

Effects of Contaminants on Reproductive Success of Aquatic Birds Nesting at Edwards Air Force Base, California

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Abstract. Contamination by organochlorine pesticides (OCs), polychlorinated biphenyls, metals, and trace elements at Edwards Air Force Base (EAFB), located in the Mojave Desert, could adversely affect nesting aquatic birds, especially at the sewage lagoons that comprise Piute Ponds. Estimates of avian reproduction, in conjunction with analyses of eggs and avian foods for contaminant residues, may indicate the potential for negative effects on avian populations. From 1996 to 1999, we conducted studies at the Piute Ponds area of EAFB to evaluate the impacts of contaminants on nesting birds. Avian reproduction was evaluated in 1999. Eggs were collected for chemical analyses in 1996 and 1999, and African clawed frogs (*Xenopus laevis*), a likely food source, were collected for chemical analyses in 1998. Avian species occupying the higher trophic levels—black-crowned night-heron (*Nycticorax nycticorax*), white-faced ibis (*Plegadis chihi*), and American avocet (*Recurvirostra americana*)—generally bioaccumulated higher concentrations of contaminants in their eggs. Reproductive success and egg hatchability of night-herons and white-faced ibises in the Piute Ponds were similar to results observed at other western colonies. Deformities were observed in only one embryo in this study, but concentrations of contaminants evaluated in this ibis embryo were considered insufficient to have caused the deformities. Because clawed frogs, a primary prey item for night-herons at Piute Ponds, had no detectable levels of any OCs, it is likely that OCs found in night-heron eggs were acquired from the wintering grounds rather than from EAFB. The presence of isomers of dichlorodiphenyltrichloroethane (DDT) in ibis eggs indicated recent exposure, but invertebrates used for food by ibises were not sampled at Piute Ponds, and conclusions about the source of OCs in ibis eggs could not be made. Concentrations of contaminants in random and failed eggs of individual species were not different, and we concluded that contaminants did not cause the observed egg failures.

Environmental contaminants, including organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), metals, and

trace elements are present at many sites at Edwards Air Force Base (EAFB) (United States Air Force Flight Test Center 1994), but the effects of these contaminants on avian reproduction have not been evaluated. Reproductive success, coupled with chemical residue analyses, can indicate the likelihood of adverse effects of environmental contaminants (Kendall *et al.* 1990).

Edwards AFB is located in the northwestern Mojave Desert in Kern and Los Angeles counties, CA (Fig. 1). In this arid region, large natural bodies of water are uncommon, and artificial ponds used for sewage treatment, storm water retention, or other purposes may be attractive to aquatic birds as stopover sites during migration, as wintering sites (Henny *et al.* 1994) or ash nesting areas (Piest and SOWLS 1985). Because of the paucity of nearby wetland feeding habitats, aquatic birds nesting in ponds at EAFB likely feed in nearby ponds. Therefore, residues in eggs of these species should generally reflect exposure to contaminants acquired at EAFB. Exceptions would include elements that are slow to accumulate or that have long retention times (*e.g.*, cadmium) (Scheuhammer 1987; Stickel *et al.* 1977).

Black-crowned night-herons (*Nycticorax nycticorax*) breed at EAFB, have a high trophic position in the food web, bioaccumulate contaminants, have a wide geographic distribution, and nest in large colonies (Custer *et al.* 1991). Therefore, this species is well suited as an indicator of environmental contamination and was selected to be the focal species for this study.

The diet of the black-crowned night-heron, a member of the family Ardeidae, includes a wide variety of prey, including fish, insects, crayfish, mussels, amphibians, lizards, snakes, rodents, birds, and eggs (Davis 1993). Lafferty and Page (1997) observed three other Ardeids—great blue herons (*Ardea herodias*), a great egret (*Ardea alba*), and a green heron (*Butorides virescens*)—feeding on African clawed frogs (*Xenopus laevis*) an introduced species in the Santa Clara River estuary (Ventura County, CA). On several occasions in 1996, we observed adult night-herons feeding on these frogs at Piute Ponds, a series of sewage lagoons located in the southwestern part of EAFB. In addition, we observed in 1996 a night-heron chick in one of the ponds regurgitate the partial carcass of a clawed frog. We also found numerous clawed frog

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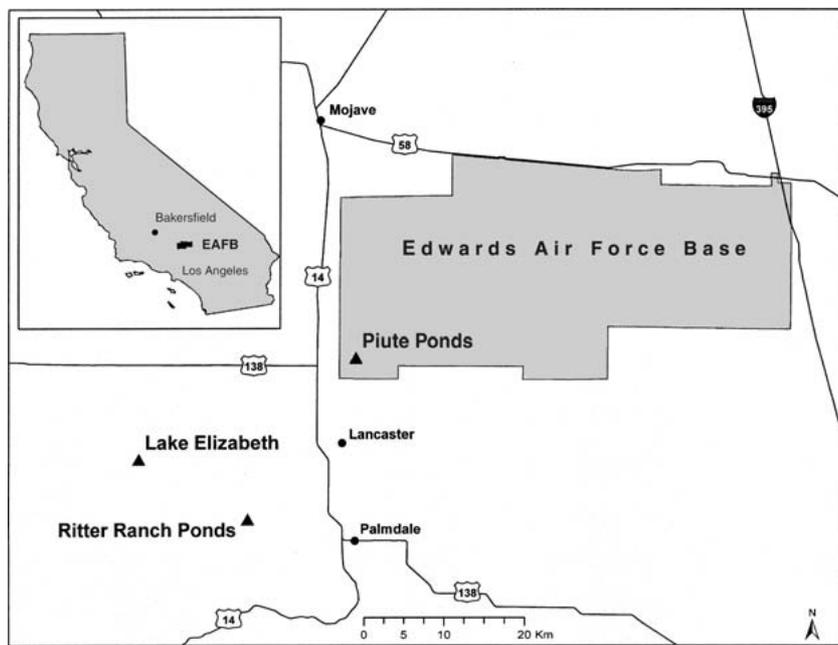


Fig. 1. The location of EAFB (Los Angeles and Kern counties, Southern California) and the locations of the study sites (enlarged map): Piute Ponds and the two reference sites, Elizabeth Lake and the Ritter Ranch Ponds

bones in bird pellets beneath trees used for nesting by great blue herons on the periphery of Piute Ponds (Crayon and Hothem 1998). The results of our seining and trapping indicated that fish were not plentiful in Piute Ponds, but clawed frogs were abundant in all ponds. We hypothesized—based on their abundance, their proximity to nesting areas, and their observed presence in chick diets—that clawed frogs comprised a significant portion of the diet of adult and nestling night-herons at Piute Ponds.

