PCB CONTAMINATION, REPRODUCTIVE SUCCESS AND POPULATION TRENDS OF THE COMMON TERN (*Sterna hirundo*) IN THE RHINE-MEUSE-SCHELDT DELTA

Thesis for MSc. Ecotoxicology of Natural Populations,
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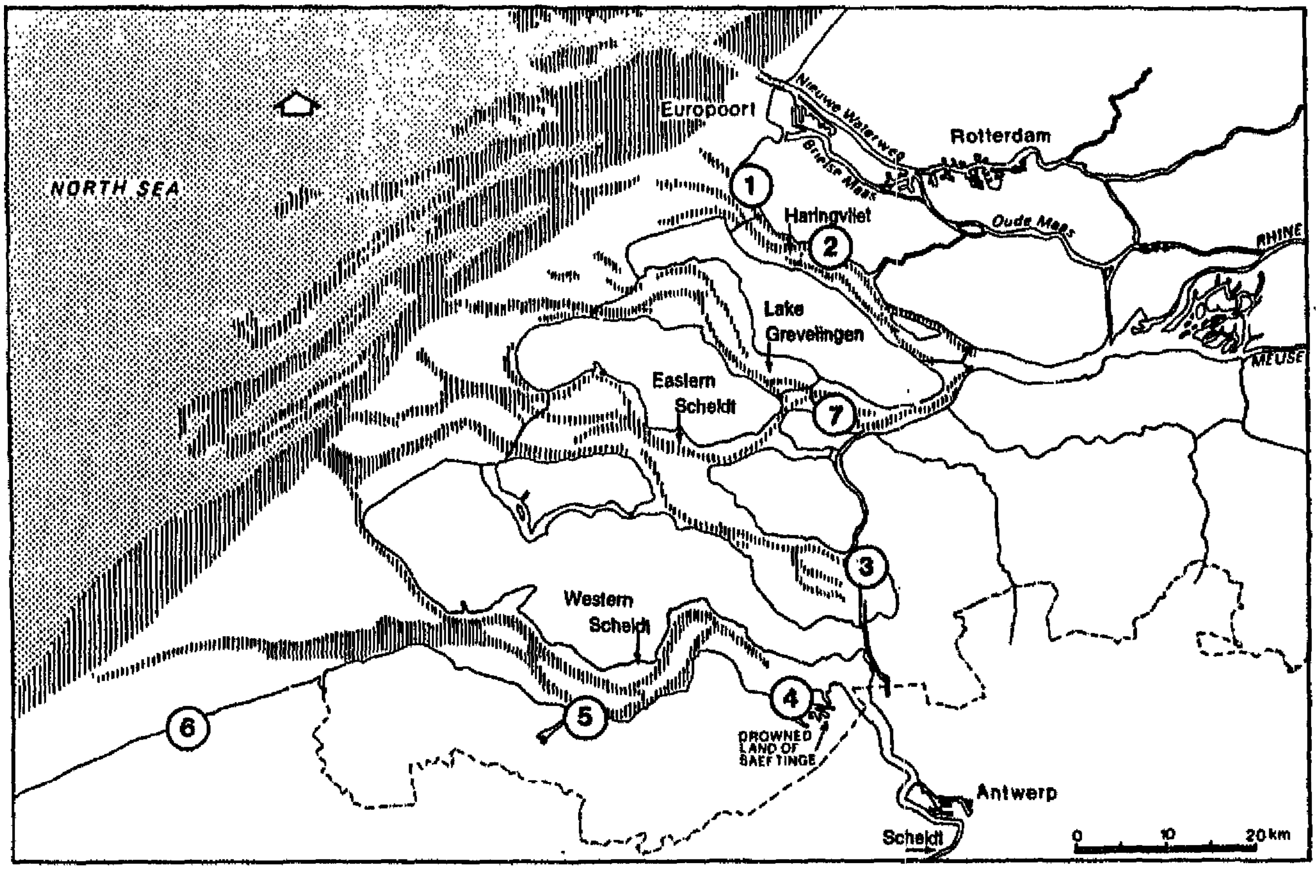

Figure 1 The Delta region in SW-Netherlands. The basins of the Rhine- Meuse- Scheldt Delta cover a wide range of contamination. The Haringvliet and the eastern part of the Western Scheldt are contaminated while the Eastern Scheldt is relatively clean.
Numbers indicate the investigated colonies of the Common Terns:
1. Westplaat, 2. Slijkplaat, 3. Prinsesseplaat, 4. Saeftinge, 5. Terneuzen, 6. Zeebrugge and 7. Philipsdam.

1. INTRODUCTION

Since the 1930s the physical structure and chemical quality of the environment in The Netherlands have changed considerably and resulted in loss of biotopes and in toxic effects on flora and fauna (Hekstra & van Linden, 1991; VROM-RIVM, 1991).
To set quantitive and verifiable ecological objectives for the Dutch management of the North Sea and inland waters the AMOEBA-approach has recently been developed (Ministry of Transport & Public Works, 1989: Ten Brink *et al.*, 1991). A selection of 60 target variables has been made, mostly species, representing a cross-section of the entire ecosystem. The aim of the water management is to achieve "a sustainable development in the marine and brackish waters in The Netherlands" and will be considered successfull when the numbers of the selected species are within 75%-200% of the "reference levels". The period round about the year 1930 is taken as a reference. It represents a situation with limited human interference in the environment, while sufficient information is available.

One of the target variables for the North Sea and Waddensea is the Sandwich Tern (*Sterna sandvicensis*) and in addition the Common Tern (*S. hirundo*) for the Delta region in SW-Netherlands. These are fish eating birds and regarded as good environmental indicators for several reasons.
First of all, much is known about their ecology worldwide (e.g. Burger & Gochfeld, 1991; Nisbet *et al.*, 1984). In The Netherlands their numbers have been censused since the 1920s so a long term trend is known (Stienen & Brenninkmeijer, 1992). These observations are important because a population decline is often the first signal of deleterious effects of e.g. toxic compounds that is recognized (Ratcliffe, 1980; Sarokin & Schulkin, 1992). Second, Terns are susceptible for human influences like the pollution with organochlorine pesticides (Koeman, 1971) or PCBs (Kubiak *et al.*, 1989; Environmental Canada, 1991) because they are specialized predators at the top of the foodchain (Walker, 1990). Third, they have a social appeal in our society. Finally, with regard to Common Terns, the species is to a certain extend accessible for measurements which also makes them a good target variable.

This MSc. thesis discusses the ecotoxicology of polychlorinated biphenyls (PCBs) with particular reference to the Common Tern in the Delta region (Fig. 1). The Delta region forms the delta of the rivers Rhine, Meuse and Scheldt. Here, a strong decline in the numbers of breeding pairs occurred in the 1950s and 1960s. It coincided with an increase in pollution and at the same time a changing physical structure of the area due to land reclamation for industrial and

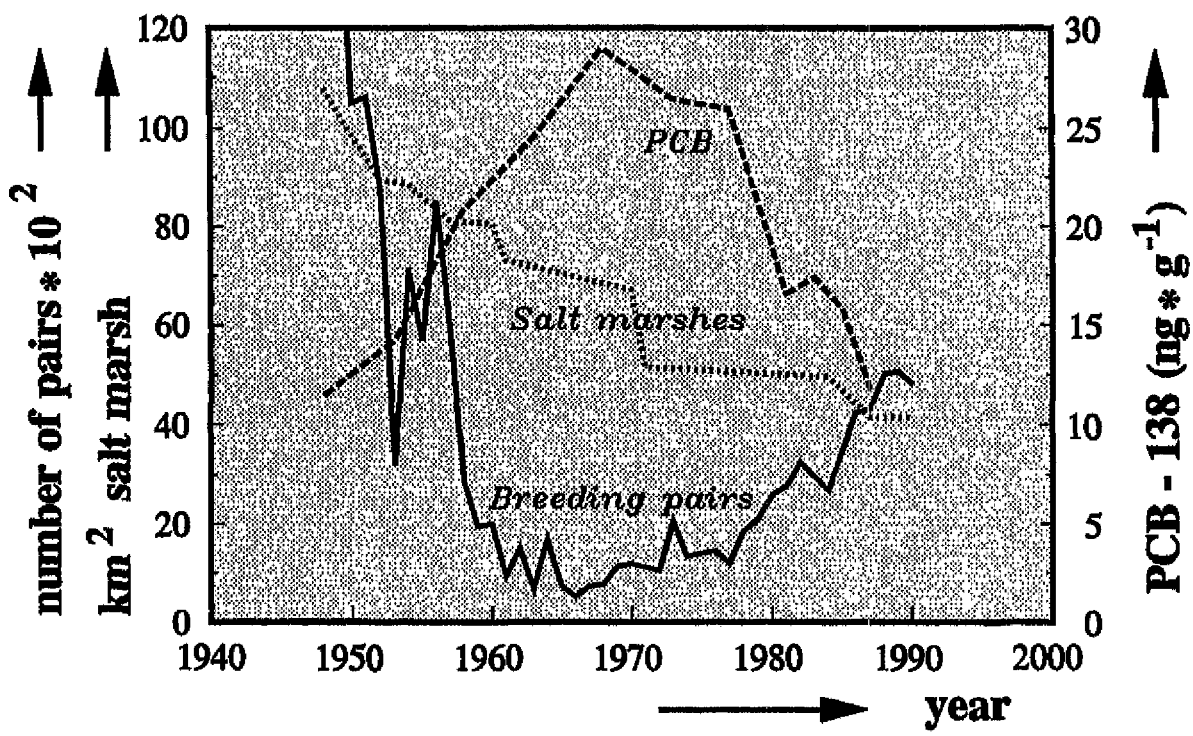

Figure 2 The changes in suitability of the Delta region as a breeding area for Common Terns over the past 40 years occurred against a background of changing conditions of pollution and physical structure of habitats. The trend in pollution is illustrated by concentrations of PCB congener IUPAC no. 138 in a sediment core from a salt marsh in the Western Scheldt (van Eck & de Rooij, in prep.). The area of saltmarshes represent the changes imposed by land reclamation and Delta Works. See further comment in text.

agricultural purposes and the large scale construction of dams. The latter are the so called "Delta Works" and were initiated after the devastating stormflood in 1953.
The trends are illustrated in a suggestive combination in Figure 2. There is an apparant relation between the tern population size and both the PCB levels and the area of saltmarshes. Concentrations of PCBs in sediment illustrate the history in environmental contamination very well and assuming a more or less equilibrium between tidal sidement, water and biota, the data also may represent a trend of PCBs in fish. Present concentrations are equal to those around the year 1950. However, it should be stressed that the contamination with organochlorine insecticides (in particular dieldrin, endrin and telodrin), or polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) from PCB mixtures may have a closer (causal) relation with toxic effects and consequently with numbers of breeding pairs. Likewise, the area of saltmarshes represent only one type of breeding habitat. Other breeding sites also fell into disuse (e.g. sandy beaches) or alternatively came available (e.g. industrial waste grounds) (Meininger *et al.*, 1992).

This thesis discusses issues concerning the contamination of Common Terns with PCB, their population trends and breeding success in the Delta region. First, data on concentration of PCB in eggs and tissues of Common Terns from the Eastern Scheldt and Western Scheldt are analyzed with regard to (a) the uptake of PCBs from the aquatic environment, (b) differences between the colonies, (c) differences in body burden between the sexes and (d) distribution of PCB congeners in several organs. Second, the trend in the size of the breeding population is analyzed in the context of estimates of mortality rates and fecundity of the Common Tern reported in literature. Third, to uncover a possible relation between the exposure of the breeding colonies with PCBs and reproductive success, data obtained in an integrated field and laboratory study in 1991 (Rossaert *et al.*, in prep.; Bosveld *et al.*, 1992) have been used to correlate the hatching success with concentrations of PCB in the yolksac of hatchlings.

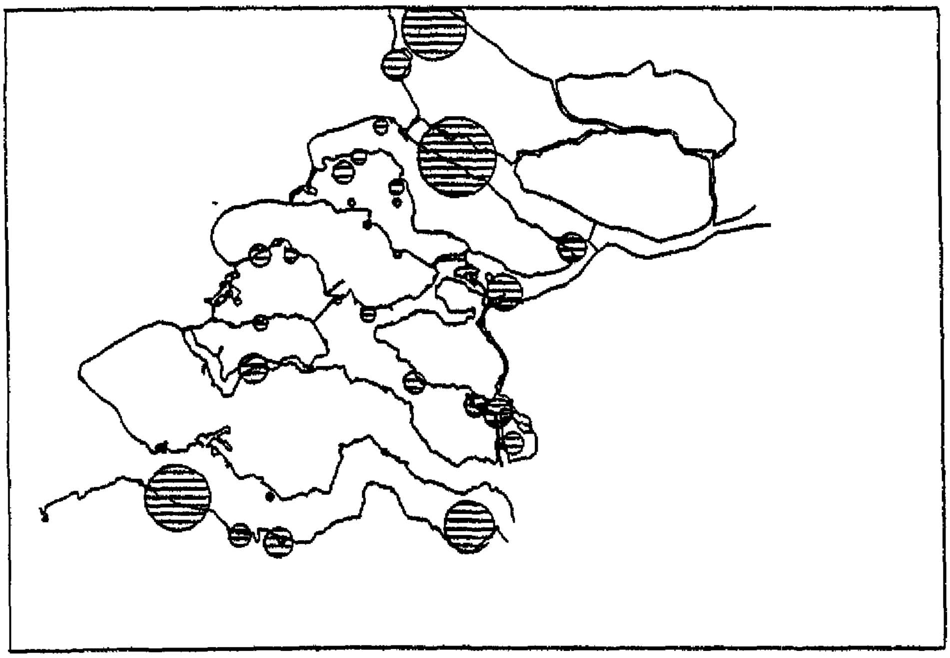

Figure 3 Breeding colonies in the Delta region in 1991 expressed in relative size compared to colony Slijkplaat with highest numbers of breeding pairs (1100) in the area.
From Meininger et al. (1992).

2. BACKGROUND

2.1. A species account

The Common Tern (*Sterna hirundo*) is a protected species in the EC (see Annex 1 of the EC Birds Directive 1979). In Europe the Common Tern is widely distributed (Cramp, 1985) with an estimated population of 91.400 pairs of a total world population of 250.000-500.000 (Lloyds *et al.*, 1991). In 1991 there were *c.* 11.000 breeding pairs in The Netherlands (Stienen & Brenninkmeijer, 1992) of which *c.* 5000 are present in the Delta region (Meininger *et al.*, 1992). The distribution and size of the colonies in the region is presented in Figure 3. There are three other species of Terns that breed in the Delta region: Sandwich Tern (*S. sandvicensis*), Little Tern (*S. albifrons*) and Arctic Tern (*S. paradisaea*), but they are less abundant (Meininger *et al.*, 1992).

The diet of the Common Tern consists mainly of small clupeids like sprot (*Clupea sprattus*) and herring (*Clupea harengus*) and crustacea like the brown shrimp (*Crangon crangon*). Terns arrive in the Delta region in April and lay their eggs within a few weeks time. During that period the female builds up a fat reserve for egg formation by consumption of small clupidea during courtship feeding. They prefer open areas as breeding sites but also breed in salt marshes (Burger & Gochfeld, 1991). By the end of the summer they leave for their wintering areas in W-Africa as has been confirmed by ringing (Meininger *et al.*, 1992).

