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Contamination of and biomarkers in the Great Blue Heron, *Ardea herodias*, indicator species for the St. Lawrence River, 2001–2002

L. Champoux, S. Trudeau, G. Fitzgerald, P. A. Spear, and D. C. G. Muir

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Contamination of and biomarkers in the Great Blue Heron, *Ardea herodias*, indicator species for the St. Lawrence River, 2001–2002

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ABSTRACT

In the St. Lawrence Vision 2000 Action Plan, the Great Blue Heron was selected as an indicator species for the State of the St. Lawrence Monitoring Program. The first testing under this program was conducted in 1996 and 1997. This report presents the results of the second round of testing, conducted in 2001–2002. Eggs as well as blood samples and feathers from nestlings were collected at eight colonies, and contaminants as well as physiological parameters were analyzed. Polybrominated diphenyl ethers (PBDEs), non-*ortho*-PCBs, and toxaphene were analyzed for the first time in Great Blue Heron eggs from the St. Lawrence River. PBDE concentrations were of the same order of magnitude as *p,p*'-DDE and total PCBs, and toxaphene concentrations were of the same order of magnitude as chlordane. Although non-*ortho*-PCBs were present in low concentrations, they accounted for 67% on average of the toxic equivalent. Mercury levels in eggs as well as in blood and feathers were unchanged since the previous survey. Concentrations of total PCBs decreased in eggs but not in blood, and concentrations of organochlorine pesticides showed little difference. Concentrations of total PCBs, non-*ortho*-PCBs, dioxins and furans, the toxic equivalent, total PBDEs, total toxaphene, and several organochlorine pesticides in eggs showed no significant differences between regions, whereas differences were observed in concentrations of several contaminants in plasma. Many blood clinical variables—thyroid hormone free T4 (free thyroxine), plasma retinol, and 3,4-dehydroretinol as well as stable isotopes of carbon and nitrogen—showed significant differences among colonies and between regions. The nestlings from colonies in the freshwater section of the river were more contaminated than those from the estuary or reference colonies. The level of contamination in the St. Lawrence River was generally below the levels associated with toxic effects for the Great Blue Heron; however, plasma retinol levels in nestlings from the freshwater colonies remain low, and this could affect their development and survival. More efforts should be devoted to research in order to achieve a better understanding of the many factors that can influence the status of the Great Blue Heron population and the health of the ecosystem.

RÉSUMÉ

Dans le cadre du Plan d'Action Saint-Laurent Vision 2000, le Grand Héron a été retenu comme espèce indicatrice du réseau de suivi de l'état du Saint-Laurent. Une première campagne d'échantillonnage dans le cadre de ce réseau a été effectuée en 1996 et 1997. Le présent rapport présente les résultats de la seconde campagne d'échantillonnage effectuée en 2001-2002. Des œufs, de même que du sang et des plumes des jeunes ont été récoltés à huit colonies et des analyses de contaminants de même que des mesures physiologiques ont été effectuées. Les polybromodiphényléthers (PBDE), les non-*ortho*-BPC (no-BPC) et le toxaphène ont été analysés pour la première fois dans les œufs de Grand héron du Saint-Laurent. Les concentrations de PBDE mesurées sont du même ordre de grandeur que celles de *p,p*'-DDE et de BPC total, tandis que celles de toxaphène sont du même ordre de grandeur que celles de chlordane. Les no-BPC, bien que présents en faibles concentrations, contribuent en moyenne à 67 % de l'équivalent toxique. Depuis la campagne d'échantillonnage précédente, le niveau de mercure est demeuré inchangé tant dans les œufs que dans le sang et les plumes. Le BPC total a diminué dans les œufs mais pas dans le sang, tandis que les pesticides organochlorés montrent peu de différence. Le BPC total, les no-BPC, les dioxines et furannes, l'équivalent toxique, les PBDE totaux, le toxaphène total et plusieurs pesticides organochlorés mesurés dans les œufs ne montrent pas de différence significative entre les régions, tandis qu'on observe des différences dans plusieurs des contaminants mesurés dans le plasma. Plusieurs variables cliniques sanguines, l'hormone thyroïdienne T4-libre, le rétinol et le 3,4-déhydrorétinol plasmatiques et les isotopes stables de carbone et d'azote montrent des différences significatives entre les colonies et entre les régions. Les jeunes des colonies situées en eau douce sont plus contaminés que ceux des colonies de l'estuaire et des colonies témoins. Le niveau de contamination dans le fleuve Saint-Laurent se situe en général en deçà des niveaux d'effets toxicologiques pour le Grand Héron. Cependant, les niveaux de rétinol sanguin des jeunes hérons des colonies en eau douce demeurent bas, ce qui pourrait affecter leur développement et leur capacité de survie. Davantage d'efforts devraient être consacrés à la recherche afin de mieux comprendre l'influence des nombreux facteurs pouvant affecter l'état de la population du Grand Héron ainsi que la santé des écosystèmes.

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1. INTRODUCTION

The St. Lawrence River, which is a vast and complex ecosystem, runs more than 3000 km from its source in the Great Lakes to the Atlantic Ocean. Gateway to the continent and a vector for development, it has been under intense pressure from anthropogenic activities. Nevertheless, programs instituted in the Great Lakes and the St. Lawrence River over the last 20 years have started to bear fruit. One of the goals of the St. Lawrence River Vision 2000 Action Plan is to develop and implement a network to monitor the state of the St. Lawrence so that changes can be tracked and the effectiveness of protection and conservation measures can be assessed.

Colonial birds have many advantages as bioindicators of contamination, and they are commonly used for this purpose, largely because of their wide distribution, the ease with which samples can be collected from them, their position in the food chain (often at the top), and their regionally restricted feeding range (Mineau et al. 1984; Fox and Weseloh 1987; Gilbertson et al. 1987; Kushlan 1993). Studies done during the development of the network led to the selection of the Great Blue Heron (*Ardeas herodias*) as an indicator species for the State of the St. Lawrence Monitoring Program (Champoux et al. 2000, 2002, 2004; Rodrigue et al. 2005; Champoux et al. 2006a). The Great Blue Heron has been widely used as a bioindicator because it is found in both saltwater and freshwater environments, its colonies are accessible, and it is at the top of the food chain (Elliott et al. 1989; Custer et al. 1997; Thomas and Anthony 1999; Jenssen et al. 2001).

Monitoring chemical contamination alone does not provide information on the bioavailability, persistence, and toxicity of these substances and therefore does not provide information on the ecosystem's health. The effects that contamination in the tissues of sentinel wildlife species has on the animals' biological systems must also be determined (Fox 1993). The toxicity of chemical substances generally results from interference by the compound itself or one of its metabolites in the biochemical sequence of homeostatic control of physiological processes, such as growth, reproduction, energy production, and osmoregulation. Under normal conditions, these physiological processes are controlled by hormones, vitamins, and other signal transmitters. Early indications of toxicity can therefore be obtained by studying the interference of toxic substances with the endocrine system (Brouwer et al. 1990). Many chemical products that are released into the environment—several of which can be found in the tissues of Great Blue Herons from the St. Lawrence River—are capable of disrupting endocrine system functions

(Colborn et al. 1993; Fry 1995; De Guise et al. 1995; Colborn 1998; Ankley and Giesy 1998; Campbell and Hutchinson 1998; Grasman et al. 1998).

An initial round of testing using biomarkers selected in a previous study was carried out under this program in 1996 and 1997 (Champoux et al. 2004). This current report presents data on the state of the population, contamination of eggs and nestlings, and data on biomarkers measured in nestlings in a selection of Great Blue Heron colonies in the second round of testing (2001–2002). Links are drawn between the toxic substances and the biological variables, and the results are compared with those from studies carried out in 1991–1993 and 1996–1997 (the initial survey). Finally, the results are discussed in the context of the State of the St. Lawrence Monitoring Program.

2. MATERIALS AND METHODS

2.1 COLLECTION OF SAMPLES AND INVENTORY

Most of the known Great Blue Heron colonies were inventoried in June 2001 by the Société de la Faune et des Parcs du Québec (now the Ministère des Ressources naturelles et de la Faune, MRNF) as part of its five-year aerial survey (Desrosiers et al. 1998; Desrosiers 2003). The total number of active nests (occupied by eggs, young, or adults) was counted. Nesting success is the average number of young per nest; breeding success is the proportion of nests that contained young at the time of the aerial survey or during the collection of samples.

In 2001, eggs and samples from nestlings were collected from four Great Blue Heron colonies, three on the St. Lawrence River and a reference colony located outside the St. Lawrence. The colonies on Île Dickerson, Île aux Hérons, and Grande Île are located in the freshwater part of the St. Lawrence River, while the Île Steamboat colony (which served as the reference colony) is located in Lac Wayagamac, close to La Tuque. In 2002, three other colonies—Île de la Corneille, Île aux Basques, and Île aux Oeufs, all in the saltwater section of the river (estuary)—were tested. The colony at Île aux Oeufs served as the reference colony that year. Île Manowin was also visited, but no nests were found. Later in the season, the colony on Île Laval was selected to replace Île Manowin, so no eggs were collected from Île Laval. The colonies are located from Cornwall and Lac Saint-Francois downriver to Baie-Trinité on the North Shore of the Gulf of the St. Lawrence (Figure 1).

The colonies were visited initially between the end of April and the end of May to collect eggs in order to test them for contaminants and retinoids. The geographic distribution of the colonies makes it necessary to travel long distances, but it also means that the collection of the samples can be spread out, since the difference in latitude between the source of the St. Lawrence River and the mouth of the estuary further north means there is a lag between the laying dates of birds at the various colonies. As Great Blue Heron nests are located in the tops of trees, the collection of the eggs was entrusted to a professional climber; two eggs were collected per nest, one to be tested for contaminants and one for retinoid analyses. In general, nine nests were visited per colony. The climber put all of the eggs from each nest into a foam-lined case with depressions in it to hold the eggs and then lowered the case with a rope. The length and width of the eggs were

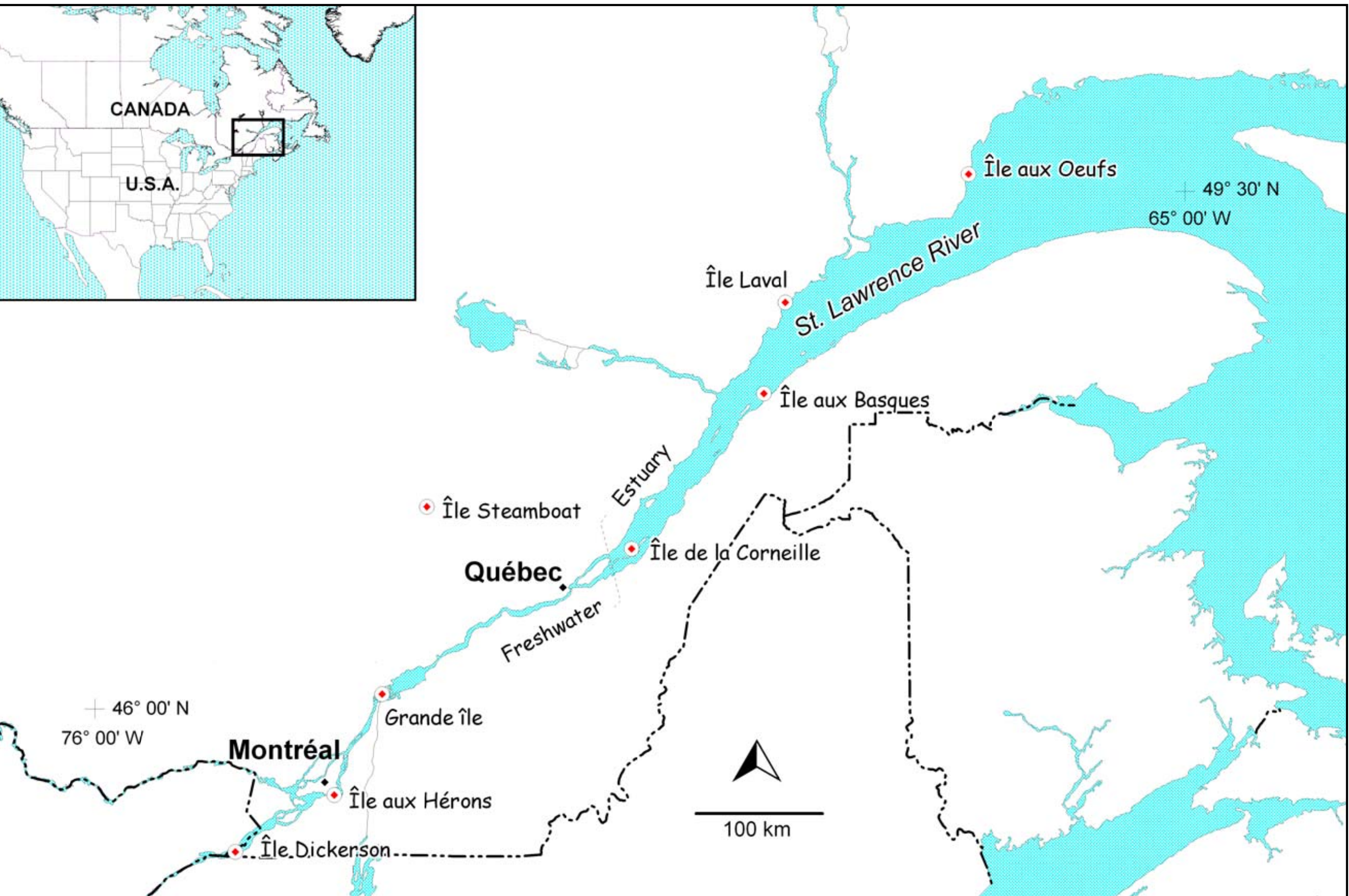


Figure 1. Location of Great Blue Heron colonies studied in 2001 and 2002

measured with a dial calliper, and the eggs were weighed using a Pesola scale (100 g). The stage of development was estimated by placing the egg in a pot filled with water and estimating buoyancy (Hays and Lecroy 1971). The heaviest egg was retained for the contaminant test and a second egg that matched the desired stage of development was kept for the retinoid test. These eggs were placed in labelled Whirl-Pak bags. The first egg was placed in a foam-lined case, and the second was kept in a cooler on dry ice in order to arrest development. The other eggs were returned to the nest.

The colonies were visited a second time seven to eight weeks later, between mid-June and mid-July, to take samples from the young. Samples from nine nestlings, usually from the same nests from which the eggs had been collected, were collected in each colony. The nestlings were banded and weighed using a Pesola scale (5 kg). The age of the young was estimated using tarsus measurements taken with a calliper and Quinney's growth equation (Quinney 1982). Feathers (the fifth primary, the fifth secondary, and two coverts from each wing; one rectrix; four from under the tail; and four tail feathers) were then cut using scissors and kept on ice in a plastic bag (Whirl-Pak). About 10 mL of blood were extracted from the ulnar vein using a 25 gauge needle rinsed with heparin. The blood was collected in two Vacutainer blood collection tubes containing heparin and was kept on ice. The bird was then put back into the nest. Plasma and hematocrit were prepared at the end of each field day. Capillary tubes were filled and centrifuged (five min. at 7000 rpm) to measure hematocrit. A 5-mL tube was centrifuged (five min. at 5000 rpm), and aliquots of 1 mL of plasma were placed in liquid nitrogen for the biochemical analyses. The samples collected for the analysis of contaminants and biochemical parameters were sent to the National Wildlife Research Centre (NWRC) laboratories, where they were stored at appropriate temperatures.

2.2 BIOLOGICAL ANALYSES

2.2.1 Clinical blood analyses

Analyzing basic biochemical parameters of blood can provide valuable supplementary information on an animal’s overall health (Suber 1989). Hematocrit (the ratio of the volume of red blood cells to the total volume of blood) was determined in the hours following collection by centrifuge in a microhematocrit tube. Calcium, glucose, total proteins, albumin, globulins, the

ratio of albumin to globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and plasma concentrations of uric acid were analyzed in the laboratories of the Faculté de médecine vétérinaire of the Université de Montréal using an automated clinical system, the Synchron CX5 (Beckman Coulter, Mississauga, Ontario).

2.2.2 Retinoids

Vitamin A (retinol) is a fat-soluble vitamin essential to vision and reproduction. Over 95% of the body's retinol reserves are stored in the liver as retinyl palmitate following esterification with the corresponding fatty acid. Retinol can be measured in the blood, where it circulates bound to transport proteins. NWRC used the MET-BMK-VIT-02A analytical method (available on request) with the following modifications: samples were analyzed using a high peformance liquid chromatograph (HPLC) from Varian (9010-2641, 9100-2778) equipped with a UV/Vis detector (9050-0664). The Zorbax octadecylsilane (ODS) analytical column (4.6 mm interior diameter × 15 cm; serial number G0013456) used to separate the compounds was protected by an ODS Spheri-5 guard column (4.6 mm × 3 cm; Brownlee lot 03B1-50640). 3,4-Dehydroretinol (F. Hoffman-La Roche Ltd.; Vitamins and Fine Chemicals Division-R & TD; CH-4070 Basel; product synthesis reference number R04-3791) was used as a standard to determine retention time according to our chromatographic conditions. The internal standard method was used to calculate the levels of retinol and 3,4-dehydroretinol. Various retinol standards were included in each analytical sequence, and every plasma sample was fortified with a known quantity of retinol acetate, which makes it possible to monitor extraction efficiency.

Retinoids were measured in the Great Blue Heron eggs in the TOXEN laboratories at the Université du Québec à Montréal. The stage of development of the embryos was determined using the chronology established for the Herring Gull (Williams and Ludwig, unpublished). Unfertilized eggs and eggs whose stage of development was below 18 or above 35 were eliminated from further analysis in order to target the period in which retinoids are the most stable. A portion of the yolk was dehydrated and vitamin A compounds were extracted with hexane. After centrifuging, an aliquot was evaporated with nitrogen gas and the retinoids were

resuspended in ether and acetonitrile. The retinoid concentrations were determined by reverse phase high peformance liquid chromatography (HPLC) (Boily et al. 1994).

2.2.3 Thyroid hormones

Radioimmunoassay of free thyroxine (FT4) and total thyroxine (TT4) is based on competition between the hormone labelled with I^{125} and an unlabelled antigen for antibody binding sites. Total T4 was analyzed first, followed by free T4 (FT4), using the MET-BMK TT4-01A and MET-BMK-FT401A methods, respectively. RIA (radioimmunoassay) methods were adapted from the protocols of the supplier, ICN, and validated by the NWRC laboratory. Specific T4 antibodies were incorporated into the walls of incubation tubes (Quanticoat, Kallestad Diagnostics). Known amounts of T4 were added, followed by the labelled T4. After incubation, the bound hormone was separated from the free hormone by precipitation and the precipitate's radioactivity was counted for one minute using a Canberra-Packard gamma counter, model E-5002 (serial number 423345). For each series of tests, a calibration curve was calculated using six amounts of standard T4 measured in duplicate, and the concentrations of T4 in the samples were determined. Two commercial controls were analyzed and the results were compared to the target values provided by the manufacturer. In addition, a "drift" sample (a commercial control) was used at the end of the series to verify the stability of the measurements. A QA/QC section is an integral part of the computer program; a detailed report was provided after each test series.

2.2.4 Stable isotopes of carbon and nitrogen

Stable carbon and stable nitrogen isotopes were measured in heron plasma to assess the birds' trophic position and their habitat. Tests of $\delta^{13}C$ and $\delta^{15}N$ were done at the Sinlab laboratory (University of New Brunswick) using a Thermo-Finnigan Delta Plus XP continuous flow isotope ratio mass spectrometer and a Carlo Erba NC2500 elemental analyzer. Four IAEA standards, three element standards, and one internal standard were measured at the same time as the samples. Results are accurate to within approximately 0.20‰.

2.3 CHEMICAL ANALYSES

2.3.1 Mercury

The NWRC laboratory analyzed total mercury in eggs, blood, and feathers. The standard procedure without acid digestion was the same for all three tissues, as described in the manual of the Laboratory Services, under MET-CHEM-AA-03E (Neugebauer et al. 2000). Mercury was analyzed using an advanced mercury analyzer (AMA-254, ALTEC, Czech Republic) using direct combustion of the sample in an oxygen-rich atmosphere. Test accuracy was determined using NIST standard reference material (Bovine Blood 966, Level 1 and Level 2) and NRC standard reference materials DOLT-2 and DORM-2 (National Research Council Canada, Ottawa, Canada). Some randomly selected samples were also analyzed in duplicate. Recovery of mercury from reference materials was within certified values. The detection limit was 2 ng/g.

