

PRODUCTIVITY, EMBRYO AND EGGSHELL CHARACTERISTICS, AND CONTAMINANTS IN
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Abstract—Chlorinated hydrocarbon concentrations in eggs of fish-eating birds from contaminated environments such as the Great Lakes of North America tend to be highly intercorrelated, making it difficult to elucidate mechanisms causing reproductive impairment, and to ascribe cause to specific chemicals. An information-theoretic approach was used on data from 197 salvaged bald eagle (*Haliaeetus leucocephalus*) eggs (159 clutches) that failed to hatch in Michigan and Ohio, USA (1986–2000). Contaminant levels declined over time while eggshell thickness increased, and by 2000 was at pre-1946 levels. The number of occupied territories and productivity increased during 1981 to 2004. For both the entire dataset and a subset of nests along the Great Lakes shoreline, polychlorinated biphenyls (Σ PCBs, fresh wet wt) were generally included in the most parsimonious models (lowest-Akaike's information criterion [AICs]) describing productivity, with significant declines in productivity observed above 26 μ g/g Σ PCBs (fresh wet wt). Of 73 eggs with a visible embryo, eight (11%) were abnormal, including three with skewed bills, but they were not associated with known teratogens, including Σ PCBs. Eggs with visible embryos had greater concentrations of all measured contaminants than eggs without visible embryos; the most parsimonious models describing the presence of visible embryos incorporated dieldrin equivalents and dichlorodiphenyldichloroethylene (DDE). There were significant negative correlations between eggshell thickness and all contaminants, with Σ PCBs included in the most parsimonious models. There were, however, no relationships between productivity and eggshell thickness or Ratcliffe's index. The Σ PCBs and DDE were negatively associated with nest success of bald eagles in the Great Lakes watersheds, but the mechanism does not appear to be via shell quality effects, at least at current contaminant levels, while it is not clear what other mechanisms were involved. Environ. Toxicol. Chem. 2010;29:1581–1592. © 2010 SETAC

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INTRODUCTION

The North American Great Lakes ecosystem has received chemical pollution from decades of surrounding industrial and agricultural activities. During the late 1960s and into the 1970s, instances of reproductive failure, developmental anomalies, and population declines were reported for species of raptorial and fish-eating colonial water birds. Chemical analysis of eggs revealed relatively high concentrations of persistent contaminants, such as organochlorine (OC) insecticides and polychlorinated biphenyls (PCBs) [1,2]. With legislated restrictions on usage and other source controls for most of the chlorinated environmental contaminants, exposures decreased dramatically and health parameters for many aquatic birds improved, leading to partial recovery of populations [3]. However, at some locations there were persistent reports of poor nesting success associated with biochemical and developmental abnormalities [4–7]. The observed effects were referred to as GLEMEDS (Great Lakes Embryo Mortality Edema and Deformities Syndrome) because of the similarity to chick edema disease

of domestic poultry exposed to dioxins [8]. The cause was considered to be contamination by polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and in particular the dioxin-like non-*ortho* PCB congeners, all of which exert their effect primarily via the aryl hydrocarbon receptor (AhR) to cause dioxin-like toxicity.

The bald eagle (*Haliaeetus leucocephalus*) was among the aquatic bird species virtually extirpated from the Great Lakes during the 1950s through the early 1970s [9–12]. Like the other aquatic-based avian predators, by the late 1980s populations were increasing [13,14], but continued to have relatively high burdens of organochlorine pesticides and PCBs, particularly at nests located along the Great Lakes shoreline [15]. As with colonial waterbirds, evidence of GLEMEDS in eagles including reports of developmental abnormalities was attributed mainly to dioxin-like PCBs [16,17].

Many factors influence reproductive success of wild birds, including food supply, weather, age, predation, persecution, and habitat destruction [18]. Further complicating investigations of contaminant impacts, including those of sea eagles (genus *Haliaeetus*), the various chlorinated hydrocarbon compounds are intercorrelated across moderate spatial scales, and relationships among biological and chemical parameters often remain equivocal, even with careful statistical analysis and modeling

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(e.g., [19]). Recently the information-theoretic approach has emerged as an alternate statistical tool designed to extract biologically relevant variables from a large set of possible candidate variables in noisy datasets [20]. Therefore, to further examine relationships between chlorinated hydrocarbon contaminants and biological parameters, we analyzed data for bald eagle nests in Michigan and Ohio, USA, over a 15-year period (1986–2000), where unhatched eggs had been salvaged for contaminant analysis and measurement of shell parameters, and reproductive success consistently measured. The information-theoretic approach was used to address the following questions: Which contaminants best explain variation in productivity, eggshell quality, and in ovo abnormalities among bald eagles nesting in the Great Lakes? Is any single contaminant responsible for reduced reproductive success, or do several operate together or through independent mechanisms toward this common adverse outcome? Is reproductive success reduced through mortality of embryos or other egg-related problems? What mathematical models best describe relationships between contaminants and observed effects?

MATERIALS AND METHODS

Field data

Bald eagle productivity surveys followed the guidelines contained in the Supplemental Data, Appendix D, of the Northern States Bald Eagle Recovery Plan. During March and April 1963 to 2000 (Michigan, Upper Peninsula, USA), 1964 to 2000 (Michigan, Lower Peninsula) and 1974 to 2000 (Ohio), observers conducted aerial surveys of all known and suspected breeding territories to determine the occupancy status by the breeding pairs. Ground observations were also conducted from 1961 to 1963 in Michigan. An occupied territory was defined as having an adult in incubation posture on the nest, a nest with visible repairs/enlargement/relining from the previous breeding season, or a nest with one or both adults attending in close proximity. The goal of the first survey was to determine if incubation had occurred and which nest, of several existing alternate or new nests, was in use. A second aerial survey in mid May through early June determined the success or failure of all occupied breeding territories. Observers counted the number of pre-fledge eaglets in nests and noted the presence of dead young or unhatched eggs. Based on the above site visits, productivity was calculated as the number of young (at or close to fledging age) per occupied breeding territory.

Intact, unhatched bald eagle eggs were collected either by climbing nests identified as abandoned and containing an egg by aerial surveyors, or by recovering eggs at the time of banding of nearly fledged young (Fig. 1). Unhatched eggs were often buried in the nest lining as a result of the normal activities of nestlings and adults, and banders encountered these eggs by digging through the nest lining when handling nestlings. At the time of collection the egg was individually wrapped in aluminum foil, sealed in a labeled whirl-pak bag, and placed in a sturdy container with shredded newsprint or other shock-absorbent materials. Eggs were transported back to the lab on ice and refrigerated.

Egg samples

In the laboratory the exterior of the egg was cleansed of attached nest debris and excrement by rinsing under a stream of cold tap water and gentle scrubbing with a plastic dish pad. When dry, the egg was weighed and the length and width (maximum and three random widths) measured using a digital

caliper precise to ± 0.01 mm. The egg was opened by scoring a line around the equator (midline) of the egg with a hacksaw blade and continuing the cut until only the shell membranes remained intact. These membranes were cut with a scalpel and the contents transferred to a chemically cleaned jar.