The natural habitat of the Common Tern is very dynamic and in one year breeding success may be high, in another year it may fail completely as a result of low food availability, bad weather conditions, flooding or predation by westely herring gulls (*Larus argentatus*), rats, minks etc., as have been reported for e.g. the German Bight Waddensea (Becker, 1991) Shetlands (Monaghan & Alley, 1989) and Western Scheldt (Beyersbergen, 1991). The most critical factor controlling the population size for seabirds is probably food supply during the breeding season (Birkhead & Furness, 1984). In this respect the Common Tern is more adaptable than e.g. the Arctic Tern (Monaghan & Uttley, 1989).

A population can be defined as "birds belonging to the same species living in a specified geographical area" (Lloyds *et al.*, 1991). The Delta region as a whole can be regarded as such an area, because very little migration to or from surrounding areas have been observed (pers. comm. P.L. Meininger). At a population level it is more likely that numbers will fluctuate less strongly in time than at a colony level, because local differences regarding predation, disturbance by man, flooding and migration from and to neighbouring colonies are excluded.

For the same reasons it is therefor difficult to ascribe differences in breeding success between colonies to only one factor eg. pollution.

For long living animals like the Common Tern with a maximal lifespan of *c.* 25 years, it is known that small changes in adult mortality rate can have a marked influence on the size of the breeding population. This has been reported for Shags (*Phalacrocorax aristotelis*) at Farne island after a mass mortality of adults due to paralytic shellfish poisoning caused by a red tide (*Gonyaulax tamatensis*) (Potts, 1980). In contrast, effects of breeding productivity for one or even several years may have little effect on breeding numbers as has been reported for the long term reduction in breeding success of the Puffin (*Fratercula arctica*) in Norway due to food shortages (Lid, 1981).

The number of specimens in a population can be described by the following equation:

$$N_{t+1} = N_t + B - D + I - E \qquad [1]$$

where N_t = population size at time t, N_{t+1} = population size at time t+1, B = number of individuals born each year, D = number of individuals that die each year, I = number if immigrants each year and E = number of emigrants each year.

Data obtained from banding and trapping Common Terns in the Delta region have revealed that breeding adults show very little exchange between colonies and young breeders are often found within a small range from the colony they were born (Meininger *et. al.*, 1992).
Common Terns in general join the breeding population at the age of three, this is in the fourth calander year. Some individuals breed at a younger age but with a lower success (Nisbet *et al.*, 1984). They reproduce once a year and have an age-specific fecundity F and mortality M. In this case the Leslie matrix model (Leslie, 1945) is a useful tool to describe population dynamics. This approach has major limitations as it does not take into account 1.density dependent processes and 2. migration. Nevertheless, a Leslie matrix can illuminate on the influence on population size of PCB related changes in fecundity and mortality.

2.2. Polychlorinated biphenyls

PCBs have been manufactured since the 1930s for a wide spectrum of applications, in particular for its nonflammability and heat-resistant properties. PCBs were first discovered in the marine environment by Jensen (1966) and

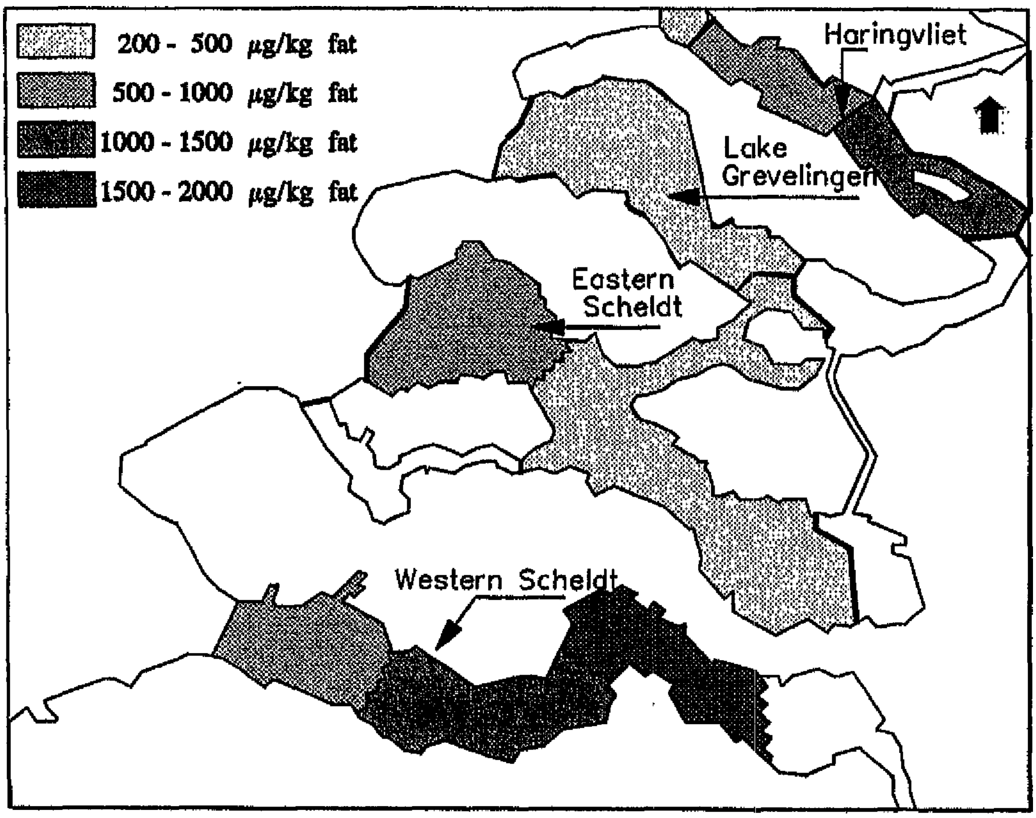

Figure 4 The distribution of PCB-153 in blue mussel (Mytilus edulis) from Lake Grevelingen, Eastern and Western Scheldt (Stronkhorst, subm.) and in Zebra mussel (Dreissna polymorphis) from Haringvliet (Hendriks & Pieters, subm.) in 1987-1990. The highest concentrations are observed in the eastern parts of the Western Scheldt and Haringvliet but in the Eastern Scheldt concentrations do increase in seaward direction.

soon after that the global character of the contamination became clear as, for instance, high levels were found in eggs of seabirds from remote places (Risebrough *et al.*, 1968). PCBs were banned in Japan in 1973 after an incident causing toxic effects in humans. In Europe however, the production continued until 1987. The global production of the various technical mixtures is estimated at *c.* 2 million tons with 70% still in use nowadays in so called 'closed systems' (transformers etc.). However, in reality PCBs continue to enter the environment by e.g. leaching from hydraulic systems in mines or shipwrecks. By now, 1-10% have reached the oceans. An increase of PCBs in marine environment is expected for decades to come (Tateya *et al.*, 1989).

PCBs are a group of 209 theoretically synthesizable compounds (congeners). The numbering is according to the degree and positions of substitution of the biphenyls with chlorine (Ballschmiter & Zell, 1980). The introduction of gaschromatography with electron capture detectors and capillary columns in the 1980s made it possible to analyze the individual congeners. PCBs strongly bioaccumulate. As a hydrophobic compound the molecules form an envelop of water around it because of the polarity of water molecules. Near a lipid (apolar) phase the envelop disintegrates and the PCB molecule diffuses into the lipid phase of for instance a fish. This process is called bioconcentration. Through biomagnification, i.e. the intake of the compound through the food, fish eating birds therefore can experience a high exposure. Bioaccumulation, the elevated concentrations as a result of both processes (Connell, 1988), occurs in particular with congeners that are only slowly metabolized and have an high tendency to concentrate in lipids. The latter expressed as Kow, the octanol-water partition coefficient, increases with increasing chlorination of the biphenyl rings. PCB-153, for instance, has a log K_{ow} of 6.92 and is hardly metabolized and therefore shows a very strong tendency to bioaccumulate. It is one of the most dominant congener in biota (de Voogt, 1990) and therefore selected for statistical analysis and calculations in this thesis. Higher chlorinated congeners like PCB-170 and PCB-180 might pass the membranes less easily due to steric hinderance (Opperhuizen *et al.*, 1985).
Although the pattern of PCB congeners in one type of material is quite constant there is not always a good correlation between PCB-153 and other congeners (de Voogt, 1990).

The distribution of PCBs in the aquatic environment of the Delta region is presented in Figure 4. Relative high concentrations occur in mussels from the Haringvliet and brackish zone of the Western Scheldt. Here, as a result of the sedimentation of contaminated fluvial suspended matter, "geo-accumulation" took place for many years and will continue to form a source for many years to come.

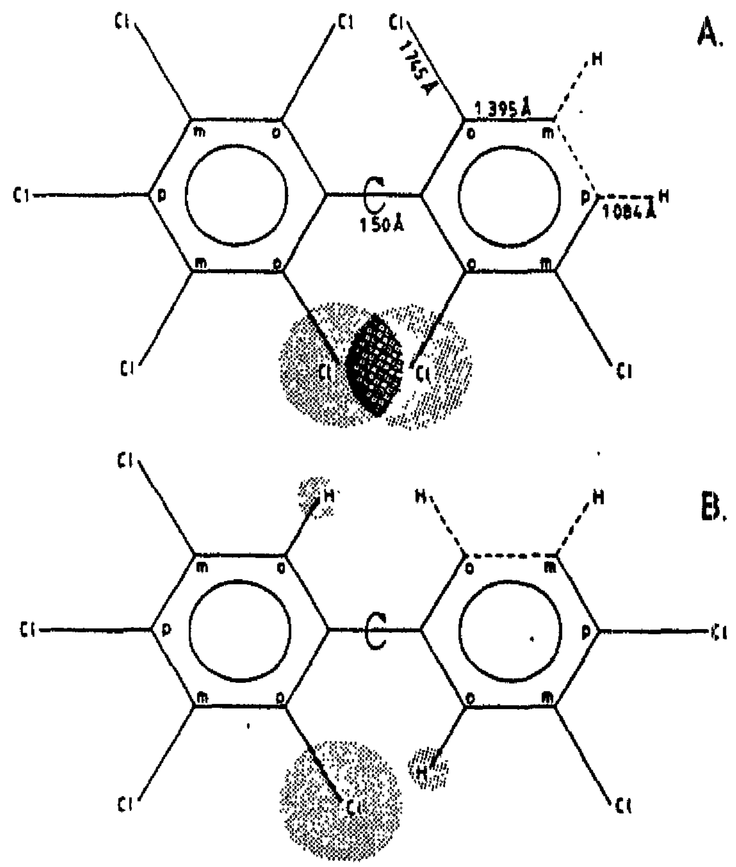

Figure 5 Structural features of PCB-congeners influencing enzymatic metabolism (in female harbour seals). Areas where the principal enzymatic reaction occurs are given by dotted lines.
-A- Vicinal H-atoms in a meta-para position. Overlapping covalent radii for two ortho-Cl indicate that a planar configuration is highly improbable when three or four ortho-Cl are present.
-B- Vicinal H-atoms in a ortho-meta position. One or two ortho-Cl will not oppose each other. Non-overlapping covalent radii of Cl and H show that a planar configuration is not sterically hindered in this case. However, the introduction of a second ortho-Cl opposite to a vicinal H-atom apparently prevented interaction with biotransformation enzymes in seals.
Boon & Eijgenraam (1988).

PCBs accumulate through the foodchain according to the availability of adjacent positions not substituted with chlorine (Braune & Norstrom, 1989): no adjacent unsubstituted positions (B group) congeners have the highest accumulation, meta-ortho unsubstituted (M group) congeners are intermediate and congeners with para-meta unsubstituted positions (P group) have the lowest accumulation. PCBs with chlorine atoms bound to at least two out of three of the lateral positions and no ortho chlorine substitution on each benzene ring are the most toxic congeners (PCB IUPAC no. 77, 81, 126 and 169). These are called the planar congeners. Less toxic, but still more toxic than the di-, tri- and tetra ortho substituted congeners are the mono-ortho congeners (e.g. PCB 105, 118, 156). These groups of molecules can rotate around the C-C bond towards a planar configuration (Fig. 5). Because they have stereochemically similarity to 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDD) they exert similar biochemical activity. The toxicity of these planar compounds therefore is regarded to be additive and can be expressed as 2, 3, 7, 8 TCDD Toxicity Equivalence Factors (TEFs). For instance, the TEF of PCB-77, PCB-126 and PCB-105 is 0.1, 0.05 and 0.001 respectively (Safe, 1990). De Voogt (1990) multiplied the environmental concentrations and TEFs and concluded that PCB-105, PCB-126, possibly PCB-118 and PCB-156 have the greatest toxic potential.
In a recent study Tillett *et al.* (1992) show that the relative potency of PCB residues in eggs is three- to fourfold greater than of PCB technical standards.

2.3. *Monooxygenase and its role in PCB metabolism*

As PCBs lack features like ester bonds or eg. nitro-groups no other detoxification enzyme system than monooxygenase (MO) can metabolise these molecules. The monooxygenase system consists of a NADPH-cytochrome P450 reductase and cytochrome P450 isozymes. In vertebrates it is mainly present in liver microsomes (Walker & Ronis, 1989). In fish eating seabirds this enzyme system is poorly developed (Fig. 6). Besides species variation, marked inter-species differences have recently been reported for gulls (Yamashita , 1992).
The in vivo metabolism of dosed PCBs was determined by Harris & Osborn (1981) in wild Puffins (*Fratercula arctica*) and showed the high biological persistance of PCBs. The half life in fat tissues was about 14-18 months. Depending on the molecular structure some congeners are hardly broken down at all, others can be metabolized at a very low rate (phase I reaction) and then conjugated and excreted (phase II reaction). Boon *et al.* (1989) and Borlakoglu *et al.* (1989) have shown that congeners with meta-para unsubstituted adjacent carbon atoms can be metabolized successfully by seabirds. Persistent PCB-congeners in seabirds are the ones with ortho-meta unsubstituted adjacent carbon atoms.

