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# EPIDEMIOLOGY OF DISEASES IN FLOUNDER (*PLATICHTHYS FLESUS* L.) IN DUTCH COASTAL WATERS, WITH PARTICULAR REFERENCE TO ENVIRONMENTAL STRESS FACTORS



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## ABSTRACT

In order to investigate potential links between marine pollution and fish diseases, a long-term epidemiological study was conducted in the Netherlands during 1983-89. This study concentrated on grossly identifiable diseases of flounder (*Platichthys flesus*), an abundant species which is highly susceptible to disease.

Flounder were found to be affected with the viral skin disease lymphocystis and by skin ulcers of bacterial origin. Overall prevalences of these two diseases in fish ≥ 2 years old were 14.1% and 2.6% respectively. Also notable was the presence of neoplastic nodules (tumours) in the livers of 0.8% of the population, prevalences rising steeply with age and locally attaining values of up to 30 % in 6+ year old fish.

Spatial and temporal patterns of disease occurrence were analysed statistically using log-linear models which incorporated significant effects of length, age and sex. Temporal variation showed little correspondence between the three diseases, but their spatial distributions showed striking similarities. In the case of lymphocystis and skin ulcers, there was also a significant association in individual fish.

The observed spatial and temporal patterns showed no apparent correlation with the condition factor of the fish or with concentrations of contaminants in sediments or in tissues. However, spatial variation was strongly correlated with salinity and with fishing activity (and hence possibly damage by fishing gear). In view of the differing aetiologies of the three diseases, the similarities in spatial patterns may indicate a general underlying mechanism of disease causation, perhaps acting via an immunosuppresive effect. At the same time, migratory behaviour might explain some aspects of the spatial patterns of liver neoplasia.

The findings of this field study have led to the formulation of several hypotheses that need further investigation and testing. It is clear that on the basis of these findings alone, the significance of pollution in disease causation cannot be clearly demonstrated, due to the apparent importance of other factors such as salinity, fishing activity, and migration. Experimental work will be needed to test the various hyposeses, and a large-scale experimental study, now in progress on the isle of Texel, will test for the effects of pollution acting in isolation.

# 1 INTRODUCTION

## 1.1 GENERAL

With growing concern about marine pollution, interest in the potential of fish diseases for monitoring environment quality has increased considerably in recent years (Vethaak and ap Rheinallt 1992). In the North Sea area, many studies have focussed on diseases of dab, *Limanda limanda*) (eg Bucke et al. 1984, Dethlefsen et al. 1987, Vethaak and Meer 1991), but numerous other species including flounder *Platichthys flesus*, have also been studied. At present there appears to be no general consensus of opinion about the importance of pollution as a causative factor for disease in this area, although a small number of studies appear to provide convincing evidence for a relationship (see recent review by Vethaak and ap Rheinallt 1992). This situation contrasts with that prevailing on the other side of the Atlantic, where an impressive body of evidence has been assembled, showing clearly the existence of a link between liver abnormalities and pollution, particularly by PAHs (eg Malins et al 1988).

In the North Sea area, studies to date show that dab and flounder are both susceptible to diseases with easily recognizable signs, frequently affecting a large proportions of the population. This together with their abundance and widespread distribution, has led to the recommendation of these two species for use in disease monitoring programmes (Dethlefsen et al. 1986, Anon 1989).

Early studies of flounder in the North Sea and associated waters such as the Irish sea, drew largely negative conclusions about the influence of pollution on disease in this species (Perkins et al 1972, Shelton and Wilson 1973, Newell et al 1979, McArdle et al 1982, Bucke et al 1983a, 1983b, Reiersen and Fugelli 1984). Some of these negative findings can be attributed to methodological shorthcomings. More recently, a small number of more thorough studies have been published.

Extensive studies of diseases of flounder in the Elbe estuary and the German Wadden Sea were carried out by Möller and colleagues (Möller 1990, Möller and Anders 1992). The prevalence of most of the diseases observed was highest at intermediate stations in the estuary even though pollution levels increased inwards towards Hamburg. The major factor triggering disease was considered to be fluctuating salinities in the central estuary, which led, via decreased production and hence a reduced food supply, to starvation and an associated increase in susceptibility to disease. Although the possible existence of other relationships was acknowledged, pollution was not considered to be a major factor causing disease.

Results of a special survey of flounder diseases in the Dutch Wadden Sea in 1988 have been published recently (Vethaak 1992). Particularly high prevalences of skin ulcers (20 to 50%) were observed at certain localities in the SW Wadden Sea, corresponding to discharges of fresh water, although in the freshwater bodies behind the sluices prevalences were very low. It was suggested that bacterial infection, osmotic stress, and anaerobic sediments, as well as perhaps crowding and nutritional deficiencies, could all be involved in disease causation (Vethaak 1992).

In contrast to the above, a comprehensive series of studies of liver pathology in flounder from the Elbe provides convincing evidence a causal relationship between disease and pollution, most significantly by chlorinated hydrocarbons (Peters *et al.* 1987, Köhler 1989). Data revealed that major liver damage was confined almost exclusively to fish from the polluted river, being absent in fish from the neighbouring Eider, a relatively clean river. Neoplastic liver nodules were found in other species from the Elbe, though they did not occur in flounder. Experimental studies confirmed the field data, flounder showing signs of liver regeneration when kept in clean water and fed contaminant-free diet (Köhler 1989).

## 1.2 BIOLOGY OF THE FLOUNDER

The flounder is a common species on soft substrata in the coastal zone, estuaries, and large fresh water bodies in the Netherlands, where it is frequently exposed to high levels of pollution. It was therefore considered appropriate for use in the present study.

In order to understand and evaluate disease data, knowledge of the population biology of the flounder is required. In particular, the interpretations of spatial and temporal patterns of disease occurrence depends critically on the movement patterns of the target species.

Information on the migration of flounder in this area is largely confined to Dutch language publications or unpublished data from the Netherlands Institute for Fishery Investigations (RIVO) (Vethaak and Rijnsdorp 1989).

The flounder is an euryhaline species: it spawns in offshore marine waters but both the nurseries and the feeding grounds of the adults are concentrated in or near to estuaries. In Dutch waters some young flounder grows up under marine conditions (Wadden Sea, Eastern Scheldt), but the the majority occur in brackish and fresh waters (Rijnsdorp and Vethaak 1989). The importance of rivers as nursery grounds for 0- and 1-group flounder has recently been demonstrated, with smaller fish preferring less saline waters (Kerstan 1991). It appears that during the first years of a flounder's life there is a downstream migration, with 4 year old and older fish seldom occurring above the estuary mouth (author's unpublished observations). In terms of pollution, the condition of flounder captured in the vicinity of a particular estuary can therefore be regarded as reflecting the status of the estuarine/river system as a whole.

The migratory habits of flounder in German and Dutch waters were studied by Redeke (1907) Ehrenbaum (1908) and Veen (1971). Using tagging methods, these authors found that flounder from inshore waters, estuaries and fresh water migrate offshore in the winter to spawn. The study of Veen in particular is important, since this shows strong homing behaviour with respect to the feeding grounds. Only exceptionally were tagged flounders observed to migrate back inshore to a different location the following year.

Because of their migration pattern, only during the summer can flounder captured at a given location be considered to have been feeding there for any length of time. In particular the condition of flounder captured in September, as in the present study, can be expected to reflect local conditions over the previous 3 to 4 months or so.

## 1.3 SCOPE OF THE PRESENT STUDY

The final stage of the present study comprised an inventory of internal and external lesions in flounder, dab, and plaice *Pleuronectes platessa* in Dutch coastal waters, carried out during 1983-84 (Vethaak 1985). From this it emerged that the flounder was the most suitable species for monitoring pollution in near-shore and estuarine waters. Three principal diseases were thought to be potentially useful for this purpose, namely lymphocystis (viral), skin ulcers (associated with bacteria) and liver nodules (aetiology less clear). Other frequently encountered diseases included fin rot, but although reported in the literature as possibly associated with pollution, its use was abundant because of diagnostic problems related to mechanical damage (Vethaak 1992).

The study continued to 1989, with additional sites being incorporated into the programme. The principal objective was to map the occurrence of grossly identifiable diseases in flounder populations inhabiting various areas with differing degrees of pollution. Interim reports have already been published (Marquenie and Vethaak 1989, Vethaak 1987, 1991), and show that disease prevalence are significantly influenced by season, year, length or age of the fish, and sex of the fish. These reports were also the first to record the presence of epizootics of macroscopically observable liver nodules, corresponding to histologically identified neoplastic and putative pre-neoplastic disorders (Vethaak 1985, 1987).

This paper constitutes a final report on the survey programme. In it, the epidemiology of three major diseases, lymphocystis, skin ulcers, and liver neoplasia, is described in detail. Associations between certain potential risk factors (fish condition, salinity, fishery impact, pollutant concentrations) and spatial and temporal variation of disease occurrence are examined in an attempt to evaluate the impact of pollution on flounder diseases in this area.

# 2 METHODS

## 2.1 SAMPLING SITES

The choice of the sampling areas was made on the basis of data available through the various environmental monitoring programmes carried out by the Public Works Department (RWS) of the Dutch Ministry of Transport and Public Works, as well as, on the basis of the occurrence of the target species.

From 1983 until 1987 surveys were limited to three sites, two in polluted coastal waters (Sites 5 and 6), and a reference site in the Eastern Scheldt (Site 4), known to suffer relatively little from pollution. During 1987-89, however, the programme was extended to include six additional sites: two in the coastal waters (Sites 3 and 7), one in the Wadden Sea (Site 8), and a further three sites in two different estuaries, the Western Scheldt (Sites 1 and 2) and the Ems-Dollard (Site 9) (Fig 1).

The characteristics of each site and the main sources of pollution are described in Table 1.

## 2.2 SAMPLING AND INSPECTION OF FISH FOR DISEASE

Most fishing was carried out by the two research vessels RV Octans (1983-85) and RV Small Agt (1985-1989). The gear used was a 6-meter beam trawl fitted with 2-4 tickler chains, and a standard sole net having a a 7 cm mesh and a cod-end mesh of 4 cm.

Each site was visited once in September of each year and a number of tows were made within a deliminated area (Fig 1). The same tows were repeated every year until the desired sample size was attained. Each tow lasted between 35 and 80 min, and at each site a minimum of 4 tows were made, but the number was usually greater and varied depending on the size of the catch. Some additional samples were taken by local shrimp trawlers or by standing nets at Sites 1, 2 and 9. The depth fished at each site was noted (Table 1).

