CHAPTER 4

EFFECTS OF POLYHALOGENATED AROMATIC HYDROCARBONS AND RELATED CONTAMINANTS ON COMMON TERN REPRODUCTION; INTEGRATION OF BIOLOGICAL, BIOCHEMICAL AND CHEMICAL DATA*

ABSTRACT

In eight Dutch or Belgian common tern (*Sterna hirundo*) colonies, breeding biology and food choice were determined, and 15 second eggs were collected from three-egg clutches for artificial incubation, biochemical analysis and analysis of yolksac PHAH levels. Results from these analyses were combined with biological data from the eggs remaining in each clutch. In some breeding colonies severe flooding, rainy and cold weather, and extreme predation caused extensive losses of eggs and chicks. A relationship was found between yolksac mono-*ortho*-(mo-)PCB levels and main food species (fish or insects) of the adult terns before egg-laying. Colony average breeding data differed only slightly, and were difficult to relate to PHAH-levels. When the colonies were grouped after yolksac PHAH-patterns and main food species, significant differences in average egg laying date, incubation period, egg volume and chick weight could be related to differences in yolksac PHAH and retinoid levels, and hepatic EROD activity. The data from all colonies also were combined into one data-set and correlated with the biochemical parameters and PHAH levels. In summary greater yolksac PHAH levels or hepatic EROD-activity correlated with later egg laying, prolonged incubation period and smaller eggs and chicks. Lesser yolksac retinoid- and plasma thyroid hormone levels, and a greater ratio of plasma retinol over yolksac retinoids correlated with later egg laying, prolonged incubation periods and smaller chicks and eggs.

The dynamic environment of the terns had more obvious detrimental effects on breeding success than PHAHs. However, the more subtle effects observed for PHAHs could still be of importance during specific stress circumstances. To monitor site-specific reproduction effects, tree-nesting birds feeding on relatively big and non-migrating fishes would be most suitable. The use of specific biomarkers for exposure and effect is recommended to establish a causal relationship between a certain class of pollutants and an adverse biological effect.

**Based on: A.J. Murk, T.J. Boudewijn, P.L. Meininger, A.T.C. Bosveld, G. Rossaert, T. Ysebaert, P. Meire, S. Dirksen (1996): Effects of Polyhalogenated Aromatic Hydrocarbons and Related Contaminants on Common Tern Reproduction: Integration of Biological, Biochemical and Chemical Data. Arch. Environ. Contam. Toxicol. 31:128-140*

INTRODUCTION

Empirical field studies and toxicological studies suggest that birds can be affected by lipophilic persistent pollutants, such as polyhalogenated aromatic hydrocarbons (PHAHs) and pesticides, that accumulate through the aquatic food chain. In birds PHAHs have been associated with adverse effects such as impaired reproduction, growth retardation, morphological abnormalities, behavioural changes and alterations in vitamin A and thyroid hormone metabolism (Gilbertson and Fox 1977; Koeman *et al.* 1973; Hoffman *et al.* 1987; Gilbertson 1989; Gilbertson *et al.* 1989; Kubiak *et al.* 1989; Spear *et al.* 1990; Walker 1990; Fox *et al.* 1991; Fox 1993; Murk *et al.* 1994a; Van den Berg *et al.* 1995). Although the use and release into the environment of polychlorinated -biphenyls (PCBs), -dibenzofurans (PCDFs) and -dibenzo-*p*-dioxins (PCDDs) is restricted now, levels in depositional zones of the North Sea and in top predators have hardly decreased (Cummins 1988; Klamer *et al.* 1991; Becker *et al.* 1992; Evers *et al.* 1993). Additionally, other PHAHs such as polybrominated diphenyl ethers and polychlorinated terphenyls, have been shown to exert similar effects, probably through the same mechanism (Safe *et al.* 1990 1994; Murk *et al.* 1996b). This means there is still concern for PHAH-effects on top-predators.

To set quantitative and verifiable ecological objectives for the Dutch management of the North Sea, adjacent salt waters and inland waters, 60 indicator species have been selected that are vulnerable to various ecological disturbances and represent a cross section of the ecosystem (Ten Brink *et al.* 1991). The common tern (*Sterna hirundo*) has been recently added, being a specialized top predator of the aquatic food-chain in estuarine areas. Literature data suggest that terns are sensitive for effects of PHAH contamination. For Forster's terns positive correlations have been observed between levels of non- and mono-*ortho*-PCBs and decreased hatchability, increased incubation period and decreased parental attentiveness (Kubiak *et al.* 1989), and between hepatic arylhydrocarbon hydroxylase (AHH) activity (as a measure of PHAH exposure) and weight loss and abnormal functioning of the thyroid gland (Hoffman *et al.* 1987). Becker *et al.* (1991) reported a reduced hatching success for common terns which correlated with increased PCB levels. This reduction was, however, not statistically significant. Common terns offer the advantage of easily accessible nests, as they breed on the ground. Within a few weeks after they arrive from their wintering sites in West and South Africa (Cramp 1985), the terns store reserves for the breeding season. They forage mostly within 10 km from their breeding place (Stienen and Brenninkmeijer 1992; Rossaert *et al.* 1993), therefore their diet is expected to reflect the local degree of contamination. The PCB-118 levels in common tern eggs from the brackish zone of the Dutch Western Scheldt estuary

(0.1-0.7 $\mu g \cdot g^{-1}$ WW, Stronkhorst *et al.* 1993) partially overlap the range reported for Green Bay (0.32-1.56 $\mu g \cdot g^{-1}$ ww) where 75% reproductive impairment was observed (Kubiak *et al.* 1989). Therefore deleterious effects cannot be excluded.

An integrated ecotoxicological study was carried out in 1991 to study the possible effects of PHAHs on the reproduction and development of common tern chicks. Eight colonies were studied, which were expected to cover a gradient in pollution levels. The colonies were (Figure 4.1): Saeftinge, Terneuzen and Zeebrugge in the Western Scheldt area, situated along a gradient towards the North Sea; Prinsesseplaat near the Eastern Scheldt; Slijkplaat and Westplaat in the mouth of the rivers Rhine and Meuse; the isle of Griend in the Wadden Sea and Zeewolde, a relatively clean 'fresh-water colony'. For these colonies the food species, feeding areas and fate of eggs and young were studied. From all colonies the second egg of 15 three-egg-clutches were collected for artificial incubation and chemical and biochemical analysis. More detailed results of the Cytochrome P450IA activity, plasma thyroid hormone levels, T4-glucuronyltransferase (UGT)-activity, plasma and yolk-sac vitamin A and yolk-sac PHAH levels, laboratory incubation period and correlations are described by Murk *et al.* (1994b) and Bosveld *et al.* (1995). This paper focuses on the relationship between the breeding biology in the field, food species before egg laying, and biochemical data and PHAH levels from the (artificially incubated) second egg of the same clutch. More detailed additional observations in the colonies have been reported by Rossaert *et al.* (1993).

