IMPACT OF 2,3,7,8-TCDD EXPOSURE ON SURVIVAL, GROWTH, AND BEHAVIOR OF OSPREYS BREEDING IN WISCONSIN, USA

JAMES E. WOODFORD,‡ WILLIAM H. KARASOV,‡ MICHAEL W. MEYER,‡ and LAURA CHAMBERS§
‡Department of Wildlife Ecology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, Wisconsin 53706, USA
§Wisconsin Department of Natural Resources, 107 Surfitt Avenue, Rhinelander, Wisconsin 54501, USA

(Received 2 May 1997; Accepted 20 October 1997)

Abstract—Osprey (Pandion haliaetus) eggs collected from areas (Castle Rock and Petenwell Flowages) 7 km downstream from two bleached-kraft mill facilities from 1992 to 1996 contained much higher levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (range = 29 to 162 pg/g wet weight, n = 18) than eggs collected from two reference areas upstream (range = below detection limits to 23.8 pg/g, n = 15). Levels in eggs of the remaining planar halogenated hydrocarbon congeners and other nonplanar organochlorines were not statistically different between the contaminated and upstream areas (p > 0.05). We placed eggs from the contaminated area into nests at both reference areas (group A) and eggs from the reference areas into nests at the contaminated area (group B). No significant differences in egg hatching or chick fledging rates were observed between these groups and nests left unmanipulated at both reference areas (group C) and the contaminated area (group D). Mass increase rates of chicks differed significantly (p = 0.03), with the highest rates from group C and the lowest rates from group B. This difference cannot be easily attributed to differences in parental nest attentiveness or food provisioning, which were greater at the contaminated area. We conclude that although current planar halogenated hydrocarbon exposure levels were not affecting hatching and fledging rates, they may have affected chick growth.

Keywords—Osprey Tetrachlorodibenzo-p-dioxin Egg exchange Growth Behavior

INTRODUCTION

Ospreys (Pandion haliaetus) are common breeders at the Wisconsin River’s Castle Rock and Petenwell system (CR/P), adjoining reservoirs contaminated with planar halogenated hydrocarbons (PHHs). Planar halogenated hydrocarbons are lipophilic organochlorines (OCs) that undergo significant biomagnification as they progress through increasing trophic levels within a food chain [1]. Hence, top predators merit special attention because they provide information on the impact of maximum exposure levels in an ecosystem and because the toxic effects might manifest only in these species. Here we report measures of PHH exposure and tests for toxic effects in ospreys, a top predator piscivore.

Sediments from the CR/P system are known to be contaminated with relatively high levels of PHHs, such as polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (PCBs). Two bleached-kraft pulp mills, located 7 and 12 km upstream of the CR/P, historically have discharged PHHs, via effluents, into the Wisconsin River [2]. Elevated levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in fish sampled from the CR/P, along with warnings against human consumption of certain species, predicted a significant risk to piscivorous birds foraging at the CR/P. Concentrations of PCDDs and PCDFs in fish sampled from the CR/P that represented those preyed on by ospreys were approx. 30 to 100 times higher than levels in similar species and size classes collected upstream of the kraft mills (M.W. Meyer et al., unpublished data). Overall toxicity levels of the PHHs were calculated using toxic equivalency factors, based on in vitro and in vivo studies, relative to the toxicity of TCDD and expressed as 2,3,7,8-TCDD equivalents (TCDD-Eq) [3].

Previous studies have correlated elevated PHH exposure in piscivorous birds to reproductive impairment, growth retardation, and wasting syndrome [4–9]. Two field studies with birds have suggested an association between reproductive impairment and egg PCDD/PCDF concentrations comparable to levels occurring in osprey eggs from the CR/P [4–10]. These correlational studies, although suggestive, are not conclusive proof of a direct link between the toxins and the effect [11]. Wiemeyer et al. [12] and Kubiak et al. [9] attempted to test experimentally the cause–effect link of OC exposure to reproductive impairment in free-living birds. In both studies, an egg exchange between contaminated eggs and relatively contaminant-free eggs was used to test for a cause–effect relationship. Although both studies reported an apparent direct effect of OC exposure on embryo survival, the analyses of reproductive rates were possibly confounded by other uncontrollable factors (i.e., predation, food abundance, and parental behavior). On the basis of these findings, we designed our study to investigate the impact of current PHH exposure levels to osprey breeding on the CR/P.

