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ß-carotene and retinoids in eggs of Great Blue Herons (*Ardea herodias*) in relation to St Lawrence River contamination

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The potential use of retinoids and ß-carotene as biomarkers in the eggs of the Great Blue Heron was investigated. In the spring of 1991, 65 eggs were collected from nine heronries (seven along the St Lawrence River and two reference sites). A method was specifically developed for the extraction and analysis of ß-carotene and the retinoids in heron egg yolks by reversed-phase HPLC. When results were expressed either as the molar ratio of retinol : retinyl palmitate or as retinyl palmitate concentration, significant differences were found between colonies; however, retinyl palmitate concentration was deemed the better biomarker because it was not significantly influenced by embryonic stage of development. Retinyl palmitate concentrations in freshwater colonies were negatively related to PCB congeners Nos 105 and 118 as well as their TCDD-EQ values ($p < 0.02$, $r^2 = 0.78$). Egg tetrachloro-mono-*ortho* biphenyl concentrations were also negatively related to retinyl palmitate ($p < 0.005$, $r^2 = 0.90$). With the exception of the two mono-*ortho* co-planar congeners detected in the present study, the contamination levels found in heron eggs were well below those found for other bird species in the Great Lakes area and, so far, no detrimental effects have been reported in Great Blue Heron populations in Quebec. These results suggest that retinyl palmitate may be useful as a sensitive and non-invasive biomarker for monitoring organochlorine contaminant effects in the Great Blue Heron in freshwater sites.

Keywords: biomarkers; retinoids; ß-carotene; heron; PCBs

Introduction

The retinoids comprise a group of compounds which includes vitamin A-active forms such as retinol and retinoic acid. The principal role of carotenoids, such as ß-carotene, is considered to be that of metabolic precursors to the retinoids. As animals do not synthesize these compounds *de novo*, an adequate dietary supply is important. Some retinoids and carotenoids are typically stored in the tissues of animals and may be mobilized and converted to active forms depending upon physiological demand.

The vitamin A-active retinoids are essential to several aspects of avian reproduction and development. Retinoic acid is a morphogen in the developing embryo (Thaller and

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Eichele 1987). Nutritional excess or deficiency is associated with changes in secondary sexual characteristics, testes weight, spermatogenesis, egg laying, egg size, embryo survival, incubation time, hatchability and deformities of bone and cartilage (Moore 1957; Thompson 1970, 1976). Inadequate vitamin A status is also related to immunosuppression and susceptibility to disease and cancers in humans and laboratory mammals, while little is known of these interactions in birds.

During the reproductive cycle in avians, retinoids are normally mobilized from internal body stores of the adult female, transported via the blood to the oviduct and incorporated into the forming egg yolk. Retinoids and carotenoids in the egg yolk represent stores available to the developing embryo. The measurement of biomarkers in bird eggs is appealing because in-field sampling procedures are rapid, inexpensive, technically uncomplicated and entail a minimum of disturbance during the breeding period.

The capacity of adult Great Lakes' Herring Gulls (*Larus argentatus*) to store retinoids is apparently diminished by chemical contamination (Spear *et al.* 1986, 1992) and retinoids (molar ratio of retinol : retinyl palmitate) in gull eggs are strongly correlated with levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (Spear *et al.* 1990). Laboratory studies with Ring Doves (*Streptopelia risoria*) have demonstrated the potential for a co-planar PCB congener, 3,3′,4,4′-tetrachlorobiphenyl to decrease liver retinoid stores in the adult, raise serum retinoid levels and alter yolk retinoid concentrations (Spear *et al.* 1986, 1989). Consequently retinoids have been considered in the context of effects biomarkers for birds (Peakall 1992).

The object of the present study was to investigate retinoids and ß-carotene in the eggs of the Great Blue Heron, a possible sentinel species for the St Lawrence River. The Great Blue is widely distributed in Quebec and the largest colonies are located along the St Lawrence River (Bélanger and Tremblay 1989; DesGranges 1993). Aspects of the breeding and foraging behaviour have been well-studied (DesGranges and Laporte 1983; DesGranges 1993). With respect to its suitability as a sentinel species in ecotoxicology studies, this species lives a relatively long time (more than 15 years), occupies a niche at the end of its food chain and feeds almost exclusively on aquatic animals within a short distance from the nest site (Benoit *et al.* 1993; Gibbs *et al.* 1987; Gibbs 1991). This species was found to be a suitable indicator of habitat quality in that clutch size and fledging success reflected localized decreases in ecosystem productivity as a result of human activities (Powell and Powell 1986). The heron colonies investigated in the present study are thought to be exposed to contaminants transported downstream from the Great Lakes and to contaminants released locally.

Materials and methods

In the late spring of 1991, eggs of the Great Blue Heron were collected in nine heronries, all situated on islands (see Fig. 1). Seven of those sites are located along the St Lawrence River while two others (numbers 5 and 6 in Fig. 1) served as reference sites. Because we needed two eggs per nest (one for chemical analysis and one for retinoids) only nests approaching the maximum clutch size of five eggs were selected. In several of the larger colonies, additional nests were sampled giving a total of 65 eggs for retinoid analysis. In the field, each egg was identified, measured and weighed. Eggs for chemical analysis

