ABSTRACT

Title of dissertation: CONTAMINANT EXPOSURE, FOOD WEB TRANSFER AND POTENTIAL EFFECTS ON OSPREYS (*Pandion haliaetus*) IN CHESAPEAKE BAY

Rebecca Saul Lazarus, Doctor of Philosophy, 2015

Dissertation directed by: Professor Mary Ann Ottinger and
Adjunct Professor Barnett A. Rattner
Department of Animal and Avian Sciences
Marine-Estuarine Environmental Sciences Program

The last large-scale ecotoxicological study of ospreys (*Pandion haliaetus*) in Chesapeake Bay was conducted in 2000-2001 and focused on the U.S. Environmental Protection Agency designated Regions of Concern (ROCs; Baltimore Harbor/Patapsco, Anacostia/middle Potomac, and Elizabeth Rivers). From 2011-2013, ROCs, Susquehanna River and flats, James and Back Rivers were evaluated to determine spatial and temporal trends in osprey productivity and contaminants in eggs. Concentrations of *p,p'*-DDE were below the threshold associated with eggshell thinning. Total PCB concentrations in eggs from the Anacostia/middle Potomac were lower in 2011 than 2000, but remained unchanged in Baltimore Harbor. Polybrominated diphenyl ether flame retardants declined across study sites and five alternative brominated flame retardants were detected at low levels in osprey eggs. Concentrations of oxidized DNA (biomarker of oxidative stress) were slightly elevated on the Anacostia/middle Potomac and Baltimore Harbor/Patapsco Rivers, but no univariate contaminant predictors correlated with DNA damage. Pharmaceuticals and personal care products were also examined. An integrative modeling approach was used to evaluate bioaccumulation potential of pharmaceuticals using hypothetical screening-level exposure scenarios. A

first-order kinetic exposure model was applied to estimate the average daily and cumulative 45-day dose of pharmaceuticals received by a nestling osprey. To complement the exposure model, water, fish and osprey nestling plasma samples were analyzed for 23 pharmaceuticals and an artificial sweetener (sucralose). Of the 18 analytes detected in water, 8 were found in fish plasma, and 1 in osprey nestling plasma (antihypertensive diltiazem). Diltiazem was detected at concentrations approximately 21.6 times greater in fish plasma than water and 4 times greater in osprey nestling plasma than fish. Diltiazem was found in all 69 osprey plasma samples (540–8,630 ng/L), with 41% of these samples exceeding maximum concentrations found in fish. Diltiazem levels in fish and osprey were below the human therapeutic plasma concentration (30,000 ng/L). Effect thresholds for diltiazem are unknown in ospreys at this time, and there was no evidence to suggest adverse effects. Overall, findings document continued recovery of the osprey population, declining levels of select persistent halogenated compounds, and modest evidence of oxidative genetic damage in nestlings.

CONTAMINANT EXPOSURE, FOOD WEB TRANSFER AND POTENTIAL EFFECTS ON OSPREYS (*Pandion haliaetus*) IN CHESAPEAKE BAY

By

Rebecca Saul Lazarus

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Advisory Committee:

Professor Mary Ann Ottinger, Chair/Co-advisor
Adjunct Professor Barnett A. Rattner, Co-advisor
Professor Bryan D. Watts
Dr. Beth L. McGee
Professor William W. Bowerman, Deans Representative

Dedicated to my Mother, Father and Friends

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LIST OF ABBREVIATIONS

8-OHdG = 8-hydroxy-2'-deoxyguanosine
BCF = bioconcentration factor
BMF = biomagnification factor
BWt = body weight
CAFO = concentrated animal feeding operations
DI = daily intake
DNA = deoxyribonucleic acid
dw = dry weight
e = 2.71
FIR = food intake rate
GSSG = glutathione disulfide (reduced glutathione)
GSH = glutathione (oxidized glutathione)
HTD = human therapeutic dose
k = half-life elimination constant
LOAEL = lowest-observed-adverse-effect-level
lw = lipid weight
MDA = malondialdehyde
MDL = method detection limit
MDG= million gallons per day
PPCB = pharmaceuticals and personal care product
POCIS = polar organic chemical integrative samplers
PUFA = polyunsaturated fatty acids
ROC = region of concern
ROS = reactive oxygen species
$t_{1/2}$ = half-life
TBARS = thiobarbituric acid reactive substances
TEF= toxic equivalent factor
TEQ = toxic equivalent
ww = wet weight
WQLS = water quality limited segment
WWTP = wastewater treatment plant
US EPA = United States Environmental Protection Agency

CHAPTER 1

INTRODUCTION

The Osprey as a Sentinel

The Chesapeake Bay is the largest estuary in the United States and supports a diversity of avian species. At the same time, increasing human population and related activities in this region have resulted in deterioration of both water and habitat quality (Ernst, 2003; Phillips, 2007). Degradation of habitat quality from agricultural, industrial and urban pollution continues to impact the Chesapeake ecosystem, threatening sensitive wildlife species in vulnerable portions of the estuary, and adversely affecting fish and wildlife (e.g., Blazer et al., 2007; Rattner and McGowan, 2007; Iwanowicz et al., 2009).

Ospreys (*Pandion haliaetus*) have often served as sentinels of ecosystem health (Furness et al., 1993; Grove et al., 2009). The Chesapeake Bay is home to the largest nesting osprey population in the world (Poole, 1989; Watts et al., 2004; Watts and Paxton, 2007). This population has been extensively studied in the Bay since the 1970's (e.g., Wiemeyer, 1971; Henny et al., 1974; Wiemeyer et al., 1975; Watts et al., 2004). Golden and Rattner (2003) developed a system for ranking higher order terrestrial vertebrate species for their utility as a bioindicator and their vulnerability to environmental contaminants. Out of 25 vertebrate species, ospreys ranked third for their utility in monitoring persistent organic pollutants and fourth for their vulnerability to this group of compounds. Previous studies have used the osprey to monitor exposure and potential adverse effects of toxics on wildlife, document spatial and temporal ecotoxicological trends, and understand bioaccumulation and biomagnification of contaminants (e.g., Wiemeyer et al., 1975; Elliot et al., 1998, 2000, 2001; Henny et al., 2003, 2009, 2010; Rattner et al., 2004, 2008).

Ospreys provide an excellent index of ecohealth for their environment. Grove and colleagues describe ospreys as a species that are tolerant to short-term disturbance for

sample collection, opportunistically nest on artificial structures, and are adaptable to human landscapes. Pairs have nest-site fidelity and nests can be selected randomly for large-scale studies to document spatial patterns in contamination (Toschick et al., 2005). Nests are conspicuous and easily accessed on navigation markers, platforms, and other structures found in large bodies of water. Ospreys are strictly piscivorous and feed at a high trophic level, which makes them ideal for monitoring bioaccumulation and biomagnification of lipophilic organic contaminants in the food web. Although ospreys are long-lived, no relationship has been detected between age and body burdens of organochlorine contaminants (Ewins et al., 1999; Elliott et al., 2007). Taken together, the osprey is an ideal species for large studies to address questions of climate, ecotoxicology risk and population monitoring.

Contaminant accumulation in prey items directly relates to spatial variation in exposures at the nesting grounds. Ospreys typically spend one month on the breeding grounds before laying eggs. Lipid soluble compounds may accumulate in prey items, where the foraging adult female deposits ingested contaminants into the lipid rich eggs, thereby exposing the highly sensitive developing embryo. Nestlings are exclusively fed fish that the parents capture generally within 1-5 km of the nest site; however, locations with limited nestling nests may require birds to forage up to 15 km (Poole, 1989). Elliot and coworkers (1998) reported that polychlorinated dibenzo-*p*-dioxins (PCDDs) accumulated at several orders of magnitude higher in eggs downstream of pulp and paper mills compared to upstream nests, reflecting patterns in localized contaminant situations. Other less lipophilic contaminants (e.g., metals) are also readily detected in the blood and nestling's feathers (Rattner et al., 2008).

Exposure of Ospreys to Legacy and Emerging Contaminants and Health Effects

Legacy contaminants

Ospreys are sensitive to the effects of persistent lipophilic organic contaminants; most notably the metabolites of the organochlorine pesticide DDT (dichlorodiphenyl-trichloroethane). Exposure to the metabolite of DDT (*p,p'*-DDE, dichlorodiphenyldichloroethylene) resulted in population declines of osprey and other raptors during the 1960-70's due to eggshell thinning (Anderson and Hickey 1972; Wiemeyer et al., 1975, 1988). Following the ban of DDT in 1972, raptor populations across the Northeastern U.S. have shown significant recovery (e.g., Watts et al., 2004).

Even though concentrations of DDT, its metabolites and other organochlorine pesticides have declined in response to increased regulations, polychlorinated biphenyl (PCB) concentrations continued to remain elevated in the Chesapeake Bay (Wiemeyer et al., 1975, 1988; Rattner et al., 2004). Rattner and colleagues (2004), collected osprey egg samples from Chesapeake Bay Regions of Concern (ROCs) and found greater total PCB concentrations in osprey eggs collected in 2000 from Baltimore Harbor/Patapsco River and the Anacostia and Potomac Rivers (7.25 and 9.28 μg/g wet weigh, ww respectively), compared to the South River near Annapolis, MD (4.91 μg/g ww). These findings were similar to the 1972 data collected by Wiemeyer and colleagues (1975), who recorded mean PCB concentrations of 7.81μg/g ww, from randomly collected addled eggs along the Potomac River in Maryland. Similarly, black-crown night heron (*Nycticorax nycticorax*) eggs collected from the Fort Carroll colony in Baltimore Harbor (Rattner et al. 2001), were compared to eggs collected 7 years earlier at the Baltimore Gas & Electric Colony (Rattner et al., 1993). PCB concentrations decreased only slightly (3.83 μg/g ww

in 1991 vs. 3.40 µg/g ww in 1998). Interestingly, the magnitude of the most toxic coplanar congeners did exhibit a decline over time.

PCBs continue to be the principal driver of human fish consumption advisories for a large portion of the watershed including the ROCs. Fish species listed under consumption advisories (e.g., carp, *Cyprinus carpio* and catfish, *Ictaluridae sp.*) are consumed by high trophic level piscivorous waterbirds including ospreys and bald eagles (*Haliaeetus leucocephalus*) (MDE, 2014; VDH, 2013). As such, the presence of PCBs is likely to impact piscivorous colonial waterbird populations (Tillit and Giesy, 2012). Furthermore, gaining an understanding of the health effects of contaminants on wildlife populations has important implications for human health and is critical information for natural resource managers given the close interlocking relationship between ecosystem and human health (Harris and Jones, 2008).

PCBs are of concern for avian species, given a number of different health effects shown in both laboratory and field studies (Kubiak et al., 1989; Tillitt and Giesy, 2012). The toxicity of PCBs depends on the specific suite congeners in the mixture (Toxic Equivalency Quotient (TEQ) quantifying the potencies of PCB congeners to 2,3,7,8-tetrachlorodibenzo-para-dioxin, TCDD), and their induction patterns of EROD in hepatocyte cultures from a suite of species. Only a small subset of the most potent PCBs (77, 89, 126 and 169) can be directly embryotoxic (Kennedy et al., 1996). Hoffman and coworkers (1998) orally dosed kestrel nestlings with PCB 126 at 50, 250, and 1000 ng/g body weight. At the higher dosed groups, they documented decreased bone growth, thyroid lesions, increased EROD activity, endocrine, and immunological effects in the nestlings. However, if exposed to other congeners that may not be directly embryotoxic,

other subtle endocrine disruption effects (i.e., thyroid, cardiovascular or lipid impacts) may occur. In a similar manner, Fernie and coworkers (2003) reported developmental, behavioral, and endocrine-thyroid effects in maternally exposed American kestrel (*Falco sparverius*) hatchlings. Cardiovascular developmental effects have been reported in laboratory studies in embryonic Japanese quail (*Coturnix japonica*) and chickens (*Gallus gallus domesticus*) exposed increasing doses of an air-cell injected PCB congener mixture (Carro et al. 2013a). Specifically, heart abnormalities occurred across all treatments with elongation and expansion of heart tube, improper looping and indentations in the emerging ventricular wall during embryo development (and even ventricular thinning, dilation and absence of the trabeculated layer; Carro et al., 2013a, 2013b).

Polybrominated diphenyl ethers

Polybrominated diphenyl ether (PBDE) flame retardants have been detected in the eggs of avian species in both the Chesapeake and other estuaries worldwide (Chen and Hale, 2010). These compounds are additive (i.e., covalently bound) and chemicals can leach out into the environment and pose a hazard to wildlife. Despite the recent discontinuation of manufacture and use of the penta-BDE and octa-BDE formations in the U.S., and an agreement to phase out BDE-209 (the deca- formulation) by the end of 2012, PBDEs will continue to enter the environment from in-service and discarded products.

Rattner and colleagues (2004) measured eight PBDE congeners (BDE 28, 47, 49, 99, 100, 153, 154 and 183) in osprey eggs collected in 2000-2001. The Anacostia/middle

Potomac River study sites had significantly higher total PBDE concentrations (geometric mean 0.725 μg/g ww) compared to the South River (geometric mean 0.176 μg/g ww). The BDE-47 congener accounted for 65% (0.114 μg/g out of 0.176 μg/g) of the total found in the South River and 66% (0.480 μg/g out of 0.725 μg/g) in Baltimore Harbor/Patapsco River. On a spatial scale, high PBDE levels in bird eggs have been measured in industrialized regions and large urbanized compared to remote rural areas (Norstrom et al., 2002; Chen et al., 2008; Potter et al., 2009). Similar patterns in congener accumulation have been described for several additional piscivorous waterbird species, including the brown pelicans (*Pelecanus occidentalis*), and double-crested cormorants (*Phalacrocorax auritus*).

Total PBDE concentrations in herring gull (*Larus argentatus*) eggs in the Great Lakes increased exponentially over 17 years (1983-2000), with an estimated doubling time of 2.2-3.1 years (Norstrom et al., 2002). Park and colleagues (2009) found levels of PBDEs in California peregrine falcon (*Falco peregrinus*) eggs tripled with each decade from 1986-2007. This suggests that, PBDE levels could be increasing in Chesapeake Bay waterbird populations. In contrast, others have reported that PBDE concentrations are beginning to level off. Gauthier and coworkers (2008) examined data from herring gull eggs in the Great Lakes and found residues of PBDEs in eggs were beginning to level off post-2000.

In order to interpret the potential effects of PBDEs found in Chesapeake Bay osprey eggs, McKernan and coworkers (2009) conducted an egg injection study in which a suite of endpoints were examined across several different avian species including chickens, mallards (*Anas platyrhynchos*), and American kestrels. American kestrels were

found to be the most sensitive, with decreased pipping and hatching success reported at egg concentrations of 10 and 20 µg DE-71/g egg. Measurement of the amount of the injected PBDE that entered the egg contents from the injection site in the air cell indicated a lowest-observed-adverse-effect-level (LOAEL) of 1.8 µg/g ww (~32 µg/g lipid weight, lw; McKernan et al., 2009, 2010). Other studies also documented increased oxidative stress, delay in time to hatch, altered glutathione levels, changes in Vitamin A, and decreased thyroxine concentrations in nestling kestrels as well as altered courtship behavior in adults (Fernie et al., 2005, 2006, 2008; Rattner et al., 2013). Comparative toxicity studies indicated that mallard embryos and common tern (*Sterna hirundo*) appear relatively insensitive to PBDEs (McKernan et al., 2009; Rattner et al., 2013).

The LOAEL of 1.8 µg DE-71/g ww was approached by PBDE concentrations in eggs of several wild raptor species (Rattner et al., 2004, Chen et al., 2008, She et al., 2008, Henny et al., 2009). Henny and colleagues (2009) documented reduced reproductive performance in osprey pairs exposed to PBDEs. The number of young per occupied nest was reduced to 0.83 per nest with eggs containing over 1.0 µg/g PBDE ww compared to 1.43 young/occupied nest for eggs containing <250 ng/g PBDE ww. Although similar to the LOAEL reported by McKernan and coworkers, a more recent study (Henny et al. 2011), suggests ospreys can tolerate higher levels of PBDEs. Birds laying eggs containing >1.0 µg total PBDE/g ww did not show a significant relationship between PBDEs and reproductive success. Henny and colleagues (2011) suggested that additional monitoring is warranted to understand effects of PBDEs on reproductive success. There is no current evidence for large-scale population level effects from

PBDEs in wild birds (Chen and Hale, 2010) as have been documented with other contaminants, including DDT, methyl mercury and PCBs.

Additional brominated flame retardants

With the phase out of traditional PBDEs, the use of alternative brominated flame retardants (Alt-BFRs) has increased. These compounds include, α, β and γ hexabromo-cyclododecane (HBCD), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), di(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH), 2-ethylhexyl 2,3,4,5-tetrabromo-benzoate (TBB) and decabromodiphenyl ether (DBDPE). As with PBDEs, these BFRs are additive flame retardants that can leach into the environment and have been detected in many matrices including sewage (La Guardia et al., 2010). Measurable concentrations are also found in breast milk, food and household furniture (Roosens et al., 2009; Stapleton et al., 2009) as well as in peregrine falcon eggs (Guerra et al., 2012). HBCD is produced in high volumes for use in polystyrene foams, upholstered furniture and textiles, and electrical appliances (US EPA, 2010). HBCD ranks third in production to TBBPA and deca-BDE with 16,700 metric tons produced per year (Morose, 2006).

Among the replacement flame-retardants, the commercial HBCD formulation was tested for effects in reproductively active American kestrels (e.g., Marteinson et al., 2011, 2012). Birds received a dietary dose of 0.51 µg HBCD per g kestrel for 75 days; resulting in the dosed birds having enlarged testes, elongated spermatids, a moderate increase in plasma testosterone, reduced free and total T_4, and reductions in courtship behaviors of male and female dosed kestrels compared to the controls. Other flame retardants including BTBPE (a replacement for octa-BDE) and DBDPE have been

detected in eggs from glaucous gulls (*Larus hyperboreus*) in the Arctic (Verreault et al., 2007) and in some peregrine falcon eggs (Guerra et al., 2012).

Metals

Many metals are found in the Chesapeake Bay region, often below the thresholds for adverse effects for waterbird populations. These levels of metals have not been associated with population declines in ospreys and bald eagles (Rattner and McGowan, 2007; Rattner et al., 2008). Golden and coworkers (2003) found higher concentrations of lead (Pb) in the feathers of black-crowned night-herons in samples from Baltimore Harbor compared to herons nesting at a remote island site (Holland Island, Maryland) in Chesapeake Bay. Nestling ospreys showed a similar pattern in their feather concentrations of Pb (Rattner et al., 2008). Interestingly, Pb concentrations were slightly elevated in Baltimore Harbor, compared to the South, West and Rhode River reference sites; whereas significantly greater concentrations in blood and feathers occurred in the Elizabeth River.

Cadmium (Cd) is another metal of potential concern as it has been detected in livers of waterfowl including mute swans (*Cygnus olor*) and long-tailed ducks (*Clangula hyemalis*) at concentrations up to 94 μg/g and 81 μg/g dry weight respectively (Beyer et al., 1998). However, the threshold of toxicity for Cd has not been established for waterbirds; additional information would be needed to assess any adverse effects. Total Maximum Daily Loads (TMDLs) have been established for metals, but have yet to be related to effects on wildlife. In summary, heavy metals (e.g., Cd, Hg, Pb) have been detected in osprey nestling blood in ROCs; however, concentrations were well below

toxicity thresholds and, as such, did not adversely affect nestling survival, and therefore may not be a primary concern for fish eating birds.

Pharmaceuticals and personal care products

The application of newer analytical technologies that allow the detection of picogram quantities of contaminants has revealed the presence of over 600 pharmaceuticals and personal care products (PPCPs) compounds in a variety of environmental matrices including sediments, sewage sludge, biosolids, drinking water and fish tissue (Brooks et al., 2005; Alavarez et al., 2009; Iwanowicz et al., 2009; Ramirez et al., 2009; Küster and Adler, 2014). The term "PPCP" encompasses veterinary, over the counter and illicit human drugs, and personal care products including cosmetics, food supplements and fragrances, as well as their respective metabolites (Daughton and Ternes, 1999; US EPA, 2009). With this in mind, it is quite possible that these compounds have been continuously entering the environment for many years without notice via somewhat unregulated sources (e.g., WWTP effluent, improper disposal of expired drugs, flooding events and veterinary drugs in concentrated animal feeding operation (CAFO) run-offs) resulting in potential chronic exposure and accumulation across a range of organisms (Nikolaou et al., 2007). Exposure to a mixture of PPCPs could result in synergistic impacts, potentially compromising the health of fish and wildlife (Bean et al., 2014).

A recent study investigating the occurrence of PPCPs in fish and liver samples from five streams near sewage treatment plant effluent discharge sites revealed measurable concentrations of antidepressants including fluoxetine (Prozac®), its

metabolite norfluoxetine, and sertraline (Zoloft®) at 3.2-4.0 ng/g in fish and 19-70 ng/g in liver, respectively (Ramirez et al., 2009). Other pharmaceuticals detected in fish fillet and liver samples included the antihistamine diphenhydramine used in Benadryl®, the antihypertension medication diltiazem, the antiseizure medication carbamazepine and the anitilipemic gemfibrozil (Ramirez et al., 2009). Although pharmaceuticals have been detected in water bodies at low levels, Kolpin and coworkers (2002) suggest that these compounds are not removed by traditional wastewater treatment and are resistant to degradation, with the potential of degrading into more persistent compounds.

Of particular concern is exposure of non-target organisms to these pharmaceuticals with unknown effects from low-level chronic exposure. Much of the focus on aquatic organisms (e.g., Brodin et al., 2014; Du et al., 2014), has recently shifted to wildlife species and bioaccumulation across trophic levels in higher order vertebrates (Arnold et al., 2013; Shore et al., 2014). Current questions include the behavioral effects of these pharmaceuticals in birds. Most well-known and widely studied is the case study of Asian vultures that were poisoned after feeding on carcasses of cattle that had been fed diclofenac (Oaks and Watson, 2011). This is the only well-known instance for a veterinary pharmaceutical having a population effect on wildlife. Little is known about the occurrence of these compounds in wildlife in the Chesapeake Bay. Their presence, adverse effect levels and potential for food web transfer in wildlife have yet to be established.

Transfer of Contaminants in the Water-Fish-Osprey Food Web

Ospreys are unique in that they feed at a high trophic level, and represent the fate of bioaccumulated and biomagnified persistent lipophilic organic contaminants in the aquatic ecosystem. Biomagnification studies integrate the source of prey (fish) with the predator (osprey) and allow us to follow a contaminant (or its metabolites) though the food web. By identifying individual prey species specific to salinity and geographic location, we can closely reconstruct the fish-osprey food web. Biomagnification factors (BMFs) can help predict exposure of high risk or endangered species to contaminants (Henny et al., 2003). Henny and coworkers calculated BMFs from whole fish to osprey eggs in 1993 and 2001 (Henny et al., 2003, 2009) using the fish osprey food web on the Columbia River (total PCBs BMF: 12-13 lw, organochlorine pesticides (e.g., *p, p*'- DDE BMF: 112-113 lw), and mercury (Hg) BMF: 0.60 dw). BMFs were also calculated for PCDDs (e.g., BMF: 20 lw) and polychlorinated dibenzofurans (PCDFs) (e.g., BMF: 18 lw). In 2009, Chen and coworkers (2009) calculated BMFs for total PCB congeners (BMF 23.9 lw), total PBDEs (BMF: 25.1 lw) and *p,p'*-DDE (BMF: 18 lw) in the James River fish-osprey food web. Chen and coworkers (2010) noted that a BMF greater than 5 indicates that there is a potential for the contaminant to biomagnify up the food web.

Oxidative DNA Damage

In the toxicological literature, there are several prominent classes of xenobiotics that increase reactive oxygen species (ROS) in cells. These compounds have damaging effects, impair cellular defense mechanisms (Mitchelmore and Chipman, 1998) and exacerbate the production of free oxyradicals. Electrophilic oxyradicals can bind to nucleophilic sites on molecules including DNA, lipids and proteins leading to production

of harmful products including intermediates that can bind to the DNA to form adducts. Polyunsaturated fatty acids (PUFAs) are most susceptible to ROS since H atoms can easily be abstracted from C-H bonds. As with redox cycling compounds, attack of lipids by ROS initiates a chain reaction. As the lipid hydroperoxides (polyunsaturated fatty acids) break down, they form malondialdehyde (MDA) (Hoffman et al., 2011), which is carcinogenic in its own right. It can react with the DNA bases deoxyguanosine and deoxyadenosine (Marnett, 1999). In Oakes and Van Der Kraak (2003), increases of thiobarbituric acid (TBARs) activity was measured in white suckers (*Catostomus commersoni*) residing downstream from pump mill effluent discharge, indicating that redox active compounds were present in this toxic chemical mixture.

Oxidation of guanosine (8-hydroxy-2'-deoxyguanosine, 8-OHdG) can be used as a sensitive biomarker of oxidative injury to DNA bases and occurrence of DNA damage. In the literature, one widely used assay to detect DNA strand breaks and damage across a variety of taxa is the comet assay (i.e., single-cell gel electrophoresis) described by Singh and coworkers (1988). When DNA is damaged, the disruption results in the appearance of a "tail" similar to a comet trailing in the gel. The head of the comet represents intact and undamaged DNA. A 1998, a spill releasing toxic mine waste containing high levels of heavy metals (Ar, Cu, Pb, Zn) occurred in western Spain. A series of studies conducted by Pastor and coworkers (2004) documented genotoxic effects of metals on local bird populations near the spill. Blood samples from white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) collected over four years showed that birds from contaminated sites had consistently higher tail moments compared to controls. An increasing pattern of genotoxic damage was observed throughout the four subsequent

years of monitoring. High genotoxic damage was also observed in nestlings in follow up studies indicating potential multi-generational long-term consequences and possibly heritable damage to DNA (Pastor et al., 2004).

In the Chesapeake Bay watershed, Pinkney and coworkers (2004a) described fish, particularly the brown bullhead (*Ameiurus nebulosus*) living in highly polluted areas (i.e., in proximity to combined sewer overflows) on the Anacostia River, as having large skin tumors containing high levels of PAHs. Further DNA adducts were observed from isolated DNA from fish livers through a ^{32}P post-labeling assay. PBDEs have an oxidative mechanism of action and evoke mild oxidative stress in American kestrels (Fernie et al., 2005), observed as DNA damage in common tern and kestrel hatchlings (Rattner et al., 2013). Consistent with other endpoints examined and previous work (e.g., McKernan et al., 2009), DE-71 treated kestrel hatchlings had significantly higher levels of hepatic concentrations of oxidized glutathione (GSSG) and also 8-OH-dG; however, instead of depleting reduced glutathione (GSH), as was observed in other studies (e.g., Fernie et al., 2005), a slight increase was observed in GSH (Rattner et al., 2013). To complement the assays for glutathione status, hepatic thiols, which protect against the effects of oxidative stress and lipid peroxidation, a manifestation of oxidative damage, were measured (Hoffman et al., 2005). Although no statistically significant differences were observed, total sulfhydryl, protein bound sulfhydryl, and TBARS concentrations were slightly elevated in treated kestrels. This supports the contention that exposure conditions induced mild oxidative stress in kestrels and may evoke similar effects in other species. Other studies have reported DNA damage after exposure to the BDE-47

and BDE-99 congeners in rats (e.g., He et al., 2008; Albina et al., 2010; Pellacani et al., 2012) which may lead to epigenetic effects in later populations.

Chesapeake Bay Regional Waterways of Concern

Anacostia/middle Potomac River

The Potomac River watershed encompasses four states and drains 37,995 km^2. Stretches of the Potomac in District of Columbia, VA and MD have segments listed on the 303d list as impaired waters listed under the Clean Water Act. In these listed water bodies, the current pollution controls are insufficient to maintain water quality standards (US EPA, 2012a, 2012b). Due to the listing, a TMDL (total maximum daily load) for toxics such as PCBs have been established for most of the Anacostia and middle Potomac Rivers (Haywood and Buchanan, 2007). The TMDL sets a maximum loading amount for PCBs on the river and includes inputs from both point and non-point sources in its allocations. On the Potomac, PCBs enter the river through a combination of both point and non-point sources, with most of the inputs coming from direct drainage from areas adjacent to the river (Haywood and Buchanan, 2007). Inputs of PCBs can be attributed to direct discharge from WWTPs (e.g., Blue Plains) and power plants, as well as non-point sources flowing in from the Anacostia's combined sewer overflows, landfills, and re-mobilization events where contaminated sediments are transported further down river. These latter non-point sources are most difficult to regulate and may offer a continued input of sediment bound toxics into the food web. As the river flows out of the District, land use changes to primarily suburban uses (Interstate Commission on the Potomac River Basin 1997). Consequences of chemical input can be reflected in fish health in this

watershed. Reproductive data for fish indicates reports of intersex in male smallmouth bass (*Micropterus dolomieu*) near wastewater treatment effluent point sources (Blazer et al., 2007; Iwanowicz et al., 2009).

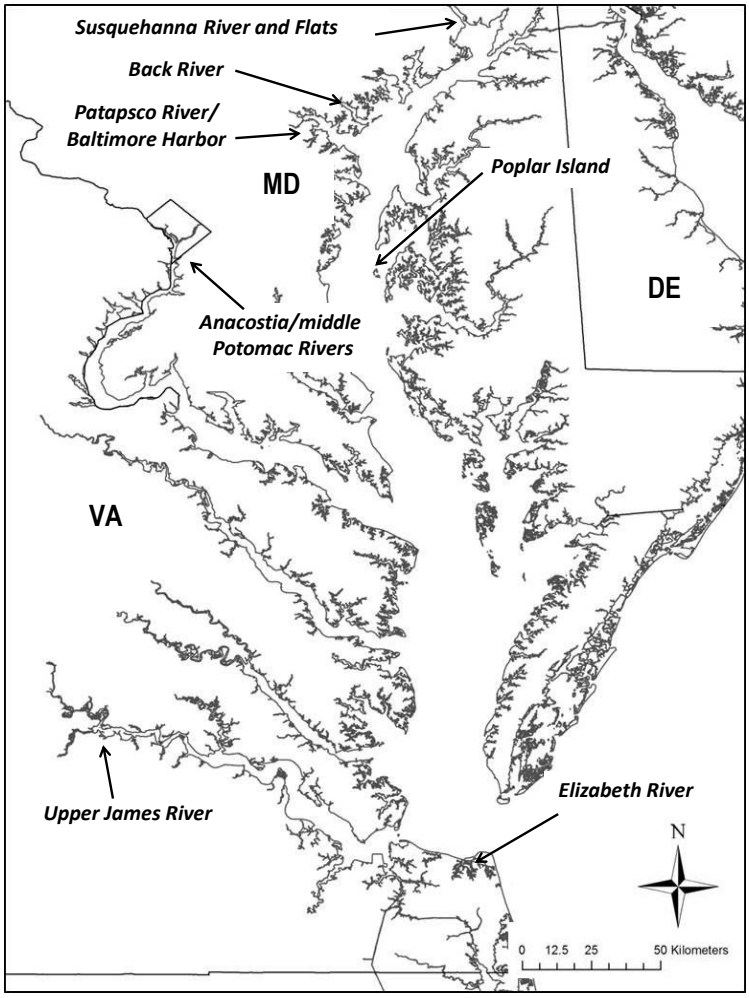


Figure 1. Map of study sites in Chesapeake Bay (including Chesapeake Bay Regions of Concern).

Prevalence of tumors in brown bullheads, increased cytochrome P450 activity, increased bile PAH metabolites and elevated concentrations of organochlorine contaminants have been documented in the tidal portions of the Potomac River, including Quantico, and the Anacostia River (Pinkney et al., 2001, 2004a). These effects indicate

the presence of a suite of redox active contaminants that produce ROS, which affect fish populations. Polar organic chemical integrative samplers (POCIS) detected galaxolide (955 ng/POCIS), tonalide (515 ng/POCIS) and other emerging contaminants at the Blue Plains and Conococheague downstream study sites (e.g., Iwanowicz et al., 2009). Collaborator Vicki Blazer and coworkers have conducted a significant amount of research on fish health on the Potomac, particularly near Blue Plains WWTP; however, the extent to which the pharmaceuticals are bioaccumulating in the food web and their effects on wildlife are unknown. Legacy contaminants continue to drive fish consumption advisories on the Potomac. Subsequently, the investigation of the transfer of compounds in the fish osprey food web and their impact on osprey productivity and health is warranted (MDE, 2009).

Since last reported in 2000-2001, levels of total PBDE congeners in the Anacostia and middle Potomac River were approaching observed effect thresholds (Henny et al., 2009, McKernan et al., 2009). The highest residues of total PBDEs in osprey eggs on this tributary were 928 ng/g ww. This value approaches the LOAEL for pipping and hatching success in American kestrels reported by McKernan and coworkers (2009) of 1.8 µg/g ww. As mentioned earlier, other adverse effects of PBDEs in kestrels (the laboratory raptor model) include changes in reproductive behavior in adults, increased oxidative stress, and decreased thyroxine concentrations (Fernie et al., 2005, 2006, 2008). Evidence of increased sensitivity of kestrels to PBDEs has also been documented in McKernan et al., 2009, where reduced pipping and hatching success as observed at the highest injection dose and in Rattner et al. (2013), in which significant effects were

reported for increased oxidative stress and DNA damage endpoints in American kestrel embryos.