Only flounder with a total length of 20 cm or more were retained and examined, smaller individuals being virtually absent from coastal sites at the time of sampling. Fish from the catch were divided into 3 length classes: 20-24.9 cm; 25-29.9 cm; and 30 cm and over. The objective at each site was to collect at least 150 fish in each length class. A total sample of 450 fish per site allows the detection of a disease having a prevalence of at least 1.0% with a confidence level of 95% (Munro et al. 1983). Subsequent data analysis was, however, confined to diseases with observed prevalences (considerably) greater than this value.

Although the target sampling size for the middle length class (25-29,9 cm) was usually reached at each site, some difficulty was experienced in collecting enough large fish (30 cm+) at Site 9, but more often in collecting small fish

(20-24.9 cm) at some coastal sites, especially Sites 3 and 6 (Table 2). For analysis, therefore, data for the small and middle length class were pooled.

Every fish was inspected externally by a combination of visual and tactile examination, lasting approximately 30 to 60 s per fish. On each occasion, fish were examined by a small group of observers in close communication, and in addition there was a considerable degree of continuity from year to year.

Inspection for disease signs concentrated on three major conditions, as follows:

1. Lymphocystis. This viral disease can be recognised by the presence of nodules, sometimes creamy-white in colour, but occasionally pigmented, on the skin covering the body and/or fins. Occasionally nodules are found in the gill or oral cavity. The presence of a single nodule on the body or fins was taken to be the minimum for recording this disease, and the position of the nodules on the body (upperside, underside, or both sides) was noted. Cases where the diagnosis was doubtful were checked by histological examination.

   The signs of lymphocystis were divided into 3 categories in order of increasing severity:

   Grade I: 1 to 10 cysts or clusters of cysts not exceeding a total diameter of 5 mm;
   Grade II: lesions covering a surface area up to twice that of the outspread caudal fin;
   Grade III: lesions covering a larger area.

2. Skin ulcers. These are usually circular open lesions surrounded by a whitish border, but when in the process of healing the periphery develops darker pigment. Healed ulcers can be identified as areas of dark pigment with irregular distribution of scales, but were not included in the data analysis. Only lesions larger than 2 mm in diameter were recorded, and the position on the body noted as for lymphocystis.

3. Liver neoplasia. Nodular lesions with well-defined margins and a diameter of 2 mm or more, situated on the surface of the liver, were included in this category. Frequently these nodules are lighter or darker in colour than the rest of the liver. All nodules were sampled for further histopathological investigation, and only cases histologically confirmed as neoplasia were retained for data analysis.

Recording of gross lesions, visible with the naked eye, permitted epidemiological investigation of large numbers of fish, as in earlier studies (Vethaak and Meer 1990). Because of the time and costs involved, it would not have been possible to attain such large samples sizes using histological investigation alone.

All 14041 individuals captured during 1983-89 were inspected externally for the presence of lymphocystis and skin ulcers. All 12670 individuals captured during 1985-89 were also inspected internally for liver nodules (Table 2).

In addition, the occurrence of skeletal deformities was noted, but the prevalence of this condition was very low (less than 0.3% overall) and it was therefore omitted from further analysis. Fin rot has already been referred to above.

## 2.3 AGE DETERMINATION

During 1987 and 1988, otoliths were taken from a random subsample of 10 healthy individuals per cm length group at each site, and also from all diseased fish. After the fish had been aged, length age-keys were determined separately for healthy and diseased fish at each site, following the method described by Van Leeuwen and Vethaak (1988). A summary of the growth curves at the 9 sites used in the present study is provided by van Leeuwen and Vethaak (1988).

Using the length-age keys, and the individually determined ages of the diseased fish, the 1987 and 1988 samples could then be reclassified into three age categories (2, 3, and $4^+$ year old fish) for statistical analysis.

## 2.4 OTHER POTENTIAL RISK FACTORS

### 2.4.1 Fish condition factor

A gutted condition factor (C) was determined for a sub-sample of at least 40 fish (females 20-29.9 cm length) at each site every year. For this purpose fish were stored on ice after removal of the viscera, and later weighed in the laboratory.

C was calculated as: 100 x somatic weight (mg) x total length ($mm^{-3}$).

### 2.4.2 Salinity

For each site the observed range of salinities (Table 1) is based on mean values derived from a routine monitoring programme carried out by RWS during 1983-89.

### 2.4.3 Fishing activity

An index of total fishing activity was calculated as the total boat-days spent within each area by beam trawlers (< 300 HP) and shrimp boats in 1987, using statutory fishing returns (RIVO, unpublished data).

### 2.4.4 Contaminant concentrations

Sediment

Concentrations of PCB-C153, PAHs (benz(b)fluoranthene, benz(k)fluoranthene, and benz(a)pyrene), and the heavy metals Pb and Cd were measured in the <63 µm) fraction of superficial sediments from each site. Some sites (Sites 2, 4-9) were sampled in November 1986 and in the summer of 1987, but sampling was repeated in 1990 and 1991 during the same periods in order to cover all the sites (data from special surveys of RWS).

Analysis of contaminant concentrations was carried out according to standard methods described by Joint Monitoring Group of ICES.

Because of the wide variation of the organic content of sediment from site to site (2.0% - 7.5%) the concentrations of these contaminants, known to be attached principally to organic matter, were expressed in terms of organic content.

Flounder livers

Concentrations of polychlorinated biphenyls (PCBs) were measured in pooled samples of 20 male and 20 female livers (length 25-29.9 cm) collected at each site in 1987 during the fish disease survey (author's own data). PCB concentrations were determined using high resolution gas chromatography. Lipid content were determined by soxhlet extraction with hexane. Details of the analytical methods are given in Stronkhorst (1992).

Concentrations of PCBs were expressed as the sum of 6 congeners (C52, C101, C118, C138, C153, and C180). As an alternative, the concentration of C153 was expressed separately: this congener normally makes up about 10% of the total PCB concentration.

## 2.5 DATA PRESENTATION AND STATISTICAL METHODS

The disease data were analysed using a log-linear model with disease occurrence as the dependent variable and site, year, length (or age), and sex as independent variables. The logit model used predicts the log odds logits) for the dependent variable as an additive function of the other variables. If, in a population exposed to a certain risk factor, the number of fish which have a disease is A, and the number not having the disease is B, then the prevalence is A/A+B, but the odds are A/B.

This measure of association is commonly used in retrospective epidemiological studies and seems equally suitable for the analysis of data collected from fish disease surveys (Rhodes et al. 1987, Vethaak 1992, Vethaak et al. 1992).

The method allows the estimation of the odds ratio, which is a measure of the degree of association of explanatory factors (such as site, sampling occasion, length, age or sex) with the occurrence of disease.

The odds ratio, estimated from the parameter coefficients of the logit analysis, is defined, as:

(A/B)/(C/D)

where A and B are defined as above, and C and D are the numbers with and without disease in a population not exposed to the risk factor (C/D being the odds for this population). In the present study, where risk factors are not formally defined, the population represented by C and D is selected as follows. Odds ratios for site were expressed relative to Site 4 (reference site), for year relative to the first year of sampling (1983 or 1985), for length and age relative to the smallest (20-24.9 cm) or youngest (age 2) group, and for females relative to males.

The parameters were estimated using the statistical package GLIM (Baker and Nelder 1978), assuming a binomial error structure. Since the numbers of fish analysed for each combination of independent factors, the design is unbalanced and therefore each main effect was tested by comparing two models, one with all the main effects included and the other with that particular main effect excluded. Interaction effects were tested by comparing the model with all main effects and the interaction effect under consideration.

Because the statistical analysis involved numerous tests (> 50; the limit according to the Bonferroni inequality), the level of significance for each test separately was kept low (a=0.01), in order to ensure a low experiment-wise error rate (preferably not exceeding 0.05).

The following data sets were analysed separately:

1. For spatial trends, data on all three diseases for 1987-89.

2. For temporal trends, data on lymphocystis and skin ulcers for 1983-89 and data on liver nodules for 1985-89 at 3 sites.

3. For effects of age, data on all three diseases for 1987-88.

Multiple regression analysis was then used to seek correlations between the estimated odds ratios for site or year and other potential risk factors such as condition factor, salinity, fishing activity, and chemical concentrations.

## 3 RESULTS

### 3.1 CRUDE DISEASE PREVALENCES

In Table 3 observed disease prevalences (irrespective of size, sex, and year) are presented for each site. It can be seen that lymphocystis was the predominant disease with an overall prevalence of 14.1%, followed by skin ulcers with 2.6%. The overall prevalence of liver neoplasias was 0.8 %.

### 3.2 EFFECTS OF LENGTH, SEX, SITE AND YEAR

#### 3.2.1 Spatial patterns:1987-89 data set

Lymphocystis
For lymphocystis, the logit analysis showed that there were significant differences between sites, years, length groups and sexes. However, there were no significant two-way interactions and thus the final model chosen was that including the four main effects only (Table 4a).

Relative to the reference site, all other sites showed significantly higher estimated odds for lymphocystis (Table 5). Odds ratios increased along the coastal transect going from Site 3 to Site 7. The odds ratio for flounder from the Wadden Sea (Site 8) was comparable to that at Site 3, while odds at the estuarine sites (Sites 1,2 and 9) were lower, though still higher than at the reference site.

Large flounder were more likely than small ones, and males more likely than females, to have lymphocystis (Table 5).

Skin ulcers
For skin ulcers, only site and year were significant main effects, indicating that length and sex did not affect the occurrence of the disease. Again, two-way interactions were not significant (Table 4a).

Relative to the reference site, only 4 sites had significantly higher or lower estimated odds. At 3 coastal sites (Sites 5, 6, 7) the odds for skin ulcers were higher than at Site 4, whereas in the Ems-Dollard (Site 9) it was lower (Table 5).

Liver nodules
For liver nodules, no good fit could be achieved with any model when all four explanatory variables (site, year, length, sex) were used. This was due largely to low prevalences and the presence of zero values in many cells of the table. In order to avoid this problem, data from both sexes were pooled and Sites 1 and 9, where the disease was not observed (Table 2) were omitted.

A reasonably good fit was then obtained by the model including all three main effects but no two-way interactions (Table 6a).

Estimated odds ratios were significantly higher at the coastal sites (Sites 3,5,6 and 7) but not at the Wadden Sea site (Site 8) The estimated odds ratios for Sites 6 and 7 were particularly high (Table 5). Large flounder were more likely to have liver nodules than small ones (Table 5).

Although the effect of sex on the prevalence of liver nodules was ignored, a comparison of the crude data indicated that female flounder more frequently had liver nodules than males, by a factor of about 2 (Fig 3).