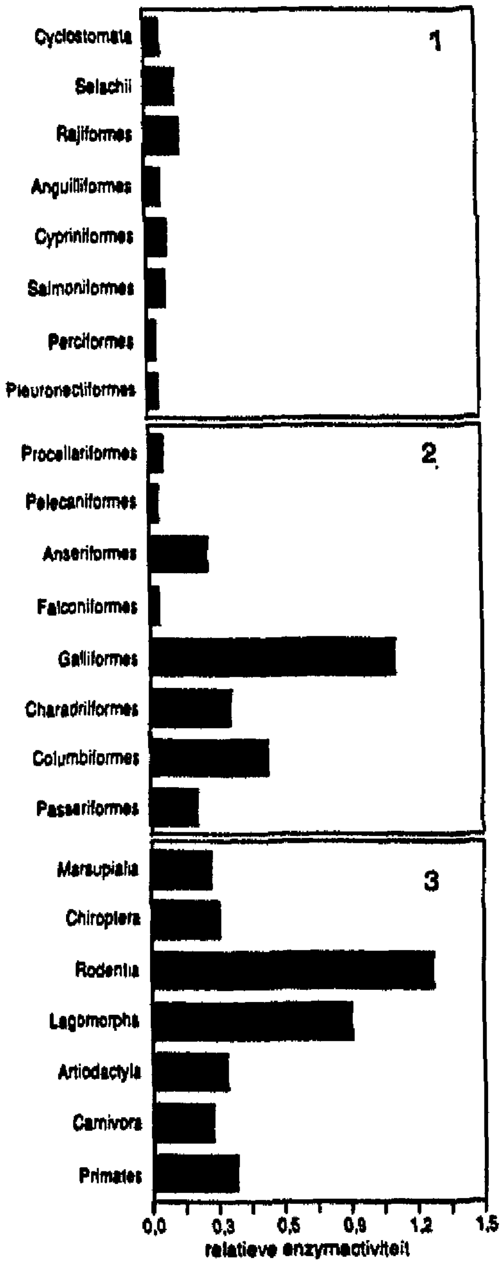

Figure 6 Species variation in relative enzyme activity of the monooxygenase enzyme system in liver microsomes of several orders of fish (1), birds(2) and mammals(3). From Van Straalen & Verkleij (1991), after Walker (1980).
Fish eating birds (Charadriiformes, Procellariformes and Pelecaniformes) have a relatively low MO activity.

Normally the MO system is involved in the regulation of steroids, vitamin D and fatty acids but it can be induced by xenobiotic compounds. Nebert & Gonzalez (1987) developed a phylogenetic system based on cytochrome P450 induction properties. Here, induction means the proccess of a. binding with the Ah receptor, b. transport into the nucleus and c. increase transcription capacity of mRNA to form more P450 isozymes (Safe, 1990). Because PCBs are very persistent molecules in an organism, i.e. have a low clearance rate, they can cause a long term induction of the MO system. Enzymes of the cytochrome P450-IA1 subfamily are inducible by 3-methyl cholanthrene but also by xenobiotics like the planar PCBs. P450-IA1 is also found in birds and regarded as a dangerous line because it metabolizes e.g. PAH (Malins & Collier, 1981) into carcinogens. EROD (ethoxyresorufin-O-deethylase) and AHH (aryl hydrocarbon hydroxylase) are representatives of the cytochrome P450-IA1. The H4IIE rat hepatoma cell bioassay is another technic to evaluate the catalytic activity of P450-IA1.

Recently, in addition to the Ah theory, the TTR (transthyretin) concept has been presented (Brouwer *et al.*,1990) with the aim to describe retinol and thyroxine disfunction caused by planar PCBs. TTR, together with RBP (retinol-binding protein) form a transport protein complex of plasma retinol and thyroxin. The OH metabolite of PCB-77, generated by the MO system, strongly binds with this complex. It therefore competes for free binding sites resulting in an increased excretion of retinol and thyroxin through the kidney and bile. However, not all wildlife species may have the "ability" to do so. It is suspected that planar congeners PCB-77 and PCB-126 can be metabolized only by mammals e.g. seals, cetaceans and polar bears but probably not by seabirds (Boon *et al.*, 1989).

2.4. Effects of PCB on sea birds

PCBs are regarded as the most threatening and wide spread contaminant in the marine environment for top predators like dolphins and seals (Sarokin & Schulkin, 1992). Immuno-incompetence and reproductive impairment are the most likely consequences. The seabird wreck in the Irish Sea in 1969, when thousands of dead guillemots were washed ashore, is a case of a possible link with PCB poisoning (Holdgate, 1971). In the 1970s mortality and reproductive impairment of Cormorants (*Phalacrocorax carbo*) from the Biesbosch (Rhine-Delta), The Netherlands were related to contamination with PCBs (Koeman *et al.*, 1973). During the last decade the Dutch population of Cormorants recovered from hunting and pollution but the colony in the Biesbosch still experiences reproductive impairment due to PCB (Dirksen *et al.*, 1991; van der

Gaag *et al.*, 1991).

At the current levels of PCBs in the aquatic environment in general it is very unlikely that any acute lethal effect on birds will occur (Peakall, 1986). Among the many sublethal effects of PCB on birds are: weight loss, loss of feathers, liver damage, porphyria, congenital malformations, embryo toxicity and altered reproductive success. Terns have shown to be sensitive indicators for effects of PCB contamination. The highest incident of birth defects (1.3%) ever reported is that of Common Tern chicks from Lake Ontario in the early 1970s (Environment Canada, 1991). Nisbet & Reynolds (1984) reported impairment of reproductive success of Common Terns in Massachusetts. In a study with Forster's Tern (*Sterna forsteri*) Kubiak *et al.* (1989) have shown that reproductive success was influenced by planar and mono ortho PCBs in Green Bay, Lake Michigan due to both embryo toxicity and parental behaviour (Table 1). The latter aspect is not taken into account in this thesis.

Table 1. Egg-exchange experiment with Forster's Tern (*Sterna forsteri*) in 1983 have revealed the influence of intrinsic effects (embryo toxicity) as well as extrinsic effects (parental care) on hatching rate (Kubiak *et al.*, 1989). However, some results are not consistent with on another. From Environmental Canada (1991).

adult	egg	hatching rate (%)	type of result
clean	clean	88	normal reproduction
clean	dirty	94	intrinsic effect (?)
dirty	clean	11	extrinsic effect
dirty	dirty	55	in- and extrinsic
artificial	clean	75	normal reproduction
artificial	dirty	37	intrinsic effect

AHH activity in Forster's Terns from Green Bay and a clean reference site show a correlation with weight loss, deformation, abnormal functioning of the immunosystem and thyroid gland (Hoffman *et al.*, 1987). On the other hand, many reports reveal no observable effects of PCBs on Terns (e.g. Becker, 1991). In the Delta region the little related work on other species of birds that have been carried out raised no concern over effects of PCBs. Breeding success of Black-headed gulls (*Larus richibundus*) along the Western Scheldt does not seem to be affected by PCBs (Ysebaert & Meire, 1989; Stronkhorst *et al.*, in prep.). Starved Oystercatcher (*Haematopus ostralegis*) found dead in the Eastern and Western Scheldt had elevated PCB residues but not at a lethal level (Lambeck *et al.*, 1991).

Indeed, at lower levels of contamination the effects are less clear and more sensitive methods are required. Unfortunately, no biomarkers are available yet to determine effects of a chemical on reproduction of wildlife (Peakall, 1992). For instance, thyroid hormone and vitamin A have a key function in the reproductive process and polychlorinated biphenyls may interfere with one of the pathways.
The changes in parental behaviour like nest-attentiveness may be related to hypo- or hyperthyriodism. Deficiency of vitamin A has been associated with decrease in hatchability, chick survival and birth defects (Thompson, 1970; Robbins, 1983). However, in both cases the homeostasis is a highly regulated process and therefore the effects of a single factor (e.g. PCB contamination) is difficult to detect.

There is no evidence that PCB's cause eggshell thinning (Peakall, 1986). The eggshells of eggs of Common Terns from the Delta region are not affected by pp'DDE residues. They have the same thickness as those from the pre-DDT period (Dirksen & Boudewijn, 1991).

3. MATERIALS AND METHODS

3.1. Distribution of PCBs in the Common Tern

Sampling

Eggs and adults were collected on June 16th 1987 and chicks on July 1st and 2nd 1987 in Saeftinge in the brackish zone of the polluted Scheldt estuary and at Philipsdam in the less polluted Eastern Scheldt (Fig. 1). Table 2 lists the sampling and analysis numbers.

The sampling method, dissection and storage are described by Marteijn & Meininger (1987). Adults were caught on their nests 2with Öttenby traps. All three eggs were collected from the same nests. Adults and 2-3 weeks old chicks were sacrificed, wrapped in tissue paper and stored in plastic at -20 °C for two days. After thawing they were weighed, sexed and stomach content was determined. Liver of chicks and liver, brain and kidney from adults were prepared, weighed and stored at -20 °C in cleaned glass jars (2 N chloride acid, demiwater and dried at 130 °C) and sealed with aluminum foil.

Table 2. Number of eggs, chicks and adult Common Terns collected in Saeftinge and Philipsdam (1987).

	Saeftinge		Philipsdam	
	sampled	analyzed	sampled	analysed
adults	6 (5♀, 1♂)	all	6 (3♀, 3♂)	all
chicks	6	1	5	1
eggs	18 (6 clutches)	12[1]	14 (5 clutches)	9[2]

1 three complete clutches plus one egg from three others clutches.
2 two complete clutches plus one egg from three others clutches.
3 one male and female (number 59A and B) are a pair.

Near Saeftinge two pooled samples of clupeids, probably herring (*Clupea harengus*), were collected on June 13th 1987 and of shrimp (*Crangon crangon*) on August 24th 1987. Additionally, herring and flounders (*Platichtus flesus*) were sampled on May 15th and June 1st 1989 for intra-species comparison. Near Philipsdam shrimps were collected in June 1987, but clupeids could not be obtained.

In June 1991 the second laid egg (B-egg) from 9-10 clutches in six colonies in the Delta region (Fig. 1) and a colony in the Waddensea (Griend) and Lake Yssel (Zeewolde) was collected and transported to the laboratory of the Research Institute Toxicology (RITOX) of the University of Utrecht, Utrecht. The eggs were incubated at 37.5 °C and the chicks were sacrificed within 12 hours after hatching.

Westplaat (colony 1) is a new (1988) man-made sandflat for birds with *c.* 150 pairs of Common Terns in 1991. Foraging areas include coastal North Sea, Rotterdam harbour and near the Haringvliet sluices, where water of the Rhine/Meuse is discharged during low tide.
Slijkplaat (colony 2) is a bird island with *c.* 1.100 pairs and located in the western part of the Haringvliet. Fouraging takes place at the western side of the Haringvliet sluices.
Prinsesseplaat (colony 3) is located on a former tidal flat near (freshwater) Lake Zoom and inhabit *c.* 300 pairs in 1991. The birds find their food in the relatively less contaminated tidal Eastern Scheldt and (freshwater) Lake Zoom.
The salt marsh Saeftinge (colony 4) in the contaminated brackish part of the Scheldt estuary has a breeding population of *c.* 500 pairs in 1991. Here, the most extreme tidal amplitude of The Netherlands occurs. Birds are foraging in the estuary.
Colony 5 near Terneuzen with *c.* 145 pairs is situated near sluices of the polluted Gent-Terneuzen Channel on a plateau (95x25 m) with gravel. There is hardly any predation and the loss of chicks is low because a wire-netting is placed around the site during the breeding season. The birds experience much disturbance by passing ships etc. Fouraging areas are in the direct neighbourhood (near sluices, Gent-Terneuzen Channel or Scheldt estuary).
Finally, colony 6, also a fast growing colony with *c.* 650 pairs in 1991, is located on a large open plane near the harbour of Zeebrugge, Belgium. Foraging takes place in the North Sea.

Chemical analysis

The analyses of the 1987-study were performed at the laboratory of the Tidal Waters Division in Haren (Netherlands). Methods are described in detail by de Jong & Smedes (1989). The following material has been analyzed: pooled samples of food items (shrimp, herring), the liver, brain and kidney of five

females from Saeftinge, livers of females and males from Philipsdam and single livers of the male from Saeftinge and of one chick from each colony, individual carcasses and individual eggs.
The carcasses were crushed after the feathers had been stripped. All samples were homogenized (Ultra Turrax TP 18/10) and dried (Virtis Unitrap 2, 10-mr-st dry-freezer). Samples were extracted according to the hot Soxhlet method with pentane for 4 hours. The extract is reduced by Kuderna-Danish evaporation and further evaporated with a N_2 stream and the residue is weighed to determine the lipid content. After the residue is dissolved in hexane the PCBs were separated from lipids on a SiO_2 (5% H_2O) column with pentane. After reducing the volume by evaporation the PCBs were eluted on a Al_2O_3 (dried at 180 °C column with 3% diethyl ether in pentane. PCB IUPAC no. #18, #28, #52, #49, #44, #101, #118, #138, #153, #180 and #170 were analyzed on a HP 5880 GC-ECD equipped with two columns. One column is coated with SE 54 and the other with CP Sil 19CB (both 50 m: 0.32 mm DD 0.2 μm filter).
PCB IUPAC no. 29 and 155 were added before analysis and used to determine the recovery of the procedure and PCB IUPAC 143 is used as an internal standard and was added just before GC analysis.
The percentage dry weight (DW) of carcasses and organs have not been determined so for the calculation of body burdens estimates of 40 % and 30 % r6espectively were used instead.

Yolksacs of hatchlings of the 1991-study were analyzed at the laboratory of RITOX. The methods and results have been reported by Bosveld *et al.* (1992). Samples were extracted with 50 ml CH_2Cl_2 for 24 hours, cleaned up in a alumina column and PCBs IUPAC no. #105, #118#, #156, #157, #167 (the mono ortho planar congeners), #28, #52, #101, #138, #153 and #180 were analyzed on a Carlo Erba Mega 536 GC-ECD.

Concentrations are expressed on a lipid basis. However, for comparison with effect concentrations reported in literature the levels in eggs from Saeftinge and Philipsdam are expressed on a wet weight basis (see Discussion).

3.2. Breeding success

Clutch size, hatching success (number of hatchlings per clutch) and hatching rate (fraction of egg hatched) were investigated from May-July 1991 in six colonies described earlier. The methods and results for this and other breeding biological parameters are reported in detail by Rossaert *et al.* (in prep).

Hatching success of the Common Tern is depending on (1) the clutch size, (2) the loss of eggs due to physical disturbance like washing out, (3) predation and (4) the fraction of eggs that failed to hatch. The categories 1 and 4 are most relevant with regard to effects of PCBs as it includes dead embryos. Conform the k-factor analysis introduced by Varley & Gradwell (1960) the relative importance of these factors on hatching success in the Delta region was investigated (Rossaert *et al.*, in prep.). The k-values, sometimes referred to as "killing power", is simply $\log_{10} n_i - \log_{10} n_{i+1}$ were n_i = numbers in phase i and n_{i+1} = numbers in the next phase. Here, four k-factors have been defined for the phases between egg-laying and hatching: $k_{egg\ production}$, $k_{predation}$, $k_{disapperance}$ and $k_{embryo\ mortality}$.

The comparison between PCB concentrations in the individual hatchlings and the average hatching rate of the two eggs from the clutch they originated makes it possible to exclude the influence of variation between individuals and between nests.

3.3. Population dynamics

Under the assumption that migration is not of any importance equation [1] can be simplified to:

$$N_t = N_{t+1} + B - D \qquad [2]$$

and so $\triangle N = N_t - N_{t+1} = B - D \quad [3]$

For a breeding population with N_t individuals and a breeding interval of $\triangle t$ the per capita rate of increase r is:

$$r = (\triangle N/\triangle t)/N_t \qquad [4]$$

From the data on breeding pairs in the Delta region between 1925-1991 (Stienen & Brenninkmeijer, 1992; Meininger *et al.*, 1992) three periods are selected to determine r: 1925-1939 with an increase in numbers, 1950-1965 with a decline from *c.* 16.000 to 600 and 1975-1991 with an increase from 1200 to 5800. Data of numbers during World War II have been omitted because they are probably biased as censusing was limited in those days. Alternatively, numbers may indeed have decreased due to shooting and egg-collection as Stienen & Brenninkmeijer (1992) suggest.