During a preliminary study in Piute Ponds in 1996, contaminant concentrations in eggs of the primarily herbivorous American coot (*Fulica americana*) were generally low (See Results and Discussion sections). Concentrations of OCs and metals in night-heron eggs, however, were high enough to warrant further investigation. Thus, in 1998, we collected African clawed frogs from Piute Ponds and from two reference sites for contaminants analyses, and, in 1999, we evaluated contaminant concentrations in avian eggs from black-crowned night-herons, American avocets (*Recurvirostra americana*), white-faced ibis (*Plegadis chihi*), mallards (*Anas platyrhynchos*) and ruddy ducks (*Oxyura jamaicensis*). We also assessed the effects of contaminants on the reproductive success of black-crowned night-herons and white-faced ibis.

The objectives of this study were (1) to estimate the exposure of nesting aquatic birds to contaminants at EAFB, (2) to evaluate the impacts of contaminants on avian reproduction, and (3) to evaluate contaminant levels in African clawed frogs.

Materials and Methods

Study Area

Piute Ponds is a series of four unlined sewage lagoons in the southwest corner of Edwards Air Force Base, Los Angeles County, CA (34°N 47' 30", 118°W 06' 50") (Fig. 1). The largest pond, Big Piute (53.5 ha), was created in 1961. The Buffer Pond (North Piute Pond;

28.9 ha) was created sometime between 1967 and 1979. In 1991, the United States Air Force, in cooperation with Ducks Unlimited, constructed the North Ducks Unlimited Pond (NDU) and the South Ducks Unlimited Pond (SDU), adding an additional 68.8 ha of surface water to the pond complex. The primary source of water for these ponds is secondarily treated and disinfected effluent from the Los Angeles County Sanitation District No. 14 Lancaster Water Reclamation Plant.

Two presumably uncontaminated sites, Elizabeth Lake and ponds on the Ritter Ranch, were selected for collection of reference African clawed frogs. Elizabeth Lake (38°N 40' 08", 118°W 24' 36") (Fig. 1) is a 59-ha natural lake (California Department of Water Resources 1987) located approximately 30 km southwest of Piute Ponds. The Ritter Ranch site consists of a series of spring-fed ponds (34°N 35' 56", 118°W 15' 36") near the confluence of Rogers and Amargosa creeks, approximately 15 km southeast of Elizabeth Lake and 23 km southwest of Piute Ponds. Both reference sites are located in the San Gabriel Mountains of Los Angeles County, CA.

Egg Collection

In 1996, we collected one egg at random from each of 10 randomly selected night-heron and 8 American coot nests at Big Piute Pond for chemical analysis; reproduction was not assessed in 1996. Five eggs from each species were analyzed for metals and trace elements; five other night-heron eggs and three coot eggs were analyzed for OCs and PCBs.

From April to July 1999, we monitored nests of black-crowned night-herons, white-faced ibises, cattle egrets (*Bubulcus ibis*), American avocets, redhead (*Aythya americana*), cinnamon teal (*Anas cyanoptera*), mallard, gadwall (*Anas strepera*), and ruddy duck. We conducted early-morning searches of appropriate habitats at Piute Ponds every 7 to 10 days to discover new nests and to monitor previously marked nests. The status of eggs and chicks in each marked nest was recorded, and nests were rechecked every 7 to 10 days to estimate nest success. Signs of reproductive impairment or predation were noted, and causes of chick mortalities were estimated based on postmortem examinations. Where available, stomach contents of predated chicks were examined to estimate their food habits.

In 1999, we collected 1 egg at random from each of 10 randomly selected night-heron nests at Big Piute Pond, 6 American avocet nests at Big Piute Pond and 4 at the South Ducks Unlimited Pond, 10 white-faced ibis nests at Big Piute Pond, 4 mallard nests from the Buffer Pond and 1 from Big Piute Pond, and 12 ruddy duck nests at the Buffer Pond and 1 at Big Piute Pond. We also collected 12 failed eggs for analysis, including 4 night-heron eggs from Big Piute Pond, 5 ibis eggs from Big Piute Pond and 1 from the Buffer Pond, and 2 mallard eggs from the Buffer Pond.

Because intraclutch variability of contaminant concentrations is generally low (Custer *et al.* 1990), we assumed that contaminant concentrations in the collected egg provided a valid estimate of contamination in the clutch. We kept collected eggs on ice or refrigerated them and processed them within 7 days of collection. We measured, weighed, and dissected each egg to assess the fertility, stage of embryo development (Caldwell and Snart 1974), and condition of the embryo (*i.e.*, viability and normality). Egg contents were stored frozen in chemically-clean jars until selected eggs could be analyzed for contaminants. Forty-eight random and 12 failed eggs were analyzed for metals and trace elements; 20 random and 10 failed eggs were analyzed for OCs and PCBs.

Estimate of avian Reproductive Success

When possible, we followed nests from laying to fledging. The incubation stage of each nest found with an incomplete clutch was estimated by back-dating, assuming that night-herons lay one egg every other day (Tremblay and Ellison 1980). Incubation stages in certain nests found with complete clutches were estimated based on intervals between nest searches or by examination of embryos in collected eggs. Hatching dates were predicted based on these criteria or based on estimated ages of chicks in the nests (Klett *et al.* 1986; McVaugh 1972). A nest was considered successful if at least one egg hatched (Mayfield 1961, 1975). We classified eggs that disappeared before the hatching date as unknown unless we found evidence of their fate. Nests with cold eggs after the predicted hatching date were considered abandoned. Evidence of predation included destroyed nests, partially eaten eggs, and dead chicks with wounds attributed to predators.

