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DIFFERENTIAL INVESTMENT IN EGGS BY ARCTIC-BREEDING GLAUCOUS GULLS (*LARUS HYPERBOREUS*) EXPOSED TO PERSISTENT ORGANIC POLLUTANTS

Nanette Verboven,[1] Jonathan Verreault,[2] Robert J. Letcher,[2] Geir W. Gabrielsen,[3] and Neil P. Evans[1,4]

[1]*Division of Cell Sciences, Institute of Comparative Medicine, University of Glasgow Veterinary School, 464 Bearsden Road, Glasgow G61 1QH, Scotland, United Kingdom;*
[2]*Wildlife and Landscape Science Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, Ontario, K1S 5B6, Canada; and*
[3]*Norwegian Polar Institute, 9296 Tromsø, Norway*

Abstract.—Although egg size is a widely studied life-history trait in evolutionary ecology, it is largely unknown whether exposure to persistent organic pollutants (POPs) can influence the allocation of resources to avian eggs and, if so, how. It is well established that female birds exposed to POPs transfer these compounds to their eggs. However, little is documented with regard to contaminant-related changes in egg quality, such as egg mass, albumen mass, yolk mass, lipid, and water content. We report positive correlations between the concentrations of several major classes of POPs (organochlorines, brominated flame-retardants, and metabolically derived products) in plasma of Arctic-breeding Glaucous Gulls (*Larus hyperboreus*) and in the yolk of the last-laid egg of their clutches. The contributions of the different POP classes to the summed POP concentration were also positively correlated between female plasma and egg yolk. In addition, Glaucous Gulls with a relatively high concentration of sum (Σ) chlordanes and total-(α)-hexabromocyclododecane in their plasma laid smaller eggs. Eggs into which females had deposited a relatively low concentration of ΣPCB and a relatively high concentration of ΣDDT were also smaller. The POP patterns of yolk and maternal plasma were associated with changes in water and lipid content of the yolk. These results suggest that egg quality—and, thus, offspring performance—may be affected not only by the direct transfer of contaminants from the female to the egg, but also through associated changes in egg size and composition. *Received 3 March 2008, accepted 23 August 2008.*

Key words: egg mass, Glaucous Gull, *Larus hyperboreus*, Norwegian Arctic, persistent organic pollutants, yolk.

Investissement différentiel dans les œufs par des *Larus hyperboreus* nichant dans l'Arctique et exposés à des polluants organiques persistants

Résumé.—Bien que la taille des œufs soit un élément de l'histoire naturelle très étudié en écologie évolutive, on ne sait pas si l'exposition à des polluants organiques persistants (POP) peut influencer l'allocation des ressources aux œufs d'oiseaux et, si tel est le cas, de quelle façon. Il est admis que les femelles d'oiseaux exposées aux POP transfèrent ces composés à leurs œufs. Cependant, il y a peu de documentation concernant les changements dans la qualité des œufs reliés aux contaminants, tels que la masse des œufs, de l'albumen et du vitellus et les contenus en lipides et en eau. Nous présentons des corrélations positives entre les concentrations de plusieurs classes importantes de POP (organochlorés, ignifuges bromés et produits dérivés métaboliquement) dans le plasma de *Larus hyperboreus* et dans le vitellus du dernier œuf pondu. Les contributions des différentes classes de POP aux concentrations totales de POP étaient aussi positivement corrélées entre le plasma des femelles et le vitellus des œufs. De plus, les individus ayant une concentration cumulative (Σ) relativement élevée en chlordanes et en (α)-hexabromocyclododécane dans leur plasma ont pondu de plus petits œufs. Les œufs comportant des concentrations relativement faibles en ΣBPC et une concentration relativement élevée en ΣDDT étaient également de plus petite taille. Les patrons de POP du vitellus et du plasma maternel étaient associés aux changements dans les contenus en eau et en lipides du vitellus. Ces résultats suggèrent que la qualité des œufs—et donc la performance des jeunes—peut être affectée non seulement par le transfert direct des contaminants de la femelle aux œufs mais également par des variations associées de la taille et de la composition des œufs.

[4]Address correspondence to this author. E-mail: n.evans@vet.gla.ac.uk

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EGG FORMATION IN birds is a costly process, which can influence the subsequent performance of parents and offspring (Monaghan and Nager 1997). Because the developing embryo is entirely dependent on the resources present in the egg, the amount and quality of the material allocated to eggs by female birds can have profound and long-lasting effects on the phenotype and viability of offspring (Bernardo 1996). Larger eggs are generally considered to be of better quality, because of their higher absolute nutrient content (Blem 1990, Williams 1994). In addition, larger eggs may be better equipped with quantitatively minor components that are, nevertheless, important for offspring performance—for example, antioxidants (Blount et al. 2000, Surai 2003), immune factors (Gasparini et al. 2001, Saino et al. 2002), and maternal hormones (Schwabl 1993, Groothuis et al. 2005). As a consequence, egg size is often positively correlated with hatchling size and subsequent offspring growth and survival (e.g., Dzialowski and Sotherland 2004, Wagner and Williams 2007). Although larger eggs may contain more yolk and more albumen, the relative contribution of each component may vary with egg size (Romanoff and Romanoff 1949, Carey et al. 1980). Albumen is the major source of water for the developing chick and contains the bulk of the egg protein, whereas the yolk typically contains a large proportion of lipids. Studies in which the amount of yolk or albumen were experimentally manipulated have demonstrated that these egg components have different effects on offspring performance. For example, a reduction in the amount of albumen delayed hatching, reduced hatchling size, and lowered offspring viability (Ferrari et al. 2006, Bonisoli Alquati et al. 2007). By contrast, experimental removal of yolk caused a reduction in nutrient reserves (i.e., smaller yolk sacs) without affecting hatchling size (Finkler et al. 1998).

Although maternal investment in eggs can profoundly influence offspring growth and survival, little is known about the sources of inter-individual variation in egg size and composition (reviewed in Christians 2002). Female investment in eggs is known to be influenced by a number of maternal and environmental factors, including body condition (Houston et al. 1983), age (Bogdanova et al. 2006), ambient temperature (Nager and Van Noordwijk 1992), availability and quality of food (Reynolds et al. 2003), predation risk (Saino et al. 2005), mate quality (Cunningham and Russell 2000), and social environment (Verboven et al. 2005). Climate change and other environmental perturbations may also influence avian egg size and composition (Hayward and Wingfield 2004, Tryjanowski et al. 2004).

