows the San Gabriel River (left star) and the Seal Beach National Wildlife Refuge (right star); bottom square shows San Diego Bay (bottom star). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

SDB turtles have among the highest ΣPCBs detected in blood plasma compared to previous SDB samples and other turtles worldwide, with studies detecting ΣPCBs as low as 2.84 pg/g blood plasma and as high as 5.38 ng/g blood plasma compared to 30.11 ng/g ΣPCBs found in an SDB turtle (Camacho et al., 2014; Keller et al., 2014a; Komoroske et al., 2011; Lewison et al., 2011; Swarthout et al., 2010; van de Merwe et al., 2010b, 2010a). Conversely, SBNWR green sea turtles had similar or lower ΣPCB levels in their blood than other studies worldwide (Keller, 2013). Additional samples collected in 2011–2013 from green sea turtles inhabiting the SGR had higher PCB concentrations than SBNWR turtles. Sediment samples from previous research found very low (0–1.77 μg/kg dw) PCB concentrations near the SGR and SBNWR (Dodder et al., 2016), possibly due to differences in food items available between the locations (eelgrass in SBNWR and algae in the SGR) (Crear et al., 2016). Green sea turtles sampled from the SGR were similar in size to the current study's SBNWR green sea turtles, except for one adult turtle (93 cm, CCL), which had PCB levels analogous to other adult turtles from SDB samples. While the sample size was low for SGR turtles

Table 4
Summary of persistent organic pollutants (ΣPOPs, ΣPCB, ΣOCP) concentrations in recaptured turtles, as well as their change in POP concentrations since their first capture. No turtle was caught more than twice. N.D. signifies that no analytes were detected. ΣPOPs, ΣPCB, and ΣOCP concentrations are in ng/g blood plasma lipid. (%Δ) Refers to the percent change in analyte concentrations from first capture to recapture.

ID	Location	Date captured	ΣPCBs	ΣOCPs	ΣPOPs	%ΔPCBs	%ΔOCPs	%ΔPOPs	Δ Time
GK-33	SBNWR	25/08/2015	37.31	16.42	53.73	383.71	330.82	367.55	1 year, 4 months
GK-33	SBNWR	7/12/2016	180.49	70.73	251.22				
GK-23	SDB	1/06/2016	635.56	37.78	673.33	74.29	1.81	70.22	5 months
GK-23	SDB	10/11/2016	1107.69	38.46	1146.15				
LB-326	SDB	15/12/2015	3645.21	38.36	3683.56	−24.41	110.02	−23.01	6 months
LB-326	SDB	15/06/2016	2755.56	80.56	2836.11				
LB-337	SDB	27/08/2015	173.58	N.D.	173.58	240.35	NA	248.30	1 year, 3 months
LB-337	SDB	2/11/2016	590.80	13.79	604.60				

(n = 6) and the foraging locations are within 8 km of each other, the current study's data indicate that turtles from SGR may have higher PCB exposure and ensuing risk of PCB accumulation than turtles from SBNWR. Overall, the current study's results indicate that PCB exposure risk in SDB turtles may have increased since previous studies and that the SDB location may represent one of the highest PCB-contaminated green turtle populations studied to date (Finlayson et al., 2016; Gardner and Oberdorster, 2005; Keller, 2013). Nonetheless, previous research has shown that blood plasma PCB concentrations can vary with time and between samples (Lewison et al., 2011).

4.4. Variance from lipid mobilization

Previous research has shown that the mobilization of adipose or recent dietary exposure could also temporarily increase POP concentrations in blood plasma, indicating that POP concentrations can vary depending on whether green sea turtles are captured before or after breeding migrations that require fasting or recent feeding events (Hamann et al., 2002; Keller et al., 2004). The current study's ΣPCB, ΣOCPs, and ΣPOPs concentrations were lipid-normalized to account for these lipid mobilization events. Four green sea turtles were recaptured, three from SDB and one from SBNWR, with three out of the four turtles having increased ΣPOPs (+74%, +240%, +383% ng/g lipid) since their initial capture (Table 4). Of note, three out for four recaptured green sea turtles had higher ΣPOPs in ng/g lipid in the fall/winter months than the spring/summer months, suggesting that these green sea turtles POP concentrations continually change even when accounting for increased lipid in blood plasma. Previous research that has repeated sampling of individuals in SDB found that POP concentrations can vary with time of sampling and even within samples of the same individual collected on the same day (Lewison et al., 2011). The results are also supported by additional blood samples collected from 2011 to 2013, which further indicate that SDB green turtle POP concentrations continuously fluctuate over time. These samples had higher PCB and OCP concentrations compared to current (2015–2017) SDB turtle samples and those found in 2009 (Komoroske et al., 2011). Only four SDB turtles were analyzed as recaptures, which makes it difficult to suggest that the whole aggregation is reflected in the analysis. However, these samples and trends may suggest that time of year and/or temperature may be affecting ΣPOP concentrations found in the blood of these green sea turtles.

