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Organochlorine concentrations in diseased vs. healthy gull chicks from the northern Baltic

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"Capsule": *Elevated DDE/PCB ratio correlates with a high rate of chick diseases in the endangered nominate lesser black-backed gull.*

Abstract

The population decline of the nominate lesser black-backed gull *Larus fuscus fuscus* in the Gulf of Finland (northern Baltic) is caused by an exceedingly high chick mortality due to diseases. The chick diseases include degeneration in various internal organs (primarily liver), inflammations (mainly intestinal), and sepsis, the final cause of death. The hypothesis of starvation causing intestinal inflammations (leading to sepsis) was tested by attempting to reproduce lesions in apparently healthy herring gull *L. argentatus* chicks in captivity. The herring gull chicks were provided a similar low food-intake frequency as observed for the diseased chicks in the wild. However, empty alimentary tract per se did not induce the intestinal inflammations and therefore, inflammations seem to be innate or caused by other environmental factors in the diseased lesser black-backed chicks. They had very high concentrations of PCB in their liver; but the concentrations were not significantly higher than those of the healthy herring gull chicks, indicating a common exposure area for both species (i.e. the Baltic Sea). When compared to NOEL and LOEL values for TEQs in bird eggs our TEQ levels clearly exceed most or all of the values associated with effects. Compared with published data on fish-eating waterbirds, the DDE concentrations in the diseased lesser black-backed chicks were well above the levels previously correlated with decreased reproduction, while the residues in apparently healthy herring gulls were below those levels. The DDE/PCB ratio in lesser black-backs was significantly elevated, indicating an increased exposure to DDTs as compared with most other Baltic and circumpolar seabirds. The possible exposure areas of DDT in relation to differential migration habits of the two gull species are discussed.

Keywords: Chick mortality; Diseases; Endangered gull; POPs; Baltic Sea

1. Introduction

Over the past three to four decades, the Fennoscandian subspecies of the lesser black-backed gull, the nominate *Larus fuscus fuscus*, has experienced drastic population declines over most of its range (reviewed by Hario et al., 1998). In the central Gulf of Finland, the fledging rate averaged only 0.13 during 1977–2002; i.e. only one in seven pairs managed to fledge an offspring. 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). As a consequence, the Finnish coastal population has been declining by an average of 8% per year during 1986–2002. 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 earlier studies on chick mortality in three different gull species from the Gulf of Finland (lesser black-backed gull, herring gull *L. argentatus*, and common gull *L. canus*), we demonstrated a high frequency of chick diseases due to degeneration and inflammation of various internal organs (Hario and Rudbäck, 1996,

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1999). In the Gulf of Finland, 70% of the chicks of the nominate lesser black-backed gull die of diseases (Hario and Rudbäck, 1996). The etiological infectious agent involved remains unknown. Degeneration and inflammation of various internal organs leading to bacteria entering blood circulation, and finally sepsis, have been determined as the cause of death. Possibly, the degenerated liver is not capable of detoxifying the bacteria. In our studies, death was always preceded by a marked weight loss.

Organochlorines have been detected in elevated concentrations in lesser-blacked back gull chicks in our study areas, and previous studies suggest an association between organochlorine levels and chick mortality (Hario et al., 2000). However, the causal relationship between organochlorine residues and chick diseases is poorly understood. The biochemical pathway between contaminant burden, pathologic changes in the host, and outbreak of diseases is unknown. High frequency of chick deaths in the wild was the initial observation of our study. We collected carcasses of freshly dead chicks in gulleries of the Gulf of Finland and the Bothnian Bay and conducted feeding experiments to investigate the following two questions:

1. Can lack of food explain the high occurrence of chick deaths in gulls, as is commonly believed, i.e. can the empty alimentary tract *per se* induce the inflammations leading to sepsis and early death;
2. To what extent do the different organochlorine body burdens parallel with the occurrence of diseases in gull chicks, i.e. is the promoting factor in chick deaths persistent organic compounds rather than food deficiences?

2. Materials and methods

2.1. Ecological data and pathological studies

The main study colony is located at the Söderskär Game Research Station, central Gulf of Finland (60 07 N/25 25 E) (Fig. 1). In 1995, reproductive parameters from 52 lesser black-backed gull nests and 13 herring gull nests were monitored during the entire breeding season, from April until early August. Nests were marked as soon as they were located, usually at the time of laying of the first egg. Eggs and chicks were marked with their ordinal number in the clutch (from hereafter: A, B, and C). Egg viability, hatching success, chick losses, weight gain of chicks, and fledging success were recorded during daily visits to colonies (weather permitting).

Egg mortality due to "eggs addled" and "died at pipping" was distinguished from other losses caused by predation, egg disappearances, desertion of nests, accidents (chicks fallen in crevices, entangled in vegetation, drowned) etc. By gross examination, the addled eggs showed no embryonic development, but because of difficulties in distinguishing between infertile bird eggs and those in which embryo mortality had occurred at an early stage (Birkhead et al., 1995) they were all classified addled.

The fates of the chicks were followed by (1) ringing each chick soon after hatching (with a steel ring), (2) locating rings of predated chicks in territories of predatory gulls (in food remnants), (3) weighing each chick each time it was found (with a spring balance to the nearest gram), and (4) collecting carcasses of dead chicks for further postmortem studies and chemical analyses.

Another lesser black-backed gull colony was used as a reference study population. The reference colony was located near the Tankar Bird Station in the northern Bothnian Bay (63 55 N/22 56 E), approximately 450 kilometres northwest of Söderskär (Fig. 1). Here, monitoring was conducted during the hatching period and the first half of the chick rearing period of 1995 in order to obtain information on egg viability and early chick mortality (see later).

