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Contamination and Biomarkers in the Great Blue Heron, an Indicator of the State of the St. Lawrence River

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Abstract. In 1996–1997, nine breeding colonies of the great blue heron on the St. Lawrence River and its estuary (Québec, Canada) were investigated in the framework of a biomonitoring program. Fledglings from colonies in freshwater were more contaminated with mercury, PCBs and many organic contaminants than those from estuarine colonies. The level of contamination in the St. Lawrence River is generally below the levels of toxicological effects for the great blue heron. The molar ratio of retinol: retinyl palmitate in heron eggs was correlated with total PCBs ($r = 0.79$) and mirex ($r = 0.90$). In plasma, all biochemical parameters were significantly different between freshwater and marine colonies. Plasma retinol concentrations at the Dickerson and Héron colonies were significantly lower compared with those at Grande Ile ($p < 0.05$) and Steamboat ($p < 0.001$). Based on retinoid and β-carotene concentrations in eggs, low plasma retinol was not associated with possible dietary deficiency. Plasma retinol was negatively correlated with many PCB congeners, total PCBs ($r = -0.78$), *p,p′*-DDE, *trans*-nonachlor and α-HCH. Similarly, the hormone T3 was correlated with many PCB congeners, total PCBs ($r = -0.69$) and the same organochlorine chemicals. Plasma LDH concentrations were different among freshwater colonies, Grande Ile and Héron colonies having LDH values significantly greater than those of Steamboat (respectively, $p < 0.05$ and $p < 0.01$). Globally, the health status of the St. Lawrence great blue heron population was judged to be acceptable, however, several biomarkers indicated positive responses to contaminants.

Keywords: contaminants; vitamin A; thyroid hormones; great blue heron; St. Lawrence River

Introduction

Over the last decade, many authors have published reviews on the use of biomarkers in studies in wildlife toxicology (Colborn and Clements, 1992; Peakall, 1992; Fossi and Leonzio, 1993; Peakall and Shugart, 1993; Peakall and Walker, 1994). A biomarker can be defined as "a biological response to a chemical or chemicals that gives a measure of exposure and sometimes, also of toxic effect" (Peakall and Walker, 1994). Biomonitoring and

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research on wildlife health has also concentrated on the effects of endocrine disrupting persistent organic pollutants (POPs) (Colborn et al., 1993; Fox, 1993; Grasman et al., 1998).

Many of these POPs are present in tissues of wildlife along the St. Lawrence River (DeGuise et al., 1995), which drains the highly industrialized Great Lakes. In addition to this important input, this major River also receives toxic chemicals from agricultural, industrial and urban sources along its shores and from the atmosphere over its 1600 km course to the Atlantic Ocean.

The great blue heron (*Ardea herodias*) has been widely used as an indicator species (DesGranges, 1979; Elliott et al., 1989, 2001a; Custer et al., 1997; Thomas and Anthony, 1999) because of its distribution in both marine and freshwater environments, the accessibility of its colonies and its strategic position at the top of the food chain. Between 1991 and 1993, we conducted studies to select indicators of exposure and effects of sublethal concentrations of contaminants in the St. Lawrence River in order to develop a biomarker-based biomonitoring program. Following this initial study, the great blue heron was selected as an indicator species in the biomonitoring program of the St. Lawrence River (Champoux et al., 2002; Rodrigue et al., 2005). The goals of this program are: (1) to detect spatial variations in bioavailable contaminants in indicator species; and (2) to determine if toxic substances are present at concentrations sufficiently high to affect the health of St. Lawrence biota.

Here, we present data on the contamination and biomarkers in the great blue heron from colonies visited in 1996–1997. We examine the linkages between the toxic substances and the biological variables measured and discuss the usefulness of the biomarkers that appeared the most indicative and appropriate for a biomonitoring program of toxic exposure and effects for the St. Lawrence River.

Methods

The great blue heron breeds in many colonies along the St. Lawrence River. In 1996 and 1997, nine colonies were sampled in this study (Fig. 1). They were selected to cover as much as possible the physical–chemical and ecological variability along this large ecosystem. Three colonies are situated in the more developed freshwater part of the River, between Cornwall and Québec (Ile Dickerson, Ile aux Hérons and Grande Ile) and three in the less-developed estuarine part of the River (Ile de la Corneille, Ile du Bic and Ile Manowin). The inland reference colonies (Petit Lac Jacques-Cartier, Ile Steamboat and Ile Matane) were chosen outside of the most developed regions concentrated along the River, but not necessary out of reach of atmospheric pollution.

In each colony, eggs and fledglings were sampled for chemical and biochemical analysis. Professional climbers were contracted to climb trees and to collect eggs and chicks. One fresh egg from nine nests per colony was taken for contaminant analyses and kept on ice. Another egg from six of the same nests was taken for retinoid analyses, kept on dry ice and rapidly sent to the laboratory. One fledgling from nine nests per colony was collected, as much as possible from the nest where the eggs were collected. The fledglings were weighed and the length of their tarsus and beak measured. Age was estimated from the tarsus length using the equation of Quinney (1982). Feathers (fifth primary and fifth secondary flight feathers and two covert feathers from each wing, one rectrice and eight body feathers) were cut and placed in plastic bags on ice for mercury analyses. A 10 ml blood sample was collected from the brachial vein with a 10 ml syringe equipped with a 25 G needle and pre-rinsed with heparin. Blood was transferred to two vacutainers and kept on ice. Birds were banded and returned to their nest. Capillary tubes were filled with blood and centrifuged for 5 min at 7000 rpm for hematocrit measurements. One 5 ml vacutainer was centrifuged (5 min at 4000 rpm) and 1 ml aliquots of plasma were transferred to cryovials and stored in liquid nitrogen for biomarker analyses. The remaining whole blood was kept on ice until all samples were sent to the laboratory where it was stored at −40 °C for chemical analyses.

