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Varying chick mortality in an organochlorine-"strained" population of the nominate Lesser Black-backed Gull *Larus f. fuscus* in the Baltic Sea

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Severe reproductive failure has contributed to a drastic population decline of the nominate Lesser Black-backed Gull (*Larus fuscus fuscus)* in its breeding grounds in the Gulf of Finland and the Bay of Bothnia. In intensive field studies in the central area of the Gulf of Finland, roughly 65–70% of chicks died in their nest in the 1980s and 1990s due to innate diseases. In the 1990s, the fledging rate was only 0.02. The diseases consisted mainly of liver degeneration and various inflammations, and most chicks died of sepsis. The hepatic concentrations of legacy organochlorines (OC) in dead chicks were high, and they correlated with the proportion of dead chicks in a brood. During the 2000s, however, the occurrence of diseased chicks in the Gulf of Finland decreased to 48%, which helped to achieve a fledging rate supposedly sustaining the population (0.52). At the same time, the trends in hepatic levels of certain legacy OCs in chicks decreased, especially the DDE, HCB, β-HCH, and *trans*-nonachlor levels. In spite of skewed sampling (only dead chicks were available), our results indicate an enhancing health status among the present population, probably due to a less contaminated diet during the non-breeding period.

1. Introduction

During the last three to four decades, the nominate Lesser Black-backed Gull (*Larus fuscus fuscus*) has experienced drastic population declines over most of its range, coupled with severe breeding failures. In the Gulf of Finland, in the northern Baltic Sea, the fledging rate of *L. f. fuscus* has averaged only 0.18 per clutch during 1980–1993; i.e., only one in six pairs managed to fledge an offspring (Hario 1994). This figure represents one-third of the calculated hypothetical minimum output that is required for a Lesser Black-backed Gull population to remain self-sustaining (Hario 1994). In Sweden, the fledging result varied between 0.02 and 0.16 in 2003–2005 in the two largest colonies of the Swedish Baltic coast (Lif *et al.* 2005, Capandegui 2006). At two sites in coastal Norway, no chicks fledged in the mid-1980s (Bevanger & Thingstad 1990), but a new research program has indicated enhanced fledging rates at the same sites (Bustnes 2008). In all these areas, populations decreased by 50–70% since the 1970s. The subspecies has disappeared from Denmark and only 50–200 pairs are left in Estonia. Only in Russian Karelia, the population is still thriving (Cherenkov

et al. 2007). Currently, nominate *fuscus* belongs to the Red Data Books of Finland, the Åland Islands, Sweden, Norway, East Fennoscandia, and Estonia, i.e., it is endangered over its entire range.

In our studies on chick mortality in Lesser Black-backed Gulls at the Söderskär Game Research Station, the Gulf of Finland, we demonstrated a high frequency of chick diseases due to degeneration and inflammation of various internal organs (Hario & Rudbäck 1996, 1999). In most cases, sepsis was the final cause of death. It may be that the degenerated liver is not capable of detoxifying bacterial flora entering the blood system via the open umbilicus at hatching. Organochlorines (OC) have been detected in elevated concentrations in dead Lesser Black-backed Gull chicks in our study areas, and previous studies suggest an association between legacy OC levels and chick mortality (Hario *et al.* 2000, 2004) (*legacy* applied to chemicals that are banned or restricted; AMAP 2009).

However, the biochemical pathway between contaminant burden, pathologic changes in the host, and outbreak of diseases is largely unknown (Hario & Rudbäck 1996). Liver lesions are not necessarily specific and may be pathognomic for various disorders in e.g. vitamin mobilisation in the body (e.g., Hoffman & Leighton 1985, Wobeser & Kost 1992). However, the disease syndrome of our gull chicks is different from what was recently described on the thiamine deficiency in Baltic marine birds (Balk *et al.* 2009). Also, the mortality of Lesser Black-back Gull chicks was recorded already in the 1970s and it has been constantly high since then.

The liver lesions recorded in our gull chicks (lipid accumulation, swollen hepatocytes, necrotic hepatocytes characterized by pycnotic nuclei, and intense diffuse vacuolation) were, nevertheless, similar to those described in studies on fish-eating waterbirds from the Great Lakes in North America, where environmental chemicals are considered the prime suspect to lowered breeding success (e.g., Gilbertson 1983, Gilbertson *et al.* 1991, Ewins *et al.* 1992, Fry 1995, Hebert *et al.* 1999). Adverse effects on eggs and chicks are documented with bioaccumulation of dioxins and selenium, both of which are more toxic to embryos than to adults and which bioaccumulate into egg yolk. The hepatic dioxin-like non-ortho-CP congeners, expressed as TEQ values (toxic equivalents), in our Lesser Black-backed Gull chicks and hatchlings in the 1990s amounted to 3100 pg/g w.w. (Hario *et al.* 2004), a value exceeding most known LD_{50} values for wild bird embryos (cf. Giesy *et al.* 1994, AMAP 1998).

It is also worth noting that the innate diseases in our Lesser Black-backed Gull chicks are not an outcome of under-nourishment or systematic expelling of the youngest sibling during feeding by the parent birds (the so-called brood reduction hypothesis), which was proven by our feeding trials and video recordings at nest (Hario & Rudbäck 1996, 1999). Our study is exceptional in that post mortem examination for cause of death was performed on many of the corpses taken to chemical analysis and that parental behaviour and feeding conditions prior to death were known. We conclude that starvation, exposure, and neglect were not involved.

