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Yolk PCB and Plasma Retinol Concentrations in Shag (*Phalacrocorax aristotelis*) Hatchlings

K. M. Murvoll,[1] J. U. Skaare,[2] V. H. Nilssen,[1] C. Bech,[1] J. E. Østnes,[1] B. M. Jenssen[1]

[1] Department of Zoology, Norwegian University of Science and Technology, N-7034 Trondheim, Norway
[2] The State Veterinary Laboratories, PO Box 8156 Dep., N-0033 Oslo, Norway

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Abstract. To evaluate the possibilities of applying plasma retinol as a biomarker of response in seabirds exposed to chronic low levels of organochlorines, the relationship between yolk content of polychlorinated biphenyls (PCBs) and plasma retinol levels were studied in newly hatched shag chicks (*Phalacrocorax aristotelis*) from the coast of central Norway. The mean concentration of 29 PCB-congeners (ΣPCB) in the yolk sac was 1.22 µg/g ww (wet weight basis) (SD = 0.57, n = 10), or 17.99 ng/g lw (lipid weight basis) (SD = 6.26, n = 10). Expressed as TCDD-equivalents (ΣTEQ), the exposure in the yolk sac was 43.9 pg/g ww (SD = 19.5, n = 10), or 637.1 pg/g lw (SD = 240.8, n = 10), considerably lower than the levels that have been associated with clear-cut lethal and sublethal effects such as egg mortality, hatchability, or live deformity in *Phalacrocoracidae* species. There were significant negative correlations between ΣPCB ww and the variables egg volume, yolk mass, and hatchling mass. We suggest that these relationships are passive causes of a higher lipid concentration in small eggs, rather than the PCB affecting the variables. Analyses showed that there was a borderline significant positive correlation between ΣPCB lw in yolk and plasma retinol concentration. Although the results indicate that plasma retinol level alone is a poor indicator of PCB exposure in shag hatchlings, the result may be related to the low level of contaminant exposure and the low sample size of the study.

The production of polychlorinated biphenyls (PCBs) was at its maximum at the end of the 1960s. However, these chemicals are still found in vast quantities in the environment (Philips and Rainbow 1993). The largest deposits of PCBs are located in sediments along the coast line and in open seas (Tanabe 1988). This makes seabirds vulnerable to the various effects of these substances (Safe 1994) because the birds have a low capacity in metabolizing such chemicals (Tanabe 1988; Tanabe and Tatsukawa 1992) and because of their position in the food web (Elliott *et al.* 1992).

Being top predators, seabirds and other fish-eating birds have often been used as indicator species to assess and monitor pollutants load in coastal marine environments (Elliott *et al.* 1992). However, information on body burdens does not give information on the biological harmful effects these chemicals may cause. During the past decade, the concept of biomarkers has been introduced to assess biological effects of pollutant exposure (Peakall 1992). The term *biomarker* has been defined as ''biological responses to a chemical, or chemicals, that gives a measure of exposure and sometimes, also, of toxic effect'' (Peakall 1994).

Negative correlations between PCB levels and vitamin A (retinoid) status have been reported in rodents (Brouwer *et al.* 1983), seals (Brouwer *et al.* 1989; Jenssen *et al.* 1995) as well as in fish-eating birds (Spear *et al.* 1986; Boily *et al.* 1994). Thus, vitamin A, or retinoid, status has been suggested as a biomarker for exposure to PCBs (Peakall 1992). Since a normal regulation of vitamin A is important for a wide range of biological functions, such as growth, cell differentiation, reproduction, and immunofunction (Blomhoff 1994), this biological variable may also function as a biomarker of response and of susceptibility to PCBs.

Species belonging to the family *Phalacrocoracidae* have been relatively widely used throughout the northern hemisphere for monitoring levels and effects of organochlorine compounds (Vermeer and Peakall 1977; Wilson and Early 1986; Somers *et al.* 1993; Yamashita *et al.* 1993; van den Berg *et al.* 1994; Sanderson *et al.* 1994; Dirksen *et al.* 1995; Larson *et al.* 1996). In The Netherlands, several studies have been performed on *Phalacrocorax carbo sinensis* (Craane *et al.* 1990; van den Berg *et al.* 1994; Dirksen *et al.* 1995). For the sake of comparison, more studies should ideally be carried out on *P. carbo.* However, of the two Norwegian *Phalacrocoracidae* species, the chicks of the Norwegian cormorant (*P. carbo*) are more vulnerable to predators than the chicks of the Norwegian shag (*P. aristotelis*) when studies are performed in the field because of the cormorant's open nests. Thus, in the present study, shag hatchlings were chosen to study relationships between PCBs and retinol.