MATERIALS AND METHODS

Colonies Studied

Saeftinge is a typical brackish water tidal area in the eastern part of the Western Scheldt estuary (Figure 4.1). There is a high tidal amplitude of 4.5. The birds mainly foraged in the Western Scheldt. The colony of Terneuzen is situated in the middle of a sluice-complex. There is no danger of flooding. The terns in Terneuzen were more aggressive towards intruders than in any of the other colonies. The sluice complex and the Western Scheldt were the main foraging areas. The Zeebrugge colony is situated in the harbour of Zeebrugge and holds over 50% of the Belgian breeding population of the common tern. Most of the common terns foraged in the North Sea, but during early breeding season several terns were also foraging in adjacent fresh water areas. The Prinsesseplaat is a tidal flat which separates the freshwater lake 'Zoommeer' from the Eastern Scheldt. The common terns bred in two (sub-)colonies. Generally the birds foraged in the Eastern Scheldt, but in early May many terns were foraging on emerging Chironomids in the

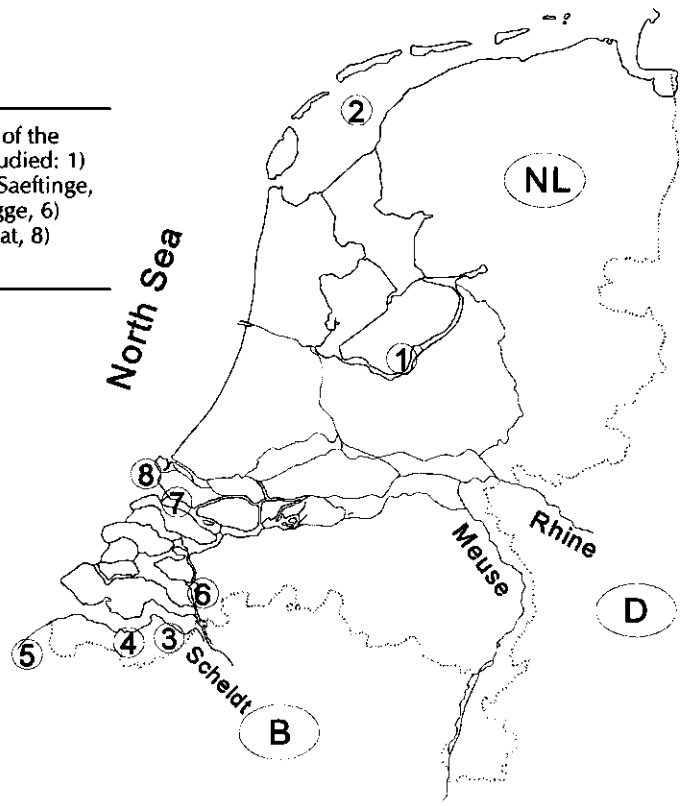

Figure 4.1 Locations of the common tern colonies studied: 1) Zeewolde, 2) Griend, 3) Saeftinge, 4) Terneuzen, 5) Zeebrugge, 6) Prinsesseplaat, 7) Slijkplaat, 8) Westplaat.

Zoommeer. The Slijkplaat is a sandy, shallow flat, situated in the western part of the Haringvliet, a freshwater area with a tidal amplitude of 30-40 cm. The Haringvliet-sluices is the most important foraging area, especially during low tide when (fresh) water of the Haringvliet is discharged into the sea. Westplaat is a man-made bird island along the North Sea. During spring tides the lower parts of the island are flooded. Foraging took place in the surroundings of the Westplaat and near the Haringvliet-sluices when these were discharging into the sea. The Zeewolde colony is situated on the top of a sand depot along a dyke, ca. 2.5 m above surface level. The common terns foraged in the fresh-water lakes Wolderwijd and Veluwemeer around the colony. Shortly before egg-laying, hundreds of common terns were seen foraging on emerging chironomids. Griend is a small island in the western part of the Dutch Wadden Sea. The terns mainly forage within the Wadden Sea in water above tidal flats and in gullies (Klaassen 1992).

Weather Conditions

Data on daily precipitation, temperature and wind speed for the period May 1st-July 31st 1991, were extracted from monthly reports of the Royal Netherlands Meteorological

Institute (KNMI). May 1991 was one of the coldest Mays of this century in The Netherlands, with an average temperature of 10°C. Night frost occurred on a large scale until the end of the month. There were 153 hr of sunshine compared to an average of 205 hr. June 1991 was extremely wet, very cold and exceptionally cloudy. It was the wettest Dutch June of the century with 122 mm of precipitation, against 62 mm normally. There were 119 hr of sun compared to 207 hr normally. Due to this combination it was considered the worst June of this century, resulting in very unfavourable conditions for young birds. July 1991 was warm, sunny and generally dry, although there were several days with heavy showers during the first half of the month.

Field Procedures and Number of Visits

The observations in the colonies started late April-early May 1991 during pair-formation and ended in the second half of July when chicks had fledged. Nests were marked with numbered wooden stakes placed 20-30 cm from the nests. During each visit new eggs were marked, and the number of eggs, damage and/or mortality and number of hatched eggs were recorded. During the peak of egg-laying the nests were checked daily (Saeftinge, Terneuzen and Zeebrugge) or every two days in the other colonies. Before and after this peak the colonies were checked less frequently. The visits were carefully planned in order to avoid disturbance and temperature stress of eggs and chicks as much as possible. During visits in and after the hatching period, young terns were searched and checked for developmental abberations, total weight, wing length and total head length. They were banded, or marked with a little picric acid when they were too small for banding. Wherever possible observations on feeding behaviour and prey selection were made.

Recorded Breeding Data

The date the first egg appeared was used to index the laying date of the clutch. In some cases the laying date was calculated from the exact laying date of another egg using the average laying period for an egg, or from the hatching date of the first hatched egg using the average incubation period per egg. To minimize the chance of underestimating the clutch size due to predation or the fact that clutches might not yet have been completed, the content of all marked nests was recorded during all visits. The egg volume was calculated using the Hoyt (1979) formula: volume (ml) = 0.509 x length (cm) x $(\text{width})^2(\text{cm}^2)$. The length and width were measured with sliding callipers to the nearest 0.1 mm. The incubation period of every individual egg was calculated as the time (days) between completion of the clutch and hatching, provided that the exact laying and hatching dates were known.

Laboratory Incubation and Analyses

In each colony, the second egg of 15 three-egg clutches of which the exact laying date of every egg was known, was collected after at least 7 days of incubation. In the laboratory the eggs were weighed and incubated at 37.5°C and a relative humidity of 50-60%. Eggs were turned automatically every 30 min. Within 12 hr after hatching the young terns were weighed and sacrificed. Livers and yolk-sacs were weighed immediately, snap frozen in liquid nitrogen and stored at -70 °C. Blood was centrifuged and the plasma was stored at - 20°C. Of the hatched eggs the eggshell thickness was measured at the equator of the egg after the shell membranes were removed, using a Mitutoyo micrometer.

Yolk-sac PCB, PCDD and PCDF residues were analyzed in yolk-sacs by GC-MS (Bosveld *et al.* 1995). Total levels of mono-*ortho*-(mo-)PCBs were used, or the individual PHAH levels were transformed into Toxic Equivalents (TEQs), using Toxic Equivalency Factors (TEFs) as proposed by Safe (1990, 1994). Di-*ortho*-PCBs were not used in TEQ calculations. Liver microsomes were prepared as described in Bosveld *et al.* (1995). Protein content was analyzed according to the method described by Bradford (1976). 7-ethoxyresorufin *O*-deethylation (EROD) and 7-pentoxyresorufin *O*-depenthylation (PROD) activities were analyzed fluorimetrically according to the method of Rutten *et al.* (1987).

