Reproduction of Black-crowned Night-Herons Related to Predation and Contaminants in Oregon and Washington, USA

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Abstract.—We studied reproductive characteristics of Black-crowned Night-Herons (*Nycitcorax nycticorax*) at 4 colonies in south central Washington and 1 colony in north central Oregon in 1991. Nest success, adjusted using the Mayfield method, was significantly different between colonies and ranged from 12-84% to hatching and 12-73% to 14 days post-hatching. The mean number of young surviving to 14 days of age in each colony ranged from 0.47-1.94 per nesting female (includes recycling efforts that involve laying more than 1 clutch). There were marked intercolony differences in clutch size and incidence of recycling. Predation (primarily avian) was a major factor that adversely affected nest success in 3 colonies and was relatively unimportant in 2 colonies. Residues of DDE, total polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and other compounds in eggs were generally low and apparently had little influence on reproductive success at any of the colonies. Mean eggshell thinning ranged from 7-11% in comparison to a pre-1947 norm for eggs measured in museum collections. Cytochrome P450 enzyme (EROD, PROD, and BROD) induction in livers of pipped embryos by colony ranged from low to average in comparison with other colonies throughout the U.S. Average EROD and BROD activities were highest at Sand Dune Island and were lowest at Potholes Reservoir which was designated the reference colony. In relation to our study of 3 of the 5 colonies in the early 1980s, residues of DDE and several related compounds appeared to decline, nest predation rates increased, and nest success decreased at all 3 colonies. *Received 12 September 1996, accepted 2 February 1997.*

Key words.—Black-crowned Night-Heron, clutch size, corvid predation, cytochrome P450, dioxin, eggshell thickness, *Nycticorax nycticorax*, Oregon, organochlorines, reproduction, Washington.

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The Black-crowned Night-Heron (*Nycticorax nycticorax*) has been the subject of a number of studies related to effects of contaminants on reproductive success, eggshell thinning, adult survival, and other factors (Custer *et al.* 1983, Henny *et al.* 1984; Hoffman *et al.* 1986; Rattner *et al.* 1993, 1994). By the early 1950s, DDT was probably related to a decline of Black-crowned Night-Herons in the northeastern United States that was associated with eggshell thinning of 18% (Peterson 1969, Anderson and Hickey 1972). The problems seemed to dissipate after the ban on DDT in 1972, but high levels of DDE (a metabolite of DDT) in eggs persisted until 1980 in some colonies in the western United States and resulted in reduced productivity, particularly in the colony at Ruby Lake, Nevada (Henny *et al.* 1984).

Environmental contaminants are considered to pose a hazard to biota in the Columbia River system, particularly in the lower stretch where Bald Eagles (*Haliaeetus leucocephalus*) are experiencing decreased productivity (Anthony *et al.* 1993), and mink (*Mustela vison*) are nearly extirpated (Henny *et al.* 1996). Further upstream, contaminants of agricultural and industrial origin are still of concern. For example, a white paper mill at Wallula, Washington utilized chlorine in the bleaching process; some of the most toxic dioxins and furans are formed in the presence of chlorine (Clement *et al.* 1989). The objectives of this study were to study the basic breeding biology of the Black-crowned Night-Heron at a colony near the white paper mill at Wallula and at 4 other colonies in the region and to determine potential effects of contaminants on reproductive success, eggshell thinning, and other toxicological responses.

METHODS

Five nesting colonies of Black-crowned Night-Herons were selected for study in 1991 (Fig. 1). Colonies were located in Washington at Crescent Island near Wallula, 46°05'N, 118°55'W; Foundation Island near Burbank, 46°05'N, 118°57'W; Potholes Reservoir (designated reference site) near Moses Lake, 47°03'N, 119°23'W; and Sand Dune Island near Paterson, 45°55'N, 119°37'W; and in Oregon at Threemile Island near Boardman, 45°50'N, 119°59'W. The study was initiated on 12 April at Crescent Island, 13 April at Sand Dune Island and Threemile Island, 16 April at Foundation Island, and 26 April at Potholes Reservoir. The primary purposes of the site visits were to find and mark nests, to collect eggs and young; and to determine nest fate. Nests were individually marked with numbered strips of plastic tape or the number was written on the tree near the nest. Nest trees were marked with plastic flagging. Nest contents as well as predation, abandonment, recycling, and other information were recorded at each visit. Clutches in active nests were classified as complete when there was no change in the number of eggs for ≥ 1 wk. An aluminum leg band was placed on 61 young captured when ≥ 2 wk of age in order to provide some information on their survival to the end of our study.

Eggs collected for chemical analysis (2 from Crescent Island and 3 from each of the other 4 colonies) were stored in a refrigerator (~4°C) for ≤ 2 days until they were weighed, volumed, and measured. Each egg was opened at the equator, and the contents were transferred to a chemically-cleaned jar. The samples were stored in a freezer (~ -4°C) until they were analyzed. Eggshells were rinsed in tap water and thoroughly dried. Eggshell thickness (including shell membranes) was measured at 3 sites on the equator of the shell with a micrometer graduated in units of 0.01 mm; the 3 measurements were then averaged. Pipped eggs collected in the field, as well as eggs to be artificially incubated until pipping, were placed in a Koolatron® where humidity and temperature were controlled to maintain viability of the embryos until they were processed. The shell of the pipped egg was opened, and the mass of the whole body, including the yolk sac, was determined. The embryo was then euthanized by decapitation. The entire liver and yolk sac were excised from the body, and the mass of each was determined. The liver was placed in a

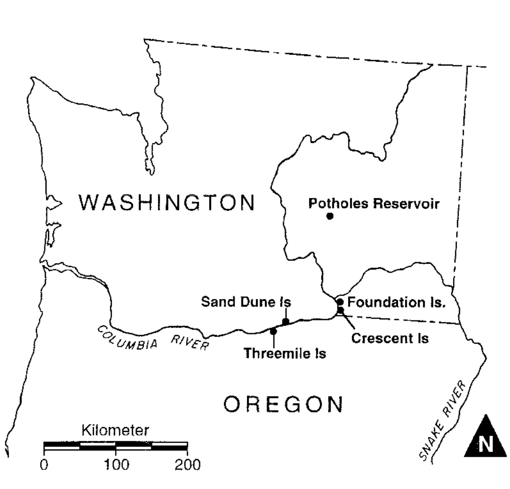

Figure 1. Map of Washington and Oregon showing locations of the 5 nesting colonies of Black-crowned Night-Herons, 1991.