### 3.2.2 Temporal patterns: 1983-89 data set

Significant main effects of some or all of site, year, length and sex were found, depending on the disease (Tables 4b, 6b). No significant two-way interaction effects between these four variables were detected. The final models chosen were similar to those obtained by analysing the spatial data set (Table 4a, 6a).

Table 7 presents estimated odds ratios of different years, length groups and sexes, derived from the final models chosen.

For lymphocystis, odds were significantly higher in 1984-88 (relative to 1983), and peaked in 1986. However, the odds for the disease in 1989 were not significantly different from those in 1983. Estimated odds ratios for large fish and male fish (Table 7) approximate to values obtained by analysing the spatial data (Table 5).

For skin ulcers, the odds ratios show considerable temporal variation, peaking in 1984 and 1988 but statistically indistinguishable from 1983 in 1986 and 1987 (Table 7).

Odds ratios for liver nodules showed a very clear peak in 1987, being 4.3 times higher than in 1985. As expected, large fish were much more likely to have liver nodules than small fish, the odds ratio being 4.9 (Table 7).

## 3.3 AGE AS AN EXPLANATORY VARIABLE

### 3.3.1 Length-age relationships

Growth rates of flounder appeared to be considerably higher at the coastal sites than at the estuarine sites (Table 8). There appeared to be no difference in length-age relationships between healthy and those with lymphocystis or skin ulcers. However, flounder with liver neoplasias showed slower growth rates than apparently healthy ones, thus, justifying the use of separate length-age keys for diseased and healthy fish in this case.

### 3.3.2 Spatial patterns: 1987-88 data set

Results of the logit analysis gave broadly similar results to the analysis based on length, with all main effects significant in the case of lymphocystis and liver neoplasias (Table 9). Similarly, the estimated odds ratios did not clearly alter the patterns obtained previously, although estimates were less precise, particularly in the case of skin ulcers and liver neoplasias for which prevalences were relatively low (Table 10). Note that because effects of year were ignored; the effects of sex on the occurrence of liver nodules could be included on this occasion.

An examination of the age composition of fish at each site (Fig 2) shows trends which are not apparent from the statistical analysis, particularly noticeable is the lack of old fish at estuarine sites (Site1, 2, and 9), particulalrly at Site 9 where only 2 and 3 year old fish were caught. Fig 2 also clearly indicates that most of the fish affected with liver nodules belong to the 6+ age group. The occurrence of this disease in fish as young as 2 years old is restricted to the sites with the highest prevalences, particularly Site 6.

With age, the prevalence of lymphocystis shows a steady increase in both males and females until they are about 5 years old, and then levels off (Fig 3). In contrast, the prevalence of skin ulcers, although increasing until the age of 4 years, decreases in older fish (Fig 3). This latter trend was not detected in the logit analysis.

Age-specific prevalences for liver nodules (Fig 3) confirm the pattern of Fig 2, with a steep increase in 6 year old fish. The scarcity or absence of liver nodules from some sites may be explained in terms of the age structure, with older fish being absent. At Site 9, for example the sample was compost almost exclusively of 2 year old fish (Fig 3). On the other hand, 3.2% of the 156 2 and 3 year old fish examined at Site 6 had liver neoplasia, whereas none of the much larger number of similarly aged fish from Sites 1 and 2 were affected.

## 3.4 SEVERITY OF DISEASE SIGNS

The degree of severity of lymphocystis and skin ulcer at each site, categorised as described in Section 2, was investigated using the spatial (1987-89) and the temporal data (1983-89) sets. No statistical analysis was attempted on these data because of the relative low numbers of fish per cell and the complexity of the analysis. For neither disease were there any marked differences between sites or discernible trends from year to year. Thus the degree of severity did not appear to be linked to the prevalence. Percentages of affected fish making up each of the three grades of severity were as follows:

Lymphocystis: Grade 1, 77%; Grade 2, 15%; Garde 3, 8%.

Skin ulcers: 1 ulcer, 57%; 2 or 3 ulcers, 15 %; 4 or more ulcers, 23 %

Of flounder with lymphocystis, 39% had them on the underside and 18 % on the upperside, with 43% having lymphocystis on both sides of the body. For skin ulcers the corresponding percentages were 68% (underside), 24% (upperside) and 8% (both sides). For both diseases most sites showed a similar trend, except Sites 1 and 3 for skin ulcers.

## 3.5 ASSOCIATIONS BETWEEN DISEASE CONDITIONS

It was found that 3.6 % of the fish affected by lymphocystis also had skin ulcers, whereas the corresponding percentage was only 2.3% in fish unaffected by lymphocystis. The association was highly significant (Table 11). This is in agreement with the overlap in their spatial patterns. Liver nodules, on the other hand, were not significantly associated with either lymphocystis or skin ulcers (Table 11).

## 3.6 RELATIONSHIP WITH OTHER POTENTIAL RISK FACTORS

### 3.6.1 Condition factor

There was considerable temporal variation of the condition factor in the study area. At Sites 4, 5 and 6 temporal differences over 7 years were largely consistent with a maximum in 1987, falling to low values in 1988 and 1989. This fall to low values towards the end of the study was also visible at the other sites (data for only 3 years), with the exception of the Wadden Sea (Site 8) and the Ems-Dollard estuary (Site 9) where values remained fairly constant (Fig 4).

No clear spatial trend emerged, although values at Sites 1, 2, and 7 were lower than at Sites 8 and 9, at least in 1988 and 1989.

### 3.6.2 Salinity

A highly significant positive correlation was found between the occurrence of liver nodules (using log (odds)) and mean salinity at each site ($r=0.908$, $p< 0.001$). Correlations of salinity with lymphocystis and skin ulcers were not significant ($P= 0.22$ and 0.08 respectively). It should be noted, however, that the distribution of sites was heavily biased towards high salinity values with only Sites 1, 2 and 9 having medium or low salinities. It is clear from the data that these low salinity sites were associated with low prevalences of lymphocystis and skin ulcers as well (Table 5).

### 3.6.3 Fishing activity

Fig 5 indicates the relative intensity of fishing at each site in 1987. The pattern in 1988 and 1989 was considered to be similar (RIVO unpublished data).

Fishing intensity was found to be significantly positively correlated with the occurrence of lymphocystis ($r=0.80$, $p< 0.01$), skin ulcers ($r=0.75$; $p< 0.02$) and liver nodules ($r=0.80$, $p< 0.04$).

### 3.6.4 Contaminant concentrations

Concentrations of PCBs in the liver were similar in males and females, with the exception of Site 2 where females showed concentrations almost twice as high as males (Fig 6). Concentrations were higher at Sites 2, 3 and 5 than at the other sites, which appeared to differ little among themselves (Fig 6). The high values at Site 2, and perhaps also at Site 3, probably indicate a local source of pollution originating from the Western Scheldt, whereas Site 5 is affected by discharges from the Rhine.

The spatial patterns of all four groups of contaminants in sediments were rather similar in 1986-87 and 1990-91, but concentrations of Pb and especially Cd were higher at Site 6 on the latter occasion (Fig 7).

When the complete data set was analysed, no significant correlations were detected between the different contaminants and the estimated log odds for lymphocystis, skin ulcers and liver nodules. When the analysis was restricted to the 6 marine sites, in order to eliminate a possible bias due to salinity, Pb in sediment then showed positive correlations with the occurrence of skin ulcers and liver nodules (Table 12).

Concentrations of PCBs in the sediments, although significantly correlated with each of the three other contaminant groups (Table 12), showed no apparent correlation with PCB concentrations in the liver of the fish. This discrepancy is partly due to high concentrations in the liver at Site 3, which might indicate movement of fish to this site from Site 2 or elsewhere.

# 4 DISCUSSION

## 4.1 SPATIAL AND TEMPORAL PATTERNS OF DISEASE

The results reveal considerable similarities in the spatial distribution of the three diseases studied. Despite their different aetiologies, this suggest that a common causal mechanism may contribute to their development, particularly as two of the diseases (lymphocystis and skin ulcers) were associated in individual fish as well. Temporal variation, however, showed little similarity between the diseases.

The observed spatial patterns did not correspond to the nutritional status of the fish (as assessed by the condition factor) or on the whole to observed contaminant concentrations. Instead, disease prevalence was statistically correlated with salinity and fishing activity, with sites of low salinity (Table 1) and little fishing activity (Fig 5) having the lowest disease prevalences.

Nevertheless, the results show for the sites with high salinities (Sites 3 - 8; Table 1), that prevalences of all the diseases were considerably lower at the Eastern Scheldt reference site (Site 4) than at the other sites (Table 5). The only exception to this was skin ulcers at Site 8 in the Wadden Sea (Table 5). This would appear to justify the choice of Site 4 as reference site. On the basis of contaminant levels in sediments and fish tissue, however, Sites 8 or 9 would have been equally suitable as reference sites (Fig 6, 7). In addition, an influence of fishing activity on disease prevalences at these sites cannot be discounted, and is discussed below.

## 4.2 SALINITY

The finding that salinity could be an important factor affecting skin diseases of flounder is in agreement with earlier studies on the same species in the Elbe (Möller 1990) and the Dutch Wadden Sea (Vethaak 1992). At the same time, the absence of liver neoplasia in fish from waters of low salinity agrees with the findings of Peters et al (1989) in the polluted Elbe estuary.

The nature of the possible reduced disease risk associated with low salinity is not known. The occurrence, activity or pathogenicity of the lymphocystis virus or of bacteria associated with skin ulcers could show a positive relationship with salinity, but this is unlikely to apply to he liver neoplasia and other factors may be involved as well.

## 4.3 FISHING ACTIVITY

Flounders have a low commercial value in the Netherlands and they are frequently discarded from catches. At most of the coastal sites there are intensive flatfish and shrimp fisheries, and fresh or healed catch wounds are commonly observed in flounder from these areas. Fishing gear could damage the protective mucous layer of the fish, making them more vulnerable to infectious pathogens.

In general, the sites with most fishing activity (Sites 3, 5, 6) also had highest disease prevalences, and the reference site (Site 4) was unique among the coastal sites in having almost no fishing (Fig 5). Changes in fishing pressure can not explain, however, observed temporal variation in disease prevalence. For example, the peak of lymphocystis in 1986 did not coincide with any substantial increase of fishing pressure during that year (RIVO unpublished data). Moreover, the prevalence of skin ulcers was particularly low during the same year.

Furthermore, it is difficult to imagine how fishery activity could have an influence on the development of liver neoplasia, and thus at best it could help to explain the spatial pattern of only 2 out of the 3 diseases studied.

