ORGANOCHLORINE CONTAMINANTS AND REPRODUCTIVE SUCCESS OF DOUBLE-CRESTED CORMORANTS FROM GREEN BAY, WISCONSIN, USA

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Abstract—In 1994 and 1995, nesting success of double-crested cormorants (Phalacrocorax auritus) was measured at Cat Island, in southern Green Bay, Lake Michigan, Wisconsin, USA. Sample eggs at pipping and unhatched eggs were collected and analyzed for organochlorines (including total polychlorinated biphenyls [PCBs] and DDE), hepatic microsomal ethoxyresorufin-O-dealkylase (EROD) activity in embryos, and eggshell thickness. Of 1,570 eggs laid, 32% did not hatch and 0.4% had deformed embryos. Of 632 chicks monitored from hatching to 12 d of age, 9% were missing or found dead; no deformities were observed. The PCB concentrations in sample eggs from clutches with deformed embryos (mean = 10.2 µg/g wet weight) and dead embryos (11.4 µg/g) were not significantly higher than concentrations in sample eggs from nests where all eggs hatched (12.1 µg/g). A logistic regression of hatching success versus DDE, dieldrin, and PCB concentrations in sibling eggs identified DDE and not dieldrin or PCBs as a significant risk factor. A logistic regression of hatching success versus DDE and eggshell thickness implicated DDE and not eggshell thickness as a significant risk factor. Even though the insecticide DDT was banned in the early 1970s, we suggest that DDE concentrations in double-crested cormorant eggs in Green Bay are still having an effect on reproduction in this species.

Keywords—Organochlorines Double-crested cormorant Polychlorinated biphenyls Reproduction DDT

INTRODUCTION

A correlation between polychlorinated biphenyl (PCB) concentrations and egg mortality was reported in double-crested cormorants (Phalacrocorax auritus) nesting in the Great Lakes [1]. In that study, egg mortality and contaminant concentrations were recorded during 1986 through 1988 at double-crested cormorant colonies in Lake Huron, Lake Michigan, Lake Superior, Lake Ontario, and a reference site, Lake Winnipegosis, Manitoba, Canada. In contrast, using a sample-egg approach [2], Larson et al. [3] found that PCB concentrations were not associated with internal differences in hatching success or survivorship in double-crested cormorant chicks at Spider Island, a colony in Lake Michigan, Door County, Wisconsin, USA.

A limitation of both the Tillitt et al. [1] and Larson et al. [3] studies was that exposure to DDE was not measured. Dichlorodiphenyldichloroethylene was considered to be the major contaminant responsible for the decline of double-crested cormorants in the Great Lakes between the 1940s and the 1970s [4]. Mean residues in double-crested cormorant eggs collected in the early 1970s varied from 6.4 µg/g wet weight in Lake Erie to 14.5 µg/g in Lake Huron [4]. Elevated DDE concentrations in double-crested cormorant eggs caused severe shell thinning leading in some cases to egg breakage during incubation [5,6]. Data from post-1947 eggshells suggest that double-crested cormorants were among the species to have suffered the greatest degree of eggshell thinning [7]. Because DDE concentrations are often highly correlated with PCB concentrations [2,5,8], the recently reported relationship between PCBs and nesting success [1] possibly could have been in response to DDE and not PCBs. This raises the question whether the reproductive problems in double-crested cormorants in the Great Lakes since the early 1970s [1] were an expression of PCB effects rather than of the continuing effects of DDE.

Conflicting evidence also exists regarding the possible cause of embryonic and chick abnormalities in double-crested cormorants. Abnormalities in colonial waterbirds have been well documented in the Great Lakes [9,10]. A correlation was reported between the frequency of embryonic deformities in double-crested cormorants and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents (TEQs) from seven colonies on Lakes Michigan, Huron, Superior, and Winnipegosis [11,12]. In contrast, Larson et al. [3], working on individual nests within in a single Lake Michigan colony, found that clutches with deformed embryos did not have significantly higher PCB or TEQ concentrations than clutches where no chicks were defecated. At the regional level, Ryckman et al. [13] found no correlation between the frequency of bill deformities and total PCB concentrations among colonies from different regions in the Great Lakes. In addition, when double-crested cormorant eggs were injected with PCB congener 126 or TCDD, no significant increases in developmental abnormalities were observed in relation to increased dose [14,15].