Lesser Black-backed Gull chicks from the Gulf of Finland and from the Bay of Bothnia had very high concentrations of PCB in their livers, but the lipid-based concentrations were not significantly higher than those of the healthy Herring Gull *Larus argentatus* chicks, indicating a common PCB exposure area for both species, i.e., the Baltic Sea. In contrast, the DDE concentrations in the diseased Lesser Black-backed Gull chicks in the 1990s were within the levels previously correlated with decreased reproduction, while the residues in apparently healthy Herring Gulls were below those levels (see Hario *et al.* 2004). The DDE/PCB ratio in Lesser Black-backed Gulls was significantly elevated, indicating an increased exposure to DDTs as compared with most other Baltic and circumpolar seabirds (reviewed by Hario *et al.* 2004). A similarly high DDE/PCB ratio in *fuscus* was obtained in Norway in a study comparing residues of OCs in nominate *fuscus* with those in *L. f. intermedius* and other greyish-mantled gull populations on the Norwegian coast (Bustnes *et al.* 2006).

The nominate Lesser Black-backed Gulls spend the winter in equatorial Africa, in western Rift Valley lakes, mainly in Uganda and to a lesser extent in Ethiopia, Kenya and Tanzania (Kilpi & Saurola 1984), and in the Congo River basin in the west (Kylin *et al.* 2010). They travel long distances with apparent ease and use the Black Sea

and the Mediterranean as stop-over sites (Kube *et al.* 2000). We do not know to what extent adults of Lesser Black-backed Gulls become exposed to toxicants in Africa; for example, not much is known of their feeding ecology there (but see Hario 2006).

This paper updates the OC situation in Lesser Black-backed Gulls of the northern Baltic Sea and explores the possible connection between residue burdens and varying levels of reproduction during three decades of monitoring at one site in the Gulf of Finland and during two decades at two sites in the Bay of Bothnia. This is the final report from the Gulf of Finland because the Söderskär Game Research Station was put out of business in 2008, and the numbers of local Lesser Black-backed Gulls are currently too low to allow for an adequate chick monitoring program. At the start of the study in 1978, there were 250 pairs in the area, now only 10–15 pairs (an annual mean of 8% reduction). Grossly similar reduction applies to most of the Gulf of Finland (see Hario *et al.* 2009).

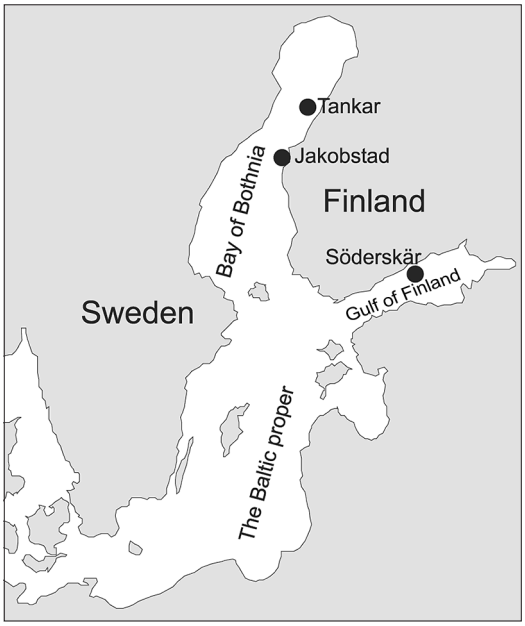

Fig. 1. Location of the study sites along the Finnish coast.

2. Material and methods

2.1. Ecological data and pathological studies

In the Gulf of Finland, we collected carcasses of freshly dead chicks of Lesser Black-backed Gulls from gulleries in the Söderskär Game Research Station study area (60°07'N, 25°25'E; Fig. 1). During the breeding seasons of 1985, 1986 and 1988 (henceforth, "the 1980s"), 1994–1996 ("the 1990s"), and 2002–2004 and 2006–2007 ("the 2000s"), all dead chicks were removed from the nests, and those suitable for examination were stored in a freezer for subsequent analysis. Nest monitoring in three adjacent colonies (results combined) was done each year on a daily basis during the whole chick rearing period, and the checks consisted of assessing egg losses, egg viability, hatching success, chick body weight, chick losses and fledging success. The chicks were ringed on the day of hatching. Predation of chicks by Herring Gulls was verified by locating chick rings at the predatory individuals' nesting sites in pellets and food remnants (detailed in Hario 1990, 1997).

In 1995, corpses at the Söderskär site were taken for pathological studies at the National Veterinary and Food Research Institute in Helsinki for post-mortem examination. Cause of death was determined by routine necropsy followed by histological evaluation of tissues. Bacteriological examination was always performed on samples collected from lungs and intestines and on other organs based on gross findings (for details, see Hario & Rudbäck 1996). A total of 14 Lesser Black-backed Gull chicks were necropsied, i.e., 47% of the 30 carcasses found in 1995 in the Söderskär study area (see later). The small sample size resulted from logistical difficulties. The intestines decomposed rapidly, within a few hours, when the carcass was exposed to hot sunny weather, especially during the afternoon hours. Their transport to Helsinki, a 25 km-long sea voyage, resulted in a further delay. In the present study, only chicks younger than one week were analysed for chemical residues.

In the Bay of Bothnia, carcasses were collected in 1995 from the Tankar Bird Station (63°55'N, 22°56'E), and in 2003, from two adjacent colonies in Jakobstad (63°41'N, 22°35'E; Fig. 1). They were subjected to chemical analysis only; comprehensive nest checks such as those performed at Söderskär were not conducted at the Bay of Both-

nia sites, and no ecological data are reported here. However, the fledging result in the Bay of Bothnia appeared far better than that in the Gulf of Finland, with a lower rate of chicks found diseased (whereas the egg mortality did not deviate; Wistbacka 2000, Hario *et al.* 2004).

