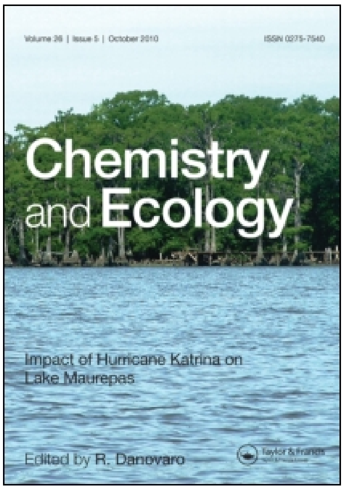

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FISH DISEASE MONITORING IN THE DUTCH PART OF THE NORTH SEA IN RELATION TO THE DUMPING OF WASTE FROM TITANIUM DIOXIDE PRODUCTION

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Gross pathologies were recorded in a total of 5942 individual common dab (*Limanda limanda* L.) at 5 sites in and around Dutch coastal waters, in spring, from 1986 to 1988. Two of these sites (an offshore dumping ground and an inshore site influenced by direct river discharge) received large quantities of diluted acids of titanium dioxide waste (TDW); the other three were selected as reference sites for comparison.

The main diseases recorded were epidermal hyperplasia/papilloma, lymphocystis, liver nodules (pre-neoplastic and neoplastic lesions), and infections caused by the protozoan *Glugea* sp. Frequencies of disease were analysed using a logit model. There was a consistently high prevalence of epidermal hyperplasia/papilloma in dab from the two sites that received TDW when compared to the other sites. However, no clear relationships were found between the prevalence of epidermal hyperplasia/papilloma and dumping-associated heavy metals or other relevant environmental and biological factors. No significant spatial trend was revealed for liver nodules, although there was a statistically significant association between the occurrence of epidermal hyperplasia/papilloma or of lymphocystis and that of liver nodules in individual fish. Prevalences of lymphocystis were usually higher at offshore sites than in inshore areas, while prevalences of *Glugea* showed the opposite trend.

Although at first sight the pattern of disease prevalence would appear to furnish a strong case for a cause-and-effect relationship between TDW and epidermal hyperplasia/papilloma, interpretation of the data is complicated by interference from riverine inputs, long-distance dispersion of discharged wastes, local hydrographic conditions, and possible local migration of dab. On the basis of present results, therefore, the possibility that discharges of TDW contributed to the occurrence of this disease cannot be proven or discounted.

KEY WORDS Fish pathology, Titanium dioxide wastes, Bio-monitoring, *Limanda limanda* L.

1. INTRODUCTION

Titanium dioxide waste acids (TDW) contain mainly sulphuric acid and iron sulphates with smaller proportions of magnesium, titanium, vanadium and chromic sulphates (Table 1). Studies of biological effects of TDW on the aquatic ecosystem, including adverse effects on fish health, are reported throughout the literature (e.g. Lethinen, 1980; Knutzen, 1983; Pickaver, 1981).

Table 1 Composition of the waste acids discharged at the Dutch offshore dumping ground (based on data from Public Works Department, North Sea Directorate).

Compound	*Concentration* (g/l)
Sulphate	855
Iron	90.8
Titanium	10.6
Vanadium	1.20
Chromium	0.54
Zinc	0.125
Nickel	0.012
smaller quantities of other heavy metals	

TDW from German industries has been discharged in a restricted area 25 miles west of the Dutch coast in the North Sea since 1961 with an average yearly contribution of ca. 840,000 tonnes during the period 1981 to 1989, with little variation from year to year. Dumping was discontinued at the end of 1989. Although the Dutch offshore dumping site is located opposite the Rhine and Meuse estuaries, the site is outside the plume of these estuaries, and river discharges are not a significant source of pollution in the offshore area. The rivers, however, provide a highly significant input of heavy metals and organic micro-pollutants and nutrients to coastal waters. The Rhine also provided in the past a discharge of TDW with an average yearly contribution of 112,400 tonnes during the period 1981 to 1989.

Since 1980, Dethlefsen and co-workers, working in the German Bight, have regularly reported elevated prevalences of epidermal hyperplasia/papilloma (referred to through the text as epidermal hyperplasia) in common dab (*Limanda limanda* L.) in a TDW dumping area, by comparison with surrounding stations. This disease is characterized by the occurrence of jelly-like or whitish raised areas, up to several centimetres in diameter, on the body and fins (Figure 1). In the German studies, disease prevalence was correlated with concentrations of heavy metals (iron and chromium) associated with the wastes in both water and in sediments. The correlation was interpreted as circumstantial evidence of a causal link between disease and the waste materials (Dethlefsen and Watermann, 1980; Dethlefsen, 1984). More recent work established a relationship linking high levels of iron and chromium in the epidermis, and chromium in liver tissue, with the occurrence and intensity of the disease. This strengthened the hypothesis of a cause-effect relationship between disease and waste material (Dethlefsen *et al.*, 1987).