Plasma thyroid hormones levels, total thyroxin (TT4), total triiodothyronine (TT3) and free thyroxin (FT4), were determined in, respectively, 10, 25, and 25 μl aliquots of plasma, by chemiluminescence immunoassay using commercially available kits (Amerlite assay kits, Amersham Internat. plc., Amersham, UK). Plasma retinol and yolk-sac retinylpalmitate were extracted from 25 μl aliquots with methanol/diisopropylether and analyzed with HPLC as described by Murk *et al.* (1994b). The ratio plasma retinol over retinylpalmitate was calculated as a measure of mobilisation of retinol to the circulation or decreased retinoid ester storage.

Statistical Methods

Differences between clustered data were tested with one-way ANOVA. In addition to comparing colony averages, the data from all colonies were combined into one data set and correlated with the biochemical and chemical parameters from the artificially incubated chicks. The biochemical and chemical data from the artificially incubated chicks were divided into 4 groups based on the data for breeding biology in the field. Attention was paid to an equal distribution of the numbers of biochemical and chemical data over the four groups. The acceptance level was set at $P<0.05$. Clear, but not statistically significant, differences that could be related to differences in PHAH contamination are mentioned as 'trends'.

RESULTS

Comparison of parameters for breeding biology

The colony average breeding biology parameters measured in the field are summarized in Table 4.1. The colonies are arranged in order of increasing colony average yolk-sac mono-*ortho*-(mo-) PCB levels. Although the onset of egg laying was well synchronized among the colonies (13-15 May, only Saeftinge and Zeebrugge somewhat later; data not shown) the average laying date of the first egg showed more variation. Westplaat was the latest of all colonies. The average clutch size was largest in Terneuzen and smallest in Kleine Prinsesseplaat and Westplaat. The average egg volume is only calculated of three-egg clutches, because egg volume partially depends on the number of eggs already layed. The eggs were largest in Terneuzen and smallest in Saeftinge, Westplaat and Prinsesseplaat (Table 4.1). Most colonies showed a decrease of egg volume in clutches which were started later (data not shown, Rossaert *et al.* 1993). The average laying period needed to complete a three-egg clutch varied between 2.59 days (Zeewolde) and 3.50 days (Westplaat) (data not shown). The average incubation period of three-egg clutches was longest on Terneuzen and shortest on Griend. The observed differences in colony averages in breeding biology could not be related to differences in average yolk-sac PHAH-levels.

Large differences in hatching success of three-egg clutches were found between Kleine Prinsesseplaat (0.00) or Saeftinge (0.64) compared to Zeebrugge (2.88) and Terneuzen (2.62) (Table 4.2). However, adverse climatological effects and sometimes extreme predation negatively influenced reproductive success in several colonies (Table 4.2). In the Prinsesseplaat colony heavy showers produced a layer of up to 10 cm of water which sometimes took days to disappear, resulting in massive loss of eggs and chicks. Strong winds in combination with spring tide caused additional losses of clutches in Saeftinge and Griend. Severe predation by oystercatchers (*Haematopus ostralegus*) was observed in the Prinsesseplaat colony. A major problem in the Slijkplaat colony was the rapid growth of thistles. Nests got completely overgrown and were difficult to relocate by the observers and sometimes deserted by the birds. In the Zeebrugge, Terneuzen and Westplaat colonies the adverse factors were not extreme and did not result in abnormal losses. In none of the colonies studied hatching failure was a major cause of egg-loss.

The adverse factors mentioned above make it difficult to relate average breeding biology data to differences in PHAH-contamination. Therefore, from here on calculations are restricted to the three-egg clutches that were not reduced by the factors mentioned

Table 4.1 Breeding biology (average ± SD): colony size, date of laying of the first egg, clutch size, egg volume and incubation period of the first egg of all monitored clutches. The colonies are arranged in order of colony average yolksac mono-ortho (mo-) PCB levels, going from relatively low (6 μg/g lipid in Zeewolde) to high (40 μg/g lipid in Slijkplaat) levels.

Colony	Colony size (clutches studied)	Laying date first egg[a] (± days) (month-day)	Clutch size[a] (eggs)	Egg volume[b] (ml)	(N)	Incubation period[b] first egg (days)	(N)
Zeewolde (Z)	317 (317)	6-4[c] ± 17.8▲^{T}	2.55 ± 0.66	19.74 ± 1.40	(337)	21.83 ± 1.18	(60)
Prinsesseplaat (P)	181 (181)	6-1[d] ± 12.0	2.44 ± 0.67	19.23 ± 2.03	(29)	22.00 ± 0.00	(2)
Kleine Pr.plaat (K)	64 (64)	5-21 ± 3.5	2.11 ± 0.78▼^{ZPBATS}	19.92 ± 1.32	(55)	--	--
Griend (G)	1500 (30)	5-18 ± 1.2	2.37 ± 0.49	19.69 ± 1.20	(33)	21.11 ± 0.88▼^{ZBATWS}	(19)
Zeebrugge (B)	650 (245)	5-25 ± 4.4	2.49 ± 0.79	19.73 ± 1.40	(462)	21.88 ± 0.94▼^{W}	(186)
Saeftinge (A)	107 (107)	5-25 ± 1.7▼^{ZT}	2.60 ± 0.66	19.33 ± 1.42▼^{ZKBTS}	(221)	22.89 ± 0.93▲^{ZB}	(92)
Terneuzen (T)	145 (145)	5-31 ± 9.8	2.76 ± 0.56▲^{ZPKGBWS}	20.12 ± 1.53▲^{ZPCBAWS}	(321)	23.38 ± 1.31▲^{ZGBWS}	(132)
Westplaat (W)	195 (195)	6-8 ± 19.0▲^{ZATS}	2.14 ± 0.75▼^{ZPBATS}	19.25 ± 1.47▼^{ZKBTS}	(183)	22.39 ± 1.50	(33)
Slijkplaat (S)	420 (420)	5-26 ± 10.3▼^{ZT}	2.54 ± 0.63	19.59 ± 1.43	(278)	22.00 ± 1.67	(44)

[a] All clutches; [b] 3-clutches only; [c] Two distinct peaks of laying; [d] Settled late, from K
▼^{x} Significantly less than colony x
▲^{x} Significantly more than colony x
-- No incubation completed

in Table 4.2, and from which the second egg was collected for laboratory incubation. The field data from these clutches can easily be compared with the laboratory data of the collected second egg. Additionally, these three-egg clutches were layed during the peak periods of laying in each colony, which is a limited period of time. This is important as egg volumes, clutch size and laying period were found to be related (Rossaert *et al.* 1993). Of these selected three-egg clutches colony averages in breeding biology data were also calculated (data not shown). The average laying dates of the first egg were significantly earlier for Zeewolde, Griend and Slijkplaat compared to the other colonies, but these differences could not be related to differences in PHAH levels. The average incubation period of the first egg was significantly shortest (21 days) on Griend and longest in the Slijkplaat colony (25 days). The average egg volumes and average eggshell thickness (1.3-1.4) did not differ significantly between the colonies. No morphological abberations were observed in the field nor for the eggs that were artificially incubated.

