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Rebecca S. Lazarus
US Geological Survey

Barnett A. Rattner
US Geological Survey, brattner@usgs.gov

Peter C. McGowan
US Fish and Wildlife Service

Robert C. Hale
Virginia Institute of Marine Science

Natalie K. Karouna-Renier
US Geological Survey

See next page for additional authors

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Authors

Rebecca S. Lazarus, Barnett A. Rattner, Peter C. McGowan, Robert C. Hale, Natalie K. Karouna-Renier, Richard A. Erickson, and Mary Ann Ottinger

CHESAPEAKE BAY FISH–OSPREY (*PANDION HALIAETUS*) FOOD CHAIN: EVALUATION OF CONTAMINANT EXPOSURE AND GENETIC DAMAGE

Rebecca S. Lazarus,†‡ Barnett A. Rattner,*† Peter C. McGowan,§ Robert C. Hale,‖
Natalie K. Karouna-Renier,† Richard A. Erickson,# and Mary Ann Ottinger‡††
†Patuxent Wildlife Research Center, US Geological Survey, Beltsville, Maryland, USA
‡Marine-Estuarine Environmental Sciences Program and Department of Animal and Avian Sciences, University of Maryland,
College Park, Maryland, USA
§Chesapeake Bay Field Office, US Fish and Wildlife Service, Annapolis, Maryland, USA
‖Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia, USA
#Upper Midwest Environmental Sciences Center, US Geological Survey, La Crosse, Wisconsin, USA
††Department of Biology and Biochemistry, University of Houston, Houston, Texas, USA

Abstract: From 2011 to 2013, a large-scale ecotoxicological study was conducted in several Chesapeake Bay (USA) tributaries (Susquehanna River and flats, the Back, Baltimore Harbor/Patapsco Rivers, Anacostia/ middle Potomac, Elizabeth and James Rivers) and Poplar Island as a mid-Bay reference site. Osprey (*Pandion haliaetus*) diet and the transfer of contaminants from fish to osprey eggs were evaluated. The most bioaccumulative compounds (biomagnification factor > 5) included p,p'-dichlorodiphenyldichloroethylene (DDE), total polychlorinated biphenyls (PCBs), total polybrominated diphenyl ethers (PBDEs), and bromodiphenyl ether (BDE) congeners 47, 99, 100, and 154. This analysis suggested that alternative brominated flame retardants and other compounds (methoxytriclosan) are not appreciably biomagnifying. A multivariate analysis of similarity indicated that major differences in patterns among study sites were driven by PCB congeners 105, 128, 156, 170/190, and 189, and PBDE congeners 99 and 209. An integrative redundancy analysis showed that osprey eggs from Baltimore Harbor/Patapsco River and the Elizabeth River had high residues of PCBs and p,p'-DDE, with PBDEs making a substantial contribution to overall halogenated contamination on the Susquehanna and Anacostia/ middle Potomac Rivers. The redundancy analysis also suggested a potential relation between PBDE residues in osprey eggs and oxidative DNA damage in nestling blood samples. The results also indicate that there is no longer a discernible relation between halogenated contaminants in osprey eggs and their reproductive success in Chesapeake Bay. Osprey populations are thriving in much of the Chesapeake, with productivity rates exceeding those required to sustain a stable population. *Environ Toxicol Chem* 2016;35:1560–1575. Published 2016 Wiley Periodicals Inc. on behalf of SETAC. This article is a US Government work and, as such, is in the public domain in the United States of America.

Keywords: Biomagnification   Chesapeake Bay   Genotoxicity   Ospreys   Wildlife toxicology

INTRODUCTION

Ospreys (*Pandion haliaetus*) are a well-known ecotoxicological sentinel species. Many studies have utilized this charismatic fish-hawk as a bioindicator to increase our knowledge of spatial and temporal trends in contaminants [1,2]. For example, ospreys feed at a high trophic level and bioaccumulate lipophilic contaminants. Ospreys are also strictly piscivorous, which makes their diet easy to characterize.

The decline and recovery of the osprey population in the Chesapeake Bay (mid-Atlantic coast of the USA) was intertwined around the use of the pesticide dichlorodiphenyltrichloroethane (DDT) and the metabolite dichlorodiphenyldichloroethylene (p,p'-DDE) [3–7]. A thriving osprey population was described through the 1930s, and ospreys were a common nesting species in many upper reaches of the western shore [7]. The population began to decline during the 1960s because of p,p'-DDE–induced eggshell thinning. Birds could only be found in the main stem of the Chesapeake Bay. After the ban on DDT in 1972, and also with the construction of osprey platforms, the osprey population began to rebound.

Birds could be found nesting further up Bay tributaries and in more urbanized and industrialized areas. Now, the Chesapeake Bay provides a breeding habitat to the largest osprey population in the world, because of its shallow waters and high productivity [3].

Several studies have characterized osprey diet and foraging ecology in the Chesapeake Bay [8–10]. These investigators documented that energy-rich menhaden (*Brevoortia tyrannus*) are a dominant osprey prey item in polyhaline sites such as the lower James River. However, ospreys nesting in oligohaline areas on the upper James and York Rivers currently consume catfish (Ictaluridae) species and gizzard shad (*Dorosoma cepedianum*) [9]. Studies investigating the biomagnification of contaminants from fish tissue to osprey eggs are scarce. A few studies have addressed this issue by examining components of the fish osprey food chain to estimate biomagnification factors [11–14].

The last large-scale ecotoxicological study of ospreys in the Chesapeake Bay was conducted in 2000 to 2001 [15]. That study focused on the US Environmental Protection Agency (USEPA) locations that have been designated as Regions of Concern (Anacostia/middle Potomac, Baltimore Harbor/ Patapsco, and Elizabeth Rivers) as a result of poor water quality and environmental contamination. In 2011 to 2012, we re-evaluated contaminants in ospreys nesting in Chesapeake Bay Regions of Concern and found that concentrations of

halogenated pollutants in eggs had declined [16]. Nonetheless, there was evidence of increased concentrations of 8′-hydroxy-deoxyguanosine (8-OH-dG), a biomarker of genetic damage, in osprey nestlings found in the most industrialized regions [16]. In addition to the Regions of Concern, many other locations in the Bay warrant further study. An examination of the Contaminant Exposure and Effects–Terrestrial Vertebrates Database indicated that there are limited ecotoxicological data for wildlife in the northern regions of the Bay [17]. This includes the Susquehanna River, the largest freshwater inflow to the Chesapeake Bay.

Between 2011 and 2013, we studied dietary exposure to persistent bioaccumulative toxicants, food chain transfer, and potential effects on ospreys nesting in the Susquehanna, James, and Potomac Rivers. In addition, pollutant exposure was also monitored in Back River (MD, USA; northeast of Baltimore Harbor and the Patapsco River). Relative to other sites in the Chesapeake, sediment from Back River contains large quantities of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) [18], and preliminary observations indicate poor osprey productivity in the vicinity of the Back River wastewater treatment plant (WWTP; R.S. Lazarus, unpublished data).

In the present study, we describe the findings of an examination of contaminant concentrations and their transfer (biomagnification) between whole fish and osprey eggs. Reproductive success, eggshell thickness, and oxidative genetic damage in nestlings were used to investigate potential effects at various biological levels (population, individual, and molecular) among several Chesapeake Bay tributaries.

MATERIALS AND METHODS

Study sites

From 2011 to 2013, 3 major Chesapeake Bay tributaries (lower Susquehanna River and flats, middle Potomac River, and James River), the Back River, and Poplar Island as a mid-Bay reference site were studied (Figure 1). In 2011, whole fish, osprey eggs, and nestling blood samples were collected along a 45-km stretch of the Anacostia/middle Potomac River (Frederick Douglass Bridge, Washington, DC to Mattawoman Creek, MD, USA). In 2012, similar sampling was conducted along a 60-km segment of the James River (Richmond to Milton, VA, USA). Finally, in 2013, sampling was undertaken along a 20-km stretch of the Susquehanna River and flats (Aberdeen, MD to the I-95 Millard E. Tydings Memorial Bridge, USA). In 2013, we also studied an 11-km section of the Back River (Back River WWTP to Hart Miller Island). Based on results from previous studies indicating low levels of organic contaminants in common tern (*Sterna hirundo*) eggs, the Paul S. Sarbanes Ecosystem Restoration Project at Poplar Island (MD, USA) was used as a reference site for all 3 yr of the study [10,16,19].

Osprey reproduction and foraging activity

All procedures involving fish and ospreys were conducted under approval of the Institutional Animal Care and Use Committees of the US Geological Survey (USGS) Patuxent Wildlife Research Center and the University of Maryland, and with appropriate Federal and state scientific collection permits. Starting in late March, osprey nests were visited every 7 d to 10 d to determine the number of eggs laid and hatched and young present at ≥ 40 d. These data were used to calculate productivity [20–22]. Additional nests were monitored at each

site as a potential source of blood samples in the event that the selected study nest failed.

During the osprey reproductive period, dietary preferences of adults were monitored using a variety of techniques [9,23]. Game camera (Bushnell 8MP Trophy Cam) images of prey items captured, direct identification of fish scraps found in nests, and photographic observations of prey deliveries (Nikon D3100 DSLR camera, AF VR-Nikkor 80-400 mm lens; Nikon) were used to characterize osprey diet. Using these observations ($n = 1662$), we determined the 2 to 3 dominant prey items (based on the highest percentage of catch) to sample for the food chain component of the present study.

Osprey egg sample collection

Using the sample egg collection technique [24], after completion of a clutch (3 or more eggs), 1 fresh egg was randomly sampled from each study nest (Susquehanna River and flats, $n = 10$; Anacostia/middle Potomac, $n = 13$; James River, $n = 12$; Back River, $n = 5$; Poplar Island, $n = 12$). Eggs were transported to the USGS Patuxent Wildlife Research Center, cleaned, and weighed, and the length and width were measured to the nearest 0.01 mm [16]. A 2.4-mm hole was drilled into the blunt end of the egg (MultiPro® 7.2V, model 770; Dremel®), and distilled water was injected into the air cell to return contaminant concentrations to that of a freshly laid egg [25]. Each egg was opened, and the contents (excluding shell membrane) were transferred to a chemically clean jar (I-CHEM; VWR Scientific), examined, weighed, and then stored at –80 °C. Shells were dried for 3 mo to 4 mo at room temperature, and thickness measurements were taken at 3 points along the equator using a micrometer (model 1010M; L.S. Starrett).

