Contaminant Exposure and Reproductive Success of Ospreys (*Pandion haliaetus*) Nesting in Chesapeake Bay Regions of Concern

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Abstract. The Chesapeake Bay osprey population has more than doubled in size since restrictions were placed on the production and use of DDT and other toxic organochlorine contaminants in the 1970's. Ospreys are now nesting in the most highly polluted portions of the Bay. In 2000 and 2001, contaminant exposure and reproduction were monitored in ospreys nesting in regions of concern, including Baltimore Harbor and the Patapsco River, the Anacostia and middle Potomac Rivers, and the Elizabeth River, and a presumed reference site consisting of the South, West and Rhode Rivers. A "sample egg" from each study nest was collected for contaminant analysis, and the fate of eggs remaining in each nest (n=14-16/site) was monitored at 7-10 day intervals from egg incubation through fledging of young. Ospreys fledged young in regions of concern (observed success: 0.88-1.53 fledglings/active nest), although productivity was marginal for sustaining local populations in Baltimore Harbor and the Patapsco River, and the Anacostia and middle Potomac Rivers. Concentrations of *p,p'*-DDE and many other organochlorine pesticides, total PCBs, some arylhydrocarbon receptor-active PCB congeners and brominated diphenyl ether congeners, and perfluorooctane sulfonate were often greater in sample eggs from regions of concern compared to the reference site. Nonetheless, logistic regression analyses did not provide evidence linking marginal productivity to *p,p'*-DDE, total PCBs or arylhydrocarbon receptor-active PCB congener exposure in regions of concern. In view of moderate concentrations of total PCBs in eggs from the reference site, concerns related to new and emerging toxicants, and the absence of ecotoxicological data for terrestrial vertebrates in many Bay tributaries, a more thorough spatial evaluation of contaminant exposure in ospreys throughout the Chesapeake may be warranted.

Introduction

The Chesapeake Bay provides critical habitat for many species of waterbirds, including one of the largest breeding populations of ospreys (*Pandion haliaetus*) in the entire world. Agricultural, industrial, and urban pollution, and loss of submerged aquatic vegetation are recognized as the critical factors causing the deterioration of habitat that support waterbirds in this ecosystem (Erwin *et al.* 1993). Because of its high trophic level, tendency to bioaccumulate contaminants, widespread distribution, nest site fidelity, and synchrony of nesting, the osprey is generally recognized as an excellent sentinel of environmental contamination in estuarine ecosystems (Golden and Rattner 2003).

The decline of the mid-Atlantic osprey population during the post-World War II era is one of several well-documented instances in which DDT exposure was linked to impaired reproductive success (Henny *et al.* 1977; Spitzer *et al.* 1978; Wiemeyer *et al.* 1975, 1988). In the late 1960's, the Chesapeake osprey population appeared to be declining at a rate of 2-6% annually (Henny and Ogden 1970; Reese 1991), and substantial eggshell thinning (up to 35%) was apparent through the 1970's (Wiemeyer *et al.* 1988). In 1973, the Chesapeake Bay osprey population was estimated at 1450 nesting pairs. Only seven of these nesting pairs were observed in the northwestern portion of this estuary (western shore from Chesapeake Bay Bridge to the Susquehanna River, including the Patapsco River and Baltimore Harbor) and only two pair were nesting in the James River and in nearby tributaries (including the Elizabeth River) (Henny *et al.* 1974). No published data on nesting osprey are available for the Anacostia and middle Potomac River from the 1970's.

With restrictions on the use of DDT and other organochlorine pesticides, reproductive performance of Chesapeake Bay ospreys has markedly improved and the size of the population had more than doubled to an estimated 3473 pairs by 1995 and 1996 (Watts *et al.* 2003). Coincident with this recovery, ospreys are now nesting in the most highly polluted tributaries of Chesapeake Bay, identified as regions o concern by the U.S. Environmental Protection Agency (US EPA). These regions of concern (US EPA 1994; MDE 1996) include Baltimore Harbor in Maryland, the Anacostia River in Washington, D.C., and the Elizabeth River in Virginia, all of which exhibit ambient water quality conditions that are toxic to several species of invertebrates and finfish larvae (US EPA 1994; MDE 1996; Hall *et al.* 2002). In Baltimore Harbor and the Patapsco River, sediment concentrations of chlordane, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and some metals exceed the adverse effect thresholds for aquatic organisms (NOAA 1994; McGee *et al.* 1999). Elevated concentrations of total PCBs and arylhydrocarbon (*Ah*) receptor-active PCB congeners, and cytochrome P450 induction have been found in black-crowned night-heron (*Nycticorax nycticorax*) eggs and pipping embryos (Rattner *et al.* 1997, 2001). In the Anacostia River, sediment concentrations of chlordane, PCBs, PAHs, lead and zinc exceed probable effects levels (NOAA 1994), and a high incidence of histopathological lesions and tumors have been reported in finfish (Pinkney *et al.* 2001). In the Elizabeth River, sediment concentrations of PCBs, PAHs, lead and zinc exceed probable effect levels, and numerous reports have documented histopathological lesions, impaired immune function and cytochrome P450 induction in finfish (NOAA 1994; US EPA 1994). Human health advisories are in effect for the consumption of several shellfish and finfish species from all three regions of concern (US EPA 1994).

The last large-scale ecotoxicological investigation of ospreys nesting in Chesapeake Bay was conducted over 15 years ago. In 2000 and 2001, a study was undertaken to determine exposure to traditional organochlorine pollutants (*e.g.*, pesticides, total PCBs, PCB congeners) and mercury, and more contemporary contaminants including brominated diphenyl ethers

(PBDEs), alkylphenols and ethoxylates, and perfluorinated compounds, in osprey eggs collected from nests in or near these regions of concern. The fate of the eggs remaining in sampled nests was also monitored through fledging of young to examine potential adverse effects of environmental contaminants on reproduction.

Material and Methods

Study sites, sample collection and reproduction

Ospreys nesting on navigational markers, duck blinds and other structures were studied over the course of their breeding season (March through August). In 2000, study areas included Baltimore Harbor and Patapsco River in Maryland and the Anacostia and middle Potomac Rivers in Washington, D.C. and Virginia, and a presumed reference site, the South River near Annapolis, Maryland (Figure 1). In 2001, study areas included the Elizabeth River in Virginia, and an expanded reference site consisting of the South, West, and Rhode Rivers near Annapolis, Maryland.

Sample nests were selected based upon accessibility and proximity (>500 m) to other study nests. Once a nest contained three or more eggs, a random sample egg was collected for organic contaminant and mercury analysis, and the remaining eggs were numbered with a non-toxic marker (Forestry Supplies, Jackson, MS). The fate of the eggs in each nest was monitored at 7-10 day intervals through hatching (35-43 days of incubation). Unhatched eggs remaining in the nest 10 days after predicted hatch date were collected for contaminant analysis. Nestlings were monitored at a similar interval. At 40-45 days of age, one nestling was removed for 10 minutes or less for determination of body weight and culmen length, visual inspection of condition, and palpated to determine the size of crop contents. Body feathers (< 0.5 g) and a brachial blood sample (3-5 mL) were collected for metal, metalloid and trace element analysis (data to be presented in a separate manuscript). If a freshly caught fish was present in the nest, a small portion of its musculature was collected for organic analysis. Nests were monitored until young fledged (about 55-60 days of age). In addition to sampled nests, we also monitored reproductive activity at other nests in the study areas.