2.3.2 Halogenated organic compounds

The method the NWRC uses to analyze polychlorinated biphenyls (PCBs) and organochlorine compounds is described under MET-CHEM-OC-04C in Won et al. (2001). In the first phase, lipids were extracted from eggs and plasma using a 1:1 mixture of dichloromethane (DCM) and hexane. In the second phase, the chemical substances of interest were extracted and separated from the lipids and biogenic compounds via gel permeation chromatography (GPC). The extracts obtained were then purified via Florisil column chromatography and finally analyzed using a quadrupole mass spectrometer coupled to a gas chromatograph (GC/MSD). The compounds being sought were 1,2,4,5- and 1,2,3,4-tetrachlorobenze; pentachlorobenzene; hexachlorobenzene (HCB); α, β, and γ-HCH (hexachlorocyclohexane); octachlorostyrene (OCS); heptachlor epoxide (HE); oxychlordane; *trans*- and *cis*-chlordane; *trans*- and *cis*-nonachlor; *p,p'*-DDD; *p,p'*-DDE; *p,p'*-DDT; dieldrin; mirex; photomirex; TCPM; and 67 PCB congeners (IUPAC numbers 17, 18, 16/32 (co-eluted), 22, 28, 31, 33/20, 42, 44, 47/48, 49, 52, 56/60, 64, 66, 74, 70/76, 85, 87, 92, 95, 97, 99, 101/90, 105, 110, 118, 128, 130, 137, 138, 141, 146, 149, 151, 153, 156, 157, 158, 171, 172, 174, 176, 177, 178, 179, 180, 183, 187, 170/190, 194, 195, 200, 201, 202, 196/203, 206, 207, 208). The total reported concentration of PCBs is the sum of these congeners. The detection limit for the target compounds varies but was typically below 0.001 mg/kg. The NWRC quality control program includes an analysis of a sample of

reference material of known concentration. Congeners of polychlorinated bornanes (toxaphene) were measured in eggs at the National Water Research Institute (NWRI, Burlington, Ontario) via GC/MS using gas chromatography-mass spectrometry in electron capture negative ion (GC-ECNIMS) mode, as described in Muir et al. (2004). Analysis was performed using an Agilent 6890 GC/5973 MS in selected ion monitoring mode with a HP5-MS gas capillary column 30 m × 0.25 mm (internal diameter) and internal film thickness of 0.25 µm. Quantification of total toxaphene was verified against a technical toxaphene standard. Individual congeners, referred to as either Parlar ("P") numbers or bornanes ("B"), were quantified by a series of authentic external standards of each compound obtained from Ehrenstorfer GmbH (Germany). The minimum detection limit (MDL) for total toxaphene was 1 ng/g; for individual toxaphene congeners the MDL ranged from 0.02 to 0.5 ng/g. Additional contaminants were measured in a composite sample of nine eggs from each colony. Dioxins, furans, and non-*ortho*-PCBs were analyzed in eggs at NWRC using a high-resolution double-focusing mass spectrometer (VG AutoSpec) coupled to a high-resolution gas chromatograph (HP 5890 series II) following the MET-CHEM-PCDD-01C method (Simon and Wakeford 2000). After inoculating samples with BDE solutions and a BDE performance standard solution (EO-4981, MBDE-MXB, and EO-4151, Cambridge Isotopes Solutions), polybrominated diphenyl ethers (PBDE) in the eggs were analyzed using a mass spectrometer coupled to a double-focusing gas chromatograph (VG AutoSpec) operated at 7000 resolution in the electron ionization mode (70 ev), following the method described by Norstrom et al. (2002).

2.4 DATA PROCESSING

While the biological measurements were all done on individual samples, chemical analyses were done on composite samples (pools), grouped in threes, at the rate of three analyses of three individuals per colony. The non-homogeneity of these data needed to be taken into consideration during processing. The general statistics were initially calculated for all of the biological and chemical variables for each colony and tissue. Comparisons of samples grouped by colony and region were then done for each biological and chemical variable using analysis of variance of rank-transformed (non-parametric) data, as described in Quinn and Keough (2002). When a difference was found, colonies were then compared using Tukey's tests (Zar 1984). The biological variables were grouped in threes according to the corresponding composite groups of

chemical variables in order to check for correlations between the chemical and biological variables. These groups were used for all of the subsequent analyses. Spearman's non-parametric correlations were calculated to determine the relationship between the biological variables and chemical variables, and stepwise multiple regressions were done to determine the chemical variables that best accounted for the variations in the levels of the biological variables (Scherrer 1984). Cluster analysis and principal component analyses were done to determine those biological and chemical variables that best accounted for the distribution of the groups of birds and colonies. Comparisons with previous rounds of testing were also analyzed using analysis of variance of rank-transformed data. When a significant difference was found, multiple comparisons using the Tukey-Kramer adjustment were done on the least mean squares (when sample sizes were different) or using Tukey's test (when sample sizes were the same). The tests were done using SAS software (SAS Institute 1989) or JMP software (SAS Institute 1999).

3. RESULTS

3.1 INVENTORY OF COLONIES

The number of active (occupied) nests surveyed in June 2001 ranged from 39 to 1310 (Table 1). According to previous data, five colonies appeared to be getting larger and three colonies appeared to be getting smaller (Desrosiers 2003; CWS, unpublished data). The average number of eggs per nest during the first visit to the colonies was 3.9 (SD 0.6). Breeding success ranged from 68 to 100%, while nesting success ranged from 1.7 to 3.0 (average = 2.4) (SD 0.4). Figure 2 summarizes the changes in nesting success over the years. Average productivity, i.e., the number of young fledged per egg, was 59.3% (SD 13.5). For some colonies, MRNF data were used because the number of nests inventoried during our visits to collect samples was low.

3.2 BIOLOGICAL ANALYSES

3.2.1 Retinoids in the eggs

Only one of the eggs gathered at Île aux Oeufs could be analyzed for retinoids; it was not included in the analyses. (The other eggs collected from Île aux Oeufs fell outside the target stages of development or were infertile.) There were no significant differences among colonies in the weight, length, or width of the eggs used for retinoid analyses, nor did the stage of development of the embryos differ (Tukey's test, $P > 0.05$; Table 2). Retinol, retinyl palmitate, and the ratio of retinol to retinyl palmitate all showed a significant difference among colonies ($P < 0.05$; Figure 3). The highest levels of retinol were found in the Grande Île colony; the highest levels of retinyl palmitate were found in the Île Steamboat colony. Comparisons between regions showed that the levels of retinol and the ratio of retinol to retinyl palmitate were higher in freshwater colonies than in colonies located in the estuary.

Table 1. Inventory of Great Blue Heron colonies tested in 2001–2002

Colony	No. of active[1] nests (growth rate)	Average no. of eggs per nest (*n*; SD)	Breeding success[2] (% of nests with young)	Nesting success[2] (average no. of young per nest) (*n*; SD)	Productivity[4] (%)
Île Dickerson	221 (+7%)	4.5 (10; 0.5)	90	2.3 (10; 1.3)	51.1
Île aux Hérons	170 (−21%)	4.0 (24; 0.9)	nd	2.1[3] (45; nd)	63.3
Grande Île	1310 (+5%)	3.7 (23; 0.9)	68	1.7 (2; 1.4)	47.7
Île Steamboat[R]	41 (−62%)	4.4 (9; 1.0)	100	2.5[3] (13; nd)	55.0
Île de la Corneille	79 (+20%)	3.4 (19; 1.1)	71	2.3[3] (43; nd)	58.8
Île aux Basques	49 (+88%)	4.6 (10; 0.5)	82	2.4 (11; 1.4)	51.9
Île Laval	47 (−33%)	nd	100	2.4[3] (18; nd)	nd
Île aux Oeufs[R]	39 (+160%)	2.9 (11; 1.9)	91	3.0[3] (16; nd)	87.5

n = number of nests.
[R] = reference colony.
nd = not determined.
[1] Active nests = occupied by eggs, young, or adults.
[2] At the time of the aerial inventory or during visits to collect samples.
[3] Data from the MRNF aerial inventory.
[4] Number of young fledged per egg.

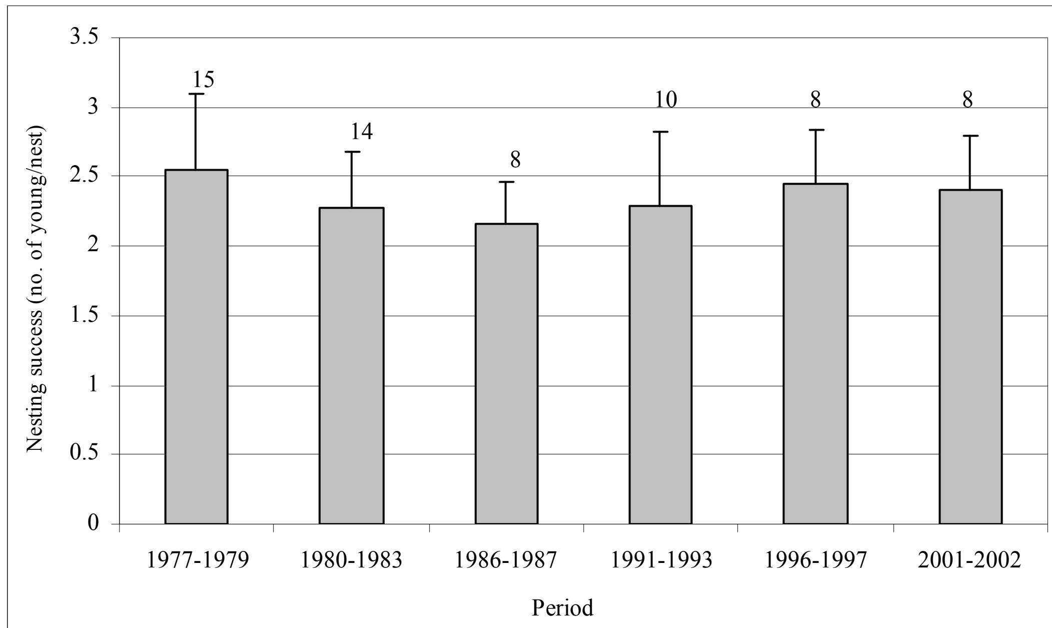


Figure 2. Changes in nesting success (average number of young per nest) of Great Blue Heron colonies in the St. Lawrence from 1977 to 2002 (the figures above the bars indicate the number of colonies used for the calculations)

Table 2. Morphometrics and the stage of development of Great Blue Heron eggs and retinoids in eggs, showing average, standard deviation, and range

Colony	No. of eggs	Egg weight (g)	Length (mm)	Width (mm)	Stage	Retinol (µg/g)	Retinyl Palmitate (µg/g)	Ratio of retinol to retinyl palmitate
Île Dickerson	5	74.6	67.8	46.2	27	3.20[ab]	0.20[b]	22.8
		6.5	2.0	1.5	2	1.44	0.15	18.4
		69–85	65.0–70.6	44.0–47.7	24–30	2.0–5.4	0.1–0.4	8.8–54.0
Île aux Hérons	7	67.3	64.0	46.0	29	3.16[b]	0.31[b]	18.6
		9.1	2.4	2.1	5	1.14	0.25	18.1
		58–80	59.9–67.4	42.1–47.9	20–33	2.1–5.5	0.1–0.7	5.0–55.3
Grande Île	8	66.9	64.5	44.9	24	5.41[a]	0.62[ab]	9.3
		4.3	2.7	0.7	5	2.07	0.28	3.0
		61–73	58.5–67.6	43.6–45.7	19–33	2.5–7.6	0.3–0.9	6.3–15.2
Île Steamboat[R]	4	71.0	66.0	45.8	28	2.45[b]	1.24[a]	2.9
		8.0	4.5	1.4	3	0.63	0.90	1.7
		61–78	60.9–71.8	44.3–47.6	25–31	1.7–3.1	0.5–2.5	0.7–4.4
Île de la Corneille	5	63.8	63.0	44.3	32	2.02[b]	0.83[ab]	3.5
		6.9	4.2	1.1	8	0.85	0.53	2.6
		53–70	56.5–67.1	42.6–45.4	21–35	1.3–3.5	0.3–1.7	0.7–7.6
Île aux Basques	3	64.0	62.8	44.3	28	1.83[b]	0.91[ab]	2.1
		1.0	2.4	1.6	12	0.15	0.29	0.6
		63–65	60.0–64.4	43.0–46.0	22–33	1.7–1.9	0.6–1.2	1.6–2.8
Region								
Freshwater	20	69.0	65.1	45.6	27	4.07[a]	0.41[b]	15.9[a]
		7.3	2.8	1.6	5	1.92	0.29	14.5
		58-85	58.5-70.6	42.1-47.9	19-33	2.0-7.6	0.1-0.9	5.0-55.3
Estuary	8	63.9	62.9	44.3	28	1.95[b]	0.86[a]	3.0[b]
		5.1	3.4	1.2	6	0.66	0.43	2.2
		53-70	56.5-67.1	42.6-45.4	21-35	1.3-3.5	0.3-1.7	0.7-7.6
Reference colonies	4	71.0	66.0	45.8	28	2.45[ab]	1.24[a]	2.9[ab]
		8.0	4.5	1.4	3	0.63	0.90	1.7
		61-78	60.0-71.8	43.0-47.6	22-33	1.7-3.1	0.5-2.5	0.7-4.4

[R] = reference colony.
Values in a single column with the same letter are not significantly different according to Tukey's test ($P > 0.05$)

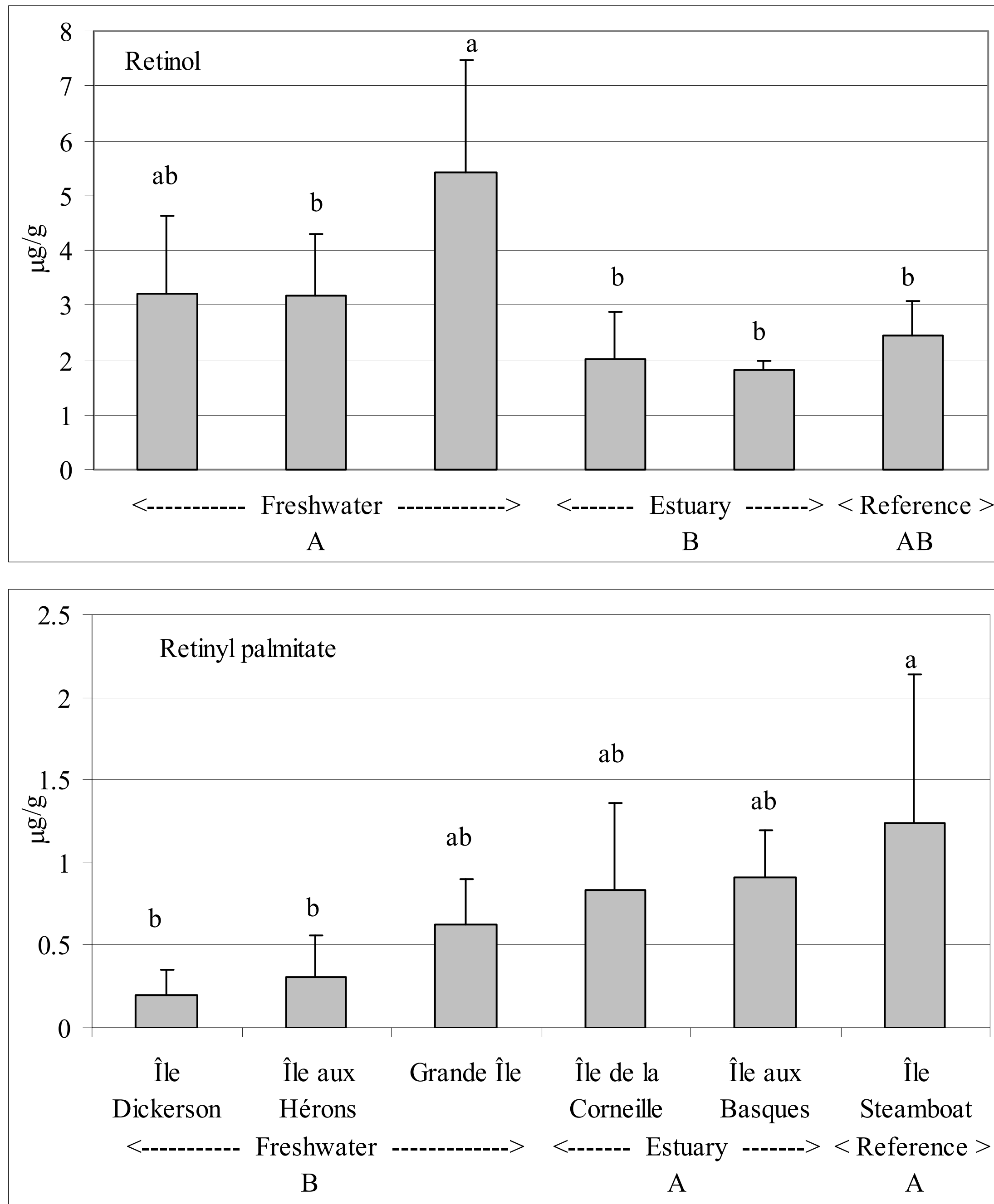


Figure 3. Levels of retinol and of retinyl palmitate in Great Blue Heron eggs (colonies or regions with the same letter are not significantly different according to Tukey's test; $P > 0.05$)

3.2.2 Morphological observations

The weight of young herons varied significantly among colonies, as did tarsus length and age, which was estimated from tarsus length (Tukey's test, $P < 0.5$; Table 3). Young herons from Île aux Oeufs weighed more than those from Île aux Hérons; young herons from Grande Île, Île de la Corneille, and Île aux Basques had longer tarsi and were older than those from Île Laval. The weight of young herons also varied significantly between regions ($P < 0.05$): herons from freshwater colonies were smaller than those from reference colonies.

Table 3. Morphometrics and age of young Great Blue Herons, by colony and region, showing average, standard deviation, and range

Colony	Year	No. of eggs collected	No. of young measured	Weight (kg)	Right tarsus (cm)	Age (days)
Île Dickerson	2001	18	9	1.7 ± 0.2^{ab} (1.3–2.0)	15.2 ± 1.4^{ab} (13.3–17.0)	36 ± 4^{ab} (31–41)
Île aux Hérons	2001	18	9	1.5 ± 0.5^{b} (0.5–2.1)	14.2 ± 3.3^{ab} (7.9–17.8)	34 ± 9^{ab} (17–43)
Grande Île	2001	18	9	1.8 ± 0.3^{ab} (1.0–2.2)	17.1 ± 1.9^{a} (13.0–19.0)	42 ± 5^{a} (31–47)
Île Steamboat[R]	2001	18	9	1.8 ± 0.4^{ab} (1.2–2.3)	15.5 ± 3.0^{ab} (10.0–19.0)	37 ± 8^{ab} (23–47)
Île de la Corneille	2002	18	9	2.1 ± 0.3^{ab} (1.6–2.5)	17.3 ± 0.9^{a} (15.7–18.4)	42 ± 7^{a} (38–45)
Île aux Basques	2002	18	9	2.0 ± 0.2^{ab} (1.8–2.5)	16.6 ± 1.8^{a} (14.2–19.2)	40 ± 5^{a} (34–47)
Île Laval	2002	0	9	1.6 ± 0.3^{ab} (1.1–2.1)	12.7 ± 2.2^{b} (9.4–16.1)	30 ± 6^{b} (21–39)
Île aux Oeufs[R]	2002	18	9	2.2 ± 0.5^{a} (1.2–2.8)	16.0 ± 3.4^{ab} (9.9–19.5)	39 ± 9^{ab} (23–48)
Region						
Freshwater				1.67 ± 0.4^{b} (0.5–2.2)	15.5 ± 2.6 (7.9–19.0)	37 ± 7 (17–47)
Estuary				1.88 ± 0.3^{ab} (1.1–2.5)	15.5 ± 2.6 (9.4–19.2)	37 ± 7 (21–47)
Reference colonies				1.98 ± 0.5^{a} (1.2–2.8)	15.8 ± 3.1 (9.9–19.5)	38 ± 8 (23–48)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

3.2.3 Clinical analyses and blood biomarkers

A number of the variables measured in young herons' blood showed significant differences among colonies and between regions ($P < 0.05$). The thyroid hormone free T4 (free thyroxine) and retinol were lower in freshwater colonies but 3,4-dehydroretinol was higher (Table 4; Figures 4 and 5). Stable C and N isotopes also showed significant differences among colonies and between regions ($P < 0.05$)—they were both lower in reference colonies. Several variables in the clinical blood analyses also showed significant differences ($P < 0.05$) among colonies, between regions, or both (Table 5).

