



Eggshell thinning and decreased concentrations of vitamin E are associated with contaminants in eggs of ivory gulls

Cecilie Miljeteig^{a,b,*}, Geir Wing Gabrielsen^b, Hallvard Strøm^b, Maria V. Gavrilo^c, Elisabeth Lie^d, Bjørn Munro Jensen^a

^a Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway

^b Norwegian Polar Institute, Framsenteret, 9296 Tromsø, Norway

^c Arctic and Antarctic Research Institute, St. Petersburg, Russia

^d Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, PO Box 8146 Dep., 0033 Oslo, Norway

ARTICLE INFO

Article history:

Received 27 October 2011

Received in revised form 3 May 2012

Accepted 7 May 2012

Available online 4 June 2012

Keywords:

Pagophila eburnea

Retinol

α -Tocopherol

Organochlorines

Brominated flame retardants

Perfluorinated alkyl substances

ABSTRACT

The ivory gull is a high Arctic seabird species threatened by climate change and contaminant exposure. High levels of contaminants have been reported in ivory gull *Pagophila eburnea* eggs from Svalbard and the Russian Arctic. The present study investigated associations between high levels of contaminants (organochlorinated pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), perfluorinated alkyl substances (PFASs) and mercury (Hg)) and three response variables: eggshell thickness, retinol (vitamin A) and α -tocopherol (vitamin E). Negative associations were found between levels of OCPs, PCBs and BFRs and eggshell thickness ($p < 0.021$) and α -tocopherol ($p < 0.023$), but not with retinol ($p > 0.1$). There were no associations between PFASs and mercury and the three response variables. Furthermore, the eggshell thickness was 7–17% thinner in the present study than in archived ivory gull eggs (≤ 1930). In general, a thinning above 16 to 20% has been associated with a decline in bird populations, suggesting that contaminant-induced eggshell thinning may constitute a serious threat to ivory gull populations globally.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

The ivory gull *Pagophila eburnea*, a high Arctic seabird species associated with sea ice throughout the year, is one of the most poorly known seabird species in the world (Mallory et al., 2008). On average, the ivory gull is the northernmost breeding bird species (Blomqvist and Elander, 1981), with scattered colonies in the Canadian Arctic, Greenland, Svalbard and Russia (Strøm, 2006). Recent population estimates suggest between 8000 and 11,500 breeding pairs globally, with about 86% breeding in the Russian Arctic (Gilchrist et al., 2008), making it a rare species. In the Canadian Arctic, the ivory gull population has declined by 80% since the 1980s (Gilchrist and Mallory, 2005) and is at risk of local extirpation across much of its breeding range (Robertson et al., 2007). The population also seems to be declining in the southern parts of its Greenland breeding range (Gjlg et al., 2009), whereas the population status in northern Greenland, Svalbard and Russia is uncertain due to lack of historical data. The ivory gull is classified as near threatened on the IUCN Red List of Threatened Species. Global warming and pollution have been identified as the major threats to the species (IUCN, 2010).

The ivory gull, feed on fish and amphipods, and on carcasses and remains from polar bear kills (Mallory et al., 2008), and has a relatively high trophic position during part of the year (Karnovskiy et al., 2009). The species therefore accumulates high levels of persistent contaminants through biomagnification (Fisk et al., 2001; Hobson et al., 2002; Buckman et al., 2004; Braune et al., 2007; Miljeteig et al., 2009). Indeed, the levels of organochlorinated pesticides (OCPs), polychlorinated biphenyls (PCBs) and mercury (Hg) in ivory gulls are among the highest ever reported in Arctic seabirds (Braune et al., 2006; Miljeteig et al., 2009). The levels of brominated flame retardants (BFRs) and perfluorinated alkyl substances (PFASs) reported in ivory gull eggs from the Norwegian and Russian Arctic were similar to those reported in other Arctic seabirds (Miljeteig et al., 2009).

Contaminants that originate from human activity, such as halogenated organic compounds (HOCs) and heavy metals, interfere with a range of cellular and physiological processes in living organisms (Kendall et al., 2001). Depending on the dose, these effects cause reduced fitness, and may thus affect population dynamics and ecosystem structure and function. In several Arctic top predators, high levels of anthropogenic contaminants have been linked to endocrine, reproductive and behavioural effects (Bustnes et al., 2001; Bustnes et al., 2002; Haave et al., 2003; Olsen et al., 2003; Braathen et al., 2004; Verreault et al., 2004; Villanger et al., 2011). High levels of OCPs have also been associated with eggshell thinning in Greenlandic peregrine falcons *Falco peregrinus tundrius* (Falk et al., 2006). Anthropogenic contaminants are therefore considered to

* Corresponding author at: Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway. Tel.: +47 73595000; fax: +47 73596311.
E-mail address: cecilie.miljeteig@bio.ntnu.no (C. Miljeteig).

be a major threat to Arctic top predators (Jenssen, 2006; Gabrielsen, 2007; Letcher et al., 2010).

Eggshell thinning is a well-documented ecological significant effect of contaminant exposure (e.g. Ratcliffe, 1967; Cooke, 1973; Blus et al., 1997). Eggshell thinning has been attributed to a range of contaminants, such as OCPs, PCBs, BFRs and mercury (e.g. Cooke, 1973; Wiemeyer et al., 1984; Mason et al., 1997; Pain et al., 1999; Fernie et al., 2009). The effects of PFASs on eggshell thickness appear to be unknown. As eggshell thinning significantly reduces the survival of the embryos and the hatchability, contaminant-induced eggshell thinning resulted in major population declines among birds of prey in Europe and North America after 1945 (Walker et al., 2001). Thus, contaminant-induced eggshell thinning is considered to be a major threat to populations of avian top predators. Ivory gulls have high levels of a range of contaminants (Miljeteig et al., 2009), and several of these have been reported to have eggshell thinning properties.

Anthropogenic contaminants have also been reported to cause developmental effects and high embryonic and chick mortality in birds (Barron et al., 1995; Fernie et al., 2003). Many of these effects have been linked to the endocrine disruptive properties of contaminants. Vitamin A (retinoids) and vitamin E (tocopherols) have essential roles in embryonic development (Blomhoff, 1994), and HOCs have been reported to interfere with the homeostasis of these vitamins in bird embryos and chicks (e.g. Rolland, 2000; Champoux et al., 2006; Murvoll et al., 2006b). Many of the contaminants that are found in high concentrations in ivory gull eggs (Miljeteig et al., 2009) have been associated with vitamin disruptive effects (Zile et al., 1997; Champoux et al., 2006; Murvoll et al., 2007). Thus, the high levels of HOCs reported in ivory gull eggs may affect the homeostasis of these important vitamins, and thereby affect hatchability and chick survival.

The aim of the present study was to examine if the high levels of OCPs, PCBs, BFRs, PFASs and mercury reported in ivory gull eggs from the Norwegian and Russian Arctic cause eggshell thinning and disruption of embryonic vitamin A and E homeostasis. Contaminant-induced effects on these variables may be important in explaining the apparent decline in ivory gull populations (Gilchrist and Mallory, 2005; Robertson et al., 2007; Gilg et al., 2009). Contaminant concentrations in ivory gull eggs were reported in Miljeteig et al. (2009). In the present study we have applied these results to investigate effects on the ecological significant response variables eggshell thickness and embryonic vitamin A and E status.

