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Dirksen, S.; Boudewijn, T.J.; Slager, L.K.; Mes, R.G.; van Schaick, M.J.M.; de Voogt, P.

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REDUCED BREEDING SUCCESS OF CORMORANTS (*Phalacrocorax carbo sinensis*) IN RELATION TO PERSISTENT ORGANOCHLORINE POLLUTION OF AQUATIC HABITATS IN THE NETHERLANDS

S. Dirksen,* T. J. Boudewijn,* L. K. Slager,‡ R. G. Mes§

Bureau Ecoland, Hoofdstraat 178, 2351 AR Leiderdorp, The Netherlands

M. J. M. van Schaick¶ & P. de Voogt‖

Institute for Environmental Studies, Free University, De Boelelaan 1115, 1081 HV Amsterdam, The Netherlands

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Abstract

*Cormorants (*Phalacrocorax carbo sinensis*) breeding in the heavily contaminated sedimentation area of the rivers Rhine and Meuse have a severely reduced breeding success as compared to several other Dutch colonies. A detailed analysis of reproductive performance in combination with chemical analysis of eggs and food from colonies in differently contaminated aquatic habitats is presented. The differences in breeding success between colonies are caused mainly in the egg-stage of breeding. Eggshell thinning and increased embryonic mortality cause the differences in hatching success. The observed effects seem to be related to chlorinated hydrocarbons. Significant correlations are found for concentrations of DDE with eggshell thinning and for concentrations of PCBs with hatching and breeding success. The correlations between concentrations of chlorinated hydrocarbons in eggs and biological effects measured in the field are established both on colony and individual clutch level.*

INTRODUCTION

Throughout the last decades, the West European rivers Rhine and Meuse have been carrying large loads of pollutants downstream. A large proportion has been deposited in the southwestern part of The Netherlands, where both rivers flow together into a delta. Especially in the northern part of this delta the concentrations of chlorinated hydrocarbons and heavy metals in the sediment exceed Dutch quality standards over a large surface. The pollution in this area, including Biesbosch, Hollandsch Diep, Haringvliet and several riverbranches is considered a major environmental problem (Bijlsma & Kuijpers, 1989).

Chlorinated hydrocarbons have been associated with a number of negative effects on organisms of terrestrial as well as aquatic ecosystems. In aquatic ecosystems especially, fish eating birds and mammals have been the conspicuous victims of contamination with DDT (and related compounds like DDE), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Studies on gulls, terns, cormorants and raptors have linked (some of) these contaminants with reproductive failure through eggshell thinning and subsequent breaking of eggs (Ratcliffe, 1967, 1970; Anderson *et al.*, 1969; Anderson & Hickey, 1972; Koeman *et al.*, 1972; Cooke, 1979; Pearce *et al.*, 1979; Moriarty *et al.*, 1986; Lundholm, 1987), mortality of embryos and little chicks (Fox, 1976; Morris *et al.*, 1976; Vermeer & Peakall, 1977; Gilbertson, 1983; Kubiak *et al.*, 1989), and morphological aberrations in chicks (Hays & Risebrough, 1972; Gochfeld, 1975; Gilbertson *et al.*, 1976). The combination of the latter two types of effects has been reviewed extensively and named Great Lakes Embryo Mortality, Edema and Deformities Syndrome (GLEMEDS) (Gilbertson *et al.*, 1991).

The only fish-eating bird species for which some data are available on possible effects of contaminants in the Rhine ecosystem is the cormorant (*Phalacrocorax carbo sinensis*). Already in 1961 the cormorant had disappeared as a breeding bird along the Dutch branches of the Rhine and Meuse, including the Biesbosch area.

This paper is dedicated to the memory of Geert van Urk.

Present addresses:
*Bureau Waardenburg bv, P.O. Box 365, 4100 AJ Culemborg, The Netherlands.
‡Institute for Environmental Studies, Free University, de Boelelaan 1115, 1081 HV Amsterdam, The Netherlands.
§Provincie Zuid-Holland, P.O. Box 90602, 2509 LP's Gravenhage, The Netherlands.
¶Gemeente Weesp, Nieuwstraat 41, 1381 CS Weesp, The Netherlands.
‖Department of Environmental and Toxicological Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands.

Both human persecution and water pollution were supposed to be responsible (Coomans de Ruiter, 1966). In 1970 adults found dead in the Biesbosch contained levels of PCBs high enough to cause their death (Koeman *et al.*, 1972, 1973). From 1978 onwards several colonies (re-) settled in The Netherlands, amongst which was a colony in the Dordtse Biesbosch, in the centre of the sedimentation area of the rivers Rhine and Meuse (Rooth, 1985). In contrast to other new colonies which were rapidly increasing, numbers in the Dordtse Biesbosch were fluctuating and remained relatively low. Incidental visits to the colony lead to the suspicion of a low breeding success (D. Bruijsters & J. van der Neut, pers. comm.; Gebuis, 1987).

Initial research in 1987 in the Dordtse Biesbosch confirmed that breeding success was low. Furthermore, some of the phenomena observed strengthened the suspicion of a relation to contaminants. As a next step a comparative setup was chosen, in which possible correlations between levels of contaminants and ecological parameters were investigated. Seven colonies in areas with different degrees of contamination, within and without the direct reach of the rivers' sedimentation, were selected. In all these colonies ecological studies, including an analysis of breeding success at the level of individual clutches, were carried out. These were combined with the results of chemical analyses of eggs and regurgitated fish.

STUDY SITES

Seven colonies of cormorants (Fig. 1) were chosen according to the (assumed) relative degree of contamination of the surrounding aquatic habitat. Direct observations of feeding flights (not reported in detail here) were carried out to establish the feeding areas of the cormorants. All colonies were situated in willow or alder, standing in or near water.

The Dordtse Biesbosch colony (198 and 125 pairs in 1987 and 1988) is situated in a former freshwater tidal area. Since the closing of two major outlets to the sea with dams (with sluices) the tidal movement has been largely reduced. Because of the lower flow rates, large quantities of highly polluted sediment have been deposited since then. Thus, the feeding areas for this colony are situated in a polluted aquatic habitat.

Four colonies are situated upstream along branches of the Rhine: Haaften (59 pairs in 1988), Pannerden (195 pairs), Olst (70 pairs) and Wijhe (12 pairs). The cormorants from these colonies feed in the winter bed of the river, both in the main stream and in ditches in the forelands. These feeding sites are, generally speaking, less contaminated than those in the river delta.

The Oude Venen colony (480 pairs) is situated in a region of lakes in a former peat bog. The cormorants use the surrounding lakes and canals for feeding. These can be characterized as relatively clean compared to the Biesbosch area.

The Brede Water colony (1000 pairs) is located in a dune lake close to the North Sea coast. From there, cormorants feed on fish from salt water (North Sea, Lake Grevelingen) as well as on fish from several freshwater areas. The degree of contamination of these waters ranges from clean (Lake Grevelingen) to polluted (Haringvliet). The majority of birds forages in relatively clean areas.