Egg and fish samples were transported to the Chesapeake Bay Field Office of the U.S. Fish and Wildlife Service in Annapolis, Maryland for processing. Egg samples were individually weighed and measured (length and breadth). Each egg was then opened along the equator, and the embryo, if present, was morphologically examined and developmental stage was noted. The contents of each egg and fish samples were stored in chemically clean jars (ICHEM Research, New Castle, DE) at -10°C.

Contaminant Analysis of Egg Samples

Organochlorine pesticides and metabolites, and total PCBs, aroclors, and PCB congener analyses (Qian *et al.* 1998; Sericano *et al.* 1998) were conducted by the Geochemical and Environmental Research Group at Texas A&M University (College Station, TX) under the rigorous quality assurance and quality control guidelines of the Patuxent Analytical Control Facility. Egg and fish samples were individually homogenized, mixed with anhydrous sodium sulfate and extracted with dichloromethane. An aliquot of the extract was concentrated to dryness and weighed for lipid determination. The remaining lipid extract was then concentrated and dissolved in hexane, fractionated by alumina/silica gel chromatography, and quantified by high resolution gas chromatography with an electron capture detector. The lower limit of detection was about 2 ng/g wet weight for 26 organochlorine pesticides, industrial chemicals and metabolites, and between 20 and 30 ng/g for toxaphene and total PCBs (sum of all congeners).

The lower limit of detection for the 15 *Ah* receptor-active PCB congeners was between 90 and 130 pg/g wet weight. Four eggs collected from the Elizabeth River near its confluence with the James River were also analyzed for kepone at a limit of detection of 50 ng/g. Residues in 10% of the samples were confirmed by gas chromatography/mass spectrometry (GC/MS) in electron impact mode.

All egg samples were quantified for mercury by cold vapor atomic absorption spectroscopy by Trace Element Research Laboratory (College Station, TX). The detection limit was 25 ng/g wet weight. A subset of egg homogenates (consisting of samples with low, intermediate and high concentrations of total PCBs from each study site) was also analyzed for PBDEs, alkylphenols and ethoxylates, and perfluorosulfonates and perfluorocarboxylic acids. Brominated diphenyl ether compounds were quantified as described by Hale and coworkers (2001). Briefly, samples were lyophilized and PCB congener 204 (Ultra Scientific, North Kingstown, RI) was added as a surrogate standard. Dried samples were subjected to accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA). The procedure employed two 5 min extraction cycles with methylene chloride (Burdick and Jackson, Muskegon, MI) using a 60% vessel flush at 100°C and 68 atm followed by chromatographic isolation of halogenated pollutants on an Envirosep size exclusion column (Phenomenex, Torrance, CA). The column was eluted with methylene chloride at 5 mL/min. The resulting fraction of interest was further purified on a 2000 mg silica gel solid-phase extraction column. The column was initially eluted with 3.5 mL hexane, which was discarded, followed by 6.5 mL of 60:40 hexane:methylene chloride, which contained the halogenated compounds of interest. Following solvent exchange to hexane, the halogenated compounds in the purified extracts were separated on a GC (Varian 3400, Palo Alto, CA), equipped with a 60 m DB-5 column (J&W Scientific, Folsom, CA; 0.25 m film, 0.32 ID). Carrier gas was He and injections were made in the splitless mode. Initial column temperature was held at 90°C for 2 min, programmed to 320°C at 4°C/min, then held 320°C for 10 min. Pentachlorobenzene was used as an internal standard. Data were corrected based on recovery of PCB congener 204 in each sample (mean $\pm$ standard deviation, 75.8 $\pm$ 12.8%). Quantitation was accomplished by GC/MS in the full scan electron ionization mode (Varian 4D ion trap; Palo Alto, CA) by summing the areas of the three major ions of each congener (BDE 47, 49, 99, 100, 153 and 154; Cambridge Isotope Laboratories, Andover, MA) versus the area of the surrogate standard PCB congener 204. The limit of quantification was approximately 100 pg/g wet weight.

Alkylphenol and ethoxylate analyses (Schmitz-Afonso *et al.* 2003) entailed drying egg homogenates mixed with sodium sulfate, accelerated solvent extraction of the homogenate (ASE-Dionex Corp., Salt Lake City, UT) using organic-free acetonitrile (Burdick and Jackson UV/Pesticide Grade, Muskegon, MI), roto-evaporation of the extract up to 4 mL, cleanup on a 500 mg C-18 cartridge (J.T. Baker, Phillipsburg, NJ), and elution with acetonitrile. The extract was then concentrated under nitrogen evaporation and the solvent was exchanged to 60:40 methanol:water for high performance liquid chromatography (HPLC) MS/MS analysis. Alkylphenols and ethoxylates were separated by reversed-phase HPLC (Shodex, MSpak GF 310 4D column, Showa Denko K.K., Tokyo, Japan) and quantitated using MS/MS detection (Waters 2690 Alliance HPLC system and Micromass Quattro LC triple stage quadrupole MS with an electrospray interface). All ethoxylates were detected in positive ionization mode, whereas both octylphenol and nonylphenol were detected in negative ionization mode. Analytical standards were obtained commercially (Aldrich Chemical Co., Inc., Milwaukee, WI; Chem Services, Chester, PA; Huntsman Corporation, Salt Lake City, UT; Schenectady International, Schenectady, NY), as gifts from other researchers (Lee Ferguson, State University of New York

at Stony Brook), or were purified by silica gel chromatography (Datta *et al.* 2002; Loyo-Rosales *et al.*, 2003). Quantitation was performed by the internal standard method (6-point calibration curve in solvent) utilizing the ^{13}C ring-labeled nonylphenol and nonylphenol ethoxylates as internal standards. Method detection limits were generally <4 ng/g wet weight, although only values above the method quantification limit (4-12 ng/g wet weight) are reported.
Egg homogenates were analyzed for perfluorosulfonates and perfluorocarboxylic acids (C8-C12) by Exygen Research (State College, PA). Sample aliquots were extracted with methanol, followed by the addition of activated carbon. The samples were filtered, and then analyzed in duplicate by liquid chromatography MS/MS electrospray on a Hewlett Packard 1100 (Avondale, PA) interfaced to a Micromass Quattro Ultima (Beverly, MA). A six-point calibration curve (C8-C12 perfluorocarboxylic acids and C8 perfluorosulfonate) was included at the beginning, throughout, and at the end of the analytical sequence. Spike recoveries of C8-C12 acids ranged from 70 to 100%, although recoveries of C8 sulfonates were more variable because concentrations in several samples were higher than the standards. The limit of detection was approximately 0.5 ng/g wet weight, and the limit of quantification was 10 ng/g wet weight. Because the stage or state of egg samples (*e.g.*, freshly laid, embryonated, addled or failed to hatch) varied, the volume of each egg was estimated and the concentrations of organochlorine contaminants in eggs were adjusted to account for moisture loss during incubation (Stickel *et al.* 1973).

Toxic equivalents of PCB congeners

The potential toxicity of *Ah* receptor-active PCB congeners in each egg sample was estimated by summing the products of their measured concentrations and toxic equivalency factors (Van den Berg *et al.* 1998). The potential toxicity of the congeners present in each sample is expressed as a toxic equivalent (TEQ) in pg/g wet weight.

Eggshell thickness measurements

Eggshells were rinsed with distilled water and air dried for several months at room temperature. Eggshell thickness (including membranes) was measured at three sites on the equator with a rounded contact point micrometer (model 1010M, L.S. Starrett Co., Athol, MA) and values were averaged.