2. Materials and methods

2.1. Sample collection and preparation

A total of 35 eggs were sampled from individual ivory gull nests within four colonies in Svalbard: Svenskøya (78°47'N, 26°36'E; n=10) in 2007, and in north-western Russia in 2006; Nagurskoe (80°48'N, 47°37'E; n=6) and Cape Klyuv (81°39'N, 62°11'E; n=7) in Franz Josef Land and Domashny (79°30'N, 91°05'E; n=12) in Severnaya Zemlya (Fig. 1). One egg was sampled from each nest and clutch size was noted for all sampled nests. However, to minimise disturbance of the nesting birds, the egg laying sequence was not determined and eggs were taken randomly from each nest. The eggs were individually wrapped in aluminium foil and stored frozen until further analyses. During preparation of the samples, the eggshell was thoroughly removed and the embryo was removed from the thawed egg and weighed when present. The mass of the embryos varied from 0 g (when not present) to 27.5 g. Due this large variation, the mass of the embryos were used as a proxy for their developmental stage. Subsequently, the whole egg content (including the embryo) was homogenised individually using a food blender (Melissa, Adexi group, Risskov, Denmark or Waring Commercial Laboratory Blender, Waring Laboratory, Torrington, CT, USA). The homogenates were separated

into aliquots for different analyses and stored at -20°C until analysed at the respective laboratories. Homogenates for vitamin analyses were kept in cryo tubes wrapped in aluminium foil to prevent light degradation of vitamins.

2.2. Eggshell thickness and vitamin analyses

The inner membrane of the eggshell was removed from the shell using running tap water and the eggshell was left to dry at room temperature for a minimum of two weeks. Eggshell thickness was measured at or near the equator using a spring-loaded micrometer with an accuracy of 0.01 mm (Vikøren and Stuve, 1996). The mean of four different measurements along the equator was recorded as the eggshell thickness. The coefficient of variation (CV%) for eggshell thickness was below 4% for the four parallel measurements.

The vitamin analyses presented herein are only for the eggs sampled in Russia (n=25). The vitamin analyses were conducted at the Department of Biology, Norwegian University of Science and Technology (Trondheim, Norway). The extraction of retinol and α -tocopherol was conducted in red light to prevent light degradation of the vitamins. Egg homogenate was extracted with hexane and retinyl acetate (internal standard). A stainless steel bead (d=5 mm; Qiagen GmbH, Hilden, Germany) was added to the sample for further homogenisation with a Qiagen TissueLyser (3 min; Qiagen GmbH). The sample was sonicated on a high intensity ultrasonic processor GEX400 (four microtips; 38% amplitude; Sonics and Materials, Inc., Newtown, CT, USA) set to give pulses for 2 s, followed by 0.5 s with no pulse, with a total pulse time of 1.5 min. After sonication the sample was centrifuged and the hexane layer was transferred to a minisorb tube. Hexane and retinyl acetate were added to the sample and the extraction procedure repeated twice, thus, each sample was extracted three times. The extract was evaporated to dryness and mobile phase (98:2% methanol:water) was added.

The concentrations of retinol and α -tocopherol were determined by high-performance liquid chromatography (HPLC; PerkinElmer 200 Series, Waltham, MA, USA). The detection limit was defined as three times the background noise level with two times standard deviation (SD), and was 37.2 $\mu\text{g/L}$ for retinol and ranged from 177 to 348 $\mu\text{g/L}$ for α -tocopherol. All samples were above the detection limit. All samples were extracted and analysed in duplicate or triplicate and the coefficient of variation (CV%) for the parallel samples was <20% and <15% for retinol and α -tocopherol, respectively. The retinol data is based on one extraction series and one HPLC run, thus with no between-run variation. The α -tocopherol data is based on three runs. A hen egg control sample, analysed in minimum two parallels per run, was used as an interassay control and gave a CV% of <9%. More details on the extraction procedure, quantification and quality control are given in Murvoll et al. (2005).

2.3. Contaminant analyses

Following homogenisation and extraction, concentrations of organochlorinated pesticides (dichlorodiphenyltrichloroethane [*p,p'*-DDE], *p,p'*-DDT], chlordanes [oxychlordane, *trans*-nonachlor], β -hexachlorocyclohexane [β -HCH], mirex, hexachlorobenzene [HCB] and toxaphene [CHB-26, -40, -41, -44, -50 and -62]), polychlorinated biphenyls [PCB-28, -47, -52, -66, -74, -99, -101, -105, -114, -118, -128, -137, -138, -141, -149, -151, -153, -156, -157, -170, -180, -183, -187, -189, -194, -196 and -206], brominated flame retardants (hexabromocyclododecane [sum of α -, β - and γ -HBCD] and polybrominated diphenyl ethers [BDE-28, -47, -99, -100, -153 and -154]), perfluorinated alkyl substances (perfluorononanoate [PFNA], perfluorodecanoate [PFDA], perfluoroundecanoate [PFUnA], perfluorododecanoate [PFDoA], perfluorotridecanoate [PFTriA], perfluorotetradecanoate [PFTeA], perfluoropentadecanoate [PFPeDA],

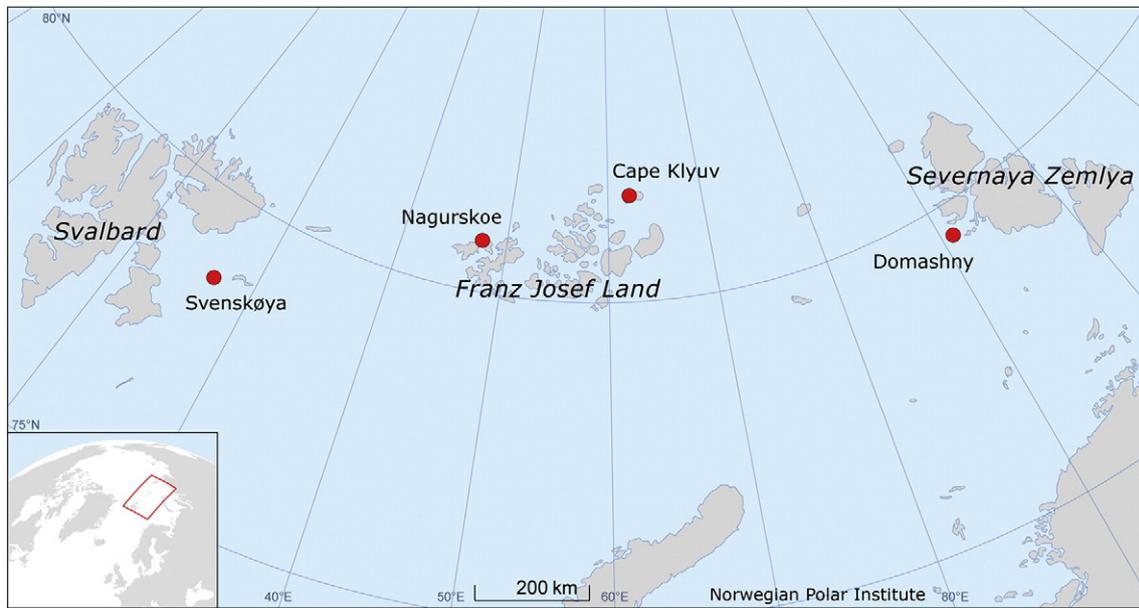


Fig. 1. Map of Svalbard (Norway) and the western Russian Arctic. Ivory gull *Pagophila eburnea* eggs were sampled from colonies on Svinskøya in Svalbard, Nagurskoe and Cape Klyuv in Franz Josef Land and Domashny Island in Severnaya Zemlya.

perfluorohexane sulfonate [PFHxS], perfluorooctane sulfonate [PFOS] and perfluorodecane sulfonate [PFDCS] and mercury were determined.