Eggs from nests in Michigan (1986–2000) and Ohio (1986–1997) were collected ($n = 197$). Eggshell thicknesses were measured using a dial micrometer precise to ± 0.01 mm. The mean of eight measurements (one per quarter arc per shell half) was used for analysis. The absence or detachment of one or both membranes was corrected by adding 0.03 mm if the inner membrane was absent and 0.13 mm if both were absent (S.N. Wiemeyer, personal communication). Dry shell weight was determined after > 10 d of air-drying. Ratcliffe Index (RI) was calculated as: $RI = \frac{S_{dry\ wt}}{L \cdot W}$, where W is maximum egg width, L is egg length, and $S_{dry\ wt}$ is shell dry weight [21]. Prior to analyses, contaminant concentrations were corrected for desiccation (to fresh wet wt) by multiplying by $D_i = M \cdot V^{-1}$ [19], where M is the mass (in g) of the egg contents and V is the maximum whole egg volume. V was selected as the maximum of either the formula $3.73 \cdot W \cdot L - 35.3$ [22], where W is maximum egg width and L is egg length, or the volume as measured by the displacement of water by the egg (where possible). The desiccation-corrected fresh wet-weight values were then ln-transformed to obtain normality for statistical purposes.

Egg contents were examined and described by R. Balander (Animal Science Department, Michigan State University) for the period 1986 to 1993 and by the lead author, 1994 to 2000. All embryos were examined for evidence of visible abnormalities. Eggs without a clearly discernible embryo were classified as infertile or early death. An incubation period of 35 d was assumed and embryo age extrapolated from a 21-d incubation period for chickens.

Chemical analyses

All samples were submitted for analysis of OC pesticides, p,p' -DDE, o,p' -DDE, p,p' -dichlorodiphenylchloroethane (DDD), o,p' -DDD, p,p' -dichlorodiphenyltrichloroethane (DDT), o,p' -DDT, *cis*-nonachlor, *trans*-nonachlor, alpha-chlordane, beta-chlordane, gamma-chlordane, oxychlordane, alpha-hexachlorocyclohexane (HCH), beta-HCH, hexachlorobenzene, heptachlor epoxide, toxaphene, endrin, dieldrin and mirex, and Σ PCBs. Because *c*-nonachlor values were missing for 13 eggs, total nonachlor values were calculated for those eggs from *t*-nonachlor values (Total nonachlor = $1.28 \cdot t$ -nonachlor + 0.02; $R^2 = 0.99$, based on the data from the eggs with both *c*- and *t*-nonachlor measured). Briefly, eggs were individually homogenized (large embryos were first cut up with a chemically cleaned scalpel), mixed with anhydrous sodium sulfate (quantity 25 times the sample weight), and Soxhlet-extracted with hexane. The extract was concentrated to dryness and weighed for lipid determination. The lipid extract was dissolved in petroleum ether and processed by Florisil cleanup to remove fatty interferences for subsequent gas chromatographic analysis. Silica gel chromatography was used to separate organochlorine pesticides from PCBs [23]. The pesticides and PCBs in each of the final fractions were quantified with a gas-liquid chromatograph equipped with a 63 Ni electron capture detector. Residues in 10% of the samples were confirmed by gas chromatography/mass spectrometry. The results were reported to two significant digits in 1986 to 1996, and to three significant digits in 1996 to 2000. The nominal lower limit of detection was 0.01 $\mu\text{g/g}$, wet weight, for OC pesticides



Fig. 1. Locations of territories ($n = 159$) where bald eagle eggs ($n = 197$) were salvaged in Michigan and Ohio, USA, 1986 to 2000.

(except toxaphene), and $0.05 \mu\text{g/g}$, wet weight, for ΣPCBs and toxaphene, based on a 10-g aliquot. In all years except those listed below the analyses were performed by the U.S. Fish and Wildlife Service (USFWS), Patuxent Analytical Control Facility (PACF, 1990, 1996–2000) and contract laboratories to PACF, including Mississippi State Chemical Laboratory (1986–1988, 1990, 1993–1996), Texas A&M Research Foundation (1989), and Hazelton Environmental Services (1992). In 1988 two samples from Ohio were analyzed by the Wisconsin Department of Natural Resources using comparable methodology. In 1991 all samples were analyzed by the USFWS, National Fisheries Contaminant Research Center. Quality assurance/quality control of results from all the participating laboratories was monitored by PACF through the analysis of duplicate samples, matrix and reagent blanks, spiked samples, calibration checks, standard reference material samples, method blanks, and gas chromatography/mass spectrometry confirmation. Dieldrin Equivalents were calculated assuming an additive model [24] and using the formula:

$$\begin{aligned} (\text{Dieldrin}_{\text{EQ}}) = & \text{Dieldrin} \cdot 1.0 + \text{Endrin} \cdot 4.0 \\ & + (\text{Chlordanes} + \text{Nonachlors}) \cdot 0.8 \\ & + \text{Heptachlor Epoxide} \cdot 0.5 \end{aligned}$$

Statistical analyses

All analyses were completed in the statistical package R 3.2.1. For productivity analyses, four sets of analyses were completed, depending on the anticipated relationship between contaminant concentrations and observed effects. First, a linear relationship was considered and constructed using a general linear model. Then, based on suggestions from the literature [19,25] three dose-dependent formulae were considered:

$$Y = \frac{h \exp(a + bx)}{1 + \exp(a + bx)} \quad (1)$$

$$Y = \frac{h}{1 + x^n c^{n-1}} \quad (2)$$

$$Y^k = a + \frac{b}{1 + \frac{x^n}{c^n}} \quad (3)$$

where Y is the effect (productivity), b , c , h , k , and n are model parameters and x is the concentration of a given contaminant in the egg. Models for endpoints such as shell thickness, desiccation index, presence of an abnormality, were fit on ln-transformed data, except for productivity analyses because we wished to compare among models according to the formulae given above,

without including $\ln x$ terms. We constructed forward stepwise models that used Akaike's information criterion (AIC) scores to progressively add independent variables; whenever independent variables were added, models including all possible interaction terms were also tested. The same observations were included in all AIC analyses (i.e., missing data were dropped for all contaminants). Because the ratio of observations to variables could be below 40, we calculated cAIC (small sample unbiased AIC), as well as AIC, to determine whether criterion affected results [20].

Because only unhatched eggs were collected, our dataset is potentially biased; since successful pairs may be underrepresented, average productivity may be underestimated and average contaminant levels may be overestimated [9]. Those factors were minimized by redoing analyses excluding the collection year; five-year means include only the two years before and after collection. Because this made no difference to model selection, we retained all five years in the analyses presented here. These potential biases were assumed to be minimal, and did not affect the strength of association between contaminants and other parameters. The lack of demonstrable biases may be because many of the unhatched eggs were derived from successful nests, particularly in later years.

Our measurements were not expressed on a lipid-weight basis because decomposition of lipids in unhatched eggs would bias those measurements. A result at or below the nominal detection limit was recorded as 0.5 times the detection limit. Prior to using normal statistics, we tested whether \ln -transformed data were normally distributed (Shapiro–Wilk). A geometric mean concentration was calculated for each nest where two or three unhatched eggs were collected.