Equation [2] can alternatively be written as:

$$N_{t+1} = N_t - B + D = N_t + r * N_t = (1 + r) * N_t = r' * N_t \qquad [5]$$

The Eigenvalue in the Leslie matrix model represents the average rate of

increase r'. If $r' > 1$ there is a population increase, if $r' = 1$ the population is stable and $r' < 1$ represents a declining population.
To apply the Leslie matrix model estimates of survival of juveniles S_j, subadults S_s and adults S_a are required as well as estimated of fecundity F. These have been collected from literature. A constant F is taken for adult birds and zero for younger birds. F, defined as chicks raised to fledging per pair per year, is a very difficult measure to determine in the field (Nisbet *et al.*, 1990) and consequently has a relative high error. But also reported estimates of survival are hard to obtain and moreover do show a wide natural variation.

The Leslie matrix model is formulated by M. van Boven (Tidal Waters division) in a mathematical package on PC.

3.4. Statistics

The difference in concentrations is determined with the t-test or in case of PCB-153 in eggs from Saeftinge and Philipsdam a nested analyses of variance has been used. Simple (non-)linear regression analysis and one way ANOVA was used to correlate concentrations of PCB-153 in eggs *vs* females and PCB-153 in the yolksac of hatchlings *vs* hatching success, hatching rate and $k_{embryo\ mortality}$.
The statistical analyses were performed with the statistical package SYSTAT (Wilkinson, 1990) and all tests were carried out at the 5% significance level unless otherwise indicated.

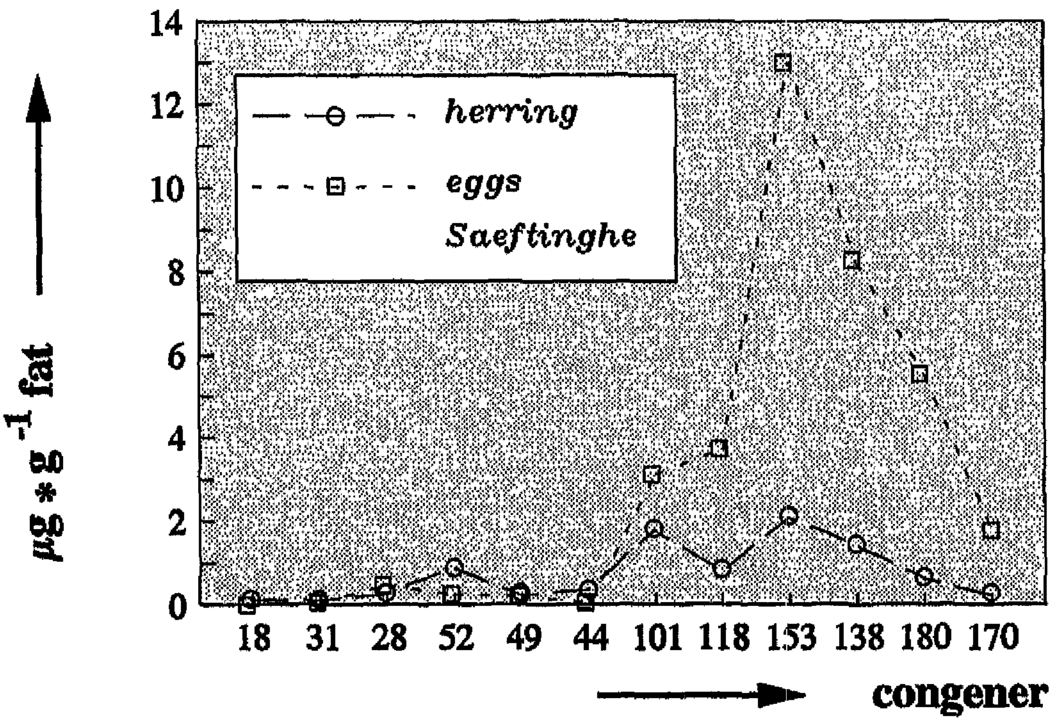

Figure 7 Pattern in geometric mean concentration of PCB congeners in herring and eggs of Common Terns from Saeftinge. The most persistent congener, PCB-153, shows the largest biomagnification factor.

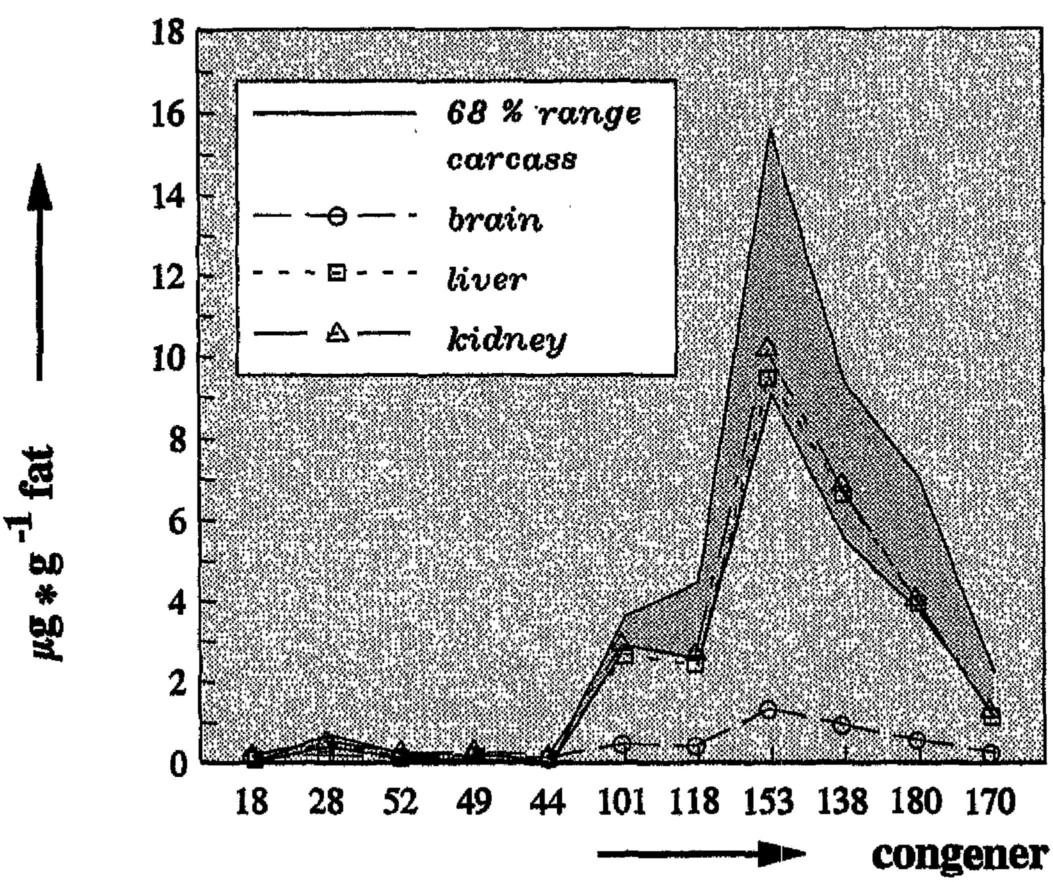

Figure 8 Pattern in concentration of PCB congeners in carcasses of female Common Terns from Saeftinge (n=5) and pooled samples of liver, kidney and brain. Levels in the brain are significant lower than in other tissues. Concentration in carcasses expressed as 68 % confidence interval of the mean.

4. RESULTS

4.1. PCBs in tissues and eggs of the Common Tern

Basic parameters and PCB concentrations in the carcasses of adults and chicks and in organs are presented in the appendices 1 (Saeftinge) and 2 (Philipsdam). Data of eggs and food items are shown in the appendices 3 (Saeftinge) and 4 (Philipsdam).

Figure 7 shows the PCB congeners analyzed in herring and eggs from Saeftinge. Concentrations of the lower chlorinated congeners are higher in fish than eggs. The dominant congener is PCB-153 with a biomagnification factor of six.

Concentrations in carcass, liver and kidney, when expressed on a lipid basis, are comparable but in the brain the higher chlorinated congeners are at a lower level (Fig. 8). Again, PCB-153 is the dominant congener.

Average concentrations of PCB-153 in total bodies of adults and chicks and in eggs are presented in Table 3, as well as the calculated body burden or egg content.
The average concentration of PCB-153 shows the following sequence:

male > female ≥ egg > chick.

The sequence in the body burden is:

male > female > chick > egg.

In females from Seaftinge the total body burden of PCB-153 is mainly (98.4 %) present in the carcass while brain, liver and kidney contain only 0.1 %, 1.3 % and 0.2 % respectively.

A comparison between the sexes on basis of body burden is made for data of Philipsdam exclusively as there is only one observation of males from Saeftinge. The concentration in males is approximately 1.4 times higher than in females. On average, the body burden in males have an excess of 48 μg PCB-153 compared to female Terns. This suggests the excretion of PCBs from the female body by laying eggs. Indeed, when the mass of PCB-153 in a clutch is added to the burden in females, no significant difference between the sexes is found. Moreover the concentration in eggs from both colonies do correlate with levels in the matching female (Fig 9).

The differences between the two colonies is most clearly demonstrated for

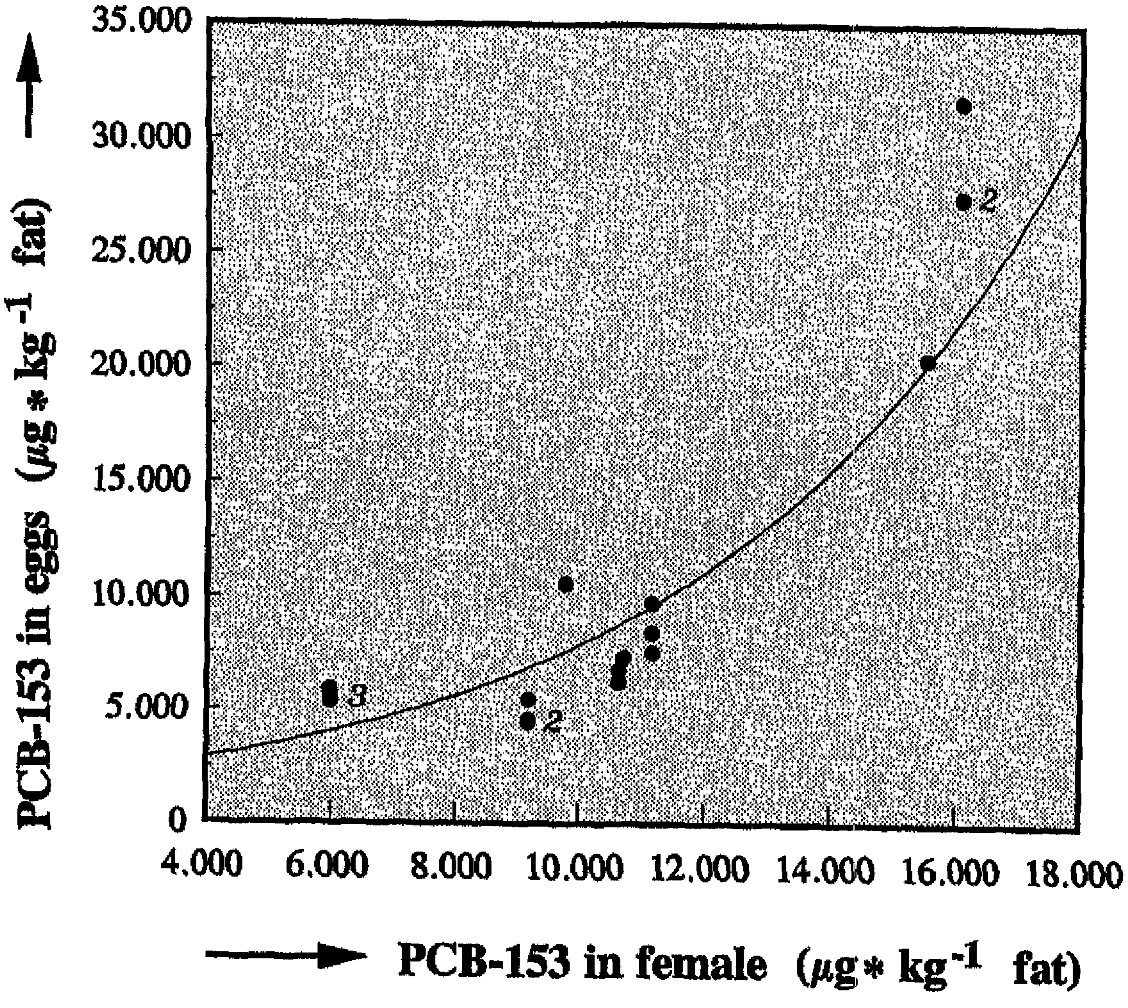

Figure 9 Concentrations of PCB-153 in eggs increase with increasing concentration of the female. ($Y = 1446 * e^{0.00017 * X}$; $R^2=0.80$, $n=8$, $P<0.01$). Numbers in the graph indicate numbers of eggs with equal concentrations.

concentrations in eggs. PCB-153 concentration in Seaftinge are 2.4 times higher than at Philipsdam (nested ANOVA: $P<0.01$).

Table 3. Concentration of PCB-153 (μg/g fat) and total body burden (μg) in adults, chicks and eggs from Saeftinge and Philipsdam (1987) (AVG = average, SD = standard deviation, n = number of observations).

	Concentration		Total Burden		
	AVG	SD	AVG	SD	n
Saeftinge					
- female	12.3 [d]	2.9	266	58	5
- male	16.9	-	288	-	1
- chick	5.0	-	52	-	1
- egg	15.2 [c,d]	8.1	24	9	6
- clutch of known female	-	-	67	28	5
Philipsdam					
- female	9.1 [a]	2.2	160 [b]	28	3
- male	12.8 [a]	1.6	215 [b]	33	3
- chick	1.6	-	32	-	1
- egg	6.2 [c,e]	0.6	12	2	5
- clutch of know female	-	-	35	6	3

a: significant difference between females and males ($0.05<P<0.10$)
b: significant difference between females and males ($0.01<P<0.05$)
c: significant difference between colonies ($P<0.01$)
d: no significant difference between females and eggs ($P>0.10$)
e: significant difference between females and eggs ($0.01<P<0.05$).

4.2. PCB-153 in yolksacs of hatchlings *vs* hatching success

Table 4 contains the data that have been used to compare the average breeding success per colony with the level of contamination with PCBs. The concentration of PCB-153 per hatchling with the hatching rate from the matching clutch are presented in Appendix 5.