Comparison of Food Choice between Colonies

Due to circumstances differing between colonies, the quality and quantity of the data on food and foraging is different for each colony. However, the data enable at least a qualitative description for all colonies. In general, common terns from coastal colonies mainly foraged on clupeids (herring (*Clupea harengus*) and sprat (*Sprattus sprattus*)), smelt (*Osmerus eperlandus*), sandeel (*Ammodytes spec.*), goby (*Gobius spec.*), whiting (*Merlangius merlangus*), and to a lesser extent on small flatfish, three-spined stickleback (*Gasterosteus aculeatus*), Ruffe (*Gymnocephalus cernuus*) and crustaceans. Especially in the Zeewolde, but also in the Prinsesseplaat colony, emerging chironomids were an important prey in the beginning of the breeding season. For the Prinsesseplaat colony the fish species were not identified. Figure 4.2 presents the main food choice of adults in each colony in the period before egg laying, combined with the average yolk-sac mo-PCB levels. The average yolk-sac mo-PCB levels were least when adults had foraged on large amounts of insects. Yolk-sac mo-PCB levels were highest in the Westplaat and Slijkplaat (significant) colonies where smelt and herring were the main food items. Clupeids were the main food species in the four colonies with intermediate PCB-levels.

Clustering of Colonies Based on Food Choice and PHAH-Pattern

Based on the PCDD/F patterns in yolk-sacs established by principal component analysis (PCA, Bosveld *et al.* 1995), three groups of colonies can be distinguished coinciding with mainly smelt, clupeids, or insects as food choice before egg laying. This PCA revealed that the Slijkplaat and Westplaat colonies, were clearly distinct from all other colonies, and the Zeewolde colony could be distinguished from the other colonies. The five

Table 4.2 Hatching success (average ± SD) and adverse climatological and ecological factors that influenced the reproductive success.

Colony	Hatching success of 3-clutches	(N)	Specific climatological and ecological factors
Zeewolde (Z)	2.24 ± 1.69	(82)	Many dead young due to cold and rainy wheather, many second clutches
Prinsesseplaat (P)	1.88 ± 1.34▼^{ZS}	(32)	Losses of eggs and young through flooding due to rainfall in June
Kleine Pr.plaat (K)	0.00 ± 0.00▼^{ZPGBATWS}	(11)	Heavy egg predation (mainly by a few oystercatchers)
Griend (G)	2.50 ± 0.58	(4)	Losses of young through flooding by high tide (6-13), predation by gulls
Zeebrugge (B)	2.88 ± 0.33▲^{ZPKAW}	(72)	– (some predation by black-headed and herring gull)
Saeftinge (A)	0.64 ± 1.02▼^{ZPGTWS}	(58)	Massive loss of clutches through flooding by high tide (6-13)
Terneuzen (T)	2.62 ± 0.79▲^{ZPKAW}	(90)	– (disturbance through human activity on sluices)
Westplaat (W)	1.76 ± 1.30▼^{ZS}	(29)	– (some nests were washed away or covered under sand)
Slijkplaat (S)	2.46 ± 0.95	(50)	Rapid growing thistles covered many nests, some were abandoned

– No specific adverse climatological or ecological circumstances that resulted in abnormal losses
▼^{x} Significantly less than colony x
▲^{x} Significantly more than colony x

remaining colonies (Prinsesseplaat, Griend, Zeebrugge, Saeftinge and Terneuzen) exhibited a similar PCA-pattern. The average data were recalculated for three groups of colonies: Slijkplaat/Westplaat, Zeewolde and 'Rest'. In contrast to the colony averages, the group-averages show clear differences in both breeding, biochemical and chemical data (Table 4.3). In the Zeewolde colony eggs are layed significantly earlier and are significantly bigger than in the other two groups. Yolk-sac PHAH levels (expressed either as TEQs or as mo-PCBs) and hepatic EROD activity are significantly least and yolk-sac retinylpalmitate levels and vitamin A2 levels are significantly greatest in the Zeewolde colony. In the Slijkplaat/Westplaat group incubation period is significantly longer, chickweight significantly less, and yolk-sac PHAH and hepatic EROD activity significantly greater than in the other two groups. Plasma FT4 levels are greatest in the Zeewolde group and least in the Slijkplaat/Westplaat group, but these differences were not statistically significant.

Clustering of Data Based on Parameters for Breeding Biology

Laying Date. The biochemical and chemical data grouped after laying date of the first egg are presented in Table 4.4. Later layed eggs contained slightly (38%) more PHAH-residues (expressed either as mo-PCBs or as TEQs) than early layed eggs. Earlier eggs (on average 137 days after January 1st) contained significantly (82%) greater yolk-sac retinyl

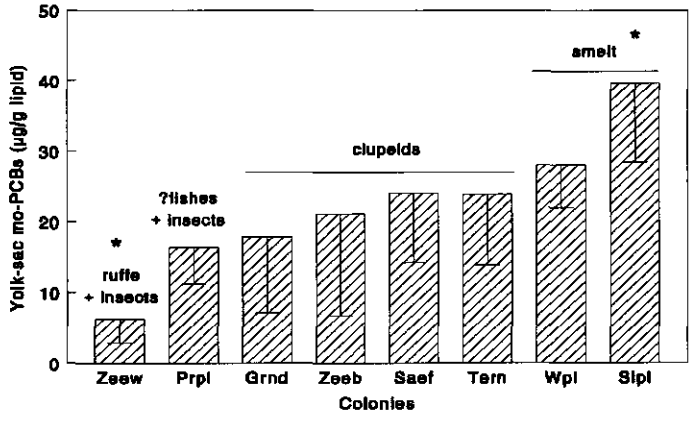

Figure 4.2 Colony average yolksac mo-PCB levels with main food items before egg-laying written above the bars: ruffe (*Gymnocephalus cernuus*); smelt (*Osmerus eperlanus*); clupeids: herring (*Clupea harengus*) and sprat (*Sprattus sprattus*); insects (mainly chironomids) and ?fishes (fish species unknown).
*: mono-ortho (mo-) PCB levels significantly different from the other colonies.

Table 4.3 Group averages (± SD) of breeding data (a) and chemical and biochemical data (b) for colonies grouped after their PHAH-pattern.

A

Colony	Laying date first egg ± SD (days)	Incubation period first egg (days)	Egg volume second egg (ml)	Chick weight second egg (g)
Zeewolde (Z)	127.2 ± 3.6▼^{RS}	21.6 ± 1.2	20.7 ± 1.2▲^{RS}	14.5 ± 1.3
Rest (R)	144.7 ± 4.3	22.0 ± 1.3	19.7 ± 1.4	14.2 ± 1.2
Slijk-/ Westplaat (S)	141.4 ± 3.4	23.1 ± 1.9▲^{ZR}	19.2 ± 1.3	13.4 ± 0.9▼^{ZR}

B

Colony	mo-PCBs µg/g lipid	TEQs ng/g lipid	EROD act. nmol/g.min	Yolks.RP (ng/g)	Yolksac. Vit A2 (ng/g)	Plasma FT4 (pmol/l)
Zeewolde (Z)	6.22 ± 3.30▼^{RS}	3.724 ± 1.763▼^{RS}	138 ± 47	1.18 ± 0.91▲^{R}	13.3 ± 5.6▲^{RS}	5.39 ± 1.54
Rest (R)	20.52 ± 10.20	10.427 ± 4.592	158 ± 78	0.62 ± 0.41	6.4 ± 2.1	4.85 ± 2.29
Slijk-/ Westplaat (S)	32.84 ± 9.80▲^{ZR}	14.112 ± 5.571▲^{ZR}	399 ± 292▲^{ZR}	0.86 ± 0.60	6.8 ± 1.7	4.78 ± 1.70

▼^{X} Significantly less than group X
▲^{X} Significantly more than group X

palmitate levels, 69% greater vitamin A2 levels and an almost 2-fold smaller ratio plasma retinol over yolk-sac retinylpalmitate, than latest layed eggs (on average 150 days). No clear trend was visible for plasma thyroid hormone levels TT4 and FT4. Plasma TT3 levels were slightly greater in earlier layed eggs. The incubation period for the earliest eggs was on average 2 days shorter compared to the latest layed eggs.