Previous authors reported differences in productivity between sites along the Great Lakes shoreline and interior sites [12,14,16,17]. Therefore, another set of analyses were completed that included only nests along the entire Great Lakes shoreline or only nests within the interior (sites inaccessible to anadromous fish in Michigan and Ohio; non-anadromous sites). Previous authors examined spatially averaged relationships [13,16]. However, here we examined the statistical relations on an individual egg or nest site basis to avoid sample size confounding goodness-of-fit with strength-of-association in AIC models. All data are available in the Supplemental Data (Digital Appendix.xls).

RESULTS

All egg contaminant concentrations (except Mirex) declined significantly over time (Fig. 2A, Supplemental Data). Eggshell thickness increased over time, and approximated historical levels (≈ 0.61 mm; Fig. 2B) by 2000. For those nest sites from which unhatched eggs were collected in multiple years, PCB (mean difference between consecutive samples = -5.8 ± 3.0 $\mu\text{g/g}$; $t_{35} = 1.60$; $p = 0.04$), DDE (-1.8 ± 0.8 $\mu\text{g/g}$; $t_{35} = 2.42$; $p = 0.01$) and dieldrin (-0.15 ± 0.06 $\mu\text{g/g}$; $t_{35} = 2.74$; $p = 0.01$) all declined over time with the magnitude of the decline correlating with the number of years between samples for DDE (slope = -0.16 $\mu\text{g/g/year}$; $p = 0.01$; $r^2 = 0.26$) and dieldrin (slope = -0.28 $\mu\text{g/g/year}$; $p = 0.005$; $r^2 = 0.34$) but not PCBs (slope = -0.08 $\mu\text{g/g/year}$; $p = 0.08$; $r^2 = 0.09$). Sites with inexperienced birds (1–2 years breeding experience) had similar levels of contamination to sites with experienced birds at both the Great Lakes (comparison with birds 3–9 years breeding experience: early period 1986–1995: PCB, $t_{26} = 0.89$, $p = 0.38$; DDE, $t_{23} = 0.15$,

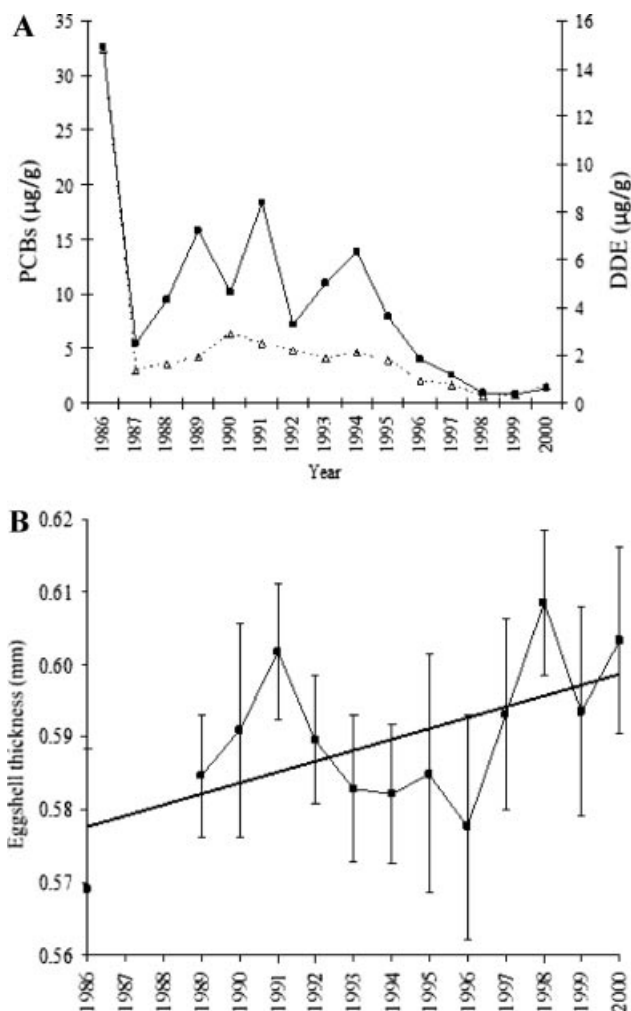


Fig. 2. (A) Polychlorinated biphenyls (■, solid line) and dichlorodiphenyltrichloroethane (Δ, dashed line) declined in bald eagle eggs collected in Michigan and Ohio, USA 1986 to 2000. (B) Eggshell thickness increased in bald eagle eggs collected in Michigan and Ohio 1986–2000 ($n = 197$). Pre-1946 eggshell thickness for the Great Lakes states and Alaska, USA = 0.61 mm.

$p = 0.88$; later period 1995–2000: PCB, $t_{15} = 1.30$, $p = 0.21$; DDE, $t_{15} = 1.44$, $p = 0.17$; comparison with birds 10+ years breeding experience: early period 1986–1995: PCB, $t_{23} = 0.19$, $p = 0.85$; DDE, $t_{23} = 1.20$, $p = 0.24$; later period 1995–2000: PCB, $t_{11} = 0.30$, $p = 0.77$; DDE, $t_{11} = 0.99$, $p = 0.34$) and interior sites (comparison with birds 3–9 years breeding experience: early period 1986–1995: PCB, $t_{16} = 1.59$, $p = 0.13$; DDE, $t_{23} = 0.89$, $p = 0.39$; later period 1995–2000: PCB, $t_{19} = 0.62$, $p = 0.54$; DDE, $t_{15} = 1.44$, $p = 0.17$; comparison with birds 10+ years breeding experience: early period 1986–1995: PCB, $t_{34} = 0.54$, $p = 0.59$; DDE, $t_{34} = 0.03$, $p = 0.97$; later period 1995–2000: PCB, $t_{30} = 0.70$, $p = 0.49$; DDE, $t_{30} = 1.29$, $p = 0.21$). The number of occupied territories and productivity in Michigan decreased through the 1960s (the increase in Fig. 3 is due to discovery of previously existing nests at the start of the study), remained stable in the 1970s and increased in 1981 to 2004 (Fig. 3). Until about 1974, mean nest success was less than 0.7 young / occupied territory, at which time it increased until leveling at ≈ 0.9 young / occupied territory, where it has remained until the present time. By 1980, six years following the 1974 improvement in nest success, the population (reflected in the number of occupied territories)

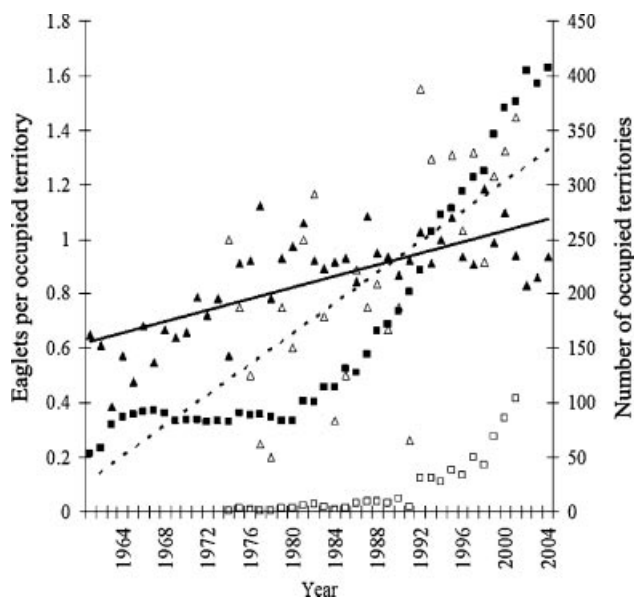


Fig. 3. The number of occupied territories (squares) and productivity (triangles and regression lines) increased in Michigan, USA, 1961 to 2004 (filled symbols, black line) and Ohio, USA, 1974 to 2001 (open symbols, dashed line). Aerial surveys did not begin in Michigan until 1963, and the increase in the number of territories during the 1960s represents discovery of new nests, rather than an increase in population size.

began to increase steadily. Similarly, the number of occupied territories and productivity also increased in Ohio 1974 to 2001 (Fig. 3).

