

# The Role of DDE, PCB, Coplanar PCB and Eggshell Parameters for Reproduction in the White-tailed Sea Eagle (*Haliaeetus albicilla*) in Sweden

The reproduction of white-tailed sea eagles was monitored in 1964–1999 in 3 differently contaminated sub-populations: Baltic Sea coast (Bp), inland central Sweden (Ip) and Lapland (Lp). 249 dead eggs from 205 clutches were obtained for analyses of DDE and PCBs and for eggshell measurements. A desiccation index ( $D_i$ ) value was calculated for each egg as a measure of water loss through the shell. In the highly contaminated Bp, *p,p'*-DDE concentrations in the eggs decreased continuously and 5-fold during the study period and PCB concentrations decreased 3-fold from the mid 1980s. The PCB pattern changed slightly over time towards more high-chlorinated congeners but the relative toxicity of the PCB mixture, expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQ), remained constant and TEQ can be assumed to have decreased in a similar way as PCB over time. Productivity (P), shell thickness ( $S_t$ ), shell index (S), and  $D_i$  increased over time in the Bp but no change in  $D_i$  or productivity occurred in the Lp, where residue concentrations were 5–8 times lower. P of the Bp was not correlated to  $S_t$  or S, but was negatively correlated to  $D_i$ , DDE and PCB. An S-shaped dose-response relationship was indicated between P and DDE. After 1988, when the PCB/DDE ratio was considerably higher than previously, PCB but not DDE concentrations were significantly higher in eggs with dead embryos as compared to undeveloped eggs, implying lethal concentrations of PCB, and a LOEL of 320  $\mu\text{g g}^{-1}$  TEQ is suggested for embryo mortality. In a subset of 21 eggs, representing productive and unproductive females, analyzed for a selection of coplanar PCB congeners, tris(4-chlorophenyl) methanol and bis(4-chlorophenyl) sulphone, there was no evidence for a correlation between P and any of these compounds. A reduction in residue concentrations in old females did not lead to increased P or improved  $D_i$ -values, indicating a remaining effect from a previous, higher exposure to contaminants. The inability to reproduce included a high rate of undeveloped eggs, indicating effects at a prezygotic stage. P showed the strongest correlation with  $D_i$ , and  $D_i$  was most strongly correlated to DDE. Thus, the remaining effect of previous exposure resulted in a stronger correlation to the symptom ( $D_i$ ) rather than to the suggested causative agent (DDE). LOEL values for depressed P were estimated at 120  $\mu\text{g g}^{-1}$  DDE and 500  $\mu\text{g g}^{-1}$  PCB (lipid basis). It is concluded that the major reason for depressed P during the study period was DDE, but that effects also from PCB were largely concealed by the effects from DDE.

## INTRODUCTION

In this paper, white-tailed sea eagle (*Haliaeetus albicilla*) and sea eagle are used synonymously.

The white-tailed sea eagle population on the Swedish coast of the Baltic Sea has been under strong influence of environmental pollution for decades. Consistent breeding failures over several years among a few well-studied pairs on the Swedish coast were reported in the early 1960s by Olsson (1) and other ornithologists, leading to the start of a nationwide survey in 1964 of the breeding population and its reproductive success. The surveys revealed a strongly reduced reproductive ability and a declining population (2). Retrospective studies of the Swedish Baltic population have demonstrated a significant decline in brood size, starting already in the first half of the 1950s, and a reduction in productivity in the second half of the 1950s and early 1960s to below half of the background level (3). A further decline in productivity to below 25% of the background level occurred during the 1970s (3, 4). Similar poor breeding results were reported for the 1970s also from the Estonian and Finnish coasts of the Baltic (5, 6), indicating a common

cause related to the Baltic ecosystem. The alarming drop in the reproductive ability of the sea eagles in the Baltic region shows a strong resemblance to the situation reported for some bald eagle *Haliaeetus leucocephalus* populations in North America (e.g. 7–12). Colborn (13) suggested a fledgling ratio (mean number of fledglings per successful territory to the mean number of fledglings per active territory) of  $> 2$  as an indicator of exposure to organochlorine chemicals affecting the productivity in the bald eagle. The fledgling ratio of the Swedish sea eagle population on the Baltic coast 1965–1984 was in the range 3.4–5.0, thus indicating a significant exposure to chemicals during that period.

Studies on the reproductive ability of individual white-tailed sea eagle females on the Swedish Baltic coast, in relation to the contamination with organochlorine and mercury residues in their eggs, indicated that 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (DDE) had the strongest negative influence on their reproductive capacity (14, 15). Following the ban of DDT use in the countries surrounding the Baltic Sea in the early 1970s, the levels of DDT and its metabolites have decreased significantly in Baltic biota (16, 17). Studies by Koivusaari et al. (18) indicated an increase in productivity on the northern Finnish Baltic coast already in 1974–1978, coinciding with a significant decrease of DDE but no change in the levels of polychlorinated biphenyls (PCB) and mercury in a small sample of sea eagle eggs from that region. In the Swedish population on the central and southern Baltic coast, residue levels in sea eagle eggs also declined, but the average productivity remained at low level throughout the 1970s. As the DDE levels in eagle eggs decreased further during the 1980s and 90s, a substantial improvement in reproductive ability was observed in this population (4, 19).

However, in spite of comparatively low levels of DDE in their eggs, some females on the Swedish coast still reproduced very poorly (4). This could be the result of a direct influence from chemical compounds other than DDE in the eggs, or from a remaining effect on the female of a previous exposure to DDE or some other anthropogenic compounds. Studies on the white-tailed sea eagle and its close relative, the bald eagle, have indicated a stronger negative correlation to productivity for DDE than for any other analyzed compound (9, 14, 15, 20–22). A close correlation between the residue levels of DDE and PCB in the samples, however, made it impossible to exclude adverse effects also from the PCBs. The need for research on the effects of specific PCB congeners to clarify their role on reproduction in relation to that of DDE has been emphasized (13).

Polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F) and especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are embryo-toxic. The same type of effect is also expressed by specific PCB congeners with one or no chlorine in the *ortho*-positions (coplanar PCBs). In the Great Lakes of North America, reproduction problems among several bird species have been linked to TCDD-like toxicity (23, 24). A symptom-complex has been described (25) where one of the symptoms is malformation of the avian bills. Such abnormalities have also been observed in nestling bald eagles (26, 27) and white-tailed sea eagles (28). This indicates that the eagles might also suffer from reproduction problems due to substances that express TCDD-like toxicity. In fact, it has been claimed that depressed reproduction in the 1990s among bald eagles on the Great Lakes shoreline and sea eagles on the Finnish Baltic coast was mainly due to the effects of coplanar PCBs (29–31).

In this paper, we investigate the role of DDE, PCB, coplanar PCBs and eggshell parameters for the reproductive ability in 3 sub-populations of white-tailed sea eagles in Sweden. To facilitate proper selection of samples, the representativeness of the sampled dead eggs is studied. We also investigate the changes in the PCB-pattern in sampled eggs between time periods. The study is based on eggs collected 1965–



1997 and productivity data from the sampled breeding territories through 1999. In addition, we report on the residue levels of 2 recently identified antropogenic substances, tris(4-chlorophenyl) methanol (TCPMeOH) (32) and bis(4-chlorophenyl) sulphone (BCPS) (33) in the sea eagle egg samples.

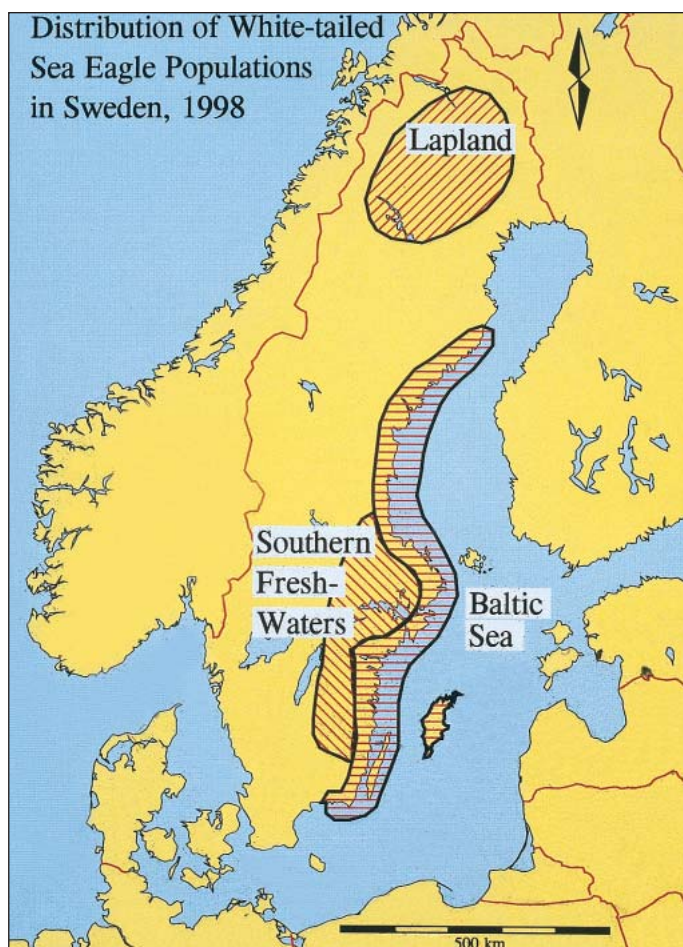
## MATERIALS AND METHODS

### Study Areas and Populations

After being extirpated from large parts of its breeding range in Sweden during the 19<sup>th</sup> and early 20<sup>th</sup> century, scattered populations remained on the brackish Baltic sea coast, roughly between lat. 57° and 65° N, and by freshwater lakes and rivers in Lapland, mainly north of the Arctic circle (66°–69° N). The populations occupying these two regions will be referred to as the Baltic and the Lapland population, respectively. Along with a recovery of the Baltic population from the 1980s to the present time, a reoccupation of breeding areas in the interior of southern and central Sweden commenced. This population, subsequently referred to as the "Interior", now comprises more than 35 territorial pairs. The Swedish Baltic population has increased from below 50 pairs in the early 1970s to about 150 in 1999. The Lapland population has increased at a slower rate but is at the present time estimated to be at least 50 pairs. Habitat characteristics and the natural circumstances for reproduction differ between Lapland and the Baltic coast; most important are the effects of a more limited food supply and, in some years, of heavy precipitation, upon the overall productivity in Lapland (3). Food supply and climatic factors in the 'new' Interior breeding areas resemble the situation on the Baltic coast. The study is based on data from annual nest surveys including 2130 breeding attempts on the Baltic coast (1964–1999), 656 in Lapland (1976–1999) and 170 in the Interior population (1985–1999).

### Productivity Data

Productivity, defined as the mean number of young produced per year, was calculated on a territory basis over a 5-yr period, consisting of the egg-sampling year  $\pm$  2 years (4, 20, 22). Breeding attempts that failed after strong human disturbance at the nest were excluded from the cal-



White-tailed sea eagle distribution and populations in Sweden.



Occupied nests are checked during May and June to verify reproductive success and collect dead eggs for investigation. Photo: K. Elmqvist.

culations of productivity, as were those years when sub-adults occurred in the mated pairs, resulting in no egg production. Years when adult pairs produced no eggs were included in the productivity calculations. If the 5-yr period around a sampling year overlapped with another 5-yr period around a sampling year representing the same female, a mean value for productivity was calculated over the period from the first egg-sampling year  $-2$  yrs to the last egg-sampling year  $+2$  yrs, and a mean value for each contaminant and for each shell parameter was calculated from all clutches within that period. This was done to avoid an over-representation of a few females in the sample.

Many territories on the Swedish Baltic coast have been carefully investigated for long periods and several individual females have been studied over many years. The continuity and turnover of individuals in the territories was based on field observations, the characteristics of moulted feathers (34) and the reading of rings on the birds.

### Egg Samples

#### Sampling

The breeding success of the Swedish white-tailed sea eagle population has been surveyed annually as described by Helander (3). New pairs that were located when populations grew were included in the surveys, aiming at complete coverage. Since the species was classified as endangered until 1990 and as vulnerable thereafter (35), sampling during incubation was unattainable. Except for 5 clutches from the Baltic coast, collected for artificial incubation in 1978–1980 (28), all eggs were sampled after failure to hatch in the nest. Most eggs were collected 4–7 weeks after expected hatching time (based on the ages of nestlings in each population). Some eggs were found crushed but still with contents usable for analyses. Since our studies of organochlorine contaminants are based on lipid weight data, the crushed eggs were included among the samples, assuming that the concentrations of the persistent, exogenous compounds in the egg lipids remained constant.

Upon collection in the nest, each egg was placed in a plastic bag, put in a hard box stuffed with cotton and transferred to the Swedish-Museum of Natural History for preparation for analyses, as described by Helander et al. (14). The entire egg sample consists of 249 eggs from the period 1965–1997, from 154 Baltic, 42 Lapland and 9 Interior clutches. Eggshells from museum and private collections from the period 1856–1961 include 154 eggs, from 44 Swedish and 33 Finnish clutches.

Due to the nonrandom sampling procedure (post-hatch, dead eggs), a possible bias in the material was investigated. Data from the Baltic

population was divided into 6 consecutive time periods. Within each period, the frequency distribution of nests containing 0, 1 and 2–3 nestlings among pairs (*i*) from which eggs were sampled compared to; (*ii*) among pairs from which no eggs could be obtained (nonsampled pairs) was tested.

#### Preparation

The egg contents were removed through a drilled hole; in eggs with large embryos a piece of the shell was removed and saved with the shell. The empty shell was rinsed clean with tap water and dried at room temperature. Egg contents were homogenized, including embryos below 60 mm of length; larger embryos were removed from the rest of the egg content for separate analyses of yolk, muscle and brain lipids (see (14)). In eggs with separately analyzed embryos, the concentrations in yolk lipids were used.

#### Lipid contents

The measured lipid concentration in each whole egg was corrected for dehydration of the egg contents using the  $D_i$ -value for the egg (see below), to obtain a calculated original lipid concentration. This value was multiplied with inside egg volume for an estimate of the original lipid mass.

Some egg contents were badly putrefied (characterized by strong smell and usually greyish-greenish color), whereas others were much less affected by putrefaction (no strong smell, whitish-yellowish colour). If a reduction in lipid contents due to putrefaction occurs, residue concentrations on a lipid weight basis would increase. To investigate this possible source of error, undeveloped eggs from the Baltic sample were selected for comparison of the lipid contents in putrefied *versus* not putrefied eggs.

#### Eggshell parameters

Length and breadth were measured at a resolution of  $\pm 0.01$  mm. The weight of the dry shell was determined at  $\pm 0.01$  g. Eggshell thickness was measured using a modified dial micrometer (Starret 1010) with a precision of  $\pm 0.01$  mm, at three points around the blowing-hole at the equator. Crushed eggs were measured at several points on the pieces. Eggshell thickness index values were calculated for all whole eggshells according to the method given by Ratcliffe (36).

As a measure of functional shell quality a desiccation index ( $D_i$ ) was calculated according to the formula  $D_i = (W - S) V^{-1}$  where  $W$  = weight (g) of the egg after sampling,  $S$  = dry shell weight (g) and  $V$  = inside volume (ml) of the egg.  $D_i$  expresses the mean density of the entire egg content where, at the time of sampling, a varying quantity of the original water content has been replaced by air. Thus, a low  $D_i$ -value indicates desiccated eggs. Assuming that the specific weight of fresh-egg content is approximately 1.0, the calculated inside volume equals weight and the initial  $D_i$ -value (at laying) is approximately 1.0. Egg volume was calculated by the formula given by Stickel et al. (37) for bald eagle eggs, adjusted here for eggshell thickness to obtain the inside volume. The suitability of this formula for white-tailed sea eagle eggs was investigated previously (14).

