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Sea turtles across the North Pacific are exposed to perfluoroalkyl substances☆

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ABSTRACT

Perfluorinated alkyl substances (PFASs) are global, persistent, and toxic contaminants. We assessed PFAS concentrations in green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles from the North Pacific. Fifteen compounds were quantified via liquid chromatography tandem mass spectrometry from 62 green turtle and 6 hawksbill plasma samples from Hawai'i, Palmyra Atoll, and the Northern Marianas Islands. Plasma from 14 green turtles severely afflicted with fibropapillomatosis, and eggs from 12 Hawaiian hawksbill nests from 7 females were analyzed. Perfluorooctane sulfonate (PFOS) predominated in green turtle plasma; perfluorononanoic acid (PFNA) predominated in hawksbill tissues. Concentrations were greater in hawksbill than green turtle plasma ($p < 0.05$), related to trophic differences. Green turtle plasma PFOS concentrations were related to human populations from highest to lowest: Hawai'i, Marianas, Palmyra. Influence on fibropapillomatosis was not evident. PFASs were maternally transferred to hawksbill eggs, with decreasing concentrations with distance from airports and with clutch order from one female. A risk assessment of PFOS showed concern for immunosuppression in Kailua green turtles and alarming concern for hawksbill developmental toxicity. Perfluoroundecanoic (PFUnA) and perfluorotridecanoic (PFTriA) acid levels were correlated with reduced emergence success ($p < 0.05$). Studies to further examine PFAS effects on sea turtle development would be beneficial.

1. Introduction

Perfluoroalkyl substances (PFASs) are a chemical family used in many industrial and commercial products (Lau et al., 2007). Their chemical makeup typically consists of a fluorinated carbon backbone (4–14 in length) and a charged functional moiety, such as a carboxylate. The carbon-fluorine bond is incredibly stable, which gives PFASs extreme persistence and both hydrophobic and lipophobic properties (Buck et al., 2011). Since their invention in the 1930s and commercialization in the 1950s, PFASs have been incorporated into every-day consumer products including coatings, fabrics, grease-proof papers, soil repellents, and aqueous film-forming foam fire suppressants (AFFFs) (Moody and Field 2000; Buck et al., 2011; Ahrens and Bundschuh 2014; Interstate Technology Regulatory Council, 2020). AFFFs used for firefighting and training at military bases and airports are large point sources of

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PFAS contamination (Schultz et al., 2004). Their nearly non-biodegradable properties, along with global distribution (Yamashita et al., 2008) render PFASs highly persistent pollutants in the environment worldwide (Lau et al., 2007) with detectable baseline concentrations in the most remote regions (Young et al., 2007).

PFASs are prominent contaminants in wildlife and human tissues, and can result in toxicological effects, including immune, developmental, hepatic, and endocrine disruptions (Lau et al., 2007; DeWitt 2015). In 2001, 3M Corporation, a major producer of perfluorooctane sulfonate (PFOS), began phasing out production of this chemistry. In 2006, eight manufacturers committed to reduce perfluorooctanoate (PFOA) production by 2015 through a US EPA Stewardship program (US EPA 2018). Additional policies have focused on eliminating further production of PFASs in Europe and Asia, but PFASs remain prominent in the environment (McCarthy 2017).

Because sea turtles are rare and protected, understanding their exposure to toxic contaminants is important for the recovery of these species (NMFS and US FWS, 1998a, b). Moreover, certain life stages of sea turtles can indicate the contaminant levels in their respective foraging grounds, because they integrate those contaminants over several years (O'Connell et al., 2010; Keller et al., 2013). The generalized long life history of green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) sea turtles includes an ontogenetic switch that separates two juvenile phases (Bolten et al., 2003). The youngest turtles forage in open ocean habitats, and transition to foraging in nearshore benthic habitats as older juveniles where they grow into subadults and adults. As adults, they migrate from foraging grounds to inter-nesting habitat where they do not eat much. The green turtle is the lowest trophic level sea turtle species (Bjorndal et al., 1997). They transition from omnivorous pelagic juveniles (Parker et al., 2011) to herbivory in the neritic stages (Arthur and Balazs 2008; Summers et al., 2017). Green turtles are highly migratory, across entire ocean basins, but have strong site fidelity to foraging grounds within a life stage (Summers et al., 2017; Shimada et al., 2020). Hawksbill sea turtles are less migratory (Gaos et al., 2020b) and continue to feed at higher trophic levels after their ontogenetic switch to eating primarily sponges in the neritic phase (Bjorndal et al., 1997; Summers et al., 2017).

Six studies have reported PFAS concentrations in tissues of six sea turtle species (Keller et al., 2005; O'Connell et al., 2010; Keller et al., 2012; Guerranti et al., 2013; Guerranti et al., 2014; Pasanisi et al., 2016), but none from turtles across the North Pacific. Most analyzed plasma (Keller et al., 2005; O'Connell et al., 2010; Keller et al., 2012), and none have analyzed eggs. Differences in concentrations among five species provide evidence that PFASs biomagnify in the turtles' distinct food webs and that the major route of exposure is diet (Keller et al., 2012). For example, PFAS concentrations are greater in carnivorous loggerhead (*Caretta caretta*) sea turtles than herbivorous green turtles from the same neritic habitat (Keller et al., 2012).

The toxicological effects of PFASs on sea turtles are still largely undetermined. Immunosuppressive contaminants have long been a suspected contributing influence in a debilitating, tumor-forming, viral disease, fibropapillomatosis (FP) (Herbst and Klein 1995; Keller et al., 2013), but to date, no convincing causal relationship between exposure to toxicants and development of FP has been made. For instance, levels of other types of persistent organic pollutants (POPs) measured in many of the same plasma samples used in this study did not relate to prevalenceof FP in Hawaiian green turtles; however, PFASs were not included in that analysis (Keller et al., 2014a). Furthermore, maternal offloading of POPs occurs during yolk deposition (Keller et al., 2013; Munoz and Vermeiren 2020), but transfer of PFASs to eggs and effects on hatching success and embryonic development are unknown.

In this study, we measured concentrations of 15 PFASs in the plasma of neritic-phase green and hawksbill sea turtles across three North Pacific regions: the Main Hawaiian Islands (MHI), the Commonwealth of the Northern Marianas Islands (CNMI), and Palmyra Atoll. Eggs from excavated hawksbill nests from MHI were also analyzed. We hypothesized that (1) PFOS will predominate like in most other studies, (2) higher trophic positioned, spongivorous hawksbills will have greater plasma PFAS concentrations than herbivorous green turtles, (3) PFAS concentrations in turtles will correlate to human population or proximity to military bases or airports (and in the lack of local point sources, remote Palmyra Atoll will represent globally diffuse contamination levels transported by air and ocean currents), (4) PFASs will be detected in the sampled hawksbill eggs, revealing maternal offloading, (5) PFAS concentrations in eggs will negatively correlate to nest success variables, and (6) eggs from successive clutches from the same female within a nesting season will have stable PFAS concentrations.

