Progress Notes 36034104 **Canadian Wildlife Service**

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Disponible également en français

No. 176, April 1988

Levels of polychlorinated dibenzodioxins and polychlorinated dibenzofurans in eggs of Great Blue Herons (*Ardea herodias*) in British Columbia, 1983–87: possible impacts on reproductive success

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Introduction

In 1982, a survey of polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) levels in Canadian wildlife revealed that Great Blue Heron (*Ardea herodias*) eggs from a colony in the Fraser River estuary contained unusually high levels of PCDDs (Norstrom and Simon 1983). In this note, we report the results of studies undertaken in 1983–87 to measure PCDD and PCDF levels in heron eggs from several colonies along the Strait of Georgia and to determine their effect on Great Blue Heron reproductive success. PCDDs are potent avian embryotoxins (Verrett 1970; Cheung *et al.* 1981). PCDDs and PCDFs are trace contaminants in chlorophenols used for wood preservation (Miles *et al.* 1985) and are formed in the manufacture of bleached kraft wood pulp (Amendola *et al.* 1987).

Methods

Sample collection

Five Great Blue Heron colonies on the Strait of Georgia were chosen for study (Fig. 1):

(1) The Crofton colony was located about 1 km from a kraft pulp and paper mill producing approximately 925 t of kraft pulp per day. The birds forage on an intertidal area near the underwater discharge pipe of the mill.

(2) A colony on Gabriola Island was near a kraft pulp and paper mill at Harmac.

(3) The third colony was located on the Endowment Lands of the University of British Columbia (UBC). These birds forage on the lower Fraser River and its estuary (Paine 1972), waters that receive wastes from the most heavily urbanized and industrialized area in British Columbia. The effluents of 13 pulp, paper, and lumber mills and 20 wood treatment operations on the lower Fraser River are discharged to the system (Garrett 1982).

(4) The Nicomekl colony is located in a predominantly agricultural setting, and the birds forage in nearby Mud Bay and Boundary Bay.

(5) The Sidney Island colony is situated about 10 km east of the town of Sidney on the southeast end of Vancouver Island and, like Boundary Bay, does not receive any direct input of industrial wastes. These herons forage mostly in a lagoon 1 km from the colony.

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One egg was collected from each of 34 nests in four colonies (Nicomekl, 7; UBC, 10; Gabriola Island, 8; Crofton, 9) in April–May 1983. In 1986, one egg was collected from each of 26 nests at Sidney Island and three of the colonies sampled in 1983 (Sidney Island, 4; Nicomekl, 5; UBC, 7; Crofton, 10). On 21 April 1987, one egg was collected from each of 10 nests in the colony at UBC; on 5 May 1987, one egg was collected from each of 10 nests at Crofton. The eggs were opened and the contents emptied into acetone-rinsed jars. The jars were then sealed with lids lined with aluminum foil, frozen, and flown to the National Wildlife Research Centre in Hull, Quebec, for analysis.

Reproductive assessment

In 1986, the number of young that fledged in nests from which eggs were taken and in undisturbed nests at the Sidney Island, Nicomekl, UBC, and Crofton colonies was counted from the ground through binoculars and telescope in late June or early July to provide a measure of reproductive success. The number of young produced per successful nest (at least one young fledged) was used as a measure of reproductive success. In 1987, we visited the Crofton colony on 21 and 29 April, 5 and 11 May, and 17 and 25 June. All eggshells on the ground were gathered. The total weight of eggshells collected was divided by the average weight of 20 complete shells to provide an estimate of the total number of eggs laid in the colony. Fledging success at the three remaining colonies (UBC, Nicomekl, and Sidney Island) in 1987 was determined as in 1986, except that all nests at UBC and Sidney Island were monitored at least once per week throughout the prefledging period. The greatest number of young seen in each nest before the nestlings began to climb out of the nest was taken as the measure of fledging success at UBC and Sidney Island.

Chemical analysis

The 1983 samples were pooled on an equal-weight basis prior to analysis; eggs from 1986 and 1987 were analyzed individually. Egg samples were homogenized and analyzed at the National Wildlife Research Centre. Samples (25 g) were ground with sodium sulfate and extracted with dichloromethane:hexane (1:1), and the lipids were removed by gel-permeation chromatography (GPC) (Norstrom *et al.* 1986). PCDDs and PCDFs were separated from other organochlorines by carbon, alumina, and Florisil chromatography and determined by gas chromatography/mass spectrometry (GC/MS) using a Hewlett-Packard 5987B GC/MS and a 30-m DB-5 capillary GC column (Norstrom and Simon 1988).