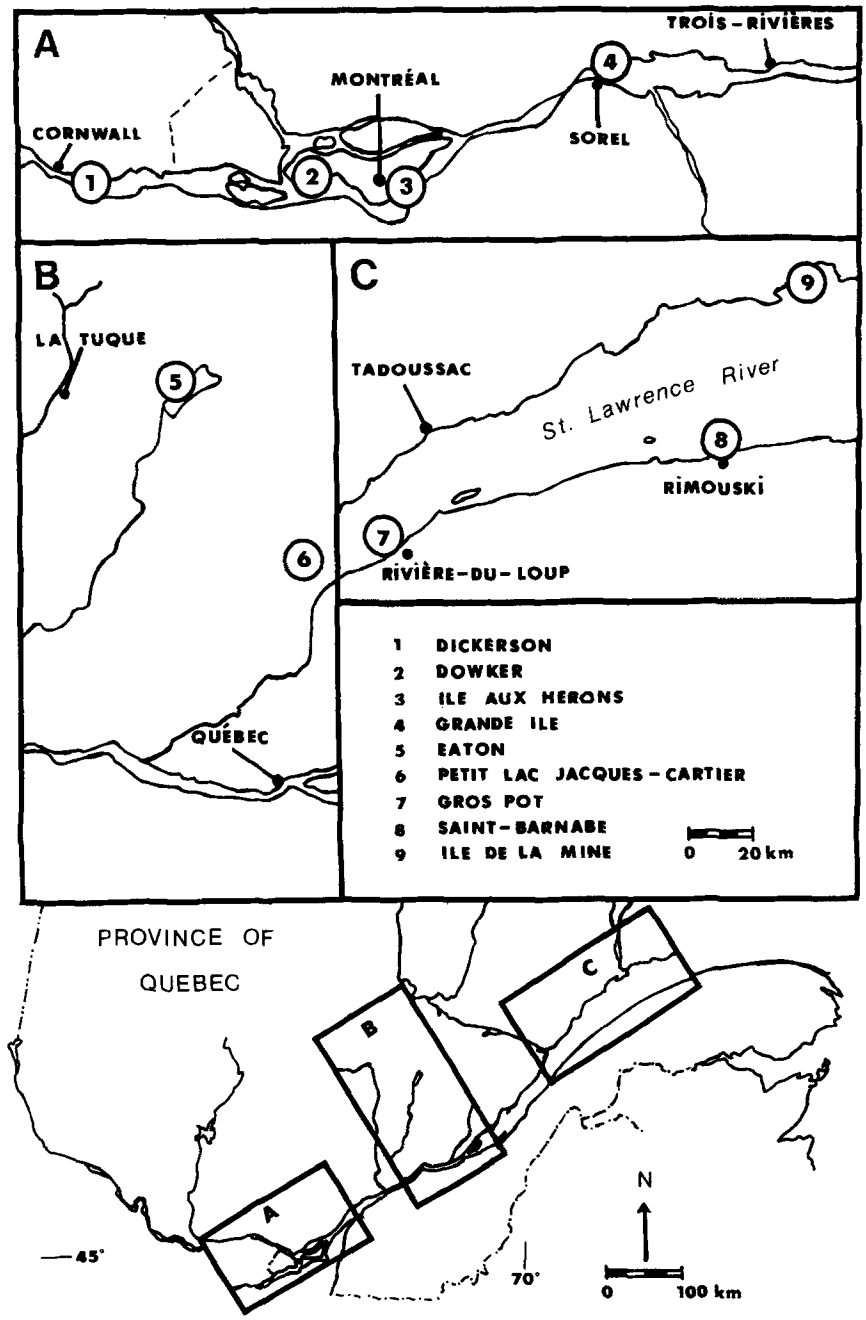


Fig. 1. Location of Great Blue Heron colonies in Quebec, eastern Canada, studied in 1991.

were kept in a cooler while those for retinoid analysis were sealed in a plastic bag, frozen on dry ice and stored at −80°C.

To prevent isomerization of the retinoids, dissection of the partially thawed eggs was conducted under yellow incandescent lights. A portion of the yolk was refrozen in 1.5ml microtest-tubes for retinoid analysis. Retinoids were determined by reversed-phase high-performance liquid chromatography (HPLC) according to a method modified from Spear *et al.* (1989). A small portion of frozen yolk was dehydrated by grinding with ten times its weight of anhydrous Na_2SO_4. A 0.4 g aliquot of the resulting powder was extracted twice by mixing (15 min) with 3 ml acetonitrile and centrifuging (5 min, 2500 *g*) to remove particles. The solvent from the two extractions was combined in a 50 ml test-tube and further extracted by vortexing with 4 ml *n*-hexane followed by 5 ml distilled-deionized water. After 15 min the organic phase was transferred to a 10 ml test tube and evaporated to dryness at 45°C under a gentle stream of nitrogen gas. The vitamin A compounds were redissolved in 60 μl diethyl ether followed by 120 μl acetonitrile.

The HPLC system consisted of an Altex 210A injector equipped with a 100 μl loop, an ultrasphere (5 μm particle size, 4.6 × 150 mm) ODS column, a model 168 diode array detector, a mode 126 pumping system and an IBM model 55 SX computer loaded with a System Gold program (all components Beckman Co.). The solvent system of 100% acetonitrile flowed at a constant 2.0 ml min^{-1}. At 2 min after injection, the solvent was linearly changed to 86.5% acetonitrile : 13.5% chloroform over a 3 min period. Retinoids were detected at 326 nm and ß-carotene at 453 nm. Retention times were 2.6, 13.0 and 14.5 min for retinol, ß-carotene and retinyl palmitate, respectively. All chemicals and solvents used in the extraction and analysis were HPLC grade or the highest quality available. External standards used for quantitation were purchased from Sigma Chemicals (St Louis, MO). Recovery of ß-carotene, retinol and retinyl palmitate were, respectively, 72, 112 and 68%. The coefficient of variation for ß-carotene was 16%, retinol, 12% and retinyl palmitate, 14%.