Nestling blood samples and morphological endpoints

Blood samples were collected from osprey nestlings (40–45 d old) at each study nest or a nearby nest in the event of reproductive failure ($n = 46$ accessible nests from the Susquehanna, Potomac, and James Rivers and Poplar Island; $n = 3$ nestlings for Back River because there were no nearby replacement nests). Briefly, 1 nestling/nest was removed for approximately 10 min. After physical examination, body weight, crop contents (size of food contents present in crop determined via palpation), and culmen length were measured. A 5-mL to 7-mL brachial blood sample was collected using a 23-gauge, 25.4-mm needle in a heparinized syringe (Sarstedt International). Approximately 100 μL of blood were immediately transferred to a microcentrifuge tube, frozen on dry ice, and stored at –80 °C for subsequent DNA damage assays. The remainder of the blood sample was centrifuged, and plasma was harvested for pharmaceutical [10] and stable isotope analyses.

Fish sampling

In each year of the study, fish sample collection was undertaken in early July, with target fish ranging from 25 cm to 35 cm in length (preferential prey size for ospreys [3]). On the Anacostia/middle Potomac and the James Rivers, gizzard shad and catfish were caught by electroshocking. On the Susquehanna River, a combination of electroshocking and hook and line were used to capture gizzard shad and catfish. At Poplar Island, menhaden and striped bass were caught using a midwater trawl and a commercial pound net. Because of the limited number of osprey nests on the Back River, only a small sampling of menhaden, gizzard shad, and white perch (*Morone americana*) were collected via electroshocking. In total, 201 fish

Susquehanna River and Flats

Back River

Poplar Island

Chesapeake Bay

Anacostia/Potomac

James River
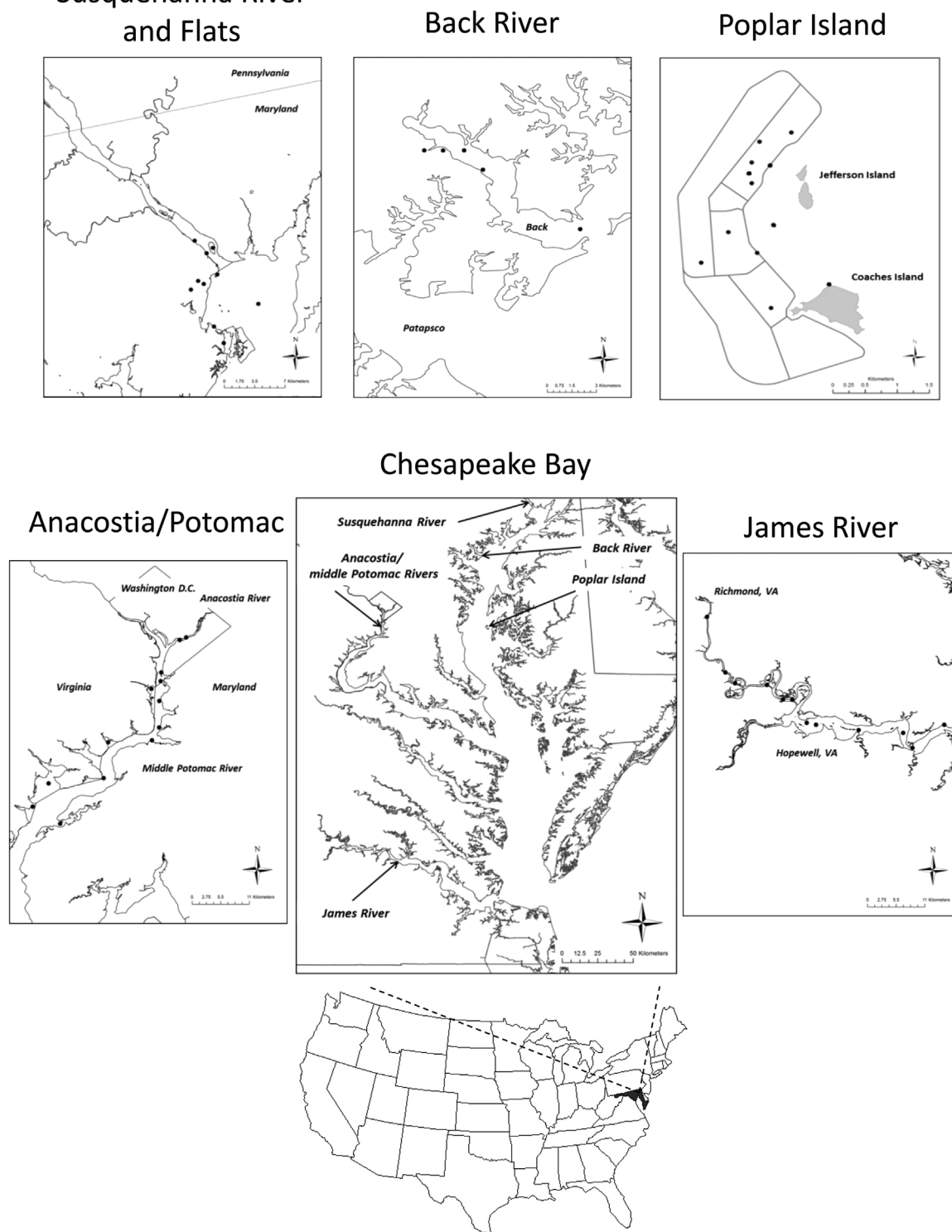
Figure 1. Locations of osprey nests sampled from Chesapeake Bay and Poplar Island reference site (USA); a solid dot indicates a sampled nest.

were collected, stored at –20 °C, and then composited ($n = 3$ fish/composite) by location, species, and size.

Chemical analysis of osprey eggs and whole fish, and quality assurance

Whole fish and osprey egg contents were analyzed for 129 PCB congeners, 44 organochlorine pesticides and metabolites, methoxytriclosan, 11 polybrominated diphenyl ethers (PBDE) congeners, and 5 alternative brominated flame retardants (alt-BFRs: α, β, γ hexabromocyclododecane [HBCD], 1,2-bis (2,4,6-tribromophenoxy) ethane [BTBPE], di(2-ethylhexyl)-2,3,4,5-tetrabromophthalate [TBPH], 2-ethyl-hexl 2,3,4,5-tetrabromobenzoate [TBB], and decabromodi-phenyl ether [DBDPE]). In the analysis for PBDEs, 1 osprey egg sample from the Back River was lost during sample processing.

Chemical analyses were conducted at the Virginia Institute of Marine Science based on previously described approaches [16,26–29]. Briefly, egg contents were homogenized, lyophilized, and spiked with surrogate PCB standards

(congeners 30, 65, and 204; Ultra Scientific,[13]C-PCB-126) and 2,3,4,4′,5,6-hexabromodiphenyl ether (BDE-166; Cambridge Isotope Laboratories). Sodium sulfate blanks were analyzed coincidentally. Dried samples underwent enhanced solvent extraction (ASE 200; Thermo Fisher Scientific) using methylene chloride (DCM) at $100\,^{\circ}C$ and 68 atm. Extracts were purified by size exclusion chromatography (Envirosep-ABC, 350-mm × 21.1-mm column; Phenomenex). Each post–size exclusion chromatography extract was reduced in volume, added to the top of a silica gel glass solid phase extraction column (Isolute; International Sorbent Technology), and eluted with 3.5 mL hexane (to waste), followed by 6.5 mL of 60/40 hexane/DCM and 8 mL DCM. The latter 2 fractions were combined and then divided, with one-half going for coplanar PCB analysis. Coplanar PCBs were separated from nonplanar PCBs by elution through a Supleclean ENVI-Carb solid phase extraction column (Sigma-Aldrich). The column was first eluted with 15 mL hexane (to waste). The coplanars were obtained by elution with 20 mL hexane/toluene (99/1) and 20 mL toluene. The pooled eluent was reduced in volume, spiked with p-terphenyl (Ultra Scientific) as an internal standard, and analyzed by gas chromatography–mass spectrometry (GC–MS) on an Agilent 5975C, in electron impact mode and selected ion monitoring. A 60-m DB-5 GC–MS column (Agilent, 0.32-mm inner diameter × 0.1-μm thickness) was used.

The second half of the silica solid phase extraction fraction retained was spiked with decachlorodiphenyl ether (Ultra Scientific) as the internal quantitation standard. Identification and quantitation of noncoplanar PCBs was conducted by GC–MS in the electron ionization mode on a Varian 2200 GC–MS (Varian now owned by Agilent Technologies). The organochlorine pesticides and methoxytriclosan were analyzed similarly by GC–MS on a Varian 4D MS. Both analyses used 60-m DB-5 columns (0.32-mm inner diameter × 0.25-μm thickness). Data for noncoplanar PCBs and organochlorine pesticides were corrected based on recoveries of the PCB 204 surrogate. The PBDEs and alt-BFRs were separated by ultra-performance liquid chromatography (UPLC; Waters) and analyzed by atmospheric pressure photoionization tandem mass spectrometry (APPI–MS/MS; Q-Trap3200 MS, AB Sciex [29]).

Method detection limits (MDLs) on a wet weight basis were converted to a lipid weight basis using the following formulas: MDL dry = MDL wet/(1-fraction of sample that is water) and MDL lipid = (MDL dry/% lipid). The percentages of lipids and moisture used in this calculation were from samples exhibiting the lowest lipid and water values to allow for the most conservative estimates. The MDLs for organochlorine pesticides and noncoplanar PCB congeners for osprey eggs were 0.4 μg/kg wet weight and 11.9 μg/kg on a lipid weight basis, and for fish they were 0.2 μg/kg wet weight and 7.2 μg/kg lipid weight. The MDLs for coplanar PCB congeners were 0.04 μg/kg wet weight and 1.19 μg/kg lipid weight in osprey eggs, and 0.02 μg/kg wet weight and 0.72 μg/kg lipid weight in fish. The MDLs for PBDEs and alt-BFRs were 0.4 μg/kg wet weight and 11.9 μg/kg lipid weight in eggs, and 0.2 μg/kg wet weight and 7.2 μg/kg lipid weight in fish.

