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ON THE OCCURRENCE OF LIVER TUMORS IN FLAT FISH IN DUTCH WATERS

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Abstract

Data are presented and discussed on spatial and temporal variability in the occurrence of liver nodules/tumors (putative neoplastic and neoplastic lesions) in dab (*Limanda limanda*) and flounder (*Platichthys flesus*) from Dutch waters. Spatial variability in the prevalence of liver nodules/tumors in both species, but particularly in flounder, suggests an association with pollution. Nevertheless, the aetiology of the disease is probably complex, with chemical pollution only one of many causal factors.

Introduction

Recently, several European researchers have described the occurrence of hepatocellular changes in two species of flatfish, dab (*Limanda limanda*) and flounder (*Platichthys flesus*), and have discussed the possible association between these changes and the presence of chemical contaminants in the North Sea (see for review Vethaak and ap Rheinallt, in press). Similar changes have been described in other flatfish from polluted North American waters, and convincing evidence has been provided to implicate PAHs as an important causal agent (Malins et al. 1988).

This paper describes epidemiological characteristics of liver abnormalities in dab and flounder captured in Dutch coastal waters between 1985 and 1989. An attempt was made to relate disease prevalence (in the number of individuals showing signs of the disease at a moment in time) to environmental pollution.

The host species

Dab and flounder belong to the family Pleuronectidae, but they usually occupy different habitats.

Dab are widely distributed throughout the North Sea in both coastal and offshore habitats, their feeding and spawning grounds overlapping. Recent studies have shown that dab might undertake extensive migrations (Rijnsdorp et al. in press); this is in contrast to the general assumption that the species is fairly stationary (e.g. Dethlefsen et al. 1987; Vethaak and Meer, 1991).

Flounder spawn in offshore areas but grow up and feed in estuaries and fresh water: after spawning in winter they usually return to the same feeding areas as the previous year, and move little during the summer.

The tendency to remain faithful to the same feeding areas, together with their abundance and wide distribution, and susceptibility to disease, has resulted in both species being recommended for studies of fish disease in relation to pollution (Anon., 1990). However, recent findings of migratory behaviour of dab make it necessary to re-assess the suitability of this species in the implementation of monitoring programmes.

The disease

Histologically, liver abnormalities observed in dab and flounder correspond fairly to those recorded in American flatfish, and include a spectrum ranging from foci of cellular alteration through to hepatocellular adenoma and occasionally hepatocellular carcinoma. However, in order for a wide-ranging epidemiological study to be meaningful, it is necessary to collect data from a very large number of individual fish, and enormous resources would be required to carry out a histopathological examination of every liver. Effort has therefore been devoted to attempting to find gross lesions which are reliable indicators of the above histological changes. It has been found that recording only distinct nodules on the liver, with a diameter exceeding 2 mm, produces the best correspondence (Vethaak, unpublished data).

Epidemiology

Over 5 years, a total of 7833 dab (minimum length 15 cm) were examined for liver nodules at 5 sites in the Dutch coastal waters during late winter, and a total of 9938 flounder (minimum length 20 cm) were examined during late summer at 9 sites in that area, which included some estuaries and a fresh water lake (see Figure 1). The livers of a considerable proportion of these fish were also subjected to a histopathological examination (Vethaak, unpublished data). The epidemiological and statistical methods are described earlier by Vethaak and Meer (1991).

Interestingly, the prevalence of liver nodules in both species increased with length and, at the same time, females of in particular larger size groups tended to have nodules more frequently than did males (see Figures 2 and 3). The increase in prevalence with length is not linear: instead, nodules are concentrated in the largest fish, suggesting - as growth is asymptotic - that the occurrence of the disease is related to age rather than length.