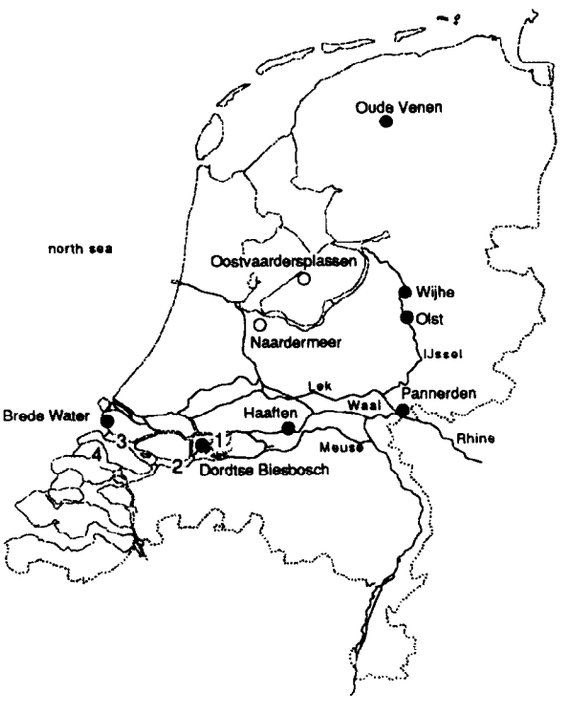

Fig. 1. Map of The Netherlands showing the geographical position of the colonies where cormorants were studied and some other areas mentioned in the text. Colonies: DB: Dordtse Biesbosch, in sedimentation area; H: Haaften, P: Pannerden, O: Olst and W: Wijhe, all along river-branches; OV: Oude Venen, at inland lakes; BW: Brede Water, mainly coastal. Other areas: 1: Biesbosch, 2: Hollandsch Diep, 3: Haringvliet, 4: Lake Grevelingen and 5: Naardermeer.

MATERIALS AND METHODS

Chemical analysis

After homogenising the egg as a whole, an aliquot of the homogenate of approx. 10 g was dried with sodium sulphate. Approximately 10 g of the dried material was transferred to a separatory funnel, containing a glass wool plug, and 5 ml of *n*-hexane was added. After standing for 1 h, the *n*-hexane was collected. Another 50 ml of *n*-hexane was added to the residue and eluted with a flow 2–3 $ml \cdot min^{-1}$. This was repeated a further four times. The total eluate of approx. 255 ml was transferred to a Kuderna–Danish evaporator and concentrated, an aliquot was used for determining the lipid content by evaporating until constant weight and the remainder was transferred to the clean-up column.

The clean-up of the *n*-hexane extracts was carried out according to the method described in detail by De Voogt *et al.* (1986), using a combined column containing deactivated (5% water) aluminium oxide and deactivated (0·5% water) silica.

For the determination of PCB congeners, columns were eluted with 30 ml iso-octane. For the analysis of chlorinated pesticides the columns were first eluted by 25 ml *n*-hexane; and this fraction was discarded. Next, 40 ml of a mixture of *n*-hexane/diethylether (9/1 v/v) were used to elute the chlorinated pesticides. Aliquots of the concentrated eluates were used for analysis after spiking with an internal standard.

Fish samples were filletted, homogenized, and chemically dried using Na_2SO_4. About 10 g of the dried material was Soxhlet extracted during 6 h using 150 ml *n*-pentane/dichloromethane (1 : 1, v/v, cf. De Boer, 1988). The extract was cleaned up using the methods described above.

Chlorobiphenyl (PCB) congeners 28, 52, 101, 138, 153 and 180, and *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD and dieldrin were determined quantitatively by capillary gas chromatography (GC) using splitless injection (2 μl) and an electron capture detector. The analytical column was a 50 m × 0·2 mm 5% phenyl methylsilicone gum coated fused silica capillary column (HP-5 Hewlett Packard, Waldbronn, Germany). Dichlorobenzyl heptylether (7-DCBE, cf. Wells *et al.*, 1988) was used as the internal standard.

GC-MSD (electron impact, selected ion monitoring) was used for confirmation of the presence of DDE isomers, since a small shift in relative retention times was observed in the sample extracts compared to those in the standards.

All reagents used were of analytical grade (solids) or HPLC grade (solvents, eluents) quality.

Ecological (field-)studies

In 1988 the colonies were visited once every fortnight, whenever possible within the same week, between 28 March and 21 July. Dordtse Biesbosch was visited almost every week in 1987, but not before 21 April. Each visit lasted 2–3 h at most, in order to limit disturbance. Normally, after entering the colony, birds with eggs or (small) young returned quickly to nests more than 30–50 m from the observers. This 'free circle' remained around the observers while going through the colony, so probably hardly any individuals that were incubating or brooding were kept off their nests for more than 2 h.

All trees with nests in a selected area were marked. The nests often were situated high up in the trees, up to 20 m in Olst, Wijhe and Dordtse Biesbosch. Ladders, climbing gear and a mirror mounted on a 8 m telescope pole were used to inspect the contents of the nests.

The number of eggs and young was determined during each inspection of a nest. Length and width of eggs within reach were measured to the nearest 0·1 mm with callipers. The eggs were marked on both ends.

In Olst regular inspection of a representative sample of the clutches was not possible. Therefore, only the presence of large young could be scored for most of the clutches. The data of intermediate stages (given between brackets in Table 5) were not included in statistical testing.

The area underneath the nests was searched for eggshells, which could often be related to known clutches by their numbers, dead young, pellets and regurgitated fish. Dead young were measured and weighed as described above, if still possible. All samples were put in plastic bags, marked with date, colony and tree number and stored at –18°C.

For each clutch laid a number of data was retrieved from the nest records. Assuming a laying interval of 2·5 days between two eggs and an incubation period of, at most, 26 days after the last egg was laid (Cramp & Simmons, 1977; own obs. TJB/SD), the laying date (and week) of the first egg was calculated. Clutch size, being the maximum number of different eggs recorded, and the number of eggs hatching were derived directly from the observations. The number of young still present at an age of 4–5 weeks was assumed to be the number of young fledging. This was necessary since young, although still unable to fly, start to leave the nests at an age of 5–6 weeks and spread out over the trees. It seemed justifiable as well, as the mortality of young between 5 weeks and the age of fledging (7–8 weeks) is almost negligible: very few were found dead in or near the colonies (see Table 6).

For measuring eggshell thickness, parts of the girth of the egg were taken, the shell membranes removed, gently washed and allowed to dry. Thickness was measured directly at nine points to the nearest 0·001 mm with a micrometer. Eggshell thickness of eggs in museum collections was measured including membranes, taking one measurement opposite the hole in the egg. Thickness of the membranes was determined by measuring eggshell thickness with and without membranes of a sample of eggshells.

Egg volume was calculated using Hoyt's (1979) formula:

$$\text{volume} = 0{\cdot}509 \times \text{length} \times (\text{width})^2$$

In each colony eggs were collected for chemical analysis. Due to the height of the nests and the quality of the trees, the sample remained small in some colonies. For Dordtse Biesbosch, eggs from 1987 had to be added to the three eggs that could be collected in 1988.

Some of the regurgitated fish were analysed for contaminants: pooled samples of eel (*Anguilla anguilla*) from six colonies and a pooled sample of cyprinids from Dordtse Biesbosch.

The pellets, which contained several undigested parts of fish, such as pharyngeal bones and otoliths, were used to analyse the diet of the cormorants in the colonies. Cormorants in all colonies mainly fed on cyprinids, of which roach (*Rutilus rutilus*) was the most important prey. Percids and eel were eaten in smaller amounts. A detailed analysis will be published elsewhere.

Statistics

Values below detection limits were taken to be 0 in calculating means. One-way Anova and Tukey's multiple range test were used to identify statistically significant differences in mean pesticide and PCB concentrations between locations. The homogeneity of the variances was tested. In case of non-homogeneity, a log transformation was applied. In the analysis of breeding biology, logarithmic transformations were used where data

were suspected not to be distributed normally. If the original variable could be zero, the logarithm of the variable +1 was taken. A square root transformation was used for the calculation of confidence limits to the numbers of young hatching and fledging, respectively, in successful nests (nests with at least one young hatching or fledging). One-way Anova and Duncan's multiple range test were used to identify statistically significant differences in breeding biology data between locations. All computations were done using SPSS/PC+ (Norusis, 1986). When a difference is referred to as 'statistically (not) significant' in the text without further details, the necessary information can be found in the legend of the relevant table or figure.