It is recognized that some anthropogenic substances that are released into the environment can affect avian egg production. Exposure of female birds to dichlorodiphenyltrichloroethanes (DDTs), for example, is known to reduce egg-shell thickness (Ratcliffe 1970, Lundholm 1997). Persistent organic pollutants (POPs) are anthropogenic substances or unintentionally generated byproducts of various industrial processes. They are lipid soluble and, as a consequence, accumulate in fatty tissues of humans and wildlife. In some cases, POPs biomagnify from species to species in food webs and result in biological effects and influence the health of exposed individuals. It is known that female birds can deposit a substantial part of their POP burden into their eggs (Barrett et al. 1996, Braune et al. 2001, Pusch et al. 2005). However, the potential effects of maternal POP exposure on other aspects of egg quality, such as size and composition, are not well documented.

Because of their structural similarity to endogenous hormones, certain POPs are known to interfere with the functioning of the endocrine system (Giesy et al. 2003). Because the process of egg formation is tightly regulated by hormones, in particular estrogens, it could be hypothesized that POP exposure might affect the egg production process and, thus, egg quality. Support for such a proposal comes from reports that Great Black-backed Gulls (*Larus marinus*) from northern Norway that exhibited high blood concentrations of certain POPs produced smaller eggs toward the end of the laying sequence (Helberg et al. 2005). Moreover, captive American Kestrels (*Falco sparverius*) fed a diet supplemented with certain polychlorinated biphenyls (PCBs) produced eggs similar in size to eggs of controls, yet their eggs contained more yolk and less albumen (Fernie et al. 2000). Also in this experimental study, American Kestrels were exposed to a limited number of PCB congeners. In the field, however, birds are exposed to a complex mixture of PCBs, as well as numerous other POP-like compounds and their metabolites that may have antagonistic, additive, or synergistic effects.

Because the Arctic acts as a sink for POPs produced and environmentally released elsewhere in the world (De Wit et al. 2004), and because POPs tend to accumulate in higher concentrations within organisms that forage at higher trophic levels in the marine food web (Hop et al. 2002), top-predators like the Glaucous Gull (*L. hyperboreus*) are exposed to high levels of POPs. Indeed, high blood residues of a variety of POPs have been reported in adult Glaucous Gulls (reviewed in Gabrielsen 2007) and in their eggs (Verreault et al. 2005b, 2006b, 2007b). Exposure of Glaucous Gulls to POPs has been associated with adverse effects on a range of different biological endpoints in parents and offspring, including ecological (Bustnes et al. 2003, 2005a), behavioral (Bustnes et al. 2001a), developmental (Bustnes et al. 2002), immunological (Sagerup et al. 2000, Bustnes et al. 2004), endocrinological (Verreault et al. 2004, 2006a, 2008), energetic (Verreault et al. 2007a), and genotoxic (Østby et al. 2005, Krøkje et al. 2006) effects. This study tested the hypothesis that maternal exposure to a complex mixture of POPs, including PCBs, major chlorinated pesticides, brominated flame-retardant additives, and metabolically derived products will affect the eggs laid by Glaucous Gulls. Given the importance of egg size and composition for parental and offspring fitness, our objective was to examine the intra-individual relationship between contaminant exposure of female Glaucous Gulls and their eggs and to investigate whether contaminant patterns in females and in eggs are associated with variation in egg size and composition (i.e., yolk, albumen, shell, water, and lipid content).

METHODS

Sample collection.—Field work was done on Bjørnøya (74°21′N, 19°05′E), an island in the Svalbard archipelago, Arctic Norway, in spring and summer 2006. The Glaucous Gull population on Bjørnøya comprises ~800 breeding pairs (H. Strøm pers. comm.). During the egg-laying period, Glaucous Gull nests were visited once every two days and new eggs were marked as they were laid to determine laying order. Between 30 May and 6 June, 31 eggs were collected from randomly chosen nests. Eggs were collected fresh (i.e., 1–2 days after they were laid). Hence, embryo development should not have affected egg composition. To minimize the effects of egg collection on the Glaucous Gull population, only one

egg per clutch was collected. It was replaced by a dummy egg, so that egg collection did not interfere with normal incubation behavior. Glaucous Gulls normally produce clutches of three eggs (Gilchrist 2001). Egg size and composition may differ among eggs laid at different positions of the laying sequence (Alisauskas 1986, Meathrel et al. 1987, Nager et al. 2000). To standardize the comparison among eggs of different clutches, we always collected the third, last-laid egg of the clutch.

During the first half of the 27- to 28-day incubation period, a subsample of females ($n = 11$) from which an egg had been collected were captured on the nest. The trap consisted of a snare placed on the edge of the nest bowl, which was triggered from a distance using a radiotransmitter. Upon capture, the birds were weighed and head-bill length was measured to confirm the sex of the bird (individuals with a combined head and bill length of <142 mm are females; Verreault et al. 2004). Determination of POP concentrations in female blood plasma is considered to be a reliable quantification of the momentary POP burden of an individual (Henriksen et al. 1998; Bustnes et al. 2001b, 2005b). Therefore, a blood sample (~10 mL) was collected from the wing vein for contaminant analysis (see below). Permission to collect Glaucous Gull eggs and to capture breeding birds was granted by the Governor of Svalbard (2004/00481-12) and the Norwegian Animal Research Authority (2006/16056).