Most studies on the use of biomarkers in birds have been conducted on populations that live in or close to industrialized areas. The aim of the present study was to examine the possible relationship between PCBs and plasma retinol concentrations in a fish-eating seabird species that lives in an area with relatively low levels of PCBs. Thus, to evaluate the possibilities of

Correspondence to: B. M. Jenssen

applying plasma retinol as a biomarker in seabirds exposed to chronic low levels of organochlorines, the relationship between yolk content of PCBs and plasma retinol levels were studied in newly hatched shag chicks from the coast of central Norway.

Materials and Methods

Ten newly hatched shag chicks (age less than 12 h) were sampled from 10 different nests at Sklinna (65°12' N, 11°00'E), an island situated approximately 50 km off the coast of central Norway. All chicks hatched on 21 June 1995. Because both organochlorine and retinol levels vary as a function of the age of the embryo/chick (Parrish *et al.* 1951; Spear *et al.* 1990; Jones *et al.* 1994), it is necessary to standardize the sampling time when studying relationships between PCB levels and retinoid concentrations. Thus, as has been the procedure in many recent studies (Murk *et al.* 1994a, 1996; van den Berg *et al.* 1994; Dirksen *et al.* 1995; Elliott *et al.* 1996), the present study was carried out on 1-day-old hatchlings.

Prior to sampling, the length and width of the eggs were measured to the nearest 0.1 mm with a caliper, allowing calculation of egg volume using Hoyt's formula (Hoyt 1979). The eggs were marked with a pencil for later recognition of the hatchlings. After hatching, the body mass of each chick was determined using a spring balance to nearest 0.01 g, and the skull length and the tarsus length were measured to the nearest 0.1 mm using calipers. Following ether anesthesia, blood samples were taken (Venoject with sodiumheparine, Terumo, Leuven, Belgium), and after sacrificing the yolk sac was removed and its mass determined using an electronic balance to the nearest 0.01 g. The blood samples were centrifuged (15 min, 3,000 rpm) and the plasma transferred to polyethylene vials and kept frozen at −18°C until analysis of retinol. To prevent photodegradation of retinol, all procedures involving the blood samples were carried out in dull light. For the same reason, the plasma filled vials were covered with aluminum foil immediately.

PCB Analysis

Concentrations of 34 PCB congeners (IUPAC numbers after increased retention time: CB-31, 28, 52, 47, 74, 66, 56, 101, 99, 87, 136, 110, 151, 149, 118, 114, 153, 105, 141, 137, 138, 187, 128, 183, 156, 157, 180, 170, 199, 196, 189, 194, 206, and 209) were analyzed in the yolk sac of the shag hatchlings at the Environmental Toxicology Laboratory at the Norwegian College of Veterinary Medicine, Oslo, Norway, using gas chromatography (GC).

After thawing, the yolk sacs were homogenized using a metal spoon and scalpel. For extraction of lipids, acetone and cyclohexane were used. After spiking, the organic layer, containing fat and PCBs, was concentrated by evaporation. One aliquot of each sample was transferred into weighed and marked glasses, and evaporated to dryness in a sand bath. After evaporation, the glasses were weighed again, and the fat percent was calculated. The rest of the organic layer was used in the identification of the CB congeners by GC. A more detailed description of the determination of PCB congeners, the methods used for extraction, clean-up, identification, and quantification is given in Gabrielsen *et al.* (1994), where methods for quality assurance also are included.

Vitamin A Analysis

The concentration of vitamin A was analyzed in plasma of the shag hatchlings at Department of Ecotoxicology, Arts and Science Research Foundation (Allforsk), Trondheim, by using high-performance liquid chromatography (HPLC).

The extraction of vitamin A (retinol) from the plasma samples were conducted in green light because of the rapid degradation of retinol in exposure to visible light. Following thawing, aliquots (150 µl) of plasma were spiked with 150 µl ultraclean water and 300 µl pure ethanol with internal standard (2 µg/L retinylacetate; vitamin A-acetate stab. oily concentrate, Biochemica, Fluca Chemie, Buchs, Switzerland). The aliquots were extracted with 300 µl hexane. Aliquots (150 µl) of the hexane layer, containing the retinol, were concentrated by evaporation under pure N_2 (40°C/3 min) and dissolved in 100 µl of 85% methanol. Aliquots (80 µl) were analyzed on a reversed-phase silica C_{18} column (Chromosphere 5 C-18, 100 mm/4.6 mm, Middelburg, The Netherlands) with methanol/water (85:15) as eluent. The HPLC equipment was delivered by Pharmacia LKB, Uppsala, Sweden. The fluorescence detector had excitation and emission wavelengths of 330 nm and 408 nm, respectively. The retention time for retinol and retinylacetate (internal standard) was 5.6 and 6.5 min, respectively. The quantification limit for retinol was 50 µg/L.