Statistical and Geographic Analyses

For each study site, the area of open water was estimated using the X-Tools extension in ArcView 3.2 (Redlands,CA), and the density of active osprey nests per km^2 was calculated. Reproductive data and observed nest success (hatching and fledging success, and productivity) were compared among study sites by Fisher's Exact Test (two-tailed). In addition, survival rate to hatching and fledging, and nest success, were estimated using the Mayfield method (Mayfield 1961, 1975; Bart and Robson 1982). Morphological measurements, contaminant concentrations and TEQs were tested for homogeneity of variance using the F-max test. Contaminant values and TEQs were $\log_{10}$ transformed to stabilize variances for statistical analysis. For organochlorine pesticides and metabolites, and PCBs, a value one-half the lower limit of detection was assigned to samples with undetectable contaminant concentrations if detectable quantities were present in more than half of the samples from the study site. Because only a subset of samples was analyzed for PBDEs, alkylphenols and ethoxylates, and perfluorinated compounds, statistical analyses were only conducted when quantifiable amounts were detected in all samples. For 2000, differences between study sites and other comparisons were conducted by

one-way analysis of variance and Tukey's HSD method of multiple comparison (Kirk, 1968). For 2001, sites were compared with a t-test, which gives identical results to an ANOVA in the case of two study areas.

The relation of eggshell thickness and the concentration of *p,p'*-DDE, total PCBs and TEQ was examined by analysis of covariance (ANACOVA) and Pearson product-moment correlation. The relation between concentrations of *p,p'*-DDE, total PCBs and TEQ in the sample egg and hatching and fledging success of each nest was evaluated by logistic regression (Hosmer and Lemeshow 1989), and logistic analysis of covariance (LANACOVA) to expand this analysis to test for site differences (Nisbet *et al.* 1998).

Simple linear regression was employed to examine potential geographic gradients of contaminant exposure in regions of concern and the reference site using *p,p'*-DDE and total PCB concentrations in sample eggs. The independent variable was the distance north and south of the nest with highest concentration and the dependent variable was the concentration of *p,p'*-DDE and total PCBs in that sample egg.

Concentrations of organochlorine contaminants were compared in a subset of 10 nests that were sampled in both 2000 and 2001 from the South River using a paired t-test. It is presumed that these sample eggs were laid by the same hen (Poole 1989), thus permitting an analysis of year to year variation in contaminant concentrations.

Results

Reproduction of Ospreys Nesting in the Chesapeake Bay Regions of Concern

In 2000, 25 osprey nests were identified along a 12-km stretch of the South River to the main stem of the Chesapeake. A total of 26 nests was located along a 20-km stretch from Baltimore Harbor through the Patapsco River. Eight of these nests were found north of the Francis Scott Key Bridge (5 on the Patapsco River in the area of the Harbor, and 3 in Curtis Bay and Creek). Just south of the Francis Scott Key Bridge, one nest was observed on Bear Creek and 5 nests were observed on poles at Sparrows Point on the facilities of Bethlehem Steel. A total of 20 nests was found along a 32-km segment of the Anacostia River and middle Potomac River. Despite the presence of 6 platforms and 2 old abandoned nest sites, only one active osprey nest was present on the Anacostia in 2000. Osprey nesting density, based on water surface area, on the South River (1 nest/0.8 km^2) appeared to be greater than observed in Baltimore Harbor and the Patapsco River (1 nest/4.7 km^2) and the Anacostia and the Potomac Rivers (1 nest/2.3 km^2) study sites.

Based upon location and nest accessibility, 14-16 nests from each of these three study areas were selected for sampling and intensive study (Figure 1). Egg loss during the course of incubation was substantial at all three study sites (Table 1). The causes of egg loss were generally unknown, although in a few instances there was evidence of predation. Four nests on floating channel markers in the Potomac River failed apparently due to instability of these structures in strong winds and current associated with storms. Hatchability of eggs remaining in nests through the course of incubation was high (90-100%), and loss of nestlings was generally modest. There were no differences among study sites in the number of eggs laid in active nests ($p > 0.2$), the percent of active nests with hatched eggs ($p > 0.50$) and the percent of successful nests (*i.e* , one or more young fledged) ($p > 0.10$). It is worthy to note that 2 of 4 nests failed in or near the highly industrialized Curtis Bay area of Baltimore Harbor, and 3 of 4 nests failed near the Naval Research Laboratory and Reagan National Airport on the middle Potomac River. Fledglings produced per active nest at the three study sites ranged from 0.88-1.20. Mayfield method estimates of survival rate during egg incubation and nestling periods were also used to

examine overall reproductive success (Table 1), and productivity estimates were found to be quite comparable (0.80 to 1.21 fledglings/active nest).

In 2001, a total of 41 osprey nests or nesting attempts were identified along the South River, West and Rhode Rivers in the vicinity of Annapolis, Maryland, and 22 nests were located along the Elizabeth River (east, west and south branches) and a nearby tributary (Lafayette River) in Norfolk, Virginia. All nests sampled in this area were successful, despite heavy industrial and recreational boat and air traffic, and proximity to the Norfolk Naval Station in Willoughby Bay (1 nest), Craney Island U.S. Army Corps of Engineers disposal area (6 nests), the Norfolk Naval Shipyard (1 nest), and historic creosote and fertilizer manufacturing plants (1 nest). Nesting density of ospreys on the South, West and Rhode Rivers was 1 nest/1.1 km^2 compared to 1 nest/4.7 km^2 on the Elizabeth River and nearby tributaries. Sample eggs were collected from 15 nests at each of these study sites (Figure 1). Compared to the year 2000, egg loss during incubation was modest (Table 1). The number of eggs in active nests, the percent of active nests with hatched eggs, and the percent of successful nests did not differ between study sites ($p > 0.20$). The average number of fledglings per active nest was remarkably similar between sites (1.47 for the South, West and Rhode River versus 1.53 for the Elizabeth River). In 2001, Mayfield method estimates of reproductive success (1.37 and 1.53 fledglings/active nest) closely approximated observed nest success. It is noteworthy that a 45 day old nestling from the Elizabeth River had a fish hook imbedded in its shank with monofilament line tangled around its leg; the hook and line were removed and this bird successfully fledged its nest. Tangled fishing line was removed from one other nestling from the Elizabeth River.

Some investigators routinely adjust osprey productivity for removal of a sample egg because they have noted higher productivity in unsampled nests (e.g., addition of 0.17 young/active nest; Henny and Kaiser, 1996). In the present study, Mayfield method estimates were calculated to account for this potential difference (Table 1, see mean number of young surviving to 53 days), and were in general agreement with this approach. However, for the South, West and Rhode Rivers in 2001, productivity of sampled nests was compared to that of unsampled nests ($n = 16$), and no difference was found (1.37 versus 1.38 fledglings/active nest). Similar trends were found for other regions, however sample sizes were small. These data suggest that a productivity adjustment may not be appropriate for this data set.

Eggshell Thickness and Morphological Observations in Nestlings

For comparative purposes, osprey eggshell thickness prior to the introduction of DDT has been reported to average 0.505 mm (Anderson and Hickey, 1972). In 2000, shell thickness of sample eggs (almost exclusively fresh or early embryonated eggs) appeared to be slightly thinner in the Anacostia and middle Potomac Rivers study site (mean $\pm$ SD; 0.461 $\pm$ 0.034 mm) compared to the South River (0.492 $\pm$ 0.059 mm) and Baltimore Harbor and Patapsco River (0.499 $\pm$ 0.022 mm) sites, although this apparent difference was not statistically significant ($p = 0.09$). Eggshell thickness of two of the three eggs that remained in nests during the entire course of incubation but failed to hatch (Baltimore Harbor: 0.447 mm; middle Potomac River: 0.447) were about 9% below site means, but neither had elevated concentrations of *p,p'*-DDE or other contaminants. During blood and feather collection (days 40-45 of age), all nestlings were found to be in good flesh, and body weight and culmen length were comparable ($p > 0.10$) among sites (South River: 1566 $\pm$ 161 g, 30.05 $\pm$ 1.18 mm; Baltimore Harbor and Patapsco River: 1506 $\pm$ 156 g, 29.39 $\pm$ 1.37 mm; Anacostia and middle Potomac Rivers: 1453 $\pm$ 28.12 g, 28.94 $\pm$ 0.84 mm).