We monitored chicks after hatching to estimate fledging success, setting 15 days of age as the cutoff date for successful fledging of night-herons and 10 days for cattle egrets and white-faced ibis. Fledging success was not estimated for ducks and avocets because the precocial young were too difficult to find after hatching.

African Clawed Frogs

We used a bag seine to collect adult frogs at NDU and SDU and minnow traps baited with beef liver at Big Piute, the Buffer Pond, and Elizabeth Lake, where deep water and dense shoreline vegetation made seining difficult. D. Muth, of LSA Associates, Inc, used similar traps to collect clawed frogs from Ritter Ranch in June 1998. Sufficient frogs for chemical analyses were collected in one evening of trapping at each site.

Because funding limited the numbers of African clawed frogs that could be analyzed, we chose to decrease potential bias by sex by analyzing only females. The carcasses of five female clawed frogs of similar size were randomly chosen for chemical analyses from the total collected at each of the four Piute Ponds and the two reference sites. In addition, five randomly chosen adult male frogs were analyzed from the Buffer Pond to estimate sex-related differences in contaminant concentrations. Clawed frogs were placed in a cooler on wet ice, transported to the laboratory, and then killed with carbon

dioxide in a cooler with dry ice. Samples were stored frozen until they could be processed. Using clean laboratory techniques, each specimen was thawed and rinsed in deionized water. Total length, measured dorsally from the tip of the snout to the cloaca, and weight were determined, and each frog was examined for gross abnormalities. The digestive tract was removed and the stomach contents saved for later identification. Digestive tracts were thoroughly flushed with deionized water, combined with the appropriate carcass in a chemically cleaned jar, and stored frozen pending chemical analyses.

Chemical Analyses

Analyses were by laboratories under contract to the United States Fish and Wildlife Service. In 1996, Hazleton Environmental Services, Inc, (Madison, WI) conducted all of the analyses. In 1999, samples were analyzed for metals and trace elements by the Research Triangle Institute (Research Triangle Park, NC); OC analyses were conducted by Mississippi State Chemical Laboratory (Mississippi State, MS).

Samples were analyzed for: percentage lipid, percentage moisture, hexachlorobenzene (HCB), benzene hexachloride (α -BHC), β -BHC, δ -BHC, γ -BHC, oxychlordane, α -chlordane, γ -chlordane, *cis*-nonachlor, *trans*-nonachlor, dieldrin, endrin, heptachlor epoxide, mirex, toxaphene, total PCBs, and metabolites of DDT (*p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, *o,p'*-DDD, *o,p'*-DDE, and *o,p'*-DDT). Individual samples were homogenized, thoroughly mixed with anhydrous sodium sulfate, and Soxhlet extracted with hexane. The extract was then concentrated to dryness for lipid determination. The weighed lipid sample was dissolved in petroleum ether and extracted four 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 Florisil. The column was eluted with 200 ml 6% diethyl ether and 94% petroleum ether (fraction I) followed by 200 ml 15% diethyl ether and 85% petroleum ether (fraction II). Fraction II was concentrated to appropriate volume for quantification of residues by packed or capillary column electron-capture gas chromatography (ECGC). Fraction I was concentrated and transferred to a silicic acid chromatographic column for additional cleanup required for separation of PCBs from other OCs. Three fractions were eluted from the silicic acid column. Each was concentrated to the appropriate volume for quantification of residues by packed or megabone column for ECGC. HCB and mirex were in fraction I; PCBs were in fraction II, and the rest were in fraction III. Gas chromatographic determinations were run on a Varian 3600 GC with a Varian Star Data System and a Varian 200 Autosampler (Varian Instruments, Walnut Creek, CA, USA). PCBs were determined by shooting Fraction II on a Varian 3400 GC. Total PCBs were calculated by adding the sum of Aroclor 1242, 1248, 1254, and 1260.

OCs and PCBs are reported on a wet-weight basis for frogs and on a fresh wet-weight basis (corrected for moisture loss) for eggs (Stickel *et al.* 1973). The limit of detection (LOD) was 0.01 $\mu\text{g/g}$ for OCs and metabolites and 0.05 $\mu\text{g/g}$ for toxaphene and total PCBs. Residues in at least 10% of the samples, or 1 per matrix, were confirmed by GC-mass spectrometry.

The precision of the methods, as measured by duplicate sample analyses, was acceptable for all analytes. The accuracy of the analytical methods, as measured by spike recovery, was between 80% and 100% average recovery, which was acceptable for all analytes, except for δ -BHC in eggs. Recovery of this analyte was slightly lower than normal (56.3%), but this was not considered problematic because α -BHC, β -BHC, γ -BHC, and δ -BHC were not detected in any frogs or eggs in this study. Results of the analysis of procedural blanks indicated that no analytes were added during sample processing.

Samples analyzed for metals and trace elements were homogenized using a food processor, and a portion of the homogenate was freeze-dried for moisture determination and ground to 100 mesh with a mill.

Table 1. Reproduction by aquatic birds at Piute Ponds, EAFB in 1999

Reproduction factors	American avocet	Cattle egret	Ducks ^a	White-faced ibis	Black-crowned Night-heron
Nests found	17	7	22	58	122
Nests observed hatched	0	4	7	36	76
Percent hatched (%)	0.0	57.1	31.8	62.1	62.3
PreHatch unknowns	11	3	5	9	28
Total prehatch losses	6	0	10	13	18
Predation	6	0	6	10	16
Destroyed, other	0	0	2	0	0
Abandoned	0	0	2	3	2
Nests observed fledged	0	1	0	20	42
% fledged of hatched	0.0	25.0	–	55.6	55.3
% fledged of total	0.0	14.3	–	34.5	34.4
Posthatch unknowns	0	2	7	12	17
Total Posthatch predation	0	1	0	4	17
Total observed losses	6	1	10	17	35
Total unknowns	11	5	12	21	45

^a Cinnamon teal ($n = 3$), Redhead (1), Mallard (13), Gadwall (2), and Ruddy duck (3).