Statistical Analyses

The statistical calculations of the data were conducted using Statview 4.0 (Abacus Concepts, Berkeley, CA) and a Macintosh computer. The results are presented as mean values ± standard deviation (SD). The range (r), *i.e.*, the minimum and maximum value, of the results are also presented. Unless stated otherwise, the sample size (n) is 10. The data material was tested for normal-distribution using Z-score histogram. The level of significance was defined as $p < 0.05$, and $0.05 > p > 0.1$ was defined as borderline significance, or a trend. Where multiple tests of the variables were conducted, sequential Bonferroni-correction was applied (Rice 1989).

Results

Birds

Prior to hatching the mean egg volume was 48.28 cm^3 (SD = 3.97,r = 41.53–53.23), and the mean hatching body mass was 36.68 g (SD = 4.25, r = 29.60–42.35). The mean mass of their yolk sac was 3.65 g (SD = 0.63, r = 2.55–4.35), hence the mean yolk free body mass was 33.03 g (SD = 3.81, r = 27.05–38.95). The mean liver mass was 0.88 g (SD = 0.12, r = 0.75–1.15), corresponding to a mean relative liver mass of 2.39% (SD = 0.26, r = 2.06–2.72). The mean skull length was 3.30 cm (SD = 0.06, r = 3.22–3.34) whereas the mean tarsus length was 1.31 cm (SD = 0.04, r = 1.25–1.36). The extractable lipid content of the yolk was 6.75% (SD = 1.78, r = 4.51–9.42).

All the above biometrical data, except for the liver mass and the relative liver mass, were normally distributed. As shown in Figure 1, analysis of correlations between the variables revealed that following sequential Bonferroni correction for six tests, there were significant relationships between egg volume and the variables, hatching mass ($r^2 = 0.715$, $p = 0.0013$), yolk free body mass ($r^2 = 0.652$, $p = 0.0029$), yolk mass ($r^2 = 0.606$, $p = 0.0048$) and fat % ($r^2 = 0.537$, $p = 0.0096$).

PCBs

The recovery of the controls in the PCB analyses was between 79–111% for all PCB-congeners, except for CB-209, which had