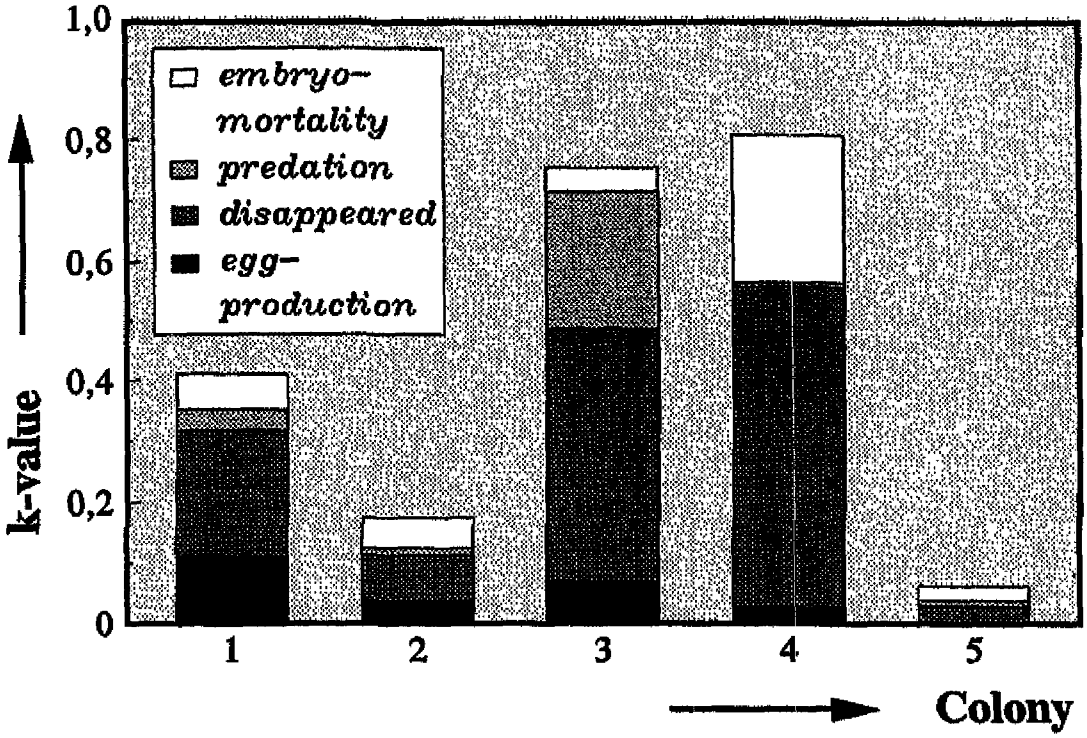

Figure 10 Lower egg production, physical disturbance, predation and embryo mortality all contribute to a reduction in hatching success. The $k_{egg\ production}$ indicates the deviation of the observed clutch size from the (maximum) clutch size of 2.76 in the colony Terneuzen. Flooding and rainfall (physical disturbance) are the major causes of loss.
(After: Rossaert et al, in prep.)

Table 4. Average clutch size, hatching success (1/clutch) and hatching rate (%) and PCB-153 concentration in yolksacs of hatchlings (ug/g fat) from colonies in the Delta region. Clutch size shows little variation but the hatching success shows marked differences between colonies. On average there is only a 3 fold variation in PCB levels.

	ALL CLUTCHES						3-EGG CLUTCHES				CLUTCHES USED FOR ANALYSES			
COLONY	CLUTCH SIZE		HATCHING SUCCESS		HATCHING RATE		HATCHING SUCCESS		HATCHING RATE		HATCHING RATE		PCB-153	
	avg.	n	avg.	n	avg.	n	avg.	n	avg.	n	avg.	n	avg.	n
1.	2.14	194	1.07	102	0.58	258	1.76	29	0.63	135	0.89	9	85.8	9
2.	2.54	420	1.84	103	0.84	500	2.46	50	0.86	377	0.92	7	147.1	7
3.[1]	2.44	245	0.82	126	0.40	359	1.40	43	0.57	208	0.00	4	46.2	4
4.	2.60	107	0.43	90	0.16	260	0.64	58	0.19	206	0.00	3	64.1	3
5.	2.76	145	2.39	113	0.86	360	2.62	90	0.88	315	0.81	8	67.8	8
6.	2.49	245	-	0	-	0	2.88	72	0.96	259	1.00	6	65.1	6
weighed avg.	2.48		1.33		0.61		2.09		0.73		0.73		84.1	

1) weighed average of 2 sub-colonies

No significant differences in clutch size between the colonies were found. Hatching success and hatching rate in the colonies Prinsesseplaat and Saeftinge were strongly depressed due to flooding after intensive rainfall and a high tide respectively (Rossaert *et al.*, in prep.). Because none of the 89 artificially incubated "Delta" eggs were unfertile (pers. comm. Bosveld), the occurrence of sterile eggs seems of little importance and therefore the observed hatching failure of eggs that were not washed out or predated is probably caused by embryo mortality.
The highest PCB-153 concentration in yolksacs of hatchlings is found for colony Slijkplaat and the lowest concentration for colony Prinsesseplaat. The concentrations in hatchlings from the colonies Saeftinge, Terneuzen and Zeebrugge are comparable.

No correlation was found between the average concentration of PCB-153 in yolksacs of hatchlings and the colony-averaged hatching success.

The influence on hatching success of 1. egg production, 2. physical disturbance, 3. predation and 4. embryo mortality is presented in figure 10. Data of colony Zeebrugge could not be used as only 3-egg clutches were investigated. The k-

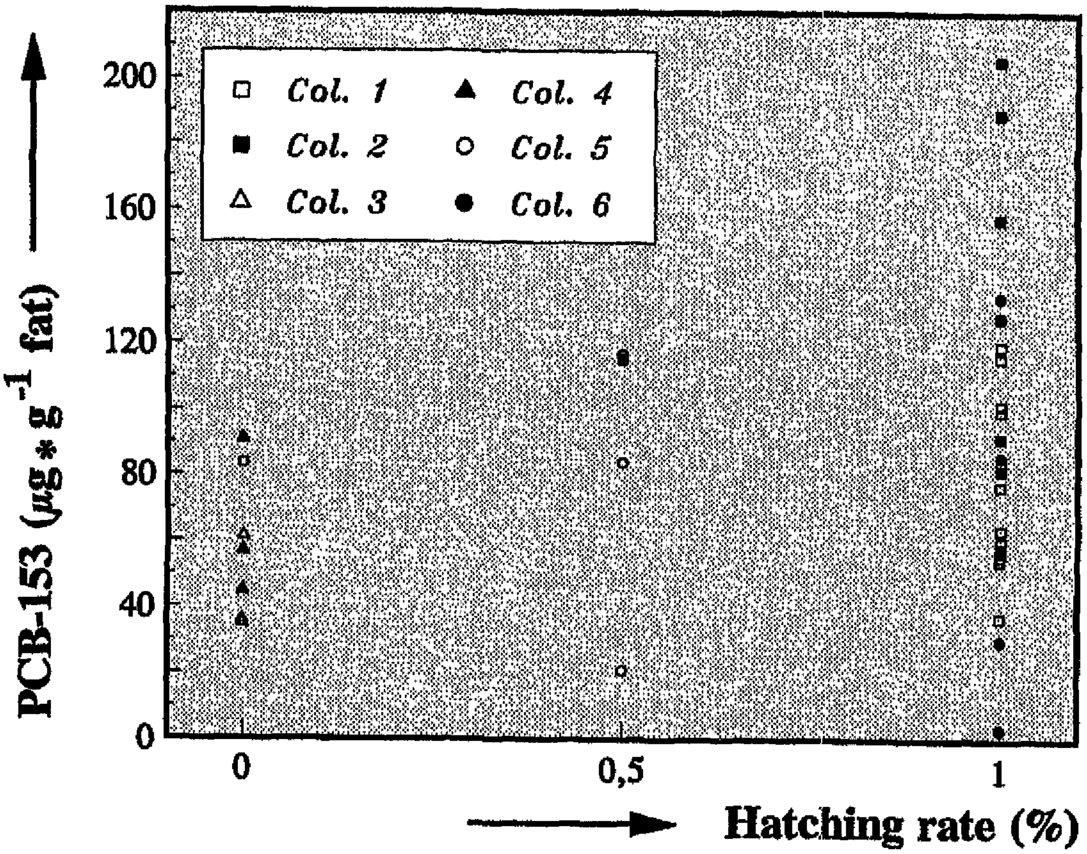

Figure 11 A comparison between the hatching rate of 2 remaining eggs and the concentration of PCB-153 in yolk sac of hatchlings from the same clutch. Data from Rossaert et al (in prep.) and from Bosveld et al (1992). No decrease in hatching rate is observed in clutches with an increasing exposure to PCB-153.

values of colony Prinsesseplaat is a number-weighed average from the two sub-colonies.
Indeed, the most important factor is the disappearance and damage by flooding or for unknown causes. No correlation was found between PCB-153 and $k_{embryo\ mortality}$.

The comparison between PCB-153 in the individual hatchlings and the average hatching rate of the two eggs from the clutch they originated makes it possible to exclude the influence of variation between clutches. Only those clutches that were not washed out could be used. The hatching rate of the two remaining eggs show no correlation with the concentration of PCB-153 in the matching hatchling (Fig. 11).

4.3. Population parameters

The estimates of survival and fecundity reported in literature are summarized in Table 5. No estimates of S_e have been found in literature and are therefore derived indirectly using the following data on survival between fledging until the age of maturity: 7-14% (DiConstanzo, 1980), 38% (Großkopf ,1964) and 25-35% (for Sandwich Tern; Veen & Faber, 1989). On average, this is *c.* 25%. Assuming that S_j = 0.5 (for Sandwich Tern; Veen & Faber, 1989) than S_e = $\sqrt{(0.25/0.5)}$ = 0.71. The maximum and minimum value of S_e is based on a survival until breeding age of 32 % and 15 % respectively.
The data show that survival of juveniles is low, higher for sub-adults and highest for adults until their maximal age of 25 years.

The estimates of S_j, S_e, S_a and F lead to a stable population size (r' = 0.995). Table 5 also present average rates of increase for several other combinations of the parameters. A maximal fecundity of 1.4, for instance, result in a 2% yearly increase. An improvement in survival of juvenile and subadults to e.g. 0.6 and 0.7 has a great influence on population size because the absolute numbers of individuals is high.

Figures 12 and 13 present the estimated population size in the Delta region between 1925-1991 and the calculated per capita rate of change (r). Between 1950-1965 the average rate of decrease is 0.15 per year, excluding the very low numbers in 1953 which is probably related to the loss of breeding sites after the storm flood. In the period 1925-1939 and 1975-1990 the average rate of increase is 0.06 and 0.10 per year respectively. In 1977 the high rate

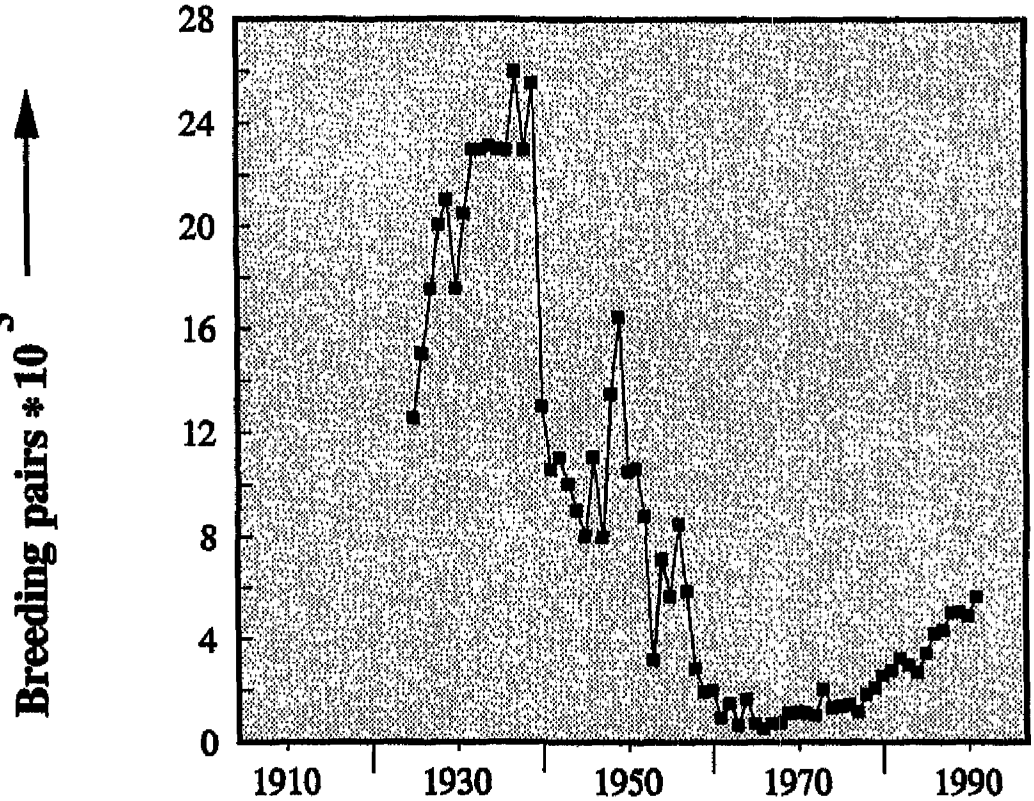

Figure 12 The number of breeding pairs of Common Tern in the period 1925-1991. The numbers in the 1920s and 1930s are rough estimates and during WOII unreliable. Major events are the increase between 1925-1939 and 1975-1991 and the decline between 1950-1966. After Kwint (1992) and Meininger et al. (1992).

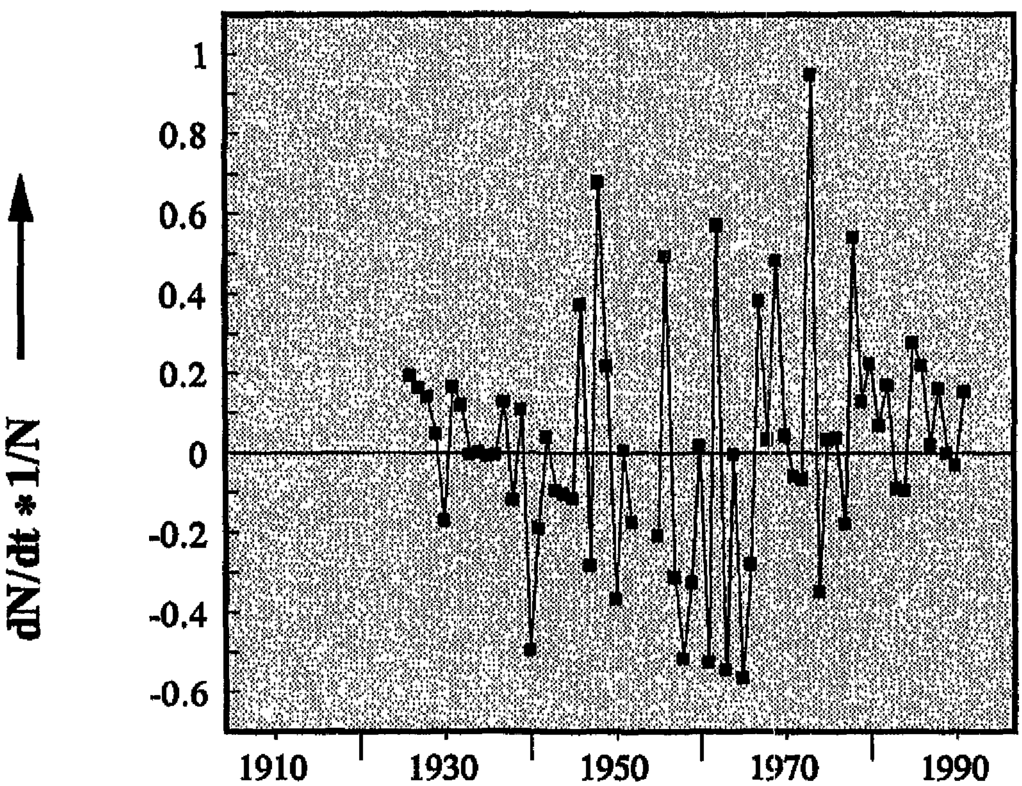

Figure 13 The capita rate of increase does not show a trend over time. In the three selected periods the average rates are +0,06 (1925-39), -0,15 (1950-66) and 0,07 (1975-1991 excluding 1977).