Samples analyzed for Hg, Se, and As were prepared by digesting a freeze-dried sample in a microwave oven in a capped Teflon vessel in the presence of nitric acid for 3 minutes at 120 watts, for 3 minutes at 300 watts, and for 15 minutes at 450 watts. The residue was then diluted with laboratory-pure water. Se and As were determined using graphite furnace atomic absorption (GFAA) using a Perkin-Elmer Zeeman 3030 or 4100ZL atomic absorption spectrometer (AAS). Mercury was determined using cold-vapor atomic absorption (CVAA) with a Leeman PS200 Hg Analyzer and SnCl₄ as the reducing agent. Other metals and trace elements were determined using inductively coupled plasma spectrophotometry (ICP) using a Leeman Labs Plasma Spec I sequential or ES2000 simultaneous spectrometer.

Results of eggs and frogs analyzed for Al, As, B, Ba, Be, Cd, Cr, Cu, Fe, Mg, Mn, Hg, Mo, Ni, Pb, Se, Sr, V, and Zn are reported on a dry-weight basis. LODs for metals and trace elements in eggs included 0.02 µg/g for Hg; 0.1 µg/g for Be and Cd; 0.2 µg/g for Sr; 0.4 µg/g for Mn; 0.5 µg/g for As, Ba, Cr, Cu, Mo, Ni, Pb, Se, and V; 1.0 µg/g for Zn; 2.0 µg/g for B; 5.0 µg/g for Al; and 10 µg/g for Fe and Mg. LODs for clawed frogs were slightly higher and included 0.12 µg/g for Hg, Be, and Cd; 0.2 µg/g for Sr, 0.5 µg/g for Mn; 0.6 µg/g for As, Ba, Cr, Cu, Mo, Ni, Se, and V; 1.2 µg/g for Pb and Zn; 2.5 µg/g for B; 6.0 µg/g for Al; and 12 µg/g for Fe and Mg.

The precision of the methods for eggs, as measured by duplicate sample analyses, was within normal limits for all analytes. However, for clawed frogs, the average relative percent differences for Cr (50.1%) and Sr (54.0%) were higher than normal, but this was assumed not to have an effect on interpretation of the data. The accuracy of the methods, as measured by spike recovery, was within acceptable limits (80% to 120%) for frogs and eggs for all analytes. Results of the analysis of certified reference materials for both frogs (dogfish muscle) and eggs (lobster hepatopancreas) indicated that the methods worked with naturally incorporated analytes.

Statistical Analyses

Rates of nest success, fledging success, and cause-specific failure rates were calculated using the computer program MICROMORT (Heisey and Fuller 1985; Ohlendorf *et al.* 1989). Nests terminated when found or deserted on the day of the first visit were not included. Methods of calculating exposure days (Mayfield 1961) were done according to Johnson (1979) and Klett *et al.* (1986). Adjusted clutch sizes were calculated by subtracting from full clutch sizes all randomly collected eggs and eggs that disappeared from nests before the

predicted hatching dates. Failed eggs did not include those abandoned, destroyed by predators, lost, or unknown. Hatchability (egg success) for each species was calculated by dividing total hatched eggs by the adjusted clutch size for successful nests. We evaluated avian reproductive success and compared it with literature values of similar species from uncontaminated areas. Concentrations of contaminants in collected eggs were compared among species and with literature values.

To improve homogeneity of variances, contaminant concentrations were transformed to common logarithms, and geometric means were calculated when a contaminant was detected in at least 50% of the samples. When means were calculated, a value equal to one-half the LOD was assigned to any not-detected values before logarithmic transformation. We used student *t* test to compare two species or contaminants. For multiple comparisons among sites, we used one-way analysis of variance (ANOVA) when samples had normal distributions ($p > 0.05$, based on Kolmogorov-Smirnov test for goodness of fit) and equal variances ($p > 0.05$, based on Levene Median test). If either the test of normality or equal variance failed, then we conducted Kruskal-Wallis one-way ANOVA on ranks. If mean values differed ($p < 0.05$), we used Student-Newman-Keuls method for multiple comparisons.

Results and Discussion

Avion Reproductive Success

We monitored 226 nests at Piute Ponds in 1999 (Table 1); 54% were black-crowned night-herons. We also monitored nests of white-faced ibis, cattle egret, American avocets, and five species of ducks, including redhead, cinnamon teal, mallard, gadwall, and ruddy duck.

Of the nests monitored in Piute Ponds, 54.4% hatched; 20.8% failed before hatching; and 24.8% had unknown hatching success. Of the nests that hatched, 51.2% fledged at least one chick; 17.9% were destroyed by predators; and 30.9% were unknown. Included in the prehatch unknown nests (Table 1) were 23% of the ducks and 65% of the avocets. Overall, 30.9% of all nests were unknown, but that included all of the avocets and ducks that had unknown fledging success. Unknowns included lost nests, nests with unknown fate, and

nests not revisited at the end of the breeding season (especially avocets).

Sufficient data were acquired to evaluate reproductive success in only the white-faced ibis and the black-crowned night-heron. The two species had similar observed hatching and fledging success (Table 1). Using the Mayfield method (Mayfield 1961, 1975), estimates of nesting success (and 95% confidence limits) for night-herons ($76.0\% \pm 10.2\%$) and ibis ($75.5\% \pm 13.2\%$) were not different ($\chi^2 = 0.004$; $p = 0.95$). However, the fledging success for ibis ($91.2\% \pm 8.2\%$) was higher than night-herons ($75.8\% \pm 10.0\%$) ($\chi^2 = 5.479$; $p = 0.02$). Overall success for ibis ($68.9\% \pm 13.5\%$) and night-herons ($57.6\% \pm 10.9\%$) were not different ($\chi^2 = 1.620$; $p = 0.20$). Reproductive success of these two species was similar to that observed at other western colonies (Blus *et al.* 1991; Hothem *et al.* 1995; Hothem and Hatch 2004; Ryder and Manry 1994; Taft *et al.* 2000).