Embryo mortality induced by TCDD (or similarly structured OC) has been identified as one of the most sensitive and the only common toxic endpoint measurable in birds [1,8,13,14]. Our study experimentally tested the hypothesis that variation in the hatching rate is caused by direct exposure of the embryo to the toxicants (an intrinsic mechanism), altered parental care (an extrinsic mechanism), or both. In addition,
Fig. 1. Locations of study areas in Wisconsin, USA: Castle Rock and Petenwell Flowages (contaminated area), Mead Wildlife Area (reference area), and Rainbow Flowage (reference area).

by following chick survival and growth through fledging age, we tested the hypothesis that chicks fed a PHH-contaminated diet were fledging and growing at a slower rate than those fed a less contaminated diet. Our goal was to test the effect of PHH exposure in the field while controlling for, or at least measuring, other relevant factors that could affect our measurable endpoints. For example, Clum [15] and Steidl and Griffen [16] concluded that low food availability was responsible for the low productivity in the osprey populations they studied. Also, Poole [17] reported that young, inexperienced breeders (caused by high turnover or low recruitment rates) had much lower reproductive success than experienced adults. To account for these confounding effects, we collected observational data on specific behavioral activities that occur during the nesting period.

MATERIALS AND METHODS

Study areas

All three study areas are at or near artificial impoundments built along the Wisconsin River, Wisconsin, USA (Fig. 1). These impoundments were created for the production of hydroelectric power and to control spring floods [2]. The Rainbow Flowage (Rainbow) reference area is upstream of all known PHH point sources. Our second reference area, the Mead Wildlife Area, is upstream of the two bleached-kraft mill facilities but downstream of other industrial discharges.

Exposure assessment

We collected the third egg of freshly laid (3-4-egg) clutches during early May 1992 (n = 5), 1993 (n = 4), 1994 (n = 5), and 1996 (n = 4) from the CR/P, in 1992 (n = 6) and 1993 (n = 6) from the Rainbow Flowage, and in 1996 (n = 3) from the Mead Wildlife Area. All eggs were refrigerated after collection until they were opened. When opened, the contents of each egg were homogenized, placed into a chemically clean glass jar, and frozen at −30°C in preparation for shipment to the laboratories for analysis. Eggs collected in 1992 and 1993 were analyzed at Wright State University (Dayton, OH, USA) for 17 PCDDs and PCDFs, total PCBs, dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyltrichloroethane (DDT). Eggs collected in 1992 and 1996 were analyzed for the same 17 PCDDs and PCDFs and three coplanar PCBs (PCBs 77, 126, and 169) at the Geochemical and Environmental Research Group laboratory with high-resolution gas chromatography-mass spectrometry. Mass spectrometer resolutions were 10,000 or greater for all monitored functions during the analysis, and all iso- topic ratios were within the methods' specified quality control limits. Internal standard isotope recoveries ranged from 59 to 117% in all samples run for PCDDs and PCDFs and 41 to 123% for the coplanar PCBs.

Experimental tests for effects

Egg exchange. To measure the effect of PHH exposure on osprey embryo survival and postembryonic growth at the CR/P, and to detect a possible cause–effect link, we conducted our egg-exchange experiment in a manner similar to those described by Kubiak et al. [9] and Wiemeyer et al. [12]. Spitzer [18] also concluded that ospreys are very amenable to egg exchanges because of their tolerance of human activity around the nest and the conspicuous locations of nests.

In late April and early May, nests at our study areas were monitored every 2 d to estimate when incubation began. Nests were entered and eggs collected for switching only after the entire clutch was present (6-8 d after initiation). Collected eggs were temporarily replaced by chicken eggs painted to resemble osprey eggs to prevent nest abandonment by the adults. All eggs were transported between study areas in a heated chest (Koolatron model P34A, Brantford, ON, Canada). Temperatures inside the chest ranged from 31 to 36°C.

Group A eggs, taken from the contaminated area, were matched with similarly aged eggs from nests at both reference areas and switched as entire clutches. The switched reference eggs (group B) were then transported back to the CR/P and placed in the same nests where the group A eggs had originated. Unswitched eggs from the reference areas served as controls (group C). We switched 12 group C eggs among themselves to determine if our switching method biased hatching success. In 1996, eggs from both group C and group D, unswitched eggs from the contaminated area, were monitored. After switching, all nests were monitored weekly until they neared their estimated hatch date, at which time they were checked every 2 d until each egg's outcome was known. Eggs that remained unhatched 5 d beyond their estimated hatch date were collected and opened immediately to check content status. Unhatched or missing eggs were categorized as added, infertile, or having no explanation. If at any time a nest appeared abandoned or the adults exhibited aberrant behavior, the nest was immediately entered for examination.