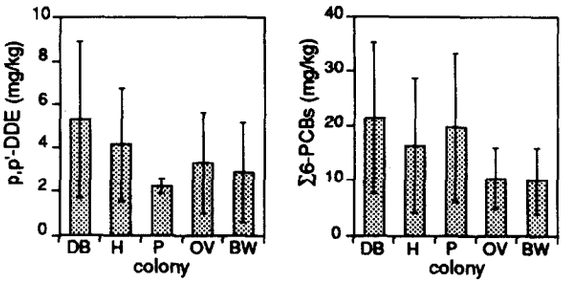

Fig. 2. Mean concentrations (mg kg^{-1} fresh weight, ±s.d.) of *p,p'*-DDE and the sum of the six PCBs analysed (28, 52, 101, 138, 153, 180) in eggs per colony. W (only 1 egg) is excluded from the figure. Abbreviations: see Fig. 1.

RESULTS

Chemical analysis of eggs and fish

Chlorinated pesticides in eggs

Table 1 presents the results of the analysis of chlorinated pesticides in Cormorant eggs. None of the samples analysed contained *o,p'*-DDE in concentrations above the limit of detection. Only for dieldrin a statistically significant lower mean concentration was found for the breeding colony in Oude Venen as compared to that in Dordtse Biesbosch ($p < 0.05$). For all other pesticides, there was no statistically significant difference in the mean concentrations between locations. Relatively large variations were found between eggs from the same colony. However, a general trend exists for all pesticides found to be present in the eggs, consistent with the finding for dieldrin. The lowest means are found in Oude Venen or Brede Water and the highest means in Dordtse Biesbosch. As an example, for *p,p'*-DDE, the pesticide present in the highest concentrations, the trend is illustrated in Fig. 2. The relative low mean of *p,p'*-DDE for Pannerden is an exception to the general trend.

PCB congeners in eggs

Table 2 presents the results of the analysis of six PCB congeners in the eggs, based on fresh weight, respectively. The highest concentrations of PCB congeners were invariably found in eggs from Dordtse Biesbosch. Figure 2 presents the mean concentrations and standard deviations per colony for the sum of the six congeners analysed. The means for PCB 52 and 101 were significantly ($p < 0.05$) higher in eggs from Dordtse Biesbosch as compared to eggs from Brede Water and Oude Venen. For PCB 28, the mean concentration found in eggs from Dordtse Biesbosch was significantly ($p < 0.05$) higher than in eggs from Brede Water. For the more highly chlorinated PCBs (no. 138, 153 and 180) no significant differences could be found. Large egg to egg variations within colonies were found for all PCBs.

Chlorinated pesticides and PCB congeners in fish

Table 3 presents the results of the analysis of pesticides and PCBs in regurgitated fish. Neither *o,p'*-DDE nor *o,p'*-DDT were found in concentrations above the limit of detection. The data confirm the assumed relative differences in contamination of the feeding grounds corresponding to the colonies, although eel from Haaften has higher concentrations than Dordtse Biesbosch, and eel from Olst has lower concentrations than the other colonies along the branches of the Rhine. In Fig. 3 the mean concentrations of the sum of all six PCBs and *p,p'*-DDE in fish and eggs are plotted against each other. The figure shows that the differences in contamination of the food are reflected in the eggs, even though eel only forms part of the cormorant's food.

Table 1. Chlorinated pesticides (mean ± standard deviation, $\mu g\ kg^{-1}$ wet weight) and lipid content in eggs of cormorants, 1987–88. All levels of *o,p'*-DDE were below the detection limit (8 $\mu g\ kg^{-1}$ wet weight)

Colony[a] and region[b]	Dieldrin mean ± s.d.	*p,p'*-DDE mean ± s.d.	*p,p'*-DDD mean ± s.d.	*o,p'*-DDT mean ± s.d.	*p,p'*-DDT mean ± s.d.	% lipid mean ± s.d.	*n*
DB s	480 ± 530	5300 ± 3600	33 ± 28	130 ± 120	470 ± 400	4·5 ± 0·9	9
H r	230 ± 220	4100 ± 2600	18 ± 22	48 ± 23	250 ± 110	3·7 ± 1·6	4
P r	77 ± 8	2200 ± 350	14 ± 12	71 ± 69	180 ± 140	3·8 ± 0·6	3
W r	970	8300	48	56	210	3·7	1
OV il	58 ± 150	3300 ± 2300	5 ± 9	42 ± 31	120 ± 120	3·5 ± 1·0	7
BW c	110 ± 71	2900 ± 2300	12 ± 13	45 ± 46	170 ± 120	3·8 ± 0·6	6
d.l.[c]	8	8	8	10	8		

[a]Colony names: DB, Dordtse Biesbosch; H, Haaften; P, Pannerden; O, Olst; W, Wijhe; OV, Oude Venen; BW, Brede Water. See also Fig. 1.
[b]Region: s, sedimentation area delta; r, upstream along river branches; il, inland lakes; c, coastal.
[c]d.l.: detection limit.

Table 2. PCB congeners (mean ± standard deviation, $\mu g\ kg^{-1}$ wet weight) in eggs of cormorants, 1987–88. For lipid contents and abbreviations: see Table 1

Colony and region	PCB28 mean ± s.d.	PCB52 mean ± s.d.	PCB101 mean ± s.d.	PCB138 mean ± s.d.	PCB153 mean ± s.d.	PCB180 mean ± s.d.	Σ6-PCBs mean ± s.d.
DB s	500 ± 400	450 ± 420	1200 ± 900	6600 ± 4200	8500 ± 5400	4100 ± 2600	21000 ± 14000
H r	190 ± 87	170 ± 72	640 ± 470	5700 ± 4500	6600 ± 5000	3100 ± 2200	16000 ± 12000
P r	300 ± 260	150 ± 67	560 ± 280	7100 ± 5100	8100 ± 5700	3500 ± 2600	20000 ± 14000
W r	290	140	540	6100	7200	3800	18000
OV il	240 ± 160	100 ± 120	300 ± 370	3400 ± 1700	4300 ± 2000	2100 ± 1400	10000 ± 5500
BW c	62 ± 86	51 ± 16	180 ± 160	3500 ± 2600	4400 ± 2300	1700 ± 900	10000 ± 5600

Table 3. Chlorinated pesticides (mg kg^{-1} lipid weight)[a], PCB congeners ($\mu g\ kg^{-1}$ lipid weight) and lipid content in fish, regurgitated by cormorants, 1988. Abbreviations: see Table 1

	p,p'-DDE	dieldrin	*p,p'*-DDD	*p,p'*-DDT	PCB congeners 28	52	101	138	153	180	% lipid
Eel											
DB s	0·9	0·5	0·8	0·3	73	560	1100	1900	2300	1200	20·6
H r	1·5	0·8	1·9	0·4	100	1100	1600	2500	2900	1500	23·8
P r	0·3	0·2	0·3	0·2	110	660	970	1900	1800	740	25·5
O r	0·4	0·2	0·3	0·2	15	130	230	640	570	280	22·5
OV il	0·3	0·4	<dl	<dl	130	240	150	310	380	420	21·1
BW c	0·8	1·2	1·1	0·2	44	70	140	420	460	150	21·2
Cyprinids											
DB s	2·5	0·9	1·8	0·6	780	2800	5700	5400	6200	2600	1·7
d.l.	0·10	0·10	0·10	0·10							

[a] *o,p'*-DDE (d.l. 0·10) and *o,p'*-DDT (d.l. 0·20): levels all below d.l.