The chemical analyses of organochlorines (OCs) and BFRs were conducted at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science (Oslo, Norway). The laboratory is accredited for the analyses by Norwegian Accreditation (Kjeller, Norway) according to NS-EN ISO/IEC 17025 (Test 137), and the analytical quality of the laboratory has been approved in several international intercalibration tests. The analyses of PFASs were performed by the Analytical Environmental Chemistry Unit at Stockholm University (Sweden). The analyses of mercury were conducted by the National Veterinary Institute (Oslo, Norway). Full details on the contaminant analyses and the results are reported in Miljeteig et al. (2009).

2.4. Statistical analyses

The results were analysed using principal component analysis (PCA) based on the contaminant variables on wet weight (w.w.) values. To examine the association between the contaminants and the three response variables (eggshell thickness, retinol and α -tocopherol), associations were tested between the PCs and the response variables using Spearman's rank correlation.

3. Results and discussion

Two PCA analyses based on the contaminant variables on wet weight values were conducted, one with the data from Svalbard and the Russian Arctic combined, and one with data only from the Russian

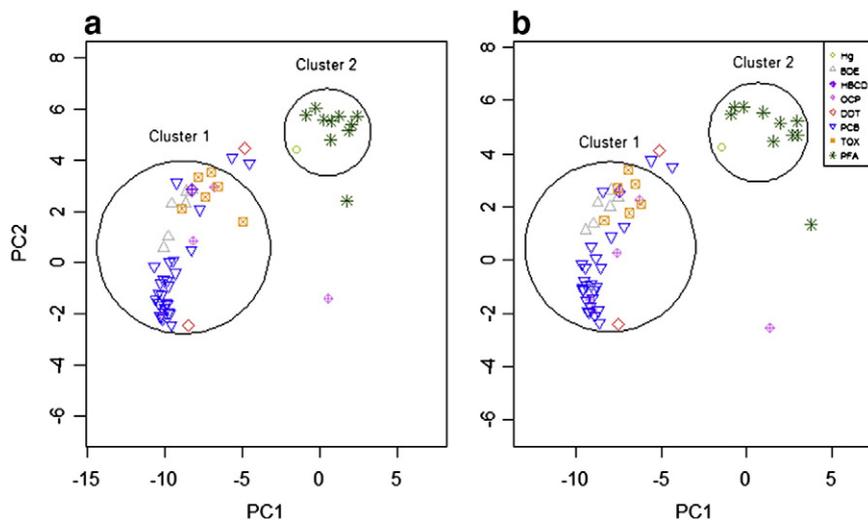


Fig. 2. Principal component analysis (PCA) of contaminants measured in ivory gull *Pagophila eburnea* eggs from (a) Svalbard (Norway) and the Russian Arctic ($n = 35$) and (b) the Russian Arctic ($n = 25$) used for testing associations with eggshell thickness and vitamins, respectively. In (a) PC1 explained 52% and PC2 explained 17% of the variance and in (b) PC1 explained 55% and PC2 explained 19% of the variance. The analysis is based on wet weight values. Low loadings on PC1 are associated with high concentrations of organochlorines and brominated flame retardants. High loadings on PC2 are associated with high concentrations of PFASs. Cluster 1 (a and b) contains *p,p'*-DDE, oxychlorodane, *trans*-nonachlor, mirex, HCB, CHB-26, -40, -41, -44, -50 and -62, PCB-28, -47, -66, -74, -99, -101, -105, -114, -118, -128, -137, -138, -141, -149, -153, -156, -157, -170, -180, -183, -187, -189, -194, -196 and -206, HBCD, BDE-28, -47, -99, -100, -153 and -154. Cluster 2 (a and b) contains PFDCa, PFUnA, PFDoA, PFTriA, PFTeA, PFPeDA, PFHxS, PFOS, PFDCs and mercury. β -HCH, PFNA, *p,p'*-DDT and PCB-52 and -151 are found outside the main clusters.

Table 1

Arithmetic mean with standard deviation (SD) and ranges (min-max) for eggshell thickness (mm) and retinol and α -tocopherol (ng/g) concentrations analysed in ivory gull *Pagophila eburnea* eggs from the Svenskøya colony (Svalbard) and the Nagurskoe, Cape Klyuv and Domashny colonies (Russian Arctic). na denotes not analysed.

		Svenskøya (n = 10)	Nagurskoe (n = 6)	Cape Klyuv (n = 7)	Domashny (n = 12)
Eggshell thickness (mm)	Mean \pm SD	0.246 \pm 0.018	0.234 \pm 0.013	0.261 \pm 0.045	0.245 \pm 0.013
	Min-max	0.206–0.270	0.221–0.251	0.220–0.356	0.226–0.270
Retinol (ng/g)	Mean \pm SD	na	1.8 \pm 0.4	1.5 \pm 0.6	1.2 \pm 0.2
	Min-max	na	1.4–2.3	1.0–2.6	0.9–1.6
α -Tocopherol (ng/g)	Mean \pm SD	na	17.9 \pm 8.4	11.0 \pm 10.2	23.7 \pm 13.7
	Min-max	na	3.6–28.2	1.5–23.4	1.6–50.4

Arctic (Fig. 2). The three first principal components (PCs) were extracted in both PCAs. In the PCA for Svalbard and the Russian Arctic birds combined, PC1 explained 52%, PC2 explained 17% and PC3 explained 9% of the variance in the data set. In the PCA for only the ivory gulls from the Russian Arctic, PC1 explained 55%, PC2 explained 19% and PC3 explained 9%.

3.1. Eggshell thickness

The eggshell thickness of the ivory gull eggs from the four colonies in the Norwegian and Russian Arctic is given in Table 1. There was a significant correlation between eggshell thickness and PC1 (Spearman correlation; $r_s = 0.389$, $p < 0.021$, $n = 35$) (Fig. 3). No relationship

was found between eggshell thickness and PC2 or PC3. The majority of the detected PCBs (except for PCB-52 and -151), OCPs (except for β -HCH, CHB-40 and p,p' -DDT) and all BFRs had a high loading along PC1, whereas PFASs, mercury and p,p' -DDT had a high loading along PC2. Thus, eggshell thickness was negatively associated with most OCs and BFRs. No association was found between eggshell thickness and PFASs or mercury (PC2). An attempt was made to separate the effects of the different compounds on eggshell thickness using projection to latent structure (PLS, Unscrambler version 9.2, Camo AS, Oslo, Norway). However, because the covariations among the OCPs, PCBs and BFRs were strong, no significant models were found. Thus, it was not possible to separate the effects of the individual compounds.