Because of cost limitations, contaminants were measured in eggs and in fledgling tissues pooled of three individuals, to obtain three pools per colony. Mercury, 21 organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) were analysed at the National Wildlife Research Centre (NWRC,

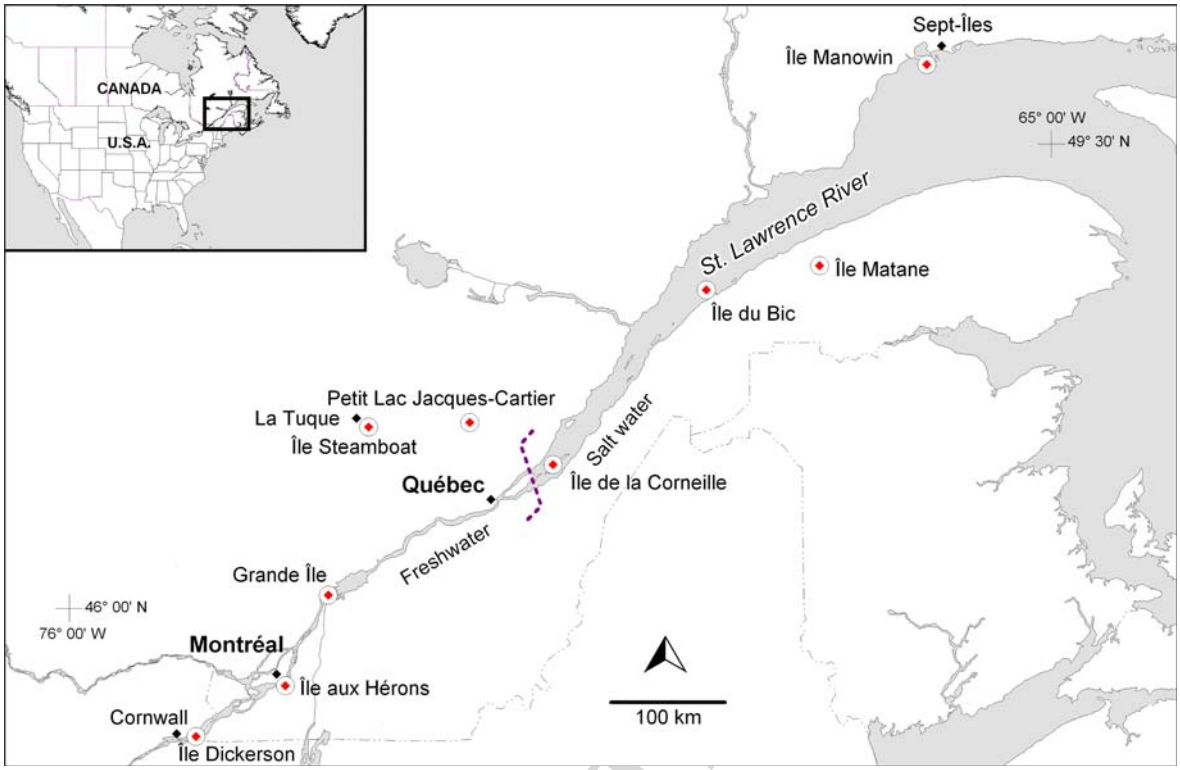

Figure 1. Great blue heron colonies studied along the St. Lawrence River in 1996–1997.

Ottawa, Canada). Total mercury was analysed by cold vapour with an atomic absorption spectrometer 3030 (Perkin–Elmer), according to CWS method No. MET-CHEM-AA-03C (Neugebauer et al., 2000). The detection limit for mercury was 1.0 μg/g dry weight for all tissues. The analytical method used by NWRC for PCBs and OCs is described under MET-CHEM-OC-04 in Won et al. (2001). Starting in 1997, all analytical data were determined using a quadrupole mass selective detector (MSD) coupled to the gas chromatograph (GC) instead of the electron capture detector (ECD) system. This change has allowed the identification of a larger number of compounds. As a first step, lipids were extracted from the egg tissues with DCM/hexane extraction. Blood specimens were extracted using a method slightly modified from Mes (1987). Whole blood specimens from 1996 were extracted with toluene and those from 1997 with DCM:hexane (1:1 by volume). The chemicals of interest in the extracts were then separated from lipids and biogenic compounds by gel permeation chromatography (GPC) and cleaned up on Florisil column chromatography. Finally, the OC and PCB levels were determined via high resolution gas chromatography coupled to a mass spectrometry detection system. Blood samples from 1996 were analysed with the previous method where the gas chromatograph (GC) was coupled with an electron capture detector (ECD) system. Although this method separates most major OCs and PCB congeners when the contaminants are split into three fractions, some PCBs that co-elute cannot be separated and determined correctly. The comparison of the quantities of the different congeners detected by the two methods in a known mixture of Aroclors 1242, 1254 and 1260 indicated that differences are usually lower than 20% for most of the major congeners. Total PCB concentrations are reported as the sum of 59 (42 with the previous method) congeners using IUPAC numbers. The detection limit of each sample varies among compounds due to varying background noise but is typically lower

than 0.001 ppm. The quality control program of NWRC includes the analysis of an aliquot of a reference material of known concentration.

The vitamin A profile was measured in eggs following the procedure described in Boily et al. (1994). Only samples from viable embryos with development stage between 18 and 32 were used for statistical analyses. The age of the embryo was estimated based on developmental chronology specific to the herring gull (*Larus argentatus*) (M.G. Williams and Dr. J.P. Ludwig, unpublished data) and a previous study on frozen herring gull eggs (Spear et al. 1990). Total thyroid hormones thyroxine (T4) and triiodothyronine (T3) were measured in fledgling plasma by a radioimmunologic method based on competition of $^{[125]}$I-marked hormone with non-marked hormone for linking sites on the antibodies. Blood clinical chemistry (mineral and chemical elements, proteins and enzymes) was assessed in the Toxicology Laboratory of University Laval Hospital Centre using standard methods. Amylase was measured with an Ektachem 700XR (Kodak) while triglycerides and cholesterol were measured with a Technicon RA-500 (Miles). Plasma retinoids were extracted with organic solvents and analysed by non-aqueous reversed phase HPLC with UV or fluorescent detection as described in Elliott et al. (2001b).