Eggs were analysed for major organochlorines and specific biphenyl congeners at the National Wildlife Research Center at Hull. Non-*ortho* biphenyls were not analysed in the present study. Contaminants were determined in pooled samples of five eggs collected from each of the colonies. Whole egg contents were homogenized prior to pooling on an equal weight basis. Egg homogenates (20 g) were ground with a 5-fold excess of sodium sulphate, extracted with dichloromethane : hexane (1 : 1) and the lipids removed by gel-permeation chromatography. PCBs were recovered after carbon and Florisil column treatments. Contaminants were quantified using a gas chromatography/mass spectrometry system (Hewlett-Packard 5987B) equipped with a 30-m DB-5 capillary GC column. The numbering of PCB isomers are based on IUPAC.

The age of the embryo was estimated based on developmental chronology specific to the Herring Gull (M.G. Williams and Dr. J.P. Ludwig, unpublished data) and a previous study on frozen Herring Gull eggs (Spear *et al.* 1990). Only samples from heron embryos between stages 18 and 32 were subjected to statistical analysis. Stage 18 represents the minimum distinguishable embryonic development in our samples. Earlier stages could not be discerned from freshly-laid infertile eggs under the dissecting microscope. Stage 32 is characterized by conspicuous feather germs, especially on the thigh, neck and spinal area, the mean embryo length (4 cm), the beak length (7 mm) and the tarsus length (8 mm). Embryos older than stage 32 were eliminated because retinoid concentrations

tend to fluctuate widely in the latter half of incubation as the yolk is gradually incorporated into the embryo.

ß-Carotene, retinol, retinyl palmitate and the ratio of retinol : retinyl palmitate for each of the nine colonies were compared using one-way analysis of variance (ANOVA). When significant differences were found using ANOVA, data were treated by the Student-Newman–Keuls (SNK) multiple comparison test ($p < 0.05$).

Data for mono-*ortho* co-planar PCBs (105 and 118) found in eggs were transformed to dioxin toxic equivalents (TCDD-EQs) using values of Brunström *et al.* (1990) and Bosveld *et al.* (1992) for EROD activity (ED_{50}) in chicken embryos. Even though these congeners are mono-*ortho* substituted they are recognized inducers of EROD and are sometimes referred as a 'co-planar analogue' (e.g. Tanabe *et al.* 1989). The relative toxicity factor of the congener 3,3′,4,4′-tetrachlorobiphenyl (No. 77) was taken to be 0.02 as reported by Bosveld *et al.* (1992). The ED_{50} for congener No. 77 was divided by those for the congeners 2,3,3′,4,4′-pentachlorobiphenyl (No. 105) and 2,3′,4,4′,5-pentachlorobiphenyl (No. 118) as reported by Brunström *et al.* (1990) to give the values 0.013 and 0.001. These values were then multiplied by the relative toxicity factor of No. 77 to give estimated factors relative to TCDD. Thus, the relative toxicity factors (relative to TCDD) were 0.00026 for congener No. 105 and 0.00002 for congener No. 118.

The correlation between contaminants and retinoids (arithmetic means) were examined by the Pearson method. The relationship between contaminants and retinoids were investigated using linear regression analysis. Due to the fact that contaminant residues showed a skewed distribution, data were transformed to common logarithms ($\log_{10}$).

Results and discussion

To examine the potential use of retinoids and ß-carotene as biomarkers in the eggs of the Great Blue Heron, the data in Table 1 were first evaluated for geographic differences between colonies. The concentrations of retinol and ß-carotene were not statistically different between colonies and are therefore considered to be unsuitable. When retinoids were expressed as the ratio of retinol : retinyl palmitate, significant differences ($p < 0.0003$) were obtained. The retinoid ratio was lowest in the estuarine colonies of Saint-Barnabé and Gros Pot followed by the reference colonies. The levels of retinyl palmitate showed even greater differences ($p < 0.0001$) between sample sites with the reference colonies, Jacques-Cartier and Eaton, having the highest retinyl palmitate concentrations. These results were generally consistent with those obtained previously with eggs of Great Lakes' Herring Gulls. In that study, retinol concentration varied slightly, but significantly, between colonies while highly significant differences between colonies were obtained for both the retinyl palmitate concentration and the retinoid ratio (Spear *et al.* 1990).

Classic studies of poultry nutrition have demonstrated that, to a certain extent, dietary vitamin A intake by laying hens is reflected in the vitamin A content of the eggs. Our field and laboratory investigations indicate that environmental contaminants may decrease vitamin A storage in fish (Spear *et al.* 1992; Tessier *et al.* submitted; unpublished results) and, thus, affect the vitamin A status of breeding birds. However, if the between-site differences in the retinol : retinyl palmitate molar ratio and retinyl palmitate in heron eggs were attributable to nutritional factors, one would expect that the concentration of retinol and ß-carotene, a retinoid precursor, would show the same

Table 1. ß-carotene and retinoids in Great Blue Heron eggs collected along the St Lawrence River