Reproductive performance of cormorants: A comparison between colonies

Breeding success is the final result of a series of different stages a breeding bird has to pass. When measuring breeding success it is useful to gather data on these intermediate stages as well. This enables a more precise identification of the stage(s) from which differences in breeding success originate, and the factors that can be responsible. In this section, the data on all these breeding biology parameters will be presented chronologically, and be investigated for inter-colony differences. This comparison will focus on the 1988 data. For comparison, the Dordtse Biesbosch 1987 data are given in the tables as well.

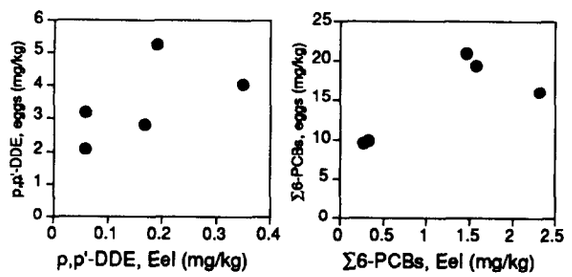

Fig. 3. Plots showing the relationship between (left) *p,p'*-DDE and (right) the sum of six PCBs analysed in eggs and in regurgitated fish from the same colony. Data taken from Tables 1–3, all expressed as mg kg^{-1} fresh weight.

Laying date and clutch size (Table 4)

Laying date for the first 25% of all clutches was day 89–93 in colonies Pannerden, Olst, Oude Venen and Brede Water. Colonies Wijhe, Dordtse Biesbosch and Haaften were 5–14 days later. Mean clutch size varied between 2·8 (Wijhe) and 3·6 (Pannerden) eggs. The relatively low value for Wijhe, a newly settled colony where egg laying was relatively late, is probably exceptional: in the nearby Olst colony clutch size was much higher. Excluding Wijhe, a significant difference was found only between Oude Venen and Dordtse Biesbosch, Dordtse Biesbosch having a smaller mean clutch size. These differences are not independent, because mean clutch size decreases with laying date (Fig. 4). Therefore, differences in clutch size seem to be related to differences in laying date.

Egg volume (Table 4)

Only a small number of eggs could be measured in some of the colonies. There were neither significant differences in mean egg volume per clutch between colonies nor in mean egg volume for all individual eggs measured (data not given here). Means per clutch ranged between 44·43 cm^3 (Dordtse Biesbosch) and 47·13 cm^3 (Oude Venen).

Eggshell thickness (Table 4)

The mean eggshell thickness of all egg samples measured is given here, instead of the mean per clutch. Only minor differences exist between these two values

Table 4. Comparison of breeding biology data: Egg-laying and eggs.
Abbreviations: (see Table 1), nd, not determined; cl, confidence limits. Data of the colonies investigated in 1988 were tested for inter-colony differences using Duncan's multiple range test (Norusis, 1986), except for laying date. Colonies sharing the same letter are significantly different ($p < 0.05$). Data between brackets (colony Olst) are given but not included in testing because of probable bias in sample (see Methods). Dordtse Biesbosch 1987 and 1988 were compared using the *t*-test; none of the between-year comparisons in this table were significantly different ($p > 0.05$)

Colony and region,	Year	Date 25% clutches started	Clutch size no. of eggs mean	±95% cl	*n*	Egg volume[a] cm^3 mean	±95% cl	*n*	Eggshell thickness[b] mm mean	±95% cl	*n*
DB s	1987	nd	3·1	±0·3	55	46·1	±2·6	9	0·324	±0·006	169
DB s	1988	100	3·3 A	±0·3	52	44·4	±7·3	4	0·324 AB	±0·010	44
H r	1988	106	3·2	±0·3	31	46·1	±7·3	4	0·326 CD	±0·011	31
P r	1988	89	3·7 B	±0·5	22	45·8	±13·4	3	0·337 E	±0·014	15
O r	1988	92	3·6	±0·4	18			—	(0·377	±0·025	8)
W r	1988	97	2·8 BC	±0·9	8	47·1		1	0·334	±0·163	2
OV il	1988	92	3·6 AC	±0·2	104	47·1	±1·9	19	0·360 ACE	±0·009	79
BW c	1988	93	3·3	±0·2	51	45·3	±1·5	8	0·356 BD	±0·008	29

[a]Mean of means per clutch, only data for eggs from identified clutches.
[b]Mean of all eggshell samples measured.

within colonies, but the sample size of the mean per clutch is much smaller in some of the colonies. Dordtse Biesbosch and Haaften have significantly thinner eggshells than Oude Venen and Brede Water, and Pannerden has significantly thinner eggshells than Oude Venen. No significant differences were observed within the two groups Dordtse Biesbosch, Haaften and Pannerden on the one hand, and Oude Venen and Brede Water on the other.

Incubation period
Due to the relative long intervals between visits in 1988 as compared to the incubation period, only a few direct measurements of the duration of incubation are available for Dordtse Biesbosch 1987. The mean incubation period (24·8 days, s.d. 2·71, $n = 6$) is in agreement with data from the literature (Cramp & Simmons, 1977).

Hatching success (Table 5)
The number of eggs hatching per clutch ranged from 1·1 (Dordtse Biesbosch) to 2·4 (Brede Water). In Dordtse Biesbosch significantly fewer eggs hatched than in Oude Venen and Brede Water, while the value for Haaften was significantly lower than that for Brede Water. The low figure found for Dordtse Biesbosch resulted from two causes. First, Dordtse Biesbosch had a lower percentage of clutches being successful (= having at least one egg hatching) than all other colonies. Secondly, the number of eggs hatching in a successful clutch was comparable in Dordtse Biesbosch, Haaften, Pannerden and Wijhe (1·7–2·0), but higher in Oude Venen and Brede Water (2·4–2·6).

Especially in Dordtse Biesbosch, a relatively large proportion of eggs not hatching disappeared from the clutch before completion of the incubation period (34%, $n = 121$ eggs not hatching, 1987).

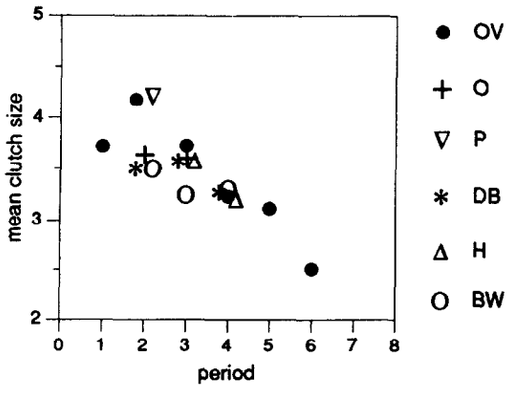

Fig. 4. Decrease in clutch size as a function of laying date (in periods of two weeks) during the breeding season, 1988. Period 1 = week 11 + 12, period 2 = week 13 + 14, etc. Only values based on 5 clutches or more are plotted. For abbreviations see Fig. 1.

Survival of young (Tables 5, 6)
Survival of young is expressed as the mean of the percentage of young hatched per clutch that reached fledging age. This survival was lowest in Dordtse Biesbosch (54%) and Pannerden (60%), while the other colonies had around 80% survival. Dordtse Biesbosch was significantly different from Haaften, Oude Venen and Brede Water, while Pannerden was significantly different from Oude Venen.