In June 1997, a census of most colonies was made by helicopter and number of active nests was counted (Desrosiers et al., 1998). Colony productivity was estimated from a selection of nests in the visited colonies, including the nests where eggs were collected, by counting the number of chicks produced per active nest. Since they nest on top of trees, monitoring great blue heron productivity is only feasible on a small number of nests because of risk of disturbance and nest access difficulties.

Biochemical parameters in plasma were tested for differences between sites using 1-way analysis according to the General Linear Model (GLM). When significant, colonies were compared to their respective reference site by Dunnett's test. In the case of two-way analysis of variance, least squared means were compared by the Tukey multiple range test. When the residuals were not normally distributed, data were transformed to base 10 logarithm (thyroxine, T4 data) or square root (plasma retinol data). Certain data having sample numbers too small for parametric analysis were treated by the Kruskal–Wallis test followed by the Q test (Zar, 1984), when appropriate, to determine which colonies were different. Relationships between variables were calculated using non-parametric Spearman's rank correlations. All statistics were calculated using JMP^{TM} (SAS Institute, 1999).

Results

Levels of mercury, total PCBs, PCB congeners and organochlorines in the eggs did not indicate significant differences among colonies, although there was a tendency for PCBs (Fig. 2) and mirex to decrease downstream ($p > 0.05$; Table 1). In blood, however, most of these chemicals indicated significant differences among colonies ($p < 0.05$; Table 2; Fig. 2): mercury was higher in birds from Ile Steamboat while total PCBs were higher in birds from Ile Dickerson and OCs (*p,p′*-DDE, *trans*-nonachlor, *cis*-nonachlor, HCB) in those from Ile aux Hérons. The pesticide metabolite *p,p′*-DDE was the most abundant OC, both in eggs and in blood. With respect to feathers, birds from Ile Steamboat again had the highest mercury levels. The major PCB congeners in eggs, which represented 53% of total PCBs, were # 153 > 138 > 180 > 118 > 187. The pattern in blood was different, more congeners being needed to make 50% of total PCBs (# 138 > 153 > 118 > 49 > 52 > 99 > 180). The presence of CB-49 and CB-52 in blood reflects recent uptake from the diet. These congeners are biotransformed more rapidly than the other congeners in birds (Drouillard et al., 2001) and are therefore not normally found in eggs, which reflect long-term exposure. Less chlorinated congeners were also present in higher proportions at the more contaminated freshwater sites.

The reference colony of Petit Lac Jacques-Cartier was the smallest of the colonies visited (10 nests) and failed to produce young. It was replaced by Ile Steamboat for the sampling of fledglings. The other colonies ranged from 20 active nests (Ile Matane) to 1250 (Grande Ile), had a good reproductive success (range 80–98%) and produced a mean of 2.47 young per active nest (range 2.08–2.77) (CWS, unpublished data). The weight of the fledglings did not vary among colonies (mean 1.75 kg; s.d. 0.35; $n = 70$; $p = 0.12$) while the tarsus

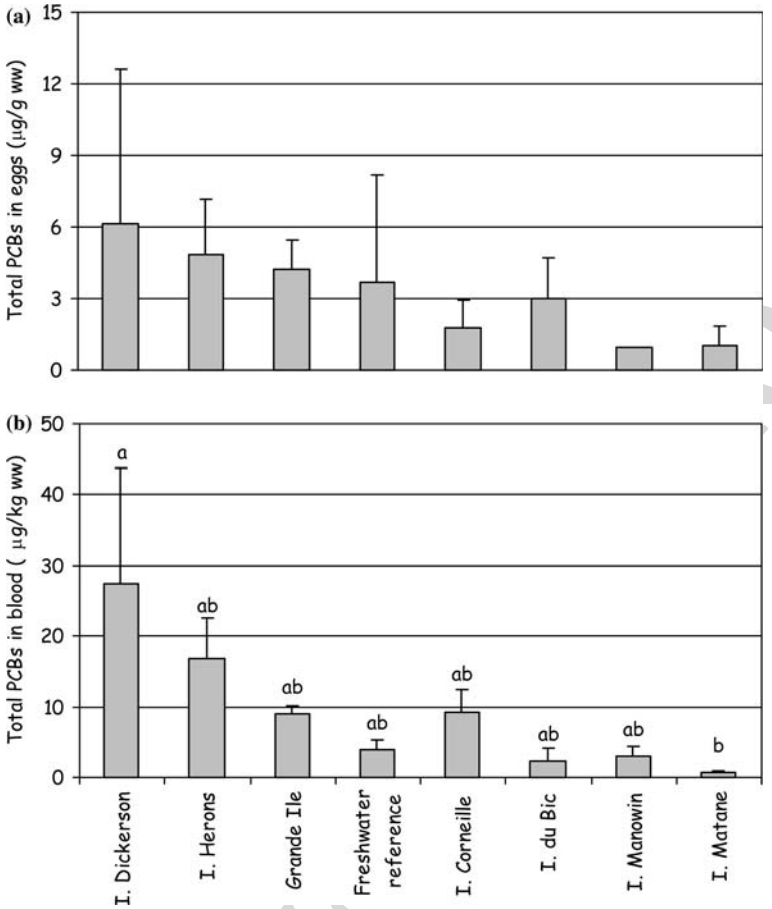

Figure 2. Total PCB concentrations (a) in eggs of the great blue heron and (b) in the blood of great blue heron fledglings from colonies along the St. Lawrence River. Colonies with same letter are not significantly different according to a Q test ($p > 0.05$).

length (mean 15.3 cm; s.d. 2.4; $n = 68$) and estimated age (mean 38 days; s.d. 6.3; $n = 68$) indicated significant differences ($p < 0.05$): birds from Ile de la Corneille were slightly smaller and younger, while those from Ile du Bic were larger and older.

Considering morphological parameters of the heron eggs (Table 3) no differences among sampling sites were identified (egg weight $F_{6,21} = 0.77$ $p = 0.60$; shell weight $F_{6,21} = 0.29$ $p = 0.94$; egg length $F_{6,21} = 0.59$ $p = 0.74$; egg width $F_{6,21} = 1.17$ $p = 0.36$).

