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URBAN PROXIMITY WHILE BREEDING IS NOT A PREDICTOR OF PERFLUOROALKYL SUBSTANCE CONTAMINATION IN THE EGGS OF BROWN PELICANS

Bradley P. Wilkinson[1]*, Anna R. Robuck[2], Rainer Lohmann[2], Heidi M. Pickard[3], Patrick G. R. Jodice[4]

[1]Department of Forestry and Environmental Conservation, South Carolina Cooperative Fish and Wildlife Research Unit, Clemson University, Clemson, SC 29634, USA

[2]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA

[3]Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA

[4]U.S. Geological Survey South Carolina Cooperative Fish and Wildlife Research Unit, Department of Forestry and Environmental Conservation, Clemson University, Clemson, SC 29634, USA

*Corresponding author: bpwilki@g.clemson.edu

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ABSTRACT

Identifying sources of exposure to chemical stressors is difficult when both target organisms and stressors are highly mobile. While previous studies have demonstrated that populations of some organisms proximal to urban centers may display increased burdens of human-created chemicals compared to more distal populations, this relationship may not be universal when applied to organisms and stressors capable of transboundary movements. We examined eggs of brown pelicans (*Pelecanus occidentalis*), a nearshore seabird with daily movements ranging from local to 50 km and annual migrations ranging from year-round residency to 1,500 km. Thirty-six eggs from three breeding colonies located at increasing distances to a major urban center (Charleston, South Carolina, USA) were analyzed for concentrations of per- and polyfluoroalkyl substances (PFAS). Areas of high use for each colony during the breeding season were also assessed via the tracking of adult pelicans from each colony using GPS-PTT satellite transmitters and overlapped with measures of relative urbanization via land cover data. We report potentially significant ∑PFAS concentrations in the eggs of pelicans (175.4 ± 120.1 ng/g w wt. SD), driven largely by linear perfluorooctane sulfonate (n-PFOS) (48 – 546 ng/g w wt.). Residues of the precursor compound perfluorooctane sulfonamide (FOSA) were also present in pelican eggs, suggesting continued exposure of local wildlife beyond implemented phaseouts of some PFAS. For most analytes, egg concentrations did not exhibit a significant spatial structure despite some differentiation in high-use areas unlike similar data for another regional apex predator, the bottlenose dolphin (*Tursiops truncatus*). We suggest that the partially migratory nature of brown pelicans during the non-breeding season, combined with daily ranges that may extend to 50 km from local point sources, may have homogenized exposure across individuals. Charleston likely remains a major source for PFAS in the overall region, however, given the high concentrations observed as well as known releases of PFAS in the nearshore environment.

KEYWORDS

Brown pelican; perfluoroalkyl substances; urban; tracking; GPS; seabird

1.1 INTRODUCTION

Ranging behaviors of highly mobile organisms can expose these species to lethal and sublethal stressors not experienced by more sedentary organisms (Jodice & Suryan 2010, Mello et al. 2016, Odsjö 1975). The risks to vagile organisms are amplified when the stressors themselves are also mobile in nature, capable of affecting organisms across relatively broad spatial or temporal scales (Cabrera-Cruz et al. 2018, Henkel et al. 2012). The opportunity for individuals far from local sources of exposure to encounter the stressor should be greater when both organism and stressor are capable of frequently moving among systems, compared to organisms which occupy a distinct spatiotemporal distribution removed from the stressor or for which the stressor is relatively concentrated in a given area. Proximity to sources of environmental stressors may therefore only be a good predictor of exposure for relatively sedentary populations or those with distinct, consistent, or local ranges, and may not be as relevant for highly mobile species interacting with a highly mobile environmental stressor (Adams et al. 2008, Power et al. 2020).

Anthropogenic chemicals, including compounds of emergent interest such as per- and polyfluoroalkyl substances (PFAS), can act as mobile stressors because they are capable of long-range dispersal from point sources (Lohmann et al. 2007). PFAS are widespread chemicals that are persistent in both marine and terrestrial environments worldwide (Houde et al. 2006a). Manufactured for their stability and ability to repel both oily and aqueous substances, PFAS have been used for coating paper and packaging products, non-stick cookware, stain-resistant carpet and clothing, as industrial surfactants, and in fire-fighting foams (Sunderland et al. 2019). In production since the 1940s, PFAS contamination in the environment has occurred globally via both direct release and remote transport

47 (Armitage et al. 2009). Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), two of the
48 most commonly-detected PFAS, have been observed to be pervasive in the blood of both wildlife and
49 human populations, and are associated with harmful and diverse biological effects across taxa (Fenton
50 et al. 2020, Houde et al. 2006a, Houde et al. 2011, Sunderland et al. 2019).
51 Exposure to PFAS can vary by physicochemical properties of the compound, toxicokinetic and
52 ecological qualities of the organism at risk, or characteristics of the ecosystem within which the
53 organism resides. For example, PFAS bioaccumulate and biomagnify in apex predators via direct
54 consumption of contaminated prey, making them particularly harmful to species that occupy upper
55 trophic levels (Houde et al. 2006b). Individual exposure can also be affected by intrinsic properties of the
56 ecosystem in which the species forages as well as the behavior of the organism itself. For example,
57 large-scale boundary habitats (i.e. coastal systems) which integrate pollution inputs from both marine
58 and terrestrial domains may present a higher risk to individuals that forage there as opposed to
59 individuals that forage in systems that tend to function as isolated units or have less input from adjacent
60 systems (i.e. pelagic habitats or upland systems) (Crain et al. 2009). Furthermore, exposure potential
61 may not be spatially predictable within an ecosystem, and different aspects of the abiotic environment
62 may serve to collect or distribute risk. For example, although areas with high levels of urban
63 development can concentrate anthropogenic stressors such as toxic pollutants (Adams et al. 2014,
64 Gewurtz et al. 2016), the transport capabilities of many ecological toxicants can result in high levels of
65 exposure even to organisms relatively far from source inputs (Robuck et al. 2020). The long-range
66 broadcasting of risk may thus create a heterogenous exposure landscape that is not defined simply by
67 the location of the source.
68 Our goal was to assess PFAS concentrations in the eggs of a highly mobile apex predator
69 breeding near an urbanized landscape. Charleston, South Carolina, USA is a rapidly developing city
70 located within a complex coastal morphology of rivers, estuaries, and nearshore marine environments.

Prior research suggests that habitats in the Charleston region have significantly elevated levels of PFAS relative to other regions (Keller et al. 2005, Houde et al. 2006b, Vander Pol et al. 2012, Bangma et al. 2017). For example, White et al. (2015) reported sediment PFAS concentrations from estuarine habitats in and around Charleston Harbor in excess of any other previously examined U.S. city, with approximately half of tested sites within the study area above the global median concentration for PFOS (0.54 ng/g d wt.). Bottlenose dolphins (*Tursiops truncatus*) resident within the harbor possess plasma PFAS levels comparable to occupationally exposed humans and are some of the highest recorded in marine mammals globally (Houde et al. 2005, Houde et al. 2006b, Fair et al. 2013, Fair & Houde 2018). Several fish species frequently consumed by both humans and wildlife in the Charleston area also were commonly above recommended levels for safe consumption by mammals, posing a potentially significant health risk (Fair et al. 2019).

Here we assess concentrations of 24 PFAS in 36 eggs of a locally abundant seabird, the Eastern brown pelican (*Pelecanus occidentalis carolinensis*). Pelicans nest colonially on only 2-3 islands within the vicinity of Charleston in any given year, and these islands and the colonies on them vary in both distance from the urban center (~ 2 – 35 km) as well as in the number of breeding adults (~ 250 – 3000 pairs). We hypothesized there would be an inverse relationship between distance to Charleston Harbor and ΣPFAS, with birds breeding closer to the urban center and therefore also closer to likely point sources acquiring greater toxicity burdens. Therefore, we sought to (i) assess the presence of PFAS in pelican eggs from the Charleston Harbor region relative to published values for other seabird eggs collected from other locales and (ii) investigate the influence of urban habitat use on concentrations of PFAS in pelican eggs using movement data from an additional subset of GPS-tracked adult pelicans from each colony.

METHODS

95 **2.1 Sample collection and processing**

96 Eggs for contaminant analysis were collected from three breeding colonies of Eastern brown pelicans
97 located at progressively greater distances from urban Charleston (Figures 1 & 2). Castle Pinckney (32°
98 46’ 26’’ N, 79° 54’ 40’’ W) is an urban seabird colony centrally located on a small shell island within the
99 harbor and has hosted approximately 250 breeding pairs of brown pelicans near-annually since
100 individuals first started nesting in 1999 (Jodice et al. 2007). Bird Key Stono (32° 38’ 00’’ N, 79° 58’ 04’’ W)
101 is a larger sand island located at the mouth of the Stono River approximately 17 km to the southwest of
102 Charleston Harbor. This island is a regionally important nesting site for brown pelicans, with
103 approximately 3,000 nesting pairs annually since recolonization in 2014 (Jodice et al. 2007, F. Sanders
104 2021). Deveaux Bank (32° 32’ 46’’ N, 80° 11’ 30’’ W) has hosted annual breeding pairs of brown pelicans
105 since 1989, with an average count of 1,300 nests per year (Jodice et al. 2007). Deveaux Bank is located
106 approximately 37 km southwest of Charleston Harbor at the outflow of the North Edisto River.

107 Thirty-six eggs were collected in total, with efforts split evenly among colonies (*n* = 12 per
108 breeding site). All eggs were collected between 10 May 2019 and 15 May 2019, with procedures
109 approximating those of Vander Pol et al. (2012). Briefly, eggs were floated to estimate approximate age,
110 with an effort made to collect eggs in as early a stage of incubation as possible. Brown pelicans typically
111 lay a clutch of three eggs, and we aimed to collect first-laid eggs as these tend to have higher
112 concentrations of maternally transferred chemical compounds than second- and third-laid eggs (Vicente
113 et al. 2015, Parolini et al. 2021). The laying order of eggs was based on visual inspection of shell
114 cleanness. Only eggs which sank in water were collected for analysis, with resting angles ranging from
115 approximately 0°- 60° relative to the bottom of the floating vessel (Rush et al. 2007). Only one egg was
116 collected per nest, and an attempt was made to distribute the collection throughout the spatial
117 footprint of the colony (~ 0.01 km^2).

Eggs were transported from the colony to an off-site refrigerator (4°C) until homogenization. Egg contents were separated from the shell and homogenized using a bag mixer (BagMixer 400 W, Interscience Laboratories, Inc.) in non-filter 400 mL polyolefin blender bags (BagLight PolySilk, Interscience Laboratories, Inc.). Aliquots of homogenized sample (15 mL) were then transferred to polypropylene vials via individual transfer pipettes and stored at -80°C until sample extraction and analysis (March 2020).

2.2 Sample preparation and analysis

Sample preparation and analysis followed a modified protocol based on Chu & Letcher (2008). Sample aliquots were thawed at room temperature, and 0.5 g of homogenate were weighed into polypropylene centrifuge tubes and spiked with 20 µL of isotopically labeled internal standard (0.5 ng/µL). Samples were extracted with 4 mL 10 mM potassium hydroxide (KOH) in methanol (MeOH) and vortexed. Following sonication (20 min) and centrifugation (2 min x 4000 rpm), the resulting supernatant was transferred to 15 mL polypropylene tubes. Remaining pellets received a secondary wash of 4 mL 10 mM KOH in MeOH, sonication, and centrifugation (10 min x 4000 rpm), with supernatant decanted and added to the prior fraction.