Statistical treatment

Residue data are reported as arithmetic means and standard deviations. After conversion of the data to common logarithms, we used Statistical Program for Social

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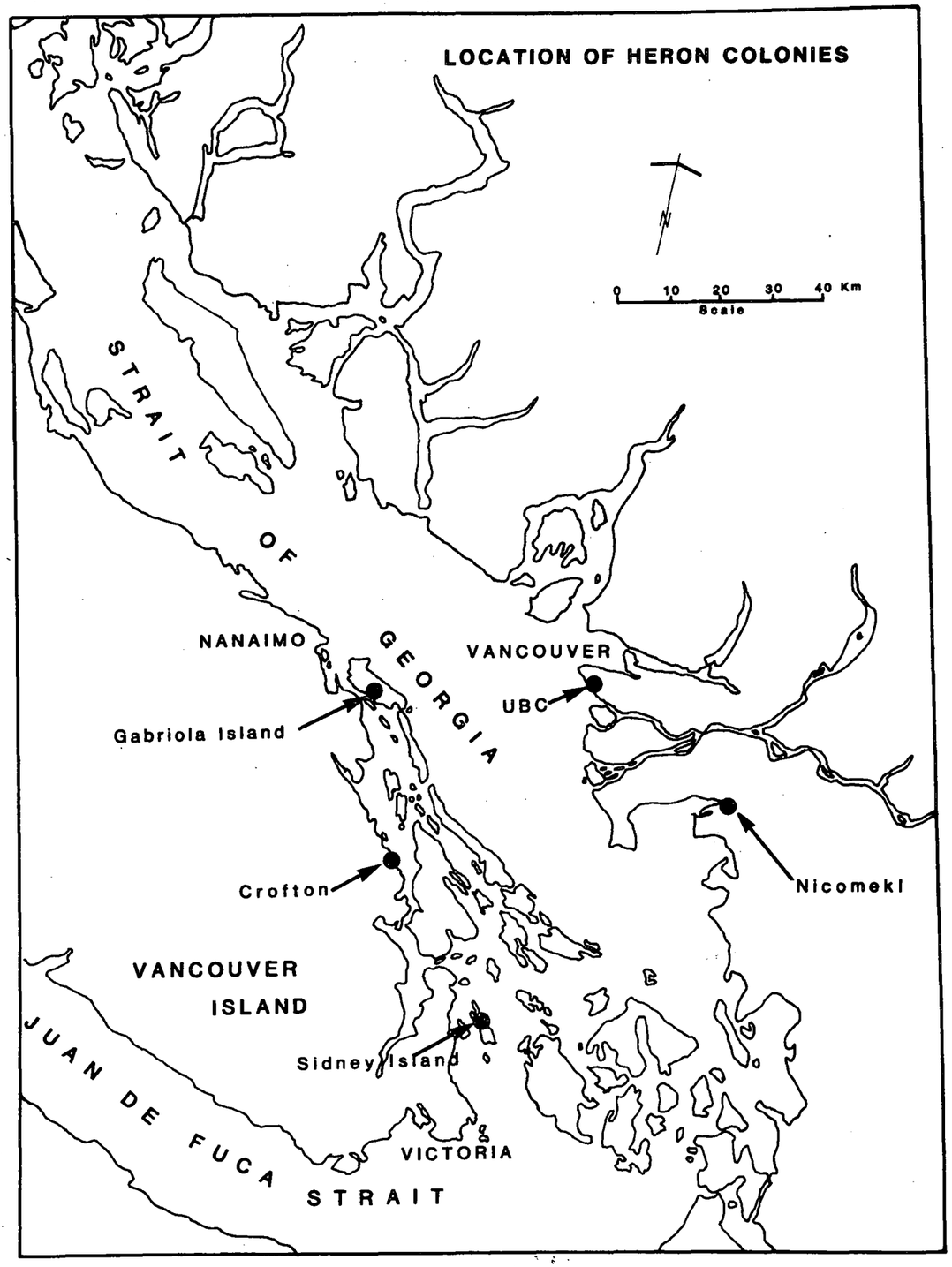
Figure 1
Map of Great Blue Heron colony sites in the Strait of Georgia, BC, from which eggs were collected



Sciences routines to perform a one-way analysis of variance followed by Tukey's Alternate Range Test to determine the significance of differences in residue levels among colonies. Significance of differences in residue levels between years was determined by *t* tests. Unless otherwise indicated, a significance level of $p < 0.05$ was applied to all statistical tests.

