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Organochlorine Pesticides, PCBs, and Mercury in Osprey *Pandion haliaetus* eggs from the Upper Volga River, Russia

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ABSTRACT

The Osprey population associated with Darwin Nature Reserve and the Rybinsk Reservoir increased from only a few pairs prior to the creation of the reservoir in the late 1940s, to about 45-50 pairs in 1994. Productivity rates were excellent in 1988 and 1989 (1.38 young/occupied nest), but extremely low in 1987 (0.47 young/occupied nest). A chemical spill into the Volga River in early 1987 resulted in a massive fish kill, which was believed responsible for low production that year. With the exception of the year of the chemical spill and 1992 (the year an egg was collected from 10 of 11 nests studied), production was comparable to rates observed in stable or increasing populations in Byelorussia, Finland, and Sweden. The p.p'-DDE (DDE), polychlorinated biphenyl (PCB), and mercury concentrations from eggs collected in 1992 were below known effect levels and eggshell thickness showed only 6.3% thinning -an amount not associated with reproductive problems.

INTRODUCTION

At the World Conference on Birds of Prey, Galushin (1977) noted that it had been one decade since birds of prey became legally protected in the U.S.S.R. in 1964. There was some evidence of a continued decline in the Osprey which is included in Red Data Book of the Russian Federation (1983). It was indicated that pesticides may be implicated although destruction of habitat, human disturbance, occasional shooting and nest destruction may be important. Despite much information published recently on environmental contamination in the former USSR (Yablokov 1990 and Khabibullov 1991, and others), not so much is known of raptor contamination in this country. Under Area V of the U.S.-Russia Environmental Agreement, studies were designed to evaluate contaminant burdens in eggs of birds of prey from Russia. In 1991, eggs were collected from Peregrine Falcons *Falco peregrinus* on the Kola Peninsula of Arctic Russia (Henny *et al.* 1994). Henny, Galushin and Kuznetsov collected Osprey eggs within Darwin Nature Reserve in May 1992. The eggs were taken to the United States for analysis of chemical residues and eggshell thickness.

STUDY AREA AND METHODS

Darwin Nature Reserve, a protected Natural Area created in 1945, is 300 km north of Moscow at the western end of Rybinsk Reservoir. The Reserve occupies over 1100 km^2 including about 700 km^2 of terrestrial habitats. The 4650 km^2 reservoir was created in the late 1940s by a dam on the Volga River. The terrain is generally flat and consists of thick tills and sandy soils covered with hardwood forest. Most small adjacent lakes (2 to 200 ha) were in forested catchments without dwellings or roads.

Nemtsev (1988), Kuznetsov and Nemtsev (1992) provided a review of raptors nesting at the Darwin Nature Reserve including Osprey, White-tailed Eagle *Haliaeetus albicilla*, and other species. The number of nests monitored annually since 1987 has varied because of helicopter availability. If available, two annual helicopter flights are made, one in May during the period with eggs present (e.g., 22 May 1992), and another flight later to count large young in the nests. However, the early survey was not conducted in 1990, 1991, and 1993. Therefore, the number of young produced per occupied nest was not available for each year.

As a general survey for environmental contaminants from the Darwin Nature Reserve, we elected to evaluate eggs of Ospreys for organochlorine (OC) pesticides, PCBs, and mercury. Our concern about DDE, other OC pesticides, PCBs and mercury centres on their toxicity, persistent nature, and propensity for bioaccumulation.