Supernatant samples were diluted with 80 mL of Milli-Q (MQ) water prior to solid phase extraction (SPE). Waters Oasis WAX cartridges (Waters Corp.) were preconditioned with 4 mL 0.1% ammonium hydroxide (NH_4OH) in MeOH, 4 mL MeOH, and 4 mL MQ water. Samples were then loaded onto cartridges at an approximate flow rate of 1 drop/sec. Cartridges were then allowed to dry under vacuum for 5 min and eluted with 4 mL MeOH and 4 mL 0.1% NH_4OH in MeOH. Eluent was collected in 15 mL polypropylene tubes containing 200 mg ENVI Carb sorbent. Following vortexing and centrifugation (10 min x 4,000 rpm), the resulting supernatant was transferred to 50 mL polypropylene tubes. The ENVI Carb sorbent was rinsed with MeOH, centrifuged, and the resulting supernatant was decanted and combined with the prior sample fraction. Samples were evaporated to dryness, and

reconstituted using 50:50 water:MeOH with 2 mL ammonium acetate. Solutions were microcentrifuged at 15,000 rpm for 15 min and transferred to autosampler vials for analysis.

Sample extracts were analyzed for 24 PFAS using an Agilent (Santa Clara, CA, U.S.A.) 6460 triple quadrupole liquid chromatograph tandem mass spectrometer (LC-MS/MS) equipped with an Agilent 1290 Infinity Flex Cube online SPE, following previously published methods with slight modifications (Weber et al. 2017). A 100 µL aliquot of each sample extract was injected and loaded onto an Agilent Zorbax SB-Aq (4.6 x 12.5 mm; 5 µm) online SPE cartridge with 0.85 mL of 0.1% formic acid at a flow rate of 1 mL min^{-1}. Following sample loading, analytes were eluted from the SPE cartridge and loaded onto an Agilent Poroshell 120 EC-C18 (3.0 x 50 mm; 2.7 µm) reversed-phase HPLC column using ammonium acetate (2 mM) in MQ water (A) and ammonium acetate (2mM) in MeOH (B) at a flow rate of 0.5 mL min^{-1} and a column temperature of 50°C. Initial gradient conditions were 97% A and 3% B. From 0.85 to 3.5 min the gradient was linearly increased to 54% B and from 3.5 to 15 mins, linearly increased to 85% B, before increasing to 100% B and maintaining at 100% B from 15.5 to 16.5 mins. Sample analytes were introduced to the tandem mass spectrometer after being ionized with an electrospray ionization source operated in negative ion mode at a temperature of 300°C, gas flow rate of 13 L min^{-1}, and nebulizer pressure of 45 psi.

2.3 Quality assurance and quality control

Matrix spikes and procedural blanks were included with the sample set to monitor matrix effects, process recovery, and background contamination. Matrix effects were addressed using a 7-point matrix-matched curve, made up of chicken egg homogenate extracted in an identical fashion to egg samples, and spiked with native and isotope-labelled standards directly prior to analysis. The chicken egg matrix used for the curve contained trace levels of n-PFOS and was corrected for background n-PFOS using the average of triplicate chicken egg samples taken through the extraction. Recoveries for detected compounds ranged from 27 - 150% for FOSA, perfluorotridecanoate (PFTrDA), and

166 perfluorotetradecanoate (PFTeDA) having the lowest recoveries due to predictable loss of these
167 analytes during sample preparation (Taniyasu et al. 2005). Excluding these outliers, average analyte
168 recovery ranged from 63 - 150%, with an average recovery of 78%. Data reported in this study were not
169 blank corrected, due to low levels of process contamination identified in procedural blanks. Method
170 detection limits (MDLs) were defined as procedural blank levels of a given analyte plus 3 times the
171 standard deviation. In the absence of quantifiable blank concentrations, the lowest curve point (0.25
172 ng/mL) was deemed the method detection limit. Values below MDLs were considered zero for
173 summation purposes. Summary statistics and group comparisons were derived using uncensored data
174 analyzed using the *cenfit* function in the R package *NADA* version 1.6 - 1.1 (Lee 2020) to account for
175 artifacts of left-censored data (Helsel 2011). Significant differences in contaminant concentrations
176 among colonies were assessed using both uncensored and censored log-transformed data. The *cendiff*
177 function in the R package *NADA*, which uses Kaplan-Meier (KM) model estimates, was used to evaluate
178 group differences via Peto & Peto modification of the Gehan-Wilcoxon test. Left-censored data was also
179 assessed for significant differences by habitat and compound using Kruskal-Wallis tests followed by
180 post-hoc application of Dunn's test for multiple comparisons.

181 **2.4 GPS Tracking and Spatial Analysis**

182 Movements of representative adult brown pelicans were ascertained via GPS satellite tracking
183 during the nesting period. GPS-equipped pelicans were not the same individuals from which eggs were
184 collected; therefore comparisons between contaminant exposure and movement are population-based
185 (i.e., at the level of the colony) and not individual-based. For the purposes of contaminant exposure, we
186 also assume that habitat use before and after egg laying is approximately equivalent. Adult pelicans
187 typically spend 2-3 weeks at the colony engaged in courtship activities (e.g. nest site selections, mate
188 advertisement, nest construction) prior to egg laying (Schreiber 1977) and during incubation and chick-
189 rearing forage within the vicinity of the colony while mates trade-off incubation, nest attendance, and

provisioning duties. A total of 68 solar-powered GPS-PTT units (GeoTrak Inc., North Carolina, USA) were deployed annually in spring/summer from 2017-2020 on adult pelicans during incubation or early (i.e. 2-4 weeks post-hatch) chick-rearing (Castle Pinckney, *n* = 20; Bird Key Stono, *n* = 25; Deveaux Bank, *n* = 23). Transmitters weighed ~65 g (10 x 3.3 x 3 cm) and were ≤3% body mass of instrumented pelicans (range = 2475 – 4350 g). Adult pelicans were captured at the nest with either a leg or neck lasso and equipped in the field. Transmitters were attached dorsally via a backpack-style harness system as described in Lamb et al. (2017a), and were programmed to record 12 GPS positional fixes per day at 90 min intervals between the hours of 10:00 – 02:30 GMT (fixes limited by power availability). Unit error was assumed to be approximate to that of Lamb et al. (2017b), i.e. 4.03 ± 2.79 m. Equipped pelicans were typically released within 20 mins of capture and 50 m of the nest site.

We used a recursive detection algorithm in the R package *recurse* (Bracis et al. 2018) to identify nest-site attendance of instrumented pelicans for delimiting breeding locations. Exact nest coordinates were extracted from release locations, with a 250 m radius buffer established around each nest. Regular nest attendance was defined as the presence of locational fixes within the 250 m radius buffer separated by ≤168 hrs. This relatively conservative time cutoff was chosen to balance the infrequency of locational fixes compared to the amount of time an adult may spend at the nest, which decreases as chicks age (Sachs & Jodice 2009), with the observation that pelican chicks may be able to survive without provisioning for at least 2 – 3 wks (Shields 2020). All GPS points were then extracted from initial deployment to the last date of nest attendance for each individual. For pelicans that remained near the nest site beyond the breeding season (i.e. non-migratory individuals), a 90-day cutoff was imposed for adults that were initially instrumented with chicks and a 120-day cutoff for adults initially instrumented with eggs, corresponding to the maximum recorded time to successfully raise offspring (Lamb et al. 2017b, Shields 2020). We included telemetry data from both incubation and chick-rearing stages in spatial analyses, as the majority of locations were collected during chick-rearing. It should be noted that

214 home ranges tend to decrease in size as chicks age, so estimates of overlap in high-use areas by colony
215 may be somewhat biased towards increased segregation (Geary et al. 2019). However, home range size
216 reduction is driven by increased foraging site fidelity, so that habitats used during chick-rearing are
217 derived from those used during incubation (Geary et al. 2019).

218 Breeding movements included *n* = 22,274 locational fixes and ranged from 12 May – 21 October
219 within each year (mean duration = 34.4 ± 27.8 days). To identify high-use areas for each colony, we
220 utilized a grid-cell based approach based on the number of GPS fixes per cell. To reduce spatial bias
221 introduced by time spent at the nest, all points within 250 m of the relevant breeding colony were
222 removed. A 2.25 km^2 grid was then imposed over the study area, and the number of locations in each
223 cell was calculated using ArcMap version 10.1 (ESRI, Redlands, California, USA). For each colony, the
224 upper quartile (25%) of grid cells containing the most points was defined as the area of high use and
225 subsequently mapped. The upper quartile was chosen in part because the majority of cells above this
226 threshold contained multiple relocations, indicating high use; additional grid cells beyond this level were
227 populated almost exclusively by single relocations which is likely not reflective of frequent use at the
228 population level.

229 We used the boundaries of 8-digit watersheds along the coastline of South Carolina to describe
230 potential differences in urban habitat use by pelicans from each colony. We chose to use watershed
231 boundaries not only because they are ecologically meaningful for coastal birds, but also because each
232 watershed likely has a varying contaminant profile based on differences in source inputs. Hydrologic unit
233 levels are defined by the U.S. Geological Survey and represent the standard units of measurement for
234 describing watersheds. These definitions correspond to regional, subregional, accounting, and
235 cataloging levels (nested from largest to smallest in size, respectively). 8-digit watersheds correspond to
236 the cataloging level, and are therefore of relatively high resolution. Watershed boundaries were
237 obtained from the S.C. Watershed Atlas (SCDHEC 2020a). Within ArcMap, we calculated the relative

238 percentages of dominant land cover types by watershed following the Anderson Level I Land Use
239 classification system (Anderson 1976) using data from the 2016 USGS National Land Cover Database (Jin
240 et al. 2019). We also calculated the number of facilities with a National Pollutant Discharge Elimination
241 Discharge (NPDES) permit registered in each watershed (SCDHEC 2020b). Finally, the percentage of high-
242 use grid cells for each pelican colony that occurred in each watershed was calculated as a measure of
243 overlap with urbanized habitats, for the purpose of making qualitative comparisons in urban habitat use
244 between colonies. In this way, we expected that eggs from pelican colonies linked to highly urbanized
245 habitat use (i.e., a large percentage of high-use grid cells occurring in watersheds dominated by urban
246 land cover) would contain greater concentrations of PFAS than eggs from pelican colonies linked to
247 lower urban habitat use if urban exposure was indeed a reliable predictor of PFAS contamination (e.g.,
248 Adams et al. 2008) .
249
250 **3.1 RESULTS AND DISCUSSION**
251 Of the 24 PFAS analytes assessed (Table S1), 15 were measured above detection limits in ≥ 50%
252 of pelican eggs sampled across colonies (Table 1). Perfluorohexanesulfonic acid (PFHxS), PFOS, PFOA,
253 perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA),
254 perfluorododecanoic acid (PFDoA), and PFTeDA were found in 100% of tested samples. When averaged
255 by colony location, eggs from Deveaux Bank contained the highest mean ∑PFAS concentration (202 ±
256 148 ng/g w wt, n = 12), followed by Castle Pinckney (192 ± 137 ng/g w wt, n = 12), and Bird Key Stono
257 (132 ± 46 ng/g w wt, n = 12), although these differences were not statistically significant likely due to the
258 high variability among samples within colonies (Figure 3). The most abundant compound across all
259 samples was n-PFOS (mean = 127.5 ± 17.5; range = 48 – 546 ng/g w wt, n = 36). After n-PFOS, the
260 following most abundant compounds included PFDA (12.7 ± 0.8; 3 – 25 ng/g w wt), PFUnDA (7.5 ± 0.5; 2
261 – 14 ng/g w wt), PFTrDA (6.2 ± 0.5; 0 – 15 ng/g w wt), and PFNA (4.1 ± 0.2; 1 – 7 ng/g w wt). Of these,

only PFNA exhibited significant differences in concentrations among colonies, being higher at Deveaux Bank compared to Castle Pinckney (Figure 4). Other analytes found to significantly differ in concentration among colonies were FOSA, perfluoropentanoic acid (PFPeA), and PFOA although the pattern of differences among colonies differed among analytes (Figure 4). Concentrations of all remaining analytes examined did not differ significantly among colonies. Although few statistical differences were found, we should note some caution may be warranted given the relatively small number of sampled eggs and potential limitations of statistical power.