Table 4. Concentrations (average, standard deviation, and range) of thyroid hormones, retinol, and stable isotopes in the blood of young Great Blue Herons, by colony and region

Colony	Total T4 (ng/mL)	Free T4 (ng/L)	3,4-dehydro-retinol (µg/L)	Retinol (µg/L)	$\delta^{13}C$	$\delta^{15}N$	C/N
Île Dickerson	17.0 ± 6.1 (8.4–26.1)	6.9 ± 3.1[c] (2.6–11.2)	299 ± 112[a] (144–489)	528 ± 180[c] (315–841)	−21.8 ± 1.0[abc] (−23.0 to −20.6)	13.6 ± 0.5[a] (13.1–14.2)	3.34 ± 0.01[ab] (3.33–3.34)
Île aux Hérons	17.2 ± 4.2 (8.7–22.4)	7.8 ± 2.1[c] (5.3–10.5)	186 ± 68[ab] (80–288)	782 ± 262[bc] (443–1270)	−21.0 ± 0.4[ab] (−21.3 to −20.5)	14.1 ± 0.6[a] (13.4–14.7)	3.36 ± 0.01[a] (3.36–3.37)
Grande Île	19.1 ± 5.9 (11.9–30.7)	8.7 ± 4.2[c] (1.5–17.1)	184 ± 80[ab] (89–286)	646 ± 330[c] (264–1175)	−23.6 ± 0.6[bc] (−24.3 to −22.9)	13.2 ± 0.3[a] (12.9–13.5)	3.37 ± 0.01[a] (3.36–3.37)
Île Steamboat[R]	19.2 ± 5.6 (5.8–24.6)	9.8 ± 2.0[bc] (7.8–13.5)	148 ± 81[ab] (13–287)	1081 ± 296[ab] (637–1551)	−28.8 ± 0.5[d] (−29.2 to −28.2)	9.1 ± 0.1[c] (9.0–9.2)	3.36 ± 0.30[a] (3.34–3.40)
Île de la Corneille	20.4 ± 5.0 (15.4–31.4)	15.3 ± 1.6[a] (13.3–18.3)	88 ± 53[b] (25–169)	1282 ± 219[a] (926–1629)	−24.7 ± 2.6[c] (−28.5 to −19.5)	11.1 ± 1.7[b] (9.2–14.3)	3.32 ± 0.02[b] (3.29–3.36)
Île aux Basques	23.4 ± 7.1 (12.7–35.0)	13.2 ± 2.2[ab] (8.9–15.8)	178 ± 230[ab] (25–781)	1290 ± 306[a] (889–1829)	−20.2 ± 3.4[a] (−28.5 to −17.7)	13.6 ± 1.6[a] (9.9–15.0)	3.32 ± 0.04[b] (3.28–3.40)
Île Laval	22.6 ± 7.1 (15.3–34.1)	10.9 ± 3.3[bc] (4.6–14.8)	149 ± 97[ab] (74–311)	1033 ± 195[ab] (650–1277)	−23.2 ± 3.3[abc] (−27.6 to −18.2)	11.3 ± 1.6[b] (9.5–13.8)	3.34 ± 0.04[ab] (3.30–3.43)
Île aux Oeufs[R]	21.7 ± 6.9 (13.9–36.0)	8.3 ± 3.4[c] (3.8–14.5)	60 ± 65[b] (25–181)	1241 ± 220[a] (886–1503)	−24.7 ± 1.1[c] (−26.7 to −23.0)	10.3 ± 0.5[bc] (9.4–10.9)	3.37 ± 0.04[a] (3.30–3.42)
Region							
Freshwater	17.8 ± 5.4 (8.4–30.7)	7.8 ± 3.2[b] (1.5–17.1)	224 ± 102[a] (80–489)	647 ± 274[b] (264–1270)	−22.1 ± 1.3[a] (−24.3 to −20.5)	13.6 ± 0.6[a] (12.9–14.7)	3.36 ± 0.01[a] (3.33–3.37)
Estuary	22.1 ± 6.4 (12.7–35.0)	13.1 ± 3.0[a] (4.6–18.3)	138 ± 148[b] (25–781)	1202 ± 264[a] (650–1829)	-22.7 ± 3.5[a] (−28.5 to −17.7)	12.0 ± 2.0[b] (9.2–15.0)	3.32 ± 0.04[b] (3.28–3.43)
Reference colonies	20.4 ± 6.2 (5.8–36.0)	9.1 ± 2.8[b] (3.8–14.5)	107 ± 85[b] (13–287)	1161 ± 266[a] (637–1551)	−26.9 ± 2.3[b] (−29.2 to −23.0)	9.6 ± 0.7[c] (9.0–10.9)	3.37 ± 0.03[a] (3.30–3.42)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

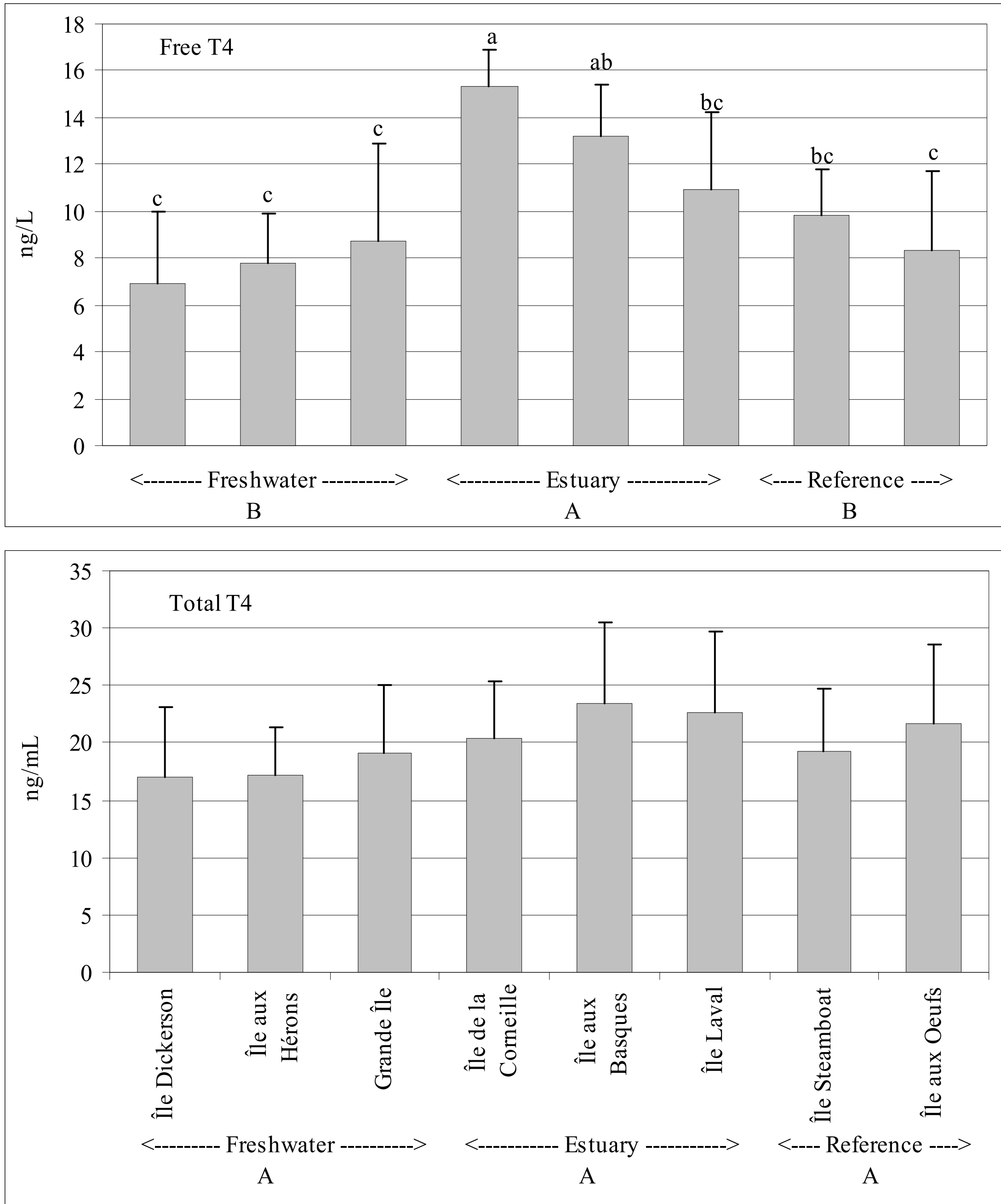


Figure 4. Levels of the hormones free T4 and total T4 in the plasma of young Great Blue Herons (colonies or regions with the same letter or no letter are not significantly different according to Tukey's test, $P > 0.05$)

Table 5. Clinical blood analyses (average, standard deviation, and range) of young Great Blue Herons, by colony and region

Colony	Hematocrit (%)	Proteins (g/L)	Albumin (g/L)	Globulin (g/L)	Ratio of albumin to globulin	Glucose (mmol/L)
Île Dickerson	33 ± 5 (29–43)	16.5 ± 2.2^{cde} (12.0–19.4)	12.9 ± 1.3^{ab} (10.7–14.3)	3.6 ± 2.2^{bc} (1.0–8.7)	4.8 ± 2.7 (1.2–11.0)	15.0 ± 1.7^{bc} (13.2–18.4)
Île aux Hérons	33 ± 3 (28–36)	17.5 ± 2.8^{bcde} (13.2–22.7)	13.6 ± 0.8^{ab} (12.3–15.0)	4.0 ± 2.8^{bc} (0.1–8.4)	23.2 ± 45.2 (1.7–131.0)	14.6 ± 1.1^{bc} (13.6–16.8)
Grande Île	32 ± 2 (29–35)	14.4 ± 2.7^{e} (10.9–18.4)	12.1 ± 0.9^{b} (10.6–13.9)	2.3 ± 2.7^{c} (0.3–7.0)	14.2 ± 10.7 (1.6–35.3)	13.5 ± 1.4^{c} (11.2–15.3)
Île Steamboat[R]	33 ± 2 (29–36)	16.4 ± 3.0^{de} (13.0–21.8)	13.9 ± 1.1^{a} (12.5–15.7)	2.5 ± 2.9^{c} (0.0–8.7)	16.3 ± 22.4 (1.5–68.5)	15.7 ± 2.4^{abc} (13.6–19.7)
Île Corneille	35 ± 3 (31–40)	20.0 ± 2.1^{abc} (16.6–23.3)	12.2 ± 1.1^{b} (10.8–14.2)	7.8 ± 1.1^{a} (5.8–9.1)	1.6 ± 0.1 (1.3–1.9)	15.5 ± 1.3^{bc} (13.2–17.5)
Île aux Basques	35 ± 3 (30–40)	22.6 ± 2.3^{a} (17.8–25.8)	13.1 ± 0.9^{ab} (11.2–14.5)	9.6 ± 1.4^{a} (6.6–11.3)	1.4 ± 0.2 (1.2–1.7)	15.8 ± 1.2^{ab} (13.8–17.3)
Île Laval	34 ± 1 (32–36)	21.1 ± 1.9^{ab} (18.5–24.0)	13.1 ± 0.8^{ab} (12.1–14.6)	8.0 ± 1.3^{a} (6.3–10.2)	1.7 ± 0.2 (1.4–2.0)	15.0 ± 0.8^{bc} (13.8–16.3)
Île aux Oeufs[R]	33 ± 2 (31–38)	19.6 ± 2.0^{abcd} (17.4–23.0)	12.9 ± 1.1^{ab} (11.6–14.7)	6.7 ± 1.6^{ab} (3.3–8.3)	2.1 ± 0.9 (1.6–4.3)	17.9 ± 1.7^{a} (15.4–20.3)
Region						
Freshwater	33 ± 3^{b} (28–43)	16.1 ± 2.8^{b} (10.9–22.7)	12.8 ± 1.2 (10.6–15.0)	3.2 ± 2.6^{b} (0.1–8.7)	13.7 ± 25.9^{a} (1.2–131.0)	14.3 ± 1.5^{c} (11.2–18.4)
Estuary	35 ± 3^{a} (30–40)	21.2 ± 2.3^{a} (16.6–25.8)	12.8 ± 1.0 (10.8–14.6)	8.4 ± 1.5^{a} (5.8–11.3)	1.6 ± 0.2^{b} (1.2–2.0)	15.4 ± 1.2^{b} (13.2–17.5)
Reference colonies	33 ± 2^{ab} (29–38)	17.9 ± 3.0^{b} (13.0–23.0)	13.4 ± 1.2 (11.6–15.7)	4.5 ± 3.1^{b} (0.0–8.7)	9.2 ± 17.0^{ab} (1.5–68.5)	16.8 ± 2.3^{a} (13.6–20.3)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

Table 5 (cont'd)

Colony	AST (U/L)	CK (U/L)	LDH (U/L)	ALP (U/L)	Calcium (mmol/L)	Uric acid (µmol/L)
Île Dickerson	192 ± 96 (124–429)	4 541 ± 2 259[b] (610–7 260)	645 ± 164[abc] (474–950)	391 ± 88[bc] (249–528)	2.52 ± 0.13[ab] (2.39–2.78)	456.1 ± 172.3 (170.0–716.5)
Île aux Hérons	153 ± 21 (122–188)	5 674 ± 1 448[b] (4 490–8 610)	762 ± 229[ab] (473–1 110)	363 ± 59[bc] (267–429)	2.47 ± 0.11[b] (2.33–2.64)	487.9 ± 142.0 (268.0–682.2)
Grande Île	341 ± 521 (137–1 730)	4 952 ± 1 796[b] (2 010–7 800)	580 ± 142[bcd] (414–772)	393 ± 131[abc] (162–605)	2.26 ± 0.09[c] (2.08–2.41)	571.3 ± 193.2 (347.7–928.0)
Île Steamboat[R]	135 ± 17 (115–169)	5 034 ±1 063[b] (3 770–6 700)	532 ± 117[cd] (345–672)	335 ± 75[c] (256–463)	2.45 ± 0.10[b] (2.34–2.63)	616.8 ± 183.1 (260.0–793.0)
Île de la Corneille	150 ± 21 (119–177)	8 151 ± 1 217[a] (5 680–9 810)	803 ± 165[a] (636–1 110)	535 ± 169[a] (342–913)	2.51 ± 0.12[ab] (2.32–2.67)	1 850.0 ± 2 855.8 (336.2–9 420.0)
Île aux Basques	148 ± 25 (105–188)	6 079 ± 1 152[ab] (3 920–7 700)	650 ± 87[abc] (518–765)	504 ± 103[ab] (297–614)	2.55 ± 0.14[ab] (2.21–2.66)	910.4 ± 207.4 (561.2–1 242.0)
Île Laval	123 ± 14 (105–152)	5 022 ± 1 152[b] (3 240–7 150)	521 ± 101[cd] (354–623)	359 ± 63[bc] (279–443)	2.59 ± 0.08[ab] (2.48–2.75)	1 677.4 ± 2 309.2 (681.0–7 810.0)
Île aux Oeufs[R]	129 ± 57 (5–208)	5 026 ± 1 159[b] (3 520–6 410)	400 ± 140[d] (241–678)	419 ± 69[abc] (316–518)	2.63 ± 0.09[a] (2.51–2.76)	2 579.4 ± 4 561.3 (683.0–14 730.0)
Region						
Freshwater	231 ± 311 (122–1 730)	5 032 ± 1 864[b] (610–8 610)	659 ± 188[a] (414–1 110)	383 ± 96[b] (162–605)	2.42 ± 0.16[b] (2.08–2.78)	505.8 ± 172.1 (170.0–928.0)
Estuary	141 ± 23 (105–188)	6 417 ± 1 739[a] (3 240–9 810)	658 ± 166[a] (354–1 110)	466 ± 139[a] (279–913)	2.55 ± 0.12[a] (2.21–2.75)	1 479.3 ± 2082.4 (336.2–9420.0)
Reference colonies	132 ± 41 (5–208)	5 030 ± 1 079[b] (3 520–6 700)	466 ±142[b] (241–678)	377 ± 82[b] (256–518)	2.54 ± 0.13[a] (2.34–2.76)	1 598.1 ± 3 290.3 (260.0–14 730.0)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

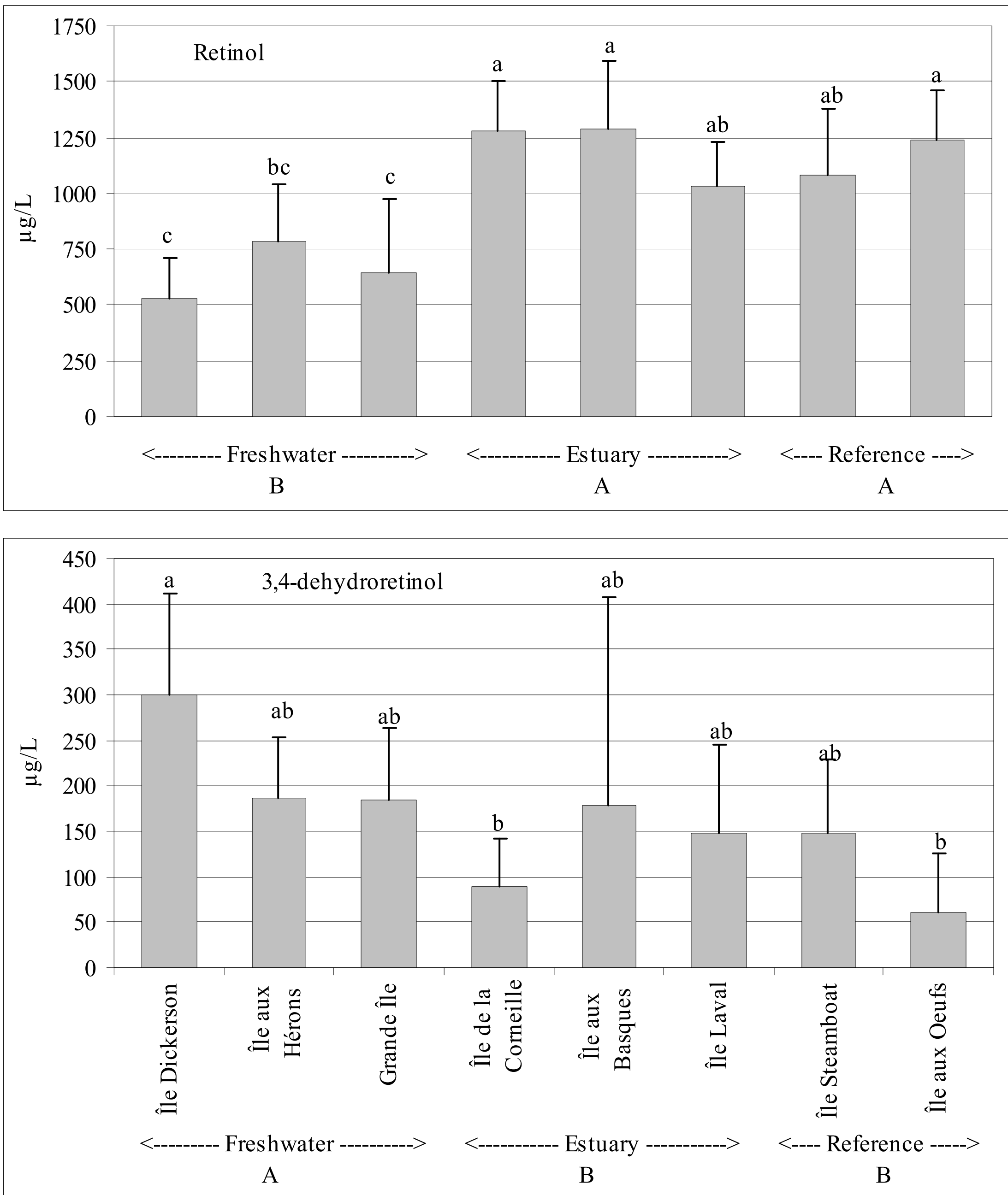


Figure 5. Levels of retinol and 3,4-dehydroretinol in the plasma of young Great Blue Herons (colonies or regions with the same letter or no letter are not significantly different according to Tukey's test, $P > 0.05$)

3.3 CHEMICAL ANALYSES

3.3.1 Mercury

Concentrations of mercury in eggs and blood did not vary significantly among colonies ($P > 0.05$; Table 6; Figure 6). However, there were significant differences in the concentrations of mercury in feathers ($P < 0.01$). The Île Steamboat colony, where levels of mercury were high, differed significantly from the Île aux Basques colony, where they were low. There were no significant differences among the colonies located in the St. Lawrence River. There were no differences in mercury concentrations between the regions in any of the three tissue types analyzed ($P > 0.05$).

3.3.2 Halogenated organic contaminants

Concentrations of total PCBs, the principal congeners, the chlorine compounds, and the *ortho* compounds analyzed in the eggs did not vary significantly among the colonies, except for congener PCB 206 and the nonachloro-PCBs (Table 7; Figure 7). The principal PBC congeners present in the eggs, accounting for 56% of average of total PCBs (Figure 8), were congeners 153 > 138 > 180 > 118 > 206 > 187. The other congeners of less importance that showed differences among colonies were 56/60, 66, 74, 105, 158, 176, 179, and 194. Comparisons between regions yielded similar results: congeners 47/48, 56/60, 66, 74, 105, 141, 194, and 206, as well as the tetra-, octa-, and nonachloro-PCB groups, showed significant differences.

Non-*ortho*-substituted congeners (non-*ortho*-PCBs) as well as dioxins and furans (Figure 9) were measured in a single composite sample of nine eggs per colony. A toxic equivalent (TE) based on the relative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was calculated based on toxic equivalent factors published by Van den Berg et al. (1998) for the PCB congeners for which equivalents were available for birds, i.e., the mono-*ortho*-substituted congeners (105, 118, 156, and 157), the non-*ortho*-PCBs (77, 81, 126, and 169), and the dioxins and furans (Table 8; Figure 10). Non-*ortho*-PCBs accounted for over 67% on average of total TE. As only one sample per colony was used in the analysis of the variables from which the TE was calculated, it was not possible to determine whether there was a significant difference among colonies. However, the

highest value was reported in the Île aux Hérons colony. No significant difference was observed between regions ($P > 0.05$).