Egg composition analysis.—Upon arrival in the laboratory, frozen eggs were removed from sealed plastic bags, thawed, and weighed to the nearest 0.01 g, after which yolk, albumen, and shell were separated. The shell, including the shell membrane, was rinsed with distilled water, air-dried, and weighed. Yolk wet mass was also determined. Inevitably, a small fraction (~4.2%) of the albumen that adhered to the yolk and to the inside surface of the shell was lost when egg components were separated. To overcome this discrepancy, we subtracted shell mass and yolk wet mass from whole egg mass and used this value as a measure for albumen wet mass. Weighed aliquots of yolk and albumen were oven-dried to constant mass at 60°C and then weighed again to determine the water content. Because most POPs have lipophilic properties and because most egg lipids are contained in the yolk (Ricklefs 1977, Carey 1996), POP concentrations were determined in yolk samples rather than in homogenized whole eggs (see below). As part of the sample extraction and clean-up routine preceding chemical analysis, bulk lipids were separated from the organic contaminants by gel permeation chromatography (GPC) and the total extractable yolk lipid content was quantified gravimetrically.

Chemical analysis.—The POPs quantified in the present study (Table 1) were hexabromocyclododecane (total-[α]-HBCD), polybrominated diphenyl ethers (PBDE; $n = 38$ congeners), methoxylated-PBDE (MeO-PBDE; $n = 15$ congeners), PCBs ($n = 58$ congeners), chlorinated benzenes (CBz; $n = 4$ congeners), chlordanes (CHL; $n = 6$ compounds), DDTs ($n = 3$ compounds), and octachlorostyrene (OCS). The analytical methods for the determination of POPs in eggs and blood plasma have been described in detail previously (Verreault et al. 2005a, b, 2006b). Briefly, chlorinated and brominated compound quantification was performed using a gas chromatograph–mass spectrometer (GCMS) (Agilent 6890; Agilent Technologies, Palo Alto, California) operating in the electron impact (EI) mode (for CBzs, DDTs, CHLs, OCS, and PCBs) or electron-capture negative ionization (ECNI) mode (for total-[α]-HBCD, PBDEs, MeO-PBDEs). The GCMS(EI) separation was completed using a fused silica DB-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, California), whereas a fused silica DB-5 HT capillary column (15 m, 0.25 mm i.d., 0.10 μm film thickness; J&W Scientific) was used for GCMS(ECNI) separation. The GCMS(ECNI) used methane as a buffer gas. The GCMS(EI) and GCMS(ECNI) analyte determinations were accomplished in the selected ion-monitoring (SIM) mode. The analytes were identified on the basis of their retention times on the DB-5 columns, and verified by matching retention times with authentic standard mixtures. An external standard method was used for CBz, DDT, CHL, OCS, and PCB quantification using a spiked recovery surrogate mixture of six ^{13}C-labeled PCB congeners and three tetra- through hexa-CBzs. An internal standard (IS) approach was used for quantification of total-(α)-HBCD, PBDEs, and MeO-PBDEs (IS was BDE71).

Quality-assurance and quality-control procedures included method blanks, duplicate extractions and injections of authentic standards, standard reference material (Polar Bear plasma pool #01-2004) from Environment Canada, National Wildlife Research Centre, and cleaned-up Glaucous Gull egg yolk extracts for each block of six samples to monitor for quantitative reproducibility and instrument sensitivity. Blank samples showed negligible background contamination for all analyte classes; therefore, background correction was not necessary. The duplicate extractions and injections demonstrated, on average, 10% and 5% analytical variation of selected compound concentrations, respectively. The analyte-specific method limits of quantification (MLOQs) were set as a signal, being 10× the standard deviation of the noise.

TABLE 1. Mean concentrations (ng g^{-1} wet weight; ± SE) of eight classes of POPs in egg yolk and blood plasma of female Glaucous Gulls from Bjørnøya.

	Yolk ($n = 31$) (mean ± SE)	Plasma ($n = 11$) (mean ± SE)
ΣPCB[a]	5,771.2 ± 856.1	262.4 ± 66.5
ΣDDT[b]	4,243.0 ± 670.3	108.6 ± 16.2
ΣCHL[c]	705.1 ± 104.6	22.0 ± 3.4
ΣCBz[d]	314.6 ± 29.7	13.0 ± 2.8
ΣPBDE[e]	162.4 ± 13.6	8.0 ± 1.9
Total-(α)-HBCD	19.8 ± 2.2	2.7 ± 0.7
ΣMeO-PBDE[f]	20.2 ± 2.7	0.9 ± 0.3
OCS	8.1 ± 0.7	0.5 ± 0.1

[a] Sum of CB22, 28/31, 20/33, 41/64, 42, 44, 47/48, 49, 52, 56/60, 66, 70/76, 74, 85, 87, 90/101, 92, 95, 97, 99, 105, 110, 114, 118, 128, 130, 137, 138, 141, 146, 149, 151, 153, 156, 157, 158, 167, 170/190, 171, 172, 174, 176, 177, 178, 179, 180, 183, 187, 189, 194, 195, 196/203, 199, 200, 202, 206, 207, and 208.

[b] Sum of p,p′-DDT, p,p′-DDE and p,p′-DDD.

[c] Sum of heptachlor epoxide, oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor, and cis-nonachlor.

[d] Sum of 1,2,4,5-TeCBz, 1,2,3,4-TeCBz, PnCBz, and HxCBz.

[e] Sum of BDE17, 25, 28, 47, 49, 54, 66, 75, 77, 85, 99, 100, 116, 119, 138, 139, 140, 153, 154/BB153, 155, 156, 171, 180, 181, 183, 184, 190, 191, 196, 197, 201, 202, 203, 205, 206, 207, 208, and 209.

[f] Sum of 6′-MeO-BDE17, 4′-MeO-BDE17, 2′-MeO-BDE28, 4-MeO-BDE42, 5-MeO-BDE47, 6-MeO-BDE47, 3-MeO-BDE47, 4′-MeO-BDE49, 6′-MeO-BDE49, 2′-MeO-BDE68, 6-MeO-BDE85, 6-MeO-BDE90, 6-MeO-BDE99, 2-MeO-BDE123, and 6-MeO-BDE137.

Statistical analysis.—We analyzed POPs as sums (Σ) of closely related congeners and compounds based on similarities in their chemical structure (Table 1). Prior to summing the concentrations of closely related congeners and compounds, samples with concentrations below the MLOQ were arbitrarily assigned a random value between zero and the compound-specific MLOQ. The summed concentrations were $\log_{10}$-transformed prior to analysis to approximate a normal distribution.