This paper presents epidemiological data derived from disease monitoring surveys carried out in and near Dutch coastal waters from 1986 to 1988. The study was conducted as part of a special monitoring programme aiming to assess possible biological effects of offshore TDW dumping (Bos, paper presented at 8IODS Dubrovnik). The common dab was chosen as a species to monitor both because it is abundant in the area under consideration and because it is particularly susceptible to disease (Vethaak, 1985).

General patterns of disease occurrence are discussed below and the results are compared with those available from the German Bight TDW dumping site.

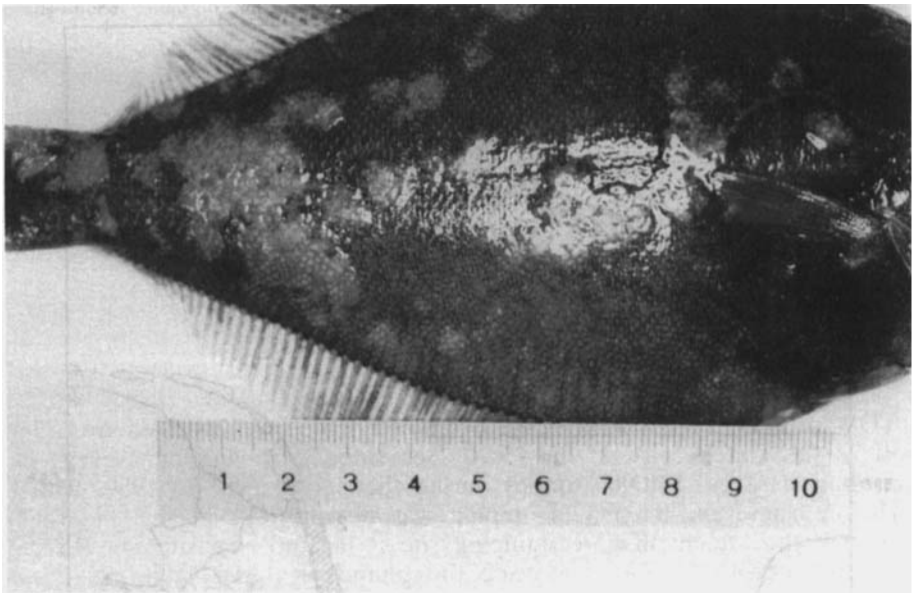


Figure 1 A dab (*Limanda limanda* L.) affected with epidermal hyperplasia/papilloma.

Reference is also made to the interpretation of a causal relationship between disease prevalence and offshore disposal of TDW, and to the limitations of using fish diseases to monitor the effects of disposal.

MATERIALS AND METHODS

Study Sites

The main characteristics of the sampling sites (Figure 2) are summarized in Table 2. In 1988 only, Site 2, an inshore site, and Site 5, situated on the English continental shelf, were included as additional reference sites. Because of the existence of TDW sources in Belgian waters, it was not possible to find suitable reference areas south of the Dutch dumping ground.

Fish Sampling

Fish samples were taken in spring (March and April). This is the period when dab usually shows highest prevalence of disease (Vethaak, 1985). A 6-m beam trawl with a mesh diameter of 7 cm and a cod-end mesh diameter of 4 cm was used, fitted with 2 to 4 tickler chains. Each tow lasted between 35 and 60 minutes. The number of hauls made at each site depended on the size of the catch, with a minimum of 3 hauls. The fish were divided into 3 length classes: 15–19 cm; 20–24 cm; > 24 cm, and fishing continued until 150 individuals in each length category were captured. A random sample of the first three hauls provided size