No lesser black-backed gull chicks were sacrified for this study. The chicks collected for investigations were all found dead in the study colonies during nest checks. The carcasses were first transported for postmortem evaluation to the National Veterinary and Food Research Institute in Helsinki. Cause of death was determined by routine necropsy followed by histological

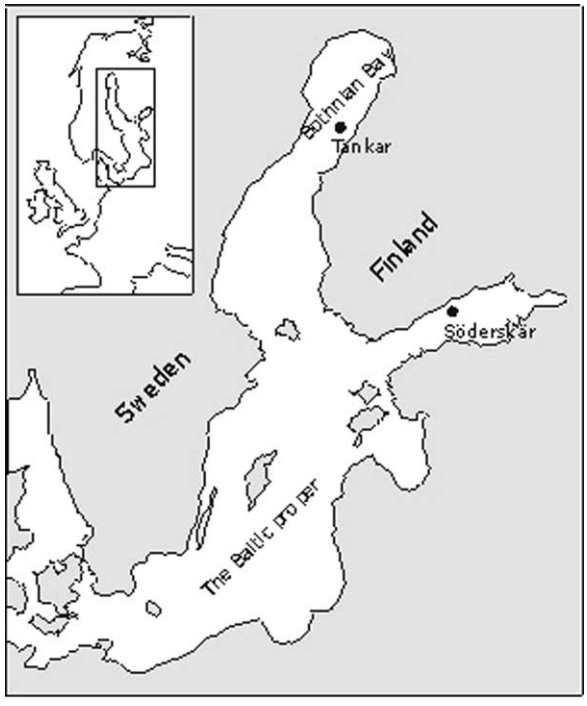

Fig. 1. Location of the study sites along the Finnish coast.

evaluation of tissues. Bacteriological examination was always performed on samples collected from lung and intestine, and on other organs based on gross findings (Hario and Rudbäck, 1996).

A total of 14 lesser black-backed gull chicks/hatchlings were necropsied, i.e. 41% of the 34 carcasses found in the study areas (Table 1). The small sample size resulted from logistical difficulties. The intestines decomposed rapidly, within a few hours, when the carcass remained in terrain exposed to hot sunshine, especially during afternoon hours. The shipping to Helsinki, a 25 km long sea voyage, resulted in a further delay.

2.2. Feeding trials

From our earlier studies we knew that growing herring gull chicks doubled their body mass during the first four days of life (from a mean of 66 g to a mean of 135 g; Hario and Rudbäck, 1996), whereas diseased lesser black-backed chicks of the same age lost weight, or at best, kept their weight constant (around 50 g). Consequently, due to the presence of a dilution effect (Lemmetyinen et al., 1982; Niemi et al., 1986) the organochlorine concentrations of lighter-bodied gull chicks tend to be markedly higher than those of heavier-bodied chicks even when the actual tissue burdens were equal (Hario et al., 2000).

To allow for comparisons of organochlorine concentrations between healthy herring gull chicks and diseased lesser black-backed chicks, we exposed herring gull chicks to a feeding trial in which we aimed to hinder their rapid weight gain. We also wanted to know whether the lowered feeding frequency per se would lead to an outbreak of diseases and elevated mortality, as has been suggested in many gull studies in which an empty alimentary tract and a low body weight have been considered as proof of food deficiences in the environment (e.g. Bevanger and Thingstad, 1990; Strann and Vader, 1992; Pons and Yésou, 1997).

Under a special licence from the Ministry of the Environment (no. 16/4342/91), seven herring gull C-chicks were removed from their nest on the day of hatching and reared in captivity for four days. They were kept in an outdoor aviary and provided free access to shelter from adverse weather. Water was supplied *ad libitum*. They were fed twice a day (at 09 am and at 12 pm) with ground herring (*Clupea harengus*) mixed with water. The amount of fish fed daily (averaging 30 ml) was adjusted to suffice only for the maintenance but not for the growth [amounts derived from the energy requirement formula provided by Kahru and Keskpaik (1983)]. Chicks were weighed before and after every meal. At night, they were moved indoors to a roosting temperature of 27–30 °C to ensure normal thermoregulation (Robbins and Ballew, 1984). On day 5 of the experiment, the chicks were euthanized with chloroform, and patholocigal studies were conducted in the same manner as on wild chicks found dead. Their older siblings (A and/or B) were monitored in the wild and were weighed every day up to the day 17, when they were euthanized and necropsied as well. Thus, from the point of species conservation, only seven out of the 464 herring gull broods (1.5%) over the entire Söderskär area were sacrified for this study, but none of the 62 local lesser black-backed gull broods.

Table 1
Breeding parameters of the gulls studied at Söderskär, Gulf of Finland, and at Tankar, northern Bothnian Bay, in 1995

	Lesser black-backed gull		Herring gull
	Söderskär	Tankar	Söderskär
No. of nests	52	28	13
Eggs laid	150	⩾64	34
Mean clutch size	2.89	–	2.62
Addled eggs (%)	4 (2.7)	5 (7.8)	2 (5.9)
Died at pipping (%)	8 (5.3)	4 (6.3)	3 (8.8)
Hatching rate	68.7%	–	61.8%
Egg mortality	8.0%	14.1%	14.7%
Chicks hatched	103	54	20
Chicks disappeared (%)	57 (55.3)	–	0
Found diseased			
at 0–4 days (%)	14 (13.6)	3 (5.5)	0
at >4 days	16 (16.5)	–	1 (5.0)
Verified as preyed-on (%)	14 (13.6)	0	1 (5.0)
Fledglings/nest	0.02	–	1.15

2.3. Chemical analysis

After necropsy, the carcasses were stored at −20 °C until chemical analyses. The following organochlorines were analyzed in 11 lesser black-backed chick livers and 16 herring gull chick livers by the laboratory of Finnish Environment Institute's Research Department, Helsinki: α-, β-, γ-, and δ-hexachlorocyclohexane (HCH), α- and γ-chlordane, oxychlordane, *trans*-nonachlor, hexachlorobenzene (HCB), *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, and total PCBs. Total PCBs comprised the following 21 congeners (IUPAC nos.): 8, 18, 28, 31, 52, 66, 101, 110, 77, 149, 118, 153, 105, 138, 187, 129, 126, 156, 180, 170, and 169.

Each sample was placed in a beaker and extracted with 25 ml of acetone:hexane (1:1, v/v) for 60 min with an Ultrasonic Cell Disruptor (Branson Sonifer B-15). About 17 ml of the extract was evaporated until dry in a rotavapor (Buchi RE-121) 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 into isooctane following fat purification with concentrated sulphuric acid.

OCPs and PCBs were analyzed with 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 a HP 7673 automatic sampler. The residues are given as μg/g of liver lipid weight unless otherwise stated.