plastic cryotube containing glycerol, the cryotube was inverted several times to thoroughly coat the tissue. The liver was then snap frozen and stored in liquid nitrogen until the cryotubes were shipped on dry ice to the Patuxent Wildlife Research Center. Upon arrival, they were placed in an ultra-cold freezer (-80°C) until cytochrome P450 associated activities of monooxygenases including benzyloxyresorufin-O-dealkylase (BROD), ethoxy resorufin-O-dealkylase (EROD), and pentoxyresorufin-O-dealkylase (PROD) were measured. Monooxygenase activity was measured following methods described by Rattner *et al.* (1993). Enzyme activity was calculated by linear regression, and values were converted to picomoles of product formed per minute per milligram (pmol min^{-1} mg^{-1}) of microsomal protein. The lower limit of detection for these assays was ≤0.5 pmol min^{-1} mg^{-1} microsomal protein. The Potholes Reservoir Colony was designated as a reference colony since cytochrome P450 activities were among the lowest in the United States (Rattner *et al.* 1993).

Residue analysis of 14 eggs (collected from active nests with clutch sizes ≥ 2) was conducted at the Mississippi State Chemical Laboratory. All residues are adjusted to fresh wet weight based on egg volume (Stickel *et al.*, 1973). For anaylses of organochlorine pesticides and polychlorinated biphenyls (PCBs), 10 g of egg contents were thoroughly mixed with anhydrous sodium sulfate and soxhlet extracted with hexane for 7 h. The extract was concentrated by rotary evaporation; transferred to a tared test tube, and further concentrated to dryness for lipid determination. The weighed lipid sample was dissolved in petroleum ether and extracted 4 times with acetonitrile saturated with petroleum ether. Residues were partitioned into petroleum ether which was washed, concentrated, and transferred to a glass chromatographic column containing 20 g of Florisil. The column was eluted with 200 ml 6% diethyl ether/94% petroleum ether (Fraction I) followed by 200 ml 15% diethyl ether/85% petroleum ether (Fraction II). Fraction II was concentrated to appropriate volume for quantification for residues by packed or capillary column electron capture gas chromatography. Fraction I was concentrated and transferred to a silicic acid chromatographic column for additional cleanup required for separation of PCBs from other organochlorines. Three fractions were eluted from the silicic acid column; each was concentrated to appropriate volume for quantification of residues by packed or megabore column, electron capture gas chromatography. PCBs were found in Fraction II. The lower limit of sensitivity was 0.5 μg/g for total PCBs and 0.01 μg/g for organochlorine pesticides and their metabolites.

Analysis of the 14 eggs for residues of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) was conducted at the Geochemical & Environmental Research Group, Texas A & M University. Matrix specific extraction, analyte specific cleanup, and high resolution gas chromatography combined with high resolution mass spectroscopy (HRGC/HRMS) were used. In this process, selected cleanup procedures were used to eliminate interference. A specified amount of the sample matrix was spiked with a solution containing each isotopically (13C12) labeled dioxin or furan. The sample was then extracted according to a matrix specific extraction procedure. Aqueous samples that were judged to contain 1% or more solids, and solid samples that show an aqueous phase were filtered. The solid phase and the aqueous phase then were extracted separately, and the extracts combined before cleanup. Following a solvent exchange step, the extracts were cleaned up by column chromatography on alumina, silica gel, and AX-21 activated carbon on silica. The preparation of the final extract for HRGC/HRMS analysis was accomplished by adding 2 isotopically (13C12) labeled recovery standards.

Two µL of the concentrated extracts were injected into an HRGC/HRMS system capable of performing selected ion monitoring at resolving powers of at least 10,000 (10% valley definition). The identification of the 2,3,7,8-substituted isomers was based on their elution at their exact retention time and the simultaneous detection of the 2 most abundant ions in the molecular ion region. Confirmation is based on a comparison of the ratios of the integrated ion abundance of the molecular ion species to their theoretical abundance ratios. Quantitation of the individual congeners is achieved in conjunction with the establishment of a multipoint calibration curve for each homologue, during which each calibration solution is analyzed once. The lower limit of sensitivity for TCDD and TCDF was approximately 1 pg/g.

Statistical analysis using SAS computer programs (SAS, Institute, Inc. 1994) included 1-way ANOVA; Tukey's HSD method for separation of multiple means when differences were significant, Fisher's exact Chi-square (χ^2) test, and regression analysis for determining the relation of DDE with eggshell thickness. We made comparisons of clutch size, nest initiation dates, young surviving to 14 days, and effect of collection of 1 egg on production of young by using χ^2 tests. Clutch size was separated into 2 groups (2 to 3 eggs or 4 to 5 eggs). Size distribution of first clutches laid as well as all clutches (including replacements) were used in the overall χ^2 test and comparison of each pair of colonies.

Nest initiation dates (date of first egg laid in the first clutch in each nest) were compared to the overall median Julian date of 111 (21 April) derived from data from all 5 colonies. Nests were separated into 2 groups (< 111 and > 111) that were used in the overall χ^2 test and comparison of each pair of colonies.

Young per nest that survived to 14 days of age were separated into 2 groups (0 to 1 young and 2 to 4 young) that were used in the overall χ^2 test and comparison of each pair of colonies. The number of young per nest that survived to 14 days of age was also compared on the basis of egg collection (0 or 1 egg or from first clutches) in the overall χ^2 test and intracolony comparisons.

The Mayfield procedure (Mayfield 1961, 1975; Johnson 1979) was used to calculate daily survival rates (DSR) of marked nests in the 5 colonies; statistical differences were tested using the program CONTRAST. Using ANOVA and Tukey's HSD test, survival rates of nests were calculated from laying of the first egg to hatching (0-27 days); from hatching to 14 days after hatch, when young first being leaving the nest (28-41 days), and for both periods (0-41 days), we also compared success of nests with or without egg collections using ANOVA and Tukey's HSD test. Statistical significance was considered as $P \leq 0.05$.