All data were corrected based on the recovery of surrogate standards in each fractionated extract and for moisture loss back to a fresh weight basis. The average recoveries of the surrogate standard PCB 204 from the organochlorine pesticide analyses were (mean ± standard deviation [SD]) 81.8 ± 14.0% from eggs and 84.7 ± 10.7% in fish. Mean recoveries of surrogate PCB 204 from the noncoplanar PCB analyses were comparable: 87.9 ± 18.0% in eggs and 84.0 ± 21.2% in fish. For coplanar PCBs, the surrogate

standard PCB 126 average recoveries were 87.8 ± 20.7% in eggs and 96.1 ± 24.9% in fish. Recoveries of surrogate standard BDE 166 for the PBDE analyses averaged 101.1 ± 25.0% in eggs and 104.4 ± 30.7% in fish. Overall, mean moisture content in eggs was 83.9 ± 0.5%, and it was 74.7 ± 3.5% in fish.

Biomagnification factors

Biomagnification factors (BMFs) were calculated by relating the concentrations of detected chemicals in prey (whole fish) to those in the predator (osprey). Beyer and Biziuk [30] present a simple formula for calculation of BMFs as the concentration of the chemical in the organism (CB) to the concentration of the chemical in its prey (CA): BMF= CB/CA. To calculate BMFs for the present study, we applied the model presented by Elliott et al. [31]. This equation adjusts residues based on diet composition:

$$Y = \mathrm{BMF}\,[F1(X1) + F2(X2) + \ldots + Fn(Xn)]$$

where Y represents the geometric mean contaminant concentration in the predator, Fn represents the percentage of each prey species in the diet, and Xn reflects the geometric mean contaminant residue per species consumed. Biomagnification factors were calculated on both a wet weight and a lipid weight basis for the major groups of contaminants analyzed. The lipid weight of each sample was calculated by dividing the dry weight recovery corrected values by the percent lipid. For those contaminant residues presented as a Kaplan–Meier range of means, the minimum and maximum BMFs were calculated.

Stable isotopes

Stable isotope analyses were performed at the Colorado Plateau Stable Isotopes Laboratory at Northern Arizona University (Flagstaff, AZ, USA) to determine ^{13}C and ^{15}N content. Approximately 1 mL of osprey nestling plasma was freeze-dried and analyzed using a Thermo-Electron Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific). The isotope ratio mass spectrometer was configured through the Finnigan CONFLO III (Thermo Finnigan) using a Carlo Erba NC2100 elemental analyzer (CE Elantech). Carbon and nitrogen stable isotope compositions were obtained in a single run. The Colorado Plateau Stable Isotopes Laboratory used biological standards for calibration and raw data normalization from the National Institute of Standards and Technology and the International Atomic Energy Agency [32]. Stable isotope values for $\delta^{13}C$ were reported per mille (parts per thousand; ‰) according to the Vienna Pee Dee belemnite standard, and $\delta^{15}N$ values were reported relative to atmospheric air. Uncertainty factors were ≤0.10 ‰ for $\delta^{13}C$ and ≤0.20 ‰ for $\delta^{15}N$.

DNA damage assays

Whole blood samples ($n = 49$; 1 excluded from the James River because of consistently large % coefficient of variation [CV] after multiple reruns) were analyzed for 8-OH-dG as an indicator of DNA damage (assay methods and validation also described in Lazarus et al. [16] and Rattner et al. [19]). Briefly, samples were analyzed using the DNA/RNA oxidative damage enzyme immunoassay kit (Cayman Chemical). Plates included blanks, and all samples were analyzed in duplicate. Standard curves were fit using a 4-parameter model ($R^2 > 0.998$; MARS Data Analysis Software 2.10; BMG Labtech). Intra-assay variation (precision of duplicate determinations; $CV \pm$ SD) was 3.5 ± 3.0% in 2011 and 7.4 ± 4.2%

for samples collected in 2012 and 2013. Any samples with a $CV > 20\%$ were reanalyzed. Inter-assay variation among plates for reference samples was $4.1 \pm 11.5\%$ in 2011 and $9.93 \pm 11.3\%$ for samples collected in 2011 and 2013. Those samples collected in 2011 on Poplar Island and the Anacostia/middle Potomac Rivers and samples from the Susquehanna and James Rivers were analyzed at different times. Because of variations in performance between manufacturing lots, however, we were unable to quantitatively compare data from these 2 assays. The limit of detection for this assay was 1.03 pg/µg DNA determined by evaluating the mean minus 3 SDs from the standard curve [19].

Statistical analyses

Descriptive statistics were generated for continuously distributed variables (eggshell thickness, morphological endpoints, DNA damage, and contaminant residues). Variables were tested for normality and homogeneity of variance, and were log-transformed as necessary [33]. Analysis of variance was used to detect overall differences among sites, and specific comparisons were conducted using Tukey's honest significant difference test ($\alpha = 0.05$). If the assumptions for an analysis of variance (ANOVA) were not met, Wilcoxon nonparametric statistics were used followed by a Bonferroni correction to adjust for multiple comparisons. For contaminants with residues < MDL in <50% of samples, the Kaplan–Meier method was used to estimate the extremes of the mean [34]. Fisher's exact test was used to compare site-specific differences in productivity endpoints. In osprey eggs, concentrations of aryl hydrocarbon (Ah) receptor active PCB congeners were multiplied by toxic equivalency factors (TEFs) to estimate toxic equivalence (TEQs) [35]. A correlation analysis was conducted to examine relationships among all variables. A logistic analysis of covariance was first used to examine site-specific relationships between egg residues (p,p'-DDE, PCBs, and PBDEs) and osprey nest success. If there were no site-specific differences, data were combined to evaluate overall differences in productivity and contaminant residues.

Comparisons among patterns of PBDE and PCB congeners were made among sites using analysis of similarity (ANOSIM), a multivariate analysis of variance to test patterns among groups [36]. One-half the MDL was used for the nondetects for the purpose of this analysis. Data were then standardized and log-transformed for composition analysis. As described by Custer et al. [37], distributions of patterns among sites were based on R test statistics and a p value < 0.05. Differences in patterns are evident when $R > 0.4$, and there is some support for pattern differences when R is ≥ 0.3 and < 0.4. When pattern differences were identified, the similarity percentage subroutine was used to identify which congeners contributed to the observed differences.

Redundancy analysis was conducted using data from Lazarus et al. [16] and from the present study to increase statistical power. This included data from 13 additional nests from Baltimore Harbor/Patapsco River and the Elizabeth River. All predictor variables (egg contaminant concentrations, DNA damage, and stable isotope data) were log-transformed to obtain normality and homogeneity of variance [33]. The δ^{13}C isotope data were $-\log(x)$ transformed because values were negative. Redundancy analysis [38] was used to assess whether concentrations of PCB, PBDE, and p,p'-DDE; DNA damage; and carbon and nitrogen stable isotope measurements differed by study site:

$$PCB + PBDE + DDE + C + N + DNA \sim Site$$

The TEQs for PCBs were considered for inclusion in the model; however, they were highly correlated with total PCBs and DDE. We used redundancy analysis rather than a more generalized distance-based redundancy analysis because Euclidian distance was appropriate for our data. We specifically examined whether axes explained more variability than would be expected by chance alone using a permutation-based ANOVA. Year was used as a blocking factor, because DNA damage assays from 2011 versus 2012 and 2013 were not comparable [16]. This analysis was conducted using the redundancy analysis function from the Vegan package in R [39,40]. Interaction terms between contaminant concentrations were also evaluated, and then a mixed effects linear regression was used to examine whether any pollutant data affected DNA damage (sample site random effect).

RESULTS

Productivity

For the 5 study sites, productivity ranged from 1.17 fledglings/active nest to 1.80 fledglings/active nest (Table 1). There were no site-related differences in productivity (eggs laid, eggs lost, hatching, fledging and nest success; Fisher's exact test, $p > 0.60$; Table 1) among the Susquehanna, Potomac, and James Rivers and Poplar Island. On average, of the 47 nests sampled, 3.10 eggs were laid per nest, 68.5% hatched, 96.9% of the nestlings that hatched fledged, and 77.7% of the active pairs fledged young.

On the Back River, there were only 5 active nests. Hatchability was adequate (83.3%) and fell within range of the other study sites (78.9–90.0%). However, of these intensively studied nests, only 1 fledgling was produced per active nest, and 60.0% of the successful pairs fledged young. These reproductive parameters for the Back River are seemingly lower compared with other study sites.

Eggshell thickness

On the Anacostia/middle Potomac Rivers, eggshells were thinner ($p = 0.003$) compared with the reference site (Anacostia/middle Potomac Rivers mean $\pm$ SD, 0.49 ± 0.05 mm; Poplar Island reference site, 0.55 ± 0.05 mm). Eggshell thickness on the Back River was significantly greater than in eggs from the Potomac (0.55 ± 0.03 mm; $p = 0.048$), but was similar to the Poplar Island reference site. In addition, there were no differences in eggshell thickness among the other sites (Susquehanna: 0.52 ± 0.03 mm; James: 0.52 ± 0.04 mm).

Nestling body weight and culmen length

On Poplar Island (reference site), there were no significant differences in body weight and culmen length of 40- to 45-d-old nestlings among sampling years (2011, 2012, and 2013; $p > 0.49$), and thus measurements were combined. Overall, there were no differences in body weight across all study sites ($p = 0.23$; mean weight 1590.0 ± 157.2 g). Culmen length did not vary among sites and averaged 30.1 ± 1.3 mm. Overall, nestlings appeared to be in good condition.