2.4. Statistical analysis

Organochlorine residues are presented as arithmetic mean values with standard deviations and ranges unless otherwise stated. To calculate the sum PCB (SPCB), samples where chemicals were not detected were assigned a value at half of the congener-specific detection limit. Due to the small sample sizes and the low frequency of detectable concentrations in some of the chemicals measured, the statistical evaluation is based on the use of nonparametric tests. We used a Mann–Whitney U-test for examining the residue differences between species and sites, and χ^2 test for comparing proportions. For the chick weight data, the similarity of variances was first tested with Bartlett's test and when found homoscedastic, the data were analysed with one-way ANOVA. Tests are two-tailed and significance is set at 0.05. All computations were done using SYSTAT 5.0 package.

3. Results

3.1. Overall breeding success

At Söderskär, hatching rate (percentage eggs hatched from the initial number of eggs layed, Table 1) did not differ between the two gull species ($\chi^2 = 0.601$, df = 1, $P = 0.438$). In the reference area, Tankar in the Bothnian Bay, the initial number of eggs laid is not known due to the shortened period of monitoring, and therefore, the final hatching rate was not calculated. Egg mortality, i.e. the combined percentage of addled eggs and eggs that died at pipping, were equal among sites ($\chi^2 = 2.505$, df = 2, $P = 0.286$) as well as among gull species at Söderskär ($\chi^2 = 1.486$, df = 1, $P = 0.223$). Fertility of the addled eggs could not be assessed, but the percentage of eggs that died at pipping did not deviate between sites nor species ($P > 0.1$).

The final fledging result of lesser black-backed gulls at Söderskär in 1995 was 0.019 fledglings/pair and that of herring gulls 1.15 fledglings/pair.

3.2. Chick mortality

The above mentioned difference in breeding success among species was due to differences in chick mortality rates. The majority of the lesser black-backed chicks merely disappeared (Table 1), which is a common phenomenon in all field studies on ground-nesting larids. Of the verified losses, the category "found diseased" refers to chicks found dead. Their carcasses were always intact, with no injuries to the body nor signs of pecking attributable to adult aggression during territorial clashes (adult intraspecific aggression on chicks does not exist in lesser black-backed gulleries at Söderskär, whereas predation on chicks by larger gulls is very common, see Hario, 1994). The rate of documented predation on lesser black-backed chicks at Söderskär was 14% (Table 1)—a fairly high proportion when based merely on ring retrieval from nests of predatory gulls. It lies within the range of a long-term yearly variation (10–37%) of this phenomenon at Söderskär (Hario, 1994; Hario and Rudbäck, 1999). Most chicks were taken as downy young, and their rings were located in pellets and food remnants in local herring gull territories.

In the following sections, only the "found diseased" category will be treated. As in previous years, the lesser black-backed chicks of this category could be divided into two distinct "death waves" (see Hario and Rudbäck, 1996):

1. those that died in the nest within 4 days of hatching, showing poor weight gain or none at all, and
2. those that first grew normally, but at approximately 1–3 weeks of age showed similar symptoms, and died within 4–5 days after weight loss was first observed.

Growth curves for 1995, clearly illustrating this distinction are presented in Fig. 2. Chick death was always preceded by a marked reduction in body weight. At Söderskär, lesser black-backed chicks of the first death wave lost, on average, 15.7% of their body weight (SD = 10.6, median 17.0, $n = 11$) during the three days they stayed alive after the first signs of weight loss (mean 2.7 ± 0.8 days, median 3, $n = 11$). The corresponding figures for the second death wave were 21.8 ± 10.5% (median 22.8, $n = 9$) and four days (4.1 ± 2.0, median 4, $n = 9$).

The median age at the time of death in the first wave was three days (mean 2.8 ± 1.4, range 0–4, $n = 14$) whereas it was 10 days in the second one (mean 11.3 ± 3.0, range 6–16, $n = 16$). Only one herring gull chick was found diseased, at the age of 10 days. In the following, the data on chick diseases and contaminant loads are ranked according to these two death waves.

At Söderskär, no differences existed in the occurrences of the "found diseased" category among siblings of lesser black-backed gulls (A:B:C = 10:10:8; $\chi^2 = 0.446$, df = 2, $P = 0.800$).

3.3. Chick diseases

Forty-one percent of chicks found dead in this study (Table 1) were suitable for postmortem examination