#### Inter- and intra-clutch variation

As in most studies on residue concentrations in eggs of threatened eagles, this study is largely based on data from only one egg from each clutch. This is a consequence of the sampling procedure (post-hatch, abandoned nests, etc.). When studying relationships between residue concentrations, eggshell parameters and reproduction, the variation within and between clutches and different females are important factors. The variation of individual eggs in relation to the clutch mean (within-clutch variation) in each of 15 Baltic clutches was investigated. This was compared to the variation between the means of the 15 individual clutch means, in relation to the mean value for all these clutches (among-clutch variation). Secondly, in another set of samples, 38 Baltic clutches representing 12 different females were investigated for variation between clutches from each female in relation to the mean for all her clutches (within-female variation between years). This was compared to the variation between these different females, based on each female-mean value in relation to the mean value of all female-means (among-female variation).

#### Selection of Samples

Out of the 249 analyzed eggs, different samples were selected for the various tests. Selections are specified for each test under Results.

Due to metabolism the lipid content in the egg has been found to decrease during embryonic development, leading to an increase in the concentrations of organochlorines when expressed on a lipid weight basis (38, 39). The main reduction in lipid concentration and an approximate doubling of the residue concentrations occurred after the embryo was half-grown. Thus, to avoid a significant influence from embryonic metabolism on the residue concentrations in the egg lipids, eggs containing half-grown or larger embryos ( $> 75$  mm) were excluded, except for studies on embryo mortality and on the possible influence of embryo growth on the eggshell thickness.

#### Chemical Analysis

##### Annually analyzed samples

Eggs collected at the annual surveys have been analyzed more or less annually since 1971 at the Institute of Applied Environmental Research, ITM (formerly the National Environmental Protection Board Special Analytical Laboratory, NSL) in Stockholm. Eggs sampled and analyzed between 1965 and 1970 were re-analyzed in the late 1970s. Until 1991 the samples were analyzed according to the method described by (40) and the results have partly been reported elsewhere (4, 14, 15). The extraction and clean-up method has in principle been kept the same over the years. In 1991, the gas chromatography (GC) with electron capture detector (ECD) analysis was changed from packed column to capillary column chromatography (16), to enable quantification of specific individual PCB congeners instead of a total PCB determination as previously measured. In this paper, the calculated total PCB concentration from packed column GC analysis is denoted tot-PCB.

In 1991 and 1992, GC-analyses on 13 white-tailed sea eagle eggs were performed on both packed and capillary columns to relate tot-PCB concentration to concentrations of individual PCB congeners. On packed column chromatography CB-138 is the major constituent of the tenth PCB peak (#10). The ratio of #10 / CB-138 for the 13 eggs was found to be 1.33 (sd = 0.044). The ratio of #10 to tot-PCB in eggs of white-tailed sea eagle analyzed between 1973 and 1990 from the Baltic coast and Interior populations is 0.161 (sd = 0.0093,  $n = 142$ ) and for the Lapland population 0.146 (sd = 0.0089,  $n = 29$ ). The tot-PCB concentrations in eggs from 1993 to 1997 were estimated by multiplying the concentration of CB-138 with a factor  $(1.33 / 0.161) = 8.22$  for eggs from the Baltic and Interior populations and a factor  $(1.33 / 0.146) = 9.06$  for the Lapland population.

DDE was detected in all samples while 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT) was not found in any of the samples analyzed. The relative amount of 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD) in samples collected in the 1990's was less than 4% of the amount of DDE. In samples from the 1960s and 1970s the DDD amounts were in a few cases as high as 17% of the DDE amounts. In this study the focus is on the effects of DDE and a sum concentration was not calculated, as opposed to previous studies (4, 14, 15).

##### Analysis of coplanar PCB congeners

Samples from the eggs selected for analysis of coplanar PCBs were analyzed at the Department of Environmental Chemistry in Stockholm. The samples were homogenized and extracted according to (40) except that methyl *tert*-butyl ether (MTBE) was used instead of diethyl ether. Surrogate standards were added to the samples before they were homogenized. For PCB congener analysis 2,2',5,6'-tetrachlorobiphenyl (CB-53, CB numbers according to (41)) was used as a surrogate standard, for 1-*ortho*-PCBs 2,3,3',4,5,5'-hexachlorobiphenyl (CB-159) and for non-*ortho*-PCBs <sup>13</sup>C-labelled 3,3',4,4'-tetrachlorobiphenyl (CB-77), 3,3',4,4',5-pentachlorobiphenyl (CB-126) and 3,3',4,4',5,5'-hexachlorobiphenyl (CB-169) (Cambridge Isotope Laboratories, Woburn, MA, USA) were used. 3'-methylsulphonyl-4'-methyl-2,3,4,5,5'-pentachlorobiphenyl (42) was used as a surrogate standard for quantification of tris(4-chlorophenyl)methanol (TCPMeOH) and bis(4-chlorophenyl)sulphone (BCPS) purchased from Larodan Fine Chemicals AB (Malmö, Sweden) and Aldrich Chemical CO. (Milwaukee, WI, USA), respectively.

After extraction an aliquot of 10% of the sample extract was taken for lipid amount determination. This aliquot, dissolved in *n*-hexane, was partitioned with concentrated sulphuric acid. The purified sample was analyzed for PCBs and DDE utilising a 3400 Varian GC-ECD instrument. As external standard for the quantification of PCB congeners, the technical PCB product Clophen A50 (Bayer, Germany) was used. The relative amount of different PCB congeners in Clophen A50 as reported by (43) was used for quantification. The sum of PCB congeners are



**Table 1. Recoveries of 9 model PCB congeners<sup>#</sup> after completed analytical clean-up procedure, determined in the 3 fractions after PYE-column separation. Included are also recoveries of surrogate standards determined in the samples. Recoveries are given in % with S.D. in parentheses.**

	2-4- <i>ortho</i> -PCB fraction			1- <i>ortho</i> -PCB fraction			0- <i>ortho</i> -PCB fraction		
	CB-53	CB-138	CB-153	CB-118	CB-156	CB-159	CB-77	CB-126	CB-169
<b>Method control</b>									
High dose (n = 5)	70 (2)	88 (5)	86 (4)	78 (8)	90 (7)	79 (7)	67 (5)	74 (5)	92 (12)
Low dose (n = 5)	94 (6)	86 (5)	109 (19)	104 (36)	77 (15)	72 (11)	67 (18)	Imp	84 (15)
<b>Surrogate standards</b>									
Sample (n = 21)	99 (11)*				82 (7)		75 (13)	83 (16)	88 (22)

<sup>#</sup>The PCB congeners analyzed are abbreviated according to (41)

\* Determined in the 10% aliquot for PCB and DDE quantification  
imp = no recovery due to obvious contamination

denoted ΣPCB to be distinguished from tot-PCB.

The remaining 90% of the sample was partitioned with potassium hydroxide (0.5 M) to remove potential acidic components in the eggs like polychlorophenols and hydroxylated metabolites of PCB. This fraction was not further analyzed in the present study.

The organic phase, after partitioning with potassium hydroxide, was transferred to an open column with a stationary phase consisting of conc. phosphoric acid: silica (1:2 w.w.). Twenty g gel was used and the sample was transferred to the top of a dry column. The silica gel (MN-Kieselgel 60, 70–230 mesh from Merck Nagel F.R.G.) was activated in 180°C for 24 hrs before used in the different clean-up steps included in the present method. The mobile phases used were; n-hexane:toluene (1:1, 50 ml), followed by n-hexane:toluene:MTBE (9:9:2, 70 ml). Two fractions were taken, 20 ml and 80 ml, respectively. The first fraction eluted contained nonpolar components like PCB and DDE, the second fraction contained e.g. TCPMeOH and BCPS.

Fraction 2 was evaporated to dryness (in 75°C water-bath with a slight stream of nitrogen gas), and the residue was dissolved in n-hexane (12 ml). The n-hexane was partitioned with DMSO (1 ml) 3 times (44). The TCPMeOH and BCPS that partitioned into the DMSO fraction were recovered in n-hexane by diluting the DMSO with water. TCPMeOH and BCPS were quantified by comparison to the authentic reference standards by GC-ECD.

Fraction 1 contained traces of lipids and the solvent was therefore evaporated to dryness, the residue was dissolved in a small volume of n-hexane and transferred to a column containing concentrated sulphuric acid: silica (1:2 w.w., 1 g). The PCBs were eluted with n-hexane (8 ml) and the solvent volume was then reduced to 50 µl. The sample analytes were basically fractionated by HPLC according to (45), in a system equipped with two 150 x 4.6 mm I.D. Cosmosil 5-PYE columns (Nacalai Tesque, Kyoto, Japan) linked together. The columns were held at 0°C temperature (ice-water bath) during separation. Four fractions were collected: 1 containing 2-4-*ortho*-PCBs (not further analyzed), 1 with 1-*ortho*-PCBs, with the fraction limits set by the elution times of CB-118 and CB-157 and finally 1 fraction of non-*ortho*-PCBs, limited by the elution times of CB-77 and CB-169. After the third fraction the flow direction was reversed and any remaining substances were collected in a back-flush fraction (fraction 4). Since the separation of the analytes was performed at 0°C it was possible to achieve baseline separation between CB-157 and CB-77. This is in line with the reported improved separation of polycyclic aromatic hydrocarbons when the PYE-column is held at a low temperature (46).

The 1-*ortho*-PCB fraction was analyzed and the PCB congeners were quantified, relative synthesised standards by GC-ECD. The non-*ortho*-PCBs were analyzed by GC-mass spectrometry (the MS instrument was a Finnigan TSQ, ThermoQuest, Germany), electron ionization (70 eV), operating in selected ion monitoring mode. Of all analytes 2 ions in each molecular ion cluster were detected and the quantification was done in relation to the added <sup>13</sup>C-labelled compounds using multilevel calibration curves (47).

In order to investigate the reliability of the method described above 10 samples (approx. 10 g each) were prepared from a homogenate of 2 hen eggs. Nine PCBs (including non-*ortho*-PCBs) were added to the samples, in 5 samples at a low dose and in 5 with a dose 10 times higher. The amount of the 9 PCB congeners in the low dose was approximately 10 ng each, except for CB-77, CB-126 and CB-169 of which only ap-

proximately 2 ng was added, respectively. The samples were worked-up and analyzed as described above and the relative amount of each substance was determined (Table 1). Also the recoveries of the surrogate standards added to the white-tailed sea eagle samples were determined (Table 1).

### Toxic Equivalent Estimation

Different toxic equivalence factors (TEFs) have been used to estimate critical levels in *Haliaeetus* species to TCDD like toxicity (29, 30, 31, 48). We chose to use the TEFs most often used in these studies. Thus, the TEFs according to Tillit et al. (49) were used for calculation of TCDD like toxic equivalents (TEQ) of the coplanar PCBs analyzed. The calculations of TEQs are based on the assumption that the toxicity of individual dioxin-like compounds can be added to give a total TEQ. The PCB congeners used for calculation of TEQ for non-*ortho*-PCBs were CB-77, CB-126 and CB-169. To estimate a PCB derived TEQ-value, TEQ for CB-105, CB-118, CB-156 and CB-157 (all being 1-*ortho*-PCBs) was calculated. The sum of TEQ for these PCB congeners and the non-*ortho*-PCBs gives a PCB based TEQ, hence called TEQ<sub>PCB</sub>.

### Weight-loss of Chicken Eggs

For comparison to data on the weight-loss of developing chicken eggs during natural incubation (50), we measured the weight-loss of chicken eggs with no embryonic development. The eggs were bought in a grocery store approximately one week after being laid. The eggs were kept in the office at room temperature, 1 sample (n = 3) on the window sill and thus exposed to sunlight and higher temperatures during the day and the other sample (n = 3) on a shelf away from the sun. Weight-loss was recorded gravimetrically at 0.001 g over a period of 90 days.

### Statistical Analysis

The distribution of concentrations of organochlorines measured in the environment can be assumed to be right skewed (51). This was also found in the present investigation of eagle eggs. Consequently, geometric means (gm) have been used to represent average values for concentrations whereas shell variables have been reported as arithmetic mean values (am) both in the comparisons among groups and in the time series. Parametric tests - one-way Analysis of Variance (ANOVA) - have generally been applied, except in cases where suspected outliers have been present where the nonparametric Kruskal-Wallis or Mann-Whitney's U-test were applied to detect differences between the groups studied. For multiple comparisons among means, Scheffé's method has been applied following the ANOVA where more than 2 groups have been studied (52). The reported confidence intervals (Table 5 and Table 11) were calculated with allowances for repeated tests.

Simple log-linear regression analyses have been used to detect systematic changes over time for several variables. Multiple regression has been used to investigate the relative importance of various variables for reproduction and desiccation index.

If contaminants like DDE or PCB influence the productivity of sea eagles one would expect a dose-response relationship, with a minimum contaminant level below which no effect can be detected and a maximum level above which no production takes place. Presuming this is the case a nonlinear function  $Y = h(I + x^n c^{n-1})^{-1}$  was tentatively applied, where  $h$  is the maximum productivity,  $c$  is the point of inflection and  $n$

regulates the degree of steepness. The line was fitted to the data using the Marquard method (53). The Coefficient of Determination ( $r^2$ ) was estimated as the proportion of the variance explained by the function vs the total variance. The significance of the sigmoid function was tested by a one way ANOVA.

In order to detect significant differences between groups in the frequency of produced fledglings (cf. under Sampling), contingency table analysis using the G-test of independence (52) was applied (Table 2).

A 'P'-value less than 0.05 (two-tailed) in the various tests has been considered statistically significant.

## RESULTS

### The Egg Matrix

#### Bias of samples

The offspring production in sampled vs nonsampled sea eagle pairs is summarized in Table 2. There was no statistically significant difference between sampled and nonsampled pairs during 1970–1974, 1980–1989 and 1995–1999. The sampled pairs produced significantly better than the non-sampled pairs in 1975–1979, whereas the nonsampled segment of the population produced significantly better than the sampled pairs in 1990–1994. The difference in 1990–1994 was mainly due to a higher frequency of broods containing 2 or 3 young in the nonsampled group; the proportion of successful pairs was about equal in both groups. In 1975–1979, the percentage successfully breeding pairs was substantially higher in the sampled group.

#### Lipid contents in different egg categories

Corrected lipid concentrations in undeveloped, whole eggs from the Baltic were significantly and negatively correlated to increased egg volume ( $p < 0.001$ ,  $n = 97$ ). Lipid mass was not correlated to volume in this sample set. This implies that the allocation of fat for yolk formation was less variable than the allocation of proteins and water for the formation of the albumen. This has been reported also for the herring gull (*Larus argentatus*) (54). Comparisons of calculated original fat contents were therefore based on lipid mass. There was no significant difference in the lipid contents of putrefied (gm = 5.20 g, 95% c.i. = 4.72–5.74,  $n = 17$ ) vs not putrefied eggs (gm = 5.06 g, 95% c.i. = 4.76–5.38,  $n = 80$ ).

Undeveloped eggs from the Baltic contained on average (gm and 95% c.i.) 5.08 g lipids (4.82–5.36,  $n = 97$ ) whereas undeveloped eggs from Lapland averaged 4.52 g (4.11–4.97,  $n = 29$ ). The distribution of lipid contents was skewed to the right in both groups, so log-transformed data were used for testing between means. This difference was statistically significant ( $p < 0.05$ ). Thus, the eggs from Lapland contained on average about 11% less fat than eggs from the Baltic.

We also tested the Baltic sample for a possible difference in lipid content in the sample of undeveloped eggs ( $n = 97$ , see above) vs eggs with up to half grown (20–62 mm) embryos (gm = 4.39 g, 95% c.i. = 4.08–4.73,  $n = 33$ ). The difference was statistically significant ( $p < 0.01$ ), indicating some influence from metabolism on the lipids even at these earlier stages of development.