In 2001, shell thickness of sample eggs was also similar ($p > 0.50$) between the South, West and Rhode Rivers site (0.493 ± 0.041 mm) and the Elizabeth River site (0.497 ± 0.024 mm). Shell thickness of two eggs that failed to hatch that remained in nests throughout incubation did not exhibit evidence of shell thinning (South River: 0.467 mm; Elizabeth River: 0.510 mm). These addled eggs were not chemically analyzed. During blood and feather collection (days 40-45 of age), all nestlings were generally found to be in good flesh. A single nestling from the Elizabeth River died while being handled, and necropsy revealed that it apparently was asphyxiated from aspirated ingesta. During sample collection, another nestling from the Elizabeth River was found tangled in monofilament fishing line with a hook in its leg. The hook was removed and this bird eventually fledged. There was a difference ($p < 0.01$) in body weight and culmen length between nestlings from the South, West and Rhode River study site (1639 ± 204 g, 30.41 ± 1.39 mm) and comparably aged young from the Elizabeth River (1436 ± 127 g, 28.54 ± 1.46 mm).

Organochlorine Pesticides and Metabolites, and Mercury in Eggs

In 2000, 17 of 27 organochlorine pesticides and metabolites were detected in more than half of the sample eggs from at least one of the three study sites. Concentrations of *p,p'*-DDE, heptachlor epoxide, a-chlordane, *cis*-nonachlor, *trans*-nonachlor and oxychlordane were greatest ($p < 0.05$) in Anacostia and middle Potomac River eggs compared to the South River, and were often elevated in samples from Baltimore Harbor and the Patapsco River (Table 2). Low levels (≤0.1 ?g/g wet weight) of *p,p'*-DDD, *p,p'*-DDT, *o, p'*-DDD, and *o,p'*-DDT were present in most samples from each site, and concentrations were generally greater in regions of concern. Detectable quantities (<0.02 ?g/g) of dieldrin, endrin, ?-chlordane, pentachloro-anisole, HCB and 1,2,4,5-tetrachlorobenzene were more frequently detected, and values were larger, in eggs from Baltimore Harbor and the Patapsco River, and Anacostia and middle Potomac River compared to the South River study site. Mirex was detected at very low concentrations in all samples. Aldrin, *o,p'*-DDE, heptachlor, a-HCH, ß-HCH, ?-HCH, ?-HCH, endosulfan II, 1,2,3,4-tetrachlorobenzene and toxaphene were rarely detected.

In 2001, only 12 of the 27 organochlorine pesticides and metabolites were detected in more than half of the sample eggs from the South, West and Rhode Rivers study site or the Elizabeth River. Concentrations of *p,p'*-DDE, *p,p'*-DDD, and a-chlordane were greater ($p < 0.05$) in eggs from the Elizabeth River compared to the South, West and Rhode Rivers (Table 2). Low levels (≤0.1 ?g/g wet weight) of *o,p'*-DDT, *o, p'*-DDD, dieldrin, heptachlor epoxide, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane were present in all samples, and concentrations were similar between study sites. The HCB metabolite 1,2,4,5-tetrachlorobenzene was detected (<0.02 ?g/g) in all samples from the South, West and Rhode River study site but not in eggs from the Elizabeth River. Mirex was detected at very low concentrations in all samples. Aldrin, *o,p'*-DDE, *p,p'*-DDT, endrin, heptachlor, ?-chlordane, a-HCH, ß-HCH, ?-HCH, ?-HCH, endosulfan II, pentachloro-anisole, HCB, 1,2,3,4-tetrachlorobenzene and toxaphene were rarely detected. Kepone was not detectable in the four egg samples collected from the Elizabeth River near its confluence with the James River.

Total mercury concentrations in eggs from Chesapeake Bay were relatively low (geometric means <0.07 ?g/g wet weight), and did not differ among study sites in either 2000 or 2001 (Table 2).

Total PCBs, Aroclors, and Ah Receptor-active PCB congeners in eggs

In 2000, total PCB concentrations were greater ($p < 0.05$) in Baltimore Harbor and the Patapsco River, and the Anacostia and middle Potomac Rivers sample eggs compared to those collected from the South River (Table 3). However, total PCB levels for South River eggs were not as low as expected, and the range of values overlapped those in regions of concern. The profiles of PCB congeners in eggs were principally Aroclors 1254 and 1260, and were in a ratio averaging 1:2.70 for the South River, 1:1.87 for the Baltimore Harbor and the Patapsco River, and 1:1.87 for the Anacostia and the middle Potomac Rivers. There was relatively more ($p < 0.05$) Aroclor 1260 than 1254 in the South River eggs compared to the other study areas in 2000. Concentrations of 9 of the 11 less potent *Ah* receptor-active congeners were greater ($p < 0.05$) in both regions of concern compared to the South River. However, concentrations of the most toxic *Ah* receptor-active congeners (numbers 77, 81, 126 and 169) and TEQs did not differ among study sites. Congener 114 was not detected in any egg samples.

Concentrations of total PCBs, *Ah* receptor-active congeners, and TEQs did not differ between the Elizabeth River and the South, West and Rhode Rivers study sites in 2001 (Table 3). However, for the Elizabeth River the concentration of Aroclor 1254 was greater ($p < 0.05$), and the concentration of Aroclor 1260 was less ($p < 0.05$), than that observed in the South, West and Rhode Rivers. The relative quantity of Aroclors 1254 and 1260 in 2001 averaged 1:4.90 for the South, West and Rhode Rivers that was different ($p < 0.05$) than the 1:2.18 ratio for eggs from the Elizabeth River. Aroclors 1242 and 1248 were not detected in eggs from either study site.

Brominated Diphenyl Ethers, Alkylphenols and Ethoxylates, and Perfluorinated Contaminants in Eggs

Brominated diphenyl ether congeners 47, 99, 100, 153 and 154 were detected in all egg samples analyzed in 2000 and 2001, with congeners 47, 99, and 100 being predominant. Compared to egg samples collected from the South River in 2000, concentrations of BDE congeners 47, 99, 100 and 153, and total PBDEs were greater ($p < 0.05$) in eggs from the Anacostia and middle Potomac Rivers, while concentrations were intermediate in eggs from Baltimore Harbor and the Patapsco River (Table 4). In 2001, concentrations of individual BDE congeners and total PBDEs in the Elizabeth River did not differ from the South, West and Rhode Rivers study site.

Nonylphenol was detected in all eggs, but quantifiable in a few of the samples (Table 4). Nonylphenol ethoxylates, octylphenol, and octylphenol ethoxylates were not detected in any samples, with the exception of 14.6 ng/g wet weight of octylphenol ethoxylate (1EO) in an egg collected from the West River in 2001.