## 4.4 CONTAMINANTS AND SEDIMENT-ASSOCIATED FACTORS

Although there was some indication of a relationship between contaminant concentrations and spatial disease patterns for the marine sites alone (Table 12), the evidence is weak and unconvincing.

A statistical analysis of PCB C138 and Cd in the liver tissue and of Hg in the muscle tissue of flounder from Sites 2 and 9 was carried out by Stronkhorst (1992). Only for Cd at Site 9 was there any significant increase in concentrations from 1985 to 1990. These findings could not explain the observed temporal patterns of disease at these sites.

However, the observation that skin ulcers and lymphocystis occur more frequently on the blind site of the fish suggest a determining role of factors associated with sediment. These could include irritation by sediment particles alone or with associated micro-contaminants, particularly as flounders prefer soft muddy substrata and turbid habitats. On the other hand, the muddiest and most turbid sites, broadly speaking, were in estuaries where disease prevalences were lowest.

## 4.5 NUTRITIONAL STATUS

In principle, a low condition factor may indicate malnutrition associated with local feeding conditions, which could increase disease risk, as proposed by Möller (1990) for Elbe flounder.

However, statistical analysis of the present data indicates that malnutrition is unlikely to play an important role in the development of disease at the sites studied. This factor was, however, considered important for flounder populations near drainage sluices in the Wadden Sea (Vethaak 1992). Although estuarine sites were included in the present study (Sites 1, 2, 9), they were not comparable to those studied by Vethaak (1992) in terms of salinity fluctuations, population density, and other factors. Prevalences of skin ulcers were considerably lower in the present study.

The possible influence of the nature, as well as the quantity, of food should also be taken into account when attempting to explain patterns of disease prevalence. in a study carried out in 1989, considerable spatial variation was found in flounder diets, fish at some sites feeding almost exclusively on crustaceans, those at other sites on bivalves, and yet others having mixed diets with a high proportion of polychaetes (Haver and Vethaak, unpublished data). Such differences could be important in terms of the lipid content of the prey that might affect contaminant accumulation, or other food-related risk factors. This hypothesis remains purely speculative for the moment, however.

## 4.6 OTHER POSSIBLE CAUSAL FACTORS

Although temperature fluctuations and temperature extremes may interfere with the immune system of fish and affect the occurrence and virulence of pathogens, differences between the various sites in the present study were small (2 to 4 deg C at most) and are unlikely to have affected observed prevalences. Similarly high population densities could increase prevalences, but in the present study those sites with highest disease prevalences (Sites 3, 6, 7) also had the lowest population densities (author's unpublished data).

Some of the disease patterns could be explained in terms of spawning and associated migration. During spawning, flounder are subjected to stress which may lower their resistance to infections. The high prevalence of lymphocystis in male flounder by comparison with females may find its origin in spawning stress in combination with a high population density on the offshore spawning grounds, where males remain for considerable longer periods than females (Rijnsdorp and Vethaak 1989).

Conversely, the higher risk of developing liver neoplasia in female fish may be linked with reproduction, perhaps via sex hormones or risk factors related to the depletion of lipid stores in the liver. A similar sex-related difference in prevalence was found in dab (Vethaak and Meer 1991), but does not appear to have been reported elsewhere in the literature.

A little-known factor which may be related to temporal variation in particular is the history of the cohorts examined. Fluctuating environmental conditions affecting larval and post-larval stages may lead to different selection pressures, thus indirectly affecting disease prevalence in later life. For example, the weaker members of a cohort could be eliminated by a particularly cold winter or higher predation pressure occurring at age 0+, thus decreasing the apparent prevalence of disease in future years.

## 4.7 LIVER NODULES

Since lymphocystis and skin ulcers are infectious diseases, any effect of pollution can only be indirect, for example via immunosuppression or via activation or increased virulence of a pathogen. By contrast the liver neoplasia observed in flounder could have a direct chemical aetiology without the involvement of infective agents. Although this has not been established for flounder in particular, chemical contaminants in the livers of other fish species are thought to act as carcinogens (Mix 1986, Malins et al 1989, Vethaak and ap Rheinallt 1992),

The finding of high prevalences of liver neoplasia at some of the coastal sites thus merits closer examination. Spatial variation in the prevalence of this disease was large but very consistent from year to year. The highest prevalences were found near the mouths of heavily polluted estuaries, with significantly lower values at the reference site further removed from direct estuarine discharges. As stated above, differences in the intensity of fishery activity are unlikely to offer a satisfactory explanation for this spatial pattern. However, the disease was absent from fresh and brackish waters irrespective of pollution status, suggesting that a factor related to salinity is necessary for its development.

For neoplasia as well as the two skin diseases, the observed increase in prevalences with length and age of the fish are well known. The increase in prevalence with length is approximately linear for both lymphocystis and skin ulcers, but cases of liver neoplasia are concentrated in the very largest fish. Since growth is asymptotic, this suggests that the occurrence of neoplasia is related to age rather than length.

Neoplastic nodules were found mainly in individuals older than 6 years of age, which were absent from some estuarine sites. However, at Sites 5, 6 and 7 neoplasia were also observed in younger flounder (2 and 3 years old), suggesting extreme exposure to causal agents (Fig 3). A more detailed recent survey of Site 6, near the North Sea Canal and in the vicinity of an iron works, has revealed the occurrence of higher prevalences of liver nodules in young flounder (20-24 cm; age 1) than in older individuals (author's unpublished data). This suggests both unusually high levels of a causative factor or factors, as well as early mortality from the disease. It seems possible that the factor involved is pollution, and further investigation are planned at Site 6.

It should also be noted that marked temporal variation in the occurrence of liver neoplasia was observed, with a peak in 1987. This cannot be explained in terms of salinity, fishing activity, or contaminant concentrations.

As well as probably having a different aetiology, liver neoplasia also differs from the other two diseases studied in having a more lengthy development period. For a chronic disease, long-term movements and migratory patterns are likely to be important in explaining spatial variation. In the present case, the downstream migration of flounders as they grow explains why the disease is absent or has only a low prevalence at some estuarine sites, as above. In addition, large fish at some of the coastal sites may have been exposed to pollution under estuarine conditions at an earlier stage in their lives. For example, flounder at Site 6 and 7 may have grown up in the North sea canal, those at Site 5 in the Rhine, and those at Site 3 in the Western or Eastern Scheldt.

Histologically, the liver lesions observed in this study correspond to those described in American flatfish species. A publication dealing with the histological aspects is in preparation (Vethaak and Wester, in prep). There are, however, some differences between the findings reported here and published American findings on liver neoplasia in fish (Mix 1986, Malins 1988). In contrast to the latter, neoplasia appears to be present in fish from less polluted sites as well as in those from heavily polluted sites. However, it must be admitted that background prevalences of this diseases in flounder from genuinely 'clean' sites are unknown, and migratory patterns still need clarification.

## 4.8 GENERAL DISCUSSION

The present study demonstrates that disease occurrence is influenced by host-related factors such as length, age and sex. Although corrections can be made for these factors, the same does not apply to pollution or to other environmental factors, such as salinity and fishing activity. The last two factors are positively correlated, with fishing activity greatest in coastal waters where salinity is highest. Thus it is not possible to separate out their effects, or indeed to consider the effect of pollution acting in isolation. It can be said, however, that in certain estuaries, the negative effect of low salinity or absence of fishing on disease prevalence appear to override any potential positive effects of high pollution levels.

It is in any case apparent that comparison of disease prevalences in flounder along a transect of a river or estuary must be considered invalid from the point of view of pollution-related studies. A better approach would be to compare cohorts of flounders at the mouths of different estuaries. These cohorts probably consist of fish which have migrated downstream during the first years of their life.

One or more of salinity, fishing activity, and pollution may help to explain the observed spatial patterns of disease, but temporal variation appears to have a different explanation. Not only did the three diseases studied show different patterns of temporal variation, but in addition none of the factors studied are likely to vary over time in the same way as disease prevalence. The cause of the observed variation is thus unknown.

# 5 CONCLUSIONS

In flounder, the apparent association between the three diseases studied may indicate a general underlying mechanism of disease causation.

However, the findings of the study do not indicate a clear and universal relationship between pollution and disease prevalence, but suggest instead that disease causation is complex. Effects of salinity, fishing activity, and migratory behaviour may interact with pollution to produce observed spatial patterns of the diseases studied, the evidence for a relationship is perhaps strongest for liver neoplasia. However, there are too many uncontrolled factors for any firm conclusion to be possible.

Nevertheless, this study has produced several hypotheses which can be tested further. Experimental work will be particularly important in this context, and a large-scale mesocosm study now in progress on the island of Texel (Vethaak 1992) is designed to test for the effects of pollution acting in isolation.