Five watersheds contained at least 10% of high-use grid cells for any of the three pelican colonies, including the Edisto River, St. Helena Island, Cooper River, Bulls Bay, and Stono River watersheds. Of these, the most highly urbanized watershed was the Cooper River (17.3% developed land), which also contained nearly 4 times the number of NPDES-registered facilities (68) as the next nearest watershed (Table 2). All remaining watersheds contained < 10% developed land cover, and < 20 NPDES facilities. Pelicans from Castle Pinckney used the Cooper River watershed the most frequently (58.8% overlap), while use by individuals from Bird Key Stono was infrequent (8.9%) and use by individuals from Deveaux Bank was absent(Table 2). Individuals from Bird Key Stono instead used all five watersheds at relatively similar levels (range = 8.9 – 28.3%), while over half of the high-use grid cells for individuals from Deveaux Bank occurred within the Edisto River watershed.

3.2 Potential Sub-lethal Effects

Brown pelican eggs from the Charleston region displayed relatively elevated levels of ∑PFAS (175.4 ± 120.1 ng/g w wt) compared to published values of ∑PFAS from eggs of other seabirds (Table S2). These high concentrations were driven in large part by PFOS loads in individual eggs. Exposure to PFAS may precipitate reproductive impacts for seabirds, including pelicans. Critically, it remains unclear exactly which PFAS analytes or mixtures of analytes may induce reproductive impairment and at what concentrations these effects begin to manifest (Custer 2021). Research examining reproductive impacts

286 to wild populations in field setting is especially limited (Custer 2021). Tree swallows (*Tachycineta*
287 *bicolor*) at a contaminated location experienced a detectable reduction in hatching success when PFOS
288 levels in eggs were as low as 148 ng/g w wt, and a 50% reduction in hatching success compared to the
289 average rate throughout the USA with PFOS levels of 494 ng/g w wt (Custer et al. 2014). In the current
290 study, 5 of 36 pelican eggs were above the 148 ng/g value and 2 of 36 were above the 494 ng/g value.
291 Tartu et al. (2014) reported a correlation between plasma PFDoA concentrations and reduced hatching
292 success in black-legged kittiwakes (*Rissa tridactyla*) from the Arctic. Additional research on tree
293 swallows as well as great tits (*Parus major*) has suggested a possible association between reduced
294 hatching success and elevated levels of PFDA at concentrations similar to those found in pelican eggs
295 from this study (Groffen et al. 2019, Custer 2021). Taken together, these results suggest that further
296 study of hatchability in relation to concentrations of PFAS may be warranted at pelican colonies in the
297 region.

298 **3.3 FOSA Contamination and Recent Exposure**

299 The concentrations of the semi-volatile precursor compound FOSA measured in brown pelican
300 eggs (mean = 1.0 ± 0.1, range = 0 – 3 ng/g w wt) suggest relatively recent inputs of PFAS into the
301 Charleston system extending beyond the phase-out period for this compound (Robuck et al. 2020). As
302 avian consumers may have the capacity to biotransform FOSA *in vivo* to more stable compounds (e.g.
303 PFOS; Gebbink et al. 2009), significant concentrations of precursor compounds may indicate that the
304 metabolic capacity for transformation has been exceeded as a result of continued, elevated exposure to
305 FOSA or other FOSA-precursors (Gebbink et al. 2016, Robuck et al. 2020). For example, over the period
306 1990-2010, Gebbink et al. (2011) were unable to detect FOSA in herring gull (*Larus argentatus*) eggs
307 from the Great Lakes after 2006 which is consistent with industrial PFAS phase-outs during that same
308 time period. Importantly, FOSA generally declined throughout the two decades of study, with
309 concentrations never exceeding 1.7 ng/g w wt (Gebbink et al. 2011). A follow-up study also was unable

310 to detect FOSA and other precursor compounds from eggs of herring gulls in the same area (Letcher et
311 al. 2015). These patterns suggest that the occurrence of FOSA in our samples may be due to continued
312 exposure and not to historic exposure, particularly given that we found brown pelican eggs with
313 maximum concentrations of FOSA approaching 3 ng/g w wt (Table 1).

314 FOSA was also one of four compounds with significant differences in concentrations among
315 colonies, and was most elevated in eggs from Castle Pinckney. Foraging pelicans from this urban colony
316 consistently showed frequent use of the Cooper and Ashley Rivers during the breeding season compared
317 to pelicans from Bird Key Stono and Deveaux Bank, which both had relatively low overlap of high-use
318 areas with the Cooper River watershed (Table 2). Together with the ability of FOSA to be
319 biotransformed, and therefore the increased likelihood of relatively recent exposure, the spatial
320 segregation of daily breeding-season movements found here suggest that differences in habitat used for
321 foraging during reproduction may at least partially contribute to the loads of this precursor compound.
322 Establishing interannual trends of FOSA concentrations from urban colonies such as Castle Pinckney may
323 therefore assist efforts to determine changes in regional production or use that may drive changes in
324 FOSA or FOSA precursor concentrations in the environment.

325 **3.4 Other Differences in Analytes**

326 While FOSA is likely influenced primarily by recent inputs of FOSA or its precursors into the local
327 environment, observed differences in PFNA, PFPeA, and PFOA concentrations between colonies are
328 likely influenced not only by freshwater industrial sources of these perfluorocarboxylic acids (PFCA).
329 Most likely, the latent transport, oxidation, and accumulation of PFCA precursors will have contributed
330 to the observed PFCA in the marine environments and biota (Ellis et al. 2004, Thackray et al. 2020). For
331 example, Zhang et al. (2019) observed higher than expected bioaccumulation of PFPeA in marine
332 plankton off the northeastern Atlantic coast of the United States, and attributed this to the *in situ*
333 biotransformation of precursors. Several studies have implied that the consumption of marine prey is

causing a PFAS profile enriched in longer-chain PFCAs, including PFNA (Dassuncao et al. 2017, Robuck et al. 2020). Indeed, longer chain PFCAs have been increasing linearly with time in seabird eggs globally (Gebbink et al. 2011, Miller et al. 2015, Pereira et a. 2021), perhaps as a result of an increased bioaccumulation ability of longer-chain compounds or an increase in their anthropogenic use. Pelican eggs from the current study contained high concentrations of several long-chain PFCAs (e.g. PFDA and PFUnDA) compared to shorter-chain analytes, and this may be a result of their highly marine diet.

3.5 Similarities in Contamination Profiles Among Colonies

A thorough assessment of contaminant profiles within an ecosystem is possible only when multiple species and temporal points are considered. For example, Adams et al. (2008) examined PFAS contamination in plasma of bottlenose dolphins from the Charleston region and suggested a positive relationship between contaminant concentrations and urban habitat use immediately following industrial PFAS phaseouts, which was consistent with our initial prediction. While the overall pattern of analyte abundance in the plasma of dolphins was similar to that found in pelican eggs during our study (PFOS > PFDA > PFUnDA > PFNA > PFOA), dolphins residing primarily in or near the harbor exhibited significantly higher concentrations of PFOS, PFDA, and PFUnDA compared to those living in a less urbanized environment (i.e., the Stono River estuary; Adams et al. 2008). No differences were found spatially for PFOA and PFNA (Adams et al. 2008). In contrast, we found no differences in levels of PFOS, PFDA, or PFUnDA among pelican colonies based on the same land cover and watershed classifications, while reporting significant differences for PFOA and PFNA (Fig. 3). Of note is that pelicans from Deveaux Bank, which primarily used the Edisto River watershed, had the highest concentrations of PFOA and PFNA in sampled eggs. Two non-exclusive hypotheses explaining the spatial structuring found in Adams et al. (2008) compared to our results are that (i) the dolphin study reflected the direct release of PFAS from local point sources before industrial phaseouts in comparison to our study that occurred after phaseouts were implemented or that (ii) dolphins in the region may have displayed a higher degree of

358 fidelity to specific locations compared to pelicans, especially across the annual cycle (i.e. a lack of
359 migration in dolphins). The contrast between our results and those of Adams et al. (2008) highlights the
360 need to examine multiple apex predators with different life histories and at different temporal points
361 when investigating contaminant profiles for a given region.
362 Indeed, the relatively broad similarities in concentrations of the majority of PFAS analytes
363 among the three pelican colonies in our study suggest that the frequency of using highly urbanized
364 watersheds by foraging adults cannot reliably predict PFAS concentrations in eggs of brown pelicans.
365 Lamb et al. (2020) made a similar conclusion when assessing concentrations of polycyclic aromatic
366 hydrocarbons (PAHs) in blood samples of adult brown pelicans from the northern Gulf of Mexico. There,
367 it was expected that PAHs would differ among regions of the Gulf based on differing background levels
368 of oil and gas activity but the data did not consistently support that supposition. Lamb et al. (2020)
369 posited that other inputs unrelated to the level of oil and gas activity and extensive ranging patterns in
370 individuals may have contributed to the lack of consistent regional differences. Similarly, Newtoff &
371 Emslie (2017) were unable to find differences in methylmercury concentrations in pelican eggs between
372 two estuarine complexes with differing intensities of anthropogenic influence, contrary to expectations.
373 While some tissues (e.g. blood) reflect relatively local contamination due to their high turnover times,
374 and therefore tend to minimize the influence of migratory and non-breeding areas in determining
375 source locations (Miller et al. 2020 but see Leat et al. 2013), eggs primarily reflect the contamination
376 levels of the nutrient sources that were used to create them (Bond & Diamond 2010). Individuals may
377 mobilize nutrients for egg production from energy reserves acquired while on migratory or non-
378 breeding areas (*capital* strategy) or through the rapid conversion of local resources obtained at the
379 breeding grounds (*income* strategy) (Drent & Daan 1980). Capital and income strategies are best
380 represented, however, not as dichotomous alternatives but as two endpoints on a spectrum containing
381 many intermediates (Meijer & Drent 1999). While the balance of endogenous versus exogenous

382 nutrients involved in egg deposition in brown pelicans remains unclear, it is likely to be a combination of
383 sources rather than one or the other in totality.