To study the relative contribution of different contaminants, independent of the total amount of POPs quantified in a sample, the concentrations of the eight POP classes were summed and principal components (PC) extracted from arcsine-transformed proportions of each of the POP classes, based on a correlation matrix. Principal components with eigenvalues greater than one were considered to account for a significant portion of the total variance according to the latent root criterion (Hair et al. 1998). Factor loadings and factor scores were used to interpret variation in the proportion of different POP classes. Three separate principal component analyses (PCAs) were conducted, as appropriate: one for yolk samples ($n = 31$), one for female plasma samples ($n = 11$), and one in which yolk samples were combined with maternal plasma samples ($n = 2 \times 11$). The latter was used for within-individual comparisons of POP patterns of females and their eggs.

To investigate the relationship between egg size and egg composition, we examined the exponent b of the allometric relationship $y = a \times x^b$, in which x represents whole egg mass and y the mass of an egg component (wet yolk, wet albumen, dry yolk, dry albumen, extractable yolk lipids, and shell). The value of b, which was obtained from log-log regressions, indicates whether or not the mass of an egg component increases in direct proportion to whole egg mass ($b = 1$). If the component increases less than in proportion, b has a value <1.0 and, if the component increases more than in proportion, then b has a value >1.0 (e.g., Ricklefs 1984).

Variation in whole egg mass was analyzed in relation to plasma and yolk POP patterns using linear regression models with egg mass as dependent variable, PC scores as independent explanatory variables, and laying date (i.e., number of days since 1 May) or female body mass as covariates. When the relationship between the water content of yolk or albumen was analyzed, we controlled for yolk or albumen wet mass, respectively, so that the analysis effectively represented the proportion of water in yolk or albumen. Pearson product-moment correlations were used to investigate similarities between the concentrations and proportions of the POP classes in maternal plasma and yolk. Significance levels for the statistical tests on POP class concentrations were adjusted using a sequential Bonferroni procedure (Rice 1989), because several measurements were obtained from the same sample. All tests are two-tailed, and means are reported ± SE.

RESULTS

Egg size and composition.—The mean weight of Glaucous Gull eggs was 108.44 ± 1.45 g ($n = 31$). Whole egg mass was not related to the laying date of the egg (linear regression, $F = 1.44$, df = 1 and 29, $P = 0.24$). Yolk wet mass, albumen wet mass, and shell mass constituted, on average, 26.4 ± 4.0% ($n = 31$), 66.3 ± 4.0% ($n = 31$), and 7.3 ± 0.1% ($n = 31$) of whole egg mass, respectively. In all cases, the exponent of the allometric relationship between egg component mass and whole egg mass did not differ significantly from 1.0 (Student's t test, all $P > 0.073$; Table 2), and, thus, the mass of the separate egg components varied in proportion with whole egg mass.

TABLE 2. Exponents (b) and 95% confidence intervals (CI) of the allometric relationships ($y = a \times x^b$) between the mass of egg components and the mass of whole Glaucous Gull eggs ($n = 31$).

	b	CI	t [a]	P
Wet yolk	0.836	0.429 to 1.243	–0.804	0.428
Dry yolk	0.564	0.079 to 1.048	–1.857	0.073
Wet albumen	1.068	0.901 to 1.234	0.859	0.397
Dry albumen	1.745	0.749 to 2.740	1.520	0.139
Yolk lipids	0.640	0.188 to 1.092	–1.630	0.114
Shell	0.990	0.652 to 1.327	–0.061	0.952

[a]Student's t test for a difference from 1.

POP concentrations in yolk and female plasma.—In both yolk and plasma samples, ΣPCB and ΣDDT were the POP classes present in the highest concentrations, followed by ΣCHL, ΣCBz, and ΣPBDE. The POP classes ΣMeO-PBDE, total-(α)-HBCD, and OCS were present in the lowest concentrations (Table 1). Within individuals, POP concentrations (per g wet weight) were, on average, 22.8 ± 3.4 ($n = 8$ POP classes) times higher in yolk than in female plasma. With the exception of total-(α)-HBCD, the yolk concentrations of all POP classes included here were positively correlated with their concentrations in the plasma of the egg-laying females (Pearson product-moment correlation, all $P < 0.027$; Table 3).

The PCAs used to describe patterns of contamination in yolk samples, plasma samples, and yolk plus plasma samples each resulted in three PCs with eigenvalues greater than one. The three PCs explained ≥78% of the total variance (Table 4). In all cases, the first principal component (PC1) had a general character with moderately high, predominantly positive factor loadings for several POP classes (ΣCBz, ΣPBDE, total-[α]-HBCD, ΣMeO-PBDE, and OCS). The second principal component (PC2) was associated with relatively low proportions of ΣPCB combined with relatively high proportions of ΣDDT, except when only female plasma samples were analyzed, in which case high scores of PC2 were associated with relatively high proportions of ΣPCB and relatively low

TABLE 3. Pearson correlation coefficients and 95% confidence intervals (CI) that resulted from a within-female comparison of POP concentrations in maternal blood plasma and yolk ($n = 11$).

	r	CI	P
ΣPCB	0.881	0.593 to 0.969	<0.001 [a]
ΣDDT	0.740	0.247 to 0.928	0.009 [a]
ΣCHL	0.771	0.315 to 0.938	0.005 [a]
ΣCBz	0.849	0.504 to 0.906	0.001 [a]
ΣPBDE	0.796	0.372 to 0.956	0.003 [a]
Total-(α)-HBCD	0.422	–0.212 to 0.817	0.196
ΣMeO-PBDE	0.773	0.320 to 0.939	0.005 [a]
OCS	0.661	0.097 to 0.904	0.027

[a]Statistically significant after sequential Bonferroni correction for a table-wide $\alpha = 0.05$.

TABLE 4. Factor loadings of principal components (PCs) extracted from arcsine-transformed proportions of the concentrations of eight POP classes in relation to the summed concentration of all POP classes: (A) blood plasma of female Glaucous Gulls, (B) yolk of Glaucous Gull eggs, and (C) combination of female Glaucous Gulls with their respective eggs. The percentage of the explained variation of the PCs is indicated in parentheses.