Table 5. Survival rates of juveniles (S_{j}), subadults (S_{s}), adults (S_{a}) and fecundity F (fledglings per pair) reported in literature. The estimates lead to a stable population size. Possible combinations of parameters are presented which result in a yearly population increase of 7% and 15% which occured in the Delta region in the period 1925-1939/1975-1991 and 1950-1966 respectively. The average rate of increase r' is calculated with a Leslie matrix model assuming that there is no density dependency, no migration, the first year of breeding is at 3 years and a maximum lifespan of 25 years.

	F	Sj	Ss	Sa	r'
estimate	1.1[ab]	0.5[e]	0.71[a,e,f]	0.88	0.995
range	0.6[c]-1.4[g]	0.25[a]-0.77[f]	0.53[a]-0.8[f]	0.82[f]-0.92[a]	
improved F	1.4	0.5	0.71	0.88	1.020
improved S_j and S_s	1.1	0.6	0.8	0.88	1.041
improved S_a	1.1	0.5	0.71	0.92	1.026
increasing Delta population	1.1	0.6	0.8	0.92	1.073
decreasing Delta population	0.6	0.25	0.53	0.86	0.853

a DiConstanzo (1980)
b Nisbet (1978)
c Becker (1991)
d Nisbet *et al.* (1984)
e Veen & Faber (1989)
f Großkopf (1964)
g Burger & Gochfeld (1991)

of increase suggest that immigration has taken place. Without this observation the average r between 1975-1991 is 0.07. For the three selected periods r is plotted against number of pairs (Fig. 14). The first period shows density dependence. No density dependence is observed between 1950-1966 and 1975-1991.

Unfortunately no data on S_j, S_s, S_a and F are available for the Delta region in neither period. Many combinations of long term reduction of fecundity and

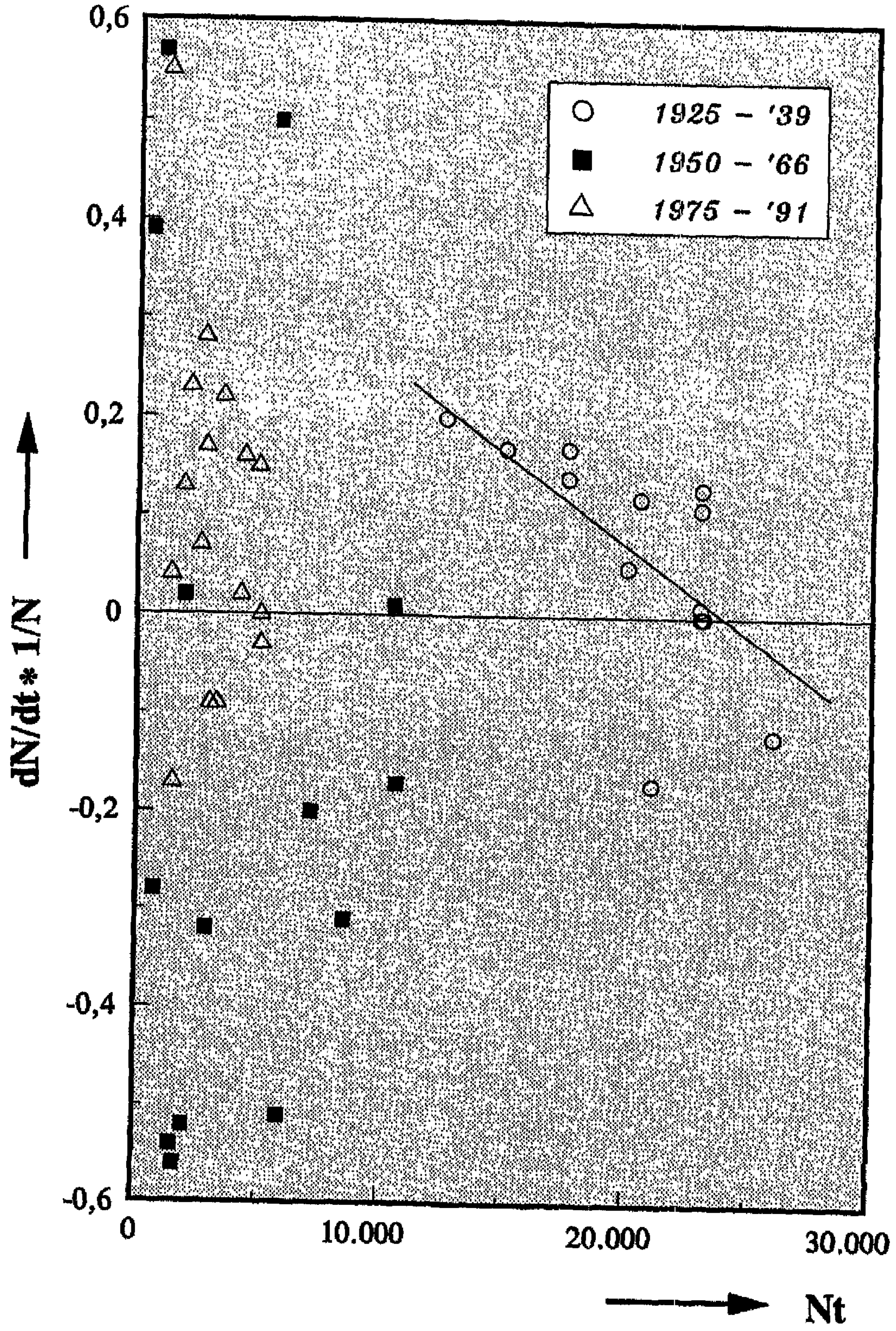

Figure 14 Investigation of density dependance in number of breeding pairs of Common Terns in the Delta region. The rough estimates of breeding numbers between 1925-1939 do show a significant relation ($P<0.01$). The carrying capacity of the region, at $y=0$, is 23.000 pairs.

survival can explain the downward trend in the period 1950-1966. For instance, the selected combination in table 5 of very low fecundity and maximum mortality of juveniles, subadults and adults results in the average r' of 0.85. When in the period 1975-1990 the survival rates Sj, Ss and Sa are at their estimate values, only an increase in fecundity up to 1.9 fledgling can explain the increase in breeding numbers. As this is above the expected maximal fecundity, survival must be higher than the estimated values. An alternative combination in given in table 5 were fecundity is at the estimate value and survival is at a maximal level.

5. DISCUSSION

5.1. PCB contamination

<u>the relation with the aquatic environment</u>

Although there has been a reduction in pollution with PCBs in the Rhine (Japerga *et al.*, 1990) and Scheldt (van Eck & de Rooij, in prep.) over the last 10-15 years these rivers still contain substantial levels in the abiotic compartments. Due to the hydrophobic and persistent character of PCBs and the processes of bioconcentration and biomagnification high concentrations do occur in tissues of fish eating birds like the Common Tern.
PCBs levels in eggs and hatchlings reflect the contamination in the aquatic environment (Custer *et al.*, 1985; Becker, 1991). In the Western Scheldt a seaward decrease in PCB concentration in water (van Zoest & van Eck, 1990) as well as in eggs of Terns and Black-headed Gulls (Stronkhorst *et al.*, in prep.) has been reported. Concentrations of PCBs in hatchlings from Saeftinge, Terneuzen and Zeebrugge, however, do not show this gradient (Bosveld *et al.*, 1992). Likewise, the difference in PCB-153 in eggs from Saeftinge and Philipsdam in 1987 is more pronounced than the difference observed in 1991 between the levels in hatchlings from both areas.

A female tern builds up a considerable PCB burden by consumption of small clupidea during courtship feeding, despite the fact that these young fish in the brackish and marine zone of the Rhine - Meuse - Scheldt Delta are less contaminated than fish with a long residence time. Connell (1988) pointed out that for compounds with a high log K_{ow} no equilibrium can occur between the ambient water and fish during its lifespan. Applying his model for PCB-153 with a log K_{ow} of 6.92, the estimated concentration in fish fat at 99% equilibrium is reached after 119 weeks. At an estimated average concentration in water of 0.045 ng/l (van Zoest & van Eck, 1989) and assuming that the bioconcentration factor BCF = log K_{ow} (Bruggeman, 1981) the calculated concentration in fish is 4.5 ug/g fat.
The bioconcentration of total PCBs in O-group herring was studied by ten Berge and Hillebrand (1974) in the Waddensea. Concentrations of total PCBs in fish were 44.1 mg/kg on March 12*th*, 210 mg/kg on April 13*th*, 1103 mg/kg on June 25*th*, 668 mg/kg on August 24*th* and 523 mg/kg on September 10*th* 1973. This suggests that during courtship feeding, which is taking place in April, the food items of Common Terns are relatively less contaminated. On a lipid basis, however, concentrations were 9.5, 22.8, 21.3, 28.5 and 17.5 mg/kg respectively. The authors concluded that no accumulation occurred due

to the fattening of the fish in the growth season.
In May 1990 the concentration of PCB-153 in herring which just immigrated from the North Sea and were caught near Saeftinge was 1.3 μg/g fat but the level in flounders which overwintered in the brackish zone was as high as 5.2 μg/g fat. This probably points out the difference in residence time, although it can not be excluded that the bottom living flounder, despite the intensive tidal mixing in the estuary, is exposed to higher ambient water concentrations.

the distribution in bird's tissue

Levels of PCBs in eggs show a correlation with concentrations in females. The great variation that is found in PCB levels in different clutches from one colony indicate the individual difference in female birds. More attention for inter-species variation is indeed required (Depledge, 1990). As this variation in eggs is identified for PCB-153 it probably is related to behavioral aspects of food selection while differences in metabolic activity between individuals (Walker, 1980) is expected to be of minor importance for this congener.
The difference in body burden between male and female Common Tern is explained mainly by the excretion in eggs. A clutch of 3 eggs contain approximately one third of the body burden of the female. Based on a autoradiographic investigation of the distribution of [^{14}C] HCB in Puffins (*Fratercula arctica*) Ingebrigtsen *et al.* (1984) suggested that the uropygial gland could be another important excretory pathway for lipophilic xenobiotics.

In conformity with other investigations (e.g. Renzoni *et al.*, 1986) lowest levels of the higher chlorinated PCBs have been observed in brain. Concentrations of PCB-153 in brain lipid are *c.* 12 times lower than in eggs. This is probably related to the fact that brain tissue contains more phosphorlipids (Boon *et al.*, 1989).

the critical phase

Concentrations in eggs show the strong biomagnification potency of PCBs. The bio-magnification factor (eggs:herring) from Saeftinge is *c.* 6 and is equal to the factor reported by Nisbet & Reynolds (1984). During incubation PCBs tend to accumulate in the yolksac. In yolksacs of hatchlings from Saeftinge and Prinsesseplaat the average PCB-153 concentration in 1991 was 64 μg/g fat (n=3) and 46 μg/g fat (n=4) respectively. These concentrations are 4-7 times higher than in eggs collected in Saeftinge and Philipsdam in 1987.
In an analyzed chick from Saeftinge the concentration of PCB-153 is comparable with the average of 5,01 μg/g fat in 2-4 weeks old chicks from the German Waddensea (Scharenberg, 1991). In Saeftinge and Philipsdam the concentration

in chicks is lower than in eggs which, according to Lemmetyinen & Rantamäki (1980), Lemmetyinen *et al.* (1982) and Scharenberg (1991), is the result of 'dilution' by growth. It is therefore likely that the impact of PCBs on reproductive success of the Common Tern is most pronounced directly after hatching when the chick starts to absorb the yolksac content.

risk assessment on basis of concentrations in eggs

Correlations between field observations of reproduction success of Forster's Tern (*Sterna forsteri*) and concentrations of AHH-active PCB congeners in eggs presented by Kubiak *et al.* (1989) can serve as an indication for the effect on Terns in the Delta region. PCB-105 and PCB-126 accounted for 90% of the calculated TCDD-equivalents based on the AHH induction potency. The comparison with the data presented here is limited to PCB-118 as this is the only AHH-active congener we analyzed, but could be false when congener-patterns in the comparing egg samples are not the same. Kubiak *et al.* (1989) reported no reproduction failure in the unpolluted colony Lake Poygan (USA) with a PCB-118 level of 0.08-0.30 μg/g WW. A reproductive impairment of 75% was observed in a polluted colony in Green Bay (Lake Michigan, USA) with a wide range in PCB-118 of 0.32-1.56 μg/g WW and a median value of 1.1 μg/g WW.

Eggs of Common Terns from Saeftinge contain 0.11-0.85 μg PCB-118/g WW with median value of 0.3 μg/g WW and eggs from Philipsdam contain 0.14-0.18 μg/g WW. This suggests that breeding success of terns at Philipsdam is not affected by PCB contamination, but may cause a deleterious effect on reproduction at the colony Saeftinge as it overlaps the range reported for Green Bay.

5.2. Effects of PCPs on hatchlings and reproductive success

effects at a cellular level

At the site of action the capacity of PCBs to actually cause a biochemical lesion depends first of all on the ability to combine with a receptor. At this level the EROD activity indicates the specific effects of inducing the monooxygenase (P450-IA1) system by planar PCBs and dioxins. However, it does not indicate a toxic effect but merely an adaptation to exposure.