DNA damage

Assays of DNA damage for samples collected in 2011 were conducted separately from those collected in 2012 and 2013. Because of variation in assay performance among test kit lots, the results could not be quantitatively compared among years. Results were generated for 48 nestling blood samples ($CV < 20\%$), with 1 sample from the James River excluded

Table 1. Reproductive success of ospreys nesting in Chesapeake Bay (USA) regional waterways and Poplar Island reference site[a]

Site	Poplar Island[a] 2011–2013 No.	%	Susquehanna River and Flats 2013 No.	%	Anacostia/middle Potomac River[a] 2011 No.	%	James River 2012 No.	%	Back River 2013 No.	%
Active nests sampled	12		10		13		12		5	
Eggs laid	36		31		41		38		18	
Sample eggs collected	12		10		13		12		5	
Eggs relaid because of predation	0		0		3		0		0	
Eggs naturally incubated	24		21		31		26		13	
Fate of eggs										
Unknown or predation[b]	5		1		9		6		7	
Crushed	0		0		1		1		0	
Failed to hatch[c]	3		2		4		4		1	
Hatched	(16/24)	66.7	(18/21)	85.7	(17/31)	54.8	(15/26)	57.7	(5/13)	38.5
Hatchability[d]	(16/19)	84.2	(18/20)	90.0	(17/21)	80.9	(15/19)	78.9	(5/6)	83.3
Fate of nestlings										
Disappeared	0		0		1		1		2	
Found dead	0		0		0		0		0	
Fledged	(16/16)	100	(18/18)	100	(16/17)	94.1	(14/15)	93.3	(13/15)	100
Successful pairs (fledged young)	(9/12)	75.0	(10/10)	100	(9/13)	69.2	(8/12)	66.7	(3/5)	60.0
Fledglings/active nest	(16/12) 1.33		(18/10) 1.80		(16/13) 1.23		(14/12) 1.17		(13/12) 1.08	
Fledglings/successful nest	(16/9) 1.77		(18/10) 1.80		(16/9) 1.77		(14/8) 1.75		(5/3) 1.66	
Mayfield method estimates										
Egg laying and incubation period	n = 12		n = 10		n = 13		n = 12		n = 5	
Daily survival rate ± standard error	0.991 ± 0.005		1.000		0.989 ± 0.005		0.991 ± 0.005		0.960 ± 0.027	
Survival rate to hatching (A)	0.711		1.000		0.650		0.711		0.204	
Nestling period	n = 8		n = 10		n = 9		n = 8		n = 3	
Daily survival rate ± standard error	1.000		1.000		1.000		1.000		1.000	
Survival rate to fledging (B)	1.000		1.000		1.000		1.000		1.000	
Nest success (A × B)	0.711		1.000		0.650		0.711		0.204	
Probability of an egg hatching given that the nest is successful (C)	0.889		0.857		0.895		0.833		0.556	
Probability of young living to 53 d given that the nest is successful (D)	1.000		1.000		0.941		0.933		1.000	
Mean clutch size (E)	3.00		3.10		3.15		3.17		3.60	
Mean number of young surviving to 53 d (A × B × C × D × E)	1.90		2.66		1.72		1.75		0.410	
Mean number of young surviving to 53 d less sample egg (A × B × C × D × [E − 1])	1.26		1.80		1.18		1.20		0.300	

Egg success (A × B × C × D): Poplar Island 0.632; Susquehanna River and Flats 0.857; Anacostia/middle Potomac River 0.547; James River 0.553; Back River 0.114.

[a] A subset of these data has been published previously [16].
[b] Considered to be lost during the egg stage.
[c] Eggs in nest >45 d or abandoned.
[d] Hatchability (eggs hatched/[eggs laid − eggs that disappeared or sampled before hatching]) represents the percentage of eggs that remain in the nest through hatch.
A = daily survival rate to the 39th power to account for a 39-d to 43-day incubation period [62]; B = daily survival rate to the 53rd power to account for 50-d to 55-d nestling period; C = number of eggs that hatched in successful nests divided by total number of eggs in successful nests; D = number of nestlings that fledged in successful nests divided by the total number of nestlings in successful nests; E = mean clutch size.

because of poor precision in 3 separate assays. In 2011, nestlings from the Anacostia/middle Potomac Rivers (33.9 ± 6.1 pg/μg DNA) exhibited greater DNA damage than those from Poplar Island (26.6 ± 2.9 pg/μg DNA; $p = 0.04$; Figure 2). There were no differences ($p = 0.15$) in oxidative DNA damage in nestling blood samples from Poplar Island between 2012 and 2013, and thus data for these 2 yr were combined (27.6 ± 12.6 pg/μg DNA). The DNA damage in nestling blood samples did not differ among the Susquehanna, James, and Back Rivers and Poplar Island. The nestling sampled closest to the Back River WWTP had the greatest concentration of 8-OH-dG (78.1 pg/μg DNA) of all samples.

Diet characterization

Over the 3-yr study, 1662 osprey prey items (15 fish species) were documented (Supplemental Data, Table S1). On Poplar Island, ospreys predominantly fed on striped bass (*M. saxatilis*; 47.8% of diet) and Atlantic menhaden (44.3% of diet). On the Susquehanna, Anacostia/middle Potomac, and James Rivers, osprey fed predominantly on catfish and gizzard shad (*D. cepedianum*). Mean ^{15}N content in nestling plasma, which is a proxy for trophic level, was similar on the Anacostia River (19.4‰) and Poplar Island (18.2‰; $p = 0.02$, $\alpha = 0.008$ for Bonferroni correction), but was slightly lower on the Susquehanna (17.5‰) and James Rivers (15.3‰; $p < 0.006$). This indicates that ospreys were feeding at fairly similar trophic levels across study sites. A complete reconstruction of osprey diet was not the specific goal of

the present study; however, these basic dietary observations were used to identify the fish species to sample for the food chain component.

Contaminants in fish

The average length and weight of all fish collected were 284.1 ± 52.3 mm and 256.6 ± 96.6 g, respectively. Organochlorine pesticides, PCBs, PBDEs, and alt-BFRs were measured in the dominant fish species in osprey diet (Supplemental Data, Table S2). Of the chemical analytes quantified, 19 of 44 organochlorine pesticides, 111 of 129 PCB congeners, 6 of 11 PBDE congeners, and 5 of 7 alt-BFRs were detected in fish. Fish from the Anacostia/middle Potomac Rivers had a mean concentration of p,p'-DDE (39.1 ng/g wet wt) that was more than 4 times greater than Poplar Island (9.24 ng/g wet wt; $p < 0.0001$), with values on the Susquehanna and James Rivers in the intermediate range. Similarly, fish from the Anacostia/middle Potomac Rivers contained the greatest total PCB residues (481.2 ng/g wet wt, ranging up to 1145.2 ng/g wet wt) compared with Poplar Island (49.1 ng/g wet wt, ranging up to 102.3 ng/g wet wt; $p < 0.0001$). Similar to p,p'-DDE, total PCBs on the Susquehanna and James Rivers exhibited intermediate values. The only coplanar PCB congener detected in fish samples was PCB 77, and there were no differences among sites ($p = 0.72$). There were no differences in total PBDE residues in fish among study sites, and only low levels of alt-BFRs were detected across study sites (<8.3 ng/g wet wt).
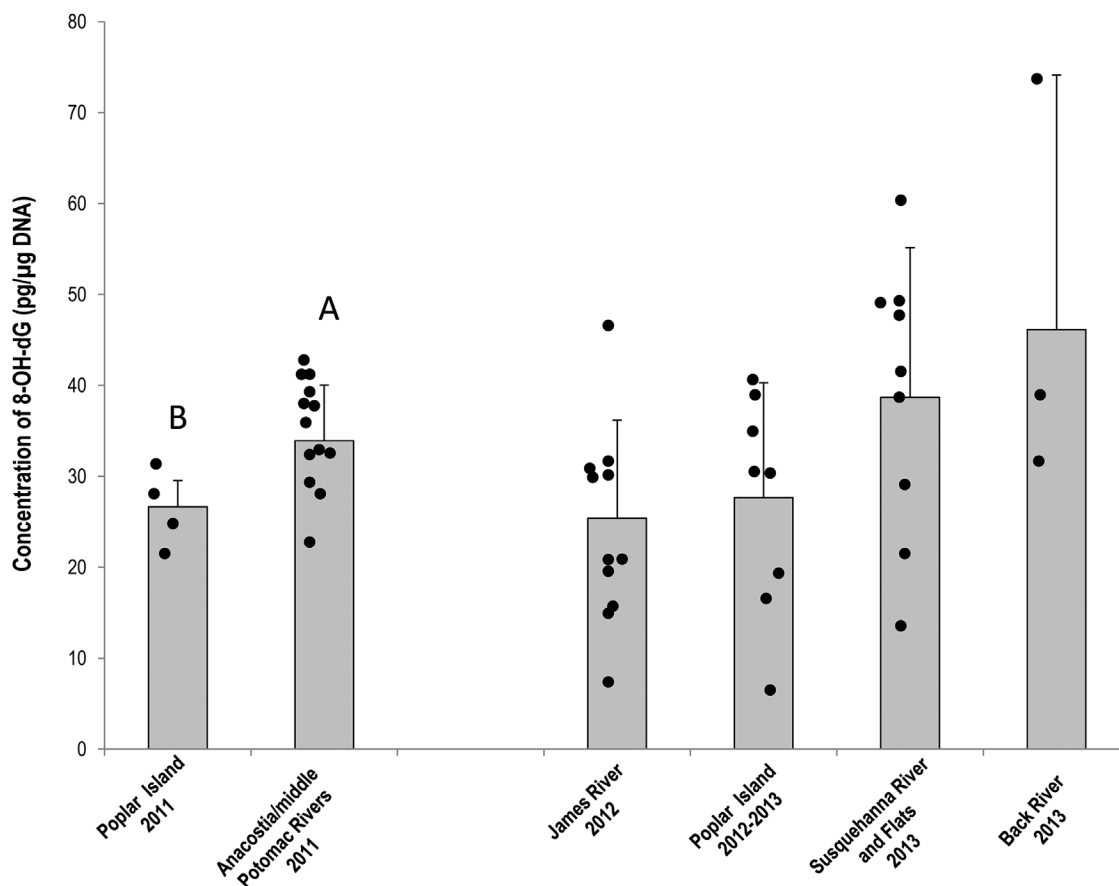

Figure 2. Concentrations of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in osprey nestling whole blood. Capital letters indicate a statistically significant difference ($p < 0.05$) using Tukey's honest significant difference test. Means (gray bars), standard deviations (whiskers), and individual values (solid dots) are presented. Data from 2011 and 2012/2013 were analyzed separately.

Because of the small sample size, contaminant residues in fish from the Back River were not statistically evaluated. Qualitatively, p,p'-DDE residues were similar to those on the Anacostia/middle Potomac Rivers, whereas total PCB and PBDE concentrations in gizzard shad were the greatest compared with the other study sites.