Young found dead in the colonies were aged using regression lines of structural size measurements against age, derived from young of known age (Boudewijn *et al.* in prep.). The fledging period was divided into three age classes, and the frequency of young found dead was calculated for each age class (Table 6). In the colonies Haaften, Pannerden and Dordtse Biesbosch a relatively high proportion of the young found dead were in their first two weeks of life. In Dordtse Biesbosch young died significantly younger than in Oude Venen (X^2, 0–14 days vs 15 days and older, 1 df, $p < 0.05$).

Breeding success (Table 5)
The data on breeding success (the number of young at fledging) are presented in the same way as those on hatching success. At fledging, similar but more pro-

Table 5. Comparison of breeding data: hatching and fledging. Abbreviations: see Table 1 and 4. Sample sizes given are for the foregoing column(s). Means of the number of eggs hatching, percentage of young surviving and young fledging per clutch of the seven colonies investigated in 1988 were tested for inter-colony differences using Duncan's multiple range test (Norusis, 1986), after logarithmic transformation. Colonies sharing the same letter are significantly different ($p < 0{\cdot}05$). Data between brackets (colony Olst) are given but not included in testing because of probable bias in sample (see Methods). Means of the number of eggs hatching, percentage of young surviving and young fledging per clutch in the Dordtse Biesbosh 1987 and 1988 were compared using the t-test after logarithmic transformation; none of the between-year comparisons was significantly different ($p > 0{\cdot}05$). Confidence limits for proportions of clutches being successful were derived from Table 1.2(a) in Neave (1978). Confidence limits for means per successful clutch were computed after square root transformation

Colony and region	Year	Hatching eggs						Survival young		
		Eggs hatching/clutch mean		% clutches successful mean	(95% cl)	*n*	Eggs hatching/ successful clutch mean	(95% cl)	% young/ successful clutch mean	*n*
DB s	1987	0·7		42	(28–56)	55	1·7	(1·3–2·0)	69	23
DB s	1988	1·1	AB	55	(41–68)	53	2·0	(1·7–2·3)	54 ABC	29
H r	1988	1·6	C	77	(58–90)	31	2·0	(1·6–2·3)	81 A	24
P r	1988	1·6		90	(68–99)	20	1·7	(1·3–2·0)	60 D	18
O r	1988	(2·9		88	(48–100)	8	3·3	(2·4–4·2)	71	7)
W r	1988	1·4		75	(35–97)	8	1·8	(1·1–2·6)	83	6
OV il	1988	2·1	A	80	(71–87)	106	2·7	(2·4–2·8)	82 BD	85
BW c	1988	2·4	BC	98	(89–100)	44	2·4	(2·0–2·6)	82 C	43

Colony and region	Year	Breeding success						
		Young fledging/ clutch mean		% clutches successful mean	(95% cl)	*n*	Young fledging/ successful clutch mean	(95% cl)	*n*
DB s	1987	0·5		31	(19–45)	55	1·7	(1·3–2·0)	17
DB s	1988	0·6	ABCDE	34	(23–47)	64	1·6	(1·3–1·8)	22
H r	1988	1·3	AF	66	(47–82)	32	2·0	(1·5–2·3)	21
P r	1988	1·2	B	65	(39–86)	17	1·8	(1·3–2·3)	11
O r	1988	1·7	C	72	(59–83)	61	2·4	(2·0–2·6)	44
W r	1988	1·3		63	(25–92)	8	2·0	(1·2–3·0)	5
OV il	1988	1·7	D	74	(64–82)	106	2·4	(2·1–2·5)	78
BW c	1988	2·2	EF	84	(60–97)	19	2·6	(1·9–3·1)	16

nounced differences existed between colonies as compared to hatching. Colony Dordtse Biesbosch had a 2–4 fold lower breeding success than the other colonies. All differences between Dordtse Biesbosch and other colonies, except for Wijhe, are statistically significant. Wijhe, Haaften and Pannerden had an intermediate breeding success, whilst Olst, Oude Venen and Brede Water had the highest figures. Of these differences only the one between Haaften and Brede Water is statistically significant.

Table 6. Age of young found dead

Colony	Age of young (days) found dead (%) 1–14	15–28	29–42	*n*
Dordtse Biesbosch	82	18	—	11
Haaften	75	25	—	4
Pannerden	60	40	—	5
Oude Venen	31	54	15	13
Brede Water	50	50	—	4

Table 7. Breeding success of cormorants in several colonies in The Netherlands, 1930–1987

Site	Year(s)	Breeding success	Reference
Several colonies	1930s	1·5–1·8	Kortlandt, 1942
Wanneperveen	1938–40	ca. 2·5	Van Dobben, 1952
Naardermeer	1971	1·9	De Boer, 1972
Idem	1982	1·7	Voslamber, 1988
Idem	1984	2·2	Denneman & De Vries, 1985
Oostvaardersplassen	1982	2·7	Voslamber, 1988
Idem	1983	1·0[a]	Voslamber, 1988
Brede Water	1984–87	>2–2·6	Lok & Bakker, 1988
Several colonies	1987–88	0·5–2·2	this study

[a]Due to weather conditions.

In Table 7 the data available from other studies on breeding success of cormorants are summarized. Excluding the minimum of 1·0, since it was caused by severe weather conditions in the period of feeding young (Voslamber, 1988), a range from 1·5 to 2·6 young fledging per clutch is found. This is a confirmation that the breeding success in the present study, especially in Dordtse Biesbosch but also in the colonies Haaften, Pannerden and Wijhe, is relatively low.

Summary of differences

From the data presented above, it is clear that large differences exist in breeding performance between the seven colonies of cormorants studied. The low breeding success in Dordtse Biesbosch (and to a lesser extent in the colonies Haaften, Pannerden and Wijhe) is the cumulative consequence of:

- — a later onset of egg laying and a related decrease in clutch size;
- — a reduction of eggshell thickness, probably to a level low enough to cause breakage of eggs (see below);
- — a high embryonic mortality, resulting in a reduced hatching percentage;
- — a high mortality of young, especially in their first weeks.

It can be concluded that the main differences originate in the egg stage of the breeding process. The similarity within Dordtse Biesbosch between the years 1987 and 1988 indicates that the low breeding success in this colony was not an incident, and was caused in the same stage of breeding in both years.

Correlations of chlorinated hydrocarbons and ecological parameters

Figure 5 shows the significant correlation found between the concentration of *p,p'*-DDE and eggshell thickness in individual eggs.

For the colonies from which data are available, a negative correlation between PCB concentrations in the egg and hatching success can be shown (Fig. 6). A direct correlation of PCB concentration and hatching success of the egg involved cannot be made: the egg was taken away from the nest, so its biological fate is unknown. However, if the egg is accepted to be representative for the clutch from which it originates, the PCB concentrations can be compared with the reproductive performance of the remaining clutch. In Fig. 7 the number of eggs hatching in the remaining clutch is plotted against the concentration of PCB-101, PCB-180 and Σ6-PCBs, respectively, for eggs from all the colonies. The explained variance was highest when the relationship between the number of eggs hatching and the concentration of a PCB was expressed as a log–log relationship. For all six congeners, as well as for the sum of these, the correlation is statistically significant (all correlations given in legend to Fig. 7).