#### Intra- and inter-clutch variation in DDE and PCB levels and eggshell parameters

The selection of clutches was restricted to the period 1973–1982, to minimize an influence on the analysis from a change over time in the studied parameters (Table 4 and Fig.1). Eggs with no embryonic development from the Baltic sample set were used. Results are given in Table 3. The variation within clutches was smaller than among clutches for all measured parameters. The coefficients of variation (CV) were much higher for desiccation index ( $D_i$ ), DDE and PCB, than for eggshell thickness ( $S_i$ ) and

**Table 2. Number of nests containing 0, 1 and 2–3 young among sea eagle pairs on the Swedish Baltic coast from which dead eggs were obtained for contaminant analyses (= sampled pairs) and among nonsampled pairs, with average productivity and % successfully breeding pairs.**

	No of young			Level of significance	Productivity	% success
	0	1	2–3			
<b>1970–1974</b>						
sampled	55	12	2	$p < 0.8$	0.23	20
nonsampled	89	15	5		0.24	18
<b>1975–1979</b>						
sampled	63	26	5	$p < 0.02^*$	0.38	33
nonsampled	103	15	8		0.25	18
<b>1980–1984</b>						
sampled	36	11	2	$p < 0.7$	0.31	27
nonsampled	149	41	16		0.36	28
<b>1985–1989</b>						
sampled	53	28	9	$p < 0.12$	0.53	41
nonsampled	128	42	32		0.53	37
<b>1990–1994</b>						
sampled	20	23	4	$p < 0.001^{***}$	0.66	57
nonsampled	172	87	105		0.84	53
<b>1995–1999</b>						
sampled	7	10	8	$p < 0.5$	1.04	72
nonsampled	192	160	224		1.09	67

**Table 3. Variation within and among clutches, and within and among females, in residue concentrations of DDE and PCB, eggshell thickness ( $S_i$ ), eggshell index ( $S_i$ ) and desiccation index ( $D_i$ ), expressed as coefficient of variation (CV), and as ratio between highest and lowest value (H/L);  $n$  = sample size.**

a) Variation within and among 15 clutches representing 15 sea eagle females

	Within clutches			Among clutches		
	n	CV	H/L	n	CV	H/L
DDE	32	20.4	1.74	15	43.0	3.22
PCB	32	20.2	1.72	15	39.8	2.65
$S_i$	31	6.7	1.24	14	15.9	1.42
$S_i$	31	6.0	1.23	14	14.6	1.44
$D_i$	24	34.2	2.94	10	68.9	3.72

b) Variation within females between years, and among females, based on 38 clutches representing 12 sea eagle females.

	Within females			Among females		
	n	CV	H/L	n	CV	H/L
DDE	38	30.9	3.10	12	66.0	3.51
PCB	38	31.6	2.94	12	73.3	3.13
$S_i$	38	19.3	2.14	12	20.9	1.47
$S_i$	31	13.0	1.51	9	21.7	1.52
$D_i$	24	48.2	3.25	8	63.9	3.77



Clutch of 2 dead eggs in a white-tailed sea eagle nest, May 1980. Photo: B. Helander.

**Table 4. Eggshell thickness ( $S_t$ ), eggshell index ( $S_i$ ), desiccation index ( $D_i$ ) and residue levels of DDE and PCB in different periods in 3 sea eagle populations. Number of clutches or records of productivity are given in parentheses. Eggshell parameters are arithmetic means with standard deviation [ ]. Residue concentrations are geometric means with 95% confidence limits [ ]. For sample sizes < 4, the range [ ]\* is replacing the S.D. or confidence limits. Eggs with embryos exceeding 75 mm are excluded.**

Collection period	Eggshell parameters			Residues ( $\mu\text{g g}^{-1}$ l.w.)		Productivity
	$S_t$ (mm)	$S_i$	$D_i$	DDE	tot-PCB	
<b>Baltic coast</b>						
<b>Finland:</b>						
1882–1935	0.61 (32) [0.053]	3.21 (33) [0.48]				
<b>Sweden:</b>						
1856–1916	0.61 (22) [0.052]	3.20 (27) [0.24]				$\geq 1.07^1$
1948		3.28 (1)				
1957–1964	0.53 (3) [0.48–0.60]*	2.93 (3) [2.63–3.16]*				0.70 <sup>2</sup>
1965–1969	0.51 (2) [0.49–0.52]*	3.09 (3) [2.29–3.59]*	0.68 (1)	743 (6) [430–1300]	770 (6) [390–1500]	0.33 (168)
1970–1974	0.50 (27) [0.036]	2.58 (19) [0.15]	0.34 (19) [0.14]	710 (27) [590–840]	1100 (27) [940–1300]	0.24 (178)
1975–1979	0.55 (30) [0.11]	2.81 (28) [0.46]	0.43 (28) [0.19]	560 (31) [460–660]	1000 (31) [860–1200]	0.31 (220)
1980–1984	0.54 (17) [0.056]	2.76 (13) [0.26]	0.45 (10) [0.26]	400 (18) [340–480]	1000 (18) [870–1200]	0.35 (255)
1985–1989	0.54 (30) [0.047]	2.82 (26) [0.25]	0.59 (25) [0.21]	250 (31) [220–290]	740 (31) [650–840]	0.53 (292)
1990–1994	0.56 (15) [0.087]	2.76 (13) [0.28]	0.74 (13) [0.17]	170 (17) [140–210]	540 (17) [450–650]	0.82 (411)
1995–1997	0.58 (4) [0.069]	2.88 (3) [2.40–3.37]*	0.78 (3) [0.63–0.86]*	110 (5) [70–160]	390 (5) [260–590]	1.10 (606) <sup>3</sup>
<b>Lapland</b>						
1867–1909	0.59 (7) [0.059]	3.14 (8) [0.46]				–
1966				250 (1)	310 (1)	–
1977–1979	0.53 (2) [0.50–0.55]*	2.78 (2) [2.64–2.92]*	0.83 (2) [0.73–0.93]*	130 (3) [86–190]*	250 (3) [190–380]*	0.46 (68)
1980–1984	0.53 (9) [0.047]	2.85 (9) [0.27]	0.85 (9) [0.15]	57 (10) [33–98]	200 (10) [130–320]	0.63 (111)
1985–1989	0.62 (5) [0.054]	3.10 (5) [0.24]	0.84 (5) [0.014]	33 (5) [26–42]	130 (5) [70–250]	0.68 (127)
1990–1994	0.56 (10) [0.052]	2.84 (10) [0.24]	0.80 (10) [0.034]	20 (10) [13–30]	76 (10) [46–120]	0.69 (155)
1995–1997	0.60 (5) [0.011]	3.04 (5) [0.12]	0.85 (5) [0.033]	14 (6) [5.9–32]	51 (6) [23–110]	0.61 (195) <sup>3</sup>
<b>Interior</b>						
1867–1915	0.62 (4) [0.044]	3.19 (6) [0.12]				–
1985–1989	0.58 (3) [0.53–0.66]*	2.98 (2) [2.73–3.23]*	0.45 (2) [0.36–0.54]*	110 (3) [43–190]*	310 (3) [120–500]*	0.79 (19)
1990–1994	0.61 (3) [0.53–0.68]*	3.14 (3) [3.06–3.26]*	0.85 (3) [0.81–0.87]*	49 (4) [28–83]	190 (4) [110–330]	1.02 (43)
1995–1997	0.58 (1)	3.02 (1)	0.89 (1)	78 (2) [30–200]*	560 (2) [140–990]*	0.78 (108) <sup>3</sup>

\* range

<sup>1</sup> from (96)

<sup>2</sup> 1951–1965, calculated from (3)

<sup>3</sup> including data from 1998–1999

eggshell index ( $S_i$ ), both within and among clutches. Similarly, the variation was smaller within females between years, as compared to that among different females, for  $D_i$ , DDE and PCB but not for  $S_t$  and  $S_i$ . The highest CV was found for  $D_i$  both within and among clutches and within females.

#### Egg volume, eggshell thickness and eggshell index correlation

Calculated outside egg volumes in the Swedish Baltic samples averaged 118.7 ml in 1856–1916 (sd = 9.1, n = 27 clutches) and 120.3 in 1965–1997 (sd = 11.4, n = 124). The difference between means was not significant. In the Lapland sample, egg volume averaged 122.0 in 1867–1909 (sd = 9.1, n = 8) and 115.0 in 1976–1997 (sd = 11.8, n = 40). This difference between Lapland means was substantially larger than for the Baltic samples, but still not significant ( $0.05 < p < 0.10$ ).

In the Baltic sample, individual eggs with embryonic development were on average significantly larger ( $x = 121.5$ , sd = 11.9, n = 59) than undeveloped eggs ( $x = 117.2$ , sd = 12.0, n = 101) ( $p < 0.05$ , Mann-Whitney U-test). In the Lapland sample, eggs with embryo averaged 117.1 ml (sd = 10.7, n = 11) and undeveloped eggs averaged 114.9 (sd = 11.8, n = 33), but this difference was not statistically significant. Comparing egg volumes from the Baltic and Lapland samples, no significant difference was found between undeveloped samples or between samples with embryo.

A significant correlation between egg size and eggshell thickness (and index) was reported in the guillemot (*Uria aalge*): eggs from second clutches were significantly smaller than eggs from first clutches and had significantly thinner shells (55). There was no statistically significant correlation between eggshell thickness and egg volume or eggshell index and egg volume in the sea eagle egg samples (undeveloped eggs; Baltic 1969–1997, thickness:  $r^2 = 0.01$ , n = 81; index:  $r^2 = 0.00$ , n = 78. Lapland 1977–1997, thickness:  $r^2 = 0.12$ , n = 26; index:  $r^2 = 0.11$ , n = 27). Thus, eggshell thickness and index values were not corrected for an influence of egg volume in this study. Eggshell index was strongly correlated with eggshell thickness, as might be expected (Baltic 1969–1997,  $r^2 = 0.79$ , n = 100; Lapland 1977–1997,  $r^2 = 0.72$ , n = 31).

A possible correlation between embryo length and eggshell thickness and index was investigated in embryo eggs collected in 1975–1994 in the Baltic, Lapland and Interior populations. There was no significant difference between the study areas in thickness or index means in undeveloped eggs during that period so the sample sets were combined (n = 53). Embryo lengths ranged from 10 to 150 mm. There was no significant correlation between embryo length and eggshell index. For shell thickness to embryo length the p-value obtained was 0.06 ( $r^2 = 0.07$ ). Thus, no relation between embryo length and shell thickness could be validated in this material.



## Organochlorine Concentrations, Egg-shell Parameters and Reproduction

### DDE and PCB levels, eggshell parameters and productivity over time

Residue concentrations are given on a lipid weight basis unless indicated otherwise. Data on eggshell thickness, eggshell index, desiccation index, concentrations of DDE and PCB, and productivity in different periods from historic to recent time are summarized in Table 4.

Since the egg volume was significantly larger in eggs with an embryo compared to nondeveloped eggs in the Baltic sample set, a possible correlation between egg volume and the concentrations of DDE or PCB was investigated. No significant correlation was found for DDE or PCB ( $n = 199$ ).

The temporal distribution of the concentrations of DDE and PCB in sea eagle eggs from the Baltic coast, Lapland and Interior populations is illustrated in Figure 1. Both DDE and PCB decreased significantly over time in the Baltic and Lapland samples. In the Baltic and Lapland sample sets the DDE levels appear to have decreased continuously over the study period. The average annual decrease in DDE 1977–1997 was 8.6% in the Baltic sample ( $n = 90$ ,  $p < 0.001$ ) and 10% in the Lapland sample ( $n = 17$ ,  $p < 0.001$ ). The annual decrease in PCB during 1977–1997 averaged 5.5% in the Baltic sample ( $n = 90$ ,  $p < 0.001$ ) and 8.6% in the Lapland sample ( $n = 17$ ,  $p < 0.001$ ). For PCB there is a clear indication of roughly constant concentrations throughout the 1970s. The small sample from the Interior population revealed no statistically significant change in residue levels during the shorter sampling period available (1985–1997). It appears from Table 4 that the strongest reduction in shell thickness in the Baltic sample occurred in the period 1965–1974, coinciding with the highest DDE-levels in the eggs (no samples from Lapland are available from that period). For comparison of the mean values for shell thickness and shell index between specific time periods, the samples from Table 4 were distributed among 4 groups: 1856–1916, 1965–1974, 1975–1984 and 1985–1997. There was a significant difference between groups for eggshell thickness in the Baltic sample ( $p < 0.001$ , four periods) and in the Lapland sample ( $p < 0.05$ , 3 periods), and for eggshell index in the Baltic sample ( $p < 0.001$ ), but not for eggshell index in the Lapland sample. The results from pair-wise comparisons between mean values of the various time periods are given in Table 5. The average reduction in shell thickness and shell index in the Baltic material 1965–1974, as compared to the historic sample, was 18% and 17%, respectively. There was no significant difference between adjacent time periods in the Baltic sample, but a significant increase for  $St$  occurred between the periods 1965–1974 and 1985–1997. Still,  $St$  averaged 10% thinner and  $Si$  12% lower in the sample from 1985–1997 than in the sample from 1856–1935. In the Lapland sample, a significant increase occurred in  $St$  between 1977–1984 and 1985–1997, approximately back to the levels from 1867–1909. The difference in  $St$  between the Lapland samples from 1867–1909 and 1977–1984 was of the

Figure 1. Concentrations of DDE and tot-PCB ( $\mu\text{g g}^{-1}$  lipid weight) over time in eggs from 3 white-tailed sea eagle populations in Sweden. Large dots = annual means, small dots = individual clutches, vertical lines = 95% confidence limits (for sample sizes  $> 3$ ).  $n$  = sample size,  $r^2$  = squared regression coefficient for the period 1977–1997.

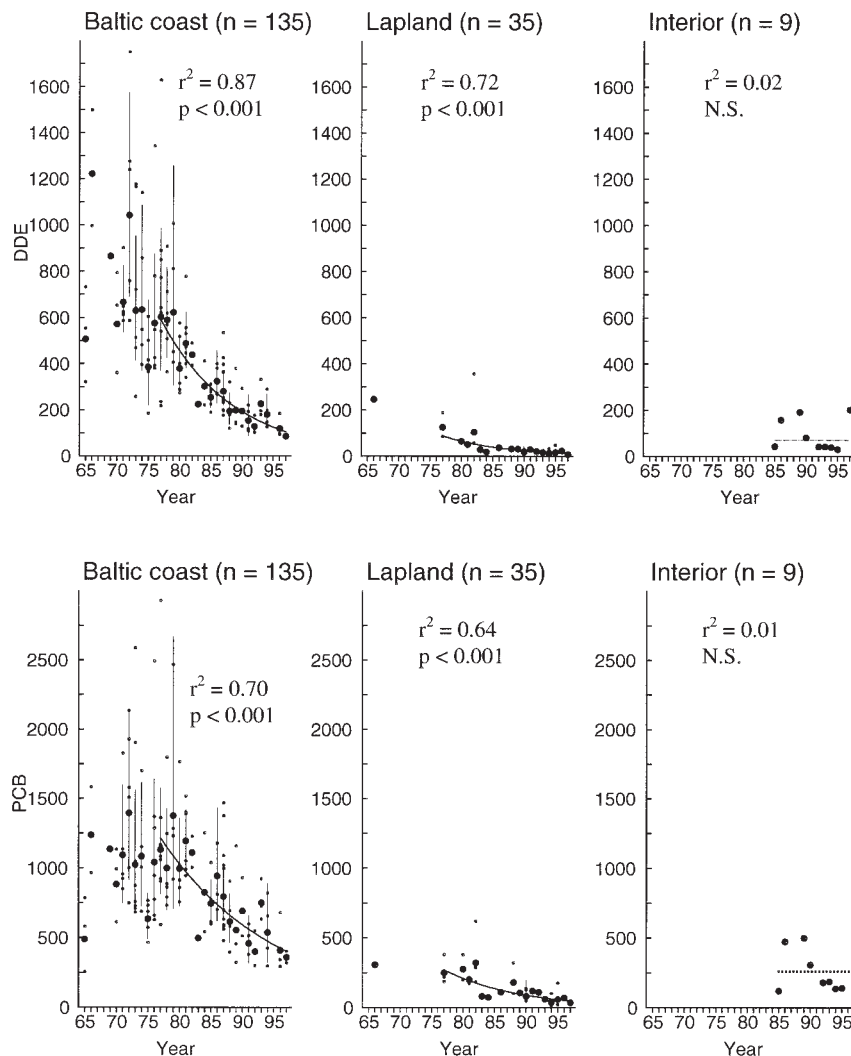


Table 5. Pair-wise comparisons between time periods for mean eggshell thickness ( $S_t$ ) and for mean eggshell index ( $S_i$ ) in 2 white-tailed sea eagle populations. \* = significantly ( $p < 0.05$ ) different from zero. Confidence intervals are according to Scheffe's method (52).