	ß-carotene ($\mu g\ g^{-1}$)	Retinol ($\mu g\ g^{-1}$)	Retinyl palmitate ($\mu g\ g^{-1}$)	Ratio of retinol to retinyl palmitate ($\mu g\ g^{-1}$)
1 Dickerson ($n = 6$)	1.60 ± 0.38	2.99 ± 1.17	0.39 ± 0.16[a]	8.68 ± 4.62[a]
2 Dowker ($n = 4$)	2.48 ± 0.68	2.69 ± 1.32	0.61 ± 0.32[a]	7.11 ± 7.80[ab]
3 Ile aux Hérons ($n = 8$)	3.34 ± 3.40	3.85 ± 2.66	0.52 ± 0.32[a]	7.60 ± 3.45[a]
4 Grand Ile ($n = 9$)	1.56 ± 0.34	3.68 ± 1.50	0.80 ± 0.30[ab]	5.35 ± 3.73[ab]
5 Eaton ($n = 3$)	2.11 ± 0.41	5.84 ± 1.82	2.29 ± 0.39[c]	2.52 ± 0.45[abc]
6 Jacques-Cartier ($n = 10$)	2.22 ± 0.79	2.64 ± 2.37	1.44 ± 0.83[bc]	2.32 ± 1.72[abc]
7 Gros Pot ($n = 5$)	1.84 ± 0.77	2.39 ± 3.03	0.97 ± 0.42[ab]	1.94 ± 2.12[c]
8 Saint-Barnabé ($n = 4$)	2.07 ± 0.71	1.88 ± 1.84	1.18 ± 0.42[bc]	1.87 ± 1.86[bc]
9 Ile de la Mine[d] ($n = 1$)	1.94	3.53	1.62	2.18
Between-colony variation (F-value)	1.03 NS	1.90 NS	8.18 $p < 0.001$	5.19 $p < 0.001$

Values are mean ± SD. Sample size shown in parentheses.
NS, non significant.
[a,b,c]Mean values that do not share common superscript letters are significantly different. Statistical significance determined by SNK multiple range test ($p < 0.05$) (log-transformed data).
[d]Data for this site were not included in analysis (see text).

pattern. As this was not the case, we consider that vitamin A intake in food was not a major factor affecting retinoid variation between sites.

Retinoid concentrations in yolk are known to vary according to the embryonic stage of development. The levels are typically constant in the first half of the incubation period and tend to decrease in the latter half of incubation as lipids are transferred to the embryo (Parrish *et al.* 1951; Joshi *et al.* 1973; Noble 1987; Spear *et al.* 1990). Ideally sample collection should be conducted in the first half of incubation to minimize variation associated with the embryonic stage of development. In the present study, however, the size of the survey area, the almost simultaneous laying time at different colonies within the survey area and the logistics of obtaining heron eggs from tree-tops precluded such a sampling strategy. Alternatively, the data for retinyl palmitate and the retinoid ratio were subjected to one-way analysis of variance. The results of these tests indicate that the retinoid ratio was significantly influenced by developmental stage (ANOVA $p < 0.001$), whereas retinyl palmitate was not (ANOVA $p > 0.05$). The

overriding logistical difficulties of conducting a detailed field collection of heron eggs introduced a compounding variable – developmental stage – which did not influence the retinol palmitate concentration in this study. We therefore judged retinyl palmitate concentration to be the better potential biomarker for the Great Blue Heron. Furthermore, retinyl palmitate concentration showed highly significant variation between colonies ($p < 0.0001$) which is essential for a good biomarker.

The chemical analysis of eggs for organochlorine contaminants (42 specific biphenyl congeners plus 17 other organochlorines, dioxins, furans and non-*ortho* biphenyls not included) indicated a moderate level of contamination. Of the 17 organochlorine contaminants analysed in the present study, mirex, photomirex and octachlorostyrene were significantly correlated with retinyl palmitate in the Great Blue Heron eggs (Table 2). The accumulated levels of these contaminants were relatively low compared with Herring Gull eggs collected from the Great Lakes. Mean concentrations of mirex in the heron eggs varied from 0.012 to 0.14 $\mu g\ g^{-1}$ (Table 2). Egg mirex levels have typically ranged from 0.1 to a maximum of approximately 5.0 $\mu g\ g^{-1}$ in Great Lakes gull colonies (Gillman *et al.* 1977, 1979; Mineau *et al.* 1984; Ellenton *et al.* 1985; Boersma *et al.* 1986). The injection of Herring Gull eggs with this maximum level, 5 $\mu g\ g^{-1}$ mirex, failed to cause mortality and embryonic liver enzyme activities were not consistently altered (Boersma *et al.* 1986). Interspecific differences in sensitivity notwithstanding, mirex levels in the present study are probably well below the threshold for effects on egg retinoids. Similarly, the photomirex values of 0.003–0.038 $\mu g\ g^{-1}$ (Table 2) are relatively low. Photomirex in Herring Gull eggs from the Great Lakes have been reported in the range from 0.02 to 0.96 $\mu g\ g^{-1}$ (Ellenton *et al.* 1985; Braune and Norstrom 1989).

In the heron eggs, octachlorostyrene values were $< 0.0002\ \mu g\ g^{-1}$ in a reference colony and the maximum value was 0.009 $\mu g\ g^{-1}$ in a St Lawrence River colony (Table 2). This value is considerably lower than the 0.04 $\mu g\ g^{-1}$ reported for Great Lakes' Herring Gull eggs (Braune and Norstrom 1989). Octachlorostyrene is thought to be highly bioaccumulative having a $\log_{K_{ow}}$ of 7.7 (Tarkpea *et al.* 1985), although its chronic toxicity to birds is not known. The NOEL (no observable effects level) for liver and thyroid histopathology in rats is estimated to be 0.5 $\mu g\ g^{-1}$ in the diet (Chu *et al.* 1982) which indicates low chronic toxicity. Whether octachlorostyrene affects egg retinoids is a matter of speculation. Toxic interactions or additivity of contaminants is possible. Equally possible are spurious correlations between a given contaminant and egg retinoids attributable to the intercorrelation of contaminants which has been observed previously by many authors (c.f. Laporte 1982; Boersma *et al.* 1986).