Productivity declined with increasing contaminant concentrations (Table 1; Supplemental Data, Table S1, Fig. 4), but this did not appear due to reduced eggshell thickness. There was no relationship between the Ratcliffe Index or eggshell thickness and productivity (Supplemental Data, Table S1). Polychlorinated biphenyls and DDE were most closely related with reduced productivity (Table 1). A combined dose-dependent model for ΣPCBs and DDE had the lowest AIC value ($r^2 = 0.178$; Table 2), and ΣPCBs were generally included in all candidate models with low AIC values (Tables 1

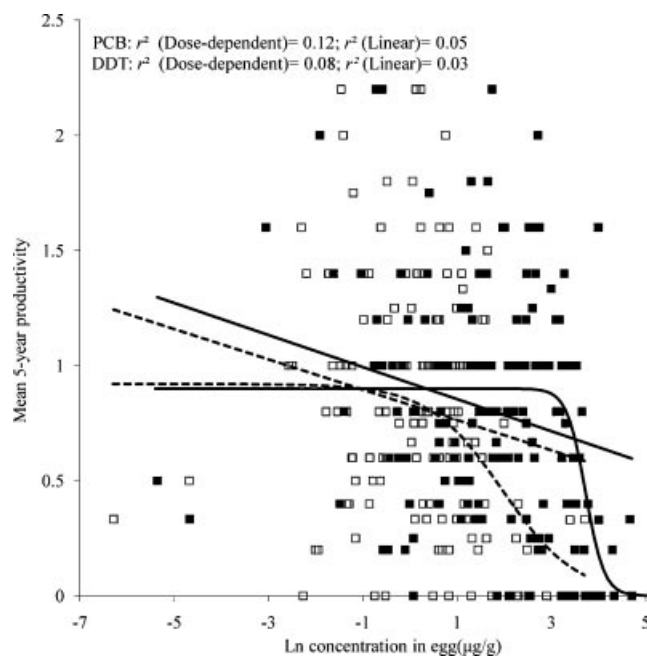


Fig. 4. Five-year mean productivity decreases with increasing polychlorinated biphenyl and dichlorodiphenyltrichloroethane concentrations for nests where bald eagle eggs were collected in Michigan and Ohio, USA, 1986–2000 ($n = 159$).

and 2). The effects (inflection point in the univariate models) were seen at approximately $\ln \Sigma\text{PCB} = 3.25$ ($\approx 26 \mu\text{g/g}$) and $\ln \text{DDE} = 1.5$ ($\approx 4.5 \mu\text{g/g}$) as shown in Figure 4; similarly, an effect level of 0.7 young per occupied nest was seen at concentrations of 26 to 30 $\mu\text{g/g}$ PCB and 6.5 to 7.4 $\mu\text{g/g}$ DDE (Table 2). When the data were partitioned into Great Lakes and interior sections, ΣPCBs continued to have a much lower AIC value than other contaminants at the nests on the Great Lakes shoreline (Table 2; Supplemental Data, Table S2), whereas at interior sites, which had relatively low contaminant burdens, there was little support for any relationship between contaminants and productivity. (Table 2; Supplemental Data, Table S2).

Table 1. ΔAIC and ΔcAIC values for regression of five-year mean bald eagle productivity (second and third column), eggshell thickness (fourth and fifth column), and presence of visible embryo (final six columns) and eggshell thickness in 197 salvaged bald eagle eggs (159 clutches) from Michigan and Ohio, USA, 1986 to 2000

Model	Productivity (All)		Eggshell (All)		All sites		Shoreline		Interior	
	ΔAIC	ΔcAIC	ΔAIC	ΔcAIC	ΔAIC	ΔcAIC	ΔAIC	ΔcAIC	ΔAIC	ΔcAIC
PCB + DDE (Model 1)	0.00	0.00								
PCB + Dieldrin _{EQ} (Linear)	1.59	1.59								
PCB + Dieldrin _{EQ} (Model 1)	1.68	1.68								
PCB + Mirex			0.00	0.00						
PCB + Mirex + Dieldrin _{EQ}			1.48							
DDE + Mirex			1.86							
PCB + DDE + Mirex			1.98							
Dieldrin _{EQ} + DDE + Dieldrin _{EQ} · DDE					0.00	0.00				
Dieldrin _{EQ} + PCB + PCB · Dieldrin _{EQ}					0.84	0.84				
PCB + DDE + PCB · DDE							0.00	0.00		
Dieldrin _{EQ} + Mirex									0.00	0.00
Dieldrin _{EQ}									0.63	0.63
Dieldrin _{EQ} + DDE									1.52	1.52

1 = embryo visible, 0 = embryo not visible on log-transformed contaminant values. All regressions except productivity used a logit link. Models with ΔAIC < 2.0 are shown. Candidate models were selected stepwise by selecting all single-parameter models with ΔAIC < 5.0, and including additional parameters if ΔAIC < 2.0. The procedure was repeated for the 86 eggs from the Great Lakes shoreline and the 111 eggs from interior sites.

Table 2. Dose-dependent formulae for the relationship between productivity and contaminant levels

		h	a	b	Critical value
DDE	Great Lakes	1.4	10	-4.9	7.4
	All nests	0.9	10	-5.1	6.5
PCB	Great Lakes	1.4	18	-4.9	30
	All nest	0.9	19	-5.1	26

Values for parameters for Model 1 (see text) given, as well as the critical contaminant value ($\mu\text{g/g}$) at which productivity = 0.7 young per occupied nest. Other critical values can be determined from the formulae; note that a value of 1.0 would never occur for the entire dataset (all)-average productivity is always lower than 1.0 young per occupied territory.

Eagles nesting along the Great Lakes shoreline had significantly higher concentrations of PCBs, dieldrin, DDE, ΣDDT , nonachlor, oxychlorane, heptachlor epoxide, and mirex compared to nests at interior sites (Table 3). Productivity and eggshell parameters for eggs collected at inland (non-anadromous), inland (anadromous), and Great Lakes sites in Michigan and Ohio, 1986 to 2004, were compared (Table 4). Employing ANOVAs using post hoc tests no difference in abnormalities, presence of a visible embryo, RI, or eggshell thickness were found among sites, but there was a significant difference in average productivity between Great Lakes and interior sites ($t = -1.77$, $df = 36$, $p = 0.04$), but not between anadromous sites and either of the other site categories (Table 4). Productivity increased with breeding pair experience for the first three years at the interior but not the Great Lakes shoreline sites (Fig. 5).