In the case of biochemical parameters measured in eggs, the Ile Manowin colony with a sample number of two eggs was excluded from statistical treatment. Retinyl palmitate concentrations were different among colonies ($F_{6,21} = 7.54$ $p < 0.001$); more precisely, this retinoid was greater in eggs of the Matane colony (pairwise comparison, Tukey $p < 0.01$) compared with the other sites (Table 3). Retinyl palmitate was significantly correlated with egg width ($r = 0.56$ $p < 0.01$), but was not influenced by embryonic stage of development ($F_{6,20} = 0.06$ $p = 0.80$). No among-colony differences were apparent for retinol concentrations ($F_{6,21} = 0.26$ $p = 0.95$) or the ratio of retinol to retinyl palmitate ($F_{6,21} = 0.59$ $p = 0.73$). However, retinol was correlated with both egg width ($r = 0.46$ $p < 0.05$) and stage of development ($r = -0.50$ $p < 0.01$) while the ratio of retinoids correlated with stage of development ($r = -0.48$ $p < 0.01$).

The concentrations of β-carotene in eggs of freshwater colonies (Dickerson, Hérons, Grande and Jacques-Cartier) were significantly different ($F_{1,26} = 7.24$ $p = 0.012$) from those of marine colonies (Corneille, Bic and Matane) (Table 3).

Table 1. Mean (standard deviation*) concentrations (μg/g wet weight) of mercury, total PCBs and selected organochlorines in great blue heron eggs collected in 1996–1997 from colonies along the St. Lawrence River

Colony	Hg	Total PCBs	p,p′-DDT	p,p′-DDD	p,p′-DDE	Mirex	*t*-nonachlor	*c*-nonachlor	Oxychlordane	Dieldrin	HCB
I. Dickerson	0.23 (0.16)	6.1 (6.5)	0.009 (0.005)	0.015 (0.017)	1.362 (1.171)	0.068 0.083	0.042 (0.023)	0.018 (0.014)	0.017 (0.004)	0.040 (0.029)	0.004 (0.001)
I. aux Hérons	0.15 (0.01)	4.9 (2.3)	0.021 (0.003)	0.053 (0.055)	2.0 (0.3)	0.047 0.028	0.096 (0.011)	0.035 (0.010)	0.029 (0.002)	0.060 (0.034)	0.006 (0.002)
Grande Ile	0.16 (0.03)	4.2 (1.2)	0.012 (0.011)	0.009 (0.007)	1.5 (1.5)	0.019 0.009	0.095 (0.030)	0.032 (0.013)	0.024 (0.009)	0.033 (0.015)	0.005 (0.001)
Petit Lac J.-Cartier[R]	0.25 (0.02)	3.7 (4.5)	0.006 (0.005)	0.005 (0.003)	0.6 (0.3)	0.019 0.017	0.032 (0.019)	0.011 (0.006)	0.018 (0.008)	0.028 (0.019)	0.005 (0.001)
I. de la Corneille	0.23 (0.04)	1.8 (1.2)	0.007 (0.002)	0.006 (0.000)	0.5 (0.1)	0.020 0.014	0.036 (0.021)	0.011 (0.007)	0.010 (0.004)	0.013 (0.005)	0.004 (0.003)
I. du Bic	0.14 (0.03)	3.0 (1.7)	0.021 (0.015)	0.065 (0.092)	1.8 (1.0)	0.010 0.002	0.128 (0.063)	0.032 (0.020)	0.047 (0.034)	0.064 (0.041)	0.009 (0.002)
I. Manowin	0.13	0.96	0.016	0.012	0.4	0.005	0.030	0.008	0.008	0.012	0.008
I. Matane[R]	0.22 (0.09)	1.0 (0.8)	0.017 (0.003)	0.010 (0.006)	1.3 (0.4)	0.016 0.012	0.039 (0.031)	0.012 (0.010)	0.013 (0.018)	0.041 (0.038)	0.004 (0.001)

Hg: mercury; HCB: hexachlorobenzene.
*n = three pools of three except for Ile Manowin, one pool of three.
[R]Reference colony.

Accordingly, the freshwater and marine sites were considered separately. There was a tendency for β-carotene to be different ($F_{3,12} = 2.09$ $p = 0.15$) among the freshwater colonies, and the levels at Ile aux Hérons were significantly lower compared with the reference site, Jacques-Cartier (Dunnett $p < 0.05$). Among the marine colonies, β-carotene concentrations were influenced by the colony ($F_{2,6} = 16.69$ $p = 0.0035$), the stage of development ($F_{1,6} = 25.87$ $p = 0.0023$) and the combined effect of these two factors (colony×stage of development $F_{2,6} = 17.23$ $p = 0.0033$). Thus, 86.9% of the variation in β-carotene concentration is explained. Without the interaction term, however, the results are not significant (and only 12% of the variation would be explained): this suggests that differences cannot be attributed to among-colony differences alone.

In plasma, all biochemical parameters were significantly different between freshwater and marine colonies, therefore possible effects of contaminants were examined within these two groupings. Plasma retinol was significantly different among the freshwater colonies (Table 4; $F_{3,31} = 14.16$ $p < 0.001$) and was correlated with body weight ($r = 0.58$ $p < 0.001$). A two-way analysis of variance indicated that body weight and colony together explained 72% of the variation associated with plasma retinol (no interaction between these two factors; $F_{3,1,28} = 1.66$ $p = 0.19$). The two-way model explored using Tukey multiple comparisons demonstrated that plasma retinol concentrations at the Dickerson and Héron colonies were significantly lower compared with those at Grande Ile ($p < 0.05$) and Steamboat ($p < 0.001$). Plasma LDH (lactate dehydrogenase) concentrations were also different among freshwater colonies ($F_{3,36} = 3.66$ $p < 0.05$), Grande Ile and Héron colonies having LDH values significantly greater than those of Steamboat (respectively, $p < 0.05$ and $p < 0.01$). The levels of plasma T4 showed a tendency to be different among freshwater colonies ($F_{3,33} = 2.61$ $p = 0.068$) with the Grande Ile colony having greater plasma T4 compared with Steamboat (Dunnett's test $p = 0.027$).