Contaminants in osprey eggs

In ospreys, 24 of 44 organochlorine pesticides, 110 of 129 non-coplanar PCB congeners, 4 of 4 coplanar PCB congeners, 8 of 11 PBDE congeners, and 5 of 7 alt-BFRs were detected in eggs. In total, the same 19 organochlorine pesticides, 6 PBDE congeners, 4 alt-BFRs, 1 coplanar PCB, and 77 non-coplanar PCBs were detected in both fish and osprey samples. Residues of organochlorine pesticides were greater on the Anacostia/middle Potomac Rivers and Back River for α-chlordane (*cis*-chlordane), *cis*-nonachlor, and *trans*-nonachlor ($p < 0.001$) compared with other study sites (Table 2). Residues of p,p'-DDE in osprey eggs on the Anacostia/middle Potomac ranged up to 1.00 μg/g, which is 2.5 times greater than the maximum value on Poplar Island (0.414 μg/g wet wt). Methoxytriclosan (a metabolite of the antimicrobial agent triclosan) was detected infrequently (Poplar Island, 1 of 12, range <MDL–1.49 ng/g wet wt; Susquehanna River, all <MDL; Anacostia/middle Potomac, 12 of 12, range 0.40–6.29 ng/g wet wt; James River, 1 of 12, range <MDL–1.49 ng/g wet wt; and Back River, 1 of 5, range <MDL–3.77 ng/g wet wt).

Total PCBs were greater at all study sites compared with Poplar Island ($p < 0.0001$) (Table 3). There were no significant differences in PCB concentrations among the other 4 sites ($p > 0.47$). Congener 169 was detected most frequently on the James and Anacostia/middle Potomac Rivers. The TEQs for all study sites were greater compared with the Poplar Island reference site ($p < 0.003$).

Total PBDE concentrations in eggs were greatest on the Susquehanna and Anacostia/middle Potomac Rivers compared with other sites ($p < 0.002$; Table 4). The geometric mean of total PBDE concentrations in eggs from the Back River was similar to the Anacostia River and Susquehanna River and flats ($p > 0.9$), but marginally greater than the James River ($p = 0.08$). The maximum PBDE residue (801.8 ng/g wet wt) was detected in the vicinity of the Blue Plains WWTP on the middle Potomac River. Congener 47 followed a similar pattern among sites and was the dominant component of total PBDEs (55–72% of total PBDEs across all study sites). Congeners 99, 100, and 154 followed a slightly different pattern. Concentrations in osprey eggs were greatest on the Susquehanna and Back Rivers, followed by the James, Anacostia/middle Potomac, and Poplar Island. Residues of BDE 153 on the Susquehanna, Anacostia/middle Potomac, Back, and James Rivers were all significantly greater compared with the reference site ($p < 0.004$). Congeners 183 and 209 were less frequently detected. Congener 183 was present in 10 of 13 samples collected on the Anacostia/middle Potomac Rivers, whereas congener 209 was present in 10 of 12 samples on the James River and in ≤50% of all samples at the other study sites. Congeners 47, 100, 153, and 154 followed a similar pattern, with values being greater on the Susquehanna and middle Potomac Rivers compared with the other sites. Inspection of these data revealed that α-HBCD, BTBPE, and DBDPE were most frequently detected on the Anacostia/middle Potomac Rivers, with 5 of the 7 alt-BRFs present in eggs. Generally, alt-BFR values were 2 orders of magnitude lower than PBDE concentrations. Neither β- nor γ-HBCD was detected in any osprey egg samples.

Analysis of similarity revealed that PCB congener patterns differed among sites (Global ANOSIM $R = 0.41$, $p < 0.001$; Figure 3). For PCB congener concentrations, there was strong support ($R > 0.4$) for differences in patterns among various tributaries (James and Potomac Rivers, James River and Poplar Island, Potomac River and Poplar Island, and Potomac and Susquehanna Rivers; Supplemental Data, Table S4). There was some support for differences between Poplar and Susquehanna ($R = 0.34$). The PCB congeners that contributed most toward dissimilarities between the James and Potomac Rivers and between the James and Susquehanna Rivers included PCB 156 and 189 (Supplemental Data, Table S4). In contrast, the congeners that contributed most toward dissimilarities between the James River and Poplar Island, the Potomac River and Poplar Island, and the Susquehanna River and Poplar Island were congeners 170/190, 105, 156, and 128. For the Potomac and Susquehanna Rivers, PCB congeners 189, 156, and 118 accounted for most of the dissimilarities.

The pattern of PBDE congeners also differed across study sites (Global ANOSIM $R = 0.56$, $p < 0.001$; Figure 3). For concentrations of PBDEs, there was strong support ($R > 0.4$) for differences among tributaries (Susquehanna and James Rivers, Susquehanna River and Poplar Island, James and Potomac Rivers, James River and Poplar Island, and Potomac River and Poplar Island; Supplemental Data, Table S4). There was some support for differences between the Susquehanna and Potomac Rivers ($R = 0.307$). Specifically, the BDE congener 209 contributed the most toward the differences between the Susquehanna and James Rivers and between the James and Potomac Rivers. However, BDE congener 99 contributed the most toward differences between the Susquehanna and Potomac Rivers, Susquehanna River and Poplar Island, James River and Poplar Island, and James and Potomac Rivers. Despite the aforementioned site differences, logistic regression failed to reveal significant relations between egg contaminant concentrations (organochlorine pesticides, PCBs, and flame retardants) and osprey productivity in Chesapeake Bay ($p > 0.22$).

Biomagnification factors for osprey eggs

Biomagnification factors were used to relate contaminant residues in fish to those in osprey eggs (Table 5; Supplemental Data, Table S5). All dietary ratios were rounded to the nearest whole number. Using dietary observations, the ratio of predominant fish prey species on Poplar Island (menhaden and striped bass) and the Susquehanna River (catfish and gizzard shad) was approximately 1:1. Common carp (*Cyprinus carpio*), catfish, and gizzard shad were the most dominant species in osprey diet on the Anacosita/middle Potomac Rivers. Difficulties were encountered collecting enough carp that were approximately 25 cm to 35 cm in length. Three carp caught at our downriver site on the Potomac River within the osprey foraging size range were compared with 3 channel catfish (*Ictalurus punctatus*) at this same site. Contaminant residues were low and similar between these 2 species (no more than 8-fold difference). Thus, we chose to use catfish, which were consistently sampled in an adequate size range across all sampling sites on the Anacostia/middle Potomac Rivers. The 2 dominant fish species sampled on the Anacostia/middle Potomac Rivers were catfish and gizzard shad, and they were consumed at a 3:2 ratio.

Prey species of ospreys on the James River were similar (catfish and gizzard shad) but were consumed at a slightly

Table 2. Osprey egg concentrations (µg/g wet wt) of organochlorine pesticides and metabolites from Chesapeake Bay (USA) regional waterways and Poplar Island reference site[a]

Contaminant	Poplar Island[b] 2011–2013 ($n = 12$)	Susquehanna River and Flats 2013 ($n = 10$)	Anacostia and middle Potomac Rivers[b] 2011 ($n = 13$)	James River 2012 ($n = 12$)	Back River 2013 ($n = 5$)
p,p'-DDE					
Geometric mean	0.160 C	0.410 B	0.628 A	0.315 B	0.432 A,B
Extremes	0.090–0.414	0.325–0.777	0.370–1.00	0.241–0.500	0.191–0.667
No. detected	12/12	10/10	13/13	12/12	5/5
p,p'-DDD					
Geometric mean	0.010 C	0.025 A,B	0.034 A	0.018 B	0.046A
Extremes	0.006–0.037	0.018–0.034	0.019–0.072	0.008–0.040	0.014–0.103
No. detected	12/12	10/10	13/13	12/12	5/5
Dieldrin					
Geometric mean[c]	—	—	0.0149–0.0150	—	0.0522–0.0523
Extremes	<MDL	<MDL	<MDL–0.035	<MDL	<MDL–0.203
No. detected	0	0	11/13	0	3/5
Heptachlor epoxide					
Geometric mean	—	—	0.035	—	—
Extremes	<MDL–0.006	<MDL–0.009	0.014–0.096	<MDL–0.010	<MDL
No. detected	2/12	1/10	13/13	1/12	0
α-Chlordane (*cis*)					
Geometric mean	—	—	0.015 A	0.005 B	0.023 A
Extremes	<MDL–0.018	<MDL	0.004–0.041	0.002–0.006	0.011–0.050
No. detected	2/12	0	13/13	12/12	5/5
γ-Chlordane (*trans*)					
Geometric mean[c]	—	—	0.0019–0.0021	—	—
Extremes	<MDL–0.004	<MDL	<MDL–0.005	<MDL	<MDL–0.004
No. detected	1/12	0	8/13	0	1/5
cis-Nonachlor					
Geometric mean[c]	0.0035–0.0036 C	—	0.033 A	0.008 B	0.05 A
Extremes	<MDL–0.010	<MDL–0.014	0.010–0.058	0.008–0.012	0.017–0.160
n detected	9/12	4/10	13/13	12/12	5/5
trans-Nonachlor					
Geometric mean[c]	0.0028–0.0029 C	0.0043–0.0044 B,C	0.017 A	0.005 B	0.022 A
Extremes	<MDL–0.014	<MDL–0.011	0.005–0.037	0.002–0.007	0.011–0.033
n detected	8/12	7/10	13/13	12/12	5/5
Oxychlordane					
Geometric mean	—	—	0.016	—	—
Extremes	<MDL	<MDL	0.005–0.051	<MDL	<MDL
No. detected	0	0	13/13	0	0
Mirex					
Geometric mean[c]	0.0011–0.0013 B	0.0041–0.0043 A,B	0.003 A	0.0030–0.0031 A,B	0.0052–0.0053 A,B
Extremes	<MDL–0.003	<MDL–0.009	0.002–0.005	<MDL–0.007	<MDL–0.013
No. detected	6/12	6/10	13/13	9/12	3/5

[a]A dash (—) indicates that no mean was calculated because the contaminant was detected in fewer than half the samples. Extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's honest significant difference method of multiple comparisons ($p < 0.05$) or a generalized Wilcoxon nonparametric test followed by pairwise comparisons using a Bonferroni correction.
[b]A subset of these data has been previously published [16].
[c]If nondetects were present in fewer than half of the samples, the Kaplan Meier method was used to estimate the extremes of the mean.
p,p'-DDE = *p,p*'-dichlorodiphenyldichloroethylene; *p,p*'-DDD = *p,p*'-dichlorodiphenylodichloroethane; MDL = method detection limit.

different ratio (4:1). Using these proportions of prey species consumed by nestlings, the BMFs averaged 17.7 for *p,p*'-DDE, 28.5 for total PCBs, approximately 19.6 for total PBDEs, and 14.9 to 25.7 for BDE congeners 47, 99, and 100. Values were approximately in the same ratios when calculated on a wet weight and lipid weight basis. Many of the compounds had a BMF < 1 (*trans*-nonachlor, *cis*-nonachlor, α-chlordane [*cis*-chlordane], *trans*-chlordane, and methoxytriclosan), indicating no biomagnification in the upper trophic levels in Chesapeake Bay.