384 According to traditional life-history theory, species with large body sizes or those undertaking
385 relatively short migrations are likely to favor a capital breeding strategy (Klaassen et al. 2006). Brown
386 pelicans are one of the largest avian species in North America and exhibit a facultative partial migration
387 that can range from completely sedentary to highly migratory (Lamb et al. 2017b). However, brown
388 pelicans also lay relatively small eggs compared to other seabirds and a full clutch may comprise < 8 %
389 body mass of an average adult (Bartholomew & Goldstein 1984). Pelicans may therefore pay a relatively
390 low energetic cost for producing eggs, suggesting a reduced need to build energetic reserves for this
391 purpose. The local estuarine systems inhabited by pre-breeding pelicans are also likely relatively
392 productive, unlike more temperate or polar systems favored by capital breeders that may not be as
393 predictably productive during pre-breeding for individuals returning from wintering areas (Schelske &
394 Odum 1962, Hahn et al. 2011, Hupp et al. 2018). Results from Geary et al. (2020) indicated that adult
395 pelicans begin the reproductive cycle foraging in suboptimal habitats relative to the surrounding
396 environment, foraging in optimal habitats only as chicks age and energetic costs rise. This suggests that
397 local productivity is not a limiting factor when considering resource acquisition immediately following
398 egg laying, and that pre-breeding conditions are likely capable of providing the energy necessary for egg
399 formation as well.

400 If brown pelicans are therefore capable of using local resources for egg production, their
401 reliance on foraging habitats at the interface of actively dynamic and complex estuarine systems near
402 Charleston may pose a significant risk for PFAS contamination, as the potential for the release,
403 transport, and accumulation of harmful anthropogenic compounds appears high. Prior investigations
404 into both abiotic and biotic PFAS concentrations centered on the estuarine regions of Charleston suggest
405 that the surrounding aquatic environment, particularly the Cooper River watershed, may indeed be

406 more heavily contaminated than other comparable urbanized estuaries (White et al. 2015, Fair & Houde
407 2018, Fair et al. 2019). Identifying specific source inputs of PFAS in the Charleston region, however, is
408 difficult. Candidate sources include PFOS-contaminated groundwater associated with relatively recent
409 releases of aqueous film-forming foams (AFFF) from Joint Charleston Air Force Base near the Ashley
410 River (U.S. Army Corps of Engineers 2018), as well as older AFFF events from the former Charleston Navy
411 Base on the Cooper River (operational from 1901-1996) (White et al. 2015). Wastewater treatment
412 plants (WWTP) discharging effluent into Charleston Harbor have also been identified as potential
413 sources, with tested effluent containing relatively large amounts of both PFOS and PFOA (Houde et al.
414 2006b). Other suggested point sources include commercial container ships entering the Port of
415 Charleston as well as various anthropogenic activities along freshwater inputs, especially the Cooper
416 River, which aggregates discharge from numerous industrial facilities indicated by NPDES permit
417 registries (White et al. 2015, Leads & Weinstein 2019) (Figure 1). Importantly, increasing concentrations
418 from 2004-2012 of some compounds in estuarine sediments from the Charleston area suggest
419 continuing inputs into the system despite widespread production bans in the early 2000s (White et al.
420 2015). Although the Cooper River watershed contained the highest levels of urban development as well
421 as the most NPDES facilities, no watersheds examined were completely free of development or
422 discharge facilities, indicating the widespread potential for PFAS exposure throughout the entirety of the
423 study area.
424 However, if egg production is reliant instead on resources acquired during the non-breeding
425 season or while migrating, local point sources of PFAS in urban Charleston may have a reduced impact
426 on observed egg concentrations. Linking overwintering areas with contaminant exposure in brown
427 pelicans is difficult and compounded by the relatively broad range occupied at the population level,
428 driven by variation in post-breeding movements at the level of the individual (Poli 2015). For example,
429 pelicans from colonies in the northern Gulf of Mexico did not exhibit uniform migratory strategies

among individuals but instead displayed a range of behaviors from complete sedentarism to long-distance migrations (e.g., ~1500 km; Lamb et al. 2017b). Preliminary observations of GPS-tracked pelicans from our study colonies in South Carolina, as well as earlier tracking work by Poli (2015), suggest that high-use areas during the non-breeding season occur in coastal Georgia, Florida Bay, and Cuba, as well as along the central and southern coast of South Carolina (i.e., our study area). Each of the aforementioned regions is likely to have a discrete contaminant profile based on anthropogenic activity, local abiotic factors, and regional transport mechanisms (O'Connell et al. 2010, Robuck et al. 2020). The highly variable nature of pelican migratory destinations, both within and between individuals, may therefore have homogenized contaminant exposure between breeding colonies over relatively long temporal scales. This study highlights the need to resolve the relative importance of endogenous versus exogenous resources in eggs when examining contaminants in avian species for making assessments about where contamination may occur during the annual cycle.

A limitation of the current study was that we were unable to assess local habitat use for the same individual pelicans from which eggs were collected, due to logistical difficulties, instead relying on colony-level assessments of both movement and contaminant levels. The conclusions made are therefore applicable at the level of the colony, and may not reflect how individual-specific habitat use and movement patterns contributes to PFAS levels. Future studies may better resolve potential associations between habitat use and PFAS contamination by tracking and assaying the same individual.

4.1 Conclusion

Our results indicate that potentially impactful ∑PFAS concentrations exist in brown pelican eggs from the Charleston region. Taken together with previous studies as well as known releases of PFAS in the region (i.e. AFFF exposure from military installations), it appears that Charleston may act as a significant source for these contaminants in the nearshore environment. Impacts of this contamination remain unclear but the potential for reproductive or physiological impairment at current exposure levels

appears to be possible based on previous avifaunal studies (Custer 2021). Contrary to expectations, we were unable to find a relationship between PFAS contamination and use of urbanized habitats for the majority of analytes studied. We therefore suggest that proximity to likely point sources for environmental contaminants may not always act as a reliable proxy for exposure when both stressor and organism are capable of transboundary movement, and that individuals even relatively distant from likely sources may still show elevated risk. Given that brown pelicans were previously listed under the Endangered Species Act largely as a result of interactions with anthropogenic contaminants (Wilkinson et al. 1994), continued monitoring of this species for PFAS contamination may be particularly valuable (Vander Pol et al. 2012).

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REFERENCES

Adams, J., Houde, M., Muir, D., Speakman, T., Bossart, G., & Fair, P. (2008). Land use and the spatial distribution of perfluoroalkyl compounds as measured in the plasma of bottlenose dolphins (Tursiops truncatus). *Marine environmental research*, *66*(4), 430-437.

Adams, J., Speakman, T., Zolman, E., Mitchum, G., Wirth, E., Bossart, G. D., & Fair, P. A. (2014). The relationship between land use and emerging and legacy contaminants in an Apex predator, the bottlenose dolphin (*Tursiops truncatus*), from two adjacent estuarine watersheds. *Environmental Research*, *135*, 346-353.

Anderson, J. R. (1976). *A land use and land cover classification system for use with remote sensor data* (Vol. 964). US Government Printing Office.

Ankley, G. T., Cureton, P., Hoke, R. A., Houde, M., Kumar, A., Kurias, J., ... & Sample, B. E. (2020). Assessing the Ecological Risks of Per-and Polyfluoroalkyl Substances: Current State-of-the Science and a Proposed Path Forward. *Environmental Toxicology and Chemistry*.

Armitage, J. M., MacLeod, M., & Cousins, I. T. (2009). Comparative assessment of the global fate and transport pathways of long-chain perfluorocarboxylic acids (PFCAs) and perfluorocarboxylates (PFCs) emitted from direct sources. *Environmental science & technology*, *43*(15), 5830-5836.

Bangma, J. T., Bowden, J. A., Brunell, A. M., Christie, I., Finnell, B., Guillette, M. P., ... & Wilkinson, P. M. (2017). Perfluorinated alkyl acids in plasma of American alligators (*Alligator mississippiensis*) from Florida and South Carolina. *Environmental Toxicology and Chemistry*, *36*(4), 917-925.

Bartholomew, G. A., & Goldstein, D. L. (1984). The energetics of development in a very large altricial bird, the brown pelican. In Respiration and metabolism of embryonic vertebrates (pp. 347-357). Springer, Dordrecht.

Bond, A. L., & Diamond, A. W. (2010). Nutrient allocation for egg production in six Atlantic seabirds. *Canadian Journal of Zoology*, *88*(11), 1095-1102.

Bracis, C., Bildstein, K. L., & Mueller, T. (2018). Revisitation analysis uncovers spatio-temporal patterns in animal movement data. *Ecography, 41*(11), 1801-1811.

Cabrera-Cruz, S. A., Smolinsky, J. A., & Buler, J. J. (2018). Light pollution is greatest within migration passage areas for nocturnally-migrating birds around the world. *Scientific reports*, *8*(1), 3261.

Chu, S., & Letcher, R. J. (2008). Analysis of fluorotelomer alcohols and perfluorinated sulfonamides in biotic samples by liquid chromatography-atmospheric pressure photoionization mass spectrometry. *Journal of Chromatography A*, *1215*(1-2), 92-99.

Crain, C. M., Halpern, B. S., Beck, M. W., & Kappel, C. V. (2009). Understanding and managing human threats to the coastal marine environment. *Annals of the New York Academy of Sciences*, 1162, 39–62.

Custer, C. M., Custer, T. W., Dummer, P. M., Etterson, M. A., Thogmartin, W. E., Wu, Q., ... & McKann, P. C. (2014). Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. *Archives of environmental contamination and toxicology*, *66*(1), 120-138.

Custer, C. M. (2021). Linking field and laboratory studies: reproductive effects of perfluorinated substances on avian populations. *Integrated Environmental Assessment and Management*.

Dassuncao, C., Hu, X. C., Zhang, X., Bossi, R., Dam, M., Mikkelsen, B., & Sunderland, E. M. (2017). Temporal shifts in poly-and perfluoroalkyl substances (PFASs) in North Atlantic pilot whales indicate large contribution of atmospheric precursors. *Environmental science & technology*, *51*(8), 4512-4521.

Drent, R. H., & Daan, S. (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea*, *68*(1-4), 225-252.

Ellis, D. A., Martin, J. W., De Silva, A. O., Mabury, S. A., Hurley, M. D., Sulbaek Andersen, M. P., & Wallington, T. J. (2004). Degradation of fluorotelomer alcohols: a likely atmospheric source of perfluorinated carboxylic acids. *Environmental science & technology*, *38*(12), 3316-3321.