With respect to marine colonies, significant between-colony differences were identified for T3 ($F_{3,29} = 2.99$ $p < 0.05$), retinol ($F_{3,29} = 6.99$ $p < 0.001$), total protein ($F_{3,26} = 2.95$ $p < 0.05$) and LDH ($F_{3,26} = 4.50$ $p < 0.05$). Consistent with the

Table 2. Mean (standard deviation*) concentrations of mercury in feathers (μg/g dry weight) and mercury (μg/g wet weight), total PCBs and selected organochlorines (μg/kg wet weight) in whole blood of great blue heron fledglings collected in 1996–1997 from colonies along the St. Lawrence River

Colony	Feather Hg	Blood Hg	Total PCBs	p,p′-DDE	*t*-nonachlor	*c*-nonachlor	HCB	Heptachlor epoxyde	Dieldrin	Mirex	Penta-CB	α-HCH
I. Dickerson	6.3 ^{ab}	0.35 ^{ab}	27.3 ^{a}	3.1^{ab}	0.60 ^{ab}	0.23 ^{ab}	0.13 ^{ab}	0.27	1.03	0.17	0.001	0.53
	(1.2)	(0.07)	(16.5)	(0.6)	(0.36)	(0.21)	(0.06)	(0.06)	(0.55)	(0.06)	(0.000)	(0.21)
I. aux Hérons	7.4 ^{ab}	0.53 ^{ab}	16.7 ^{ab}	4.9 ^{a}	0.83 ^{a}	0.30 ^{a}	0.33 ^{a}	0.23	0.73	0.07	0.001	0.37
	(2.2)	(0.11)	(5.8)	(1.0)	(0.31)	(0.10)	(0.25)	(0.12)	(1.10)	(0.06)	(0.000)	(0.06)
Grande Ile	5.6 ^{ab}	0.35 ^{ab}	8.9 ^{ab}	1.9 ^{ab}	0.43 ^{ab}	0.13 ^{ab}	0.10 ^{ab}	0.17	0.10	0.03	0.001	0.30
	(1.2)	(0.04)	(1.3)	(1.5)	(0.06)	(0.06)	(0.00)	(0.12)	(0.00)	(0.06)	(0.000)	(0.26)
I. Steamboat^{R}	10.1 ^{a}	0.70 ^{a}	3.9 ^{ab}	1.0 ^{ab}	0.13 ^{ab}	0.001 ^{b}	0.10 ^{ab}	0.30	0.80	0.001	0.001	0.57
	(0.3)	(0.07)	(1.3)	(0.4)	(0.06)	(0.000)	(0.00)	(0.27)	(1.21)	(0.000)	(0.000)	(0.12)
I. Corneille	7.2 ^{ab}	0.41 ^{ab}	9.1 ^{ab}	2.0 ^{ab}	0.28 ^{ab}	0.18 ^{ab}	0.15 ^{ab}	0.06	0.22	0.11	0.030	< 0.001
	(1.1)	(0.16)	(3.3)	(1.5)	(0.10)	(0.05)	(0.04)	(0.03)	(0.13)	(0.14)	(0.005)	(0.000)
I. du Bic	5.1 ^{ab}	0.26 ^{ab}	2.4 ^{ab}	0.4 ^{b}	0.15 ^{ab}	0.16 ^{ab}	0.17 ^{b}	0.15	0.16	0.05	0.082	< 0.001
	(1.3)	(0.05)	(1.8)	(0.2)	(0.08)	(0.11)	(0.09)	(0.01)	(0.17)	(0.01)	(0.079)	(0.000)
I. Manowin	3.1 ^{b}	0.17 ^{ab}	3.1 ^{ab}	0.5 ^{ab}	0.30 ^{ab}	0.18 ^{ab}	0.14 ^{ab}	0.41	0.69	< 0.001	0.036	< 0.001
	(1.0)	(0.05)	(1.3)	(0.1)	(0.18)	(0.12)	(0.01)	(0.53)	(0.80)	(0.000)	(0.007)	(0.000)
I. Matane^{R}	4.5 ^{ab}	0.16 ^{b}	0.8 ^{b}	0.6 ^{ab}	0.06 ^{b}	0.04 ^{ab}	0.11 ^{ab}	0.05	0.07	< 0.001	0.047	< 0.001
	(0.7)	(0.02)	(0.1)	(0.1)	(0.02)	(0.03)	(0.03)	(0.02)	(0.01)	(0.000)	(0.018)	(0.000)

^{R}Reference colony.
*n = three pools of three.
Hg: mercury; HCB: hexachlorobenzene; penta-CB: pentachlorobenzene; α-HCH: alpha-hexachlorocyclohexane.
Means in same column followed by same letter are not significantly different according to a Q test ($p > 0.05$).

results for freshwater colonies, plasma retinol was significantly correlated with body weight ($r = 0.58$ $p < 0.001$) and the combined influence of body weight and colony (no interaction $F_{3,1,25} = 1.33$ $p = 0.29$) explained 62% of the variance in plasma retinol. This statistical model revealed that the Bic colony had greater retinol levels compared with either Corneille (Tukey, $p < 0.05$) or Matane ($p < 0.01$). Similarly, plasma protein and LDH values were both significantly greater at the Bic colony compared with the reference site, Matane (Dunnett's test, LDH $p < 0.01$; protein $p < 0.05$).

Several significant correlations were observed between contaminants and biomarkers in the eggs. The molar ratio of retinol: retinyl palmitate in heron eggs was significantly correlated with total PCBs ($r = 0.79$, $p = 0.02$) and mirex ($r = 0.90$, $p < 0.01$) while β-carotene was negatively correlated with total PCBs ($r = -0.79$, $p = 0.04$) and to PCB congeners #66, 105, 138, 153, 170, 180 and 187. In blood, retinol showed negative relations ($p < 0.001$) with many PCB congeners, total PCBs ($r = -0.78$; Fig. 3), *p,p′*-DDE ($r = -0.78$), *trans*-nonachlor ($r = -0.75$) and α-HCH ($r = -0.60$, $p < 0.01$). The hormone T3 showed negative relations with many PCB congeners, total PCBs ($r = -0.69$, $p < 0.01$) and the same OCs. The clinical variables hematocrit, protein, creatinin, phosphorus, sodium and calcium also were correlated ($p < 0.05$) with a few congeners and OCs. Among clinical parameters, the strongest correlation was found between protein and α-HCH ($r = -0.82$, $p < 0.001$).