2. Materials and methods

2.1. Study sites

Samples were collected on beaches and coastal waters from three Pacific Island regions: the Main Hawaiian Islands (MHI), the Commonwealth of the Northern Marianas Islands (CNMI), and Palmyra Atoll (Fig. 1). The MHIs have roughly 1.4 million people, three international airports, and 11 military bases (US Census Bureau 2019a). CNMI has about 57,000 people (United Nations Dept. of Economic and Social Affairs, 2019), three international airports, and no active military bases, but a historically large military presence from World War II. Palmyra Atoll is a remote U.S. National Wildlife Refuge with less than 20 staff, no commercial airport, and a decommissioned military base.

2.2. Sample collection

Cryogenically archived sea turtle samples were selected from the NIST Biorepository's Biological and Environmental Monitoring and Archival of Sea Turtle tissues project (BEMAST). Sample collection and processing methods are detailed in Keller et al. (2014b). Sample metadata are provided (Tables S1, S2). Plasma samples were selected to investigate spatial, species, and health status differences and included 62 samples from 61 free-ranging green sea turtles from across three study sites (Table 1, Fig. 1): 39 from MHI (n = 13 from three sites), 12 from CNMI (n = 6 from Saipan and Tinian), and 10 from Palmyra Atoll (n = 2 from each segment of the refuge, with one turtle sampled twice over two years). Plasma from 14 severely tumored, MHI-stranded turtles were also analyzed. All MHI plasma samples came from the same turtles that were analyzed previously for other POPs (Keller et al., 2014a), representing four groups of green turtles with varying prevalence of FP: Kiholo Bay (0% FP), Kailua Bay (low to moderate FP 0%–5%), Kapoho Bay (higher FP 35%) and tumored-stranded (100% FP). All six hawksbill plasma samples available in the BEMAST inventory from CNMI (n = 4, two each from Saipan and Tinian) and Palmyra (n = 2) were included. All 12 hawksbill nests available in BEMAST inventory at the time were included. They were from beaches on Maui, Hawai'i, and Kauai (Fig. 1) in 2012 and represent seven nesting females (Table S2). Unhatched eggs were collected from each nest upon excavation after hatchling emergence. Egg contents from at most three unhatched eggs per nest were pooled, homogenized, aliquoted, and cryogenically archived. The number of eggs laid, days of incubation, hatching success, and

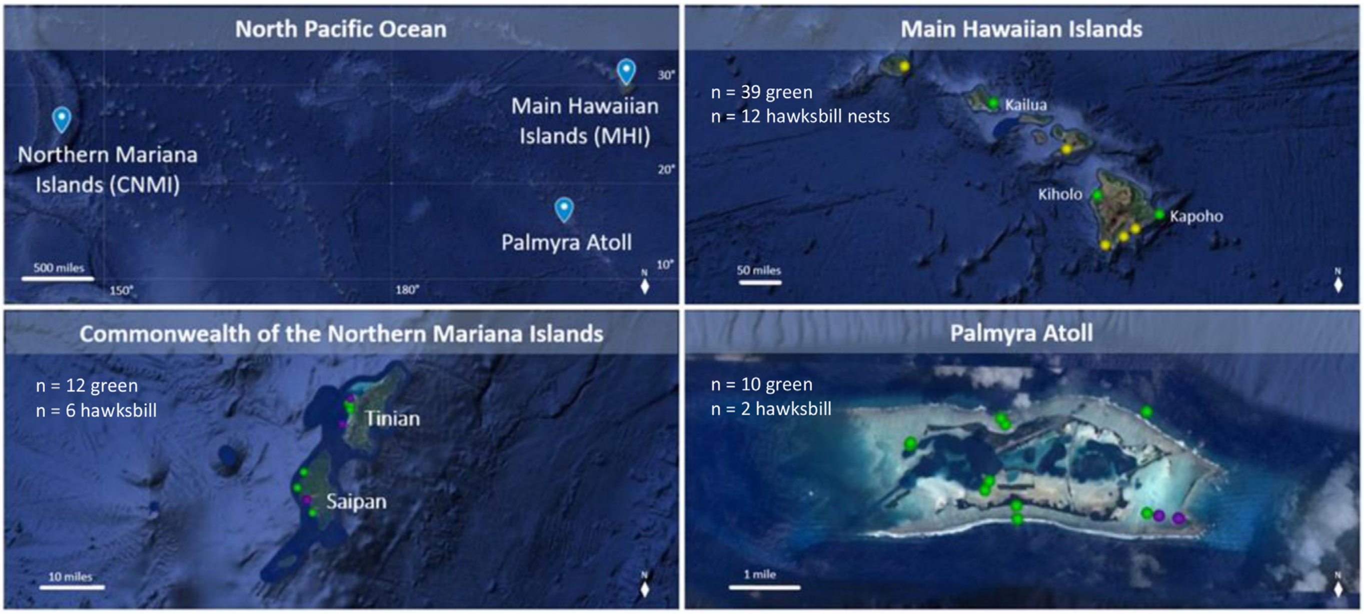

Fig. 1. Green and hawksbill sea turtle sampling sites and sample sizes. Dots show capture sites of free-ranging green (green) and hawksbill (purple) turtles and excavation sites of hawksbill nests (yellow). Fourteen stranded green turtles from MHI severely afflicted with FP plasma are not mapped. Base map data from Google, DigitalGlobe 2015. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

emergence success were calculated per nest according to Miller et al. (1997) to inspect the fitness of each nest in relation to PFAS concentrations. The percent of eggs laid that developed to at least stage 23 of development, as defined by Miller et al. (2017) was also calculated (Table S2).

2.3. PFAS measurements

Detailed methods are provided in Supplemental Information. Samples were measured using methods modified from Keller et al. (2012). Concentrations of 15 PFASs were quantified using the internal standard approach (Table S3). Field blanks were prepared with the same lot number of blood collection supplies (Table S4). Samples were extracted via sonication with acetonitrile and purified on 250 mg Envi-carb columns (Supelco, Bellefonte, Pennsylvania). Methanol extracts (20 μL) were injected on an Agilent Zorbax Eclipse Plus C18 column using a liquid chromatograph (Agilent 1100 HPLC, Palo Alto, CA) negative electrospray ionization tandem mass spectrometer (API 4000, Applied Biosystems-MDS Sciex, Foster City, CA). The mobile phase consisted of methanol and 20 mmol/L ammonium acetate in water. Two transitions for PFOS were monitoring (499 → 99 and 499 → 80) to ensure bile acids did not interfere. Concentrations are totals of linear and branched isomers. Reporting limits (RLs) were determined according to O'Connell et al. (2010). The mass fractions of PFASs measured in NIST SRM 1957 Organic Contaminants in Non-Fortified Human Serum and SRM 1947 Lake Michigan Fish Tissue were within the uncertainty of the reference values (Table S5).