Baltic population				
Period	$S_t$	Period	$S_t$	Difference (95% c.i.)
1856–1935 (n = 56)	<b>0.607</b>	1965–1974 (n = 29)	<b>0.504</b>	-0.103 (-0.14 – -0.061)*
1856–1935 (n = 56)	<b>0.607</b>	1985–1997 (n = 49)	<b>0.553</b>	-0.054 (-0.090 – -0.018)*
1965–1974 (n = 29)	<b>0.504</b>	1975–1984 (n = 47)	<b>0.544</b>	0.039 (-0.083 – 0.004)
1965–1974 (n = 29)	<b>0.504</b>	1985–1997 (n = 49)	<b>0.553</b>	0.049 (0.006 – 0.092)*
1975–1984 (n = 47)	<b>0.544</b>	1985–1997 (n = 49)	<b>0.553</b>	0.009 (-0.047 – 0.028)
$S_i$		$S_i$		
1856–1935 (n = 61)	<b>3.20</b>	1965–1974 (n = 22)	<b>2.65</b>	-0.55 (-0.80 – -0.30)*
1856–1935 (n = 61)	<b>3.20</b>	1985–1997 (n = 42)	<b>2.80</b>	-0.40 (-0.60 – -0.20)*
1965–1974 (n = 22)	<b>2.65</b>	1975–1984 (n = 41)	<b>2.80</b>	0.14 (-0.12 – 0.41)
1965–1974 (n = 22)	<b>2.65</b>	1985–1997 (n = 42)	<b>2.80</b>	0.15 (-0.12 – 0.41)
1975–1984 (n = 41)	<b>2.80</b>	1985–1997 (n = 42)	<b>2.80</b>	0.008 (-0.21 – 0.23)
Lapland population				
Period	$S_t$	Period	$S_t$	Difference (95% c.i.)
1867–1909 (n = 7)	<b>0.586</b>	1977–1984 (n = 10)	<b>0.528</b>	-0.058 (-0.14 – 0.005)
1867–1909 (n = 7)	<b>0.586</b>	1985–1997 (n = 20)	<b>0.584</b>	-0.002 (-0.058 – 0.054)
1977–1984 (n = 10)	<b>0.528</b>	1985–1997 (n = 20)	<b>0.584</b>	0.056 (0.007 – 0.105)*
$S_i$		$S_i$		
1867–1909 (n = 8)	<b>3.14</b>	1977–1984 (n = 11)	<b>2.84</b>	-0.30 (-0.66 – 0.054)
1867–1909 (n = 8)	<b>3.14</b>	1985–1997 (n = 20)	<b>2.96</b>	-0.18 (-0.50 – 0.14)
1977–1984 (n = 11)	<b>2.84</b>	1985–1997 (n = 20)	<b>2.96</b>	0.12 (-0.17 – 0.41)

same magnitude as between 1977–1984 and 1985–1997, but was not statistically significant, probably due to smaller sample size.

Productivity data over time for the Baltic, Lapland and Interior populations, including 1998 and 1999, are shown in Figure 2. In the Baltic population, productivity dropped from the early 1950s (3) and reached a bottom level during 1965–1985 (mean productivity = 0.30). A significant increase occurred during the 1980s and 1990s coinciding with the observed decrease in DDE and PCB levels in the egg. In the Lapland and Interior populations the inter-annual variation in productivity was considerably larger than on the Baltic coast, with no significant trends over time.

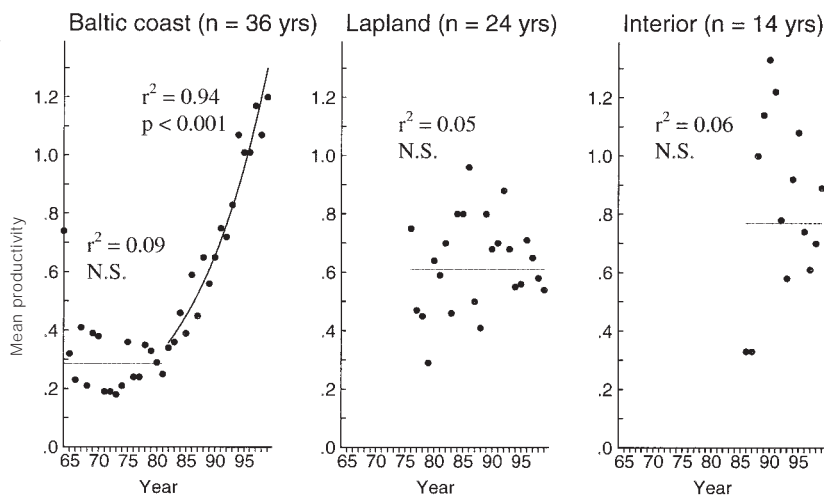
#### Reproduction in relation to organochlorine compound levels

Figure 3 illustrates the mean productivity within different concentration intervals (on a log-scale) of DDE and PCB, for 82 females from the Baltic and Interior populations 1965–1997. We used interval steps of 50 from 0 up to 200  $\mu\text{g g}^{-1}$ , interval steps of 100 from 201 up to 1000  $\mu\text{g g}^{-1}$ , and the steps 1001–1200, 1201–1500, 1501–2000 and 2001–2500  $\mu\text{g g}^{-1}$ . The data were fitted to a nonlinear sigmoid function as described under Statistical Analyses. An S-shaped dose-response relationship is indicated, in particular for DDE with a point of inflection at about 210  $\mu\text{g g}^{-1}$  and no reproduction at concentrations exceeding 900  $\mu\text{g g}^{-1}$  DDE.

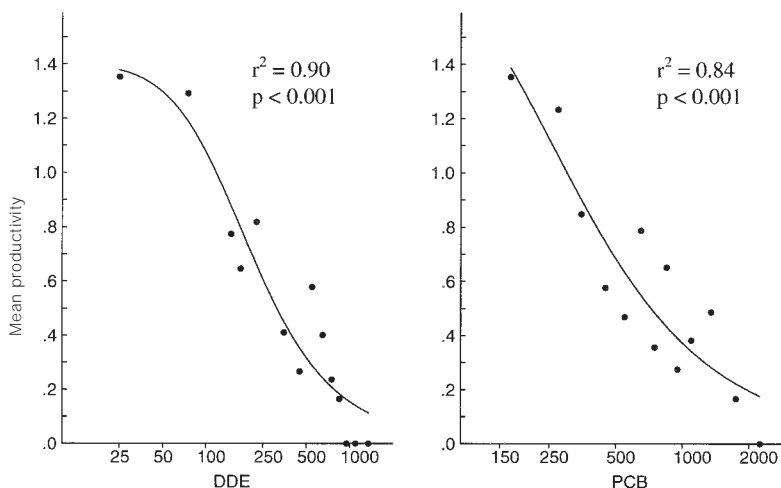
The individual productivity of these 82 sea eagle females in relation to the concentrations of DDE and PCB in their eggs is illustrated in Figure 4. For the period 1965–1988 ( $n = 65$ ) a highly significant relationship with productivity was observed for DDE ( $p < 0.001$ ) and a significant relationship also for PCB ( $p < 0.05$ ). For the period 1989–1997, when the DDE levels in the eggs had decreased about twice as much as the PCB levels, there was still a significant negative correlation for productivity to both DDE and PCB ( $p < 0.05$ ;  $n = 17$ , 2 clutches from old females with low Di-values excluded). A considerable variation in productivity is observed over a wide range of residue burdens of both DDE and PCB. In fact, about one third of the material from 1965–1988 is productivity records of zero, over a range of residue burdens including DDE concentrations at 200–400  $\mu\text{g g}^{-1}$ . These are DDE levels where other sea eagle females reproduced fairly well (4).

In order to study whether poor reproductive ability could be related to specific PCB-congeners, a selection of eggs were re-analyzed primarily for coplanar PCBs. The concentrations of a subset of 2-4-ortho CBs, 1-ortho-CBs and non-ortho-CBs,

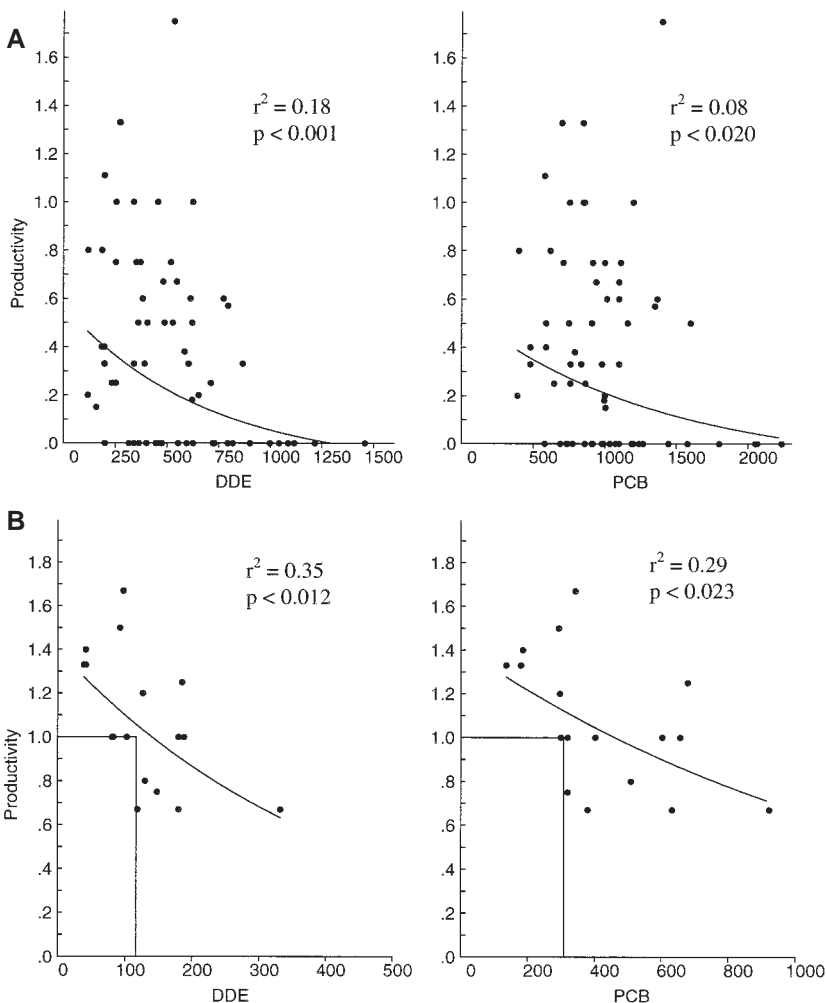
**Figure 2. Annual mean productivity over time in 3 white-tailed sea eagle populations in Sweden.  $n =$  sample size,  $r^2 =$  squared regression coefficients for the periods 1964–1981 and 1982–1999.**



**Figure 3. Mean 5-year productivity vs concentrations of DDE and tot-PCB ( $\mu\text{g g}^{-1}$  lipid weight) in the clutches of 82 white-tailed sea eagle females from the Swedish Baltic coast and Interior, grouped into 14 concentration intervals.  $r^2 =$  squared regression coefficient. Sigmoid function given under Statistical Analysis.**



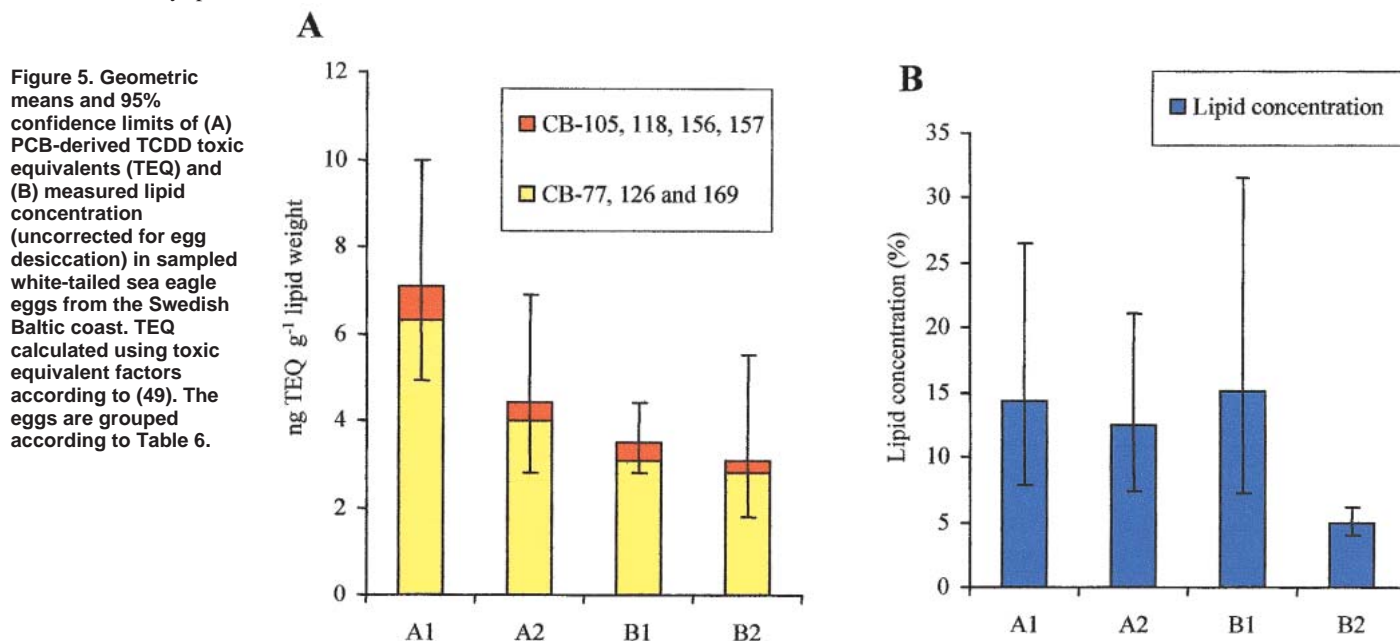
**Figure 4. Individual mean 5-yr productivity vs concentrations of DDE and tot-PCB ( $\mu\text{g g}^{-1}$  lipid weight) in the clutches of 82 white-tailed sea eagle females from the Swedish Baltic coast. A = 1965–1988 ( $n = 65$ ), B = 1989–1997 ( $n = 17$ ).  $r^2 =$  squared regression coefficient.**





TCPMeOH and BCPS, as well as of DDE and  $\Sigma$ PCB, in 21 white-tailed sea eagle eggs are given in Table 6. The eggs were selected from a period (A) of high DDE-contamination (1970s): A1 = eggs from females with no reproduction, A2 = eggs from females that managed to reproduce, and a period (B) of lower DDE-contamination (late 1980s and 1990s): B1 = eggs from females with no reproduction, B2 = eggs from females with good reproduction. The sample represents 20 different females over a 21-yr period.

Significantly ( $p < 0.05$ ) higher concentrations of DDE, PCB, CB-138, CB-153, CB-180 and TCPMeOH were found for group A1 ( $n = 7$ ) as compared to group A2 ( $n = 5$ ). None of the analyzed organochlorine contaminants differed significantly between group B1 ( $n = 4$ ) and B2 ( $n = 5$ ). There was no significant difference for the levels of  $\Sigma$ PCB or any of the individual PCB congeners, or for TCPMeOH, between group A2 and B1.



**Figure 5.** Geometric means and 95% confidence limits of (A) PCB-derived TCDD toxic equivalents (TEQ) and (B) measured lipid concentration (uncorrected for egg desiccation) in sampled white-tailed sea eagle eggs from the Swedish Baltic coast. TEQ calculated using toxic equivalent factors according to (49). The eggs are grouped according to Table 6.

**Table 6.** Concentrations of DDE,  $\Sigma$ PCB, selected PCB congeners<sup>1</sup>, bis(4-chlorophenyl)sulfone (BCPS) and tris(4-chlorophenyl)methanol (TCPMeOH) in white-tailed sea eagle eggs from the Swedish Baltic coast. The eggs are grouped according to sampling period and productivity. Geometric mean concentrations are given for each group.