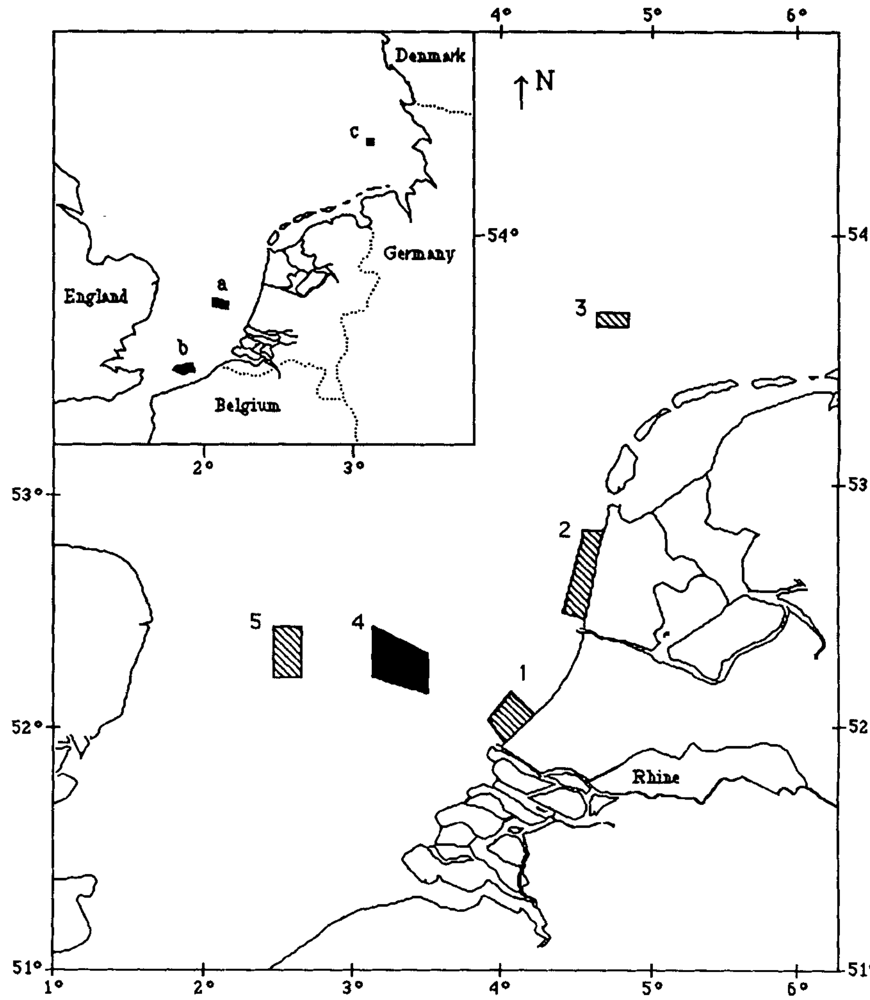


Figure 2 Map showing the geographic position of the sampling sites. Site 1 = coast near Hook of Holland/Rhine estuary; Site 2 = coast near IJmuiden; Site 3 = offshore area NW of Wadden Islands; Site 4 = offshore TDW-dumping ground; Site 5 = offshore area West of TDW-dumping ground. Inset shows (a) Dutch, (b) Belgian, and (c) German TDW dumping grounds.

and age distribution and population density, but from subsequent hauls length-stratified samples were taken. A total sample of 450 fish per site allows the detection of a disease prevalence of at least 1.0% with a confidence level of 95% (see Munro *et al.* 1983). Subsequent data analysis was confined to diseases with observed prevalences considerably greater than this value.

Each fish was measured to the nearest millimeter, sexed, and inspected externally and internally for visible signs of disease.

To reduce sampling bias, DECCA equipment was used to ensure that the same tows were revisited each year, and the fish were examined by the same observers throughout the sampling period.

Table 2 Data on the sampling sites; position, principal pollution sources, range of water depth, and index of fishing activity.

(No) Site	*Geographic position*	*Main sources of pollution*	*Depth (m)*	*Sediment fraction* <63 *µm (%)*	*Fishing activity*[a]
(1) Hook of Holland	52°04′N 03°57′E 52°10′N 04°08′E 52°06′N 04°14′E 52°00′N 04°04′E	– polluted water from the Rhine and the Meuse including discharge of TDW (Tioxine BV) – dumping of harbour dredging spoil – discharge of sewage	7–14	0.44	1.9
(2) IJmuiden	52°28′N 04°32′E 52°29′N 04°24′E 52°53′N 04°36′E 52°52′N 04°42′E	– pollution originating from Site 1 – Port of Amsterdam approach with iron works – dumping of harbour dredging spoil	7–21	0.12	1.7
(3) Area NW of the Wadden islands	53°29′N 04°39′E 53°42′N 04°39′E 53°39′N 04°51′E 53°42′N 04°51′E	– pollution from Belgian and Dutch coastal waters	27–33	1.93	7.8
(4) TDW dumping ground	52°14′N 03°07′E 52°26′N 03°07′E 52°20′N 03°31′E 52°10′N 03°31′E	– TDW dumping – pollution from Belgian coastal waters including TDW	28–40	0.05	11.3
(5) Area West of TDW dumping area	52°15′N 02°30′E 52°25′N 02°30′E 52°25′N 02°41′E 52°15′N 02°41′E	– pollution from the English Channel	34–49	0.08	2.1

[a] Beam trawl fishing efforts in all sampling areas in uncorrected fishing hours per square kilometer for different seasons during 1981 and 1982 (based on Welleman, 1989).