545 Fair, P. A., Romano, T., Schaefer, A. M., Reif, J. S., Bossart, G. D., Houde, M., ... & Peden□Adams, M.
546 (2013). Associations between perfluoroalkyl compounds and immune and clinical chemistry
547 parameters in highly exposed bottlenose dolphins (*Tursiops truncatus*). *Environmental Toxicology*
548 *and Chemistry*, *32*(4), 736-746.
549
550 Fair, P. A., & Houde, M. (2018). Poly-and Perfluoroalkyl Substances in Marine Mammals. In *Marine*
551 *Mammal Ecotoxicology* (pp. 117-145). Academic Press.
552
553 Fair, P. A., Wolf, B., White, N. D., Arnott, S. A., Kannan, K., Karthikraj, R., & Vena, J. E. (2019).
554 Perfluoroalkyl substances (PFASs) in edible fish species from Charleston Harbor and tributaries,
555 South Carolina, United States: Exposure and risk assessment. *Environmental Research*, *171*,
556 266–277.
557
558 Fenton, S. E., Ducatman, A., Boobis, A., DeWitt, J. C., Lau, C., Ng, C., ... & Roberts, S. M. (2020). Per-
559 and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge
560 and Strategies for Informing Future Research. *Environmental Toxicology and Chemistry*.

561 Geary, B., Walter, S. T., Leberg, P. L., & Karubian, J. (2019). Condition-dependent foraging strategies in
562 a coastal seabird: evidence for the rich get richer hypothesis. *Behavioral Ecology*, 1–8.

563 Geary, B., Leberg, P. L., Purcell, K. M., Walter, S. T., & Karubian, J. (2020). Breeding brown pelicans
564 improve foraging performance as energetic needs rise. *Scientific Reports*, *10*(1), 1-9.

565 Gebbink, W. A., Hebert, C. E., & Letcher, R. J. (2009). Perfluorinated carboxylates and sulfonates and
566 precursor compounds in herring gull eggs from colonies spanning the Laurentian Great Lakes of
567 North America. *Environmental Science & Technology*, *43*(19), 7443-7449.

568 Gebbink, W. A., Letcher, R. J., Hebert, C. E., & Weseloh, D. C. (2011). Twenty years of temporal change
569 in perfluoroalkyl sulfonate and carboxylate contaminants in herring gull eggs from the Laurentian
570 Great Lakes. *Journal of Environmental Monitoring*, *13*(12), 3365-3372.

571 Gebbink, W. A., Bignert, A., & Berger, U. (2016). Perfluoroalkyl acids (PFAAs) and selected precursors in
572 the Baltic Sea Environment: Do precursors play a role in food web accumulation of PFAAs?.
573 *Environmental science & technology*, *50*(12), 6354-6362.

574 Gewurtz, S. B., Martin, P. A., Letcher, R. J., Burgess, N. M., Champoux, L., Elliott, J. E., & Weseloh, D. C.
575 (2016). Spatio-temporal trends and monitoring design of perfluoroalkyl acids in the eggs of gull
576 (Larid) species from across Canada and parts of the United States. *Science of The Total*
577 *Environment*, *565*, 440-450.

578 Groffen, T., Lasters, R., Lopez-Antia, A., Prinsen, E., Bervoets, L., & Eens, M. (2019). Limited
579 reproductive impairment in a passerine bird species exposed along a perfluoroalkyl acid (PFAA)
580 pollution gradient. *Science of the Total Environment*, *652*, 718-728.

581 Hahn, S., Loonen, M. J., & Klaassen, M. (2011). The reliance on distant resources for egg formation in
582 high Arctic breeding barnacle geese Branta leucopsis. Journal of Avian Biology, 42(2), 159-168.

583 Helsel, D. R. (2011). *Statistics for censored environmental data using Minitab and R* (Vol. 77). John Wiley
584 & Sons.

585 Henkel, J. R., Sigel, B. J., & Taylor, C. M. (2012). Large-scale impacts of the Deepwater Horizon oil spill:
586 can local disturbance affect distant ecosystems through migratory shorebirds?. BioScience, 62(7),
587 676-685.

588 Houde, M., Wells, R. S., Fair, P. A., Bossart, G. D., Hohn, A. A., Rowles, T. K., ... & Muir, D. C. (2005).
589 Polyfluoroalkyl compounds in free-ranging bottlenose dolphins (*Tursiops truncatus*) from the Gulf of
590 Mexico and the Atlantic Ocean. *Environmental Science & Technology*, *39*(17), 6591-6598.

591 Houde, M., Martin, J. W., Letcher, R. J., Solomon, K. R., & Muir, D. C. G. (2006a). Biological monitoring of
592 polyfluoroalkyl substances: A review. *Environmental Science and Technology*, *40*(11), 3463–
593 3473.
594
595 Houde, M., Bujas, T. A., Small, J., Wells, R. S., Fair, P. A., Bossart, G. D., ... & Muir, D. C. (2006b).
596 Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (*Tursiops truncatus*) food
597 web. *Environmental Science & Technology*, *40*(13), 4138-4144.
598
599 Houde, M., De Silva, A. O., Muir, D. C., & Letcher, R. J. (2011). Monitoring of perfluorinated compounds
600 in aquatic biota: an updated review: PFCs in aquatic biota. *Environmental Science & technology*,
601 *45*(19), 7962-7973.
602
603 Hupp, J. W., Ward, D. H., Soto, D. X., & Hobson, K. A. (2018). Spring temperature, migration chronology,
604 and nutrient allocation to eggs in three species of arctic□nesting geese: Implications for resilience
605 to climate warming. *Global Change Biology*, 24(11), 5056-5071.
606
607 Jin, S., Homer, C., Yang, L., Danielson, P., Dewitz, J., Li, C., ... & Howard, D. (2019). Overall
608 methodology design for the United States national land cover database 2016 products. *Remote*
609 *Sensing*, *11*(24), 2971.
610
611 Jodice, P. G., Murphy, T. M., Sanders, F. J., & Ferguson, L. M. (2007). Longterm trends in nest counts of
612 colonial seabirds in South Carolina, USA. *Waterbirds*, *30*(1), 40-51.
613
614 Jodice, P. G., & Suryan, R. M. (2010). The transboundary nature of seabird ecology. In *Landscape-scale*
615 *conservation planning* (pp. 139-165). Springer, Dordrecht.
616
617 Keller, J. M., Alava, J. J., Aleksa, K., Young, B., & Kucklick, J. R. (2005). Spatial trends of polybrominated
618 diphenyl ethers (PBDEs) in loggerhead sea turtle eggs and plasma. *Organohalogen Compounds*,
619 *67*, 610-611.
620
621 Klaassen, M. R. J., Abraham, K. F., Jefferies, R. L., & Vrtiska, M. (2006). Factors affecting the site of
622 investment, and the reliance on savings for Arctic breeders: the capital-income dichotomy
623 revisited. *Ardea*, *94*(3), 371-384.
624
625 Lamb, J. S., Satgé, Y. G., Fiorello, C. V., & Jodice, P. G. (2017a). Behavioral and reproductive effects of
626 bird-borne data logger attachment on Brown Pelicans (*Pelecanus occidentalis*) on three temporal
627 scales. *Journal of Ornithology*, *158*(2), 617-627.
628
629 Lamb, J. S., Satgé, Y. G., & Jodice, P. G. (2017b). Influence of density-dependent competition on
630 foraging and migratory behavior of a subtropical colonial seabird. Ecology and evolution, 7(16),
631 6469-6481.
632
633 Lamb, J. S., Satgé, Y. G., Streker, R. A., & Jodice, P. G. R. (2020). Ecological drivers of brown pelican
634 movement patterns, health, and reproductive success in the Gulf of Mexico. New Orleans (LA):
635 US Department of the Interior, Bureau of Ocean Energy Management. 234 p. Report No.: BOEM
636 2020-036. Contract No.: M12PG00014.
637
638 Leads, R. R., & Weinstein, J. E. (2019). Occurrence of tire wear particles and other microplastics within
639 the tributaries of the Charleston Harbor Estuary, South Carolina, USA. *Marine Pollution Bulletin*,
640 *145*, 569-582.
641

642 Lee, L. (2020). NADA: Nondetects and Data Analysis for Environmental Data. R package version 1.6-1.1.
643 https://CRAN.R-project.org/package=NADA
644
645 Letcher, R. J., Su, G., Moore, J. N., Williams, L. L., Martin, P. A., de Solla, S. R., & Bowerman, W. W.
646 (2015). Perfluorinated sulfonate and carboxylate compounds and precursors in herring gull eggs
647 from across the Laurentian Great Lakes of North America: temporal and recent spatial
648 comparisons and exposure implications. *Science of the Total Environment*, *538*, 468-477.
649
650 Lohmann, R., Breivik, K., Dachs, J., & Muir, D. (2007). Global fate of POPs: current and future research
651 directions. *Environmental pollution*, *150*(1), 150-165.
652
653 Meijer, T., & Drent, R. (1999). Re□examination of the capital and income dichotomy in breeding birds.
654 *Ibis*, *141*(3), 399-414.
655
656 Mello, F. V., Roscales, J. L., Guida, Y. S., Menezes, J. F., Vicente, A., Costa, E. S., ... & Torres, J. P. M.
657 (2016). Relationship between legacy and emerging organic pollutants in Antarctic seabirds and
658 their foraging ecology as shown by δ13C and δ15N. *Science of the Total Environment*, *573*,
659 1380-1389.
660
661 Miller, A., Elliott, J. E., Elliott, K. H., Lee, S., & Cyr, F. (2015). Temporal trends of perfluoroalkyl
662 substances (PFAS) in eggs of coastal and offshore birds: increasing PFAS levels associated with
663 offshore bird species breeding on the Pacific coast of Canada and wintering near
664 Asia. *Environmental toxicology and chemistry*, *34*(8), 1799-1808.
665
666 Miller, A., Elliott, J. E., Wilson, L. K., Elliott, K. H., Drouillard, K. G., Verreault, J., ... & Idrissi, A. (2020).
667 Influence of overwinter distribution on exposure to persistent organic pollutants (POPs) in
668 seabirds, ancient murrelets (Synthliboramphus antiquus), breeding on the Pacific coast of
669 Canada. *Environmental Pollution*, *259*, 113842.
670
671 Newtoff, K. N., & Emslie, S. D. (2017). Mercury exposure and diet in brown pelicans (*Pelecanus*
672 *occidentalis*) in North Carolina, USA. *Waterbirds*, *40*(1), 50-57.
673
674 O'Connell, S. G., Arendt, M., Segars, A., Kimmel, T., Braun-Mcneill, J., Avens, L., … Keller, J. M. (2010).
675 Temporal and spatial trends of perfluorinated compounds in juvenile loggerhead sea turtles
676 (Caretta caretta) along the east coast of the United States. *Environmental Science and*
677 *Technology*, *44*(13), 5202–5209.
678
679 Odsjö, T. (1975). Toxic chemicals in sedentary and migratory birds in Fennoscandia and the Baltic Area.
680 *Ornis Fennica*, *52*, 74-82.
681
682 Parolini, M., Cappelli, F., De Felice, B., Possenti, C. D., Rubolini, D., Valsecchi, S., & Polesello, S. (2021).
683 Within-and Among-Clutch Variation of Yolk Perfluoroalkyl Acids in a Seabird from the Northern
684 Adriatic Sea. *Environmental Toxicology and Chemistry*, *40*(3), 744-753.
685
686 Pereira, M. G., Lacorte, S., Walker, L. A., & Shore, R. F. (2021). Contrasting long term temporal trends in
687 perfluoroalkyl substances (PFAS) in eggs of the northern gannet (*Morus bassanus*) from two UK
688 colonies. *Science of the Total Environment*, *754*, 141900.
689
690 Poli, C. (2015). Variability in movement patterns and habitat use of two species of Pelecaniformes.
691 Thesis. Clemson, SC: Clemson University.
692
693 Power, A., White, P., McHugh, B., Berrow, S., Schlingermann, M., Tannian, M., ... & O'Hea, L. (2020).
694 Persistent pollutants in Northern Gannet *Morus bassanus* eggs in Ireland: Levels and colony
695 differences. *Environmental Pollution*, *268*, 115723.
696

Robuck, A., Cantwell, M. G., McCord, J. P., Addison, L. M., Pfohl, M., Strynar, M. J., ... & Lohmann, R. (2020). Legacy and Novel Per-and Polyfluoroalkyl Substances (PFAS) in Juvenile Seabirds from the US Atlantic Coast. *Environmental Science & Technology, 54*(20), 12938-12948.