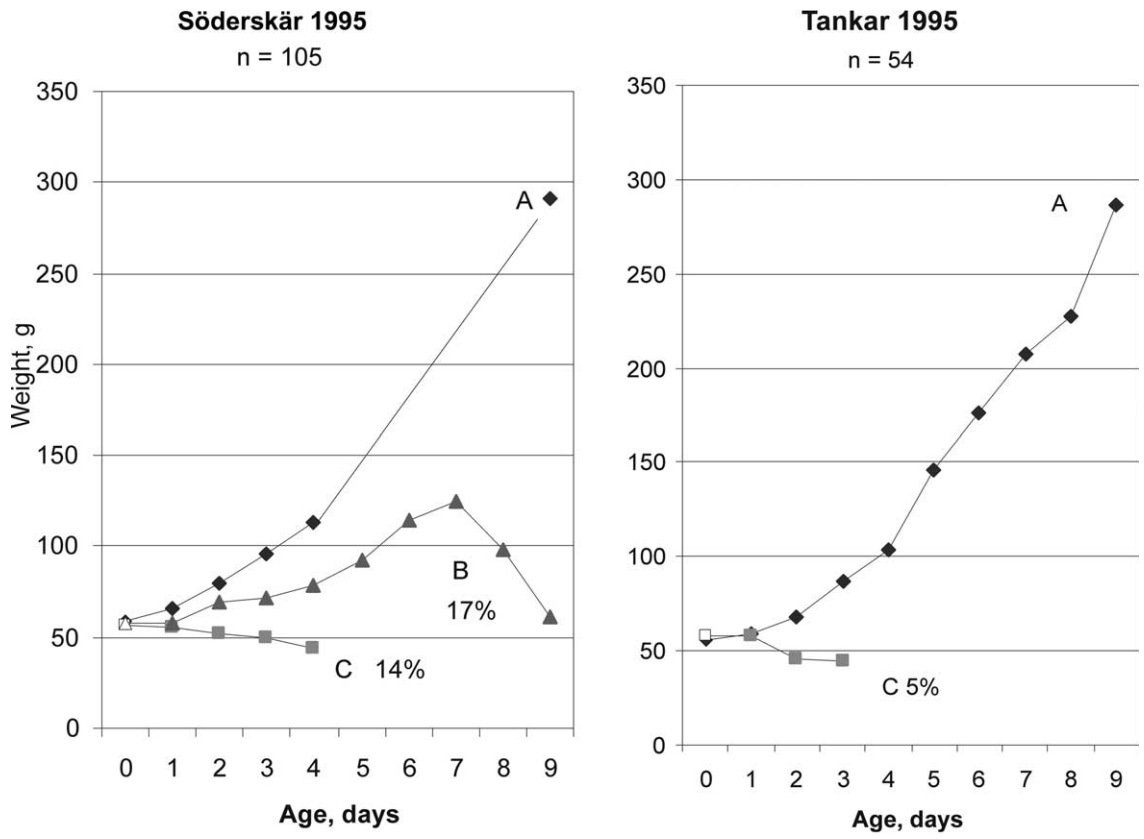

Fig. 2. Daily means of body weight of lesser black-backed gull chicks from the Gulf of Finland (Söderskär) and from the northern Bothnian Bay (Tankar) in 1995. Graphs indicate healthy (A) and the two "found diseased" groups: B = died at >4 days; C = died at 0–4 days. There was no B group at Tankar. Percentages of the total (*n*) in each group are also indicated.

($n = 14$). They were all lesser black-backed chicks from Söderskär. Irrespective of age and position (A, B, or C) in the brood, all had similar pathological findings: (1) degenerations in the liver, cardial 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.

Chicks had no signs of depleted lipids except that their yolk sac had disappeared by day 4. Emaciation, with a marked reduction in body weight through dehydration, was typically seen in the terminal stages.

Hand-reared herring gull chicks that were exposed to the feeding trial had different pathological findings as compared with the diseased lesser black-backed chicks from the wild. Despite the low body weight (Fig. 3), empty alimentary tract and signs of anemia (probably caused by the low feeding frequency), no inflammations nor degenerations in internal organs were observed. At the time of euthanasia on day 5, the herring gull chicks appeared healthy, and no sepsis was discernible at necropsy. Thus, empty alimentary tract per se did not induce intestine inflammation, and we consider the herring gulls in this experiment healthy. Their 17-day-old siblings, collected for chemical analysis from the wild, appeared equally healthy, with no signs of inflammations or degenerations. Their growth also had been very rapid (Fig. 3).

3.4. Organochlorine concentrations in diseased vs. healthy chicks

Concentrations (ng/g, w.w.) of the following organochlorines were below detection limits (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).

Also, the PCB congeners no. 8, 18, 31, 129 and 169 were not included in the statistical analysis as their levels were below the detection limits. Comparisons between groups (according to species, age group and location) are based on congener nos. 28, 52, 118, 138, 153, 180 (a.k.a. 6PCB), which together amount to about 80% of the sum concentrations of the 21 congeners measurable (so called sum-PCB or SPCB) ($79 \pm 4\%$, $n = 30$ samples). In order to study whether chick deaths could be related to specific PCB-congeners, the data were partitioned according to differentially chlorinated groups of CBs. Di-*orto*-CBs comprise congener nos. 153, 138, 180 and 170; mono-*ortho*-CBs congener nos.

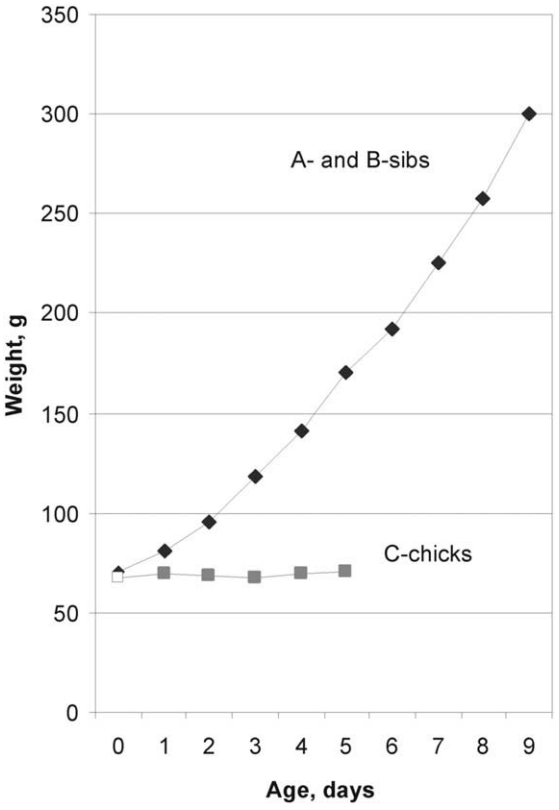

Fig. 3. Mean body weight of the healthy herring gull chicks exposed to feeding trials (C-chicks) and that of their siblings in the wild (A- and B-sibs).

66, 118, 105, and 156, and non-*ortho*-CBs congener nos. 77 and 126.

Two sets of toxic equivalency factors (TEFs) were used to calculate toxic potencies of coplanar PCBs, viz. those established by the World Health Organization (WHO, 1993) and those proposed by Safe (1990). The latter TEQs are expressed on a wet weight basis to allow for comparisons with published data.

Measurable amounts of other organochlorines were detected in all samples. In the following, all residues are presented in μg/g both on a wet weight basis and on a lipid weight basis.

3.4.1. PCB concentrations

On a lipid weight basis, PCB levels in black-backed chicks from Söderskär and in herring gull chicks of the same age were very similar due to the fact that the liver lipid content is three times lower in the herring gulls (Table 2, 0–4-day-old). On a wet weight basis, there is a difference (Mann–Whitney $U=7.00$, $P=0.05$); yet, since organochlorines are sequestered in lipids, the comparison should be based on lipid weights and not on wet weights.

Between sites, diseased lesser black-backed chicks from Tankar had suggestively lower 6PCB concentrations in their liver than diseased lesser black-backed chicks from Söderskär ($U=20.00$, $P=0.1$), whereas the concentrations equalled to those of healthy herring gulls of the same age ($P > 0.1$). The 17-day-old herring gull chicks had lowest concentrations of all, but this is largely due to the dilution effect.