2.2. Chemical analysis

After necropsy, the carcasses were stored at –20°C until chemical analysis. The presence of the following organochlorines in chick livers was analyzed by the laboratory of the Finnish Environment Institute, Helsinki: α-, β-, γ- and δ-hexachlorocyclohexane (HCH), α- and γ-chlordane, *trans*-nonachlor, hexachlorobenzene (HCB), *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT and total PCBs. Total PCBs comprised the following 19 congeners (IUPAC nos.): 8, 18, 28, 31, 52, 66, 77, 101, 105,110, 118, 128, 138, 149, 153, 156, 180, 187, and 170.

In each sample batch, a blank sample and control samples, i.e., certified organochlorine pesticides (QOR03CA) and chlorobiphenyls (QOR0 2CA) standard solutions in isooctane, and fish muscle sample obtained from QUASIMEME, were measured with the same method as the samples. The results for each compound in the control samples were within the extended measurement uncertainty (36%) of the method. Depending on the compounds the method detection limits are between 0.02–0.18 ng/g (wet weight basis).

Each sample was placed in a beaker and extracted with 80 ml of acetone:hexane (1:1, v/v) for 60 minutes with an ultrasonic bath (Branson 5510). About 40 ml of the extract was evaporated with nitrogen until dry (Zymark TurboVap II), and the content of fat residue was weighed for calculation of fat%. The internal standard (PCB congener 53) was added, and the sample was redissolved in isooctane following fat purification with concentrated sulphuric acid.

Organochlorine pesticides (OCPs) and PCBs were analyzed with a gas chromatograph (Hewlett-Packard GC, model 5890 Series II, Waldbronn, Germany) equipped with two ^{63}Ni electron capture detectors (ECDs) and two capillary columns HP-1701 & HP-5 (60 m × 0.25 mm i.d., film thickness 0.25 μm) and an HP 7673 automatic sampler. The residues are given as μg/g or ppm unless otherwise stated.

2.3. Statistical analysis

Organochlorine residues are presented as arithmetic mean values (± standard error) to allow for comparisons with European seabird studies. For comparisons with North American results, we use geometric means (see Discussion). To calculate the sum PCB (SPCB), samples where chemicals were not detected (IUPAC nos. 8, 18, 31, 101) were assigned a value at half of the congener-specific detection limit (0.04–0.06 ng/g). We used ANOVA and associated Tukey post hoc tests on log-transformed data to examine the temporal and geographical variation in the organochlorine concentrations. The Mann-Whitney *U*-test was used for examining the inter-decade variation in the weight loss of chicks. To compare proportions in breeding results, the χ^2 test was used. The tests were two-tailed and the critical alpha level set at 0.05. All computations were done using the SYSTAT 10.2 package.

3. Results

3.1. Overall breeding success

No statistically significant difference emerged in the total egg losses between the three decades in the Gulf of Finland ($\chi^2_2 = 4.860$, $P = 0.176$; Table 1). The proportion of addled eggs became significantly larger ($\chi^2_2 = 13.754$, $P = 0.002$), while that of egg deaths at hatching ("died at pipping") became suggestively smaller by the 2000s ($\chi^2_2 = 6.411$, $P = 0.082$). The total egg losses of the 2000s (20%) were suggestively smaller than those of the 1990s (31%) ($\chi^2 = 4.399$, $P = 0.072$).

Total chick losses significantly differed between the three decades ($\chi^2_2 = 32.797$, $P < 0.001$; Table 1). This was mainly due to the varying proportion of chicks found diseased ($\chi^2_2 = 18.715$, $P < 0.001$). In the 1980s, 12% of the chicks were found diseased, while the corresponding proportion was 29% in the 1990s ($\chi^2 = 8.890$, $P = 0.006$). In the 2000s, only 8% were found diseased, representing a highly significant decrease from the 1990s ($\chi^2 =$

Table 1. Breeding parameters of the Lesser Black-backed Gulls studied at Söderskär, Gulf of Finland, over three decades (the 1980s, 1990s and 2000s).

Parameter	1980s	1990s	2000s
Number of nests	60	52	43
Eggs laid	150	150	107
Mean clutch size	2.48	2.89	2.48
Addled eggs (%)	6 (4.0)	4 (2.7)	14 (13.1)
Died at pipping (%)	9 (6.0)	8 (5.3)	0
Other losses (%) [a]	30 (20.0)	35 (23.3)	7 (6.5)
Total egg losses (%)	45 (30.0)	47 (31.3)	21 (19.6)
Chicks hatched	105	103	86
Chicks disappeared (%)	57 (54.3)	57 (55.3)	49 (57.0)
Verified as preyed-on (%)	6 (5.7)	14 (13.6)	3 (3.5)
Found diseased (%)	13 (12.4)	30 (29.1)	7 (8.0)
Other losses (%) [b]	5 (4.8)	1 (1.0)	1 (1.2)
Total chick losses (%)	81 (77.1)	102 (99.0)	59 (68.6)
Fledglings/nest (mean ± SD)	0.37 ± 0.21	0.02 ± 0.01	0.52 ± 0.17

a = Disappearances, presumed predation, nest desertion, accident
b = Entangled, fallen into crevices, etc.