The sum of PCB congeners was significantly correlated with egg yolk retinoids (Table 2). Total PCB values ranged from 1.6 $\mu g\ g^{-1}$ in a reference colony to 7.8 $\mu g\ g^{-1}$ in a St Lawrence River colony. These values are marginally lower than the mean colony levels of 5.9 to 10.6 $\mu g\ g^{-1}$ reported for the same species collected from various Quebec colonies in 1979 (Laporte 1982). Comparable values in the low p.p.m. range were detected in Great Blue Heron eggs from the Tennessee Valley and Texas (southern United States) as well as British Columbia (west coast of Canada) (Mitchell *et al.* 1981; Fleming *et al.* 1984; Bellward *et al.* 1990).

In light of the fact that we did not observe other adverse effects, these exposure levels seem to be below the threshold for gross reproductive effects in the Great Blue Heron. Similarly, normal reproduction of a St Lawrence River estuarine colony of Black-crowned Night Herons (*Nycticorax nycticorax*) was documented despite accumulated

Table 2. Values of selected organochlorine contaminants in relation to retinyl palmitate concentration in eggs of the Great Blue Heron

Site	Contaminants[a]						
	Mirex ($\mu g\ g^{-1}$)	Photomirex ($\mu g\ g^{-1}$)	OCS[b] ($\mu g\ g^{-1}$)	Sum of PCBs ($\mu g\ g^{-1}$)	PCB 105 ($ng\ g^{-1}$)	PCB 118 ($ng\ g^{-1}$)	TCDD-EQs[c] ($ng\ kg^{-1}$)
1 Dickerson	0.090	0.038	0.0048	3.482	99	459	34.9
2 Dowker	0.057	0.026	0.0044	4.123	97	458	34.5
3 Ile aux Hérons	0.054	0.018	0.0036	3.448	75	419	27.9
4 Grand Ile	0.033	0.011	0.0029	2.300	39	228	14.7
5 Eaton	0.012	0.003	0.0002[e]	1.757	31	208	12.1
6 Jacques-Cartier	0.014	0.004	0.0017	1.600	29	179	11.1
7 Gros Pot[d]	0.047	0.022	0.0089	7.818	169	859	61.4
8 Saint-Barnabé[d]	0.034	0.019	0.0050	4.770	97	532	35.8
9 Ile de la Mine[d]	0.145	0.013	0.0086	7.159	175	917	63.9
Correlation coefficient (r)	−0.931[f]	−0.941[f]	−0.971[f]	−0.825[g]	−0.811[g]	−0.776[g]	−0.885[g]

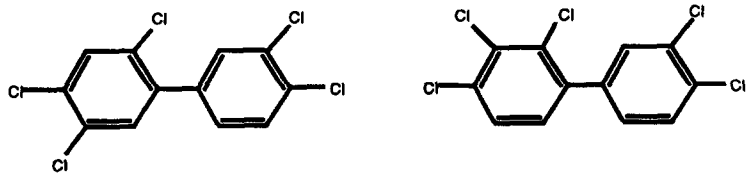
[a]Organochlorine analyses of a pooled whole egg sample (wet weight) from each colony.
[b]Octachlorostyrene.
[c]TCDD-toxic equivalents (sum of PCB 105 and PCB 118).
[d]Data for those sites were not included in correlation analysis (see text).
[e]Minimum detectable amount.
[f]$p < 0.01$.
[g]$p < 0.05$.

levels of PCBs averaging 10.9 μg g^{-1} in their eggs (Tremblay and Ellison 1980). Field observations indicate, however, that detrimental effects do occur at slightly higher exposures in some species. Slightly higher PCB levels of 22.0 μg g^{-1} were associated with reproductive failure in Forster's Tern (*Sterna forsteri*) in the Green Bay area of Lake Michigan (Kubiak *et al.* 1989). Extremely high PCB levels have been associated with reproductive failures in Great Lakes' Herring Gulls (52.0 to 142.0 μg g^{-1}) in the mid-1970s (Gilman *et al.* 1977).

The definition of exposure–effects relationships in wild bird populations may also be expressed as TCDD-EQs rather than total PCBs (Gilbertson *et al.* 1991; Jones *et al.* 1993). Although a complete congener-specific analysis of co-planar PCBs was not attempted in the present study, two mono-*ortho* co-planar PCBs were detected. The concentrations of 2,3,3′,4,4′-pentachlorobiphenyl (No. 105) and 2,3′,4,4′,5-pentachlorobiphenyl (No. 118) were found to be relatively high compared with other case studies. For example, co-planar PCBs were calculated to contribute 90% of TCDD-EQs in Forster's Tern eggs from Green Bay, Lake Michigan, where various reproductive effects presently occur (Kubiak *et al.* 1989). In the study by Kubiak and coworkers, 0.56 μg g^{-1} of congener 105 plus 1.12 μg g^{-1} of congener 118 were detected. Compared with Green Bay terns, the levels of these two congeners accumulated in Great Blue Heron eggs from Ile de la Mine in the present study were 30 and 82%, respectively. We do not presume to have estimated the total TCDD-EQs which should take into account the contribution of dioxins, furans and all co-planar PCBs, however it is interesting to note what appear to be relatively high levels of congeners 105 and 118 and expressed as their TCDD-EQs, these congeners were correlated with egg retinyl palmitate (Table 2).