Results

PCDD and PCDF residue levels in heron eggs

Structures and numbering systems of the dibenzo-p-dioxins and dibenzofurans are given in Fig. 2. Major PCDD contaminants identified and determined in heron eggs (Table 1) were (in descending order of concentration) 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (123678-HxCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (12378-PnCDD), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (2378-TCDD). The only exception to this order occurred at UBC in 1987, when levels of 2378-TCDD were greater than those of 12378-PnCDD. All eggs and egg pools contained measurable levels of the three major PCDD congeners. Lower concentrations of 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (1234678-HpCDD) and octachlorodibenzo-p-dioxin (OCDD) were found in most eggs (Tables 1 and 2). The only PCDFs consistently present were 2,3,7,8-tetrachlorodibenzofuran (2378-TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (23478-PnCDF).

The ranking of colonies according to degree of PCDD contamination was the same in each of the years for which there were data: Crofton > Gabriola Island > UBC > Nicomekl = Sidney Island (Table 1). The last two colonies can be considered representative of the least contaminated environments in the Strait of Georgia. There were no significant differences between 1983 and 1986 in levels of the major PCDDs in the Nicomekl colony; however, OCDD levels dropped by a factor of 10.

Levels of 2378-TCDD in Sidney Island and Nicomekl eggs were significantly lower ($p < 0.01$) than in Crofton and UBC eggs in 1986. The difference between Crofton and UBC was not significant. Levels of 2378-TCDD in individual heron eggs from Crofton and UBC are plotted in Fig. 3. The ratio of 12378-PnCDD to 123678-HxCDD was not dependent on concentration and varied little among colonies and years, ranging from 0.38 at Crofton in 1983 to 0.71 in the same colony in 1986. The mean ratio ± SD for all colonies and years was 0.57 ± 0.11. This suggests a common source for these two PCDDs in the Strait of Georgia. Considerably more variation was observed in levels of 2378-TCDD relative to 123678-HxCDD: from 0.04 at Crofton in 1983 to 0.79 at UBC in 1987. The ratio of 2378-TCDD to 123678-HxCDD increased with time at all colonies, mainly because of a consistent increase in 2378-TCDD levels between 1983 and 1987. In 1987, 2378-TCDD levels were three to four times higher at Crofton than at UBC ($p < 0.01$); indeed, all levels of PCDDs were significantly higher at Crofton than at UBC that year. Levels of 2378-TCDD in eggs from Crofton were three times higher in 1987 than in 1986 ($p < 0.01$). Levels doubled in eggs from UBC between 1986 and 1987, but the increase was not significant.

Figure 2
Chemical structure and carbon numbering system of dibenzo-p-dioxin and dibenzofuran: the most toxic polychlorinated derivatives are those substituted with chlorine at all four of the 2, 3, 7, and 8 positions

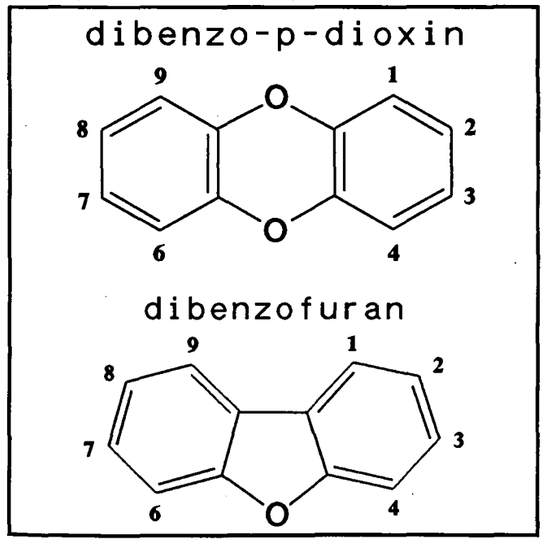


Figure 3
Levels of 2378-TCDD in individual Great Blue Heron eggs from Crofton and UBC, 1986 and 1987