RESULTS

Colony Characteristics

Four of the colonies were located on islands in the Columbia River and 1 was located on a reservoir (Fig. 1). At Potholes Reservoir, most nests were located in willows (*Salix* spp.) that were widely scattered along the edges of the reservoir. We marked 54 nests that were located from 1-6 m above the ground or water. We made no attempt to obtain a complete census, but Fitzner *et al.* (1979) indicated that about 1,000-1,500 pairs of Black-crowned Night-Herons were nesting at Potholes Reservoir in 1978.

Using dredge spoil and other fill, Crescent Island was constructed, as a mitigation measure in the 1980s. The Black-crowned Night-Heron colony probably was established in 1991 because there was no record of their nesting in 1990 (Al Sutlick, U.S. Corps of Engineers, pers. comm.). The nests were 1-3 m above the ground in young willows that had a maximum height of about 4 m. The 31 nests that we located and marked essentially represented a complete count.

The Foundation Island Colony was located along the north shore of the Columbia River. Black cottonwoods (*Populus trichocarpa*), mulberries (*Morus* spp.), and willows were found on part of the island. Nearly all of the Black-crowned Night-Heron nests were in mulberry trees with only a few in willows; height of nests above ground ranged from 3-9 m. We marked 82 nests; the total number of nests on Foundation Island was estimated at 200.

The Sand Dune Island colony was located near the middle of the Columbia River on the Umatilla National Wildlife Refuge. The colony was established in the 1980s after willows reached sufficient height to support the nests. We marked 69 nests, and this represented essentially a complete count. Nests were located from 2-9 m above the ground.

The Threemile Island colony was located in Oregon near the south shore of the Columbia River. We marked 54 nests, all of which were located in mulberry except for 1 in big sagebrush (*Artemisia tridentata*). We located and marked most nests on the island. Nest height ranged from 2-7 m above ground.

Clutch Size

Complete clutches in the 5 colonies ranged from 2-5 eggs (Table 1). There were

Table 1. Variation in the distribution of clutch size of Black-crowned Night-Herons, 5 Oregon or Washington Colonies, 1991.

	Clutch size					
	1st clutch[1]			All clutches[2]		
Colony	N	Mean±SE	Range	N	Mean±SE	Range[3]
Sand Dune Island	58	3.93±0.101	2-5A	61	3.92±0.097	2-5A
Threemile Island	44	3.50±0.100	2-5B	59	3.51±0.082	2-5B
Foundation Island	58	3.52±0.082	2-5BC	82	3.37±0.075	2-5BC
Crescent Island	15	3.33±0.126	3-4BC	18	3.33±0.114	3-4BC
Potholes Reservoir	33	3.09±0.118	2-4C	40	3.10±0.106	2-4C

[1]Overall χ^2_4 = 18.327, P = 0.001.
[2]Overall χ^2_4 = 21.535, P = 0.001.
[3]Distribution of clutch sizes (2 to 3 eggs or 4 to 5 eggs) in each column that are significantly different from one another do not share the same letter.

significant differences in clutch size distribution by colony. The largest incidence of 4 to 5 eggs in apparent first clutches or all clutches (including replacements) occurred at Sand Dune Island and the lowest at Potholes Reservoir. The incidence of 5-egg clutches varied from 23% at Sand Dune Island, 2% at Foundation and Threemile Islands, and 0% at Crescent Island and Potholes Reservoir.

Replacement Clutches

The incidence of replacement clutches varied by colony. At Foundation Island, 25 second-clutches, 7 third-clutches, and 3 fourth-clutches were observed in the 82 nests. At Threemile Island, the 54 nests had 15 second-clutches and 2 third-clutches. Second clutches were noted at Crescent Island (3 in 31 nests), Potholes Reservoir (9 in 54 nests), and Sand Dune Island (4 in 69 nests).

Eggshell Thickness

Mean thickness of eggshells ranged from 0.246 mm at Foundation Island to 0.261 mm at Threemile Island; shell thickness means for each of the 5 colonies ranged from 5.1-10.5% less than the pre-1947 mean of 0.275 mm (Henny *et al.* 1984). The only significant differences were between the pre-1947 mean and the means at Foundation Island and Potholes Reservoir (Table 2). Several eggs had shells that were 28% thinner than the pre-1947 norm. We found dented and cracked eggs in nests from Foundation Island (10 dented), Threemile Island (5 dented, 1 cracked), and Sand Dune Island (1 dented). In 17 clutches in marked nests with a dented or cracked egg, 9 produced ≥ 1 young that survived to 14 days of age.

Nesting Phenology

Each colony was visited from 11 to 18 times during the nesting season. There were significant intercolony differences in phenology of nesting based on the estimated date that the first egg was laid in each nest (first clutches only, Fig. 2). Initial visits ranged from 12-26 April when all colonies except 1 had nests with eggs. There were no nests on Crescent Island on 12 April, and only a few nests were established there by 3 May. The approximate peak of egg laying was 16-21 April at Foundation Island, 21-25 May at Crescent Island, 26 April-2 May at Potholes Reservoir, 1-6 May (major peak) and 15-20 May (secondary peak) at Threemile Island, and 17-20 April at Sand Dune Island. The earliest hatching occurred between 20 April-1 May at Sand Dune Island. The approximate peak of hatching was 7-10 May at Foundation Island, 12-17 June at Crescent Island, 2-8 May at Potholes Reservoir, 29 May-6 June at Threemile Island, and 6-10 May at Sand Dune Island. The nesting season was essentially complete at Sand Dune Island on 18 June, when only a few young were noted in nests. The season was extended to 9 July at

Table 2. Shell thickness of Black-crowned Night-Heron eggs, 5 Oregon or Washington Colonies, 1991, compared with one another and with the pre-1947 norm.

Sample	N	Shell thickness Mean±SE[1]	Range	% thinning
Pre-1947[2]	142	0.275±0.002A	0.240-0.323	
Threemile Island	15	0.261±0.029AB	0.197-0.310	5.1
Crescent Island	8	0.256±0.022AB	0.227-0.297	6.9
Sand Dune Island	13	0.254±0.008AB	0.222-0.313	7.6
Potholes Reservoir	19	0.253±0.006B	0.197-0.300	8.0
Foundation Island	17	0.246±0.007B	0.200-0.283	10.5

[1]Means sharing a common letter were not significantly different (Tukey's HSD test).
[2]Data from the Northwestern United States (Henny *et al.* 1984).