Table 6. Concentrations of mercury (average, standard deviation, and range) in Great Blue Heron eggs and in the blood and feathers of young herons, by colony and region

Colony	Eggs Hg (µg/g wet weight)	Blood Hg (µg/g wet weight)	Feathers Hg (µg/g dry weight)
Île Dickerson	0.33 ± 0.16	0.41 ± 0.05	5.22 ± 0.36[ab]
	(0.15–0.46)	(0.35–0.45)	(4.98–5.64)
Île aux Hérons	0.19 ± 0.08	0.42 ± 0.03	6.09 ± 0.57[ab]
	(0.12–0.27)	(0.40–0.45)	(5.43–6.47)
Grande Île	0.21 ± 0.05	0.39 ± 0.05	4.53 ± 0.02[ab]
	(0.17–0.26)	(0.34–0.44)	(4.51–4.54)
Île Steamboat[R]	0.42 ± 0.13	0.73 ± 0.09	9.04 ± 0.32[a]
	0.28–0.50	(0.65–0.84)	(8.77–9.40)
Île de la Corneille	0.25 ± 0.10	0.70 ± 0.36	6.51 ± 2.57[ab]
	(0.16–0.35)	(0.32–1.04)	(3.83–8.95)
Île aux Basques	0.22 ± 0.10	0.32 ± 0.29	2.71 ± 1.23[b]
	(0.14–0.34)	(0.14–0.66)	(1.62–4.04)
Île Laval	na	0.31 ± 0.20	4.99 ± 2.69[ab]
	–	(0.14–0.53)	(2.08–7.39)
Île aux Oeufs[R]	0.21 ± 0.07	0.44 ± 0.05	5.24 ± 1.05[ab]
	(0.16–0.30)	(0.40–0.49)	(4.03–5.85)
Region			
Freshwater	0.24 ± 0.11	0.40 ± 0.04	5.37 ± 0.75
	(0.12–0.46)	(0.34–0.45)	(4.51–6.47)
Estuary	0.23 ± 0.09	0.44 ± 0.32	4.73 ± 2.56
	(0.14–0.35)	(0.14–1.04)	(1.62–8.95)
Reference colonies	0.32 ± 0.15	0.59 ± 0.17	7.14 ± 2.19
	(0.16–0.50)	(0.40–0.84)	(4.03–9.40)

[R] = reference colony.
na = not analyzed.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

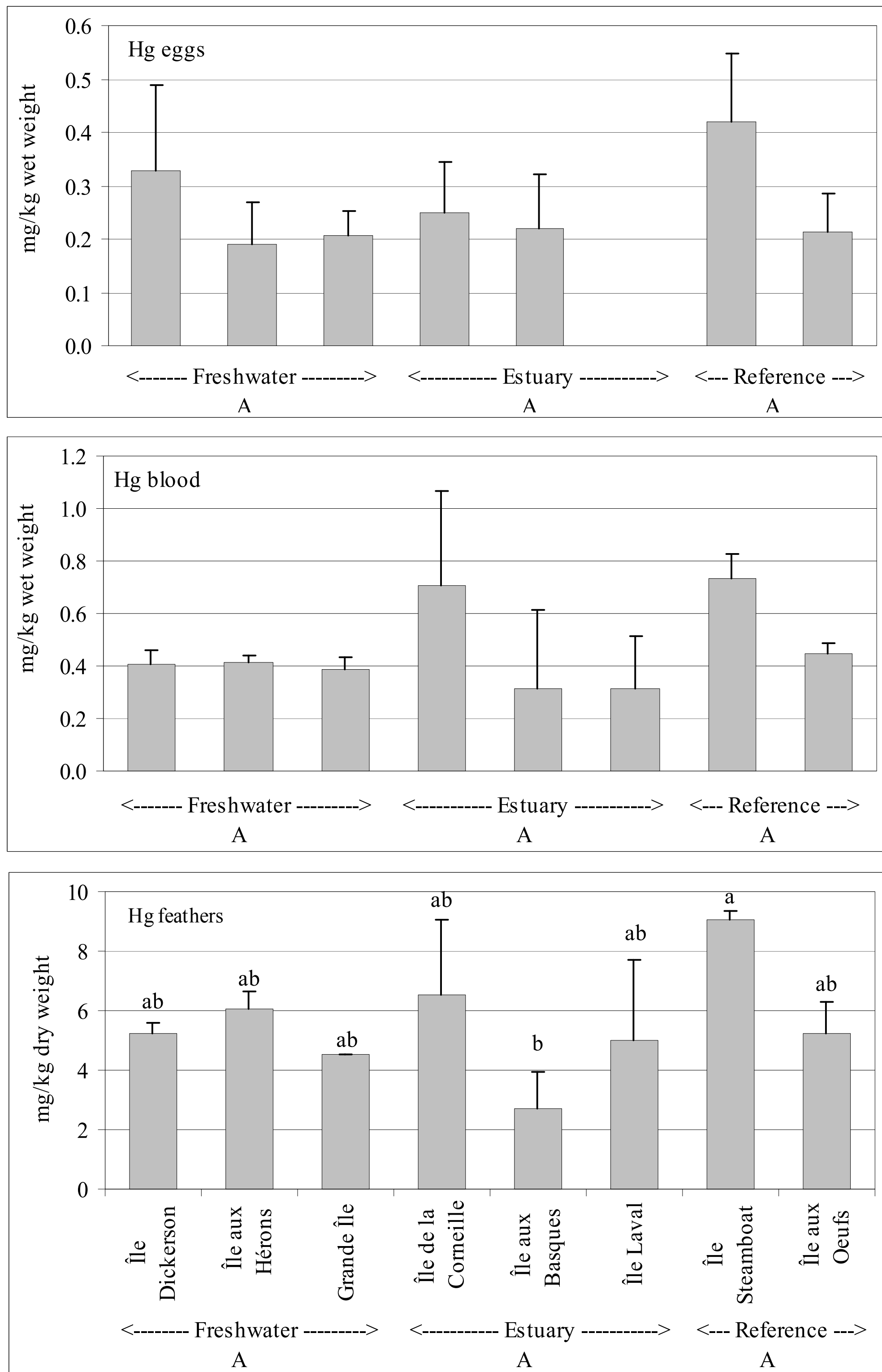


Figure 6. Concentrations of mercury in Great Blue Heron eggs and in the blood and feathers of young herons (colonies or regions with the same letter or no letter or not significantly different according to Tukey's test, $P > 0.05$)

Table 7. Average concentrations (standard deviation and range) of total PCBs and the main PCB congeners in Great Blue Heron eggs (mg/kg wet weight), by colony and region

Colony	Total PCBs	118	138	153	180	187	206
Île Dickerson	0.875 (0.352) (0.66–1.28)	0.057 (0.021) (0.04–0.08)	0.088 (0.042) (0.05–0.14)	0.129 (0.069) (0.07–0.20)	0.081 (0.036) (0.05–0.12)	0.040 (0.014) (0.03–0.06)	0.070[abc] (0.019) (0.05–0.09)
Île aux Hérons	3.47 (3.19) (1.41–7.15)	0.224 (0.155) (0.13–0.40)	0.440 (0.411) (0.17–0.91)	0.548 (0.514) (0.22–1.14)	0.356 (0.405) (0.12–0.83)	0.175 (0.181) (0.06–0.38)	0.194[a] (0.150) (0.10–0.37)
Grande Île	2.26 (0.45) (1.83–2.72)	0.142 (0.017) (0.12–0.15)	0.275 (0.070) (0.20–0.34)	0.348 (0.069) (0.30–0.43)	0.230 (0.067) (0.16–0.30)	0.122 (0.038) (0.08–0.15)	0.172[a] (0.048) (0.13–0.23)
Île Steamboat[R]	1.20 (1.24) (0.28–2.61)	0.093 (0.096) (0.02–0.20)	0.132 (0.125) (0.03–0.27)	0.177 (0.167) (0.05–0.37)	0.074 (0.050) (0.02–0.12)	0.045 (0.045) (0.01–0.10)	0.171[ab] (0.225) (0.04–0.43)
Île de la Corneille	1.15 (0.36) (0.83–1.53)	0.079 (0.022) (0.06–0.10)	0.147 (0.049) (0.10–0.20)	0.258 (0.084) (0.18–0.34)	0.110 (0.048) (0.08–0.17)	0.051 (0.019) (0.03–0.07)	0.011[bc] (0.004) (0.01–0.01)
Île aux Basques	0.99 (0.78) (0.47–1.88)	0.063 (0.031) (0.03–0.09)	0.127 (0.098) (0.06–0.24)	0.206 (0.174) (0.10–0.41)	0.085 (0.076) (0.04–0.17)	0.058 (0.057) (0.03–0.12)	0.011[c] (0.008) (0.01–0.02)
Île aux Oeufs[R]	0.87 (0.57) (0.46–1.53)	0.050 (0.015) (0.04–0.07)	0.095 (0.054) (0.05–0.16)	0.195 (0.149) (0.07–0.36)	0.103 (0.095) (0.02–0.21)	0.035 (0.032) (0.01–0.07)	0.014[c] (0.015) (0.01–0.03)
Region							
Freshwater	2.203 (1.974) (0.66–7.15)	0.141 (0.107) (0.04–0.40)	0.268 (0.259) (0.05–0.91)	0.342 (0.319) (0.07–1.14)	0.222 (0.238) (0.05–0.83)	0.112 (0.110) (0.03–0.38)	0.145[a] (0.098) (0.05–0.37)
Estuary	1.069 (0.547) (0.47–1.88)	0.071 (0.025) (0.03–0.10)	0.137 (0.070) (0.06–0.24)	0.232 (0.125) (0.10–0.41)	0.098 (0.058) (0.04–0.17)	0.055 (0.038) (0.03–0.12)	0.011[b] (0.005) (0.01–0.02)
Reference colonies	1.034 (0.882) (0.28–2.61)	0.072 (0.066) (0.02–0.20)	0.113 (0.089) (0.03–0.27)	0.186 (0.142) (0.05–0.37)	0.089 (0.070) (0.02–0.21)	0.040 (0.035) (0.01–0.10)	0.093[b] (0.167) (0.01–0.43)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test (*P* > 0.05).

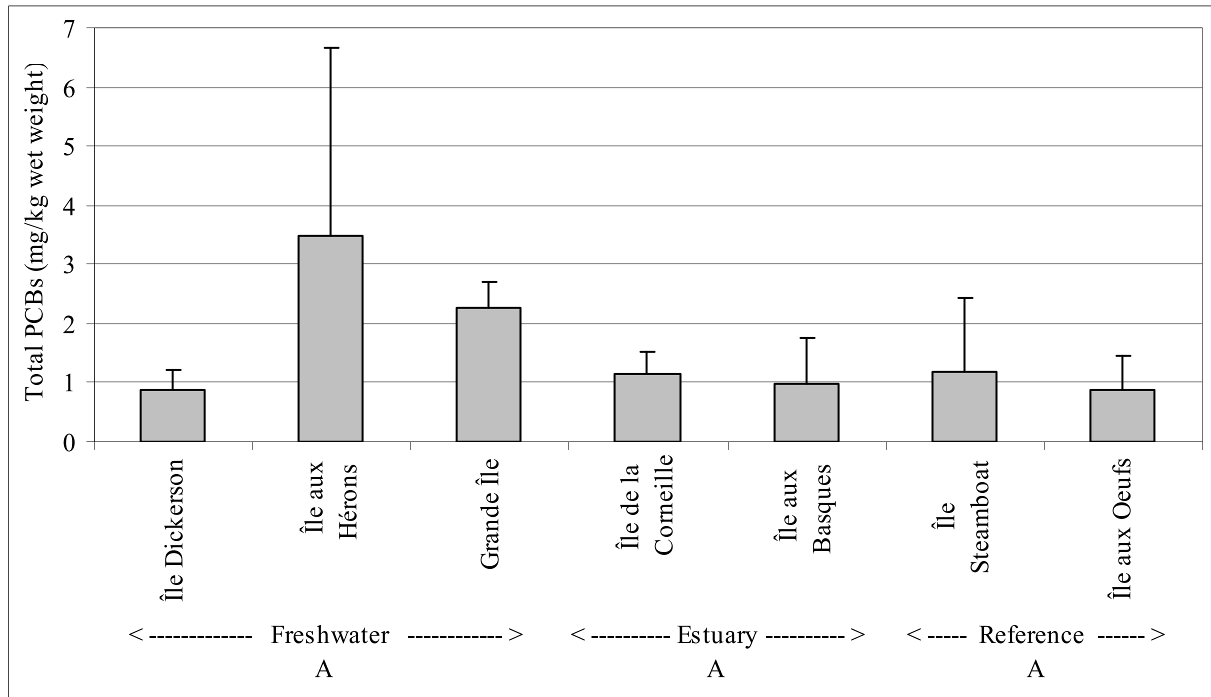


Figure 7. Concentrations of total PCBs in Great Blue Heron eggs (colonies or regions with the same letter or no letter are not significantly different according to Tukey's test, $P > 0.05$)

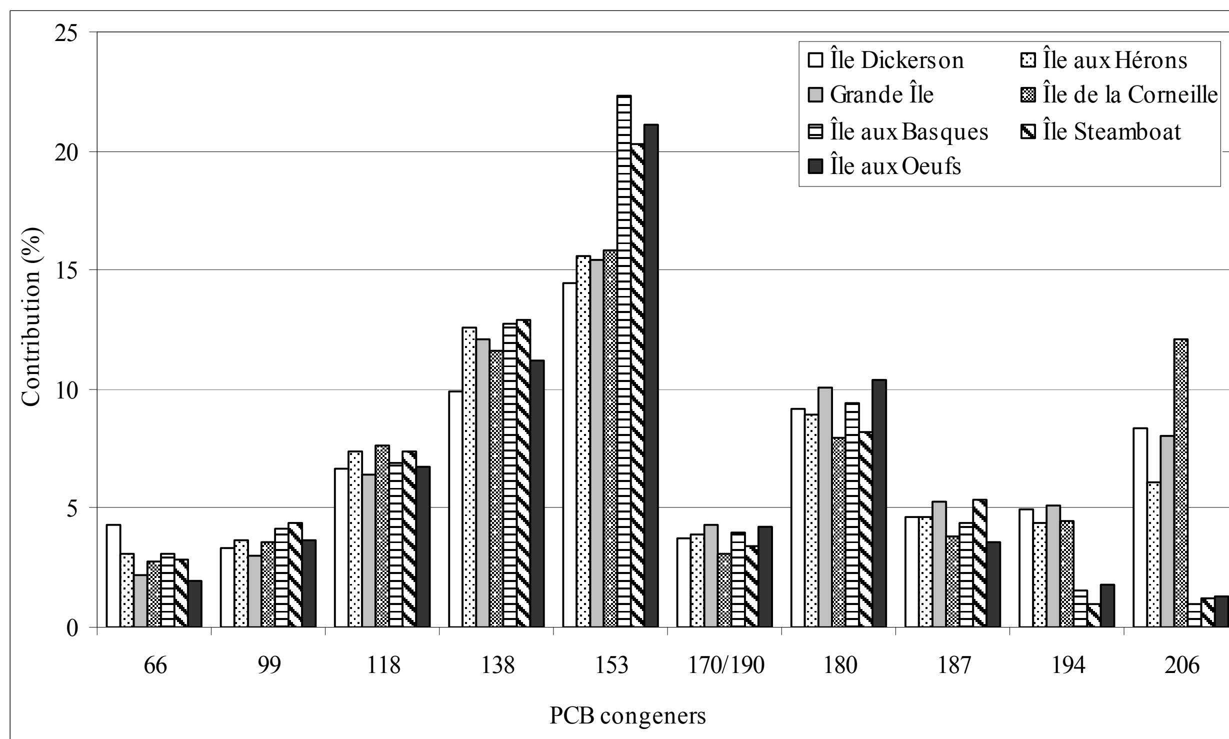


Figure 8. Contribution of the main PCB congeners to total PCBs in Great Blue Heron eggs

Table 8. Average concentrations (standard deviation) of mono-*ortho*-PCBs, non-*ortho*-PCBs, dioxins and furans, and toxic equivalents in the eggs of Great Blue Herons, by colony and region

Colony	Mono-*ortho*-PCBs (mg/kg wet weight)	Non-*ortho*-PCBs (ng/kg wet weight)	Dioxins and furans (ng/kg wet weight)	Toxic equivalent (ng/kg wet weight)
Île Dickerson	0.093 (0.035)	535.8	23.02	56.17
Île aux Hérons	0.369 (0.268)	1264.3	31.67	135.70
Grande Île	0.236 (0.036)	894.3	26.83	100.82
Île Steamboat[R]	0.141 (0.144)	694.3	26.37	76.51
Île de la Corneille	0.129 (0.036)	349.3	45.50	43.58
Île aux Basques	0.105 (0.050)	280.5	12.37	32.95
Île aux Oeufs[R]	0.085 (0.026)	260.4	39.06	36.46
Region				
Freshwater	0.233 (0.138)	898.1 (364.2)	27.17 (4.34)	97.56 (39.87)
Estuary	0.117 (0.017)	314.9 (48.6)	28.93 (23.42)	38.27 (7.52)
Reference colonies	0.113 (0.040)	477.4 (306.8)	32.72 (8.97)	56.49 (28.32)

Mono-*ortho*-PCBs = sum of congeners 105, 118, 156, and 157.
Non-*ortho*-PCBs = sum of congeners 77, 81, 126, and 169.
[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

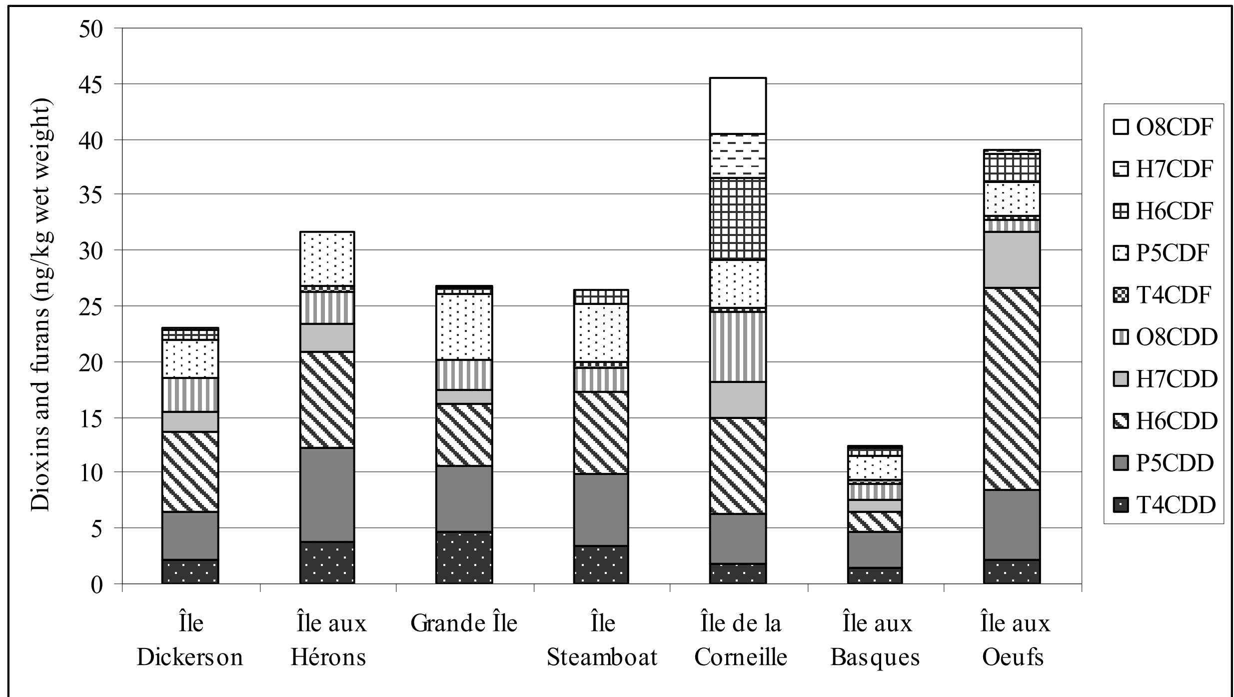


Figure 9. Contribution of dioxin and furan isomers to total dioxins and furans in Great Blue Heron eggs

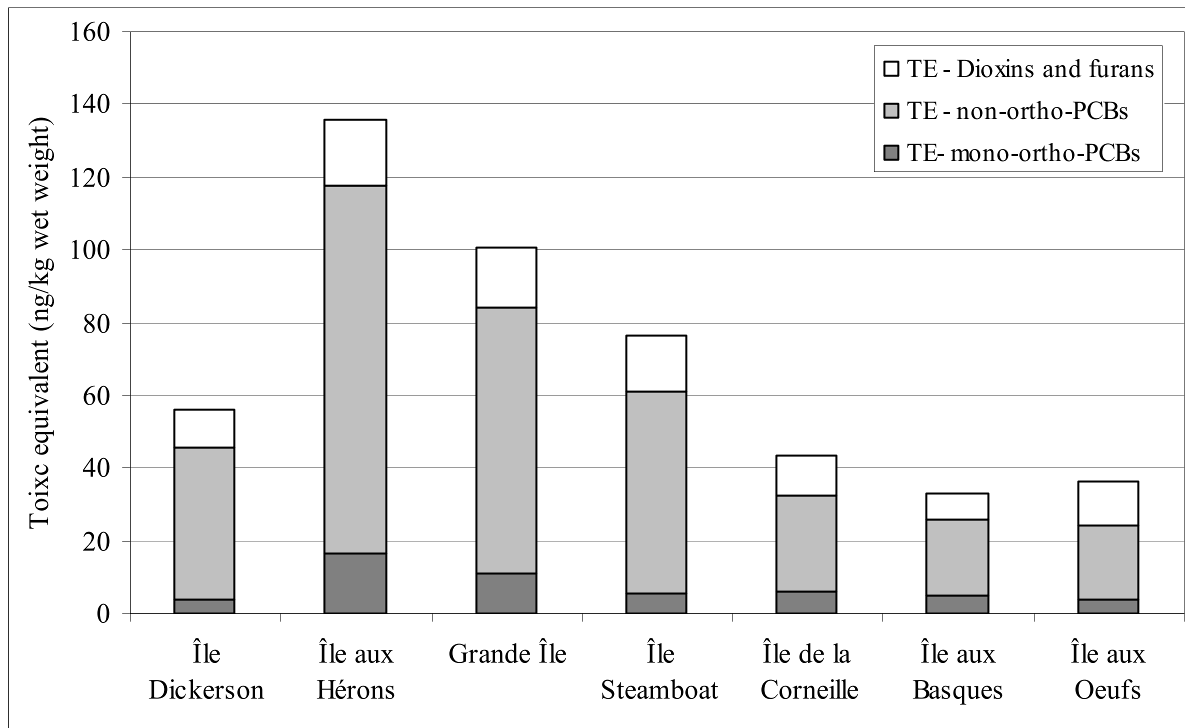


Figure 10. Contribution of mono-*ortho*-PCBs, non-*ortho*-PCBs, and dioxins and furans to total toxic equivalents in Great Blue Heron eggs

The concentrations of total polybrominated diphenyl ethers (polybromodiphenyl ethers) (PBDE) and of the seven principal congeners are shown in Table 9 and Figure 11. On average, these 7 congeners account for 98.4% of total PBDEs (28 congeners were measured). As PBDEs were analyzed in only one sample per colony, it was not possible to determine whether there was a significant difference among colonies. However, the highest value was in the Île aux Hérons colony. There was no significant difference between the regions ($P > 0.05$).

