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Correlation between the level of PCB's and the presence of diseases in common dab (Limanda limanda L.)

by

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Abstract.

Livers from diseased and healthy dab collected at three locations in the North Sea were analysed for the presence of different organochlorine compounds.
The difference in the PCB content of the diseased and healthy fish was not found to be statistical significant. However, significant difference was demonstrated between the sexes for liver weight, liver lipid content and for ΣPCB.
This investigation demonstrated the need for differentiation between the sexes when carrying out analysis of organochlorines in livers of flatfish.

Introduction.

Since Snieszko (1974) pointed out the importance of environmental stress on outbreaks of fish diseases, a series of studies of the impact of pollutants on fish health have been initiated (e.g. Bucke et al. 1983; Dethlefsen 1980, 1984; Dethlefsen et al. 1987; Vethaak 1985).

Most frequently, outbreaks of fish diseases have a multifactorial background. Therefore, it has only in a few instances been possible to get clear correlations between certain pollutants and the outbreak of a specific fish disease (Grizzle et al. 1984; Lindesjöö & Thulin 1990; McDermott-Ehrlich et al. 1977). However, increased susceptibility to viral diseases has been demonstrated in birds fed a PCB-containing diet (Friend & Trainer 1970).

The aim of this investigation was to examine if a correlation between the content of PCBs and the presence of the viral diseases, lymphocystis and epidermal papillomas in common dab could be established.

The common dab seems to be a suitable fish species for such an examination as it is a relatively stationary, bottom dwelling fish. This is of importance as contaminated sediment seems to be the main contributor to the uptake of PCBs in fish (Courtney & Langston 1980).

Material and methods.

Sampling of fish.

For PCB analysis, common dab (Limanda limanda, L.) were sampled in May 1987 during a fish disease survey with H/S Dana using a standard fishing trawl (Nymplex fishing trawl, Star model). The trawl was rigged either with 12" rubber disks or with 10" bobbins on the footrope - depending on bottom conditions in the area of research and fitted with a footrope chain. The mesh size in the cod end was 40 mm. The fishing took place at a number of stations

where haul tracks had been available from commercial fishermen (Fig. 1.). Standard one hour hauls were taken at a speed of three knots.

The total catch was sorted into species and the dabs were subjected to further investigation. A sample of 150-250 specimens corresponding to 15-20 kg per haul was examined. Subsamples were taken at random if the total catch of dab exceeded 20 kg.
For all fish examined, length, weight and sex were registered and otoliths removed for ageing. The dabs were examined for the presence of the fish diseases, lymphocystis, epidermal papillomas and skin ulcers using recommended procedures for detection (Dethlefsen et al., 1986).

For the present investigation, fish of a size of 20-25 cm corresponding to an age range between 3 and 6 years were sampled. Livers for analysis were removed immediately after the catch was onboard wrapped in alufoil and frozen.

The fish were sampled at three different stations. Two of the stations (2 and 10), were characterized by having shown high disease rates during previous investigations (Mellergaard & Nielsen 1985). The third station (102), were chosen as a reference station situated between the two other stations.
At station 2, livers from 44 fish, 15 diseased and 29 healthy fish, were removed and frozen separately. At station 10, livers from ten healthy and from ten diseased fish were removed and frozen in two pools and at station 102, livers from 18 randomly selected fish were pooled.

Analytical procedure.
The samples were kept at -25°C until analysis. After thawing, the livers were weighed. Each liver was cut into small pieces with a scalpel and ground in a mortar with 20 g anhydrous sodium sulphate. The pooled samples were homogenized before grinding. Following soxhlet extraction for 6 hours with 100 ml n-hexane/acetone (4:1) and vacuum evaporation. The extractable materials

were weighed (the n-hexane/acetone extract contains neutral lipids and some phospholipids).

The lipid was dissolved in iso-octane with 50 ng hexabromobenzene per ml added as a volume standard after which the samples were cleaned up using sulphuric acid treatment.