Predation was the primary factor limiting avian reproduction at Piute Ponds during 1999 (Table 1). Overall, 26.5% of all the monitored nests were destroyed by predators, 38 nests before hatching and 22 more after hatching but before fledging. Predators destroyed 67% of the cinnamon teal, 35% of the avocet, 33% of the ruddy duck, 23% of the mallard, 17% of the ibis, and 13% of the night-heron nests before hatching. They also destroyed 25% of the cattle egret, 22% of the night-heron, and 11% of the ibis nests that hatched. Overall, predation losses of the two most abundant species, the ibis (24%) and night-heron (27%), were similar. Predator species observed during the study that were assumed to have caused some predation included great horned owls (*Bubo virginianus*), barn owls (*Tyto alba*), common ravens (*Corvus corax*), coyotes (*Canis latrans*), and feral dogs (*Canis familiaris*).

Only 3.1% of the nests monitored in the present study were abandoned. The average abandonment rate for night-herons at Alcatraz Island from 1990 to 2002 was 3% (Hothem and Hatch 2004), approximately twice the 1.6% found at Piute Ponds. In the study at the Lower Klamath National Wildlife Refuge (NWR), 4.8% of the ibis nests were abandoned before hatching, and 0.9% were abandoned after hatching (Taft *et al.* 2000). The abandonment rate for ibis at Piute Ponds was 5.2%.

Eight of the 179 (4.5%) night-heron eggs followed to hatching failed; causes of the failures were not determined. Thus, hatchability for night-heron eggs was 95.5%, which compares favorably with the mean (94.5%; range 89.1% to 97.6%) observed during a 13-year Alcatraz study (Hothem and Hatch 2004) when 3,388 eggs were observed to hatching.

For the ibis, 90.6% of the 67 eggs in successful nests hatched, but 1 embryo among the 7 failed eggs had multiple gross deformities. This 22-day-old embryo had a truncated upper mandible (maxillary micrognathia); its brain protruded from the braincase (exencephaly), and it had no apparent eyes (anophthalmia). These abnormalities were similar to those observed in aquatic birds exposed to increased concentrations of selenium at Kesterson Reservoir in the early 1980s (Ohlendorf *et al.* 1988). Ibis hatchability at Piute Ponds was similar or better than the 3% to 94% reported by Ryder and Manry (1994) from 24 Utah colonies in the 1970s and the 77% to 86% hatchability reported from Lower Klamath NWR (Taft *et al.* 2000).

Avian Food Habits

Avian food habits were estimated based on food items in stomachs of 4 ibis and 11 night-heron chicks killed by predators during the study. The only invertebrates found in night-heron stomachs were beetles (order Coleoptera) found in one chick. Unidentified mammal parts (hair or bones) were found in 8 night-heron stomachs (72.7%); clawed frogs were found in 3 (27.3%) stomachs; and fish were found in 1 of the stomachs (9.1%). Stomach contents indicated that ibis chicks fed exclusively on invertebrates, including aquatic insects, isopods, and snails.

Contaminants

Avian eggs. Of all the OCs included in the analyses, only *p,p'*-DDE was detected in every egg both years, and, in 1996, it was the only OC detected in coot eggs (Table 2). The geometric mean (GM) concentration of *p,p'*-DDE in night-heron eggs (1.74 $\mu\text{g/g}$) was approximately 45 times higher (student *t* test, $p = 0.003$) than that in coot eggs (0.038 $\mu\text{g/g}$). All night-heron eggs also had detectable PCBs (GM = 0.617 $\mu\text{g/g}$), and three of five eggs had detectable residues of *p,p'*-DDT (GM = 0.0185 $\mu\text{g/g}$) and dieldrin (GM = 0.0197 $\mu\text{g/g}$).

The OCs not detected in any sample were α -BHC, α -chlordane, β -BHC, γ -BHC, γ -chlordane, δ -BHC, mirex, *o,p'*-DDD, *o,p'*-DDE, and toxaphene. OCs detected in <50% of samples were *cis*-nonachlor (0.036 $\mu\text{g/g}$ in one random night-heron egg); endrin (0.017 $\mu\text{g/g}$ in one random night-heron egg); HCB (0.011 $\mu\text{g/g}$ in one random night-heron egg, 0.048 $\mu\text{g/g}$ in a random ibis egg, and 0.015 $\mu\text{g/g}$ in a failed ibis egg); heptachlor epoxide (0.023 $\mu\text{g/g}$ in a random night-heron egg and 0.012 $\mu\text{g/g}$ in a failed ibis egg); and *o,p'*-DDT (0.046 $\mu\text{g/g}$ in one random ibis egg and 0.051 and 0.032 $\mu\text{g/g}$ in two failed ibis eggs).

The only OC detected in ruddy ducks, avocets, and mallards was *p,p'*-DDE. The highest residue of *p,p'*-DDE observed in this study was 7.6 $\mu\text{g/g}$ in a randomly collected ibis egg. This was the only egg in this study that exceeded the 4 $\mu\text{g/g}$ in two failed ibis eggs) threshold for reproductive impairment found by Henny and Herron (1989) in white-faced ibis at Carson Lake, NV. Based on one-Way ANOVA, differences in GM values among species were significant for *p,p'*-DDE ($p = 0.026$). Using the Student-Newman-Keuls Method of pair-wise multiple comparisons, we determined that the mean concentration of *p,p'*-DDE in random ibis eggs (1.24 $\mu\text{g/g}$) was greater than that in both ruddy ducks (0.109 $\mu\text{g/g}$) and mallards (0.056 $\mu\text{g/g}$). There were no differences in mean concentrations between ibises and the other species, but the means of night-herons (0.499 $\mu\text{g/g}$) and avocets (0.765 $\mu\text{g/g}$) were not statistically different from any other species for *p,p'*-DDE (Table 2). The GM concentrations of *p,p'*-DDE in random night-heron eggs were compared between years, but the difference was not significant (student *t* test, $p = 0.253$).