ANALYTICAL METHODS

Egg contents were analyzed at the Geochemical and Environmental Research Group, Texas A&M University, College Station, Texas. Egg samples for organics were extracted by the NOAA Status and Trends Method (MacLeod *et al.* 1985) with minor revisions (Brooks *et al.* 1989; Wade *et al.* 1988). Briefly, the egg samples were homogenized with a Teckmar Tissumizer. A 1 to 10-gram sample wet weight (w/w) was extracted with the Techmar

Tissumizer by adding surrogate standards, Na_2SO_4 and methylene chloride in a centrifuge tube. The tissue extracts were purified by silica/alumina column chromatography to isolate the pesticide/PCB fractions. The pesticide/PCB fraction was further purified by HPLC in order to remove interfering lipids. Eggs were analyzed for toxaphene, alpha BHC, beta BHC, gamma BHC, delta BHC, HCB, heptachlor epoxide, oxychlordane, gamma chlordane, alpha chlordane, *trans*-nonachlor, *cis*-nonachlor, aldrin, dieldrin, endrin, mirex, DDE, DDT, DDD, and total PCBs. The quantitative analyses were performed by capillary gas chromatography (GC) with electron capture detector for pesticides and PCBs (Wade *et al.* 1988). The pesticides and PCBs are initially analysed on a DB-5 capillary column. The analyte identity and concentrations are confirmed on a DB-17 capillary column. In addition, PCB congeners 77, 126, and 169 were analysed individually with high resolution GC-Mass Spectrometry.

Mercury was determined by EPA method 245.5 with minor revisions (EPA 1980). The sample is weighed into a 50 ml polypropylene centrifuge tube. 2.5 ml of concentrated sulfuric acid (H_2SO_4) and 1.5 ml of concentrated nitric acid ($HN0_3$) were added and the samples heated in a water bath at 90 C for 15 min. After cooling 10 ml of distilled water and 15 ml of mixture of 3.3% (w/w) potassium permanganate ($KMnO_4$), and 1.7% (w/w) potassium persulfate ($K_2S_20_8$) were added to each tube and the samples heated in a water bath at 90 C for 30 min. After cooling 5 ml of 10% (w/w) hydroxylamine hydrochloride (NH_2OH HCl) was added to reduce excess permanganate and the volume brought to 35 ml with distilled water. Mercury is determined by a modification of the method of Hatch and Ott (1968). A portion of the digest solution is placed in a sealed container. To this is added 0.4 ml of 10% (w/w) stannous chloride ($SnCl_2$). Mercury is reduced to the elemental state and aerated from solution into an atomic absorption spectrophotometer where its concentration is measured.

We converted contents of eggs to an approximately fresh w/w using egg volume (Stickel *et al.* 1973); all organochlorines and mercury in eggs were expressed on a fresh w/w basis.

RESULTS

Osprey Population and Nesting Success

Five to seven pairs of Ospreys nested in the Darwin Nature Reserve prior to the creation of the Rybinsk Reservoir (Belko 1985; Kuznetsov & Nemtsev 1992). However, the population gradually increased to about 45-50 pairs in the Darwin Nature Reserve and vicinity in 1994 (Kuznetsov 1995).

Table 1. The success of Ospreys nesting on the Darwin Nature Reserve, Russia, 1987-1993 (Kuznetsov, unpublished data).

Category	1987[b]	1988	1989	1990	1991	1992[c]	1993	Total
Number Occupied Nests Visited[a]	17	16	26	NA	NA	11	NA	70
Number of Successful Nests	6	15	21	8	18	6	19	93(48)[d]
Number of Large Young	8	24	34	9	34	8	34	151(74)[d]
Young/Successful Pair	1.33	1.60	1.62	1.13	1.89	1.33	1.79	1.62
Young/Occupied Nest	0.47	1.50	1.31	NA	NA	0.73	NA	1.06[e]

[a] The number of nests visited each year does not reflect population size. Nests with a pair present (occupied nest) may include some pairs not laying eggs; and no early survey was made for occupied nests in 1990, 1991, and 1993.

[b] A chemical spill from the Chervopovets Metallurgy Plant into the Volga River upstream from the study area killed many fish in Rubinsk Reservoir.

[c] One egg was taken from 10 of the 11 nests studied.

[d] ( ) Number of successful nests and number of large young in nests checked early in season for occupancy.

[e] Includes only years when nests checked for occupancy early in season.