Incubation Period. When clustering data based on incubation period in the field, significantly greater yolk-sac mo-PCB or TEQ levels (100%), or hepatic EROD activities (60%) were found in eggs needing 24 days or more for incubation compared to eggs needing on average 20 days (Table 4.5). Because of the small number of eggs for which both incubation period and yolk-sac retinoids were measured, these data were divided over only two groups. Yolk-sac retinylpalmitate and vitamin A2 levels were (not statistically significant) greater in the shortest incubation group. The ratio plasma retinol over yolk-sac retinylpalmitate, however, was significantly greater (3.2-fold) in the longest incubation group. Plasma thyroid hormone (TT3, TT4, FT4) levels were all significantly (50-70%) less in the two longest incubation groups compared to the two shortest incubation groups. The eggs from the shortest incubation group were layed significantly earlier (on average 4 days) than the eggs from the longest incubation group. The chicks (Table 4.5) and eggs (data not shown) from the 2 longer incubation groups were slightly smaller compared to the 2 other groups.

Figure 4.3 shows the ratio of the incubation period of the first egg of a clutch in the field over the incubation period of the second egg in the laboratory incubator. This ratio increases significantly with the incubation period in the field.

Egg Volume and Chick Weight. As average egg volume (whole clutch) and chick weight (second egg) are closely related, these parameters are presented in combination. Yolk-sac PHAH-levels (expressed either as mo-PCBs or as TEQs) were slightly greater (36%) in the smallest eggs and chicks compared to the bigger eggs (Table 4.6a) and chicks (data not shown). Slight differences in average EROD activity were only observed for different chick weight groups (Table 4.6b) but not for different egg volume groups (data not shown). Yolk-sac retinoid levels were slightly greater and the ratio plasma retinol over yolk-sac retinylpalmitate levels less in the biggest eggs. Not enough data were available to make a grouping for yolk-sac retinoid levels based on chick weight. Differences in plasma thyroid hormone (TT4, TT3, FT4) levels that could be related to PHAH contamination, were only observed between groups clustered after chick weight. These differences were only significant for FT4. Egg volume, chick weight and liver weight were significantly correlated with each other. The ratio liver weight over chick weight was on average 2.66-2.73 %, and did not differ between any of the groups.

Table 4.4 Chemical and biochemical data (average ± SD) grouped after egg laying dates (days after January 1st).

Parameter	Group 1 (early) [137.2-137.6][a]		Group 2 [140.1-141.5][a]		Group 3 [143.2-147.3][a]	Group 4 (late) [145.6-150.3][a]	N
mo-PCBs (μg/g)	18.96 ± 10.77		23.07 ± 17.84		23.94 ± 8.19	25.55 ± 11.49	12/13
TEQs (pg/g)	8508 ± 4851		11229 ± 7259		12217 ± 3702	11640 ± 4603	9/10
EROD act. (nmol/g.min)	162 ± 144		294 ± 260		185 ± 77	186 ± 88	18/19
ys ret.palmitate (ng/g)	0.89 ± 0.36	▲[3,4]	1.06 ± 0.47	▲[3,4]	0.42 ± 0.23	0.49 ± 0.42	9/10
ys vit A2 (ng/g)	9.90 ± 2.43	▲[2,3,4]	6.32 ± 1.34		6.13 ± 1.36	5.86 ± 2.06	9/10
plasma retinol (ng/ml)	96.9 ± 53.2		98.1 ± 34.7	▲[3,4]	79.7 ± 22.7	76.9 ± 29.1	23/24
ratio pl.re./ys.r.palm	118 ± 98	▼[4]	146 ± 133		202 ± 98	230 ± 82	9/10
plasma TT3 (nmol/l)	3.32 ± 1.49		3.22 ± 1.51		2.88 ± 1.48	2.78 ± 0.97	22/23
incubation period (days)	21.6 ± 1.84	▼[4]	21.6 ± 1.02	▼[4]	22.3 ± 0.82	23.4 ± 1.49	12/13

[a] The average group-laying dates are not the same for all parameters, as the grouping is performed per parameter, and the total number of data available per parameter varies.

▼[x] Significantly smaller than group x

▲[x] Significantly larger than group x

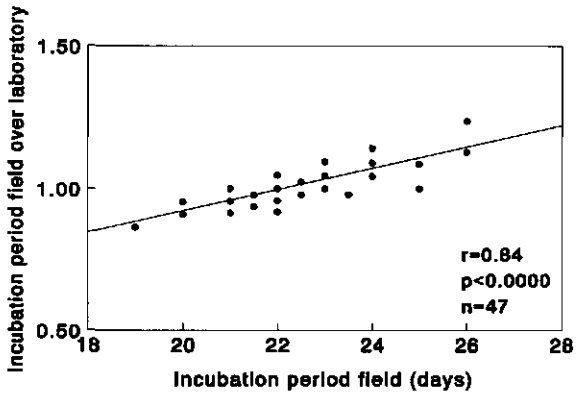

Figure 4.3 The ratio of the egg incubation period in the field over the incubation period in the laboratory incubator, plotted against the incubation period in the field (n = 47).

Discussion

Due to their way of nesting on bare ground close to the shore, common terns are relatively vulnerable to climatological influences and predation if compared to, for example, tree-nesting cormorants. Massive flooding, severe predation and extreme rainy and cold weather had strong negative effects on clutch size, and on hatching and fledging success, and masked possible adverse effects of PHAH-exposure on these parameters. Because of these strong natural influences, only information on egg laying dates, egg volumes and incubation periods could be used for estimating contaminant effects.

Although colony average breeding data could not clearly be related to PHAH-levels, colonies clustered after PCDD/F patterns and main food choice, showed significant differences in both breeding, biochemical and chemical data. Using the data from all colonies combined into one data-set, the breeding parameters could be correlated with yolk-sac PHAH-levels, EROD-activity, yolk-sac retinoid-, and plasma retinol and thyroid hormone levels. These correlations were either statistically significant or clear trends.

Food Choice

On the analogy of observations in North America (Nisbet 1973) female terns could build up a considerable PHAH burden during courtship feeding, when returning from their wintering grounds in Africa to breed in The Netherlands. This period shortly before egg laying is probably the most important period for accumulating PHAH-levels in the eggs.