There were eight embryos with visible abnormalities among the 73 eggs ($8/73 = 11\%$) with a visible embryo (Table 5). All abnormalities were observed prior to 1996, when contaminant levels were higher (Table 5). There were three bill-related abnormalities, and two of those abnormal embryos had particularly high contaminant concentrations (Table 5). Embryos with detectable abnormalities had significantly higher concentrations of dieldrin equivalents than those without abnormalities, and ΔAIC values for those contaminants were somewhat

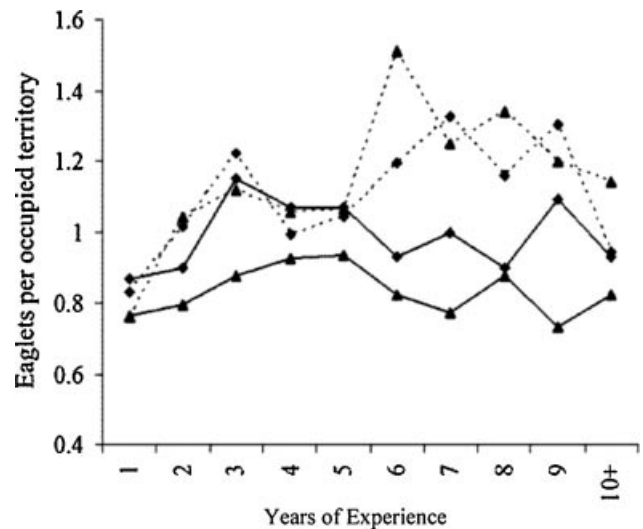


Fig. 5. Productivity in bald eagles from Michigan and Ohio, USA, increased with experience (number of years the nest site had been occupied) at interior (dashed line) but not Great Lakes shoreline (solid line) sites during the early (1986–1995; triangles) and late (1996–2004; diamonds) time period. See text for details.

better (Supplemental Data, Table S3, Fig. 6). Nonetheless, there was no support for a logistic model of contaminant concentrations and abnormalities in the embryos above the null model. When only 1986 to 1990 were included in analyses (to avoid transient effects due to changing contaminant levels over time), the three eggs with abnormalities had two to three times the contaminant levels of eggs without abnormalities. During 1991 to 1995 the five eggs with abnormalities had 0.6 to 1.0 times the contaminant levels of eggs without abnormalities. There was no difference in productivity among nests that had an abnormality (0.72 young per year) and those that did not (0.79, $p = 0.31$). This was also true when only 1986 to 1990 data were included ($p > 0.5$).

Eggs with a visible embryo had significantly higher concentrations of all contaminants than those without visible embryos (Supplemental Data, Table S4). For the combined

Table 3. Contaminant concentrations ($\mu\text{g/g}$) in eggs collected at interior (non-anadromous; 111 eggs) and Great Lakes shoreline (86 eggs) sites in Michigan and Ohio, USA (1986–2000)

Site	PCBs	Dieldrin	DDE	ΣDDT	Nonachlor	Oxychlorane	HE	Mirex
Great Lakes	26.4 ± 2.7	0.39 ± 0.05	5.6 ± 0.8	5.9 ± 0.8	0.60 ± 0.03	0.098 ± 0.012	0.077 ± 0.010	0.065 ± 0.018
Interior	4.3 ± 0.5	0.06 ± 0.01	1.39 ± 0.24	1.4 ± 0.2	0.14 ± 0.01	0.021 ± 0.003	0.014 ± 0.003	0.018 ± 0.004

Contaminant levels are all significantly higher at Great Lakes shoreline sites (t test).

Table 4. Embryo, eggshell, and productivity parameters (\pm standard deviation) for eggs collected at inland (non-anadromous), inland (anadromous) and Great Lakes shoreline sites in Michigan and Ohio, USA (1986–2000)

Site	No. eggs	% Eggs without visible embryo	% Eggs with abnormalities	Ratcliffe Index	Eggshell thickness (mm)	Productivity (young/occ terr)
Great Lakes shoreline	86	54 (46)	5.8 (5)	2.92 ± 0.30	0.58 ± 0.04	0.88 ± 0.15
Inland	111	71 (79)	2.7 (3)	3.04 ± 0.41	0.60 ± 0.05	1.02 ± 0.10
Anadromous	0					0.93 ± 0.24

Values in parentheses refer to actual number observed. Using χ^2 or t tests we found no difference in abnormalities, infertility, Ratcliffe Index, or eggshell thickness among sites, but there was a significant difference in average productivity between Great Lakes and Inland sites ($t = -1.77$, $df = 36$, $p = 0.04$), but not between anadromous sites and either of the other sites.

Table 5. Description of visibly abnormal embryos ($n = 8$ out of 73 eggs with an embryo present) from eggs salvaged from bald eagle nests in Michigan and Ohio, USA (1986–2000) along with individual and mean concentrations of major chlorinated hydrocarbon contaminants

Location	Collection Date	Embryo age (d)	Description	Contaminant concentrations					
				PCBs	DDT	Σ Chlordanes	Σ Cyclodienes	Mirex	Dieldrin
Shakey River	May 1986	13-14	Bill skewed to right	29.3	15.9	1.47	2.41	0.06	0.92
Ossineke/South Point	June 1986	19-20	Bill skewed to right	95.6	38.4	3.27	5.53	0.31	2.21
Vanderbilt/Fontinalis Club	June 1987	22-23	Skull and left leg poorly developed	1.9	1.1	0.14	0.21	0.01	0.07
Fishdam River Mouth	May 1991	25	Missing top of skull, body small and poorly calcified	27.2	7.6	0.68	1.06	0.03	0.38
Fence Lake	June 1992	31-32	Body laterally compressed	4.9	0.9	0.14	0.23	0.00	0.09
Chalk Hill	June 1995	30-32	Bills do not align	8.2	1.7	0.27	0.35	0.01	0.07
Fishdam R Mouth	May 1995	22-25	Head appears crushed	22.2	8.5	0.76	1.12	0.00	0.36
Lower Hemlock Rapids	June 1992	34	Left side of head appears crushed	1.5	0.7	0.11	0.15	0.01	0.03
Mean ($n = 8$), abnormality present				26.3 \pm 29.5	9.6 \pm 12.7	0.9 \pm 0.9	1.46 \pm 1.52	0.06 \pm 0.10	0.56 \pm 0.72
Mean ($n = 65$), abnormality absent				19.8 \pm 22.7	5.2 \pm 6.3	0.7 \pm 0.6	1.02 \pm 1.13	0.04 \pm 0.09	0.32 \pm 0.40

dataset, the relationship between contaminant concentration and probability of a visible embryo was strongest for dieldrin equivalents (Supplemental Data, Table S4, Fig. 6). There was little difference between logit, probit, and log-log link functions, so it appeared that the mathematical form of the logistic relationship was unimportant biologically (Supplemental Data, Table S4). When eggs were split into Great Lakes shoreline and interior groups, DDE/ Σ DDT became the best predictor in the Great Lakes shoreline dataset (Table 1) and dieldrin equivalents in the interior dataset (Table 1). When only 1986 to 1990 were included in analyses, there was no difference in contaminant levels for birds with and without visible embryos ($p > 0.05$), although eggs with visible embryos had slightly higher levels of most contaminants. There was no difference in productivity among nests that had a visible embryo (0.71) and those that did not (0.83, $p = 0.15$). This was also true when only 1986 to 1990 were included ($p > 0.5$).