In the study on Common Terns in The Netherlands Bosveld *et al.* (1992) found a significant correlation between EROD activity in livers of hatchlings and the concentration of mono-ortho PCBs. The mono ortho PCBs however explain only 16 % of the variation in EROD. The results suggest that the P450-IA1 in Common Tern is only inducible to a limited extent. Additional analysis of the

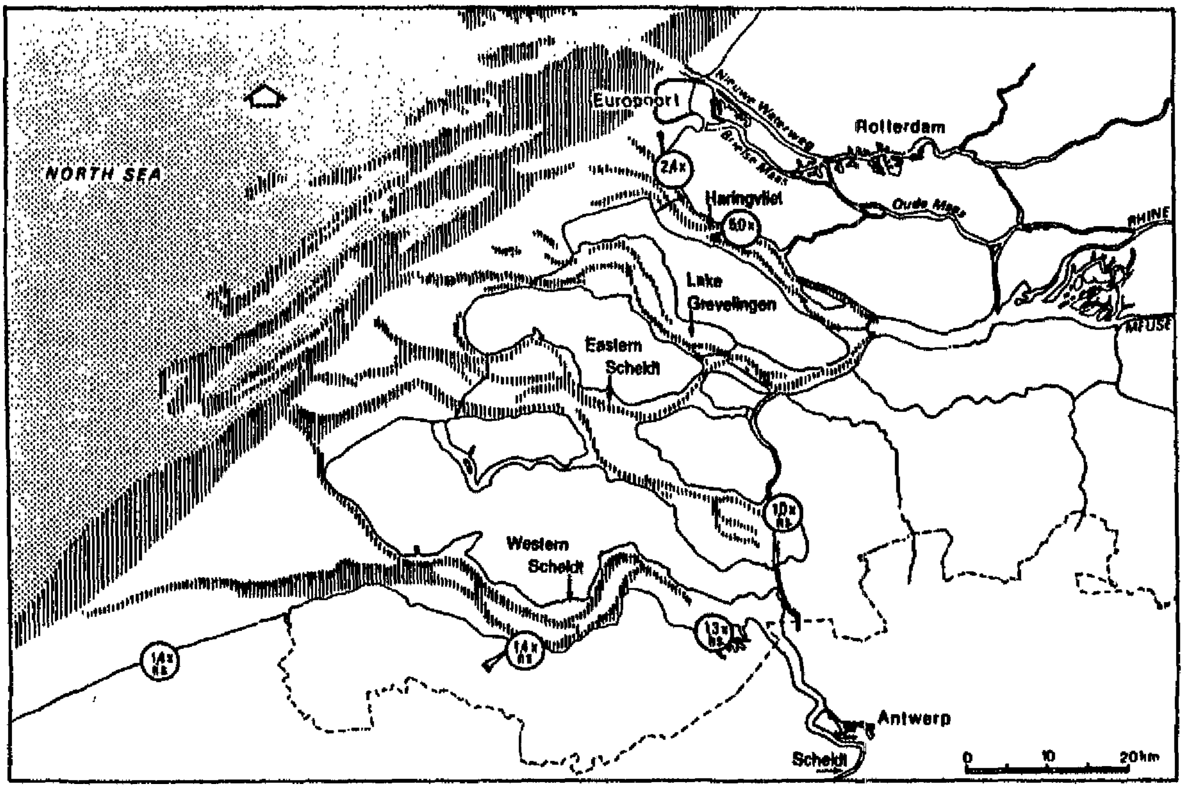

Figure 15 EROD activity in liver microsomes of hatchlings from colonies of Common Terns in the Delta region relative to a reference value of 130 pmol/min/mg protein. After Bosveld et al (1992). On average, only hatchlings from the colonies Slijkplaat and Westplaat show a significant induction of the P450-IA1 system.

planar PCBs and dioxins may improve the correlation.

In the colony Zeewolde the lowest PCB level in yolksacs of hatchlings (19 μg PCB-153/ g fat) was found with an average EROD activity of 130 pmol/min/mg protein. Comparable low levels have been reported for adult Black-tailed Gull in Japan (Yamasihita *et al.*, 1992), but is four times lower than in Cormorants from an uncontaminated site in The Netherlands (van der Gaag *et al.*, 1991). Regarding this EROD activity as a reference value the observations in the Delta region range from the reference level at Prinsesseplaat to 5 times reference activity at Slijkplaat (Fig. 15).

effects at a physiological and morphological level

After the biochemical lesion the effect can become physiologically or morphologically manifest but precise links are often unknown or not unique (Timbrell, 1991).

An elevated metabolism in eggs of Cormarants (*Phalacrocorax carbo*) was found for the PCB contaminated colony Biesbosch, The Netherlands (van der Gaag *et al.*, 1991). The same measurements were carried out in eggs of the Common Tern during their incubation in the laboratory (Bosveld *et al.*, 1992) but no effects on oxygen consumption and carbon dioxide production was found.

Terns have shown to be sensitive indicators for PCB-induced malformations (Environment Canada, 1991) but in the Delta region no malformation were observed in the field or in hatchlings in the laboratory (Bosveld *et al.*, 1992; Rossaert *et al.*, in prep).

the relation between PCB contamination and embryo mortality/hatching rate

Tillitt et al. 1992 recently reported a highly significant linear relation between H4IIE bioassay-derived TCDD-equivalents in eggs of the double-crested cormorant (*Phalacrocorax auritis*) and field observations of hatching success in colonies from the Great Lakes. Total PCB concentrations and egg mortality, however, correlated poorly. This suggest that the lack of correlation between concentrations of PCB-153 and hatching success of the Common Tern in the Delta region is due to the fact that PCB-153 does not represent the congeners with a dioxin-like activity. The determination of dioxin equivalents is currently under way. However, even if dioxin equivalent concentrations are available and would appear to be above a no-observable effect level for Common Terns, the change of finding a significant relation seems rather small because hatching rate was strongly influenced by physical disturbances. For instance, hatching rate in the most contaminated but undisturbed colony Slijkplaat in 1991 was 84%

while in the low contaminated colony Prinsesseplaat hatching rate was only 40% due to flooding after a period of rainfall (Rossaert *et al.*, in prep.). In colony Terneuzen hatching rate was as high as 86% but in the near-by colony of Saeftinge with equally contaminated hatchlings the hatching rate is only 16% due to flooding during a spring tide.

The k-factor analysis showed that embryo mortality in the field is of minor importance. No significant correlation between the concentration of PCB-153 and the $k_{embryo\ mortality}$ was established. During incubation in the laboratory embryo mortality only occurred in 3% of the eggs from the Delta region (pers. comm. B.A.T.C. Bosveld) indicating that an intrinsic toxic effect is absent.

As mentioned earlier, the variation in PCB contamination between clutches of one colony is high. The inter-colony variation was excluded by correlating the concentration of PCB-153 in individual hatchlings with the hatching rate of two remaining eggs in the clutch they originated. The fact that the two remaining eggs in the clutches in the colonies Prinsesseplaat and Saeftinge all failed to hatch, is probably a delayed effect of flooding (pers. comm. S. Dirksen). Again, no significant relation between PCB contamination and hatching rate was found.

In conclusion, these findings do not indicate toxic effects of PCBs at the present level of contamination of the Delta waters. The absents of any deleterious effects of PCB have also been reported for Common Terns in the German Waddensea and contaminated Elbe estuary (Becker, 1991) and for Forster's Tern in a contaminated Texas bay (King *et al.* , 1991). Nowadays, Terns are probably only affected by PCBs near hot-spots like the Green Bay, USA (Kubiak *et al.*, 1989).

5.3. Trend in breeding population

<u>reconstruction of the population decline in the Delta region</u>

Finally, PCBs can result in changes in population size when it causes mortality or when reproduction is affected. This is the ultimate level of interest in ecotoxicology. However, there are many factors controlling population size. So, while the ecological significance increases with progression from the biochemical level to population level the uncertainty of the causal relation with pollution increases in the same order (Sheehan *et al.*, 1984; Peakall, 1992). Moriarty (1990) also stated that effects of toxicants are often hard to discover in nature because they are comparable with natural stress or occur with a great natural biological 'noise'.

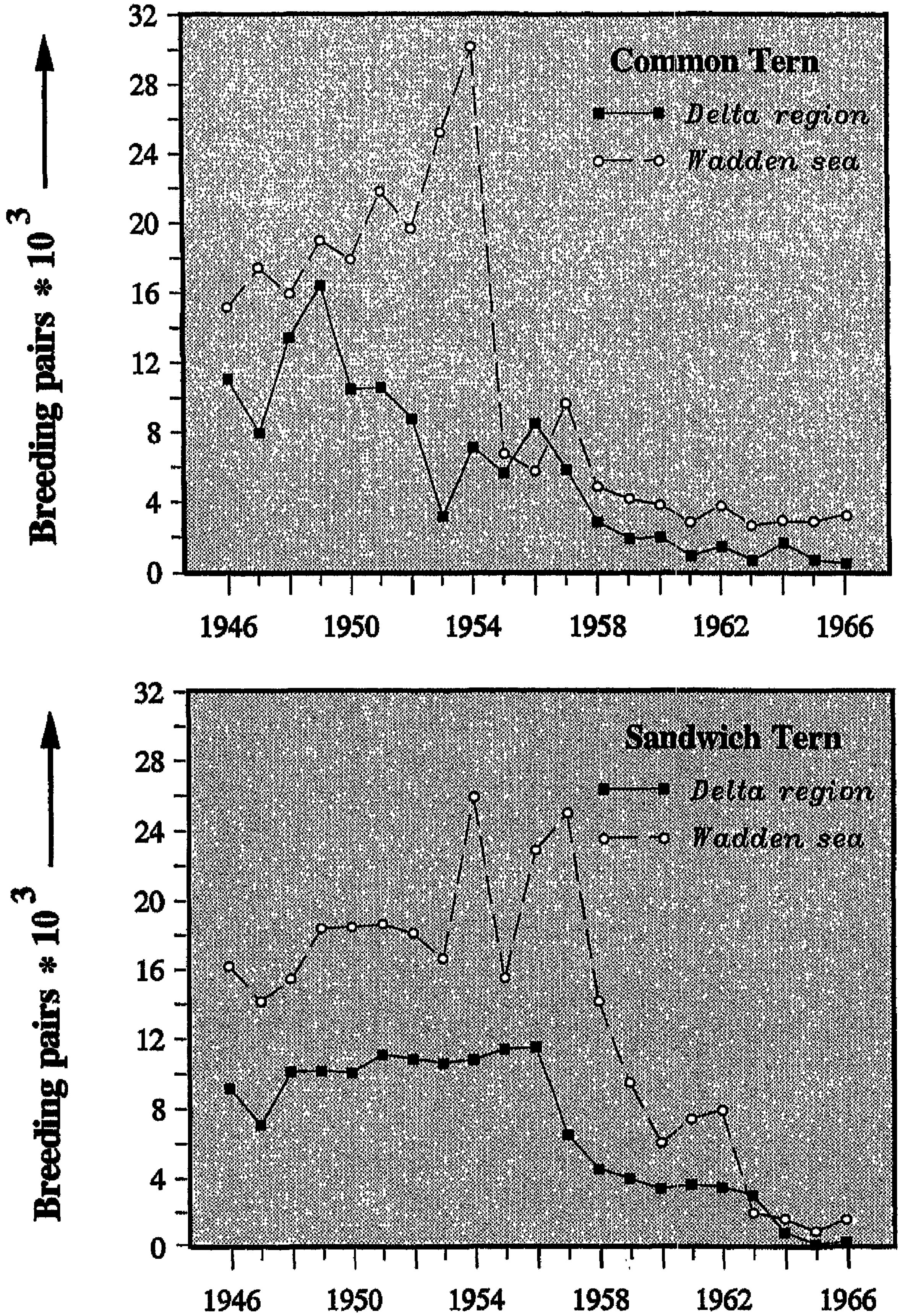

Figure 16 The numbers of breeding pairs of the Common Tern and Sandwich Tern in the Delta region and Wadden Sea between 1946 and 1966 (data from Stienen & Brenninkmeijer (1992) and Brenninkmeijer & Stienen (1992)).

The decline in the numbers of Common Terns in the Delta region occurred nearly simultaneous with a decline in the Waddensea and also with a drop in numbers of Sandwich Terns in both areas (Figure 16). Looking at these trends in more detail there are some remarkable differences noticeable.
Between 1946-1954 the Common Tern population in the Delta region fluctuated strongly while the Waddensea population showed a constant increase. Meanwhile, the population of the Sandwich Tern in the Delta remains stable.
In 1950 the Delta population dropped for elusive reasons because 1. the loss in breeding sites in that year (salt marshes of Zuid-Sloe) affected only a small (*c.* 100 pairs) colony, 2. organochlorine pesticides were not in mass production until 1954 and 3. PCB levels in sediment were about the same level as in 1991 and therefore indicate no effects.
In 1955, at a time the Delta-population was in the process of recovery from the loss of breeding sites caused by the storm flood of 1953, the Waddensea-population suffered a steep decline from *c.* 30.000 to *c.* 7000 pairs. The reasons for the decline are unknown (Stienen & Brenninkmeijer, 1992). After 1956 both Common Tern and Sandwich Tern in the Delta region show a downward trend, which also occurred in the Waddensea but started one year later.

It is difficult to reconstruct the environmental history of the Delta region in which the decline of the Terns between 1950 and 1965 has taken place. Here, causes of reproductive impairment or increased adult mortality have not been documented. In the Waddensea the decline of Sandwich Terns is ascribed to the discharge of telodrin and dieldrin from a production plant near Rotterdam (Koeman, 1971). The Waddensea was contaminated via a water linked transport by the north-bound residual current along the Dutch coastal zone. Data of both insecticides in blue mussels (*Mytilus edulis*) in 1965 presented by Koeman (1971) indicate that the average level in the Delta region did not differ significantly from the level in the Waddensea (0.47 and 0.41 mg dieldrin toxicity equivelent/kg WW respectively). Other sources may be involved as supported by the detection of dieldrin and telodrin in the top 10 cm of the saltmarsh sediment in Saeftinge in 1991 (up to 11 and 12 μg/kg DM respectively). The contamination with organochlorine pesticides may have increased the mortality of not only adults but also of young chicks. The Leslie matrix analysis showed that the decline with an average rate of decrease of 15 % between 1950-1966 can only be explained by a combination of low fecundity and low survival rates. According to the PCB analysis in sediments (Fig. 2) concentrations between 1965-1975 were twice as high as around 1950 and the late 1980s. For colonies like Saeftinge a doubling of PCB concentrations in eggs certainly would have implied reproductive impairment at the level that is observed in Green Bay

(Kubiak *et al.*, 1989).

In conclusion, the decline of the Common Tern could have been the consequence of pollution with organochlorine pesticides between 1957-1965 (increased mortality rates) followed by a suppression of a recovery due to PCBs in the late 1960s and early 1970s (decreased fecundity).

the recovery

After *c.* 1965 numbers of breeding pairs remained low for a decade and started to increase since *c.* 1975 at a time when pollution levels started to drop. According to Becker (1991) the lack of suitable habitats is the main limiting factor which jeopardizes an increase of the Tern population in the Waddensea at the moment. This seems not to be the case for Delta region, as there are still many suitable breeding sites available that can be occupied (pers. comm. P.L. Meininger).

Although immigration might have occurred during the recent period of increase (e.g. in 1977) it is thought that the recovery is the cumulative result of an improved fecundity and increased survival of fledglings, juveniles and adults. The Leslie matrix analysis shows that an improvement in survival of juveniles results in a relatively strong increase in numbers of breeders. This also indicates the importance of the conditions for Common Terns in the overwintering areas in W-Africa. Moreover, as Newton (1989) pointed out, the ultimate determinant of reproductive success in general is not the egg production or number of young that survive but the breeding lifespan which integrates the lifespan of a female, the rate of fertilization, frequency of mating and ability to give birth. Some 80% of the recruitment appeared to be produced by only 20% of the females, so population size relies on very small number of individuals (Newton, 1989).