	(A) Female plasma samples ($n = 11$)			(B) Yolk samples ($n = 31$)			(C) Plasma and yolk samples ($n = 22$)		
	PC1 (33%)	PC2 (30%)	PC3 (18%)	PC1 (43%)	PC2 (27%)	PC3 (13%)	PC1 (32%)	PC2 (30%)	PC3 (16%)
ΣPCB	−0.318	0.541	−0.118	0.136	−0.643	0.050	0.220	−0.587	−0.112
ΣDDT	0.247	−0.562	−0.022	−0.258	0.566	−0.127	−0.288	0.538	0.004
ΣCHL	−0.010	−0.308	0.614	0.037	0.371	0.698	−0.202	0.220	0.626
ΣCBz	0.460	−0.092	−0.435	0.478	0.165	−0.263	0.270	0.376	−0.458
ΣPBDE	0.530	0.085	−0.127	0.411	0.261	−0.002	0.376	0.396	−0.068
Total-(α)-HBCD	0.250	0.173	0.568	0.469	0.025	0.114	0.376	−0.036	0.537
ΣMeO-PBDE	0.336	0.386	0.283	0.263	−0.137	0.568	0.456	0.068	0.297
OCS	0.412	0.320	0.011	0.476	0.121	−0.299	0.515	0.118	−0.068

proportions of ΣDDT. The third principal component (PC3) was associated with relatively high proportions of ΣCHL and total-(α)-HBCD, except when only yolk samples were analyzed, in which case high scores for PC3 were associated with relatively high proportions of ΣCHL and ΣMeO-PBDE (Table 4).

Female POP class proportions were not related to the date of clutch completion (linear regression, all $P > 0.45$). However, the pattern of yolk POP class proportions changed throughout the season. Eggs that were laid later in the season had significantly lower scores for yolk PC1 (linear regression, $F = 11.22$, df = 1 and 29, $P = 0.002$). Yolk PC2 and PC3 did not vary with the laying date of the egg, though there was a tendency for yolk PC2 scores to be higher toward the end of the laying season (linear regression, yolk PC2: $F = 3.57$, df = 1 and 29, $P = 0.069$; yolk PC3: $F = 2.65$, df = 1 and 29, $P = 0.11$).

The relative contributions of the eight POP classes in yolk and plasma samples were more similar within than among females. This was demonstrated by positive correlations between POP patterns of female plasma and the yolk of their eggs. On the basis of PCAs of matched samples (Table 4C), positive correlations were found between plasma and yolk PC2 and PC3 (Pearson product-moment correlation, PC2: $r = 0.86$, $P < 0.001$; PC3: $r = 0.70$, $P = 0.018$). Yolk and plasma scores for PC1 were not correlated ($r = 0.53$, $P = 0.090$; Fig. 1).

Egg size and composition in relation to yolk POPs.—Of the three principal components used to describe the contaminant pattern of the yolk, only PC2 was related to whole egg mass, such that eggs with a higher score for yolk PC2 were smaller (linear regression, $F = 10.71$, df = 1 and 29, $P = 0.003$; Fig. 2). No relationship was found between whole egg mass and either yolk PC1 (linear regression, $F = 1.70$, df = 1 and 29, $P = 0.203$) or yolk PC3 (linear regression, $F = 1.17$, df = 1 and 29, $P = 0.289$).

When yolk composition was analyzed, it was found that the water content of the yolk was related to the pattern of POP class proportions in the yolk. Controlled for yolk wet mass, a positive relationship was found between the amount of water in the yolk and yolk PC1 (multiple regression, $F = 5.62$, df = 1 and 28, $P = 0.025$). The amount of water in the yolk was not associated with yolk PC2 (multiple regression, $F = 2.16$, df = 1 and 28, $P = 0.152$) or yolk PC3 (multiple regression, $F = 0.14$, df = 1 and 28, $P = 0.715$).

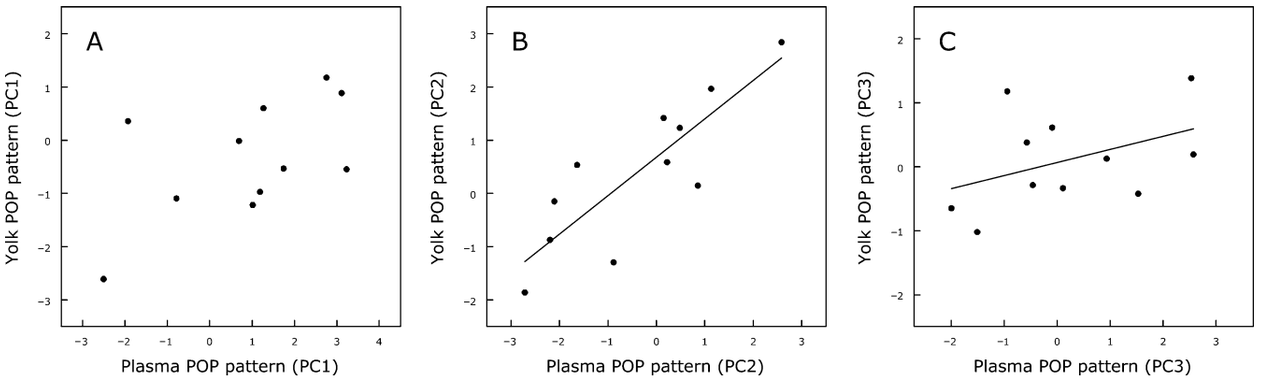

FIG. 1. Factor scores from principal component analysis (PCA). (A) PC1, (B) PC2, and (C) PC3 describe the persistent organic pollutant (POP) pattern of yolk plotted against factor scores describing the POP pattern of maternal plasma (within-female comparison) for Glaucous Gulls nesting on Bjørnøya. High scores for PC1 ($r = 0.53$, $P = 0.090$, $n = 11$) indicate relatively high concentrations of ΣMeO-PBDE and OCS. High scores for PC2 ($r = 0.86$, $P < 0.001$, $n = 11$) indicate relatively high concentrations of ΣDDT and relatively low concentrations of ΣPCB, whereas high scores for PC3 ($r = 0.70$, $P = 0.018$, $n = 11$) were associated with relatively high concentrations of ΣCHL and total-(α)-HBCD (see Table 4C for details of the PCA).