When partitioned into subsets of congeners of different planarity (Table 3), none of the congener-specific comparisons between species and sites gave significantly deviating results. The same applied to the WHO-TEQs based on lipid weights. Thus, no differences between gull groups emerged in the non-*ortho*-CBs (77 and 126), which are the most toxic dioxin-like coplanar PCBs ($P>0.2$ in all three comparisons).

The wet weight based TEQs for non-*ortho*-CBs (SAFE-TEQs) gave deviating results in that the diseased lesser black-backed chicks from Söderskär had significantly higher levels than the healthy herring gull chicks ($U=2.00$, $P=0.004$). The same applied to the comparison between the Tankar gulls and the healthy herring gulls ($U=3.00$, $P=0.036$), whereas no difference emerged in diseased lesser black-backed chicks among sites ($U=21.00$, $P=0.182$).

3.4.2. DDE concentrations

The diseased lesser black-backed chicks from Söderskär had significantly higher lipid weight DDE concentrations than healthy herring gull chicks of the same age (Table 2; $U=40.00$, $P=0.048$). Between sites, diseased lesser black-backed chicks from Tankar tended to have lower DDE concentrations than those from Söderskär ($U=21.00$, $P=0.186$). These differences held when comparisons were made on a wet weight basis, too. No difference was found between Tankar gulls and similar-aged healthy herring gull chicks from Söderskär (based on lipid weight: $U=15.00$; $P=0.850$).

In healthy herring gull chicks, the DDE levels were less than half of the PCB levels in the same individual, whereas in lesser black-backed chicks the concentrations of DDE and PCBs were of equal magnitude (Table 2).

The total concentrations of PCBs and DDE were highly significantly correlated over the entire data set (Spearman $r=0.905$, $n=27$, $P<0.001$).

3.4.3. HCB, oxychlordane, trans-nonachlor

No significant differences were found in HCB, oxychlordane and *trans*-nonachlor concentrations among chick groups. There was a highly significant correlation between HCB and DDE concentrations over the entire data set ($r_s=0.885$, $P<0.001$).

4. Discussion

In herring gull chicks, the early outbreak of the diseases did not seem to be promoted by the empty alimentary

Table 2
Hepatic concentrations of organochlorines (μg/g, w.w. and l.w.), body weight at death (g), and lipid-% in diseased lesser black-backed chicks and healthy herring gull chicks from the Gulf of Finland (Söderskär) and from the Bothnian Bay (Tankar) in 1995. 0-4–day and 17–day refer to the age groups of the chicks. Arithmetic mean±SD (range) are given.

	Diseased lesser black-backed chicks		Healthy herring gull chicks	
	Söderskär	Tankar	Söderskär	
	0–4-day, $n=7$	0–4-day, $n=4$	0–4-day, $n=7$	17-day, $n=9$
6PCB, w.w.	16.7±11.9	6.4±3.5	4.7±1.8	0.1±0.04
	(3.7–36.2)	(3.6–11.0)	(2.8–7.4)	(0.07–0.1)
6PCB, l.w.	158.5±109.9	94.6±88.6	130.8±63.5	3.8±0.9
	(32.8–375.9)	(31.5–222.5)	(69.3–258.6)	(2.2–5.2)
DDE, w.w.	16.6±10.5	5.4±4.3	2.0±0.9	0.06±0.03
	(1.8–30.0)	(1.3–11.0)	(1.1–3.4)	(0.02–0.1)
DDE, l.w.	170.9±108.8	80.7±84.7	56.1±28.7	1.9±0.7
	(15.9–309.0)	(14.1–198.8)	(25.9–110.3)	(0.7–2.7)
HCB, w.w.	0.10±0.09	0.08±0.03	0.05±0.02	0.003±0.001
	(0.03–0.3)	(0.05–0.1)	(0.01–0.09)	(0.001–0.005)
HCB, l.w.	1.01±0.99	1.16±0.90	1.20±0.47	0.10±0.03
	(0.24–3.11)	(0.54–2.48)	(0.43–1.96)	(0.05–0.13)
oxychlordane, w.w.	0.07±0.08	0.06±0.03	0.04±0.01	0.002±0.001
	(0.01–0.27)	(0.03–0.09)	(0.03–0.06)	(0.001–0.002)
oxychlordane, l.w.	0.67±0.83	0.99±1.14	1.24±0.56	0.05±0.01
	(0.11–2.39)	(0.29–2.69)	(0.72–2.28)	(0.03–0.06)
trans-nonachlor, w.w.	0.08±0.07	0.06±0.05	0.01±0.007	0.001±0.001
	(0.02–0.2)	(0.02–0.12)	(0.001–0.02)	(nd–0.002)
trans-nonachlor, l.w.	0.76±0.77	0.62±0.31	0.28±0.16	0.04±0.01
	(0.14–2.39)	(0.26–0.93)	(0.03–0.51)	(0.02–0.05)
DDE/SPCB	0.93±0.48	0.61±0.22	0.35±0.04	0.40±0.11
	(0.23–1.41)	(0.29–0.79)	(0.31–0.39)	(0.10–0.49)
SPCB, w.w.	20.7±14.6	8.0±4.5	5.7±2.2	0.2±0.05
	(4.7–44.3)	(4.4–13.9)	(3.4–9.0)	(0.1–0.3)
SPCB, l.w.	196.4±133.3	119.0±112.0	158.9±75.5	5.07±1.27
	(41.1–460.0)	(39.5–280.5)	(84.0–309.9)	(3.0–6.74)
Body weight	49.5±8.8	49.0±5.5	67.0±8.0	571±115
	(38–72)	(45–53)	(56–77)	(400–710)
Lipid-%	10.4±2.9	9.3±4.3	3.8±1.0	3.2±0.6
	(6.2–15.9)	(3.3–12.9)	(2.6–5.4)	(2.4–4.2)

Table 3
Hepatic concentrations of differentially chlorinated groups of CBs (μg/g, l.w.) in 0–4-day-old gull chicks from the Gulf of Finland (Söderskär) and from the Bothnian Bay (Tankar) in 1995