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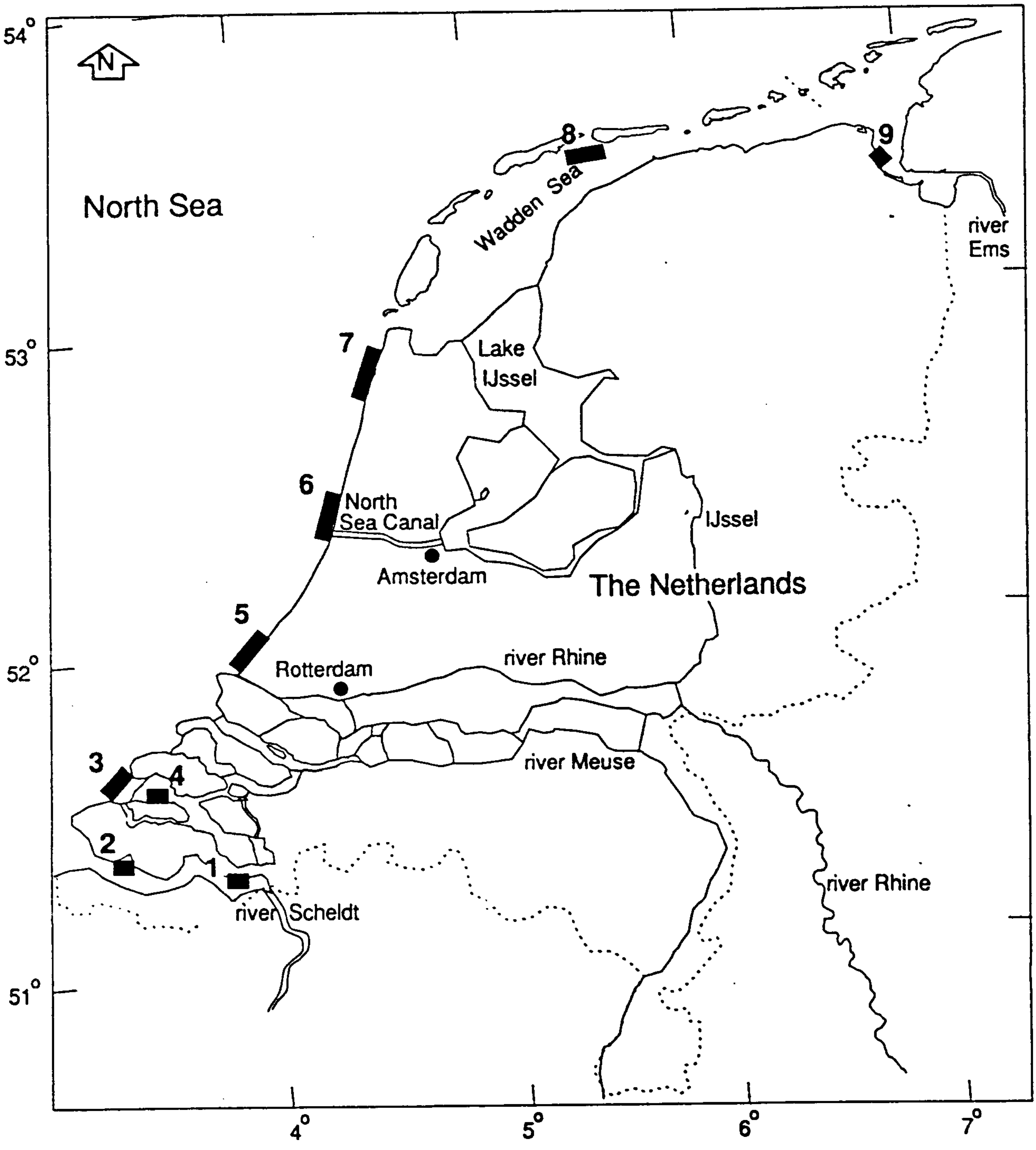


Fig. 1 Map showing the geographic position of the sampling sites.

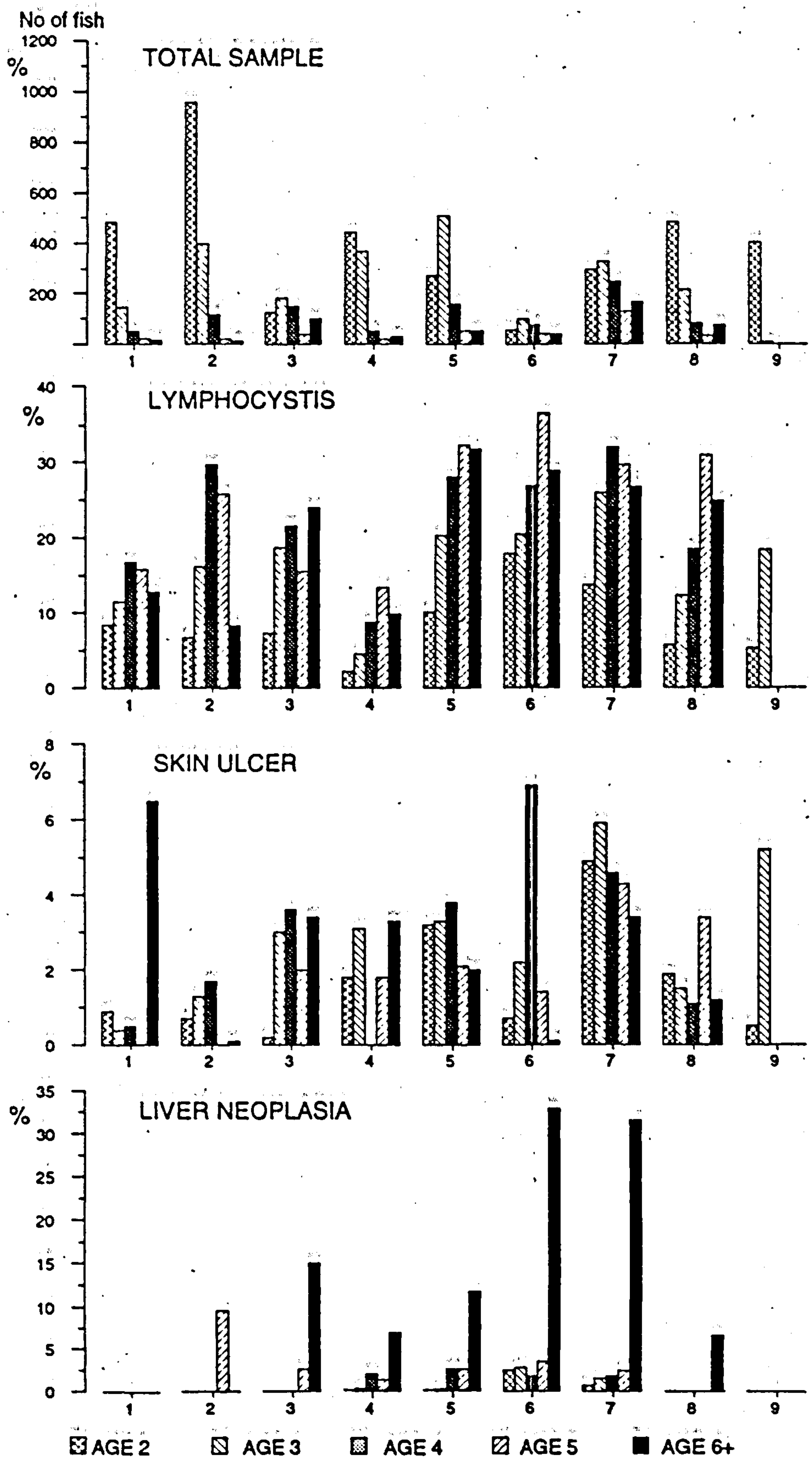


Fig. 2 Age distribution of all flounder sampled, and prevalences by age group of lymphocystis, skin ulcers and liver nodules at each sampling site in 1987-88 (data for the 2 years pooled).

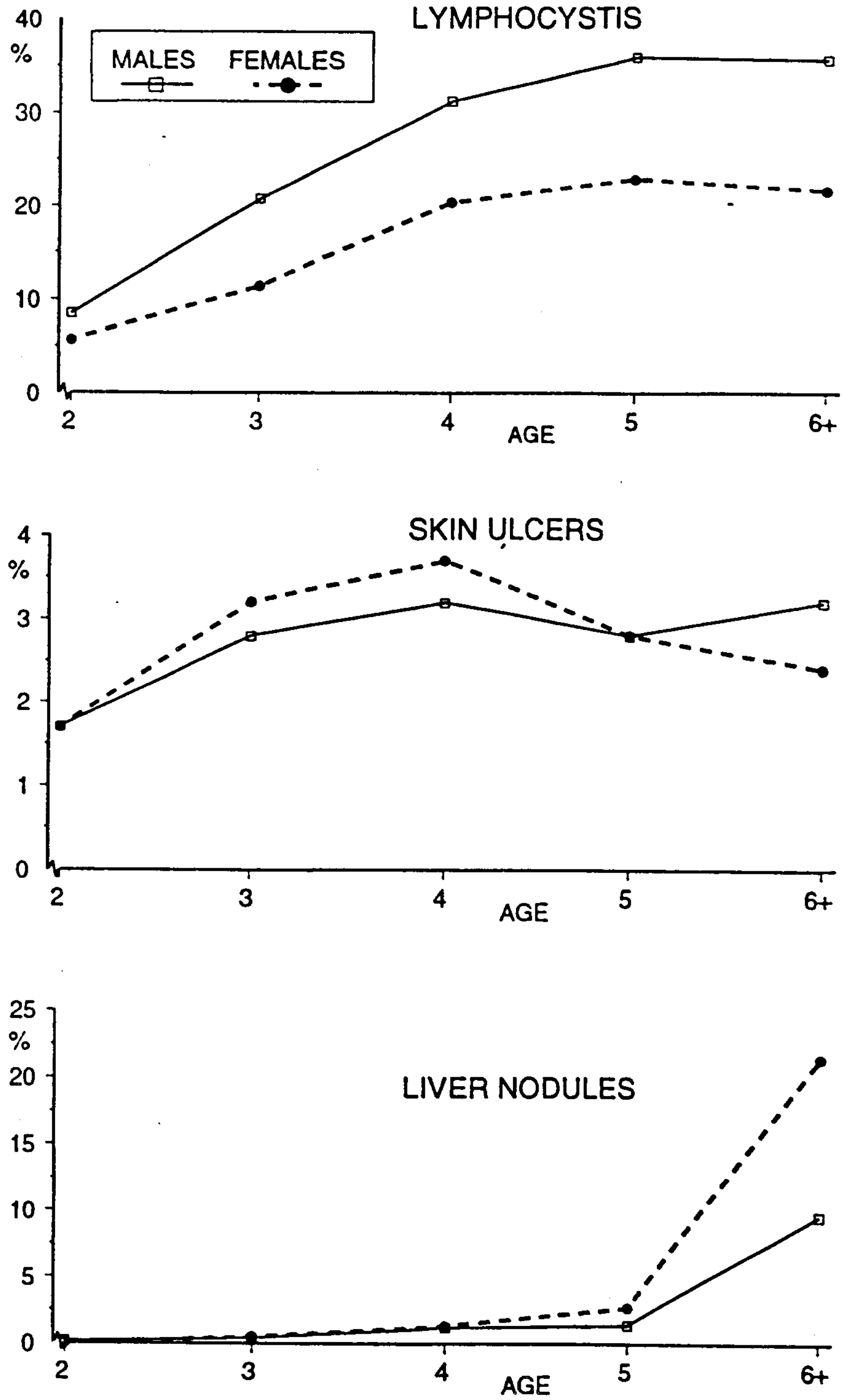


Fig 3. Age-specific observed prevalences of lymphocystis, skin ulcers and liver nodules in male and female in 1987-88 ( data from all sites pooled).