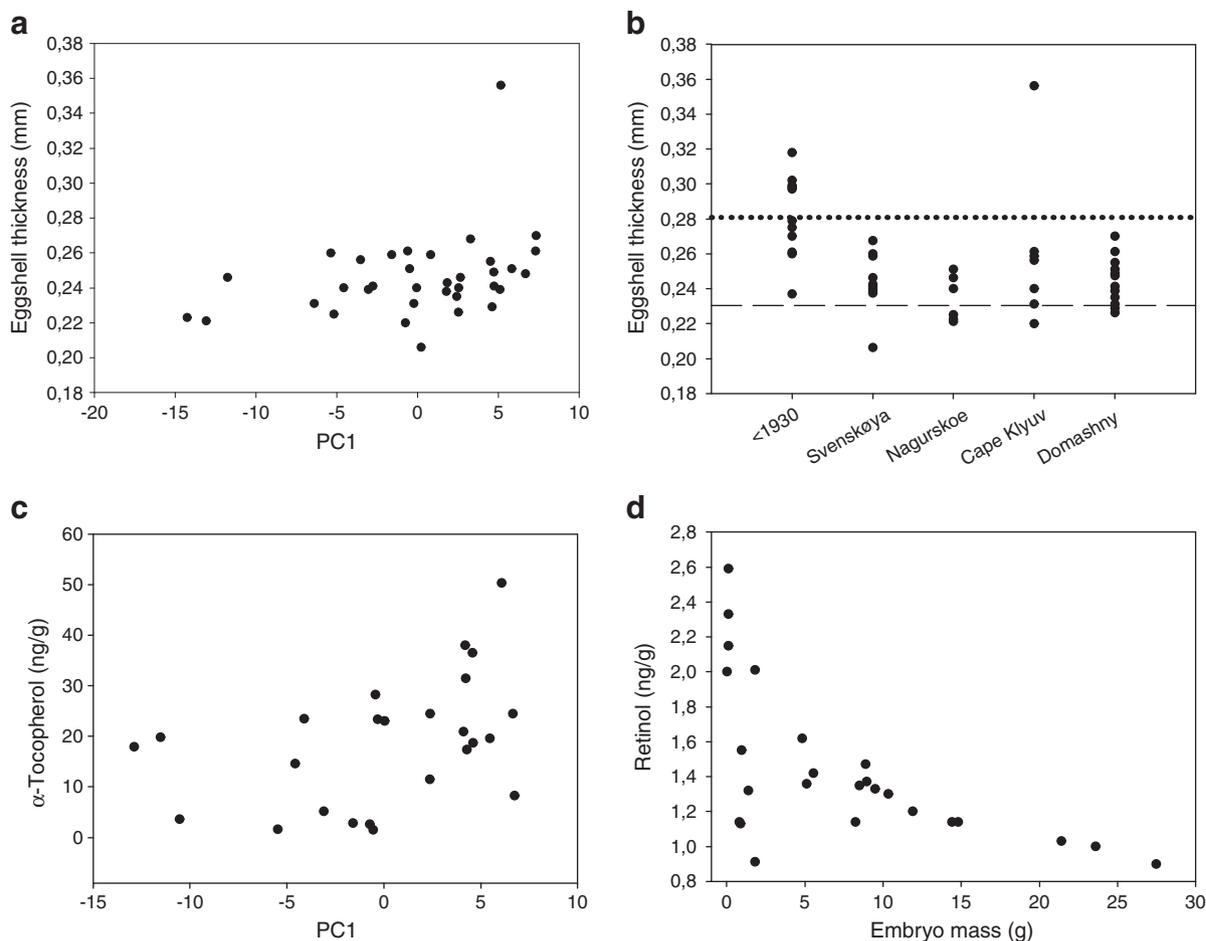


Fig. 3. a) A significant association between eggshell thickness and principal component 1 (PC1; see Fig. 2a) in ivory gull *Pagophila eburnea* eggs from the Norwegian and Russian Arctic was found (Spearman correlation; $r_s = 0.389$, $p < 0.021$; $n = 35$). b) Eggshell thickness in archived ivory gull eggs (Western Foundation of Vertebrate Zoology; ≤ 1930) and in ivory gull eggs from four colonies in the Norwegian and Russian Arctic (2007 and 2008). The dotted line indicates the mean of the archived eggs, the dashed line indicate 18% eggshell thinning below the average of the archived eggs. c) A significant association between α -tocopherol concentrations and PC1 (see Fig. 2b) in ivory gull eggs from the Russian Arctic was found (Spearman correlation; $r_s = 0.457$, $p < 0.023$; $n = 25$). d) A significant negative association between retinol concentrations and embryo mass (as proxy of developmental stage) in ivory gull eggs from the Russian Arctic was found (Spearman correlation; $r_s = -0.619$, $p < 0.00096$, $n = 25$).

Several of the contaminants with high loading along PC1, such as PCBs, *p,p'*-DDE, dieldrin, HCB, mirex and oxychlorane, have been shown to be associated with eggshell thinning (Ratcliffe, 1967; Cooke, 1973; Wiemeyer et al., 1984; Lowe and Stendell, 1991; Mason et al., 1997; Nygård, 1999). Thus, the results of the present study are in accordance with numerous laboratory and field studies.

For some bird species, the eggshell thickness changes in relation to the stage of incubation. This should therefore be taken into consideration when determining eggshell thickness in relation to contaminant exposures (Peterle, 1991). In the present study we did not have information on the stage of incubation. However, embryo mass was recorded, and varied from 0 g (when not present) to 27.5 g. In the present study, embryo mass was therefore used as a proxy for developmental stage. There was no association between eggshell thickness and embryo mass (Spearman correlation; $p > 0.8$). It should also be noted that there were no associations between embryo mass and egg volume (as calculated according to Hoyt, 1979; Spearman correlation; $p > 0.5$), or between eggshell index and egg volume (Spearman correlation; $p > 0.5$). However, although the results indicate that there was no association between the developmental stage and the eggshell thickness in ivory gulls, this needs to be verified.

Eggshell formation is a complicated process with many steps where disruption may lead to alterations in eggshell thickness. *p,p'*-DDE, which had a high loading along PC1, is believed to influence eggshell thickness through inhibition of prostaglandin synthesis in the eggshell gland mucosa and thereby reduce the transfer of bicarbonate and calcium to the eggshell gland lumen (Lundholm, 1997), the site of the shell calcification (Cooke, 1973). Calcium availability is essential in eggshell formation, and any substances disrupting calcium homeostasis from absorption in the gut to deposition in the eggshell gland lumen may potentially influence the eggshell thickness (Cooke, 1973). Several steps in egg laying and eggshell formation are under strong hormonal control. Thus, compounds influencing oestrogens, androgens and thyroid hormones may also alter the eggshell thickness (Cooke, 1973). PCBs, BDEs and several OCPs have been demonstrated to be endocrine disrupters (Colborn, 1993; Hewitt and Servos, 2001) and may thus influence eggshell thickness through these pathways. However, it is generally agreed that the main compound responsible for eggshell thinning in birds is *p,p'*-DDE (Lundholm, 1997).