	Diseased lesser black-backed chicks		Healthy herring gull chicks
	Söderskär	Tankar	Söderskär
	0–4-day, $n=7$	0–4-day, $n=4$	0–4-day, $n=7$
non-*ortho*	1.064±1.210	0.690±0.636	1.096±0.396
	(0.301–3.664)	(0.235–1.587)	(0.567–1.840)
WHO-TEQ	0.019±0.013	0.011±0.012	0.013±0.005
SAFE-TEQ (w.w.)	0.0031±0.0026	0.0011±0.0006	0.0008±0.0002
mono-*ortho*	29.67±26.43	17.87±18.73	28.25±13.57
	(7.69–83.77)	(5.37–45.04)	(13.71–51.33)
WHO-TEQ	0.006±0.005	0.005±0.004	0.006±0.003
SAFE-TEQ (w.w.)	0.0031±0.0028	0.0012±0.0007	0.0010±0.0005
di-*ortho*	150.74±99.88	90.54±83.46	120.55±58.26
	(30.02–345.65)	(30.45–211.00)	(64.50–239.91)

Non-*ortho*-CBs comprise congener nos. 77 and 126, mono-*ortho*-CBs congener nos. 66, 118, 105, and 156, and di-*orto*-CBs congener nos. 153, 138, 180 and 170. The concentrations of instrumental TEQs for coplanar PCBs are calculated from the two sets of TEFs (WHO, 1993; Safe, 1990). SAFE-TEQs are expressed on a wet weight basis. Arithmetic mean±SD (range) are given.

tract per se, as was shown by the feeding trials. Thus, the diseased chicks were not victims of food deficiences or parental neglect (i.e. no adaptive brood reduction *sensu* Lack, 1954; Mock, 1994). This conclusion has further been supported by our video-recordings at nests (Hario and Rudbäck, 1999). Diseased lesser black-backed chicks were prudently offered food by their parents; yet, the moribund chicks could not digest the food but vomited it out or just sat at the food bolus without eating. They appeared too sick to digest, and they died by the fourth day showing no weight gain despite having free access to food. At necropsy, they had similar pathological changes that were documented in the present study.

It may be suggested that comparison between two different species is not a valid approach to assess potential effects of food deprivation on survival, because of the possible differences in tolerance between the species. This suspicion applies both to the conclusion of missing effects of empty alimentary tract and to the different organochlorine levels between species (see below). However, a gull chick refraining from eating is highly abnormal, and most of our lesser black-backed chicks under surveillance never ate during their short lives. The median age at death was only three days. We feel this is too short a period for starvation effects to emerge as the yolk sac only disappeared by day 4. The healthy herring gull chicks instead were seemingly hungry and vigorously begged for food, their behaviour clearly deviating from the apathetic behaviour of the lesser black-backed chicks. We conclude that lesser black-backed chicks were sick from the beginning and their diseases were innate, not induced by the empty alimentary tract and certainly not by food deficiences in the environment. This is contrary to the common belief that factors affecting gull chick mortality are predominantly ecological in character (e.g. Parsons, 1975; Hahn, 1981; Hebert and Barcley, 1986; Pierotti and Bellrose, 1986; Strann and Vader, 1992; Hamer et al., 1993; Royle and Hamer, 1998), mostly in accordance with the theory of adaptive brood reduction or sibling rivalry (for reviews see Forbes, 1994; Stenning, 1996). Yet, no earlier studies made attempts to investigate the causes of death by detailed post-mortem evaluations of carcasses.

4.1. PCB concentrations

Innate diseases are not unique in the wild, however. For a fatal sepsis (bacteria entering the blood circulation), the dysfunction of a degenerated liver is probably significant. Because the diseased lesser black-backed chicks were not able to eat, all their organochlorine burden must originate from maternal transfer, i.e. from the female via the yolk sac into the liver.

In the present study, the hepatic PCB levels in chicks with the liver degeneration were not significantly higher than those in healthy chicks. This might indicate a low connection between residues and diseases. Indeed, our PCB levels in diseased lesser black-backed chicks are within the wide range of levels found *not* to affect the embryonic development of various fish-eating waterbirds (ranging 3–105 ppm wet weight, see Larson et al., 1996). Yet, there are a number of other studies indicating acute toxicity of PCBs to bird embryos at lower levels than referred to here (see Giesy et al., 1994; Barron et al., 1995; AMAP, 1998).

Some bird species seem to be more resistant to organochlorines than others, and there is a great deal of individual variation also within species and populations. Among 20 clutches of well-reproducing (1–1.8 nestlings) white-tailed eagles (*Haliaeetus albicilla*) from Sweden, 12 had PCB levels exceeding the suggested LOELs of 25 ppm w.w. affecting embryo mortality (LOEL = lowest-observed-effect-level; here recalculated from ppm in lipid weight × 0.20) (Helander et al., 2002). At the extreme, this exceedance level was 6-fold. The relative sensitivity of nominate lesser black-backed gulls in this respect is not known.

4.1.1. Sample-chick technique: correlating PCBs and effects

To increase sample size we added the 1991 PCB data of lesser black-backed chicks from Söderskär (Hario et al., 2000) into the present data and got a significant negative correlation between PCB concentrations and the number of healthy siblings within the brood ($r_s = -0.512$, $n = 19$, $P = 0.02$). This result 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). However, we refrain from making definite conclusions from these data due to our small sample sizes in chemical analyses. The corresponding comparison on DDE yields an equally negative correlation (−0.134) though not a significant one (sample size only seven broods). Moreover, the DDE and PCB levels were highly correlated, making further separation of their effects difficult.

It is noteworthy, that among the PCB congeners of differential planarity, the non-*ortho*-CBs were not disproportionately elevated in our most contaminated chicks from Söderskär. While most PCB congeners are relatively non-toxic, some of them are potent dioxin-like toxicants, such as PCB 126 and PCB 77 (WHO, 1993), both of which are included in the non-*ortho*-group of our study. Non-*ortho*-CBs represent a vast majority of the known TEQ load (toxic equivalents) in fish-eating waterbirds although their contribution to total PCBs is very small, in our data ranging 0.07–0.1%, and in Great

Lakes' colonial waterbirds 0.02–0.2% (Frank et al., 2001). The TEQ values of non-*ortho*-CBs in the present study were nontheless high. When compared to LOEL and NOEL values for TEQs in bird eggs (pg/g, w.w.; see Giesy et al., 1994; AMAP, 1998) they clearly exceed all of the values associated with effects. Furthermore, the levels in Söderskär lesser black-backed chicks (3100 pg/g w.w. for non-*ortho*-CBs) exceed all known LD_{50} values for wild birds (LD_{50} = lethal-dose-with-50%-mortality).