The chromatographic analyses were performed on a Varian 3500 gas chromatograph equipped with an on-column injector and a linearized ^{63}Ni electron capture detector using a 25 m x 0.33 mm Hewlett Packard HP-5 capillary column with 0.5 μm coating. The determinations included seven chlorinated biphenyls (IUPAC nos. 28, 52, 101, 118, 138, 153, and 180 as recommended by ICES (1988)), ΣDDT(p,p'-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) and its metabolites p,p'-DDE, p,p'-DDD), α-hexachlorocyclohexane(α-HCH), γ-HCH and hexachlorobenzene(HCB). The reproducibility of the analyses was tested by dividing eight livers in two parts and analysing each part. The livers might not be homogeneous with respect to lipid contents and the relative standard deviations for the lipid contents varied from 2-36 %. However, based on contents in μg/g lipid average relative standard deviations of 2.5% for ΣPCB (4 double determinations), 5.7% for ΣDDT, 11% for α-HCH, 11% for γ-HCH and 12% for HCB (8 double determinations) were recorded.

Parts of the analytical procedure have been intercalibrated within the framework of the ICES and the Helsinki Commission.

Results.

Fish infected with lymphocystis were the only diseased fish present among the fish in the size group between 20-25 cm.
Table 1 lists the mean concentrations of the different organo-chlorines (μg/kg wet weight(ww)) with the relative standard deviations (RSD) in livers of healthy and diseased males and females, respectively, in the individually sampled fish from station 2. The mean weight of the livers of diseased and healthy male dabs was 0.80 g and 0.95 g, respectively. The similar

figures for diseased and healthy females were 2.00 g and 2.27 g, respectively. The lipid content of the livers was 13.6% for diseased and 16.8% for healthy males. In females, the lipid content was 3.5% for diseased and 4.7% for healthy specimens. The concentration of ΣPCB in diseased males was found to be 228 µg/kg liver (ww) and in healthy males 325 µg/kg liver (ww). For females, the similar figures were 34 µg/kg and 39 µg/kg liver (ww), respectively.

An analysis of the variance (anovar) of the liver weight, the liver lipid content and the ΣPCB, respectively, among diseased and healthy males and females, respectively, and diseased and healthy fish (sexes combined) gave the following F-values (df):

	Liver weight	Lipid content	ΣPCB
Diseased/healthy males ($P_{.01}$=8.53)	0.86 (1,16)	0,89 (1,16)	4.28 (1,16)
Diseased/healthy females ($P_{.01}$=7.82)	0.33 (1,24)	1,37 (1,24)	0.21 (1,24)
Diseased/healthy fish ($P_{.01}$=7.28) (sexes combined)	3.28 (1,42)	0.20 (1,42)	0.26 (1,42)

It can be seen that there is no significant difference of the liver weight (ww), the lipid content of the livers and the ΣPCB between diseased and healthy males and females, respectively, or between diseased and healthy fish (sexes combined).
However, marked differences were observed between males and females what concerned liver weight, lipid content and ΣPCB. Males had approximately half the liver weight (ww), four times higher lipid content in the liver and almost ten times as much ΣPCB compared with females. Analysis of the variance (anovar) of these figures among males and females showed significant difference between the sexes for the liver weights; F=29.60 (df=1,42), for the lipid content; F=49.14 (df=1,42) and for the ΣPCB; F=117.61 (df=1,42). Also for HCB and HCH's the concentration in males is several times higher than in females.

These marked differences in the concentrations of the organochlorines between the sexes are reduced when the figures are

based on the lipid content of the livers. These figures are also entered in Table 1. The concentration of organochlorine compounds based on the lipid content of the liver of males is only twice as high as observed in females. However, an analysis of the variance of the ΣPCB, based on the lipid content, between males and females demonstrated a significant difference between the sexes; $F=26.31$ $(df=1,42)$ $P_{.01}=7.82$.

Because of the marked differences in the concentration of organochlorines between male and female dab livers, mixed samples with unknown sex distribution are difficult to evaluate. This is partly the case for station 10 with a pooled sample of 10 livers from diseased dabs and 10 livers from healthy fish and station 102 with a pooled sample of 18 livers. However, the relative sex distributions for all dabs sampled at the stations are known. The concentration of organochlorines based on lipid contents of livers of males, females and a combination of both sexes from station 2, a combination of diseased and healthy fish from station 10 and the pooled sample from station 102 are presented in Table 2. Although, there are minor variations in the sex distribution of the fish between the three stations, the organochlorine content is almost at the same level. Due to the limitation of data, no statistical analysis was carried out.