Potholes Reservoir and to 22 or 23 July at Foundation Island, Crescent Island, and Threemile Island when a few nestlings were recorded at the last visit.

Breeding Success

Using the Mayfield method (Mayfield 1961, 1975; Johnson 1979), there were significant differences in nest success to hatching (0-27 days), from hatching to 14 days post-hatching (28-41 days), and for combined periods (0-41 days). Sand Dune Island was the most successful colony and Potholes Reservoir the least successful (Table 3). Most of the losses occurred pre-hatching with the daily survival rate (DSR) ranging from 0.9240-0.9934 for 0-27 days compared to DSRs ranging from 0.9782-1.0 for 28-41 days. Nest success at the 5 colonies ranged from 12- 84%

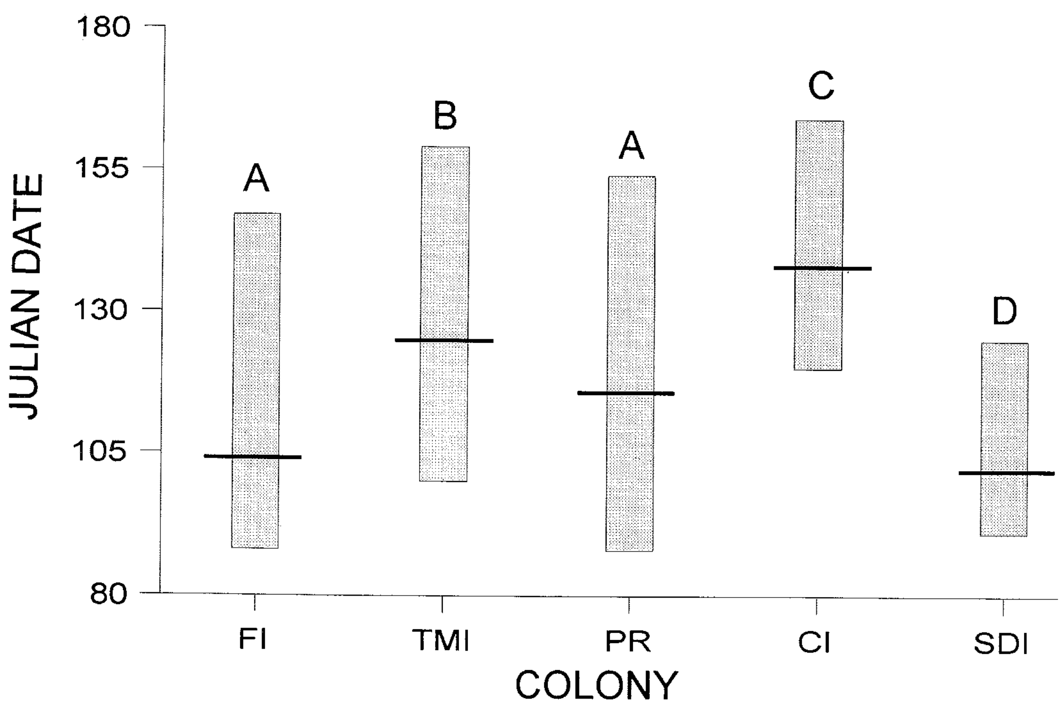

Figure 2. Ranges and median Julian dates of first egg laid in nests (first clutches only) of Black-crowned Night-Herons in 5 Oregon or Washington colonies, 1991. Black lines indicate median dates. Distribution of laying dates of first egg by colony (above or below the overall median of 111 days) that are significantly different from one another do not share the same letter. Overall $\chi^2_4 = 96.785$, P = 0.001.

Table 3. Nest success[1] of Black-crowned Night-Herons in 5 Oregon or Washington Colonies, 1991.

	0-27 days		28-41 days		0-41 days	
Colony	% success	DSR	% success	DSR	% success	DSR
Sand Dune Island	83.6	0.9934A	88.1	0.9910A	72.8	0.9923A
Threemile Island	56.1	0.9788B	75.5	0.9801A	42.0	0.9791BC
Crescent Island	52.8	0.9766ABC	100	1.000B	62.4	0.9886AB
Foundation Island	36.5	0.9634C	86.5	0.9897A	30.3	0.9713C
Potholes Reservoir	11.8	0.9240D	73.4	0.9782A	11.8	0.9493D

[1]Calculations made using the Mayfield Technique with comparison of pairs of means using CONTRAST; DSR equals the mean daily survival rate during 0-27 days, 28-41 days, and 0-41 days post-hatching. Means in each column for DSR that share a common letter are not significantly different.

from 0-27 days, 74-100% from 28-41 days, and 12-73% over the entire 41-day period.

In comparing the distribution of young (0 to 1 and 2 to 4) surviving to 14 days post-hatching, there were significant intercolony differences (χ^2 tests, Table 4). The number of young per marked nest at Sand Dune Island was usually significantly higher than the other colonies for all categories including first clutches (eggs only when marked) with either 0 or 1 egg collected, all first clutches including those with young present when marked, and all clutches including replacements. The mean number of young per marked nest was ca. 2 at Sand Dune Island and ca. 0.5 at Potholes Reservoir. In every colony but 1, the number of 14-day-old young produced per successful nest was greater when no eggs were collected compared to those with 1 egg collected for analysis or artificial incubation. The differences ranged from -14% at Potholes Reservoir to +5% at Threemile Island, with a mean difference of -9%. When collection of pipped eggs

Table 4. Variation in the distribution of survival of young Black-crowned Night-Herons to 14 days post-hatching in 5 Oregon or Washington colonies, 1991.

	Mean (±SE) young per marked nest[1]				
	First clutch only				
Colony	No eggs collected[2,3]	1 egg collected[2,4]	All[5,6]	All clutches[7,8]	All clutches[9,10]
Sand Dune Island	1.65±0.26A[11]	2.05±0.21A	1.79±0.15A	1.94±0.15A	1.83±0.15A
Foundation Island	0.91±0.16AB	0.89±0.23BC	0.96±0.13B	1.14±0.13B	0.80±0.10B
Crescent Island	0.67±0.49AB	1.44±0.20AB	1.48±0.17A	1.61±0.16A	1.47±0.17A
Threemile Island	0.61±0.21B	1.16±0.25B	0.83±0.16B	1.09±0.16B	0.83±0.13BC
Potholes Reservoir	0.29±0.17B	0.38±0.14C	0.51±0.09C	0.47±0.11C	0.41±0.10C

[1]Nests with fate determined.