Table 2. Clutch size, young produced, eggshell thickness and contaminant residues (µg/g, wet wt.) for Ospreys at Darwin Nature Reserve, Russia 1992.

Nest No.	Location[a] Collected	Egg Size	Clutch	Young Fledged	PCB	DDE	DDD	§BHC	Hg	Eggshell Thickness (mm)
HT-1	N208	May 8	3[b]	2	1.00	0.31	0.02	0.01	0.09	0.493
HT-2	N201	May 8	2[b]	0[c]	0.64	0.13	0.02	0.03	0.18	0.434
HT-3	N200	May 8	2[b]	0[c]	2.93	0.54	0.06	0.03	0.09	0.447
HT-4	N211	May 11	3	1	0.35	0.09	0.01	0.01	0.15	0.520
HT-5	N212	May 13	3[b]	0[d]	0.65	0.23	0.04	0.02	0.12	0.446
HT-6	N212	May 13	3	1	0.31	0.27	0.04	0.01	0.02	0.484
HT-7	N212	May 13	3	0[d]	2.95	0.19	0.03	0.01	0.06	0.521
HT-8	N212	May 13	3	0[c]	3.31	0.51	0.09	0.02	0.08	0.531
HT-9	Uteskovo Lake	May 16	3	1	4.55	0.25	0.06	0.01	0.05	0.502
HT-10	N208	May 20	3	1	0.49	0.27	0.02	0.01	0.18	0.490
Mean[e]			2.80	0.60	1.11	0.25	0.03	0.02	0.09	0.487

Note: DDT-(0.01 to 0.03 µg/g) was detected in 3 eggs, cis-nonachlor (0.01) in 1 egg, and dieldrin (0.01) in 1 egg.

[a] Refer to squares of 1 km X 1 km dimension.

[b] Collected egg was fresh (no development), therefore, clutch size may have been incomplete. Note, one egg was collected from each nest.

[c] Nest deserted.

[d] Nest fell down.

[e] Residue concentrations (geometric means).

This represents an 8-10X increase since the late 1940s.

Nesting success at the Darwin Nature Reserve was variable, but 69 percent of the nesting attempts were successful between 1987 and 1993 and 1.06 large young were produced per occupied nest (Table 1). An average of only 0.60 young were fledged from the 10 nests with an egg collected in 1992 (Table 2). Two nests fell down, and three nests were deserted, including the two 2-egg clutches with fresh eggs at the time of collection.

Prey Species

Prey remains from the nests and observations of Ospreys catching fish were used to evaluate fish species captured by Ospreys. The most common 6prey were Blue Bream (*Abramis ballerus*), Bream (*Abramis brama*), Roach (*Rutilus rutilus*), Perch (*Perca fluviatilus*) and Pike (*Esox lucius*). Sometimes Ospreys take fish with helmintes (*Ligula sp.*), but they never eat them. The great majority of Osprey pairs (≈90-95%) at Darwin Nature Reserve fish at the Rybinsk Reservoir. Only 2 or 3 inland pairs in the north of the reserve entirely fish at large lakes (more than 1 km in diameter). In addition, about 10 pairs (also in the north) could fish both at the reservoir and largest lakes. Small lakes were not used for fishing because of the shortage of fish, and lakes with brown water were not used because fish (mostly Perch) were small and scarce. Even Arctic Loons *Gavia arctica*, which nest near small lakes, fish at the reservoir. Osprey at the 10 nests where an egg was collected in 1992 all fished in Rybinsk Reservoir.

Table 3. AHH-active PCB congeners (ng/g, wet weight) in Osprey eggs from Darwin Nature Reserve, Russia, 1992.

	PCB 77	PCB 126	PCB 169
TEF[a]	0.01	0.10	0.05
Nest No.			
HT-1	0.55	1.88	0.30
HT-2	2.64	1.94	0.68
HT-3	0.44	6.30	ND[b]
HT-4	0.97	0.69	0.41
HT-5	0.38	1.57	0.11
HT-6	1.50	0.69	0.18
HT-7	1.62	4.71	0.25
HT-8	1.09	4.68	0.17
HT-9	0.62	1.86	0.41
HT-10	0.53	1.17	0.20
Geometric mean	0.85	1.96	0.18

[a] Toxic Equivalency Factors (TEFs) from Safe (1990).
[b] ND = non-detection (<0.01).