2.4. Data analyses

R software package, NADA, was used whenever possible when samples were <RL as suggested by Helsel (2005), otherwise JMP (SAS Institute Inc. Cary, NC) was used with values < RL substituted with half the RL. Significance was determined as $p < 0.05$. Data were assessed for assumptions of normality and equal variances. Non-parametric ANOVAs in NADA tested differences among sites for green turtle plasma PFAS concentrations, followed by Steel Dwass non-parametric multiple comparison tests using JMP. Wilcoxon t-tests in JMP compared hawksbill plasma PFAS concentrations between Palmyra and CNMI, except PFUnA required NADA's Wilcoxon *t*-test. Non-parametric ANOVAs in NADA tested differences among sites and FP incidence (grouped as in Keller et al. (2014a)) for green turtle plasma PFOS concentrations, followed by Steel Dwass non-parametric multiple comparison tests using JMP.

Species differences were assessed with CNMI data.

Amount of PFASs offloaded into each nest was estimated by multiplying the total number of eggs laid by the average grams of each pooled egg contents from that nest, and by the measured PFAS concentration (ng/g). The nanograms of PFASs offloaded were then compared across clutches from one nesting season of a mother using linear regressions. PFAS concentrations in the eggs were compared to nest success variables using Kendall's tau correlations in NADA.

To assess toxicological risk, estimated margins of safety (EMOS) were calculated as described in Keller et al. (2012), as the ratio of the average plasma, serum, or egg PFOS concentration in laboratory-exposed animals in the lowest observed adverse effect level (LOAEL) dose group to either the average or maximum sea turtle plasma or egg PFOS concentration. EMOS below 100 were considered of concern (Faustman and Omenn, 1996).

3. Results and discussion

3.1. Plasma PFAS concentrations

PFAS mass fractions (hereafter called concentrations) in ng/g wet mass for each turtle plasma sample are provided (Table S6). Summary statistics for all possible groupings of Pacific turtles are compared to turtles along the Eastern U.S. (Table S7).

3.1.1. Green turtle plasma PFAS concentrations

PFOS comprised >96% of the ∑PFAS concentrations in green turtle plasma regardless of capture location (Table 1; Fig. 2 and S1),

Table 1
Samples sizes and mass fractions (ng/g wet mass) of predominant perfluoroalkyl substances (PFASs) in plasma and eggs of North Pacific green and hawksbill sea turtles.

Species	Tissue	Grouping	Year	SCL range (cm)	n	TOTAL PFASs					PFOS					PFNA				
						Median	Mean	SD	Min-Max	% >RL	Median	Mean	SD	Min-Max	% >RL	Median	Mean	SD	Min-Max	% >RL
Green turtle	Plasma	MHI Live captured, no FP stranded	2011–2012	35.5–83.4	39	0.447	1.14	1.29	<0.063–4.52	92.3	0.447	1.14	1.29	<0.063–4.52	92.3				<0.188 - <1.18	0
Green turtle	Plasma	Kiholo (live captured)	2011–2012	43.7–65.1	13	0.126	0.183	0.121	<0.063–0.560	76.9	0.126	0.183	0.121	<0.063–0.560	76.9				<0.188 - <1.03	0
Green turtle	Plasma	Kapoho (live captured)	2011–2012	39.2–83.4	13	0.407	0.796	0.734	0.196–2.19	100	0.407	0.796	0.734	0.196–2.19	100				<0.239 - <0.893	0
Green turtle	Plasma	Kailua (live captured)	2011–2012	42.9–80.3	13	2.11	2.45	1.32	0.865–4.52	100	2.11	2.45	1.32	0.865–4.52	100				<0.233 - <1.18	0
Green turtle	Plasma	MHI FP stranded	2011–2012	35.5–70.2	14	0.242	0.902	1.04	<0.054–2.77	85.7	0.242	0.816	0.912	<0.054–2.52	85.7				<0.162 - <1.21	0
Green turtle	Plasma	CNMI	2013		12	1.74	0.562	1.12	0.141–4.11	100	0.634	0.524	0.987	0.141–3.65	100				<0.301 - <0.377	0
Green turtle	Plasma	Saipan	2013	46.3–85.6	6	0.295	0.926	1.56	0.175–4.11	100	0.295	0.850	1.37	0.175–3.65	100				<0.326 - <0.377	0
Green turtle	Plasma	Tinian	2013	47.7–63.7	6	0.204	0.199	0.045	0.141–0.270	100	0.204	0.199	0.045	0.141–0.27	100				<0.301 - <0.369	0
Green turtle	Plasma	Palmyra Atoll	2012–2013	44.3–87.6	10	0.155	0.171	0.051	<0.085–0.265	80.0	0.155	0.171	0.051	<0.085–0.265	80.0				<0.273 - <8.70	0
Green turtle	Plasma	Core Sound, NC, USA (Keller et al., 2012)	2006	25–70	10	2.92	3.39	1.74	0.871–6.09	100	1.12	2.41	2.37	0.871–3.87	100				<0.070–0.182	10
Hawksbill turtle	Plasma	CNMI	2013	35.1–48.3	4	1.74	1.99	0.951	1.19–3.29	100	0.634	0.654	0.285	0.334–1.01	100	0.972	1.08	0.516	0.582–1.79	100
Hawksbill turtle	Plasma	Palmyra	2013	54.4–55.5	2		4.18	N/A	3.373–4.978	100		0.300	N/A	0.253–0.348	100		3.40	N/A	2.90–3.90	100
Hawksbill turtle	Plasma	Juno Beach, FL, USA (Keller et al., 2012)	2006	25–70	5	33.5	44.1	23.5	24.8–79.0	100	11.9	11.9	6.27	5.45–21.2	100	17.0	17.3	1.21	3.87–30.8	100
Hawksbill turtle	Egg contents	MHI	2012	unknown	7*	31.0	35.0	34.1	5.46–106	100	2.92	16.3	34.2	0.862–93.8	100	1.28	7.44	8.90	0.756–22.8	100