4.2. DDE concentrations

Our DDE concentrations of 16.6 ppm (w.w.) in diseased lesser black-backed chicks from Söderskär rank well above the minimum DDE concentrations in waterbird eggs that were associated with decreased reproduction. These "effect levels" have varied between 2.5 and 8.0 ppm (w.w.) in eight fish-eating bird species in North America (Custer et al., 1999). For white-tailed eagle eggs from the Swedish Baltic coast, a reduction in eggshell thickness was noted at DDE levels exceeding 2.5 ppm (w.w.), but eggshell thickness was not correlated with productivity (Helander et al., 2002). Productivity (measured as number of nestlings) was not affected until DDE concentrations in eggs rose to 100 ppm lipid weight (l.w.; roughly 5 ppm in w.w.), and it dropped to an average of 0.7 young at 210 ppm (10.5 in w.w.) and down to zero at 900 ppm (45 in w.w.). The LOEL figure is thus set at 100–120 ppm l.w. (5-6 ppm w.w.). In bald eagles (*H. leucocephalus*) DDE concentrations exceeding 15 ppm (w.w.) and 20 ppm (w.w.) in eggs were associated with almost complete breeding failure (Nisbet and Risebrough, 1994 according to Helander et al., 2002). Our figures in chick livers approached these, although no data exist on sensitivity variation within species in gulls. In white-tailed eagles from Sweden, normal productivity was still observed at levels 3–5 times higher than LOEL in some cases (Helander et al., 2002).

We need to convert our liver residues into residues in eggs in order to make more direct comparisons, however. Our DDE figures on a liver tissue basis for the lesser black-backed gulls from Söderskär rank among the highest in seabirds currently known to us (see AMAP, 1998; Custer et al., 1999; Frank et al., 2001), with the exception of the sea eagles. However, comparison with the above mentioned levels in eagles is hampered by the differential pattern of lipid concentrations in eggs and chicks. The concentrations in eagle egg samples, mentioned above, were analyzed in unhatched/putrefied eggs with up to half-grown embryos and were expressed on a lipid basis. During incubation, a reduction of 50% of lipids occurs in a herring gull egg through metabolism, mainly during the latter half of the incubation (Peakall and Gilman, 1979), after which the residue concentrations have doubled due to residues being concentrated into a smaller lipid mass (see also Newton and Bogan, 1978). This makes the comparison between eggs and chicks awkward.

For instance, newly-laid herring gull C-eggs from the German coast had lipid weight DDE concentrations of an average 2.6 times lower than A-siblings (5-day-old) of the same brood, as their lipid content was 11% as compared to only 2% in chicks (Becker and Sperveslage, 1989: Table 1). Pollutant levels do not increase in avian eggs, and fresh eggs should have higher or equal organochlorine loads than hatchlings and very young chicks. Indeed, when converted into wet weight basis the difference in German eggs turns into the opposite so that DDE concentrations were on average 1.8 times higher in eggs than in chicks (residue in lipid weight divided by the ratio 100/lipid-%, yet, the sequential increase of 13% from A-egg to C-egg not taken into account, see Becker et al., 1989). This sounds reasonable because the dietary exposure of DDTs in the German coast was very low throughout the chick growing period, having little effect on young chicks (that still partially rely on the yolk sac energy reserves). Applying this ratio 1.8 to our lesser black-backed gull livers, the mean of 17 ppm (on a wet weight basis) in 3-day-old chicks (from Söderskär) corresponds to a hypothetical 31 ppm in freshly laid eggs. This figure compares well to the critical levels found in sea eagle eggs.

Despite the high degree of correlation between PCBs and DDE, Custer et al. (1999) believe that DDE, and not PCB, is responsible for egg mortality in double-crested cormorants (*Phalacrocorax auritus*) in Green Bay, USA (and this was not due to eggshell thinning). The same applied to the Swedish white-tailed eagles (Helander et al., 2002). Yet, in observational studies, based on correlation of residue levels of a sample egg with the hatching success of the remaining clutch, the effects of correlated contaminants cannot be unequivocally separated (Custer et al., 2001). Our residues, too, were highly significantly correlated, not only PCB and DDE but also the fungicide hexachlorobenzene (HCB) and DDE.

4.3. DDT/PCB ratio

Since the ban of PCBs and DDT use in the 1970s, the residues have been declining in circumpolar countries, especially the DDT levels. The decrease of DDT started earlier and has been more rapid than that of PCB, resulting in a reverse ratio of the substances (Bignert et al., 1995; Olsson et al., 2000). In the Baltic seals, the DDT/PCB used to be above 2.0, whereas it is currently below 0.5 (Nyman et al., 2002). For Finnish inland (lake areas) lesser black-backed gulls from the early 1970s, the ratio was 1.47 (Paasivirta et al., 1981). For white-tailed eagles, the ratio decreased from 1.0 in the late

1960s to 0.3–0.4 in the mid-1990s in Sweden (Helander et al., 2002) and to 0.2 by mid-1980s in Finland (calculated from Table 1 in Tarhanen et al., 1989). Currently, the DDT/PCB ratio is far below 1.0 in nearly all studies on circumpolar seabirds, the only exception being mollusc-feeding eiders in Norway and Canada (ratio 1.0; AMAP, 1998: Table 6 A16).

In our herring gull chicks, the ratio conforms well with the general trend, being 0.35 (Table 2), whereas it is still 0.93 in lesser black-backed chicks from Söderskär (median 1.18; $U=1.500$, $P=0.006$) and 0.61 in those from Tankar. This implies that lesser black-backed gulls receive additional exposure from areas that are not shared by the other fish-eating waterbirds of the Baltic (with the osprey *Pandion haliaeetus* being a notable exception, see later). All the northern gulls for which data exist show significantly lower DDT/PCB ratios than lesser black-backs from the Gulf of Finland: herring gulls from Norway and Russia (median 0.23; $U=5.00$, $P=0.03$), glaucous gulls (*L. hyperboreus*) from Norway, Canada and Russia (0.33, $U=13.00$, $P=0.06$), kittiwakes (*Rissa tridactyla*) from Norway, Canada and Russia (0.125, $U=1.00$, $P<0.0005$) (data derived from AMAP, 1998: Table 6. A16).