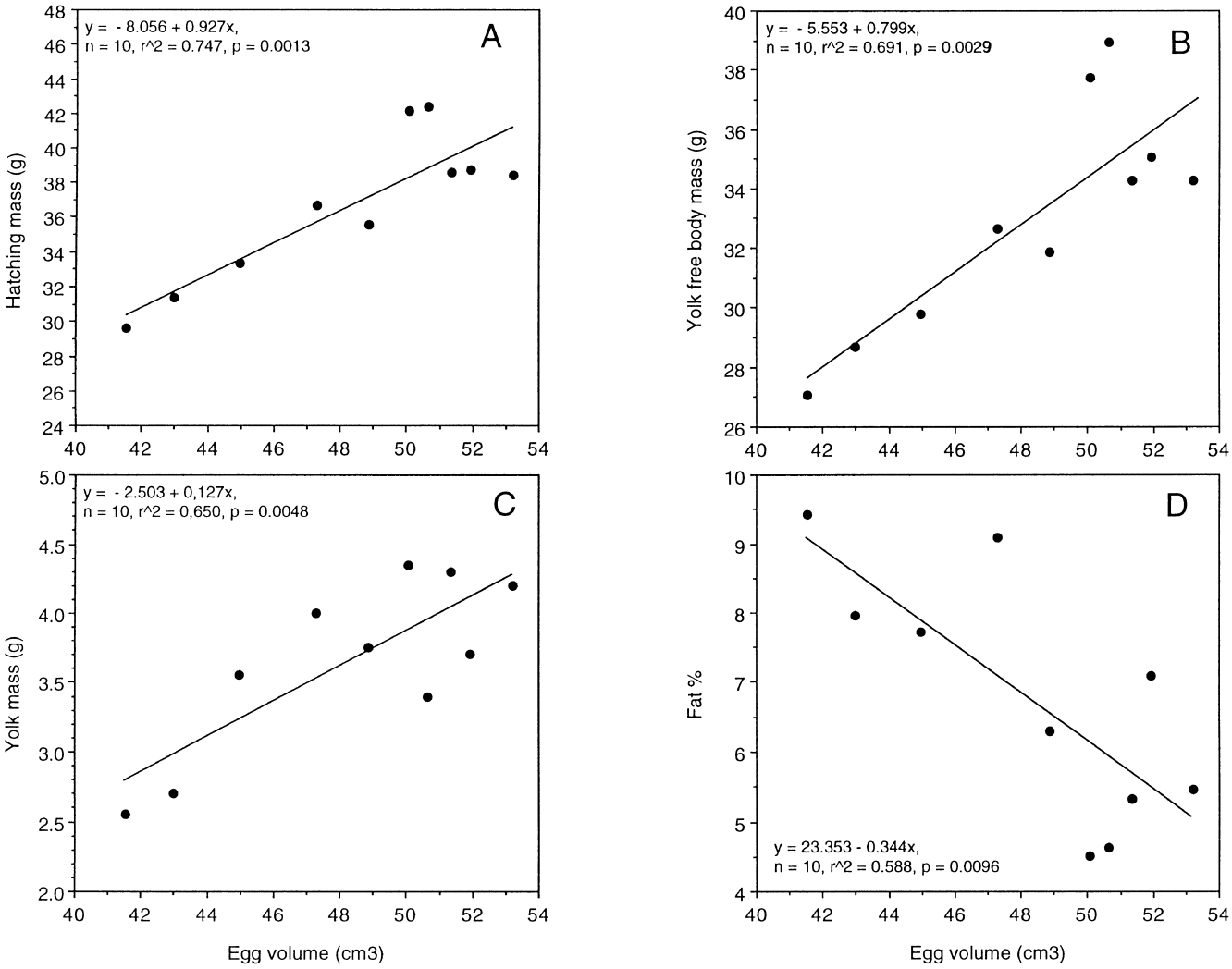

Fig. 1. Relationships between egg volume and hatching mass (A), yolk free body mass (B), yolk mass (C), and fat % in yolk (D) in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna on the coast of central Norway

a recovery of less than 70%. However, since this congener only was detected at low levels in few samples, no attempts were made to correct for the low recovery. The values obtained from the control samples were all within the accepted limits, and the compounds detected and quantified had concentrations within the linear part of the calibration curve. The drift in the output signal from the analyzing equipment during the analysis was also within accepted limits, *i.e.*, between 96 and 106%. For all series of analyses carried out at the laboratory in 1995, the measuring bias was between 7 and 28% CV.

Of the 34 CB congeners, 18 congeners (CB-31, 28, 74, 66, 101, 99, 151, 149, 118, 114, 153, 105, 138, 187, 128, 156, 180, and 170) were detected in all hatchlings. Six congeners were not detected in quantifiable concentrations (CB-52, 87, 136, 132, 183, and 199). Six mono-orthochlorinated PCBs (CB-118, 114, 105, 156, 157, and 189) and seven di-orthochlorinated PCBs (CB-153, 137, 138, 128, 180, 170, and 194) were detected.

The mean concentration of the 29 detected PCB-congeners (ΣPCB) was 1.22 µg/g ww (wet weight basis, SD = 0.57, r = 0.43–2.26), or 17.99 ng/g lw (lipid weight basis, SD = 6.26, r = 7.90–25.90). The data material was normally distributed, and as depicted in Figure 2, after sequential Bonferroni correction for 12 tests, there were significant negative correlations between ΣPCB ww and the variables yolk mass ($r^2 = 0.808$, $p = 0.0004$), egg volume ($r^2 = 0.753$, $p = 0.0011$), and hatchling mass ($r^2 = 0.689$, $p = 0.0030$). There were no significant correlations between ΣPCB lw and any of the biometrical variables.

As shown in Figure 3, which illustrates the concentrations of the different CBs relative to the most abundant congener, CB-153, CB-138 was present at the second highest concentration, followed by CB-118 and 180. The concentrations of the individual CBs 153, 138, 118, and 180, represented 28.4% (SD = 1.8), 16.0% (SD = 0.8), 12.9% (SD = 0.7), and 6.4% (SD = 0.8) of ΣPCB, respectively.

TEQ values were estimated for the congeners CB-118, 114, 105, 156, 157, 189, 180, and 170 using TEF values given by Ahlborg *et al.* (1994), and these values are presented in Table 1. ΣTEQ was 43.9 pg/g ww (SD = 19.5, r = 15.1–82.8), or 637.1 pg/g lw (SD = 240.8, r = 285.1–921.9). The six mono-orthochlorinated congeners (CB-118, 114, 105, 156, 157, 189)