[2]Includes nests marked initially when eggs present.

[3]Overall χ^2_4 = 15.199, P = 0.004.

[4]Overall χ^2_4 = 25.171, P = 0.001.

[5]Includes nests marked initially when eggs or young present.

[6]Overall χ^2_4 = 47.866, P = 0.001.

[7]We assumed that replacement clutches in the same nest were laid by the same female; therefore, success was based on the nest—0 young if all clutches failed or the number of young surviving to 14 days if 1 clutch was successful (we did not record 2 successful clutches in the same nest).

[8]Overall χ^2_4 = 43.598, P = 0.001.

[9]We assumed that a different female laid each replacement clutch in the same nest; therefore, success was based on each clutch.

[10]Overall χ^2_4 = 61.336, P = 0.001.

[11]Distribution of χ^2 Values for survival of young in each column (0 to 1 young or 2 to 4 young) that are significantly different from one another do not share a common letter.

was included in the sample of nests with 1 egg collected, the average number of 14-day-old young produced per successful nest averaged 13% less (colony averages were 4-22% lower) than nests with no collections. The distribution of young produced in first clutches in all nests which contained eggs when first marked, indicated more young were produced in nests with 1 egg collected compared to those with no collections (χ^2_9 = 44.085, P = 0.001), but the intracolony comparisons were significant only for Threemile Island (χ^2_1 = 4.131, P = 0.046).

Considering mortality from 14 days post-hatching to fledgling, 13.1% of banded young (8 of 61) were later found dead; but most mortality occurred at Threemile Island where 43% (6 of 14) were recovered. In a radiotelemetry study of Black-crowned Night-Heron young in Virginia, Erwin *et al.* (1996) listed posthatching mortalities of 0-20% (14-40 days) and 40-75% (40-60 days). We determined cause of death in only a few instances. Two intact young with severely pecked heads were found on the ground at Threemile Island, and a young on Crescent Island apparently died of starvation after jumping from the nest into dense herbaceous cover at our approach. Responses of older young to our approach were highly variable; some fell or jumped up to 8 m to the ground when we were as far as 15 m from the nest tree; whereas, most larger young climbed into the tree canopy.

The major factor that adversely affected breeding success was avian predation, primarily of eggs but of young as well. Most nests that were apparently depredated exhibited no signs except that the eggs and, infrequently, young disappeared. American Crows (*Corvus brachyrhynchos*) were seen in most colonies, and they nested in the same trees with Black-crowned Night-Herons at Threemile Island, Sand Dune Island, and Foundation Island. The number of depredated eggs in 260 nests in the 5 colonies was relatively low (33 eggs in 14 nests). Crows were seen flying with what appeared to be whole eggs of Black-crowned Night-Herons on several occasions, and remains of depredated eggs were found immediately under the nests and at some distance from the nearest nest. Crows were noted frequently at Threemile Island (≥7 nests and 50 birds in a post-breeding flock) and Foundation Island, but only a few crows were observed at Sand Dune Island. A Black-billed Magpie (*Pica pica*) nested over water in willows near several Black-crowned Night-Heron nests at Potholes Reservoir, and Common Ravens (*Corvus corax*) were also seen at Potholes Reservoir. We observed only 1 nest with depredated eggs at each colony on Sand Dune Island and Crescent Island.

Body and Yolk Sac Mass

On an intracolony basis, pipped eggs obtained either in the field or from the incubator were not significantly different in relation to mass of the whole body, yolk sac, or body less the yolk sac. Thus, all data were combined for intercolony comparisons which, in turn, showed no significant differences (Table 5) in mass of whole body ($F_{1,16}$ = 1.07, P = 0.32), or body less the yolk sac ($F_{1,16}$ = 0.88, P = 0.36).

Cytochrome P450-associated Monoxygenase Activity

Hepatic cytochrome P450 activity (BROD and EROD) in pipping embryos of Black-crowned Night-Herons (Fig. 3) varied significantly among the 5 colonies ($F_{4,75}$ = 3.17, P = 0.02 and $F_{4,75}$ = 4.93, P = 0.001, respectively). The lowest mean for each of the enzymes was found at the designated reference site, Potholes Reservoir, and the highest activity was usually found at Sand Dune Island. Inspection of individual observations from Sand Dune Island revealed that >50% of the BROD values and 67% of the EROD values were >2 SD above the Potholes Reservoir means. At the other 3 colonies, 25-35% of the BROD values and 38-55% of EROD values were >2 SD above the Potholes Reservoir means. Means for PROD did not differ significantly among sites ($F_{4,75}$ = 1.11, P = 0.36), and these values were not included in Fig. 3.

Table 5. Mean yolk sac and body mass of pipping embryos of Black-crowned Night-Herons in 5 Oregon or Washington colonies, 1991.

		Mass (g)[1]±SE		
Colony	N	Whole body	Yolk sac	Body-yolk sac
Threemile Island	19	30.2±0.56	5.8±0.42	24.4±0.51
Crescent Island	13	29.9±1.01	5.7±0.33	24.2±0.87
Foundation Island	17	28.9±0.81	5.3±0.28	23.6±0.80
Sand Dune Island	17	28.8±0.71	5.2±0.33	23.7±0.55
Potholes Reservoir	12	28.2±0.89	5.6±0.60	22.7±0.87

[1]Means in each column are not significantly different from one another (Tukey's HSD test).

Developmental Abnormalities

No developmental abnormalities were noted in embryos, in addled eggs, or in viable eggs at various stages of incubation (including pipping).