Perfluorooctane sulfonate was present in all egg samples, and concentrations were significantly greater ($p < 0.05$) in Baltimore Harbor and the Patapsco River, and the Anacostia and middle Potomac River, compared to the South River in the 2000 collection (Table 4). Perfluorooctane sulfonate values did not differ between the Elizabeth River and the reference site in 2001. Concentrations of perfluorodecane sulfonate appeared to be higher in Baltimore Harbor and the Patapsco River, and the Anacostia and middle Potomac River, in 2000 compared to the reference site. The C9, C10, C11 and C12 perfluorocarboxylic acids were detected in all samples, but quantifiable only in a few eggs (values were <30 ng/g wet weight). The C9, C11 and C12 perfluorosulfonates and the C8 perfluorocarboxylic acid were not quantifiable in any of the egg samples that were analyzed.

Organochlorine Pesticides and Metabolites, and Total PCBs in Fish

In 2000, too few fresh fish samples were found in osprey nests to warrant analysis. However, in 2001, minimally adequate numbers of fish samples (including flounder, *Paralichthys* spp., shad, *Alosa* spp., Norfolk spot, *Leiostomus xanthurus*) were available in nests from the South, West and Rhode Rivers (n=3) and the Elizabeth River (n=3). Concentrations of organochlorine pesticides and metabolites were generally low (geometric means: total *p,p'*-DDT homologs <0.05 ?g/g wet weight; other cyclodiene insecticides and metabolites < 0.01 ?g/g), while levels of total PCBs were moderate (< 0.25 ?g/g). However, there were no significant differences in concentrations between the Elizabeth River and the reference site ($p > 0.05$) for this very small set of samples.

Relation between Contaminants and Reproduction

Concentrations of *p,p'*-DDE, total PCBs and TEQs were positively associated ($r^2 = 0.74$-0.86; $p < 0.001$), but there were site effects ($p < 0.001$) and site by contaminant interactions ($p < 0.05$) which complicate interpretation. ANOCOVA was also performed to compare eggshell thickness versus *p,p'*-DDE, total PCBs and TEQs. For *p,p'*-DDE and total PCBs there were no site effects or site by contaminant interactions; simple linear regression indicated a weak negative relationship between shell thickness and the relatively low levels of *p,p'*-DDE ($r^2 = 0.05$, $p < 0.05$), but not a significant relation between shell thickness and total PCBs. For shell thickness versus TEQs, there were significant site effects ($p < 0.05$) and site by TEQs interactions ($p < 0.05$), but in this model shell thickness was not related to TEQs.

Likelihood ratio tests that were used in the LANOCOVA determined that the best model for examining the relation for *p,p'*-DDE and total PCBs with hatching success was that of unequal slopes. A significant negative relation ($p < 0.001$) was found between *p,p'*-DDE and hatching success, however, there was no significant relation for total PCBs. Likelihood ratio tests determined that the best model for hatching success versus TEQs was the simplest model with no site effects (logistic regression), and a significant negative relation was found ($p < 0.005$).

There was no significant relation between *p,p'*-DDE and fledging success using the equal slopes LANOCOVA model, the most appropriate model for this comparison. Logistic regression was the most appropriate model for total PCBs and TEQs, and both had significant ($p < 0.01$) negative relations with fledging success.

Spatial Relationships within Study Regions

Based on visual inspection of the total PCB concentrations in sample eggs, there appeared to be geographic trends for Baltimore Harbor and Patapsco River (highest concentrations at the mouth of the Patapsco), Anacostia and middle Potomac Rivers (highest concentrations near the Naval Research Laboratory), and the South River (highest concentrations south of the Riva Road Bridge). However, there were no statistically significant ($p > 0.05$) geographic gradients for either total PCBs or *p,p'*-DDE concentrations in eggs from regions of concern and the reference site. In 2001, no apparent spatial trends in concentrations of *p,p'*-DDE or total PCBs were apparent for either the Elizabeth River or the reference site.

South River Nests in 2000 versus 2001

With the exception of three PCB congeners (77, 126, 166) and dieldrin, concentrations of organochlorine contaminants in sample eggs did not differ ($p > 0.15$) between years for 10 nests studied in the South River in both 2000 and 2001. The differences in concentrations of congeners 77, 126 and 166 between years may reflect the lower ratio of Aroclor 1254: Aroclor 1260 in 2000 compared to 2001.

Discussion

Reproduction of Ospreys

Ospreys reproduced successfully in all three Chesapeake Bay regions of concern and at the South, West and Rhode Rivers reference site. Although a substantial number of eggs disappeared from nests in all study areas, hatchability generally exceeded 90% and a large percentage of active nests successfully fledged young. Productivity of active osprey nests in Chesapeake Bay regions of concern in 2000 and 2001 (observed nest productivity: 0.88 to 1.53 fledglings/active nest) was generally greater than observed in 1970 and 1971 (*e.g.*, Potomac River, 0.17 to 0.92 fledglings, Wiemeyer 1971, 1977; Tidewater Virginia, 0.69 to 0.96 fledglings; Kennedy 1977), just prior to the banning of DDT and some other organochlorine compounds. Based upon observed nest success calculations and Mayfield method estimates (Table 1), the number of fledglings produced per active nest met or exceeded 0.8, which is the breeding rate needed to maintain population stability in New England (Spitzer 1980). However, Poole (1989) suggests a productivity rate of 1.15 young fledged per active nest may be required to sustain the population in an area like Chesapeake Bay, where the initial breeding age of ospreys may occur slightly later in life than in New England due to competition for nest sites. This suggests that the stability of local osprey populations in Baltimore Harbor and Patapsco River, and for the Anacostia and middle Potomac Rivers, is marginal. The only other recent avian ecotoxicological investigations with reproductive endpoints in Chesapeake Bay regions of concern revealed that productivity at the black-crowned night-heron colony in Baltimore Harbor appeared to be adequate to maintain a stable population (Rattner *et al.* 2001).

Impaired reproduction of Chesapeake Bay ospreys in the 1960's and 1970's was undoubtedly related to eggshell thinning (Wiemeyer *et al.* 1975, 1988). In the present study, mean eggshell thickness of osprey eggs from Baltimore Harbor and the Patapsco River, the Elizabeth River, and the South, West and Rhode Rivers reference site ranged from 0.492 to 0.499 mm, which closely approximates the pre-1947 mean of 0.505 mm (Anderson and Hickey 1972). Although not statistically different from other sites, mean shell thickness of eggs from the Anacostia and middle Potomac River study site in 2000 (*i.e.*, 0.461 mm) averaged 8.7% less than the pre-1947 value, and fell in the range of mean values (0.402-0.468 mm) for osprey eggs from the lower Potomac River between 1968 and 1977 (Wiemeyer *et al.* 1975, 1988). Notably, *p,p'*-DDE concentrations in these eggs from the Anacostia and middle Potomac Rivers were the greatest of all sites studied in 2000 and 2001; 9 of 16 values fell within the 95% confidence interval associated with 10% percent eggshell thinning in osprey eggs (Wiemeyer *et al.* 1988).

Osprey nesting density was markedly greater in the reference area compared to the regions of concern. This difference is probably a function of many factors, including water depth and clarity, nest site availability, and anthropogenic activity and disturbance. Despite an abundance of potential nest sites on pilings and channel markers, parts of Baltimore Harbor and the Elizabeth River are deep channel ship ports with poor water clarity and compromised aquatic invertebrate and fish populations. The middle Potomac Rivers probably provide the most suitable nesting habitat for ospreys. The density of ospreys nesting on the middle Potomac might be further enhanced by the installation of additional fixed nest platforms, as four nests that were precariously built on floating channel markers (possibly by reproductively inexperienced ospreys) failed. In contrast, there was only one osprey that nested on the Anacostia River in 2000, despite the presence of seven nest platforms. Factors other than the availability of nesting structures (*e.g.*, poor water clarity and quality, human disturbance) probably account for its limited use. Since 2000, the number of active osprey nests on the Anacostia River has increased slightly (two pair in 2001 and 2002, and 3 pair in 2003).