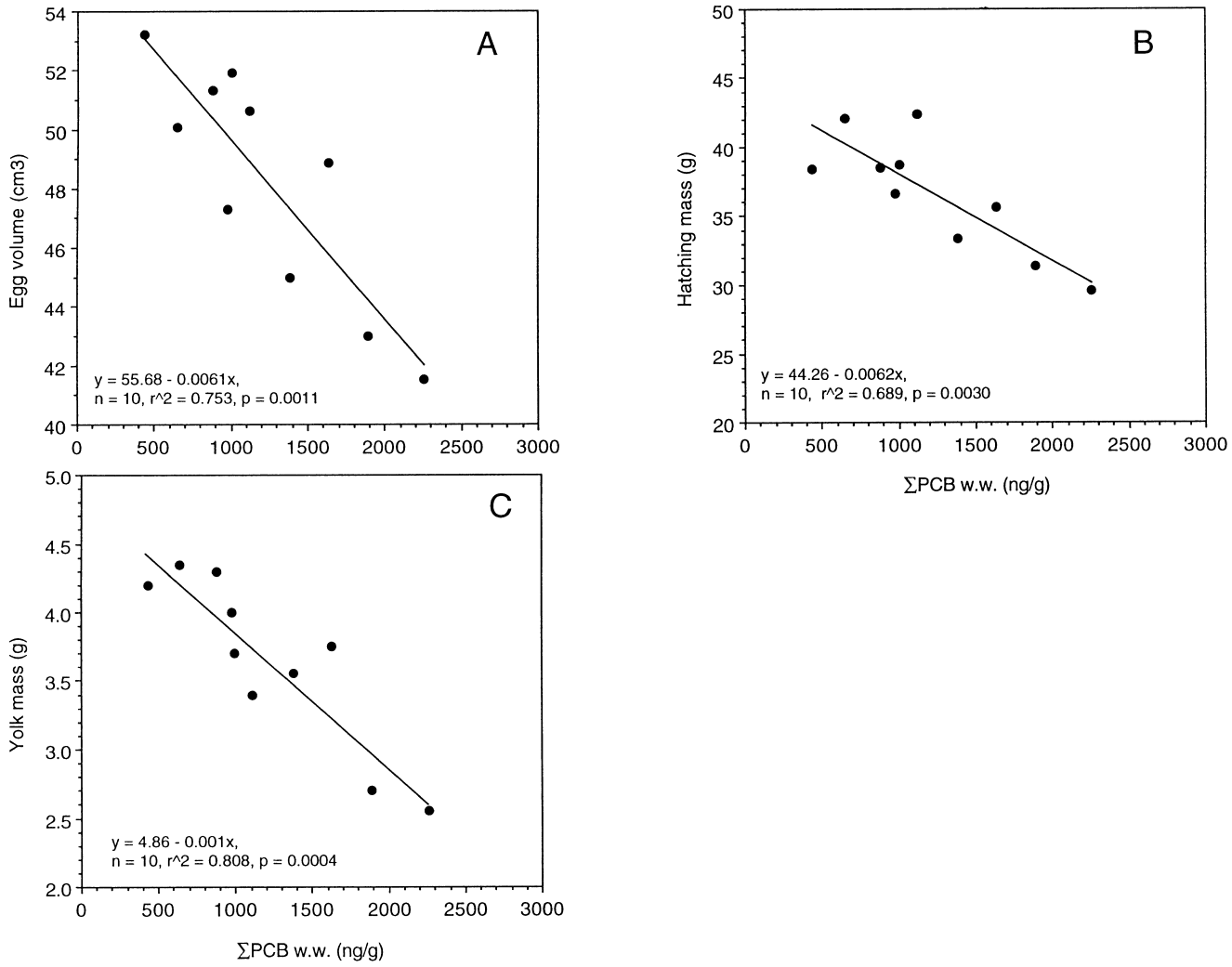

Fig. 2. Relationships between ΣPCB ww in yolk sac and egg volume (A), hatching mass (B), and yolk mass (C), of shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna on the coast of central Norway

contributed to 90.4% of ΣTEQ. There was a significant positive correlation between ΣPCB and ΣTEQ both when expressed on lipid weight and on wet weight basis ($r^2 = 0.982$, $p < 0.0001$ and $r^2 = 0.992$, $p < 0.0001$, respectively).

Retinol

The extraction efficiency of retinol from the plasma samples was 87.7%. Analysis of retinol content in the certified control material showed that the retinol concentration was underestimated by 6%, and that there was a low variability in the sample analysis (SD = 5 µg/L).

Plasma samples of eight chicks were analyzed for retinol concentrations. Because there were low volumes of some plasma samples, we were only able to analyze duplicate samples from four chicks. The CV in duplicate analysis of each of these samples were between 1.3 and 3.3%. The CV of five standards was 2.7%.

The mean plasma retinol concentration was 214 µg/L (SD = 37, r = 158–262, n = 8), and analysis showed that the data set was normally distributed. There were no relationships between the plasma retinol concentration and any of the biometrical variables, but analysis showed that there was a borderline significant positive correlation ($r^2 = 0.459$, $p = 0.065$) between ΣPCB lw and retinol (Figure 4). There was no correlation between ΣPCB ww and retinol.

Discussion

Due to differences in analytical procedures and matrixes, care should be taken when comparing results on levels of PCBs from different studies (*e.g.*, Eaganhouse and Gosset 1991). However, the concentrations detected in the shag hatchlings can with confidence be compared with the PCB data presented by Barret *et al.* (1996) in their study on organochlorines in seabird eggs from northern Norway. The analyses in both studies were conducted at the Norwegian College of Veterinary Medicine, Oslo, Norway, using the same analytical procedures. Barret *et al.* (1996) reported that ΣPCB (*i.e.*, the sum of 21 congeners) in shag eggs were highest in east Finnmark (0.71 µg/g ww,