Abbreviations: Commonwealth of the Northern Marianas Islands (CNMI), Main Hawaiian Islands (MHI), North Carolina (NC), Florida (FL), % >R = percent of samples above the reporting limit, straight carapace length (SCL), standard deviation (SD).
*Eleven nests from seven mothers were analyzed. Multiple nests from the same mom were averaged before the summary statistics shown here were calculated for all seven moms. NC and FL data taken from Keller et al. (2012).

similar to previous reports for green, leatherback, loggerhead, and Kemp's ridley turtles from the Eastern U.S. (O'Connell et al., 2010; Keller et al., 2012). PFOS concentrations in green turtle plasma were significantly different among the Pacific Island regions and increased with human population (Fig. 3a). This trend continues when including previously published PFOS concentrations in green turtles from Core Sound, North Carolina (mean = 2.41 ng/g (Keller et al., 2012); Fig. 3a), a watershed with ≈6,390,000 people (O'Connell et al., 2010). The MHI with 1,400,000 people had the next highest mean PFOS concentration at 1.14 ng/g and significantly greater than CNMI (57,000 people) at 0.524 ng/g and Palmyra (20 people) at 0.155 ng/g. This relationship between PFAS plasma concentration and human population corroborates previous relationships for sea turtle contaminant exposure (O'Connell et al., 2010; Alava et al., 2011), is expected for these man-made chemicals, and suggests that local or regional sources add to the diffuse, globally distributed PFAS levels in nearshore marine organisms.

Green turtles from the MHIs were categorized into four groups based on their capture location and prevalence and severity of FP using the same grouping as Keller et al. (2014a). These were No FP (Kiholo), Low FP (Kailua), High FP (Kapoho), and FP stranded. The FP stranded turtles were alive, severely afflicted with FP tumors, and exceedingly emaciated because of the disease. Significant differences in PFOS plasma concentrations were noted among the groups ($p = 5e\text{-}9$), but not in a dose-dependent manner if PFOS was contributing to FP (Figure S2). The low FP Kailua group (mean = 2.45 ng/g), from the most urbanized area sampled, had significantly higher concentrations than the other three groups and was relatively similar to those in Core Sound, NC (2.41 ng/g) (Table 1, Table S7) (Keller et al., 2012). This is likely due to O'ahu being a heavily populated island (974,563 people), and hosting numerous military bases, airports, and other point-sources (US Census Bureau 2019a). The Kailua sampling site is adjacent to Marine Corps Base Hawai'i, with an active airstrip and firefighting training sites. Additionally, the Kawainui watershed empties into Kailua Bay at this sampling site and contains 19.1% urban development, while Kapoho and Kiholo watersheds had 7.8% and 0% urban development, respectively (Parham et al., 2008a, b, c). Given that the MHIs are one of the most isolated archipelagos in the world, approximately 4000 km away from the nearest continent (National Academy of Sciences 2004), air and ocean circulation transport a baseline level of globally diffuse PFASs to Hawaiian waters. The differences among turtle groups here indicate that local sources compound global sources of PFASs in Hawai'i.

The lack of a dose-dependent relationship between PFASs and FP is similar to results for other POPs that were measured in these same turtles (Keller et al., 2014a) in which Kailua turtles with low FP prevalence had higher concentrations than the other two locations. What differs between the two studies is the relative difference in the FP stranded turtles. For protein-associating PFOS, the FP stranded turtles had levels that were close to average among the MHI turtles (Table 1). For lipophilic POPs, the FP stranded turtles had elevated levels compared to the other MHI turtles (Keller et al., 2014a). This can be explained by the different distribution of these compounds during weight loss and lipid mobilization. The lipophilic POPs flooded into the blood of the emaciated FP stranded group upon weight loss. With no lipid to associate within the thin turtles, those POPs continued to circulate in the blood at high levels. Conversely, PFOS preferentially associates with proteins in the blood and liver. Upon weight loss, protein concentrations in these tissues do not change as much as lipid levels. Thus, PFOS remains associated with proteins, even in emaciated animals, resulting in less mobilization of PFOS into the blood upon weight loss.

Green and hawksbill turtles inhabit the coral reefs of CNMI (Summers et al., 2017), a U.S. Commonwealth composed of 14 islands. They face several threats, including illegal harvest and marine debris entanglement (Summers et al., 2018). Sampling sites were on the archipelago's two most populated islands, Saipan with 48,200 residents and Tinian with 3056 residents (Department of Commerce Central Statistics Division, 2017). Green turtles from Saipan had significantly greater plasma PFOS and ΣPFAS concentrations than from Tinian ($p = 0.02$); (Table 1), corroborating other human population trends.

Palmyra Atoll is extremely remote in the Central Pacific at 5.9° N 162.1° W, with a history of extensive human disturbance during World War II in the 1940s when construction of a U.S. naval base necessitated the remodeling of the small islets (Collen et al., 2009). Construction of >100 buildings and two airstrips occurred between 1940 and 1944. Dredging in the West Lagoon, as well as causeway construction across multiple reef flats, roughly doubled the land area and resulted in major hydrodynamic changes in the lagoon (Collen et al., 2009). By 1945, most military activity had ceased and personnel were evacuated, which prompted many natural changes in the atoll's topography (Collen et al., 2009). Since 2001, the atoll has been protected as a U.S. Fish and Wildlife Refuge.

No uses or disposal of PFASs are known on Palmyra Atoll. AFFFs were invented 15 years after the U.S. Navy abandoned the atoll (Moody and Field 2000). A cursory spatial comparison using green turtle plasma PFAS concentrations showed no evidence of a significant point source on the atoll (see Supplemental Information). The comparison was confounded by life stage with the greatest PFAS burden in a young green turtle that recently recruited from the pelagic carnivorous stage into the nearshore herbivorous stage. New recruits favor the eastern region of Palmyra (Sterling et al., 2013), and may carry a greater PFAS burden to Palmyra, which dilutes with growth thereafter (Keller et al., 2013). The current study suggests that remote Palmyra Atoll is a control reference site with little to no local point sources of PFASs, reflecting baseline globally diffuse concentrations. The first study to measure chemical contaminants in Palmyra sea turtles found elevated aluminum and iron (McFadden et al. (2014).

3.1.2. *Hawksbill plasma PFAS concentrations*

Perfluorononanoic acid (PFNA) predominated in hawksbill plasma from CNMI ($n = 4$) and Palmyra ($n = 2$), with means of 1.08 ng/g and 3.40 ng/g, respectively (Figs. 2 and 3b). The same PFAS profile was seen in hawksbills from Juno Beach, FL (a typo in Keller et al. (2012) should read 17.3 ng/g for mean PFNA). It is interesting that hawksbill turtles from such distant regions have similar PFAS profiles (PFNA predominating), while green turtles from these disparate regions show PFOS dominating. The reasons for the species differences in PFAS profiles are difficult to explain, especially because marine mammals stranded in Hawai'i show still another profile (perfluoroundecanoic acid (PFUnA) dominated (Kurtz et al., 2019)). Differences may be explained by elimination mechanisms, prey selection, or migratory pathways.