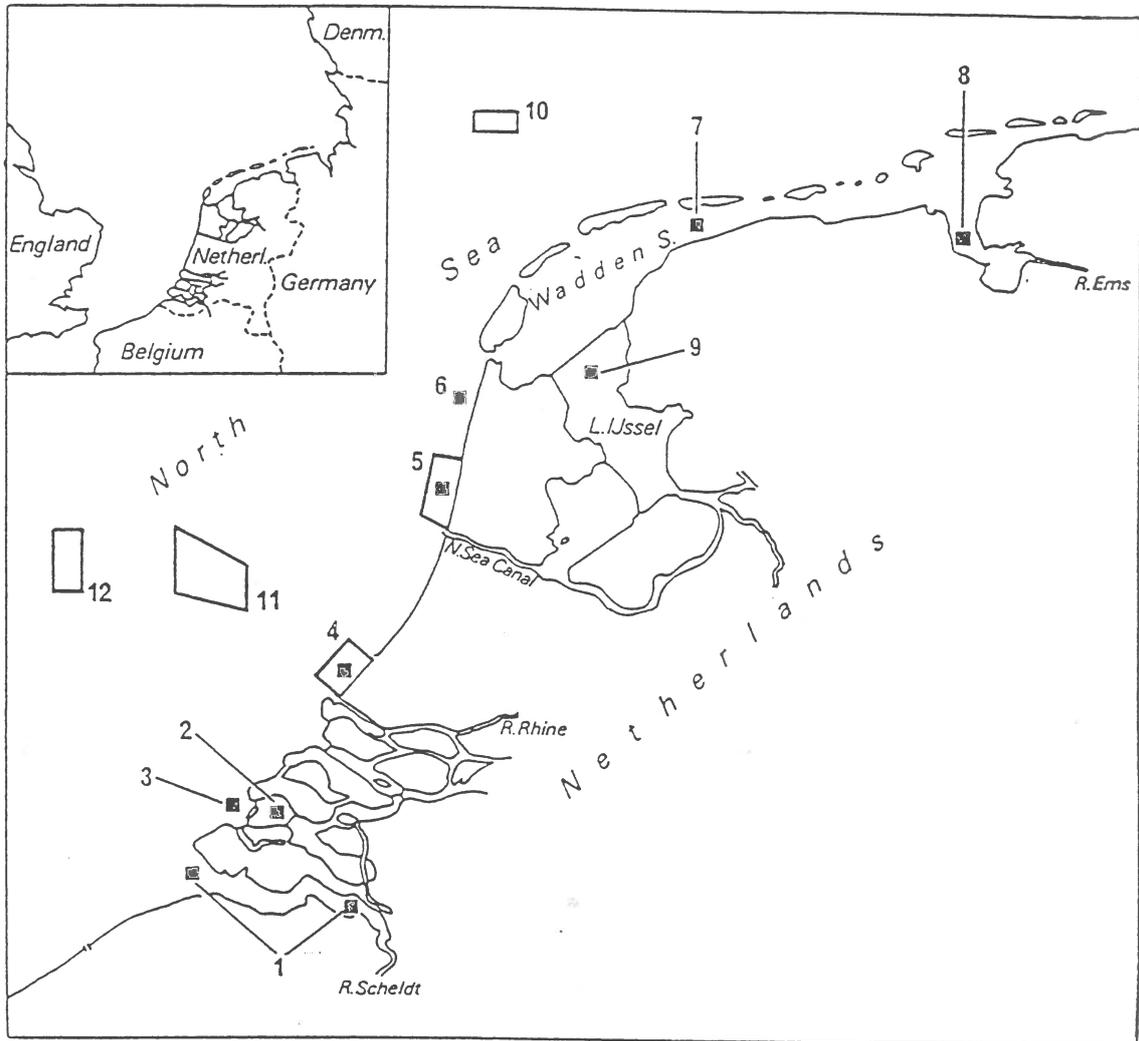


Figure 1: Position of sampling sites: ■ flounder □ dab.