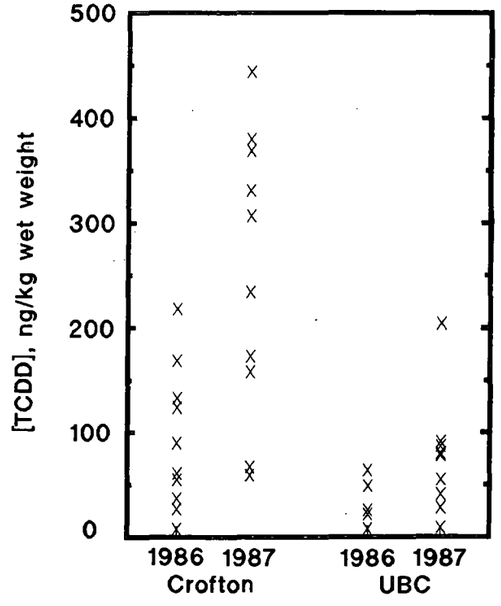


Table 1
PCDD and PCDF levels in Great Blue Heron eggs from British Columbia, 1983–87

Location	Year	n (in pool)	2378-TCDD	12378-PnCDD	123678-HxCDD	123789-HxCDD*	1234678-HpCDD	OCDD	2378-TCDF	23478-PnCDF
			Residue level (ng/kg, wet weight), mean ± SD (range in parentheses)							
Nicomekl	1983	(7)	7	33	70	ND†	6	20	3	15
	1986	5	11 ± 2 (7–12)	32 ± 12 (14–44)	60 ± 35 (21–110)	3 ± 2 (ND–5)	5 ± 4 (ND–9)	2 ± 1 (ND–3)	ND	8 ± 4 (2–10)
Sidney Island	1986	4	11 ± 8 (3–18)	31 ± 22 (9–59)	60 ± 77 (6–174)	5 ± 6 (ND–13)	1 ± 1 (ND–2.5)	ND	1 ± 1 (ND–1)	3 ± 3 (ND–6)
UBC	1983	(10)	15	59	104	ND	6	27	11	48
	1986	7	31 ± 19 (7–63)	94 ± 77 (28–255)	136 ± 119 (40–385)	10 ± 8 (1–23)	18 ± 23 (ND–70)	11 ± 16 (2–47)	5 ± 4 (ND–10)	21 ± 16 (6–51)
	1987	10	71 ± 54 (9–204)	55 ± 28 (23–109)	90 ± 36 (39–158)	6 ± 4 (2–12)	7 ± 4 (2–12)	8 ± 7 (2–19)	22 ± 16 (5–50)	16 ± 5 (9–25)
Crofton	1983	(9)	40	347	911	46	8	25	24	46
	1986	10	92 ± 68 (8–218)	388 ± 315 (11–1018)	544 ± 427 (13–1298)	35 ± 28 (1–89)	4 ± 4 (ND–11)	6 ± 6 (ND–21)	5 ± 5 (ND–14)	30 ± 26 (ND–68)
	1987	10	253 ± 135 (67–444)	314 ± 204 (73–728)	505 ± 293 (110–1052)	31 ± 16 (9–58)	3 ± 1 (ND–4)	4 ± 1 (ND–5)	5 ± 5 (ND–17)	45 ± 26 (10–104)
Gabriola Island	1983	(8)	22	153	339	23	4	7	11	44

*1,2,3,7,8,9-hexachlorodibenzo-p-dioxin.
†ND, no peak observed (signal/noise < 3).