CNMI and Palmyra hawksbill plasma had lesser PFAS concentrations than Juno Beach hawksbills, in line with the drastic differences in human population (Fig. 3b). This finding is congruent with the green turtle spatial differences described above and those in loggerhead turtles from the U.S. East coast (O'Connell et al., 2010). Together these findings support the idea that PFAS levels, biomagnifying in marine organisms inhabiting developed nearshore regions, are influenced more from local or regional land-based sources than diffuse sources from global air and ocean currents. Juno Beach, FL, is within the St. Lucie-Loxahatchee Watershed which has 6,400,000 human residents (South Florida Water Management District, 2009; US Census Bureau, 2019b, c). Surprisingly, Palmyra hawksbills (only 20 human residents) had a greater mean concentration of PFNA, PFUnA, and $\sum$PFASs than those in

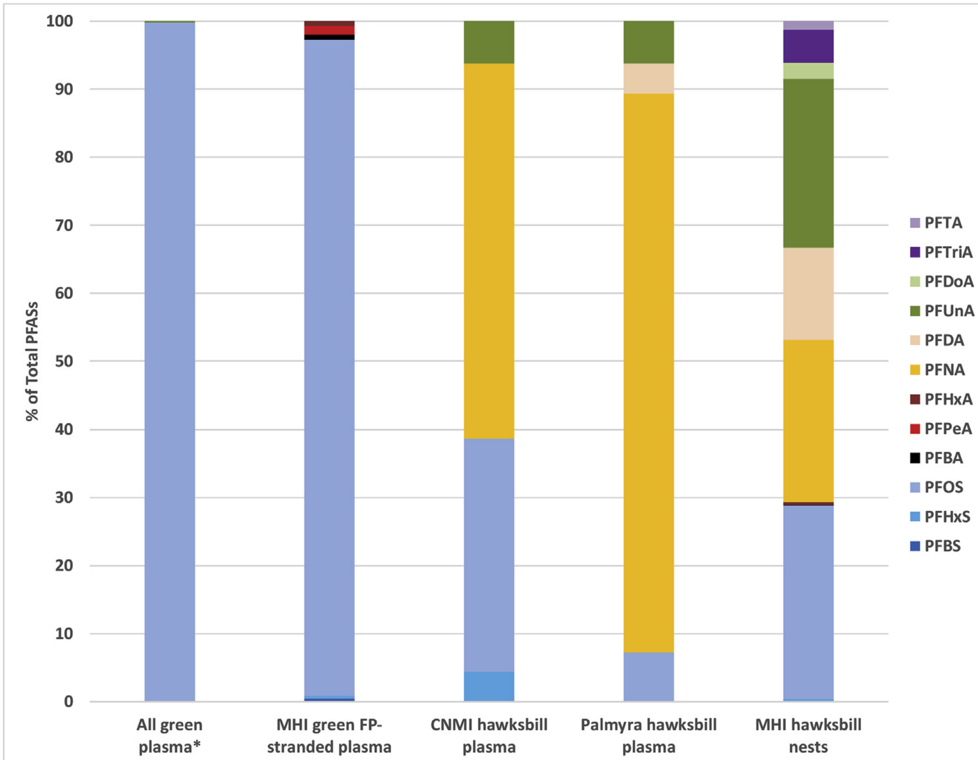

Fig. 2. PFAS profiles detected in Pacific Island sea turtles. MHI = Main Hawaiian Islands, CNMI = Commonwealth of the Northern Marianas Islands. *All green turtles from MHI, CNMI and Palmyra were combined excluding the MHI FP-stranded green turtles. Perfluoroalkyl sulfonates (blue shades) are visually distinguished from perfluoroalkyl carboxylates (other colors). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

CNMI, but low sample size prevented statistical analyses. The reasons for the observed spatial differences could include prey selection, migratory pathways, or sources of contamination.

3.1.3. Species comparisons of plasma PFAS concentrations

The two hawksbill turtles from Palmyra were both captured in the eastern region of the atoll, and had PFAS concentrations one order of magnitude greater than the Palmyra green turtle mean (Tables 1 and S6). In CNMI, ∑PFAS concentrations in hawksbill plasma was significantly greater than green turtles (Figure S3). These results corroborate previous results showing Eastern U.S. hawksbills had greater plasma PFOS levels than three other species, including green turtles (Keller et al., 2012). Similarly, in Australia and Japan, hawksbills had greater concentrations of POPs (PFASs were not tested) than green turtles (Hermanussen et al., 2008; Malarvannan et al., 2011). The trophic position of the two species may explain greater biomagnification of PFASs in hawksbills. Omnivorous hawksbills primarily prey on sponges, but also forage on other invertebrates and marine vegetation, placing them higher on the food web than the herbivorous stage of green turtles studied (Bjorndal et al., 1997).

3.2. Egg PFAS concentrations

Eleven PFAS compounds were detected in at least one hawksbill nest (Tables 1 and S2). Other POPs are known to be deposited into eggs from mother sea turtles during egg production, rather than crossing the eggshell in the nest environment (Keller et al., 2013; Munoz and Vermeiren 2020). Therefore, POP concentrations in eggs represent the mother's exposure from her foraging grounds (Alava et al., 2011). It is expected that PFASs deposit into eggs in a similar fashion as maternal offloading has been documented in other egg-laying species (Wilson et al., 2021). The presence of PFASs in hawksbill eggs reveals, for the first time, offloading from females to their eggs and, to our knowledge, is the first report of PFAS offloading in any reptile species. These data fill an important gap in understanding exposure of adult female hawksbills and their developing embryos to PFASs.

∑PFAS concentrations were greatest in the nest laid in Wailua, Kauai, and lowest in the four nests from three mothers in Pohue, Hawai'i (Figure S4). Nest PFAS concentrations were negatively, significantly ($p = 0.030$) correlated with the distance over water from the nests to the nearest international airport (Figure S4). This preliminary finding may be explained by airports and military bases being some of the largest point sources of PFASs due to firefighting training (Schultz et al., 2004; Houtz et al., 2016). The relationship was driven by the Wailua nest, and a larger sample size would be useful when analyzed with future satellite tracks of females.