Lipid %	Concentrations ( $\mu\text{g g}^{-1}$ lipid)		2-4-ortho-CBs			1-ortho-CBs				0-ortho-CBs			Polar components		
	DDE	$\Sigma$ PCB	CB-138	CB-153	CB-180	CB-105	CB-118	CB-156	CB-157	CB-77	CB-126	CB-169	TCPMeOH	BCPS	
<b>A. Period of higher contamination (1970–1976)</b>															
<b>A1. Eggs from eagles with no reproduction (mean productivity = 0)</b>															
#1/71 <sup>A</sup>	12.8	510	550	90	120	33	8.4	26	8.0	1.2	.027	.18	.024	2.8	n.d.
#2/72	47.5	1100	1100	170	220	61	19	54	17	3.0	.17	.44	.064	4.8	.016
#3/72	22.8	760	800	130	170	35	15	44	13	2.4	.10	.37	.064	6.1	.0046
#4/72	10.9	730	840	130	170	51	16	44	14	2.5	.073	.32	.042	4.8	n.d.
#5/73	4.82	500	560	89	110	38	8.6	20	7.4	1.0	.018	.18	.013	2.6	n.d.
#6/73	6.17	470	440	68	80	24	8.2	23	6.5	.90	.036	.16	.020	14	n.d.
#7/76	28.0	1000	1300	210	280	77	22	73	22	3.0	.16	.56	.072	4.7	.018
mean	14.4	690	750	120	150	42	13	37	11	1.8	.062	.28	.036	4.9	
<b>A2. Eggs from eagles that managed to reproduce (mean productivity = 0.45)</b>															
#8/70	14.3	300	380	54	68	23	6.2	16	5.5	.90	imp.	.16	.018	1.5	.0038
#9/70	29.3	780	890	150	190	50	15	47	14	2.5	.16	.41	.058	5.0	.0079
#10/71	11.1	380	420	61	69	21	7.1	20	5.9	1.0	.036	.15	.019	1.1	n.d.
#11/75	12.0	400	430	67	70	18	10	25	8.2	1.2	.061	.19	.012	2.3	.0024
#12/75	5.59	170	280	41	53	24	3.8	11	4.4	.60	.017	.099	.0074	.77	n.d.
mean	12.6	360	440	67	80	25	7.6	21	7.0	1.1	.050	.18	.018	1.7	
<b>B. Period of lower contamination (1987–1991)</b>															
<b>B1. Eggs from eagles with no reproduction (mean productivity = 0)</b>															
#13/87	8.22	290	580	110	150	35	6.7	21	6.5	1.3	.066	.20	.035	4.0	.024
#14/87	33.7	190	430	67	90	23	5.0	17	5.2	1.0	.013	.12	.018	1.7	.39
#15/88	24.5	120	250	38	55	17	4.4	14	5.6	.88	.037	.13	.045	1.6	.61
#16/89	7.60	130	330	50	69	23	4.5	14	5.8	.92	.023	.13	.022	1.9	.13
mean	15.1	170	380	61	85	24	5.1	16	5.8	1.0	.029	.14	.028	2.1	.17
<b>B2. Eggs from eagles with good reproduction (mean productivity = 0.90)</b>															
#17/87	4.10	500	900	140	190	52	14	43	14	2.7	.099	.38	.081	4.9	.51
#18/88	3.47	100	230	35	43	15	imp.	9.3	3.2	.49	.0075	.079	.010	.71	.11
#19/89	6.10	140	240	37	45	16	3.2	9.3	3.7	.57	.011	.088	.016	.81	.091
#20/90	5.75	120	290	45	65	21	4.0	13	5.3	.83	.023	.13	.025	imp.	.40
#21/91	5.90	120	270	46	63	14	3.7	12	3.5	.66	.021	.093	.015	.75	.0074
mean	4.9	160	330	52	68	21	5.1	14	5.0	0.84	.021	.13	.022	1.2	.11

<sup>1</sup> The PCB congeners analyzed are abbreviated according to (41).

<sup>A</sup> Year of collection. Imp.= quantification problem due to impurity. n.d. = below detection limit ( $1 \text{ ng g}^{-1}$  l.w.)

The TCPMeOH/DDE ratio was significantly different ( $p < 0.05$ ) among the 4 groups, with the highest ratio in B1. Thus, although there was no significant difference in TCPMeOH or DDE concentrations between group B1 and B2, the ratio of TCPMeOH/DDE was apparently higher for group B1. BCPS was found in much lower concentrations, or was not detected, in the samples from the 1970s as compared to the 1980s. Thus, of all the analyzed contaminants, only BCPS increased over time.

The geometric mean  $TEQ_{PCB}$  values are given in Figure 5 for the 4 groups (A1-2, B1-2).  $TEQ_{PCB}$  values were derived from the PCB congeners presented in Table 6. Figure 5 also includes the TEQ derived only from the non-ortho-CBs (CB-77, CB-126 and CB-169). In the eggs, CB-126 contributed to 61% of the PCB derived TEQ (range 60-62%). The calculated  $TEQ_{PCBS}$  could not explain the depressed productivity of group B1. The only parameter that differed clearly between group B1 and B2 was the percentage lipids, uncorrected for dehydration, in the collected eggs. Most eggs in groups A1, A2 and B1 had elevated lipid concentrations, indicating a high rate of desiccation, whereas none of the eggs from the normally reproducing females in group B2 showed signs of excess desiccation (Table 6 and Fig. 5).

#### Desiccation of sampled eggs and chicken eggs

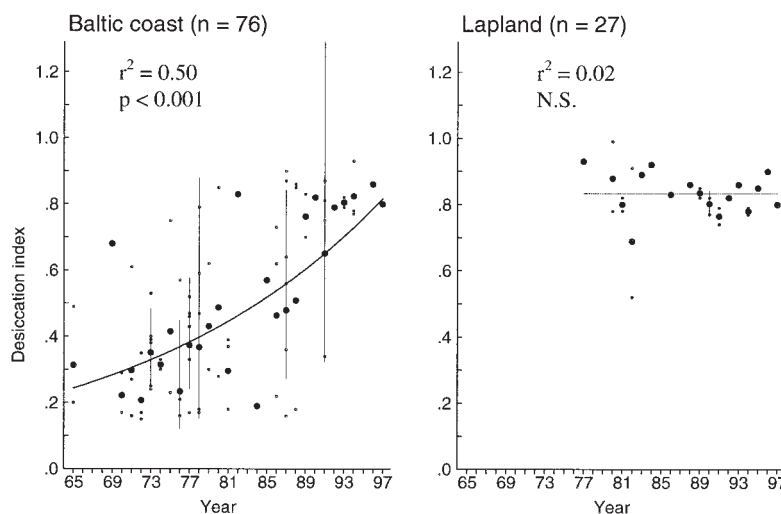
The distribution over time of desiccation index ( $D_i$ ) values in undeveloped eggs from Lapland and from the Baltic coast is shown in Figure 6. There was no change over time in  $D_i$  in the Lapland egg samples ( $n = 27$ ). In the Baltic egg samples, a significant increase occurred over time ( $p < 0.001$ ,  $n = 76$ ). After 1988, only 1 clutch in the Baltic sample had an obviously low  $D_i$ -value.

The  $D_i$ -values from all undeveloped Lapland eggs, and all undeveloped Baltic and Interior eggs from the period 1988-1997 excluding 1 outlier (c.f. above), averaged 0.82 ( $sd = 0.085$ ,  $n = 31$ ) and 0.82 ( $sd = 0.058$ ,  $n = 21$ ), respectively. The  $D_i$ -values for eggs with embryo averaged 0.59 ( $sd = 0.030$ ,  $n = 13$ ) in the Baltic sample from 1988-1997 and 0.72 ( $sd = 0.021$ ,  $n = 11$ ) in the Lapland sample. A lower rate of water loss from undeveloped eggs compared to embryo eggs has been demonstrated and is to be expected, due to the lower permeability of the shell membranes in eggs with no embryo (56). As illustrated in Figure 7 all but one egg with residue concentrations below  $170 \mu\text{g g}^{-1}$  DDE and  $500 \mu\text{g g}^{-1}$  PCB had  $D_i$ -values in the range 0.70-0.93; a sharp drop to around 0.20 on the low side of the range occurred when residue concentrations exceeded these levels. No  $D_i$ -values above 0.70 were found when DDE and PCB concentrations exceeded  $400 \mu\text{g g}^{-1}$  and  $1000 \mu\text{g g}^{-1}$ , respectively.

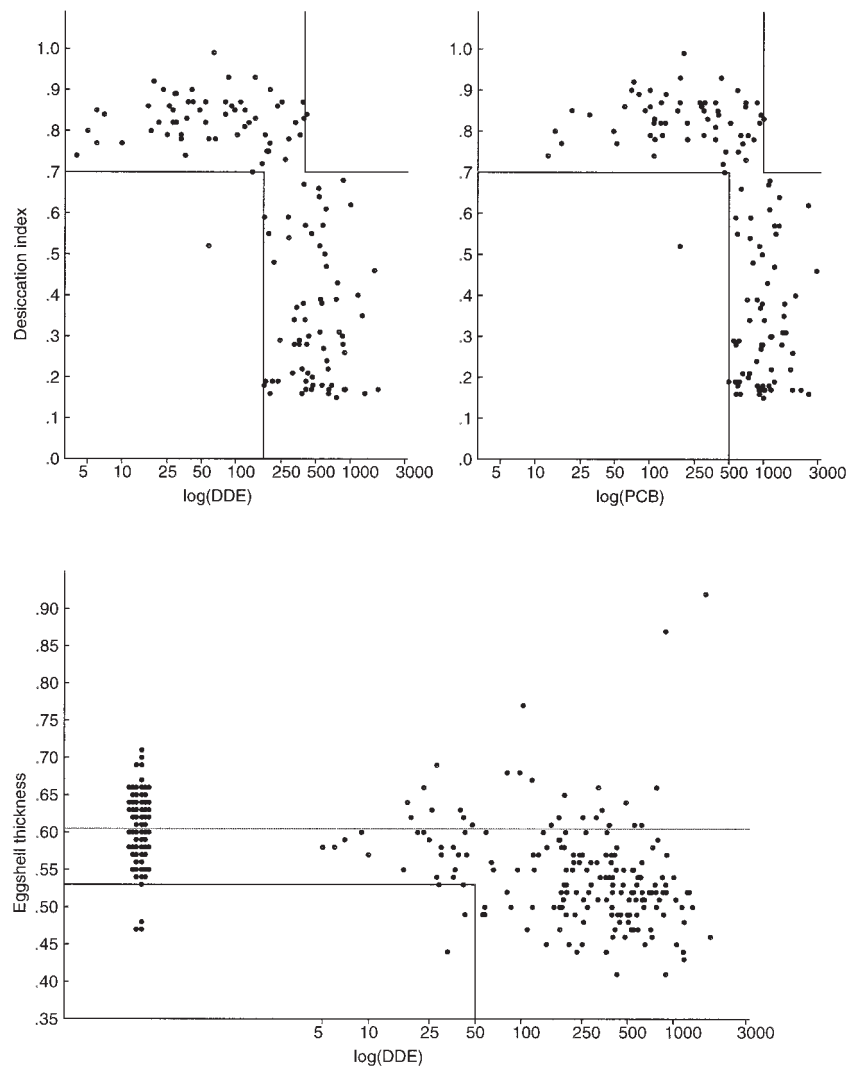
Dehydration of the egg leads to an elevated lipid concentration, so inherently uncorrected lipid concentrations in the sampled eggs were significantly correlated to  $D_i$ -values ( $p < 0.001$ ,  $n = 91$ ). 84% of the variation in lipid concentrations was explained by the  $D_i$ -values. However, lipid concentrations were not significantly correlated to eggshell thickness ( $n = 96$ ) or eggshell index ( $n = 113$ ). Thus, shell index and thickness values were not indicative of eggshell functionality in this respect.

The weights of infertile chicken eggs decreased at an approximately constant rate over the study period. There was no difference in average daily weight-loss of the exposed sample, kept by the window ( $0.19 \text{ g day}^{-1}$ ), and the sample kept away from the sun ( $0.19 \text{ g day}^{-1}$ ). Over 21 days, corresponding to the incubation period of the chicken (50), total egg weight decreased from an average of 60.10 g ( $sd = 1.62$ ,  $n = 6$ ) to 55.91 g ( $sd = 1.88$ ). Excluding the eggshell weight of 5 g (57) gives a weight loss of the egg contents of

**Figure 6.** Desiccation index over time in white-tailed sea eagle eggs without embryo from the Baltic coast and from Lapland. Large dots = annual means, small dots = individual clutches, vertical lines = 95% confidence limits (for sample sizes > 3).  $n$  = sample size,  $r^2$  = squared regression coefficient.



**Figure 7.** Desiccation index ( $D_i$ ) in relation to DDE and PCB ( $\mu\text{g g}^{-1}$  lipid weight) in 126 undeveloped white-tailed sea eagle eggs from the Swedish Baltic coast 1969-1997, and eggshell thickness ( $St$ ) in relation to DDE in 185 white-tailed sea eagle clutches from the Swedish Baltic, Interior and Lapland populations 1969-1997. Vertical and horizontal lines indicate suggested threshold levels as discussed in the text. Dashed line indicates mean  $St$  based on reference samples from 1856-1935 ( $n = 68$ ).





**Table 7. Residue levels of DDE and PCBs ( $\mu\text{g g}^{-1}$  lipid weight), desiccation index ( $D_i$ ) and productivity ( $P$ ) among 14 sea eagle females from 7 territories (T) on the Swedish Baltic coast. F1 = female no1 (etc), [ ] = female year of birth, n = number of clutches, ranges in italics, – = not measured.**

T	Female	Period	n	DDE		tot-PCB		$D_i$		P
I	F1 [ $\leq 1968$ ]	1972–77	4	<b>1200</b>	<i>780–1600</i>	<b>2000</b>	<i>1300–2900</i>	<b>.33<sup>A</sup></b>	<i>.23–.46</i>	<b>0</b>
	F1 [ $\leq 1968$ ]	1984–88	2	<b>200</b>	<i>180–220</i>	<b>570</b>	<i>540–610</i>	<b>.18</b>	<i>.18–.19</i>	<b>0</b>
II	F1 [ $\leq 1968$ ]	1971–75	2	<b>1400</b>	<i>1200–1800</i>	<b>2000</b>	<i>1900–2100</i>	<b>.26</b>	<i>.17–.40</i>	<b>0</b>
	F1 [ $\leq 1968$ ]	1986–90	2	<b>370</b>	<i>370–380</i>	<b>1100</b>	<i>1100–1200</i>	<b>.64</b>	<i>.63–.65</i>	<b>0</b>
	F2 [ $\leq 1988$ ]	1992–96	–	–	–	–	–	–	–	<b>1.60</b>
III	F1 [ $\leq 1972$ ]	1977–80	2	<b>710</b>	<i>570–890</i>	<b>1000</b>	<i>890–1200</i>	<b>.17</b>	<i>.17–.18</i>	<b>0</b>
	F1 [ $\leq 1972$ ]	1989–93	1	<b>220</b>	–	<b>520</b>	–	<b>.34</b>	–	<b>0</b>
	F2 [ $\leq 1990$ ]	1994–97	–	–	–	–	–	–	–	<b>1.50</b>
IV	F1 [1960/61]	1968–77	2	<b>380</b>	<i>360–390</i>	<b>690</b>	<i>610–770</i>	<b>.36</b>	<i>.29–.44</i>	<b>0.50</b>
	F2 [1973/74]	1986–90	1	<b>120</b>	–	<b>400</b>	–	<b>.85</b>	–	<b>0.80</b>
V	F1 [ $\leq 1964$ ]	1967–77	2	<b>690</b>	<i>550–870</i>	<b>1000</b>	<i>950–1100</i>	<b>.67</b>	<i>.66–.68</i>	<b>0.50</b>
	F2 [ $\leq 1975$ ]	1986–95	4	<b>200</b>	<i>150–230</i>	<b>570</b>	<i>430–700</i>	<b>.85<sup>A</sup></b>	<i>.75–.93</i>	<b>0.91</b>
VI	F1 [1976]	1981–83	1	<b>460</b>	–	<b>1200</b>	–	<b>.66</b>	–	<b>1.00</b>
	F2 [1986]	1991–94	2	<b>220</b>	<i>200–240</i>	<b>740</b>	<i>730–760</i>	<b>.71</b>	<i>.70–.72</i>	<b>1.20</b>
VII	F1 [ $\leq 1967$ ]	1971–79	5	<b>620</b>	<i>500–760</i>	<b>990</b>	<i>770–1200</i>	<b>.31</b>	<i>.15–.47</i>	<b>0.11</b>
	F2 [ $\leq 1983$ ]	1988–92	1	<b>130</b>	–	<b>510</b>	–	<b>.82</b>	–	<b>0.80</b>

<sup>A</sup>  $n = 3$

7.6% in this sample, as compared to about 16.5% in chicken eggs with an embryo (calculated from data given by Rahn et al. (50) and Taylor (57)).