14.912, $P < 0.001$). Also, the occurrence of verified predation tended to be lower in the 2000s (4%) than in the 1990s (14%; $\chi^2 = 5.845$, $P = 0.032$). The level of chick disappearances did not change between the three decades ($\chi^2_2 = 0.157$, $P = 0.925$).

3.2. Pathological studies

The pathological findings of the 14 necropsied chicks from the Gulf of Finland in 1995 have been detailed in Hario *et al.* (2004). Briefly, all had (1) degenerations in the liver, cardiac muscle and/or kidneys, (2) various inflammations (mostly in the intestine) and (3) sepsis. The alimentary tract was empty, and the final cause of death was sepsis. We consider these chicks diseased (see Hario & Rudbäck 1999). They had no signs of depleted lipids except that their yolk sac had disappeared by day four. Emaciation, with a marked reduction in body weight through dehydration, was typically seen in the terminal stages.

The necropsied chicks of 1995 had lost, on average, 21.8 ± 3.2% (median 22.2; $n = 11$) of their body weight during the three days they stayed alive after the first signs of weight loss (mean 2.7 ± 0.6 days, median 3). The corresponding figures for the combined group of the 1980s' and the 2000s' chicks were 15.5 ± 3.8% (median 14.6, $n = 7$, $U = 53.5$, $P = 0.348$) and 1.8 ± 0.4 days (median 2, $U = 59.5$, $P = 0.102$; there was no significant difference in the age at death between these two groups, $U = 24.00$, $P = 0.385$).

Over the entire Söderskär data set, the age at death and the hepatic lipid% correlate ($r_s = –0.462$, $n = 31$, $P = 0.020$), but there was no significant difference in the lipid% between the three decades ($F_{2, 28} = 0.095$, $P = 0.910$). Based on the consistent course of events in the field (the rapid death at the nest) and the similar body weight loss, we consider that the chicks in the 1980s' and in the 2000s' samples were diseased as well.

3.3. Contaminant residues

Concentrations (ng/g, w.w.) of the following organochlorines were below detection limits (given in parentheses) in all samples or were only detected in some of the samples and not frequently enough for statistical analysis: α-, γ- and δ-HCH (0.45), α- and γ-chlordane (0.75), *p,p'*-DDD (1.8) and *p,p'*-DDT (2.1).

The PCB congeners nos. 31, 101 and 110 were not included in the statistical analysis as their levels were often below the detection limits. Comparisons between groups are based on congener nos. 28, 52, 118, 138, 153, 180 (a.k.a. 6PCB), which together amount to about 80% of the sum concentra-

tions of the 19 congeners measured (so-called sum-PCB or SPCB).

Measurable amounts of other organochlorines were detected in all samples. In the following, test statistics are given for comparisons based on log-transformed lipid-based values.

3.3.1. The PCBs

The PCB residues in livers of the chicks from the Gulf of Finland showed no significant trend over the three decades ($F_{2, 28} = 1.018$, $P = 0.374$), although a tendency towards diminishing total organochlorine burdens is seen (Table 2). The PCB residues tended to peak in the 1990s, but this tendency was not statistically significant. The residues from the Bay of Bothnia seemed smaller than those from the Gulf of Finland, but a meaningful intra-decade difference emerged only in the 2000s ($F_{1, 10} = 4.891$, $P = 0.046$; Table 2).

3.3.2. DDE and the remaining OCs

The decrease in the DDE concentrations in the Gulf of Finland was significant between the 1990s' and the 2000s' samples only ($F_{1, 21} = 4.674$, $P = 0.042$). No intra-decade differences between these marine areas emerged, and no temporal trend was seen in the Bay of Bothnia (Table 2).

During the 1980s in the Gulf of Finland, the DDE levels in an individual were of the same magnitude as the PCB levels of the same individual, whereas in the 2000s this DDE/PCB ratio more than halved due to lesser DDE within an individual ($F_{2, 28} = 2.654$, $P = 0.089$; Table 2). In the Bay of Bothnia, the ratio increased, but not statistically significantly so. However, the very high ratio for the 2000s is noteworthy.

A significant decrease in HCB ($F_{1, 28} = 7.224$, $P = 0.003$) and in *trans*-nonachlor ($F_{2, 28} = 5.847$, $P = 0.008$) was found in the 2000s' samples in the Gulf of Finland. Also, the residues of these compounds in the Bay of Bothnia tended to decrease from the 1990s to the 2000s, but, as in most comparisons, the sample sizes are too small to detect statistical significance. The β-HCH levels also differed between the 1990s' and the 2000s' samples in the Gulf of Finland ($F_{1, 21} = 5.089$, $P = 0.026$, Table 2), but there were no intra-decade differences between the Bay of Bothnia and the Gulf of Finland for any of these compounds.