A limitation in all of the field studies reported to date is the small number of analytical samples that have been used to establish relationships between contaminants and effects. This limitation is imposed by the high cost of chemical analysis. Because of the large variation often present in biological samples, small sample size often compromises the power of statistical tests to detect relationships. Two relatively inex-
pensive biomarkers correlated with contaminants of concern in this study were available to overcome this analytical limitation. Hepatic microsomal ethoxyresorufin-O-dealkylase (EROD) activity in avian embryos was correlated with dioxin-like chemicals, which include specific PCB congeners [16,17]. Additionally, eggshell thickness is often correlated with DDE concentrations in eggs [5–7].

An opportunity to further test the relationship between PCBs and reproductive success in individual double-crested cormorants within a single colony presented itself in 1993. Before 1993, double-crested cormorants nested in tall trees on Cat Island in the southern end of Green Bay, Wisconsin, USA (Fig. 1) [18] and were not accessible for detailed study. In 1993, because of the loss of standing trees on the island, about 200 double-crested cormorant pairs began nesting on the ground. High concentrations of PCBs have been recorded in nesting colonies of cormorants in southern Green Bay, including Cat Island [19,20] and other birds [17,21–25] in the same area of the USA, where double-crested cormorant eggs were collected and reproductive success was monitored in 1994 and 1995.

The objective of this study was to determine whether organochlorines, specifically PCBs and DDE, were significant factors in determining reproductive success of double-crested cormorants nesting on Cat Island.

MATERIALS AND METHODS

A selected number of double-crested cormorant nests with eggs were marked on Cat Island (44°34′N, 88°00′W; Fig. 1) in 1994 and 1995 and monitored about once per week until eggs began to hatch and then every other day during the chick period. Seventeen visits were made each year to the colony between May 3 and June 28, 1994, and between May 4 and July 7, 1995. Nests were marked while proceeding on a sinuous but systematic path through the colony. The nest number was marked on eggs with a felt-tipped permanent ink pen when first observed. We did not individually mark chicks. To limit disturbance, we limited our time in the colony per visit to less than 1 h.

Nest success during the incubation period and chick period was estimated using the Mayfield method [26,27]. The incubation period was divided into incubation and chick periods, where the date on which the first egg was laid was day 0 of the incubation period and the date on which the first egg hatched was day 0 of the chick period. For computational purposes, 28 d were allocated to the incubation period (T. Custer, unpublished data) and 12 d to the chick period. To facilitate statistical comparisons between years, variance estimates and comparisons of daily survival rates followed the method of Hensler and Nichols [28].

Average clutch size (the number of eggs per clutch) was estimated using completed clutches. A clutch was considered completed when the same number of eggs was present in the nest on more than one visit to the colony.

We attempted to collect one egg at pipping from each of the marked nests. This was not always possible because of nest failure or because all viable eggs in some nests hatched between colony visits and we missed the pipping stage. Eggs that remained in the nest and did not hatch were also collected.

Pipping and unhatched eggs were collected under appropriate state and federal permits. Within 3 h after collection, eggs were taken to the laboratory and weighed on an electronic balance (±0.1 g) and the length and width were measured with a caliper (±0.01 mm). Egg volume was estimated based on the equation: volume = 0.51 length × width^2 [29]. If the embryo was alive, it was removed from the shell, examined for deformities, weighed (±0.1 g), the bill measured (±0.01 mm), and then the embryo killed by decapitation at the base of the hatching muscle on the upper neck. After decapitation, 48 of 247 heads were opened at the skull cap for use in a study of brain asymmetry and placed in a 10% buffered formalin solution and refrigerated.

Immediately after death of the embryo, the liver was removed and weighed (±0.001 g). Portions (<1 g) of the liver were placed into a cryotube with one to two drops of glycerin added, quick frozen in liquid nitrogen, stored in a Revco Ultra Low Freezer (Asheville, NC, USA) at −85°C and analyzed within 6 months for EROD activity. The remainder of the out liver (without liver and sometimes without head) was put into a chemically clean jar and frozen at −20°C. Eggs without live embryos were opened and the contents put into a chemically clean jar and frozen at −20°C. If dead embryos were present, they were examined for deformities.

A subset of nests was selected to compare the percent of eggs in a clutch that hatched with EROD activity, eggshell thickness, and organochlorine concentrations. One egg from each nest, where eggs were collected, was used in the analysis. If more than one egg was collected from a nest, eggs collected at pipping were selected first in order to maximize the number of samples with EROD activity.