DISCUSSION

PCB levels in cormorant eggs: A comparison with literature data

Literature data on the levels of PCBs in eggs of several cormorant species have been summarized in Table 8. It is surprising that still so little information is available for individual PCBs. Hence, a comparison of concentrations of individual congeners is impossible. Therefore, data had to be taken from papers reporting the total PCB content on the basis of packed column chromatography as well as high resolution capillary chromato-

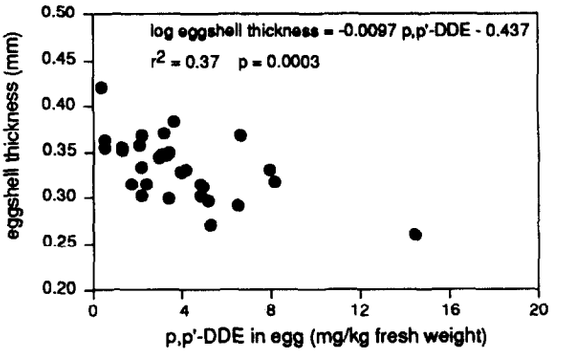

Fig. 5. Relationship between eggshell thickness (mm) and concentration of *p,p'*-DDE in the egg (mg kg^{-1} fresh weight). The line given is: log eggshell thickness = –0·0097 *p,p'*-DDE –0·437, r^2 = 0·37, p = 0·0003.

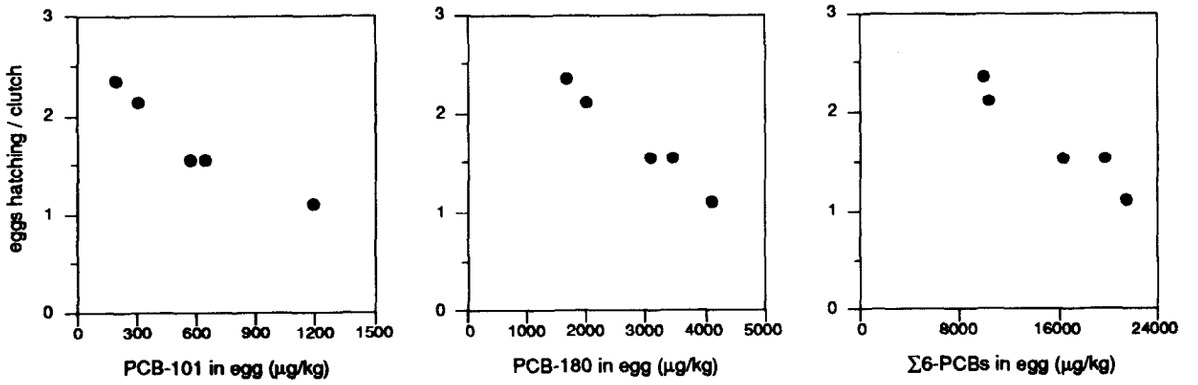

Fig. 6. Relationship of the mean concentration of PCB-101, PCB-180 and Σ6-PCBs respectively in eggs (µg kg^{-1} fresh weight) per colony and the mean number of eggs hatching in the same colony.

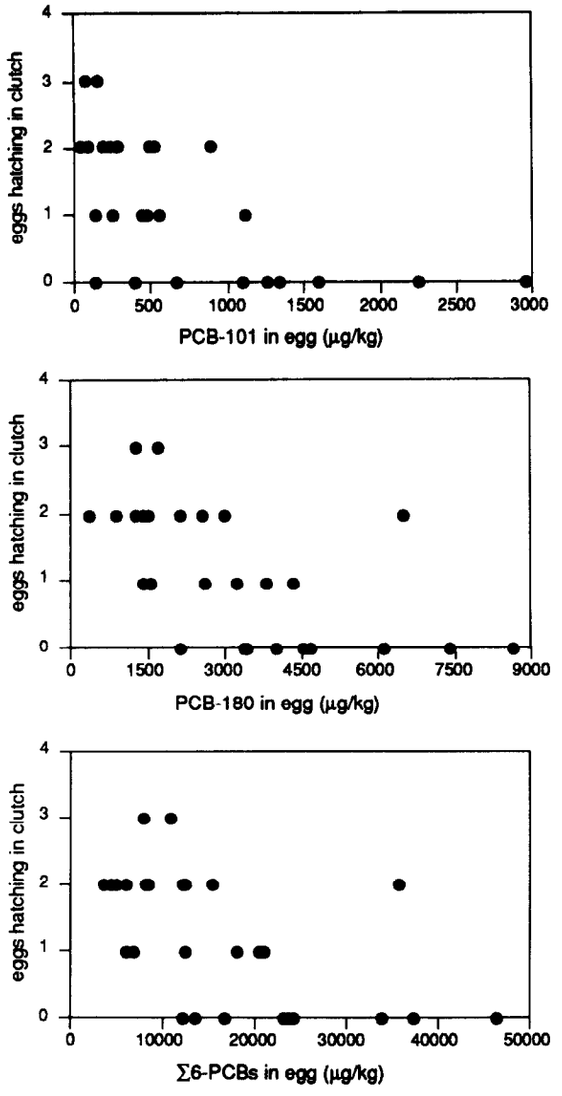

Fig. 7. Correlations of the concentration in the egg ($\mu g\ kg^{-1}$ fresh weight) of PCB-101 PCB-180 and Σ6-PCBs respectively and the number of eggs hatching in the remaining clutch. Eggs from all colonies were used in these analyses. Correlation formulae of the type: log (eggs hatching + 1) = *a* log (PCB) + *b* were calculated for all PCBs analysed:

	a	*b*	r^2	*p*
PCB-28	0·224	0·789	0·37	0·0007
PCB-52	0·251	0·807	0·34	0·0015
PCB-101	0·289	1·030	0·42	0·0003
PCB-138	0·402	1·742	0·33	0·0019
PCB-153	0·465	2·017	0·37	0·0008
PCB-180	0·440	1·773	0·41	0·0003
Σ6-PCBs	0·452	2·143	0·39	0·0005

graphy, and using different quantification methods (pattern comparison using commercial mixture standards, as well as individual congener analysis and subsequent summation). For the studies reporting individual PCBs, an artifice had to be applied to arrive at total PCB concentrations. Accordingly, for the data from the present study, the sum of the concentrations of the six PCBs was multiplied by 2, assuming that the six PCBs accounted for approximately 50% of the total PCB content. This assumption was based on the relative contribution of the six selected congeners to the total response in the gas chromatograms. Similarly, for the results of Focardi *et al.* (1988) it was assumed that summation of the available contents of 20–25 PCBs accounted for approximately 100% of the total PCB content of the eggs. The results of Vermeer and Peakall (1977) were expressed on lipid weight basis. To calculate the fresh weight concentrations, a lipid percentage of 4% was assumed, corresponding to the mean percentage found in the present study (Table 1). The possible error that can be introduced by the above assumptions can probably be neglected compared to the errors which are known to occur when packed column and/or pattern recognition technology is used for the quantitation of PCB levels (see, e.g. De Voogt *et al.*, 1984). The highest concentrations mentioned in Table 8 largely originate from the period before 1980. The analytical methods used in that period generally lead to an overestimation of the real PCB levels due to inclusion of false-positive peaks in the procedure applied. All cormorants are fish eaters, and differences in food composition within species may be almost as great as differences between species. A broad comparison between species can therefore be done without taking detailed information on food choice into account; a more detailed interpretation would only be possible including such data.

The data in Table 8 show that there is a general decrease in the period 1970–1989 of levels in both *P. carbo* and *auritus* in Western Europe and North America, respectively. Nevertheless, the total PCB levels in eggs of *P. carbo* found in this study belong to the highest ever recorded for all the cormorant species.

What determines differences in reproductive performance?

Chlorinated hydrocarbons are well known to be related to reproductive impairment in fish eating birds (see Introduction). As the effects described above seem to be very similar to those presented in the literature, this possibility will be discussed first. Thereafter, other possibilities will be examined as well.