Organochlorine Contaminants and Mercury in Osprey Eggs

Concentrations of *p,p'*-DDE and other DDT metabolites in osprey eggs from the Anacostia and middle Potomac Rivers were markedly greater than found in other regions of concern and the reference site. Current values were less than one-half of the levels observed during the 1960's and 1970's from the Potomac River, while values at the highly industrialized shipping ports of Baltimore Harbor and the Elizabeth River, and the South, West and Rhode Rivers reference site, were nearly an order of magnitude lower than historic peak values for the Chesapeake Bay (Wiemeyer *et al.* 1975, 1988). Geometric means for these compounds in Chesapeake Bay study sites in 2000 and 2001 were not unlike values reported for osprey eggs collected from Delaware Bay in 1998 (Clark *et al.* 2001). However, *p,p'*-DDE values in the present study were consistently lower than observed in osprey eggs collected during the 1990's from the Great Lakes (Martin *et al.* 2003) and the Pacific Northwest (Elliott *et al.* 2000), for which many samples exceeded the 4.2 ?g/g wet weight threshold associated with 15% shell thinning.

Relative concentrations or the frequency of detection of endrin, heptachlor epoxide and several components and metabolites of chlordane were consistently greater in eggs collected from the Anacostia and middle Potomac Rivers, and Baltimore Harbor and the Patapsco River compared to the reference site, although this tendency was not borne out for egg samples collected from the Elizabeth River. Concentrations of dieldrin, heptachlor epoxide, and *cis*-nonachlor at all sites were lower than historic values for osprey eggs from the Potomac River (Wiemeyer *et al.* 1975, 1988). However, such temporal comparisons for other organochlorine pesticides and metabolites (*e.g.*, *trans*-nonachlor, oxychlordane, mirex, HCB) are difficult because many samples in these earlier studies had undetectable residues due to poorer analytical sensitivity limits than currently achieved (*i.e.*, 0.05 versus 0.01 ?g/g wet weight). Levels of dieldrin, heptachlor epoxide, and components of chlordane in Chesapeake Bay samples collected in 2000 and 2001 were similar to those found in osprey eggs from Delaware Bay (Clark *et al.* 2001) and the Pacific Northwest (Elliott *et al.* 2000) collected during the 1990's, and generally lower than reported in the Great Lakes (Martin *et al.* 2003). Concentrations of these and other organochlorine pesticides and metabolites were well below levels known to adversely affect reproduction in those species of wild birds that have been studied in detail.

Concentrations of total PCBs in osprey eggs from Baltimore Harbor and the Patapsco River, and the Anacostia and middle Potomac Rivers, were greater than observed for the South River, but unlike organochlorine pesticides and metabolites, values were only slightly diminished compared to total PCB concentrations in Potomac River osprey eggs from the 1970's (Wiemeyer *et al.* 1988). Total PCB levels in eggs from the Elizabeth River, a highly industrialized area that includes one of the largest military naval ports in the world, did not differ from that observed at the South, West and Rhode Rivers. However, total PCB concentrations for the South, West and Rhode Rivers (Table 3, 4.91 ?g/g in 2000 and 4.30 ?g/g in 2001) were by no means low when compared to historic data from remote areas of Chesapeake Bay (Wiemeyer *et al.* 1975, 1988), and to contemporary data from several locations in North America (*e.g.*, Delaware Bay 2.53 ?g/g wet weight, Clark *et al.* 2001; Ogoki Reservoir in northern Ontario 1.65 ?g/g, Martin *et al.* 2003; many study sites in the Fraser and Columbia River drainage systems averaged <2.5 ?g/g, Elliott *et al.* 2000; Crane Prairie Reservoir in Oregon <0.2 ?g/g, Henny *et al.* 2003). This leads one to suspect that ospreys nesting in the so-called reference area also transferred significant PCB burdens into their eggs. Low to moderate levels of PCBs were detected in the 3 relatively fresh

fish muscle samples from the Elizabeth River and South, West and Rhode Rivers in 2001 (geometric means of 0.24 and 0.12 ?g/g wet weight, respectively), and values were quite similar to those reported for white perch (*Morone americana*) and spot (*Leiostomus* spp.) collected from the South River in 2000 by the Maryland Department of the Environment (Joseph Beaman, personal communication). Recently, estimates of fish to osprey egg biomagnification factors (BMF) have been derived for many chlorinated hydrocarbon contaminants using data from the Willamette River in Oregon (Henny *et al.* 2003). The BMF for total PCBs was estimated to be 11 (Henny *et al.* 2003), and when applied to the fish samples collected from the Elizabeth River and South, West and Rhode Rivers in 2001, underestimated the concentration of total PCBs detected in osprey eggs by 24-70%. This could be due to a number of factors, including differences in forage fish, small sample size (only 3 fish samples/site), sample processing (composite samples versus muscle), and possibly differences between Willamette and Chesapeake ospreys in contaminant burdens acquired during their migration and on their wintering grounds.

Although there were no significant differences in the concentrations of the most potent *Ah*-receptor active PCB congeners (*i.e.*, congeners 77, 81, 126 and 169) and TEQs in osprey eggs from Baltimore Harbor and the Patapsco River, Anacostia and middle Potomac Rivers, and the South River in 2000, values are several times greater than observed in osprey eggs from Delaware Bay (Clark *et al.* 2001) and the Pacific Northwest (Elliott *et al.* 2000; Henny *et al.* 2003) and not unlike levels reported in the Great Lakes Basin (Martin *et al.* 2003). It is noteworthy that the concentration of these congeners and TEQs in osprey eggs from Baltimore Harbor and the Patapsco River are 1.8 to 10.7 times greater than recently observed in eggs of black-crowned night-herons nesting in Baltimore Harbor (Rattner *et al.* 2001), presumably reflecting the higher trophic feeding level of the osprey. Compared to osprey eggs collected in 2000, distinctly lower concentrations of *Ah* receptor-active congeners and TEQs were found in samples from the Elizabeth River and South, West and Rhode Rivers in 2001. Although total PCB concentrations were comparable at the reference site in both 2000 and 2001, the exposure pattern in 2001 was predominated by Aroclor 1260 which has lower quantities of congeners 77 and 126 than Aroclor 1254 (Table 3) (Cogliano 1998).

In the present study, concentrations of mercury in osprey eggs were similar among study sites in Chesapeake Bay, and are remarkably similar to historic values reported for eggs collected in 1973 from the Potomac River and Smith Island (Wiemeyer *et al.* 1988). Values in the Chesapeake Bay osprey eggs were moderately lower than noted in Great Lakes (Hughes *et al.* 1997) and Delaware Bay (Clark *et al.* 2001), and well below the thresholds associated with decreased hatchability in controlled exposure studies (Wiener *et al.* 2003).

Other Contaminants in Osprey Eggs

Three groups of emerging contaminants, PBDEs, alkylphenols and their ethoxylates, and perfluorinated compounds, were measured to determine if detectable quantifies were transferred into eggs of ospreys nesting in Chesapeake Bay. From an environmental monitoring perspective, such concentration data can assist in exposure evaluations should the production, use or release of these compounds change, and may also be of value in risk assessments in the event that adverse effects are detected. Unfortunately, toxicity thresholds for these compounds in fish-eating birds are not available.