Baltimore Harbor/Patapsco River and Back River

During the DDT era, there were no ospreys nesting in Baltimore Harbor/Patapsco River and few pairs in 2000-2001 (Henny et al., 1974, Rattner et al., 2004). The United States Environmental Protection Agency (US EPA) categorized this tributary as an ROC due to poor water quality conditions and elevated concentrations of pesticides, PCBs and metals (Chesapeake Bay Program, 1994). With the banning of DDT and other compounds, osprey populations rebounded and now have moved into some of the most highly polluted areas of the Bay including Baltimore Harbor (Rattner et al., 2004, Watts et al., 2004). Egg samples showed that *p,p*'-DDE and other organochlorine pesticide levels were decreasing in these areas, while PCB levels remained unchanged. Fish consumption advisories recommend avoiding several major fish species due to PCB contamination including channel and white catfish (*Ictalurus punctatus* and *Ictalurus catus*), carp, and white perch (*Morone americana*) (MDE, 2009). These high concentrations of PCBs may be indicative of a suite of potential adverse effects mentioned in the earlier section including endocrine-thyroid, behavioral, developmental and cardiovascular effects in developing embryos and nestlings that solely feed on fish caught in highly contaminated areas in Baltimore Harbor. The sediments in Sparrow's Point/Bear Creek area in Baltimore Harbor and the Inner Harbor itself remain heavily contaminated (McGee et al., 1999). Due to the large increase in the osprey population, birds are now nesting in these areas. Surveys conducted in 2011 in Baltimore

Harbor/Patapsco River revealed that at least three osprey pairs are now nesting in Bear Creek where only one pair was observed in the 2000-2001 study (Rattner et al., 2004).

In nearby Back River, elevated sediment PBDE concentrations were detected at Cox's Point. Back River receives effluent from the state's largest WWTP. At Cox Point, total BDE levels were 4,735 ng total BDE/g dw and the BDE-209 congener made up 93% of the total (Klosterhaus, 2007). Few ospreys have been reported nesting in Back River, but the pairs that do may be at risk of exposure to high levels especially since many of the PBDE congeners can biomagnify at all levels in the food web (Klosterhaus, 2007; Chen et al., 2009). As seen on the Potomac River, values of PBDE congeners were high near the Blue Plains WWTP at levels that may have the potential to exert adverse effects, not just on avian survival, but also induce sublethal effects including oxidative DNA damage and behavioral effects (e.g., Fernie et al., 2006, 2008, 2009; Rattner et al., 2013).

Elizabeth River

The Elizabeth River was listed as an US EPA Chesapeake Bay ROC in the 1990's due to issues with toxic contamination and poor water quality and currently is identified in the Chesapeake Bay Executive Order as an area of concern (Chesapeake Bay Executive Order, 2009). In 1973, when the osprey population was surveyed at 1,450 pairs of nesting birds, only two pairs were located in the James River and nearby tributaries (Henny et al., 1974; Rattner et al., 2004). The Elizabeth is the location of the largest naval shipyard in the United States and also many manufacturing plants; thus, the Elizabeth faces significant pollution and sewage discharge issues. In fact, as far back as 1983, the US EPA singled out the Elizabeth as the most highly polluted river in the Bay

Watershed (Elizabeth River Project, 2008). Its highly contaminated toxic sediment has resulted from major creosote spills (Atlantic Wood Industries Inc.). In addition, it also contains high concentrations of PAHs, metals and PCBs (US EPA, 1994). Both histopathological lesions and the increased presence of DNA adducts have been reported in mummichog (*Fundulus heteroclitus*) populations in this area (Rose et al., 2000). DNA adducts are a characteristic effect of PAH exposure and are indicative of a high cancer rate that was found in fish (e.g., identified by the presence of skin ulcerations and preneoplastic lesions). Fish consumption advisories remain in effect for PCBs and dioxins. These advisories warn against eating a suite of fish species including gizzard shad, carp, and several catfish species (VDH, 2009).

Many of these species are readily consumed by of the osprey (Glass and Watts, 2009) and the birds nesting in these areas are consuming PCB contaminated fish on a regular basis. As discussed above, there is a wide range of many sublethal effects of PCBs and developing embryos and nestlings in this area may be at risk. Other species in the area exhibit increased sublethal effects such as the DNA damage in mummichung. Many efforts have been underway to clean up the Elizabeth River and restore some of the most highly contaminated areas. Recently, the Elizabeth River Project has initiated efforts in areas on the South Branch, such as Money Point, where sediments have been dredged and wetlands and oyster reefs are now restored (Elizabeth River Project, 2008). Interestingly, a decade ago there were only a few pairs of osprey on the South Branch, a sign of the severe ecosystem degradation. The presence of successful nesting pairs, their contaminant burden and health of ospreys nesting in these degraded areas will serve as sentinel of the health of the entire ecosystem.

James River

The James River flows entirely through the state of VA and serves as a major tributary to Chesapeake Bay. The Chesapeake Bay Executive Order called for ongoing monitoring efforts to understand the extent of contaminants on the James River. Historically, chlorine was responsible for two major fish kills in 1973 and 1974. Subsequently, the larger James River and Boat Harbor Plants were identified as potential sources of wastewater contaminants along with the smaller Fort Eustis and Williamsburg plants (Bellanca and Bailey, 1977) further downstream. Upriver, there are inputs from the Richmond WWTP, Falling Creek WWTP, Proctor's Creek WWTP and Hopewell WWTP. The James River has a large osprey population that has been well-studied (Watts et al., 2004). Osprey nests can become very dense and plentiful especially in the high productivity areas near Hopewell and further upriver near Presquile National Wildlife Refuge. The suite of WWTPs on the James River has the potential to be hotspots for PBDEs (Chen and Hale, 2010) to enter the food web and exert potential adverse effects on wildlife as discussed for the Anacostia/middle Potomac River.

Along with inputs of nitrogen and phosphorous, the James River health was compromised by Kepone® contamination from its manufacturing source in Hopewell as early as the mid 1960's and was followed by the decline of nesting ospreys in this area (Kennedy, 1977). Luellen and coworkers (2006) reported overall concentrations of the insecticide Kepone® were decreasing in fish, but samples still contained detectable levels. Sediment burial continues to be the primary source of removal of Kepone® from the environment; however, turbidity and sediment re-suspension could continue to result in hotspots of contamination. In 2001, osprey egg samples collected off of Craney Island

in the Elizabeth River were analyzed for Kepone®, but this insecticide was not detected (Rattner et al., 2004).

Although Kepone® advisories are still in effect, the fish consumption advisories are primarily driven by PCBs (Harris and Jones, 2008; VDH, 2009). The highest levels of PCBs were detected in carp and blue catfish in Richmond, Hopewell and Bailey's Creek, VA, all exceeding thresholds for suitable fish consumption set by the US EPA. A number of sewage treatment plants are clustered around the major cities (Richmond, Falling and Proctor's Creek, Hopewell, Williamsburg and Norfolk). At Richmond, the river is highly industrialized and narrow, until below Hopewell where the predominant land use is for agriculture. Much of this area is listed as a priority agriculture watershed for inputs of both nitrogen and phosphorous (Wolf, 2009). Although the osprey population has been studied extensively on the James River (e.g., Glass and Watts, 2009; Watts et al., 2004), there is limited contaminant information on legacy contaminants, replacement brominated flame-retardants (e.g., HBCD, TBBH, and TBB), and no information the occurrence of pharmaceuticals in wildlife.

Lower Susquehanna River and flats

As the largest tributary to the Chesapeake, the Susquehanna empties into the northern Bay and provides 50% of the freshwater input. The river flows down from New York (NY), Pennsylvania (PA) and Maryland (MD), encompassing a drainage basin of 71,200 km^2 (Susquehanna River Basin Commission, 2006). Human activities continue to contribute toward habitat loss and degradation. The basin yields some of the most productive and heavily used agricultural lands, and transports 66% of the nitrogen and

40% of phosphorous non-point nutrient inputs into the Bay on average per year, which directly affect habitat and water quality for fish and wildlife (Langland, 1998). In addition, flooding, storm water run-off and effluents from urban, agricultural and industrial sources contribute 25% of sediment loads to the Bay. In 1996, the lower river basin was placed on Maryland's 303d list for nutrients, sediments, Cd and PCBs (MDE, 2005). Although there are historic data related to health, contaminant exposure and associated hazards for fish in Chesapeake Bay (Pinkney et al., 2001, 2004a, 2004b; Blazer et al., 2007), considerably less is known about waterbirds particularly in northern tributaries (Rattner and McGowan, 2007). Due to the distribution of osprey nests on the Susquehanna in 2013, sampling efforts were concentrated at the mouth of the Susquehanna and the Susquehanna flats. The Susquehanna flats are located at the northernmost region of the Bay (Havre de Grace, Maryland).

Purpose of this Research and Scientific Objectives

In 2000, the Chesapeake Executive Council Toxics Strategy highlighted the ongoing toxic contamination issues US EPA ROCs and called for ongoing efforts for monitoring, clean up and remediation by 2010 (Chesapeake Bay Program, 2000). Though progress has been made, goals have not been met. Therefore in May 2009, the Chesapeake Bay Executive Order 13508 (Chesapeake Bay Executive Order, 2009) was signed into action, and placed an expanded emphasis on the restoration and protection of the Bay and its biota. Specifically, contaminants are addressed under goals developed to restore clean water in an effort to reduce contaminant exposure to fish and wildlife. Under this Executive Order goal, the US EPA, Department of the Interior (DOI) and the National Oceanic and Atmospheric Administration (NOAA) are called on to work with

state and local governments in understanding the extent of the contaminant issues in the Bay and also develop reduction goals by 2013 along with strategies for reducing contaminants by 2015. New threats will also be addressed including emerging contaminates in 2011 and 2012. In order to address goals and outcomes laid out in the Executive Order, the U.S. Geological Survey (USGS) in collaboration with other organizations has expanded its research and monitoring activities to assist Federal and State management agencies, and nongovernment organizations to improve the health and quality of the Bay.

This project will increase our scientific knowledge of the occurrence and effects of contaminants in fish and wildlife populations in two major tributaries of concern identified by the Executive Order (Potomac and James Rivers) with continued monitoring of the Chesapeake Bay ROCs (Figure 1). Summary data from the Chesapeake Bay Program indicate that waters in these tributaries are impaired by PCBs, metals and unidentified toxics (Weinberg, 2010). Sampling will also include the Susquehanna River and flats, at the mouth of the largest tributary to the Chesapeake Bay, where limited contaminant exposure data on wildlife has been collected (Figure 1). Although fish-eating birds have been used in previous Chesapeake Bay ecotoxicological studies, there are limited contaminant exposure records for Chesapeake Bay in the "Contaminant Exposure and Effects – Terrestrial Vertebrates" (CEE-TV) database for these locations since 2000-2001 and no information on the occurrence of pharmaceuticals and personal care products (Rattner and McGowan, 2007). A decade has elapsed since the last large-scale ecotoxicological study of Chesapeake Bay wildlife was undertaken. Bioaccumulative replacement flame-retardants are being produced in large-volumes and

have shown potential reproductive effects in American kestrels (Martinienson et al., 2011, 2012). They have also shown up in a suite of biological matrices (Covaci et al., 2010). Pharmaceuticals and personal care products have been detected in water and fish samples from wastewater effluent dominated streams including the Potomac River (Iwanowicz et al. 2009); however, it is unknown to what extent they bioaccumulate through the food web and are bioavailable to piscivorous species.

In this study ospreys were used as a sentinel to monitor a suite of regional waterways and tributaries to Chesapeake Bay where there are limited ecotoxicological data for wildlife, or locations that are hotspots for contaminants and have been historically impaired and identified by the US EPA as ROCs. Ospreys have been well studied in the Chesapeake Bay, and this work will help us understand patterns in contaminants over time across a large spatial scale and help managers identify new contaminants that may be entering the food web which are important from both a human and wildlife health perspective. Our studies focused on the spatial and temporal trends of contaminant exposure and effects on ospreys in Chesapeake Bay and placed both historical and emerging contaminants into a food web perspective.

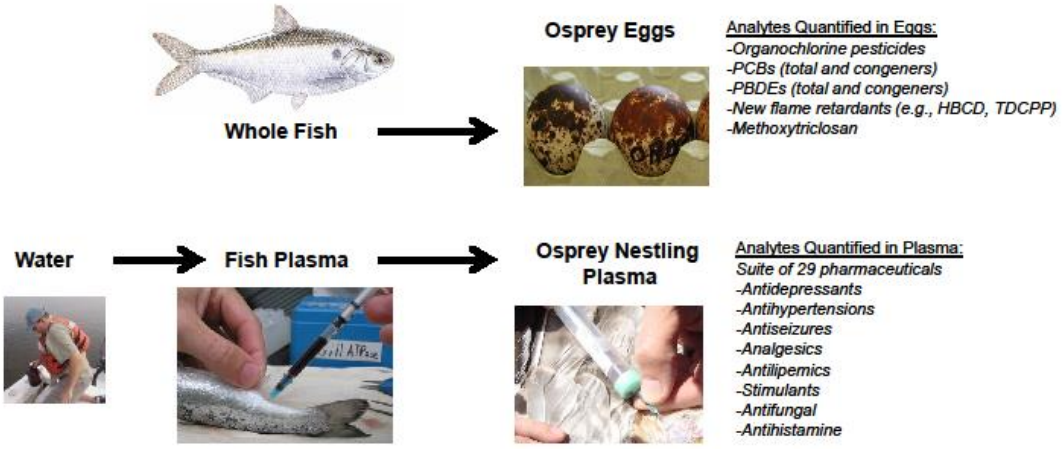


Figure 2. A schematic of food web transfer matrices and overview of the major groups of contaminants quantified in the following studies.

1. The first study, "Decadal re-evaluation of contaminant exposure and productivity in ospreys nesting in Chesapeake Bay Regions of Concern" had two main objectives:

    a) Measured contaminants in osprey egg samples, and conducted morphological (culmen length and nestling body weight), reproductive endpoints to assess their relationship to contaminant exposure in Chesapeake Bay ROCs.

    b) Looked at spatial and temporal trends in osprey productivity and contaminant exposure in Chesapeake Bay ROCs.

2. The second study, "Chesapeake Bay fish-osprey food web: contaminant exposure and potential genetic damage" had four objectives:

    a) Reconstructed osprey dietary preferences to account for variation in dietary exposure in order to relate contaminant concentrations of organochlorine pesticides PCBs, and PBDEs in whole fish to osprey eggs. This was done by calculation of biomagnification factors (Figure 2) and a comparison of the calculated factors to others found in the literature.

    b) Measured oxidative DNA damage in osprey nestlings across all study sites as a biomarker of contaminant effects.

    c) Conducted an analysis investigating the relationships between contaminants and stable isotopes by site to DNA damage.

3. The third study, "Exposure and food web transfer of pharmaceuticals in ospreys: predictive model and empirical data" had two main objectives:

a) Conducted a screening-level exposure assessment for 117 pharmaceuticals in ospreys.

b) Analyzed 23 pharmaceuticals and an artificial sweetener in the water-fish-osprey food web (Figure 2).

The following chapters describe a series of three studies conducted to evaluate contaminant exposure and effects in ospreys nesting in Chesapeake Bay. The first study described in Chapter 2 consisted of a decadal re-evaluation of contaminants in Chesapeake Bay ROCs, spatial and temporal patterns in contaminant exposure and potential effects at the genetic, individual and population levels. The next chapter (Chapter 3) described a study investigating contaminant exposure from a food web perspective. Here we conducted an integrative sampling scheme and calculated biomagnification factors from fish to osprey for both legacy and emerging contaminants. This study also evaluated effects at a genetic, individual and population level (looking at changes in DNA damage, body weight, culmen length and overall osprey productivity). The final study (Chapter 4) evaluated a suite of 23 pharmaceuticals and an artificial sweetener (sucralose) in the water, fish osprey food web. A screening-level risk assessment was conducted to predict those compounds, which may reach the human therapeutic dose in a single day or over a 40-45 day nestling period. This assessment was complemented by empirical measurements of pharmaceuticals in water, fish and osprey nestling plasma samples from several tributaries in the Bay.

CHAPTER 2

A DECADAL RE-EVALUATION OF CONTAMINANT EXPOSURE AND EFFECTS ON OSPREYS (*Pandion haliaetus*) NESTING IN CHESAPEAKE BAY REGIONS OF CONCERN

Introduction

The Chesapeake Bay is the largest estuary in the United States and supports a diversity of avian species. Degradation of habitat quality by a mixture of agricultural, industrial and urban pollution continues to threaten the most vulnerable portions of the estuary, and jeopardize fish and wildlife health. Globally, the largest nesting osprey (*Pandion haliaetus*) population is found in the Chesapeake Bay, which has been nicknamed the "osprey garden of the world" (Poole, 1989). Ospreys are used as ecological sentinels of environmental health due to their high trophic level, widespread distribution and nest-site fidelity (Grove et al., 2009; Henny et al., 2010).

The Chesapeake Bay osprey population has been extensively studied since the 1970s. In 1973, the osprey population was estimated to be only 1450 nesting pairs, and only seven pairs were observed north of the Chesapeake Bay Bridge (near Annapolis, MD) to the Susquehanna River (Henny et al., 1974). With the banning of the pesticide DDT in 1972, the osprey population rebounded both numerically and spatially. A bay-wide survey in 1995–1996 estimated 3500 nesting pairs, and population growth was rapid in the tidal freshwater tributaries and areas north of the Chesapeake Bay Bridge (Watts et al., 2004).

For decades, impaired water quality and toxic chemicals in sediment, water and biota have been found in highly industrialized and urbanized areas of Chesapeake Bay including Baltimore Harbor and the Anacostia and Elizabeth Rivers. These three sites have been designated by the U.S. Environmental Protection Agency (US EPA) as Regions of Concern (ROCs) (US EPA, 1994). Rattner et al. (2004) reported that osprey productivity in these regions was marginally adequate to sustain local populations.

Although concentrations of *p,p*'-DDE and other organochlorine pesticides declined in eggs, PCB levels remained elevated especially in Baltimore Harbor compared to reference sites. Polybrominated diphenyl ethers (PBDEs) were detected in osprey eggs from all sites, with values being some of the greatest found in North America. All ROCs continue to have human health advisories on the consumption of several fish and shellfish species due to contamination with PCBs and other pesticides (VDH, 2009; MDE, 2014).

In May 2009, the Chesapeake Bay Executive Order 13508 was signed, which placed emphasis on continued monitoring of ROCs and the restoration and protection of the Bay (Chesapeake Bay Executive Order, 2009). Just over a decade has elapsed since the last large-scale ecotoxicological monitoring study of Chesapeake Bay wildlife, during which time there were limited exposure data for Bay avifauna (Rattner and McGowan, 2007; Chen et al., 2010; US EPA, 2012a). As part of a larger study examining contaminant exposure, food web transfer, and potential effects on ospreys in Chesapeake Bay, the three historical ROCs were re-visited to examine temporal and spatial changes in osprey productivity and concentrations of legacy and more contemporary pollutants.

Materials and Methods

Study sites

Ospreys nesting on navigational markers, platforms, duck blinds, and other accessible structures were sampled during their nesting seasons from March through July of 2011 and 2012. Sampling was conducted in Chesapeake ROCs including (i) Baltimore Harbor/Patapsco River in MD (2011, n = 7), (ii) the Anacostia/middle Potomac Rivers in parts of Washington, D.C., Maryland, and Virginia (2011, n = 9), and (iii) the Elizabeth

River in Virginia (2012, n = 6) (Figure 3). These sites encompassed urban and industrial gradients along a 20–25 km stretch of each river. The Paul S. Sarbanes Ecosystem Restoration Project at Poplar Island, MD (hereafter, Poplar Island), a remote mid-Bay location, was used as a reference site (2011–2012, n = 4 eggs/year). Notably, results from a 2010 common tern (*Sterna hirundo)* egg collection at Poplar Island indicated low levels of organochlorine pesticides, non-coplanar PCBs, and PBDEs (Rattner et al., 2013), making it a more suitable reference site than the South, West and Rhode Rivers that were used in 2000–2001 (Rattner et al., 2004). In 2011–2012, nests in ROCs were strategically selected near those nests previously studied. All procedures involving ospreys were conducted under approval of the Institutional Animal Care and Use Committees of the USGS-Patuxent Wildlife Research Center (USGS-PWRC) and the University of Maryland, and with appropriate Federal and State scientific collection permits.

Egg sample collection

Using the sample egg technique described in Blus (1984), osprey eggs were collected for residue analysis (n = 30 total). After each clutch was complete (three or more eggs), a random egg was sampled within a week for subsequent contaminant analysis. Eggs were transported to the USGS-PWRC, cleaned with distilled water, weighed and their length and breadth were measured to the nearest 0.01 mm. A 2.4-mm hole was drilled into the blunt end of the egg (Dremel® MultiPro® 7.2 V, Model 770, Mt. Prospect, IL, USA).

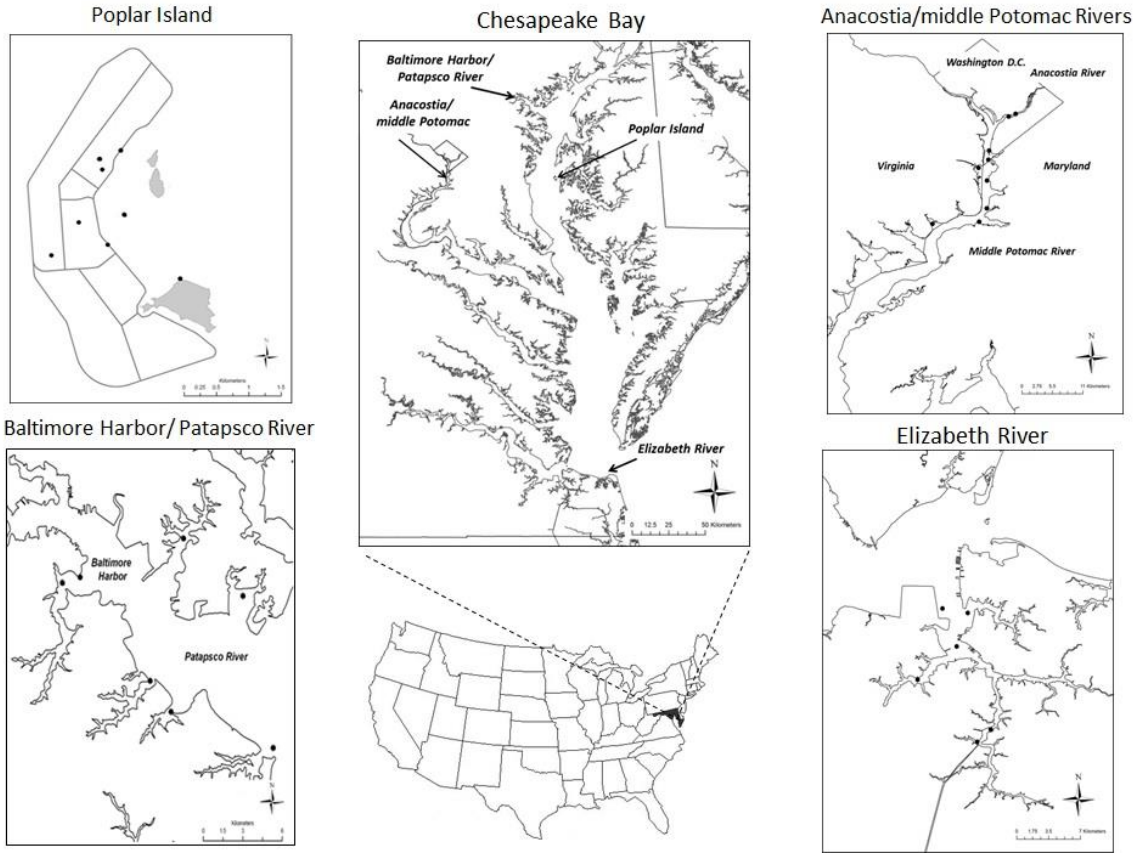


Figure 3. Locations of sample nests in Chesapeake Bay Regions of Concern and Poplar Island reference site, (●) indicates a sampled nest.

Moisture loss during incubation concentrates contaminants in the egg. Distilled water was injected into the air cell to return moisture content and contaminant concentrations to those in the egg when it was freshly laid (Heinz et al., 2009a). Eggs were weighed, opened to determine fertility and developmental stage, and contents transferred into a chemically clean jar (I-CHEM, VWR Scientific, Radnor, PA, USA), stored at −80 °C, and eventually transported to the Virginia Institute of Marine Science (VIMS),

Gloucester, VA, USA. Eggshells were dried for 3–4 months at room temperature and measured for thickness at three sites on the equator using a micrometer (Model 1010M; L.S. Starrett Co., Athol, MA, USA) and averaged.

Osprey reproductive success

Osprey productivity was monitored following the definitions and traditional methods described by Postupalsky (1977) and more recently Steenhof and Newton (2007). Initial nest visits were made in March to locate study nests and determine breeding status. Additional nests were monitored to have back-up locations for sampled nests if the targeted nests failed. Nests were visited at 7–10 day intervals to determine the fate of the nest including the numbers of eggs laid, eggs hatched, and young present at advanced age (>45 days) to fledging. Other observations including evidence of predation or disturbance were noted.

Osprey nestling blood samples and morphological endpoints

A blood sample was collected from a 40–45-day-old nestling at each nest from which a sample egg was collected. In the event the target nest failed (8/30 instances), the sample was drawn from a nestling residing in a nearby nest (within 2.5 km). Specifically, before fledging, one nestling per nest (n = 30) was removed from the nest for about 10 min. Following physical examination, culmen length and body weight were measured and the crop was palpated to determine level of crop filling. A 5–7-mL brachial blood sample was drawn using a 23-gauge 1-inch needle into a heparinized syringe (Sarstedt International, Newton, NC, USA). About 100 μL of fresh osprey nestling blood was

transferred to a microcentrifuge tube, frozen on dry ice, and stored at −80 °C for the DNA damage assay. The remainder of the blood sample was saved for a concurrent study (Lazarus et al., 2015b).

Analysis of contaminant residues in osprey eggs

Osprey egg analyses for 11 PBDE congeners, 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-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), and decabromodiphenyl ether (DBDPE)], 129 PCB congeners, 44 organochlorine pesticides and methoxytriclosan were conducted based on the methods of Chen et al. (2008) and La Guardia et al., 2007 and La Guardia et al., 2010. Egg contents were homogenized, lyophilized and then spiked with surrogate standards of PCB 30, 65 and 204 (Ultra Scientific, North Kingstown, RI, USA), ^{13}C-methoxytriclosan, ^{13}C-PCB-126 and 2,3,4,4′,5,6 – hexabromodiphenyl ether (BDE 166; Cambridge Isotope Laboratories, Inc., Andover, MA, USA). Blanks, consisting of sodium sulfate (baked at 450 °C overnight), were analyzed to evaluate possible laboratory contamination. Dried samples underwent accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA, USA) using methylene chloride (DCM) at 100 °C and 68 atm.

Extracts were purified by size exclusion chromatography (SEC, Envirosep-ABC®, 350 × 21.1 mm column; Phenomenex, Torrance, CA, USA). Each post-SEC extract was reduced in volume, added to a solid-phase 2-g silica glass extraction column (Isolute, International Sorbent Technology, Ltd., Hengoed Mid Glamorgan, UK) 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 two fractions were combined, pooled and then split, with half going for coplanar PCB analysis. Coplanar PCBs were separated from nonplanar PCBs by elution through a Supleclean ENVI-Carb SPE column (Sigma–Aldrich Co., St. Louis, MO, USA). The column was first eluted with 15 mL hexane (to waste). The coplanar congeners 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, North Kingstown, RI, USA) as an internal standard, and analyzed by gas chromatography/mass spectrometry (GC/MS) on an Agilent 5975C instrument (Agilent Technologies, Santa Clara, CA, USA), in electron impact mode and selected ion monitoring. A 60 m DB-5 MS GC column (Agilent, 0.32 mm ID × 0.1 μm thickness) was used.

The second half of the silica SPE fraction retained was spiked with decachlorodiphenyl ether (Ultra Scientific) as the internal quantitation standard. Identification and quantitation of non-coplanar PCBs was conducted by GC/MS in the electron ionization mode on a Varian 2200 GC/MS (Agilent Technologies). 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 ID × 0.25 μm thickness).

The PBDEs and alt-BFRs were analyzed using this same fraction. PBDEs and alt-BFRs were separated by ultra-performance liquid chromatography (UPLC, Waters Corp., Milford, MA, USA) and analyzed by atmospheric pressure photoionization tandem mass

spectrometry (APPI/MS/MS, Q-Trap3200 MS, AB Sciex, Framingham, MA, USA). Further details of the UPLC-APPI/MS analysis can be found in La Guardia et al. (2013).

Quality control and assurance

Instrumental analysis and laboratory QA/QC for method development are described in Chen et al. (2008). The method detection limit (MDL) for organochlorine pesticides and non-coplanar PCB congeners was 0.4 μg/kg ww, coplanar PCBs was 0.04 μg/kg ww, and PBDEs and alt-BFRs was 0.4 μg/kg ww. Data were corrected based on the recovery of surrogate standards in each sample. For quantification of organochlorine pesticides and methoxytriclosan, average recovery of the surrogate standard PCB-204 (similar physiochemical properties to the organochlorine pesticides) and ^{13}C-methoxytriclosan from the eggs were (mean ± SD) 85.9 ± 11.0%. Recoveries of the surrogates for the non-coplanar PCBs (PCB-204) averaged 94.9 ± 13.7%, coplanar PCBs 97.9 ± 0.13%, and brominated flame retardants (BDE 166) 98.6 ± 24.1%. Overall, moisture content (adjusted to fresh weight) in eggs averaged 83.7 ± 1.37%.

Due to changes in analytical methodologies and laboratories used between 2000–2001 (Rattner et al., 2004) and 2011–2012, a subset of 11 egg homogenates (three on the Anacostia/middle Potomac Rivers, five from Baltimore Harbor/Patapso Rivers, and three on the Elizabeth River) from 2000–2001 were re-analyzed with current samples. Based on data from our earlier work (Rattner et al., 2004), we selected those samples containing a range (relatively low, intermediate and high) of *p,p′*-DDE and total PCB residues. A comparison of the major stable groups of contaminants (*p,p′*-DDE, total PCBs, and sum of PBDE congeners 47, 99, 100, 153 and 154) was conducted and the percent differences

between each analysis was calculated (Supplemental Table S1). Individual PCB congener patterns were not evaluated on a temporal perspective due to substantial differences in analytical methodologies. Percent difference of moisture content of these samples in 2000–2001 and 2011–2012 changed by only 1.48%, indicating that sample moisture had not changed appreciably. However, in 2000–2001, moisture correction was determined by mathematically adjusting to the volume of the entire egg (Rattner et al., 2004), while in 2011–2012 we physically adjusted by the addition of distilled water (Heinz et al., 2009a).

In 2000–2001, PCBs were analyzed at the Geochemical and Environmental Research Group (GERG) at Texas A&M using an Aroclor profile-based analysis. Wet samples were mixed with sodium sulfate, spiked with surrogates and extracted with DCM in a Teckmar Tissumizer. Extracts were cleaned up on a silica/alumina column and purified by HPLC to remove interfering lipids. Identification/quantification was by GC in concert with electron capture detection (ECD) using an Aroclor profile-based approach (Rattner et al., 2004). In contrast, VIMS conducted a congener-based analysis using GC/MS in 2011–2012 (Chen et al., 2008). Thus, some differences in results between the two analytical methods were expected. Total PCB concentrations from 11 samples were comparable using the two methodologies, with some 2011–2012 estimates being greater and others being less (average percent difference of 23.7 ± 20.5%) than values reported by GERG in 2000–2001. This seems quite reasonable, as others (Turle et al., 1991) reported that total PCB concentrations from Aroclor-based analyses were on average 46% greater than congener-based analyses.

The VIMS conducted PBDE analyses of the 2000–2001 egg samples using GC/MS (Rattner et al., 2004) and the re-analysis of a subset of five of these samples using ultra-performance liquid chromatography (UPLC)/MS 10 years later. Of the five samples reanalyzed in 2011–2012, average percent difference between the original samples and their re-analysis was 17.9 ± 13.4% (with some 2011–2012 measurements higher and some lower compared to GERG values from 2000–2001). Perhaps most surprisingly, the re-analysis of 11 archived samples for *p,p′*-DDE varied by 44.3 ± 24.5% (Supplemental Table S1). A GC/ECD method was used by GERG for the original 2000–2001 analyses, whereas a GC/MS method was employed for the re-analysis by VIMS. All surrogate recoveries within the 2011–2012 samples were acceptable (84.9 ± 11.0%). Thus, due to variation between labs, years and sample storage, quantitative comparisons between *p,p′*-DDE values from this current study with published values (Rattner et al., 2004) are not justified. Notably, values for *p,p′*-DDE in osprey eggs in 2011–2012 were well below the threshold for eggshell thinning (9 of 11 were <1.0 μg/g ww; Wiemeyer et al., 1988).

Toxic equivalents for PCB congeners

The World Health Organization toxic equivalency factors (TEFs) were used to estimate the toxic equivalent (TEQ) for birds (van den Berg et al., 1998). Since 1998, TEFs have changed slightly for mammals, but not for avian species (van den Berg et al., 2006). The TEQ of *Ah*-receptor active PCB congeners was calculated by multiplying the congener concentration (pg/g ww) by the TEF and summing individual values.

DNA damage assay

Whole blood from osprey nestlings was analyzed for the presence of 8-hydroxy-2′-deoxyguanosine (8-OH-dG), a biomarker of oxidative DNA damage, using the DNA/RNA Oxidative Damage EIA Kit (Cayman Chemical, Ann Arbor, MI, USA). This analysis followed the protocol and assay validation described in Rattner et al. (2013). The limit of detection for this assay was 1.03 pg/μg DNA. Plates included blanks and reference samples to account for inter-assay variability. Samples were analyzed in duplicate.

Standard curves were fitted and concentrations determined using a 4-parameter model ($R^2 > 0.998$; MARS Data Analysis Software 2.10 R3, BMG Labtech). Samples collected in 2011 and 2012 were assayed separately. Precision of duplicate determinations (intra-assay variation, coefficient of variation, CV ± SD) was 3.8 ± 2.7% for samples collected in 2011 and 5.1 ± 3.1% for samples collected in 2012. Duplicate analyses with a CV greater than 20% were re-analyzed. Inter-assay variation (CV among assay plates) for reference samples was 9.9 ± 11.3%. Due to a number of factors (e.g., DNA degradation in samples stored for varying durations, observed variation in assay performance among test kit lots), we were unable to justify statistical comparisons of results between the 2011 and 2012 collections.