Because of the low number of contaminated eggs available per year, our experiment required 2 years of manipulations to provide adequate statistical power. In 1995, we monitored egg hatching, chick fledging, and chick growth rates for groups A, B, and C (Fig. 2). In 1996, we followed the hatching and fledging rates of eggs and chicks in groups C and D and growth rates for chicks in group D. Therefore, our experiment tested the intrinsic and extrinsic mechanisms singly in 1995 and the combined effect of both mechanisms in 1996. Specifically, comparisons of group A to group C tested for a contaminant effect via a mechanism intrinsic to the eggs, and the group B to group C comparison tested differences via mechanisms ex-
trinastic to the eggs. The comparison of results of group D with those of group C tested the combined extrinsic/intrinsic mechanism. We also compared the outcomes of Group D with those of each group followed in 1995.

**Growth rates.** After hatching, all surviving chicks within groups A, B, and D, along with a portion of those in group C, were individually marked and measured every 7 d (±1 d because of storm events). Chicks were weighed to the nearest gram with a 300-g spring scale, to the nearest 5 g with a 1,000-g scale, and to the nearest 25 g with a 2,000-g scale as their mass increased. Culmen length, recorded from the cere to the tip of the beak [19], was measured with dial calipers to the nearest 0.1 mm. Chicks, still unable to fly, may leap from the nest when disturbed after reaching 50 d of age [20]. Nests were sampled until the oldest chick reached this age.

A large raptor chick’s mass can be greatly overestimated as a result of crop capacity and content [21,22]. To account for this, each chick’s crop size was scored as full, half full, or empty at weighing. Schaeidt and Bird [20] consistently found that 10 to 12% of an osprey chick’s total mass was due to crop content (when full). We corrected field measurements by subtracting 10% of an individual’s measured mass when weighed with a full crop and 5% from individual’s weighed with a half-full crop. Each chick was examined for physical anomalies and ear parasites.

Osprey chicks become sexually dimorphic for mass when chicks reach 28 to 34 d of age [17,20]. Previous studies of ospreys have found that the most common and reliable field criterion for determining sex of chicks is asymptotic mass [17,20]. However, we could not use mass to sex chicks in groups A and B because PHH exposure can reduce chick mass gain [23]. We corrected this oversight in 1996 by determining the genetic sex of all chicks monitored. This was done by chromosome karyotyping of growing feather pulp [24]. We assumed chicks from group C, exposed only to background concentrations of PHHs, had normal mass gains and asymptotes for this region. Only individuals that survived through 7 weeks of age (fledging age) were included in the growth analyses.

**Behavioral tests for effects**

To test for differences in adult behavior between sites, we quantified prey delivery rates and adult nest attendance through direct observations and video recordings. We chose these behaviors because they can influence nest success and growth rates of osprey chicks [25-27]. Direct observations were made from a blind located 50 to 200 m from each nest. One person, equipped with a spotting scope, tripod, binoculars and field notebook, conducted each observation. Each nest observation lasted 4 h, with activities quantified in 5-min time blocks. Each nest 5-min block, the observer entered a code describing the primary activity of each bird that was within sight of the observer. The 4-h observation periods were defined as early morning (sunrise–4 h later), midmorning (09:00–13:00), afternoon (13:00–17:00), and evening (17:00–dark). Each nest was visited at least six times, with one or more visits occurring in all observation periods.

Osprey chicks eat only food provided by the parents. Hence, the number and estimated size of prey deliveries to the nest can provide an index of adult provisioning rates. Prey sizes were estimated as 0 to 16 cm, 16 to 31 cm, 31 to 46 cm, and greater than 46 cm. During both the incubation and nestling periods, we quantified adult nest attendance and prey deliveries per hour to the nest. Nest attendance was defined as the percentage of time that at least one adult was present at the nest during an observation.

**Statistical analysis**

As in previous egg-exchange experiments, the egg was used as the experimental unit [9,28]. Hatching and fledging rates were statistically compared between groups with a 2 × 2 contingency table (in a binomial comparative trial) using the Yates correction for continuity [29]. Culmen length and mass increases were analyzed using a repeated-measures two-factor analysis of variance (ANOVA) (the factors were group and brood size, plus interactions). If a significant difference due to group affiliation was found, multiple individual t-tests were performed between groups A and C, B and C, and D and C for each measurement week to determine whether a specific age or group comparison was responsible. When these tests were performed, the level of significance was adjusted using Bonferonni’s procedure [30]. Mass increase of chicks in groups C and D (through week 7) was analyzed using a repeated-measures three-factor ANOVA (the factors were group, brood size, and gender, plus interactions). In all multiple-factor ANOVAs, if an interaction was not significant (p > 0.05), it was removed and the test was repeated.