In 1999, isomers of DDT were found only in some of the ibis eggs (Table 2). The presence of DDT generally indicates recent exposure to this pesticide. Unfortunately, foods of the ibis, primarily invertebrates, as based on stomach contents in chicks

Table 2. GM ($\mu\text{g/g}$ fresh wet weight) of OC pesticides and PCBs (and range) in eggs of American coot, black-crowned night-heron, white-faced ibis, mallard, ruddy duck, and American avocet from EAFB in 1996 and 1999

Species	Egg type	<i>n</i>	Dieldrin	Oxychlorthane	<i>p,p'</i> -DDD	<i>p,p'</i> -DDE	<i>p,p'</i> -DDT	PCB-total	<i>trans</i> -nonachlor
Coot	1996 Random	3	ND ^a	ND	ND	0.038 (0.03–0.05)	ND	ND	ND
Night-heron	1996 Random	5	0.020 (ND–0.09)	ND	ND	1.74 (0.28–5.6)	0.019 (ND–0.07)	0.617 (0.31–0.97)	ND
Night-heron	1999 Random	3	0.0089 (ND–0.018)	0.0148 (ND–0.073)	ND	0.499 AB ^b (0.21–2.7)	ND	0.395 (0.20–0.99)	0.0187 (0.005–0.075)
	Failed	3	ND	0.0086 (ND–0.013)	ND	0.303 (0.073–1.2)	ND	0.341 (.22–0.44)	0.0129 (0.008–0.075)
Ibis	1999 Random	5	ND	ND	NC ^c (ND–0.061)	1.24 A (0.33–7.6)	0.0457 (ND–0.48)	NC (ND–0.095)	0.0136 (ND–0.031)
	Failed	5	ND	NC (ND–0.008)	0.0215 (ND–0.140)	1.77 (0.68–3.5)	0.0174 (ND–0.092)	ND	0.0171 (ND–0.043)
Mallard	1999 Random	3	ND	ND	ND	0.056 B (0.040–.078)	ND	ND	ND
	Failed	2	ND	ND	ND	0.066 (0.063–0.068)	ND	ND	ND
Ruddy duck	1999 Random	6	ND	ND	ND	0.109 B (0.029–2.0)	ND	ND	ND
Avocet	1999 Random	3	ND	ND	ND	0.765 AB (0.28–40)	ND	ND	ND

^a Not detected; all residues were less than LOD; for PCBs LOD = 0.05 $\mu\text{g/g}$; for all other OCs LOD = 0.01 $\mu\text{g/g}$. See text for OCs not detected or detected in 50% of the samples species.

^b GMs of random eggs sharing a capital letter are not significantly different by Student-Newman Keuls method of pairwise multiple comparisons.

^c Not calculated because >50% of the sample residues were less than the LOD.

from this study, were not collected for analysis. Therefore, it is not known whether the ibis acquired the DDT at the Piute Ponds or if they obtained these contaminants elsewhere and then deputed them through their eggs at Piute Ponds. Although *p,p'*-DDT was not detected in random or failed night-heron eggs in 1999, three of five random eggs collected in 1996 contained this contaminant (GM = 0.019 $\mu\text{g/g}$).

We compared random and failed night-heron eggs to evaluate the potential effects of each contaminant on hatchability (Table 2). Although we detected no statistical differences between failed and random eggs for oxychlorthane, *p,p'*-DDE, total PCBs, or *trans*-nonachlor ($p > 0.56$), sample sizes for both random and failed eggs were small ($n = 3$), making detection of differences difficult.

AL, As, Be, Cd, Pb, and V were not detected in coot or night-heron eggs in 1996. The GM concentration of Se (Table 3) in night-heron eggs (5.41 $\mu\text{g/g}$) was higher than in the coots, but it was lower than the lowest adverse-effect concentration for hatchability found in laboratory studies of night-herons (Smith *et al.* 1988) and mallards (Heinz 1996). The mean concentration of Sr was higher in coots (students *t* test, $p < 0.0001$); Cu was higher in night-herons (students *t* test, $p = 0.003$), and B and Ba were detected in all five coot eggs but only in one night-heron egg. However, the means of the other trace elements and metals (Cr, Fe, Hg, Mg, Mn, Ni, and Zn) were not different between species.

In 1999, As, B, Be, Cd, Cr, Ni, and V were not detected in any egg. Al was not detected in >50% of the random eggs of any species, but it was present in both of the failed mallard

eggs and >50% of the failed night-heron and ibis eggs (Table 2). Pb was detected in three random night-heron eggs, in one random ibis egg, and in three failed ibis eggs, but it was not detected in >50% of the random eggs at any site. Mo was detected in one random night-heron egg in 1996 (2.38 $\mu\text{g/g}$). In 1999, Mo was detected in >50% of the random night-heron eggs (GM = 0.546 $\mu\text{g/g}$), in one failed night-heron egg (1.2 $\mu\text{g/g}$), and in one failed ibis egg (1.1 $\mu\text{g/g}$), but it was not detected in any of the other eggs (Table 3).

One-way ANOVA on the Fe and Se results for random eggs found a significant difference ($p < 0.05$) among sites for Se but not for Fe. Pairwise multiple comparison using Student-Newman-Keuls method revealed that night-herons and ibis had higher mean concentrations than the mallards and avocets. Kruskal-Wallis one-way ANOVA on ranks was performed on the Ba, Cu, Hg, Mg, Mn, Sr, and Zn because either they failed the normality test or did not have equal variances. Too few eggs had detectable Al, Mo, and Pb to allow statistical comparisons. As was found in 1996, Sr was significantly lower in the night-herons than in any of the other species, and Ba was detected in all randomly collected eggs of the other species, but was detected in only 2 of 10 night-heron eggs. With these 2 exceptions, ibises and night-herons generally had higher concentrations of metals and trace elements than the ducks and avocets (Table 3). This can likely be attributed to dietary differences, with ibises and night-herons feeding at higher trophic levels.