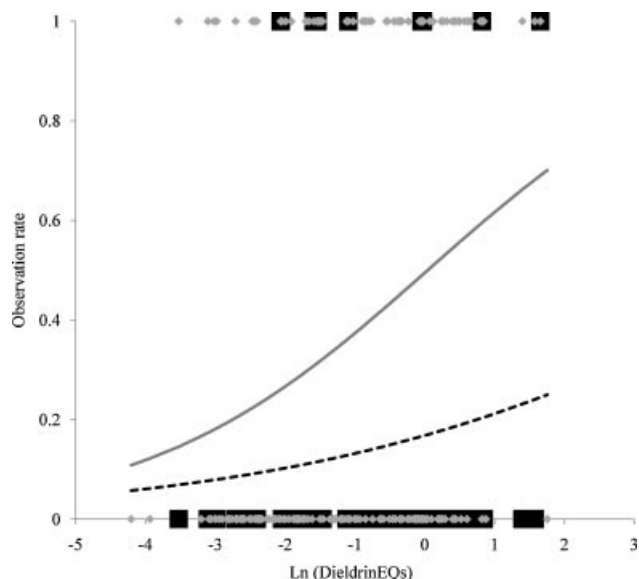


Fig. 6. Rate of embryo abnormalities (black) and visible embryos (gray) in Michigan and Ohio, USA (1986–2000) in bald eagle eggs ($n = 197$) increases with dieldrin_{EQ} concentrations.

There were significant negative correlations between eggshell thickness and most contaminants (Supplemental Data, Table S5, Fig. 7), with Σ PCB + Mirex being the most parsimonious model (Δ AIC = 1.31; Table 1). Dose-dependent formulae for eggshell thickness generally had higher AIC values than linear models (Supplemental Data, Table S5). Σ PCB was the best single-parameter linear model, with Σ DDT the second-best model at Δ AIC (compared to PCB alone) = 2.68. There was no support for either linear or dose-response relationships ($r^2 < 0.01$; $p > 0.3$; lowest AIC for null model) between contaminants and RI .

Concurrent to the decreases in contaminant concentrations over time, eggshell parameters and embryo presence and condition also tended to change temporally. Abnormalities per embryo (slope = -0.016 ± 0.010 units \cdot year $^{-1}$; $t = -1.63$; $p = 0.13$; $r^2 = 0.13$) and proportion of eggs with a visible

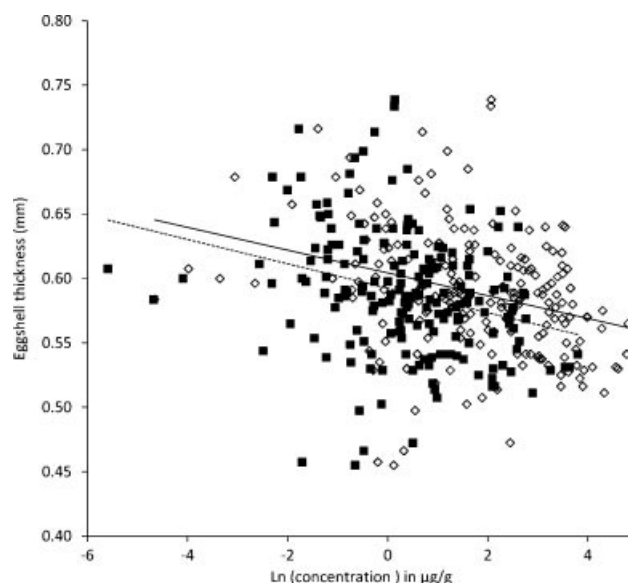


Fig. 7. Eggshell thickness for bald eagle eggs ($n = 197$) from Michigan and Ohio, USA (1986–2000) decreases with polychlorinated biphenyls (■, solid line) and total dichlorodiphenyltrichloroethane (◇, dashed line) concentration.

embryo (slope = -0.025 ± 0.016 units \cdot year $^{-1}$; $t = -1.57$; $p = 0.14$; $r^2 = 0.11$) declined over time (1986–2000), although the relationship was not significant, whereas *RI* (slope = -0.019 ± 0.021 units \cdot year $^{-1}$; $t = -0.87$; $p = 0.40$; $r^2 = -0.02$) showed little change over the study period.

DISCUSSION

In 1986, when this study was initiated, the bald eagle was federally listed as endangered in Ohio and threatened in Michigan. Regulatory actions in the early 1970s, prior to the species' federal listing in 1978, initiated source controls on the most problematic chlorinated hydrocarbon chemicals. Reductions in contamination and persecution combined with increased protection of critical habitat and release programs [10,11,14] meant that by the late 1980s, as apparent in the data presented here, many populations experienced a period of exponential growth in the number of breeding pairs. By 1995, having met recovery targets, the bald eagle was federally reclassified to threatened in Ohio and delisted in both states in 2007 [26]. Under state law the bald eagle was delisted in Michigan in 2009, but remains a threatened species in Ohio. In the present study we are assessing, therefore, whether chlorinated hydrocarbon contaminants continued to impact productivity of bald eagles during a period of rapid population rebound.

There were initial sharp decreases in concentrations of all contaminants, followed by a period of no significant temporal trends in the data, possibly because factors such as variation in contaminant levels due to differences in the trophic levels of the diverse prey items [27] obscured any temporal trends. Based on reports of other species from the Great Lakes or nearby ecosystems (e.g., herring gulls [3]; lake trout [28]), concentrations of chlorinated hydrocarbons were relatively stable during the major portion of our study period (1990–2000) and much lower than in the Organochlorine Era, from the late 1940s to the early 1980s [10,11].

Contaminants and populations

In analyzing a similar set of data collected over the period 1969 to 1984 from 15 U.S. states, Wiemeyer et al. [9] concluded that DDE was the contaminant most closely correlated to both productivity and egg shell thickness, accounting for 30% of the variance in the data. At that time there was no statistical correlation of PCBs with reduced productivity over the 15 states studied. They hypothesized that if environmental residues of DDE declined faster than PCBs, the effects of PCBs could then become evident, a scenario which appears to have developed during the 1990s in the Great Lakes [15,16]. For the entire sample, and for the shoreline subset, PCBs had the clearest relationship with productivity, and the best relationship was dose-dependent and included PCBs and DDE as factors. For our total sampled population the best model was PCB+DDE ($r^2 = 0.18$), while PCBs accounted for 12% of the variability in productivity. That increased to 15% for the subset of 65 territories located along the Great Lakes shoreline. Additive effects of DDE and PCBs have also been reported for chick survival in laboratory studies [29]. Compared to a single compound such as DDE, one would expect greater variance in the data for PCBs or TEQs, given the complex mixtures involved, including significant polychlorinated dibenzo-*p*-dioxin (PCDDs) and/or dibenzofuran (PCDFs) contribution at some sites combined with species specific variation in TEFs (Steve Bursian, pers. commun.). Nonetheless, except situations where local sources of PCDDs and PCDFs are relatively high com-

pared to PCBs, (e.g., [30]), dioxin-like toxicity in wild birds has mostly commonly been related to PCB congeners, and Σ PCBs provide a reasonable surrogate [31]. Polychlorinated biphenyl-126 and PCB-77, along with some major mono-*ortho* PCBs, contributed most of the overall TEQ load in Great Lakes fish-eating birds [5] and bald eagles from a PCB-contaminated area with similar reproductive problems [32].