Chemical analysis

Double-crested cormorant eggs were selected for chemical analysis based on three categories of nest fate: nests with dead embryos (n = 39), nests with deformed embryos (n = 6), and nests where all eggs hatched and none of the embryos were deformed (n = 30). All eggs with deformed embryos were analyzed. Eggs from the other two categories were randomly selected from the total available using a random number table.

The following organochlorines were analyzed in 75 double-crested cormorant eggs by Mississippi State Chemical Laboratory, Mississippi State University, Mississippi State, Mississippi, USA: α-, β-, γ-, and δ-hexachlorocyclohexane.
Organochlorines and cormorant reproduction

Environ. Toxicol. Chem. 18, 1999 1211

(HCH); α- and γ-chlordane; oxychlordane; cis-nonachlor; trans-nonachlor; dieldrin; endrin; hexachlorobenzene (HCB); heptachlor epoxide; mirex; toxaphene; α,p′-dichlorodiphenyldichloroethane (DDD); α,p′-dichlorodiphenylchloroethane (DDE); α,p′-dichlorodiphenyltrichloroethane (DDT); p,p′-DDD; p,p′-DDE; p,p′-DDT; and total PCBs. Total PCBs were estimated based on Aroclor equivalents. Samples were homogenized, mixed with sodium sulfate, and Soxhlet-extracted to remove the liver (concentration = 0.65 mg liver, 2.5 mg microsomal protein). EROD activity was assayed by the method of Burke and Mayer [32] as pmol product per min per mg microsomal protein.

Organochlorines and cormorant reproduction

Environ. Toxicol. Chem. 18, 1999 1211

Ethoxyresorufin-O-dealkylase activity

Ethoxyresorufin-O-dealkylase activity was determined in hepatic microsomes from pipping embryos [31]. Microsomes were prepared from homogenates of thawed liver samples by differential centrifugation. The 11,000-g supernatant was centrifuged at 40,000 rpm for 60 min to obtain the microsomal pellet. Each 100,000-g pellet was resuspended in 2.0 ml/g of tissue weight of 0.05 M Na/KPO₄ and 0.001 M disodium ethylenediaminetetraacetate, pH 7.6. Ethoxyresorufin-O-dethylyase was assayed by the method of Burke and Mayer [32] as adapted to a fluorescence microwell plate scanner [31]. The 200-μl total assay volume contained microsomes equivalent to 0.65 mg liver, 2.5 μM substrate, and 0.125 mM NADPH in 0.066 M Tris buffer, pH 7.4. Protein concentrations were determined by a 50% reduced volume Lowry assay [33] and EROD activity was calculated as pmol product per min per mg microsomal protein.

Eggshell thickness

Eggshells were gently washed with tap water before opening to remove attached debris and the chalky accumulation often found on cormorant eggs. Sometimes it was necessary to scrape the chalky material off using a fingernail or scissors edge. Eggshell thickness was measured to the nearest 0.001 mm with a micrometer after the empty shells had dried at room temperature for at least 1 month. Three measurements of the shell and shell membranes were taken at the equator; a mean thickness value for each egg was derived from these three measurements.

Statistics

Linear correlation, using Pearson correlation coefficients, was used to identify tentative relationships among variables. The response of hatching success to contaminant and bioindicated variables was modeled using logistic regression [34]. This statistical approach was chosen because egg success was a binary dependent variable (i.e., either an egg hatched or it did not). We included the possibility of extrabinomial variance, which is greater variance than in the binomial distribution, by estimating an extrabinomial scale parameter as the deviance divided by its degrees of freedom [35]. For the binomial distribution, this scale parameter is exactly one, and larger values indicate the presence of extrabinomial variation in the data. Our tests of significance are adjusted for this scale parameter. Logistic regression allows multiple variables to be evaluated simultaneously in relation to the dependent variable, in this case egg success. Logistic regression was also used to fit a curvilinear response function between each variable and egg success. The Hosmer and Lemeshow goodness-of-fit test [34] was used to determine whether the data adequately fit the logistic function; a p value >0.05 indicates an adequate fit.

For comparisons of DDE and PCB concentrations among nest success categories, contaminant concentrations were log transformed (using base 10 logarithms) to satisfy the homogeneity of variance assumption of analysis of variance. The Bonferroni multiple comparison method was used to determine differences among means. Unless otherwise stated, the probability level determining significance was p < 0.05.