Concentrations of total toxaphene (polychlorinated bornanes) and of individual chlorinated homologues of toxaphene are shown in Table 10; the contributions made by the 10 principal chlorobornane congeners out of the 21 analyzed are illustrated in Figure 12. There was a difference among the colonies for only deca-toxaphene; there were differences between regions for nona-toxaphene and toxaphene B8-1412.

Table 9. Average concentrations of total PBDEs and the principal PBDE congeners in the eggs of Great Blue Herons (ng/g wet weight), by colony and region (standard deviation in parenthesis)

Colony	Total PBDEs	PBDE-47	PBDE-49	PBDE-99	PBDE-100	PBDE-153	PBDE-154	PBDE-183
Île Dickerson	134.6	33.9	0.2	37.8	29.0	22.1	9.0	0.9
Île aux Hérons	1377.4	484.6	0.8	283.5	237.5	260.1	88.1	9.0
Grande Île	776.9	194.2	2.2	191.7	159.5	157.3	57.3	5.7
Île Steamboat[R]	371.9	77.1	0.7	118.9	70.9	66.9	30.2	1.8
Île de la Corneille	172.4	29.6	0.5	38.1	38.5	38.3	24.5	0.6
Île aux Basques	116.4	29.5	1.0	20.7	28.9	18.3	14.6	0.4
Île aux Oeufs[R]	70.7	21.1	< 0.001	9.2	14.2	16.2	7.9	0.3
Region								
Freshwater	762.9 (621.5)	237.6 (228.5)	1.1 (1.0)	171.0 (124.2)	142.0 (105.4)	146.5 (119.4)	51.5 (39.9)	5.2 (4.1)
Estuary	144.4 (39.6)	29.5 (0.04)	0.8 (0.3)	29.4 (12.3)	33.7 (6.8)	28.3 (14.1)	19.6 (7.0)	0.5 (0.2)
Reference colonies	221.3 (213.0)	49.1 (39.6)	0.4 (0.5)	64.0 (77.5)	42.5 (40.0)	41.6 (35.8)	19.1 (15.7)	1.0 (1.0)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

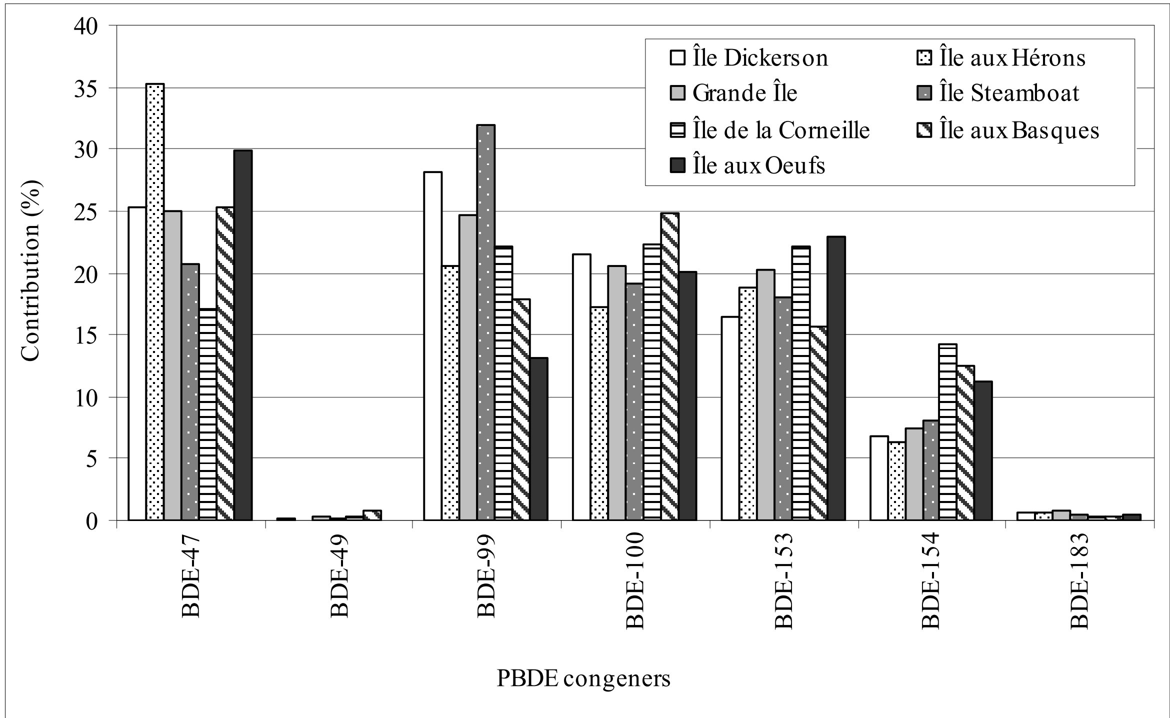


Figure 11. Contribution of the principal PBDE congeners to total PBDEs in Great Blue Heron eggs

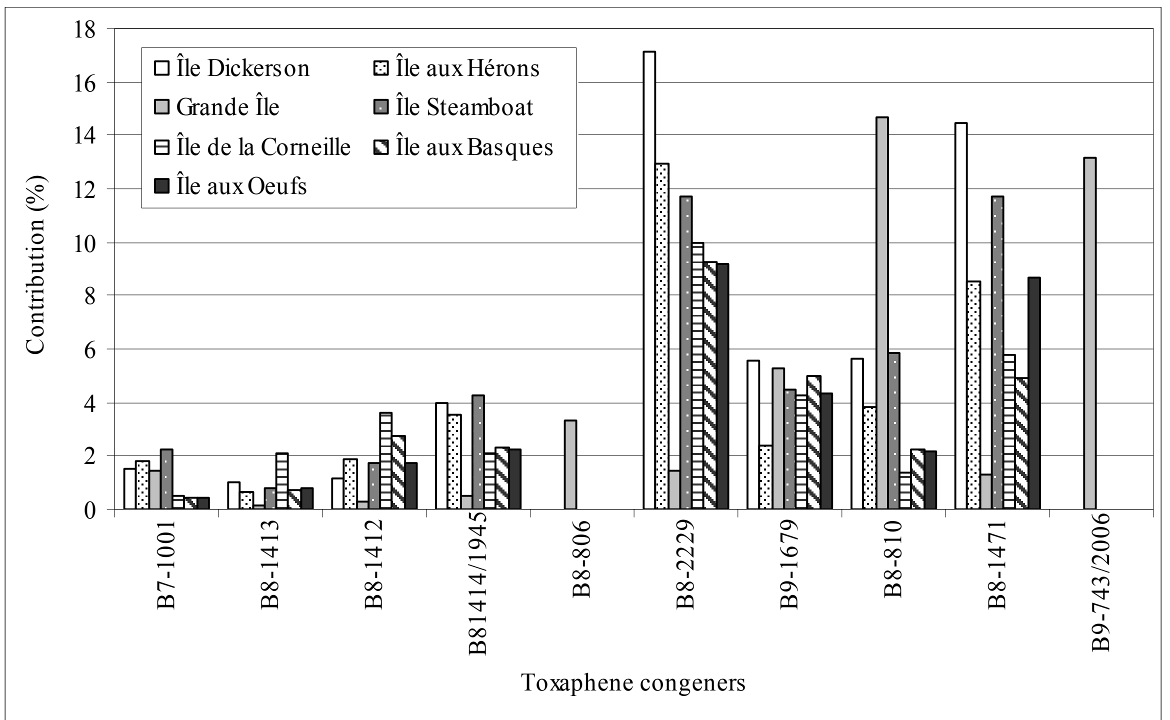


Figure 12. Contribution of the principal toxaphene congeners to total toxaphene in Great Blue Heron eggs

Table 10. Concentrations of toxaphenes (average, standard deviation, and range) in the eggs of Great Blue Herons (ng/g wet weight), by colony and region

Colony	Toxaphene					
	Total	hexa	hepta	octa	nona	deca
Île Dickerson	20.2 ± 23.3 (5.7–47.1)	nd nd	5.1 ± 6.9 (1.1–13.1)	11.8 ± 13.2 (3.9–27.1)	3.3 ± 3.3 0.7–7.0)	nd[b] nd
Île aux Hérons	33.0 ± 39.1 (6.4–77.9)	nd nd	12.6 ± 19.1 (1.0–34.6)	17.9 ± 17.8 (4.7–38.2)	2.5 ± 2.4 (0.6–5.1)	nd[b] nd
Grande Île	159.1 ± 238.1 (4.2–433.3)	0.6 ± 0.6 (<dl–1.1)	73.1 ± 116.1 (0.9–207.0)	78.1 ± 114.1 (2.9–209.4)	7.3 ± 7.8 (0.3–15.7)	nd[b] nd
Île Steamboat[R]	26.9 ± 18.4 (15.9–48.1)	nd nd	7.9 ± 9.0 (2.2–18.2)	15.4 ± 8.1 (10.5–24.7)	3.5 ± 1.4 (2.3–5.0)	0.1 ± 0.1[a] (<dl–0.3)
Île de la Corneille	32.5 ± 12.5 (24.2–46.9)	0.06 ± 0.06 (<dl–0.13)	6.8 ± 3.1 (4.9–10.3)	18.8 ± 9.7 (13.1–30.0)	6.9 ± 1.2 (6.0–8.2)	nd[b] nd
Île aux Basques	52.0 ± 3.4 (48.3–54.9)	0.3 ± 0.2 (<dl–0.4)	13.4 ± 1.8 (11.4–14.9)	26.1 ± 5.3 (22.1–32.1)	12.2 ± 1.8 (11.0–14.3)	nd[b] nd
Île aux Oeufs[R]	55.0 ± 42.0 (19.5–101.4)	nd nd	12.5 ± 8.4 (4.9–21.5)	30.7 ± 24.9 (10.1–58.3)	11.8 ± 8.9 (4.5–21.6)	nd[b] nd
Region						
Freshwater	70.8 ± 138.3 (4.2–433.3)	0.21 ± 0.40 (<dl–1.1)	30.3 ± 67.2 (0.9–207.0)	36.0 ± 66.2 (2.9–209.4)	4.4 ± 4.9[b] (0.3–15.7)	nd nd
Estuary	42.2 ± 13.4 (24.2–54.9)	0.16 ± 0.17 (<dl–0.4)	10.1 ± 4.3 (4.9–14.9)	22.5 ± 8.1 (13.1–32.1)	9.7 ± 3.2[a] (6.0–14.3)	nd nd
Reference colonies	40.9 ± 32.9 (15.9–101.4)	nd nd	10.2 ± 8.2 (2.2–21.5)	23.0 ± 18.5 (10.1–58.3)	7.7 ± 7.3[ab] (2.3–21.6)	0.07 ± 0.1 (<dl–0.3)

[R] = reference colony.
dl: detection limit.
nd: not detected.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test (*P* > 0.05).

Of the 21 organochlorine pesticides measured in the eggs, 12 were detected in over 50% of samples and 8 were detected in all samples (Table 11). Concentrations of *p,p'*-DDD, *p,p'*-DDT, *trans*-nonachlor, *cis*-chlordane, oxychlordane, photomirex, and pentachlorobenzene analyzed in the eggs differed significantly among colonies ($P < 0.05$). Concentrations of heptachlor epoxide, *trans*-nonachlor, *cis*-nonachlor, *p,p*'-DDT, *p,p'*-DDD, *cis*-chlordane, oxychlordane, photomirex, and pentachlorobenzene were significantly higher in the freshwater region ($P < 0.05$).

In blood, concentrations of total PCBs, the main PCB congeners, the chlorine compounds, and the *ortho* compounds showed significant differences among colonies ($P < 0.05$; Table 12; Figure 13). Total PCBs appeared to be significantly higher in the freshwater portion than in the estuary or in the reference colonies. The pattern of the main PCB congeners in blood was similar to the pattern in eggs, with only one additional congener being needed to reach 56% of total PCBs (118 > 153 > 138 > 180 > 105 > 187 > 99) (Figure 14). In blood, only seven organochlorine pesticides were detected in over 50% of the samples. Four were detected in all samples (hexachlorobenzene (HCB), *p,p*'-DDE, *trans*-nonachlor, and *cis*-nonachlor). 1,2,4,5-Tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, *p,p*'-DDE, *p,p'*-DDD, *p,p'*-DDT, heptachlor epoxide, and *cis*-nonachlor showed significant differences among colonies as well as between regions, except for pentachlorobenzene and *p,p*'-DDE ($P < 0.05$; Table 12). Concentrations were generally higher in freshwater colonies than in the estuary or the reference colonies.

3.4 CORRELATIONS BETWEEN CONTAMINANTS AND BIOLOGICAL VARIABLES

3.4.1 Correlations among variables in eggs

An initial analysis of the correlations was done by matching the biological data from the eggs with the contaminants measured in the eggs from the same nests grouped according to the composite samples for the contaminants measured in the groups of three eggs, for a total of 15 groups of eggs. In this set, there were several significant correlations between retinol and organochlorine pesticides, PCBs, retinyl palmitate, and the ratio of retinol to retinyl palmitate (Table 13). A second analysis was then done using an average value per colony for the biological variables in order to calculate correlations with contaminants measured in a single composite sample per colony. Only retinol was positively correlated with non-*ortho*-PCBs and toxic equivalent (Table 14). Stepwise multiple regressions were done to find out which variables best accounted for the variability in the level of retinol in the eggs. The best result was obtained with the developmental stage and the log-transformed PCB congener 118 (adjusted $r^2 = 0.4131$, $P = 0.016$).

Table 11. Concentrations (average, standard deviation, and range) of the principal organochlorine pesticides in the eggs of Great Blue Herons (µg/kg wet weight), by colony and region

Colony	Dieldrin	Heptachlor epoxide	Hexachloro-benzene (HCB)	Mirex	*trans*-nonachlor	*cis*-nonachlor
% detected	**95**	**100**	**100**	**90**	**100**	**100**
Île Dickerson	27 ± 6 (<0.1–31)	16 ± 17 (4–36)	3 ± 1 (2–4)	24 ± 20 (<0.1–38)	39 ± 26 (18–68)	13 ± 9 (5–23)
Île aux Hérons	100 ± 99 (14–208)	34 ± 23 (9–55)	7 ± 8 (2–16)	11 ± 1 (10–12)	267 ± 299 (91–612)	86 ± 98 (23–199)
Grande Île	75 ± 53 (15–113)	11 ± 2 (9–13)	4 ± 1 (3–5)	13 ± 11 (6–26)	113 ± 67 (67–189)	34 ± 19 (21–56)
Île Steamboat[R]	66 ± 90 (10–170)	27 ± 37 (4–69)	4 ± 1 (3–5)	5 ± 4 (2–9)	102 ± 128 (13–249)	30 ± 40 (3–76)
Île de la Corneille	14 ± 7 (9–22)	5 ± 1 (4–5)	4 ± 1 (3–4)	7 ± 7 (<0.1–12)	24 ± 7 (17–30)	7 ± 2 (5–9)
Île aux Basques	11 ± 4 (8–16)	5 ± 3 (3–8)	4 ± 1 (3–5)	4 ± 3 (2–7)	21 ± 8 (16–30)	8 ± 5 (5–14)
Île aux Oeufs[R]	20 ± 20 (4–42)	6 ± 3 (3–9)	7 ± 6 (2–14)	4 ± 4 (1–8)	13 ± 15 (7–32)	7 ± 4 (3–10)
Region						
Freshwater	64 ± 67 (<0.1–208)	21 ± 18[a] (4–55)	5 ± 4 (2–16)	14 ± 11 (<0.1–38)	140 ± 184[a] (18–612)	44 ± 59[a] (5–199)
Estuary	13 ± 5 (8–22)	5 ± 2[b] (3–8)	4 ± 1 (3–5)	5 ± 4 (<0.1–12)	22 ± 7[b] (16–30)	8 ± 4[b] (5–14)
Reference colonies	43 ± 64 (4–170)	17 ± 26[ab] (3–69)	6 ± 4 (2–14)	5 ± 3 (1–9)	62 ± 93[ab] (4–249)	19 ± 28[ab] (3–76)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey’s test ($P > 0.05$).