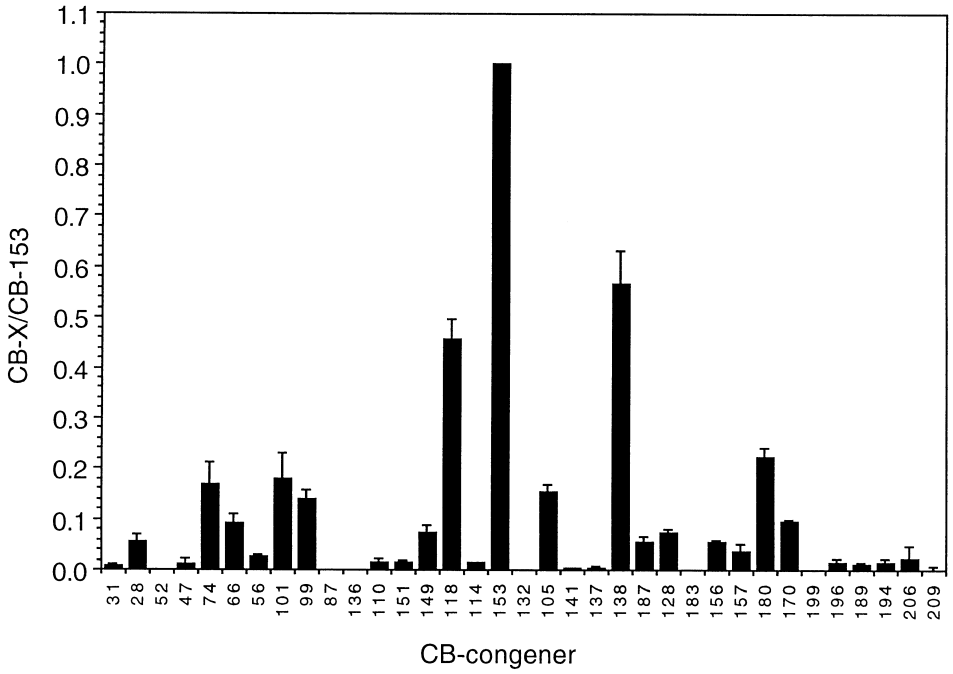

Fig. 3. Concentrations of CB-congeners relative to the concentration of CB-153 in yolk sacs of shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna on the coast of central Norway

Table 1. TEQ-values (pg/g) estimated for various CB-congeners analyzed in yolk of 10 shag (*Phalacrocorax aristotelis*) hatchlings from the coast of central Norway

Congener	TEF	Mean TEQ	SD	Min	Max
CB-118	0.0001	0.01596	0.00708	0.03030	0.00830
CB-114	0.0005	0.00218	0.00084	0.00350	0.00100
CB-105	0.0001	0.00523	0.00220	0.00980	0.00290
CB-156	0.0005	0.00937	0.00412	0.01700	0.00500
CB-157	0.0005	0.00681	0.00411	0.01500	0.00000
CB-189	0.0001	0.00036	0.00014	0.00060	0.00020
CB-180	0.00001	0.00077	0.00032	0.00135	0.00040
CB-170	0.00001	0.00321	0.00123	0.00550	0.00180
ΣTEQ		0.04392	0.01945		

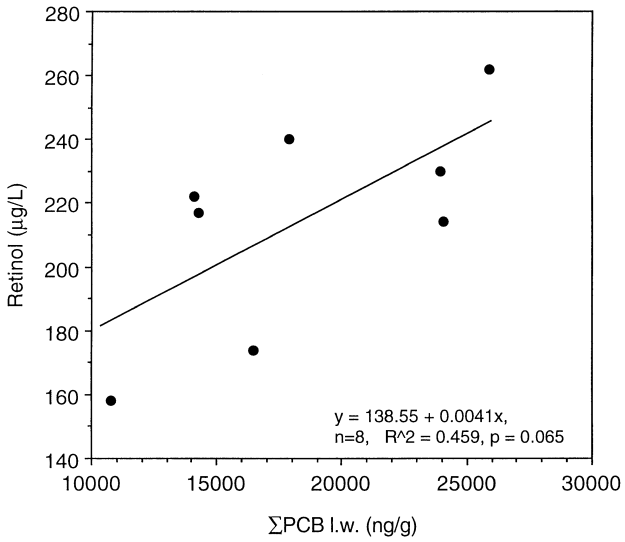

Fig. 4. Relationship between ΣPCB lw in yolk sac and plasma retinol concentration of shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna on the coast of central Norway

SD = 0.23, n = 5), intermediate in Troms/Nordland (0.48 µg/g ww, SD = 0.19, n = 5), and lowest in Lofoten (0.28 µg/g ww, SD = 0.16, n = 5). In comparison, the concentration of ΣPCB (*i.e.*, the sum of 29 CB congeners; 1.22 µg/g ww, SD = 0.57; 17.99 µg/g lw, SD = 6.26) in the yolk sac of the shag hatchlings from Sklinna was somewhat higher. The differences in the ΣPCB concentrations in the two studies are probably mainly caused by differences in fat content in the matrixes that were analyzed. Barret *et al.* (1996) reports an extractable fat content of approximately 4.5% in shag eggs, whereas it was somewhat higher (6.75%) in the yolk sacs of our hatchlings. The differences in ΣPCB concentrations may also be caused by the fact that central Norway has a larger industrial activity and is situated closer to European pollution sources than northern Norway. Skaare (1995) found higher concentrations of PCBs in harbor porpoise (*Phocoena phocoena*) and harbor seal (*Phoca vitulina*) in southern parts of Norway than in nothern parts.