Residues in Eggs and Eggshell Thickness

A random intact egg was obtained from 10 different Osprey clutches in 1992 (Table 2). Mean clutch size was 2.80 eggs; however, four of the clutches (2,2,3, and 3 eggs) contained fresh eggs (no development). Therefore, the clutch size may be slightly underestimated if additional eggs were laid after our collection was made; however, the clutch sizes reported for 1976-90 in Byelorussia (2.82), and for southern and central Sweden prior to 1947 (2.84) and 1973 (2.80) were similar (Tishechkin & Ivanovsky 1992; Odsjö & Sondell 1973).

Eggshell thickness in 1992 was 0.487 ± 0.034 mm (mean ± SD) (Table 2), compared with 0.52 ± 0.01 (mean ± 95% C.L.) for Osprey in Scandinavia in 1874-1941 (Anderson & Hickey 1974); this represents a reduction of 6.3%. The pre-DDT era Scandinavia shell thickness information was the geographically closest data available.

DDE concentrations were low (see Wiemeyer *et al.* 1988); the geometric mean was 0.25 µg/g (fresh w/w) with no concentrations above 0.54 µg/g (Table 2). Other organochlorine pesticides were found at very low concentrations. Total PCBs were higher (geometric mean 1.11 µg/g), with concentrations from some eggs about triple the mean. Three PCB congeners, which include some of the more potent inducers of hepatic aryl hydrocarbon hydroxylase, were quantified (Table 3). The geometric means for PCB congeners 77, 126 and 169 (0.85, 1.96, and 0.18 ng/g) differed slightly from those found in six Peregrine Falcon eggs collected on the Kola Peninsula of Russia in 1991 (1.5, 1.3, and 0.31 ng/g, respectively). Mercury was detected in all eggs, but no concentrations were above 0.18 µg/g.

DISCUSSION AND CONCLUSIONS

Osprey population numbers at Darwin Nature Reserve have increased since the Rybinsk Reservoir was created in the 1940s, which is logical since the reservoir is the primary source of fish for the Ospreys. Population increases of nesting Ospreys associated with reservoirs are also common throughout the western United States (Henny *et al.* 1978). Two years (1987 and 1992) showed lower production rates than the other years. The Cherepovets Metallurgical Plant, which is about 30 km upstream on the Volga River, had a major chemical spill into the river in February 1987 that killed many fish (Bordenkov 1988). The loss of fish probably accounts for the low productivity in 1987, whereas the collection of a sample egg from 10 of the 11 nests studied in 1992 partially accounts for the lower production that year. The two 2-egg clutches with a fresh egg collected were both deserted (probably investigator caused). Production rates (excluding 1987 and 1992) were excellent (see Henny & Wight 1969) at 1.38 young/occupied nest, and the average remained within an acceptable range (1.06 young/occupied nest) with the anomalous years of 1987 and 1992 included. Within the general region of northern Europe, the numbers of occupied Osprey territories in a well-studied area (2000 Km^2) in southern Finland in 1962, 1972, and 1982 was 46, 37, and 43 (Saurola 1986). Saurola believed these figures indicate that the population decrease, which began in the 1950s, stopped in the 1970s, and the population is now recovering. Osprey population trends are similar in the most parts of European Russia (Galushin 1991, 1994). From 1971 to 1985, the population of southern Finland produced 1.37 large young/occupied territory with no obvious pattern of change over time. Tishechkin and Ivanovsky (1992) reported 1.29 young fledged per occupied nest or 1.59 young per active (eggs known to be laid) nest in northern Byelorussia between 1986 and 1990. The Byelorussia population was estimated at 100-120 pairs and productivity and nesting success were similar to those observed in stable populations in Finland (Saurola 1986) and Sweden (Odsjö and Sondell 1976). The pattern of eggshell thickness over time in Ospreys from northern Europe is best illustrated by the shell thickness index data of Odsjö (1971) for Sweden and Finland. Compared to the pre-1947 norm, eggs collected from 1947 to 1953 showed 7.8% thinning, eggs from 1954 to 1959 12.0% thinning, and eggs from 1960 to 1967 15.9% thinning. Ospreys nesting in the Darwin Nature Reserve now show only 6.3% shell thinning, which is considerably less than the generally accepted 18% shell thinning (Lincer 1975) associated with declining raptor populations with problems related to DDT and its metabolites.