Discussion

Great blue heron fledglings from Ile Steamboat had higher levels of mercury than those from other colonies, while PCBs were higher at Ile Dickerson and OCs at Ile aux Hérons. The fact that the reference colony (Ile Steamboat) situated outside of the St. Lawrence displays the highest mercury levels probably reflects the fact that this contamination comes for a large part from atmospheric sources. In addition, soils and lakes in this region are sensitive to acid precipitation, a factor which favours the transfer of mercury in the trophic web (Meyer et al., 1998).

Compared to the previous sampling period of 1991–1993, mercury in eggs was significantly lower by 67% in 1996–1997, while no difference was

Table 3. Morphometry, β-carotene and retinoids in eggs of great blue heron from colonies along the St. Lawrence River

	Stage of development	Shell weight (g)	Weight of egg (g)	Length (mm)	Width (mm)	β-carotene (μg/g)	Retinol (μg/g)	Retinyl Palmitate (μg/g)	Molar ratio* Ret./Pal.
Ile Dickerson (n=5)									
Mean	25	7.57	67.94	65.31	44.61	0.70 ^{abcd}	5.44	0.41 ^{a}	24.8
Stand. deviation	5	0.19	3.08	2.49	1.01	0.37	2.72	0.04	13.5
Ile aux Hérons (n=4)									
Mean	21	7.77	70.87	63.80	45.75	0.39 ^{bc}	5.55	0.40 ^{a}	24.1
Stand. deviation	4	0.25	5.50	3.43	2.75	0.14	3.83	0.06	14.0
Grande Ile (n=3)									
Mean	22	7.49	67.20	64.87	45.00	0.44 ^{cd}	4.61	0.47 ^{a}	17.6
Stand. Deviation	4	0.18	5.60	6.36	1.00	0.24	2.49	0.07	7.9
Petit L. J.-Cartier^{R} (n=4)									
Mean	20	7.60	65.86	61.84	45.90	0.96 ^{ad}	5.31	0.48 ^{a}	22.3
Stand. deviation	1	0.38	2.67	1.10	1.32	0.52	2.59	0.10	13.8
Ile de la Corneille (n=5)									
Mean	24	7.39	65.85	64.40	44.95	1.24 ^{ac}	3.64	0.34 ^{a}	19.4
Stand. deviation	5	0.82	6.41	3.96	1.47	0.65	1.37	0.06	5.2
Ile du Bic (n=3)									
Mean	29	7.48	64.48	64.40	44.65	2.10 ^{ac}	3.67	0.44 ^{a}	15.0
Stand. Deviation	1	0.22	0.98	1.10	0.30	2.49	0.97	0.06	3.1
Ile Matane^{R} (n=4)									
Mean	25	7.56	65.35	62.69	47.18	1.50 ^{d}	6.06	1.06 ^{b}	9.9
Stand. Deviation	6	0.51	5.87	2.17	2.45	0.85	1.68	0.13	2.2

^{R}Reference colony.
*Molar concentration of retinol divided by the molar concentration of retinyl palmitate.
Means in same column not sharing a common superscript are significantly different according to Tukey or Dunnett tests ($p < 0.05$).
Vertical lines indicate difference (ANOVA $p = 0.012$) between freshwater and marine colonies for β-carotene.

observed in blood and feather mercury levels or in PCB levels in eggs and blood (Champoux et al., 2002). Despite this decline, Hg levels in eggs were still higher or comparable to other published levels for great blue heron eggs from various locations in North America (Elliott et al., 1989; Custer et al., 1997; Thomas and Anthony, 1999). Mercury levels in blood were also high compared to other

Table 4. Biochemical parameters (means and standard deviation in parentheses) measured in plasma of great blue heron fledglings from colonies along the St. Lawrence River

Colony	Total T3 N g/ml	Total T4 N g/ml	Retinol μg/l	Hematocrit %	Protein g/l	Cholesterol mmol/l	Triglycerids mmol/l	LDH U/l	Calcium mmol/l
Freshwater colonies									
I. Dickerson	1.94 (0.40)	31.70^{ab} (5.26)	464^{a} (250)	36 (3.2)	25 (2.4)	4.96 (0.53)	1.39 (1.08)	1390 ^{ab} (358)	2.57 (0.16)
I. aux Hérons	1.64 (0.36)	31.84^{ab} (6.00)	700^{a} (335)	34 (6.3)	27 (3.3)	4.48 (0.60)	1.25 (0.45)	1672 ^{a} (726)	2.46 (0.19)
Grande Ile	1.68^{b} (0.39)	38.75^{b} (6.03)	770^{b} (332)	31 (3.2)	25 (1.3)	4.59 (0.70)	1.47 (0.63)	1605 ^{a} (363)	2.52 (0.10)
I. Steamboat^{R}	1.84 (0.41)	31.70^{a} (8.79)	1098^{b} (258)	32 (3.8)	25 (1.6)	4.96 (1.72)	0.72 (0.24)	1018 ^{b} (237)	2.47 (0.11)
Marine colonies									
I. Corneille	2.18^{ab} (0.85)	32.54 (6.93)	969^{b} (258)	35 (1.8)	30 ^{ab} (2.6)	5.64 (0.64)	1.53 (0.71)	1682 ^{b} (521)	2.35 (0.15)
I. du Bic	3.32^{ab} (1.1)	29.79 (7.2)	1553^{a} (231)	38 (2.3)	33 ^{a} (4.4)	5.06 (0.8)	1.91 (0.8)	2190 ^{a} (415)	2.30 (0.10)
I. Manowin	3.59^{b} (1.10)	32.94 (4.41)	1238^{ab} (416)	37 (2.6)	29 ^{ab} (1.6)	5.23 (0.32)	1.42 (0.31)	1547 ^{ab} (303)	2.30 (0.13)
I. Matane^{R}	3.13^{a} (1.05)	26.82 (5.41)	1158^{b} (249)	36 (2.7)	29 ^{b} (1.3)	4.97 (0.70)	1.65 (1.23)	1358 ^{b} (432)	2.25 (0.06)

^{R}Reference colony.
LDH: lactate dehydrogenase.
Means in same column not sharing a common superscript are significantly different according to Tukey or Dunnett tests ($p < 0.05$).
Comparisons either among freshwater or marine colonies.