Based on the mono-*ortho* co-planar congeners 105 and 118, toxic equivalents calculated from Brunström *et al.* (1990) and Bosveld *et al.* (1992) gave values between 11 and 64 ng kg^{-1} (Fig. 2a; see Materials and methods). Jones *et al.* (1993) calculated TCDD-EQs in Forster's Tern eggs from Green Bay to be in the 50–100 ng kg^{-1} range; their calculation used different equivalency factors and included the contribution of non-*ortho* biphenyls, dioxin and furan congeners. One should note that although the values for Forster's Terns and the Great Blue Herons are similar, the choice of equivalency factors exerts a large influence on the outcome of such calculations. Brunström–Bosveld factors are used in the present study because they permit the estimation of a factor for congener 118 for birds, whereas a factor for this congener is not given by Jones *et al.* (1993). Figure 2(a) illustrates that the lowest TCDD-EQs are associated with the reference colonies. The toxic equivalents tend to decrease between sites 1 and 4 with distance downstream in freshwater colonies. Interestingly, the most contaminated colonies are those of the marine estuary, sites 7 and 8. Toxic equivalents of approximately 200 ng kg^{-1} were associated with reproductive impairment in Great Blue Herons from British Columbia in 1986–1987 (Bellward *et al.* 1990); in that case, a large dioxin and furan component of the calculated toxic equivalents was the result of direct pulp mill discharges.

Figure 2(b) depicts retinyl palmitate concentrations which show the opposite tendency to contaminant levels. That is, retinyl palmitate levels increased with distance downstream in the freshwater colonies. Whether ecological factors along a gradient with distance downstream influenced egg yolk retinoids is not known. Eggs collected from the marine estuarine colonies contained relatively high retinyl palmitate concentrations possibly owing to the greater availability of carotenoids and retinoids in the temperate marine ecosystem compared with the inland freshwater sites. Temperate water marine



2,3',4,4',5-pentachlorobiphenyl
(PCB 118)

2,3,3',4,4'-pentachlorobiphenyl
(PCB 105)

a)

Fig. 2a. Sum of PCB congeners 105 and 118 expressed as TCDD toxic equivalents (egg pooled samples).

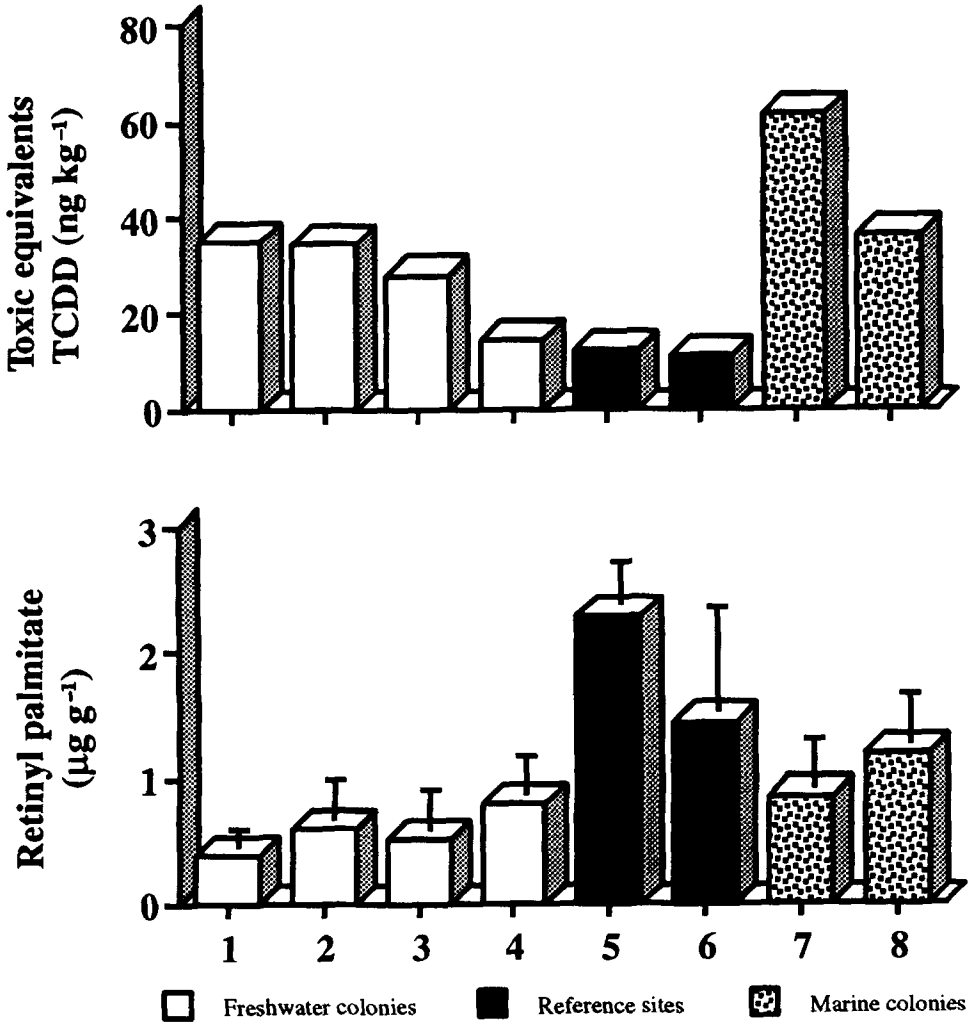


Fig. 2b. Mean concentration of egg yolk retinyl palmitate at eight sites along the St Lawrence River.

fish and the extracted fish oils such as cod liver oil have been used as a dietary supplement of lipid-soluble vitamins A and E. High liver retinoid levels were found in breeding Herring Gulls from a coastal colony in New Brunswick, eastern Canada, compared with inland colonies (Spear *et al.* 1986). The higher retinyl palmitate levels in heron eggs from the estuarine colonies, therefore, are not unexpected.