Eggshell thinning was first discovered by comparing eggshells in birds of prey from the 1950s and 1960s with eggshells from 1900 to the 1940s (Ratcliffe, 1967). A dramatic decrease in eggshell thickness in birds of prey was found around 1945–47, coinciding with the introduction of DDT into general use and increase in use of PCBs (Ratcliffe, 1967). For comparison, data on eggshell thickness in ivory gulls prior to 1930s collected throughout the species' breeding range were obtained from the collections of the Western Foundation for Vertebrate Zoology (WFVZ, <http://www.wfvz.org/>, pers. comm. R. Corado). The eggshell thickness of the ivory gull eggs in the present study was 7–17% thinner than in the WFVZ archived ivory gull eggs ($n = 11$) (Fig. 3). It is possible that the differences between the eggshell thicknesses reported for the present ivory gulls and those reported for the gulls in the WFVZ collection are due to differences in measuring techniques. The eggshell thicknesses of the eggs in the WFVZ collection were measured through the blowing holes. However, according to WFVZ, most of the old collectors removed the inner membrane from all their eggs, which also was done in the present study. Thus, although there are limitations in comparing the eggshell thicknesses of the present ivory gulls with archived eggs, we suggest that the present study strongly indicates that the eggshell thickness of ivory gulls in the present study is lower than reported prior to the intensive use and global spreading of persistent organic pollutants.

The ivory gull eggs from the Nagurskoe colony, which had the highest concentrations of contaminants (Miljeteig et al., 2009), were the eggs with the thinnest eggshells (17% thinner than that in the archived eggs). Eggs from Cape Klyuv had the thickest eggshells in the

present study (7% thinner than that in the archived eggs). However, the low degree of thinning in the eggs from Cape Klyuv was mainly explained by one egg that had a considerably thicker eggshell than the other eggs (Fig. 3). When excluding this egg, the mean eggshell thickness of eggs from Cape Klyuv was similar to that of eggs from Svenskøya and Domashny, which both had eggshells that were 13% thinner than the archived eggs.

In general, eggshell thinning above 16 to 18% has been associated with declining bird populations (Walker et al., 2001). Others have suggested a critical eggshell thinning of approximately 20% for significant reductions in reproductive success (Keith and Gruchy, 1972). There seems to be no information on critical eggshell thicknesses in gulls. In herring gull (*Larus argentatus*) eggs from the Lake Erie, Canada (1978–79) with DDE concentrations from 32 to 113 $\mu\text{g/g l.w.}$, the average eggshell thinning was 6.7%, and not associated with reproductive effects (Weseloh et al., 1990). In white-tailed eagles (*Haliaeetus albicilla*) eggshell thinning became obvious when DDE levels exceeded 50 $\mu\text{g/g l.w.}$, and an 18% reduction in eggshell thickness was associated with a DDE level of 720 $\mu\text{g/g l.w.}$ in the eggs (Helander et al., 2002). Furthermore, a lowest observed effect level (LOEL) for embryo mortality in white-tailed eagles of about 120 $\mu\text{g/g l.w.}$ was suggested (Helander et al., 2002). Since gulls are moderately resistant to eggshell thinning compared to other species (Peakall, 1975) it is likely that higher loads of contaminants are required to cause severe eggshell thinning. It should therefore be noted that the median *p,p'*-DDE concentration in ivory gull eggs from the Nagurskoe colony, which had the thinnest eggshells, was 29800 $\mu\text{g/g l.w.}$ This is considerably higher than DDE levels associated with eggshell thinning in other species.

In the present study, the eggshell thinning approached the critical limit of 16–20% associated with population decline in bird populations (Keith and Gruchy, 1972; Walker et al., 2001). This indicates that a relatively large proportion of the ivory gulls in the Norwegian and Russian Arctic experience eggshell thinning due to exposure and bioaccumulation of high levels of anthropogenic contaminants. Since high levels of anthropogenic contaminants also have been reported in ivory gulls from Canada (Buckman et al., 2004; Braune et al., 2006; Braune et al., 2007) it may affect the populations of ivory gulls globally. As the critical threshold for when eggshell thinning leads to population decline has not been determined specifically for the ivory gull, the observed rates of eggshell thinning in this study (up to 17%) may already be exceeding the threshold. Hence, eggshell thinning may possibly be contributing to the observed population declines (Gilchrist and Mallory, 2005; Robertson et al., 2007; Gilg et al., 2009).

3.2. α -Tocopherol

Concentrations of α -tocopherol in ivory gull eggs from the three colonies in the Russian Arctic are given in Table 1. There was a significant correlation between α -tocopherol and PC1 (Spearman correlation; $r_s = 0.457$, $p < 0.023$) (Fig. 3), but not between α -tocopherol and PC2 or PC3. Thus, as for eggshell thickness, α -tocopherol was negatively associated with most OCPs (except for β -HCH, CHB-40 and *p,p'*-DDT) and PCB-congeners (except for PCB-52 and -151) and all BFRs. Furthermore, as for eggshell thickness, α -tocopherol was not associated with concentrations of PFASs and mercury.

The negative association between α -tocopherol and OCPs, PCBs and BFRs herein is in accordance with previous reports in birds (Murvoll et al., 2005; Murvoll et al., 2006a). An experimental study on hatchlings of ducks *Anas platyrhynchos* showed a negative association between yolk concentration of PCB-99 and hepatic α -tocopherol concentrations (Murvoll et al., 2005). In free-living European shag *Phalacrocorax aristotelis* hatchlings, a significant negative association between yolk concentrations of BDE-28 and hepatic tocopherol has been reported (Murvoll et al., 2006a). In free-living hatchlings of Brünnich's guillemot *Uria lomvia*, negative relationships were found between some OCPs in the yolk (HCB, oxychlorane and *p,p'*-DDE) and

liver α -tocopherol levels (Murvoll et al., 2007). However, the negative relationships between these OCPs and liver α -tocopherol levels in Br nnich's guillemot hatchlings became less evident when the confounding effect of liver mass was corrected for (Murvoll et al., 2007). In the present study, all these contaminants were associated with decreased concentrations of α -tocopherol in the ivory gull eggs (Fig. 3).

It should, however, also be noted that some studies on free-living hatchlings or chicks have reported no associations, or even positive associations, between hepatic concentrations of HOCs and hepatic or plasma concentrations of α -tocopherol. In 21 day old chicks of European shags, there were no associations between hepatic levels of a range of HOCs (including most of those included in the present study) and plasma α -tocopherol concentrations (Jenssen et al., 2010). A positive association between several PCBs in the yolk and α -tocopherol (liver and plasma) was reported in black-legged kittiwake *Rissa tridactyla* hatchlings (Murvoll et al., 2006b). In common eider *Somateria mollissima* hatchlings, PCB, β -HCH and oxychlorane were significantly positively related to α -tocopherol in liver (Murvoll et al., 2007). The levels of contaminants in those birds were, however, much lower than in the ivory gulls in the present study. Thus, it has been suggested that the effect of HOCs on α -tocopherol in birds may be hormetic (Murvoll et al., 2007). It is also possible that the effects of HOCs on α -tocopherol differ between tissues, such as plasma and liver. In the present study, both contaminant concentrations and α -tocopherol were analysed in whole egg content. Eggs are closed systems without excretion or uptake of any nutrients from laying till hatching, and with only gaseous exchange (Carey, 1996). Thus, the influence of contaminants on α -tocopherol concentrations may be simpler to elucidate in whole eggs than in tissues from organisms with variability in uptake and mobilisation between tissues.