Table 2
Clutch size and number of fledglings produced from sucessful (>1 young per nest) nests

Year	Parameter	Crofton Mean ± SD	Crofton n*	UBC Mean ± SD	UBC n	Nicomekl Mean ± SD	Nicomekl n	Sidney Island Mean ± SD	Sidney Island n
1986	Clutch size	3.9 ± 0.7	50	4.0 ± 0.0	6	4.4 ± 0.7	14	3.8 ± 0.5	4
	Young fledged	2.0 ± 0.7	13	2.0 ± 0.9	46	2.1 ± 0.0	12	2.2 ± 0.9	12
1987	Clutch size	–	–	4.2 ± 0.7	23	–	–	–	–
	Young fledged	0.0	57	2.0 ± 0.8	141	2.3 ± 0.7	29	2.3 ± 0.6	48

*n, number of nests

Whereas measurable quantities of 2378-TCDF were found in most Crofton and UBC eggs in 1986, only trace amounts were found in Sidney Island and Nicomekl eggs. Most eggs from Crofton and UBC contained higher levels of 23478-PnCDF than did eggs from the Sidney Island and Nicomekl colonies, but the difference was not significant.

The 2378-TCDF levels in UBC eggs were higher ($p < 0.01$) in 1987 than in 1986; levels were also significantly higher in 1987 at UBC than at Crofton. Conversely, 23478-PnCDF levels were significantly higher at Crofton than at UBC in 1987.

Reproductive studies

In 1986, all the nests monitored at Crofton, Sidney Island, and Nicomekl produced nestlings, whereas only two of seven nests at UBC fledged young. However, there were no significant differences among the four colonies in the mean number of young fledged in nests left undisturbed (Table 2). There was no significant relationship in 1986 between the number of nestlings that fledged and PCDD or PCDF levels at any of the four colonies. Heron productivity at UBC, Sidney Island, and Nicomekl was similar in 1986 and 1987; however, the colony at Crofton failed to fledge any young in 1987. During six visits to the Crofton colony, the shells of approximately 179 eggs were found on the ground below an estimated 57 active nests. The majority of the shells appeared to have been perforated in the side, rather than in a circle toward one end, as occurs with normally hatched eggs.

Discussion

Our study shows that there is widespread contamination by PCDDs and PCDFs of the Great Blue Herons feeding in estuarine and coastal areas of the southern Strait of Georgia. Eggs from the heron colony at Crofton contained the highest levels of 2378-TCDD, 12378-PnCDD, and 123678-HxCDD, but levels of 12378-PnCDD and 123678-HxCDD at the least contaminated colonies at Nicomekl and Sidney Island were still significantly higher than levels found in eggs of fish-eating birds in other parts of Canada, such as the Great Lakes (Stalling *et al.* 1986). Thus, there appears to be a unique source(s) of the 12378-PnCDD and 123678-HxCDD contamination in the aquatic environment in British Columbia. The most important potential source of PCDDs in the B.C. aquatic environment is chlorophenols.

Tetrachlorophenol and pentachlorophenol formulations have been used for several decades in Canada to preserve wood products and protect lumber for export. About 700 t of chlorophenols are used annually by the forest industry in British Columbia (Garrett and Shrimpton, 1988). Industry practice of stacking chlorophenol-treated lumber without protection from the rain is responsible for the presence of chlorophenols in stormwater runoff from mills (Krahn *et al.* 1987). As a consequence, the receiving waters near sawmills in British Columbia are often contaminated with chlorophenols (Environmental Protection Service 1979). Chlorophenol-contaminated wood chips have also been used in pulp mills.

Chlorophenols are known to contain a host of PCDDs and PCDFs, including the congeners found in this study (Hagenmaier and Brunner 1987). However, OCDD and HpCDDs predominate. Total HxCDDs are present at levels 10 to 100 times lower than those of HpCDD in commercial chlorophenols (Miles *et al.* 1985). Levels of 123678-HxCDD are a highly variable proportion of total HxCDDs, depending on the source (Hagenmaier and Brunner 1987). Levels of 12378-PnCDD are 20 to 100 times lower than those of 123678-HxCDD, and 2378-TCDD levels are six to 100 times lower than the levels of 12378-PnCDD in current formulations of chlorophenols. The relative concentration of the lower chlorinated PCDDs is therefore much higher in Great Blue Herons than in the putative source. Selective biomagnification of the less-chlorinated PCDDs may account for this (Kuehl *et al.* 1987). Van den Berg *et al.* (1987) found similar levels and relative abundance of 2378-TCDD, 12378-PnCDD, and 123678-HxCDD in livers of Grey Herons (*Ardea cinerea*) in the Netherlands and also suggested that the source of PCDDs was chlorophenols.