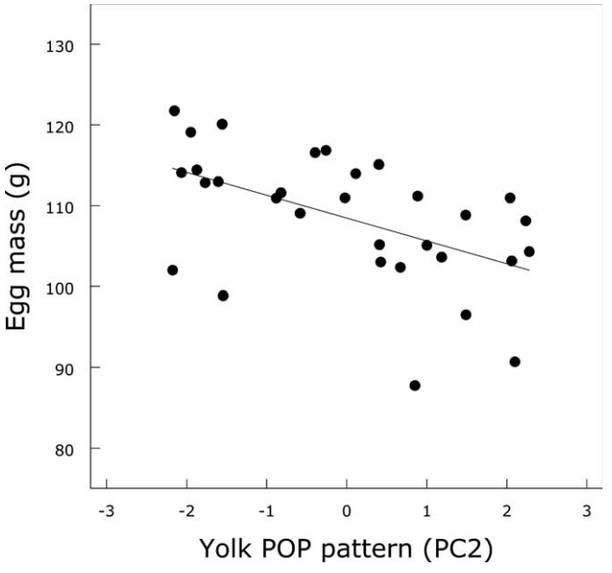

FIG. 2. Relationship between whole egg mass and factor scores (PC2) describing the persistent organic pollutant (POP) pattern of the yolk of Glaucous Gull eggs from Bjørnøya (linear regression, $F = 10.71$, df = 1 and 29, $P = 0.003$). Higher scores for yolk PC2 were associated with relatively low concentrations of ΣPCB and relatively high concentrations of ΣDDT (see Table 4B for details of the principal component analysis).

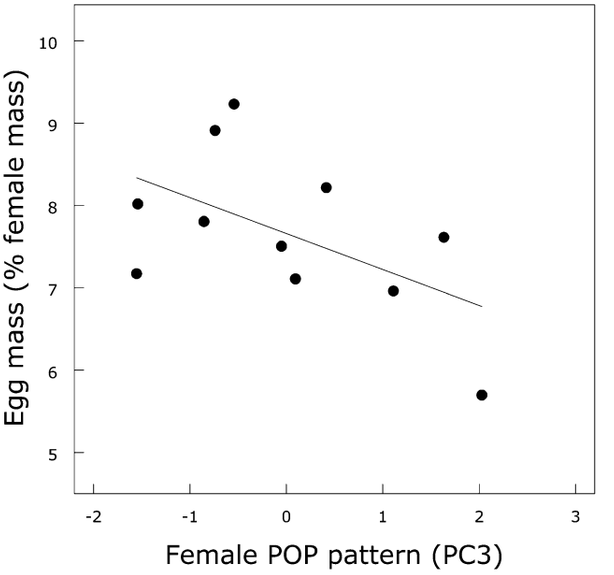

FIG. 3. Relationship between the mass of Glaucous Gull eggs, expressed as percentage of female body mass, and factor scores (PC3) describing the persistent organic pollutant (POP) pattern of maternal plasma (multiple regression, $F = 12.73$, df = 1 and 8, $P = 0.007$, controlled for female body mass). Higher scores for female PC3 were associated with relatively high concentrations of ΣCHL and total-(α)-HBCD (see Table 4A for details of the principal component analysis).

Furthermore, the positive relationship between the proportion of water in the yolk and yolk PC1 was more pronounced for smaller yolks (multiple regression, interaction yolk PC1 × yolk wet mass: $F = 9.68$, df = 1 and 27, $P = 0.004$). Controlling for laying date did not change this result ($F = 17.82$, df = 1 and 26, $P < 0.001$). No changes in albumen water content were noted in relation to yolk POP patterns (multiple regression, all $P > 0.077$).

Egg size and composition in relation to female POPs.—To investigate the relationship between egg quality and maternal contaminant exposure, we compared whole egg mass and egg composition with the plasma POP patterns of the females that had laid the eggs. This analysis revealed that whole egg mass was not associated with female PC1 (linear regression, $F = 1.28$, df = 1 and 9, $P = 0.287$) or female PC2 (linear regression, $F = 1.96$, df = 1 and 9, $P = 0.195$). Although female PC3 explained a relatively small portion (18%) of the total variance, it was negatively correlated with whole egg mass (linear regression, $F = 6.00$, df = 1 and 9, $P = 0.037$). This negative relationship remained significant when female body mass was statistically controlled ($F = 12.87$, df = 1 and 8, $P = 0.007$; Fig. 3).

The water content of the yolk was also related to the pattern of POP class proportions in female plasma. Controlled for yolk wet mass, a positive relationship was found between yolk water mass and female PC1 (multiple regression, $F = 5.72$, df = 1 and 8, $P = 0.044$; Fig. 4), but not female PC2 (multiple regression, $F = 0.33$, df = 1 and 8, $P = 0.582$) or female PC3 (multiple regression, $F = 0.91$, df = 1 and 8, $P = 0.369$). An increase in the proportion of water in the yolk was associated, though not significantly, with a decrease in lipid content (multiple regression, controlled for yolk wet mass: $F = 4.29$, df = 1 and 8, $P = 0.072$; Fig. 4). Albumen water content did not vary in relation to maternal POP patterns (multiple regression, all $P > 0.25$).

DISCUSSION

The results of the present study suggest a direct relationship between the concentrations and proportions of eight major classes of POPs in the yolk of Glaucous Gull eggs and in the plasma of egg-laying females. The results also indicated that females with relatively high proportions of ΣCHL and total-(α)-HBCD in their plasma (higher score for female PC3) laid smaller eggs. This suggests that these females allocated fewer nutrient resources to their eggs and that they invested less in egg production. Eggs into which females had deposited a relatively low amount of ΣPCB and a relatively high amount of ΣDDT (higher score for yolk PC2) were also smaller. The mass of shell, yolk, and albumen varied in proportion with whole egg mass. However, when the composition of the yolk was analyzed separately, it was found that a higher yolk water content (and, to a lesser extent, a lower yolk lipid content) was associated with contaminant patterns (both yolk and female plasma) characterized by moderately high, positive factor loadings for ΣCBz, ΣPBDE, total-(α)-HBCD, ΣMeO-PBDE, and OCS (higher score for PC1). These results suggest that female Glaucous Gulls exposed to different patterns of contamination invested differentially in egg production.