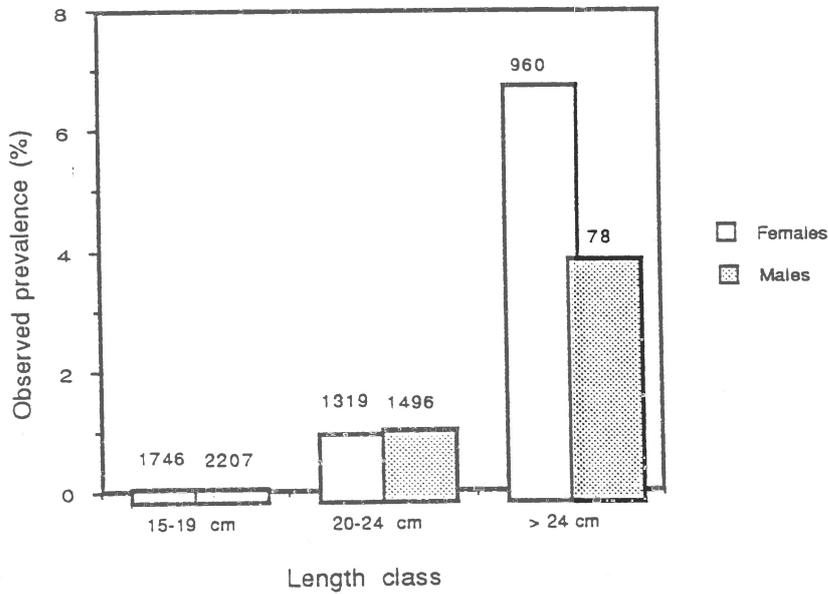


Figure 2: Dab: liver nodules > 2 mm by length class for different sexes (all sites: March-April 1986-89) with number of examined fish given on top of each histogram.

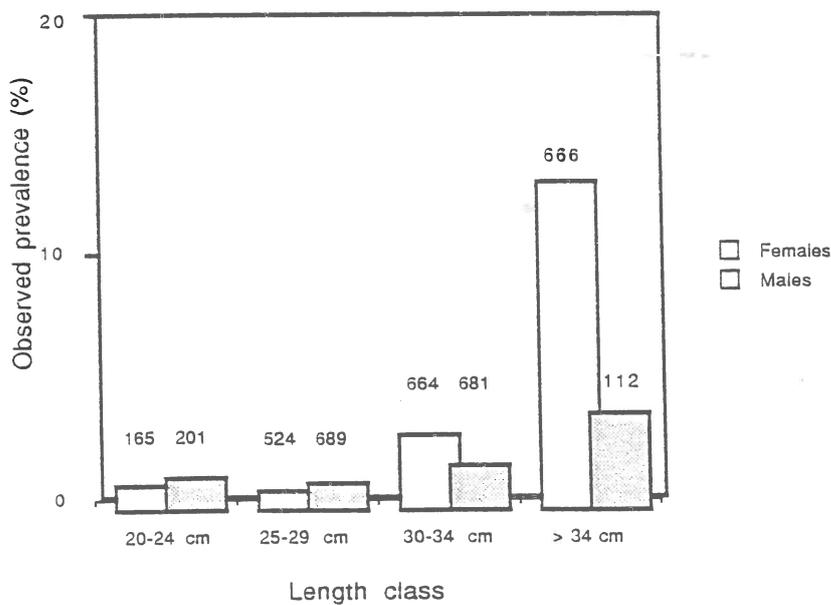


Figure 3: Flounder: liver nodules > 2 mm by length class for different sexes (Dutch coastal waters, Sites 3, 4, 5 and 6; September 1987-89) with number of examined fish given on top of each histogram.

An exception to this general trend has been discovered recently at Site 5, situated near an ironworks: a survey here showed higher prevalences of nodules in young flounder (length class 20-24 cm; age 1) than in older individuals. This suggests both extreme exposure to causal agents, in order to produce the lesions in such young fish, together with early mortality: further investigations are planned.

The temporal trend of liver nodules in dab is given in Figure 4: Adjusted prevalences of liver nodules showed a peak in male fish in 1987; in female fish they showed a steady decrease during the study period.