PFAS profiles in eggs when averaged across the seven mothers were dominated by PFOS (28.5% of ∑PFASs), PFUnA (24.7%), and PFNA (23.8%) (Fig. 2 and S1). More interestingly, the eggs from different mothers displayed drastic differences in PFAS profiles (Table S2; Figure S5). The nest laid in Wailua, Kauai, had the highest predominance of PFOS (88%) and PFTA (3.5%) and the lowest PFNA contribution (0.9%), a profile that reflects older, phased-out formulations of AFFFs (Place and Field 2012). The four nests laid by three females in Pohue, Hawai'i had an intermediate profile with PFUnA dominating, followed by either PFOS (16%–31%) or PFDA. The five nests laid by one female (ID, 19591–04) on Maui were shifted towards PFNA > PFOS (22.6%) > PFUnA > PFDA. Finally, the two nests from likely different females on Apua and Kamehame beaches on Hawai'i had a profile most different from the Kauai nest, dominated by PFNA > PFUnA > PFDA, with PFOS comprising only

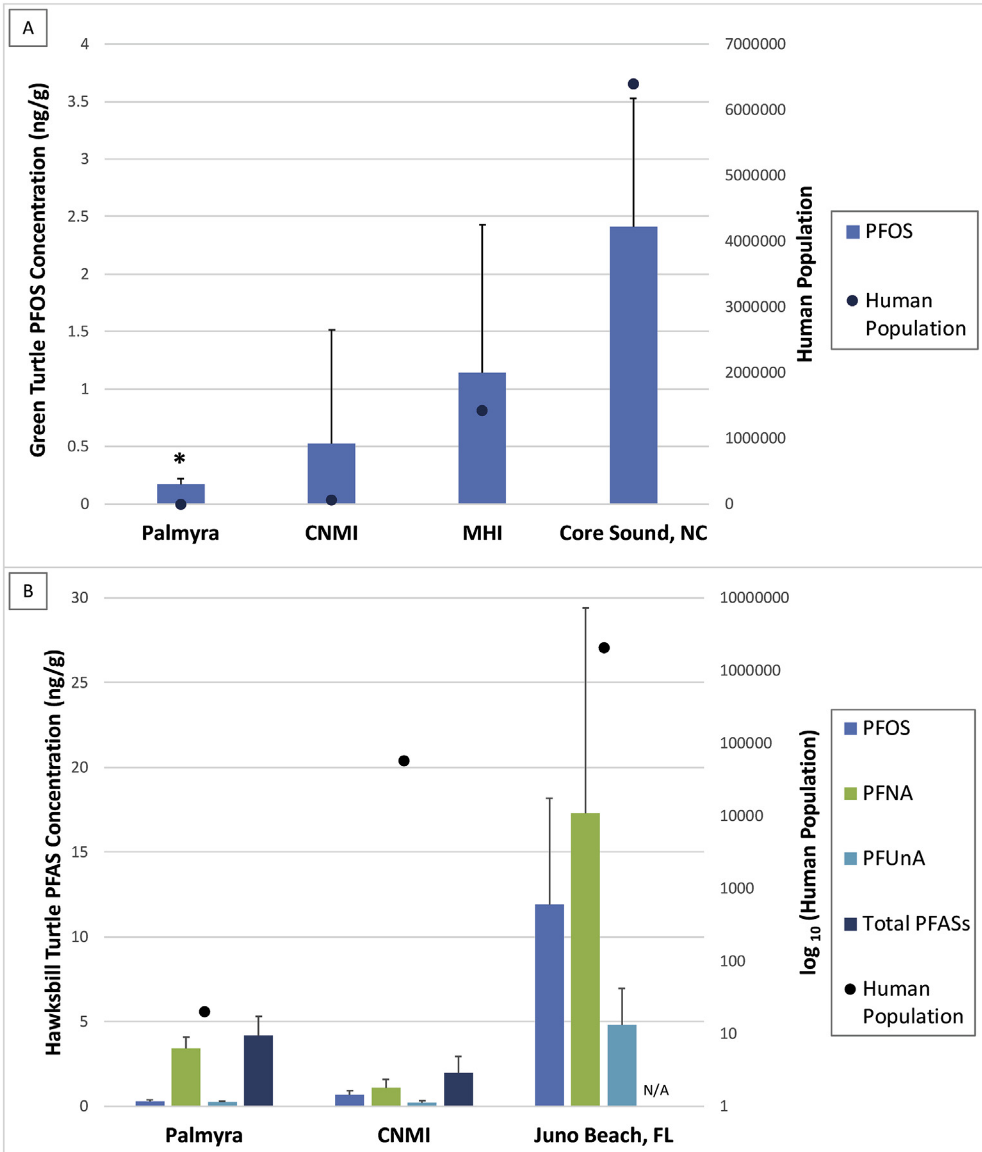

Fig. 3. Mean (one standard deviation) PFAS concentrations (ng/g) in (a) green sea turtle and (b) hawksbill sea turtle plasma (bars) in relation to human population (dots). Commonwealth of the Northern Marianas Islands (CNMI), Main Hawaiian Islands (MHI), North Carolina (NC), Florida (FL). Sample sizes were 10, 12, 39, and 10 green turtles, and 2, 4, and 5 hawksbills, respectively. NC and FL PFOS data are from Keller et al. (2012). Population data are from O'Connell et al. (2010) for NC, and summed for counties in the St. Lucie-Loxahatchee Watershed (US Census Bureau, 2019b, c) for FL. Stranded green turtles with severe fibropapillomatosis were excluded from the MHI data. The asterisk indicates a difference in PFOS concentration from other Pacific sites ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

6%. The extreme spectrum of PFOS contributions, from 88.3% in Kauai to 6% in Hawai'i, suggests that adult female Hawaiian hawksbill turtles forage in distinct areas that have different exposure profiles. This suggestion is partially explained by understanding that POP concentrations in sea turtle eggs originate from the mother's diet from her foraging grounds (Alava et al., 2011; Keller et al., 2013). Therefore, the interpretation of the extreme spectrum of PFOS contribution needs to be placed in context of hawksbill migration and foraging selection, as described next.

Though hawksbill turtles are capable of long-distance migrations (>2000 km) (Vaughan and Spring 1980), both foraging juveniles and nesting adults exhibit natal philopatry with relatively small foraging ranges (Gaos et al., 2017, 2018, 2020b; Wood et al., 2017). Hawaiian hawksbills have recently been recognized as a genetically distinct management unit, with little connectivity to other populations (Gaos et al., 2020a). Satellite tracks of nine Hawaiian hawksbills show that their nesting and foraging grounds are close, on the same or neighboring island (Parker et al., 2009). One Maui nester (ID, 19591–04) migrated to O'ahu to forage in 2004 (Parker et al., 2009). Four years later, she nested again in the same region on Maui and her post-nesting track showed the same path to the same O'ahu foraging site (Figure S6). Four more years later, she laid five nests on Maui that were sampled in the current study. This strong site fidelity to foraging grounds, relatively near the nesting beaches, with potentially different local point sources across the Main Hawaiian Islands may explain the extreme differences in PFAS profiles observed between the nests laid farthest from each other: Kauai vs. Kamehame. Future studies could test this theory by measuring PFAS concentrations and profiles in marine environmental samples, such as sediment or prey in known hawksbill foraging grounds.