Residues in Eggs

Residues of 13 contaminants were found in 14 eggs of Black-crowned Night-Herons collected at 5 colonies (Table 6). Only 2 or 3 eggs were collected per colony; therefore, all residue data were combined in analyzing effects of DDE and other compounds on eggshell thickness. Residues were low except for 1 egg from Threemile Island that contained 31 pg/g of TCDD. Residues of DDE ($F_{1,12}$ = 0.80, P = 0.39) or the other contaminants (ranges in $F_{1,12}$ = 0.01 to 3.86 and P = 0.07 to 0.92) were not significantly correlated with eggshell thickness. There were insufficient sample egg data to compare residue data with breeding success.

DISCUSSION

Although we used the same methods to study the Black-crowned Night-Heron at the 5 nesting colonies during a single breeding season, marked intercolony differences were noted. Avian predation was the major factor affecting breeding success at Threemile Island, Foundation Island, and Potholes Reservoir; it was a minor factor at Crescent Island and Sand Dune Island. The colony at Crescent Island was recently established in a shrubby growth of willows that had not yet attracted corvids. Large numbers of Ring-billed Gulls (*Larus delawarensis*) and California Gulls (*L. californicus*) that nested on the ground near the Black-crowned Night-Herons at Crescent Island and Threemile Island were not seen taking their eggs or young. Sand Dune Island was in the middle of a wide stretch of the Columbia River with only a few crows present. Black-crowned Night-Heron colonies at Sand Dune Island and Crescent Island were the most successful. The other colonies were situated on islands near the shoreline or in a reservoir where large numbers of avian predators were found. The response to nest predation seemed to vary widely; although completeness of the nesting phenology record was tempered by our schedule of visits. Birds at Foundation Island frequently recycled with up to 4 clutches laid in a single nest; whereas, the highly unsuccessful birds at Potholes Reservoir recycled far less frequently. Many nests may be lost relatively quickly under the right conditions; for example, Henny *et al.* (1984) found that a nesting pair of Common Ravens decimated a colony of Black-crowned Night-Herons that were nesting in a marsh. We concluded that most of the replacement clutches in the same nest in this study were probably laid by the same female because replacement clutches followed successful clutches in only 2 nests. We never recorded more than 1 successful clutch in any nest.

Henny (1972) calculated that a recruitment standard of approximately 2 young per nesting pair is necessary to maintain a stable population of Black-crowned Night-Herons. In this study, production of Black-crowned Night Herons at Sand Dune Island was near

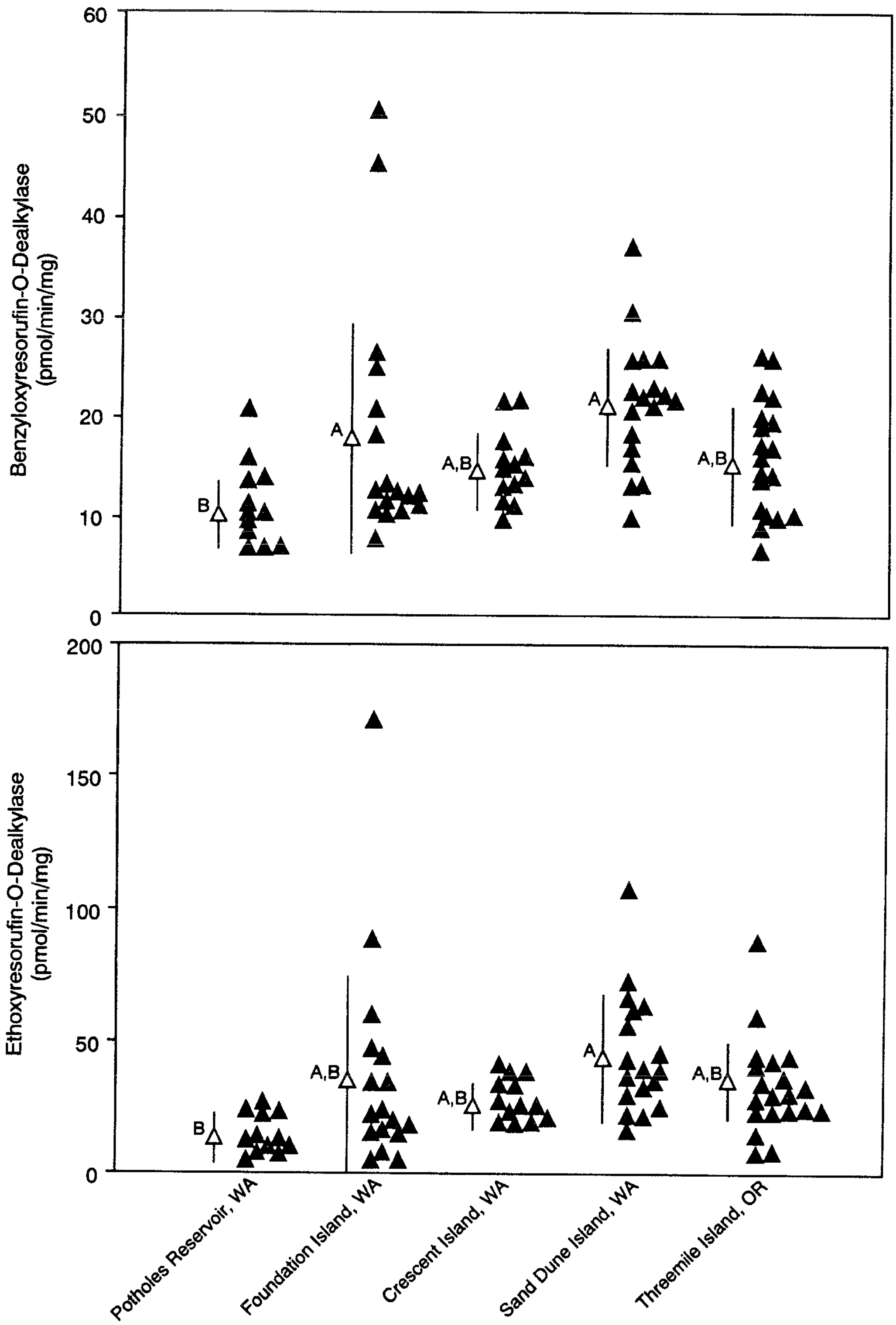

Figure 3. Hepatic microsomal BROD and EROD activities ($\overline{X} \pm SD$ and individual observations) of pipping Black-crowned Night-Heron embryos collected from 5 Oregon or Washington colonies, 1991. Sites that share a common letter are not significantly different by Tukey's HSD test.