3.3.3. Correlating residues and brood fate

We divided the Söderskär data of 3-egg broods into three categories according to the fate of the brood nest-mates: broods with no healthy siblings, broods with 1, and broods with 2 healthy siblings, "healthy" referring to chicks showing constant increase in body weight and no signs of behavioural anomalies typical of diseased chicks (Hario & Rudbäck 1999). There was a negative correlation between hepatic PCB concentrations in a dead chick and the number of healthy siblings in its brood ($r_s = –0.339$, $n = 27$, $P < 0.10$). Notwithstanding the skewed sample sizes ($n = 18$, 4 and 5 individuals analyzed in the 0-, 1-, and 2-healthy-chick broods, respectively), our data show statistically significant difference in the PCB level among groups (log-transformed data, $F_{2, 24} = 3.459$, $P = 0.048$). This is due to the residues being significantly lower in the 2-healthy-chick broods (76.3 ± 26.4 ppm l.w.) compared to the 0- and 1-healthy-chick broods ($F_{1, 25} = 6.737$, $P = 0.016$), which, on the other hand, did not deviate from each other (167.2 ± 21.5 and 173.5 ± 13.4 ppm l.w., respectively; $F_{1, 20} = 0.358$, $P = 0.556$). The corresponding comparison on DDE yields an equally negative correlation ($r_s = –0.353$) and a similar division among groups ($F_{2, 24} = 5.955$, $P = 0.008$; 25.2 ± 3.8 ppm l.w. in 2-healthy-chick broods, and 125.0 ± 26.2 and 168.5 ± 56.9 in the remaining groups; $F_{1, 20} = 0.943$, $P = 0.344$). The DDE and PCB levels were highly correlated over this entire data set (Pearson $r = 0.711$).

4. Discussion

To determine the effects of environmental contamination on a population requires multiple sampling over a longer period of time as well as thorough monitoring of the biology and reproduction rate of the population in question. Our monitoring schedule spanned 23 years, during which time we had several sampling instances, of which we pooled three units, one for each calendar decade, with the

Table 2. Hepatic concentrations of organochlorines (ppm, w.w. and l.w.), chick age at death (days), and lipid-% in diseased Lesser Black-backed Gull chicks from the Gulf of Finland and from the Bay of Bothnia over three decades. Arithmetic mean ± SE (range) are given. Comparisons between groups are based on log-transformed data on lipid-based concentrations.

	Gulf of Finland			Bay of Bothnia		
	1980s (*n* = 8)	1990s (*n* = 15)	2000s (*n* = 8)	1990s (*n* =4)	2000s (*n* = 4)	Test
Chick age at death	1.5±0.6 (0–4)	2.7±0.6 (0–6)	2.1±0.5 (0–4)	1.0±0.7 (0–3)	1.0±0.4 (0–2)	ns
% lipid	9.6±1.5 (3.3–16.0)	9.0±0.8 (5.3–15.9)	9.1±0.9 (4.8–12.0)	9.3±2.1 (3.3-12.9)	11.1±0.9 (8.5–12.0)	ns
6PCB w.w.	14.0±4.3 (3.2–33.1)	16.2±2.2 (3.7-36.2)	11.5±4.3 (1.1–39.0)	6.4±1.8 (3.6-11.0)	2.7±0.9 (0.9–4.9)	
6PCB l.w.	141.9±28.8 (28.8–239.3)	187.2±21.7 (32.8–375.9)	129.2±51.3 (14.2–463.7)	94.6±44.3 (31.5–222.5)	26.4±11.3 (7.7–58.2)	ABE
SPCB w.w.	17.6±5.4 (3.9–41.1)	19.7±2.6 (4.7–44.3)	15.4±6.4 (1.4–57.3)	8.0±2.2 (4.4-13.9)	5.9±2.1 (2.0–11.5)	
SPCB l.w.	179.0±37.4 (35.2–317.4)	227.7±26.0 (41.1–459.9)	174.6±76.6 (17.7–682.4)	119.0±56.0 (39.5–280.5)	32.6±14.0 (9.6–72.1)	ABE
DDE w.w.	20.3±13.5 (1.6–100.0)	10.6±2.3 (1.8-30.0)	5.9±2.4 (0.7–20.0)	5.4±2.2 (1.3-11.0)	4.6±2.0 (1.3–10.0)	
DDE l.w.	229.2±114.0 (16.0–833.0)	120.2±23.8 (15.9–309)	66.3±26.1 (9.6–212.8)	80.7±42.3 (14.1–198.8)	46.5±24.7 (10.8–117.6)	D
DDE/SPCB	1.04±0.33 (0.16–2.62)	0.58±0.12 (0.18–1.40)	0.43±0.08 (0.21–0.96)	0.61±0.11 (0.29-0.79)	1.4±0.4 (0.5–2.5)	BC
HCB w.w.	0.25±0.08 (0.03–0.59)	0.12±0.02 (0.03–0.30)	0.05±0.01 (0.01–0.12)	0.08±0.03 (0.05-0.1)	0.04±0.01 (0.03–0.06)	
HCB l.w.	2.44±0.54 (0.28–4.33)	1.31±0.20 (0.24–3.1)	0.52±0.12 (0.13–1.28)	1.16±0.90 (0.54–2.48)	0.39±0.11 (0.23–0.72)	AD
β-HCH w.w.	0.52±0.22 (0.06–1.90)	0.52±0.15 (0.04–2.30)	0.12±0.04 (0.01–0.34)	0.18±0.07 (0.05–0.32)	0.09±0.04 (0.01–0.20)	
β-HCH l.w.	5.07±1.77 (0.56–15.83)	6.51±1.89 (0.32–23.87)	1.21±0.37 (0.12–3.62)	3.00±1.83 (0.49–8.26)	0.88±0.53 (0.12–2.35)	ABD
trans-nonachlor w.w	0.18±0.08 (0.01–0.54)	0.10±0.02 (0.02–0.24)	0.03±0.01 (0.001–0.09)	0.06±0.05 (0.02-0.12)	0.01±0.003 (0.004–0.02)	
trans-nonachlor l.w.	1.45±0.62 (0.12–4.50)	1.29±0.26 (0.14–3.21)	0.30±0.11 (0.01–0.92)	0.62±0.31 (0.26–0.93)	0.10±0.04 (0.03–0.22)	ABD
Total OC w.w.	38.6	31.0	21.5	13.7	10.6	
Total OC l.w.	417.2	357.0	242.9	204.5	80.5	