Statistical analysis

Simple descriptive statistics were generated for measurement endpoints. Continuously distributed variables (productivity, eggshell thickness, morphometrics, DNA damage and contaminant residues) were tested for normality and homogeneity of

variance, and log transformed as necessary (SAS 9.3, SAS Institute Inc., Cary, NC, USA). Comparisons were made among tributaries using analysis of variance (ANOVA) followed by Tukey's HSD multiple mean comparison test to detect site-specific differences ($\alpha = 0.05$). For those variables that did not meet assumptions for parametric analysis, a generalized Wilcoxon non-parametric test was used followed by a Bonferroni adjustment for comparison of multiple means. A Fisher's Exact test was used to compare overall site-specific differences in productivity endpoints. For those sampling sites with residues below the MDL in <50% of the samples, the potential range of the mean was reported using the Kaplan–Meier method (Helsel, 2005). Any outliers were eliminated from the dataset using a Grubb's test.

Egg, young and nest survival probabilities were calculated using the Mayfield method (Mayfield, 1961; Bart and Robson, 1982). For those variables that were not normally distributed and/or exhibited heterogeneous variance, a generalized Wilcoxon non-parametric test was used. If this was significant, two-sample comparisons were conducted and *p*-values were adjusted using a Bonferroni correction. Results for total PCBs and PBDEs were statistically compared between the 2011–2012 and 2000–2001 from (Rattner et al., 2004) collections using a t-test.

A logistic analysis of covariance was used to examine site-specific differences in the relationship between egg residues (*p*,*p*′-DDE, total PCBs, PBDEs and the sum of *p*,*p*′-DDE + total PCBs) and productivity (nest success, egg loss). If no differences were detected, logistic regression was used to evaluate the relation between residues and productivity across sites.

Results

Reproduction of ospreys

In 2011, 36 nests were identified along a 25-km segment on the Anacostia River, from the Frederick Douglass Bridge to Dogue Creek, a tributary of the middle Potomac River. Thirty nests were found along a 20-km stretch from Baltimore Harbor (Curtis Creek) east to the mouth of Patapsco River (Bodkin Point). In 2012, 29 nests were found along a 20-km stretch of the Elizabeth River (including the Lafayette River, West and South branches). No nests were found on the East branch of the River. At Poplar Island (reference site), there were 12 active nests in 2011 and 24 in 2012. Osprey nest density based on total water surface area surveyed was 1 nest/1.60 km^2 on the Anacostia/middle Potomac Rivers, 1 nest/3.9 km^2 in Baltimore Harbor/Patapsco River, and 1 nest/3.57 km^2 on Elizabeth River. Poplar Island had the highest density of osprey nests in 2012, at 1 nest/0.19 km^2.

Productivity, the average number of fledglings per active nest, at each location ranged from 1.00 to 1.43 (Table 1). There were no site-specific differences in number of eggs laid, egg loss, hatching success, chick loss, and fledging and nest success (Fisher's Exact Test and generalized Wilcoxon non-parametric test, $p > 0.35$). On average, 3.09 eggs were laid/nest, 39.6% of eggs laid were lost, 91.5% of the eggs retained in nests hatched (hatchability), and 94.2% of the hatchlings survived to fledge. While not statistically significant, it is noteworthy that the lowest percentages of successful pairs and fledglings produced per active nest were on the Anacostia/middle Potomac (55.5% successful pairs, 1.11 fledglings/active nest) and the Elizabeth Rivers (66.7%, 1.00).

Once hatched, most chicks survived to fledge with the exception of the disappearance of one nestling on the Potomac River, and one nestling on the Elizabeth River.

Table 1. Reproductive success of osprey nesting in Chesapeake Bay Regions of Concern.

Site	Poplar Island		Baltimore Harbor and Patapsco River		Anacostia and middle Potomac Rivers		Elizabeth River	
	2011–2012		2011		2011		2012	
	Number	%	Number	%	Number	%	Number	%
Active nests sampled	8		7		9		6	
Eggs laid	24		22		29		18	
Sample egg collected	8		7		9		6	
Eggs relaid due to predation	0		0		3		0	
Eggs naturally incubated	16		15		23		12	
Fate of eggs								
Unknown or predation[a]	5		5		9		4	
Storm or wind related	0		0		0		0	
Crushed/rotten	0		0		1		0	
Failed to hatch[b]	0		0		2		1	
Hatched	(11/16)	68.8%	(10/15)	66.7%	(11/23)	47.8%	(7/12)	58.3%
Hatchability[c]	(11/11)	100%	(10/10)	100%	(11/14)	78.6%	(7/8)	87.5%
Fate of nestlings								
Dissappeared	0		0		1		1	
Storm or wind related	0		0		0		0	
Found dead	0		0		0		0	
Fledged[c]	(11/11)	100%	(10/10)	100%	(10/11)	90.9%	(6/7)	85.7%
Successful pairs (fledged young)	(6/8)	75.0%	(5/7)	71.4%	(5/9)	55.5%	(4/6)	66.7%
Fledlings/active nest	(11/8) 1.38		(10/7) 1.43		(10/9) 1.11		(6/6) 1.00	
Fledglings/successful nest	(11/6) 1.83		(10/5) 2.00		(10/5) 2.00		(6/4) 1.50	
Mayfield method estimates								
Egg laying and incubation period	8		7		9		6	
Daily survival rate ± standard error	0.989 ± 0.008		0.989 ± 0.007		0.987 ± 0.007		0.990 ± 0.007	
Survival rate to hatching (A)	0.650		0.650		0.600		0.676	
Nestling period	6		5		5		4	
Daily survival rate ± standard error	1.000		1.000		1.000		1.000	
Survival rate to fledging (B)	1.000		1.000		1.000		1.000	
Nest success (A × B)	0.650		0.650		0.600		0.676	
Probability of an egg hatching given that the nest is successful (C)	0.916		0.909		0.846		0.875	
Probability of young living to 53 d given that the nest is successful (D)	1.000		1.000		0.909		0.857	
Egg success (A × B × C × D)	0.595		0.591		0.461		0.507	
Mean clutch size (E)	3.00		3.14		3.11		3.00	
Mean number of young surviving to 53 d (A × B × C × D × E)	1.79		1.86		1.43		1.52	
Mean number of young surviving to 53 d less sample egg (A × B × C × D × (E−1))	1.19		1.26		0.97		1.01	

Note: A = daily durvival rate to the 39th power (similar to Toschick et al., 2005) to account for a 39–43 d incubation period; B = daily survival rate to the 53rd power to account for 50–55 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.

[a] Considered to be lost during the egg stage.

[b] Eggs in nest >45 days or abandoned.

[c] Hatchability = eggs hatched/(eggs laid-eggs that disappeared or sampled before hatching), represents the % of eggs that remain in the nest through hatch.

Eggshell thickness and morphological endpoints

On Poplar Island, no significant differences (t-test, $p > 0.8$) were found in osprey eggshell thickness between 2011 and 2012; accordingly, samples from both years were combined for this reference site. There was an overall difference among sites (ANOVA, $p = 0.05$). Using Tukey's HSD mean separation test, eggshell thickness on the Anacostia/middle Potomac Rivers (0.481 ± 0.049 mm) was significantly lower than for Poplar Island (0.549 ± 0.057 mm) ($p = 0.04$). While eggshell thickness was numerically lowest on the Anacostia/middle Potomac Rivers compared to the other sites (Baltimore Harbor 0.504 ± 0.040 mm and Elizabeth River 0.529 ± 0.044 mm), this trend was not significant ($p > 0.48$). Of the egg that failed to hatch on the Potomac, there was no evidence of eggshell thinning (0.503 mm).

All nestlings examined were in good body condition. One chick on the Elizabeth River had a plastic bag around its neck, but otherwise appeared to be fine. Osprey nestling body weight (body weight minus estimated weight of food in crop; Schaadt and Bird, 1993) was compared among sites. Several of the nestlings on the Elizabeth River had mites and there were instances of feather plucking indicative of sibling competition for food. There were no differences in body weight between sampling years on Poplar Island (t-test, $p > 0.6$); therefore, measurements were combined. There were no differences in osprey nestling body weight among the four sites ($p = 0.11$, ANOVA; Poplar Island 2011–2012, 1603 ± 159 g; Anacostia/middle Potomac Rivers 1480 ± 144 g; Baltimore Harbor/Patapsco Rivers 1551 ± 140 g; Elizabeth River 1365 ± 138 g). There were no differences in culmen length among sites ($p = 0.3$, ANOVA) and values ranged b

Organochlorine pesticides and metabolites

Twenty-five out of 44 organochlorine pesticides and metabolites were detected in eggs at concentrations exceeding the MDL. The Anacostia/middle Potomac Rivers and Baltimore Harbor/Patapsco River sites had greatest pesticide and metabolite residues, some exceeding Poplar Island values (2.2–11.5 times) by more than an order of magnitude (Table 2). The Anacostia/middle Potomac also had the highest frequency of detects and measureable concentrations were found in all eggs sampled at this site with the exception of *trans*-chlordane (present in 8 of 9 eggs). Of this dataset, only one egg from Baltimore Harbor had *p,p'*-DDE concentrations (1.8 μg/g ww) that fell within the 95% confidence interval (1.2–3.0 μg/g ww) associated with 10% shell thinning in osprey eggs (2.0 μg/g ww; Wiemeyer et al., 1988). There was no relation between *p,p'*-DDE concentration and eggshell thickness ($r = -0.14$, $p = 0.459$, n = 30).

Table 2. Concentrations (μg/g ww) of organochlorine pesticides and metabolites from Chesapeake Bay Regions of Concern and Poplar Island reference site. [a]

Contaminant	Poplar Island 2011–2012 (n = 8)	Baltimore Harbor and Patapsco River 2011 (n = 7)	Anacostia and middle Potomac Rivers 2011 (n = 9)	Elizabeth River 2012 (n = 6)
	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected
p,p'-DDE	0.152[B] 0.090–0.206 8	0.553[A] 0.286–1.831 7	0.725[A] 0.477–1.018 9	0.488[A] 0.240–0.873 6
p,p'-DDD	0.009[B] 0.006–0.017 8	0.046[A] 0.016–0.190 7	0.042[A] 0.022–0.072 9	0.048[A] 0.012–0.117 6
Dieldrin	– <MDL 0	0.020–0.021 <MDL-0.057 5	0.02 0.008–0.035 9	– <MDL 0
Heptachlor epoxide	– <MDL-0.006 2	0.022[B] 0.015–0.05 7	0.047[A] 0.028–0.10 9	– <MDL-0.004 1
α-Chlordane (*cis*)	– <MDL-0.002 1	0.013[A] 0.006–0.032 7	0.024[A] 0.015–0.04 9	0.0049–0.0050[B] <MDL-0.010 5
γ-Chlordane (*trans*)	– <MDL 0	0.002–0.003 <MDL-0.010 4	0.00280–0.00284 <MDL-0.005 8	– <MDL 0
cis-Nonachlor	0.004[C] 0.002–0.005 8	0.030[A,B] 0.011–0.06 7	0.045[A] 0.028–0.058 9	0.0159–0.0160[B,C] <MDL-0.035 5
trans-Nonachlor	0.00243–0.00248[C] <MDL-0.004 7	0.015[A,B] 0.007–0.031 7	0.025[A] 0.018–0.037 9	0.00670–0.00676[B,C] <MDL-0.012 5
Oxychlordane	– <MDL 0	0.0110–0.0111[B] <MDL-0.026 5	0.025[A] 0.016–0.051 9	– <MDL-0.009 1
Mirex	0.0017–0.0018[B] <MDL-0.003 6	0.00368–0.00374[A,B] <MDL-0.007 6	0.004[A] 0.003–0.005 9	0.0023–0.0024[A,B] <MDL-0.004 5

[a] – No mean calculated, as contaminant was detected in fewer than half the samples; MDL, method detection limit; extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p < 0.05$), and a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction.

[b] If non-detects were present in less than half of the samples, the Kaplan–Meier method was used to estimate the extremes of the mean.

Polychlorinated biphenyls

Total PCB concentrations were up to five times greater in Baltimore Harbor and the Anacostia/middle Potomac Rivers ($p < 0.0001$) than at the reference site (Table 3). Some of the greatest values were detected in eggs collected in Baltimore Harbor, ranging up to 35.0 μg/g ww. Of the most potent *Ah*-receptor active PCB congeners (77, 81, 126, 169), residues of PCB 77 and 126 did not differ from the reference site. However,

residues of PCB congeners 81 and 169 in eggs were greater on the Elizabeth River compared to the Anacostia/middle Potomac Rivers and Baltimore Harbor ($p < 0.01$), but were not detected at Poplar Island. In terms of *Ah*-receptor active PCB congeners, Baltimore Harbor and the Anacostia/middle Potomac Rivers had greater TEQs (16.3 and 12.8 pg/g respectively; $p < 0.04$) than the Elizabeth River and the reference site (4.91 and 2.30 pg/g ww).

Table 3. Concentrations (μg/g ww) of polychlorinated biphenyls and congeners from Chesapeake Bay Regions of Concern and Poplar Island reference site. [a]

Contaminant	Poplar Island 2011–2012 (n = 8)	Baltimore Harbor and Patapsco River 2011 (n = 7)	Anacostia and middle Potomac Rivers 2011 (n = 9)	Elizabeth River 2012 (n = 6)
	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected
Total PCBs (μg/g)	1.50^{C} 1.00–1.93 8	7.77^{A} 2.94–35.0 7	$5.57^{A,B}$ 3.88–6.53 9	$2.92^{B,C}$ 0.56–6.85 6
Congener 77 (pg/g)	$59.6^{A,B}$ 17.0–160 8	$73.8^{A,B}$ 48.0–129 7	$41.0–45.4^{B}$ <MDL-61.0 8	140^{A} 70.0–510 6
Congener 81 (pg/g)	– <MDL 0	21.9^{B} 15.0–35.0 7	$11.2–11.28^{B}$ <MDL-29.0 5	$115–128^{A}$ <MDL-380 4
Congener 126 (pg/g)	139 61–270 8	243 107–647 7	206–210 <MDL-290 8	603–610 <MDL-1080 5
Congener 169 (pg/g)	– <MDL 0	$39.3–45.0^{B}$ <MDL-91.0 6	$24.1–33.0^{A,B}$ <MDL-36.0 8	$75.0–88.3^{A}$ <MDL-140 4
Congener 105 (ng/g)	$8.54–8.60^{B}$ <MDL-16.3 7	45.0^{A} 11.9–199 7	36.7^{A} 23.8–66.1 9	$25.3^{A,B}$ 6.79–69.0 6
Congener 118 (ng/g)	59.5^{B} 32.3–79.2 8	363^{A} 138–1801 7	277^{A} 103–415 9	107^{B} 21.9–282 6
Congener 128 (ng/g)	21.1^{B} 9.28–32.0 8	141^{A} 53.9–514 7	99.1^{A} 39.0–169 9	27.7^{B} 7.20–80.2 6
Congener 138/158 (ng/g)	158^{B} 100–246 8	638^{A} 257–2091 7	538^{A} 330–975 9	$286^{A,B}$ 68.1–546 6
Congener 156 (ng/g)	7.97^{B} 3.28–14.8 8	76.4^{A} 26.9–290 7	61.2^{A} 29.7–84.5 9	11.7^{B} 2.62–25.3 6
Congener 189 (ng/g)	$1.42–1.52^{B}$ <MDL-2.51 6	14.3^{A} 4.21–62.3 7	$9.16–9.21^{A}$ <MDL-11.8 8	$2.76^{A,B}$ 0.78–7.33 6
Congener 170/190 (ng/g)	52.1^{B} 29.1–86.6 8	373^{A} 108–1619 7	307^{A} 212–412 9	95.1^{B} 24.0–239 6
Toxic equivalents (pg/g)	2.30^{B} 1.18–3.23 8	16.3^{A} 5.31–67.7 7	12.8^{A} 6.66–19.4 9	4.91^{B} 1.17–11.6 6

[a] – No mean calculated, as contaminant was detected in fewer than half the samples; MDL, method detection limit; extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p < 0.05$), and a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction.

[b] If non-detects were present in less than half of the samples, the Kaplan–Meier method was used to estimate the extremes of the mean.

Polybrominated diphenyl ether flame retardants

Congeners 47, 85, 99, 100, 153, 154, 183, 206 and 209 were detected in osprey eggs collected from one or more sites (Table 4). Penta-BDE congener 85, hepta-183, nona-206 and 209 were detected less frequently than other congeners in samples.

Congeners 28 and 66 were not detected in any of the samples. Osprey eggs from Poplar Island had the lowest PBDE congener residues; concentrations did not differ between 2011 and 2012 ($p > 0.05$) and were combined. There were significant site differences for both total PBDEs and BDE 47 ($p < 0.0001$, ANOVA). Specifically, total PBDEs were more than three times greater on the Anacostia/middle Potomac and Baltimore Harbor/Patapsco Rivers compared to the Poplar Island reference site ($p < 0.0001$). This same site pattern and magnitude of difference was also apparent for BDE congener 47 ($p < 0.0001$). The Anacostia/middle Potomac Rivers also had higher concentrations of PBDE congeners 99, 100 and 154 compared to Poplar Island and the Elizabeth River ($p < 0.01$). An egg collected near Blue Plains Wastewater Treatment Plant (WWTP) contained the greatest total PBDE levels (802 ng/g ww) found in this study. In 2000–2001, PBDE 206 and 209 were not studied. We re-analyzed 11 archived sample homogenates from 2000–2001, and BDE 209 was detected in 3 of 11 samples (range: 0.66–3.88 ng/g ww; 1/each ROC).

Table 4. Concentrations (ng/g ww) of polybrominated diphenyl ethers and alternative brominated flame retardants from Chesapeake Bay Regions of Concern and Poplar Island reference site.[a]

Contaminant	Poplar Island 2011–2012 (n = 8)	Baltimore Harbor and Patapsco River 2011 (n = 7)	Anacostia and middle Potomac Rivers 2011 (n = 9)	Elizabeth River 2012 (n = 6)
	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected	Geometric mean[b] extremes *n* detected
Total PBDEs	61.6^{C} 52.3–80.9 8	198^{B} 106–310 7	452^{A} 313–802 9	90.7^{C} 71.3–141 6
BDE congener 47	39.9^{C} 32.4–48.2 8	138^{B} 72.4–212 7	331^{A} 231–648 9	46.9^{C} 21.9–90.2 6
BDE congener 85	– <MDL-0.76 1	– <MDL 0	– <MDL 0	– <MDL-1.22 1
BDE congener 99	1.52^{C} 0.17–4.94 8	13.2^{A,B} 7.31–30.6 7	29.7^{A} 19.8–49.1 9	9.39^{B} 5.68–16.2 6
BDE congener 100	8.64^{C} 4.79–13.5 8	17.5^{B} 10.1–23.6 7	54.3^{A} 44.1–81.8 9	14.1^{B} 9.34–21.2 6
BDE congener 153	2.84^{B} 2.00–4.42 8	11.4^{A} 4.87–25.5 7	15.4–15.5^{A} <MDL-26.6 8	4.82^{A,B} 2.32–7.16 6
BDE congener 154	8.18^{B} 5.01–11.7 8	10.5^{A,B} 6.47–18.9 7	16.4^{A} 11.5–21.1 9	6.62–6.68^{B} <MDL-9.44 5
BDE congener 183	– <MDL 0	3.39^{A} 1.11–8.89 7	1.31–1.36^{B} <MDL-2.64 9	– <MDL 0
BDE congener 206	– <MDL 0	– <MDL-1.88 1	– <MDL 0	– <MDL-2.16 1
BDE congner 209	– <MDL-1.56 2	– <MDL-11.4 1	– <MDL-1.03 1	13.2–13.3 <MDL-75.5 3
α-HBCD	1.01 0.36–2.14 8	1.27 0.67–3.72 7	1.57–1.60 <MDL-3.03 7	0.84–0.98 <MDL-1.36 4
BTBPE	– <MDL-4.77 1	1.56–1.62 <MDL-3.45 5	– <MDL-28.9 4	– <MDL 0
DBDPE	– <MDL 0	– <MDL 0	– <MDL-0.89 1	– <MDL 0
TBB	– <MDL-6.42 1	1.95–2.01 <MDL-5.17 5	– <MDL-30.3 1	– <MDL-1.58 2
TBPH	– <MDL-0.66 2	0.30–0.39 <MDL-0.80 4	– <MDL-7.37 3	– <MDL-0.50 1

[a] – No mean calculated, as contaminant was detected in fewer than half the samples; MDL method detection limit; extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p < 0.05$), or a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction.

[b] If non-detects were present in less than half of the samples, the Kaplan–Meier method was used to estimate the extremes of the mean.

Other brominated flame retardants and methoxytriclosan

Five additional BFRs were quantified in osprey eggs (Table 4). Alpha-HBCD was most frequently detected (26 of 30 eggs), but tended to be at much lower concentrations than the lower brominated BDE congeners. While BTBPE, TBB and TBPH were most frequently detected in samples from Baltimore Harbor/Patapsco River, the maximum detected concentration for each of these alt-BFRs was found in samples from the Anacostia/middle Potomac Rivers. Methoxytriclosan, a moderately bioaccumulative degradate of the antibacterial agent triclosan, was detected in all 9 samples from the Anacostia/middle Potomac Rivers (1.29–7.40 ng/g ww) and in one sample from Baltimore Harbor (5.55 ng/g ww). This compound may serve as a useful marker of domestic wastewater.

The eleven historical egg homogenates were re-analyzed for these five alt-BFRs and methoxytriclosan. Alpha-HBCD was detected in 10 of 11 of the archived 2000–2001 samples with no apparent site-specific differences in concentration (Baltimore Harbor/Patapsco River, n = 5 range: 0.53–1.27 ng/g ww; Elizabeth River, n = 3 range: < MDL-1.08 ng/g ww; Anacostia/middle Potomac Rivers, n = 3, range: 0.64–1.36 ng/g ww). Of the remaining four alt-BFRs, BTBPE, DBDPE, TBB and TBPH were consistently detected in two historical samples from Curtis Bay and Shallow Creek off of the Patapsco River (0.30–6.74 ng/g ww). However, methoxytriclosan was only detected in one sample collected in 2000 on the Potomac River (4.89 ng/g ww).

Relation between contaminants and osprey productivity

Analysis of covariance revealed that there were no site-specific associations between *p,p′*-DDE and total PBDEs ($p = 0.20$), or between total PCBs and PBDEs ($p = 0.07$). However, there was a significant relation between *p,p′*-DDE and PCB residues across sites ($p = 0.01$), with the greatest correlation on the Anacostia/middle Potomac Rivers ($r = 0.77$, $n = 9$). When data for *p,p'*-DDE, total PCBs and total PBDEs were combined across sites ($n = 30$), there was a significant correlation between *p,p'*-DDE and total PCBs ($r = 0.84$, $p < 0.001$) and *p,p′*-DDE and total PBDEs ($r = 0.62$, $p = 0.0003$), and a marginal relation between total PCBs and total PBDEs ($r = 0.32$, $p = 0.08$). There were no associations (site-specific or combined sites) between egg loss or nest success and the concentrations of *p,p′*-DDE, PCBs, PBDEs or the sum of *p,p′*-DDE + PCBs ($p > 0.09$).

Temporal comparisons

Concentrations of *p,p′*-DDE, total PCBs and PBDE congeners 47, 99, 100, 153, 154 in eggs collected in 2011–2012 were compared to results from 2000–2001 (Rattner et al., 2004, Figure 4). Total PCB concentrations remained high in Baltimore Harbor/Patapsco River between decades ($p = 0.45$), but decreased on the Anacostia/middle Potomac Rivers (9.28 μg/g in 2000–2001 versus 5.57 μg/g ww in 2011–2012, $p < 0.0001$). Concentrations of PCBs remained unchanged on the Elizabeth River 10 years later ($p = 0.96$). For equitable comparison of total PBDE concentrations, the sum of congeners 47, 99, 100, 153 and 154 were examined between decades. The sum of these congeners across all ROCs declined over time ($p < 0.04$, Figure 2). A

qualitative comparison of *p,p′*-DDE levels between decades suggests that concentrations were low across all sites, and much lower than historic values (Wiemeyer et al., 1988; Rattner et al., 2004).

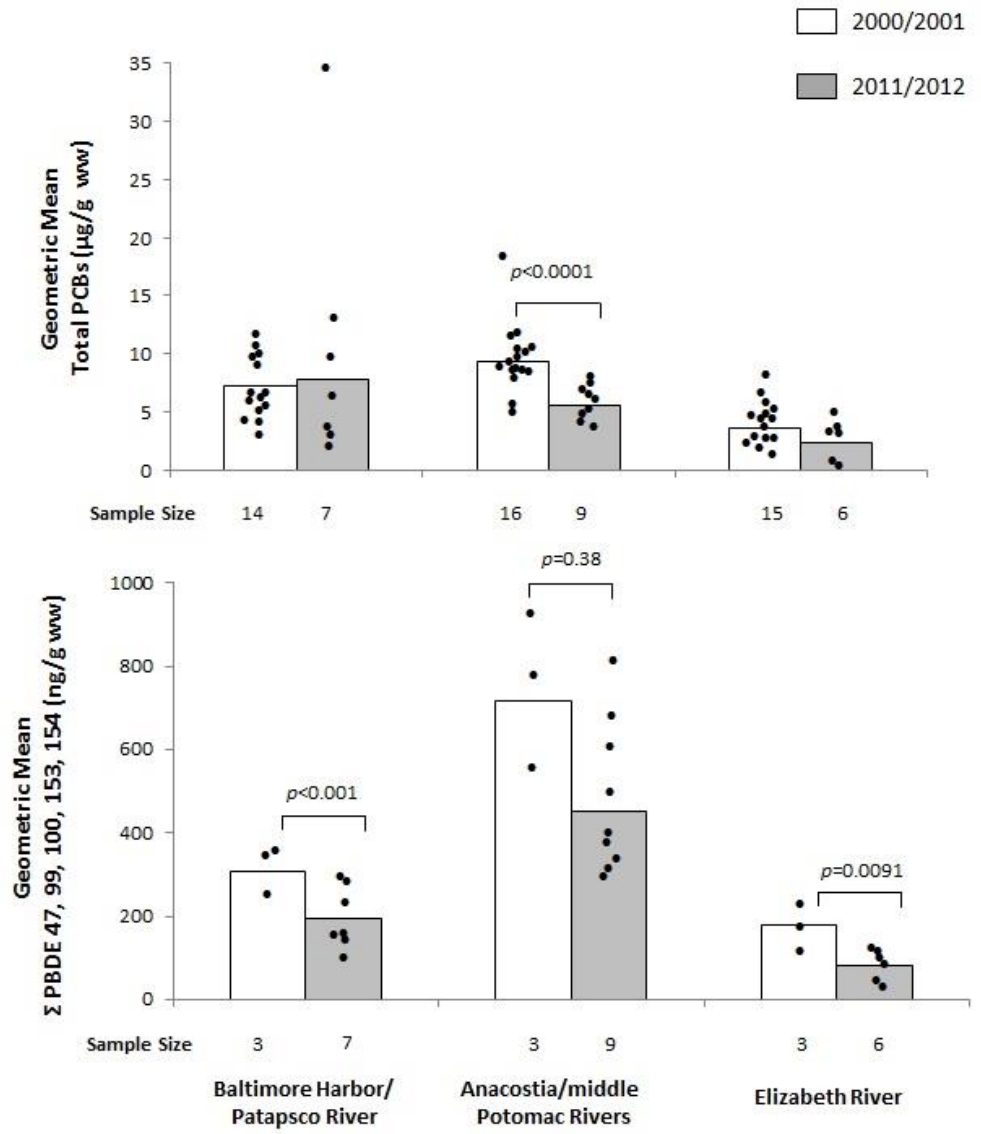


Figure 4. Temporal comparison of contaminants from Chesapeake Bay Regions of Concern in 2000-2001 (adapted from Rattner et al., 2004) and 2011-2012. Geometric means and individual data points are presented (●). At each site historical and current datasets were compared by a t-test.

DNA Damage

In 2011, Baltimore Harbor/Patapsco River had significantly greater concentrations of 8-OH-dG (36.4 pg/µg DNA) compared to Poplar Island (26.6 pg/µg DNA, $p = 0.04$) (Figure 5), but not the Anacostia/middle Potomac Rivers (35.1 pg/µg DNA, $p = 0.9$). In 2012, the concentration of 8-OH-dG in one sample collected from the Elizabeth River (entrance to the Lafayette River) was several times greater (107.5 pg/µg DNA) compared to all other values in this study. While Grubb's test identified this measurement as an outlier, there is no rationale for excluding this value from our statistical analysis. In 2012, there was no significant difference between the Elizabeth River (35.5 pg/µg DNA) and Poplar Island (34.2 pg/µg DNA; $p > 0.15$) with or without exclusion of the outlier.

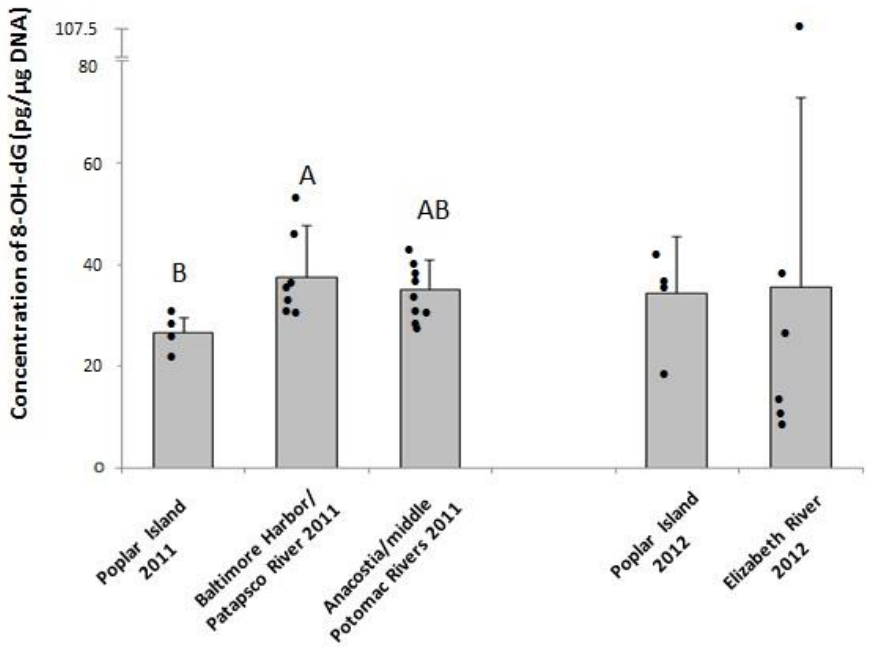


Figure 5. Concentrations of 8-hydroxy-2ʾ-deoxyguanosine (8-OH-dG) in osprey nestling whole blood samples. Means, standard deviations and individual data points are presented (●). Capital letters indicate a statistically significant difference ($p < 0.05$) using Tukey's HSD test.

Discussion

Osprey productivity in Regions of Concern

Use of the insecticide DDT, and specifically its metabolite *p,p′*-DDE, resulted in eggshell thinning and population declines of ospreys (Wiemeyer et al., 1988) and other species of piscivorous birds (Blus, 2011). In the 1970s, the Chesapeake Bay osprey population was estimated at 1450 pairs, with some population segments producing well-below normal rates (Henny et al., 1974). Following restrictions in the use of DDT and other chlorinated pesticides, the Chesapeake osprey population more than doubled by 1995–1996 (Watts et al., 2004).

To determine the recruitment rate needed to maintain a stable osprey population, information is needed on nest site competition, nest availability and age of breeding; thus, it is challenging to identify which productivity rate is best for Chesapeake Bay ROCs (Watts and Paxton, 2007). Historically, it was estimated that 0.8–1.15 fledglings/active nest are required to maintain a stable population (Spitzer, 1980; Spitzer et al., 1983; Poole, 1989), although these estimates have likely changed. While contemporary survivorship data are available for ospreys in Chesapeake Bay (Bryan Watts, unpublished data), a model has yet to be developed to predict the reproductive rate necessary to maintain a stable population. Historical recruitment rates on the southern Potomac in 1970 were 0.55 fledglings/active nest (Wiemeyer, 1971) and in 2000 the reproductive rate was 0.88 on Anacostia/middle Potomac Rivers (Rattner et al., 2004). By 2011, the reproductive rate had further increased to 1.11 fledglings/active nest on the Anacostia/middle Potomac Rivers, an increase of 0.56 fledglings/active nest over the 1970s. Based on historical estimates (Spitzer, 1980; Spitzer et al., 1983; Poole, 1989),

since 2000, productivity in Baltimore Harbor appears to be adequate to maintain a stable population. The Elizabeth River appeared to have lower productivity (1.00 fledgling/active nest) compared to the other sites, but still adequate to maintain a stable population. However, this lower productivity rate may be related to sample size (only n = 6 nests). By inclusion of data from seven additional Elizabeth River nests, the productivity estimate increased to 1.28 fledglings/active nest, which is more than adequate to maintain a stable population. Osprey productivity rates are primarily driven by food availability and brood reduction, but could also be adversely affected by *p,p'*-DDE and other contaminant burdens in osprey eggs.