#### Productivity in relation to eggshell parameters and levels of DDE and PCB

Table 7 gives data on DDE and PCB concentrations,  $D_i$  and productivity for 14 females in 7 territories from which egg samples were obtained for at least 3 yrs over 2 to 3 decades. Approximately normal individual productivity (0.80–1.20) occurred with DDE concentrations in the eggs in the range of 130–460  $\mu\text{g g}^{-1}$ , PCB levels in the range of 300–1200  $\mu\text{g g}^{-1}$  and  $D_i$  values down to 0.66.

Productivity seemed to be better correlated to  $D_i$  than to the residue levels of DDE and PCB in the eggs (Table 7). In order to investigate the relative importance of DDE, PCB,  $D_i$ ,  $S_i$  and  $S_e$  for productivity, a selection was made of clutches for which data on all 6 parameters were available. Mean values were calculated for clutches representing the same female if the 5-yr intervals around the sampling years overlapped (see Productivity Data). The 109 clutches available on these premises produced 66 sets of data representing 58 females in 47 territories. The correlation coefficients for the 6 investigated parameters are given in Table 8. Productivity was significantly correlated with  $D_i$ , DDE and PCB levels, but not with  $S_i$  and  $S_e$ . The largest correlation coefficient was found for  $D_i$ , explaining about 42% of the variation in individual productivity in this sample.  $D_i$ , DDE and PCB were also significantly interrelated.  $S_i$  and  $S_e$  were significantly correlated to DDE but not to PCB.

In order to investigate the relative importance of  $D_i$ , DDE and PCB to explain the variation in productivity, multiple regression with productivity as dependent variable was applied (Table 9). The squared multiple correlation coefficient was 0.44, showing that almost one half of the estimated variation in individual productivity may be explained by the included variables, and only  $D_i$  contributed significantly to the multiple correlation coefficient. Another multiple regression analysis was also performed, using the same sample, but with  $D_i$  as the dependent variable vs DDE, PCB. The squared multiple correlation coefficient determined in this case was 0.31, with only DDE contributing significantly to the multiple correlation coefficient.

#### PCB pattern and changes over time

A possible change in PCB composition over time in the eagle eggs might have had an effect on the reproductive success. In order to study changes in PCB patterns between 1970–1976 and 1987–1991, we used the 21 eggs for which individual PCB congeners were determined. Concentrations of different PCB congeners as well as  $\Sigma\text{PCB}$  (Table 6) and TEQ concentrations (Fig. 5) were related to CB-138; the results are given in Table 10. A general trend of decreasing concentrations in relation to CB-138 of lower chlorinated PCBs, e.g. CB-77, CB-105 and

**Table 8. Correlation coefficients for desiccation index ( $D_i$ ), concentrations of DDE and PCB, eggshell index ( $S_i$ ), eggshell thickness ( $S_e$ ) and productivity ( $P$ ).  $n = 66$ , based on 109 clutches from 58 females in 47 territories on the Swedish Baltic coast. \* indicates that  $p < 0.05$  and \*\*\* that  $p < 0.001$ .**

	$D_i$	DDE	tot-PCB	$S_i$	$S_e$
DDE	–0.553***				
PCB	–0.486***	0.858***			
$S_i$	0.015	–0.277*	–0.133		
$S_e$	0.092	–0.274*	–0.151	0.843***	
P	0.651***	–0.467***	–0.425***	0.163	0.189

**Table 9. Standard partial regression coefficients ( $B'$ ) and the corresponding probability ( $p$ ) values are given for the multiple regression analysis on (i) desiccation index ( $D_i$ ), DDE and PCB versus Productivity as the dependent variable and (ii) DDE and PCB versus Desiccation index as the dependent variable.  $n = 66$  (see Table 8).**

(i) Productivity Squared multiple regression coefficient = 0.44			(ii) Desiccation index Squared multiple regression coefficient = 0.31		
Variable	$B'$	$p$	Variable	$B'$	$p$
$D_i$	0.565	< 0.001	DDE	–0.515	< 0.014
DDE	–0.097	0.6	Tot-PCB	0.045	0.8
Tot-PCB	–0.066	0.7			

CB-118, and increasing concentrations of more persistent PCB congeners such as CB-153, CB-169 and CB-180 can be observed. No change over time was observed for the ratio of  $\Sigma\text{PCB}$  to CB138, or of CB-126 to CB-138 and, consequently, the  $\text{TEQ}_{\text{PCB}}$  to CB-138 ratio was approximately constant over time.

To enable the estimation of the concentrations of CB-126 and TEQ from either  $\Sigma\text{PCB}$ , CB-118 or CB-138, the relationship between individual PCB congener, tot-PCB,  $\Sigma\text{PCB}$  and the PCB derived TEQ were calculated. Results are given in Table 11. The relationship was calculated only for ratios with no significant difference between time periods. The relations are expressed as ratios and the variations are given both as 95% c.i. and as coefficients of variation.

#### DDE and PCB levels in eggs with embryo

In order to study a possible relation between contaminants and embryo mortality, eggs collected on the Baltic coast between 1988 and 1997 were selected for analysis. This period was chosen to limit the influence of desiccation on egg viability and embryo survival (see Desicca-

tion of sampled eggs and chicken eggs); only eggs with desiccation index ( $D_i$ ) > 0.6 were selected. Under these criteria a total of 26 eggs from different clutches were available from the Swedish Baltic coast: 9 with embryo and 17 undeveloped. Seven of the embryo-eggs and 13 of the undeveloped eggs were found in nests with a nestling. In eggs with embryos larger than 75 mm (half-grown) an attempt was made to correct the PCB and DDE concentrations for lipid metabolism (see Selection of Samples). Assuming an approximately linear relationship between the increase in residue concentrations and embryo length from 75 mm up (14) and a doubling in residue concentrations from half-grown to full-grown according to (38,39), an estimation of the original concentrations was made as *measured concentration*  $\times$  *75*  $\times$  *embryo length*<sup>-1</sup> (mm).

Mean concentrations of CB-138 and DDE in undeveloped eggs and in eggs containing an embryo are shown in Figure 8. Concentrations corrected for lipid metabolism in eggs with embryo (> 75 mm) are included. CB-138 was selected as a measure of tot-PCB. The eggs collected between 1988–1990 were analyzed by packed column, and CB-138 concentrations were therefore estimated using the ratio given in Table 11. Mean concentrations of both CB-138 and DDE were significantly higher in the eggs with embryos. The p value for CB-138 was, however, stronger than that for DDE. Based on the concentrations corrected for embryo lipid-metabolism, a significant difference between embryo eggs and undeveloped eggs was found for CB-138 but not for DDE. The concentrations of CB-138 in the embryo eggs ranged from 40–140  $\mu\text{g g}^{-1}$ .

## DISCUSSION

### Representativeness of Samples

#### Sampling bias

Nonrandom sampling might lead to biased analyses, e.g. if a more contaminated segment of a population is more frequently sampled. Wiemeyer et al. (22) reported a negative bias in the production of bald eagle pairs from eggs that were collected after hatch failure, as compared to the mean production of the whole population. When eggs were randomly collected during incubation (58), the productivity of sampled bald eagle nests did not differ from the productivity in the whole population (12).

In the present study sampled pairs did not produce significantly less offspring than nonsampled pairs in 1965–1984 (Table 2). This indicates that the entire Baltic population of sea eagles was heavily influenced by contaminants at that time. The sampled pairs made up a large proportion of the population during that period and were obviously representative for the whole population in this respect. Still, eggs that hatched might have had lower residue levels than the dead eggs available for collection and analysis. On the other hand, eggshell pieces were found in unproductive nests from as many eggs as those being hatched in other nests. Such cracked eggs might have represented the most contaminated eggs in the population. Thus, although the eggs collected between 1965–1984 were representative of the population regarding productivity, they might not have been representative regarding residue concentrations.

The higher productivity among sampled compared to nonsampled pairs in 1975–1979 might be purely coincidental. However, the difference in distributions of productive nests between the 2 groups is notably strong. It cannot be excluded that a larger proportion of more contaminated female sea eagles were present among the nonsampled pairs during this period, due to egg breakage and a higher frequency of early egg disappearance from nest desertions. In the merlin (*Falco columbarius richardsonii*), significantly lower nest defence behavior has been reported among the more highly contaminated individuals (59). In an experimental study on ring doves (*Streptopelia risoria*) fed a mixture of organochlorines, significant alterations in several aspects of the breeding performance was observed (60). Aberrant incubation behavior was also observed in one eagle pair under surveillance in 1973 in the present study.

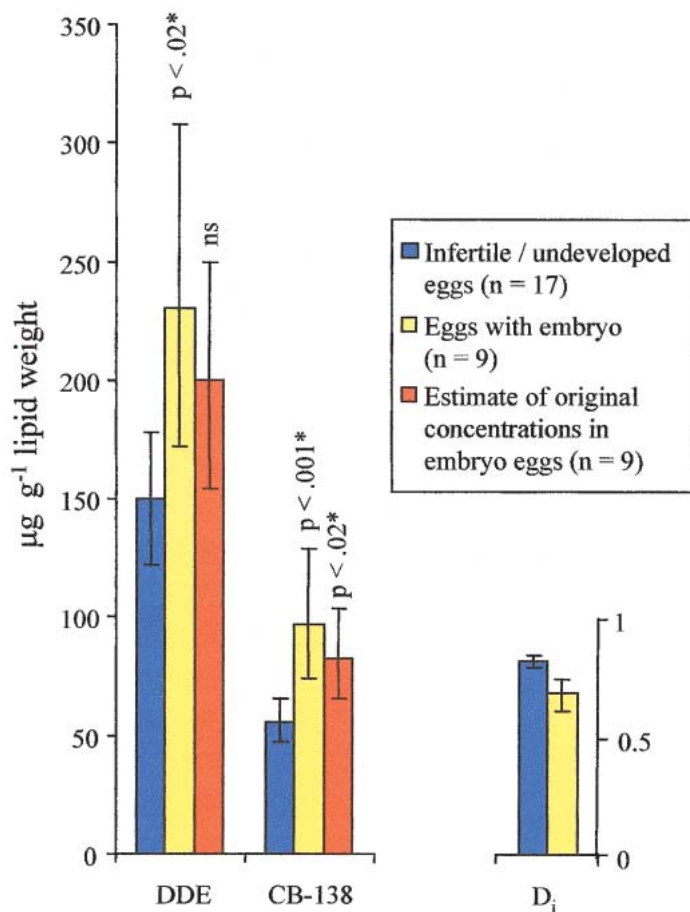
As contaminant levels decreased during the 1980s and 1990s and re-

**Table 10.** Changes between time periods (1970–1976 and 1987–1991) in composition of  $\Sigma$ PCB, TEQ and selected PCB congeners in relation to CB-138 based on 21 white-tailed sea eagle eggs (cf. Table 6). \* indicates  $p < 0.05$ . Confidence intervals are according to Scheffe's method (52).

	1970–1976 (n = 12)		1987–1991 (n = 9)		Change between periods	
	mean	S.D.	mean	S.D.	Difference (in %)	95% c.i.
$\Sigma$ PCB	6.4	.34	6.3	.44	–.14 (–2.2 %)	–.33 — .44
<b>2-4-ortho</b>						
CB-153	1.2	.089	1.4	.081	.10 (8.3 %)	.088 — .12*
CB-180	.37	.082	.40	.096	.029 (7.8 %)	.0052 — .054*
<b>1-ortho</b>						
CB-105	.11	.016	.087	.016	–.024 (–22 %)	–.025 — –.023*
CB-118	.31	.040	.27	.048	–.040 (–13 %)	–.054 — –.027*
CB-156	.10	.010	.10	.026	–.0018 (–1.8 %)	–.030 — .027
CB-157	.016	.0024	.017	.0035	.00083 (5.2 %)	–.00092 — .0026
<b>0-ortho and TEQ (ratios <math>\times</math> 1000)</b>						
CB-77	.65	.29	.49	.25	–.16 (–25 %)	–.22 — –.094*
CB-126	2.5	.31	2.4	.53	–.096 (–3.8 %)	–.49 — .30
CB-169	.30	.10	.49	.28	.18 (60 %)	–.14 — .51
TEQ	.063	.007	.060	.013	–.0024 (–3.8 %)	–.013 — .0084

\* Difference between means with confidence limits which do not include zero.

**Figure 8.** Residue concentrations (DDE and CB-138) and desiccation index ( $D_i$ ) of undeveloped and embryo eggs of white-tailed sea eagles from the Swedish Baltic coast collected between 1988 and 1997. Geometric means and 95% confidence limits. \* significantly different from the infertile/undeveloped group according to ANOVA (ns = non-significant,  $p > 0.05$ ).



production improved, the proportion of dead eggs available for sampling decreased. The lower offspring production among sampled compared to nonsampled pairs during 1990–1994 may indicate that the residue concentrations in the nonsampled, larger segment of the population were lower than in the sampled eggs, but the proportion of successfully breeding pairs was not lower in the sampled segment. Thus, residue concentrations were low enough to allow for hatching in both groups, but more eggs remained unhatched in the clutches in the sam-



pled group. A reasonable explanation for this is the range in intra-clutch variation in residue concentrations and desiccation (cf. below), with the sampled group representing birds with higher concentrations and including birds with remaining effects from a previous exposure.

#### Lipid contents

Mulhern and Reichel (61) showed experimentally that putrefaction did not cause a significant change in the recovery of DDT compounds in the eggs. It is reasonable to believe that this is also true for PCB. Helander et al. (14) found no significant difference in lipid contents between a small sample ( $n = 6$ ) of badly rotted sea eagle eggs and a sample of much less putrefied eggs ( $n = 54$ ). The results in the present study support that there is no significant change in the amount of lipid due to putrefaction in eggs collected up to 2 months after expected hatching-time. Thus, it may be concluded that residue concentrations expressed on a lipid weight basis are not significantly affected by putrefaction.

Peakall and Gilman (39) demonstrated a reduction in lipids during incubation in embryonated herring gull eggs of about 50%; the main reduction occurred during the last half of incubation. Newton and Bogan (38) reported that the residue levels of organochlorines in lipids in sparrow hawk (*Accipiter nisus*) eggs approximately doubled during incubation; the main increase seemed to occur after the embryos were half grown. The average of 0.7 g less fat in our sample of eggs with up to half grown embryos (a reduction of 13.6%) would imply that the residue levels on a lipid basis in those eggs were elevated by about that magnitude. Eggs with less than half-grown embryos made up only 22% of the sample, and were approximately randomly distributed over the study period. Thus, the slightly elevated residue levels in these embryo-eggs were considered to have a negligible effect on the distribution of residue levels in this material and no corrections were made.