RESULTS

Reproductive success

During 1994 and 1995 combined, 423 nests and 1,570 eggs were monitored from the egg-laying or incubation stages to when the first-hatched chick was 12 d of age (Table 1). For both years combined, 32% of eggs laid did not hatch. Six embryos were classified as deformed based on the presence of a crossed bill (n = 1), crossed bill and exposed spinal cord (n = 3), absence of one eye (n = 1), and an extra digit on one foot (n = 1). The main causes of egg failure were eggs that disappered from nests (68%), infertility (15%), and embryo mortality (11%) (Table 1). For both years combined, 9% of chicks that hatched died or disappeared before 12 d of age. Most (77%) of the chicks that did not survive to 12 d of age were never found; the remainder were found dead in or near the nest (Table 1).

Nest success during the incubation (Table 2, column A) and chick (Table 2, column B) periods was not significantly different between 1994 and 1995. The probability that a nest was successful through the chick stage (Table 2, column A × B) was 83% in 1994 and 75% in 1995; 59% of eggs in 1994 and 52% in 1995 were successful through the chick stage (Table 2, column A × B × C × D). The mean number of eggs per clutch was 3.7 in 1994 and 3.8 1995; the modal number of eggs per clutch was 4 in both years. The estimated mean number of chicks raised to 12 d of age per clutch was 2.2 in 1994 and 2.0 1995.

Measurements on sample eggs

Of the 423 nests monitored during incubation in 1994 and 1995, an egg at pipping or an unhatched egg was collected from 312 nests (Table 3). This sample of eggs, described in Table 3, is the basis for later linear correlations among the variables measured in eggs and embryos and included eggshell thickness, egg volume, embryo weight, embryo liver weight, EROD activity, and egg success.

From the 312 sample eggs, 75 eggs were selected for or-
ganochlorine analysis (Table 3; see Materials and Methods). The following organochlorines were not detected in any of the 75 eggs analyzed: α-, β-, γ-, and δ-HCH; γ-chlordane; o,p'-DDT; p,p'-DDE; α,p'-DDT. Trans-nonachlor (n = 4 detected), α-chlordane (n = 1), endrin (n = 1), toxaphene (n = 5), p,p'-DDE (n = 32), and p,p'-DDT (n = 15) were detected in <50% of the eggs. The remainder of the organochlorines were detected in ≥50% of the eggs (Table 3).

Reproductive success and contaminants

Egg success (the percent of eggs that hatched in a clutch) was positively correlated with eggshell thickness and negatively correlated with DDE and dieldrin concentrations in sample eggs (Table 4). The EROD activity was correlated with liver weight of the embryo at pipping and PCB concentrations in sample eggs. Eggshell thickness was positively correlated with egg volume and embryo weight and negatively correlated with DDE and dieldrin concentrations in sample eggs. Egg volume, liver weight, and embryo weight were all significantly intercorrelated. Concentrations of PCBs were negatively correlated with egg volume and embryo weight and positively correlated with EROD activity and DDE concentrations but were not correlated with egg success. Dieldrin concentrations were negatively correlated with egg success and eggshell thickness and positively correlated with DDE concentrations.

Logistic regression indicated that concentrations of DDE, but not dieldrin or PCBs, in sample eggs were a significant factor in the hatching success of double-crested cormorant eggs (Table 5 and Fig. 2). The distribution of the data conformed well to the logistic model (goodness-of-fit p = 0.55; Table 5). When DDE concentrations and eggshell thickness were analyzed by logistic regression, DDE concentrations (but not eggshell thickness) were a significant factor in hatching success of double-crested cormorant eggs (Table 5). However, evidence existed of a lack of fit (goodness-of-fit p = 0.04; Table 5), due to the presence of greater variation in the data than in the binomial distribution (our estimate of the extrabinomial scale parameter was 1.7).

Sample eggs from nests where at least one embryo died had significantly higher DDE concentrations (mean = 3.9 related with egg volume and embryo weight and positively correlated with EROD activity and DDE concentrations but were not correlated with egg success. Dieldrin concentrations were negatively correlated with egg success and eggshell thickness and positively correlated with DDE concentrations.

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μg/g; Table 6) than sample eggs from nests where one embryo was deformed (2.2 μg/g); sample eggs from nests where all eggs hatched and embryos were not deformed (2.8 μg/g) did not differ from the other categories of success. The PCB concentrations in sample eggs did not differ among nest success categories (Table 6).

**DISCUSSION**

**PCBs and reproductive success**

In contrast to an earlier study [1], our results do not support a relationship between PCB concentrations in eggs and reproductively success in double-crested cormorants. Using a sample-egg approach, no relationship was discovered between total PCBs and hatching success. Additionally, no correlation was present between EROD activity, a bioindicator of PCB and TEQ concentrations in avian embryo livers [16,17], and the hatching success of sibling eggs.