Rush, S. A., Cooper, R. J., & Woodrey, M. S. (2007). A nondestructive method for estimating the age of Clapper Rail eggs. *Journal of Field Ornithology*, *78*(4), 407-410.

Sachs, E. B., & Jodice, P. G. (2009). Behavior of parent and nestling Brown Pelicans during early brood rearing. *Waterbirds*, *32*(2), 276-281.

Schelske, C. L., & Odum, E. P. (1962). Mechanisms maintaining high productivity in Georgia estuaries.

Schreiber, R. W. (1977). Maintenance behavior and communication in the Brown Pelican. *Ornithological Monographs*, (22), iii-78.

Shields, M. (2020). Brown Pelican (*Pelecanus occidentalis*), version 1.0. In Birds of the World (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA.

South Carolina Department of Health and Environmental Control (2020, November 19). *S.C. Watershed Atlas*. https://gis.dhec.sc.gov/watersheds/

South Carolina Department of Health and Environmental Control (2020, November 19). *Interactive Maps and Geospatial Data*. https://sc-department-of-health-and-environmental-control-gis-sc-dhec.hub.arcgis.com/

Sunderland, E. M., Hu, X. C., Dassuncao, C., Tokranov, A. K., Wagner, C. C., & Allen, J. G. (2019). A review of the pathways of human exposure to poly-and perfluoroalkyl substances (PFASs) and present understanding of health effects. *Journal of Exposure Science & Environmental Epidemiology*, *29*(2), 131-147.

Taniyasu, S., Kannan, K., So, M. K., Gulkowska, A., Sinclair, E., Okazawa, T., & Yamashita, N. (2005). Analysis of fluorotelomer alcohols, fluorotelomer acids, and short-and long-chain perfluorinated acids in water and biota. *Journal of Chromatography A*, *1093*(1-2), 89-97.

Thackray, C. P., Selin, N. E., & Young, C. J. (2020). A global atmospheric chemistry model for the fate and transport of PFCAs and their precursors. *Environmental Science: Processes & Impacts*, *22*(2), 285-293.

U.S. Army Corps of Engineers. (2018). *Final Site Inspections Report of Fire Fighting Foam Usage at Joint Base Charleston-Air, Charleston County, and North Auxiliary Airfield, Orangeburg County, South Carolina*. U.S. Army Corps of Engineers, Savannah District, Savannah, Georgia.

Vander Pol, S. S., Anderson, D. W., Jodice, P. G. R., & Stuckey, J. E. (2012). East versus West: Organic contaminant differences in brown pelican (Pelecanus occidentalis) eggs from South Carolina, USA and the Gulf of California, Mexico. *Science of the Total Environment*, *438*, 527–532.

Vicente, J., Sanpera, C., García-Tarrasón, M., Pérez, A., & Lacorte, S. (2015). Perfluoroalkyl and polyfluoroalkyl substances in entire clutches of Audouin's gulls from the Ebro delta. *Chemosphere*, *119*, S62-S68.

Weber, A. K., Barber, L. B., LeBlanc, D. R., Sunderland, E. M., & Vecitis, C. D. (2017). Geochemical and hydrologic factors controlling subsurface transport of poly-and perfluoroalkyl substances, Cape Cod, Massachusetts. *Environmental Science & Technology*, *51*(8), 4269-4279.

White, N. D., Balthis, L., Kannan, K., De Silva, A. O., Wu, Q., French, K. M., ... & Fair, P. A. (2015). Elevated levels of perfluoroalkyl substances in estuarine sediments of Charleston, SC. *Science of the Total Environment*, *521*, 79-89.

Wilkinson, P. M., Nesbitt, S. A., & Parnell, J. F. (1994). Recent history and status of the eastern brown pelican. *Wildlife Society Bulletin*, *22*(3), 420-430.

Zhang, X., Lohmann, R., & Sunderland, E. M. (2019). Poly-and perfluoroalkyl substances in seawater and plankton from the Northwestern Atlantic Margin. *Environmental science & technology*, *53*(21), 12348-12356.

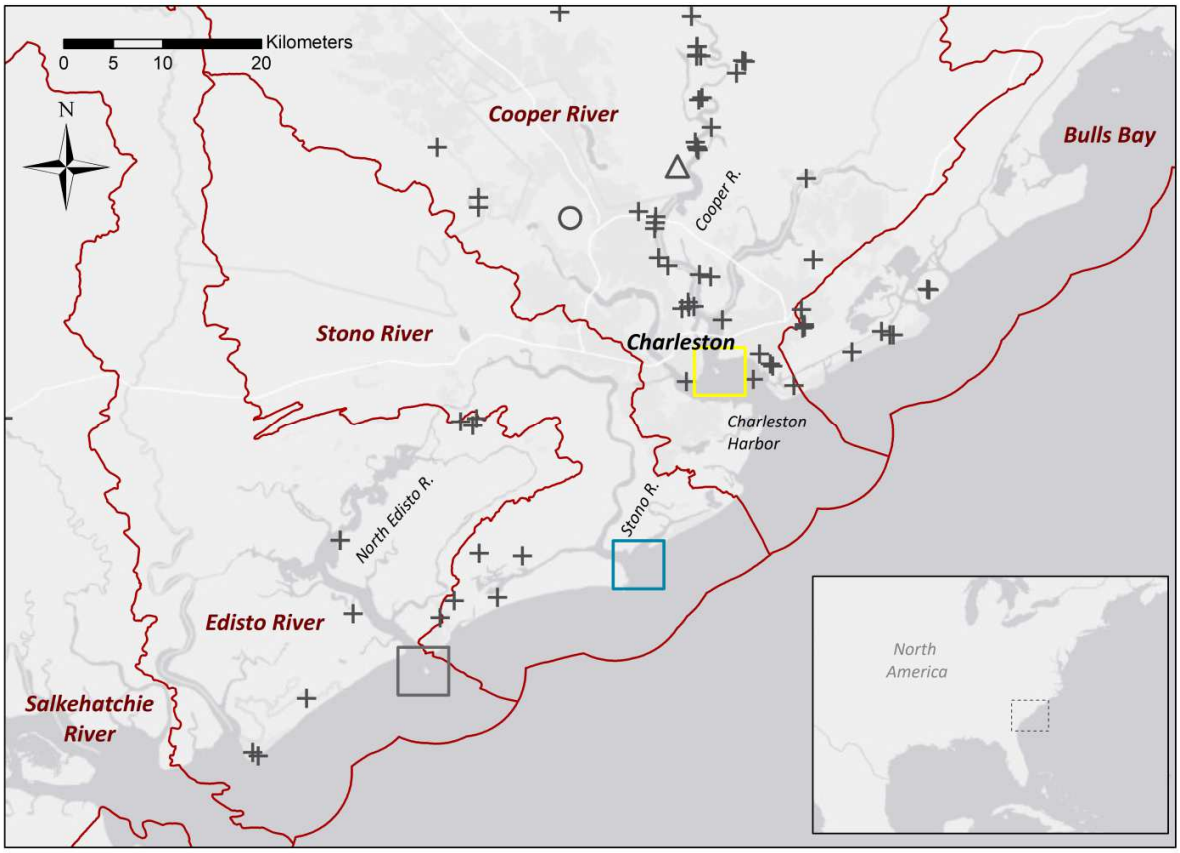


FIGURE 1

Map of the study area and relevant brown pelican colonies in coastal South Carolina, USA. Yellow, blue, and gray boxes indicate the locations of Castle Pinckney, Bird Key Stono, and Deveaux Bank, respectively. Red lines indicate eight-digit watershed boundaries with corresponding labels. Crosses indicate National Pollutant Discharge Elimination System (NPDES)-permitted discharge pipes, with the open circle indicating the location of Joint Base Charleston Air Force Base and the open triangle indicating the location of the former Charleston Navy Base.

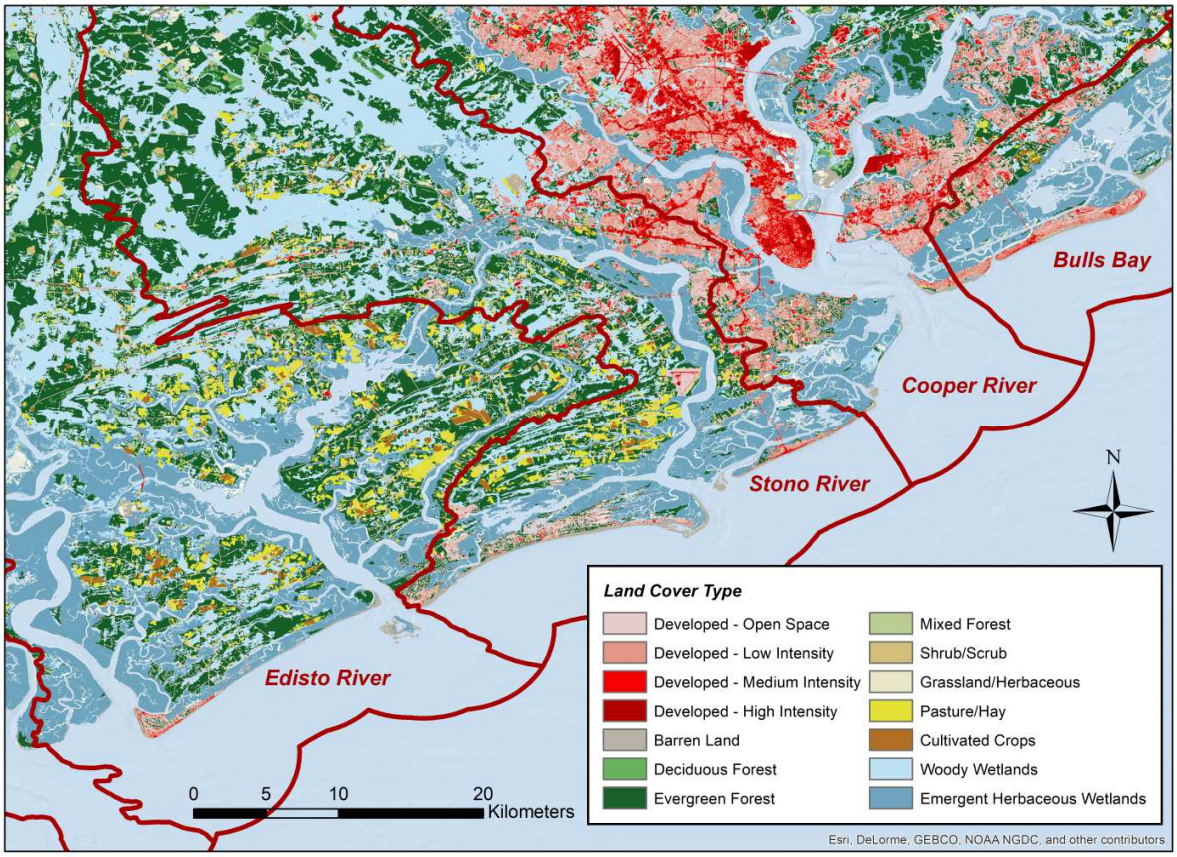


FIGURE 2

Map of the study area in coastal South Carolina, USA, with land cover types. Red lines indicate eight-digit watershed boundaries with corresponding labels. Note that specific land cover types were collated into dominant categories following the Anderson Level I Land Cover classification system for analysis.