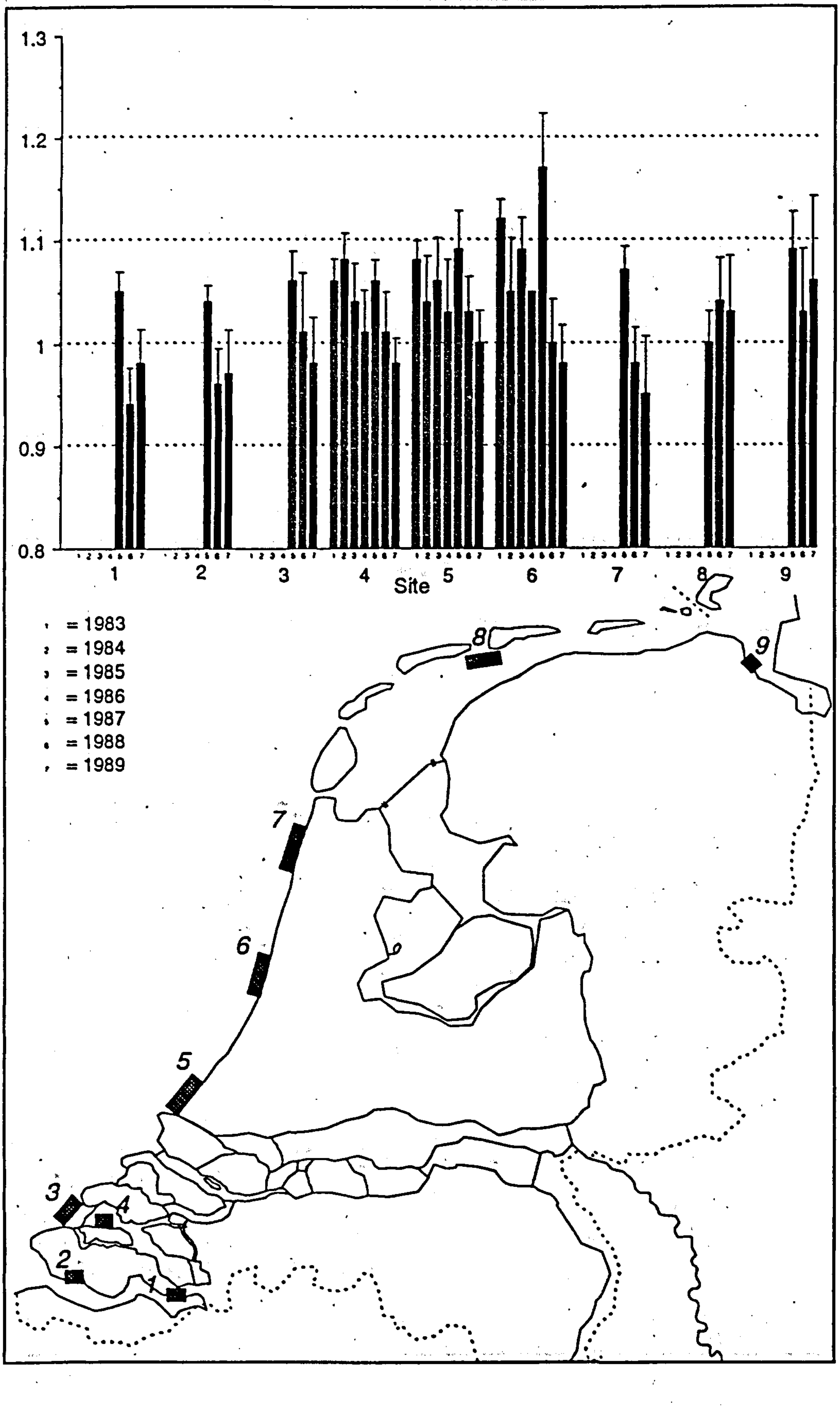


Fig. 4 Mean (SD) condition factors (+ 95% confidence interval) of female flounder 20-29.9 cm in length, plotted by site and year of capture.

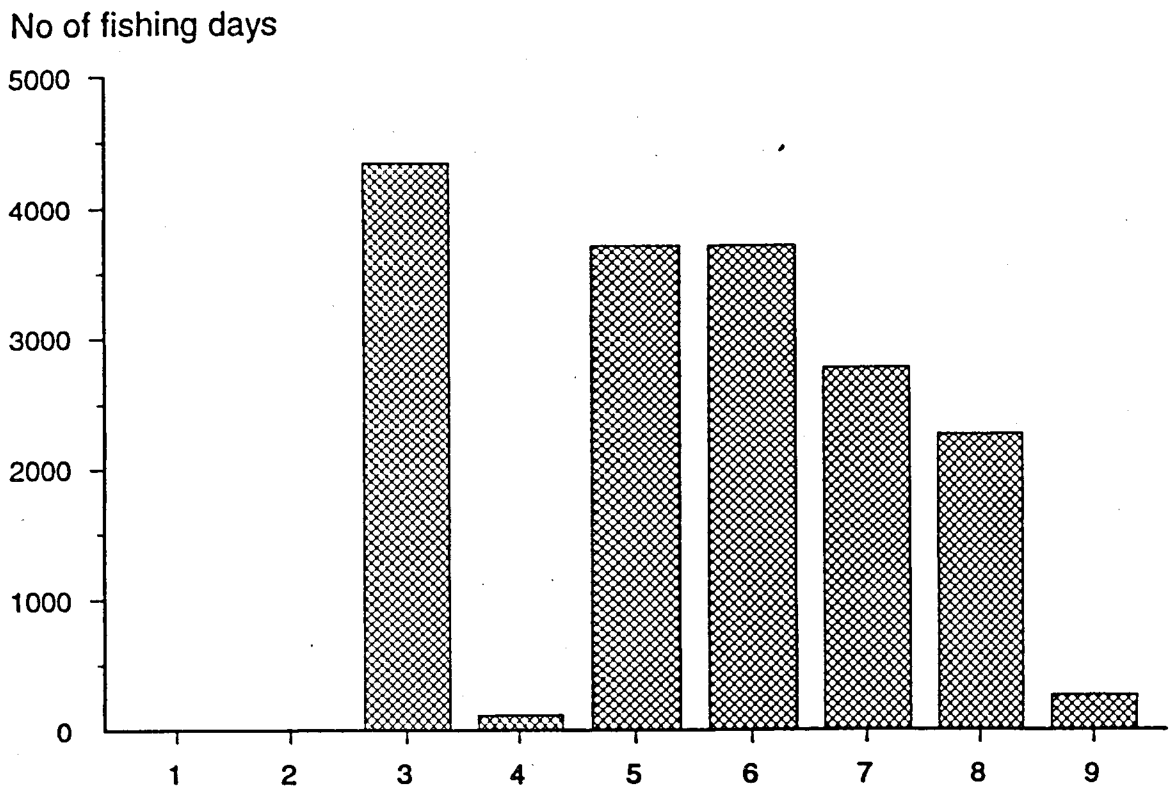


Fig. 5 Total number of fishing days fished by commercial vessels at the each sampling site in 1987.

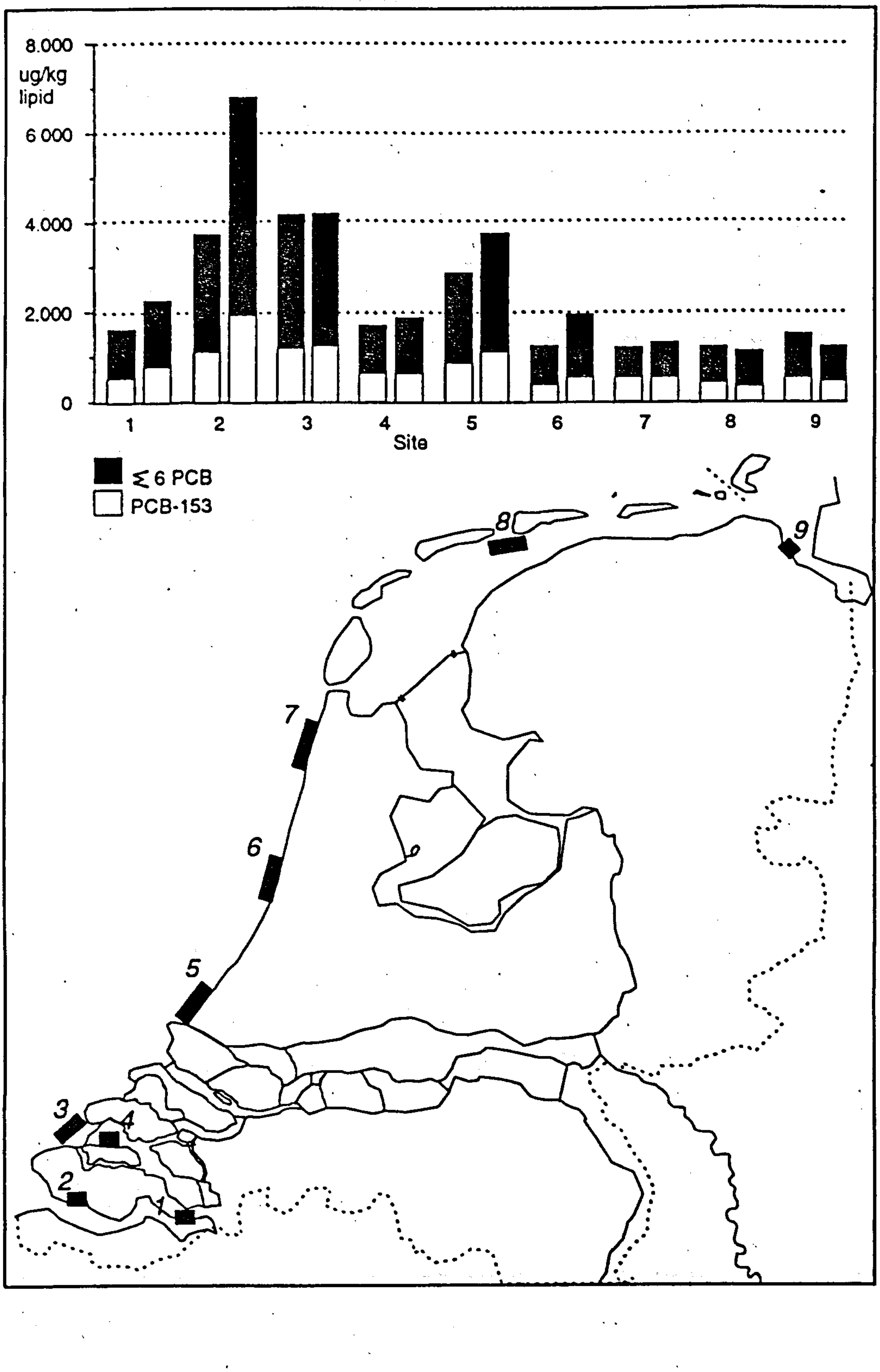


CHART09

Fig. 6 Mean concentrations (µg/kg lipid) of an individual PCB congener (PCB C153) and of the 6PCB (sum of C52, C101, C118, C138, C153, and C180) in the livers of male and female flounder 20-29.9 cm in length at each sampling site in September 1987.