4.4. Sources of pollutants

According to ring recoveries (Kilpi and Saurola, 1984), ringing controls (own obs.) and satellite tracking (Kube et al., 2000), 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 in the south. They travel long distances with apparent ease and use the Black Sea and the eastern Mediterranean as stopover sites (Kube et al., 2000). Some choose to stay in the Mediterranean (Israel and Egypt), but apparently the majority of the population follows the Rift Valley route flying to Ugandan waterbodies.There they stay from November to February/March, with the largest concentrations comprising more than 1000 individuals (Byaruhanga et al., 2001, M. Wilson in litt.). In all these East African countries, DDT is still used to a considerable degree (Crick, 1990; Nimmo and McEwen, 1994). DDT is a very effective and economical pesticide for controlling malaria-transmitting mosquitoes and tsetse flies carrying sleeping sickness in both man and cattle. DDT pesticide has a relatively weak human health risk at environmental concentrations, while it has a clearly harmful effect on wildlife, particularly on birds (Nimmo and McEwen, 1994).

We do not know to what extent lesser black-backed adults get exposed to toxicants in Africa; e.g. almost nothing is known of their feeding ecology there. However, it seems safe to conclude that they are prone to bioaccumulation from the numerous point sources of toxicants that exist in East African countries (Anon., 1999). The same applies to ospreys that also overwinter in Africa. Ospreys from Sweden used to have high DDT/PCB ratios in the 1980s (0.82; Jansson et al., 1993: Table 4). Herring gulls, on the other hand, are sedentary in the Baltic, or short-distance migrants within Europe (Kilpi and Saurola, 1984). Their contaminant burdens mostly reflect the environmental situations in these areas.

4.5. Suggestion for biomagnification

One to two weeks after their vernal migration from Africa back to the Baltic, lesser black-backed females start laying. They are fairly independent of the courtship-feeding efficiency of the male; in fact, females foraging by themselves lay proportionately larger C-eggs than those relying on male's provision (Hario, 1997). In the Gulf of Finland, male provision consists almost entirely (> 90%) of Baltic herring, and the independently foraging females presumably prey upon the same fish species. In the Bothnian Bay, they make use of ample access of offal from fur animal farms, obtaining half of their diet from anthropogenic sources (Tikkanen et al., 1998). Herring gulls from the Gulf of Finland, for their part, rely heavily on fish in their diet, just like lesser black-backed gulls. This applies both to their courtship feeding (Kilpi and Byholm, 1995) and to the chick rearing period (Hario, 1990; Hillström et al., 1994). Organochlorine levels in the Baltic herring has shown a similar decreasing trend since the 1970s as was shown for several seabirds, including the sedentary common guillemot (*Uria aalge*) which bases its diet almost exclusively on Baltic herring throughout the year (Bignert et al., 1998).

Residue concentrations in an avian egg correspond to those in the laying female on a lipid weight basis (Bargar et al., 2001; Drouillard and Norstrom, 2001). However, the range of maternal transfer into egg is difficult to determine. Chemicals within an egg come from a pool of chemicals in the maternal blood. Liver is among the organs with high perfusion by blood and may thus reflect the whole-body burden reasonably well (Becker et al., 1989). The reported maternal transfer rates in various bird species have ranged from 1 to 20% of the whole-body burden. The egg:hen ratio in experimentally dosed chickens (*Gallus domesticus*) ranged from 0.14 to 0.46 for PCB, indicating that concentrations in adults were anywhere from seven- to two-fold greater than in eggs (Bargar et al., 2001).

These varying rates may be the reason why the age effects have so far not been clearly demonstrated in birds as compared to marine mammals (see Robinson et al., 1967; Nyman et al., 2002). The age effects are marked between first-year and adult-plumaged sea

eagles (Tarhanen et al., 1989), but further connections between organochlorine levels and age have shown a great deal of scatter in bird studies (e.g. Robinson et al., 1967; Lemmetyinen et al., 1982). One reason for this may be the lack of sufficiently large data from older individuals.

If the DDT compound residues have greater impact on reproduction than PCBs, as suggested by Custer et al. (1999) and Helander et al. (2002), the implications are as follows: after completing the 6000–7000-kilometre-long spring migration journey from Africa, the lesser black-backed gulls are in a comparatively lean condition, indicating that the lipophilic substanses like DDE in females may be readily excreted from body tissues into egg yolk. Regarding the fact that lesser black-backed gulls in the Gulf of Finland have produced very few recruits during the past twenty years, one might anticipate a highly truncated age distribution towards the older age classes. In contrast, herring gulls comprise an expanding population and should have more young individuals involved. The same applies to lesser black-backed gulls from the Bothnian Bay. They produced a mean of 1.05 fledglings/pair in six monitoring areas in the 1990s (S.D. = 0.43, $n = 10$; compiled from Wistbacka, 2000) while the corresponding figure at Söderskär during the same time period was only 0.15. Clearly, the potential for recruits was far better in the Bothnian Bay.

5. Conclusions

As was shown by this study, the chick diseases are not necessarily induced by starvation. Instead, they seem to be innate by nature, reflecting the dysfunction of the degenerated liver that cannot cope with common inflammations. The organochlorine concentrations in liver of the diseased chicks were high, especially for DDE. In fact, DDE concentrations were disproportionately high considering the decreasing trend found in other biota in the Baltic since the 1970s. Compared with other species we suggest that biomagnification of DDE in lesser black-backed gulls occurs outside the Baltic to a great extent. The suggestively lower PCB levels found in gulls from the Bothnian Bay might be an outcome of a younger age distribution of gulls there and their heavier reliance on less contaminated food items during the breeding season. Yet, converted into TEQs, the PCB levels were very high in all three populations of gulls, indicating a common exposure area, viz. the Baltic Sea. However, the biochemical pathway between residues and diseases is still unknown, so we cannot rule out the possibility that other chemicals not examined here were also involved in chick deaths.

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