Table 11 (cont'd)

Colony	*p,p'*-DDT	*p,p'*-DDD	*p,p'*-DDE	*cis*-chlordane	Oxychlordane	TCPM
% detected	**100**	**100**	**100**	**95**	**100**	**67**
Île Dickerson	6 ± 2[ab] (4–8)	3 ± 3[bcd] (1–6)	468 ± 301 (174–776)	2 ± 1[ab] (1–3)	29 ± 36[ab] (8–70)	< 0.1 ± 0 nd
Île aux Hérons	13 ± 6[a] (< 0.1–6)	21 ± 14[a] (13–37)	1715 ± 1551 (527–3469)	15 ± 7[a] (10–23)	79 ± 52[a] (22–124)	6 ± 4 (2–10)
Grande Île	6 ± 1[a] (6–7)	7 ± 1[ab] (6–8)	964 ± 280 (641–1132)	5 ± 3[ab] (2–8)	32 ± 15[ab] (23–50)	4 ± 4 (< 0.1–6)
Île Steamboat[R]	5 ± 3[abc] (2–7)	7 ± 5[abc] (2–12)	509 ± 296 (328–850)	6 ± 7[ab] (0.5–14)	34 ± 36[ab] (8–75)	2 ± 3 (< 0.1–67)
Île de la Corneille	2 ± 1[bcd] (1–2)	1 ± 1[d] (0.5–2)	534 ± 404 (291–1000)	< 0.1 ± 0[b] (< 0.1–0.5)	9 ± 3[ab] (6–12)	2 ± 1 (1–2)
Île aux Basques	1 ± 1[cd] (0.5–2)	1 ± 1[cd] (0.5–2)	372 ± 171 (175–479)	1 ± 0[ab] (0.5–1)	6 ± 3[b] (4–9)	1 ± 1 (0.5–2)
Île aux Oeufs[R]	1 ± 1[d] (0.5–2)	0.5 ± 0[d] (0.5–0.5)	484 ± 156 (335–646)	1 ± 1[ab] (0.5–2)	11 ± 5[ab] (6–14)	1 ± 1 (1–2)
Region						
Freshwater	8 ± 5[a] (< 0.1–8)	10 ± 11[a] (1–37)	1049 ± 969 (174–3469)	7 ± 7[a] (1–23)	47 ± 40[a] (8–124)	3 ± 4 (< 0.1–10)
Estuary	1 ± 1[b] (0.5–2)	1 ± 1[b] (0.5–2)	453 ± 291 (175–1000)	1 ± 0[b] (< 0.1–1)	8 ± 3[b] (4–12)	1 ± 1 (0.5–2)
Reference colonies	3 ± 3[b] (0.5–7)	4 ± 4[b] (0.5–12)	496 ± 212 (328–850)	3 ± 5[ab] (0.5–14)	23 ± 26[ab] (6–75)	12 ± 27 (< 0.1–67)

[R] = reference colony.
nd = not detected.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

Table 12. Concentrations (average, standard deviation, and range) of total PCBs and the main organochlorine pesticides in the plasma of young herons (µg/kg wet weight), by colony and region

Colony	Total PCBs	Hexa-chlorobenzene (HCB)	1,2,3,4-tetra-chlorobenzene (1234-CB)	1,2,4,5-tetra-chlorobenzene (1245-CB)
% detected	**100**	**100**	**50**	**50**
Île Dickerson	36.4 ± 7.9^{ab} (26.5–44.3)	0.17 ± 0.04 (0.13–0.21)	0.02 ± 0.01^{a} (0.02–0.03)	0.06 ± 0.01^{a} (0.05–0.06)
Île aux Hérons	44.6 ± 9.0^{a} (37.2–54.7)	0.18 ± 0.04 (0.15–0.23)	0.03 ± 0^{a} (0.03–0.03)	0.28 ± 0.30^{a} (0.05–0.61)
Grande Île	56.4 ± 50.8^{ab} (24.5–115.0)	0.17 ± 0.11 (0.10–0.30)	0.02 ± 0.01^{a} (0.02–0.03)	0.06 ± 0.01^{a} (0.05–0.07)
Île Steamboat[R]	13.9 ± 9.7^{abc} (5.43–24.4)	0.17 ± 0.04 (0.13–0.21)	0.03 ± 0^{a} (0.03–0.03)	0.06 ± 0.01^{a} (0.05–0.07)
Île de la Corneille	6.9 ± 3.0^{abc} (4.1–10.0)	0.16 ± 0.04 (0.13–0.21)	< 0.01 ± 0^{b} nd	< 0.01 ± 0^{b} nd
Île aux Basques	4.3 ± 1.1^{c} (3.3–5.5)	0.23 ± 0.05 (0.19–0.28)	< 0.01 ± 0^{b} nd	< 0.01 ± 0^{b} nd
Île Laval	14.2 ± 18.5^{bc} (3.4–35.6)	0.21 ± 0.05 (0.18–0.26)	< 0.01 ± 0^{b} nd	< 0.01 ± 0^{b} nd
Île aux Oeufs[R]	2.5 ± 0.9^{c} (1.8–3.5)	0.14 ± 0.02 (0.13–0.16)	< 0.01 ± 0^{b} nd	< 0.01 ± 0^{b} nd
Region				
Freshwater	45.8 ± 27.6^{a} (24.5–115.0)	0.17 ± 0.06 (0.10–0.30)	0.03 ± 0.01^{a} (0.02–0.03)	0.13 ± 0.18^{a} (0.05–0.61)
Estuary	8.5 ± 10.4^{b} (3.3–35.6)	0.20 ± 0.05 (0.13–0.28)	0.01 ± 0^{c} nd	0.01 ± 0.01^{c} nd
Reference colonies	8.2 ± 8.8^{b} (1.8–24.3)	0.16 ± 0.03 (0.13–0.21)	0.02 ± 0.01^{b} (<0.01–0.03)	0.03 ± 0.03^{b} (<0.01–0.07)

[R] = reference colony.
nd = not detected.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

Table 12 (cont'd)

Colony	***p,p'*-DDE**	***p,p'*-DDD**	***trans*-nonachlor**	***cis*-nonachlor**
% detection	**100**	**96**	**100**	**100**
Île Dickerson	4.84 ± 5.19[ab] (1.52–10.82)	0.20 ± 0.06[ab] (0.16–0.27)	0.51 ± 0.24 (0.24–0.70)	0.21 ± 0.05[ab] (0.15–0.24)
Île aux Hérons	5.52 ± 4.01[a] (2.46–10.06)	0.39 ± 0.09[a] (0.29–0.47)	0.82 ± 0.64 (0.10–1.33)	0.44 ± 0.15[a] (0.28–0.55)
Grande Île	9.38 ± 11.05[a] (3.10–22.14)	0.23 ± 0.09[ab] (0.17–0.32)	0.93 ± 0.83 (0.39–1.88)	0.45 ± 0.36[ab] (0.24–0.87)
Île Steamboat[R]	5.36 ± 2.21[a] (2.84–6.93)	0.17 ± 0.08[abd] (0.11–0.26)	0.96 ± 0.95 (0.13–1.99)	0.41 ± 0.37[ab] (0.05–0.79)
Île de la Corneille	2.01 ± 1.13[ab] (1.27–3.31)	0.04 ± 0.05[d] (0.01–0.09)	0.30 ± 0.15 (0.16–0.47)	0.11 ± 0.05[ab] (0.05–0.14)
Île aux Basques	1.40 ± 0.16[ab] (1.26–1.58)	0.08 ± 0.01[bd] (0.06–0.09)	0.47 ± 0.10 (0.35–0.54)	0.12 ± 0.02[ab] (0.11–0.15)
Île Laval	3.09 ± 1.31[ab] (1.65–4.21)	1.31 ± 2.13[abd] (0.06–3.76)	0.39 ± 0.08 (0.34–0.48)	0.10 ± 0.04[ab] (0.06–0.13)
Île aux Oeufs[R]	1.20 ± 0.14[b] (1.12–1.36)	0.03 ± 0.00[d] (0.03–0.04)	0.26 ± 0.10 (0.15–0.35)	0.08 ± 0.02[b] (0.07–0.11)
Region				
Freshwater	6.57 ± 6.77 (1.52–22.14)	0.27 ± 0.11[a] (0.16–0.47)	0.75 ± 0.57 (0.10–1.88)	0.37 ± 0.23[a] (0.15–0.87)
Estuary	2.17 ± 1.14 (1.26–4.21)	0.48 ± 1.23[b] (0.01–3.76)	0.39 ± 0.13 (0.16–0.54)	0.11 ± 0.04[b] (0.05–0.15)
Reference colonies	3.28 ± 2.67 (1.12–6.93)	0.10 ± 0.09[b] (0.03–0.26)	0.61 ± 0.71 (0.13–1.99)	0.24 ± 0.29[b] (0.05–0.79)

[R] = reference colony.
Values in a single column with the same letter or no letter are not significantly different according to Tukey's test ($P > 0.05$).

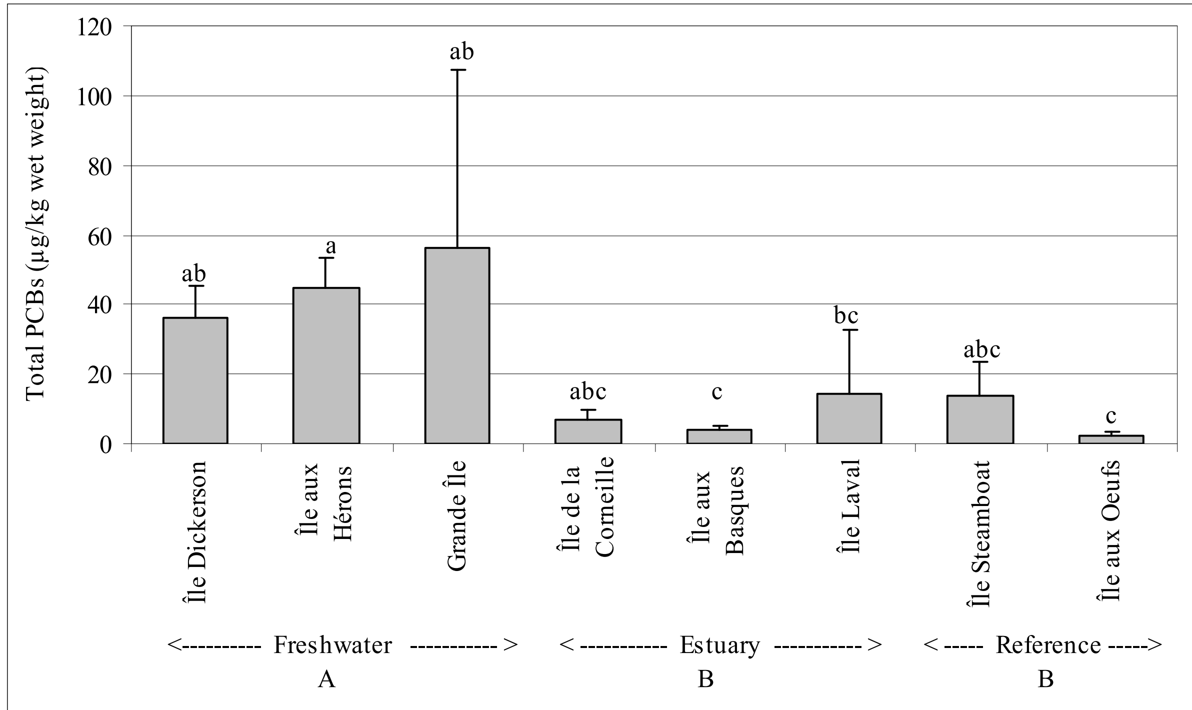


Figure 13. Concentration of total PCBs in the plasma of young Great Blue Herons (colonies with the same letter or no letter are not significantly different according to Tukey's test, $P > 0.05$)

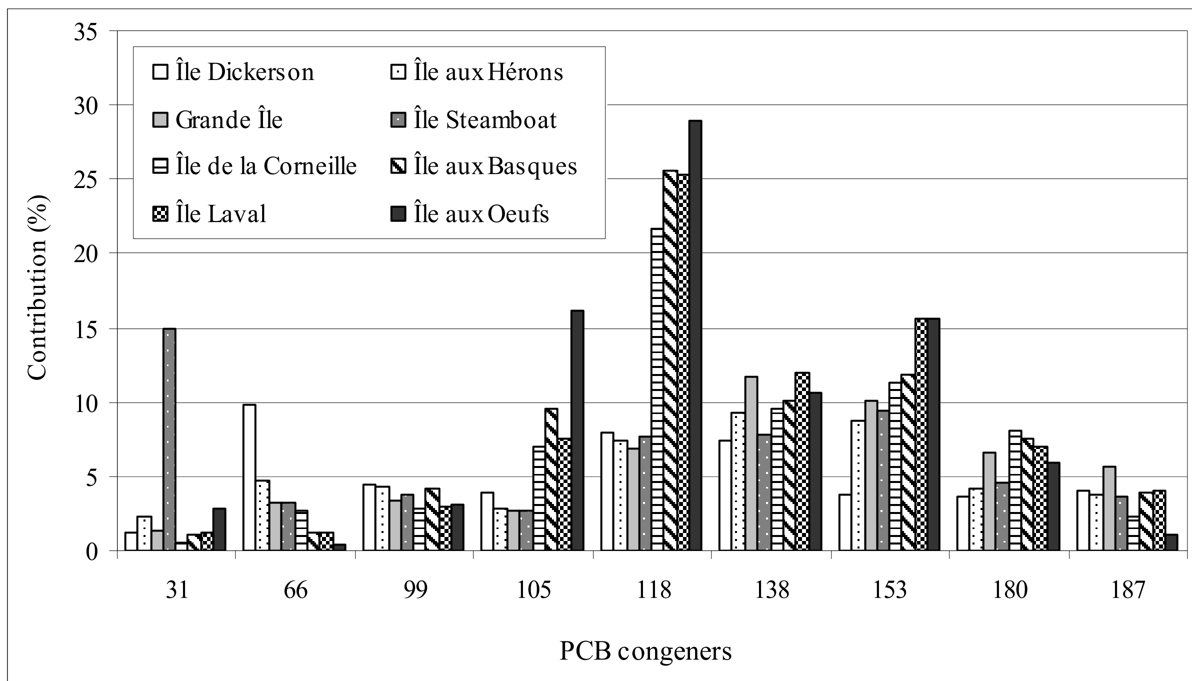


Figure 14. Contribution of the principal PCB congeners to total PCBs in the plasma of young Great Blue Herons

Table 13. Spearman rank correlations between biological variables and contaminants in Great Blue Heron eggs (n = 15)

	Egg weight	Embryo weight	Stage	Retinol	Retinyl palmitate	Ratio of retinol to retinyl palmitate
Mercury	0.48	−0.38	−0.18	−0.29	0.23	−0.33
Hexachlorobenzene (HCB)	−0.40	0.45	−0.15	0.22	0.33	−0.14
Heptachlor epoxide	−0.10	0.11	−0.15	**0.52**	**−0.54**	**0.62**
Oxychlordane	0.09	0.09	−0.38	**0.54**	−0.42	**0.54**
trans-nonachlor	−0.03	0.15	−0.19	**0.58**	**−0.56**	**0.61**
cis-nonachlor	0.01	0.18	−0.24	**0.58**	**−0.52**	**0.56**
p,p'-DDE	−0.18	0.31	−0.50	0.50	−0.09	0.30
Dieldrin	−0.28	0.33	−0.28	**0.60**	−0.25	0.40
Mirex	−0.25	−0.01	0.36	0.36	−0.50	**0.56**
PCB 118	−0.38	0.44	−0.11	**0.63**	−0.32	0.46
PCB 138	−0.39	0.51	−0.19	**0.57**	−0.24	0.35
PCB 153	−0.48	0.45	−0.19	**0.51**	−0.20	0.29
PCB 180	−0.41	0.41	−0.17	**0.59**	−0.28	0.40
Total PCBs	−0.36	0.47	−0.15	**0.61**	−0.29	0.43
Toxaphene	−0.40	0.26	−0.16	−0.06	0.43	−0.32

Bold numbers are significant at $P < 0.05$.

Table 14. Spearman rank correlations between biological variables and contaminants in Great Blue Heron eggs (n = 6)

	Egg weight	Embryo weight	Stage	Retinol	Retinyl palmitate	Ratio of retinol to retinyl palmitate
Non-*ortho*-PCBs	0.37	0.26	−0.44	**0.83**	−0.31	0.49
Dioxins and furans	−0.43	0.20	−0.32	0.26	−0.09	0.20
PBDEs	0.09	0.37	−0.35	0.66	−0.14	0.31
Toxic equivalent	0.37	0.26	−0.44	**0.83**	−0.31	0.49

Numbers in bold are significant at $P < 0.05$.

3.4.2 Correlations among blood variables

A number of biological variables measured in plasma showed significant, usually negative, correlations with organochlorine pesticides and PCBs (Table 15). Plasma retinol had a high negative correlation with total PCBs and several organochlorine pesticides, and 3,4-dehydroretinol showed several positive correlations with some organochlorines and total PCBs. Figure 15 illustrates the correlation between plasma retinol and total PCBs (df 21; $F = 24.2$; $P < 0.0001$). Stepwise multiple regression was done to determine which variables accounted for most of the variability in the levels of retinol in plasma. The best result was obtained with log-transformed total PCBs and log-transformed *p,p*'-DDD (adjusted $r^2 = 0.6876$, $P = 0.0001$).

3.5 MULTIVARIATE ANALYSIS

The results of the analyses of correlations and multiple regressions were used to select the chemical and biological variables that accounted for the differences among the colonies. The variables chosen were glucose, total proteins, retinol, $\delta^{13}C$, $\delta^{15}N$, and logarithms of *p,p*'-DDE, of 1,2,4,5-TCB, of *cis*-nonachlor, and of total PCBs. Figure 16 shows the distribution of Great Blue Heron groups according to the first two axes of the principal components, which account for 80.6% of the variability. A group analysis was done with the same variables, and the groups obtained were plotted on the principal component graph. Graph A shows the variables that account for the distribution of birds and colonies (independent variables). Along axis 1, groups are differentiated by their level of contamination and retinol level, with the group farthest to the right containing the birds that were the most contaminated and had the lowest levels of retinol. Along axis 2, the groups are primarily differentiated by their stable isotope levels, with those at the top having the highest levels of $\delta^{13}C$ and $\delta^{15}N$.

Table 15. Spearman rank correlations between biological variables and contaminants in the blood of young Great Blue Herons (n = 24)

	Mercury (Hg) in blood	**1,2,3,4, chloro-benzene (1234-TCB)**	**1,2,4,5, chloro-benzene (1245-CB)**	**Hexa-chloro-benzene (HCB)**	***p,p'*-DDE**	***p,p'*-DDD**	***trans*-nona-chlor**	***cis*-nona-chlor**	**PCB 118**	**Total PCBs**
Weight	0.03	−0.37	**−0.44**	−0.33	**−0.69***	**−0.64***	−0.56*	−0.57*	−0.58*	**−0.49**
Proteins	−0.32	**−0.71***	**−0.66***	0.36	−0.53*	**−0.43**	−0.32	**−0.64***	−0.35	−0.57*
Glucose	0.18	**−0.44**	−0.53*	−0.08	**−0.70***	**−0.66***	**−0.49**	−0.59*	**−0.70***	**−0.69***
AST	−0.12	0.30	0.40	−0.12	0.16	0.29	0.29	**0.47**	0.39	**0.46**
ALP	−0.11	−0.36	−0.33	−0.15	−0.54*	−0.53*	−0.19	−0.23	−0.18	−0.22
CK	0.15	−0.39	−0.32	0.14	−0.35	−0.28	−0.09	−0.25	−0.18	−0.18
LDH	−0.11	0.09	0.16	0.33	0.16	0.15	**0.41**	0.31	**−0.50**	**−0.50**
Albumin	0.11	0.30	0.24	0.06	0.07	0.24	0.13	0.11	−0.06	0.06
Globulin	−0.32	**−0.78***	**−0.72***	0.32	−0.54*	**−0.49**	−0.37	**−0.66***	−0.37	−0.60*
Albumin/ Globulin	0.25	**0.76***	**0.72***	−0.25	**0.64***	0.59*	**0.43**	**0.68***	**0.44**	**0.63***
Calcium	−0.25	**−0.50**	**−0.45**	0.13	−0.37	**−0.40**	−0.30	**−0.49**	−0.28	**−0.50**
Uric acid	−0.12	**−0.80***	**−0.77***	0.07	**−0.63***	**−0.71***	**−0.41**	**−0.72***	−0.53*	**−0.73***
Dehydro-retinol	−0.25	0.61*	0.59*	−0.00	**0.43**	0.54*	0.25	0.39	**0.43**	**0.62***
Retinol	0.02	**−0.68***	**−0.72***	0.05	**−0.66***	**−0.73***	−0.39	−0.60*	**−0.66***	**−0.77***
Free T4	0.02	**−0.48**	−0.52*	−0.05	−0.34	**−0.62***	−0.28	**−0.41**	**−0.42**	**−0.44**
Total T4	0.08	−0.55*	−0.53*	0.03	−0.24	−0.41*	−0.08	−0.31	−0.31	−0.55*
$\delta^{13}C$	**−0.63***	0.04	0.14	0.38	−0.11	0.12	0.26	0.25	0.54*	**0.45**
$\delta^{15}N$	−0.60*	0.20	0.29	0.37	0.07	0.35	**0.41**	0.38	**0.64***	0.57*
Carbon/ nitrogen	0.21	**0.44**	**0.48**	−0.38	0.24	0.51*	−0.06	0.20	−0.06	0.09

Numbers in bold are significant at $P < 0.05$.
* $P < 0.01$.
* and in bold $P < 0.001$.