Redundancy analysis

The redundancy analysis incorporated data from the present study ($n = 47$ excluding the Back River, with only 3 nests) and data for additional nests from Baltimore Harbor/ Patapsco and Elizabeth Rivers ($n = 13$) presented in our previous publication [16], for a total of 60 samples. Overall, the redundancy analysis model was highly significant

($F_{df = 5} = 11.53$, $p < 0.001$; Figure 4). Axes 1 and 2 explained more variability than would be expected by chance alone ($F_{df = 1} = 46.89$, $p < 0.001$ and $F_{df = 1} = 6.99$, $p < 0.001$), and axis 3 was near the threshold for significance ($F_{df = 1} = 2.54$, $p = 0.0667$; Supplemental Data, Table S3 and Figure S1). A distinct grouping emerged among sites. Halogenated contamination was greatest in quadrants 1 and 3 compared with 2 and 4 (Figure 4). Contributions of PCBs and *p,p*'-DDE represented the largest proportion of overall contamination for those sites in quadrant 1 (Figure 4, top left; Patapsco River/Baltimore Harbor, Elizabeth River). Although chlorinated hydrocarbons still remained high in quadrant 3 (Figure 4, bottom left; Anacostia/middle Potomac Rivers and the Susquehanna River), PBDEs had a substantial contribution to overall halogenated contamination. The PBDEs appeared to be most closely associated with DNA damage compared with the other chemicals. Poplar Island had the least contamination, which is consistent with it being the reference site. There were no

Table 3. Osprey egg concentrations of total PCBs and congeners from Chesapeake Bay (USA) regional waterways and Poplar Island reference site[a]

Contaminant	Poplar Island[b] 2011–2013 ($n = 12$)	Susquehanna River and Flats 2013 ($n = 10$)	Anacostia and middle Potomac Rivers[b] 2011 ($n = 12$)	James River 2012 ($n = 12$)	Back River 2013 ($n = 5$)
Total PCBs (µg/g)					
Geometric mean	1.43 B	4.15 A	4.94 A	4.19 A	4.46 A
Extremes	0.650–3.22	1.89–7.75	2.72–6.53	2.35–6.50	1.34–7.95
No. detected	12/12	10/10	13/13	12/12	5/5
Congener 77 (pg/g)					
Geometric mean[c]	96.5	103.7	113.1–116.2	176.9	189.0
Extremes	37.0–420.0	40.0–250.0	<MDL–170.0	100.0–330.0	120.0–430.0
No. detected	12/12	10/10	12/13	12/12	5/5
Congener 81 (pg/g)					
Geometric mean	—	—	—	133.1	—
Extremes	<MDL–340.0	<MDL	<MDL–80.0	40.0–250.0	<MDL–40.0
No. detected	2/12	0	6/13	12/12	1/5
Congener 126 (pg/g)					
Geometric mean[c]	197.6 B	437.5 A	534.6–537.7 A	599.2 A	598.0–606.0 A
Extremes	61.0–730.0	170.0–620.0	<MDL–830.0	480.0–750.0	<MDL–740.0
No. detected	12/12	10/10	12/13	12/12	4/5
Congener 169 (pg/g)					
Geometric mean[c]	—	—	60.0–72.3	74.0	—
Extremes	<MDL	<MDL	<MDL–100.0	30.0–140.0	<MDL
No. detected	0	0	9/13	12/12	0
Congener 105 (ng/g)					
Geometric mean[c]	8.47–8.60 B	40.1 A	31.6 A	27.4 A	45.5 A
Extremes	<MDL–33.3	12.4–228.7	9.16–66.1	20.8–37.5	12.7–97.4
No. detected	8/12	10/10	13/13	12/12	5/5
Congener 118 (ng/g)					
Geometric mean	65.2 C	118.2 B	216.5 A	118.6 B	203.9 B
Extremes	32.4–210.5	55.7–181.1	67.4–415.0	90.0–171.2	48.6–437.9
No. detected	12/12	10/10	13/13	12/12	5/5
Congener 128 (ng/g)					
Geometric mean[c]	17.7–17.8 C	51.5 A,B	95.1 A	42.2 A,B	67.8 A,B
Extremes	<MDL–31.0	21.2–91.7	39.0–154.5	28.9–63.9	14.4–153.8
No. detected	9/12	10/10	13/13	12/12	5/5
Congener 138/158 (ng/g)					
Geometric mean	183.0 B	492.4 A	482.6 A	543.0 A	603.7 A
Extremes	100.5–460.7	194.5–816.4	289.7–975.0	295.4–803.5	162.3–1074.1
No. detected	12/12	10/10	13/13	12/12	5/5
Congener 156 (ng/g)					
Geometric mean[c]	8.48–8.58 B	21.3–21.5 B	53.8 A	16.6 B	24.7–24.8 A,B
Extremes	<MDL–29.8	<MDL–50.3	28.3–84.5	5.98–27.8	<MDL–59.2
No. detected	9/12	7/10	13/13	12/12	3/5
Congner 167 (ng/g)					
Geometric mean	—	—	—	—	—
Extremes	<MDL	<MDL–15.6	<MDL–18.8	<MDL	<MDL–15.6
No. detected	0	2/10	2/13	0	2/5
Congener 189 (ng/g)					
Geometric mean[c]	0.95–1.15 C	—	8.30–8.33 A	4.1–4.2 B	—
Extremes	<MDL–2.51	<MDL–8.94	<MDL–13.0	<MDL–17.4	<MDL
No. detected	6/12	3/10	12/13	9/12	0
Congener 170/190 (ng/g)					
Geometric mean[c]	51.2–51.3 C	191.6 B	282.3 A	157.4 B	224.4–224.5 A,B
Extremes	<MDL–174.1	74.3–386.2	125.2–411.6	59.6–254.1	<MDL–492.3
No. detected	9/12	10/10	13/13	12/12	4/5
Toxic equivalents (ng/g)					
Geometric mean	0.06 B	0.12 A	0.19 A	0.13 A	0.17 A
Extremes	0.03–0.14	0.08–0.18	0.07–0.28	0.11–0.16	0.03–0.29

[a] A dash (—) indicates that no mean was calculated because the contaminant was detected in fewer than half the samples. Extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's honest significant difference method of multiple comparisons ($p < 0.05$) or a generalized Wilcoxon nonparametric test followed by pairwise comparisons using a Bonferroni correction.
[b] A subset of these data has been published previously [16].
[c] If nondetects were present in fewer than half of the samples, the Kaplan Meier method was used to estimate the extremes of the mean.
PCB = polychlorinated biphenyl; MDL = method detection limit.

univariate predictors for DNA damage (PCB $t_{df=48} = 0.445$, $p = 0.658$; PBDE $t_{df=48} = -0.087$, $p = 0.931$; p,p′-DDE $t_{df=48} = 0.915$, $p = 0.365$).

DISCUSSION

Osprey productivity

Because of the presence of the organochlorine pesticide DDT and its metabolites (primarily p,p′-DDE) in the osprey food web, the range of the Chesapeake Bay osprey population had contracted to the main stem of the Bay by the 1970s, with few nesting pairs present north of the Bay Bridge [4,41,42]. During the DDT use era, productivity rates were low (e.g., 0.55 fledglings/active nest on the middle Potomac in 1970 [42]). Spitzer and Poole [3,5] stated that ospreys producing 0.8 fledglings/active nest to 1.15 fledglings/active nest are required to maintain a stable population in the Chesapeake Bay watershed. By the mid-1990s, the osprey population had more than doubled [7]. Our productivity estimates exceeded this range at all study sites (>1.17 fledglings/active nest; Table 1),

Table 4. Osprey egg concentrations (ng/g wet wt) of PBDEs and alternative brominated flame retardants from Chesapeake Bay (USA) regional waterways and Poplar Island reference site[a]