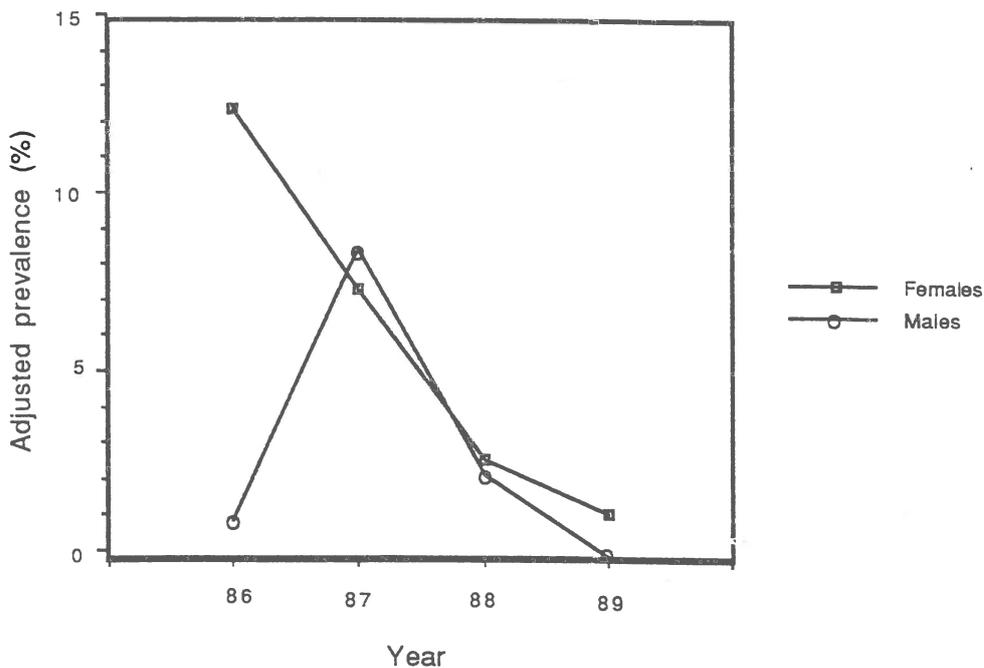


Figure 4: Temporal trends in liver nodules > 2mm in large-sized female dab (> 24 cm) histologically confirmed as putative pre-neoplastic and neoplastic lesions; based on log-linear analysis.

The spatial pattern of liver nodules in dab appears to show an association with pollution, in that the 2 sites with highest prevalences are known to be polluted, in one case by the plume of the Rhine (Site 4) and in the other by titanium dioxide wastes (Site 11) (Vethaak and Meer, 1991).

Nevertheless, a detailed statistical analysis did not reveal any significant spatial variation in prevalence. This highlights a problem of epidemiological studies where only a small proportion of the individuals examined are diseased: sample

sizes must be extremely large in order to detect spatial variation, and a shortage of the most affected size group (usually large fish) at any site will hamper the analysis.

Comparison of adjusted prevalences with sedimentary concentrations of PAHs in the same area, however, does not show any relationship.

In flounder, the temporal patterns in liver nodules in flounder show a peak in 1987. The prevalence of nodules shows considerable spatial variation, and the differences among sampling sites are very consistent form year to year (see Figure 5). During the sampling it was frequently observed that flounder affected with nodules tend to be clustered in different hauls, providing evidence that they occur in local "clusters".