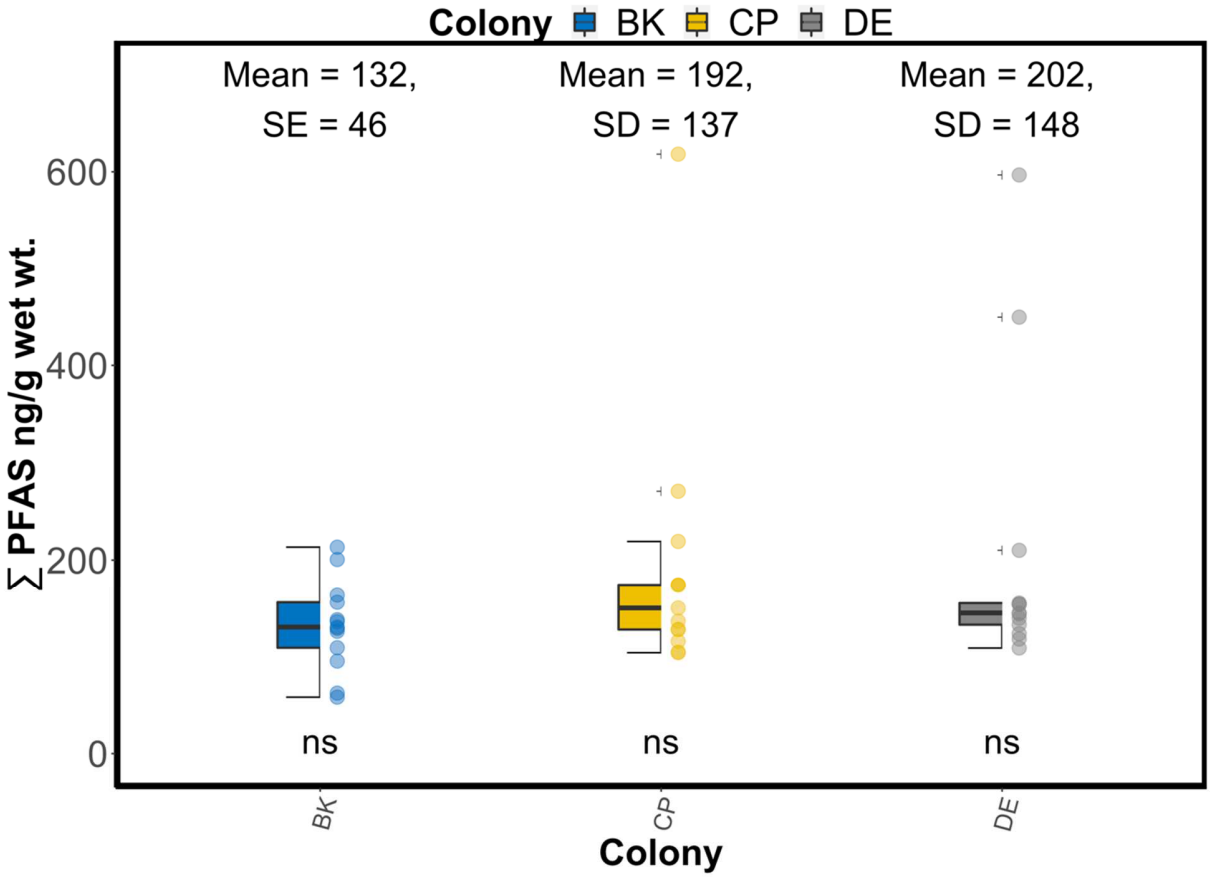


FIGURE 3

Boxplots of ∑PFAS (ng/g w wt.) representing 15 analytes found in sampled eggs from brown pelicans nesting on three colonies near Charleston, South Carolina. BK, CP, and DE signify Bird Key Stono, Castle Pinckney, and Deveaux Bank, respectively. Within the boxplots, dark lines represent the median, box limits denote the first and third quartiles, whiskers denote 1.5 times the interquartile range, and crosses denote outliers. Differences between colonies were not significant (as indicated by 'ns' notations).

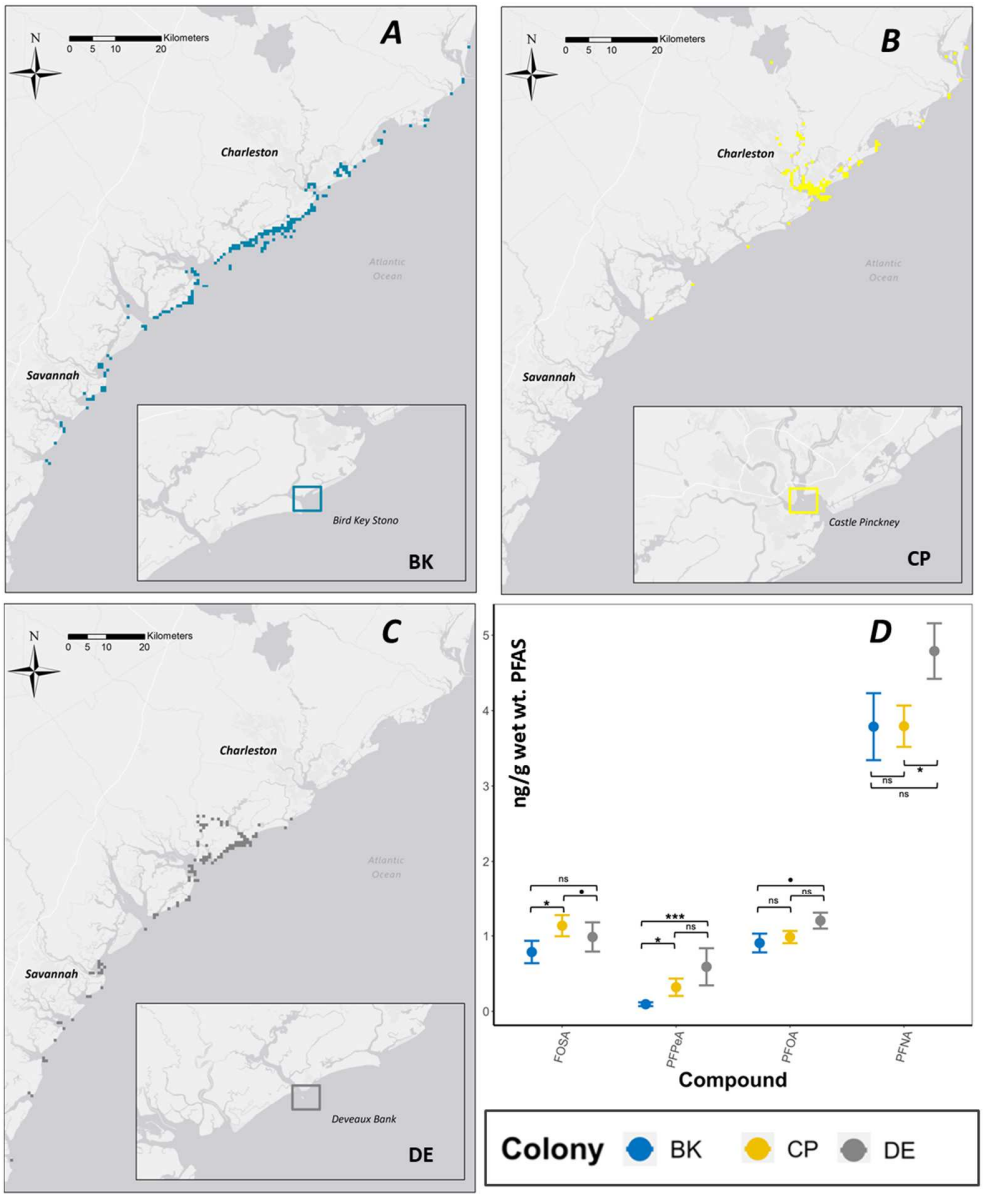


FIGURE 4

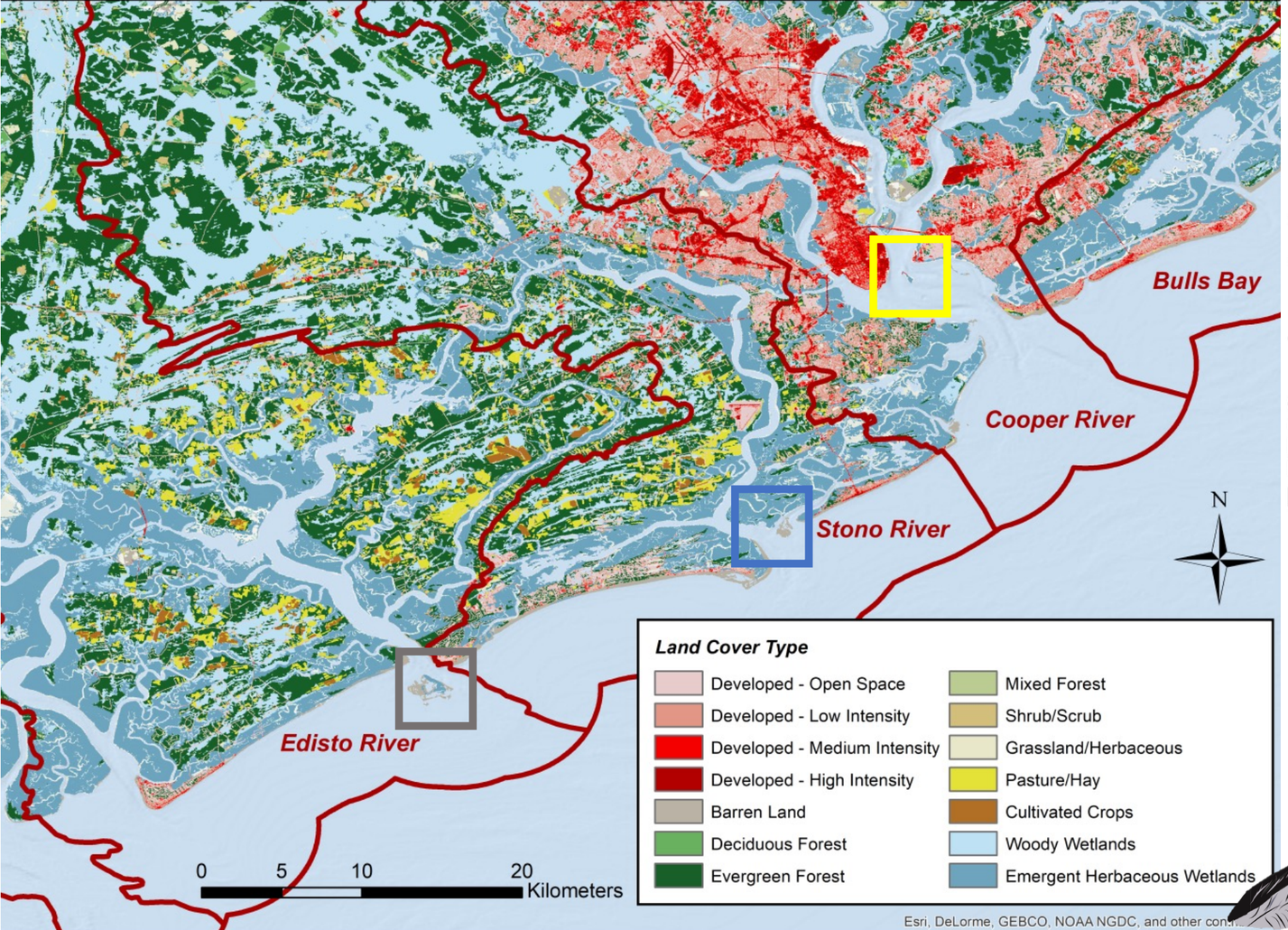
High-use areas of adult brown pelicans actively nesting on three colonies near Charleston, South Carolina, USA determined via GPS tracking. Blue squares represent high-use areas of birds from Bird Key Stono (A), yellow squares represent Castle Pinckney (B), and grey squares represent Deveaux Bank (C). Open boxes indicate colony locations following the same color scheme. Panel (D) shows points representing arithmetic means stratified by habitat, with whiskers denoting standard error. Differences between group means were determined using Dunn's test of multiple comparisons, with "ns" equal to "not significant", while * indicates $p < 0.05$, *** indicates $p < 0.001$, and • representing $p < 0.1$. BK, CP, and DE signify Bird Key Stono, Castle Pinckney, and Deveaux Bank, respectively.