α -Tocopherol is a potent antioxidant, and the proposed mechanisms for the effect of contaminants on levels of α -tocopherol are all related to the production of oxidising molecular species (Boelsterli, 2003). Tocopherols are the most abundant and efficient scavengers of hydroperoxyl radicals in biological membranes with α -tocopherol being the most important of the tocopherol homologs (Di Mascio et al., 1991). Small amounts of reactive oxygen species (ROS) are constantly generated inside cells by physiological processes, such as the mitochondrial respiratory chain (Nohl et al., 2005). Oxidative stress arises when there is an imbalance between ROS and cellular antioxidants (Boelsterli, 2003). Contaminants may enhance ROS production in several ways, i.e. by disrupting normal electron flow in the mitochondrial membrane, often leading to redox cycling, or by CYP 450 uncoupling, resulting in increased amounts of both superoxide anion and hydrogen peroxide (Boelsterli, 2003). Generally, exposure to contaminants such as PCBs is thought to lead to an increase in oxidative stress through induction of CYP 450 enzymes (e.g. Twaroski et al., 2001; Hilscherova et al., 2003; Fernie et al., 2005) and α -tocopherol supplementation has been widely shown to counteract contaminant-induced responses (e.g. Xie and Zhang, 2004; Yun et al., 2005; Banudevi et al., 2006). The scavenging of oxidising agents leads to decreased levels of α -tocopherol (Di Mascio et al., 1991), which supports the findings in the present study with decreasing concentrations of α -tocopherol with increasing levels of contamination.

The associations identified herein between the contaminants with high loadings along PC1 (e.g. PCBs, *p,p'*-DDE, oxychlorane, *trans*-nonachlor, mirex, HCB, toxaphenes and BDEs) and α -tocopherol, imply that these contaminants may have serious effects on embryo survival and even on chick survival rate in ivory gulls. However, this issue needs to be addressed in more detail before any firm conclusions can be drawn.

3.3. Retinol

Concentrations of retinol in ivory gull eggs from three colonies in the Russian Arctic are given in Table 1. No significant correlation between retinol concentrations and any of the three first PCs was

found (Spearman correlation; $p > 0.1$). However, there was a significant negative association between retinol and embryo mass (Spearman correlation; $r_s = -0.619$, $p < 0.00096$) (Fig. 3). There was no association between retinol and egg volume (Spearman correlation; $p > 0.5$) or between retinol and eggshell thickness (Spearman correlation; $p > 0.5$). Thus, retinol concentrations appeared to decrease as the embryo developed. This is in accordance with studies on great blue heron *Ardea herodias*, where negative correlations between retinol concentrations and the developmental stage of the eggs also were reported (Boily et al., 1994; Champoux et al., 2006).

Previous studies on associations between contaminant concentrations and retinol concentrations in birds have reported differing results, ranging from a borderline significant positive relationship (Murvoll et al., 1999) through lack of associations (Murvoll et al., 2006b; Murvoll et al., 2007; Jenssen et al., 2010), to negative associations (e.g. Zile et al., 1997; Kuzyk et al., 2003; Fernie et al., 2005; Champoux et al., 2006; Murvoll et al., 2006a). These differing, and apparently opposing results are most likely due to the use of different matrices (plasma, liver, yolk), indicating that effects on retinol may be tissue specific. It should also be noted that several studies have reported no associations between contaminant levels and retinol levels in eggs or yolk, but at the same time identified significant positive relationships between contaminant levels and the ratio of retinol to retinyl palmitate (e.g. Boily et al., 1994; Rolland, 2000; Champoux et al., 2006). Retinol is the mobile form of vitamin A, whereas for storage retinol is esterified to long-chain fatty acids such as retinyl palmitate (Blomhoff, 1994). Retinyl palmitate was not detectable in ivory gull eggs using the analytical method in the present study, thus, a retinol to retinyl palmitate ratio or vitamin A storage could not be evaluated. Therefore, even though no effects were reported on retinol in the present study, we cannot rule out that the vitamin A homeostasis of ivory gull embryos is affected by the examined contaminants.

3.4. Implications for the ivory gull

The ivory gull eggs in the present study contained some of the highest concentrations of OCPs and PCBs reported in Arctic seabird eggs (Miljeteig et al., 2009). The ivory gull eggshells studied herein were up to 17% (Nagurskoe) thinner than reference eggs of ivory gulls collected throughout the breeding range prior to 1930. The eggshell thickness was negatively associated with levels of several contaminants in the eggs, such as most PCBs, BDEs and toxaphenes and *p,p'*-DDE and other pesticides. Although the critical threshold level for eggshell thinning has not been determined specifically for the ivory gull, the observed eggshell thinning in the present study (up to 17%) is within or approaches the critical range (16–20%) of eggshell thinning associated with declining populations of other bird species (Keith and Gruchy, 1972; Walker et al., 2001). Since high levels of these contaminants also have been reported in other populations of ivory gulls (Buckman et al., 2004; Braune et al., 2006; Braune et al., 2007), we suggest that eggshell thinning may contribute to the observed population declines in ivory gulls. In addition, the inverse relationships between α -tocopherol and several of the contaminants in the ivory gull eggs, suggest that the high contaminant concentrations experienced by ivory gulls may induce oxidative stress to an extent that affects embryo and chick survival. However, this issue needs to be addressed in more detail before any firm conclusions can be drawn.

It is of major concern that the effects of contaminants may become more severe when the organism is under additional environmental stress (Boonstra, 2004). Thus, exposure to high levels of contaminants can act in concert with additional stress, for example that caused by climate change, to push ivory gull populations beyond their environmental tolerance limits.

Acknowledgments

We thank Vidar Bakken, Audun Igesund, Andrey Volkov and Elena Volkova for their assistance with collection of samples. We also thank Jenny Bytingsvik and Helene Mathisen for technical analytical assistance. We thank René Corado for providing the measurements of the ivory gull eggshells in the collections of the Western Foundation of Vertebrate Zoology (WFVZ). Funding for this study was provided by the Norwegian Ministry of Environment, the Norwegian Polar Institute, the Norwegian University of Science and Technology, the Norwegian Pollution Control Authority and the Governor of Svalbard.