Contaminant	Poplar Island[b] 2011–2013 ($n = 12$)	Susquehanna River and Flats 2013 ($n = 10$)	Anacostia and middle Potomac Rivers[b] 2011 ($n = 13$)	James River 2012 ($n = 12$)	Back River 2013 ($n = 4$)
Total PBDEs					
Geometric mean	84.4 C	368.8 A	343.5 A	179.5 B	342.8 A,B
Extremes	52.7–274.9	212.4–648.6	170.4–801.8	135.2–216.8	286.9–549.1
No. detected	12/12	10/10	13/13	12/12	4/4
BDE congener 47					
Geometric mean	51.5 C	224.5 A	250.3 A	100.3 B	203.4 A,B
Extremes	32.4–183.5	116.1–398.2	104.0–648.3	79.4–121.6	154.9–348.7
No. detected	12/12	10/10	12/12	12/12	4/4
BDE congener 85					
Geometric mean	—	—	—	—	—
Extremes	<MDL–0.76	<MDL	<MDL	<MDL	<MDL
No. detected	1/12	0	0	0	0
BDE congener 99					
Geometric mean	3.01 C	58.5 A	22.1 B	26.0 B	45.2 A,B
Extremes	0.17–29.9	25.1–92.1	8.88–35.7	14.4–39.1	28.5–73.0
No. detected	12/12	10/10	13/13	12/12	4/4
BDE congener 100					
Geometric mean	12.2 D	64.7 A	39.9 B	25.4 C	46.3 A,B,C
Extremes	4.79–39.5	37.8–107.0	18.0–77.1	20.4–34.1	36.2–68.6
No. detected	12/12	10/10	13/13	12/12	4/4
BDE congener 153					
Geometric mean[c]	3.11 B	14.0 A	13.47–13.51 A	11.6 A	13.6 A
Extremes	2.00–8.20	8.10–23.9	<MDL–26.6	7.38–20.2	9.00–26.5
No. detected	12/12	10/10	12/13	12/12	4/4
BDE congener 154					
Geometric mean	8.77 C	19.3 A	13.6 A,B	11.4 B,C	22.3 A
Extremes	5.01–20.7	11.7–27.9	8.37–21.1	5.98–20.6	16.5–31.9
No. detected	12/12	10/10	13/13	12/12	4/4
BDE congener 183					
Geometric mean[c]	—	—	1.24–1.33	—	—
Extremes	<MDL	<MDL	<MDL–3.68	<MDL	<MDL
No. detected	0	0	10/13	0	0
BDE congener 209					
Geometric mean[c]	—	—	—	2.95–3.02	8.40–8.60
Extremes	<MDL–24.2	<MDL–12.6	<MDL–1.21	<MDL–9.74	<MDL–20.4
No. detected	5/12	4/10	2/13	10/12	2/4
α-HBCD					
Geometric mean[c]	0.82–0.95	—	1.30–1.31	—	—
Extremes	<MDL–2.14	<MDL	<MDL–3.03	<MDL–10.2	<MDL
No. detected	8/12	0	12/13	5/12	0
BTBPE					
Geometric mean	—	—	—	—	—
Extremes	<MDL–4.77	<MDL	<MDL–28.7	<MDL	<MDL
No. detected	1/12	0	4/13	0	0
DBDPE					
Geometric mean	–	–	–	–	–
Extremes	<MDL	<MDL	<MDL–0.89	<MDL	<MDL
No. detected	0	0	1/13	0	0
TBB					
Geometric mean[c]	—	2.30–2.50	—	—	5.60–5.80
Extremes	<MDL–63.7	<MDL–7.40	<MDL–30.3	<MDL–2.14	<MDL–11.5
No. detected	2/12	5/10	1/13	1/12	2/4
TBPH					
Geometric mean[c]	—	—	—	—	2.00–2.20
Extremes	<MDL–31.3	<MDL–2.4	<MDL–7.37	<MDL–0.54	<MDL–4.30
No. detected	3/12	3/10	3/13	1/12	2/4

[a]A dash (—) indicates that no mean was calculated because the contaminant was detected in fewer than half the samples. Extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's honest significant difference method of multiple comparisons ($p < 0.05$) or a generalized Wilcoxon nonparametric test followed by pairwise comparisons using a Bonferroni correction.
[b]A subset of these data has been published previously [16].
[c]If nondetects were present in fewer than half of the samples, the Kaplan–Meier method was used to estimate the extremes of the mean.
PBDEs = polybrominated diphenyl ethers; α-HBCD = α-hexabromocyclododecane; BTBPE = 1,2-bis (2,4,6-tribromophenoxy) ethane; DBDPE = decabromodiphenyl ether; TBB = 2-ethylhexl 2,3,4,5-tetrabromobenzoate; TBPH = di(2-ethylhexyl)-2,3,4,5-tetrabromophthalate [TBPH]; MDL = method detection limit.

suggesting that osprey reproduction in the Chesapeake Bay watershed may be adequate to maintain a stable population. Unfortunately, the estimates to maintain a stable population have not been updated since the 1980s, and we are inferring that they have remained the same over the past 35 yr. Research is needed to determine contemporary productivity rates needed to maintain a stable population [43]. The large numbers in the Bay currently make this type of study difficult.
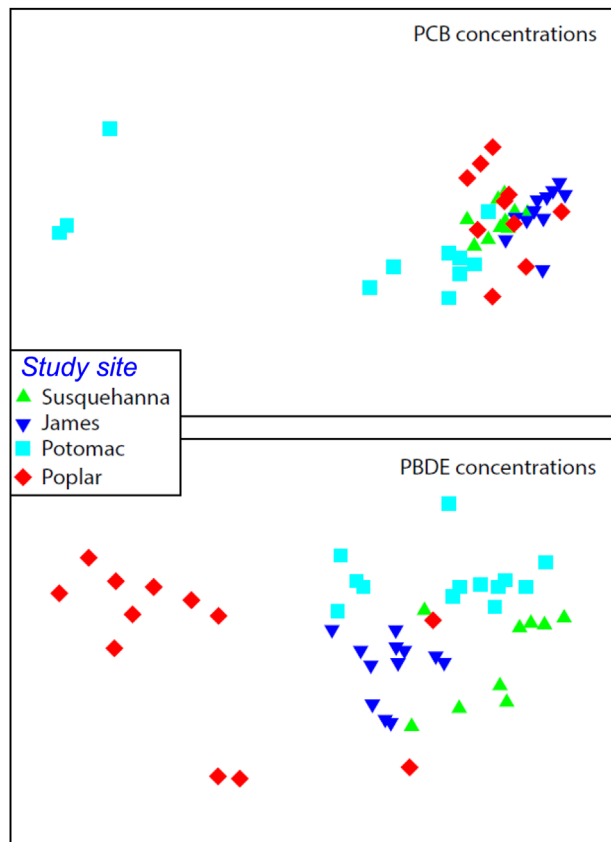
Figure 3. Nonmetric multidimensional scaling (NMDS) plot showing polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) congener concentrations across Chesapeake Bay study sites. Axes of NMDS plots do not have units.

Contaminants in ospreys

The present study further demonstrates that residues of *p,p'*-DDE in osprey eggs have declined since the 1970s (averaging 3.1 μg/g wet wt on the middle Potomac in 1971–1977 [42] to 0.63 μg/g wet wt in the present study). Current *p,p'*-DDE concentrations in eggs are well below the threshold associated with 10% eggshell thinning (2.0 μg/g wet wt [44]), and there is no apparent relation between *p,p'*-DDE and eggshell thickness at these low levels.

Total PCB concentrations were similar among the Susquehanna, Anacostia/middle Potomac, and James Rivers. Compared with historic values (Potomac River 9.8 μg PCBs/g wet wt in 1973) reported by Wiemeyer and coworkers [44], PCB concentrations have declined on the Potomac by approximately 50%. On a broader geographic scale, however, total PCB concentrations in eggs have not dramatically changed in USEPA designated Regions of Concern (Anacostia/middle Potomac, Baltimore Harbor/Patapsco, and Elizabeth Rivers) [16]. Although PCBs have been associated with many adverse effects in fish-eating birds [45], there were seemingly no effects on osprey productivity in the Chesapeake Bay. Egg TEQs in some study sites exceeded the no-observed-adverse-effect-level (NOAEL) (0.136 ng TEQ/g wet wt egg [46] and 0.037 ng TEQ/g wet wt [47]) and lowest-observed-adverse-effect-level (LOAEL) (0.130 ng TEQ/g wet wt) [47] for osprey hatching success and induction of cytochrome P450 as inferred by ethoxyresorufin-*O*-deethylase activity. Although growth rate was not examined, body weight was used as a

surrogate measure. There was no evidence of a relation between TEQs in Chesapeake Bay osprey eggs and body weight of 40-d-old to 45-d-old nestlings ($R^2 = -0.12$, $p = 0.42$).

Total PBDEs were greatest on the Anacostia and Susquehanna Rivers (368.8 ng/g and 343.5 ng/g wet wt, respectively). These values are lower than reported in Chesapeake Bay osprey eggs collected in 2000 to 2001 from Regions of Concern [15]. Manufacture of the penta-BDE commercial formulation ceased in 2004 in the United States [27]. The expanded subset of nests sampled on the Anacostia/middle Potomac Rivers revealed that PBDE egg residues decreased downstream from the Blue Plains WWTP. Such treatment facilities are documented sources of PBDEs [27,48]. Notably, the greatest residues of PBDEs on both the middle Potomac (801.8 ng/g wet wt) and Susquehanna Rivers (648.6 ng/g wet wt) were found in nests near WWTPs. Values in the present study were below the LOAEL associated with reduced pipping and hatching success in American kestrels (*Falco sparverius*; 1.8 μg/g wet wt [49]) but exceeded the NOAEL (approximately 0.18 μg/g wet wt [49]). The paucity of ecotoxicity data (i.e., environmental concentration contaminants in aquatic bird eggs and reproductive rates) for aquatic birds from the Susquehanna River makes it difficult to place the present findings into a historical perspective, but these data are valuable for future monitoring studies. Alternative-BRFs, including α-HBCD, BTBPE, DBDPE, TBB, and TBPH, were detected at low concentrations compared with the PBDE flame retardants (Table 4), but it is difficult to determine their significance because toxicity reference values have yet to be derived for these compounds in birds.

Although all study sites differed in congener concentrations, the dominant PCB and PBDE congeners that accounted for site-specific differences followed similar patterns (PCB congeners 156, 170/190, and 189 and PBDE congeners 99 and 209). Interestingly, the ANOSIM indicated that individual congeners accounted for only 36% of the difference among sites. From an ecotoxicological perspective, PCBs 156, 170/190, and 189 have relatively low TEQs and were detected at ng/g quantities. For PBDEs, congeners 99 and 209 accounted for the up to 25% of the differences among sites. These congeners were found at low concentrations in the present study, because BDE 209 is a large molecule and is not readily absorbed or distributed in adipose tissue.

Osprey diet and biomagnification of contaminants

Similar to findings of others [8,9,50], ospreys consumed Atlantic menhaden in estuarine sites, but shifted to gizzard shad and catfish in tidal freshwater tributaries. Although osprey diet varied among study sites, only slight differences in ^{15}N signatures were observed (e.g., both striped bass and catfish species are opportunistic predators, whereas gizzard shad and menhaden are both planktivores), indicating that ospreys were feeding at similar trophic levels.