Prey delivery and nest attendance data deviated substantially from normal. Consequently, we used the nonparametric Kruskal-Wallis rank sum and Mann-Whitney U tests [31] for analysis. Similar to the mass tests, when a significant study area effect resulted, multiple individual comparisons by area were performed with the level of significance adjusted. We used t tests to compare log-transformed chemical levels between study areas. For all tests except the multiple individual t tests, a 95% confidence interval (p ≤ 0.05) was used to measure significance. All tests were performed using the SYS-TAT® statistical software package [32].

**RESULTS**

**Exposure assessment**

Elevated levels of PCDDs and PCDFs (TCDD-Eq) were found in osprey eggs from the CRP, with TCDD accounting for 80 to 95% of the total PCDD/PCDF TCDD-Eq in each egg (Table 1). Log-transformed data did not differ significantly by year collected (p > 0.05), so results were pooled for comparison with eggs from the reference areas. Levels of TCDD and total PHHs (TCDD-Eq) in CRP eggs were significantly higher than levels in eggs from either reference area (p < 0.001; Table 1). However, coelomic PCBs and all other PCDD/PCDF congener levels were not different. Concentrations of TCDD and total PCDDs/PCDFs (TCDD-Eq) in eggs from the Mead Wildlife Area were not significantly greater than con-
<table>
<thead>
<tr>
<th>Study area</th>
<th>Year</th>
<th>No. eggs</th>
<th>2,3,7,8-TCDDa</th>
<th>PCDDs/PCDFs (TCDD-Eq)b</th>
<th>Coplanar PCBs (TCDD-Eq)b</th>
<th>Coplanar PCBs (TCDD-Eq)b</th>
<th>Coplanar PCBs (TCDD-Eq)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle Rock/Peterwell</td>
<td>1992</td>
<td>5</td>
<td>77 (47–162)</td>
<td>84 (52–171)</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>4</td>
<td>108 (80–148)</td>
<td>119 (88–162)</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>5</td>
<td>75 (29–148)</td>
<td>86 (64–171)</td>
<td>63 (39–87)</td>
<td>57 (34–74)</td>
<td>41 (26–59)</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>4</td>
<td>59 (54–67)</td>
<td>71 (59–94)</td>
<td>49 (40–61)</td>
<td>43 (35–57)</td>
<td>31 (23–35)</td>
</tr>
<tr>
<td>Rainbow Flowage</td>
<td>1992</td>
<td>6</td>
<td>5 (ND–24)</td>
<td>7 (6.4–27)</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>6</td>
<td>4 (1–7–19)</td>
<td>4 (2–28)</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>3</td>
<td>2 (1–3)</td>
<td>8 (7–9)</td>
<td>41 (17–80)</td>
<td>36 (15–70)</td>
<td>24 (9–47)</td>
</tr>
</tbody>
</table>

*a ND = not detected; NM = not measured; PCBs = polychlorinated biphenyls; PCDD = polychlorinated dibenzo-p-dioxins; PCDF = polychlorinated dibenzofurans; TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin, and TCDD-Eq = 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents.

*b Statistically significant difference between study areas (p < 0.05).

Outcomes for the unhatched eggs were as follows: added, n = 10 (group A, n = 3; group B, n = 1; and group C, n = 6); infertile, n = 3 (group A, n = 2; and group C, n = 1); and disappeared with no explanation n = 11 (group A, n = 1; group B, n = 3; group C, n = 5; and group D, n = 2).

The fledging rate of chicks in group B (94%) was significantly lower than that of group C chicks (67%; p = 0.03, 1 df) during our 1995 experiment (Table 3). Although this difference is significant, we predicted a lower, not higher, fledging rate for chicks with the greatest exposure to contaminants (via ingestion of contaminated fish) compared to those with the least exposure. In fact, the fledging rates for all experimental groups were greater than those of group C in either year. Results from an interyear test of fledging data were similar to results from the 1995 data alone (Table 3).

**Egg exchange.** The hatching rate of 27 eggs taken from the contaminated site that were incubated at the reference sites (group A) was 74% (Table 3). This rate was not significantly different from that of the 33 unswitched eggs incubated at the reference sites (group C, 1995, 73%; p = 0.910, 1 df). Likewise, the hatching rate of the 22 reference site eggs incubated at the contaminated site (group B, 82%) was not significantly different from that of group C (p = 0.626, 1 df). We originally switched 25 group B eggs, but three eggs from one nest were eliminated from the test after the nest was preyed upon by a raccoon (*Procyon lotor*). The hatching rate of group C eggs from 1996 did not differ statistically from that of group C in 1995 (p = 0.28, 1 df), so we pooled 1995 and 1996 group C hatching rates and repeated the tests. Results were nearly identical to those of 1995 alone. Group D eggs monitored in 1996 had a higher hatching rate (91%) than all other groups, but the difference was not significant.