Of the 12 nests sampled in 2012, five were laid during the same nesting season (22 days–25 days apart) by Turtle ID 19591–04 in Makena, Maui. Two other nests were laid by another female (ID 112) 43 days apart at Pohue, Hawai'i; it is likely this turtle laid at least one more nest between these dates but it was not sampled. Two additional Hawai'i Island nests came from turtles with unique IDs, and three nests were laid by unknown females (Table S2). The amounts of PFASs in nests of Turtle ID 19591-04 significantly declined with clutch order (Fig. 4), indicating maternally offloaded contaminant concentrations may be a function of time within a nesting season. However, a decline in transferred PFAS amounts was not apparent in the two nests from Turtle 112.

When sea turtles arrive at their nesting site, they are equipped with all lipid-rich follicles ready to become the yolk of all eggs to be laid that nesting season (Miller et al., 1997). The follicles for a single nest transit the oviduct where they are fertilized and surrounded by protein-rich albumen (Miller et al., 1997). After laying this clutch, hawksbills prepare the next clutch which is laid 14–25 days later. Because PFASs associate with serum albumin and fatty acid binding proteins (Ahrens and Bundschuh 2014) rather than lipids, they should deposit more in egg albumen than follicles. Since the albumen is deposited just before each nest is laid, and because in general mother turtles fast during nesting (Miller et al., 1997; Hays et al., 2002; Guirlet et al., 2008, 2010), the mother's body could have less PFASs to transfer through albumen into successive clutches. Theoretically, females would offload a greater portion of her body burden of PFASs into the first clutch. Turtle 19591–04 offloaded a total of 367 μg of PFASs into these five clutches in one year, with approximately 25% of that into the first clutch and 12% in the fifth clutch (Table S8). Previously, a decrease in ΣPCBs, ΣHCHs and ΣDDTs yolk concentrations from successive leatherback clutches suggested that reproductive lipid investment into eggs decreases as the maternal lipid stores decrease (Guirlet et al., 2010). The current findings suggest that PFASs are offloaded through albumen, and that Turtle 19591–04 was fasting during this nesting season while Turtle ID 112 may have been foraging. These interpretations are supported by changes in chicken egg PFAS concentrations during and after exposure of hens to PFASs in drinking water (Wilson et al., 2021).

Nest success variables were examined for relationships with PFAS concentrations (n = 11 nests). Only two significant correlations were observed ($p < 0.05$). Emergence success was negatively correlated with concentrations of two contaminants: PFUnA and perfluorotridecanoic acid (PFTriA) (Fig. 5). Few studies exist on developmental effects of PFASs, and no toxicology studies are available for reptiles. In chickens, hatching success was significantly reduced to 61.4% by a 100 ng/g injection of PFOS into eggs compared to 85.7% in controls (Molina et al., 2006). In tree swallow (*Tachycineta bicolor*) nests, hatching success was significantly, negatively correlated with PFOS concentrations, and complete nest failure was observed in three nests with concentrations at or above 150 ng/g (Custer et al., 2012). While we saw no negative correlation between egg burdens of PFOS and hatching success, PFASs may have more insidious reproductive effects for hawksbill turtles. These novel results indicate a potential consequence of PFAS maternal offloading to embryonic sea turtles, and are supported by our risk assessment. Future studies could address the developmental effects of PFASs in turtles. It is possible that the correlations in this study between emergence success and PFASs are confounded by the decline in PFASs in successive clutches, but to the authors' knowledge, no studies have examined whether emergence success increases in successive clutches of sea turtles.

3.3. Risk assessment

Using surrogate species is the only available option for sea turtle toxicology risk assessments, but because reptiles can be more sensitive than other taxa (Weir et al., 2010) a wider margin of safety (<100) should be used. Keller et al. (2012) estimated margins of safety for toxicological effects of PFOS based on plasma concentrations in five species of sea turtles along the Eastern U.S. All five species had margins of safety <100, which indicate a risk of at least immunosuppressive effects. Using the same method for PFOS

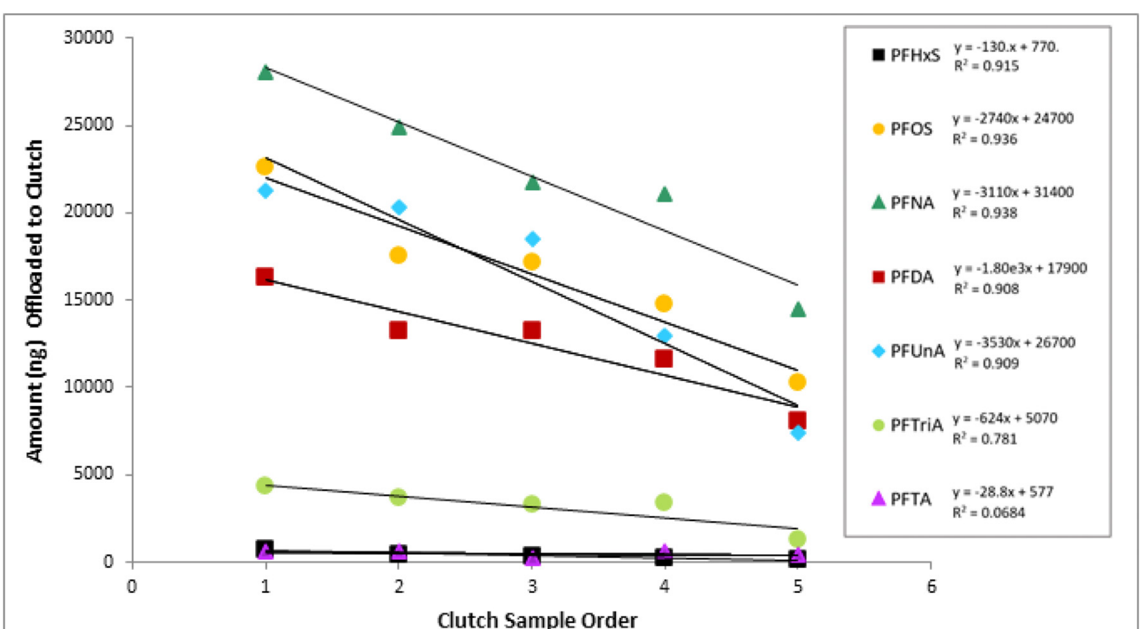

Fig. 4. PFAS quantities (ng) estimated in the entire clutch of eggs from five clutches laid by hawksbill turtle 19591–04 on Maui. Linear regression statistics for ng vs. clutch order are shown for each compound.