References

- Banudevi S, Krishnamoorthy G, Venkataraman P, Vignesh C, Aruldas MM, Arunakaran J. Role of α -tocopherol on antioxidant status in liver, lung and kidney of PCB exposed male albino rats. *Food Chem Toxicol* 2006;44:2040–6.
- Barron MG, Galbraith H, Beltman D. Comparative reproductive and developmental toxicology of PCBs in birds. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1995;112:1–14.
- Blomhoff R. Introduction – overview of vitamin A metabolism and function. In: Blomhoff R, editor. *Vitamin A in health and disease*. New York: Marcel Dekker, Inc.; 1994. p. 1–35.
- Blomqvist S, Elander M. Sabine's gull (*Xema sabini*), Ross's gull (*Rhodostethia rosea*) and ivory gull (*Pagophila eburnea*) – gulls in the Arctic: a review. *Arctic* 1981;34:122–32.
- Blus LJ, Wiemeyer SN, Bunck CM. Clarification of effects of DDE on shell thickness, size, mass, and shape of avian eggs. *Environ Pollut* 1997;95:67–74.
- Boelsterli UA. Mechanistic toxicology – the molecular basis of how chemicals disrupt biological targets. Oxon: Taylor & Francis; 2003.
- Boily MH, Champoux L, Bourbonnais DH, Desgranges JL, Rodrigue J, Spear PA. β -carotene and retinoids in eggs of great-blue herons (*Ardea herodias*) in relation to St-Lawrence-River contamination. *Ecotoxicology* 1994;3:271–86.
- Boonstra R. Coping with changing northern environments: the role of the stress axis in birds and mammals. *Integr Comp Biol* 2004;44:95–108.
- Braathen M, Derocher AE, Wiig O, Sormo EG, Lie E, Skaare JU, et al. Relationships between PCBs and thyroid hormones and retinol in female and male polar bears. *Environ Health Perspect* 2004;112:826–33.
- Braune BM, Mallory ML, Gilchrist HG. Elevated mercury levels in a declining population of ivory gulls in the Canadian Arctic. *Mar Pollut Bull* 2006;52:978–82.
- Braune BM, Mallory ML, Grant Gilchrist H, Letcher RJ, Drouillard KG. Levels and trends of organochlorines and brominated flame retardants in ivory gull eggs from the Canadian Arctic, 1976 to 2004. *Sci Total Environ* 2007;378:403–17.
- Buckman AH, Norstrom RJ, Hobson KA, Karnovsky NJ, Duffe J, Fisk AT. Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. *Environ Pollut* 2004;128:327–38.
- Bustnes JO, Bakken V, Erikstad KE, Mehlum F, Skaare JU. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. *J Appl Ecol* 2001;38:791–801.
- Bustnes JO, Folstad I, Erikstad KE, Fjeld M, Miland OO, Skaare JU. Blood concentration of organochlorine pollutants and wing feather asymmetry in glaucous gulls. *Funct Ecol* 2002;16:617–22.
- Carey C. Female reproductive energetics. In: Carey C, editor. *Avian energetics and nutritional ecology*. New York: Chapman & Hall; 1996. p. 324–74.
- Champoux L, Rodrigue J, Trudeau S, Boily MH, Spear PA, Hontela A. Contamination and biomarkers in the great blue heron, an indicator of the state of the St. Lawrence River. *Ecotoxicology* 2006;15:83–96.
- Colborn T. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 1993;101:378–84.
- Cooke AS. Shell thinning in avian eggs by environmental pollutants. *Environ Pollut* 1973;4:85–152. (1970).
- Di Mascio P, Murphy ME, Sies H. Antioxidant defense systems – the role of carotenoids, tocopherols, and thiols. *Am J Clin Nutr* 1991;53:194–200.
- Falk K, Moller S, Mattox WG. A long-term increase in eggshell thickness of Greenlandic Peregrine Falcons *Falco peregrinus tundrius*. *Sci Total Environ* 2006;355:127–34.
- Fernie K, Bortolotti G, Smits J. Reproductive abnormalities, teratogenicity, and developmental problems in American kestrels (*Falco sparverius*) exposed to polychlorinated biphenyls. *J Toxicol Environ Health A* 2003;66:2089–103.
- Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, et al. Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). *Toxicol Sci* 2005;88:375–83.
- Fernie KJ, Shutt JL, Letcher RJ, Ritchie IJ, Bird DM. Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American Kestrels. *Environ Sci Technol* 2009;43:2124–30.
- Fisk AT, Moisey J, Hobson KA, Karnovsky NJ, Norstrom RJ. Chlordane components and metabolites in seven species of Arctic seabirds from the Northwater Polynya: relationships with stable isotopes of nitrogen and enantiomeric fractions of chiral components. *Environ Pollut* 2001;113:225–38.
- Gabrielsen GW. Levels and effects of persistent organic pollutants in arctic animals. In: Orbaek JB, Kallenborn R, Tombre I, Hegseth EN, Falk-Petersen S, Hoel AH, editors. *Arctic-Alpine ecosystems and people in a changing environment*. Berlin: Springer Verlag; 2007. p. 377–412.
- Gilchrist HG, Mallory ML. Declines in abundance and distribution of the ivory gull (*Pagophila eburnea*) in Arctic Canada. *Biol Conserv* 2005;121:303–9.
- Gilchrist G, Strøm H, Gavrilov MV, Mosbech A. International Ivory Gull conservation strategy and action plan. Conservation of Arctic Flora and Fauna (CAFF) International Secretariat, Circumpolar Seabird Group (CBird), Akureyri; 2008. p. 20.
- Gilg O, Boertmann D, Merkel F, Aebischer A, Sabard B. Status of the endangered Ivory gull *Pagophila eburnea* in Greenland. *Polar Biol* 2009;32:1275–86.
- Haave M, Ropstad E, Derocher AE, Lie E, Dahl E, Wiig O, et al. Polychlorinated biphenyls and reproductive hormones in female polar bears at Svalbard. *Environ Health Perspect* 2003;111:431–6.
- Helander B, Olsson A, Bignert A, Asplund L, Litzén K. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. *Ambio* 2002;31:386–403.
- Hewitt M, Servos M. An overview of substances present in Canadian aquatic environments associated with endocrine disruption. *Water Qual Res J Can* 2001;36:191.
- Hilscherova K, Blankenship A, Kannan K, Nie M, Williams LL, Coady K, et al. Oxidative stress in laboratory-incubated double-crested cormorant eggs collected from the Great Lakes. *Arch Environ Contam Toxicol* 2003;45:533–46.
- Hobson KA, Fisk A, Karnovsky N, Holst M, Gagnon J-M, Fortier M. A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. *Deep Sea Res Part II* 2002;49:5131–50.
- Hoyt DF. Practical methods of estimating volume and fresh weight of bird eggs. *Auk* 1979;96:73–7.
- IUCN. IUCN red list of threatened species. Version 2011.1. <http://www.iucnredlist.org> 2010. Downloaded on 24 October 2011.
- Jenssen BM. Endocrine-disrupting chemicals and climate change: a worst-case combination for arctic marine mammals and seabirds? *Environ Health Perspect* 2006;114:76–80.
- Jenssen BM, Aarnes JB, Murvoll K-M, Herzke D, Nygård T. Fluctuating wing asymmetry and hepatic concentrations of persistent organic pollutants are associated in European shag (*Phalacrocorax aristotelis*) chicks. *Sci Total Environ* 2010;408:578–85.
- Karnovskiy NJ, Hobson KA, Brown ZW, Hunt GL. Distribution and diet of ivory gulls (*Pagophila eburnea*) in the north water polynya. *Arctic* 2009;62:65–74.
- Keith JA, Gruchy JM. Residue levels of chemical pollutants in North American bird life. In: Voous KH, editor. *Proceedings of the XVth international ornithological congress*. Leiden, The Netherlands: E.J. Brill; 1972. p. 437–54.
- Kendall RJ, Anderson TA, Baker RJ, Bens CA, Carr JA, Chiodo LA, et al. *Ecotoxicology*. In: Klaassen CD, editor. *Cassarett and Doull's toxicology*. New York: McGraw-Hill; 2001. p. 1013–45.
- Kuzyk ZA, Burgess NM, Stow JP, Fox GA. Biological effects of marine PCB contamination on black guillemot nestlings at Saglek, Labrador: liver biomarkers. *Ecotoxicology* 2003;12:183–97.
- Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jørgensen EH, Sonne C, et al. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Sci Total Environ* 2010;408:2995–3043.
- Lowe TP, Stendell RC. Eggshell modifications in captive American kestrels resulting from Aroclor-1248 in the diet. *Arch Environ Contam Toxicol* 1991;20:519–22.
- Lundholm CE. DDE-induced eggshell thinning in birds: effects of p,p'-DDE on the calcium and prostaglandin metabolism of the eggshell gland. *Comp Biochem Physiol C Toxicol Pharmacol* 1997;118:113–28.
- Mallory ML, Stenhouse IJ, Gilchrist G, Robertson G, Hanev JC, Macdonald SD. Ivory gull (*Pagophila eburnea*). In: Poole A, editor. *The birds of North America online*. Ithaca: Cornell Lab of Ornithology; 2008. Retrieved from the Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/175>.
- Mason CF, Ekins G, Ratford JR. PCB congeners, DDE, dieldrin and mercury in eggs from an expanding colony of cormorants (*Phalacrocorax carbo*). *Chemosphere* 1997;34:1845–9.
- Miljeteig C, Strøm H, Gavrilov MV, Volkov AE, Jenssen BM, Gabrielsen GW. High levels of contaminants in ivory gull *Pagophila eburnea* eggs from the Russian and Norwegian Arctic. *Environ Sci Technol* 2009;43:5521–8.
- Murvoll KM, Skaare JU, Nilssen VH, Bech C, Ostnes JE, Jenssen BM. Yolk PCB and plasma retinol concentrations in shag (*Phalacrocorax aristotelis*) hatchlings. *Arch Environ Contam Toxicol* 1999;36:308–15.
- Murvoll KM, Jenssen BM, Skaare JU. Effects of pentabrominated diphenyl ether (PBDE-99) on vitamin status in domestic duck (*Anas platyrhynchos*) hatchlings. *J Toxicol Environ Health* 2005;68:515–33.
- Murvoll KM, Skaare JU, Anderssen E, Jenssen BM. Exposure and effects of persistent organic pollutants in European shag (*Phalacrocorax aristotelis*) hatchlings from the coast of Norway. *Environ Toxicol Chem* 2006a;25:190–8.
- Murvoll KM, Skaare JU, Moe B, Anderssen E, Jenssen BM. Spatial trends and associated biological responses of organochlorines and brominated flame retardants in hatchlings of North Atlantic kittiwakes (*Rissa tridactyla*). *Environ Toxicol Chem* 2006b;25:1648–56.
- Murvoll KM, Skaare JU, Jensen H, Jenssen BM. Associations between persistent organic pollutants and vitamin status in Brünnich's guillemot and common eider hatchlings. *Sci Total Environ* 2007;381:134–45.
- Nohl H, Kozlov AV, Gille L, Staniek K. Endogenous oxidant-generating systems. In: Grune T, editor. *Oxidants and antioxidant defense systems*. 2 O. Berlin: Springer-Verlag; 2005. p. XII. 239 s.
- Nygård T. Long term trends in pollutant levels and shell thickness in eggs of merlin in Norway, in relation to its migration pattern and numbers. *Ecotoxicology* 1999;8:23–31.
- Olsen GH, Mauritzen M, Derocher AE, Sormo EG, Skaare JU, Wiig O, et al. Space-use strategy is an important determinant of PCB concentrations in female polar bears in the barrens sea. *Environ Sci Technol* 2003;37:4919–24.
- Pain DJ, Burneleau G, Bavoux C, Wyatt C. Levels of polychlorinated biphenyls, organochlorine pesticides, mercury and lead in relation to shell thickness in marsh harrier (*Circus aeruginosus*) eggs from Charente-Maritime, France. *Environ Pollut* 1999;104:61–8.