4.5. Future directions

Overall, both populations of green sea turtles in the current study had low to non-detectable levels of dioxin-like PCBs, a heavily studied group of PCBs (Domingo and Bocio, 2007; Srogi, 2008). Rather, the most abundant PCB congeners detected were non-dioxin-like PCBs (Table 1), such as PCB 138, 153, 187, and 180. These PCBs are known to activate the ryanodine receptor or alter dopaminergic-signaling pathways (Holland et al., 2017; Kenet et al., 2007; Pessah et al., 2006; Wigestrand et al., 2013; Yang et al., 2009). Non-dioxin-like PCB actions through these pathways have been related to induced muscle impairment, altered neuronal growth, and impaired learning and memory (Pessah et al., 2010; Wayman et al., 2012; Wigestrand et al., 2013; Yang et al., 2009). Although these previous studies were conducted in mammalian species, their findings suggest that green sea turtles could be at risk for the induction of neurotoxicity due to PCB burdens. Juvenile/hatchling green sea turtles born from SDB green sea turtles may receive high PCB burdens through maternal transfer of non-dioxin-like PCBs, potentially negatively impacting their early life stages. Given that the majority of PCBs detected in SDB and SBNWR turtles were non-dioxin-like PCBs, and the current lack of research into neurotoxic non-dioxin-like PCBs in reptiles, it would be beneficial to investigate these possible effects in the SBNWR and SDB green sea turtles (Finlayson et al., 2016; Gardner and Oberdorster, 2005). The current study did not include many other pollutants (e.g., PAHs, plastics) that could be accumulating within southern California green sea turtles, which future research could help determine risk and possible mixture interactions. New research into POPs include: health panels for investigating health effects (Banerjee et al., 2019; Keller et al., 2014a; Komoroske et al., 2011), non-targeted extraction methods to evaluate mixture effects and monitor multiple compound types (Dogruer et al., 2018; Heffernan et al., 2017; Vijayasarathy et al., 2019), and combining non-targeted extractions with cell line bioassays as measurements of contamination and toxicity (Allan et al., 2017; Finlayson et al., 2019b, 2019c, 2019a; Jin et al., 2015). These new methods and tools help link POP concentrations to various health effects and assess the effects of multiple pollutant types; and while the body of research is growing, pollutant physiological tipping points and pollutant mixture interactions are still not well understood (Cortes-Gomez et al., 2017; Finlayson et al., 2016). Until more research is completed, it is uncertain whether the amount POPs detected are high enough to have a detrimental effect on SDB and SBNWR green sea turtles' health.

5. Conclusions

Overall, evidence was found that green sea turtles from SDB accumulated higher PCB levels than green sea turtles from SBNWR. While factors such as size, and lipid mobilization events can change PCB levels in the short term (6 months to 1.5 years), the results indicate greater PCB levels in green sea turtles in SDB than those found in other parts of the world (Keller, 2013). The current study's results suggest that green sea turtles foraging within SGR are at greater risk of PCB accumulation than SBNWR turtles and similar PCB levels to SDB turtles. The most common PCBs accumulated were non-dioxin-like PCBs, indicating opportunities for future research to investigate the possible effects of non-dioxin-like PCBs impacts on green turtle physiology. Overall, considering the disparity and fluctuations in PCB accumulation patterns found, additional monitoring of turtles within the Los Angeles and San Diego areas may be necessary. Even green sea turtles foraging within proximate habitats (SBNWR vs. SGR) exhibited different PCB accumulation patterns. While the health effects of these PCBs are currently unknown, green sea turtles will continue to inhabit these urban areas for the foreseeable future due to increasingly warm waters and population recovery (Seminoff et al., 2015). Urban populations, such as the green sea turtles inhabiting critical habitat within southern California, provide clues and opportunities to elucidate how green sea turtles are affected by anthropogenic pollutants.

CRediT authorship contribution statement

Arthur D. Barraza: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing - original draft. **Lisa M. Komoroske:** Supervision, Investigation, Resources, Validation, Writing - review & editing. **Camryn D. Allen:** Investigation, Data curation, Resources, Writing - review & editing. **Tomoharu Eguchi:** Software, Validation, Resources, Writing - review & editing. **Rich Gossett:** Methodology, Resources, Writing - review & editing. **Erika Holland:** Supervision, Validation, Writing - review & editing. **Daniel D. Lawson:** Investigation, Resources, Writing - review & editing. **Robin A. LeRoux:** Investigation, Writing - review & editing. **Varenka Lorenzi:** Methodology, Validation, Resources, Writing - review & editing. **Jeffrey A. Seminoff:** Investigation, Resources, Project administration, Funding acquisition, Writing - review & editing. **Christopher G. Lowe:** Supervision, Conceptualization, Funding acquisition, Resources, Project administration, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.110979.

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