The 12 group C eggs that were switched among themselves hatched at a rate (75%) similar to those of both the group A and other group C eggs.

**Growth rates.** A significant group effect for mass increase occurred during the first 4 weeks after hatching for chicks measured in 1995 (*F* = 3.25, p = 0.04, Fig. 3). Multiple individual comparisons between groups (i.e., A and B, B and C, A and C) revealed no significant differences. When mass measurements from group D were added to the 1995 data for the first 4 weeks, the group effect became nonsignificant (*F* = 1.72, p = 0.09, Fig. 3), whereas the brood size factor became significant (*F* = 3.99, p = 0.009). Mass increase during the entire nestling period for groups C and D was significantly affected by brood size and gender (brood size: *F* = 4.74, p < 0.001; gender: *F* = 8.57, p < 0.001). No other group or interactions were statistically significant.

Chicks followed at one nest in 1996 (group D) seemed to exhibit symptoms of wasting syndrome [33]. During our visit

<table>
<thead>
<tr>
<th>Study area</th>
<th>Year</th>
<th>No. eggs</th>
<th>Total PCBsb</th>
<th>DDT</th>
<th>DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle Rock/Peterwell</td>
<td>1992</td>
<td>5</td>
<td>1,586 (1,246–1,907)</td>
<td>3.7 (1.2–9)</td>
<td>340 (79–823)</td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>4</td>
<td>1,666 (809–2,745)</td>
<td>5 (1–21)</td>
<td>521 (116–939)</td>
</tr>
<tr>
<td>Rainbow</td>
<td>1992</td>
<td>6</td>
<td>482 (285–804)</td>
<td>3.6 (0.5–18)</td>
<td>312 (176–827)</td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>6</td>
<td>478 (200–1,694)</td>
<td>1.6 (0.9–9)</td>
<td>197 (89–739)</td>
</tr>
</tbody>
</table>

*b DDE = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyldichloroethane; and PCBs = polychlorinated biphenyls.

*b Statistically significant difference between study areas (p < 0.05).
on July 4 (growth week 4), all three chicks had a mass greater than the mean mass for the entire group and appeared healthy. On our next visit (July 11), only one chick remained. This chick was emaciated and appeared to be starving. Its mass had increased 48 g, compared to the mean mass increase of 320 g for all other group D chicks during this same interval. No food was apparent, because two partially eaten prey items were present in the nest. During our next visit (July 18), the chick was in the same physical condition as the week before (mass increase = 0 g, mean mass increase = 161 g). This time one partially eaten prey item was present. No chick or carcass was found on our July 25 visit.

Culmen length increase does not become sexually dimorphic in osprey chicks [28], so we tested for an effect on culmen growth during the entire nesting period. Neither a group nor a brood size effect occurred using the 1995 data set (group: \( F_{2,194} = 1.434, p = 0.253 \); brood size: \( F_{4,196} = 1.011, p = 0.419 \)) or the 1995 and 1996 data combined (group: \( F_{2,285} = 1.597, p = 0.06 \); brood size: \( F_{3,285} = 0.883, p = 0.51 \)). Culmen growth curves for each group were mostly linear (as expected).

No external abnormalities were observed on any of the nestlings examined (\( n = 112 \)) during our study. However, nestlings raised in the Rainbow area (groups A and C in 1995 and group C in 1996) were infested with ear parasites. Bird blowfly larvae (Protocalliphora avium) were first seen in the external ear of nestlings in late June, with infestations lasting through mid-July. We suspected no direct mortality due to these parasites. Infected chicks normally had two to five pupae per ear, with the feathers around the ear smeared with dried blood caused by frequent scratching. These infestations occurred exclusively at the Rainbow study area.

Behavioral tests for effects

**Nest behavior.** Direct observations of nest behavior were conducted at CR/P (1994, \( n = 4 \); 1995, \( n = 5 \); 1996, \( n = 2 \); total observation time, 220 h), Rainbow (1994, \( n = 5 \); 1995, \( n = 4 \); total observation time, 172 h), and the Mead Wildlife Area (1995, \( n = 4 \); total observation time, 88 h). Time-lapse video accounted for 4% of the observational data.