- Peakall DB. Physiological effects of chlorinated hydrocarbons on avian species. In: Hague R, Freed VH, editors. Environmental dynamics of pesticides. New York: Plenum Press; 1975. p. 343–60.
- Peterle TJ. Wildlife toxicology. New York: Van Nostrand Reinhold; 1991.
- Ratcliffe DA. Decrease in eggshell weight in certain birds of prey. *Nature* 1967;215:208–10.
- Robertson GJ, Gilchrist HG, Mallory ML. Colony dynamics and persistence of ivory gull breeding in Canada. *Avian Conserv Ecol* 2007;2(8):1–18.
- Rolland RM. A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. *J Wildl Dis* 2000;36:615–35.
- Strøm H. Ivory gull. In: Kovacs KM, Lydersen C, editors. Birds and mammals of Svalbard. 13. Tromsø: Norwegian Polar Institute; 2006. p. 151–4.
- Twaroski TP, O'Brien ML, Larmonier N, Glauert NP, Robertson LW. Polychlorinated biphenyl-induced effects on metabolic enzymes, AP-1 binding, vitamin E, and oxidative stress in the rat liver. *Toxicol Appl Pharmacol* 2001;171:85–93.
- Verreault J, Skaare JU, Jenssen BM, Gabrielsen GW. Effects of organochlorine contaminants on thyroid hormone levels in Arctic breeding glaucous gulls, *Larus hyperboreus*. *Environ Health Perspect* 2004;112:532–7.
- Vikøren T, Stuve G. Fluoride exposure and selected characteristics of eggs and bones of the herring gull (*Larus argentatus*) and the common gull (*Larus canus*). *J Wildl Dis* 1996;32:190–8.
- Villanger GD, Lydersen C, Kovacs KM, Skaare JU, Jenssen BM. Disruptive effects of persistent organohalogen contaminants on thyroid function in white whales (*Delphinapterus leucas*) from Svalbard. *Sci Total Environ* 2011;409:2511–24.
- Walker CH, Hopkin SP, Sibly RM, Peakall DB. Principles of ecotoxicology. London: Taylor & Francis; 2001.
- Weseloh DV, Mineau P, Struger J. Geographical distribution of contaminants and productivity measures of herring gulls in the Great Lakes: Lake Erie and connecting channels 1978/79. *Sci Total Environ* 1990;91:141–59.
- Wiemeyer SN, Lamont TG, Bunck CM, Sindelar CR, Gramlich FJ, Fraser JD, et al. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs — 1969–79 — and their relationships to shell thinning and reproduction. *Arch Environ Contam Toxicol* 1984;13:529–49.
- Xie MN, Zhang CQ. Estrogenic and toxic effects of polychlorinated biphenyls on cultured ovarian germ cells of embryonic chickens. *Reprod Toxicol* 2004;19:79–86.
- Yun JS, Na HK, Park KS, Lee YH, Kim EY, Lee SY, et al. Protective effects of vitamin E on endocrine disruptors, PCB-induced dopaminergic neurotoxicity. *Toxicology* 2005;216:140–6.
- Zile MH, Summer C, Aulerich R, Bursian SJ, Tillitt DE, Giesy JP, et al. Retinoids in eggs and embryos of birds fed fish from the Great Lakes. *Environ Toxicol Pharmacol* 1997;3:277–88.