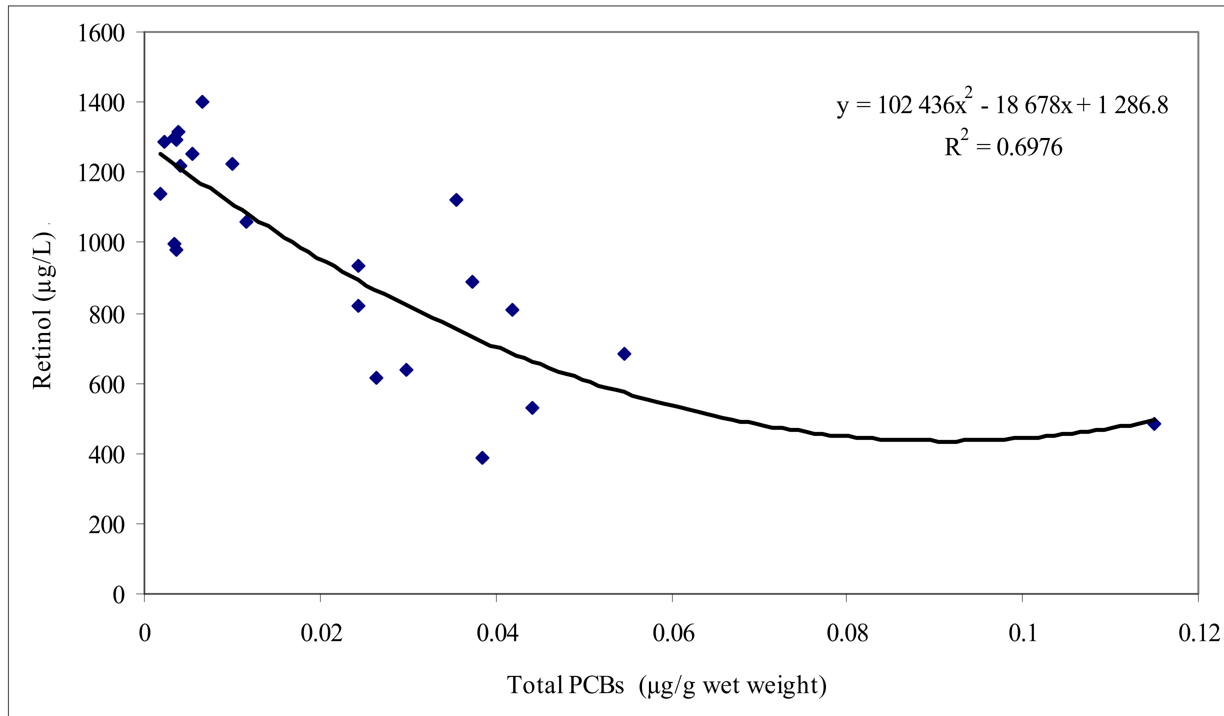


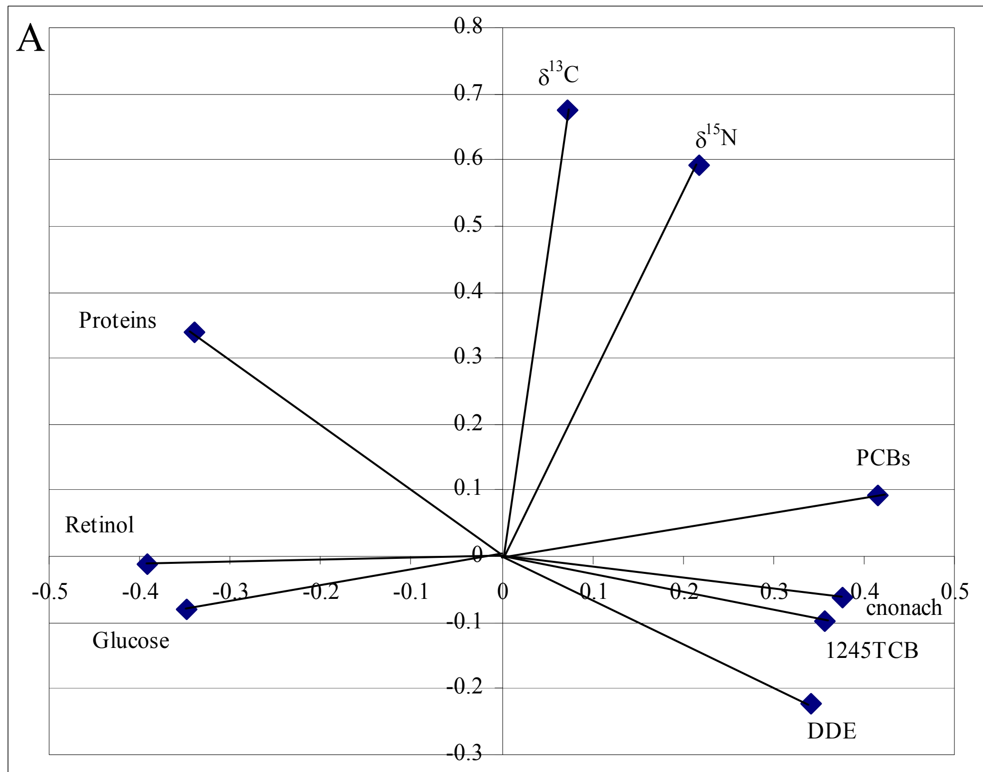
Figure 15. Relation between retinol and total PCBs in the plasma of young Great Blue Herons

3.6 COMPARISONS WITH PREVIOUS DATA

The levels of mercury in eggs, blood, and feathers of young herons did not differ significantly between the surveys (Table 16; $P > 0.05$).

The level of total PCBs in Great Blue Heron eggs tested in 2001–2002 was significantly lower than the levels recorded from the two previous periods (Table 17; $P < 0.05$). Average concentrations of *p,p*'-DDE, of *cis*-nonachlor, and of oxychlordane for the 1991–1993 period were significantly higher than in subsequent surveys (Table 17; $P < 0.05$). There was a significant difference for dieldrin between 1991–1993 and 1996–1997 only (Table 17; $p < 0.05$). Average concentrations of *trans*-nonachlor did not differ among surveys (Table 17; $P > 0.05$).

The analyses showed no significant difference in average concentrations of total PCBs, of *cis*-nonachlor, or of *trans*-nonachlor in the blood of young herons during the various rounds of testing (Table 18; $P > 0.05$). The average concentration of *p,p*'-DDE was significantly higher in 1991–1993 than in subsequent surveys (Table 18; $P > 0.05$). The level of dieldrin was significantly lower in 2001–2002 than in previous periods (Table 18; $P > 0.05$).



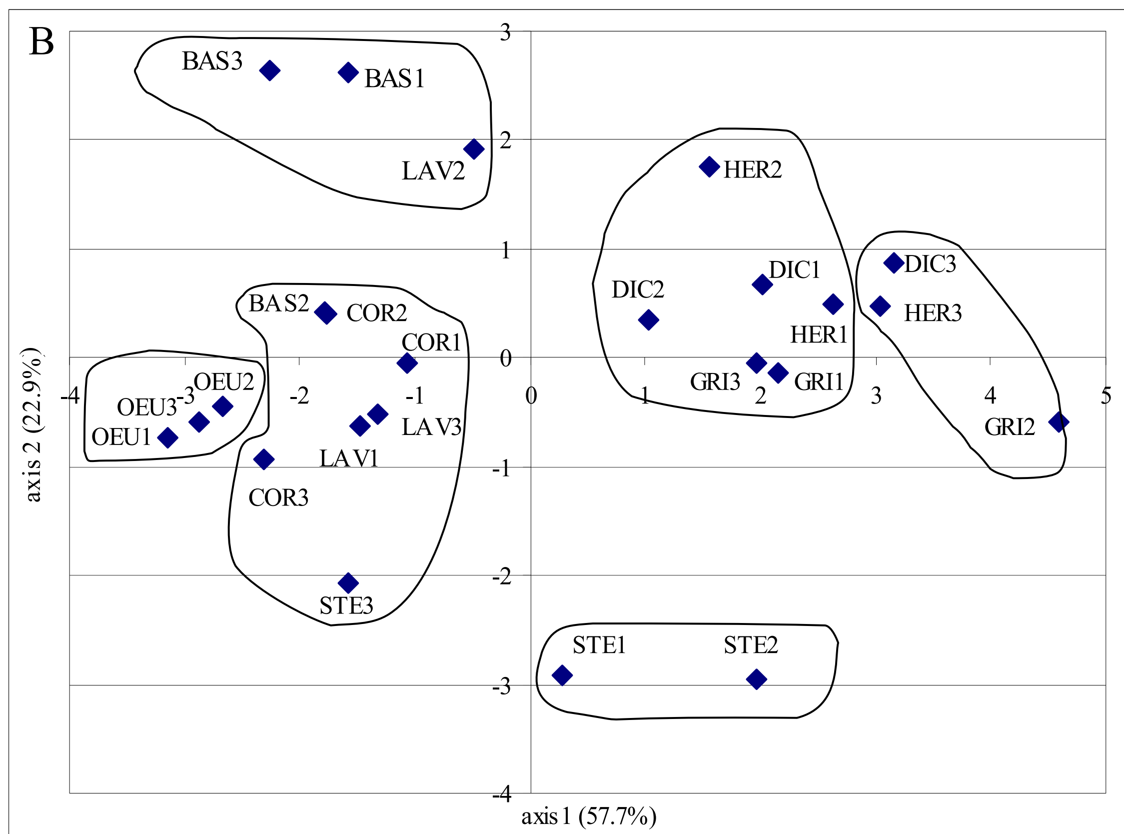


Figure 16. Dispersion of birds along the first two axes of a principal component analysis (B) using the variables in graph A

Table 16. Average concentrations of mercury in Great Blue Heron eggs and in the blood and feathers of Great Blue Herons in 1991–1993, 1996–1997, and 2001–2002

Colony	Eggs (mg/kg wet weight)			Blood (mg/kg wet weight)			Feathers (mg/kg dry weight)		
	1991–1993	**1996–1997**	**2001–2002**	**1991–1993**	**1996–1997**	**2001–2002**	**1991–1993**	**1996–1997**	**2001–2002**
Île Dickerson	0.37	0.23	0.33	0.53	0.35	0.41	6.47	6.27	5.22
Île aux Hérons	0.29	0.15	0.19	1.76	0.53	0.42	6.80	7.36	6.09
Grande Île	0.30	0.16	0.21	0.77	0.35	0.39	5.92	5.59	4.53
Petit Lac Jacques-Cartier[T]	0.43	0.25	na	0.74	na	na	6.93	na	na
Île Steamboat[R]	na	na	0.42	na	0.70	0.73	na	10.08	9.04
Île de la Corneille	0.23	0.23	0.25	0.43	0.41	0.70	5.82	7.21	6.51
Île aux Basques	na	na	0.22	na	na	0.32	na	na	2.71
Île du Bic	na	0.14	na	na	0.26	na	na	5.05	na
Île Saint-Barnabé	0.15	na	na	0.52	na	na	3.14	na	na
Île Beauséjour	0.18	na	na	0.13	na	na	1.27	na	na
Île Matane[R]	na	0.22	na	na	0.16	na	na	4.46	na
Île Laval	na	na	na	na	na	0.31	na	na	4.99
Île aux Oeufs[R]	na	na	0.21	na	na	0.44	na	na	5.24
Île Petit Caouis[R]	0.32	na	na	0.40	na	na	5.36	na	na
Île Manowin[R]	na	0.13	na	na	0.17	na	na	3.12	na
Comparison	–	–	–	–	–	–	–	–	–

[R] = reference colony.
na = not analyzed.
Columns (time periods) with the same letter or no letter for a single tissue are not significantly different ($P > 0.05$).

Table 17. Average concentrations of total PCBs and the principal organochlorine pesticides in Great Blue Heron eggs (mg/kg wet weight) in 1991–1993, 1996–1997, and 2001–2002

Colony	Total PCBs			*p,p'*-DDE			Dieldrin		
	1991–1993	1996–1997	2001–2002	1991–1993	1996–1997	2001–2002	1991–1993	1996–1997	2001–2002
Île Dickerson	3.48	6.14	0.88	1.91	1.36	0.47	0.04	0.04	0.03
Île aux Hérons	3.45	4.87	3.47	3.22	2.03	1.72	0.14	0.06	0.1
Grande Île	2.30	4.20	2.26	9.88	1.51	0.96	0.30	0.03	0.08
Petit Lac Jacques-Cartier/ Île Steamboat[R]	1.60	3.67	1.20	1.61	0.63	0.51	0.04	0.03	0.07
Île de la Corneille	3.53	1.76	1.15	1.45	0.50	0.53	0.04	0.01	0.01
Île du Bic/Île Basques	18.07	3.03	0.99	8.43	1.76	0.37	0.30	0.06	0.01
Île Manowin/Île aux Oeufs[R]	5.40	0.96	0.87	2.34	0.42	0.48	0.13	0.01	0.02
Comparison	a	a	b	a	b	b	a	b	ab

Colony	*trans*-nonachlore			*cis*-nonachlore			Oxychlordane		
	1991–1993	1996–1997	2001–2002	1991–1993	1996–1997	2001–2002	1991–1993	1996–1997	2001–2002
Île Dickerson	0.08	0.04	0.04	0.02	0.02	0.01	0.02	0.02	0.03
Île aux Hérons	0.32	0.10	0.27	0.11	0.04	0.09	0.08	0.03	0.08
Grande Île	0.23	0.10	0.11	0.05	0.03	0.03	0.15	0.02	0.03
Petit Lac Jacques-Cartier/ Île Steamboat[R]	0.13	0.03	0.1	0.05	0.01	0.03	0.05	0.02	0.03
Île de la Corneille	0.01	0.04	0.02	0.03	0.01	0.01	0.04	0.01	0.01
Île du Bic/Île Basques	0.56	0.13	0.02	0.15	0.03	0.01	0.21	0.05	0.01
Île Manowin/Île aux Oeufs[R]	0.17	0.03	0.01	0.05	0.01	0.01	0.07	0.01	0.01
Comparison	–	–	–	a	b	b	a	b	b

[R] = reference colony.
na = not analyzed.
Columns (time periods) with the same letter or no letter for a single contaminant are not significantly different ($P > 0.05$).

Table 18. Average concentrations of total PCBs and organochlorine pesticides in the plasma of young herons (µg/kg wet weight) in 1991–1993, 1996–1997, and 2001–2002 (1991–1993 and 1996–1997 data from blood were converted into equivalent values for plasma using the hematocrit values)

Colony	Total PCBs			*p,p'*DDE			Dieldrin		
	1991–1993	1996–1997	2001–2002	1991–1993	1996–1997	2001–2002	1991–1993	1996–1997	2001–2002
Île Dickerson	100.6	43.4	36.4	8.1	4.9	4.8	1.77	1.63	0.005*
Île aux Hérons	109.5	25.6	44.6	16.0	7.5	5.5	3.08	1.07	0.005*
Grande Île	39.2	12.9	56.4	11.2	2.9	9.4	2.28	0.15	0.005*
Île Steamboat[R]	na	5.8	13.9	na	1.4	5.4	na	1.18	0.005*
Petit Lac Jacques-Cartier[R]	2.8	na	na	2.6	na	na	0.005*	na	na
Île de la Corneille	33.1	14.0	6.9	14.9	3.0	2.0	0.45	0.34	0.005*
Île aux Basques	na	na	4.3	na	na	1.4	na	na	0.005*
Île du Bic	na	3.8	na	na	0.7	na	na	0.26	na
Île Saint-Barnabé	27.9	na	na	12.9	na	na	2.79	na	na
Île Matane[R]	na	1.2	na	na	1.0	na	na	0.11	na
Île Laval	na	na	14.2	na	na	3.1	na	na	0.005*
Île aux Oeufs[R]	na	na	2.5	na	na	1.2	na	na	0.005*
Île Petit Caouis[R]	2.3	na	na	6.0	na	na	0.30	na	na
Île Manowin[R]	na	4.9	na	na	0.8	na	na	1.06	na
Comparison	–	–	–	a	b	bc	a	a	b

[R] = reference colony.
na = not analyzed.
* Value that corresponds to half of the detection threshold (0.01 µg/kg wet weight).
Columns (time periods) with the same letter or no letter for a single contaminant are not significantly different ($P > 0.05$).

Table 18 (cont'd)

Colony	*trans*-nonachlor			*cis*-nonachlor		
	1991–1993	**1996–1997**	**2001–2002**	**1991–1993**	**1996–1997**	**2001–2002**
Île Dickerson	1.29	0.94	0.51	0.48	0.37	0.21
Île aux Hérons	3.54	1.28	0.82	1.08	0.47	0.44
Grande Île	3.16	0.63	0.93	0.88	0.20	0.45
Île Steamboat[R]	na	0.20	0.96	na	0.005*	0.41
Petit Lac Jacques-Cartier[R]	0.17	na	na	0.005*	na	na
Île de la Corneille	2.76	0.43	0.30	0.60	0.27	0.11
Île aux Basques	na	na	0.47	na	na	0.12
Île du Bic	na	0.24	na	na	0.25	na
Île St-Barnabé	2.06	na	na	0.59	na	na
Île Matane[R]	na	0.09	na	na	0.07	na
Île Laval	na	na	0.39	na	na	0.10
Île aux Oeufs[R]	na	na	0.26	na	na	0.08
Île Petit Caouis[R]	0.15	na	na	0.005*	na	na
Île Manowin[R]	na	0.47	na	na	0.28	na
Comparison	–	–	–	–	–	–

[R] = reference colony.
na = not analyzed.
* Value that corresponds to half of the detection threshold (0.01 µg/kg wet weight).
Columns (time periods) with the same letter or no letter for a single contaminant are not significantly different ($P > 0.05$).

4. DISCUSSION

4.1 CONTAMINATION

No difference in the level of mercury in eggs and blood was noted either among the colonies or between the regions; the level of mercury in feathers in the Île Steamboat colony, where levels were high, differed significantly from the Île aux Basques colony, where they were low. There was no difference over time, either, unlike the findings from the previous survey (Champoux et al. 2004). The reversal seems to be due to the fact that a slight increase was noted in 2001–2002, particularly in the colonies in the estuary. The homogeneity in the levels of mercury appears to indicate that the mercury is coming primarily from atmospheric sources and that the mercury from these sources is not declining. These concentrations also appear to be higher than the published data on birds (Elliott et al. 1989; Custer et al. 1997; Wolfe and Norman 1998; Thomas and Anthony 1999; Sepuvelda et al. 1999; Goutner et al. 2001; Golden et al. 2003). Mercury concentrations in the eggs and feathers of young Great Blue Herons in the St. Lawrence were in the low risk of toxic effect category; concentrations in the blood were in the moderate (0.1 to 0.3 mg/kg wet weight), elevated (0.3 to 0.4 mg/kg wet weight) or very elevated (> 0.4 mg/kg wet weight) risk of toxic effect categories, as described by Meyer et al. (1998) for the Common Loon (*Gavia immer*). According to this ranking, 4 of 24 values (17%) were in the moderate category, 6 of 24 (25%) were in the elevated category, and 14 of 24 (58%) were in the very elevated risk of toxic effect category. Levels of mercury in the blood of juvenile birds is a good indicator of the local concentration of available mercury, as the young are fed only from the resources in the region in which they were born. Almost all of the mercury in the blood is in the form of methylmercury bound to red blood cells. The half-life of methylmercury (MeHg) in bird blood is two to three months (Scheuhammer 1988), so it is one of the best matrices for determining exposure at the nesting site. Levels of mercury in the young are influenced by their age and the stage of feather growth. Since feathers are the main elimination route for mercury, the level of mercury in the blood is low during the period when the feathers are growing (Fournier et al. 2002). However, similar or higher levels of mercury have been found in other piscivorous species. Young loons tested in Quebec (Champoux and Masse 2006; Champoux et al. 2006b) had higher levels of mercury for their age than the levels in North America (Evers et al. 1998). Average mercury concentrations in the blood and feathers of Osprey (*Pandion haliaetus*)

nestlings from hydroelectric reservoirs were 1.94 and 37.35 µg/g, respectively (DesGranges et al. 1998). A significant correlation between mercury in the blood and mercury in the brain of Great Blue Herons has been demonstrated by Wolfe and Norman (1998), and Scheuhammer et al. (2008) showed that mercury levels in piscivorous birds reflecting realistic levels of environmental exposure can affect the transmission of neurochemical signals in the brain.

According to a report on the virtual elimination of priority substances (Saint-Laurent Vision 2000 (1999)), mercury discharged into the river by the industrial plants that were being monitored dropped by 88% between 1993 and 1998; however, 17 establishments were still releasing Hg (total of 44 g/d), with 11 of these 17 exceeding acceptable levels. During the same period, the discharge of PCBs was reduced by 100%, with no PCBs being reported by any of the 38 pulp and paper mills on the priority list. Dioxins and furans were reduced by 89%, with 11 establishments still releasing a total of 722 µg/d and 6 of the 11 exceeding discharge objectives. Lastly, hexachlorobenzene (HCB) was not detected in any industrial effluent. The other substances were not monitored, as they are no longer manufactured, used, or discharged by the industrial establishments.

The Cornwall-Massena area of Lac Saint-Francois has long been known for the substantial contamination of its sediments by mercury and PCBs (Pelletier and Lepage 2004), with the mercury coming from plants on the Cornwall side of the river and the PCBs coming from the GM, Alcoa, and Reynolds Metals/Alcoa sites on the Massena side. This sector, recognized by the International Joint Commission as an Area of Concern, was the focus of plans to restore sediments that were contaminated. Projects to dredge and remediate about 16 000 m^3 of contaminated sediment were undertaken at the GM and Alcoa plants in 1995; in 2001, Reynolds Metals/Alcoa dredged 65 750 m^3 of contaminated sediments containing over 10 000 kg of PCBs. No major drop in concentrations of PCBs in sediments has been observed since the 1995 dredging (Pelletier and Lepage 2004), and results from monitoring following the 2001 dredging are not yet available.

Total concentrations of PCBs in eggs did not vary among colonies or between regions, but concentrations did show a decline from previous surveys (Champoux et al. 2004). A decline was noted at the colony on Île Dickerson, in particular; this colony is located just downstream from the sites that were dredged. The decline does not appear to be linked to the dredging done in 2001, however, as the dredging was just beginning when the heron eggs were collected. The PCB

pattern varied among colonies, with congener 153 being higher in the estuary than in freshwater and congeners 194 and 206 showing the opposite pattern. Concentrations of total PCBs in Herring Gull (*Larus argentatus*) eggs collected at Strachan Island, located in the St. Lawrence River at the outlet of Lake Ontario, are about 10 times higher, but, aside from congener 206, which is higher in Île Dickerson, Île aux Hérons, and Grande Île, the patterns were similar for Great Blue Herons (Jermyn-Gee et al. 2005). de Solla et al. (2001) and Ashpole et al. (2004) found very high levels of total PCBs and non-*ortho*-PCBs (planar PCBs) in snapping turtle (*Chelydra serpentina*) eggs in the Cornwall–Massena area.