If the rate of increase calculated for 1975-1991 remains constant (*c.* 0.07) than the carrying capacity of the Delta region of *c.* 23.000 pairs will be reached in a time span of approximately 20 years. However, density dependent processes will probably slow down this fast recovery.

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APPENDIX I. Basic paramaters and PCB concentrations (AFDW and fat basis) in tissues of Common Terns (*Sterna hirundo*) from Saeftinge (1987). (M = male, F = female, P = pull, liver5 = pooled sample of five livers).

AREA	WS.	WS.	WS.	4 WS.	WS.	WS.	WS.	WS.	WS.	WS.	WS.
SAMPLE No.	Mnr3	Fnr5	Fnr1	Fnr2	Fnr4	Fnr6	Fbrain5	Fliver5	Fkidney5	P(nr4)	Pliver
total wet weight(g)	125.78	139.41	129.93	138.78	131.75	129.29	2.55	8.402	2.5503	113.02	6.6429
ash in DW (%)	10.9	11.4	11.3	11.6	10.5	12.2		6.1	4.8	8.2	6.3
ash free dry in DW (%)	87.2	87	86.3	86.6	86.8	85.1	90	89.3	87.5	89	89.1
fat in DW (%)	33.9	33	42.4	45.2	43.3	39.6	28.9	14.2	7.5	23	3.4
PCB (ug/kg avdg)											
18	10	10	12	13	9	12	10	10	10	10	10
28	175	154	280	241	288	367	28	69	41	40	10
52	32	55	66	120	47	66	12	19	15	13	10
49	69	63	66	132	56	66	24	28	18	29	10
44	10	10	12	12	10	10	10	10	10	10	10
101	1028	1091	1737	1781	1434	1684	120	419	242	329	65
118	1920	1106	2197	1358	1486	2153	103	378	219	383	63
153	6574	4232	7629	4790	4874	7481	393	1504	864	1296	282
105			855	720	652	885					
138	4491	2729	4566	2756	2903	4409	268	1045	576	829	172
187			1525	915	850	1206					
180	3865	2611	3242	1937	1733	2873	143	624	338	597	103
170	885	509	1164	697	647	1016	50	182	101	116	20
PCB (ug/kg fat)											
18	26	26	24	25	18	26	31	63	117	39	262
28	450	406	570	462	577	789	87	434	478	155	262
52	82	145	134	230	94	142	37	119	175	50	262
49	177	166	134	253	112	142	75	176	210	112	262
44	26	26	24	23	20	21	31	63	117	39	262
101	2644	2876	3535	3412	2875	3619	374	2635	2823	1273	1703
118	4939	2916	4472	2602	2979	4627	321	2377	2555	1482	1651
153	16910	11157	15528	9177	9771	16077	1224	9458	10080	5015	7390
105			1740	1379	1307	1902					
138	11552	7195	9294	5280	5819	9475	835	6572	6720	3208	4507
187			3104	1753	1704	2592					
180	9942	6884	6599	3711	3474	6174	445	3924	3943	2310	2699
170	2276	1342	2369	1335	1297	2183	156	1145	1178	449	524

APPENDIX II. Basic paramaters and PCB concentrations (AFDW and fat basis) in tissues of Common Terns (Sterna hirundo) Philipsdam (1987). (M = male, F = female, P = pull, liver3 = pooled sample of three livers).

AREA	OS.	OS.	OS.	OS.	OS.	OS.	OS.	OS.	OS.	OS.
SAMPLE No.	M 59a	M 42	M 58	M lever 3	V 59b	V 41	V 46	V lever 3	P a	P lever
total wet weight(g)	126.86	125.58	125.14	7.5656	141.87	131.03	140.22	9.7764	126.22	8.1462
ash (%)	11.7	12.3	14.8	4.4	14.7	11.9	12.6	7.4	11.4	6.7
ash free dry (%)	85.9	81	82.6	89.7	83	85.3	82.2	87.1	86	87.5
fat (%)	37.3	28.4	34.6	18	36.3	34.1	27.7	18.2	38.9	7.4
PCB (ug/kg avdg)										
18	10	11	11	10	10	14	7	10	10	10
28	47	103	91	27	90	120	66	30	23	14
52	10	12	19	10	30	13	6	10	9	10
49	40	26	44	16	37	32	17	13	26	14
44	10	10		10	10	10	10	10	10	10
101	491	489	711	152	231	435	298	94	179	37
118	1160	1065	1487	293	739	969	874	249	195	55
153	4748	4395	6255	1311	2618	4251	3604	1088	731	222
105		277	344			276	140			
138	2749	2189	3314	715	1733	2113	1886	611	371	141
187		946	1400			740	616			
180	2976	1996	2787	562	1485	1803	1462	401	245	77
170	651	701	956	178	431	632	424	130	43	23
PCB (ug/kg fat)										
18	23	31	26	50	23	35	21	48	22	118
28	108	294	217	135	206	300	196	144	51	166
52	23	34	45	50	69	33	18	48	20	118
49	92	74	105	80	85	80	50	62	57	166
44	23	29	0	50	23	25	30	48	22	118
101	1131	1395	1697	757	528	1088	884	450	396	438
118	2671	3038	3550	1460	1690	2424	2594	1192	431	650
153	10934	12535	14932	6533	5986	10634	10695	5207	1616	2625
105		790	821			690	415			
138	6331	6243	7911	3563	3963	5286	5597	2924	820	1667
187		2698	3342			1851	1828			
180	6854	5693	6653	2801	3395	4510	4338	1919	542	910
170	1499	1999	2282	887	985	1581	1258	622	95	272

APPENDIX III. Basic parameters and PCB concentrations (AFDW and fat basis) in eggs and some food items of Common Terns (*Sterna hirundo*) from Saeftinge (1988).

AREA	WS.	WS.	WS.	WS.	WS.	WS.	WS.	WS.	WS.	WS.	WS.	WS.
SAMPLE No.	E v2 1	E v2 2	E v2 3	E v5 1	E v5 2	E v5 3	E v1 1	E v3 1	E v4 1	E v6 1	E v6 2	E v6 3
total wet weight(g)	18.65	19.00	19.14	20.90	20.75	20.96	18.42	18.35	21.45	16.61	17.41	16.93
ash (%)	4	4	3.9	4.2	4.1	4	5.6	5.5	5.9	6.4		
ash free dry (%)	95	93.8	95.1	93.7	94	91.8	86.9	91.5	92.8	90.4	94.29	93.69
fat (%)	35.2	39.2	40.6	36.2	38.9	37.5	33	38.6	40.4	27.9	30.91	29.23
PCB (ug/kg avdg)												
18	10	10	10	10	10	10	10	9	9	9	4	3
28	37	54	64	252	178	134	147	230	225	293	406	331
52	77	102	114	80	68	86	61	142	54	60	97	77
49	55	79	104	62	45	57	49	63	42	56	89	70
44	10	10	10	10	10	10	10	10	10	10	9	7
101	519	662	847	1056	773	945	1150	1845	1215	1608	2071	1638
118	403	463	649	1166	972	891	2265	1980	1423	2384	3114	2444
153	1708	1871	2349	3781	3147	3468	7765	7551	4629	8494	10394	8595
105							759	911	526	953	1051	812
138	1060	1295	1410	2860	2439	2309	4670	4581	2749	5024	6611	5314
187							1418	1465	713	1374	1775	1521
180	888	844	1042	1952	1942	1569	2950	3351	1700	3483	3620	3047
170	155	157	177	698	726	316	1124	1231	551	1250	1425	1199
PCB (ug/kg fat)												
18	27	24	23	26	24	24	26	21	21	29	13	11
28	100	129	150	652	430	328	387	545	517	949	1240	1060
52	208	244	267	207	164	211	161	337	124	194	297	247
49	148	189	244	160	109	140	129	149	96	181	270	223
44	27	24	23	26	24	24	26	24	23	32	28	24
101	1401	1584	1984	2733	1868	2313	3028	4374	2791	5210	6319	5249
118	1088	1108	1520	3018	2349	2181	5965	4694	3269	7725	9500	7834
153	4610	4477	5502	9787	7605	8490	20448	17899	10633	27522	31708	27548
105							1999	2159	1208	3088	3206	2604
138	2861	3099	3303	7403	5894	5652	12298	10859	6315	16278	20167	17032
187							3734	3473	1638	4452	5415	4874
180	2397	2020	2441	5053	4693	3841	7768	7943	3905	11285	11042	9766
170	418	376	415	1807	1754	774	2960	2918	1266	4050	4347	3842
weight yolk (g)	15.71	17.22	17.26	18.86	18.85	19.18	7.46	12.9858	16.26	4.48	7.31	5.6
weight embryo(g)	0.00	0.00	0.00	0.00	0.00	0.00	9.56	3.8538	3.45	10.61	8.74	9.77
egg length (mm)	40.6	42.5	42.9	43	42.4	41.6	41.6	42.5	45.3	40.2	39.5	41
egg breath (mm)	30.1	29.9	29.9	31.3	31	31.6	30.3	29.6	30.9	29.8	30.4	29.6
shell thickness(mm)	0.155	0.167	0.161	0.170	0.167	0.157	0.139	0.154	0.165	0.141	0.143	0.144
shell weight (g)	1.07	1.03	1.03	1.24	1.24	1.12	0.99	1.03	1.07	0.87	0.94	0.87
Ratcliff (mg/mm2)	0.87	0.81	0.80	0.92	0.95	0.85	0.79	0.81	0.77	0.73	0.79	0.72

continu APPENDIX III

	WS. herring	WS. herring	WS. shrimp	WS. mussel
ash (%)	12.8		23.2	21
ash free dry (%)	82.4	85.31	70.3	71.7
fat (%)	13.0	9.8	0.5	3.2
PCB (ug/kg avdg)				
18	17	13	1	1
28	33	33	1	2
52	114	123	6	21
49	32	29	1	12
44	45	48	2	8
101	268	209	5	64
118	108	103	11	35
153	314	247	27	108
105		68		
138	198	180	22	76
187		47		
180	95	75	27	17
170	33	27	9	4
PCB (ug/kg fat)				
18	108	116	141	22
28	209	291	141	45
52	723	1072	844	471
49	203	253	141	269
44	285	417	281	179
101	1699	1818	703	1434
118	685	894	1547	784
153	1990	2146	3796	2420
105				
138	1255	1566	3093	1703
187				
180	602	649	3796	381
170	477	221	1265	90

APPENDIX IV. Basic parameters and PCB concentrations (AFDW and fat basis) in eggs of Common Terns (Sterna hirundo) from Philipsdam (1987).
(explanation sample: E=egg, v41=nest, 1=egg no.)

AREA	OS.	OS.	OS.	OS.	OS.	OS.	OS.	OS.	OS.	OS.	OS.
SAMPLE No.	E v41 1	E v41 2	E v41 3	E v59 1	E v59 2	E v59 3	E v42 1	E v46 1	E v58 1	shrimp	mussel
total wet weight(g)	19.1493	19.1185	19.8056	19.6871	18.2175	19.4737	18.6109	20.2796	19.9924		
ash (%)	3.8	4.1	4	4.1	4.1	4.1	5.6	5.3	5.2	26.1	12
ash free dry (%)	94.2	95.1	94.4	93.5	94	95	93.6	94	92.7	69.4	81
fat (%)	36.8	33.9	37.4	35.8	38.8	38.1	47.4	40.2	37.6	0.9	2.43
PCB (ug/kg avdg)											
18	11	8	16	13	10	10	9	9	9	0.5	1.8
28	70	61	83	74	77	84	56	98	95	10	3.1
52	10	11	11	13	10	11	10	5	19	3.7	5.4
49	37	37	49	44	32	38	29	40	105	4.5	5
44	10	10	10	10	10	10	10	10	10	0.5	2.6
101	276	243	326	315	279	281	325	348	618	11.5	11.2
118	771	645	795	607	708	634	654	799	649	8.6	11.5
153	2476	2233	2701	2268	2354	2168	2850	3146	2414	26	23.2
105							221	224	224	32.3	
138	1681	1412	1724	1470	1692	1487	1511	1646	1294	16.5	21.9
187							611	551	601	10.1	
180	1062	1062	1036	927	1066	882	1193	1140	768	12	
170	392	335	361	283	423	352	448	463	343	6.5	4.7
PCB (ug/kg fat)											
18	28	22	40	34	24	25	18	21	22	39	60
28	179	171	209	193	187	209	111	229	234	771	103
52	26	31	28	34	24	27	20	12	47	285	180
49	95	104	124	115	78	95	57	94	259	347	167
44	26	28	25	26	24	25	20	23	25	39	87
101	707	682	823	823	676	701	642	814	1524	887	373
118	1974	1809	2007	1585	1715	1581	1291	1868	1600	663	383
153	6338	6264	6817	5923	5703	5406	5628	7356	5952	2005	773
105							436	524	552	2491	
138	4303	3961	4351	3839	4099	3708	2984	3849	3190	1272	730
187							1207	1288	1482	779	
180	2718	2979	2615	2421	2583	2199	2356	2666	1893	925	
170	1003	940	911	739	1025	878	885	1083	846	501	157
weight yolk (g)	16.026	17.4176	17.8948	16.8546	16.7723	17.9409	16.7323	18.2396			
weight embryo(g)	0	0	0	1.2987	0	0	0	0			
egg length (mm)	42	40.1	42.9	45.2	42.3	43.6	42	45.2	40.7		
egg breath (mm)	29.9	30	30	29.7	29	30.3	29.4	29.9	31.6		
shell thickness(mm)	0.16	0.14	0.14	0.14	0.14	0.14	0.15	0.15			
shell weight (g)	1.14	0.96	1.11	0.94	0.99	0.99	0.98	1.11			
Ratcliff (mg/mm2)	0.91	0.80	0.86	0.70	0.80	0.75	0.79	0.82			

APPENDIX V. Data of PCB-153 (ug/g fat) in yolksacs of hatchlings and hatching rate (fraction of remaining two eggs that hatched) in the matching clutch.

CLUTCH	PCB-153	HATCHING RATE	COLONY No.
w12	83.385	0.0	1
w13	54.258	1.0	1
w2	99.890	1.0	1
w3	81.848	1.0	1
w4	76.911	1.0	1
w5	83.465	1.0	1
w7	119.442	1.0	1
w8	116.104	1.0	1
w9	57.161	1.0	1
h13	141.332	.	2
h15	189.446	1.0	2
h3	115.375	0.5	2
h4	128.136	1.0	2
h7	157.632	1.0	2
h8	91.811	1.0	2
h9	205.661	1.0	2
p2	60.959	0.0	3
p3	35.853	0.0	3
p4	34.728	0.0	3
p6	53.390	.	3
s1	44.825	0.0	4
s2	90.700	0.0	4
s3	56.667	0.0	4
t13	116.545	0.5	5
t1	63.669	1.0	5
t2	60.070	1.0	5
t4	83.683	0.5	5
t5	58.353	1.0	5
t6	101.816	1.0	5
t7	20.827	0.5	5
t8	37.038	1.0	5
z1	81.615	1.0	6
z2	55.534	1.0	6
z3	30.130	1.0	6
z4	134.205	1.0	6
z6	3.499	1.0	6
z7	85.902	1.0	6