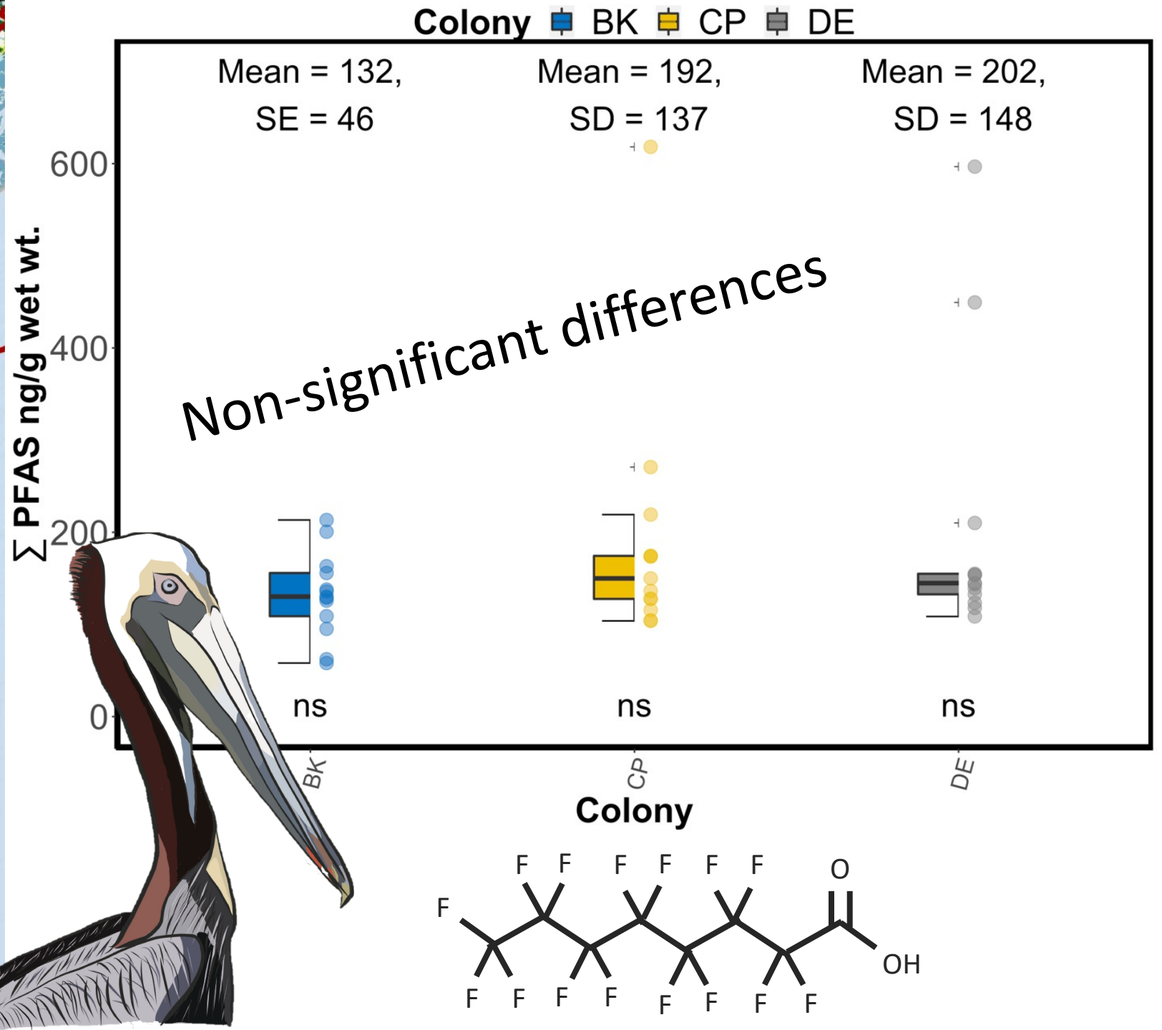
TABLE 1
Table of means (ng/g w wt.), standard errors, ranges, and % detection for compounds found in ≥ 50% of samples. Mean and standard error derived from NADA package to consider data below MDLs in estimation of summary stats. "n-" and "br-" refer to linear and branched analytes, respectively. Each colony has a sample size of (n = 12) eggs.

		Castle Pinckney				Bird Key Stono				Deveaux Bank			
Comp.	MDL	Mean	Std. Err.	Range	% Detect	Mean	Std. Err.	Range	% Detect	Mean	Std. Err.	Range	% Detect
FOSA	0.250	1.135	0.1	0.4 - 3	100.0	0.856	0.1	0 - 2	84.6	0.986	0.2	0 - 3	100.0
br - PFHxS	0.004	0.041	0.002	0 - 0.1	92.3	0.039	0.003	0 - 0.1	84.6	0.041	0.002	0.03 - 0.1	100.0
n - PFHxS	0.034	0.504	0.1	0.2 - 1	100.0	0.443	0.1	0.1 - 1	100.0	0.503	0.1	0.2 - 1	100.0
PFHpS	0.250	1.425	0.4	0 - 6	53.8	1.108	0.3	0 - 4	38.5	1.479	0.4	0 - 5	53.8
br - PFOS	0.024	7.615	1.4	0 - 16	92.3	6.788	1.3	1 - 15	100.0	7.678	1.8	0 - 28	92.3
n - PFOS	0.053	141.17	35.5	74 - 546	100.0	90.195	6.9	48 - 137	100.0	151.22	37.6	80 - 527	100.0
PFDS	0.250	2.703	0.5	1 - 8	100.0	2.391	0.5	0 - 7	84.6	2.322	0.4	1 - 5	100.0
PFPeA	0.047	0.336	0.1	0 - 2	84.6	0.105	0.0	0 - 0.3	92.3	0.590	0.2	0.05 - 3	100.0
PFOA	0.262	0.984	0.1	0.6 - 1	100.0	0.906	0.1	0.3 - 2	100.0	1.202	0.1	0.6 - 2	100.0
PFNA	0.295	3.793	0.3	3 - 6	100.0	3.787	0.4	1 - 6	100.0	4.789	0.4	2 - 7	100.0
PFDA	0.102	12.997	1.3	7 - 25	100.0	10.581	1.2	3 - 18	100.0	14.413	1.4	5 - 24	100.0
PFUnDA	0.163	8.142	0.8	4 - 13	100.0	6.637	0.8	2 - 12	100.0	7.838	0.8	4 - 14	100.0
PFDoA	0.086	2.248	0.2	1 - 4	100.0	1.956	0.3	0.5 - 5	100.0	2.091	0.2	1 - 4	100.0
PFTrDA	0.098	7.449	1.0	4 - 15	100.0	5.589	0.9	1 - 12	100.0	5.557	0.6	0 - 9	92.3
PFTeDA	0.161	0.930	0.1	0.5 - 2	100.0	0.903	0.2	0.2 - 3	100.0	0.907	0.1	0.4 - 2	100.0

Watershed	Edisto River	St. Helena Is.	Cooper River	Bulls Bay	Stono River
% Land Cover Type					
Developed	4.33	1.91	17.32	3.28	7.76
Forested	28.76	4.43	27.47	8.51	27.17
Agriculture	11.43	1.45	3.29	0.51	2.97
Wetland	38.93	22.74	34.40	31.54	36.67
Open Water	12.62	67.60	13.60	53.59	22.16
Barren Land	0.25	0.76	0.37	0.86	0.93
Shrub/Scrub	1.79	0.31	1.39	0.20	1.04
Grassland/Herbaceous	1.66	0.54	1.40	0.17	1.05
# of NPDES Permits					
Registered Facilities	18	5	68	11	4
% High Use Grid Cells					
Castle Pinckney	0.98	1.96	58.82	30.39	0.98
Bird Key Stono	12.78	14.44	8.89	13.89	28.33
Deveaux Bank	50.94	12.26	0.00	0.00	5.66

Table 2. Percent land cover type, number of National Pollutant Discharge Elimination System (NPDES)-registered facilities, and percent high use grid cell occurrence by pelican colony for five watersheds in the Charleston, South Carolina region. Each watershed listed contained at least 10% of high use grid cells for at least one colony. Land cover classification follows the Anderson Level I Land Cover system.


Bulls Bay
Cooper River
Stono River
Edisto River
N
0 5 10 20
Kilometers
Land Cover Type
Developed - Open Space
Developed - Low Intensity
Developed - Medium Intensity
Developed - High Intensity
Barren Land
Deciduous Forest
Evergreen Forest
Mixed Forest
Shrub/Scrub
Grassland/Herbaceous
Pasture/Hay
Cultivated Crops
Woody Wetlands
Emergent Herbaceous Wetlands
Esri, DeLorme, GEBCO, NOAA NGDC, and other con


Colony BK CP DE
Mean = 132,
SE = 46
Mean = 192,
SD = 137
Mean = 202,
SD = 148
Non-significant differences
∑ PFAS ng/g wet wt.
600
400
200
0
ns
ns
ns
BK
CP
DE
Colony
F F F F F F O
F
OH
F F F F F F F F