#### Variation within and between clutches and females

The substantially larger CVs obtained among clutches as compared to the CVs within clutches for all measured parameters shown in Table 3, imply that individual eggs can largely be regarded as representative of the clutch. This is in agreement with other studies (e.g. (38, 62–64)). The percentage deviation of individual eggs from their clutch means, averaged about 9% for DDE and 8% for PCB (2- and 3-egg clutches combined), with 50% of the eggs deviating < 5% and 82% deviating < 15%. Thus, most single eggs obtained can be regarded as representative of the clutch for the concentrations of these contaminants. In a few clutches, however, the residue concentrations may differ by as much as about 70% between the eggs (see H/L-ratios, Table 3).

For eggshell thickness and eggshell index, the percentage deviation from the clutch mean averaged less than 4%. The smaller variation in shell thickness within clutches as compared to among clutches agrees with the findings by Klaas et al. (65), who reported about twice as high a variance among clutches as compared to within clutches in 4 out of 5 studied species, which is contradictory to the results reported for the American peregrine falcon (*Falco peregrinus*) (66). For desiccation index (D), the percent deviation averaged 12% and the observed range shows that, in some clutches, one egg had lost about 4 times more water than the other egg over the same period. Such a difference should have a strong effect on the hatching success of individual eggs in the clutch.

The variation of DDE and PCB levels within females between years was smaller than the variation between different females. This implies that we can expect the clutches from some females to be more contaminated and those from other females to be less so, over a given period, rather than that residue concentrations would vary randomly between all clutches sampled over the period. This seems to agree with the discriminating pattern for productivity found among different females (Table 7).

#### Egg volume and shell

Mean volume for eggs with embryo from the Baltic was about the same as for eggs from Lapland from 1867–1909. At that time, eggs were collected during incubation, i.e., with no bias for availability due to hatching, and it is reasonable to assume that the majority of those eggs contained embryos. There is no evidence here that DDE or PCB significantly affected reproduction in the Lapland sea eagle population during our study period, but good evidence that this was the case in the Baltic population (Figs 2–4). If there is a difference in volume between fertile and infertile eggs, as suggested in the Baltic sample set, the lower

mean value for volume in the recent as compared to the historic sample from Lapland might stem from a bias towards infertile eggs in this sample. Such a bias would be much smaller in the Baltic sample, if a larger proportion of fertile eggs in this population failed to hatch due to contaminants, and thus became available for sampling. Another possible explanation for smaller eggs in the late Lapland sample could be a decrease in the food supply in recent years (3), but evidence for this is lacking.

No significant thinning of the eggshell due to embryo-growth could be shown in this study, although a tendency is suggested ( $p < 0.06$ ). Our investigation was not based on repeated measurements of each egg over the incubation period, but only uses one measurement from each egg in a sample with embryos of varying size. Shell thickness varies initially among eggs and a larger sample of this type would be required to minimize the influence from that variation. Shell thinning during embryo growth has been reported for some species (e.g. (67, 68) but not for others, e.g. the peregrine falcon (66). Thinning during embryo growth might be expected in bird eggs, since calcium is needed for the growth of bone-tissue in the embryo. However, a significant influence would not be expected with small embryos. Thus, the exclusion of eggs with embryos > 75 mm in length (about half of the length at the time of hatching) in our investigations of eggshell thickness and eggshell index, should minimize the possible bias from an influence of embryo growth.

#### Temporal Changes

##### PCB: concentration changes over time

It has been suggested that weathering of the PCB mixture in the environment over the years has resulted in a composition of more toxic PCB components (30). This would imply that even though the PCB concentrations in biota have decreased since the 1970s, the toxic potency of the remaining PCB congeners might have remained the same. PCB, especially coplanar PCBs, would then pose the major threat to eagle populations today, when DDE concentrations have decreased (29, 30). In order to investigate this matter in our study area, we re-analyzed eggs collected in the 1970s and compared the composition of selected PCB congeners with eggs collected in the late 1980s and 1990s. The results indicate that changes have occurred during this period, with a shift towards higher chlorinated PCB congeners (Table 10). With a balance around CB-138, the relative contribution of heavier and more persistent PCB congeners in relation to  $\Sigma$ PCB has increased and that of lower chlorinated, less persistent PCB congeners has decreased. This is a similar change of the PCB pattern as observed in herring gull eggs from the Great Lakes (69). Even though the PCB composition today contains relatively more highly-chlorinated PCB congeners, the CB-138 to  $\Sigma$ PCB ratio has remained constant. Similarly, the CB-126/CB-138 ratio has remained constant. CB-126 has the highest dioxin-like toxicity among all individual PCB congeners (49, 70, 71) and was in this study the major contributor to the PCB derived TEQ. As given in Table 11, the toxic potency expressed as TEQ<sub>PCB</sub> concentrations has, throughout the investigated period, been closely correlated to the  $\Sigma$ PCB and hence also of tot-PCB. This means that even though the composition of PCB in sea eagle eggs has changed over time, the toxic potency of the environmental PCB mixture in terms of TEQ<sub>PCB</sub> concentrations has in general followed the changes in concentration of tot-PCB,  $\Sigma$ PCB, or e.g. the concentration of CB-138, must therefore be regarded as a good indirect measure of the possible toxic effects also of coplanar PCB congeners.

**Table 11. Relations between CB-126, CB-138, CB-118, TEQ, tot-PCB and  $\Sigma$ PCB expressed as mean ratios ( $n = 21$ ). Variations are given both as 95% c.i. and the coefficients of variation (CV).**

[ng g <sup>-1</sup> ] versus [ $\mu$ g g <sup>-1</sup> ]	mean ratio	95% c.i.	CV [%]
CB-138 vs $\Sigma$ PCB <sup>A</sup>	157	153–162	6.4
CB-138 vs tot-PCB <sup>B</sup>	122	118–127	6.9
CB-126 vs CB-118	8.42	8.00–8.85	12
CB-126 vs CB-138	2.49	2.31–2.66	17
CB-126 vs $\Sigma$ PCB	.390	.365–.415	15
TEQ vs $\Sigma$ PCB	.00963	.00902–.0102	15

<sup>A</sup> sum of PCB congeners according to capillary column separation

<sup>B</sup> sum of 14 peaks according to packed column separation, in eggs from the Baltic coast ( $n = 13$ ).

### Residue concentrations, eggshell parameters and productivity

A stronger decrease over time of the DDE levels as compared to the PCB levels in the eagle eggs resulted in an altered PCB/DDE ratio, from approximately 1 in the late 1960s, to 3–4 in the mid-1990s (Table 4). A similar temporal change in ratio has been reported for guillemot eggs and herring (*Clupea harengus*) from the Baltic proper (16). The data from the few eggs available from the mid-1960s to early 1970s indicate that the eagle population had the highest DDE concentrations at that time, in agreement with reported time trends for pike (*Esox lucius*), herring and guillemot from Swedish waters (16,17). The use of DDT was banned step by step between 1972 and 1975 in Sweden and in most Baltic countries during the early 1970s. There is no indication of higher levels of PCB in the earlier eggs from the Baltic eagles, as compared to the levels determined throughout the 1970s. As has been reported for guillemot eggs and herring (16), the onset of the decrease in residue levels occurred later for PCB than for DDE also in the sea eagle eggs.

There was an approximately 5-fold decrease of the DDE concentrations and an approximately 3-fold decrease of the PCB concentrations in the sea eagle eggs from the Baltic coast during the study period (Fig. 1). Based on published data and on their own analyses, Koistinen et al. (31) stated incorrectly that DDE concentrations in Baltic sea eagle eggs had decreased by a factor of 30 from the late 1970s to the early 1990s; this error was due to confusion of concentrations given on a lipid weight basis and on a fresh weight basis. They also reported concentrations of CB-77, CB-126 and CB-169, which they stated were 5–35 times lower than in 1982; however, no concentrations were reported for individual PCB congeners in the cited paper from 1982. Applying the results from this study of the relative changes for these specific congeners (Table 10) to a 3-fold decrease of  $\Sigma$ PCB over the period, the changes would correspond to a 2-fold decrease of CB-169, a 3-fold decrease of CB-126 and a 4-fold decrease of CB-77.

BCPS is a "new" environmental contaminant that was recently detected in Baltic wildlife (33). The clean-up method used in this study was not primarily designed for analysis of BCPS. This may explain the large variations in BCPS levels as shown in Table 6. The obvious change of BCPS concentrations over time in these samples is nevertheless relevant and an interesting observation. It is obvious that BCPS is an environmental pollutant that has increased in recent years and further studies must be pursued to address this issue in detail. The structural similarities with DDT and DDE, as well as similar toxic properties as DDT against flies (72), generates BCPS a special interest, even though the concentrations presented here are very low in comparison to the DDE concentrations (Table 6).

A significant negative correlation for eggshell thickness ( $S_i$ ) to DDE has been reported from several field studies and has been demonstrated in experimental studies for a number of bird species (e.g. (20, 21, 63, 73–78)). Although an increase occurred over time in the Baltic sample in  $S_i$  and shell index ( $S_i$ ) (Table 4), eggs were still significantly thinner (–10%) and had significantly lower  $S_i$ -values (–12%) in 1985–1997 compared to 1856–1935 (Table 5). This is the same magnitude of reduction as observed in the Lapland sample from 1977–1984, which showed on average about 10% thinner shells and lower  $S_i$ -values than the Lapland sample from 1867–1909. A significant correlation for  $S_i$  and for  $S_i$  to DDE but not to PCB was found (Table 8). This is in agreement with some other reports on the influence from PCB on shell thickness (e.g. (22, 79–81)). However, Nygård and Skaare (62) reported a better correlation for  $S_i$  to PCB than to DDE for white-tailed sea eagles in Norway. The increase of desiccation index values seems to be continuous from the early 1970s to the late 1980s (Fig. 6) corresponding with the continuous decrease of DDE, but not with PCB (Fig.1).

It was previously concluded that the interannual fluctuations in breeding success and productivity of the Lapland population in 1976–1982 were mainly a result of food shortage, human disturbance and weather (3, 15). The levels of DDE and PCB were considerably lower in the Lapland samples than in the Baltic samples and it was concluded that these contaminant concentrations were not significant for the reproductive success of the Lapland population during that period (14). This is supported by the fact that, in the present study, the decrease in residue concentrations in the Lapland sample over the period 1977–1997 did not have an effect on the productivity of this population (Figs 1 and 2). The wide range in annual productivity in the Interior population is probably a result of the small population size, inexperience of pairs during first and second years of breeding (82) and of a higher rate of human disturbance at these nests as compared to the Baltic population (83).

Productivity increased significantly over time in the Baltic population in parallel with a significant reduction in DDE and PCB concentrations in the eggs (Table 4; Figs 2–4) and a significant increase in eggshell thickness, eggshell index and desiccation index (Table 5; Fig. 6). This could imply relationships between productivity and any one of these parameters. The strong interrelation between several of the parameters (Table 8) makes a more detailed analysis necessary.

### Influence of Residue Concentrations and Shell Parameters on Individual Productivity

#### Residue concentrations and productivity

Several studies have indicated that the major cause for depressed reproduction in the white-tailed sea eagle and bald eagle was contamination by DDE, but that an influence from PCB could not be excluded (for references see Introduction). In the current study there was no indication that the consistent failure to reproduce among the females of group B1 (Table 6) was directly linked to the residue concentrations in their eggs of any of the contaminants studied. In fact, the residue concentrations were quite similar and did not differ significantly between the eggs of the nonreproducing group B1 and the eggs of the normally reproducing group B2.  $TEQ_{PCB}$  concentration averaged about 10% lower in B2 compared to B1 (Fig. 5), but 20–30% higher in A2 (reproducing) compared to B1. Thus, there is no evidence here that coplanar PCBs at the concentrations found in the B groups have a significant effect on reproduction in this species. It should be noted, though, that sample sizes are small and the power of the tests to detect a difference is weak. In the regression analyses on individual productivity vs DDE and vs PCB for the period 1989–1997, when the DDE levels had decreased more than twice as much as the PCB levels compared to the situation in the 1960s and 1970s, a slightly stronger correlation was still found with DDE (Fig. 4). This further supports that the major reason for depressed productivity in the sea eagle during the study period has been DDE.

Productivity, eggshell thickness and eggshell index were all significantly correlated to DDE, while productivity was not correlated with eggshell thickness or eggshell index (Table 8). This lends support to a suggestion by Nisbet (21), based on investigations of bald eagle eggs, "that eggshell thinning may be a parallel symptom of DDE poisoning rather than a mechanism of reproductive impairment".

#### The desiccation syndrome: Remaining effects

In order to estimate expectable  $D_i$ -values for eggs with normally functioning shells, the following calculations can be made. According to Rahn et al. (65 species studied) (50), loss of water during incubation is independent of the metabolic rate of the embryo and typically leads to a reduction of about 15% of the initial egg mass (including the shell) during natural incubation, regardless of egg size. This corresponds to a reduction of about 16–17% in mass of the egg contents. Thus, a  $D_i$ -value of about 0.83 would be to consider as "normal" at the time of hatching in eggs with embryo. However, our study on undeveloped chicken eggs showed a desiccation rate that was only half of that reported by Rahn et al. (50) for embryo eggs. Assuming a total weight loss of undeveloped eggs of 7.6% over the incubation period also in the sea eagle, and an approximately steady rate of water loss in accordance with the results from the chicken eggs in this study, expected  $D_i$ -values beyond hatching time can be roughly estimated for undeveloped as well as embryo eggs. The weight of most of the eagle eggs in this study was determined approximately 100 days after they were laid (based on the median laying-dates according to Helander (3)). Over a 100-day period, the expected weight-loss of undeveloped sea eagle eggs with normal shell would amount to about 19%, corresponding to an expected  $D_i$ -value of about 0.81. Normal-shell eggs with embryo would, after 100 days, have expected  $D_i$ -values in the order 0.72–0.80, depending on whether the embryo died at full-time incubation (38 days according to (84, 85)) or as early as day 5. These are crude estimates but the best we can do on the basis of available data. Since the laying dates for collected clutches were usually not known, a correction of  $D_i$ -values for variation in sampling time was not feasible. A variation of 4 weeks in sampling time in relation to egg laying would correspond to a difference of about 5–6% in  $D_i$ .

The average measured  $D_i$ -values from eggs in the Lapland samples (undeveloped = 0.82, embryo eggs = 0.72) and in the Baltic and Interior sample of undeveloped eggs from 1988–1997 (= 0.82) are close to the estimates above and probably represent eggs with normally functioning shells. The constancy of productivity and  $D_i$ -values over time in Lapland (Figs 2 and 6) shows that the residue concentrations expe-



rienced by females in this population were generally below a threshold level for effects on this type of shell functionality. The lower  $D_i$ -values in embryo eggs from the Baltic 1988–1997 (mean value 0.59) indicates that desiccation was involved in the hatching failure of these eggs. In an experimental study on domestic chicken a dramatic drop in hatching success occurred, from 85% in control eggs which lost 12.5% in weight during incubation, to 20.5% hatching success in eggs that lost 25.1% in weight (86). The fact that hatching occurred in the clutches in group A2 (Table 6 and Fig. 5) is probably explained by the considerable within-clutch variation shown for  $D_i$  (see H/L-ratio in Table 3).

The data in Table 7 illustrate that although the residue concentrations of DDE and PCB were greatly reduced during the 1980s within 3 old females, their desiccation ( $D_i$ )-values remained abnormally low. The DDE and PCB concentrations in their latest clutches were within the range where other females reproduced quite well; still their productivity remained at zero. *This indicates a remaining effect from the previously higher contamination of those old females on their ability to produce functional shells and to produce young.* Such remaining effects probably account for the reproductive failure among the females of group B1 in Table 6. Two of those females were born in the 1960s, one before 1976 and one in 1979.

Excess desiccation is likely connected to changes in the eggshell structure and may be linked to the eggshell-thinning phenomenon. In a study on the peregrine falcon, water loss from the eggs increased with thinning (87). In this study, however, there was no correlation between  $D_i$  and  $S_i$ , or  $D_i$  and  $S_i$  (Table 8). Among the studied variables, productivity showed the strongest correlation with  $D_i$  (Table 8). Due to their high intercorrelation, the results from the multiple regression do not give a fair comparison between DDE and PCB, but DDE showed the strongest relationship with  $D_i$  (Table 9). The stronger correlation for productivity to  $D_i$  than to the residue concentrations measured in the eggs (Table 9) can be explained by the suggested remaining effect from a previous, higher contamination with DDE in some females in the sample. This is also supported by the data in Figure 6, where the increase in  $D_i$  over time was largely a result of the replacement of females in the population. Thus, such remaining effects will tend to confuse the relationships between productivity and measured concentrations of pollutants in the eggs.