A = Bay of Bothnia in the 2000s differs from Gulf of Finland in the 1980s
B = Bay of Bothnia in the 2000s differs from Gulf of Finland in the 1990s
C = Gulf of Finland in the 2000s differs from Gulf of Finland in the 1980s
D = Gulf of Finland in the 2000s differs from Gulf of Finland in the 1990s
E = Bay of Bothnia in the 2000s differs from Gulf of Finland in the 2000s.

sampling years falling approximately in the middle of each decade. The two first decadal data sets indicated no marked changes in the contamination levels or in the reproduction capability of the Söderskär study population, whereas the last instance saw diminishing DDE, HCB, β-HCH and *trans*-nonachlor levels along with increasing reproductive success. In the following, we will discuss the possible association between these events.

4.1. Egg losses

The fertilization status of the addled eggs could not be resolved due to difficulties in distinguishing between infertile bird eggs and those in which embryo mortality had occurred at a very early stage (e.g., Birkhead *et al.* 1995, but see Birkhead *et al.* 2008). Most addled eggs in our gulleries had been incubated too long, becoming putrefied and partly

cracked. Thus, we cannot judge whether their increasing proportion in the 2000s was due to infertility or elevated embryonic mortality. However, "addled" eggs were readily distinguishable from those that had "died at pipping". The occurrence of "died at pipping" decreased, partially counterbalancing the non-hatching rate of the eggs.

The proportion of "other losses" decreased in the 2000s. "Other losses" consist mainly of egg disappearances due to predation and due to parents eating their own eggs and demolishing the nest (see Hario 1990). In studies on terrestrial-nesting larids, egg disappearances are mostly related to egg predation resulting from inadequate nest occupancy (long absenteeism of parents) and thereby inadequate nest defense. This sort of aberrant behaviour has long been implicated as being OC induced (Fox *et al.* 1978, Gilbertson 1983). The OC connection has been verified by experimental studies (e.g. Hoffman *et al.* 1996), but it has been difficult to detect it under field conditions (Burger *et al.* 2001). However, at the time of the initial finding of this behavioural anomaly, the OC levels in gulls were extremely high in the Great Lakes (Gilman *et al.* 1977).

In the Arctic-dwelling Glaucous Gulls *Larus hyperboreus*, PCB and oxychlordane residues in blood of the parent birds were significantly higher in those individuals that spent a higher proportion of time away from the nest site during the off-duty pauses between incubation bouts. The effects of DDE and HCB were not significant (Bustnes *et al.* 2005). Yet, the time spent incubating was actually not affected by blood concentration of PCBs (Bustnes *et al.* 2001, 2005). There was no association between egg losses and the OC burden in the blood of the Glaucous Gull parents (Bustnes *et al.* 2003). Eggs were constantly incubated by one of the parents (Bustnes *et al.* 2005), and no nest destruction was observed (Bustnes *et al.* 2001).

In a detailed study in the mid-1990s at Söderskär, nest occupancy was 100% among Lesser Black-backed Gulls during the incubation stage (Hario 1997). Despite this high rate of occupancy, Söderskär Lesser Black-backed Gulls do suffer from egg predation by the larger-sized Herring Gull. In the 2000s, these losses, however, decreased whereas the hepatic PCB levels did not (we did not measure oxychlordane), indicating no apparent dose-response effect for PCBs. Yet, the apparent difference in the susceptibility to egg predation between Lesser Black-backed and Glaucous Gulls may simply stem from the fact that the Glaucous Gull itself is the largest top-predator, whereas the Lesser Black-backed Gull of the nominate race (the delicate *fuscu*s; see Strann & Vader 1992) is inferior to the larger-sized predatory Herring Gulls. The decadal differences in egg predation, observed at Söderskär, may be a continuation of the same phenomenon. The fall in egg predation in the 2000s may result from current predator control programs conducted on the Herring Gulls of the central Gulf of Finland (see Hario *et al.* 2009) rather than from any changes in OC levels.

4.2. Chick losses

Disappearances are the prime chick "mortality" factor in most studies on ground nesting larids (reviewed by Hario 1990). Our fairly constant rate of chick disappearances over the decades at Söderskär indicates a uniform rate of retrieval of chicks. Yet, a significantly lower proportion of chicks were found diseased in the 2000s. This is a genuine change, which parallels the diminishing rate of "died at pipping" during the same era.

Based on chick growth rates, we have previously shown that approximately 36% of the 0–5-day-old chicks of the "disappeared" category in the 1980s were in fact taken by predatory Herring Gulls, the remaining 64% being diseased chicks, lost *in situ* (Hario 1994, Hario & Rudbäck 1996). There was no intra-specific killing or cannibalism in our Lesser Black-backed Gull colonies that could obscure the role of predation, nor did the parent birds remove corpses of dead chicks from the territories (detailed in Hario 1994). Based on this calculation, the equivalent proportions of preyed-on chicks versus diseased chicks in our 1990s' sample are 34% (i.e., 36% of 57 plus the verified 14, altogether making 34% of the total 103 chicks; see Table 1) and 72%, respectively, and 24% and 44% in the 2000s' sample. The diminishing rate of preyed-on chicks stems, without doubt, from the aforementioned culling program of predatory gulls conducted over the entire central Gulf of Finland in 2004–2007 (Hario *et al.* 2009). The diminishing rate of diseased chicks, on the other hand, could result from a better health status of the

population, possibly due to lower contaminant levels.