On the assumption that the marine estuarine colonies were a distinct group with respect to dietary vitamin A intake, only the freshwater colonies were examined more closely. Further data including appropriate marine reference colonies are necessary to adequately interpret the results reported here for the marine estuarine colonies. The retinyl palmitate concentrations in the heron eggs from freshwater colonies decreased ($p < 0.02$; $r^2 = 0.78$) with increasing TCDD-EQs (Fig. 3). This regression indicates that retinyl palmitate levels in the yolk vary as a function of the co-planar analogue biphenyls, although by itself the relationship is a relatively weak proof of causality. In a laboratory study with Ring Doves, the injection of the co-planar biphenyl No. 77 in adults 1 week prior to mating resulted in a high rate of embryo mortality coinciding with several changes in yolk retinoid concentrations (Spear *et al.* 1989). One of these changes was the decrease in yolk retinyl palmitate concentrations which is consistent with the regression shown in Fig. 3. Additionally, TCDD-EQs were highly correlated with egg yolk retinoids (molar ratio of retinol : retinyl palmitate) in Great Lakes' Herring Gulls (Spear *et al.* 1990) which is consistent with the present study. In fact, the molar ratio of retinol : retinyl palmitate in heron eggs gave a relatively high correlation coefficient with TCDD-EQs ($p < 0.009$), but was confounded by the influence of developmental stage as discussed above.

An unexpected and curious finding is the relationship between egg retinyl palmitate concentration and certain non-planar PCBs. Significant negative correlations were obtained for a group of tetrachlorobiphenyls whose molecular configurations are remarkably similar. The correlations involve PCBs 2,3,4,4′-tetrachlorobiphenyl (No. 60),

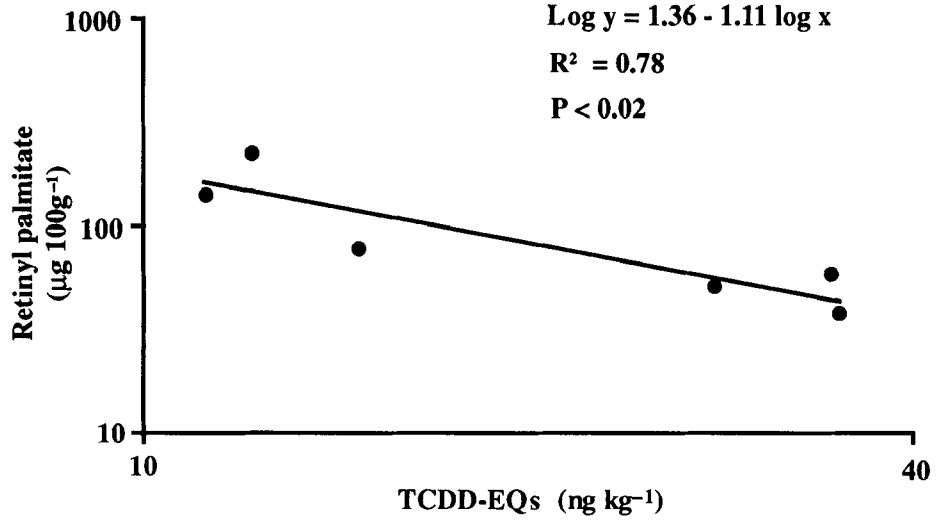


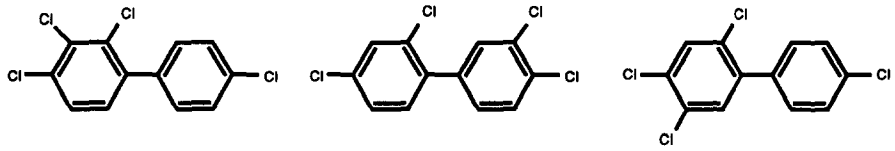
Fig. 3. Relationship of retinyl palmitate to TCDD toxic equivalents (sum of PCB congeners 105 and 118).

2,3′,4,4′-tetrachlorobiphenyl (No. 66) and 2,4,4′,5-tetrachlorobiphenyl (No. 74) which are all p,p′-substituted, mono-*ortho*-tetrachlorobiphenyls and have not been previously recognized as an environmentally significant biphenyl group (Fig. 4a). The toxicity of these particular congeners to avian species has not been reported. PCB 2,3′,4′,5-tetrachlorobiphenyl (No. 70) which is a mono-*ortho*-tetrachlorobiphenyl, although not p,p′-substituted, was tested (Brunström 1990; Brunström *et al.* 1990) in egg injection studies with the chicken. The effective dose for EROD induction by PCB No. 70 is shown in Fig. 4(a) in relation to the sum of concentrations of PCB Nos 60, 66 and 74. While the effective dose is considerably greater than the levels detected in heron eggs, the measurement of EROD activity is related to a toxic mechanism thought to be specific to the polyhalogenated co-planar biphenyls, dioxins and furans. PCB Nos 60, 66 and 74 may not bind to the *Ah* receptor, nor induce cytochrome P-450IA1 or EROD activity.

Figure 4(b) illustrates the highly significant regression ($p < 0.005$; $r^2 = 0.90$) between the sum of PCB Nos 60, 66 and 74 and retinyl palmitate concentration (log-transformed data). The strong bioaccumulative tendency of mono-*ortho*-PCBs in wildlife is well-known (Tanabe *et al.* 1989; Ankley *et al.* 1993), although the toxicity of non-co-planar congeners within this group has yet to be evaluated. Possibly the Great Blue Heron demonstrates selective accumulation of PCB congeners such as 60, 66 and 74 or these congeners have a specific effect on the retinoid metabolic pathway and dynamics in the heron.