Although sample sizes for failed eggs were small, concentrations of metals and trace elements in randomly collected and

Table 3. GMs ($\mu\text{g/g}$ dry weight) of metals and trace elements (and range) in eggs of American coot, black-crowned night-heron, white-faced ibis, mallard, ruddy duck, and American avocet from EAFB in 1996 and 1999

Species/year	Egg type	n	Al	B	Ba	Cr	Cu	Fe	Hg	Mg	Mn	Ni	Se	Sr	Zn
Coot	Random	5	ND ^a	5.39 (3.4–11.4)	1.58 (1.0–2.9)	1.35 (0.83–2.8)	3.22 ^{a,b} (2.7–3.7)	116 (103–137)	0.200 (0.13–0.40)	498 (413–569)	2.26 (1.9–3.0)	2.11 (1.1–4.2)	1.69* (1.3–1.9)	13.8* (8.6–18.5)	48.0 (41–57)
	Random	5	ND	NC (ND–3.4)	NC (ND–1.1)	1.39 (0.58–2.1)	5.56* (4.4–6.5)	124 (113–154)	0.292 (ND–0.89)	491 (425–596)	2.31 (1.9–2.4)	3.02 (0.78–7.4)	5.41* (3.8–6.7)	3.71* (1.9–8.4)	42.7 (37–50)
Night-Heron	Random	10	NC (ND–6.9)	ND	NC (ND–3.7)	ND	4.88 A (4.2–6.1)	98.1 (17–120)	0.201 (0.07–1.1)	435 AB (360–560)	1.97 (1.3–4.6)	ND	3.07 A (2.1–4.0)	2.4 B (1.4–5.7)	46.7 (41–56)
	Failed	4	7.29 (ND–9.4)	ND	0.64 (ND–1.3)	ND	6.27 (4.6–8.1)	92.3 (55–160)	0.157 (0.08–0.72)	676 (570–760)	1.66 (1.0–4.5)	ND	3.06 (2.1–4.4)	4.4 (4.0–5.4)	56.9 (34–85)
Ibis	Random	10	NC (ND–12)	ND	1.48 B (0.80–3.4)	ND	3.38 AB (2.9–4.5)	105.1 (79–150)	0.175 (0.09–0.34)	542 A (460–940)	1.28 (0.73–3.2)	ND	2.78 A (2.0–3.8)	21.4 A (12–43)	45.3 (36–66)
	Failed	6	6.29 (ND–12)	ND	1.21 (0.55–5.1)	ND	3.64 (3.0–4.5)	95.7 (43–230)	0.215 (0.07–0.42)	604 (460–920)	0.81 (ND–2.7)	ND	2.84 (2.1–3.6)	31.3 (17–76)	43.0 (26–92)
Mallard	Random	5	NC (ND–11)	ND	5.47 AB (1.0–16)	ND	3.10 ABC (2.7–3.6)	104.4 (70–140)	0.131 (0.04–0.47)	328 BC (240–640)	1.12 (0.85–1.7)	ND	1.05 B (0.90–1.4)	19.8 (7.0–72)	48.1 (41–56)
	Failed	2	6.89 (5.8–8.2)	ND	7.95 (5.4–12)	ND	2.91 (2.4–3.5)	122.2 (110–130)	0.113 (0.06–0.22)	377 (350–410)	1.29 (0.93–1.8)	ND	0.66 (ND–1.1)	46.6 (38–58)	45.6 (43–48)
Ruddy duck	Random	13	NC (ND–9.7)	ND	7.81 A (2.7–15)	ND	2.06 C (1.3–3.1)	124.0 (110–140)	NC (ND–0.10)	276 C (230–490)	1.43 (1.0–2.8)	ND	NC (ND–0.96)	20.2 (11–56)	45.7 (39–60)
	Random	10	NC (ND–5.8)	ND	4.44 AB (3.0–6.9)	ND	2.48 B (1.1–3.2)	104.5 (53–140)	0.211 (180–420)	350 ABC (0.10–0.72)	1.50 (0.84–2.0)	ND	0.82 B (0.52–1.2)	19.7 A (13–9)	40.7 (20–79)

^a Not calculated because > 50% of samples were below the LOD = Not detected; all residues were < LOD. In 1999, Mo was detected in 7 of 10 random night-heron eggs (GM = 0.546 $\mu\text{g/g}$; range 0.58–1.1) 1 failed night-heron egg (1.22 $\mu\text{g/g}$), and 1 failed ibis egg (1.14 $\mu\text{g/g}$). Mo was detected in one random night-heron egg in 1996 (2.38 $\mu\text{g/g}$). In 1999, Pb was detected in 3 random night-heron eggs (0.52–0.88 $\mu\text{g/g}$), 1 random ibis egg (1.0 $\mu\text{g/g}$) and 3 failed ibis eggs (0.72–0.94 $\mu\text{g/g}$). As, Be, Cd, and V were not detected in any eggs.

^b For eggs collected in 1996, * indicates a significant difference ($P < 0.05$) between species based on student *t* test. Within element, concentrations in random eggs from 1999 sharing a capital letter are not significantly different ($P < 0.05$) based on one-way ANOVA. Elements without capital letters were not different among species.