Although there were no differences in productivity (i.e., number of eggs laid, hatching and fledging success) among sites, there were some notable changes in osprey nesting in ROCs. In 2000, only one osprey nesting pair was observed on Bear Creek off of Baltimore Harbor/Patapsco River, which is considered to contain the most highly toxic sediments in Baltimore Harbor (McGee et al., 1999). In 2011, at least four pairs were observed nesting on channel markers and power transmission towers. On the Anacostia River, only one pair was observed in 2000, but by 2011 there were at least four nesting attempts, two of which were successful. While it is tempting to conclude that decreasing contaminants in eggs could account for differences in productivity, temporal and site-specific differences in other factors (e.g., food availability, nest site quality/availability, weather, predation, inexperienced breeders) could also contribute to fluctuations in fecundity (Levenson and Koplin, 1984; Stinson et al., 1987; Machmer and Ydenberg, 1989). Compared to 2000–2001, nest density slightly increased at all study sites (Baltimore Harbor/Patapsco River 1 nest/2.30 km^2 in 2000 vs. 1 nest/1.60 km^2 current

study; Anacostia/middle Potomac 1 nest/4.7 km^2 vs. 1 nest/3.9 km^2; Elizabeth River 1 nest/4.7 km^2 in 2001 vs. 1 nest/3.57 km^2 in 2012).

It has been suggested that the collection of a sample egg may bias productivity rates. For some datasets, a difference has been noted in productivity between sampled and unsampled nests, and some investigators (Henny and Kaiser, 1996) adjust productivity to account for sample egg collection. However, in other studies no differences in productivity between sampled and unsampled nests have been noted (Rattner et al., 2004; Toschik et al., 2005). Nests at the Poplar Island reference site from which a sample egg was collected fledged 1.38 young, while unsampled nests from this site fledged 1.31 young. Unlike Henny and Kaiser, 1996 and Henny et al., 2004, the present study limited sampling to nests containing three or more eggs, which may account for this difference.

Eggshell thickness

Eggshell thickness in Chesapeake Bay ROCs was on average 0.514 ± 0.054 mm, which is close to the average pre-DDT-era value (0.505 mm, n = 365, Anderson and Hickey, 1970). In the 1970s, eggshell thickness on the Potomac River was about 19% lower (0.402–0.416 mm) than the pre-DDT-era value (Wiemeyer et al., 1988). In the 1970s, Wiemeyer et al., 1988 found an association (r = −0.70) between *p,p'*-DDE residues in osprey eggs and eggshell thickness. Notably, concentrations of organochlorine pesticides and metabolites (Table 2) are now well below the threshold associated with 10% eggshell thinning (Wiemeyer et al., 1988). In fact, no significant relationship was observed between concentrations of *p,p'*-DDE and eggshell thickness in the 2011–2012

dataset.

Contaminant exposure in regions of concern

Residues of *p,p′*-DDE on the middle Potomac River declined about 75% between 1971–1977 and 2011 (3.1 μg/g ww in the 1970s, Wiemeyer et al., 1988; 1.16 μg/g ww in 2000, Rattner et al., 2004, 0.73 μg/g ww, present study). Concentrations of *p,p′*-DDE in eggs collected in other ROCs were low and did not seem to change in the past decade (Baltimore Harbor 0.443 μg/g ww in 2000 versus 0.553 μg/g ww in 2011; Elizabeth River 0.660 μg/g ww in 2001 versus 0.488 μg/g ww in 2012). Mean *p,p′*-DDE concentrations in Chesapeake Bay (<0.725 μg/g ww; Table 2) remain lower than those reported in the most industrialized and urbanized segment of Delaware River between C&D canal and Easton, PA (means < 1.77 μg/g ww; Toschik et al., 2005).

From a temporal perspective, PCB residues in osprey eggs from Baltimore Harbor/Patapsco and Elizabeth Rivers remained unchanged over the decade. Notably, eggs collected from Curtis Creek (Baltimore Harbor) in 2011 contained the greatest PCB residues (12.9 and 35.0 μg/g ww), and were comparable or exceeded values reported across the Northeast U.S. in the 1970s (up to 23 μg/g ww; Wiemeyer et al., 1988). However, a 40% decline in average total PCB concentrations was observed in eggs from the Anacostia/middle Potomac ROC (Figure 2). This suggests that PCB levels or their bioavailability in the osprey food web on the Anacostia/middle Potomac Rivers have decreased over the past decade. In support of this hypothesis, a reduction in total PCB concentrations in sediments has been reported for the Anacostia, where residues have declined by an order of magnitude over the past 25 years (Velinsky et al., 2011). Due to

moderate biomagnification factors from fish to osprey eggs (e.g., 11 times; Henny et al., 2003), these changes in chlorinated biphenyl concentrations in sediments may be translated up the food web.

Coplanar PCBs have been associated with embryonic deformities and adverse reproductive effects along with other toxic responses in fish-eating birds (Rice et al., 2003; Su et al., 2014). There was no evidence of embryonic deformities or reproductive impairment in the present study. Despite differences in analytical methods and detection limits between this current study and Rattner et al. (2004), all TEQs were well below the threshold associated with cytochrome P450 induction in osprey nestlings (Elliott et al., 2001).

Over the past decade, congener patterns of PBDEs did not change dramatically, with BDE 47, 99, 100, 153 and 154 being the most prominent congeners in osprey eggs across all sites. However, total PBDEs declined by about 40%. This is similar to a 55% decline reported by Henny and coworkers (2011) for osprey eggs collected on the Willamette River, Oregon. Since the last ecotoxicological study in the Chesapeake, the penta- and octa-BDE formulations have been phased out of use in the U.S. (end of 2004) (US EPA, 2014a). More recently, use of the deca-formulation has been curtailed (US EPA, 2014b). While the manufacturer and use of PBDEs in new consumer products has been banned, many long-lived goods containing these compounds remain in use and end up in landfills. Thus, it is unknown how quickly environmental levels may change in the food web in response to regulatory action (Chen and Hale, 2010; Zhang et al., 2014).

Notably, PBDE residues in osprey eggs collected along the Anacostia/middle Potomac Rivers in 2000 were over four times greater than those found at the reference

site (Rattner et al., 2004), with the maximum values detected near the Blue Plains WWTP. Other studies (Henny et al., 2011) have shown that volume of discharge and distance from a WWTP may be reflected in contaminant concentrations in osprey eggs and are presumably responsible for the high residues of PBDEs found in eggs near Blue Plains.

The detection of PBDEs in osprey eggs in ROCs in 2000–2001 stimulated a series of studies examining the effects of flame retardants on raptorial birds (e.g., Fernie et al., 2005; Fernie et al., 2006; Fernie et al., 2008; McKernan et al., 2009; Rattner et al., 2013). These studies provided a context for placing field residue data into perspective. In controlled exposure studies, the presence of PBDE flame retardants in developing American kestrel (*Falco sparverius*) embryos have been associated with oxidative stress, DNA damage, delay in hatch time, shorter humerus length, reduced total thyroid weight and reproductive and courtship behavior changes in adults. In field studies, Chen et al. (2010) have estimated a biomagnification factor of 25.1 for total PBDEs in the osprey food web. Reduced osprey productivity was associated with PBDEs at concentrations exceeding 1000 ng/g ww in eggs from Oregon and Washington (Henny et al., 2009). These findings were suggested to be equivocal in a subsequent study (Henny et al., 2011). In the Chesapeake, the greatest residues of total PBDEs in osprey eggs (0.928 μg/g in 2000 and 0.802 μg/g ww in 2011) approached the lowest-observed-adverse-effect-level (LOAEL) of 1.8 μg/g ww for pipping and hatching success (McKernan et al., 2009), but there was no evidence of impaired hatching success or overall productivity in Chesapeake Bay ospreys. Many uncertainty factors (differences in species sensitivity and metabolism, exposure to mixtures of toxicants) and knowledge

gaps for toxicity thresholds make extrapolations from the lab to the field difficult.

In place of PBDE formulations, alt-BFRs are now being manufactured and there are concerns regarding their potential persistence and bioaccumulation (de Wit et al., 2011 and Stieger et al., 2014). Like PBDEs, these BFRs can leach into the environment and have been detected in piscivorous birds (Glaucous gulls *Larus hyperboreus*, Verreault et al., 2007; herring gulls *Larus argentatus*, Gauthier et al., 2007; white-tailed sea eagles *Haliaeetus albicilla*, Eulaers et al., 2014). Among the alt-BFRs, HBCD has been shown to cause endocrine and behavioral effects in captive American kestrels (Marteinson et al., 2011; 2012). Concentrations of HBCD in Chesapeake Bay osprey eggs were much lower than the HBCD toxicity thresholds determined to date. In this current study, α-HBCD was detected most frequently in osprey egg samples, but at much lower concentrations than other locations, including the Norwegian Arctic (7.23–63.9 ng/g ww, Verreault et al., 2007 versus < MDL-3.72 ng/g ww in the present study). Our results reveal that mixtures of flame retardants are present in egg samples from the Chesapeake Bay, but the biological significance of these levels is unclear.

DNA damage

This study documents evidence of mild DNA damage in Baltimore Harbor/Patapsco and Anacostia/middle Potomac Rivers compared to the reference site, and also variations of 8-OH-dG concentrations between individuals. Xenobiotics including PCBs, PBDEs and even PAHs are known to increase reactive oxygen species in cells and can damage lipids, proteins and DNA. Mild oxidative stress has been detected in American kestrels (and to a lesser degree in common terns) in response to embryonic

exposure to the penta-BDE commercial mixture DE-71 (Fernie et al., 2005, McKernan et al., 2009 and Rattner et al., 2013). Osprey nestlings are exposed to a multitude of stressors, and may have adapted antioxidant defense mechanisms. Life history traits including trophic level and life span may influence their capability to tolerate oxidative stress leading to large variations in responses even between individuals (Costantini, 2008). Current evidence suggests that oxidative stress may impair immune function, longevity and reproduction (Costantini, 2008). Ultimately, damage to DNA may make the individual susceptible to disease progression and epigenetic effects that may be reflected in later generations (Cooke et al., 2003).

Conclusions

The present findings indicate that organochlorine pesticides and PBDE flame retardants continue to decline in osprey eggs in Chesapeake Bay, consistent with discontinuation of their use. Low levels of organochlorine pesticides were detected at all sites, well below the threshold associated with 10% eggshell thinning. Interestingly, PBDE residues in eggs were greatest near the Blue Plains WWTP on the Potomac River, which suggests that wastewater discharge and sewage sludge are a source of continued input of PBDEs to the environment (LaGuardia et al., 2010). While total PCB concentrations declined at some sites, they remain high in Baltimore Harbor/Patapsco River and continue to drive fish consumption advisories in Chesapeake Bay (MDE, 2014; VDH, 2009). Overall, there were no apparent large-scale effects of the contaminants examined here on osprey productivity. The Bay osprey population continues to increase and productivity rates are at or have exceeded the threshold to maintain a stable

population. Since reaching their nadir in the 1970s the Chesapeake Bay osprey population has demonstrated its resilience in the face of anthropogenic threats. Although osprey productivity appears stable at the population level, DNA damage assays suggest more subtle biochemical and cellular effects that could have consequences on the fitness of some individuals.

Supplemental Table S1. Concentrations of *p,p'*-DDE, total PCBs and PBDEs in archived eggs analyzed in 2000-2001 and re-analyzed in 2011-2012.

Sample ID	Site	Analytical Laboratory	*p,p'*-DDE (µg/g ww)	*p,p'*-DDE Percent Difference	Total PCBs (µg/g ww)	Total PCBs Percent Difference	Total PBDEs (ng/g ww)	Total PBDEs Percent Difference
BHRC3-1	Baltimore Harbor/Patapsco/River	Hale 2011	0.926	56.2	7.75	38.3	243	11.5
		GERG 2000	0.519		11.4		272	
BHCC1-1	Baltimore Harbor/Patapsco/River	Hale 2011	0.453	35.6	5.07	4.14	394	15.5
		GERG 2000	0.316		4.86		337	
BHORB1-1	Baltimore Harbor/Patapsco/River	Hale 2011	1.955	110.2	16.6	68.7		
		GERG 2000	0.566		8.10			
BHBP7-1	Baltimore Harbor/Patapsco/River	Hale 2011	0.758	49.9	7.94	12.0		
		GERG 2000	0.455		7.04			
BHSC5-1	Baltimore Harbor/Patapsco/River	Hale 2011	0.790	49.3	10.3	2.16		
		GERG	0.478		10.0			
PRSNRL-1	Anacostia/middle Potomac Rivers	Hale 2011	1.100	21.2	7.42	10.0		
		GERG 2000	0.889		8.20			
PRDC-2	Anacostia/middle Potomac Rivers	Hale 2011	1.720	26.8	7.01	30.6		
		GERG 2000	1.314		9.55			
PR88-1	Anacostia/middle Potomac Rivers	Hale 2011	1.513	26.2	9.44	4.58		
		GERG 2000	1.163		9.02			
ERWBP-3	Elizabeth River	Hale 2012	0.650	36.8	4.89	30.8	149	26.6
		GERG 2001	0.448		3.58		195	
ER8-1	Elizabeth River	Hale 2012	1.370	32.2	11.6	41.1	174	35.3
		GERG 2001	0.990		7.65		248	
CINW-1	Elizabeth River	Hale 2012	0.911	42.8	1.12	18.5	153	0.73
		GERG 2001	0.590		1.35		154	

[a]In 2000-2001 osprey eggs were analyzed by the Geochemical and Environmental Research Group (GERG) at Texas A&M. These eleven eggs were re-analyzed in 2011-2012 respectively by the lab of Dr. Robert C. Hale at the Virginia Institute of Marine Science. In 2000-2001, only a subset of the eggs were analyzed for PBDEs, which limited the number of samples (n = 5) that could be compared. Percent difference = (absolute difference/mean) x 100%.

CHAPTER 3

CHESAPEAKE BAY FISH-OSPREY (*Pandion haliaetus*) FOOD WEB: EVALUATION OF CONTAMINANT EXPOSURE AND GENETIC DAMAGE

Introduction

Ospreys (*Pandion haliaetus*) are a well-known ecotoxicological sentinel species. Many studies have utilized this charismatic fish-hawk as a bioindicator to increase knowledge of spatial and temporal trends in contaminants (Golden and Rattner, 2003; Grove et al., 2009). For example, ospreys feed at a high trophic level and biomagnify lipophilic contaminants. The Chesapeake Bay provides breeding habitat to the largest osprey population in the world due its shallow waters and high productivity (Poole, 1989).

The decline and later recovery of the Chesapeake Bay osprey population was associated with the patterns of use of the organochlorine pesticide DDT, in particular, the *p,p'*-DDE metabolite that induces eggshell thinning (e.g., Wiemeyer et al., 1975; Spitzer, 1980; Poole, 1989; Audet et al., 1992; Watts et al., 2004). While several studies have examined trends in osprey productivity in relation to contaminants, few have addressed this issue from an integrative food web perspective (Henny et al., 2003, 2009; Chen et al., 2010). Studies of osprey diet and foraging ecology in the Chesapeake are limited (e.g., McLean and Byrd, 1991; Glass and Watts, 2009). These investigators documented that energy rich menhaden (*Brevoortia tyrannus*) was a dominant osprey prey item in high salinity sites such as the lower James River. However, ospreys nesting in low salinity sites on the upper James and York Rivers primarily consumed catfish (*Ictaluridae*) species and gizzard shad (*Dorosoma cepedianum*).

The last large-scale ecotoxicological study of ospreys in the Chesapeake Bay was conducted in 2001-2002 (Rattner et al., 2004), and focused upon the United States Environmental Protection Agency (US EPA) designated Regions of Concern (ROCs; *viz.*, Anacostia/middle Potomac, Baltimore Harbor/Patapsco and Elizabeth Rivers). In order

to meet the requirements described in the Chesapeake Bay Executive Order 13508 (2009), federal agencies expanded their research and monitoring efforts to generate strategies to reduce toxics in the major tributaries of the Chesapeake. This entailed assessments of contaminant exposures and effects on fish and wildlife. In 2011-2012, we re-evaluated contaminants in ospreys nesting in Chesapeake Bay ROCs and found that concentrations of halogenated pollutants in eggs had declined, but there remained evidence of genetic damage in nestlings found in the most industrialized regions (Lazarus et al., 2015a).

There are limited ecotoxicological data for wildlife for the northern regions of the Bay. The Susquehanna is the largest freshwater inflow to the Chesapeake Bay (Rattner and McGowan, 2009). Between 2011 and 2013, we studied contaminant exposure, food web transfer and potential effects on ospreys nesting in the Susquehanna, James and Potomac Rivers. Due to the distribution of osprey nests on the Susquehanna River, sampling efforts focused on the lower Susquehanna River and flats. In addition, contaminant exposure was monitored in Back River, Maryland (northeast of Baltimore Harbor and the Patapsco River). Relative to other sites in the Chesapeake Bay, sediments from Back River contain very high concentrations of PCBs (McGee et al., 1999; Klosterhous, 2007), and preliminary observations (Lazarus unpublished data) indicated poor osprey productivity in the vicinity of the Back River wastewater treatment plant (WWTP). Herein, we describe findings of a study measuring contaminant concentrations and their transfer (biomagnification) between whole fish and osprey eggs. Reproductive success, eggshell thickness and oxidative genetic damage in nestlings were employed to

investigate contaminant-related effects at various biological levels (population, individual and molecular) among several Chesapeake Bay tributaries.

Materials and Methods

Study sites

From 2011 to 2013, three major Chesapeake Bay tributaries (lower Susquehanna River and flats, middle Potomac and James Rivers), Back River and Poplar Island reference site were studied (Figure 6). In 2011, whole fish and osprey eggs and nestling blood samples were collected along a 45 km stretch of the (i) Anacostia/middle Potomac River (Frederick Douglass Bridge, Washington, District of Columbia to Mattawoman Creek, Maryland). In 2012, similar sampling was conducted along a 60 km stretch of the (ii) James River (Richmond to Milton, Virginia). Finally, in 2013, sampling was undertaken along a 20 km stretch along the (iii) Susquehanna River and flats (from Aberdeen, Maryland to the I-95 Millard E Tydings Memorial Bridge). In 2013, we also studied an 11 km stretch on (iv) Back River (Back River Wastewater Treatment Plant (WWTP) to Hart Miller Island). Based on results from previous studies, the (v) Paul S. Sarbanes Ecosystem Restoration Project at Poplar Island, Maryland (Poplar Island), a mid-Bay location, was used as a reference site for all three years (Rattner et al., 2013; Lazarus et al., 2015a; 2015b).

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 U.S. Geological Survey's Patuxent

Wildlife Research Center (USGS/PWRC) and the University of Maryland, and with appropriate federal and state scientific collection permits. Starting in late-March, osprey nests were visited every 7-10 days to determine the number of eggs laid, eggs hatched and young present at ≥ 40 days. These data were used to calculate productivity (Mayfield, 1961; Postupalsky, 1977; Steenhof and Newtown, 2007). Additional nests were monitored at each site as a potential source of nestling blood samples in the event that the selected study nest failed.

During the osprey reproductive period, dietary preferences of nesting adults were monitored using a variety of techniques (Johnson et al., 2008; Glass and Watts, 2009). Game camera (Bushnell 8MP Trophy Cam, Overland Park, KS, USA) images of prey items captured by osprey, direct identification of fish scraps found in osprey nests, and direct observations of prey deliveries (photo documented Nikon D3100 DLSR camera, AF VR-Nikkor 80-400mm lens, Nikon, Tokyo, Japan) were used to characterize osprey diet. Using these observations, we determined the 2-3 dominant prey items (based on the highest percent catch) to sample for the food web component of this study.

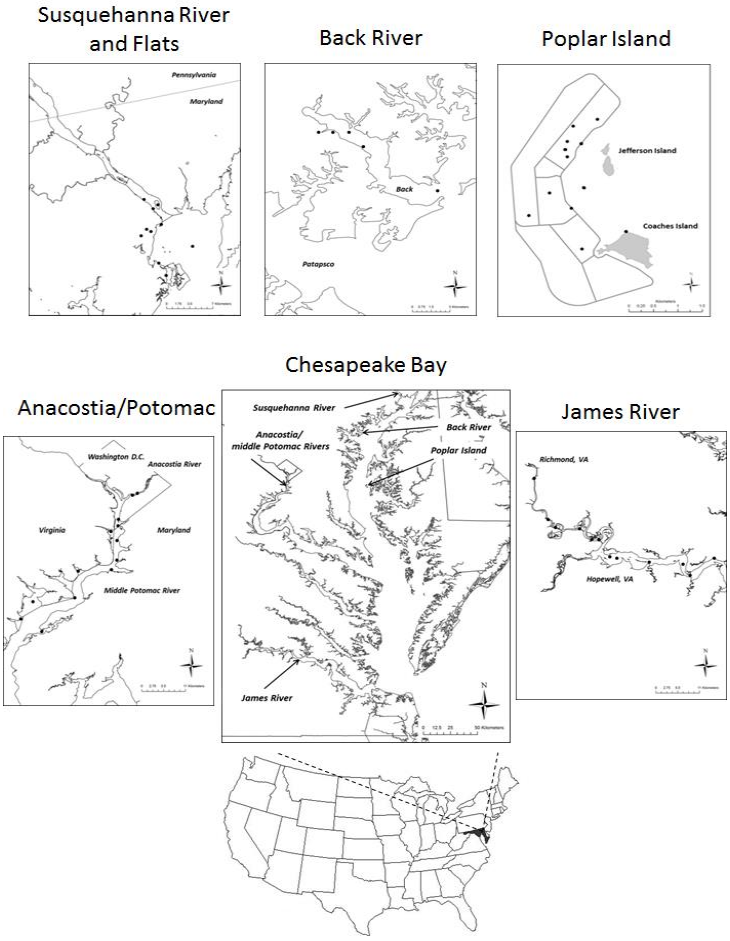


Figure 6. Locations of osprey nests sampled from in Chesapeake Bay and Poplar Island reference site, (•) indicates a sampled nest.

Osprey egg sample collection

Using egg sampling technique described by Blus (1984), upon completion of a clutch (3 or more eggs) one fresh egg was randomly collected from each study nest (Susquehanna, n=10; Anacostia/middle Potomac, n=13, James River, n=12; Back River n=5; Poplar Island, n=4/year). Eggs were transported to the U.S. Geological Survey Patuxent Wildlife Research Center (USGS/PWRC), cleaned, weighed and the length and

width were measured to the nearest 0.01-mm (Lazarus et al., 2015a). A 2.4-mm hole was drilled into the blunt end of the egg (Dremel® MultiPro® 7.2V, Model 770, Mt. Prospect, IL, USA) and distilled water was injected into the air cell to return contaminant concentrations to that of a freshly laid egg (Heinz et al., 2009). Each egg was opened, contents (excluding shell membrane) were transferred to a chemically clean jar (I-CHEM, VWR Scientific, Radnor, PA, USA), examined and weighted, and then stored -80ºC. Shells were dried for 3-4 months at room temperature and thickness measurements were taken at three points along the equator using a micrometer (Model 1010M; L.S. Starrett Co., Athol, MA, USA).

Nestling blood samples and morphological endpoints

Osprey nestling blood samples were collected from 40-45-day-old young at each sampled 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, one 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 5-7 mL brachial blood sample was collected using a 23-gauge 25.4 mm needle into a heparinized syringe (Sarstedt International, Newton, NC, USA). About 100 µL of blood was immediately transferred to a microcentrifuge tube, frozen on dry ice and stored at -80ºC for subsequent DNA damage assays. The remainder of the blood sample was centrifuged, and plasma was harvested for pharmaceutical (Lazarus et al., 2015b) 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 25-35 cm in length (preferential prey size for ospreys; Poole et al., 1989). On the Anacostia/middle Potomac and the James Rivers, fish were caught by electroshocking. On the Susquehanna, a combination of electroshocking and hook and line were used to capture live fish. At Poplar Island, fish were caught using a midwater trawl and a commercial pound net. Fish (n=201) were collected and stored at -20ºC, and then composited (n=3 fish/composite) by location, species and size. Due to the limited number of osprey nests on Back River, only a small sampling of menhaden (*Brevoortia tyrannus*), gizzard shad (*Dorosoma cepedianum*) and white perch (*Morone Americana*) were collected.

Chemical analysis of osprey eggs, whole fish and quality assurance

Whole fish and osprey egg contents were analyzed for 129 PCB congeners, 44 organochlorine pesticides, methoxytriclosan, 11 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-ethylhexl 2,3,4,5-tetrabromobenzoate (TBB), and decabromodiphenyl ether (DBDPE)]. During analysis for PBDEs, one osprey egg sample from the Back River was lost during sample processing (n reduced from 5 to 4).

Chemical analyses were conducted at the Virginia Institute of Marine Science (VIMS) following the methods described in Lazarus et al. (2015b), Chen et al. (2008) and La Guardia et al. (2007, 2010, 2013). Briefly, egg contents were homogenized,

lyophilized and spiked with surrogate standards PCB 30, 65 and 204 (Ultra Scientific, North Kingstown, RI, USA), ^{13}C-methoxytriclosan, ^{13}C-PCB-126 and 2,3,4,4',5,6-hexabromodiphenyl ether (BDE-166; Cambridge Isotope Laboratories, Inc., Andover, MA, USA). Sodium sulfate blanks were analyzed. Dried samples underwent solvent extraction using methylene chloride (DCM) at 100ºC and 68 atm. Extracts were purified by size exclusion chromatography (SEC, Envirosep-ABC®, 350 x 21.1 mm column; Phenomenex, Torrance, CA, USA). Each post-SEC extract was reduced in volume, added to the top of a 2-g silica gel glass solid phase extraction column (Isolute, International Sorbent Technology, Ltd., Hengoed Mid Glamorgan, UK) 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 two fractions were combined and then split, with half going for coplanar PCB analysis. Coplanar PCBs were separated from nonplanar PCBs by elution through a Supleclean ENVI-Carb SPE column (Sigma-Aldrich Co., St. Louis, MO, USA). 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, North Kingstown, RI, USA) as an internal standard, and analyzed by gas chromatography/mass spectrometry (GC/MS) on an Agilent 5975C (Agilent Technologies, Santa Clara, CA, USA), in electron impact mode and selected ion monitoring. A 60 m DB-5 MS GC column (Agilent, 0.32 mm ID x 0.1 µm thickness) was used.

The second half of the silica SPE fraction retained was spiked with decachlorodiphenyl ether (Ultra Scientific) as the internal quantitation standard. Identification and quantitation of non coplanar 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 ID x 0.25 µm thickness). PBDEs and alt-BFRs were separated by ultra-performance liquid chromatography (UPLC, Waters Corp. Milford, MA, USA) and analyzed by atmospheric pressure photoionization tandem mass spectrometry (APPI/MS/MS, Q-Trap3200 MS, AB Sciex, Framingham, MA. USA). Further details of the UPLC-APPI/MS analysis can be found in La Guardia et al. (2013).

Method detection limits on a wet weight basis were converted to a lipid weight basis using the following formulas: MDL dry = MDL wet/(1-% water) and MDL lipid = (MDL dry/% lipid). Percent lipid and percent moisture used in this calculation were from the lowest samples to allow for the most conservative estimates. The method detection limit (MDL) for organochlorine pesticides and non-coplanar PCB congeners for osprey eggs was 0.4 µg/kg wet weight (ww) and 11.9 µg/kg on a lipid weight (lw) basis and for fish was 0.2 µg/kg and 7.2 µg/kg lw. The MDL for coplanar PCB congeners was 0.04 µg/kg ww and 1.19 µg/kg lw in osprey eggs and 0.02 µg/kg and 0.72 µg/kg lw in fish. The MDL for PBDEs and alt-BFRs was 0.4 µg/kg ww in eggs or 11.9 µg/kg lw and 0.2 µg/kg ww and 7.2 µg/kg lw in fish.

All data were corrected based on the recovery of surrogate standards in each sample and corrected for moisture loss back a to fresh weight basis. The average recovery of the surrogate standard PCB 204 and ^{13}C-methoxytriclosan from eggs was (mean ± SD) 81.8 ± 14.0% and 84.7 ± 10.7% in fish. Mean recovery for non-coplanar PCBs (surrogate standard PCB 204) was 87.9 ± 18.0% in eggs and 84.0 ± 21.2% in fish.

For coplanar PCBs (surrogate standard PCB 126) average recovery was 87.8 ± 20.7% and 96.1 ± 24.9% in fish. Average recovery of PBDEs (surrogate standard BDE 166) was 101.1 ± 25.0% and 104.4 ± 30.7% in fish. Overall, mean moisture content in eggs averaged 83.9 ± 0.52% and in fish 74.7 ± 3.54%.

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 (2009) 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. In order to calculate BMFs in this study, we applied the model presented in Elliott et al. (2005) and used in Chen et al. (2010). This equation adjusts residues based on diet composition:

$$Y=BMF\ [(F_1(X_1) + F_2(X_2) +\ldots+ F_n\,(X_n)] \qquad (1)$$

Y represents the geometric mean contaminant concentration in the predator, F_n represents the percentage of each prey species in the diet and X_n reflects the geometric mean contaminant residue per species consumed. Biomagnification factors were calculated using both ww and lw 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 % 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 Colorado Plateau Stable Isotopes Laboratory (CPSIL) at Northern Arizona University to determine ^{13}C and ^{15}N content. Nitrogen stable isotopes indicate relative trophic level for the local food web. Approximately 1 mL of osprey nestling plasma was freeze dried and analyzed using a Thermo-Electron Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) (Thermo Fisher Scientific Inc., Waltham, MA, USA). The IRMS was configured through the Finnigan CONFLO III (Thermo Finnigan, Barkhausenstr, Germany) using a Carlo Erba NC2100 elemental analyzer CE Elantech, Inc., Lakewood, NJ, USA). Carbon and nitrogen stable isotope compositions were obtained in a single run. CPSIL uses biological standards for calibration and raw data normalization from the National Institute of Standards and Technology and the International Atomic Energy Agency (Mauldin et al., 2012). Stable isotope values for $\delta^{13}C$ were reported permille (parts per thousand) (‰) according to the Vienna Pee Dee belemnite standard and $\delta^{15}N$ were reported relative to atmospheric air (AIR). CPSIL has uncertainty factor of ≤ 0.10 ‰ for $\delta^{13}C$ and ≤ 0.20 ‰ for $\delta^{15}N$.

DNA damage assays

Whole blood samples (n=45, one excluded from the James River due to consistently high % coefficient of variation after multiple re-runs) were analyzed for 8-hydroxy-2'-deoxyguanosine (8-OH-dG), as an indicator of DNA damage (assay methods and validation also described in Rattner et al., 2013; Lazarus et al., 2015a). Briefly, samples were analyzed using the DNA/RNA oxidative damage EIA kit (Cayman

Chemical, Ann Arbor, MI, USA). 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, Cary, NC, USA). Intra-assay variation (precision of duplicate determinations, coefficient of variation, CV ± 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 re-analyzed. Inter-assay variation among plates for reference samples was 4.1 ± 11.5% in 2012 and 9.93 ± 11.3% in 2014 assays. Those samples collected in 2011 on Poplar Island and the Anacostia/middle Potomac Rivers and those samples from the Susquehanna and James Rivers were analyzed separately in 2014. However, due to variations in performance between manufacturing lots we were unable to quantitatively compare all study sites. The limit of detection for this assay was 1.03 pg/µg DNA determined by evaluating the mean minus 3 standard deviations from the standard curve (Rattner et al., 2013).

Statistical analyses

Descriptive statistics were generated for continuously distributed variables (eggshell thickness, morphological endpoints, DNA damage and contaminant residues). Variables were tested for normality, homogeneity of variance and log transformed as necessary (Quinn and Keough, 2002). Analysis of variance was used to detect overall differences among sites, and specific comparisons were conducted using Tukey's HSD test (α=0.05). If the assumptions for an ANVOA were not met, Wilcoxon non-parametric 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 (Helsel, 2005). Fisher's Exact Test was used to compare site-specific differences in productivity endpoints. In osprey eggs, concentrations of Ah-receptor active PCB congeners were multiplied by toxic equivalency factors (TEFs) to estimate toxic equivalence (TEQs, van den Berg et al., 1998). A correlation analysis was conducted to examine relationships among all variables. A logistic analysis of convariance 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.

Redundancy analysis (RDA) was conducted using data from Lazarus et al. (2015a) combined with this current study to increase statistical power. All predictor variables (egg contaminant concentrations, DNA damage and stable isotope data) were log transformed to correct for normality and homogeneity of variances (Quinn and Keough, 2002). The $\delta^{13}C$ isotope data were –log(x) transformed because values were negative. Redundancy analysis (RDA; Legendre and Anderson, 1999) was used to assess if concentrations of PCB, PBDE, *p,p'*-DDE, DNA damage and carbon and nitrogen stable isotope measurements differed by study site:

$$DNA + PCB + PBDE + DDE + C + N \sim Site \quad (2)$$

We used RDA and not a more generalized distance based-RDA because Euclidian distance was appropriate for our data. We specifically examined if axes explained more variability than would be expected by chance alone using a permutation-based ANOVA. Year was used as a blocking factor since DNA damage assays from 2011 versus 2012 and 2013 were not comparable (Lazarus et al., 2015a). This analysis was conducted

using the RDA function from the Vegan package in R (R. Core Team, 2013; Oksanen et al., 2011). Interaction terms between contaminant concentrations were also evaluated and then a mixed effects linear regression was used to examine if any contaminant data affected DNA damage (sample site random effect).

Results

Productivity

Productivity for the five study sites ranged from 1.17 to 1.33 fledglings/active nest (Table 5). There were no site-specific differences in productivity (eggs laid, eggs lost, hatching, fledging and nest success) (Fisher's Exact Test $p>0.60$, Table 1) among the Susquehanna River and flats, Potomac, 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, 77.7% of the active pairs fledged young. On 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/active nest and 60.0% of the successful pairs fledged young.

Table 5. Reproductive success of ospreys nesting in Chesapeake Bay regional waterways of concern.