Table 4.5 Chemical and biochemical data (average ± SD) grouped after incubation period in the field (first egg)

Parameter	Group 1 (short) [20.1-20.9][a]		Group 2 [21.5-21.7][a]		Group 3 [22.5-22.7][a]		Group 4 (long) [24.0-24.7][a]	N
mo-PCBs (μg/g)	12.82 ± 9.12	▼[4]	21.83 ± 11.88		18.44 ± 11.01		26.34 ± 8.72	6/7
TEQs (pg/g)	4951 ± 4080	▼[4]	10440 ± 4099		9016 ± 4851	▼[4]	12467 ± 4291	6
EROD act. (nmol/g.min)	126 ± 42	▼[4]	193 ± 114		171 ± 83		202 ± 86	8/9
ys ret.palmitate (ng/g)	1.07 ± 0.51		0.61 ± 0.34		–		–	7
ys vit A2 (ng/g)	9.31 ± 3.46		7.02 ± 1.26		–		–	7
plasma retinol (ng/ml)	83.1 ± 32.5		78.2 ± 37.2		99.0 ± 35.7		101.0 ± 44.9	10/11
ratio pl.re./ys.r.palm	63 ± 51	▼[2]	204 ± 127		–		–	7
plasma TT4 (nmol/l)	7.02 ± 1.59	▲[3,4]	8.32 ± 2.21	▲[3,4]	5.16 ± 1.58		4.67 ± 1.70	9/10
plasma FT4 (pmol/l)	6.60 ± 2.27	▲[3,4]	7.18 ± 2.10	▲[3,4]	3.67 ± 1.09	▲[4]	3.72 ± 1.04	7
plasma TT3 (nmol/l)	3.95 ± 1.55	▲[4]	4.01 ± 1.84	▲[3,4]	2.55 ± 0.77		2.51 ± 1.16	10/11
chickweight (g)	14.5 ± 1.4		14.4 ± 0.9		14.0 ± 0.8		13.8 ± 1.3	7/8
laying date (days)	140.0 ± 4.1	▼[4]	142.4 ± 4.0		142.6 ± 3.2		144.0 ± 4.6	12

[a] The average incubation periods for the four groups is not the same for all parameters, as the total number of data available per parameter varies.

▼[x] Significantly less than group x

▲[x] Significantly more than group x

High mo-PCB levels were expected based on a Dutch field study with cormorant chicks (Van den Berg *et al.* 1995) where yolk-sac levels of 184 and 58 μg mo-PCBs·g^{-1} lipid were observed in, respectively, the Biesbosch and Oude Venen colony. The average yolk-sac mo-PCB-levels in common tern chicks, however, were only 40 μg·g^{-1} lipid and 6 μg·g^{-1} lipid in, respectively, the most (Slijkplaat) and least (Zeewolde) contaminated colony. The difference in yolk-sac mo-PCB level between these two colonies was only 34 μg·g^{-1} lipid, compared to 126 μg·g^{-1} lipid for the Cormorants. The other six common tern colonies hardly differed in yolk-sac mo-PCB levels (16-28 μg·g^{-1} lipid, Figure 4.2). The relatively low mo-PCB concentrations and small differences between relatively clean and polluted sites are probably consequences of the food choice of the common terns. The terns from the salt water colonies mainly fed on migrating small clupeids, mainly small herring, that were born in the North Sea a few months earlier. Therefore, this food does not reflect the local degree of contamination, and may mask differences in contamination between locations. Small herring contain significantly less PCBs than fishes that overwinter locally, such as flounders (*Platichthys flesus*), 1.3 and 5.2 μg·g^{-1} lipid, respectively, (Stronkhorst *et al.* 1993). Cormorants mainly feed on bigger, often carnivorous, fish that mostly stay in an area with the same degree of pollution. Birds that remain in an estuary all year round, such as oystercatchers, will build up greater levels and better reflect the local degree of contamination, than species such as terns that migrate to less contaminated sites. Stronkhorst *et al.* (1993) observed for PCB-153 a biomagnification factor of 19 for the oystercatcher (egg:cockle) and for common tern only 5.5 (egg:clupeid). After hatching, the chicks are being fed with fishes that are only 1-1.5 times the bill size of the adult tern. These fishes are again much smaller and thus probably less contaminated, than the fishes fed to cormorant chicks.

Considering the relatively small mo-PCB concentrations and the small differences between relatively clean and polluted sites, it is not surprising that hardly any differences in colony average breeding data were found. However, when the colonies were clustered based on yolk-sac PCDD/F patterns, significant differences in both breeding data and biochemical and chemical data were observed. Differences in yolk-sac PCDD/F patterns may be related to the area where the breeding colonies were situated, but they could also be related to the environment the food species originated from. For example, Slijkplaat and Westplaat are the only colonies situated in the sedimentation area of the rivers Rhine and Meuse, but they are also the colonies where the terns (partially) fed on smelt. Additionally, it cannot be excluded that other factors than PHAHs, such as food availability, nutritional quality or the presence of other, unmeasured, contaminants could contribute to the observed differences in group average breeding and biochemical data.

Table 4.6 Chemical and biochemical data (average ± SD) grouped after (a) average egg volume (all eggs) and (b) chick weight (second egg)

A

Parameter	Group 1 (big) [21.3-21.6][a]		Group 2 [20.0-20.4][a]		Group 3 [19.2-19.6][a]		Group 4 (small) [17.7-18.1][a]	N	Correlation for chick weight
mo-PCBs (μg/g)	18.64 ± 13.17		20.20 ± 11.31		26.12 ± 10.79		26.84 ± 12.88	12/13	comparable
TEQs (pg/g)	9735 ± 5664		8850 ± 5281		12841 ± 4448		12599 ± 5867	8/9	comparable
ys ret.palmitate (ng/g)	0.88 ± 0.77		0.78 ± 0.41		0.68 ± 0.42		0.69 ± 0.63	9/10	not enough data
ys vit A2 (ng/g)	8.25 ± 5.50		7.33 ± 1.77		7.50 ± 3.19		6.45 ± 1.82	9/10	not enough data
ratio pl.re./ys.r.palm	135 ± 77		158 ± 97		189 ± 123		211 ± 137	9/10	not enough data
chickweight (g)	15.2 ± 0.8	▲[2,3,4]	14.3 ± 0.7	▲[3,4]	13.7 ± 0.5	▲[4]	12.8 ± 0.8	13/14	comparable
liver weight (g)	0.41 ± 0.04	▲[3,4]	0.38 ± 0.05		0.37 ± 0.03		0.35 ± 0.05	13/14	comparable

[a] The average egg volumes for the four groups are not the same for all parameters, as the total number of data available per parameter varies.
▲[x] Significantly larger than group x

B

Parameter	Group 1 (heavy) [15.1-15.5][a]		Group 2 [14.3-14.5][a]	Group 3 [13.6-13.8][a]	Group 4 (light) [12.5-12.6][a]	N
EROD act.(nmol/g.min)	175 ± 72		280 ± 224	216 ± 158	248 ± 205	9/10
plasma retinol (ng/ml)	77.8 ± 23.7		104.3 ± 33.9	99.0 ± 45.2	90.9 ± 44.2	11/12
plasma TT4 (nmol/l)	8.66 ± 6.90		8.18 ± 3.77	6.17 ± 1.82	5.68 ± 1.56	11/12
plasma FT4 (pmol/l)	9.21 ± 1.54	▲[3,4]	6.41 ± 3.28	4.26 ± 1.23	4.71 ± 1.83	8/9
plasma TT3 (nmol/l)	3.24 ± 1.55		3.86 ± 1.46	2.80 ± 1.30	2.71 ± 1.44	11/12