The main differences in breeding success between the colonies were determined in the egg stage. From the literature, several pathways leading to a reduced hatchability of eggs by chlorinated hydrocarbons can be distinguished. First, through effects on the eggshell gland of the female, eggshell thinning severe enough to cause breakage of the egg before hatching can occur. Second, chlorinated hydrocarbons accumulated by the female are transferred to the egg. There they can cause direct toxic effects and subsequent embryo or chick mortality. Furthermore, the incubation behaviour of the parents can be affected, thus decreasing the eggs' chances of hatching.

Eggshell thinning

Since Ratcliffe (1967, 1970) first related eggshell thinning to insecticides, especially to DDT and its metabolites, many authors have contributed to the knowledge of this phenomenon. It is generally accepted that

Table 8. Mean levels of total PCBs ($\mu g\ g^{-1}$ fresh weight) in eggs of several cormorant species

Country[a]	Year	Area	Reference	Phalacrocorax species				
				carbo	*aristotelis*	*auritus*	*pelagicus*	*olivaceus*
NL	1971	Naardermeer	[1]	71·1				
	1988	Dordtse Biesbosch	This study	43·0[b]				
	1988	Brede Water	This study	19·8[b]				
Ire	1982	SW Ireland	[2]	0·5	1·2			
	1982	SE Ireland	[2]	2·1	0·6			
I	198?	Thyrrenian coast	[3]	24·0[c]				
Rou	1982	Danube delta	[4]	13–24				
SA	1981–83	St· Croiz Islands	[5]	0·9				
Can	1972	Manawagonish Island	[6]			20·9		
	1972	Iles aux Pommes	[6]			29·5		
	197?	Great Lakes	[7]			17[d]		
	197?	Gulf of St. Lawrence	[7]			13[d]		
	197?	Prairie provinces	[7]			4[d]		
	197?	British Columbia	[7]				3[d]	
	1970–73	Georgian Bay	[8]			5·2–41·8[e]		
	1972–89	Lake Ontario	[8]			20·1–37·9[f]		
	1972–89	Lake Erie	[8]			22·9–45·5[f]		
	1984	Manawagonish Island	[6]			3·5		
	1984	Iles aux Pommes	[6]			11·5		
	1983–88	St. Lawrence estuary	[9]			6·0		
	1983–88	Strait of Georgia	[9]			3·8	1·9	
	1984–85	Alberta (11 colonies)	[10]			1·2–5·6		
	1988	Manitoba (L. Winnipeg)	[11]			0·8		
USA	1977–80	Green Bay, Lake Mich.	[12]			2·0–16·5		
	1980	Galveston Bay, Tx	[13]					3·6
	1981	Galveston Bay, Tx	[13]					5·0
	1986	Lake Superior/Huron/ Michigan	[11]			0·1–14·8		
	1987	Lake Superior/Huron/Michigan	[11]			4·4–12·3		
	1988	Lake Ontario/Michigan	[11]			5·3–5·5		
	1988	Lake Superior/Huron/ Michigan	[14]			3·6–7·3		

[a]NL: Netherlands, Ire: Ireland, I: Italy, Rou: Roumania, SA: South Africa, Can: Canada.
[b]Sum of six congeners, multiplied by 2 (see text for explanation).
[c]Authors indicate species name as: Shag (*P. carbo*), but Shag is *P. aristotelis*. The latin name is taken as the proper one here, although this is uncertain because of occurrence of the species. Total PCB is sum of 20–25 individual PCBs.
[d]Assuming a mean lipid weight of 4% (only lipid-weight-based data reported).
[e]Expressed as PCBs (Aroclor 1260).
[f]Expressed as PCBs (Aroclor 1254/1260).

References: [1] Koeman *et al.*, 1973; [2] Wilson & Early, 1986; [3] Focardi *et al.*, 1988; [4] Fossi *et al.*, 1984; [5] De Kock & Randall, 1984; [6] Pearce *et al.*, 1988; [7] Vermeer & Peakall, 1977; [8] unpubl. data D. V. Weseloh in Environment Canada *et al.*, 1991; [9] Noble & Burns, 1990; [10] Somers *et al.*, 1993; [11] Tillitt *et al.*, 1992; [12] Heinz *et al.*, 1985; [13] King & Krynitsky, 1986; [14] Yamashita *et al.*, 1993.

p,p'-DDE is a major factor in causing birds to lay thinner eggs, although the mechanism is not yet completely understood (e.g. Lundholm, 1987). Other compounds (e.g. PCBs, dieldrin) have also been mentioned as (potential) causes of eggshell thinning, but here the evidence is less convincing. Furthermore, Moriarty *et al.* (1986) made clear that not only thickness, measured as 'Ratcliffe's index' (weight over width times length), is important but that size and shape are involved as well. These authors do not question the role of *p,p'*-DDE in affecting eggshells, but emphasize that published data do not enable one to decide whether *p,p'*-DDE reduces shell index or shell size. They recommend direct measurements of thickness if only effects on eggshell thickness are to be investigated.

There seems to be consensus about the use of 20% thinning in comparison to pre-DDT eggshell thickness as an empirical population threshold under which breeding performance largely fails and populations are in danger (Anderson *et al.*, 1969; Anderson & Hickey, 1972; Pearce *et al.*, 1979; Weseloh *et al.*, 1983).

Table 9 presents the eggshell thickness data in comparison with data published by Koeman *et al.* (1972, 1973), including a reference value obtained from Dutch museum material (1906–1937). The correlation between the concentration of *p,p'*-DDE and eggshell thickness in individual eggs which was shown in Fig. 5, is similar to the results given by Koeman *et al.* (1972, 1973), i.e. approximately 5% thinning or more occurring at a concentration of approximately 4 mg kg^{-1} (wet weight) *p,p'*-DDE and higher. Comparable concentrations were found for the double-crested cormorant, 10 mg kg^{-1} (wet

Table 9. Eggshell thickness of cormorants' eggs from Dutch colonies, expressed as index of museum material sampled before the widespread use of DDT. Data museum material and Naardermeer from: Koeman *et al.*, 1972, 1973

Year	Location	Index
1906–1937	museum material	100·0
1948–1952	museum material	90·2
1971	Naardermeer	90·2
1987	Dordtse Biesbosch	86·5
1988	Dordtse Biesbosch	86·5
1988	Haaften	86·9
1988	Pannerden	89·4
1988	Oude Venen	94·5
1988	Brede Water	93·6

weight) being associated with 20% eggshell thinning (Pearce *et al.*, 1979). The concentrations found in the present study are comparable. It can be concluded that in all colonies eggshell thinning occurs, and that this is related to the presence of *p,p'*-DDE in the eggs in concentrations high enough to be held responsible.

In the Dordtse Biesbosch colony eggshells from clutches which disappeared from the nest before completion of the incubation period had significantly thinner eggshells than eggs from clutches which completed the incubation period (Table 10). Eggshell thickness of the first group is 79·4% of the reference value. Apparently the group of early disappearing clutches resembles colonies in danger because of reaching the 20% thinning threshold. It is likely that the eggs in these clutches were broken in the nest and subsequently thrown out by the adults, as is done with eggshells from hatched eggs as well. This effect may involve up to one third of all egg losses: in 1987, 75·8% of all eggs did not hatch, while 25·6% disappeared before completion of incubation period.

Finally, it should be kept in mind that even in eggs from the Dordtse Biesbosch colony both the mean *p,p'*-DDE level and the *p,p'*-DDE/ΣPCB ratio were much lower than in completely failing colonies of double-crested cormorants in the Great Lakes region (e.g. Weseloh *et al.*, 1983) where the failure was mainly attributed to this compound. This makes it unlikely that all the losses should be attributed to *p,p'*-DDE alone.