Site	Poplar Island[a]		Susquehanna River and Flats		Anacostia/middle Potomac River[a]		James River		Back River	
	2011-2013		2013		2011		2012		2013	
	Number	%	Number	%	Number	%	Number	%	Number	%
Active nests sampled	12		10		13		12		5	
Eggs Laid	36		31		41		38		18	
Sample egg collected	12		10		13		12		5	
Eggs relaid due to 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%	(18/19)	94.7%	(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)	83.30%
Fate of nestlings										
Dissappeared	0		0		1		1		2	
Found dead	0		0		0		0		0	
Fledged	(16/16)	100%	(18/18)	100%	(16/17)	94.10%	(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		0.9601 ± 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 x 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	
Egg success (A x B x C x D)	0.632		0.857		0.547		0.553		0.114	
Mean clutch size (E)	3.00		3.10		3.15		3.17		3.60	
Mean number of young surviving to 53 d (A x B x C x D x E)	1.90		2.66		1.72		1.75		0.410	
Mean number of young surviving to 53 d less sample egg (A x B x C x D x (E-1))	1.26		1.80		1.18		1.20		0.300	

Note: A=daily survival rate to the 39th power to account for a 39-43 day incubation period [62]; B=daily survival rate to the 53rd power to account for 50-55 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.

[a]A subset of these data have been previously published (Lazarus et al., 2015a).

[b]Considered to be lost during the egg stage.

[c]Eggs in nest >45 days or abandoned.

[d]Hatchability=eggs hatched/(eggs laid-eggs that disappeared or sampled before hatching), represents the % of eggs that remain in the nest through hatch.

Osprey eggshell thickness

Eggshells were thinner on the Anacostia/middle Potomac Rivers (p=0.003) compared to the reference site (Anacostia/middle Potomac Rivers, mean ± S.D.: 0.49 ± 0.05 mm vs. Poplar Island reference site: 0.55 ± 0.05). However, there were no differences in eggshell thickness among the other sites (Susquehanna: 0.52 ± 0.03; James: 0.52 ± 0.04 mm) compared to the Anacostia/middle Potomac and Poplar Island reference site. Back River had significantly thicker eggshells compared to the Potomac River (0.55 ± 0.03 mm; p=0.048).

Nestling body weight and culmen length

There were no significant differences in body weight and culmen length among sampling years (2011, 2012, and 2013) on Poplar Island (p > 0.49), and thus reference site measurements were combined. Overall, there were no differences in osprey nestling body weight across all study sites (p=0.18) and body weight averaged 1595.7 ± 156.7g. Culmen length did not vary among sites (p=0.34) and averaged 31.0 ± 1.1 mm. Although not included in statistics, four additional chicks on Back River were studied and their body weight averaged 1481.7 ± 28.8 g and culmen length averaged 28.6 ± 1.3 mm.

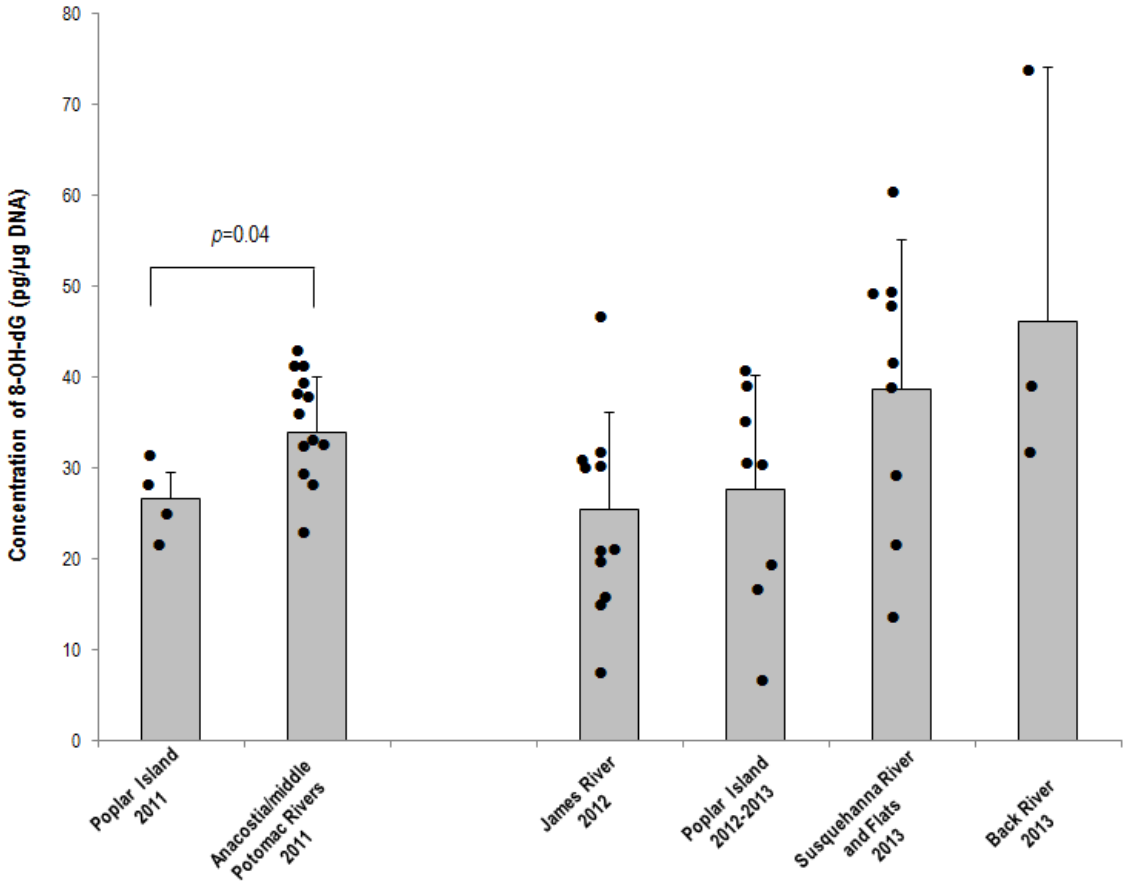


Figure 7. Concentrations of 8-hydroxy-2'-doxyguanosine (8-OH-dG) in osprey nesting whole blood. Capital letters indicate a statistically significant difference (p<0.05) using Tukey's HSD test. Means, standard deviations and individual values are presented (•). Data from 2011 and 2012/2013 were analyzed separately.

DNA damage

DNA damage assays were conducted in 2012 and 2014; however, due to variations in performance between assay kit lots, results cannot be quantitatively compared among years. Results were generated for 48 nestling blood samples (CV<20%), with one sample from the James River excluded that consistently exhibited a poor precision in three separate assays.

In 2011, nestlings from the Anacostia/middle Potomac River (33.9 ± 6.1 pg/µg DNA) exhibited greater DNA damage than those from the Poplar Island reference site

(26.6 ± 2.9 pg/µg DNA) (p=0.04, Figure 7). There was no difference (p=0.1532) in oxidative DNA damage in nestling blood samples from Poplar Island in 2012 and 2013, and thus data for these two years were combined (27.6 ± 12.6 pg/µg DNA). There were no significant differences (p=0.09) in DNA damage in nestling blood samples from the Susquehanna, James and Back Rivers and Poplar Island reference sites in 2012 and 2013. Of the three nestling blood samples collected from the Back River, 8-OH-dG averaged 46.1 ± 28.1 pg/µg DNA; the nestling sampled closest to the WWTP had the greatest 8-OH-dG value (78.1 pg/µg DNA) of all samples analyzed in this study.

Osprey diet characterization

Over the three-year study, a total of 1,662 osprey prey items (15 fish species) were documented (Supplemental Table S2). On Poplar Island osprey predominately fed on striped bass (*Morone saxatilis*) (47.8% of diet) and Atlantic menhaden (*Brevoortia tyrannus*) (44.3% of diet). On the Susquehanna, Anacostia/middle Potomac and James Rivers osprey fed predominately on catfish (*Ictaluridae sp.*) and gizzard shad (*Dorosoma 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, α = 0.008 for Bonferroni Correction), but slightly lower on the Susquehanna (17.5 ‰) and James Rivers (15.3 ‰, p<0.006). This indicates that ospreys are feeding at fairly similar trophic levels across study sites. A complete reconstruction of osprey diet was not the specific goal of this study; however, these basic dietary observations were used to identify the fish species to sample for the food web component of this study.

Contaminants in fish

Organochlorine pesticides, PCBs, PBDEs, and alt-BFRs were measured in the dominant fish species in osprey diet (Supplemental Table S3). Of the contaminants 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 mean concentrations of *p,p'*- DDE (39.1 ng/g ww) that were about 4 times greater than Poplar Island (9.24 ng/g ww) ($p< 0.0001$), with values on the Susquehanna and James Rivers in the intermediate range. Similarly, fish on the Anacostia/middle Potomac Rivers contained the greatest total PCB residues (481.2 ng/g ww, ranging up to 1,145.2 ng/g ww compared to Poplar Island (49.1 ng/g ww, ranging up to 102.3 ng/g ww) ($p< 0.0001$). Like *p,p'*-DDE, total PCBs on the Susquehanna and James 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. Alternative (alt)-BFRs were detected at low quantities across all study sites (all < 8.3 ng/g ww). Qualitatively, *p,p*-DDE residues were not unlike those on the Anacostia/middle Potomac Rivers, while total PCB and PBDE concentrations in gizzard shad were the greatest compared to the other study sites.

Contaminants in osprey eggs

Overall, there were significant relationships between contaminants and osprey productivity as indicated by logistic regression ($p>0.22$). 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 osprey eggs. In total, the same 19 organochlorine pesticides, 6 PBDE congeners, 4 alt-BFRs, 1 coplanar PCBs 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 for *p,p'*-DDE, *cis*-chlordane, *cis*-nonachlor, *trans*-nonachlor ($p<0.02$) compared to other study sites (Table 6). 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 ww). The metabolite of the antimicrobial agent triclosan, methoxytriclosan, was detected at most study sites (Poplar Island 1/12, range <MDL-1.49 ng/g ww; Susquehanna River all <MDL; Anacostia/middle Potomac 12/12, range 0.40-6.29 ng/g ww; James River 1/12, range <MDL-1.49 ng/g ww; and Back River 1/5, range <MDL-3.77 ng/g ww).

Total PCBs (sum of non-coplanar plus coplanar congeners) were elevated at all study sites compared to Poplar Island ($p<0.0001$) (Table 7). There were no significant differences in PCB concentrations in eggs among the other 3 sites ($p>0.47$). Upon inspection of the data, geometric mean PCB residues for Back River were similar to the other site means. Congener 169 was detected most frequently on the James. The TEQ on the Anacostia/middle Potomac Rivers (0.19 ng/g) was greater compared to the other study sites ($p<0.025$).

Total PBDE concentrations were greatest on the Susquehanna and Anacostia/middle Potomac Rivers compared to other sites ($p<0.002$) (Table 8). The maximum PBDE residue (801.8 ng/g ww) was detected in vicinity of the Blue Plains WWTP on the middle Potomac River. Congeners 47, 100, 153 and 154 followed a

similar pattern with values being higher on the Susquehanna and middle Potomac Rivers compared to the other study sites. Upon inspection of the data, α-HBCD, BTBPE, DBDPE, TBB, and TBPH were most frequently detected on the Anacostia/middle Potomac Rivers with 5 of the 7-alt BRFs detected in osprey eggs. Generally, alt-BFR values were two orders of magnitude lower than PBDE concentrations. Beta (β) and gamma (γ)-HBCD were not detected in any osprey egg samples.

Table 6. Osprey egg concentrations (µg/g ww) of organochlorine pesticides and metabolites from Chesapeake Bay regional waterways and Poplar Island reference site[a].

Contaminant (µg/g ww)	Poplar Island[b]	Susquehanna River and Flats	Anacostia and middle Potomac Rivers[b]	James River	Back River
	2011-2013 (n=12)	2013 (n=10)	2011 (n=13)	2012 (n=12)	2013 (n=5)
	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]
	Extremes	Extremes	Extremes	Extremes	Extremes
	n detected	n detected	n detected	n detected	n detected
p,p'- DDE	0.160[C]	0.410[B]	0.628[A]	0.315[B]	0.432[A,B]
	0.0903-0.414	0.325-0.777	0.370-1.00	0.241-0.500	0.191-0.667
	12/12	10/10	13/13	12/12	5/5
p,p'- DDD	0.010[C]	0.025[A,B]	0.034[A]	0.018[B]	0.046[A]
	0.006-0.037	0.018-0.034	0.019-0.072	0.008-0.040	0.014-0.103
	12/12	10/10	13/13	12/12	5/5
Dieldrin	-	-	0.0149-0.0150	-	0.0522-0.0523
	<MDL	<MDL	<MDL-0.035	<MDL	<MDL-0.203
	0	0	11/13	0	3/5
Heptachlor epoxide	-	-	0.035	-	-
	<MDL-0.006	<MDL-0.009	0.014-0.096	<MDL-0.010	<MDL
	2/12	1/10	13/13	1/12	0
α-Chlordane (*cis*)	-	-	0.015[A]	0.005[B]	0.023[A]
	<MDL-0.018	<MDL	0.0045-0.0409	0.002-0.006	0.011-0.050
	2/12	0	13/13	12/12	5/5
γ-Chlordane (*trans*)	-	-	0.0019-0.0021	-	-
	<MDL-0.004	<MDL	<MDL-0.005	<MDL	<MDL-0.004
	1/12	0	8/13	0	1/5
cis -Nonachlor	0.0035-0.0036[C]	-	0.033[A]	0.008[B]	0.05[A]
	<MDL-0.010	<MDL-0.014	0.010-0.058	0.008-0.012	0.017-0.160
	9/12	4/10	13/13	12/12	5/5
trans -Nonachlor	0.0028-0.0029[C]	0.0043-0.0044[B,C]	0.017[A]	0.005[B]	0.022[A]
	<MDL-0.014	<MDL-0.011	0.005-0.037	0.002-0.007	0.011-0.033
	8/12	7/10	13/13	12/12	5/5
Oxychlordane	-	-	0.016	-	-
	<MDL	<MDL	0.005-0.051	<MDL	<MDL
	0	0	13/13	0	0
Mirex	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]
	<MDL-0.003	<MDL-0.009	0.002-0.005	<MDL-0.007	<MDL-0.013
	6/12	6/10	13/13	9/12	3/5

[a]-No mean calculated, as contaminant was detected in fewer than half the samples; MDL, method detection limit; extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons (p <0.05), and a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction.

[b]A subset of these data have been previously published (Lazarus et al., 2015a).

[c]If non-detects were present in less than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean.

Table 7. Osprey egg concentrations (µg/g ww) of polychlorinated biphenyls and congeners from Chesapeake Bay regional waterways and Poplar Island reference site[a]

Contaminant	Poplar Island[b]	Susquehanna River and Flats	Anacostia and middle Potomac Rivers[b]	James River	Back River
	2011-2013	2013	2011	2012	2013
	(n=12)	(n=10)	(n=12)	(n=12)	(n=5)
	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]
	Extremes	Extremes	Extremes	Extremes	Extremes
	n detected	n detected	n detected	n detected	n detected
Total PCBs (µg/g)	1.43[B]	4.15[A]	4.94[A]	4.19[A]	4.46[A]
	0.650-3.22	1.89-7.75	2.72-6.53	2.35-6.50	1.34-7.95
	12/12	10/10	13/13	12/12	5/5
Congener 77 (pg/g)	96.5	103.70	113.1-116.2	176.9	189.0
	37.0-420.0	40.0-250.0	<MDL-170	100.0-330.0	120.0-430.0
	12/12	10/10	12/13	12/12	5/5
Congener 81 (pg/g)	-	-	-	133.1	-
	<MDL-340	<MDL	<MDL-80	40.0-250.0	<MDL-40.0
	2/12	0	6/13	12/12	1/5
Congener 126 (pg/g)	197.6[B]	437.5[A]	534.6-537.7[A]	599.2[A]	598.0-606.0[A]
	61.0-730.0	170.0-620.0	<MDL-830	480.0-140.0	<MDL-740
	12/12	10/10	12/13	12/12	4/5
Congener 169 (pg/g)	-	-	60.0-72.3	74.0	-
	<MDL	<MDL	<MDL-100	30.0-140.0	<MDL
	0	0	9/13	12/12	0
Congener 105 (ng/g)	8.47-8.60[B]	40.1[A]	31.6[A]	27.4[A]	45.5[A]
	<MDL-33.3	12.4-228.7	9.16-66.1	20.8-37.5	12.7-97.4
	8/12	10/10	13/13	12/12	5/5
Congener 118 (ng/g)	65.2[C]	118.2[B]	216.5[A]	118.6[B]	203.9[B]
	32.4-210.5	55.7-181.1	67.4-415.0	90.0-171.2	48.6-437.9
	12/12	10/10	13/13	12/12	5/5
Congener 128 (ng/g)	17.7-17.8[C]	51.5[A,B]	95.1[A]	42.2[A,B]	67.8[A,B]
	<MDL-31.0	21.2-91.7	39.0-154.5	28.9-63.9	14.4-153.8
	9/12	10/10	13/13	12/12	5/5
Congener 138/158 (ng/g)	183.0[B]	492.4[A]	482.6[A]	543.0[A]	603.7[A]
	100.5-460.7	194.5-816.4	289.7-975.0	295.4-803.5	162.3-1074.1
	12/12	10/10	13\13	12/12	5/5
Congener 156 (ng/g)	8.48-8.58[B]	21.3-21.5[B]	53.8[A]	16.6[B]	24.7-24.8[A,B]
	<MDL-29.8	<MDL-50.3	28.3-84.5	5.98-27.8	<MDL-59.2
	9/12	7/10	13/13	12/12	3/5
Congner 167 (ng/g)	-	-	-	-	-
	<MDL	<MDL-15.6	<MDL-18.8	<MDL	<MDL-15.6
	0	2/10	2/13	0	2/5
Congener 189 (ng/g)	0.95-1.15[C]	NA	8.30-8.33[A]	4.1-4.2[B]	-
	<MDL-2.51	<MDL-8.94	<MDL-13.0	<MDL-17.4	<MDL
	6/12	3/10	12/13	9/12	0
Congener 170/190 (ng/g)	51.2-51.3[C]	191.6[B]	282.3[A]	157.4[B]	224.4-224.5[A,B]
	<MDL-174.1	74.3-386.2	125.2-411.6	59.6-254.1	<MDL-492.3
	9/12	10/10	13/13	12/12	4/5
Toxic equivalents (ng/g)	0.06[B]	0.12[A]	0.19[A]	0.13[A]	0.17[A]
	0.03-0.14	0.08-0.18	0.07-0.28	0.11-0.16	0.03-0.29

[a]-No mean calculated, as contaminant was detected in fewer than half the samples; MDL, method detection limit; extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p<0.05$), and a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction.

[b]A subset of these data have been previously published (Lazarus et al., 2015a).

[c]If non-detects were present in less than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean.

Table 8. Osprey egg concentrations (ng/g ww) of polybrominated diphenyl ethers and alternative brominated flame retardants from Chesapeake Bay regional waterways and Poplar Island reference site[a].

Contaminant (ng/g ww)	Poplar Island[b]	Susquehanna River amd Flats	Anacostia and middle Potomac Rivers[b]	James River	Back River
	2011-2013	2013	2011	2012	2013
	(n=12)	(n=10)	(n=13)	(n=12)	(n=4)
	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]	Geometric Mean[c]
	Extremes	Extremes	Extremes	Extremes	Extremes
	n detected	n detected	n detected	n detected	n detected
Total PBDEs	84.4[C]	368.8[A]	343.5[A]	179.5[B]	342.8[A,B]
	52.7-274.9	212.4-648.6	170.4-801.8	135.2-216.8	286.9-549.1
	12/12	10/10	13/13	12/12	4/4
BDE congener 47	51.5[C]	224.5[A]	250.3[A]	100.3[B]	203.4[A,B]
	32.4-183.5	116.1-398.2	104.0-648.3	79.4-121.6	154.9-348.7
	12/12	10/10	12/12	12/12	4/4
BDE congener 85	-	-	-	-	-
	<MDL-0.76	<MDL	<MDL	<MDL	<MDL
	1/12	0	0	0	0
BDE congener 99	3.01[C]	58.5[A]	22.1[B]	26.0[B]	45.2[A,B]
	0.17-29.9	25.1-92.1	8.88-35.7	14.4-39.1	28.5-73.0
	12/12	10/10	13/13	12/12	4/4
BDE congener 100	12.2[D]	64.7[A]	39.9[B]	25.4[C]	46.3[A,B,C]
	4.79-39.5	37.8-107.0	18.0-77.1	20.4-34.1	36.2-68.6
	12/12	10/10	13/13	12/12	4/4
BDE congener 153	3.11[B]	14.0[A]	13.47-13.51[A]	11.6[A]	13.6[A]
	2.00-8.20	8.10-23.9	<MDL-26.6	7.38-20.2	9.0-26.5
	12/12	10/10	12/13	12/12	4/4
BDE congener 154	8.77[C]	19.3[A]	13.6[A,B]	11.4[B,C]	22.3[A]
	5.01-20.7	11.7-27.9	8.37-21.1	5.98-20.6	16.5-31.9
	12/12	10/10	13/13	12/12	4/4
BDE congener 183	-	-	1.24-1.33	-	-
	<MDL	<MDL	<MDL-3.68	<MDL	<MDL
	0	0	10/13	0	0
BDE congner 209	-	-	-	2.95-3.02	8.40-8.60
	<MDL-24.2	<MDL-12.6	<MDL-1.21	<MDL-9.74	<MDL-20.4
	5/12	4/10	2/13	10/12	2/4
α-HBCD	0.82-0.95	-	1.30-1.31	-	-
	<MDL-2.14	<MDL	<MDL-3.03	<MDL-10.2	<MDL
	8/12	0	12/13	5/12	0
BTBPE	-	-	-	-	-
	<MDL-4.77	<MDL	<MDL-28.7	<MDL	<MDL
	1/12	0	4/13	0	0
DBDPE	-	-	-	-	-
	<MDL	<MDL	<MDL-0.89	<MDL	<MDL
	0	0	1/13	0	0
TBB	-	2.30-2.50	-	-	5.60-5.80
	<MDL-63.7	<MDL-7.40	<MDL-30.3	<MDL-2.14	<MDL-11.5
	2/12	5/10	1/13	1/12	2/4
TBPH	-	-	-	-	2.00-2.20
	<MDL-31.3	<MDL-2.4	<MDL-7.37	<MDL-0.54	<MDL-4.3
	3/12	3/10	3/13	1/12	2/4

[a]-No mean calculated, as contaminant was detected in fewer than half the samples; MDL, method detection limit; extremes are defined as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p<0.05$), and a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction.

[b]A subset of these data have been previously published (Lazarus et al., 2015a).

[c]If non-detects were present in less than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean.

Biomagnification factors

Biomagnification factors were used to relate contaminant residues in fish to those in osprey eggs (Table 9). Using dietary observations, the ratio of predominant fish prey species on Poplar Island (menhaden and striped bass) and the Susquehanna (catfish and gizzard shad) was approximately 1:1. On the Anacostia/middle Potomac, birds were feeding predominately on catfish and gizzard shad (ratio of 3:2), and prey species of ospreys on the James River were similar but at a slightly different ratio (3:1). Using these proportions of prey species consumed by ospreys, the BMFs for osprey eggs (ww basis) averaged 16.5 for *p,p'*-DDE, 25.4 for PCBs, and about 17.9 for total PBDEs and 15.4-15.5 for BDE congeners 47, 99, 100 and 154. 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, *cis*-chlordane, *trans*-chlordane, and methoxytriclosan) indicating no biomagnification in the upper trophic levels in Chesapeake Bay.

Table 9. Biomagnification factors (BMFs) and a wet weight (ww) and lipid weight (lw) from whole fish to osprey egg contents by study site.

Site	Poplar Island (2011-2013)					Susquehanna River and Flats					Anacostia/middle Potomac Rivers					James River					
Contaminant	Atlantic Menhaden	Striped Bass	Diet Ratio[a] 1:1	Osprey	BMF	Catfish Sp.	Gizzard Shad	Diet Ratio[a] 1:1	Osprey	BMF	Catfish Sp.	Gizzard Shad	Diet Ratio[a] 6:4	Osprey	BMF	Catfish Sp.	Gizzard Shad	Diet Ratio[a] 3:1	Osprey	BMF	Average BMF
p,p -DDE (ng/g ww)	12.0	6.48	9.24	160.4	17.4	20.8	24.3	22.6	410.0	18.18	43.7	34.4	40.0	628.0	15.71	21.7	21.4	21.6	315.0	14.57	16.45
(ng/g lw)	108.8	224.2	166.5	3728.6	22.4	591.8	381.9	486.9	10401.5	21.4	1018.1	545.9	829.2	14896.0	18.0	418.6	317.1	393.2	6878.0	17.49	19.80
p,p -DDD (ng/g ww)	3.0	1.2	2.1	10.0	4.8	3.12	5.11	4.1	24.5	6.0	10.0	10.0	10.0	34.0	3.40	3.21-3.25	3.49	3.28-3.31	18.1	5.47-5.52	4.92-4.93
(ng/g lw)	27.5	40.8	34.2	242.2	7.1	88.70	80.2	84.5	622.7	7.4	223.2	158.4	197.3	807.9	4.1	52.3-53.0	51.7	52.2-52.7	395.7	7.51-7.58	6.53-6.55
cis -chlordane (ng/g ww)	<MDL	<MDL	-	<MDL	-	2.64	5.36-5.43	4.00-4.04	<MDL	-	20.4	33.1	25.5	15.4	0.60	2.23-2.37	<MDL	1.67-1.88	5.0	2.66-2.99	1.63-1.80
(ng/g lw)	<MDL	<MDL	-	<MDL	-	74.90	152.2-153.4	113.6-114.1	<MDL	-	474.9	526.0	495.3	622.7	1.26	42.3-43.0	<MDL	31.7-33.5	97.6	2.91-3.08	2.09-2.17
trans -chlordane (ng/g ww)	<MDL	<MDL	-	<MDL	-	1.91-2.05	2.39-2.46	2.15-2.26	<MDL	-	9.91	9.78	9.9	1.9-2.1	0.19-0.21	<MDL	<MDL	-	<MDL	-	0.19-0.21
(ng/g lw)	<MDL	<MDL	-	<MDL	-	58.2-60.5	61.7-62.9	60.0-61.7	<MDL	-	231.0	155.3	189.4	45.4-50.0	0.24-0.27	<MDL	<MDL	-	<MDL	-	0.240-0.241
cis- nonachlor (ng/g ww)	<MDL	<MDL	-	3.46-3.56	-	1.88	3.11-3.17	2.49-2.52	<MDL	-	7.92	8.86	8.30	32.9	3.96	<MDL	<MDL	-	8.0	-	3.96
(ng/g lw)	<MDL	<MDL	-	70.3-74.2	-	53.5	87.3-88.5	70.4-71.0	<MDL		184.7	140.6	167.1	784.2	4.69	<MDL	<MDL	-	184.4	-	4.69
trans -nonachlor (ng/g ww)	1.36-1.56	1.74-1.80	1.55-1.68	2.83-2.93	1.74-1.83	6.48	6.36	6.4	4.25-4.37	0.66-0.68	20.2	23.2	21.4	17.2	0.8037	3.33-3.42	4.44-4.53	3.61-3.70	5.0	1.35-1.39	1.14-1.18
(ng/g lw)	13.3-16.9	62.9-64.1	38.1-40.5	60.8-64.7	1.58-1.60	184.1	99.8	142.0	119.5-123.7	0.84-0.87	469.6	368.0	429.0	408.8	0.95	63.1-64.7	87.8-89.3	69.3-70.9	100.8	1.42-1.45	1.20-1.22
methoxytriclosan (ng/g ww)	1.22-1.36	0.22-0.42	0.72-0.89	<MDL	-	1.01	1.29	1.2	<MDL	-	8.87-8.88	11.9-12.0	10.08-10.13	3.22-3.25	0.319-0.321	6.08-6.12	7.15-7.24	6.36-6.40	<MDL	-	0.319-0.321
(ng/g lw)	12.0-14.4	7.34-9.74	9.67-12.1	<MDL	-	28.7	20.2	24.5	<MDL	-	142.8-143.6	198.0-198.7	164.9-165.6	73.7-74.6	0.447-0.450	98.4-99.2	100.1-101.6	98.8-99.8	<MDL	-	0.447-0.450
Total PCBs (ng/g ww)	49.1	41.6	45.4	1431.2	31.6	186.6	277.9	232.3	4148.7	17.9	481.7	365.9	435.4	4935.1	11.34	97.2	121.0	103.2	4187.3	40.6	25.35
(ng/g lw)	594.5	1439.5	1017.0	26979.0	26.5	5303.1	4360.1	4831.6	105353.4	21.8	11205.2	5806.8	8506.0	117128.2	13.77	1877.9	1792.4	1835.2	91413.5	49.8	27.98
Total PBDEs (ng/g ww)	<MDL	<MDL	-	84.4	-	22.7	11.5	17.1	368.8	21.6	20.7	16.1-16.2	18.86-18.90	343.5	18.17-18.21	13.9	9.90	12.9	179.5	13.9	17.89-17.90
(ng/g lw)	<MDL	<MDL	-	1455.0	-	411.5	180.0	295.8	9707.2	32.8	499.6	295.5-296.3	418.0-418.3	8766.1	20.96-20.97	137.6	73.7	121.6	1977.5	16.26	23.35-23.36
BDE 47 (ng/g ww)	<MDL	<MDL	-	51.5	-	9.68	10.9	10.3	224.5	21.8	11.2	12.5-12.6	11.7-11.8	250.3	21.2-21.4	6.72	9.44-9.52	7.40-7.42	100.3	13.5-13.6	18.8-18.9
(ng/g lw)	<MDL	<MDL		896.0	-	275.3	170.9	223.1	5701.1	25.6	259.0	200.5-202.1	235.6-236.2	5941.4	25.15-25.22	64.9	93.6-95.2	72.1-72.5	1095.1	15.1-15.2	21.95-22.00
BDE 99 (ng/g ww)	<MDL	<MDL	-	3.0	-	4.46-4.60	<MDL	2.23-2.43	58.5	24.1-26.2	4.4	<MDL	2.68	22.1	8.3	4.20	<MDL	3.18	26.0	8.2	13.5-14.2
(ng/g lw)	<MDL	<MDL		52.7	-	125.0-127.4	<MDL	62.5-67.3	1485.2	22.1-23.8	103.4	<MDL	62.0	525.7	8.5	40.6	<MDL	30.5	283.6	9.3	13.3-13.9
BDE 100 (ng/g ww)	<MDL	<MDL	-	12.2	-	3.20-3.40	<MDL	1.6-1.80	64.7	35.9-40.4	3.74	2.11-2.24	3.09-3.14	39.9	12.7-12.9	2.40	1.99-2.12	1.94-1.97	25.4	12.9-13.1	20.5-22.13
(ng/g lw)	<MDL	<MDL		212.4	-	83.4-86.9	<MDL	41.7-47.0	1644.7	35.0-39.4	87.2	35.2-37.6	66.4-67.4	948.1	14.1-14.3	23.1	21.6-24.0	22.7-23.3	277.9	11.9-12.2	20.3-22.0
BDE 154 (ng/g ww)	<MDL	<MDL	-	8.8	-	<MDL	<MDL	-	19.3		<MDL	<MDL	-	13.6	-	<MDL	<MDL	-	11.4		
(ng/g lw)	<MDL	<MDL		152.6	-	<MDL	<MDL	-	512.6		<MDL	<MDL	-	322.8	-	<MDL	<MDL	-	124.1		

Note: Biomagnification factors (BMFs) are calucated for all scenarios where the analyte was detected in greater than half of the species and site combinations. The contaminats shown above are those listed in Tables 2,3,4 and Supplemental Table S2 with sufficient information to calculate a BMF; <MDL= less than the method detection limit, analyte was detected in less than 50% of the samples and no mean was calculated. All data are presented as geometric means. For those contaminants detected in >50% of the samples, the range of the Kaplan Meier Mean was calculated and the BMF was calculated as the range of means. For those contaminants with one fish species <MDL, the value of 1/2 MDL was substituted to get an appoximate dietary intake estimation.

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

Redundancy analysis

The RDA incorporated data from samples from our previously published study (n=13; Lazarus et al., 2015a) and the present study (n=47; because there were only complete data for three nests on Back River, this site was excluded from the RDA, Figure 8). The RDA model explained more variability than would be expected by chance alone ($F_{df=5}$ =11.53, p<0.001). The first two axes (Table S3, and Figure S3) 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). A distinct grouping emerged among sites (Figure 8, Supplemental Figure S4). Axis one had the largest contribution from PBDEs, PCBs and *p,p'*-DDE, while axis 2 had the largest contributions from PBDEs. Thus, sites on the left of Figure 3 are most polluted. Those sites on the top left (Patapsco River/Baltimore Harbor, Elizabeth River) had more PCB and *p,p'*-DDE contaminations in osprey eggs, and those on the bottom left (Anacostia/middle Potomac Rivers and the Susquehanna) had more PBDEs (Axis 2). Overall, PBDEs appear most closely associated with DNA damage compared to the other chemicals. Poplar Island (furthest to the right) had the least contamination, which is consistent with it being the reference site (Figure 8, Table S4 and Figure S1). There were no 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).