[a] The average chick weights for the four groups are not the same for all parameters, as the total number of data available per parameter varies.
▲[x] Significantly larger than group x

Correlations between Breeding Biology and Biochemical and Chemical Parameters

The biological data of the first egg were compared with the biochemical and chemical data of the second egg from the same clutch. Stronkhorst *et al.* (1993) showed that the variation in contaminant levels is relatively small within the same clutch, especially if compared to the variation between clutches. In the following not only statistically significant results are discussed, but also the clear trends, as these can be a useful indication for contaminant related effects. Due to the relatively large standard deviation using natural instead of inbred laboratory species, only very strong effects will be statistically significant (Murk *et al.* 1994b). An overview of all observed correlations is presented in Figure 4.4. The arrows indicates the direction of change in biochemical and chemical parameters when the parameters for the breeding biology become more unfavourable. A clear trend is indicated with a dotted arrow, a significant effect with a solid arrow. The results for 'egg volume' and 'chick weight' are combined because the parameters are closely related. A more specific discussion is presented below.

Yolk-sac PHAH Levels and EROD-Activity

Yolk-sac PHAH levels and EROD activity are greater in eggs with more unfavourable breeding parameters (Figure 4.4). This increase is significant for a prolonged incubation period, which is similar to the results found for the eggs incubated in the laboratory (Bosveld *et al.* 1995), and in accordance with the results of Kubiak *et al.* (1989, see below). Chicks from later layed eggs contained greater yolk-sac PHAH levels. This correlation could be stronger if the eggs had been collected during the whole laying period instead of only during the peak period of laying. Additionally, (not significantly) greater PHAH levels and EROD activity were observed for smaller eggs and chicks. These correlations suggest that exposure to PHAHs may have a negative influence on breeding parameters, although a correlation with other pollutants, often present in a similar gradient (Gilbertson 1974; Vethaak 1992) cannot be excluded.

In our study no congenital deformities or bill defects were observed. Nevertheless the concentrations of the three most important mo-PCBs (#118, #156 and #105), total PCB-concentrations and total PCDD/F concentrations in common tern eggs from Slijkplaat and Westplaat were similar or slightly greater than those reported for related species (Forsters and common tern) from industrialized sites in North America where birth defects did occur (Smith *et al.* 1990; Ankley *et al.* 1993; Hoffman *et al.* 1993; Bosveld *et al.* 1995). Therefore, it cannot be excluded that those effects are partially caused by other substances, possibly also acting through the Ah-receptor, as is suggested by the results of Tillitt *et al.* (1992). In their study the hatching success of double-crested cormorant (*Phalacrocorax auritus*) eggs correlated poorly with chemically derived TEQs, but strongly

with TEQs measured in an *in vitro* H4IIE rat hepatoma EROD assay, measuring the biological potency of the total mixture of compounds acting through the Ah-receptor. The composition of mixtures of pollutants could be quite different in the north-American sites compared to those in The Netherlands, resulting in different effects of what seem to be the same levels of a certain pollutant. The hepatic EROD-activity measured in the tern chicks in our study cannot easily be compared with the EROD activity in the H4IIE rat hepatoma EROD assay performed by Tillitt *et al.* (1992), because large differences have been observed in species-specific antagonism after exposure to mixtures of PCBs (Aarts *et al.* 1995). H4IIE's only show slight antagonism, whereas nothing is known yet about occurence of antagonism in common terns.

Yolk-sac and Plasma Retinoid Levels

Later layed eggs come from clutches with significantly lesser yolk-sac retinlylpalmitate levels and significantly greater ratios of plasma retinol over yolk-sac retinylpalmitate in the second egg (Figure 4.4). Eggs that needed a prolonged incubation period came from clutches with matching chicks with a significantly greater ratio of retinol over retinylpalmitate and (not significantly) lesser yolk-sac retinyl ester levels, which is similar to the results for the eggs incubated in the laboratory (Murk *et al.* 1994b). An increased ratio plasma retinol over hepatic retinylpalmitate levels indicates an increased mobilisation of retinol to the circulation and a decreased retinoid-ester storage (Brouwer *et al.* 1988; Chen et *al.* 1992). In mammals as well as in birds disturbances in vitamin A homeostasis have been associated with PHAH-exposure. PHAH-exposure can influence vitamin A homeostasis via at least two mechanisms. PHAH hydroxy metabolites can interfere with the plasma transport of thyroid hormone bound to transthyretine (TTR) combined with retinol transport bound to retinol binding protein, resulting in increased loss of both retinol and thyroid hormone from the circulation. PHAHs can also directly increase the release of retinol from the hepatic store, resulting in decreased hepatic retinylester and increased plasma retinol levels (Spear *et al.* 1985, 1988, 1990; Brouwer *et al.* 1988; Murk *et al.* 1994a). Apart from PHAH influences, dietary intake of vitamin A is very important for vitamin A homeostasis, as are adequate stores of retinyl esters and the finely regulated release into the blood (Zile 1992). Therefore, it cannot be excluded that the observed correlations are a consequence of a weaker nutritional status of the female tern, instead of being related to PHAH-exposure.

Vitamin A is essential for normal reproduction. In chickens, vitamin A deficiency has been associated with reduced hatchability due to reduced or failing appearance of large blood vessels around the embryo (Thompson 1970). Therefore the observed greater ratio plasma retinol/retinyl palmitate in later layed eggs, eggs needing a longer incubation

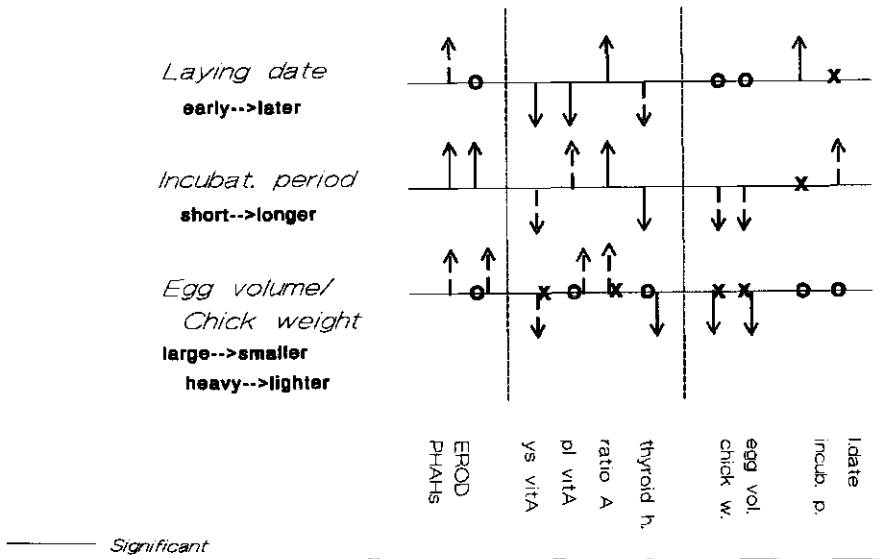

Figure 4.4 Overview of the correlations of the chemical and biochemical data with the biological data of eggs from the same clutches, when clustered on the basis of the biological data. An upwards or downwards directed arrow indicates that the chemical or biochemical parameter mentioned at the bottom increases resp. decreases when the biological parameter becomes less favourable, as mentioned at the left of the figure.

period and smaller eggs are in accordance with this information. Vitamin A also plays an important role in positioning tissues and formation of the skeleton in developing embryos (Pijnappel *et al.* 1993). Apart from development and growth, vitamin A is also essential for the immune function, the integrity of epithelia which are barriers against infections, mucous secretion, and normal vision (Friedman and Sklan 1989; Wobeser and Kost 1992).