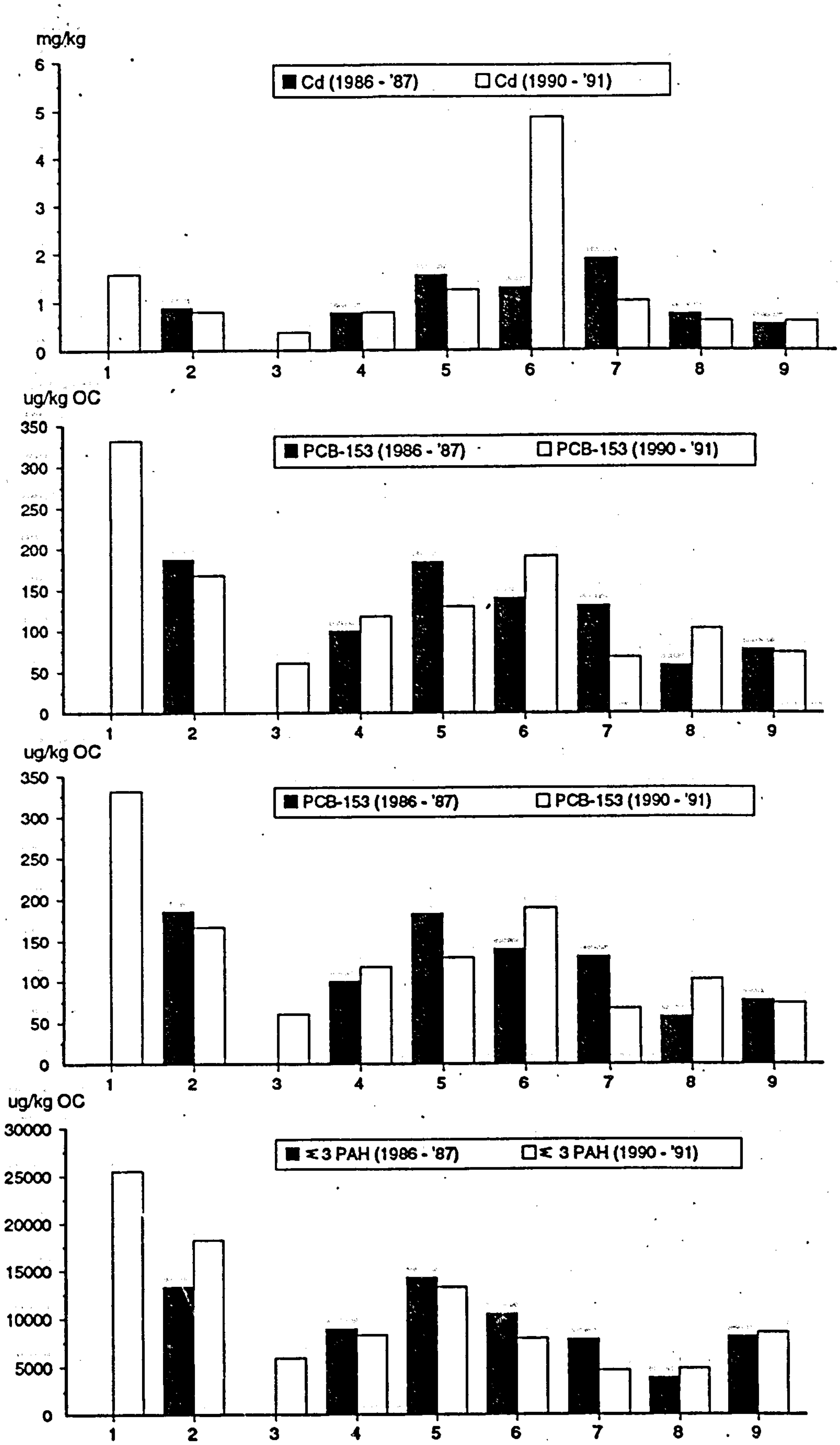


Fig. 7 Mean concentrations of Cd (mg/kg dry wt), Pb (mg/kg dry wt), PCB-C153 (µg/kg organic C), and 3PAH (benz(b)fluoranthene, benz(k)fluoranthene, and benz(a)pyrene) (µg/kg organic C) in the < 63 µm fraction of sediments at each site in 1986/87 and 1990/91.

Table 1: Characterisation of sampling sites

| | Site | Habitat | Main pollution inputs | Salinity (g/kg) | Fishing depth (m) |
|---|---|---|---|---|---|
| 1 | Western Scheldt (Saaftinge) | estuarine | River Scheldt | 1.6-8.5 | 1-5 |
| 2 | Western Scheldt (Vlissingen) | estuarine | River Scheldt and the canal from Gent to Terneuzen | 20.5-29.6 | 1-5 |
| 3 | North Sea coast (Voordelta) | coastal zone | indirectly via River Scheldt, Belgian coastal waters and Eastern Scheldt | 29.8-32.0 | 7-14 |
| 4 | Eastern Scheldt (Hammen) | estuarine, semi-enclosed | only indirectly from Hollands Diep via Volkerak and River Scheldt | 29.3-30.8 | 1-5 |
| 5 | North Sea coast (H. off Holland) | coastal zone | water from the Rhine and Meuse | 26.9-28.8 | 7-14 |
| 6 | North Sea coast (IJmuiden) | coastal zone | directly from North Sea Canal; indirectly via water from Rhine | 27.7-29.2 | 14-21 |
| 7 | North Sea coast (Callantsoog) | coastal zone | no direct discharges; indirectly via Sites 5 and 6 | 28.9-30.4 | 7-21 |
| 8 | Wadden Sea | estuarine, tidal basin | Lake IJssel and North Sea coastal waters | 28.1-30.1 | 1-22 |
| 9 | Ems-Dollard | estuarine | River Ems | 8.3-14.1 | 1-5 |

Table 2: Total number of fish examined every year at each site.

| SITE | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 |
|---|---|---|---|---|---|---|---|
| 1 | - | - | - | - | 311 | 406 | 443 |
| 2 | - | - | - | - | 386 | 392 | 590 |
| 3 | - | - | - | - | 312 | 280 | 226 |
| 4 | 458 | 602 | 362 | 238 | 625 | 283 | 855 |
| 5 | 591 | 909 | 391 | 762 | 451 | 587 | 194 |
| 6 | 306 | 951 | 337 | 570 | 188 | 125 | 216 |
| 7 | - | - | - | - | 735 | 421 | 113 |
| 8 | - | - | - | - | 560 | 325 | 169 |
| 9 | - | - | - | - | 294 | 117 | 406 |

Table 3: Observed prevalences of lymphocystis (%LYM), skin ulcers (%ULC) and liver nodules (%LNL) at each site (all sizes and years combined). N= no of fish examined.

| | EXTERNAL DISEASES | | | | LIVER NODULES | | |
|---|---|---|---|---|---|---|---|
| SITE | Years | N | %LYM | % ULC | Years | N | %LNL |
| 1 | 1987-89 | 1160 | 7.2 | 1.0 | 1987-89 | 1160 | 0.0 |
| 2 | 1987-89 | 1368 | 7.2 | 1.1 | 1987-89 | 1368 | 0.1 |
| 3 | 1987-89 | 818 | 16.1 | 2.4 | 1987-89 | 818 | 1.3 |
| 4 | 1983-89 | 3423 | 4.4 | 1.4 | 1985-89 | 2363 | 0.1 |
| 5 | 1983-89 | 3885 | 20.0 | 3.4 | 1985-89 | 2385 | 0.8 |
| 6 | 1983-89 | 2693 | 22.8 | 3.9 | 1985-89 | 1436 | 1.9 |
| 7 | 1987-89 | 1269 | 24.1 | 4.7 | 1987-89 | 1269 | 3.4 |
| 8 | 1987-89 | 1054 | 12.0 | 1.5 | 1987-89 | 1054 | 0.3 |
| 9 | 1987-89 | 817 | 4.3 | 2.0 | 1987-89 | 817 | 0.0 |
| TOTAL | | 16487 | 14.1 | 2.6 | | 12670 | 0.8 |

Table 4: Summary of the logit analysis of lymphocystis (LYM) and skin ulcers (ULC), based on (a) the 1987-89 data set (9 sites), and (b) the 1983-89 data set (3 sites): deviances with associated residual degrees of freedom (df) and significance tests (* =p<0.01). Models finally chosen are underlined. See text for explanation of significance tests.

| a) Significance tests | df | deviance | |
|---|---|---|---|
| | | Ly | Ulc |
| null model | 81 | 1367.7 | 161.7 |
| all four main effects | 71 | 71.8 | 81.7 |
| without Site | 73 | 439.8* | 123.4* |
| without Year | 77 | 184.2* | 113.5* |
| without Sex | 72 | 275.8* | 82.8 |
| without Length | 72 | 412.3* | 81.7 |
| without Length, Sex | 73 | | 82.9 |
| with Site*Year | 59 | 60.8 | 70.1 |
| with Site*Length | 69 | 65.9 | 79.0 |
| with Site*Sex | 69 | 68.5 | 78.5 |
| with Year*Length | 65 | 63.8 | 74.6 |
| with Year*Sex | 65 | 59.4 | 70.5 |
| with Length*Sex | 70 | 71.7 | 79.2 |

| b) Significance tests | df | deviance | |
|---|---|---|---|
| | | LYM | ULC |
| null model | 97 | 945.2 | 181.8 |
| all four main effects | 85 | 77.0 | 110.4 |
| without Year | 87 | 164.2* | 118.8* |
| without Site | 93 | 292.7* | 161.0* |
| without Sex | 86 | 200.1* | 110.9 |
| without Length | 86 | 324.0* | 111.1 |
| without Length, Sex | 87 | | 111.7 |
| with Site*Year | 69 | 61.5 | 93.0 |
| with Site*Length | 77 | 70.4 | 102.6 |
| with Site*Sex | 77 | 62.5 | 98.1 |
| with Year*Length | 83 | 74.7 | 109.9 |
| with Year*Sex | 83 | 69.1 | 107.3 |
| with Length*Sex | 84 | 73.4 | 110.3 |

Table 5: Estimated odds ratios (with 95% confidence intervals) for effects of site, length and sex on the occurrence of lymphovystis (LYM), skin ulcers (ULC), and liver nodules (LNL). Effects of sex were not included in the analysis of liver nodules.