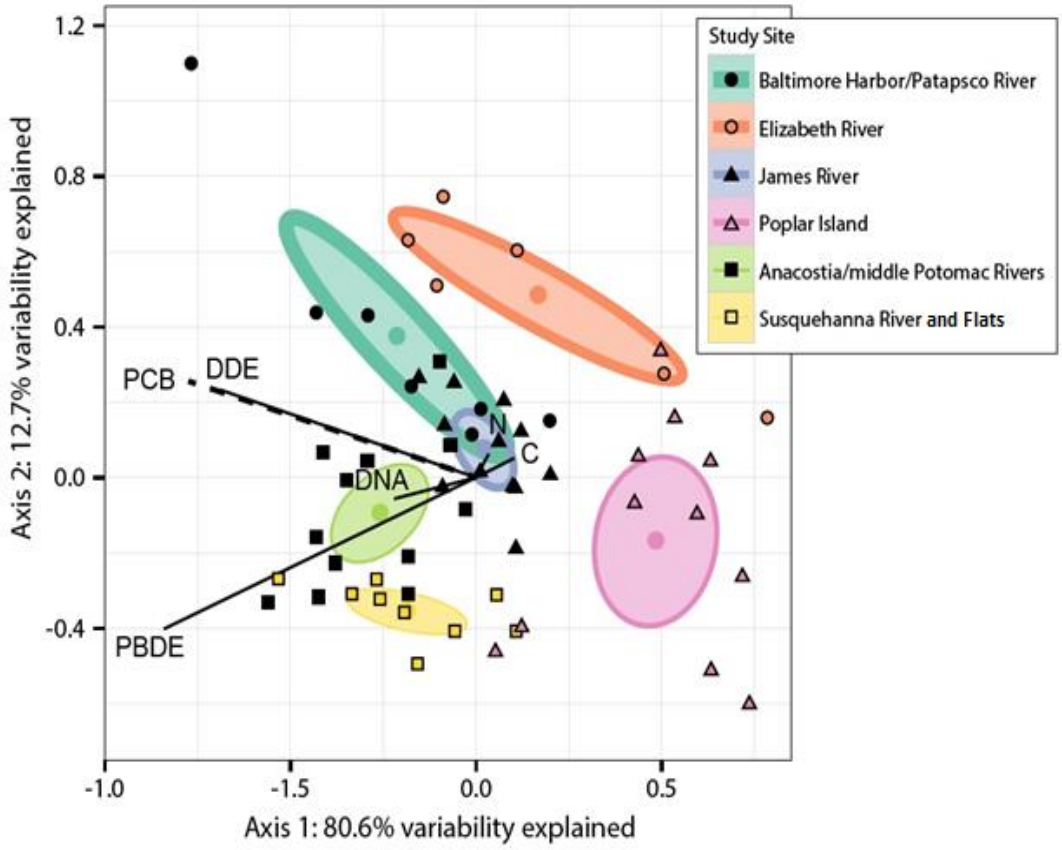


Figure 8. Redundancy analysis (RDA) biplot of DNA Damage, PCBs, PBDEs, DDE and carbon and nitrogen stable isotopes and their relationships across study sites. Data are projected onto the ordination axes. The large dots are the centroids and the shaded areas represent the 95% confidence ellipses. The vectors (black lines) represent the contribution of different variables to each axis.

Discussion

Osprey productivity

Due to the presence of the organochlorine pesticide DDT and its metabolites (*p,p'*-DDE) in the osprey food web, by the 1970's the osprey population had contracted to the main stem of the Bay with few nesting pairs present north of the Bay Bridge (Henny et al., 1974; Wiemeyer et al., 1975, 1988). During the DDT use era, productivity

rates were low (e.g., 0.55 fledglings/active nest on the middle Potomac in 1970; Wiemeyer et al., 1971). Spitzer (1980) and Poole (1989) stated that an osprey population producing 0.8-1.15 fledglings/active nest is required to maintain a stable population in the Chesapeake Bay watershed. By the mid-90's population estimates had more than doubled (Watts et al., 2004). Current productivity estimates (>1.17 fledglings/active nest, Table 1) indicate that osprey reproduction is adequate to maintain a stable osprey population in many parts of the Chesapeake (Rattner et al. 2004, Watts and Paxton, 2007; Lazarus et al. 2015a and present study).

Contaminants in ospreys

The present study further demonstrates that residues of *p,p*'-DDE in osprey eggs have declined since the 1970's (e.g., averaging 3.1 µg/g ww on the middle Potomac in 1971-1977, Weimeyer et al. 1988 to 0.63 µg/g ww in the present study). Current *p,p*'-DDE concentrations are well-below the threshold associated with 10% eggshell (i.e., 2.0 µg/g ww, Wiemeyer et al., 1988), and at these low levels there was no relationship between *p,p*'-DDE and eggshell thickness.

PCB concentrations were similar among the Susquehanna River and flats, Anacostia/middle Potomac and James Rivers. Compared to historical values reported by Wiemeyer et al., (1988) on the Potomac River in 1973 (9.8 µg/g ww), PCB concentrations in this study declined by approximately 50%. Mean total PCB concentrations at all study sites were lower than levels described in eggs from the Baltimore Harbor/Patapsco River in 2011 (geometric mean 7.77 µg/g ww, maximum 35.0 µg/g ww, Lazarus et al., 2015a). Although PCBs have been associated with many

adverse effects in fish-eating birds (Harris and Elliott, 2011), none were documented in this study as indicated by logistic regression. The TEQs found in some study sites exceeded the no-observed-adverse-effect-level for osprey nestling growth (0.136 ng TEQ/g ww egg) (Woodford et al., 1998). Although growth rate was not examined in the present study, body weight was used as a surrogate measure. There was no evidence of a relation between toxic TEQ in Chesapeake Bay osprey eggs and body weight of 40-45 day old nestlings (R^2=-0.12, p=0.42).

Average total PBDEs were greatest on the Anacostia and Susquehanna (368.8 ng/g and 343.5 ng/g ww respectively). These values are lower than those reported in Chesapeake Bay osprey eggs from 2000-2001 (Rattner et al., 2004). Manufacture of the penta BDE commercial formulation from which these congeners derive ceased in 2004 (La Guardia et al., 2007). The expanded subset of nests sampled on the Anacostia/middle Potomac River revealed that PBDE egg residues decreased with downstream distance from the Blue Plains WWTP. Such treatment facilities are documented sources of PBDEs (LaGuardia et al., 2007; Henny et al., 2011). Notably, the greatest residues of PBDEs on both the middle Potomac River (801.8 ng/g ww) and Susquehanna (648.6 ng/g ww) were found in nests in close proximity to WWTPs. Values in the present study were below the lowest-observed-adverse-effect-level associated with reduced pipping and hatching success in American kestrels (*Falco sparverius*) (1.8 µg/g ww; McKernan et al., 2009), but exceeded the recommended toxicity reference value for aquatic birds (180 ng/g ww; Zhang et al., 2014). The paucity of ecotoxicity data for aquatic birds from Susquehanna and northern Bay makes it difficult to place the present findings into a historical perspective, but these data can serve as valuable documentation for future monitoring

studies. Alternative-BRFs including, α-HBCD, BTBPE, DBDPE, TBB and TBPH were detected at some of the study sites at low concentrations compared to the PBDE flame retardants (Table 4), but it is difficult to determine their significance as toxicity reference values have yet to be derived for these compounds in birds.

Osprey diet and biomagnification of contaminants

Dietary observations documented in this study were similar to those reported in other characterizations of osprey foraging behavior in Chesapeake Bay (McLean and Bird 1991; Viverette et al., 2007; Glass and Watts, 2009). These studies similarly document that Atlantic menhaden are an important component in the diet of ospreys in much of the Chesapeake Bay. In tidal freshwater tributaries, osprey diet shifts from estuarine species to catfish and gizzard shad (Glass and Watts, 2009; Supplemental Table 1). Although osprey diet differed among study sites, only slight differences in ^{15}N signatures were observed (e.g., both striped bass and catfish species are opportunistic predators, while gizzard shad and menhaden are both planktivores).

Biomagnification of lipophilic compounds has been well studied in the field of ecotoxicology (e.g., Kelly et al., 2007). In the present study, biomagnification factor estimates are generally comparable to other reports examining the osprey food web (Henny et al., 2003, 2009; Chen et al., 2010). Overall, BMFs were similar when expressed on a ww or lw basis. The BMF for *p,p'*-DDE averaged 19.8 ng/g lw, which was comparable to Chen et al. (2010; i.e., 18 ng/g lw) for the Chesapeake Bay, but is one-fourth than that reported by Henny et al. (2003; estimated to be 85 ng/g lw in an osprey egg) on the Willamette River in Oregon. This difference may be attributable to greater

p,p'-DDE concentrations in fish that ospreys are consuming on their wintering grounds (Henny et al., 2003). Again, the BMF for total PCBs in the present study was comparable to that reported previously for Chesapeake (27.9 ng/g lw, present study vs. 25.1 ng/g lw, Chen et al., 2010), but three times greater than that on the Willamette River, OR (estimated to be 11 ng/g lw for osprey eggs, Henny et al., 2003). In general, average residues of PCBs in Chesapeake Bay osprey eggs are 6 times greater than residues along the Willamette River, while for fish total PCB concentrations were only about 2 times greater than those collected along the Willamette.

Several of the organochlorine pesticides and methoxyriclosan had a BMF < 5 indicating only modest biomagnification (*p,p'*-DDD, *cis* -chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and methoxytriclosan) (Thomann et al., 1992; Barron, 2003). No biomagnification was observed for the alt-BFRs even though their log k_{ow}'s are just as great as the hexa-, octa- and deca- BDE formulations (US EPA, 2010; Wu et al., 2011). Even though deca-BDE has been used worldwide, it was not found in great concentrations in this study. This is consistent with findings reported in Chen and coworkers (2009), where they indicate that BDE-209 was detected in peregrine falcon (*Falco peregrinus*) eggs, but not in the aquatic birds studied. Other factors may play a role including biotransformation, and the unstudied metabolites may actually be more bioaccumulative than the parent compounds (Kelly et al., 2007).

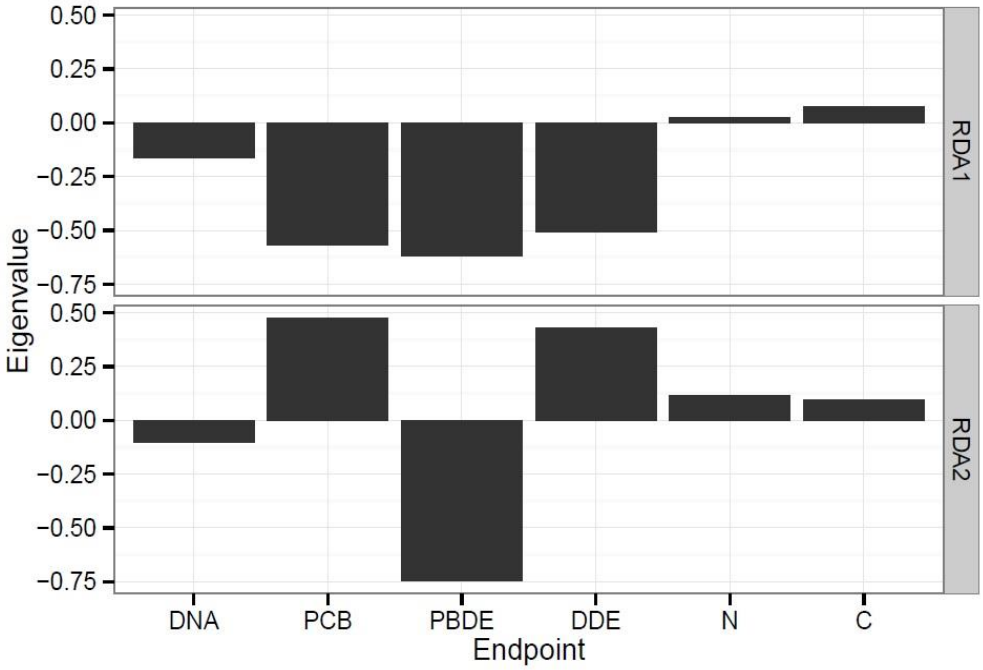
Relation of concentrations of PCBs, DDE, and PBDEs with DNA damage

The redundancy analysis indicated that DNA damage was most closely related to PBDE concentrations in osprey eggs, and presumably in nestling ospreys; however,

univariate analysis was not significant between PBDE concentrations and DNA. Although this analysis separates PBDEs as a large contributor to DNA damage, other chemicals including PAHs, PCBs, perfluorinated compounds, radiation and some metals have been shown to cause DNA damage in songbirds and Japanese quail (*Cortunix cortunix japonica*) (Custer et al, 2000; Devaux et al., 1998; Misra et al., 1998; Kim et al., 2010; Luloff et al., 2011). Damage may have been caused by some other co-occurring but unanalyzed chemical or chemicals. DNA damage has been observed in laboratory studies where American kestrels and common terns (*Sterna hirundo*) were exposed to the commercial PBDE formulation DE-71 (Rattner et al., 2013) and was elevated in the higher dose groups. The increased production of oxyradicals (above endogenous production) can lead to a variety of consequences in the body including mutations, lesions and disease progression as the body's natural repair mechanisms become overwhelmed (Valavanidis et al., 2009).

Conclusions

Ospreys in the Chesapeake Bay are now thriving and our estimates in several tributaries including historic ROCs, indicate 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. Similar to findings by Lazarus and coworkers (2015a), there is increased evidence of genetic damage in ospreys nesting in the most polluted areas.



Supplemental Figure S1. Linear contribution (eigenvalues) of predictor variables to each axis.

Supplemental Table S2. Osprey diet composition from each study site.

Family or Species	Site			
	Poplar Island	Susquehanna River and Flats	Anacostia/middle Potomac Rivers	James River
	n observations (% of total)	*n* observations (% of total)	*n* observations (% of total)	*n* observations (% of total)
	2011-2013	2013	2011	2012
American Eel (*Anguilla rostrata*)	5 (<1%)	-	-	-
Atlantic Cutlassfish (*Trichiurus lepturus*)	1 (<1%)	-	-	-
Atlantic Menhaden (*Brevoortia tyrannus*)	515 (44.3%)	-	2 (4.26%)	-
Atlantic Needlefish (*Strongylura marina*)			1 (2.13%)	-
Blueback Herring (*Alosa aestivalis*)	-	-	-	1 (<1%)
Catfish spp. (*Ictaluridae*)	-	88 (40.0%)	11 (23.4%)	179 (77.2%)
Common Carp (*Cyprinus carpio*)	-	36 (16.4%)	13 (27.6%)	1 (<1%)
Croaker (*Sciaenidae*) species	29 (2.50%)	-	1 (2.12%)	-
Gizzard Shad (*Dorosoma cepedianum*)	-	96 (43.6%)	15 (31.9%)	46 (19.8%)
Longnose Gar (*Lepisosteus osseus*)	1 (<1%)	-	-	-
Northern Snakehead (*Channa argus*)	-		1 (2.13%)	-
Other Shad spp. (*Centrarchidae*)			6 (12.77%)	2 (<1%)
Perch spp. (*Percidae*)	55(4.73%)	-	1 (2.13%)	4 (1.7%)
Speckled Seatrout (*Cynoscion nebulosus*)	1 (<1%)	-	-	-
Striped Bass (*Morone saxatilis*)	556 (47.8%)	-	-	-
Total Observations	1163	220	47	232

Supplemental Table S3. Contaminant residues in whole fish collected across all study sites[a,b].

Site	Poplar Island (2011-2013)		Susquehanna River and Flats (2013)		Anacostia/middle Potomac Rivers (2011)		James River (2012)		Back River 2013		
Species	Atlantic Menhaden	Striped Bass	Catfish	Gizzard Shad	Catfish sp.	Gizzard Shad	Catfish sp.	Gizzard Shad	Atlantic Menhaden	Gizzard Shad	White Perch
Contaminant (ng/g ww)	$n_{composite}$=6 Geometric Mean Range n detects	$n_{composite}$=6 Geometric Mean Range n detects	$n_{composite}$=6 Geometric Mean Range n detects	$n_{composite}$=6 Geometric Mean Range n detects	$n_{composite}$=9 Geometric Mean Range n detects	$n_{composite}$=9 Geometric Mean Range n detects	$n_{composite}$=9 Geometric Mean Range n detects	$n_{composite}$=9 Geometric Mean Range n detects	$n_{composite}$=3 Geometric Mean Range n detects	$n_{composite}$=2 Geometric Mean Range n detects	$n_{composite}$=2 Geometric Mean Range n detects
Average Length (mm)	317.4 ± 13.97	304.9 ± 29.23	274.7 ± 77.50	304.9 ± 48.58	302.1 ± 38.81	342.4 ± 56.33	280.4 ± 62.91	306.6 ± 53.29	205.1 ± 11.01	318.7 ± 77.44	167.5 ± 38.18
Average Weight (g)	325.9 ± 44.62	252.3 ± 63.75	229.7 ± 178.21	263.4 ± 110.34	313.9 ± 104.62	370.1 ± 147.01	181.2 ± 137.16	261.6 ± 113.54	102.6 ± 15.45	405.9 ± 220.75	115.9 ± 40.09
p,p -DDE	12.01[C,D] 5.86-22.9 6/6	6.48[D] 4.49-10.6 6/6	20.82[B,C] 9.77-79.9 6/6	24.34[A,B] 22.3-36.2 6/6	43.7[A] 26.0-67.7 9/9	34.4[A,B] 20.3-58.5 9/9	21.7[B,C] 16.1-47.6 9/9	21.4[B] 10.1-35.2 9/9	33.5 26.1-41.6 3/3	55.5 42.6-72.4 2/2	45.6 38.8-53.5 2/2
o,p -DDD					1.06-1.24 <MDL-3.78 5/9	1.73-1.87 <MDL-4.69 6/9					
p,p -DDD	3.04 1.32-5.53 6/6	1.18 0.67-2.43 6/6	3.12 1.71-11.6 6/6	5.11 3.05-8.27 6/6	10.0 4.33-26.3 9/9	9.98 4.45-18.8 9/9	3.21-3.25 <MDL-8.37 8/9	3.49 1.74-6.18 9/9	7.08 5.17-8.36 3/3	10.8 9.88-11.8 2/2	10.4 7.67-14.2 2/2
o,p- DDT		NA <MDL-1.82 1/6			1.15-1.32 <MDL-4.02 5/9	NA <MDL-6.92 3/9					
p,p -DDT			NA <MDL-2.26 1/6	0.92-1.12 <MDL-2.68 3/6	2.12-2.16 <MDL-5.82 8/9	6.32 3.23-14.3 9/9		5.75-5.93 <MDL-13.2 5/9	NA <MDL-9.97 1/3	3.71-3.91 <MDL-7.42 1/2	1.13-1.33 <MDL-2.26 1/2
p,p -DDMU			1.66-1.79 <MDL-4.95 4/6	1.84-1.97 <MDL-4.18 4/6	2.49-2.53 <MDL-5.00 8/9	NA <MDL-2.82 4/9				2.43-2.63 <MDL-4.85 1/2	
p,p -DDMS			0.59-0.72 <MDL-1.72 4/6	0.64-0.78 <MDL-2.15 4/6				4.05-4.16 <MDL-15.2 6/9			
Dieldrin					NA <MDL-8.13 3/9					35.9-36.1 <MDL-71.8 1/2	
Hepachlor epoxide					NA <MDL-7.73 2/9					4.63-4.83 <MDL-9.25 1/2	
α-chlordane (*cis*)	NA <MDL-12.0 2/6	NA <MDL-2.13 1/6	2.64[B] 0.71-10.5 6/6	5.36-5.43[B] <MDL-10.9 5/6	20.4[A] 6.61-61.8 9/9	33.1[A] 16.1-86.5 9/9	2.23-2.37[B] <MDL-5.99 6/9	NA <MDL-7.23 4/9	19.5-19.6 <MDL-40.8 2/3	48.1-48.3 <MDL-96.2 1/2	74.5 57.6-96.4 2/2
γ- chlordane (*trans*)			1.91-2.05[A] <MDL-4.47 4/6	2.39-2.46[B] <MDL-5.20 5/6	9.91[B] 3.00-22.9 9/9	9.78[B] 4.89-18.7 9/9	NA <MDL-2.36 2/9	NA <MDL-0.98 1/9	7.83 5.63-12.3 3/3	27.9 17.1-45.4 2/2	14.4 10.4-20.1 2/2
cis -nonachlor		NA <MDL-1.98 2/6	1.88[B,C] 0.68-6.76 6/6	3.11-3.17[B,C] <MDL-6.66 5/6	7.92[A,B] 3.74-19.3 9/9	8.86[A] 5.88-15.1 9/9	NA <MDL-2.03 3/9	NA <MDL-3.23 3/9	9.27 5.76-12.4 3/3	17.8 11.7-27.0 2/2	21.0 16.0-27.4 2/2
trans -nonachlor	1.36-1.56 <MDL-3.17 3/6	1.74-1.80 <MDL-3.31 5/6	6.48 3.98-15.3 6/6	6.36 3.15-15.7 6/6	20.2 7.65-57.0 9/9	23.2 12.0-30.5 9/9	3.33-3.42 <MDL-7.53 7/9	4.44-4.53 <MDL-11.7 7/9	8.24-8.38 <MDL-15.18 2/3	28.6-28.8 <MDL-57.1 1/2	76.1 61.4-94.2 2/2

Supplemental Table S3 cont'd. Contaminant residues in whole fish collected across all study sites[a,b].

Mirex					NA <MDL-1.26 1/6						
MC 1 (chlordane component)			NA <MDL-1.77 2/6		3.22-3.31 <MDL-8.50 6/9	3.08-3.22 <MDL-8.07 6/9			5.15-5.28 <MDL-8.66 2/3	11.8 9.00-15.6 2/2	6.70-6.90 <MDL-13.4 1/2
MC 2 (chlordane component)			NA <MDL-0.63 1/6		2.73-2.77 <MDL-4.82 8/9	3.66 1.73-6.44 9/9			5.57 3.66-9.04 3/3	10.9 7.56-15.6 2/2	12.0 7.75-18.5 2/2
MC 3 (chlordane component)			NA <MDL-0.24 1/6		4.32-4.36 <MDL-6.54 8/9	3.57 1.97-8.53 9/9			5.88 3.83-8.49 3/3	9.96 6.29-15.8 2/2	8.03 5.58-11.6 2/2
MC 5 (chlordane component)		0.54-0.68[C] <MDL-1.56 4/6	1.82[B,C] 0.79-3.66 6/6	3.28[A,B] 1.46-7.17 6/6	8.38[A] 2.87-27.3 9/9	12.93[A] 8.61-17.0 9/9	NA <MDL-1.49 4/9	NA <MDL-3.89 3/9	9.56 6.47-14.0 3/3	28.4 18.6-43.5 2/2	33.3 24.5-45.2 2/2
MC 6 (chlordane component)					NA <MDL-2.24 2/9	NA <MDL-3.06 1/9					
methoxytriclosan	1.22-1.36 <MDL-2.30 4/6	0.22-0.42 <MDL-0.55 3/6	1.01 0.71-1.53 6/6	1.29 0.94-2.00 6/6	8.87-8.88 <MDL-20.6 8/9	11.9-12.0 <MDL-39.0 8/9	6.08-6.12 <MDL-11.1 8/9	7.15-7.24 <MDL-13.9 7/9	15.25 11.7-17.6 3/3	14.1 12.0-16.5 2/2	
PCB 77 (pg/g)	- <MDL-2380 1/6	- <MDL-360 1/6		- <MDL-90 1/6	485.6-503.3 <MDL-1050 5/9	- <MDL-2590 4/9	NA <MDL-20.0 1/9	67.8-72.2 <MDL-120.0 8/9	1401.50 630.0-2300.0 3/3	2415.8 1720.0-6210.0 3/3	2410.7 1740.0-3340.0 2/2
Total PCBs (ng/g)	49.1[C] 13.2-102.3 6/6	41.6[C] 24.3-97.9 6/6	186.6[A,B] 107.6-665.8 6/6	277.9[A,B] 136.5-708.6 6/6	481.7[A] 185.2-1145.2 9/9	365.9[A] 210.3-780.6 9/9	97.2[B] 38.5-283.3 9/9	121.0[B] 73.4-334.9 9/9	356.5 245.5-447.5 3/3	1277.8 351.2-5602.4 3/3	640.5 632.4-648.8 2/2
Total PBDEs	NA <MDL-3.40 1/6	NA <MDL-4.61 1/6	22.8 3.37-16.9 6/6	11.5 4.12-39.6 6/6	20.7 8.03-64.3 9/9	16.1-16.2 <MDL-37.6 8/9	13.94 7.05-24.4 9/9	9.9 1.87-23.6 9/9	44.8 30.0-64.8 3/3	60.6 41.2-88.9 3/3	16.0 11.7-20.3 2/2
BDE 47	NA <MDL-2.04 1/6	NA <MDL-3.71 1/6	9.68 3.37-53.3 6/6	10.9 4.12-29.0 6/6	11.2 3.34-36.7 9/9	12.5-12.6 <MDL-27.8 7/9	6.72 3.25-12.9 9/9	9.44-9.52 <MDL-16.5 7/9		12.9-13.1 <MDL-21.4 2/3	10.9 8.57-13.3 2/2
BDE 99	NA <MDL-0.71 1/6		4.46-4.60 <MDL-12.2 4/6		4.44 1.00-16.6 9/9	NA <MDL-5.07 2/9	4.2 1.68-6.84 9/9	NA <MDL-2.21 4/9			
BDE 100	NA <MDL-0.64 1/6	NA <MDL-0.90 1/6	3.20-3.40 <MDL-12.2 3/6	NA <MDL-10.6 1/6	3.74 1.68-10.8 9/9	2.11-2.24 <MDL-4.79 6/9	2.40 1.45-3.66 9/9	1.99-2.12 <MDL-5.15 6/9		3.27 1.96-4.57 3/3	1.15-1.35 <MDL-2.29 1/2
BDE 153							NA <MDL-1.37 4/9	NA <MDL-0.79 1/9			
BDE 154					NA <MDL-2.82 3/9					2.51-2.65 <MDL-5.95 2/3	1.66-1.86 <MDL-3.32 1/2
BDE 209					NA <MDL-2.25 1/9	NA <MDL-3.03 3/9		0.60-0.78 <MDL-1.87 5/9	44.8 30.0-64.8 3/3	39.8 18.8-58.7 3/3	1.64-1.84 <MDL-3.27 1/2
TBB					NA <MDL-2.33 2/9	NA <MDL-8.29 2/9					
TBPH					NA <MDL-0.54 2/9	NA <MDL-0.35 3/9					
DBDPE							NA <MDL-5.04 1/9	NA <MDL-0.90 1/9			
alpha-HBCD	NA <MDL-4.20 1/6				NA <MDL-7.34 2/9	NA <MDL-2.56 2/9					
beta-HBCD						NA <MDL-3.48 2/9					

[a]– no mean calculated, as contaminant was detected in fewer than half the samples; MDL, method detection limit; extremes are definied as the minimum and maximum values in the dataset. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons (p <0.05), and a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction.

[b]If non-detects were present in less than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean.

Supplemental Table S4. Amount of variance explained by each axis as well as the proportion and cumulate proposition of variance explained.

Axis	Variance	Proportion of variance	Cumulative proportion explained	Degrees of freedom	F	P
1	0.1396	0.814	0.814	1	46.9	<0.001
2	0.0207	0.121	0.935	1	6.99	<0.001
3	0.0076	0.044	0.979	1	2.54	0.0667
4	0.0032	0.019	0.997	1	1.08	0.337
5	0.0004	0.002	1	1	0.147	0.938

CHAPTER 4

EXPOSURE AND FOOD WEB TRANSFER OF PHARMACEUTICALS IN OSPREYS (*Pandion haliaetus*): PREDICTIVE MODEL AND EMPIRICAL DATA

Introduction

In parallel with human population growth and a myriad of veterinary and human health uses, pharmaceuticals and their metabolites primarily enter the environment through waste- water from bulk drug production, sewage plants and septic systems, and in biosolids applied to agricultural lands (Kolpin et al., 2002; Ramirez et al., 2009). The development of advanced analytical techniques and widespread monitoring has revealed the presence of pharmaceuticals in a variety of environmental matrices (sediments, sewage sludge, water, and fish). Pharmaceuticals may not be completely removed by traditional wastewater treatment systems, and with constant wastewater inputs, even labile compounds may exhibit pseudo-persistence in surface waters (Daughton and Ternes, 1999; Celiz et al., 2009). Their detection in the environment has raised concerns about bioaccumulation, transfer through the food web, and potential effects that pharmaceutical "cocktails" may elicit on ecosystems.

Understanding ecological risks of pharmaceuticals to free-ranging wildlife (amphibians, reptiles, birds, and mammals) remains a major research need (Boxall et al., 2012), the one exception being the nonsteroidal anti-inflammatory drug (NSAID) diclofenac used to treat livestock. Diclofenac use resulted in nontarget poisoning and endangerment of several species of Asian vultures feeding on carcasses of cattle that had been treated with this drug (Oaks and Watson, 2011). The catastrophic effects of diclofenac on old world vultures resulted in detailed investigations of several NSAIDs in birds. A recent workshop evaluated the risk of pharmaceuticals to wildlife and identified major information gaps including the need to conduct food web exposure modeling and environmentally realistic risk assessments (Arnold et al., 2013). Hernout et al. (2011) also

suggested that prioritizing chemicals (e.g., metals, pesticides, pharmaceuticals) in a food web framework before intensive and costly investigation would be beneficial to natural resource managers and policymakers.

Ospreys (*Pandion haliaetus*) are a high trophic level species that have served as sentinels of ecosystem health and environmental change (Grove et al., 2009). Their eggs and blood are excellent matrices to document spatial and temporal trends and to elucidate exposure, bioaccumulation, and biomagnification of contaminants. Ospreys are strictly piscivo- rous and this aspect makes their diet easy to monitor and link to sources of localized contaminant exposure. Their diet can vary with salinity (Glass and Watts, 2009), prey availability and trophic position and can range from anadromous fish in polyhaline regions to nonmigratory fish in oligohaline waters. Ospreys are adaptable to human landscapes and can be found nesting in highly industrialized and urbanized areas and even in proximity to wastewater treatment plants (WWTPs).

To date, no studies have examined the bioaccumulation of pharmaceuticals and their fate in the water-fish-osprey food web. This study describes a framework and the findings of a screening-level exposure assessment to estimate the daily and cumulative 40 day intake of pharmaceuticals that are being analyzed by some environmental research laboratories (Du et al., 2012; Furlong et al., 2014). This was complemented by empirical analyses of 23 compounds and an artificial sweetener analyzed in water and blood plasma of fish and osprey nestlings from sites located along potentially impaired waterways in Chesapeake Bay.

Materials and Methods

Screening-level exposure model: daily intake

The daily intake (DI) of 113 pharmaceuticals, metabolites, and an artificial sweetener (Table A2) by an adult female osprey was calculated to determine which compounds reached or exceeded the human therapeutic dose (HTD) (assumes comparable intestinal absorption for both ospreys and humans) (Figure 9). These compounds are quantified at the US Geological Survey National Water Quality Laboratory (Furlong et al., 2014) and some of these compounds are analyzed in fish plasma (Du et al. 2012). Three hypothetical exposure regimes (10, 100, and 1000 ng/L) were chosen (ranging from "dilution dominated" high flow to "wastewater effluent dominated" low flow) (Brooks et al., 2006) and modeled across 3 pH values (pH 6, pH 7, pH 8) that are representative of surface water gradients at field study sites. The pH consideration is important, because the drugs examined are all potentially ionizable and their bioconcentration factors (BCFs) are pH-specific and dependent on log D (a measure taking into account ionized and un-ionized forms of a molecule) (Meylan et al. 1999; Fu et al. 2009). These factors can influence bioaccumulation and toxicity in fish (Valenti et al. 2009, 2011, 2012), and ultimately, their absorption (bioaccessibility) in the gastrointestinal tract of birds. Predicted BCFs for each substance (ACS, 2014) were used to calculate the quantity of a pharmaceutical accumulated in a generic fish in 24 hours (Berninger et al., 2011).

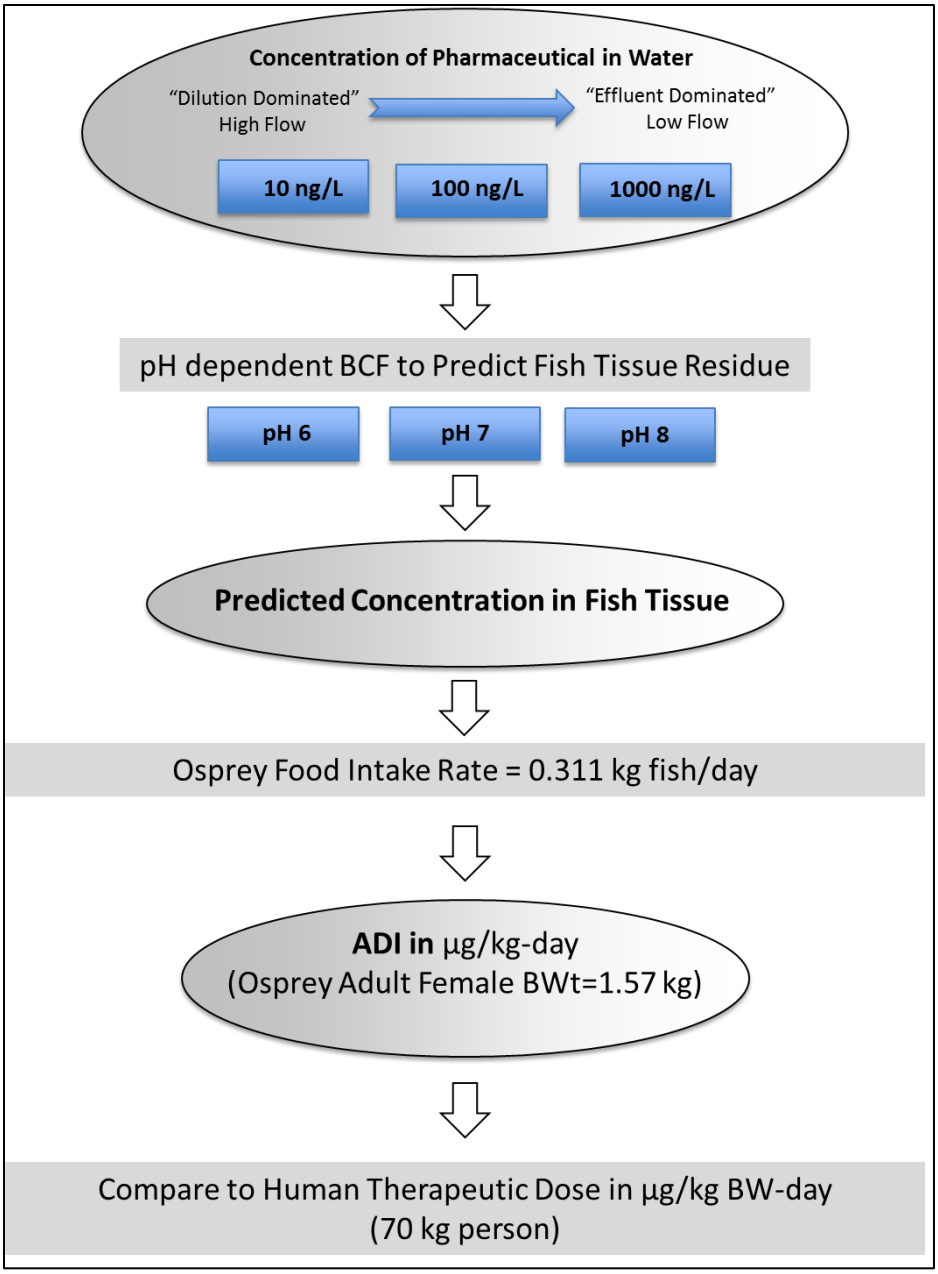


Figure 9. Theoretical screening-level exposure assessment framework used to model the daily intake (DI) for an osprey.