Fish made up 100% of prey items identified. Prey were estimated to a size class in 237 of 272 (87%) total deliveries. The greatest percentage of deliveries to nests at each study area were of the 16- to 31-cm size class. Smelts (Lepeomis spp.; 23%) and yellow perch (Percula flavescens; 20%) represented the greatest proportions of prey identified (68% of total deliveries) at CR/P nests. Prey delivery rates were not significantly different between broods of one, two, or three (\( \chi^2 = 0.575, 2 \text{ df}, p = 0.750 \)) at any of our study areas, so they were pooled by study area for the remaining analyses (Table 4). First, we tested for year and study area effects. Overall, delivery rates from 1994 were significantly higher than rates observed in 1995 during both the incubation and nesting periods (incubating: \( \chi^2 = 9.404, 2 \text{ df}, p = 0.009 \); incubation: \( \chi^2 = 8.537, 1 \text{ df}, p = 0.003 \)). Observational data collected from the CR/P in 1996 were used only to test for a change in rates at the CR/P between study years. This test showed that delivery rates to CR/P nests were not significantly different by year of study. When we tested between years for the Rainbow area alone, rates from 1994 were significantly higher than rates observed in 1995 during the incubation period (\( \chi^2 = 6.876, 1 \text{ df}, p = 0.009 \)). The same test during the nesting period was nearly significant (\( \chi^2 = 4.484, 1 \text{ df}, p = 0.034 \); level of significance adjusted to \( p < 0.03 \)). Thus, the overall decline in delivery rates from 1994 to 1995 was largely due to the decline observed at the Rainbow Flowage (which may be attributable to an observer effect). Results of all other between-year tests were not significant. Comparisons between study areas by year of study were not statistically different (1994: \( \chi^2 = 0.162, 1 \text{ df}, p = 0.803 \); 1995: \( \chi^2 = 4.68, 2 \text{ df}, p = 0.007 \)).

Statistical tests revealed no difference between years in the rate of adult nest attendance within each study area (Fig. 4). This finding was true for both the incubating and incubation periods. A Kruskal-Wallis test of nest attendance showed a significant difference by area of study (\( \chi^2 = 6.047, 2 \text{ df}, p = 0.049 \)) for the 1995 data. Multiple individual Mann-Whitney \( U \) test comparisons revealed that adult attendance at CR/P nests was significantly greater than attendance at Mead Wildlife Area nests in 1995 (CR/P vs Mead Wildlife Area: \( \chi^2 = 6.488, 1 \text{ df}, p = 0.011 \)). No results of other multiple individual tests between study areas were statistically significant.

**DISCUSSION**

What effect have recent levels of PHH contamination had on ospreys breeding in a contaminated reservoir system, and

**Table 3. Effects of elevated 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on egg-hatching and chick-fledging rates of ospreys in Wisconsin, USA, 1995-1996**

<table>
<thead>
<tr>
<th>Group</th>
<th>Year</th>
<th>No. eggs</th>
<th>Hatched eggs (%)</th>
<th>Hatched eggs that fledged (%)</th>
<th>Significance tests&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Matrix of comparisons of hatching rates (p)</th>
<th>Matrix of comparisons of fledging rates (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1995</td>
<td>27</td>
<td>74</td>
<td>73</td>
<td>0.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>1995</td>
<td>22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82</td>
<td>84</td>
<td>0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>1995</td>
<td>33</td>
<td>73</td>
<td>67</td>
<td>0.77</td>
<td>0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44</td>
</tr>
<tr>
<td>D</td>
<td>1996</td>
<td>21</td>
<td>86</td>
<td>50</td>
<td>0.77</td>
<td>0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44</td>
</tr>
</tbody>
</table>

<sup>a</sup> *p* values found by the \( \chi^2 \) test with 1 df.

<sup>b</sup> *Group A = contaminated eggs, reference adults; B = reference eggs, contaminated adults; C = reference eggs, reference adults; and D = contaminated eggs, contaminated adults.*

<sup>c</sup> *Extrinsic mechanism of action could have been responsible.*

<sup>d</sup> *Intrinsic or extrinsic mechanism of action could have been responsible.*

<sup>e</sup> *Three eggs from one nest were not included because of raccoon predation.*
what might be the possible mechanisms of action? Our goal was to answer these questions experimentally by testing three possible hypotheses. On the basis of a literature review and our own experience with ecotoxicological studies of this nature, we predicted which mechanisms were the most probable and designed our experiment and direct observations to address them. The following discussion considers these hypothetical effects and the possible mechanisms responsible.

Our first hypothesis was that current PHH exposure levels were affecting embryo survival and hatching success by either an intrinsic direct mechanism or an extrinsic mechanism. To test this hypothesis, we examined one intrinsic and several extrinsic mechanisms that could have been occurring. In seven other avian studies, in both the laboratory and the field, elevated levels of PHHs in eggs were associated with reduced embryo survival [9, 10, 34–38]. This association was not apparent in our study.