In light of the fact that between-colony differences in contaminant burden were evident in the heron eggs, one is tempted to conclude that the eggs reflect regional differences in environmental contamination. The St Lawrence River herons are known to spend approximately 8 months in the nesting areas. However, their winter migration to the southeastern coast of North America and as far south as Cuba and Jamaica, may also influence the body burden of contaminants. Individuals are known to disperse widely during the wintering period (Hancock and Kushlan 1984). Although little information is currently available concerning the influence of migration on contaminant accumulation, Hebert *et al.* (1990) detected higher levels of organochlorine contaminants in a resident duck population on Lake Erie compared with migratory ducks. The extent to which contaminant accumulation during the winter period influences the levels deposited in eggs is not known for sure. The herons arrive in the Montreal area in late March and feed locally for approximately 1 month before egg-laying. Presumably contaminants derived from wintering grounds may be mobilized from lipid stores during the migration which means that there is sufficient time to renew lipid/contaminants stores at the nesting site prior to egg production.

The results of the present study indicate that retinyl palmitate levels in heron eggs may be a useful effects biomarker. Where sampling logistics allow the collection of eggs in the first half of the incubation period, the molar ratio of retinol : retinyl palmitate may also be used as a biomarker. The data suggest that marine colonies should be treated as a separate entity and further research is needed to validate this question. In the case of freshwater colonies egg yolk retinoid concentration was negatively correlated with the level of mirex, photomirex, sum of PCBs, octachlorostyrene, TCDD-EQs based on two mono-*ortho* co-planar PCB congeners and a group of mono-*ortho*-tetrachlorobiphenyls.



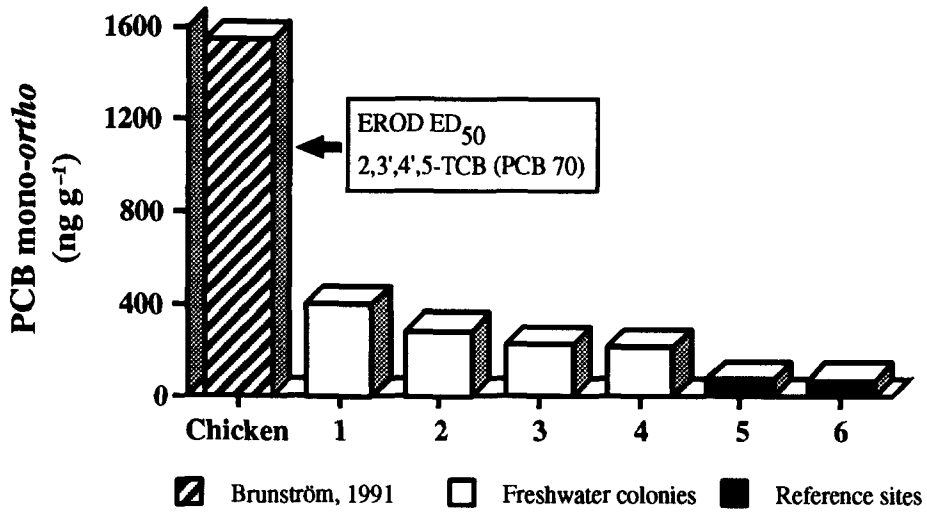
2,3,4,4'-tetrachlorobiphenyl (PCB 60)

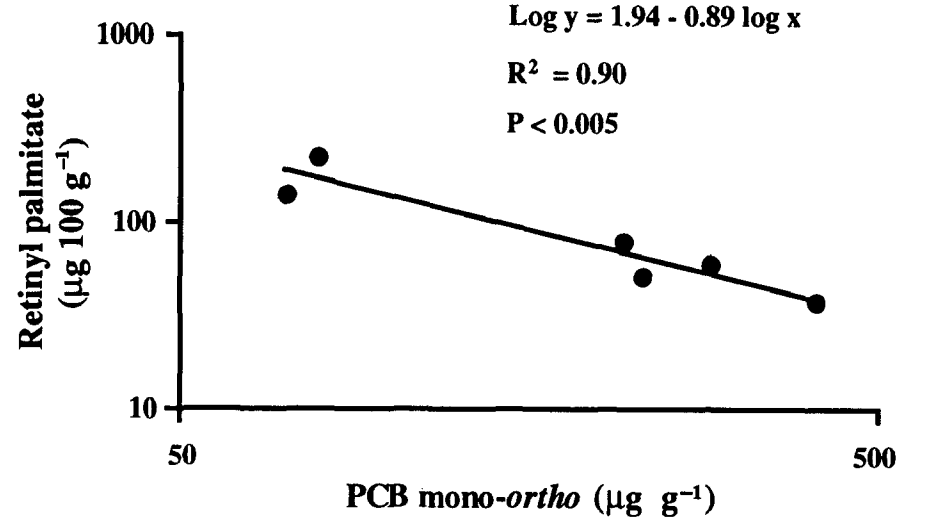
2,3',4,4'-tetrachlorobiphenyl (PCB 66)

2,4,4',5-tetrachlorobiphenyl (PCB 74)

a)

Fig. 4a. Concentration of mono-*ortho* congeners found in eggs of the Great Blue Heron (freshwater sites) compared to the dose found in chicken for EROD induction.





b)

Fig. 4b. Relationship of retinyl palmitate to mono-*ortho* PCBs (sum of congers 60, 66 and 74).

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