The only available data on levels of PCBs in shags from outside of Norway, is from Ireland, where Wilson and Early (1986) reported concentrations in eggs to be approximately 1.2 µg/g ww in southwest Ireland and approximately 0.6 µg/g ww in southeast Ireland, *i.e.*, values in the same range as those reported in the present study.

Levels of PCBs have also been studied in a range of other members of the family *Phalacrocoracidae.* In cormorants (*P. carbo sinensis*) from The Netherlands, the contaminant levels have been shown to vary depending on the breeding location relative to the River Rhine. Thus, ΣPCB in eggs varied from about 10 to 21 µg/g ww depending on their breeding location (Dirksen *et al.* 1995), whereas in yolk sacs of hatchlings ΣPCB varied from about 60 µg/g ww (Σ of eight CB congeners, 10% lipid weight) to 137 µg/g ww (Σ of eight CB congeners, 10% lipid weight) (van den Berg *et al.* 1994). In double-crested

cormorants (*P. auritus*) from the Great Lakes in North America, concentrations of ΣPCB in eggs varied from about 15 µg/g ww in the most contaminated areas, to 0.1 µg/g ww in the most pristine areas (Tillitt *et al.* 1992). Thus, the PCB exposure of the shags on the coast of central Norway is in the lower range of that previously reported in *Phalacrocoracidae*, which is consistent with the general view that biota along the Norwegian coast can be regarded as inhabiting a relatively pristine environment with regard to contamination by organochlorines.

In the present study, mono-orthochlorinated and di-orthochlorinated CBs were present in largest concentrations. CB-153 was the congener present at the highest concentration, followed by the congeners CB-138, 118, and 180. This is similar to the CB pattern previously reported in fish-eating birds (Boumphrey *et al.* 1993; Bosveld *et al.* 1995; Henriksen *et al.* 1996). CB-153, 138, 118, and 180 are all characterized by a high bioavailability and a high rate of accumulation (McFarland and Clarke 1989). The similar CB-congener pattern in different species of seabirds, reflects a similarity of the enzyme composition of their P450 system, which is responsible for biotransformation of PCBs. It is, however, of interest to mention that in yolk sacs from cormorant hatchlings from The Netherlands the concentrations of CB-180 were much higher than CB-118 (van den Berg *et al.* 1994), whereas the opposite was found in the present study. This may reflect a different biotransformation capacity of the two *Phalacrocoracidae* species, or it could be due to that CB-180 represents a more significant pollutant in The Netherlands.

In several studies on effects of organochlorine compounds on fish-eating birds, so-called 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalencies (TEQ) have been applied to express the toxic significance of the compounds. The different CB-congeners have been assigned different toxic equivalence factors (TEFs) (Ahlborg *et al.* 1994), which express their affinity to the Ah-receptor as compared to TCDDs affinity to the Ah-receptor. In the present study, mono-orthochlorinated and di-orthochlorinated CBs accounted for 90.4% and 9.6% of the estimated TEQ, respectively. As previously shown for other birds (Boumphrey *et al.* 1993), the mono-orthochlorinated congeners CB-118 and 156 made the highest single contributions to ΣTEQ. Obviously, since the non-orthochlorinated CB congeners, which generally constitute the majority of the TEQs, were not measured, some information about the total TEQs in the hatchlings is lost.

The ΣTEQ in the yolk sac of the shag hatchlings was 43.9 pg/g ww (SD = 19.5). This value is considerably lower than levels that have been associated with reduced hatching success in double crested cormorants in Lake Michigan (>200 pg/g ww in eggs [Tillitt *et al.* 1992]; 138 pg/g ww in eggs [Larson *et al.* 1996]), and occurrence of live deformity (>400 pg/g ww in eggs [Yamashita *et al.* 1993]). Thus, the levels of PCBs in shags in central Norway does not seem to be sufficiently high to cause clear-cut lethal and sublethal effects such as egg mortality, hatchability, or live deformity.

In the present study, ΣPCB (ww) correlated significantly negatively with the egg volume (Figure 2A), and this is in accordance with previous studies (Bosveld *et al.* 1995). If increased pollutant load causes a decreased egg volume, or if the pollutant load is related to egg volume is, however, unclear. In the present study we found a significant negative relationship between the egg volume and the fat content in the yolk sac (Figure 1D). The high ΣPCB in the small eggs could therefore be a result of the high lipid content in these eggs. Our conclusion is supported by the fact that when the PCB data are presented on a lipid weight basis there were no correlation between the two variables.