Eight BDE congeners were quantified in osprey eggs from study sites in Chesapeake Bay, with mean total PBDE concentrations ranging from 176-725 ng/g wet weight. Unlike polybrominated biphenyls, whose commercial production as a fire retardant has been curtailed, PBDEs lack extensive regulation worldwide, and their global use in electronic devices, furniture and textiles has grown to more than 67,000 metric tons (Hale *et al.* 2001). According to 1999 data, 98% of the global demand for the commercial penta-BDE flame retardant mixture, which contains the congeners most commonly reported in the environment (BDE 47, 99, 100, 153 and 154), resdies in North America. In several countries, accumulation of these compounds in aquatic and terrestrial wildlife has increased steadily since the 1970's. Mean total PBDEs in herring gull eggs from the Great Lakes increased from 9-23 ng/g wet weight in 1981 to 509-1331 ng/g in 2000, with doubling times of 3-5 years (Norstrom *et al.* 2002). Concentrations of total PBDEs in eggs from Norwegian birds of prey ranged from 7-732 ng/g wet weight (Herzke *et al.* 2001). In these studies and the present investigation, BDE congener 47 was the dominant congener in fish-eating birds, which is consistent with findings in other aquatic organisms, including fish sampled from freshwater sources in Virginia (Jansson *et al.* 1993; Hale *et al.* 2001). In Chesapeake Bay samples, the relative contribution of congener 47 was remarkably constant across study sites, accounting for 62-66% of total PBDEs. Though toxicity thresholds in eggs of wild birds are unknown, controlled exposure studies with laboratory species have documented alterations in hepatic and endocrine function, neurodevelopment and reproduction (Hale and La Guardia 2002). This potential toxicity and increased detection of PBDEs may warrant further study, particularly on the Anacostia and middle Potomac Rivers, where concentrations were more than four times those of the reference site.

Alkylphenol and their ethoxylates are high production nonionic surfactants used in a wide variety of industrial and manufacturing processes, and enter the aquatic environment by wastewater discharge (Talmage 1994). These compounds and their metabolites have low to moderate bioaccumulation potential, exhibit slight to high toxicity in aquatic invertebrates and fish, and may evoke weak estrogenic activity (Staples *et al.* 1998). Monitoring studies in the United States have generally detected low levels in fish (briefly reviewed in Schmitz-Afonso *et al.* 2003), and only one report describes concentrations of nonylphenol, nonylphenol monoethoxylate and nonylphenyl diethoxylate (1.2, 2.1 and 0.35 ?g/g dry weight) in muscle tissue of mallards (*Anas boscas*) from the Chriesbach River in Switzerland (Ahel *et al.* 1993). Nonylphenol, octylphenol and their ethoxylates were present in Chesapeake Bay osprey eggs, albeit at considerably lower concentrations (<20 ng/g wet weight). The limited controlled exposure data available in birds indicates that these compounds have low acute toxicity (LD50 >5000 ppm for nonylphenol ethoxylate (9EO) in a 5 day dietary study using bobwhite quail, *Colinus virginianus*; E. Mihaich, personal communication).

Perfluorinated surfactants are found worldwide, as a result of their extensive commercial and industrial use as surfactants, surface treatments, and insecticide precursors, and because of their stability in environmental and biological matrices. The 3M Company voluntarily

discontinued manufacture of perflurooctanoic acid in 2000, although production by other companies continues in the United States and elsewhere. These compounds have been detected in several species of birds around the world, principally in blood plasma and liver tissue, with highest concentrations found near urban centers (Kannan *et al.* 2002a,b). Concentrations in blood plasma ranged from below the limit of quantification up to 2220 ng/mL in a bald eagle (*Haliaeetus leucocephalus*) (Kannan *et al.* 2001). Concentrations in liver tissue in birds ranged from below the limit of quantification up to 1780 ng/g wet weight in a Brandt's cormorant (*Phalacrocorax penicillatus*), with concentrations as high as 959 ng/g in osprey (Kannan *et al.* 2001). In the present study, perfluorooctanesulfonate concentrations tended to be greatest in eggs collected from Chesapeake Bay tributaries near the urban centers of Washington, D.C. and Baltimore, with values being slightly higher than in the only other published avian egg analyses (double-crested cormorant, *Phalacrocorax auritus*; 157 ng/g wet weight, n=4 egg yolks; Kannan *et al.* 2001). Toxicity of these compounds has yet to be examined in birds, however studies with laboratory rodents indicate that perfluorooctanesulfonate has moderate toxicity following acute oral exposure, and reduced body weight and delayed sexual maturation has been noted following prolonged dietary exposure, which is some cause for concern.

Spatial Variability in Osprey Egg Contaminant Concentrations within Chesapeake Bay Regions of Concern

Numerous studies have examined water quality and sediment toxicity in Chesapeake Bay regions of concern (*e.g.*, US EPA 1994; MDE 1996; McGee *et al.* 1999; Hall *et al.* 2002). Despite considerable spatial variability and toxic hotspots within Baltimore Harbor, and the Anacostia and Elizabeth Rivers, contaminant and toxicity gradients are apparent at some scales. Although levels of *p,p'*-DDE and total PCBs in osprey eggs differed among some study sites in 2000, no concentration gradients or spatially distinct trends in concentrations were apparent within a given study area (ranging in length from 12-32 km). This absence of an exposure gradient may be attributable to the relatively large foraging range of osprey (up to and occasionally >10 km; Poole 1989; Lohmus, 2001) and the highly mobile nature of their prey, among other factors. Nonetheless, on larger spatial scales, exposure gradients for *p,p'*-DDE and PCBs in osprey eggs and nestling blood plasma have been well documented (*e.g.*, Elliott *et al.* 2000; Martin *et al.* 2003; Henny *et al.* 2003).

Are Contaminants Affecting Osprey Reproduction in Chesapeake Bay Regions of Concern?

Unfortunately, our findings do not clearly and completely resolve this question. In 2000, productivity of nesting ospreys in Baltimore Harbor and the Patapsco River, and those on the Anacostia and middle Potomac Rivers, was marginally adequate to sustain the local population in these tributaries. LANOCOVA and logistic regressions found some negative relations between *p,p'*-DDE, total PCBs, and TEQs with hatching and fledging success. The observed reproductive effects may be due to confounding factors other than individual contaminants in these highly industrialized regions of concern (*e.g.*, water clarity, disturbance, combinations of contaminants). With the possible exception of modestly elevated *p,p'*-DDE and some evidence of eggshell thinning on the Anacostia and middle Potomac Rivers, concentrations of *p,p'*-DDE, total PCBs, TEQs, and other contaminants in sample eggs did not cause direct and biologically significant toxic effects on osprey reproduction in Chesapeake Bay regions of concern.

Further monitoring of contaminant exposure and potential reproductive responses of Chesapeake Bay ospreys and other fish-eating birds may be justified for a number of reasons. Although the present study documented that concentrations of many lipophilic organochlorine

pesticides and metabolites have decreased substantially in the Chesapeake Bay since the 1970's, concentrations of total PCBs appear to have diminished only slightly. Furthermore, total PCB concentrations in osprey eggs from the South, West and Rhode Rivers in 2000 and 2001, a presumed reference site, were by no means low, and our knowledge of these and other contaminants in fish-eating birds in much of Chesapeake Bay is quite limited (Cohen *et al.* 2003). In view of the present findings, the existence of significant ecotoxicological data gaps for terrestrial vertebrates in the Chesapeake Bay, and concerns related to emerging contaminants whose concentrations in the environment appear to be steadily increasing (*e.g.*, PBDEs), a more thorough spatial evaluation of contaminants in ospreys throughout Chesapeake Bay may be justified.