Using a sample-egg approach, a 2-year study [3] also reported that PCB concentrations were not related to double-crested cormorant reproduction at Spider Island. In that study, total PCB (7.6 μg/g) and TEQ (134 pg/g) concentrations in sample eggs from clutches where all eggs produced fledged

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**Table 3. Organochlorine concentrations and other characteristics of 312 double-crested cormorant eggs and embryos collected from Cat Island, Green Bay, Wisconsin, USA, in 1994 and 1995 combined**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Range</th>
<th>n</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PCBS (μg/g)</td>
<td>13.6</td>
<td>2.01–71.85</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>DDE (μg/g)</td>
<td>3.9</td>
<td>0.96–10.82</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>Dieldrin (μg/g)</td>
<td>0.25</td>
<td>0.03–1.29</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>Heptachlor epoxide (μg/g)</td>
<td>0.08</td>
<td>0.01–0.45</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>Oxychlorane (μg/g)</td>
<td>0.05</td>
<td>0.01–0.18</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>cis-Nonachlor (μg/g)</td>
<td>0.04</td>
<td>1nd–0.13ª</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>Mirex (μg/g)</td>
<td>0.02</td>
<td>24nd–0.21</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>Hexachlorobenzene (μg/g)</td>
<td>0.01</td>
<td>34nd–0.04</td>
<td>75</td>
<td>eggs</td>
</tr>
<tr>
<td>Eggshell thickness (mm)</td>
<td>0.410</td>
<td>0.313–0.501</td>
<td>306</td>
<td>eggs</td>
</tr>
<tr>
<td>Egg volume (ml)</td>
<td>46.8</td>
<td>37.2–56.1</td>
<td>257</td>
<td>eggs</td>
</tr>
<tr>
<td>Embryo weight (g)</td>
<td>37.2</td>
<td>29.3–44.9</td>
<td>247</td>
<td>embryos</td>
</tr>
<tr>
<td>Embryo liver weight (g)</td>
<td>0.60</td>
<td>0.31–1.04</td>
<td>233</td>
<td>embryos</td>
</tr>
<tr>
<td>Ethoxyresorufin-o-dealkylase (EROD) activityc</td>
<td>0.27</td>
<td>0.97–0.02</td>
<td>210</td>
<td>embryos</td>
</tr>
<tr>
<td>Egg success (%)d</td>
<td>73.6</td>
<td>0–100</td>
<td>312</td>
<td>nests</td>
</tr>
</tbody>
</table>

ª Variation in the mean, samples where chemicals were not detected were given half the detection limit.

b The number before “nd” is the number not detected.

c EROD activity in embryo livers is measured in pmol product per minute per milligram of microsomal protein.

d Egg success is the number of eggs hatched in a clutch divided by the number of eggs laid.

**Table 4. Correlation matrix among variables associated with double-crested cormorant eggs and embryos collected from Cat Island, Green Bay, Wisconsin, USA, in 1994 and 1995**

<table>
<thead>
<tr>
<th></th>
<th>EROD activity</th>
<th>Ethoxyresorufin-o-dealkylase (EROD)</th>
<th>Eggshell thickness</th>
<th>Egg volume</th>
<th>Embryo weight</th>
<th>Liver weight</th>
<th>PCB</th>
<th>DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg success</td>
<td>0.39</td>
<td>0.06</td>
<td>0.39</td>
<td>0.68</td>
<td>0.26</td>
<td>0.27</td>
<td>0.06</td>
<td>0.3</td>
</tr>
<tr>
<td>NC</td>
<td>0.15</td>
<td>0.03</td>
<td>0.15</td>
<td>0.68</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Egg volume</td>
<td>0.07</td>
<td>0.003</td>
<td>0.07</td>
<td>0.02</td>
<td>0.38</td>
<td>0.31</td>
<td>0.01</td>
<td>0.2</td>
</tr>
<tr>
<td>NC</td>
<td>0.31</td>
<td>0.14</td>
<td>0.31</td>
<td>0.0001</td>
<td>0.13</td>
<td>0.04</td>
<td>0.01</td>
<td>0.2</td>
</tr>
<tr>
<td>Embryo weight</td>
<td>0.12</td>
<td>0.51</td>
<td>0.51</td>
<td>0.0001</td>
<td>0.14</td>
<td>0.04</td>
<td>0.01</td>
<td>0.3</td>
</tr>
<tr>
<td>NC</td>
<td>0.27</td>
<td>0.18</td>
<td>0.18</td>
<td>0.0001</td>
<td>0.23</td>
<td>0.04</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>Liver weight</td>
<td>0.13</td>
<td>0.40</td>
<td>0.40</td>
<td>0.0001</td>
<td>0.39</td>
<td>0.04</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>PCB</td>
<td>0.13</td>
<td>0.14</td>
<td>0.14</td>
<td>0.0001</td>
<td>0.39</td>
<td>0.04</td>
<td>0.01</td>
<td>0.2</td>
</tr>
<tr>
<td>DDE</td>
<td>0.13</td>
<td>0.01</td>
<td>0.01</td>
<td>0.0001</td>
<td>0.23</td>
<td>0.04</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.04</td>
<td>0.35</td>
<td>0.35</td>
<td>0.001</td>
<td>0.16</td>
<td>0.04</td>
<td>0.001</td>
<td>0.2</td>
</tr>
</tbody>
</table>