Kraft pulp and paper mills are known to produce 2378-TCDD and 2378-TCDF in the chlorine bleaching process (Amendola *et al.* 1987). Because the Crofton heron colony is feeding near the outfall of a kraft mill, it is probable that the threefold increase observed in 2378-TCDD levels between 1986 and 1987 and the significantly higher levels compared with other colonies in British Columbia were due to increased input to the environment from the pulp mill.

Dioxins are extremely toxic to chickens, the only bird

for which there are many toxicological data. Embryo mortality, teratogenic effects, and edema can occur in chicken eggs injected with as little as 10 ng/kg of 2378-TCDD (Verrett 1970), and 6.4 ng/kg caused a 21% increase in the incidence of cardiovascular malformations (Cheung *et al.* 1981). In 1986, all the heron eggs we collected contained more than 6 ng/kg of 2378-TCDD. However, the elevated levels of dioxin did not appear to affect the productivity of the herons that year, and there was no significant difference in the number of young fledged per successful nest between the more contaminated colonies at Crofton and UBC and the less contaminated colonies at Sidney Island and Nicomekl. Apparently, the Great Blue Heron is considerably less sensitive than the chicken to the embryotoxic actions of PCDDs and PCDFs. The same has been found for the Herring Gull (*Larus argentatus*) in the Great Lakes (Stalling *et al.* 1986).

In 1987, the heron colony at Crofton failed to produce any young. At the same time, mean 2378-TCDD levels were 252 ng/kg, compared with 92 ng/kg in 1986. Whether the sharp increase in 2378-TCDD levels in 1987 precipitated the failure of the colony to fledge young is uncertain. Levels of 2378-TCDD in six of 10 eggs at Crofton in 1987 were higher than had previously been measured in individual heron eggs at Crofton or UBC (Fig. 3), whereas levels in four of 10 eggs were within the range (<200 ng/kg) of levels in eggs from nests that produced two or more young in 1986 at Crofton and UBC. These results do not show a clear dose–response embryotoxic effect of 2378-TCDD: i.e., eggs at Crofton in 1987 with 2378-TCDD levels less than 200 ng/kg might have been expected to hatch and fledge, as they had the previous year. The possibility of aberrant parental behaviour is supported by the observation that most of the shells collected at Crofton appeared to have been stabbed, rather than hatched. Deliberate stabbing of eggs has been observed in the United Kingdom in Grey Heron populations that were contaminated with high levels of polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (DDE), and dieldrin (Cooke *et al.* 1976). Egg destruction by adult herons usually occurred within a few days of laying. Predation of eggs from nests deserted by the parents as a result of chemical stresses or human disturbance must also be considered as a possible cause of loss of eggs at the Crofton heronry.

A study of embryo development and liver enzyme induction in Great Blue Herons is planned for the spring of 1988 to determine whether embryo growth, hatching success, etc. are implicated in reproductive failure. An egg exchange study to establish the possible role that parental behaviour during incubation might have played in the failure of the Crofton colony to fledge young in 1987, and a comparative study of nesting behaviour of adults at a contaminated colony and at a clean colony, will also be undertaken in 1988. As well, sources of PCDDs and PCDFs are being determined from analysis of invertebrate and sediment samples for characteristic "signature" patterns of these contaminants.

The fledging success of all heron colonies we surveyed in both 1986 and 1987 was lower than the 2.5 young per successful nest reported by Forbes *et al.* (1985) for 15 colonies in southwestern British Columbia between 1977 and 1981. A review of available information suggests that the nesting population of herons in the Strait of Georgia has not changed during the past decade (Butler in prep.). However, in a long-lived species like the heron, changes in nesting productivity would not be reflected in a decline in the size of the nesting population of adults for many years. Cooke *et al.* (1976) found that Grey Herons in the United Kingdom continued to maintain population levels during periods of high organochlorine contamination and repeated nest failures.

Acknowledgements

We thank Mary Simon and Michael Mulvihill for the chemical analysis.