Table 6. Residues of contaminants (wet weight) in eggs of Black-crowned Night-Herons in 5 Oregon or Washington colonies, 1991.

	Contaminants[1]									
Colony	DDE	DDD	DDT	Dieldrin	HE	HCB	β-HCH	TNCH	PCBs	TCDD
Sand Dune Island	1.25	0.01	0.01	0.02	0.13	0.02	ND	0.03	0.38	2.42
	0.75	0.01	0.01	ND	0.01	ND	ND	0.02	0.29	1.33
	2.18	0.01	0.02	ND	0.03	ND	ND	0.03	0.43	1.68
Foundation Island	3.29	0.05	0.03	ND	0.01	ND	0.01	0.01	0.54	2.86
	0.40	ND	ND	0.01	0.28	0.04	ND	0.02	ND	2.14
	3.47	0.01	0.07	0.02	0.06	0.01	0.21	0.02	ND	2.05
Crescent Island	2.10	0.02	0.07	0.02	0.04	0.36	0.03	0.04	ND	1.95
	4.60	ND	0.01	0.01	0.09	0.02	0.12	0.02	0.61	2.65
Threemile Island	2.09	0.12	0.15	0.05	0.10	0.02	ND	0.06	0.55	1.62
	2.87	0.01	0.03	0.01	0.07	0.01	0.01	0.04	0.40	31.00
	2.07	ND	0.01	0.01	0.04	0.01	0.01	0.04	ND	1.65
Potholes Reservoir	1.42	0.03	0.07	0.06	0.01	0.34	0.04	0.01	0.16	1.30
	1.11	0.02	0.06	0.03	0.01	ND	0.05	0.01	0.47	1.82
	0.48	ND	ND	ND	ND	ND	0.01	0.01	0.17	1.29

[1]Lower sensitivity limit = 0.05 μg/g for PCBs, 1 pg/g for TCDD and TCDF, and 0.01 μg/g for all other compounds. HE = heptachlor epoxide, HCB = hexachlorobenzene, β-HCH = beta isomer of hexachlorocyclohexane, TNCH = *trans*-nonachlor, PCBs = polychlorinated biphenyls, TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin, and ND = no residue detected. Residues of 2,3,7,8-tetrachlorodibenzofuran were not detected; One egg contained 0.43 μg/g of toxaphene, and another egg contained 0.03 μg/g of α-HCH. Residues are expressed as pg/g for TCDD and μg/g for all other contaminants.

the standard. Crescent Island was the second most successful colony, but the number of young fledged per nest was over 0.5 young below the standard. Fledging rates at the other 3 colonies were about 1 to 1.5 young below the standard.

Compared to our results in 1991, a study of several nesting colonies of Black-crowned Night-Herons in 1979 and 1980 (Henny *et al.* 1984) revealed marked differences in breeding success. Colonies at Moses Lake (Potholes Reservoir), Threemile Island, and Foundation Island produced from 2.0 to 2.2 young per nest in 1979 and 1980, and there was little evidence of serious avian predation. The reason for the increased importance of avian nest predators was probably related primarily to an increase in crow populations. Nevertheless, there was an increase in the number of active Black-crowned Night-Heron nests from 1979-1980 to 1991 at Foundation Island (41 to about 200) and Threemile Island (32 to 69). There are no data on changes in the size of the breeding population at Potholes Reservoir, and the other 2 colonies were established after the earlier study.

The factors involved in significant intercolony differences in clutch size are unknown, but are most likely related to food availability. Other possibilities include differences in nest predation rates or nest parasitism. The range of clutch size means by colony was within those summarized for the Black-crowned Night-Heron throughout its geographic range (Custer *et al.*, 1983). There is little evidence to identify factors related to significant intercolony differences in nest initiation dates. Recent establishment of the Crescent Island colony seems most likely related to late nest initiation, but there were no obvious causes for other observations, as at Sand Dune Island, where nesting started early and finished early.

The higher rate of success of active nests with 1 egg collected versus those with none collected seemed related to the fact that collections usually occurred several visits after the initial location and marking of the nest. Active nests with 1 egg collected at pipping had lower success than those with an egg collected before pipping. For successful nests, collection of an egg resulted in a 13% reduction in the number of 14-day-old young per nest when eggs collected during or before pipping were included, and a 9% reduction when including only eggs collected before pipping. Henny *et al.* (1984) calculated an average decrease of 0.44 young per successful nest when 1 egg was collected compared to nests with no collections. This was slightly higher than the approximate comparable decrease of 0.29 young per successful nest calculated from this study.

Residues of DDE in eggs of Black-crowned Night-Herons decreased from 1978-1980 (Henny *et al.* 1984) to 1991 at Foundation Island (5.8 to 2.4 μg/g), Threemile Island (3.5 to 2.3 μg/g) and Potholes Reservoir (2.2 to 1.0 μg/g); but the sample sizes in 1991 were too small to permit statistical analyses. All residues of DDE in eggs collected in 1991 were below 8 μg/g—the level where reproductive problems are first noted (Henny *et al.* 1984). Residues of several other organochlorines also apparently decreased. Contaminants probably had little influence on breeding success of Black-crowned Night-Herons during this study; this conclusion is based on residues and eggshell thinning that were generally less than known effect levels and absence of developmental abnormalities.

Although our residue analysis lumped the numerous PCB congeners into total PCBs and only included analysis for TCDD and TCDF among the many congeners of dioxins and furans, concentrations were generally much lower than those associated with adverse effects in other studies. For example, means (1 to 9 μg/g) and ranges (0.2 to 53 μg/g) of total PCBs in eggs of Black-crowned Night-Herons from throughout the U.S. (Rattner *et al.* 1994) were greater than residues found in this study. Also, rates of induction of cytochrome P450 enzymes reported by Rattner *et al.* (1994) were generally greater than found in this study, although no developmental abnormalities were noted in either study. In a study of Black-crowned Night-Herons in California, Hoffman *et al.* (1986) reported that a reduction in mass of the pipping embryo less the yolk sac was significantly correlated with total PCB residues (geometric mean of 4 µg/g and range of 1 to 52 µg/g) that were well above those found in this study.