There was a negative correlation between ΣPCB and the hatching mass (Figure 2A), and also between ΣPCB and yolk mass (Figure 2B). This is consistent with previous studies (Murk *et al.* 1994a, 1996; Sanderson *et al.* 1994; van den Berg *et al.* 1994). In these studies, the authors have argued for a cause–effect relationship between PCB and these variables. However, since the hatching mass and yolk mass covariates with the egg volume (Figure 1A and Figure 1C), we suggest that the relationship may be a passive cause of a higher lipid concentration in small eggs.

The retinol concentration reported in the present study (214 µg/L, SD = 37, n = 8) was much higher than previously reported in newly hatched cormorants from The Netherlands (25 nmol/L, 7.16 µg/L [van den Berg *et al.* 1994]). In a similar study on retinol concentration in common terns (*Sterna hirundo*) from eight locations, concentrations varied from 57 to 131 µg/L (Murk *et al.* 1996), whereas concentrations of 320 to 380 µg/L have been reported in bald eagle (*Haliaeetus leucocephalus*) chicks from British Colombia (Elliott *et al.* 1996). These marked differences in retinol plasma concentration may be due to interspecies differences. The differences may also be caused by different pollution loads in the areas mentioned. However, it is also important to be aware that retinol is rapidly degraded by exposure to light, and the differing concentrations may be a result of different treatment of the plasma samples. In the present study, careful precautions were taken to avoid plasma samples being exposed to light.

There was no correlation between plasma retinol concentration and egg volume or between retinol and any of the other biometrical variables. There was, however, a borderline significant positive correlation between ΣPCB (lw) and plasma retinol concentration. This trend is in contrast to the negative correlations between PCBs and plasma retinol previously reported in mammals (Brouwer *et al.* 1988, 1989; Jenssen *et al.* 1995). Nevertheless, previous studies have shown conflicting results regarding the effects of organochlorines on plasma concentrations of retinol. Some have also found increases in plasma levels, while others have reported no effect (Thunberg *et al.* 1979; Jensen and Zile 1988).

According to Brouwer *et al.* (1990a), the retinol-depressing effect of PCBs is attributed to be caused by PCB-hydroxy metabolites (PCB-OHs). In the plasma, retinol is transported bound to a retinol-binding protein (RBP), which is bound in a complex to the thyroxin-transporting protein transthyretine (TTR). The plasma retinol-depressing effect of PCB-OHs is a result of these congeners binding to TTR. This leads to a conformational change in TTR, so that the RBP-TTR complex not is formed (Brouwer *et al.* 1988, 1990b), and plasma retinol concentration is reduced due to glomerular filtration.

However, the plasma retinol levels may be influenced by enhanced mobilization from the liver as well. Low concentrations of hepatic retinoid levels or increased retinol to retinyl palmitate ratios have been found in eggs or hatchlings from highly polluted locations as compared to more pristine breeding locations (Spear *et al.* 1990; Boily *et al.* 1994; van den Berg *et al.* 1994). Murk *et al.* (1994b) found increasing plasma retinol

levels with increasing PCB burden in common tern hatchlings (*Sterna hirundo*). Furthermore, according to Spear and coworkers (1986, 1989) the coplanar PCB congener 77 (3,3′,4,4′-tetrachlorobiphenyl) may increase serum retinoid levels in ring doves (*Streptopelia risoria*). It is thus possible that the positive relationship between ΣPCB and plasma retinol concentration reflects an effect of PCB exposure.

According to Murk *et al.* (1994b), common tern hatchlings have a limited ability of producing PCB-OHs, and if this also is the case for shag hatchlings, this could be a contributing explanation to the increasing plasma retinol concentration with increasing PCB burden in shag hatchlings. Murk *et al.* (1994a) argued that a prolonged exposure to PCBs, which leads to a depletion of the hepatic retinoid stores, eventually also may reduce plasma retinol levels. Apparently, the shag hatchlings in the present study were not exposed to levels of PCBs that induced such a mechanism.

Even though the results from the present study on shag hatchlings from a relatively pristine environment indicate that there is a relationship between PCB in the yolk sac and plasma retinol concentration, we argue that plasma retinol level alone is an unsuitable indicator of PCB exposure in shag hatchlings. This could be related to the two opposing mechanisms influencing plasma retinol levels on exposure to polyhalogenated aromatic hydrocarbons. Further studies should be conducted to clarify the mechanistic effects of PCBs on birds, and we suggest that attention in particular should be given to the retinoid balance between different organs, such as plasma, liver, and yolk sac. Such studies could result in a more successful application of retinoids as biomarkers of response for organochlorines in birds.

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