Fish at the Darwin Nature Reserve have not been analyzed for OCs or PCBs, but Perch were analyzed in 1989 for mercury at selected smaller lakes and the large Rybinsk Reservoir (Haines *et al.* 1994). Skeletal muscle of fish from Rybinsk Reservoir and Hotavets Lake (160 ha) contained the lowest mercury concentrations (0.11 and 0.09 µg/g, w/w). This reservoir and drainage lake both had a high pH (8.1 and 8.0). The other lakes sampled were coloured seepage lakes with pH ranging from 4.6 to 4.8, and Perch contained higher mercury concentrations (0.47 to 0.80 µg/g w/w). Perhaps fortunately, the Ospreys were not catching fish in the coloured seepage lakes. All of the pairs with eggs sampled in 1992 were fishing in the Rybinsk Reservoir where mercury concentrations were the lowest. Atmospheric deposition was presumed to be the source of the acidity and mercury. Haines *et al.* (1994) concluded that acidity and colour may affect the mercury content in fish by regulating loading and the bioavailability of mercury to lower trophic levels in these lakes.

What might be expected in Osprey eggs when the adults eat fish containing 0.10 µg/g (w/w) mercury in their diet? This would depend upon the form of mercury and how long birds were in the area to accumulate mercury before laying eggs. Stickel *et al.* (1977) showed that Mallards *Anas platyrhynchos* fed methylmercury retained only 24% in liver and 19% in kidney after being off the mercury diet for 16 weeks. Therefore, if there is no mercury contamination at wintering or migration localities, Ospreys would probably return to Darwin Nature Reserve each year with little residual mercury from the previous years. Heinz (1974) fed Mallards methylmercury at 0.5 μg/g dry weight (d/w) (≈0.1 w/w); mercury concentrations in eggs plateaued at slightly less than 1μg/g (w/w) after 4-5 weeks. The dosage had no effects on Mallard reproduction. Mercury concentrations found in Osprey eggs at Darwin Nature Reserve were much lower. However, only one fish species, which was not a major prey species for Osprey, was sampled from Darwin Reserve and Ospreys were in the area for only a short time (3 or 4 weeks) before laying eggs and may not accumulate much mercury in that short time from local sources. Furthermore, Ospreys may not absorb mercury in a manner similar to Mallards fed a steady diet. Several researchers (Häkkinen & Häsanen 1980; Wiemeyer *et al.* 1984; Newton & Haas 1988) concluded that mercury concentrations in eggs below 0.5-0.6 μg/g (w/w) did not adversely affect raptor reproduction. The Osprey eggs from Darwin Nature Reserve were all below 0.5 μg/g, with the highest 0.18 μg/g. Osprey eggs representing 14 clutches analyzed from Connecticut and Maryland in 1964 and 1969 averaged 0.12 μg/g (w/w) mercury with an extreme of only 0.24 μg/g (Wiemeyer *et al.* 1975). Mercury in 16 addled Osprey eggs from central Finland and Lapland in 1970-1972 varied between 0.1 and 0.4 μg/g (w/w). (Häkkinen & Häsanen 1980). We conclude that the low concentrations of DDE, total PCBs, and mercury are not adversely affecting Osprey productivity at Darwin Nature Reserve.

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