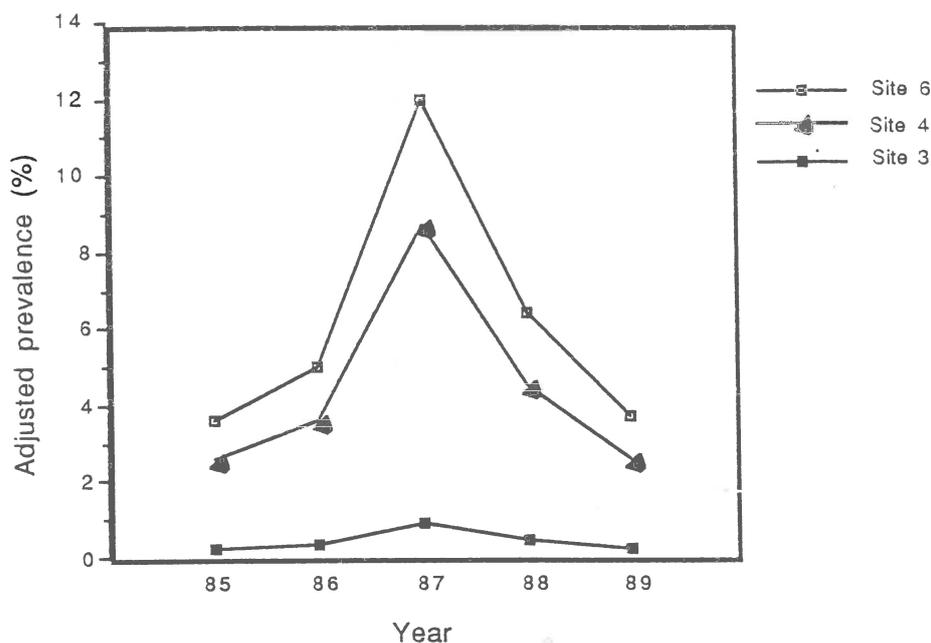


Figure 5: Temporal trends of liver nodules > 2 mm in large-sized female flounder (> 29 cm) (for three different sites, histologically confirmed as putative pre-neoplastic and neoplastic lesions; based on loglinear analysis).

Highest prevalence values of liver nodules are found along the Dutch North Sea coast near the mouths of the main estuaries, but in the Eastern Scheldt, the Dutch Wadden Sea and the estuary of the river Ems prevalences are notably lower. Particularly remarkable is the virtual absence of the disease from brackish and fresh water, which are highly polluted (Sites 1, 8, 9): the scarcity of large (and therefore old) fish in these areas only partly corrects for this.

Figure 5 shows that liver nodules in flounder in the period 1985-89 are consistently more prevalent in the polluted Dutch coastal waters than in the less polluted marine Eastern Scheldt. For 1987 the prevalences of nodules in Dutch coastal waters sites was correlated with chemical factors. A positive correlation between the prevalence of liver nodules was found with the concentration of PAH in the sediments, but not with that of PCBs in the livers of the fish themselves in 1 (Figure 6). Thus, the observed pattern of prevalences in coastal waters does not correspond to the major pathway of pollutants discharged in the waters of the rivers Scheldt and Rhine (taking PCB concentration as a indicator of general pollution). Instead, highest levels are found near Site 5 and 6, in the vicinity of the North Sea Canal, where PAH concentrations are highest.