ª CC = correlation coefficient; NC = number of clutches.

b Values of r in italic are significant at <0.05.
chicks were not significantly different from PCB (8.2 μg/g) and TEQ (134 pg/g) concentrations in sample eggs from clutches where none of the eggs hatched. One of the limitations of that study was the low sample size within each of the categories of success (n ≤ 10 nests per group), which may have compromised the power of the experiment to detect differences. On the other hand, the authors did not discount the possibility that egg success was independent of exposure to the contaminants measured in the study.

Laboratory studies by Powell and colleagues [14,15] cast further doubt on the hypothesis that field concentrations of PCBs represent a significant influence on egg mortality in double-crested cormorants. Significant embryo mortality was associated with colony means exceeding 300 to 350 pg/g TEQs based on an in vitro bioassay system, the H4IIE rat hepatoma cell bioassay [1]. In contrast, the estimated LD50 of a double-crested cormorant egg extract, derived from contaminated double-crested cormorant eggs, and injected into reference double-crested cormorant eggs was 5,000 to 26,000 pg/g TEQs [14]. These are 17 to 74 times higher than the LD50 inferred from field samples [1]. In addition, injection of the egg extract, which contained 322 pg/g TEQ, into reference double-crested cormorant eggs did not affect embryonic mortality [14].

We suspect that the earlier-reported relationship between PCBs and egg mortality of double-crested cormorants in the Great Lakes [1] was due to DDE and not to PCB concentrations. Concentrations of DDE and PCBs are often correlated in wildlife samples [2,5,8], as we found in double-crested cormorant eggs in this study ($R^2 = 0.28$; Table 4).

Dirksen et al. [36], working on a closely related species, the great cormorant (Phalacrocorax carbo), reported a correlation between the proportion of eggs hatching in a clutch and individual PCB congeners in sample eggs. The DDE concentrations were high and eggshell thinning was observed; however, DDE was not incorporated into the statistical analysis and it may have been responsible for the observed relationships.

The observed correlation between DDE and PCB in this study also has implications for interpretation of our results. Because of that correlation, it is possible that some of the effect attributed to DDE in our logistic model was caused by PCBs. However, we believe this possibility is unlikely to invalidate our principal conclusions for two reasons. First, PCB was not a statistically significant predictor ($p = 0.13$) when it alone was used to predict hatching success by using logistic regression. Second, the large difference in the $p$ values for DDE and PCB makes it unlikely that the effect ascribed to DDE might really have been induced through PCB. Still, the possibility remains because the effects of correlated predictors cannot be separated in observational studies. The only way to definitively address the relative risks associated with DDE and PCB, and any synergistic effect, is through exposure of eggs to these contaminants in a controlled factorial experimental design.

If the measure of total PCBs in this study had taken into account TEQs, a relationship between hatching success and TEQs might have been demonstrated. However, total PCBs

### Table 5. Analysis of maximum-likelihood estimates from logistic regressions of the percentage of eggs that hatched in double-crested cormorant clutches from Cat Island, Green Bay, Wisconsin, USA, in 1994 and 1995 versus (1) DDE, dieldrin, and PCB concentrations in sample eggs from the same clutch, and (2) DDE concentrations and eggshell thickness in sample eggs from the same clutch