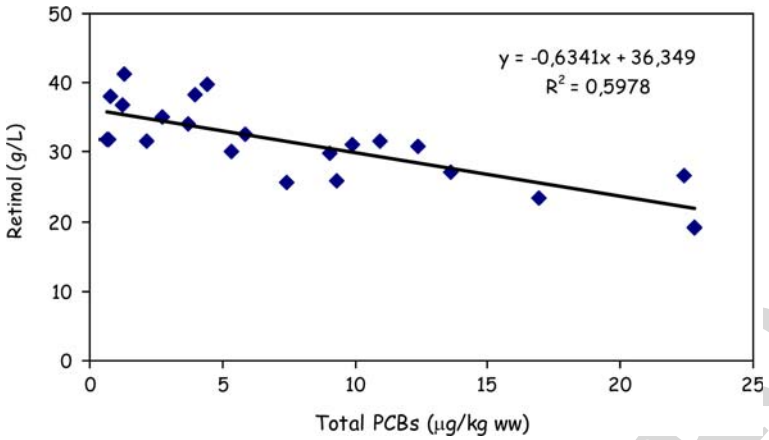

Figure 3. Relationship between retinol (square root) and total PCB concentrations in blood of great blue heron fledglings from colonies along the St. Lawrence River.

published data for nestlings of various piscivorous species (Sepuvelda et al., 1999). Few studies provide information on threshold impacts of Hg levels in blood of birds. Meyer et al. (1998) considered a level of 0.30 μg/g wet weight (w.w.) associated with lower common loon (*Gavia immer*) chick hatching and surviving, while Welch (1994) considered 0.50 μg/g w.w. as a relevant threshold in juvenile bald eagles (*Haliaeetus leucocephalus*). Wolfe and Norman (1998) reported levels of 1.2 μg/g w.w. in apparently successful heron colonies. Hg levels in feathers of herons from this study appear high compared with other published data on herons (Wolfe and Norman, 1998; Sepuvelda et al., 1999; Goutner et al., 2001). Sampling of various types of feathers in these studies could explain part of these differences.

PCBs and OC levels in heron eggs from the St. Lawrence River were comparable to those from other studies and lower than levels at which reproductive effects have been documented (Elliott et al., 1989; Blus, 1996; Hoffman et al., 1996; Custer et al., 1997; Thomas and Anthony, 1999). Levels of PCBs and most OCs in heron eggs declined by about one third since 1979 (Laporte, 1982), although levels of dieldrin and *p,p′*-DDE from Ile du Bic appear unchanged. No data could be found in the literature on levels of PCBs and OCs in the blood of great blue heron chicks. Mean PCB levels in plasma of bald eagle chicks 5–9-weeks-old from British Columbia (Elliott and Norstrom, 1998) compare with those from heron chicks from the St. Lawrence, after conversion from whole blood to plasma based on hematocrit ratio, while PCB and *p,p′*-DDE levels in plasma of bald eagle chicks from Lake Superior were higher (Dykstra et al., 1998).

According to DesGranges and Desrosiers (2005), who analysed the population trends of the great blue heron in Québec over the last 25 years, the number of young produced per active nest is sufficient to maintain stable populations. Observations on heron nests and fledglings in the present study tend to support this finding.

Most clinical parameters seemed in normal ranges known for comparable species (Fowler, 1986; Polo et al., 1994). Many blood analytes such as glucose, proteins, cholesterol and triglycerides serve as indicators of the nutritional status of wild birds and nutritional deficiency may decrease immunocompetence (Newman et al., 1997). PCBs and some organochlorines seem to interfere with the metabolism of lipids and carbohydrates (Ferrando and Andreu-Moliner, 1991; Feeley, 1995).

Because many studies have documented alterations of retinoids by PCBs and organochlorine chemicals and they are important for development, reproduction and immune function, retinoid status has been suggested as a biomarker for exposure to organochlorine chemicals (Spear et al., 1990; Peakall, 1992; Rolland, 2000). As such, correlations between the molar ratio retinol: retinyl palmitate in heron eggs and total PCBs or mirex are consistent with previous studies on egg

retinoids in freshwater and marine ecosystems (Spear et al., 1989, 1990; Boily et al., 1994; Murk et al., 1994, 1996). PCB congeners alter lecithin:retinol acyltransferase (LRAT) and retinol ester hydrolase (REH) activities in the yolk-sac membrane (Boily et al., 2003a, b) which would explain the changes in yolk retinoid concentrations. In the case of vitamin A levels in blood, the significant regression between plasma retinol and total PCBs would also suggest an effect of this group of contaminants. In addition, a correlation was obtained between egg β-carotene and total PCBs in the present study.

Alternatively, these results could reflect differences in dietary vitamin intake, since β-carotene is a precursor for retinoids and must be obtained from food (Sporn et al., 1994), and the marine environment at such latitudes is known for high availability of retinoids (Moore, 1957). In the case of the molar ratio of retinoids, the data do not support this idea. Neither egg retinol nor retinyl palmitate concentrations are significantly different between freshwater and marine colonies which indicates a lack of overall dietary (or ecosystem) effects on the molar ratio. Evidence for a marine versus freshwater dietary influence is the significantly lower levels of β-carotene in heron eggs in freshwater colonies compared with estuarine colonies. Plasma retinol was also greater in the marine colonies compared with the freshwater colonies. Therefore, for these retinoid parameters, freshwater and marine colonies were considered separately in an attempt to evaluate dietary variation between the two environments.