Inadequate parental care reportedly has caused lower hatching success in herring gulls (Larus argentatus) [39] and forster's terns (Sterna forsteri) [9] breeding on the Great Lakes (by an extrinsic effect). Had this effect occurred in our study, the three mechanisms most likely responsible would have been inexperience of the adults, altered parental care induced by PHH contamination, and low food provisioning by males to incubating females (defined in our study as the prey delivery rate). Our experimental results refute any measurable effect of these mechanisms. Furthermore, observations of nest attendance and turnover rates [40] suggest that both adult experience and parental care were, if anything, superior at the CR/P. The male osprey provides all food required for himself and the female during the incubation period [17]. Again, our observational data confirm our experimental results that, in all but one case, food provisioning rates during the incubation period were greater at CR/P nests (Table 4). Collectively, our results indicate that the no-observable-adverse-effect level for osprey embryo survival is equal to or greater than 136 pg/g wet weight for PHH TCDD-Eq.

Our second hypothesis was that current exposure levels of PHHs in ova or through posthatch ingestion were affecting chick survival. Results from our experiment detected no mean.

Table 4. Delivery rates and size class distributions of prey delivered to osprey nests in Wisconsin, USA, 1994–1996

<table>
<thead>
<tr>
<th>Nestling perioda</th>
<th>Incubation periodb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR/P (n = 12)</td>
</tr>
<tr>
<td>Fish delivered in 1994, mean (SE)</td>
<td>0.44 (0.05)</td>
</tr>
<tr>
<td>Total time observed, h</td>
<td>100</td>
</tr>
<tr>
<td>Fish delivered in 1995, mean (SE)c</td>
<td>0.37 (0.04)</td>
</tr>
<tr>
<td>Total time observed, h</td>
<td>100</td>
</tr>
<tr>
<td>Fish delivered in 1996, mean (SE)</td>
<td>0.45 (0.15)</td>
</tr>
</tbody>
</table>

* n = number of nests monitored; and NM = not measured.
* Mann–Whitney U test, Rainbow 1994 versus Rainbow 1995; p = 0.009.
* Kruskal–Wallis rank sums, 1994 versus 1995; p = 0.001.
urable effect (through either an intrinsic or extrinsic mechanism) on chick survival. Alterations of biochemical or endocrine function were not examined in this study; however, if they occurred, they did not affect chick survival.

The third hypothesis was that current exposure levels of PHHs in ova or through posthatch ingestion were affecting the rate of chick mass increase. Chicks raised at the CR/P had lower mass increase rates than reference chicks in 1995. This effect may have been caused by either an intrinsic or extrinsic mechanism or both. One intrinsic mechanism possible was that an endocrine disruption or other biochemical alteration occurred in chicks fed the more contaminated diet in 1995. A change of thyroid levels was documented in osprey chicks exposed to PHHs elsewhere; however, the physical effects of these changes were not apparent [41].

Three plausible extrinsic mechanisms that may have affected chick mass increase were low food availability, poor adult provisioning (which may have been a function of the first), and poor environmental conditions (most likely heat stress). Both the low food availability and poor adult provisioning mechanisms are eliminated by our observational data that CR/P nests, on average, received more prey deliveries per hour during the nesting period than nests from the reference areas. Additionally, prey items delivered to CR/P nests were larger than those delivered to the reference nests [40]. These results suggest that more biomass was provided to chicks raised at CR/P than those raised at reference areas. Furthermore, sibling acts of aggression were rarely observed during direct observations, consistent with typical behavior of well-fed young [42]. Finally, prey delivery rates at the CR/P were as high as or higher than those reported in other food-rich environments [43,44]. Stress from heat or exposure to the sun may have caused lower mass increases. The summer of 1995 was one of warmest recorded in Wisconsin during the past 100 years (J. Foley, personal communication). At our study areas, female ospreys provide the only shade available for the young neonates. With the female’s presence required to reduce exposure, one would predict that young from nests where the female is present most would have the lowest amount of heat stress. Results from direct observations show that adult attendance rates at the CR/P were higher than those observed at both reference areas, which suggests that heat stress was not a mechanism of lower mass increase. Clearly, further investigation of chick growth and survival after fledging is warranted.

Susceptibility of ospreys to PHHs

Levels of TCDD in osprey eggs collected at the CR/P during this study (geometric mean, 78 pg/g) were higher than levels reported in other avian studies (range, 15–37 pg/g [9,10,14,45,46]) with one exception (211 pg/g in great blue heron eggs [8]). Conversely, eggs from these other avian studies contained levels of coplanar PCBs that were higher than those from the CR/P (range, 31–63 pg/g TCDD-Eq). Consequently, CR/P osprey eggs, although relatively heavily contaminated with TCDD, had lower total PHH TCDD-Eq than eggs in the previous studies. Concentrations of DDT and DDE were five to eight times less than levels associated with 10% egg-shell thinning and reproductive impairment in osprey populations elsewhere [47].