Maternal transfer of POPs into eggs.—There is little doubt that maternal transfer is responsible for the presence of contaminants

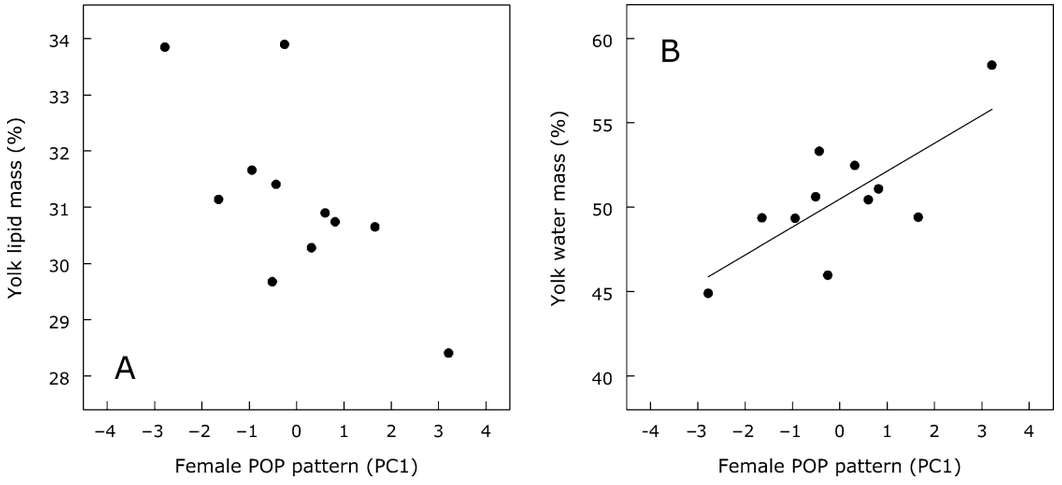

FIG. 4. Percentage of (A) extractable lipid and (B) water in the yolks of 11 Glaucous Gull eggs in relation to factor scores (PC1) describing the persistent organic pollutant (POP) pattern of maternal plasma (multiple regression, yolk lipid mass: $F = 4.29$, df = 1 and 8, $P = 0.072$; yolk water mass: $F = 5.72$, df = 1 and 8, $P = 0.044$, controlled for yolk wet mass). Higher scores for female PC1 were associated with relatively high concentrations of ΣCBz, ΣPBDE, and OCs (see Table 4A for details of the principal component analysis).

within the egg. However, how much and what part of the female contaminant burden is excreted into the egg remain poorly understood. Estimates of the proportions of the maternal contaminant burden (PCBs and DDTs) deposited in eggs vary from 1% to 22% (reviewed in Bargar et al. 2001). In experimental studies in which certain PCB congeners were administered to white Leghorn Chickens (*Gallus domesticus*) and Ringneck Doves (*Streptopelia risoria*), maternal transfer of PCBs was inversely related to the degree of chlorination and metabolic susceptibility of the chemical compounds (Bargar et al. 2001, Drouillard and Norstrom 2001). Furthermore, Verreault et al. (2006b) showed that although the concentrations of major classes of contaminants (PCBs, organochlorine pesticides and byproducts, PBDEs, and methylsulfonyl PCBs) were positively correlated between whole eggs and plasma of female Glaucous Gulls, there were marked differences in compound–congener make-up among these samples. It was found that the most recalcitrant and more highly chlorinated compounds were less readily transferred from the female to the egg. The results obtained in the present study confirm the existence of positive correlations between yolk concentrations and maternal plasma concentrations of a range of different contaminant classes with varying physico-chemical properties. This also included ΣMeO-PBDE, which was not quantified in Verreault et al.'s (2006b) study. Comparison of the proportions of these contaminant classes, rather than the specific congener–compound composition of the samples, indicated that ΣPCB and ΣDDT, the two classes of contaminants that form the bulk of the maternal contaminant burden of Glaucous Gulls from Bjørnøya, were approximately equally distributed in yolk and plasma samples. ΣCHL and total-(α)-HBCD, on the other hand, seemed to be more selectively retained by the female, given that the slope of the regression between yolk PC3 and plasma PC3 was <1.0 (Fig. 1C).

Although ΣCHL was present in the POP mixture in much lower concentrations than ΣPCB and ΣDDT, oxychlordane, which is a metabolite of *cis*- and *trans*-chlordane and a major compound of ΣCHL, was found in considerable concentrations in the brains of dead Glaucous Gulls from Svalbard and Bjørnøya (Gabrielsen et al. 1995). Moreover, it has previously been reported that among Glaucous Gulls from Bjørnøya, individuals with a relatively high plasma concentration of oxychlordane experience negative effects on many of the fitness components studied (Bustnes et al. 2005a, Bustnes 2006). This illustrates that quantitatively minor components of the POP mixture can have a large influence on an individual's reproduction and survival. In fact, quantitatively minor POP classes, such as ΣCBz, ΣPBDE, total-(α)-HBCD, ΣMeO-PBDE, and OCS, had relatively high factor loadings for PC1. This would suggest that, despite their low concentrations, these POP classes explained an important part of the total variation in contaminant burden.

Seasonal variation in yolk POP patterns.—Eggs laid at different times in the season exhibited different POP patterns (i.e., lower scores for yolk PC1 in later-laid eggs). This could be attributable to females with different contaminant burdens laying their eggs at different times of the season. For example, Great Black-backed Gulls from northern Norway with higher blood concentrations of certain PCBs laid their eggs later in the season (Helberg et al. 2005). In the present study, seasonal variation in maternal POP patterns was not observed. It is likely, however, that the intake of contaminants by Glaucous Gulls changes over time. Throughout the year, Glaucous Gulls eat fish, amphipods, and crabs (Bakken and Tertitski 2000), but during the breeding season they may supplement their diet with eggs and chicks of other seabirds, such as Common Murre (*Uria aalge*), Thick-billed Murre (*U. lomvia*), Black-legged Kittiwake (*Rissa tridactyla*), Northern Fulmar (*Fulmaris glacialis*), and Dovekie (*Alle alle*). Hence, depending on the relative timing of breeding of Glaucous Gulls and other seabirds, Glaucous Gulls may forage at different trophic levels and, thus, be exposed to different POPs in different concentrations, which could be reflected in the POP patterns found in the yolk of their eggs.