| EFFECT | Relative to | LYM | | ULC | | LNL | |
|---|---|---|---|---|---|---|---|
| SITE 1 | SITE 4 | 2.0 | (1.4-2.9) | 0.5 | (0.3-1.1) | | |
| SITE 2 | ,, | 2.4 | (1.7-3.3) | 0.6 | (0.3-1.1) | 0.6 | (0.1-6.1) |
| SITE 3 | ,, | 3.8 | (2.6-5.1) | 1.3 | (0.7-2.3) | 6.8 | (1.5-31.0) |
| SITE 5 | ,, | 4.0 | (2.3-5.5) | 1.8 | (1.1-3.0) | 6.3 | (1.4-28.4) |
| SITE 6 | ,, | 4.5 | (3.1-6.6) | 2.0 | (1.1-3.7) | 19.5 | (4.4-87.0) |
| SITE 7 | ,, | 6.0 | (4.4-8.1) | 2.7 | (1.7-4.2) | 15.7 | (3.7-65.7) |
| SITE 8 | ,, | 3.8 | (2.7-5.3) | 0.9 | (0.5-1.6) | 2.3 | (0.4-14.2) |
| SITE 9 | ,, | 1.8 | (1.2-2.8) | 0.2 | (0.1-0.4) | | |
| LARGE | SMALL | 3.2 | (2.7-3.7) | - | | 10.1 | (4.8- 21.2) |
| MALE | FEMALE | 2.1 | (1.9-2.5) | - | | | |

Table 6: Summary of the logit analysis of liver nodules (LNL) based on (a) the 1987-89 data set (9 sites), and (b) the 1985-89 data set (3 sites). Details as for Table 4.

| a) Significance tests | df | deviance |
|---|---|---|
| | | LNL |
| null model | 35 | 177.5 |
| all three main effects | 27 | 18.9 |
| without Site | 32 | 68.2* |
| without Year | 29 | 41.2* |
| without Length | 28 | 81.0* |
| with Site*Year | 17 | 15.3 |
| with Site*Length | 22 | 10.8 |
| with Year*Length | 25 | 15.2 |

| b) Significance tests | df | deviance |
|---|---|---|
| | | LN |
| null model | 29 | 88.4 |
| all three main effects | 22 | 18.0 |
| without Year | 26 | 32.1* |
| without Site | 24 | 51.4* |
| without Length | 23 | 36.5* |
| with Site*Year | 14 | 12.9 |
| with Site*Length | 20 | 12.4 |
| with Year*Length | 18 | 15.7 |

Table 7: Estimated odds ratios (with 95% confidence intervals) for effects of year on the occurrence of lymphocystis (LYM), skin ulcers (ULC), and liver nodules (LNL). Effects of sex were not included in the analysis of liver nodules. Note that for liver nodules the effect of year is expressed relative to 1985.

| EFFECT | Relative to | LYM | | ULC | | LNL | |
|---|---|---|---|---|---|---|---|
| 1984 | 1983 | 1.9 | (1.5 -2.4) | 2.3 | (1.5-3.6) | | |
| 1985 | 1983 | 1.8 | (1.4 -2.4) | 2.0 | (1.2-3.3) | | |
| 1986 | 1983 (1985) | 2.6 | (2.0 -3.2) | 1.1 | (0.6-1.9) | 1.0 | (0.4 - 2.5) |
| 1987 | 1983 (1985) | 1.5 | (1.2 -2.0) | 1.1 | (0.6-2.1) | 4.3 | (1.7 - 11.0) |
| 1988 | 1983 (1985) | 1.8 | (1.3 -2.3) | 2.4 | (1.4-4.0) | 1.7 | (0.6 -4.7) |
| 1989 | 1983 (1985) | 0.8 | (0.6 -1.2) | 2.1 | (1.2-3.6) | 1.2 | (0.4 -3.5) |
| LARGE | SMALL | 3.3 | (2.9 -3.8) | | - | 4.9 | (2.1 -11.1) |
| MALE | FEMALE | 2.4 | (2.1-2.7) | | - | | |

Table 8: Mean lengths and ages of each length class of flounder at each site (data for 1987 and 1988 pooled).

| | 20-24.9 CM | | | 25-29.9 CM | | | 30-39.9 CM | | |
|---|---|---|---|---|---|---|---|---|---|
| SITE | LEN | AGE | N | LEN | AGE | N | LEN | AGE | N |
| 1 | 22.4 | 2.2 | 99 | 27.2 | 2.2 | 99 | 33.8 | 3.7 | 243 |
| 2 | 22.6 | 2.1 | 181 | 26.9 | 2.1 | 622 | 33.8 | 4.2 | 296 |
| 3 | 22.9 | 2.0 | 57 | 27.2 | 2.7 | 186 | 34.4 | 4.9 | 319 |
| 4 | 22.6 | 2.3 | 152 | 26.9 | 2.3 | 502 | 33.9 | 4.3 | 248 |
| 5 | 22.7 | 2.1 | 156 | 27.1 | 2.9 | 437 | 34.0 | 4.8 | 419 |
| 6 | 22.4 | 2.3 | 10 | 27.2 | 2.5 | 66 | 34.2 | 4.4 | 112 |
| 7 | 23.0 | 2.1 | 118 | 27.1 | 2.7 | 389 | 34.2 | 4.7 | 595 |
| 8 | 22.4 | 2.1 | 233 | 26.5 | 2.3 | 236 | 34.4 | 4.7 | 116 |
| 9 | 21.5 | 2.0 | 200 | 26.7 | 2.0 | 85 | 33.7 | 3.3 | 8 |

Table 9: Summary of the logit analysis of lymphocystis (LYM), skin ulcers (ULC), and liver nodules (LNL), using age instead of length as main effect, based on the 1987-88 data set. Details as for Table 4.

| a) Significance tests | df | deviance | | |
|---|---|---|---|---|
| | | LYM | ULC | LNL |
| null model | 53 | 532.9 | 91.4 | 347.4 |
| all four main effects | 42 | 34.6 | 36.6 | 33.7 |
| without Site | 50 | 172.1* | 77.7* | 96.5* |
| without Sex | 43 | 92.9* | 36.6 | 52.3* |
| without Age | 44 | 208.0* | 38.5 | 143.4* |
| Site | 45 | | 38.5 | |
| with Site*Age | 26 | 17.2 | 21.3 | 17.8 |
| with Site*Sex | 34 | 21.8 | 23.4 | 28.4 |
| with Age*Sex | 40 | 32.2 | 35.9 | 29.1 |

Table 10: Estimated odds ratios (with 95% confidence intervals) for effects of site, age and sex on the occurrence of lymphocystis (LYM), skin ulcers (ULC), and liver nodules (LNL).

| EFFECT | Relative to | LYM | ULC | LNL |
|---|---|---|---|---|
| SITE 1 | SITE 4 | 2.8 (2.0 - 4.1) | 0.4 (0.2 - 0.9) | |
| SITE 2 | ,, | 3.3 (2.2 - 5.1) | 0.4 (0.2 - 0.9) | 0.4 (0.1 - 3.2) |
| SITE 3 | ,, | 3.5 (2.4 - 5.3) | 1.1 (0.6 - 2.2) | 2.2 (0.7 - 6.5) |
| SITE 5 | ,, | 4.5 (3.1 - 6.6) | 1.4 (0.8 - 2.5) | 1.8 (0.6 - 5.4) |
| SITE 6 | ,, | 5.5 (3.5 - 8.8) | 1.3 (0.6 - 3.0) | 5.0 (1.6 - 15.0) |
| SITE 7 | ,, | 5.6 (3.9 - 8.1) | 2.2 (1.3 - 3.7) | 5.1 (1.9 -1 3.7) |
| SITE 8 | ,, | 2.9 (1.7 - 5.0) | 0.7 (0.4 - 1.5) | 0.9 (0.2 - 3.2) |
| SITE 9 | ,, | 2.4 (1.4 - 4.2) | 0.3 (0.1 - 1.2) | |
| AGE (3) | AGE (2) | 2.2 (1.8 - 2.7) | - | 2.0 (0.6 - 6.7) |
| AGE (4) | AGE (2) | 4.0 (3.3 - 4.9) | - | 19.0 (6.8 - 52.3) |
| MALEALE | FEMALE | 1.8 (1.5 - 2.1) | - | 0.4 (0.2 - 0.6) |

Table 11: Contingency table showing association between lymphocystis (LYM), skin ulcers (ULC), and liver nodules (LNL) for all sites and years (1985-89) combined. Cells on the lower left show numbers of fish with different combinations of 2 diseases (+=diseased fish;-=apparently healthy fish). Cells on the upper right show associated probabilities derived from chi-square test of independence (log likelihood ratio)

| | LYM+ | LYM- | ULC+ | ULC- | LNL+ | LNL- |
|---|---|---|---|---|---|---|
| LYM+ | 1774 | - | p= 0.004 | | p= 0.063 | |
| LYM- | - | 10896 | | | | |
| ULC+ | 63 | 255 | 318 | - | p= 0.84 | |
| ULC- | 1711 | 10641 | - | 12352 | | |
| LNL+ | 22 | 85 | 3 | 104 | 107 | - |
| LNL- | 1752 | 10811 | 313 | 12250 | - | 12563 |

Table 12: Matrix of correlation coefficients between log (disease odds) and log (contaminant concentrations) for all sites (normal typeface) and for the 6 marine sites only (italics). *=p<0.05; **p=<0.01.

| | a | b | c | d | e |
|---|---|---|---|---|---|
| a PCB-C153 liver | | | | | |
| b PCB-C153 sediment | 0.203<br>*-0.272* | | | | |
| c PAH sediment | 0.318<br>*0.285* | 0.894$^{**}$<br>*0.807*$^{*}$ | | | |
| d Cd sediment | -0.176<br>*-0.509* | 0.701$^{*}$<br>*0.891*$^{*}$ | 0.418<br>*0.528* | | |
| e Pb sediment | 0.035<br>*-0.332* | 0.723$^{*}$<br>*0.695* | 0.521<br>*0.449* | 0.888$^{*}$<br>*0.888*$^{*}$ | |
| f Lymphocystis | 0.050<br>*-0.056* | -0.129<br>*0.045* | -0.335<br>*-0.148* | 0.329<br>*0.338* | 0.473<br>*0.621* |
| g Skin ulcers | 0.170<br>*-0.138* | -0.015<br>*0.482* | -0.273<br>*0.393* | 0.459<br>*0.686* | 0.453<br>*0.939*$^{**}$ |
| h Liver neoplasia | 0.380<br>*-0.027* | -0.276<br>*0.286* | -0.477<br>*0.142* | 0.149<br>*0.578* | 0.107<br>*0.830*$^{*}$ |