Among marine colonies, the egg β-carotene concentrations in the reference site are not distinct from those at the Corneille and Bic colonies, when taking into account the interaction with stage of development. With respect to freshwater sites, the difference in β-carotene between the reference colony and Ile aux Hérons is consistent with an effect of toxic chemicals, whereas the highest PCB colony, Dickerson, was not different from the reference. Thus, the results for egg β-carotene favour a between-ecosystem (freshwater versus marine) influence other than environmental contamination.

Considering plasma retinol concentrations in herons collected from the marine colonies, the results support an overall effect of environmental contaminants. Specifically, the higher plasma retinol concentration at the Bic colony compared with the Corneille colony is consistent with this hypothesis. Among freshwater colonies, plasma retinol was significantly lower at the more PCB-exposed colonies (Dickerson and Héron) compared to the reference colony. Assuming that these differences were caused by low dietary vitamin A intake, we would expect to see low carotene and low retinoid levels in the eggs of the Dickerson colony, but this is not the case. Egg retinol, retinyl palmitate and β-carotene levels in Dickerson birds are not different from these of the reference freshwater colony. Vitamin A dietary deficiency does not explain the low plasma retinol concentrations.

A wealth of studies into avian and mammalian nutrition as well as clinical medicine have demonstrated a moderate influence of dietary vitamin A on the plasma retinol levels of otherwise healthy individuals. Plasma retinol levels are maintained at the expense of body stores up to the point that plasma levels fail and deficiency symptoms begin (c.f. Moore, 1957; Underwood, 1984). The fact that plasma retinol in the Dickerson birds was only 464 μg/l (or 42% of that associated with the freshwater reference site) indicates that retinol homeostasis or metabolism had been affected.

These results compare with those from our previous study (Champoux et al., 2002), which also indicated that a reduction in plasma retinol below 800 μg/l was significantly related to a proportionally greater reduction in hepatic stores. Previous studies on effects of dioxin-like contaminants in plasma retinol levels have shown contradictory results (Spear and Bourbonnais, 2000). Murvoll et al. (1999) found a borderline significant positive correlation between PCBs in lipid weight and plasma retinol in shag (*Phalacrocorax aristotelis*) hatchlings, at PCB levels lower than levels in the great blue heron from the St. Lawrence. Murk et al. (1994) found increased plasma retinol levels with increasing PCB burden in common tern (*Sterna hirundo*) hatchlings, while Elliott et al. (1996) found no difference in 1-day-old bald eagle chick's plasma retinol among colonies with various levels of dioxin and furan contamination. However, all these results are from very young birds in which absorption of the vitellus at hatching may influence circulating retinol levels. Grasman et al.

(1996) found a strong association between reduced plasma retinol concentrations in 3-week-old Caspian tern (*Sterna caspia*) chicks and increased exposure to PCBs and *p,p′*-DDE, and a weaker but significant association in herring gull chicks. Bishop et al. (1999) found reduced vitamin A in liver of tree swallows (*Tachycineta bicolor*) in Cornwall Island, which is close to Ile Dickerson where great blue heron vitamin A levels were lowest.

In the present study, T3 levels were 53% lower in the freshwater colonies compared with marine colonies and T4 levels were 30% higher. In Champoux et al. (2002), T3 levels were not different among colonies, while T4 levels were 64% lower in freshwater colonies. In herons artificially exposed to 2,3,7,8-TCDD, no effects were observed in plasma T3 and T4 levels, at hatch or in 7-day chicks (Janz and Bellward, 1996). In adult herons, a significant increase in T4 was observed following exposure to TCDD (Janz and Bellward, 1997). A significant negative correlation was found in great cormorants (*Phalacrocorax carbo*) between mono-ortho-PCBs in the yolk sac and T4 in plasma (Van den Berg et al., 1994). A decrease in T3 and T4 was observed after treatment of chicken embryos with Aroclor 1242 and Aroclor 1254, but not after treatment with PCB congeners # 54, 77 and 80 (Gould et al., 1999). As reported by many authors, various mechanisms, sometimes contradictory, control the levels of retinol and thyroid hormones and contaminants such as PCBs, PCDDs and organochlorines may interfere in many ways in these processes (Brouwer and Van den Berg, 1986; Brouwer et al., 1990; Peakall, 1992; Fairbrother, 1993; Murvoll et al., 1999). Other non-measured contaminants such as coplanar PCB congeners and PCB metabolites may also interfere with the regulation of these systems. Although these mechanisms have not all been explained yet, it is clear that exposure to these contaminants has an effect on the retinol and thyroid hormone axis (Gould et al., 1999). Retinol is related to PCBs at levels below threshold for toxic effects, which makes it an early predictor, since a major decrease in plasma retinol may lead to compromised development, immune function or reproduction (Fox, 1993; Rolland, 2000).

Within the limits of the present study, the different biochemical parameters were tentatively evaluated for their suitability as biomarkers. Considering correlations to contaminants, differences between freshwater and marine colonies and within freshwater and marine colonies separately, confounding factors, and evidence from numerous field and experimental studies with various species, we believe that the most suitable biomarkers among those tested in this study are the molar ratio retinol:retinyl palmitate in the eggs, and retinol and T3 in the plasma of heron chicks.

Conclusion

Spatial differences among colonies were detected using measures of contaminants and biomarkers in blood of chicks. The fledglings from the upstream freshwater colonies are more contaminated than those from the downstream estuarine colonies. The level of contamination in the St. Lawrence River is generally below the documented levels of toxicological effects for great blue heron or other species. However, despite a decrease in the levels of some organochlorines, most contaminants, among them mercury and PCBs, do not show any reduction in time. Nonetheless, biomarkers used in the present study reveal PCB effects at the Dickerson and Héron colonies. The most important results were correlations between the molar ratio of retinoids in eggs and total PCBs or mirex as well as significant negative regressions between plasma retinol or T3 and contaminants. Plasma retinol levels in fledglings were strongly and negatively related to PCB concentrations and those from the freshwater colonies were very low, which could have an effect on fledgling development and survival.

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