While legacy contaminants such as DDE and PCBs were declining during the study period, other contaminants such as polybrominated diphenyl ethers (PBDEs) [33], perfluorinated compounds [34] have been increasing and may also have contributed to the variance in productivity that we measured. Ecological variables, such as age or experience of breeding birds [14], weather [35], and territory quality [36] can interact in complex ways and undoubtedly explain a substantial portion of the variance in eagle reproductive success [18].

Productivity increased with breeding experience for the first three years (mating and care of the young, a surrogate for parental age) at inland but not at shoreline territories, as has been shown previously [14], even though contaminant burdens declined over time in the shoreline populations (as reflected in egg contaminant concentrations both at a given nest and in the population mean). It appears that birds from the Great Lakes shoreline, including those immigrating from the source population in the interior, accumulated greater contaminant burdens, thus negating the positive effects of increased experience. It is conceivable that contaminant burdens were above a threshold for adverse effects on reproductive behaviors or learning, but that would need to be evaluated in specifically designed studies. Nonetheless, productivity increased with experience at the interior sites, but not along the Great Lakes shoreline, especially during the early years, and productivity was higher during the early years at interior sites compared to Great Lakes shoreline sites. Results of the present study support the hypothesis advanced previously [14] that contaminants reduced reproductive success on the Great Lakes shoreline, but not in the interior, indicating that contaminants contributed to the *sink* status of Great Lakes shoreline eagles.

Based on the univariate models, the critical values associated with a decrease in nest success below 0.7 young / occupied territory occur at about 4.5 μ g/g DDE and 26 μ g/g Σ PCBs (Table 1). Those values should be treated with considerable caution, given that they are derived from rather weak correlative data. Nonetheless, support for effect levels in those ranges comes from determinations of similar value (6 μ g/g DDE) from a North American-wide dataset for bald eagles [12]. Some support for a PCB effect level within that range also comes from a study of American kestrels (*Falco sparverius*) fed a mixture of Aroclors prior to and during reproduction, where there was a significant delay in laying time and in production of hatched and fledged young compared to controls at Σ PCBs in eggs of 34 μ g/g [37]. Lower Σ PCB thresholds in bald eagles of 13 μ g/g [9] or 20 μ g/g [13] have been suggested previously, although based on more limited datasets and/or with data heavily confounded by DDE. The r^2 values in the present study were lower than those found by others using spatial averaging [12], and the r^2 values in the current study increase threefold once spatial averaging (i.e., grouped by region) is used. Spatial averaging inflates r^2 values by confounding goodness-of-fit with strength-of-association; therefore, our values were not reported using spatial averaging. The low r^2 notwithstanding, the relationships presented herein represent a robust ecopidemiological result.

Mechanisms of contaminant effects

Contaminants affect reproductive processes in birds via a variety of mechanisms. Maternally deposited chemicals can affect the health and survival of the embryo, which have been termed *egg intrinsic* effects, and are the processes most effectively addressed in this study by examination and analysis of unhatched eggs. Second, survival of hatched chicks to fledging, which can be affected by chemicals via deficits from embryonic exposure plus dietary exposure; fledging is measured here, but nestlings were not studied directly. Postfledging survival and recruitment also can be impacted by contaminant exposure, but was not addressed in this study. Next, reproductive performance and breeding behavior of adults can be affected by contaminant exposure with secondary effects on egg and hatchling survival, which have been termed *egg extrinsic* effects, and are incorporated into our productivity measures. Finally, we suggest another process that generally has not been discussed in the avian ecotoxicology literature: the potential that adults suffer reproductive impairments as a consequence of chemical exposures which occurred during their own early developmental stages. In mammals exposed during early development to endocrine-disrupting compounds, epigenetic effects have also recurred in subsequent generations [38]. Such mechanisms were not examined directly here, but may factor into productivity through infertility, eggshell quality, and/or altered adult behaviors in relation to foraging, disturbances, or territorial defense.

Eggshell thickness was near pre-DDT era levels for most of our study period. Nonetheless, there were significant, negative relationships between most contaminants and eggshell thickness. Many published analyses contend that DDE was the main factor affecting eggshell quality in wild birds, including *Haliaeetus* [9,12,19]. Here we found almost equal evidence for PCB and DDE being the main factor, which could be interpreted as PCBs playing a role in altering eggshell quality [9,39]. Those results may be due to declines in DDE levels relative to past analyses, or, given the intercorrelation of PCBs and DDE, it may just be a random result due to chance relationships between PCB and genetic or environmental predisposition for thinner eggs. Akaike's information criterion analysis suggested that either PCB or DDE was causing eggshell thinning, but that they did not act together. More important, there were no relationships between shell parameters and nest success; thus, with some confidence we can reject shell quality mechanisms as contributing significantly to reduced productivity at the contaminant levels prevailing during this study. In the 1970s, eggshells were regularly 30% thinner and one egg was found without a shell (membrane only), suggesting that eggshell effects were much more pronounced in earlier years (S. Postupalsky, unpublished data).

Numerous reports associate dioxin-like chemicals with reduced embryonic survival and gross abnormalities, particularly of the mandibles, in both dead embryos and surviving nestlings of waterbirds and eagles around the Great Lakes [5–8,16,17]. However, disease and genetic factors may contribute to pathologies, including deformities, in avian embryos [40,41]. Indeed, some of the individual deformities reported from the Great Lakes have been observed in relatively uncontaminated waterbirds from other locations [42], suggesting that deformities may not be dose-dependent. In eagles, there were eight described abnormalities were found among the subsample of 73 embryo containing eggs or 11% of the dead embryo-containing eggs. That could be considered a high rate, 1,096 per 10,000, if

compared, for example, to the prevalence of 51 deformed cormorant nestlings per 10,000 examined at colonies in Green Bay, Michigan [43]. Nevertheless, higher rates of deformities are expected in unhatched eggs than chicks, regardless of cause [41,43]. The rapid growth of these eagle populations might argue for an increase in number or rate of abnormalities as a result of the interaction of contaminants and genetically mediated developmental processes [44]. We are unaware of any other rapidly expanding population of bald eagles in North America that has documented the level of abnormalities reported in the current study.