The TCPMeOH/DDE ratio was significantly different between the groups in Table 6 with the highest mean ratio in the B1 group. The source of TCPMeOH in the environment is uncertain so far, but one likely origin might be as a metabolite of tris(4-chlorophenyl)methane (TCPMe), which has been found as a contaminant in DDT products (88). Falandyz et al. (89) indicated that TCPMeOH has a slower elimination time in the environment than DDE. If this holds true also in the eagles, it can be speculated that the higher TCPMeOH/DDE ratio found in the B1 group may indicate a previously higher exposure to DDT and would further support that these females probably had higher DDE concentrations earlier in life.

The occurrence of desiccated eggs has been reported also for sea eagles in Germany (90). Excess desiccation of the sea eagle eggs can be expected to have a strong negative effect on the hatching success of eggs with embryo (86). However, it cannot be concluded that desiccation alone was the main cause for the strong reduction in breeding success observed in the Baltic eagle population. Most sampled eggs showed no sign of embryo, indicating an effect from prezygotic exposure. It therefore seems that excess desiccation is also a symptom in parallel with other detrimental effects on the reproductive system, probably stemming from the same causative agent(s). The relatively low  $r^2$ -value of 0.31 in the multiple regression analysis (Table 9) may indicate that other factors not included in this investigation could be important to explain the variation in  $D_i$ .

Adult white-tailed sea eagle.



Full-grown white-tailed sea eagle nestling.



#### PCB and DDE in embryo eggs

The possible effects of PCB on the reproduction of raptors have been discussed by several authors (13, 29, 30, 91). Giesy et al. (30) and Bowerman et al. (29) claimed that depressed productivity of bald eagles today is more correlated to PCB than to DDE concentrations.

The close correlation of DDE and PCB concentrations in the sea eagle eggs has greatly reduced the possibilities to distinguish any effects of PCB. As the DDE concentrations in the eggs decreased and the desiccation problem diminished from the late 1980s, new possibilities have emerged. Unhatched eggs containing dead embryos have been found since the start of the nest surveys in the mid-1960s (14). Observations of mandible and toe deformations as well as oedema on a few embryos and nestling white-tailed sea eagles have also been reported (28). These are symptoms similar to those reported among colonial fish-eating birds on the Great Lakes in North America, sometimes referred to as the "Great Lakes embryo mortality, edema and deformities syndrome" (25). PCBs and PCDD/Fs have been shown to be likely causal agents for these symptoms (23, 24). However, high embryo mortality as well as deformations has also been reported for Foster's tern (*Sterna forsteri*) from Washington, USA, even though the concentrations of PCBs and PCDD/Fs were low (92). This might indicate causes for these symptoms that are so far unknown.

In eggs collected 1988–1997 in the present study, the concentrations of CB-138 and DDE, uncorrected for metabolism of lipids by the growing embryo, were both significantly higher in eggs with embryo compared to infertile/undeveloped eggs (Fig. 8). With correction for lipid metabolism in the embryo eggs, a significant difference was found only for CB-138. This difference in results illustrates the necessity of caution when comparing residue concentrations of lipophilic substances, expressed on a lipid basis, in egg samples. Infertile/undeveloped eggs occur naturally in any bird population. The residue concentrations found in undeveloped eggs can therefore be assumed to represent the range in concentrations within the population. The significantly higher concentrations of CB-138 on a corrected lipid basis in the embryo eggs as compared to the undeveloped eggs (Fig. 8) thus might indicate embryolethal concentrations of PCB in the embryo egg sample.

Seven of the 9 embryo eggs in this sample, including 3 eggs with

the lowest CB-138 concentration, were retrieved from nests which also contained a nestling. This should exclude human disturbance as the cause for hatching failure. Four eggs contained 40, 70, 70 and 70  $\mu\text{g g}^{-1}$ , respectively, four eggs contained 90–110, and one egg contained 140  $\mu\text{g g}^{-1}$ . The mean value for the lower segment of this sample is 63  $\mu\text{g g}^{-1}$  (sd = 15). This concentration of CB-138 (lipid weight basis) is equivalent to approximately 500  $\mu\text{g g}^{-1}$  tot-PCB, or 160 ng  $\text{g}^{-1}$  CB-126 (8 ng  $\text{g}^{-1}$  fresh weight (f.w.)) (Table 10). This CB-126 concentration corresponds to 190  $\text{pg g}^{-1}$  (f.w.) TEQ (49). To estimate a total TEQ also the contribution of other PCBs and PCDD/Fs must be included. Calculations on white-tailed sea eagle egg data from the Baltic Sea gave a 60% contribution of CB-126 to the total TEQ, including PCDD/Fs (31), indicating a LOEL for embryo mortality of approximately 320  $\text{pg g}^{-1}$  f.w. TEQ.

In a study on bald eagle chicks from the Pacific coast of Canada a LOEL for enzyme induction of 210  $\text{pg TEQ g}^{-1}$  (f.w.) has been reported (48). This TEQ was estimated using TEF according to Ahlborg et al. (70). Recalculated using TEFs according to Tillitt et al. (49), this equals a LOEL of about 130  $\text{pg TEQ g}^{-1}$  (f.w.). Other authors (29, 31) have postulated a no-observable-effect-level (NOEL) for bald eagle and white-tailed sea eagle that is about 20 times lower (7  $\text{pg g}^{-1}$  TEQ), and about 45 times lower than our suggested LOEL for embryo mortality. Most of this discrepancy is made up by a safety factor of 10x, applied in the calculations of the NOEL from reported LOEL data (29). Thus, the LOEL for embryo mortality implied in this study is in the order 2.5–4.5 times higher than the LOELs reported previously for enzyme induction (29, 48).

### Effect levels

As demonstrated for other species, e.g. the bald eagle, it is obvious from the present study that the residue concentrations at which effects start to occur in the white-tailed sea eagle are different for different parameters, as well as for DDE and PCB. Eggshell thickness ( $S_i$ ) was significantly correlated to DDE but not to PCB (Table 8). The eggs in this study represent a wide range in  $S_i$ -values over a wide range of residue concentrations. As illustrated in Figure 7, a reduction in  $S_i$  is obvious as DDE levels exceed 50  $\mu\text{g g}^{-1}$  and a LOEL in the range 30–50  $\mu\text{g g}^{-1}$  (1.5–2.5 f.w.) is implied. On average, a 10% reduction in  $S_i$  was associated with a DDE level of 175  $\mu\text{g g}^{-1}$  in the Baltic sample but of only 75  $\mu\text{g g}^{-1}$  in the Lapland sample, implying that most of the effect on  $S_i$  occurs above a threshold level; a reduction in  $S_i$  by 18% was associated with a DDE level of 720  $\mu\text{g g}^{-1}$  (Baltic sample). It should be noted that in two clutches with high DDE concentrations (900 and 1600  $\mu\text{g g}^{-1}$ ) we found extremely thick shells (0.87–0.92, Fig. 7), and also extremely high shell index values (4.07 and 4.18). These  $S_i$ -values are about 20% above the upper range in the sample from 1856–1915 (Table 4) and may indicate a reverse effect of DDE in some cases. Although  $S_i$  was not correlated to productivity, eggshell thickness is nevertheless a useful “early warning” sign to be used as a tool in environmental monitoring, since it occurs already at DDE concentrations substantially lower than those where reproductive impairments start to show up.

For  $D_i$ , a strong reduction occurred among eggs from the Baltic as residue levels exceeded 170  $\mu\text{g g}^{-1}$  DDE and no normal  $D_i$ -values were found as the DDE levels exceeded 400  $\mu\text{g g}^{-1}$  (Fig. 7). In the Lapland sample 1977–1984, there was an average 10% reduction in  $S_i$  (Table 5), but still no significant change in  $D_i$ ; the DDE-levels in this sample were generally below 100  $\mu\text{g g}^{-1}$  (Table 4). These observations support that there is a higher DDE threshold level for the kind of eggshell changes causing desiccation than the threshold level for eggshell thinning. Based on the results from the multiple regression (Table 9), and the inversely corresponding temporal changes of  $D_i$  and DDE (Figs 1 and 6), we conclude that egg desiccation was most probably affected by DDE.

Productivity remained approximately normal until concentrations of DDE in individual eggs exceeded 100  $\mu\text{g g}^{-1}$  (Figs 3 and 4), dropped to on average 0.7 at 210  $\mu\text{g g}^{-1}$  (the point of inflection of the S-curve, Fig. 3) and down to zero when concentrations exceeded 900  $\mu\text{g g}^{-1}$ . From Figure 4B, an obvious reduction in productivity is indicated at DDE levels exceeding 120  $\mu\text{g g}^{-1}$ , indicating a LOEL in the range 100–120  $\mu\text{g g}^{-1}$ . This suggested LOEL is much lower than the “critical level” of about 500  $\mu\text{g g}^{-1}$ , suggested previously (14). That estimate was based on the levels in eggs collected from productive nests, but during a period when the entire Baltic eagle population suffered from depressed reproductive ability. Thus, the suggested critical level of 500  $\mu\text{g g}^{-1}$  for DDE referred more to a level for population decline than to a LOEL

for depressed reproduction. In Figure 4, the first effects on productivity from PCB appear to show up at concentrations of about 300  $\mu\text{g g}^{-1}$ , but this may be a result of covariation with DDE. The strong correlation between DDE and PCB make further interpretations of effect levels from the PCB curve (Fig. 3) unattainable.

An average productivity of 1.1 in the Baltic population 1995–1997, and a fledging ratio (13) of 1.5, was associated with mean DDE and PCB levels of 110 and 390  $\mu\text{g g}^{-1}$ , respectively, in the sampled eggs (Table 4). In sea eagle eggs from Norway, PCB levels in the range 160–400  $\mu\text{g g}^{-1}$  and a decrease in mean DDE levels during 1974–1994 from about 100 to 50  $\mu\text{g g}^{-1}$  was reported (lipid weight concentrations, recalculated from fresh weight  $\times 20$ ) (62). No discernable effect on productivity was observed during the period. This seems to agree with the LOEL-values estimated in this study. For the bald eagle, Nisbet (21) reported a steep dose-response function with most of the effect occurring in the DDE-range 2.5–5  $\mu\text{g g}^{-1}$  on a fresh weight (f.w.) basis, corresponding to about 50–100  $\mu\text{g g}^{-1}$  lipid weight. Wiemeyer et al. (22) reported a mean DDE concentration of 5.7  $\mu\text{g g}^{-1}$  (f.w.) in bald eagle eggs from unsuccessful nests, as opposed to 3.2  $\mu\text{g g}^{-1}$  in eggs from successful nests. Nisbet and Risebrough (93) reported that productivity in the bald eagle was not affected by DDE concentrations below 9–12  $\mu\text{g g}^{-1}$  (f.w.) in the eggs. DDE concentrations in bald eagle eggs exceeding 15  $\mu\text{g g}^{-1}$  (f.w.) (20) or 20  $\mu\text{g g}^{-1}$  (93) was associated with almost complete breeding failure. DDE concentrations over 30  $\mu\text{g g}^{-1}$  (f.w.) still allowed for reproduction in the sea eagle, in which complete breeding failure was associated with DDE concentrations exceeding 45  $\mu\text{g g}^{-1}$  (f.w.) ((4), this study). This indicates a considerable difference in sensitivity to DDE between bald eagles and white-tailed sea eagles, unless some other agent contributed to the low productivity in the bald eagle as suggested by Nisbet and Risebrough (93). A lower sensitivity to DDE in bald eagles than predicted from previous data was suggested by Jenkins et al. (94).

A range in individual sensitivity to DDE and PCB is indicated in the present study. During the study period, DDE in the eggs ranged up to 12 times higher than the indicated LOEL and PCB almost 6 times higher. Among 20 clutches from Baltic and Interior females with productivity in the range 1.0–1.75, 12 had DDE and PCB levels exceeding the suggested LOELs. Four clutches had DDE concentrations as high as 340–630  $\mu\text{g g}^{-1}$  and 2 had PCB concentrations of 1200 and 1400. If these eggs were representative for the clutch (Table 3), approximately normal productivity still occurred in some females at concentrations 3–5 times higher than the indicated LOEL for DDE. In experimental studies, dose response curves for CB-126 (LD50) in chicken and cormorant eggs showed differences of approximately one order of magnitude in sensitivity between individual eggs, i.e., a factor of 10 between the concentration where the first embryo dies up to 100% embryo mortality (71,95). With LOELs of about 120  $\mu\text{g g}^{-1}$  for DDE and 500  $\mu\text{g g}^{-1}$  tot-PCB for embryo mortality in the white-tailed sea eagle as suggested in this study, one would expect females with a wide range of higher concentrations that still manage to reproduce. Thus, the occurrence of DDE and tot-PCB concentrations as high as 600 and 1400  $\mu\text{g g}^{-1}$ , respectively, in eggs from white-tailed sea eagle pairs with approximately normal reproduction is not in contradiction to the postulated LOEL in this study. These observations illustrate that the acceptance of biological variation is of utmost importance in risk assessments on a population basis.

## IN CONCLUSION

Highly significant correlations were found between individual productivity and DDE during the entire study period (Fig. 4), with an indicated LOEL for depressed productivity at about 120  $\mu\text{g g}^{-1}$  (Fig. 4). There was also a significant correlation between individual productivity and PCB (Fig. 4), and a LOEL for tot-PCB at about 500  $\mu\text{g g}^{-1}$  is indicated for embryo mortality. Although slight changes occurred in the PCB composition over the study period, with a shift towards higher chlorinated congeners (Table 10), the PCB-derived TEQ has remained closely correlated to tot-PCB. The main reason for this is that the relative contribution from CB-126 remained constant.

There was no evidence in this study that the persisting reproductive failure of some females, with comparably low DDE levels in their eggs, could be attributed to the effects of coplanar PCBs (Table 6); instead, a correlation with egg desiccation was indicated (Fig. 5). Among all investigated parameters, productivity was most strongly correlated to the degree of desiccation ( $D_i$ ) of the sampled eggs, and  $D_i$  was most



strongly correlated with DDE (Tables 8 and 9; Figs 1 and 6). The relationship between egg desiccation and different organochlorines should be investigated retrospectively including also other species (e.g. the bald eagle).

Eggshell thickness and eggshell index were significantly correlated with DDE, but not with Di or with productivity. Thus, the result here supports the suggestion by Nisbet (21) that eggshell thinning may be a parallel symptom of DDE poisoning rather than a mechanism of reproductive failure.

A dramatic improvement in reproduction occurred in the white-tailed sea eagle population on the Baltic Sea coast during the investigated pe-

riod. This is most likely a result of the pronounced decrease of both DDE and PCB in the environment. The fact that abnormally high desiccation of the eggs and an inability to reproduce persisted in some old females, studied over a period when the organohalogen concentrations in their eggs decreased below the concentrations where other females reproduced normally (Table 7), indicates remaining effects from their previously high exposure to contaminants. The inability to reproduce included a high rate of undeveloped eggs, indicating effects at a prezygotic stage.

The stronger relationship for productivity with DDE than with PCB, and the strong correlation between productivity and D<sub>1</sub> and between D<sub>1</sub>

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and DDE, leads to the conclusion that the major reason for depressed productivity in the sea eagle during the study period has been DDE. However, the suggested LOEL for embryo mortality of 500 µg g<sup>-1</sup> tot-PCB is below the concentrations generally found in the Baltic population before the 1990s. This indicates that PCB also had an effect on reproduction during the study period. A higher residue concentration / LOEL ratio for DDE than for PCB throughout the 1970s explains that a PCB effect was largely concealed by DDE.

Despite some indicated effects of both PCB and DDE still today, the productivity of the white-tailed sea eagles on Baltic Sea coasts is almost back to normal and the population is clearly on the increase. Thus,

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