As demonstrated elsewhere, tolerance of PHHs by birds is species-specific [1,14]. The sensitivity of ospreys, at least those residing on the CR/P to PHHs is apparently intermediate. We reached this conclusion by comparing our study’s exposure levels and toxic effects to those from other areas that had elevated PHH exposure [8–10,14,41,45,46]. For example, exposure to PHHs at a mean level of 41 pg/g PHH TCDD-Eq (in eggs) has been associated with a lower rate of reproduction.
in cormorants (Phalacrocorax carbo) [14]. Also, White and Seginak [10] purported that wood ducks (Aix sponsa) exposed to 20 to 50 ppt of PCDDs and PCDFs (TCDD-Eq) had lower reproduction rates. In contrast, bald eagle embryos [46] exposed to 337 pg/g TCDD-Eq had no reduction in hatching rates but did exhibit induction of cytochrome P450, which is a physiological indication of exposure and not necessarily a toxic effect. Domestic poultry appear to be the most sensitive avian species studied to date (10 ppt TCDD [36]).

Egg-exchange technique

Previous experiments using egg-transfer techniques to measure contaminant effects on birds have produced mixed results [9,12]. One major limitation reported was the need for the target species to have considerable tolerance of human activity in and around its nests [48]. In addition, Kabik et al. [9] and Wiemeyer et al. [12] reported that such experiments were complex in application and logistically difficult to perform, with which we concur. For our study, the egg exchange was invaluable to test experimentally the effect of contaminants on ospreys. To obtain these results, intensive monitoring was needed to determine when each clutch was initiated. This then allowed similarly aged eggs to be interchanged between nests, minimizing any technique effect. Even though the egg exchange provided good results, our final conclusions would not have been possible had we not collected data on behavioral activities.

We are confident that our interyear comparison of egg-hatching and chick-fledging rates was justified because ospreys at the CR/P had low adult turnover [40] and similar prey physical indices during all 3 years of study (Table 4). Poole [17] reported that the most important factors influencing breeding rates, if suitable nest sites were available and predators had been controlled for, were adult age and food availability.

As stated earlier, the egg rather than the nest was used as the experimental unit in our statistical analysis. Sample independence within a clutch, an assumption we made when testing the effect of PHH exposure on hatching rates, could be questioned. We are comfortable with this assumption because raccoon predation caused three of the four complete clutch failures. In other nests where one egg failed to hatch, the other eggs did hatch. Thus, we argue that relative to the factors affecting egg hatchability (i.e., parental care, contaminant exposure, infertility, and natural mortality), these partial nest failures may indicate that natural independence exists between eggs within a nest.

Furthermore, had we used the nest as the experimental unit, our results would have been very similar, but the power to detect an effect would have been much lower. The lack of any hint of an effect on egg hatching or chick survival due to contaminant exposure indicates that a very large sample size would have been needed to detect an effect. A power analysis (with $\beta = 0.20$ and $\alpha = 0.05$) indicated that with a sample size of approx. 25 eggs in each experimental group, we could have detected a true statistical difference in egg-hatching or chick-fledging rates of approx. 30% between the reference and experimental groups. It follows that if we had used the nest as the experimental unit, our study would have required an additional 3 or more years to complete.

**SUMMARY**

Osprey eggs from the CR/P were significantly more contaminated with PHHs, particularly TCDD, than eggs from nests farther upstream on the Wisconsin River. No association between egg-hatching or chick-fledging rates and current PHH exposure levels (no-observed-adverse-effect level for osprey egg hatching $\geq 136$ pg/g total PHH TCDD-Eq) was observed. A repeated-measures test of mass increase, after hatching in 1995, indicated that nestlings exposed to elevated PHHs in ova or that grew posthatch ingestion of contaminated fish grew more slowly than those with much lower exposure. These and other data led us to conclude that, although current PHH exposure levels were not affecting hatching and fledging rates, they may have affected the normal development of osprey chicks raised at the CR/P.

Acknowledgement—We thank our field assistants, L. Gudzinski and C. Reinert, and the many volunteers who helped us throughout the project. We also thank P. Van Tuinen for determination of the genetic sex of chicks monitored in 1996. Project support came from the Zoological Society of Milwaukee County, the Wisconsin Society for Ornithology, a U.S. Fish and Wildlife Service Habitat Enhancement Grant, the Wisconsin Department of Natural Resources, and the Max McGraw Wildlife Foundation.

**REFERENCES**