Discussion.

The aim of the investigation was to assess whether there was a correlation between the concentrations of ΣPCB or the other chlorinated compounds and the presence of diseases in dabs. Some authors find indications or have proven correlations between organochlorines and fish diseases. Perkins et al. (1972) indicate a possible correlation between high disease rates in flatfish and the dumping of PCB's in the Irish Sea. McDermott-Ehrlich et al. (1977) found significantly higher DDT and PCB levels in Dover sole with fin erosions south of Los Angeles. However, the present work failed to establish a direct correlations between the PCB levels and the presence of diseases in dab.

Based on the experience from the present work, data from single fish analyses do not seem to be the most suitable information to deal with when trying to establish correlations between PCB levels and fish diseases due to the relatively pronounced individual variation. Instead, very large pools of uniform material, i.e. originating from one sex, from diseased and healthy fish, respectively, should be investigated.

Büther (1987) investigated the content of chlorinated compounds in dabs from the German Bight, the Central and Southern North Sea. The samples were collected in December 1984, at a time where the lipid contents in the livers were relatively high as the fish had not yet spawned. Each sample contained 25 female dab livers. The ΣPCB content was calculated on the basis of a technical mixture and, therefore, a direct comparison is not possible. Concentrations of ΣDDT were about 0.4 µg/g lipid in the German Bight (comparable with station 2) and the HCB content was 0.05 µg/g lipid which are at the same level as in the present survey for females with ΣDDT concentrations of 0.1-0.4 µg/g lipid and HCB 0.02-0.09 µg/g lipid. The lipid content in December 1984 was 14-19% compared with 3-7% in May 1987.

Dethlefsen and Huschenbeth (1986) have also investigated the content of chlorinated compounds in dab livers from the North Sea. The material was based on pooled samples with unknown sex distribution except for samples collected in October 1981. A sample with 6 males and 4 females collected southwest of Sild (comparable with station 2) showed a ΣDDT concentration of about 0.3 µg/g lipid and a HCB concentration of 0.07 µg/g lipid. These levels were between the levels found in males and females in the present survey. The lipid content in October 1981 was about 40% for the mixed sample compared to 3-7 % for females and 5-30 % for males, in May.

In May 1985, 25 livers from plaice were collected from station 2 in connection with an ICES baseline study (Granby, 1987). The mixed sample consisted of 72% male livers with an average weight

of 1.4 g and a relative liver lipid content of 4.4%. The contents of organochlorines in µg/g lipid compared to the sample from this survey (41% male) in brackets were for Σ5CB (nos.101, 118, 138, 153, 180) 0.95 µg/g (1.38 µg/g (Σ7CB)), ΣDDT 0.30 µg/g (0.46 µg/g), γ-HCH 0.05 µg/g (0.08 µg/g), α-HCH 0.02 µg/g (0.03 µg/g) and HCB 0.03 µg/g (0.07 µg/g). Both the lipid content and the organochlorine contents in the plaice livers collected in May 1985 were at the same level as the contents for dab livers collected in May 1987.

Although seasonal fluctuations may exist, the content of organochlorine compounds based on the liver lipid content in dab caught in the German Bight (station 2) are at the same level as demonstrated in the quoted investigations. However, it is evident that seasonal fluctuations in the liver weight due to seasonal changes in the nutritional status of the fish and especially the spawning for the females affect the content of organochlorines measured in µg/kg wet weight of the liver. The nutritional status of dabs is poor in May (Kunst 1988), where the present sampling took place. Additionally, the spawning period for dabs is from March to May. The nutritional status for dabs increases in the period from June to November (Kunst 1988). Similar observations have been made for coastal spawning flounders in the Baltic (Lapin 1976) who found the lowest fat content in livers of flounder in April. If the sampling had taken place in the period of increasing nutritional status, the PCB content per kg liver (w/w) would have been at another level. Therefore, the results of organochlorine analysis should be based on liver lipid content rather than on the wet weight of the livers.