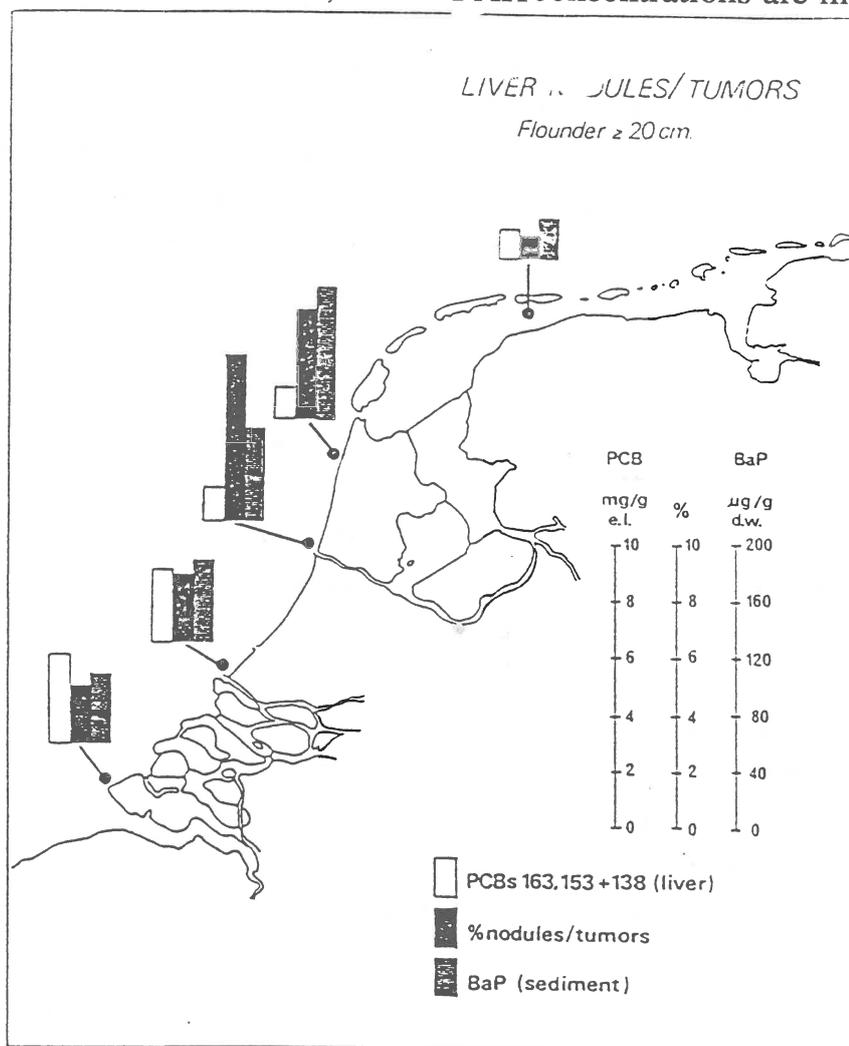


Figure 6: Correlation between the prevalence of liver nodules in flounder and various chemical factors in Dutch coastal waters in September 1987.

Discussion

It appears likely that liver nodules in both dab and flounder have a complex multifactorial aetiology, with chemical pollution acting as one of many causal factors.

A number of relevant findings still require explanation.

Firstly, the co-occurrence of high levels of liver nodules in flounder in the coastal waters near the North Sea Canal coupled with the recent observations of young flounders affected in this area, especially near the vicinity of an iron works, might provide some important clues in support of a chemical aetiology. The North Sea Canal is known to be a nursery area of flounder and therefore the observed high prevalences of liver nodules in old as well as young flounders could be related to the environmental conditions of the Canal. Next to the high environmental levels of PAHs, high levels of phenols in the water are observed here, especially near the iron works. Phenols are known to be enhancers of the effect of PAH and other carcinogens for mouse skin (Williams and Weisburger, 1986). Therefore, the extreme exposure to carcinogenic agents in this area, as mentioned earlier, could be caused by the synergistic effects of PAHs and phenols present in this area.

Secondly, the disease is absent from flounder inhabiting fresh or brackish waters, suggesting that a factor associated with salt water is necessary for its development.

Thirdly, similar temporal trends in prevalence in both dab and flounder, with a peak in 1987, over a relatively wide area, suggest that natural factors may contribute to the occurrence of the disease.

Fourthly, liver nodules are significantly associated with other diseases (epidermal hyperplasia/papilloma and lymphocystis) in dab; their aetiology must therefore have elements in common, even though lymphocystis, at least, is infectious (Vethaak and Meer, 1991).

Finally, a possible infectious aetiology of liver nodules is supported by the observation that flounder with nodules occur in local "clusters".

Histologically, the liver lesions observed in dab and flounder correspond to those described from North American flatfish species. However, in contrast to American findings, nodules appear to be present in fish from less polluted sites as well as from heavily polluted areas in the North Sea, although it should be pointed out that background prevalences of the disease in dab and flounder from

genuinely "clean" sites are unknown, and that migratory patterns especially of dab still need to be clearly identified.

American studies have assembled an impressive body of evidence to show a direct relationship between PAHs and liver abnormalities. In contrast, epidemiological studies seem to show that liver diseases in dab and flounder in Dutch coastal waters have a more complex aetiology, with chemical pollution being only one of many causal factors.

Conclusions

1. A wide-ranging epidemiological study, relying largely on recording gross anomalies, has proved to be a useful method of collecting data on spatial and temporal variability in the occurrence of liver abnormalities in flatfish from Dutch waters.
2. Spatial variability in the prevalence of liver nodules in both species, but particularly in flounder, suggests an association with pollution.
3. Nevertheless, the aetiology of the disease is probably complex, with chemical pollution only one of many causal factors.

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