Maternal POP transfer in relation to egg size and composition.—Lemmetyinen et al. (1982) suggested that the bigger the eggs a female lays, the more contaminants she can pass on to each offspring. This would be the case if the transfer of lipophilic contaminants was regulated by a passive partitioning process (Russell et al. 1999) and if egg composition, in particular the lipid content, remained constant (i.e., the amount of lipid increases in proportion with egg size). Our results suggest that egg size and maternal contaminant burden are related, because the relative contributions of ΣCHL and total-(α)-HBCD in female plasma and the relative contributions of ΣPCB and ΣDDT in yolk were associated with differences in whole egg mass. On the other hand, relatively high proportions of quantitatively minor POP classes, such as ΣCBz, ΣPBDE, total-(α)-HBCD, ΣMeO-PBDE, and OCS in yolk and plasma were associated with a shift in the lipid–water balance of the yolk. Whether or not these changes influence the partitioning of contaminants into the yolk may depend on the presence of other contaminants and their chemical interactions (Bargar et al. 2001), as well as the lipid solubility of the POPs involved. In fact, lipid solubility and protein association have been identified as important contaminant characteristics that influence the distribution of certain POP classes (e.g., hydroxylated PCBs, total-[α]-HBCD, and PBDEs) among blood and tissues of adult Glaucous Gulls from the Norwegian Arctic (Verreault et al. 2007c). Presumably, these characteristics also play a role in maternal transfer of contaminants.

POP-related changes in the egg formation process.—Certain anthropogenic chemicals are known to have estrogenic or antiestrogenic effects, and it is possible that maternal exposure to such chemicals may influence the egg formation process. During egg formation, the ovaries produce estrogens, which stimulate the liver to produce the yolk precursors vitellogenin and very low-density lipoprotein (Etches 1996, Walzem et al. 1999), which form the primary sources of yolk lipid and protein, respectively. The synthesis and secretion of albumen by the oviduct is also, in part, controlled by estrogens (Brant and Nalbandov 1956, Palmiter 1971). Disruption of the endocrine processes involved in egg production could affect resource-allocation decisions within the egg-laying female, resulting in eggs of different size and composition.

However, although contaminant-related changes in egg size or composition could be attributable to direct toxic effects, other factors associated with POP exposure cannot be ruled out. During egg formation, female birds rely on both endogenous and external resources (Houston et al. 1983, Reynolds et al. 2003). It has previously been suggested that POP exposure is associated with, for example, poor body condition (Sagerup et al. 2000, Bustnes et al. 2004), differences in foraging strategies (e.g., Bustnes et al. 2000), and variation in clutch initiation date (Helberg et al. 2005, present study). Maternal body reserves and current food intake are likely to be affected by POP exposure, and this suggests an indirect effect of POP exposure on egg size or composition through modification of the amount or quality of nutrient resources available for egg formation.

Consequences for offspring performance.—Studies in which the effect of parental rearing capacity on offspring performance has been controlled for have demonstrated that chicks that hatch from larger eggs have a developmental advantage over chicks that hatch from smaller eggs (Reid and Boersma 1990, Amundsen et al. 1996, Hipfner and Gaston 1999). Our results would thus suggest that offspring performance may be impaired by a contaminant-related reduction in egg size, over and above potential negative effects of *in-ovo* POP exposure. It is possible, however, that the effects of egg size on offspring performance are mediated through changes in nutrient content, independently of changes in egg size. The contaminant-related changes in the lipid–water balance of the yolk observed in the present study indicate that POP exposure can induce nutrient changes inside the egg that are not reflected in changes in egg size. In addition, there is evidence to suggest that POP-exposed female Glaucous Gulls from Bjørnøya also alter other egg components, for example, yolk steroids (Verboven et al. 2008), which could also influence offspring performance. Cross-foster experiments have rarely been conducted using eggs of birds that vary in POP exposure (but see Kubiak et al. 1989). Although contaminant-related adverse effects on the proportion of eggs hatched, hatchling body condition, and offspring growth have been reported in Glaucous Gulls from Bjørnøya (Bustnes et al. 2003, 2005a), it is usually not possible to disentangle adverse effects of poor egg quality from, for example, impaired incubation behavior of POP-exposed parents (Bustnes et al. 2001a, Verboven et al. 2009).

Use of eggs as bioindicators for environmental pollution.—Monitoring of POPs in the environment is of great importance because of their bioaccumulative potential and effects on the health of humans and wildlife (Vos et al. 2000). It is generally thought that contaminants in birds' eggs correspond with the contaminant burden in avian wildlife and, as such, birds' eggs have frequently been used as biomonitoring tools (Ormerod and Tyler 1990, Norstrom and Hebert 2006, Gauthier et al. 2007). Our results suggest that, although the specific compound–congener distributions may differ (Verreault et al. 2006b), Glaucous Gull eggs reflect maternal contaminant patterns, as far as proportions of major contaminant classes are concerned. Prior to contaminant quantification, whole eggs, or a pool of whole eggs, are often homogenized and contaminant concentrations are expressed either on a wet-weight basis or on a lipid-weight basis. Several studies showed that POP concentrations do not vary among different eggs from the same clutch (Custer et al. 1990, Van den Steen et al. 2006, Verreault et al. 2006b). These authors argued that in the absence of a laying-order effect, a specific strategy for egg sampling was not required and that contaminant analyses in a single egg would be representative of the entire clutch. However, egg size and yolk lipid content often vary with the position of an egg in the laying order (Alisauskas 1986, Meathrel et al. 1987, Nager et al. 2000). Moreover, our results suggest that these egg characteristics may also vary in relation to the actual pattern of contaminant exposure experienced by the egg-laying female. Therefore, extrapolation of the POP concentrations in eggs to a value for female body burden should be performed with caution, taking into account contaminant-related differences in egg size and lipid content.

Acknowledgments

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