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PCDDs AND PCDFs IN GREAT BLUE HERON AND CORMORANT EGGS, 1987

As a follow up to studies in 1982, 1983 and 1986, individual eggs of Great Blue Herons were analyzed from two colonies in British Columbia in 1987, UBC and Crofton (Table 1). The UBC and Crofton colonies had shown elevated levels of PCDDs and PCDFs in previous surveys. The UBC colony feeds in the Fraser River estuary, and the Crofton colony feeds near a pulp mill. The Great Blue Heron feeds mainly in shallow water on small fish such as sculpins, gunnels and perch, but will also feed on fish, shrimp, or any aquatic animal of suitable size. In addition to aquatic animals, Great Blue Herons in British Columbia have been known to forage for small rodents in farmer's fields. Great Blue Herons are year-round residents in Coastal British Columbia. There is, therefore, little doubt that the PCDD and PCDF contamination in their eggs is of local origin.

In addition to Great Blue Heron eggs, Double-Crested Cormorant eggs were analyzed in 1987 from a colony nesting at Crofton near the pulp mill (Table 1). Mean levels of PCDDs and PCDFs in cormorant eggs were slightly lower than those in Great Blue Heron eggs from the same site, but in the same range. Cormorants feed mainly on fish, but since they dive and chase their prey in the water, they feed more offshore in deeper water than herons. The diet of the cormorant probably overlaps that of the heron, but the size of the fish eaten may be larger. The Double-crested Cormorant is a resident species in B.C., and the PCDDs and PCDFs were therefore accumulated locally.

TABLE 1

PCDD and PCDF levels in Great Blue Heron eggs and Double-Crested Cormorant eggs, collected from B.C., 1987
Levels are in ng/kg (wet wt.)

AREA	TISSUE	USOX NO.	PCDDs								PCDFs			
			2378-T4CDD	12378-P5CDD	123478-H6CDD	123678-H6CDD	123789-H6CDD	1234679-H7CDD	1234678-H7CDD	OCDD	2378-T4CDF	23478-P5CDF	% Moisture	% Lipid
UBC	Great Blue Heron egg	37707	41	34	ND	79	4	TR	12	TR	29	13	81.9	5.49
		37709	204	109	ND	158	12	TR	10	16	20	I	81.4	6.39
		37710	9	23	4	39	7	ND	11	TR	5	10	80.9	6.36
		37711	87	42	ND	97	7	TR	11	10	50	18	82.5	5.96
		37715	78	82	ND	99	9	ND	TR	TR	19	20	82.2	5.21
		37718	80	66	ND	125	8	TR	TR	TR	7	20	80.5	7.16
		37722	28	40	TR	52	TR	TR	9	13	6	10	81.4	6.45
		37724	41	25	ND	55	TR	TR	8	10	35	12	82.0	5.02
		37727	91	55	ND	99	8	TR	10	19	39	20	83.0	6.04
		37730	55	72	ND	97	5	8	7	19	13	25	83.3	6.38
CROFTON	Great Blue Heron egg	37692	380	728	ND	799	40	ND	TR	TR	7	64	80.3	8.17
		37693	67	107	ND	185	9	ND	7	TR	1	16	82.4	6.35
		37694	444	582	ND	1052	58	ND	7	TR	3	104	81.3	5.33
		37695	173	232	ND	434	27	ND	TR	ND	3	42	81.5	6.50
		37696	59	73	ND	110	8	ND	ND	ND	1	10	80.8	6.97
		37697	234	256	ND	580	35	ND	TR	TR	3	45	82.2	5.69
		37698	331	392	ND	661	45	ND	6	TR	17	47	82.8	5.52
		37699	369	216	ND	530	39	ND	7	TR	6	34	83.5	5.50
		37700	307	264	ND	456	34	ND	TR	ND	4	46	83.6	5.49
		37701	158	291	ND	357	18	ND	8	10	3	37	82.2	5.13
	Double-Crested Cormorant egg	37702	69	105	ND	226	25	ND	TR	TR	8	20	83.1	3.86
		37703	56	97	ND	242	22	ND	11	TR	5	22	83.0	4.14
		37704	100	212	ND	602	63	ND	9	TR	11	34	77.6	5.61
		37705	58	68	ND	133	14	ND	7	10	5	11	81.3	5.07
		37706	85	275	ND	962	125	ND	11	TR	15	36	78.6	4.67
MDC signal/noise=3			1	3	4	4	4	6	6	10	1	2		

ND No peak observed (sinal/noise 2);
TR trace level below the minimum detectable concentration (MDC, signal/noise between 2 and 3);
I Interferences in the monitored ion prevented determination of the contamination level.