Bosveld and Van den Berg (1994) summarized effects of total PCBs on reproductive success of several avian species; the lowest observed adverse effect level (LOAEL) in eggs of wild birds ranged from 8 to 19 µg/g. For TCDD, the LOAEL in eggs associated with reproductive problems ranged from between 92 and 252 pg/g in Great Blue Herons (*Ardea herodias*, Elliott *et al.* 1989). Forster's terns (*Sterna forsteri*) experienced impaired reproduction when eggs averaged 37 pg/g of TCDD, although adverse effects were attributed primarily to 6-26 µg/g of total PCBs (Kubiak *et al.* 1989). Wood ducks (*Aix sponsa*) experienced a 15% decrease in hatchlings leaving the nest when their eggs contained 5-20 pg/g of 2,3,7,8-TCDD toxicity equivalents (TCDD-EQ) and a 40% decrease when TCDD-EQs reached 50 pg/g. TCDD accounted for nearly all of the TCDD-EQs in limited residue analysis of eggs of wood ducks (White and Hoffman 1995). TCDD residues in eggs of our Black-crowned Night-Herons were, with the possible exception of 1 egg, generally much lower than known or suspected effect levels; although interpretation is confounded by our limited residue analysis.

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REFERENCES CITED

Anderson, D. W. and J. J. Hickey. 1972. Eggshell changes in certain North American birds. Proceedings of the International Ornithological Congress 15: 514-540.

Anthony, R. G., M. G. Garrett and C. A. Schuler. 1993. Environmental contaminants in bald eagles in the Columbia River estuary. Journal of Wildlife Management 57: 10-19.

Bosveld, A. T. C. and M. Van den Berg. 1994. Effects of polychlorinated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans on fish-eating birds. Environmental Review 2: 147-166.

Clement, R. E., C. Tashiro, S. Suter, E. Reiner and D. Hollinger. 1989. Chlorinated-p-dioxins (CDDs) and dibinzofurans (CDFs) in effluents and sludges from pulp and paper mills. Chemosphere 18: 1-6.

Custer, T. W., G. L. Hensler and T. E. Kaiser. 1983. Clutch size, reproductive success, and organochlorine contaminants in Atlantic coast Black-crowned Night-Herons. Auk 100: 699-710.

Elliott, J. E., R. W. Butler, R. J. Norstrom and P. E. Whitehead. 1989. Environmental contamination and reproductive success of Great Blue Herons (*Ardea herodias*) in British Columbia, 1986-87. Environmental Pollution 59: 91-114.

Erwin, R. M., J. G. Haig, D. B. Stotts and J. S. Hatfield. 1996. Reproductive success, growth and survival of Black-crowned Night-Heron (*Nycticorax nycticorax*) and snowy egret (*Egretta thula*) chicks in Coastal Virginia. Auk 113: 119-130.

Fitzner, R. E., D. F. Martin and R. E. Fries. 1979. First breeding record for the Great Egret (*Casmerodius albus*) in Washington. Murrelet 60: 33-34.

Henny, C. J. 1972. An analysis of the population dynamics of selected avian species—with special reference to changes during the modern pesticide era. Wildlife Research Report No. 1, U.S. Fish and Wildlife Service, Washington, D.C.

Henny, C. J., L. J. Blus, A. J. Krynitsky and C. M. Bunck. 1984. Current impact of DDE on Black-crowned Night-Herons in the intermountain west. Journal of Wildlife Management 48: 1-13.

Henny, C. J., R. A. Grove and O. R. Hedstrom. 1996. A field evaluation of mink and river otter on the Lower Columbia River and the influence of environmental contaminants. Final Report to the Lower Columbia River Bi-State Water Quality Program.

Hoffman, D. J., B. A. Rattner, C. M. Bunck, A. Krynitsky, H. M. Ohlendorf and R. W. Lowe. 1986. Association between PCBs and lower embryonic weight in Black-crowned Night Herons in San Francisco Bay. Journal of Toxicology and Environmental Health 19: 383-391.

Johnson, D. H. 1979. Estimating nest success: the Mayfield method and an alternative. Auk 56: 651-661.

Kubiak, T. J., H. J. Harris, L. M. Smith, T. R. Schwartz, D. L. Stalling, J. A. Trick, L. Sileo, D. E. Docherty and T. C. Erdman. 1989. Microcontaminants and reproductive impairment of the Forster's Tern on Green Bay, Lake Michigan - 1983. Archives of Environmental Contamination and Toxicology 18: 706-727.

Mayfield, H. F. 1961. Nest success calculated from exposure. Wilson Bulletin 73: 255-261.

Mayfield, H. F. 1975. Suggestions for calculating nest success. Wilson Bulletin 87: 456-466.

Peterson, R. T. 1969. The contamination of food chains. Pages 529-534 *in* Peregrine Falcon populations—their biology and decline. (J. J. Hickey, Ed.). University of Wisconsin Press, Madison.

Rattner, B. A., M. J. Melancon, T. W. Custer, R. L. Hothem, K. A. King, L. J. LeCaptain, J. W. Spann, B. R. Woodin and J. J. Stegeman. 1993. Biomonitoring environmental contamination with pipping Black-crowned Night Heron embryos: induction of Cytochrome P450. Environmental Toxicology and Chemistry 12: 1719-1732.

Rattner, B. A., J. S. Hatfield, M. J. Melancon, T. W. Custer and D. E. Tillitt. 1994. Relation among Cytochrome P450, AH-activated PCB congeners and dioxin equivalents in pipping Black-crowned Night-Heron embryos. Environmental Toxicology and Chemistry 13: 1805-1812.

SAS Institute, Inc. 1994. SAS user's guide: statistics version 6.1. SAS Institute Inc., Cary, North Carolina.

Stickel, L. F., S. N. Wiemeyer and L. J. Blus, 1973. Pesticide residues in eggs of wild birds: adjustment for loss of moisture and lipid. Bulletin of Environmental Contamination and Toxicology 9: 193-196.

White, D. H. and D. J. Hoffman, 1995. Effects of polychlorinated-p-dioxins and dibenzofurans on nesting Wood Ducks (*Aix sponsa*) at Bayou Meto, Arkansas. Environmental Health Perspectives 103 (Suppl. 4): 37-39.