The two opposite mechanisms influencing plasma retinol levels upon PHAH exposure, make plasma retinol level alone an unsuitable indicator of PHAH-effects.

Thyroid Hormones

Plasma thyroid hormone levels were significantly less in chicks from eggs needing a prolonged incubation period (TT3, TT4, FT4), in smaller chicks (FT4; TT3 and TT4 a trend), and in chicks from clutches with later layed eggs (TT3 a trend). Also for chicks incubated in the laboratory a negative correlation was found between incubation period and plasma TT3 levels (trend, Murk *et al.* 1994b). Reduction in plasma thyroid hormone levels has often been associated with PHAH-exposure in *in vivo* studies with mammals,

birds and fish, both directly via influencing the metabolism and via hydroxylated PHAH metabolites as mentioned above (Leatherland and Sonstegard 1978; Brouwer *et al.* 1990; Morse *et al.* 1992; Brouwer 1991; Murk *et al.* 1994c). As thyroid hormones play an important role in various physiological processes including energy metabolism, reproduction, development, differentiation and growth (Sharp and Klandorf 1985), the observed effects could be related to reduced thyroid hormone levels.

Breeding Biology

The incubation period is significantly prolonged for eggs from clutches with later laying dates. This is not unusual, as more experienced birds lay their eggs earlier and incubate more efficiently (Hays 1978; Nisbet 1978). Additionally, a greater quality of courtship feeding by the male induces earlier egg-laying and larger eggs and clutches (Nisbet 1973, 1977). However, the observation that eggs from clutches with prolonged incubation periods in both the field and the laboratory have significantly more PHAHs and greater EROD activities suggest that PHAH contamination also plays a role. This could be through influencing vitamin A and thyroid hormone metabolism as was explained earlier. Reduced vitamin A and thyroid hormone levels in a female tern will not only influence egg formation, but also the amount of vitamin A and thyroid hormone passed on to the egg. It would interesting to know whether PHAH exposure also influences the courtship feeding behaviour of the male.

With increasing incubation period of the second egg in the laboratory, the incubation period of the first egg in the field increases even more (Figure 4.3). This suggests that apart from a factor intrinsic to the egg, an additional factor influences the incubation period in the field. This is in accordance with the observation that nest attentiveness and incubation behaviour of herring gulls were negatively correlated with the organochlorine content of the eggs (Fox *et al.* 1978). Also Kubiak *et al.* (1989) demonstrated for Forster's terns that not only factors intrinsic to the egg, but also parental attentiveness impaired reproductive outcome from a PHAH-contaminated site. In their study the differences in mean incubation periods between the contaminated and clean site were, respectively, 4.6 and 8.3 days for laboratory and field incubation. In our study these differences were less extreme, respectively, 2.0 and 4.0 days between the short and long incubation groups in the laboratory (Murk *et al.* 1994b) and the field (Table 4.5). A prolonged incubation period must be considered as adverse, as it imposes a greater risk for the eggs and costs more energy for the adult terns and chicks.

A trend is visible that eggs from clutches needing a prolonged incubation period were smaller and produced smaller chicks. Usually larger eggs need a prolonged incubation period. However, if the reduced egg volume is a consequence of PHAH-

exposure it can be expected that this exposure also increase the incubation period, possibly via decreased vitamin A and thyroid hormone levels.

Consequences for Reproduction

During our study the dynamic environment of the terns had more severe detrimental effects on breeding success than PHAHs, which is in accordance with the results of Becker *et al.* (1991). However, the observed correlations using the whole data set, and the differences in group averages found after clustering colonies after the PHAH-patterns, suggest that in the Dutch situation PHAHs or other contaminants present in the same gradient, have an adverse effect on the measured breeding biology parameters of common terns. It cannot be excluded that the more subtle correlations observed in our study will be more obvious and relatively important under circumstances without the strong adverse ecological and climatological factors. Data on hatching and fledging success are needed, integrating the effects of the quality of the egg, the incubation behaviour of the adult terns, and (with fledging success) direct effects of PHAHs from the food on the chicks. If a parallel can be drawn with mammals, it is to be expected that chicks will be more vulnerable for exposure to PHAHs *in ovo* than post-hatching, as in this period tissues are being formed and hormone levels are being 'fine-tuned'. Even a relatively small maternal dose of the PCB-mixture Aroclor 1254, has been found to result in long-term alterations in retinoid status in the offspring of rats (Morse and Brouwer, 1995) .

Choice of Species for Future Ecotoxicological Field Research

In the effect chain: PHAH-concentration --> biochemical effect in an individual --> effect on population level, the variation in the parameters measured increases continuously. Not only the PHAHs measured, but also other substances are able to act through the same mechanism of action. Biochemical responses are not only influenced by contaminants but also by factors such as physiological condition (age, sex, reproduction, etc), environmental conditions and food quality. The effect at the population level is easily masked by ecological factors such as massive predation, severe flooding, and extreme weather conditions. If the goal of a study is to establish whether effects of substances on populations occur, it is important to choose a suitable species for the specific compounds and effects of interest, but not so vulnerable for other, drastic influences. For reproduction studies, tree-nesting birds are more suitable than common terns which breed on the ground close to the waterline. Additionally, it is important that the exposure of the species studied is site specific. Species that stay in the same area all year around and/or feed on bigger fishes that are present all year around, better reflect

the local degree of pollution than species, such as the common tern, that feed on relatively small and migrating fishes.

Use of Biomarkers for Future Research

A correlation between the concentration of a certain class of chemicals and an observed adverse biological effect is just an indication that a causal relationship may exist. The use of biomarkers for exposure and effect can help to establish a causal relationship between a certain class of pollutants and an observed adverse biological effect. Biomarkers for exposure will indicate the potency of the whole mixture of, often partially unknown, compounds that an organism is exposed to. This presents a more realistic measure of the exposure, including interactions between the compounds, than measurement of some individual compounds and calculating the total toxicity. Examples are measurement of EROD-derived TEQs (Tillitt *et al.* 1992) and of luciferase produced by Ah-receptor active compounds (such as PHAHs and PAHs) using the CALUX-assay (chemical activated luciferase expression). The CALUX-assay has already been developed for small samples of blood plasma, sediment and interstitial water (Murk *et al.*, 1996b,e). Biomarkers of effect should be developed and validated in experimental studies, where hormonal and physiological consequences of different dosages of a pollutant of interest can be assessed, while all other factors are kept constant (Brouwer *et al.* 1990). The biological consequences of hormonal and physiological alterations must then be studied under field conditions. A complicating factor is that endocrine disrupters may only influence physiological functions under specific stress circumstances. Therefore, at the best, it will be possible to indicate critical levels for physiological conditions (such as vitamin A or thyroid hormone) or pollutants. Due to unpredictable and complex ecological circumstances, however, it will be impossible to foretell exact population effects if certain levels are reached.

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