Biomagnification of lipophilic compounds has been well studied in the field of ecotoxicology [51]. The BMF for *p,p'*-DDE averaged 21.4 on a lipid weight basis, which was comparable to another study of Chesapeake Bay ospreys (18 on a lipid wt basis [14]). However, this is less than observed on the Willamette River in Oregon (~87 on a lipid wt basis in an osprey egg [11]). This difference may be attributable to greater *p,p'*-DDE concentrations in eggs of ospreys nesting along the Willamette River compared with the Chesapeake Bay (geometric means, 2347 vs 378 on a wet wt basis, respectively). Concentrations of *p,p'*-DDE were comparable in fish from the present study and those sampled on the Willamette River

Table 5. Biomagnification factors greater than 5 on a wet weight and lipid weight basis from whole fish to osprey egg contents by study site[a]

| | Contaminant | | | | | | | | | | | | |
| | p,p'-DDE (ng/g) | | p,p'-DDD (ng/g) | | Total PCBs (ng/g) | | Total PBDEs (ng/g) | | BDE 47 (ng/g) | | BDE 99 (ng/g) | | BDE 100 (ng/g) | |
Site	Wet wt	Lipid wt	Wet wt	Lipid wt	Wet wt	Lipid wt	Wet wt	Lipid wt	Wet wt	Lipid wt	Wet wt	Lipid wt	Wet wt	Lipid wt
Poplar Island														
Atlantic menhaden	12.0	108.8	3.0	27.5	49.1	594.5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Striped bass	6.48	224.2	1.2	40.8	41.6	1439.5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Diet ratio 1:1[b]	9.24	166.5	2.1	34.2	45.4	1017.0	—	—	—	—	—	—	—	—
Osprey	160.4	3728.6	10.0	242.2	1431.2	26 979.0	84.4	1455.0	51.5	896.0	3.0	52.7	12.2	212.4
BMF	17.4	22.4	4.8	7.1	31.6	26.5	—	—	—	—	—	—	—	—
Susquehanna River and Flats														
Catfish sp.	20.8	591.8	3.12	88.7	186.6	5303.1	22.7	411.5	9.68	275.3	4.46–4.60	125.0–127.4	3.20–3.40	83.4–86.9
Gizzard shad	24.3	381.9	5.11	80.2	277.9	4360.1	11.5	180.0	10.9	170.9	<MDL	<MDL	<MDL	<MDL
Diet ratio 1:1[b]	22.6	486.9	4.1	84.5	232.3	4831.6	17.1	295.8	10.3	223.1	2.23–2.43	62.5–67.3	1.60–1.80	41.7–47.0
Osprey	410.0	10 401.5	24.5	622.7	4148.7	105 353.4	368.8	9707.2	224.5	570.1	58.5	1485.2	64.7	1644.7
BMF	18.18	21.4	6.0	7.4	17.9	21.8	21.6	32.8	21.8	25.6	24.1–26.2	22.1–23.8	35.9–40.4	35.0–39.4
Anacostia/middle Potomac Rivers														
Catfish sp.	43.7	1018.1	10.0	223.2	481.7	11 205.2	20.7	499.6	11.2	259.0	4.44	103.4	3.74	87.2
Gizzard shad	34.4	545.9	10.0	158.4	365.9	5806.8	16.1–16.2	295.5–296.3	12.5–12.6	200.5–202.1	<MDL	<MDL	2.11–2.24	35.2–37.6
Diet ratio 1:1[b]	40.0	829.2	10.0	197.3	435.4	8506.0	18.86–18.90	418.0–418.3	11.7–11.8	235.6–236.2	2.68	62.0	3.09–3.14	66.4–67.4
Osprey	628.0	14 896.0	34.0	807.9	4935.1	117 128.2	343.5	8766.1	250.3	5941.4	22.1	525.7	39.9	948.1
BMF	15.7	18.0	3.40	4.10	11.3	13.8	18.17–18.21	20.96–20.97	21.2–21.4	25.15–25.22	8.25	8.47	12.7–12.9	14.1–14.3
James River														
Catfish sp.	21.7	418.6	3.21–3.25	52.3–53.0	97.2	1877.9	13.9	137.6	6.72	64.9	4.20	40.6	2.40	23.1
Gizzard shad	21.4	317.1	3.49	51.7	121.0	1792.4	9.90	79.7	9.44–9.52	93.6–95.2	<MDL	<MDL	1.99–2.12	21.6–24.0
Diet ratio 1:1[b]	16.2	288.6	2.47–2.49	39.1–39.4	78.9	1387.1	9.42	87.2	5.72–5.74	55.9–56.2	2.10	20.3	1.69–1.73	16.9–17.6
Osprey	315.0	6878.0	18.1	395.7	4187.3	91 413.5	179.5	1977.5	100.3	1095.1	26.0	283.6	25.4	277.9
BMF	19.4	23.8	7.27–7.33	10.04–10.12	53.1	65.9	19.1	22.7	17.47–17.53	19.5–19.6	12.4	14.0	23.67–23.71	15.8–16.4
Average BMF	17.7	21.4	5.35–5.37	7.15–7.17	28.5	32.0	19.62–19.64	25.47–25.48	20.16–20.24	23.4–23.5	14.9–15.6	14.9–15.4	24.1–25.7	21.6–23.4

[a]Biomagnification factors (BMFs) are calculated for all scenarios in which the analyte was detected in greater than one-half of the species and site combinations. The contaminants shown above are those listed in Tables 2, 3, and 4 and Supplemental Table S2 with sufficient information to calculate a BMF. All contaminant data are presented as geometric means. If non detects were present in fewer than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean, and the BMF was calculated using the extremes. For those contaminants with one fish species <MDL, the value of one-half of the MDL was substituted to get an approximate dietary intake estimation. A dash (—) indicates that no mean was calculated because the analyte was detected in fewer than 50% of the samples.

[b]Diet ratio is the contaminant concentration calculated relative to the proportions of the 2 dominant fish species consumed at each study site.

p,p'-DDE = p,p'-dichlorodiphenyldichloroethylene; p,p'-DDD = p,p'-dichlorodiphenyldichloroethane; PCBs = polychlorinated biphenyls; PBDEs = polybrominated diphenyl ethers; <MDL = less than the method detection limit.
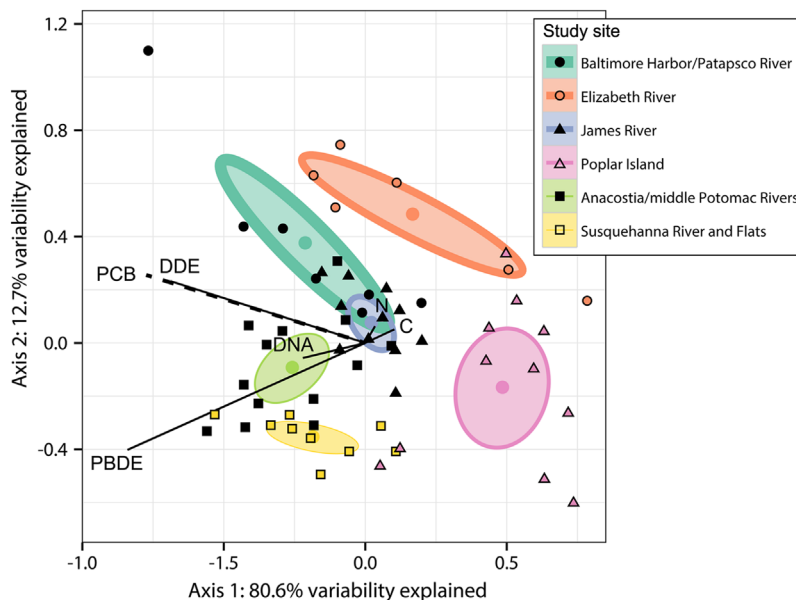
Figure 4. Redundancy analysis biplot of total polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (*p,p*'-DDE), polybrominated diphenyl ethers (PBDEs), carbon and nitrogen stable isotopes and their relationship to DNA damage. Data are projected onto the ordination axes. The large dots are the centroids, and the shaded areas represent the 95% confidence ellipses. The vectors (black lines) represent the contribution of different variables to each axis.

(<73 ng/g wet wt). Both Henny [11] and Elliott [52,53] suggest that fish captured on wintering grounds of west coast ospreys may contain greater residues of *p,p*'-DDE. Specifically, osprey on the West Coast of the United States typically spend their winters in Mexico, El Salvador, or Honduras compared with East Coast ospreys, which winter in Florida, Cuba, Venezuela, and Brazil [54]. The BMF for total PCBs in the present study was similar to other values reported for the Chesapeake Bay (28.5 in the present study vs 25.1 on a lipid wt basis in Chen et al. [14]) but 3 times greater than that on the Willamette River (estimated to be 11 on a lipid wt basis for osprey eggs [9]).

Several of the organochlorine pesticides or metabolites (*p,p*'-dichlorodiphenyldichloroethane [*p,p*'-DDD], *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor) and methoxytriclosan had a BMF < 5, indicating only modest biomagnification [55,56]. There was no evidence of biomagnification for the alt-BFRs even though their octanol–water partition coefficients are just as great as the hexa-, octa-, and deca-BDE formulations [57,58]. Although deca-BDE has been used worldwide, it was not found in great concentrations in the present study. This is consistent with findings reported by Chen et al. [14], who indicate that BDE-209 was detected in peregrine falcon (*Falco peregrinus*) eggs but not in fish-eating birds. Other factors, including biotransformation, may play a role in biomagnification, and unstudied metabolites may actually be more bioaccumulative than the parent compounds [51].

Relation of concentrations of PCBs, DDE, and PBDEs with DNA damage

Although the redundancy analysis suggested that DNA damage was most closely related to PBDE concentrations, the univariate correlation analysis did not reveal a significant relation between PBDE concentrations and oxidative DNA damage. In addition to PBDEs, other co-occurring compounds, including PAHs, perfluorinated chemicals, PCBs, and some metals, could cause DNA damage [59–64]. The increased production of oxyradicals can lead to a variety of consequences, including mutations, lesions, and disease progression [64].

CONCLUSIONS

Ospreys in the Chesapeake Bay are now thriving, and our estimates in several tributaries, including historic Regions of Concern, suggest that productivity is adequate to maintain a stable population. Both legacy and current use flame retardants had limited effects on osprey productivity across study sites and are well below established toxicity thresholds. Biomagnification factor estimates for principal contaminants are similar to those reported in other studies, and there is increased evidence of genetic damage in ospreys nesting in the most polluted areas. Such DNA damage could have subtle long-term health effects on the individual, and additional research is warranted for avian species. The present study has documented the continued recovery of ospreys in the Chesapeake Bay since the 1970s. Over the past 50 yr, the osprey population has increased from 1450 pairs in the 1970s [41] to 3500 during the mid-1990s [7]. Recent observations in several tributaries suggest that the Chesapeake Bay osprey population may be approaching 10 000 pairs (B.D. Watts, College of William and Mary, Williamsburg, VA, USA, personal communication), which is more than 6 times greater than their nadir during the era of DDT use.

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