Calculated pharmaceutical residues in fish from each scenario were used to estimate the DI for a 1568 g adult female osprey (US EPA, 1993). Due to the complexities in modeling cumulative exposure of a growing osprey nestling (e.g., logistic

growth plateauing at 40 days, changing food intake, and metabolic demands, etc.), a 40 day exposure assessment for an adult osprey was conducted. Food intake rate (FIR) was estimated 2 ways. The first estimate used osprey bite size and body weight (BWt) to calculate a FIR of 329 g of fish wet weight (ww) per day (Poole, 1985; US EPA, 1993). The second estimate used dry weight (dw) consumption rates based on the relationship between BWt and metabolic energy for birds (FIR g dw/day= $0.648BWt^{0.651}$) (Nagy, 1987; US EPA, 1993). The FIR for an adult female osprey (77.94 g fish dw/day) converted to ww (assuming 75% water content for a generic fish) was 312 g/day. These 2 estimates yielded similar results and the metabolic-based estimate was selected for use. The DI (µg pharmaceutical/kg Bwt) was calculated using Equation 1

$$DI = (\text{residue in fish})\ (FIR)/(\text{kg BWt}). \quad (1)$$

The DIs for varying degrees of absorption were compared to the oral HTD for an adult. Human therapeutic doses were obtained as the minimum daily dose to exert a therapeutic effect (RxList, 2008; FDA 2012; Drugsite Trust, 2014).

Screening-level exposure model: 40 day cumulative intake

To estimate cumulative body burden of ospreys, assumptions included that diet was the principal exposure route, BWt and FIR were constant, and intestinal absorption was comparable between ospreys and humans. Clearance was incorporated assuming a first-order kinetic elimination equation to calculate total exposure (i.e., µg/kg BWt) because the majority of ionizable pharmaceuticals follow this type of elimination (Bardal et al. 2011). Using DI (t = 1 d), exposure (E) oscillated following a saw-tooth pattern between peak (E_{peak}) and trough (E_{trough})

$$E_{peak} = (DI_{remaining})e^{-kt} + DI \text{ (just after meal)} \quad (2)$$

$$E_{trough} = (DI_{remaining})e^{-kt} + (DI)e^{-kt} \text{ (just before a meal)} \quad (3)$$

$$t_{1/2} = \ln (2)/ k \text{ (half-life elimination constant} \quad (4)$$

There are limited data on the half-lives ($t_{1/2}$) of pharmaceuticals in birds to apply in these equations. To place the 40 day exposure into perspective, the drug $t_{1/2}$ in ospreys needed to reach or exceed the HTD within 40 days at most extreme scenario (1000 ng/L concentration, pH 8, complete absorption) was back calculated. Equation (3) (daily exposure at nadir) was used to conservatively estimate cumulative daily body burden. The back calculated $t_{1/2}$ for ospreys was compared to the $t_{1/2}$ in humans (Ebadi, 2008; Wishart et al., 2008; FDA, 2012).

Empirical pharmaceutical exposure data

Study sites were selected in urbanized areas in proximity to WWTPs, combined sewer outflows, and effluent dominated low flow sites. These sites include the Susquehanna River (MD, PA), Back River (MD), James River (VA), and the US Environmental Protection Agency (USEPA) designated Regions of Concern (Baltimore Harbor [MD], Anacostia River/middle Potomac [DC, MD, VA], and the Elizabeth River [VA]), all of which appear on the 303d list for impaired waterways (Figure 10) (US EPA 2013). Sampling was undertaken during osprey nesting seasons of 2011, 2012, and 2013. The Paul S. Sarbanes Ecosystem Restoration Project at Poplar Island (MD), a remote mid-Bay location, was used as a reference site.

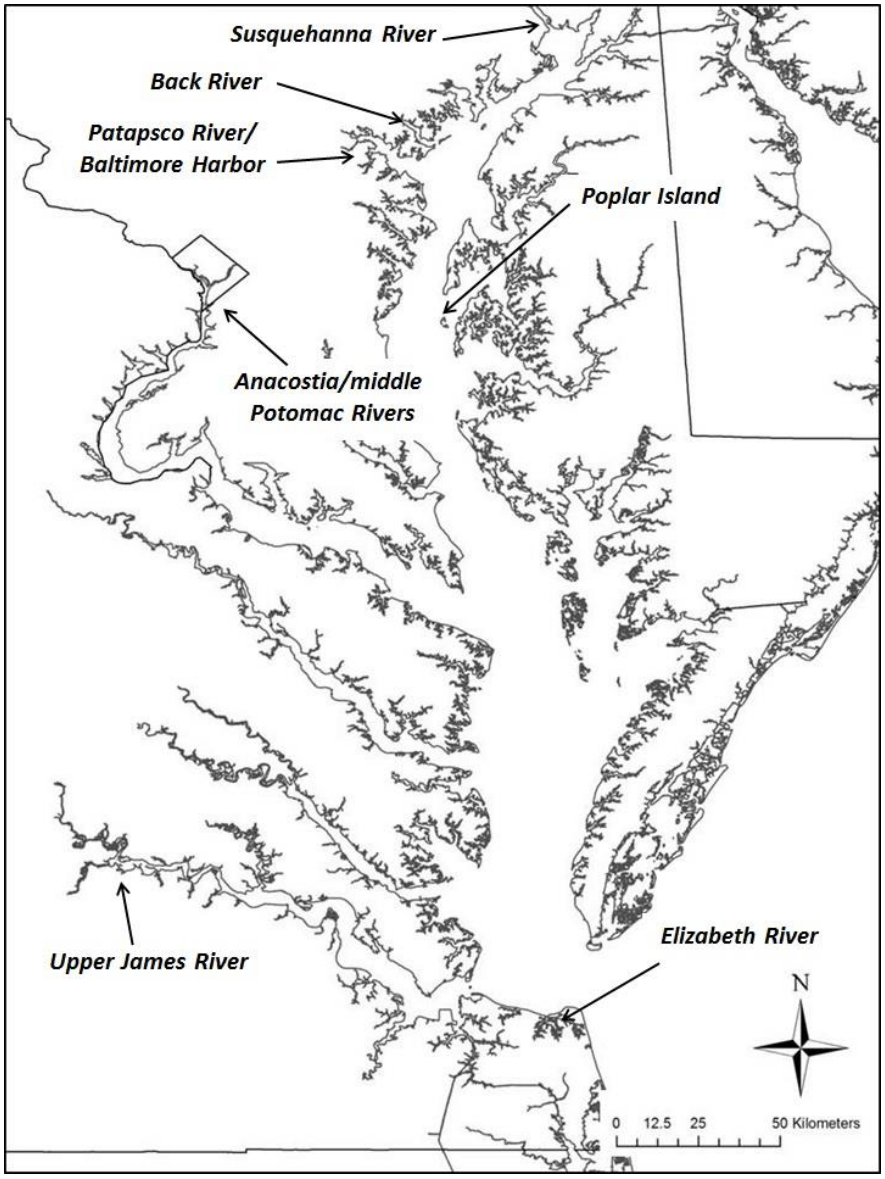


Figure 10. Map detailing the locations of Chesapeake Bay study sites.

Duplicate water samples were collected from 12 select sampling sites (2–3 locations along a stretch of the Susquehanna River, Back River, Anacostia/middle Potomac Rivers, James River, and at Poplar Island). Surface water samples were collected in clean 4 L amber glass jugs. Field blanks were taken by opening an empty jar to account for other sources of contamination. Water quality parameters (pH, dissolved

O_2, temperature, conductivity) were measured concurrently (YSI Multimeter Yellow Springs OH). Water samples were stored on wet ice and shipped overnight to Baylor University.

All procedures involving fish and ospreys were conducted under approval of the Institutional Animal Care and Use Committees of the US Geological Survey (USGS) and the University of Maryland, and appropriate scientific collection permits. Game camera (Bushnell 8MP Trophy Cam, Overland Park, KS) images of prey items delivered to osprey nests, direct observations, and identification of scraps were used to reconstruct osprey diet and identify target species for sampling. Based on osprey diet reconstruction, a combination of gizzard shad (*Dorosoma cepedianum*), catfish (blue catfish *Ictalurus furcatus*, brown bullhead *Ameiurus nebulosus*, and channel catfish *Ictalurus punctatus*), and carp (*Cyprinus carpio*) were sampled on the Susquehanna, Anacostia/middle Potomac, and James Rivers. Atlantic menhaden (*Brevoortia tyrannus*), striped bass (*Morone saxatilis*), and white perch (*Morone americana*) were sampled at the more saline Poplar Island site, and a combination of carp, catfish, gizzard shad, and white perch were sampled on Back River. These fish species reflect different trophic levels ranging from primary consumers (herbivorous) such as Atlantic menhaden and gizzard shad, to secondary consumers (carnivorous) including white perch and striped bass, to catfish and carp (omnivorous) representing a combination of both primary and secondary consumers. Fish were captured by electroshocking in upriver sites. At Poplar Island, fish were captured using a midwater trawl and a commercial pound net. Plasma was sampled from the 2 to 3 dominant prey fish species found at each site that fell within the osprey foraging size range (25–35 cm) (Poole 1989). All fish ($n = 233$) were anesthetized

(MS222, tricane methanesulfonate), weighed, measured, and 1 to 2 mLs of blood were sampled using a heparinized syringe. Fish blood was stored on wet ice and transported to the USGS-Leetown Science Center in WV. The blood was centrifuged at 2000 *g* at 4°C for 10 min on the same day of collection. Plasma was harvested for pharmaceutical analyses and stored at −80°C. Fish tissue was saved for analysis of organic contaminants as part of a concurrent study.

Osprey nests were identified in mid-March along a 25 to 35 km stretch of river. A sample egg was collected for analysis of legacy contaminants and nests were visited weekly to determine reproductive success as part of a concurrent study. Once nestlings reached 40 to 45 days of age, a single chick was briefly removed from the nest (<10 min). Body weight and culmen length were measured, and a 5 to 7 mL brachial blood sample was drawn into a heparinized syringe. Samples were stored on wet ice and centrifuged at 1500 *g* at 4°C for 10 min on the same day of collection. Plasma was harvested and samples (n = 69) were stored at the USGS-Patuxent Wildlife Research Center at −80°C. All plasma samples were shipped frozen to Baylor University for the quantification of pharmaceuticals.

Analysis of pharmaceuticals

A suite of 23 pharmaceuticals and metabolites and an artificial sweetener (Tables S5 and S6) were quantified in water and plasma samples from fish and osprey nestlings via isotopic dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS). These compounds included analgesics (acetaminophen, codeine), antibiotics (erythromycin, sulfamethoxazole, trimethoprim), an anticoagulant (warfarin),

antidepressants (paroxetine, fluoxetine, sertraline, and primary metabolites norfluoxetine and desmethylsertraline), an antihistamine (diphenhydramine), antihypertensives (atenolol, diltiazem, propranolol), anti-inflammatories (celecoxib, diclofenac), an antilipemic (gemfibrozil), an antiseizure (carbamazepine), a parasiticide (ivermectin), psychostimulants (diazepam, methylphenidate), a stimulant (caffeine), and an artificial sweetener (sucralose; conservative tracer of effluent discharges) (Soh et al., 2011).

For water, sample filtration and extraction generally followed previously described protocols (Du et al. 2014). A mixture of 24 internal standards (deuterated analogues of target compounds, except for ivermectin for which abamectin was the internal standard) and 5 mL of methanol was added to 500 mL of each water sample before extraction and acidification (pH adjusted with 100 µL of 85% [v/v] phosphoric acid) (Lajeunesse et al. 2008). Resulting concentrations of internal standards were approximately 100 ng/g. Samples were subsequently loaded onto strong cation-exchange cartridges (Strata-SCX, 500 mg; Phenomenex, Torrance, CA) preconditioned with 4 mL of methanol and 8 mL of nano-pure water. Each cartridge was washed with 4 mL of HCl (0.1 *N*) and 4 mL of methanol, followed by elution of the 5 antidepressant serotonin reuptake inhibitors with 6 mL of 5% (v/v) NH_4OH in methanol. Extraction of 19 other analytes generally followed a previously reported protocol (Vanderford and Snyder 2006). Each sample (500 mL subsample) was spiked with a mixture of internal standards and loaded onto a preconditioned HLB cartridge (200 mg, Waters, Milford, MA). These loaded cartridges were air-dried and subsequently eluted with 5 mL methanol followed by 5 mL 10:90 (v/v) methanol-methyl tertiary butyl ether. The eluate from 2 separate extractions was evaporated to dryness under a stream of N and reconstituted in 1 mL of

chromatographic mobile phase (i.e., methanol-0.1% [v/v] aqueous formic acid). Before LC-MS/MS analysis, samples were sonicated for 1 min and filtered using Pall Acrodisc hydrophobic Teflon Supor membrane syringe filters (13 mm diameter; 0.2 μm pore size; VWR Scientific, Suwanee, GA).

For plasma samples, a slightly modified extraction method was used (Fick et al., 2010a). An aliquot of fish and osprey plasma (typically 1 mL), combined with the same mixture of internal standards that was used for water, was diluted to 5 mL using 0.1% (v/v) aqueous formic acid and mixed thoroughly by sonication. The mixture was loaded on preconditioned (5 mL of methanol and 5 mL of nano-pure water) HLB SPE cartridges (200 mg, Waters). Each cartridge was air-dried and subsequently eluted with 5 mL of methanol. The eluate was reconstituted, and analytes were quantified by LC-MS/MS as previously described (Du et al. 2012).

For water and fish plasma, method detection limits (MDLs) were less than 11 ng/L with the exception of ivermectin and sucralose (Table S6). Osprey plasma from the reference site, spiked with the mixture of internal standards, was used to determine MDLs, which were similar to that of water and fish (Table S2). For quality control purposes, 1 pair of matrix spike samples and 1 method blank sample was added for each batch analysis. Spike recoveries ranged from 81% to 111% in water, 81% to 113% in fish, and 81% to 89% in ospreys.

Statistical analyses

For the daily and 40 day screening-level exposure models, DI and half-life elimination constants were estimated using Microsoft Excel. For empirical exposure data,

concentrations of pharmaceuticals and an artificial sweetener were first recovery corrected and only values above the MDL were reported. If the analyte was present in all samples, the arithmetic mean and standard deviation were obtained using SAS (SAS Institute, NC). If an analyte was detected in only 1 of the 2 duplicate water samples, one randomly selected value was included in the statistical analysis. If analytes were detected in over half (but not all) of the samples at a study location, the Kaplan-Meier method was used to estimate an interval that contains the theoretical mean (Helsel 2005, 2009).

Parametric statistics were conducted for those analytes detected in all samples. Continuously distributed analyte concentrations in water, and fish and osprey plasma were tested for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test). In 2 instances, variables were log or square root transformed to correct for normality or heterogeneous variances. A 1-way analysis of variance followed by Tukey's honestly significant difference method for multiple comparisons was performed ($\alpha = 0.05$). For those sites with nondetects, a generalized Wilcoxon nonparametric test was used (Helsel, 2005).

For all detectable compounds, a hazard quotient (HQ) was calculated by dividing the maximum concentration found in fish or osprey plasma by the human therapeutic plasma concentration (C_{max}). The larger the HQ value, the greater the potential for a compound to exert a pharmacological effect in fish or ospreys.

Results

Screening-level exposure model

Of 114 compounds, 31 had a BCF less than or equal to 1.00 (Table S6). These 31 compounds plus the bronchodilator tiotropium (no BCF available) were excluded from the model. Of the 83 remaining compounds, 15 had both a BCF greater than 100 and an estimated DI greater than 20 μg/kg-day and are predicted to have the greatest potential to bioaccumulate. The calculated DIs for these 15 compounds at concentrations of 10, 100, and 1000 ng/L in an adult female osprey are presented in Table 10. At concentrations of 1000 ng/L water at pH 8, the DI of orlistat, fenofibrate, tamoxifen, and loperamide would be 1.1 to 4.4 times greater than the oral HTD. Based on the information in Table 10, orlistat is the only compound that still exceeds the HTD even if the intestinal absorption in ospreys was only half that of humans (12 198 μg/kg BWt-day, 2.4 times greater than the HTD). Pharmaceutical concentrations in water and pH values selected in the model were environmentally realistic (analyte concentrations in Chesapeake Bay range from 0.029 to 10,249 ng/L and site pH ranged from 6.15 to 8.34) (Tables 3 and A4).

Cumulative 40 day exposure model

For the top 15 compounds (BCF > 100, DI > 20 μg/kg BWt-day), the theoretical half-life (calculated from the half-life elimination constant, k) for an osprey to reach the HTD within a 40 day period ranged from 1 to 231 days. For this subset of pharmaceuticals, the half-lives in humans ranged from 0.04 to 7 days. Notably, the half-lives in ospreys for fenofibrate, tamoxifen and ezetimibe were less than that in humans, with the HTD being exceeded in just 3 to 7 days (Table 10).

Table 10. Daily intake (DI) at three water concentrations and $t_{1/2}$ to accumulate a human therapeutic dose (HTD) within 40 days for 16 pharmaceuticals compared to human values.

Rank	Compound	BCF pH 8	DI Osprey (100% absorption) (µg/kg BW-day)[a]			HTD (µg/kg BW)	$t_{1/2}$ ospreys to exceed HTD 100% absorption[a]	$t_{1/2}$ humans (Days)
			10 ng/L pH 8	100 ng/L pH 8	1000 ng/L pH 8		1000 ng/L pH 8	
1	Orlistat	123000	244	2440	24396	5143	1.79	0.04-0.08
2	Fenofibrate	15100	29.9	299	2995	1714	0.42 (HTD in 3 days)	0.83
3	Piperonyl butoxide	2400	4.76	47.6	476	Topical	NA[b]	NA[b]
4	Tamoxifen	797	1.58	15.8	158	143	1.00 (HTD in 4 days)	5-7
5	Ketoconazole	646	1.28	12.8	128	2857	22.8	0.14
6	Ezetimibe	589	1.16	11.6	117	143	1.19 (HTD in 7 days)	0.79-1.25
7	Iminostilbene[c]	556	1.10	11.0	110	5714	34.3	0.08-2.71
8	Loratadine	538	1.06	10.7	107	143	1.27	0.35
9	Loperamide	519	1.03	10.3	103	57.1	0.67	0.45
10	Promethazine	358	0.71	7.10	71.1	357	4.07	0.67-0.79
11	Diltiazem	343	0.68	6.80	68.0	2571	231	0.12-0.18
12	Raloxifene	327	0.65	6.50	65.0	857	11.3	1.15
13	Dextromethorphan	228	0.45	4.52	45.2	571	11.3	0.05-0.16
14	Desmethylsertraline	213	0.42	4.20	42.3	NA[b]	NA[b]	2.58-4.33
15	Sertraline	142	0.28	2.82	28.2	357	11.5	1.04-1.08

[a]Shaded boxes indicate the estimated DI or $t_{1/2}$ for ospreys exceeds HTD or human $t_{1/2}$.

[b]NA=not applicable; not injested for therapeutic uses or a metabolite of a parent compound.

[c]Iminostilbenes are a group of antiseizure drugs which includes carbamazepine and oxcarbamazepine. HTD and half-lives are given based on the lowest dose in this group that is needed for a therapeutic effect.

Empirical assessment of pharmaceuticals in water, fish, and osprey

Of the 24 analytes measured, 17 were detected in water and 8 of these were also detected in fish (Tables 2, A4 and A5). Diltiazem was the only compound detected in all 3 matrices (Table 11, all values presented on a ng/L basis). When compared to the human therapeutic plasma concentration (C_{max}), all detected compounds had a HQ less than or equal to 0.08.

In water, concentrations were averaged from the 2 to 3 sampling sites per tributary. There were 73 out of 77 instances where the analyte was detected in the duplicate water samples and the median relative percent difference between samples was 12.02%. Samples were collected from the reference site each year of the study, but diltiazem was only detected in water samples in 2011 and 2012. At Back River, 18 analytes were detected in water with concentrations being 2 to 154 times greater than other sites. Carbamazepine, diltiazem, sulfamethoxazole, diphenhydramine, and caffeine were detected in water at all intensively sampled sites. Statistical analysis revealed that the Anacostia/middle Potomac Rivers had the greatest carbamazepine (log transformed) and caffeine concentrations in water (Back River excluded from analysis as there was only a single sample), followed by the James, Poplar Island, and Susquehanna ($p < 0.04$). There were no differences in diltiazem concentrations in water among all 5 sites.

Although 7 pharmaceuticals and sucralose were found in fish, detection frequency was low, rarely exceeding half of the samples per site. Thus, parametric statistical analyses could not be conducted among analytes, fish species, and sites (Table A5). By inspection of these data, diphenhydramine and diltiazem were present in fish from all study sites. Both the Anacostia/middle Potomac and Back Rivers had the largest suite of

pharmaceuticals detected. Diltiazem was found in 13% of fish samples and present in all species, with the greatest plasma concentration (2.4 ng/mL) found in a catfish collected on the Susquehanna (Table 11).

Diltiazem was detected in all 69 osprey nestling plasma samples (1.6–24.6 times greater than the MDL). Osprey nestlings on the Anacostia/middle Potomac, Baltimore Harbor, and Back River had higher ($p < 0.04$) diltiazem plasma concentrations (square root transformed) compared to the James, Elizabeth, Susquehanna Rivers, and the Poplar Island reference site. Plasma diltiazem concentrations at Poplar were higher than those on the Elizabeth and James Rivers ($p < 0.007$). For the Potomac River, which had osprey nests evenly spaced downriver from Blue Plains WWTP, diltiazem in nestling plasma did not exhibit a spatial concentration gradient ($p > 0.35$).

Table 11. Wet weight concentrations of polychlroinated biphenyls from Chesapeake Bay Regions of Concern and Poplar Island reference site[a].

Site/Class	Analgesic		Antibiotic			Anticoagulant	Antihistamine	Psychostimulant	
	Acetaminophen	Codeine	Sulfamethoxazole	Trimethoprim	Erythromycin	Warfarin	Diphenhydramine	Methylphenidate	Diazepam
Poplar Island							+		
Water			+						
Atlantic Menhaden									
Striped Bass							+		
White Perch							+		
Anacosita/middle Potomac Rivers									
Water	+		+	+	+		+	+	
Catfish sp.							+		
Gizzard Shad							+		
Carp							+		
Back River									
Water	+	+	+	+	+	+	+	+	+
Catfish sp.							+		
Gizzard Shad		+					+		
Carp							+		
White Perch							+		
James River									
Water			+	+			+		
Catfish sp.							+		
Gizzard Shad							+		
Susquehanna River									
Water			+				+		
Catfish sp.									
Gizzard Shad									

Site/Class	Antihypertensive		Anti-inflammatories			Antilipemic	Antiseizure	Artificial Sweetener	Stimulant
	Atenolol	Diltiazem	Propanolol	Celecoxib	Diclofenac	Gemfibrozil	Carbamazepine	Sucralose	Caffeine
Poplar Island									
Water		+					+	+	+
Atlantic Menhaden		+							
Striped Bass		+		+					
White Perch		+							
Anacosita/middle Potomac Rivers									
Water	+	+			+	+	+	+	+
Catfish sp.							+		
Gizzard Shad	+	+						+	
Carp		+							
Back River									
Water	+	+	+	+	+	+	+	+	+
Catfish sp.		+							
Gizzard Shad		+					+		
Carp							+		
White Perch									
James River									
Water	+	+				+	+	+	+
Catfish sp.		+							+
Gizzard Shad		+							
Susquehanna River									
Water							+	+	+
Catfish sp.		+							
Gizzard Shad									

[a]+ compound detected

Table 12. Concentrations (ng/g ww) of polybrominated diphenyl ethers, additional brominated flame retardants and methoxytriclosan from Chesapeake Bay Regions of Concern and Poplar Island reference site[a].

Site	Water (ng/L) Detects/n Mean ± std. dev. Extremes	Fish (ng/L plasma) Detects/n Mean ± std. dev. Extremes HQ[b]						Osprey (ng/L plasma) Detects/n Mean ± std. dev. Extremes HQ[b]
		Catfish	Gizzard Shad	Carp	Rockfish	Menhaden	Perch	
Poplar Island	2/3 1.06-1.14[c] <MDL-2.05	NS	NS	NS	3/17 - <MDL-1800 0.06	1/10 - <MDL-410 0.01	1/10 - <MDL-410 0.01	13/13 2199 ± 1524[C] 605-4458 0.15
Anacosita/ middle Potomac Rivers	2/3 1.08-1.62[c] <MDL-2.47	0/30 - -	3/33 - <MDL-410 0.01	1/18 - <MDL-330 0.01	NS NS	NS NS	NS NS	13/13 4517 ± 1384[A] 3503-8630 0.29
Back River	1/1 - 173	1/2 - <MDL-350 0.01	1/9 - <MDL-420 0.01	0/9 - -	NS	NS	0/5 - -	7/7 2353 ± 1207[B,C] 1049-4288 0.29
James River	3/3 5.85 ±2.51 2.96-7.49	8/27 - <MDL-770 0.03	3/27 - <MDL-570 0.02	NS	NS	NS	NS	12/12 912 ± 225[D] 537-1355 0.05
Susquehanna River	2/2 1.67 ± 0.45 1.35-1.99	9/18 - <MDL-2400 0.08	0/18 - -	NS	NS	NS	NS	10/10 1434 ± 372[C,D] 4049-2099 0.07
Additional sites where only osprey nestlings sampled								
Elizabeth River								6/6 966 ± 352[D] 564-1320 0.04
Baltimore Harbor/ Patapsco River								8/8 3786 ± 714[A,B] 2885-5110 0.17

[a]NS=Not sampled because fish species was not a large component of osprey diet at a particular site; - indicates no mean calculated, contaminant was detected in fewer than half of the samples; MDL = method detection limit. Means with different captial letter superscripts are significantly different ($p < 0.05$).

[b]Hazard Quotient (HQ) is the upper extreme concentration found in fish or osprey plasma divided by the human therapeutic plasma concentration (C_{max}) for diltiazem (30,000 ng/L).

[c]If non-detects were present in less than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean followed by a generalized Wilcoxin non-parametric test.

Discussion

Screening-level exposure assessment

Whereas several studies have examined uptake of pharmaceuticals from water by fish (Brown et al. 2007; Ramirez et al. 2009), their transfer to high trophic level wildlife has not been evaluated. The likelihood for a broad suite of potentially ionizable

pharmaceuticals to bioaccumulate in a water–fish–osprey food web was modeled using their concentration in water, pH-specific BCF and FIR of an adult female osprey. Those compounds with high pH-specific BCFs and long half-lives near low flow point sources (i.e., low dilution scenario) were predicted to exceed the HTD.

This screening-level assessment identified a subset of 15 of 114 compounds that warrant further investigation based on their potential to exceed the HTD. Over a narrow range of pH (6–8), there was little effect on the BCF of 6 of these compounds (orlistat, fenofibrate, piperonyl butoxide, ezetimibe, iminostilbene, and loratadine), with BCFs fluctuating by less than 20% (Table S1). Although ionizable at pH extremes (low or high pKa), these 6 compounds were in their neutral state from pH 6–8 and predicted to be the most bioaccumulative. It has been suggested that compounds with such characteristics could evoke pharmacological responses and possibly toxicity in invertebrates and fish at their isoelectric point (Ebadi 2008; Rendal et al. 2011). The remaining 10 compounds (mean pKa 8.37) are not ionizable until pH exceeds environmentally relevant conditions. The use of pH-specific BCFs appears to be a valuable tool to identify and prioritize pharmaceuticals and metabolites that have the greatest potential to bioaccumulate at environmentally relevant conditions.

An estimate of the half-life is required to model first-order kinetic elimination of drugs over a specific period of time and provides a measure of the persistence of a xenobiotic. Based on our screening-level exposure model, HTDs for fenofibrate, tamoxifen, and ezetimibe were exceeded in adult ospreys at theoretical half-lives (0.24–1.19 days) that were less than their half-lives in humans (0.83–1.25 days) (Ebadi 2008; Wishart et al. 2008). Such theoretical half-lives are not unreasonable. For the

aforementioned compounds, it might be possible for an osprey to accumulate a HTD within 3 to 7 days of exposure in a low-flow scenario.

Uncertainty factors and model assumptions

Eighteen pharmaceuticals and an artificial sweetener were detected in water samples from Chesapeake Bay. Frequency of detection and concentrations were greatest in water samples collected on the Back River, which receives appreciable WWTP input (180 million gallons/day from 1.3 million residents from Baltimore) (Baltimore County Watershed Management Program 2012). Despite greater input from Blue Plains WWTP and population size (330 million gallons/day from 2.1 million residents of the Washington District of Columbia metropolitan area) (District of Columbia Water and Sewage Authority 2014), concentrations were seemingly lower on the Anacostia/middle Potomac Rivers.

Sulfamethoxazole, diphenhydramine, diltiazem, carbamazepine, sucralose, and caffeine were frequently detected in water samples, and 3 of these (diphenhydramine, diltiazem, and carbamazepine) were often detected in fish plasma (Tables S3 and S4). In fish, detection frequency, concentrations, and HQs were low and far less than critical environmental concentrations hypothesized to cause pharmacological effects in fish (Schwab et al. 2005; Fick et al., 2010b; Du et al. 2014). This is not unexpected as other reports indicate that both sucralose and caffeine do not bioaccumulate in fish. Sucralose was detected at lower concentrations in fish than in water samples from the Anacostia/middle Potomac Rivers and did not bioconcentrate (maximum detected concentration in fish/maximum detected concentration in water = 0.50; Tables S3 and S4). Notably laboratory studies have reported a BCF less than 1 for sucralose in zebrafish

(*Danio rerio*) (Lillicrap et al. 2011) and is not that different from literature estimates (BCF = 1, Table S1) (ACS 2014). Of the compounds most frequently detected in fish, our estimated BCFs were within an order of magnitude compared to literature values presented in Table S1 (diphenhydramine: estimated 79.1 compared with literature value of 16.7; carbamazepine: 44.1 versus 16.2; diltiazem: 319 versus 343). Interestingly, antidepressants were not found in the present study despite being detected in many urban rivers in North America (Brooks et al. 2005; Ramirez et al. 2009; Lajeunesse et al. 2011; Schultz et al. 2010).

Diltiazem was the only analyte detected in water, fish, and biota. For diltiazem, concentrations in water were low (mean 2.44 ng/L), and with the exception of Back River (173 ng/L), was generally an order of magnitude below those found in urban inland waters of the United States and Sweden (36–1800 ng/L) (Kolpin et al. 2002, 2004; Fick, et al., 2010a; Du et al. 2014). In Chesapeake Bay, diltiazem fish plasma concentrations were 2 times greater than those observed at 3 WWTPs in Sweden (MDL-1000 ng/L) (Fick et al., 2010a). Out of 10 commonly used pharmaceuticals tested in *Daphnia magna* and Japanese medaka (*Oryzias latipes*), diltiazem exhibited the greatest acute toxicity (96 h LC50 = 8.2 and 15.0 mg/L, respectively) (Kim et al. 2007). The predicted no-effect concentration for diltiazem based on the lowest acute EC50 values was estimated to be 8.2 µg/L (Kim et al. 2007), which is over an order of magnitude greater than the maximum value observed in the Back River. Evaluating these data in a more complete assessment should also include chronic responses linked to therapeutic hazard (Brausch et al. 2012; Valenti et al. 2012). The aquatic hazards and risk of diltiazem and many other pharmaceuticals remain poorly characterized (Brooks, 2014).

Several interspecific differences in pharmaceutical bioaccumulation were found among fish species (Table S8). For example, diltiazem was detected in channel catfish, but not gizzard shad from the Susquehanna River, whereas carbamazepine was observed in blue and channel catfish, but again not gizzard shad from the Anacostia/middle Potomac Rivers. Spatial variations in fish migration patterns may explain such differences in pharmaceutical bioaccumulation. For example, anadromous gizzard shad migrate downstream to deeper waters in the winter, whereas catfish remain in upper estuarine sites where they may be continuously exposed to wastewater discharge. The influence of trophic position (e.g., herbivorous gizzard shad and omnivorous catfish) on pharmaceutical bioaccumulation in fish is not well understood.

Empirical findings in osprey nestlings

This screening-level exposure assessment suggests that only 3 of 24 analytes quantified in osprey plasma (diltiazem, sertraline, and desmethylsertraline) are likely to exceed the HTD. Of these 3, diltiazem has the highest pH-specific BCF (343 at pH 8) and was detected in all osprey nestling plasma at low concentrations (0.56–8.63 ng/mL plasma), with the maximum value being 28% of the HTD. Although present in all osprey samples, there were no overt signs (therapeutic or toxicological) observed in our companion study examining reproductive success. Ospreys are thriving in Chesapeake Bay, including the most contaminated sites, and reproduction is generally adequate to sustain stable populations (>1.15 fledglings per active nest) (Lazarus et al. 2015a).