<table>
<thead>
<tr>
<th>Logistic regression variables</th>
<th>Analysis of maximum-likelihood estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect</td>
</tr>
<tr>
<td>DDE, dieldrin, and PCBs$^a$</td>
<td>Intercept</td>
</tr>
<tr>
<td></td>
<td>DDE</td>
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<tr>
<td></td>
<td>Dieldrin</td>
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<td></td>
<td>PCBs</td>
</tr>
<tr>
<td>DDE and eggshell thickness$^b$</td>
<td>Intercept</td>
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<tr>
<td></td>
<td>DDE</td>
</tr>
<tr>
<td></td>
<td>Eggshell thickness</td>
</tr>
</tbody>
</table>

$^a$ Hosmer and Lemeshow goodness-of-fit statistic = 5.93, 7 df, $p = 0.55$.
$^b$ Hosmer and Lemeshow goodness-of-fit statistic = 15.9, 8 df, $p = 0.04$.

Table 6. Concentrations of DDE and PCBs in double-crested cormorant eggs from nests on Cat Island, Green Bay, Wisconsin, USA, in 1994 and 1995 that contained dead embryos, deformed embryos, or successful eggs without embryonic deformities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Geometric mean concentration (μg/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nest category</td>
<td></td>
</tr>
<tr>
<td>Dead embryos (n = 39)</td>
<td>3.9 A</td>
</tr>
<tr>
<td>Deformed embryos (n = 6)</td>
<td>2.2 B</td>
</tr>
<tr>
<td>Successful eggs (n = 30)</td>
<td>2.8 AB</td>
</tr>
<tr>
<td>Two-way ANOVA overall $p$</td>
<td>0.02</td>
</tr>
<tr>
<td>Year (n = 2)</td>
<td>0.16</td>
</tr>
<tr>
<td>Survive (n = 3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.16</td>
</tr>
</tbody>
</table>

$^*$ Means for each success category in the same column sharing the same letter are not significantly different.
are highly correlated with TEQs in double-crested cormorant eggs ($R^2 = 0.66$; T. Custer, unpublished data), and, as such, total PCBs would be expected to mimic TEQ concentrations. This premise is supported in the Tillitt et al. [1] study where total PCB concentrations in eggs were significantly correlated with hatching success ($R^2 = 0.32, p = 0.045$). In addition, the lack of correlation between hatching success and EROD activity in double-crested cormorant livers supports the premise that TEQs were not correlated with hatching success in our study of double-crested cormorants.

**DDE and reproductive success**

Results of logistic regression analysis suggest that DDE is still having an effect on double-crested cormorant reproduction in Green Bay. Concentrations of DDE in double-crested cormorant eggs were negatively correlated with hatching success. Even though DDT has been banned since the early 1970s, the response of double-crested cormorant reproduction on Cat Island to DDE concentrations is not uncommon among North American birds. Similar correlations of DDE with impaired reproduction have been found in the bald eagle (*Haliaeetus leucocephalus*) [8,37], brown pelican (*Pelecanus occidentalis*) [2], white-faced ibis (*Plegadis chihi*) [38], black skimmer (*Rynchops niger*) [39], black-crowned night-heron (*Nycticorax nycticorax*) [40,41], and snowy egret (*Egretta thula*) [43].

The DDE concentrations in cormorant eggs from this study (mean = 3.9 µg/g, range = 1.0 to 10.8 µg/g) are similar to concentrations that are correlated with decreased reproduction in other species. The DDE effect levels for other species studied include: bald eagle >2.5 µg/g DDE [37] and >3 µg/g [8], brown pelican >2.5 µg/g [2], white-faced ibis >4 µg/g [38], black skimmer >6 µg/g [39], black-crowned night-heron >8 µg/g [40] and >4 µg/g [41], green heron >5 µg/g [42], and snowy egret >5 µg/g [43].

**Dieldrin, eggshell thickness, and reproductive success**

The significant negative linear correlation between double-crested cormorant egg success and dieldrin concentrations was probably the result of a significant correlation between DDE and dieldrin concentrations. A logistic regression of egg success versus DDE, dieldrin, and PCB concentrations in double-crested cormorant eggs identified DDE and not dieldrin concentrations as a significant factor associated with decreased egg success. The DDE and dieldrin concentrations were also higher in eggs from unsuccessful brown pelican nests in a study by Blus et al. [2]. Because of the significant correlation ($R^2 = 32\%$) between DDE and dieldrin concentrations in brown pelican eggs, Blus et al. [2] were unable to determine whether these chemicals together or separately were responsible for decreased reproduction in that species. Bald eagle reproduction and eggshell thickness were negatively correlated with concentrations of DDE and seven other organochlorines, including PCBs and dieldrin [8]. Wiemeyer et al. [8] reported that DDE was highly correlated with these other contaminants present in bald eagle eggs but that their impact on nest success appeared minor in relation to that of DDE. Nisbet [37] reanalyzed the bald eagle data of Wiemeyer et al. [8] and also concluded that reproductive impairment in bald eagles was mainly due to DDE and not dieldrin concentrations.