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Fig. 1 Chesapeake Bay regions of concern (Anacostia River, Baltimore Harbor, and the Elizabeth River) and reference site (South, West and Rhode Rivers), including osprey nests studied in 2000 and 2001.

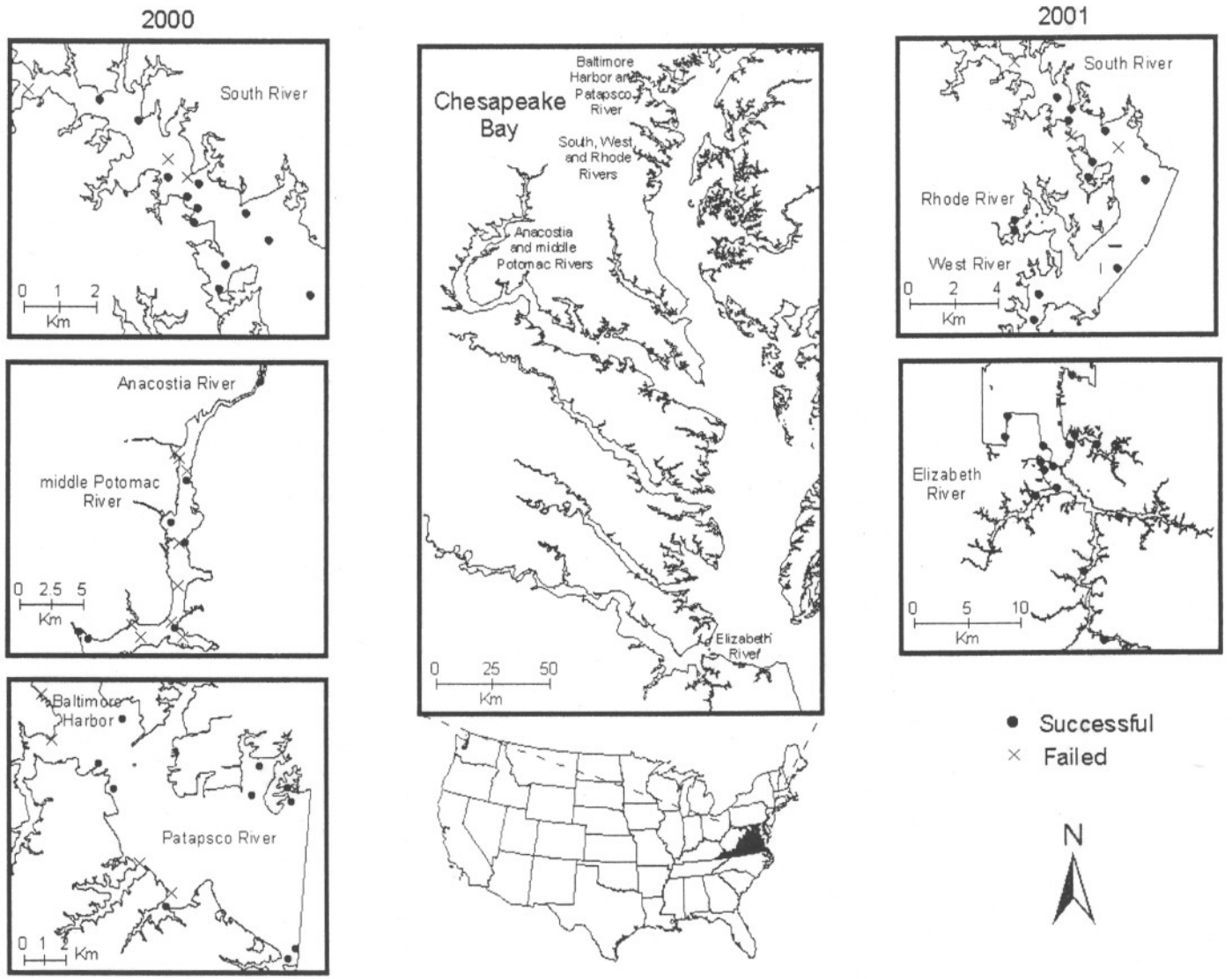
2000
South River
0 1 2
Km
Anacostia River
middle Potomac
River
0 2.5 5
Km
Baltimore
Harbor
Patapsco River
0 1
Km
Chesapeake
Bay
Baltimore
Harbor and
Patapsco
River
South, West,
and Rhode
Rivers
Anacostia
and middle
Potomac Rivers
Elizabeth
River
0 25 50
Km
2001
South River
Rhode River
West River
0 2 4
Km
Elizabeth
River
0 5 10
Km
Successful
Failed
N

Table 1. Reproductive data for osprey nests in Chesapeake Bay regions of concern

Year	2000						2001			
Location	South River		Baltimore Harbor and Patapsco River		Anacostia and middle Potomac Rivers		South, West and Rhode Rivers		Elizabeth River	
	Number	%	Number	%	Number	%	Number	%	Number	%
Active nests	15		14		16		15		15	
Eggs laid	49		42		57		48		45	
Sample egg collected	15		14		16		15		15	
Eggs naturally incubated	34		28		41		33		30	
Fate of eggs										
Unknown or predated	12		12		14		5		4	
Storm or wind related	0		0		7		0		0	
Found out of nest	0		0		0		1		0	
Failed to hatch	0		1		2		1		1	
Hatched	22	64.7	15	53.6	18	43.9 (51.5)b	26	75.8	25	83.3
Hatchability[a]	22/22	100.0	15/16	93.8	18/20	90.0 (89.5)	26/28	92.9	25/26	96.2
Active nests with hatched eggs	12	80.0	10	71.4	10	62.5	13	86.7	15	100
Fate of nestlings										
Disappeared	4		0		3		3		1	
Found dead	0		0		0		1		1[c]	
Storm or wind related	0		0		1		0		0	
Fledged	18	81.8	15	100	14	77.8	22	84.6	23	92.0
Successful pairs (fledged young)	12	80.0	10	71.4	7	43.8 (58.3)	12	80.0	15	100.0
Fledglings per active nest	1.20		1.07		0.88 (1.17)		1.47		1.53	
Fledglings per successful nest	1.50		1.50		1.86 (2.0)		1.83		1.53	
Mayfield Method Estimates										
Survival rate to hatching (A)	0.803		0.728		0.625 (0.746)		0.832		1.000	
Survival rate to fledging (B)	1.000		1.000		0.686 (0.775)		0.919		1.000	
Nest success (A x B)	0.803		0.728		0.428 (0.578)		0.764		1.000	
Probability of egg hatching in successful nests for a 39 day incubation period (C)	0.815		0.682		0.737 (0.737)		0.963		0.833	
Probability of young surviving to 53 days (D)	0.818		1.000		1.000 (1.000)		0.846		0.920	
Egg success (A x B x C x D)	0.536		0.497		0.315 (0.426)		0.622		0.767	
Mean clutch size (E)	3.267		3.000		3.563 (3.750)		3.200		3.000	
Mean number of young surviving to 53 days (A x B x C x D x E)	1.75		1.49		1.124 (1.596)		1.99		2.300	
Mean number of young surviving to 53 days minus sample egg (A x B x C X D)(E-1)	1.21		0.99		0.80 (1.17)		1.370		1.530	

[a]Hatchability = eggs hatched/(eggs laid - eggs that disappeared or were sampled before hatching)