Non-*ortho*-PCBs were measured in Great Blue Heron eggs for the first time during this study. Although present in low concentrations compared with other PCB congeners, their high toxicity—comparable to dioxins and furans—explains why they account for 67% on average of the toxic equivalent. The predominance of non-*ortho*-PCBs in the toxic equivalent has also been noted in other publications (Kannan et al. 2001; Braune et al. 2007), as has the dominant position of congener 126 among the non-*ortho*-PCBs. In samples from various species of fish from the St. Lawrence River, planar PCBs accounted for over 43% of the toxic equivalent of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in all species; this came primarily from 126 (Brochu et al. 1995). Concentrations of PCB congeners 77 and 126 varied from 17 to 1920 ng/kg wet weight and 13 to 2050 ng/kg wet weight, respectively.

Compared with the 1989–1994 period, concentrations of dioxins and furans in Great Blue Heron eggs showed little change (Rodrigue et al. 2005). Concentrations rose in some colonies but were identical in others. In 1989–1994, the highest concentration was measured in the reference colony at Petit Lac Jacques-Cartier, located in the Réserve faunique des Laurentides. The Île Steamboat reference colony, located in Lac Wayagamac, is in a neighbouring area and shows concentrations that are five times lower than those from Petit Lac Jacques-Cartier, so we can assume that levels have declined in this region. Concentrations of dioxins and furans in Great Blue Heron eggs from the St. Lawrence were similar to concentrations in British Columbia herons following changes made in the pulp and paper industry there (Elliott et al. 2001). The pattern of dioxins and furans in British Columbia was dominated by pentachlorodibenzodioxins and hexachlorodibenzodioxins, with a large amount of the toxic equivalent in urban zones coming from PCBs (Elliott et al. 2001). In herons from the St. Lawrence, pentachlorodibenzodioxins and

hexachlorodibenzodioxins also dominated in most colonies, although Île de la Corneille showed a different pattern with furans also being high.

The main sources of dioxins are pentachlorophenols (used to preserve wood), municipal incinerators, and pulp and paper mills that bleach with chlorine (Government of Canada 1990). PCBs are the main source of furans.

PBDEs were also analyzed in Great Blue Heron eggs for the first time during this study. The effects of PBDEs are not yet well known, but studies have shown impacts on neurological development, thyroid hormone homeostasis, vitamin A status, the endocrine system, and reproduction (Murvoll et al. 2006; McKinney et al. 2006a). Due to their low cost and effectiveness in reducing fire risks, brominated fire retardants are the largest group of fire retardants on the market (Birnbaum and Staskal 2004). The vast majority of PBDEs belong to three commercial PBDE mixtures, decabromodiphenyl ether (DBDE), octabromodiphenyl ether (OBDE), and pentabromodiphenyl ether (pentaBDE). While DBDEs are the most widely used worldwide, including in North America, pentaBDE is used almost exclusively in North America, largely because there has been a voluntary ban on it in Europe since 2003 (Birnbaum and Staskal 2004). The congeners BDE-47 and BDE-99 make up about 75% of the total mass of pentaBDE. According to a *Canadian Environmental Protection Act, 1999* report, the pentaBDE mixture has not been used in Canada since 2003 (CEPA 2004). Moreover, the only American manufacturer of commercial pentaBDE and octaBDE mixtures halted production in December 2004, and all Canadian companies that had been using pentaBDE and octaBDE reported complete phase-out since 2005 (Canada Gazette 2008). In June 2008, PBDEs were added to the List of Toxic Substances under Schedule 1 of the *Canadian Environmental Protection Act, 1999* (Canada Gazette 2008).

There are still relatively little data published on the presence of PBDEs in the environment in Canada or Quebec. In surface sediments in the St. Lawrence, concentrations of PBDEs rose in the mid-1980s, and concentrations in Lac Saint-Pierre were the highest among the St. Lawrence fluvial lakes, similar to Lake Michigan and second after Lake Ontario (Pelletier et al. 2006). In the trophic chain of the St. Lawrence River estuary, concentrations of total PBDEs (sum of 10 congeners) range from 13 ng/g wet weight in herring (*Clupea harengus harengus*) liver to 709 ng/g wet weight in harbour seal (*Phoca vitulina*) blubber (Law et al. 2003). In the liver of

belugas (*Delphinapterus leucas*) from the St. Lawrence River, the average concentration of PBDEs was 71 ng/g wet weight (McKinney et al. 2006b). The residue of total PBDEs (sum of 10 congeners) in blubber samples from belugas in the St. Lawrence River estuary collected from 1997 to 1999 was 377 µg/kg wet weight in adult males and 496 µg/kg wet weight in adult females. These values were 8 and 19 times higher, respectively, than the concentrations recorded in belugas tested between 1988 and 1990 (Lebeuf et al. 2004).

Reported concentrations of PBDEs in birds are as follows. In Northern Fulmar (*Fulmarus glacialis*) eggs collected from the Faroe Islands in 2000 and 2001, the concentration was 2.1 ng/g wet weight (sum of 6 congeners) (Fangström et al. 2005). In Eurasian Little Owl (*Athena noctua*) eggs collected in Belgium from 1998 to 2000, the concentration was 12 ng/g wet weight (sum of 7 congeners) (Jaspers et al. 2005). In Ivory Gull (*Pagophila eburnea*) eggs collected in the Canadian Arctic in 2004, the concentration was 4.6 ng/g wet weight (sum of 14 congeners) (Braune et al. 2007). In Great Blue Heron, Double-crested Cormorant (*Phalacrocorax auritus*), Osprey (*Pandion haliaetus*), and Leach's Storm-Petrel (*Oceanodroma leucorhoa*) eggs collected in British Columbia in 2000 and 2002, concentrations were 455 ng/g wet weight for Great Blue Heron, 62.5 ng/g wet weight for Double-crested Cormorant, 185 ng/g wet weight for Osprey, and 3.8 ng/g wet weight for Leach's Storm-Petrel (sum of 18 congeners except for the petrel, which was 6 congeners) (Elliott et al. 2005). The sum of eight PBDE congeners in the liver of Grey Herons (*Ardea cinerea*) found dead or dying in Belgium in 2003 and 2004 was 38.4 ng/g wet weight (Jaspers et al. 2006). The sum of six PBDE congeners in Black-legged Kittiwake (*Rissa tridactyla*) and European Shag (*Phalacrocorax aristotelis*) yolk sacs from Norway in 2002 was 103 and 17.2 ng/g wet weight, respectively (Murvoll et al. 2006). The concentrations of PBDEs in Great Blue Heron eggs from the St. Lawrence (average = 0.49 mg/kg or 490 ng/g) are similar to those of *p,p'*-DDE (average = 0.72 mg/kg), the organochlorine pesticide that is most abundant in Great Blue Heron eggs, and are of the same order of magnitude as those of total PCBs (average 1.55 mg/kg). What's more, the concentrations of PBDEs in Great Blue Heron eggs are comparable to those measured in Herring Gull eggs from the Great Lakes (Norstrom et al. 2002) and in Great Blue Heron eggs in British Columbia (Elliott et al. 2005). The most abundant congeners, penta-BDE, BDE-47, BDE-99, and BDE-100 (used in the manufacture of polyurethane foam in North America), are also the same.

Several of the contaminants analyzed in this study appear on the Toxic Substances Management Policy (TSMP) Track 1 list of substances (substances targeted for virtual elimination from the environment) (Environment Canada 2003), as well as on the list of 12 Persistent Organic Pollutants to be eliminated internationally (Stockholm Convention, United Nations Environment Programme) (Government of Canada 2006). They are chlordane, dieldrin, DDT, heptachlor, mirex, hexachlorobenzene (HCB), toxaphene, PCBs, and dioxins and furans (Environment Canada 2003; Government of Canada 2006). Two other pesticides—aldrin and endrin—have not been measured for many years now.

Toxaphene is a persistent pesticide that was used heavily worldwide until it was banned in 1982 in Canada and the United States and in the early 1990s in Mexico and elsewhere (Muir et al. 2002; Environment Canada 1997). Studies have shown that it induces estrogenic effects in human cells; it is not known if it also acts as an endocrine disruptor in birds (Wainwright et al. 2001). Toxaphene is not considered particularly toxic to birds as it is metabolized and excreted by the majority of species (Eisler and Jacknow 1985 *in* Wainwright et al. 2001). Toxaphene is rarely detected in bird eggs and tissue (Wiemeyer 1996), mainly because of analytical uncertainties associated with biotransformation (Muir et al. 2002). In the St. Lawrence, concentrations of total toxaphene in Great Blue Heron eggs are comparable to concentrations of chlordane. Concentrations of total toxaphene in the St. Lawrence are higher than concentrations measured in the eggs of marine birds in the Canadian Arctic (Braune and Simon 2004) and of Great Egrets (*Ardea alba*) and Black-crowned Night-Herons (*Nycticorax nycticorax*) in the Rio Grande valley in Texas but below the concentrations measured in the eggs of Green Herons (*Butorides virescens*) of the Rio Grande (4.4 mg/kg wet weight) (Wainwright et al. 2001) and Black-footed Albatrosses (*Diomedea nigripes*) in the southern Pacific Ocean (Muir et al. 2002). The colony at Grande Île, where the concentration of total toxaphene is higher than elsewhere, shows a different pattern, made up primarily of B8-810 and B9-743/2006. The other colonies have primarily B8-2229 and B8-1471. Custer et al. (1998) report average toxaphene concentrations of 300 ng/g in Great Blue Heron eggs in Indiana, whereas the average for the St. Lawrence is 54 ng/g. In the Great Lakes, Red-breasted Merganser (*Mergus serrator*) and Common Merganser (*Mergus merganser*) eggs collected from Lake Michigan in 1977 and 1978 contained on average 0.14 mg/kg and 0.27 mg/kg wet weight, respectively (Haseltine et al. 1981 *in* Rice and Evans 1984). In the St. Lawrence, toxaphene has been measured in beluga blubber. The average of the

sum of six congeners in 1999 was 1045 ng/g wet weight in females and 1864 ng/g wet weight in males (Gouteux et al. 2003).

A comparison of the concentrations of organochlorine pesticides in eggs by region highlights the differences that aren't visible among the colonies for heptachlor epoxide, *trans*-nonachlor, and *cis*-nonachlor; it also emphasizes the differences for *p,p'*-DDT, *p,p'*-DDD, *cis*-chlordane, and oxychlordane. There were no differences in *p,p'*-DDE among colonies or between regions, but levels have declined from previous surveys. One of the reasons could be the action plan of the North American Commission for Environmental Cooperation, under which Mexico stopped using DDT in 2000 (CEC 2003). The levels of the two organochlorines with the highest concentrations, *p,p'*-DDE and *trans*-nonachlor, were comparable to those in Great Blue Heron eggs in Washington state and Oregon (Thomas and Anthony 1999) and were lower than the levels associated with effects in birds of prey. Aside from concentrations of mirex, which were about 10 times higher in Herring Gull eggs from Strachan Island than in the Great Blue Heron eggs, concentrations of organochlorine pesticides are fairly similar (Jermyn-Gee et al. 2005). Using the ranking of 15 Herring Gull colonies in the Great Lakes according to their level of contamination (Weseloh et al. 2006), the eggs of Great Blue Herons from the freshwater portion of the St. Lawrence River would rank 12th for DDE, 4th for dieldrin, 16th for hexachlorobenzene (HCB), 6th for heptachlor epoxide, 14th for mirex, 16th for PCBs, and 15th for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

There were also differences in PCB patterns in plasma among the colonies. Congener 31 was highest at Île Steamboat, 105 was the highest at Île aux Oeufs, and 118 was higher in the estuary than in freshwater colonies. A comparison by region reduces the differences in plasma among colonies for *p,p'*-DDE and helps to show the overall pattern for a few others, in particular total PCBs.

Comparisons of concentrations in plasma with previous data must be done with care, since previous data have been converted. Detection percentages for some organochlorines, particularly dieldrin and the chlordanes, fell, and it is hard to say whether this reduction reflected an actual decrease in the amount in the environment or it was due to the change of method. Based on this comparison, there was no decline in PCBs, *cis*-nonachlor, or *trans*-nonachlor, while *p,p'*-DDE was lower in 2001–2002 than in 1991–1992, and dieldrin was undetectable.

4.2 BIOMARKERS

Checking the buoyancy of eggs allowed us to improve the precision with which the developmental stage of the eggs was determined, and we were therefore able to do more retinoid analyses. As was the case in one of our previous studies (Boily et al. 1994), retinyl palmitate in eggs was negatively correlated with some organochlorines but not with PCBs. However, retinol was positively correlated with PCBs and organochlorines, and this correlation seems to be new. This is also the first time that we have observed a difference among colonies in the level of retinol, with levels being higher in freshwater colonies than in the estuary. Zile et al. (1997) found that PCBs lower the retinol to retinyl palmitate ratio. Boily et al. (2003) tested for increases in retinol in eggs from females injected with PCB 77 and found that the two largest oocytes (but not the third oocyte) had higher concentrations than the controls. In incubated eggs that had been exposed to PCB 77, no trend over time for retinol was observed, but retinyl palmitate went up. According to Boily et al. (2003), a decline in the enzyme lecithin:retinol acyltransferase (LRAT) induced by PCBs should lead to lower concentrations of retinyl palmitate and higher concentrations of retinol in the yolk; however, they did not observe this phenomenon. This may be what we are seeing in St. Lawrence Great Blue Herons.

Most of the clinical blood parameters were within the normal limits usually observed in healthy birds (Fowler 1986; Polo et al. 1994); however, some showed differences from our previous study (Champoux et al. 2004). In freshwater, total proteins and lactate dehydrogenase (LDH) were lower than before, whereas alkaline phosphatase was higher. Proteins and glucose act as indicators of nutritional status, which has an impact on immunocompetence (Newman et al. 1997). Creatine kinase (CK), which can increase as a result of the stress associated with being captured, was higher at Île de la Corneille, and aspartate aminotransferase (AST) and uric acid were elevated in some individuals. These elevated values may be indicative of health problems that are not necessarily related to contaminants. Uric acid, the major end product of protein catabolism, is higher following a meal in carnivores. Hoffman et al. (2005) noted changes in AST, LDH, albumin, uric acid, and proteins in young Great Egrets that had received doses of mercury that were 10 times higher than the doses measured in St. Lawrence herons.

The stable nitrogen isotope $\delta^{15}N$ indicates the trophic level, and the stable carbon isotope $\delta^{13}C$ indicates the origin of the carbon (the primary producers in an aquatic environment have lower

$\delta^{13}C$ than terrestrial producers or those based in littoral zones) (Hebert et al. 2000). Young herons in the St. Lawrence were higher in the trophic chain than those in reference colonies, especially Île Steamboat. This may be due to the fact that the trophic chain in the St. Lawrence is probably more complex and longer. Nitrogen isotope $\delta^{15}N$ is generally considered to rise by about 3‰ per trophic level (Cabana and Rasmussen 1994). Anthropogenic contributions may also boost $\delta^{15}N$, and this may explain higher levels in more urbanized areas (Lake et al. 2001). The higher levels of $\delta^{15}N$ in the fluvial portion of the St. Lawrence River may explain in part the higher concentrations of some contaminants. Nitrogen isotope $\delta^{15}N$ also showed positive correlations with *trans*-nonachlor, PCB 118, and total PCBs. The young herons at Île Steamboat also seemed to have a more aquatic diet than the St. Lawrence River herons, which appear to feed more on land-based prey or prey with more terrestrial carbon sources.

McNabb and Fox (2003) assessed thyroid function in Herring Gull pipping embryos, prefledglings, and adults. Hatchlings at sites with the highest PCB contamination had significantly diminished T4 reserves in their thyroid glands. Despite this, hypothyroidism, indicated by a drop in plasma T4, was present in only 42% of hatchlings. In the prefledglings, there was a drop in T4 in the thyroid gland at all sites, but there was a drop in plasma T4 in only 29% of cases. Enlarged thyroid glands, indicative of a response by the hypothalamo-pituitary-thyroid (HPT) axis to the drop in T4, were found in 67%. Gentes et al. (2007) observed enhanced hormone synthesis by the thyroid gland as well as increased deiodination of T4 into T3 in Tree Swallow (*Tachycineta bicolour*) tissue, but no difference in plasma T4. T3, the most physiologically active hormone, was higher at the site contaminated by polycyclic aromatic hydrocarbons (PAHs). Displacement of T4 from its transport protein complex by PCBs should increase the amount of free T4, but it also facilitates the elimination of free T4 from the body (McNabb and Fox 2003). If the loss is not compensated for by the thyroid gland, a drop in free T4 results, and this is what was observed in young herons, principally in the freshwater colonies.

The results of several studies appear to indicate interspecific differences in the impacts of PCB on retinol, since lower concentrations of PCBs sometimes lead to greater differences in retinol than higher concentrations of PCBs (Jenssen et al. 2001; Murvoll et al. 2006). The difficulty in comparing levels of contaminants and retinol at different stages of development has also been

noted. Retinol is primarily found in marine fish; dehydroretinol is a form of retinol that is more present in freshwater fish (Lehninger 1985). However, dehydroretinol has not received much study to date due to analytical difficulties (Käkelä et al. 2002). Among young herons in the St. Lawrence, retinol and dehydroretinol followed an inverse trend, indicating possible compensation by dehydroretinol when retinol declines. Although it is difficult to establish a direct cause and effect, it appears that plasma retinol is a good indicator of the impact of PCBs and other organic contaminants on health, at least in St. Lawrence herons. This correlation was also noted in our two previous studies (Champoux et al. 2000; Champoux et al. 2004). Grasman et al. (1996) and Bishop et al. (1999) observed declines in retinol with increasing concentrations of PCB. Many studies report interference by PCBs and other organic contaminants in the endocrine system's regulatory mechanisms (Brouwer and van den Berg 1986; Gould et al. 1999; Murvoll et al. 1999).

4.3 POPULATION STATUS

Reproductive success is a critical aspect of population dynamics and may be an indicator of exposure to and the impact of contaminants, since developing and growing young are especially sensitive. Unfortunately, we have only partial data on reproductive success: due to a lack of time, only a small number of nests were observed in each colony. The average number of young produced per nest (2.4) and the average productivity rate (59%) were similar to the numbers in the previous survey and are sufficient to maintain the population at its current level. We have no new data since the report by DesGranges and Desrosiers (2006), who stated that the Great Blue Heron population is stable.

Despite the efforts made over the last 20 years to develop sensitive and relevant biomarkers as early indicators of exposure to contaminants and their impacts, it is still difficult to draw connections between the effects observed in individuals and a potential impact or ecologically significant change at the population level (Fairbrother et al. 1998; Forbes et al. 2006). Even though the level of contaminants measured in herons is relatively low compared with other studies and the reproductive success of the herons seems adequate, retinol in the freshwater colonies remains low and we do not know the long-term survival rate of young herons. The new contaminants measured in the eggs and detected at sizeable concentrations highlight the importance of remaining vigilant where emerging contaminants are concerned. Control measures

for and international pressure against the older products, such as PCBs and organochlorine pesticides, and the newer products, such as PBDEs, seem to be bearing fruit, but manufacturers continue to bring new classes of products, such as non-PBDE flame retardants, to market, and testing and monitoring methods must be developed for them (Gauthier et al. 2007).

CONCLUSION

The results of the second round of testing of Great Blue Herons provide a little better grasp of the status, spatial distribution, and changes over time of the contamination of the St. Lawrence. Overall, the results show little change in terms of contamination or the status of the Great Blue Heron population. Analysis of new contaminants in the eggs helped provide more information about the presence, concentration, and distribution of these contaminants in the St. Lawrence ecosystem. PBDE concentrations were of the same order of magnitude as concentrations of *p,p'*-DDE and total PCBs, and toxaphene concentrations were of the same order of magnitude as chlordane concentrations. Although non-*ortho*-PCBs were present in low concentrations, they accounted for 67% on average of the toxic equivalent. Since the previous round of testing, mercury levels remained unchanged in eggs, blood, and feathers. Total PCBs declined in eggs but not in blood, and there was little difference in the levels of organochlorine pesticides. The majority of contaminants measured in eggs showed no significant difference between regions, but there were differences in several of the contaminants measured in plasma. Young herons from freshwater colonies were more contaminated than those from colonies in the estuary or the reference colonies. A number of clinical blood variables, the thyroid hormone free T4, plasma retinol, and plasma 3,4-dehydroretinol, and stable carbon and stable nitrogen isotopes showed significant differences among colonies and between regions. Levels of plasma retinol in young herons from freshwater colonies remained low, and these levels could affect their development and survival. More effort must be devoted to research in order to provide a better understanding of the influence of the many factors that can have an effect on the status of the Great Blue Heron population as well as on the health of ecosystems.

The Great Blue Heron monitoring program is one of the few programs that document the contamination of wildlife, and birds in particular, not just in the St. Lawrence River and estuary but also in eastern Canada. The results presented here underline the importance of maintaining these programs and ensuring that emerging contaminants are included.

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