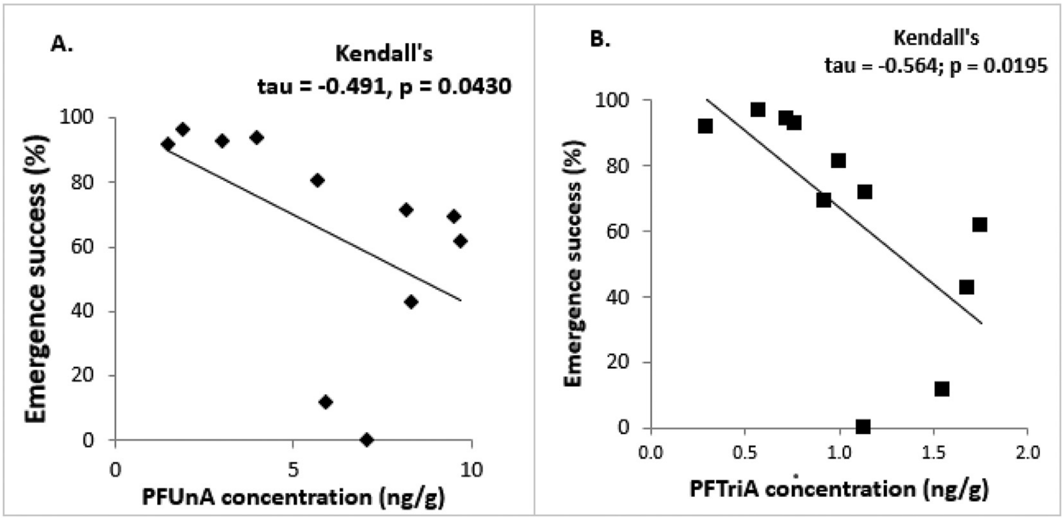

Fig. 5. Significant correlations between perfluoroundecanoate (PFUnA) (A) and perfluorotridecanoate (PFTriA) (B) concentrations (ng/g) in eggs from 11 hawksbill sea turtle nests from the Main Hawaiian Islands and emergence success.

plasma concentrations, average green turtles from Kailua Bay and the maximum green and hawksbill turtles from CNMI were at risk of immunosuppression (Table 2). Likewise for eggs, the average hawksbill nest had a margin of safety of only six, indicating heightened risk of reduced hatching success (Table 2). More concerningly, the maximum nest from Kauai was nearly equal to the PFOS concentrations (no margin of safety) that cause reduced hatching success in chickens and tree swallows (Molina et al., 2006; Custer et al., 2012). This represents the first PFAS risk assessment for embryonic stage sea turtles and suggests that sea turtles inhabiting regions close to military bases and airports, even on remote islands, could be at risk of PFAS toxicity.

4. Conclusion

Reptiles are significantly under-studied in toxicology,

Table 2
Estimated margins of safety (EMOS) for average/maximum sea turtle exposure to perfluorooctane sulfonate based on the lowest adverse effect levels in lab-exposed animals.

		Adverse effect	Neonate mortality, altered liver histology and gene expression	Increased liver weight	Altered thyroid hormones	Altered development of motor neurons	Decreased T-cell dependent IgM antibody response (immunosuppression)	Decreased hatching success
		LOEAL (ng/g or ng/mL)	85000	1500	2290	1000	91.5	100
		LOEAL in	serum	serum	serum	serum	serum	eggs
		LOEAL in	rat/mouse	rat	rat	zebrafish	mouse	chicken
		Reference	Bjork et al. (2008); Lau et al. (2003)	Curran et al. (2008)	Wang et al. (2011)	Zhang et al. (2011)	Peden-Adams et al. (2008)	Molina et al. (2006)
Species	Tissue	Turtle Grouping	Estimated margin of safety for average/maximum turtle					
Green turtle	Plasma	MHI Live captured, no FP stranded	74561/18805	1316/332	2009/507	877/221	80*/20*	
Green turtle	Plasma	Kiholo (live captured)	464481/151786	8197/2679	12514/4089	5464/1786	500/163	
Green turtle	Plasma	Kapoho (live captured)	106784/38813	1884/685	2877/1046	1256/457	115/42*	
Green turtle	Plasma	Kailua (live captured)	34694/18805	612/332	935/507	408/221	37*/20*	
Green turtle	Plasma	MHI FP stranded	104167/33730	1838/595	2806/909	1225/397	112/36*	
Green turtle	Plasma	CNMI	162214/23288	2863/411	4370/627	1908/274	175/25*	
Green turtle	Plasma	Palmyra Atoll	497076/320755	8772/5660	13392/8642	5848/3774	535/345	
Hawksbill turtle	Plasma	CNMI	129969/84158	2294/1485	3502/2267	1529/990	140/91*	
Hawksbill turtle	Plasma	Palmyra	283333/244253	5000/4310	7633/6580	3333/2874	305/263	
Hawksbill turtle	Egg contents	MHI						6**/1**

* concern of risk when EMOS <100, ** heightened concern of risk when EMOS <10.

particularly for PFASs (Reiner and Place, 2015). This is the first report of plasma PFAS concentrations in Pacific sea turtles, plus the first to document maternal offloading of PFASs into eggs of any sea turtle species. The results reveal contamination patterns similar to those documented along the Eastern U.S., with PFOS predominating in green turtles, hawksbills accumulating greater levels than green turtles, and PFAS concentrations being related to human population and specific land uses. The PFAS concentrations in Pacific turtles are generally less than those along the Eastern U.S., which can be attributed to the remoteness and smaller human populations of the islands studied. Across the study sites, islands with greater population densities and closer proximity to military bases and airports rendered greater PFAS concentrations in turtles. Prevalence and severity of FP did not relate to PFAS concentrations, so the search continues for environmental stressors that may contribute to this viral disease. No prior study has analyzed sea turtle tissues concurrently with prey items for PFAS levels; future studies on this would improve our understanding of trophic transfer. The PFAS egg concentrations are novel for reptilian species and show maternal offloading is strongest in the first clutch of a season and egg concentrations were highest in nests laid nearest international airports. Two contaminants (PFUnA and PFTriA) were related to reduced emergence success of hatchlings, which aligns with the risk assessment showing hawksbill egg PFOS concentrations are concerningly near concentrations causing developmental toxicity in birds. Future studies could address the toxicological impacts that PFASs may have on sea turtle development.

Author statement

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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.envpol.2021.116875.

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