Our results suggest that reduced hatching success in double-crested cormorant eggs is related to concentrations of DDE and not eggshell thickness. Eggshell thickness and DDE concentrations were correlated with double-crested cormorant egg success (Table 3). However, when both variables were incorporated into a logistic regression, only DDE was considered a significant factor (Table 4). The DDE concentrations were also found to be more significant than eggshell thickness in reduced hatching success of common terns (*Sterna hirundo*) [44] and bald eagles [37].

**Embryo deformities**

Our results and those of other investigations do not support the relationship reported earlier between PCBs and embryonic deformities in double-crested cormorants [11,12]. A correlation was reported between double-crested cormorant embryonic deformities and TEQ concentrations from seven colonies from Lake Michigan, Lake Huron, Lake Superior, and Lake Winnipegosis. In our study, however, PCB concentrations in deformed embryos (10.2 µg/g) were not significantly different than PCB concentrations in embryos that were not deformed (12.1 µg/g). Similar results were found by Larson et al. [3] at Spider Island near Green Bay in Lake Michigan. Eggs from clutches with deformed chicks averaged 7.3 µg/g PCBs (153 pg/g TEQs); eggs from nests where all chicks fledged averaged 7.6 µg/g PCBs (134 pg/g TEQs). At the regional level, Ryckman et al. [13] found no correlation between bill deformity rates and total PCB concentrations among double-crested cormorant colonies from different regions in the Great Lakes. Finally, when double-crested cormorant eggs were injected with PCB congener 126 and TCDD, no significant increases in developmental abnormalities were observed in relation to increased dose [14,15].

The frequency of deformities in relation to PCB contamination at Cat Island in 1994 and 1995 does not support an association between PCBs and chick deformities in double-crested cormorants. The PCB concentrations in double-crested cormorant eggs were higher at Cat Island than in other colonies in Green Bay, and yet, the frequency of deformities was noticeably lower. The PCB concentrations in double-crested cormorant eggs from Cat Island in 1994 and 1995 averaged 13.6 µg/g (this study) compared to 5.3 µg/g from Spider Island in 1988 [1] and 7.8 µg/g in 1989 through 1990 [3]. Because the TEQ to PCB ratio did not vary among Green Bay colonies in 1994 and 1995 (T. Custer, unpublished data), the TEQs would also be expected to be higher in eggs from Cat Island than from Spider Island. In contrast, the frequency of deformities in double-crested cormorant chicks (0%) at Cat Island in 1994 and 1995 was lower than those reported from other Green Bay colonies. The frequency of deformed bills in double-crested cormorant chicks from northern Green Bay was 0.5% for 1979 to 1987 [10], 0.6% for 1987 to 1991 [12], and 0.7% for 1988 to 1990 [3].

**Reproduction**

Reproductive performance of double-crested cormorants at Cat Island was generally good to excellent compared to other locations. Hatching success of eggs (61–65%, Table 2, A × C) was intermediate to other reported values [45]. Number of young produced (2.0–2.3 to 12 d of age) was also similar or greater than the 0.7 to 2.5 young per nest reported in relatively uncontaminated colonies [45]. Drent et al. [46], working on Mandarte Island off the coast of British Columbia, Canada, early in the pesticide era of 1957 to 1960, reported double-crested cormorant hatching and fledging success rates (54–67% hatching success and 2.4 young fledged per nest) very
similar to those in this study. Postupalsky [47] suggested that normal productivity should approach 2.0 large young per pair.

Conclusions

Our results do not support the hypothesis that reduced hatching success and the frequency of deformities in double-crested cormorants in lower Green Bay are caused by PCB contamination. Total PCB concentrations in double-crested cormorant eggs were not associated with hatching failure or with embryonic deformities. However, our results do suggest an association between DDE concentrations in double-crested cormorant eggs and reduced hatching success. Even though DDT was banned in the early 1970s, we suggest that DDE concentrations in eggs are still having an effect on double-crested cormorant reproduction in southern Green Bay. Based on the rapidly expanding breeding populations of double-crested cormorants in the Great Lakes [45,48], DDE contamination does not seem to be a significant risk factor to double-crested cormorant populations in this region.

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