The difference in the lipid content in male and female livers may be caused by the recent spawning as large ammounts of fat is deposited in the gonads. Parallel with the liver deposition, PCBs are also deposited in the gonads, especially in the roe. The spawning is an important "clearance factor" (Bengtsson 1980) for PCB's and the "clearance" is most pronounced in females. This may explain the differences in the PCB content between males and

females.

This work has demonstrated that to reduce the variability of PCB analyses in dab, it is important only to measure samples from one sex. Results from mixed samples may vary considerably depending of the sex distribution of the material. Sampling should preferably take place during a period where the nutritional status of the fish is optimal i.e. for dabs from October to January. To limit the variations due to differences in the nutritional status of the fish, the presented PCB levels should be based on the liver lipid content.

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Table 1. Mean content and relative standard deviation (RSD) of organochlorines in diseased and healthy dabs.

	Diseased mean	RSD		Healthy mean	RSD		sex
Physiological parameters:							
%lipid	13.6	64%	n=9	16.8	34%	n=9	MALE
	3.5	22%	n=6	4.7	54%	n=20	FEMALE
liver	0.80	1%		0.95	42%		MALE
weight(g)	2.00	56%		2.27	43%		FEMALE
length	20.7	4%		21.3	5%		MALE
(cm)	22.3	8%		23.3	10%		FEMALE
µg/kg liver wet weight:							
ΣPCB	228	49%	n=9	325	26%	n=9	MALE
	34	54%	n=6	39	68%	n=20	FEMALE
CB 138	59.8	64%		90.6	29%		MALE
	8.3	58%		9.5	62%		FEMALE
CB 153	75.7	55%		121	25%		MALE
	9.1	41%		11.8	60%		FEMALE
ΣDDT	81.7	47%		121	25%		MALE
	8.0	48%		11.4	60%		FEMALE
γ-HCH	8.7	29%		11.4	13%		MALE
	2.8	48%	3.7	42%			FEMALE
α-HCH	3.7	52%		4.9	25%		MALE
	0.8	19%	1.1	66%			FEMALE
HCB	8.6	37%		10.9	23%		MALE
	2.3	40%		2.2	54%		FEMALE
µg/g lipid:							
ΣPCB	1.96	39%	n=9	2.14	51%	n=9	MALE
	0.97	47%	n=6	0.89	69%	n=20	FEMALE
CB 138	0.49	44%		0.60	56%		MALE
	0.25	62%		0.22	69%		FEMALE
CB 153	0.66	46%		0.80	49%		MALE
	0.26	38%		0.27	70%		FEMALE
ΣDDT	0.71	38%		0.80	49%		MALE
	0.23	46%		0.26	55%		FEMALE
γ-HCH	0.096	96%		0.074	32%		MALE
	0.083	7%		0.084	26%		FEMALE
α-HCH	0.034	74%		0.030	12%		MALE
	0.022	13%		0.024	18%		FEMALE
HCB	0.092	97%		0.069	30%		MALE
	0.068	48%		0.050	46%		FEMALE

Table 2. Contents of organochlorines (μg/g lipid) in dab livers from three stations in the North Sea (relative standard deviations).

Station	2	2	2	10	102
Sex distribution	FEMALE	MALE	41% MALE	32% MALE	44% MALE
number of fish	26	18	4	20	18
% lipid	4.5 (52)	15.2 (48)	8.9 (82)	9.4	4.8
liver weight g	2.2 (45)	0.87 (38)	1.7 (62)	1.3	-
length cm	23 (10)	21 (4)	22 (9)	-	-
ΣPCB	0.91 (63)	2.05 (45)	1.38 (67)	1.00	1.38
CB 138	0.22 (66)	0.54 (51)	0.35 (74)	0.27	0.41
CB 153	0.27 (64)	0.73 (48)	0.46 (75)	0.34	0.41
ΣDDT	0.25 (53)	0.75 (44)	0.46 (74)	0.40	0.47
γ-HCH	0.084 (31)	0.085 (78)	0.084 (54)	0.061	0.065
α-HCH	0.023 (17)	0.032 (55)	0.027 (46)	0.024	0.024
HCB	0.055 (48)	0.081 (79)	0.065 (72)	0.045	0.065

Fig. 1.

Locations for sampling of dab livers for PCB analysis.