In chicks found diseased in the 2000s, the mean hepatic concentration of PCBs was not significantly smaller than previous, whereas that of DDE was. This led to a decreasing DDE/PCB ratio. This is the first record of an apparent lowering in some of the OC levels in our Lesser Black-back chicks.

4.3. Sample-chick technique: correlating residues and brood fate

Our result of a negative correlation between numbers of healthy siblings and OC residues in dead siblings is in accordance with several studies made on fish-eating waterbirds in North America and on Great Cormorants *Phalacrocorax carbo* in Europe, in which high residue levels in an egg paralleled with low hatching rate of the remaining clutch (the so-called sample-egg technique; see Dirksen *et al.* 1995, Larson *et al.* 1996, Custer *et al.* 1999). Yet, the DDE and PCB levels in this data set were also correlated, making separation of their effects difficult.

With regard to the different OC profiles, it has been difficult to decisively attribute lowered breeding success or lowered survival in wild birds and mammals to different pollutants due to the correlative nature of OCs (Hose & Guillette 1995, Bustnes *et al.* 2005). We have discussed this issue previously (Hario *et al.* 2004) and will not take a firm stance towards PCBs or pesticides as the main impactor (but see Custer *et al.* 1999, Helander *et al.* 2002, Henny *et al.* 2008). However, it needs to be stressed that along with the observed improvement in our chick disease rate at Söderskär, the compounds whose levels have reduced have been DDE and related OC pesticides, and not PCBs.

In waterbirds, there are little data on embryonic sensitivity to DDE, but it has been suggested that impairment in reproduction occurs at egg residues from 2.5 to 8 ppm w.w. in different fish-eating species (Custer *et al.* 1999). These are, however, geometric means on fresh eggs. The geometric mean of our DDE sample from the 1980s is 7.3 ppm w.w. in chick liver. As the concentrations in fresh eggs are 1.4–3.3 times lower than in pipping/hatchling stage (Drouillard *et al.* 2003), our liver figures in 0–3-day-old chicks (i.e. partly hatchlings) diminish to hypothetical 2.2–5.2 ppm w.w. This fells within the range of the above mentioned variation. The corresponding range for our 2000s' data is 0.7–1.9, figures below the postulated effect levels.

4.4. Dietary acquisition

Organochlorines are accumulated by dietary intake. Based on pellet and casting analysis and on direct observations of the courtship feeding at nests, we know that our Lesser Black-backed Gulls are predominantly fish feeders in the Gulf of Finland and that Clupeoides is the staple food (Hario 1990, 1997). However, in the Bay of Bothnia, they also forage on coastal farmlands and on fur farms (R. Wistbacka, in litt.) with food possibly being less contaminated. This may be the reason why the levels of PCBs in our data set are significantly lower in the Bay of Bothnia than in the Gulf of Finland. However, no such difference was found for DDE, even though the abundances of both legacy OCs (PCBs and DDTs) have significantly declined in Baltic fish since the 1970s (e.g., Pikkarainen & Parmanne 2006). Along the Finnish coast, PCBs are fairly evenly distributed in zooplankton, the major prey species of the Baltic herring *Clupea harengus* in the Baltic Sea (Koistinen *et al.* 2008). Thus, the most likely possibility for a higher trophic-level consumer to "reduce" the dietary intake of OCs in the Baltic is to switch from fish to a more terrestrial food. However, the DDE burden in the Bay of Bothnia did not diminish to the same extent as in the Gulf of Finland; in fact, the DDE/PCB ratio for the Bay of Bothnia sample tended to increase and was especially high in the 2000s. This may imply that there must be some extra loading of DDTs coming from outside of the Baltic biota that is highly unpredictable.

4.5. Exposure on the wintering vs. breeding grounds

The Lesser Black-backed Gulls spend 7–8 months of their year cycle outside the Baltic breeding grounds, mainly in Africa (e.g., Kilpi & Saurola

1984, Kube *et al.* 2000). In East African countries, DDT is still used to a considerable extent, especially in agriculture and horticulture but also in various other occasions like indoor spraying in houses for combating malaria (Anon. 1999). DDT is an effective and cost-efficient pesticide for controlling malaria-transmitting mosquitoes and tsetse flies carrying sleeping sickness to both man and cattle. DDT pesticide carries a relatively weak risk to human health at the environmental concentration, while it clearly has a harmful effect on wildlife, particularly on birds (Nimmo & McEwen 1994, Wiktelius & Edwards 1997). In the 1990s, DDT was used for fish poisoning as an illegal fishing practice throughout the riparian states of Lake Victoria, but this habit is now possibly vanishing due to governmental suspension of all fish export to the EU from these countries (Tanzania, Kenya, Uganda; Anon. 1999).