Of the 15 compounds identified in the screening-level model as having the greatest potential to bioaccumulate, 3 were measured in osprey plasma (Table 1;

diltiazem 11, desmethylsertraline 14, and sertraline 15), and only diltiazem was detected. The accumulation of diltiazem and other antidepressants is theoretically pH-dependent. The bioaccumulation characteristics (partition coefficients log *p* and log D at pH 8) of sertraline (log *p* = 5.08 and log D = 3.60) and its metabolite desmethylsertraline (log *p* = 4.89 and log D = 3.73), are not unlike diltiazem (i.e., log *p* = 4.73 and log D = 3.90). Thus, diltiazem may be bioaccumulating not only because of its high pH-specific BCF, but also because of other biological characteristics including specific binding mechanisms. It is clear that diltiazem concentrations were greatest in osprey nestlings followed by fish and water concentrations. Across sites, the maximum diltiazem concentrations in water, fish plasma, and osprey plasma were averaged for each matrix to approximate a biomagnification factor. Diltiazem concentrations in fish plasma were 21.6 times greater than those in water, and osprey plasma concentrations were 4.71 times greater than fish. It should be noted that the biomagnification factor from fish to osprey most certainly varies with osprey diet composition.

Conclusions

This screening-level exposure assessment identified 15 out of 113 pharmaceuticals and an artificial sweetener that warrant further investigation in fish-eating birds due to their high BCF and DI. Some of these compounds might even exceed the HTD. The antihypertensive drug diltiazem was detected in all osprey nestling plasma samples in several tributaries of Chesapeake Bay. Twelve additional analytes that were predicted to bioaccumulate, but not measured in environmental samples should receive priority for further investigation. Although diltiazem in ospreys did not exceed the HTD

and was well below the C_{max}, our findings indicate that it can bioaccumulate to levels that are over 4 times greater than values in fish plasma. Even though empirical concentrations of drugs in the present study are well-below therapeutic levels for humans, the paucity of effect threshold data for birds and lower vertebrates makes interpretation of these observations challenging. Our knowledge of mammalian pharmacology can assist in extrapolation of effects to wildlife (Huggett et al. 2003), but in some (and hopefully rare) instances, birds and other perhaps other classes of vertebrates may be sensitive to low-level environmental exposures (Oaks and Watson 2011).

Supplemental Table S5. List of 114 pharmaceutical compounds and sucralose for the screening-level exposure assessment, their CAS number, and bioconcentration factor (BCF).

Number	Compound[a]	Primary Use Class	CAS Number	BCF pH 8
1	1,7-Dimethylxanthine	Stimulant	611-59-6	1.00
2	10-Hydroxy-amitriptyline	Metabolite of amitriptyline	1159-82-6	2.51
3	Abacavir	Antiviral, reverse transcriptase inhibitor	136470-78-5	4.46
4	Acetaminophen*	Analgesic, antipyretic	103-90-2	1.34
5	Acyclovir	Antiviral	59277-89-3	1.00
6	Albuterol	β-2 adrenergic receptor agonist for asthma treatment	18559-94-9	1.00
7	Alprazolam	Benzodiazepine to treat anxiety	28981-97-7	16.8
8	Amitriptyline	Tricyclic antidepressant	50-48-6	83.2
9	Amphetamine	Psychostimulant	300-62-9	1.00
10	Antipyrine	Analgesic, antipyretic	60-80-0	1.27
11	Atenolol*	Antihypertensive	29122-68-7	1.00
12	Benztropine	Anticholinergic used in treatment of Parkinson's	86-13-5	2.47
13	Betamethasone	Synthetic glucocorticoid steroid	378-44-9	20.7
14	Bupropion	Antidepressant, smoking cessation aid	34911-55-2	30.0
15	Caffeine*	Psychoactive stimulant	58-08-2	1.00
16	Carbamazepine*	Anticonvulsant and mood stabilizer	298-46-4	16.2
17	Carisoprodol	Muscle Relaxant	78-44-4	23.2
18	Celecoxib**	Anti-inflammatory	169590-42-5	53.9
19	Chlorpheniramine	Antihistamine	132-22-9	4.54
20	Cimetidine	Antacid, Histamine H-2 receptor antagonist	51481-61-9	1.00
21	Citalopram	Antidepressant/antianxiety (SSRI)	59729-33-8	6.99
22	Clonidine	Antihypertensive	4205-90-7	16.5
23	Codeine*	Analgesic, antitussive, antidiarrheal	76-57-3	2.32
24	Cotinine	Metabolite of nicotine, tobacco constituent	486-56-6	1.00
25	Dehydronifedipine	Metabolite of the antihypertensive nifedipine	67035-22-7	6.76
26	Desmethyldiltizaem	Metabolite of diltiazem	86408-45-9	29.8
27	Desmethylsertraline**	Metabolite of sertraline	87857-41-8	213
28	Desvenlafaxine	Antidepressant, and metabolite of venlafaxine	93413-62-8	1.00
29	Dextromethorphan	Cough suppressant	125-71-3	228
30	Diazepam*	Antiseizure, antianxiety and insomnia	439-14-5	79.2
31	Diclofenac**	Anti-inflammatory	15307-86-5	1.00
32	Diltiazem*	Antihypertensive	42399-41-7	343
33	Diphenhydramine*	Antihistamine	58-73-1	16.7
34	Duloxetine	Antidepressant	116539-59-4	27.3
35	Erythromycin*	Antibiotic	114-07-8	7.73
36	Ezetimibe	Antilipemic to reduce cholesterol	163222-33-1	589
37	Fadrozole	Aromatase inhibitor	102676-47-1	6.91
38	Famotidine	Antacid, Histamine H-2 receptor antagonist	76824-35-6	1.00
39	Fenofibrate	Antilipemic to reduce cholesterol	49562-28-9	15100
40	Fexofenadine	Antihistamine	83799-24-0	1.19
41	Fluconazole	Antifungal	86386-73-4	1.29
42	Fluoxetine*	Antidepressant/antianxiety (SSRI)	54910-89-3	5.46
43	Fluticasone propionate	Corticosteroid	90566-53-3	22.1
44	Fluvoxamine	Antidepressant/antianxiety (SSRI)	54739-18-3	15.7
45	Gemfibrozil**	Antilipemic	25812-30-0	1.00
46	Glipizide	Antidiabetic	29094-61-9	1.00
47	Glyburide	Antidiabetic	10238-21-8	1.33
48	Hydrocodone	Analgesic, antitussive	125-29-1	12.30
49	Hydrocortisone	Anti-inflammatory	50-23-7	12.90
50	Hydroxyzine	Antihistamine, sedative	68-88-2	32.7
51	Iminostilbene	Intermediate for manufacture of carbamazepine	256-96-2	556
52	Ivermectin**	Parasiticide	70288-86-7	60.0[c]
53	Ketoconazole	Antifungal	65277-42-1	646
54	Lamivudine	Antiviral	134678-17-4	1.00
55	Lidocaine	Topical anesthetic	137-58-6	17.3
56	Loperamide	Anti-diarrheal	53179-11-6	519
57	Loratadine	Antihistamine	79794-75-5	538
58	Lorazepam	Anticonvulsant and muscle relaxant	846-49-1	38.0
59	Meprobamate	Carbamate derivative, anxiolytic	57-53-4	2.00
60	Metaxalone	Muscle Relaxant	1665-48-1	4.65
61	Metformin	Antidiabetic	657-24-9	1.00
62	Methadone	Analgesic	76-99-3	47.4
63	Methocarbamol	Muscle Relaxant	532-03-6	1.16
64	Methotrexate	Antimetabolite and antifoliate drug used to treat cancer	59-05-2	1.00
65	Methylphenidate**	Psychostimulant	298-59-9	1.02
66	Metoprolol	Antihypertensive	51384-51-1	1.00
67	Morphine	Analgesic	57-27-2	1.03

Supplemental Table S5 cont'd. List of 114 pharmaceutical compounds and sucralose for the screening-level exposure assessment, their CAS number, and bioconcentration factor (BCF).

68	Nadolol	Antihypertensive, used to prevent migraines	42200-33-9	1.00
69	Nevirapine	Antiviral	129618-40-2	59.7
70	Nicotine	Stimulant	54-11-5	1.00
71	Nizatidine	Antacid	76963-41-2	1.00
72	Nordiazepam	Anti-anxiety metabolite of diazepam	1088-11-5	75.8
73	Norethindrone	Contraceptive component	68-22-4	87.6
74	Norfluoxetine*	Antidepressant	83891-03-6	35.2
75	Norverapamil	Metabolite of verapamil	67018-85-3	5.99
76	Omeprazole + esomeprazole[b]	Antacid	73590-58-6	34.3
77	Orlistat	Anti-obesity	96829-58-2	123000
78	Oseltamivir	Antiviral	196618-13-0	4.92
79	Oxazepam	Anti-anxiety	604-75-1	28.4
80	Oxycodone	Analgesic and antidiarrheal	76-42-6	6.42
81	Paroxetine*	Antidepressant/antianxiety (SSRI)	61869-08-7	8.10
82	Penciclovir	Antiviral	39809-25-1	1.00
83	Pentoxifylline	Improves blood flow, cardiovascular drug	6493-05-6	1.00
84	Phenazopyridine	Analgesic	94-78-0	28.4
85	Phendimetrazine	Appetite suppressant drug	634-03-7	7.88
86	Phenytoin	Antiepileptic	57-41-0	5.23
87	Piperonyl butoxide	Pediculicides	51-03-6	2400
88	Prednisolone	Corticosteroid	50-24-8	10.3
89	Prednisone	Corticosteroid	53-03-2	9.12
90	Promethazine	Antihistamine, antiemetic, sedative	60-87-7	358
91	Propoxyphene	Analgesic	469-62-5	46.5
92	Propranolol*	Antihypertensive	525-66-6	2.60
93	Pseudoephedrine + ephedrine[b]	Decongestant, appetite suppressant, stimulant	90-82-4, 299-42-3	1.00, 1.00
94	Quinine	Antimalarial, analgesic	130-95-0	4.34
95	Raloxifene	Selective Estrogen Receptor Modulator	84449-90-1	327
96	Ranitidine	Antacid	66357-35-5	1.00
97	Sertraline*	Antidepressant/antianxiety (SSRI)	79617-96-2	142
98	Sitagliptin	Antihyperglcemic	486460-32-6	19.9
99	Sucralose**	Artifical Sweetener	56038-13-2	1.00
100	Sulfadimethoxine	Antibiotic	122-11-2	1.00
101	Sulfamethizole	Antibiotic	144-82-1	1.00
102	Sulfamethoxazole*	Antibiotic	723-46-6	1.00
103	Tamoxifen	Estrogen receopotor agonist used to treat breast cancer	10540-29-1	797
104	Temazepam	Hypnontic	846-50-4	27.1
105	Theophylline	Antiasthmatic, diuretic	58-55-9	1.00
106	Thiabendazole	Parasiticide, fungicide	148-79-8	44.4
107	Tiotropium	Bronchodilator	186691-13-4	.
108	Tramadol	Analgesic	27203-92-5	1.16
109	Triamterene	Diuretic	396-01-0	4.35
110	Trimethoprim*	Antibiotic	738-70-5	1.50
111	Valacyclovir	Antiviral	124832-26-4	1.00
112	Venlafaxine	Antidepressant	93413-69-5	3.13
113	Verapamil	Antihypertensive	52-53-9	65.7
114	Warfarin*	Anticoagulant	81-81-2	1.00

[a] *=Subset of analytes quantified at Baylor University; **=analytes quantified at Baylor but not at USGS National Water Quality Laboratry

[b]Mixture of stereoisomers.

[c]BCF predicted from SAR relationship not pH specific (Sanderson et al. 2007).

Supplemental Table S6. Analytes quantified in water and plasma from fish and ospreys and their method detection limits (MDL).

Class	Analyte	Water (ng/L)	Fish (ng/mL)	Osprey (ng/mL)
Analgesics	Acetaminophen	2.90	4.70	4.60
	Codeine	0.830	6.90	8.10
Antibiotics	Erythromycin	8.60	8.60	14.0
	Sulfamethoxazole	1.30	1.90	4.00
	Trimethoprim	1.30	2.80	0.40
Anticoagulant	Warfarin	0.78	0.93	0.62
Antidepressant	Fluoxetine	8.40	5.50	5.00
	Norfluoxetine	8.50	3.90	7.40
	Paroxetine	11.0	1.80	4.00
	Sertraline	6.10	3.70	5.00
	Desmethylsertraline	5.40	8.30	9.70
Antihistamine	Diphenhydramine	0.22	0.12	0.18
Antihypertensive	Atenolol	4.30	2.90	11.0
	Diltiazem	0.24	0.12	0.35
	Propranolol	1.80	0.58	1.90
Anti-inflammatories	Celecoxib	11.0	7.90	8.40
	Diclofenac	2.80	0.84	1.40
Antilipemic	Gemfibrozil	2.10	6.90	8.00
Antiseizure	Carbamazepine	0.53	0.53	0.74
Artificial Sweetener	Sucralose	36.0	37.00	22.0
Parasiticide	Ivermectin	93.0	61.0	140.0
Psychostimulant	Diazepam	4.60	2.80	3.50
	Methylphenidate	0.30	0.27	0.58
Stimulant	Caffeine	4.50	4.70	7.80

Supplemental Table S7. Analytes detected in water samples.

Analyte	Water Concentrations (ng/L)				
	Poplar Island (2011-13) Detects/n Mean ± std. dev. Extremes	**Susquehanna River 2013** (2 sites) Detects/n Mean ± std. dev. Extremes	**James River 2012** (3 sties) Detects/n Mean ± std. dev Extremes	**Anacostia/middle Potomac Rivers 2011** (3 sites) Detects/n Mean ± std.dev. Extremes	**Back River 2011** (1 site) Detects/n Mean Extremes
Acetaminophen				2/3 63.7-64.7[b] <MDL-174	1/1 271 -
Codeine					1/1 219 -
Erythromycin				1/3 - <MDL-11.3	1/1 461 -
Sulfamethoxazole	3/3 6.78 ± 3.51 3.67-10.6	2/2 3.26 ± 0.57 2.85-3.66	3/3 33.5 ± 10.6 21.7-42.3	3/3 35.6 ± 21.1 17.6-58.8	1/1 572 -
Trimethoprim			2/3 2.41-2.89[b] <MDL-4.23	3/3 5.00 ± 1.68 3.60-6.88	1/1 83.0 -
Warfarin					1/1 163 -
Diphenhydramine	3/3 1.56 ± 0.82 0.32-2.59	2/2 5.20 ± 6.31 0.74-9.67	3/3 1.09 ± 0.60 0.37-1.72	3/3 5.40 ± 6.53 1.20-12.9	1/1 10.9 -
Atenolol			2/3 12.1-13.6[b] <MDL-21.1	1/3 - <MDL-44.8	1/1 114 -
Diltiazem	2/3 1.06-1.14[b] <MDL-2.05	2/2 1.68 ± 0.45 1.36-2.00	3/3 5.86 ± 2.52 2.97-7.51	2/3 1.08-1.62[b] <MDL-2.47	1/1 - 173
Propanolol					1/1 245 -
Celecoxib					1/1 440 -
Diclofenac				1/1 - <MDL-12.2	1/1 20.3 -
Gemfibrozil			3/3 4.77 ± 1.62 2.97-6.11	3/3 5.57 ± 3.18 3.55-9.23	1/1 39.5 -
Carbamazepine	3/3 3.69 ± 1.31[B] 2.79-5.22	2/2 3.44 ± 0.88[B] 2.81-4.06	3/3 14.4 ± 1.85[A,B] 12.3-15.88	3/3 27.5 ± 21.7[A] 7.20-50.5	1/1 220 -
Sucralose	1/3 - ND-145		3/3 2257 ± 548 1717-2813	3/3 2644 ± 1946 699-4590	1/1 10249 -
Diazepam					1/1 177 -
Methylphenidate				1/3 - <MDL-1.05	1/1 196 -
Caffeine	3/3 22.7 ± 10.8[B] 11.7-33.3	2/2 13.5 ± 1.61[B] 12.3-14.6	3/3 55.67 ± 14.5[AB] 40.2-69.1	3/3 79.4 ± 21.5[A] 57.1-100	1/1 255 -

[a] - indicates no mean calculated, contaminant was detected in fewer than half of the samples; MDL = method detection limit. Means with different captial letter superscripts are significantly different ($p<0.05$).

[b]If non-detects were present in less than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean followed by a generalized Wilcoxin non-parametric test.

Supplemental Table S8. Concentrations of pharmaceuticals in fish plasma at each study site.

	Concentrations in Fish Plasma (ng/mL)							
Analyte	**Codeine** Detects/n Mean ± std.dev. Extremes HQ[b]	**Diphenydramine** Detects/n Mean ± std.dev. Extremes HQ[b]	**Atenolol** Detects/n Mean ± std. dev. Extremes HQ[b]	**Diltiazem** Detects/n Mean ± std. dev. Extremes HQ[b]	**Celecoxib** Detects/n Mean ± std.dev. Extremes HQ[b]	**Carbamazepine** Detects/n Mean ± std.dev. Extremes HQ[b]	**Sucralose** Detects/n Mean ± std.dev. Extremes HQ[b]	**Caffeine** Detects/n Mean ± std.dev. Extremes HQ[b]
C_{max} (ng/mL)	30	50	100	30	710	2,000	NA	2,000
Catfish								
Anacostia/middle Potomac Rivers (n=30)		2/30 - <MDL-0.42 0.01		0/30 - -		2/30 - <MDL-2.23 0.001		
Back River (n=2)		2/2 0.89±0.46 0.57-1.22 0.02		1/2 - <MDL-0.35 0.01				
James River (n=27)		1/27 - <MDL-0.51 0.01		8/27 - <MDL-0.77 0.03				2/27 - <MDL-10.9 0.01
Susquehanna River (n=18)				9/18 - <MDL-2.4 0.08				
Gizzard Shad								
Anacostia/middle Potomac Rivers (n=33)		10/33 - <MDL-1.02 0.02	1/33 - <MDL-6.82 0.07	3/33 - <MDL-0.42 0.01		0/33 - -	4/33 - <MDL-2301 -	
Back River (n=9)	4/9 - <MDL-67 2.23	8/9 0.69-0.70[c] <MDL-1.10 0.02		1/9 - <MDL-0.42 0.01		1/9 - <MDL-1.29 0.001		
James River (n=27)		4/27 - <MDL-0.39 0.01		3/27 - <MDL-0.57 0.02				
Susquehanna River (n=18)				0/18 - -				
Carp								
Anacostia/middle Potomac Rivers (n=18)		6/18 - <MDL-0.38 0.01		1/18 - <MDL-0.33 0.01		0/18 - - -		
Back River (n=9)		1/9 - <MDL-0.35 0.01		0/9 - -		1/9 - <MDL-1.58 0.001		
White Perch								
Back River (n=5)		1/5 - <MDL-0.8 0.02		0/5 - -				
Poplar Island (n=10)		3/10 - <MDL-0.31 0.01		1/10 - <MDL-0.41 0.01				
Rockfish								
Poplar Island (n=17)		5/17 - <MDL-0.98 0.02		3/17 - <MDL-1.82 0.06	2/17 - <MDL-26.3 0.04			
Atlantic Menhaden								
Poplar Island (n=10)				1/10 - <MDL-0.41 0.02				

[a] - indicates no mean calculated, contaminant was detected in fewer than half of the samples; MDL = method detection limit. Means with captial letter superscripts indicate a a significant difference ($p < 0.05$).

[b] Hazard Quotient (HQ) is the upper extreme concentration found in fish or osprey plasma divided by the human therapeutic plasma concentration (C_{max}).

[c] If non-detects were present in less than half of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean followed by a generalized Wilcoxin non-parametric test.

CHAPTER 5

OVERALL DISCUSSION

Summary of Findings

These three studies investigated spatial and temporal trends of contaminants in osprey eggs, their transfer in the food web, patterns of emerging pollutants and potential effects on ospreys. Since their population nadir in the 1970's, Chesapeake Bay ospreys have demonstrated resilience in the face of anthropogenic threats. These studies indicate that osprey productivity remains stable across three major Chesapeake Bay tributaries and Regions of Concern (greater than 0.8-1.15 fledglings/active nest). Contaminant residues in osprey eggs seem to be below adverse effect thresholds, although there was modest evidence of genetic damage in the most industrialized and urbanized areas of the Bay.

All organochlorine pesticides, PCBs, and PBDE flame retardants were below thresholds that have been documented to cause an adverse effect in laboratory and controlled exposure studies (Elliott et al., 2001; Reviewed in Beyer and Meador, 2011; Zhang et al., 2014). Eggshell thinning, which has been associated with the historical use of the pesticide DDT (specifically its metabolite *p,p*'-DDE) was not documented in this study. Current eggshell thickness measurements (0.514 ± 0.054 mm) have increased from the DDT use era (from first use in 1942 to its ban in 1972; 0.402-0.416 mm; Wiemeyer et al., 1988). Thickness measurements from 2011-2013 are similar to those reported during the pre-DDT era (0.505, n=365; Anderson and Hickey, 1970). This pattern is consistent with the discontinuation of the use of the organochlorine pesticide DDT in 1972, declining residues of *p,p'*-DDE in eggs, and the recovery of the osprey population in the Bay.

Total PCB concentrations in eggs declined slightly on the Anacostia/middle Potomac Rivers compared to 2000-2001, but remain unchanged in industrialized hotspots in Baltimore Harbor/Patapsco River. Eggs from the Anacostia/middle Potomac Rivers had total PCB concentrations in 2011 that were ~40% lower than in 2000 (9.28 µg/g ww in 2000 vs. 5.56 µg/g ww in 2011). These are similar to findings reported by Velinsky and coworkers (2011), who documented that residues in sediments declined by over an order of magnitude in the past 25 years. As Velinsky and coworkers point out, reductions can be attributed to use bans (manufacture banned in the U.S. in 1979; USEPA, 2013) and better control at point source facilities (e.g., Haywood and Buchanan, 2007). However, concentrations of PCBs in osprey eggs continued to remain elevated in industrialized hotspots in Baltimore Harbor/Patapsco River (especially in Curtis and Bear Creeks). Notably, the Coast Guard Yard in Curtis Creek was listed as a Superfund site in 2002 due to contamination with PCBs, pesticides and dioxins (US EPA, 2013). Although remediation efforts have been completed (as of 2013), PCBs continue to be localized in sediments from the areas around their discharge (Ashley et al., 2009). Physical processes in Baltimore Harbor may help explain why contaminants are retained in this location and much of the sediment material in the Harbor doesn't move (Ashley et al., 2009). Various solutions include capping or dredging the hotspots to remove contaminated sediments. However, there is still evidence in this study to indicate PCBs are biomagnifying (BMF=25.4), and it may take more time for this change to be completely reflected in the food web.

In 2000-2001, PBDE residues were reported in bird eggs on the Chesapeake and other major estuaries around the U.S. At that time, there were limited data to interpret

these values measured in the field. A laboratory study conducted by McKernan and coworkers (2009) and other work conducted by Fernie and coworkers (2003, 2005, 2006, 2008), provided information to place field data into perspective (Zhang et al., 2014). Since the last ecotoxicology studies in ROCs, PBDEs have been phased out (penta- and octa- formulations at end of 200 and deca- formulation at the end of 2009). Production of the fully brominated deca-BDE 209 ceased at the end of 2012 (US EPA, 2015). Paralleling the phase out of BDE use, there has been an overall decline of flame-retardants in ROCs. However, they have only declined slightly near WWTPs and may continue to be associated with their processing capacity, upgrade status and river dilution.

The highest relative concentration of PBDEs across study sites was detected on the middle Potomac (up to 800 ng/g ww) in the vicinity of the largest advanced WWTP in the world (Blue Plains WWTP, processing up to 370 million gallons per day, mgd). Residues on the middle Potomac followed a predictable spatial gradient. Concentrations increased starting on the Anacostia, peaked at Blue Plains and then declined downriver. Concentrations around Blue Plains were similar to maximum total PBDEs in the northern segment of Delaware Bay (up to 820 ng/g ww; Toschik et al., 2005). Similar patterns are reflected across other study sites. Residues on Back River were the highest (666.1 ng/g ww and 616.1 ng/g ww) in two of the three eggs collected in vicinity of the City of Baltimore Back River WWTP (capacity of 180 mgd). Next, followed eggs in Havre de Grace, MD on the Susquehanna River containing maximum total PBDE values of 648 ng/g ww in the vicinity of the Havre de Grace WWTP (2 mgd). The WWTP in Havre de Grace has recently completed its upgrade, so perhaps changes have not been reflected in the food web to date. Unfortunately, there are no avian monitoring data for PBDEs in this

tributary and future monitoring efforts on the Susquehanna would be valuable to track progress. PBDE residues were low in Baltimore Harbor, but there was a slight peak in 2 of the 3 nests sampled above the Key Bridge, potentially related to the Patapsco WWTP (processing 63 mgd). Overall, the James and Elizabeth Rivers had low PBDE residues compared to these other sites. This is surprising due to the abundance of WWTPs on the James River.

Methoxytriclosan was detected in every egg sample on the Anacostia/middle Potomac Rivers. Although values are low (consistent with a low BMF), they peaked around the Frederick Douglass Bridge on the Anacostia River (5.08 ng/g ww) and again downriver from the Blue Plains WWTP (7.40 ng/g ww). Even though methoxytriclosan was detected at low concentrations and rarely detected at other study sites, this "signal" may indicate contributions from additional non-point sources on the Anacostia River (i.e., combined sewer overflows). Even the antihypertensive drug diltiazem discussed in Chapter 4 peaked on the Anacostia/middle Potomac Rivers thus consistent with the "signal" of a sewage dominated system. Diltiazem was found in all 69 osprey plasma samples (540–8630 ng/L), with 41% of these samples exceeding maximum concentrations found in fish. Diltiazem levels in fish and osprey plasma were below the human therapeutic plasma concentration (30,000 ng/L). However, blood diltiazem concentrations between 2,500-8,000 µg/L have been reported in fatal case reports in humans (Roper et al., 1993). These values are much higher than detected in osprey nestling plasma samples, and at this time there is no knowledge on effect thresholds of diltiazem in wildlife.

Studies at a regional scale that can document the occurrence and concentrations of these sewage-affiliated compounds in wildlife on a spatial scale can help reveal patterns and narrow down specific contributions of these emerging contaminants. Effect thresholds for diltiazem, other personal care products, pharmaceuticals, are unknown in ospreys at this time, and there is no evidence to suggest adverse effects.

One study site that warranted special attention was Back River. The first year nests were monitored in 2011, two eggs were collected on Back River, but no nestlings were produced. Four nests were observed being built but never became active. In 2012, there was a similar situation. In 2013 a final effort was made on Back River. Only a small number of nests (n=5) were found and productivity was low (1 fledgling/active nest). This limited us from conducting a robust analysis that would be adequate to compare to other study sites. Notably, Back River sediment concentrations of PBDEs were elevated at Cox Point (Klosterhous, 2007), but we did not detect remarkable residues of contaminants in osprey eggs; however, fish (e.g., gizzard shad and carp) contained some of the greatest residues of organic contaminants and pharmaceuticals compared to other sites. Interestingly, Back River also contained an osprey nestling with high levels of DNA damage relative to other study sites. Compared to other Chesapeake Bay tributaries, Back River was an osprey ghost town. Perhaps additional contaminants that were not examined or physical factors (water clarity, food availability and quality) limited birds from nesting at this site.

In Silico Tools for Assessment

Other tools besides field studies can assist in identifying those chemicals that warrant further investigation. Empirical analyses for pharmaceuticals can be costly and timely depending on the number of analytes, types and quantities of samples. Over 1,453 drugs have been introduced going back to 1840's (Kinch et al., 2014); thus, it is critical to narrow this list down to the key pharmaceuticals that warrant closer examination. An environmentally relevant *in silico* screening-level exposure assessment was used to evaluate the bioaccumulation potential of 113 pharmaceuticals and metabolites (a subset of drugs analyzed at the U.S Geological Survey National Water Quality Laboratory), and an artificial sweetener (sucralose). Hypothetical concentrations in water reflecting "wastewater effluent dominated" or "dilution dominated" scenarios were combined with pH-specific bioconcentration factors (BCFs) to predict uptake in fish. Residues in fish and osprey food intake rate were used to calculate the daily intake (DI) of compounds by an adult female osprey. Fourteen pharmaceuticals and a drug metabolite with a BCF greater than 100 and a DI greater than 20 μg/kg were identified as being most likely to exceed the adult human therapeutic dose (HTD). These 15 compounds were also evaluated in a 40 day cumulative dose exposure scenario using first-order kinetics to account for uptake and elimination. Assuming comparable absorption to humans, the half-lives ($t_{1/2}$) for an adult osprey to reach the HTD within 40 days were calculated. For 3 of these pharmaceuticals, the estimated $t_{1/2}$ in ospreys was less than that for humans, and thus an osprey might theoretically reach or exceed the HTD in 3 to 7 days. Continuing to adapt these models based on new studies (field and lab) can assist in making robust predictions.

Examination of an Effects Hierarchy

In this study, effects of ospreys nesting in ROCs and other Bay tributaries were examined at genetic (oxidative damage to DNA), individual (body weight and culmen length) and population levels (osprey productivity number of fledglings/active nest). Historically, productivity rates have been a critical measure of monitoring effects of contaminants and declines and recovery of populations over temporal and spatial scales. Case studies of large-scale wildlife die-offs at the population level are certainly the most striking to natural resource managers and regulators. However, by the time a population reaches a tipping point (local level vs. widespread issue), it may already be too late and intense recovery efforts must be undertaken, or they may not be possible.

Based on findings in this study the osprey population in Chesapeake Bay has adequate productivity rates to maintain a stable population, but there is subtle evidence of DNA damage in the most industrialized and urbanized areas. Unfortunately, there are no data to indicate the point at which genetic damage measured in this study translates from a short term repairable response (i.e., DNA repair mechanisms kick in, or presence of adaptations to counter this damage) to an irreversible effect. Major questions emerge including: 1) how do you study effects on wildlife when the population is thriving? and 2) what do effects at the macromolecular level mean? Thus, perhaps a re-examination of the hierarchy effects is warranted. The hierarchy of effects (Figure 11) indicates how a perturbation at one level is translated to changes in higher levels.

Often it is challenging from both a research and economic standpoint to justify and show clear and meaningful effects on the left side of the hierarchy. Indicating the presence of increased DNA damage in osprey nestlings in industrialized areas is one step

towards completing the picture. Although subtle, oxidative DNA damage may have an effect in the long term health of an individual or even in subsequent generations. The application of the adverse outcome pathway (Ankely et al., 2010) may help place our findings into perspective. Research gaps remain to find a different type of canary in the coal mine, but this at sublethal levels, which is much different than the previous train of thought.

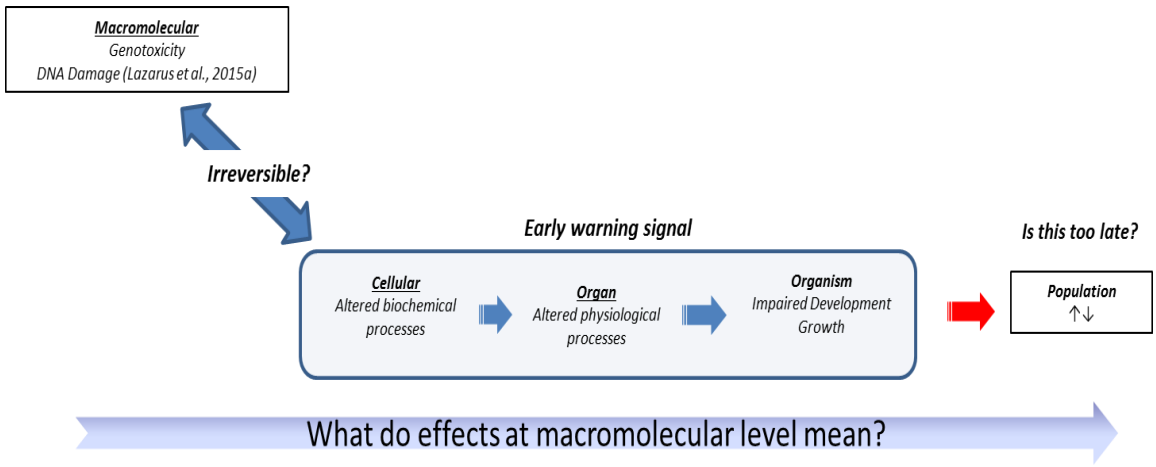


Figure 11. Gradient illustrating the hierarchy of effects and when macromolecular effects may be meaningful.

Utility of these Data

Our data can serve to assist the Chesapeake Bay Program (composed of federal, academic, state and non-profit partners) in the assessing of the progress and effectiveness of existing reduction strategies (i.e., TMDLs) and the development of new ones. These studies address many of the issues discussed in the Bay Program's 2015 Toxic Contaminants Research Outcome Management Strategy (CBP, 2015). Once these data are gathered, the Bay Program agencies can then begin to focus on reduction, remediation and prevention. The Chesapeake Bay Program not only addressed legacy contaminants,

but also new classes of contaminants including PBDEs, alt-BFRs and pharmaceuticals (CBP, 2015).

Final Conclusions

Overall, contaminants in Chesapeake Bay have declined in osprey eggs; however, issues at specific sites remain the same. Although the osprey population is stable, we have detected evidence of modest genetic damage. Unfortunately, at this time there is limited knowledge on the implications of increased oxidative DNA damage in wildlife. Additional lab and field studies may assist us in how to interpret these effects and at what point they may translate to something that is ecologically meaningful. Future directions may include the examination of complete blood counts (i.e., white blood cells and lymphocytes, Vitamin A) from those birds exhibiting the highest levels of DNA damage to indicate potential adverse effects in higher levels of the hierarchy (Figure 11).

In particular, for pharmaceuticals, limited effect threshold data for birds and other vertebrates makes this interpretation challenging. Additional *in situ* studies of pharmaceuticals in the vicinity of sewage outfalls may be warranted to identify other compounds that may be bioaccumulating either in nestling blood or osprey eggs. More robust knowledge of species sensitivities (i.e., birds compared to humans) will assist in interpretation of these data in future ecological risk assessments and development of management strategies.

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