The logistic regression of embryos with and without abnormalities showed that those with abnormalities had higher concentrations of all contaminants than those without abnormalities, but it was significant only for the cyclodiene insecticides/metabolites, nonachlor, oxychlordane, and dieldrin. Two of the three embryos described as having skewed bills were collected in 1986, and had concentrations of 29 and 96 $\mu\text{g/g}$ ΣPCBs . Experimental studies have shown that PCBs cause deformities, including those of the mandible, in chickens, with a significant increase in incidence of malformed embryos and hatchlings from chicken eggs dosed with 300 pg/g PCB-126, or 30 pg/g TEQs [45]. Beak deformities were the most common, including crossed beaks. A significant increase in malformed embryos and hatchlings of American kestrels occurred in eggs dosed with 23,000 pg/g PCB-126 or 2300 pg/g TEQs. Beak deformities involved a smaller lower mandible, presumably similar to those described for kestrel chicks from eggs with an average of 34 $\mu\text{g/g}$ of an Aroclor mixture [46]. The greater sensitivity of chickens than other birds to TCDD-like chemicals is consistent with recent findings of differences in the Ah receptor [47]. The higher threshold value in the raptor model is consistent with our finding of bill abnormalities in Great Lakes bald eagles in the first ten years of the 15-year dataset, when contaminant concentrations were generally greater. Over the same period, one foot and three bill deformities were found in nestling eagles in Michigan; three in 1993 and one in 1995 [17,40]. Dioxin-like compounds, mainly PCB-126, were a possible causal agent, given the lack of reported evidence for such effects by the other compounds including the organochlorine pesticides reported here. From 1996 through 2000 there were no further bill abnormalities in embryos or hatchlings. Analyses provided no strong link between PCBs or other chemicals with the identified abnormalities.

In a subset of white-tailed sea eagles (*Haliaeetus albicilla*) that exhibited no eggshell quality effects, once data were corrected for lipid content, eggs with a visible embryo present had significantly higher concentrations of PCB-138, possibly indicating an effect of PCBs on embryo survival [19]. In contrast, booted eagle (*Hieraaetus pennatus*) eggs showed higher concentrations of DDE and PCBs in nonembryo or infertile eggs, although there was no accounting for changes in moisture and lipid content of salvaged eggs [48]. Results of the present study are similar to those for the congeneric white-tailed sea eagle; higher contaminant concentrations were associated with the presence of more visible embryos. There was an approximate twofold higher concentration of all measured contaminants in eggs with a discernable embryo, a result that is suggestive of a toxicological linkage. However, the data did not permit establishing any rigorous link with embryo toxicity because of uncertainty over the precise classification of the large number of infertile or early dead eggs. Specifically, the data presented herein do suggest that the cohort of eggs without visible embryos likely contained some embryos that died

early in development and were completely consumed by decomposition.

The relationship between contaminants and the presence of visible embryos was strongest with the cyclodiene insecticides, as represented by the dieldrin equivalents. Polychlorinated biphenyls did not figure as a significant effect. When eggs were split into Great Lakes and interior groups, DDE and Σ DDT became the best predictor for the presence of an embryo in the Great Lakes dataset, while dieldrin equivalents were the best in the interior population dataset. The most parsimonious model for the Great Lakes shoreline dataset included an interactive term for PCBs, but PCBs as a main effect was not significant. The association of DDT-related compounds with presence of a dead embryo suggests a causal linkage separate from shell quality effects. Finding of associations between DDE and productivity and embryo mortality, but not with shell parameters, is similar to that of Helander et al. [19]. Finding of associations between cyclodienes and embryo mortality and abnormalities may be a statistical artifact resulting from a large number of eggs with low cyclodiene values and low embryo mortality during the latter half of the dataset, reducing the sum-of-squares. Laboratory studies with cyclodiene insecticides in birds have not reported evidence of direct embryo toxicity at environmentally relevant doses [24,29]. However, in the field, egg concentrations as low as 1 $\mu\text{g/g}$ dieldrin were associated with reduced nest success and population decreases in falcons but operated through poisoning of nesting adults [17,24,49]. It is conceivable, nevertheless, that such adult mortality or even decreased foraging efficiency could lead to greater rates of egg failure during incubation with a greater incidence of visible embryo eggs.

Field studies, particularly a limited number of egg swap experiments in the Great Lakes region, have suggested a role for adult reproductive behavior in low nesting success of other aquatic birds [5]. Stated or implied mechanisms assume those effects were caused by exposure of adults, with the chemicals acting on circulating androgen or estrogen levels, particularly progesterone, to negatively affect reproductive success. However, a review of laboratory studies concluded that there was limited evidence that PCBs disrupted circulating steroid levels during reproduction given the effectiveness of negative feedback compensation processes [50].

Alternately, it is conceivable that reduced productivity of Great Lakes bald eagles was not caused by real-time exposure to contaminants prior to or during the breeding season, but that some eagles suffered from reduced reproductive capability as a result of exposure to chemicals during their own critical developmental stages in ovo or shortly after hatching. There is evidence particularly from the mammalian literature demonstrating that in utero or lactational exposure to TCDD or PCBs affects neuro-endocrine and sexual development [51,52]. Strong evidence is lacking for birds, in part due to a lack of experimental studies [50]. Recent investigations related in ovo exposure to DDT or PCB compounds with later development of the brain, eggshell quality, and reproduction in field-exposed American robins (*Turdus migratorius*) [53]. Of particular relevance to the present study, white-tailed sea eagles continued to produce desiccated eggs after DDE concentrations had decreased to below apparent thresholds [18], and that if in a given territory the female was replaced, the new bird produced eggs that did not exhibit desiccation problems. They also noted that the desiccated eggs also tended to be infertile.

In the case of early developmental effects, there may not be a link between an adult breeding bird's exposure to a contaminant

in ovo and the burden in an egg produced by that bird, which is the metric for contaminant exposure in the present dataset. Bald eagles, particularly females, may disperse widely and breed some distance from their natal area [18]. A lack of connection between natal and breeding combined with patchy distribution of environmental contaminants due to the presence of many PCB hotspots around the Great Lakes may be another potential reason for the variability observed in this dataset. However, evidence from bands recovered from dead eagles in Michigan suggests that adults tend to establish territories not overly far from their natal areas.

Comparisons using AIC in the present study generally showed that all models (linear, dose-dependent, probit, log-log link) ranked contaminant effects equivalently. The similarity of various models was likely due to contaminant levels being relatively low throughout the study period. Many ecosystems are now impacted by chronic levels of many contaminants rather than acute levels of a single critical contaminant, and therefore understanding the robustness of results to model-selection when several contaminants are present is an important result. Use of cAIC or AIC gave almost identical orders for model selection, and AIC appears to be an adequate tool for contaminant studies, at least where sample sizes (60–200, depending on the analysis) and number of parameters (8–12, depending on the analysis) were similar to ours.

CONCLUSION

Over the period 1986 to 2000, reduced productivity of bald eagles nesting in the Great Lakes Basin, particularly at shoreline territories, was significantly associated with exposure to PCBs and DDE. However, no mechanistic links via reduced shell quality or increased embryo mortality were discernible at the contaminant concentrations prevailing during the study, leaving open the possibility for delayed effects of in ovo exposure of breeding adults. It seems likely that exposure to those legacy compounds will continue to decline, albeit very slowly in the more contaminated locations, and should with time become a decreasing factor influencing bald eagle productivity. However, eagles and other top predators in the Great Lakes and elsewhere are increasingly exposed to other persistent and accumulative chemicals of industrial origin, such as PBDEs [33] and per-fluorinated compounds [34]. Thus, monitoring of sentinel species, including the bald eagle, should continue as a fundamental feature of a responsible environmental policy.

SUPPLEMENTAL DATA

Supplemental Appendix. (24 MB XLS)

Supplemental Data. (103 KB DOC)

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