Without a focused study of marked or radio-tagged birds, it is difficult to assess to what extent Lesser Black-backed Gull adults become exposed to toxicants in Africa. In a study at the Ugandan Rift Valley lakes in January 2004, the wintering Lesser Black-backed Gulls were predominantly piscivorous, living in watersheds where cattle were commonly sprayed with DDT on the shoreline (Hario 2006, M. Hario unpubl. data). There is no overall high DDE contamination level in the biota of Africa but rather a wide range of various local hotspots (Ejobi *et al.* 1998). Fish fillets of the Nile Tilapia *Oreochromis niloticus* and Nile Perch *Lates niloticus* on the Tanzanian side of Lake Victoria in 1999 had DDT levels up to 3.8 ppm lipid weight, averaging 0.5. The detection of higher levels of DDT compared to its metabolite DDE implicated recent exposure of fish to DDT on the sampling sites (fish landing stations) (Henry & Kishimba 2006). On the other hand, a small sample ($n = 5$) of Nile Tilapia on the Ugandan side of Lake Victoria in 2002 averaged only 0.002 ppm wet weight (lipid weight not given; Hollamby *et al.* 2004). These figures compare to 0.05–0.13 ppm l.w. or 0.0014–0.0045 ppm w.w. in two-year-old Baltic herring in 2002 (Pikkarainen & Parmanne 2006). Thus, the upper range was 30 times higher in African fish, and the lower range was about the same magnitude as the ranges in the Baltic fish. In oceanic Capelin *Mallotus villosus*, from the Norwegian Barents Sea coast, levels of only 0.7 ppb DDE have been recorded (Savinova *et al.* 1995).

In eggs of African Fish Eagles *Haliaeetus vocifer* at Lake Kariba, Zimbabwe, in 1989–1990, mean levels of total DDT (= DDT + DDD + DDE) generally varied from 14 to 49 ppm dry weight per clutch, but up to 113–223 ppm dry weight were found in local hotspots nearby (Douthwaite 1992). These are fairly high levels (approximating 4–12 and 28–56 ppm w.w., respectively) exceeding the LOEL figure of 5–6 ppm w.w. for DDE in the eggs of Swedish White-tailed Sea Eagles *Haliaeetus albicilla* (Helander *et al.* 2002) (LOEL = lowest-observed-effect-level). However, the sensitivity variation can be large; in Swedish eagles, normal productivity was still observed at levels 3–5 times higher than LOEL in some cases (Helander *et al.* 2002).

4.6. Conclusions

It seems safe to conclude that Lesser Black-backed Gulls are prone to bioaccumulation from the numerous point sources of toxicants that exist in East African countries, but they also get legacy OCs from the still highly polluted Baltic biota. The Baltic Sea has a history of being the most polluted brackish water area in the world (e.g., Koistinen *et al.* 2008), and although the lipid-based residues in the Baltic herring are currently about 30% lower than in late 1990s (Pikkarainen & Parmanne 2006), they are still high compared to levels in other fish e.g. off the Norwegian oceanic coast. In the 1970s, at the time when the population decline of the nominate Lesser Black-backed Gull was first discovered, the Baltic biota was even more polluted (Bignert *et al.* 1998). Although not measured, the environment of the Russian *L. f. fuscus* population in the Onega Bay (representing 10% of the world population) has been thought to be less polluted than that of the remaining stock in East Fennoscandia, this possibly being one reason for the deviating population trends among these areas (Cherenkov *et al.* 2007). The migration habits of the Russian population are not known.

In the 2000s, however, the proportion of the diseased Lesser Black-backed Gull chicks at Söderskär declined along with decreasing hepatic levels of DDE, HCB, β-HCH and *trans*-nonachlor, for the first time since the commencement of

the population decline in the 1970s. This could indicate that the overall pesticide burden in Lesser Black-backed Gulls of the Baltic Sea has been decreasing, even though the data at our disposal are heavily skewed to the endpoint of the effects (dead chicks). More work is required to evaluate the role of the environmental pollutants in the chick disease etiology and in the population trajectory of the nominate Lesser Black-backed Gull. Diet constraints and diet switching are also interesting and understudied subjects that might emphasize this subspecies' susceptibility to poisoning.

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Vuosikymmenten vaihtelut selkälokin poikasten ympäristömyrkkypitoisuuksissa

Söderskärin riistantutkimusasemalla Suomenlahdella seurattiin yksityiskohtaisesti selkälokin (*Larus fuscus fuscus*) poikasten kasvua ja menestymistä kolmessa yhdyskunnassa vuosina 1978–2007. Pesimäkanta pieneni 8 % vuosivauhtia (250 parista 10–15 pariin). Lentopoikastuotto oli 1980-luvulla vain 0,4/pari ja 1990-luvulla enää 0,02/pari. Poikasista 65–70 % oli syntymästään sairaita ja kuoli sisäelinrappeutumiin, tulehduksiin ja verenmyrkytykseen. Orgaanisten halogeeniyhdisteiden pitoisuudet poikasten maksassa olivat korkeita, ja pitoisuudet korreloivat kuolevien sisarusten määräosuuteen pesyeessä. Tilanne parani 2000-luvulla siten, että tautikuolevuus oli enää 48 % ja lentopoikastuotanto nousi 0,52 poikaseen/pari. Samanaikaisesti DDE-pitoisuus poikasten maksassa oli alentunut tasolle, jolla ilmeisesti ei ole kuolevuusvaikutusta. PCB-pitoisuuksissa ei ollut merkitsevää suuntausta.

Pohjanlahden selkälokeissa (Kokkola, Pietarsaari) oli vähemmän ympäristömyrkkyjä kuin Suomenlahden, todennäköisesti ravinnon pienemmän kontaminaation johdosta. Siellä kuitenkin DDE/PCB-suhde pysyi korkeana vielä 2000-luvulla, mikä osoittanee DDT-saannon sattumanvaraisuutta selkälokin vuosikierron eri vaiheissa (pistekuormitus Afrikassa). Orgaanisten halogeeniyhdisteiden pitoisuudet ovat kuitenkin ensi kertaa osoittamassa pienenemisen merkkejä Söderskärin 30-vuotisessa aikasarjassa.

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