Increased mortality of embryos and chicks

Having shown that the differences in hatching success are correlated with PCB concentrations, both on the level of colonies (Fig. 6) and on the level of clutches (Fig. 7), this is of course no proof of a causal relationship. However, there seem to be some good reasons to believe that the correlations do indicate a kind of a causal dose–effect relationship for effects of PCBs on the reproductive performance. This is an important point and will be discussed in more detail now.

Recently, Gilbertson *et al.* (1991) have reviewed the present evidence for the causal link of high embryonic and chick mortality, edema, growth retardation and deformities in gulls, terns and cormorants in the Great Lakes system with organochlorine compounds (PCBs, PCDDs, PCDFs). These authors conclude, after evaluating the evidence meeting six epedimiological criteria, that there is a coherent linkage between the 'Great Lakes Embryo Mortality, Edema and Deformities Syndrome (GLEMEDS)' and the presence of several chick edema active compounds. The phenomena described in the detailed analysis of breeding biology in the present study closely resemble the way embryo mortality occurs in the Great Lakes. Because of this resemblance and the occurrence of the same groups of contaminants in rather high quantities in both the aquatic habitat surrounding the Dutch colonies and in the eggs analysed, this mortality is likely to be caused by the same agent(s). Not only qualitative, but also quantitative support can be drawn from the literature: the concentrations found in the eggs seem to be high enough to have caused the effects. The reproductive success of cormorant species under the influence of PCBs has not been studied experimentally. For chicken however, a level of 20 $\mu g\ kg^{-1}$ of total PCB in the diet has been shown to lead to complete hatching failure, with residue levels in eggs of between 3 and 14 $\mu g\ kg^{-1}$ (Cecil *et al.*, 1974). For the ring dove (*Streptopelia risoria*), reproduction was reduced by an amount of 10 $\mu g\ kg^{-1}$ in the diet leading to a mean PCB residue of 16 $\mu g\ kg^{-1}$ in the eggs (Peakall & Peakall, 1973).

The breeding behaviour of birds can be adversely influenced by PCBs, reducing e.g. the attentiveness at the nest of the birds (Peakall & Peakall, 1973; Peakall & Fox, 1987; Scholten *et al.*, 1989). This is difficult to separate from egg mortality caused by contaminant concentrations, without behavioural observations in the colony or egg change experiments between colonies (Kubiak *et al.*, 1989). Both methods were not used in the present study. Therefore an effect through parental behaviour cannot be excluded.

Other factors

Factors not related to pollution can also reduce hatching and/or breeding success. The age of the adults can be an important factor determining breeding performance. Young adults have a lower breeding success

Table 10. Comparison of eggshell thickness between eggs according to completion of the incubation time, Dordtse Biesbosch 1987. The difference between the two groups is statistically significant (*t*-test, $p < 0{\cdot}002$).

	Eggshell thickness (mm) mean	s.d.	*n*	Index to 1906–1937
Incubation time not completed	0·292	0·041	12	79·4
Incubation time completed	0·328	0·039	64	87·4

than older birds (Davis, 1975; Finney & Cooke, 1978; Mills, 1979; Thomas, 1983). Observations revealed that it was unlikely that proportionally more young birds nested in the Dordtse Biesbosch colony as compared to other colonies. During the study there were no signs of (mass) predation of eggs or young by crows or other predators. Neither were there signs of large scale interference by human activity like destruction of nests or egg collection. Several authors (Anderson & Keith, 1980; Fetterolf, 1983; Anderson, 1988; Boellstorff *et al.*, 1988) have shown that research may cause serious effects on the breeding results of birds. During our research the frequency and duration of the visits were comparable in all colonies. Therefore differences in breeding performance between colonies could not be caused by differences in research activity. The high breeding success in Oude Venen and Brede Water shows that research had no or at most a small influence on breeding performance. For the Dordtse Biesbosch, it could be shown that food availability was not limiting (Boudewijn & Dirksen, in press). In conclusion, other factors seem to be of little importance in causing differences between colonies.

Final remarks

In the paragraphs above we have reasoned why and how chlorinated hydrocarbons, presumably *p,p'*-DDE and PCBs, can cause the differences in reproductive success between the colonies investigated. We presented evidence of effects measured in free-living birds under field conditions and were able to correlate the concentrations of chlorinated hydrocarbons in eggs with these effects, both at colony and individual clutch level. In the present study, six individual PCB congeners have been analysed. For all six PCBs analysed a kind of dose–effect relationship appears to exist for effects observed in the field (Figs 6 and 7). This, in itself already an important finding since so few data are available for effects observed in the field at an individual level, is the more interesting when we take into account the current knowledge regarding PCB toxicity. It is now well established that, for *in vivo* and *in vitro* systems, toxicity varies significantly between PCB congeners (see, e.g. Goldstein & Safe, 1989; De Voogt *et al.*, 1990; Walker, 1990).

From what is known about the relationship between structure and toxicity of PCBs (Tanabe, 1988; De Voogt *et al.*, 1990), it seems appropriate to relate adverse effects of PCBs to the presence of the most significant PCBs in the pertaining samples. Only few comparative examples are available, mostly applying to a two-group comparison. For example in Forster's terns (*Sterna forsteri*), Kubiak *et al.* (1989) found 11-fold higher TCDD equivalents (in which two PCB congeners accounted for more than 90% of the total) in eggs from a colony with a low hatching success in a heavily contaminated area as compared to eggs from a colony in a relatively clean area where reproduction was normal. In this study, a significant correlation between effect and concentration was found for all six non-planar PCBs analysed, although the correlation coefficients were rather low. Probably this correlation might improve further if Toxicological Equivalent Concentrations (TEC) were used. On a colony level, Tillitt *et al.* (1992), did indeed find a correlation between TECs and egg mortality in double-crested cormorants.

One would expect that the ratio between the concentrations of active or planar PCBs (pPCBs) and the sum of the six PCBs analysed in this study (Σ6-PCBs), expressed as ΣpPCB/Σ6-PCBs or ΣTEC/Σ6-PCBs, is not necessarily equal for each area or food type investigated: the (ratio between) principal sources of the PCB contamination, such as atmospheric deposition, river sedimentation, may differ. If so, the relationship between certain observed effects and the concentration of any of the six congeners in this study will be less strong than a dose–effect relationship in which the TEC was used. The results obtained here, however, demonstrate the existence of such a dose–effect relationship (correlation) for all six non-planar PCBs. One may therefore speculate that the ratio mentioned above is more or less equivalent for all the colonies. Experimental confirmation of this speculation, e.g. by analysing the levels of planar and mono-*ortho* substituted PCBs in sediments, fish and eggs is required. The importance of the above reasoning lies in the fact that, if true, (1) older data for several environmental compartments can be revaluated to estimate their TEC, and (2) the highly demanding and expensive analysis of the planar PCBs will not (always) be required in order to obtain information on the toxic potential of the amount of PCBs present in a sample.

The data presented above strongly suggest a relation between persistent organochlorine contamination of aquatic habitats and breeding success of Cormorants. Two directions of further research have been developed. One direction is to get a better insight by establishing whether the suspected compounds are indeed responsible for the observed effects, and by identifying the mechanisms through which they operate in the organism. With this goal in mind, an integrated ecotoxicological research project was performed in 1989. Ecological measurements were combined with chemical and biochemical/toxicological measurements in young from eggs of Dordtse Biesbosch and Oude Venen hatched artificially (Craane *et al.*, 1990; Van den Berg *et al.*, 1992). The second direction is to develop a system in which breeding biology parameters of Cormorants can be used as biological indicators: the probable existence of a dose–effect relationship enables the use of ecological data as indicators in monitoring effects of pollution in aquatic habitats (Boudewijn & Dirksen, in press).

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