Table 4. Among-site comparisons of GMs ($\mu\text{g/g}$ dry weight) of metals and trace elements detected in female African clawed frogs at four ponds on EAFB (Big Piute, NDU, SDU, and Buffer Pond), male frogs at the Buffer Pond, and female frogs at two reference sites (Lake Elizabeth and Ritter Ranch) in 1998; $n = 5$ at all site^a

Site	Al	Ba	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Ni	Se	Sr	Zn
Lake Elizabeth	24.7	4.63 A ^b	0.111	1.79	12.0 AB	498 B	NC ^c	1040 B	12.5 A	0.826 B	1.19 C	23.5 A	93.1 B
Ritter Ranch	18.0	4.16 A	0.131	1.98	27.7 A	567 B	0.578 A	1410 A	7.96 B	NC	4.96 A	5.37 B	154 A
Big Piute	30.4	4.89 A	NC	1.89	6.90 B	921 A	0.273 B	1350 A	6.91 C	2.04 A	2.08 B	23.5 A	108 B
NDU	19.7	1.71 B	NC	1.19	6.91 B	356 BC	NC	883 B	4.33 D	0.593 B	1.57 BC	12.1 AB	85.6 B
SDU	17.3	1.41 B	NC	1.14	6.27 B	288 C	ND ^d	854 B	4.04 D	NC	1.58 BC	10.5 AB	90.9 B
Buffer Pond females	29.1	1.47 B	NC	2.56	5.65 B	393 C	ND	928 B	6.12 C	0.965 B	0.730 D	15.5 AB	142 B
Buffer Pond Males	19.5	2.10	ND	2.06	4.17	279	ND	1210*	6.63	NC	0.624	133*	108

^a For females frogs one-way ANOVA and Student-Newman-Keuls method used for Ba, Cr, Cu, Fe, and Mg; Kruskal-Walshs one-way ANOVA on ranks and Student-Newman-Keuls method used for Ni, Mn, Sr, Al, Se, and Zn; student *t* test used for Cd and Hg.

^b Within element, concentrations in female frogs not sharing the same letter are different among sites ($p < 0.05$).

^c Not calculated because $>50\%$ of samples were below the LOD.

^d Not detected because all residues were less than the LOD.

* Mean concentrations of these metals in males and female frogs from Buffer Pond were different ($p < 0.05$) based on student *t* test.

failed eggs were generally similar, including those elements (*i.e.*, Se and Hg) known to cause reproductive impairment in birds. Therefore, egg failure is not likely to be driven by concentrations of these elements.

Only one embryo, a white-faced ibis, had gross deformities. This embryo, which had multiple deformities similar to those caused by Se (Ohlendorf *et al.* 1988), was analyzed for trace elements, metals, and OCs. The Se concentration in this embryo (2.93 $\mu\text{g/g}$) was similar to the means for both the randomly collected (2.78 $\mu\text{g/g}$) and failed (2.84 $\mu\text{g/g}$) ibis eggs in this study. The Se level was also lower than the 3.0 $\mu\text{g/g}$ (wet weight) (approximately 12 $\mu\text{g/g}$ dry weight) cited by Heinz (1996) as being the threshold for reproductive impairment. Concentrations of other metals in the embryo were lower than those shown to cause deformities.

African clawed frogs. OC pesticides and PCBs were below the LOD for all African clawed frogs, with the exception of one from the Ritter Ranch, which had 0.054 $\mu\text{g/g}$ *p,p'*-DDE.

Based on students *t* test, the 5 male African clawed frogs collected from Buffer Pond had higher GM concentrations of Mg ($p = 0.008$) and Sr ($p < 0.001$) than female frogs. However, neither Mg nor Sr is commonly considered a contaminant that adversely affects avian reproduction. The eight other elements that could be compared were not different (Table 4). We concluded that, with the exceptions of Sr and Mg, comparisons of contaminants in female frogs adequately represented contamination in the species at Piute Ponds and the reference sites.

The two elements not detected in any analyzed frog were Be and V. However, B was not found in $>50\%$ of the frogs at any site; As was only found in $>50\%$ of the frogs at Ritter Ranch; Cd was found in $>50\%$ of the frogs at only the two reference sites; and Mo and Pb were found in $>50\%$ of the frogs only at Lake Elizabeth. When mean contaminant concentrations in clawed frogs from the four Piute Ponds and the two reference sites were compared, only Fe and Ni were higher in ≥ 1 Piute Ponds (Table 4). Mean concentrations of the other elements were either higher at one or both reference sites or were not different ($p > 0.05$).

Se, a potential contaminant of concern, was significantly higher at Ritter Ranch than at any of the Piute Ponds; it was lowest in clawed frogs from the Buffer Pond. The mean concentration of Hg, another contaminant of concern, was higher at Ritter Ranch than at Big Piute, the only two sites with $>50\%$ of the samples with detectable amounts of Hg. Of the 4 Piute Ponds, Big Piute had higher concentrations of Ba, Fe, Mg, and Ni; no other pond had significantly higher concentrations of any of the elements (Table 4).

Conclusions

Avian species occupying the higher trophic levels, *i.e.*, night-herons, ibis, and avocets, tended to accumulated higher concentrations of contaminants than the ducks. Reproductive success of black-crowned night-herons and white-faced ibises in the Piute Ponds was similar to results observed elsewhere, with predation being the most limiting factor for both species at Piute Ponds. Hatchability of both night-heron eggs (95.5%) and ibis eggs (90.6%) was similar to other comparable sites.

One ibis embryo had multiple gross deformities, but the cause of the deformities was not confirmed; Se and other contaminants were not especially increased in this embryo. With the exception of Be and V, all metals and trace elements were detected in at least one clawed frog from Piute Ponds and from the reference sites, but concentrations of five of the elements were higher at one or both of the reference sites, whereas only two were higher at Piute Ponds. Ten elements were either not different or were detected too rarely to be tested.

Night-herons bioaccumulate contaminants from prey items, but based on our findings, none of the African clawed frogs had detectable levels of any OCs, thus eliminating them as a source of OC contaminants. Therefore, it is likely that OCs in night-heron eggs were acquired from a location other than Piute Ponds, probably on the wintering grounds. The presence of DDT in ibis eggs may have reflected recent exposure to this contaminant, but ibis foods were not collected from EAFB, and thus the sources of DDT in ibis eggs could not be confirmed.

Random and failed eggs of individual species were not different for OCs, metals, or trace elements, including those elements (*i.e.*, Se and Hg) known to cause reproductive impairment in birds. Therefore, we conclude that egg failure was not related to contaminants.

Avian reproduction at Piute Ponds was similar to other monitored sites; concentrations of environmental contaminants measured in clawed frogs and avian eggs from the Piute Ponds were not significantly elevated; and there was no detected relationship between avian reproduction and the presence of environmental contaminants in eggs or frogs at Piute Ponds.

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