During 1987 and 1988, otoliths were taken from a subsample of 10 individuals per cm length group and length-age keys were determined for each site following the method described by Leeuwen and Vethaak (1988).

Pathological Inspection

The fish were screened for the following seven groups of gross pathology:

(1) Epidermal hyperplasia/papilloma: lesions with a diameter larger than 2 mm;

(2) Lymphocystis: the presence of a single cyst on the body surface was taken as a minimum for recording the presence of this disease;

(3) Skin ulcers: open and healing lesions with a diameter larger than 2 mm;

(4) Fin rot;

(5) Skeletal deformities: externally visible lordosis or scoliosis only;

(6) Liver nodules with a diameter larger than 2 mm, assumed to correspond to pre-neoplastic or neoplastic lesions and confirmed as such histologically;

(7) Cysts of *Glugea* sp. in the intestine.

Lesions due to epidermal hyperplasia were classed in three groups as follows:

grade I: one lesion (2–10 mm);

grade II: lesions covering a surface area of up to twice that of the spread-out caudal fin;

grade III: lesions covering a larger area than this.

For lymphocystis a similar grading system was used, except that grade I corresponded to 1 to 10 cysts or clusters of cysts not exceeding a total diameter of 5 mm.
Degrees of severity of *Glugea* infection were classed as:

grade I: 1–25 cysts;

grade II: 25–100 cysts;

grade III: over 100 cysts.

For liver nodules the diameter of lesions was used:

grade I: 2–5 mm;

grade II: 6–10 mm;

grade III: > 10 mm.

The criteria for the recognition of these pathological conditions have been described earlier by Vethaak (1985). In general, cases with doubtful diagnosis were checked by histological examination.

Calculation of the Fish Condition Factor and Population Density Index

A gutted condition factor (C) was determined for a subsample of apparently healthy female fish between 20 and 30 cm in length. After removal of the viscera, the fish were stored on ice and later weighed in the laboratory, and C was calculated as 100× somatic weight in mg/(total length in mm)^3.

The catch per hour data, based on the first three hauls, were used to estimate the relative population density of dab for each site.

Chemical Analysis

Chemical analysis of four heavy metals (Fe, Cr, Va, Ti) associated with TDW were conducted on samples of sediment (1988, 1989) and hermit crab abdomens (1986, 1987, 1988) were made by means of Atomic Absorption Spectrometry by the Laboratory for Analytical Services (Leiden) and TNO (IJmuiden) respectively.

Statistics

A logit model, which belongs to the class of generalized linear models (Nelder & Wedderburn, 1972), was assumed.

$$\ln \frac{p}{1-p} = lp \qquad (1)$$

in which p is the probability that a fish has the disease and lp, the linear predictor, is a linear function of independent factors, e.g.

$$lp = \alpha + \beta_i + \gamma_j + \delta_k + \varepsilon_l \qquad (2)$$

where α is a constant, β_i represents the effect of locality i, γ_j the effect of year j, δ_k the effect of sex k, and ε_l the effect of length class l. Interaction effects can also be included in the model. Alternatively the model can be written as:

$$p = \frac{e^{lp}}{1 + e^{lp}} \qquad (3)$$

The parameters (α, β_i, γ_j, etc) of the logit model were estimated using the statistical package GLIM (Baker & Nelder, 1978). A binomial error structure was assumed. There are unequal numbers of fish analysed for each combination of independent factors. To cope with this unbalanced design, each main effect was tested by comparing the model without that main effect, but with all other main effects included, to the model with all main effects included. Interaction effects were tested by comparing the model with the interaction effect and all main effects included, to the model with only the main effects included. Because the statistical analysis involved numerous tests, the level of significance for each test separately was kept low ($\alpha = 0.01$), in order to compromise between the experiment-wise error rate growing too large, and the power becoming too small.

The term adjusted prevalence used in the text refers to a disease probability based on the logit analysis. The model can also provide estimates of prevalence sites/years not sampled.

RESULTS

Fish Population Characteristics and Overall Disease Pattern

Altogether, 5942 dab were examined. Figure 3 shows the length and sex composition and the total number of fish examined each year at each site. Due to adverse weather conditions and limited availability of the research vessels, it was not always possible to sample every site and to obtain sufficient numbers of fish from each size class. Hence, although the target sample size for each site was usually reached, large fish in particular were not always caught in sufficient number.

Data on length/age composition, relative population density and condition factor for 1988 are provided in Table 3.

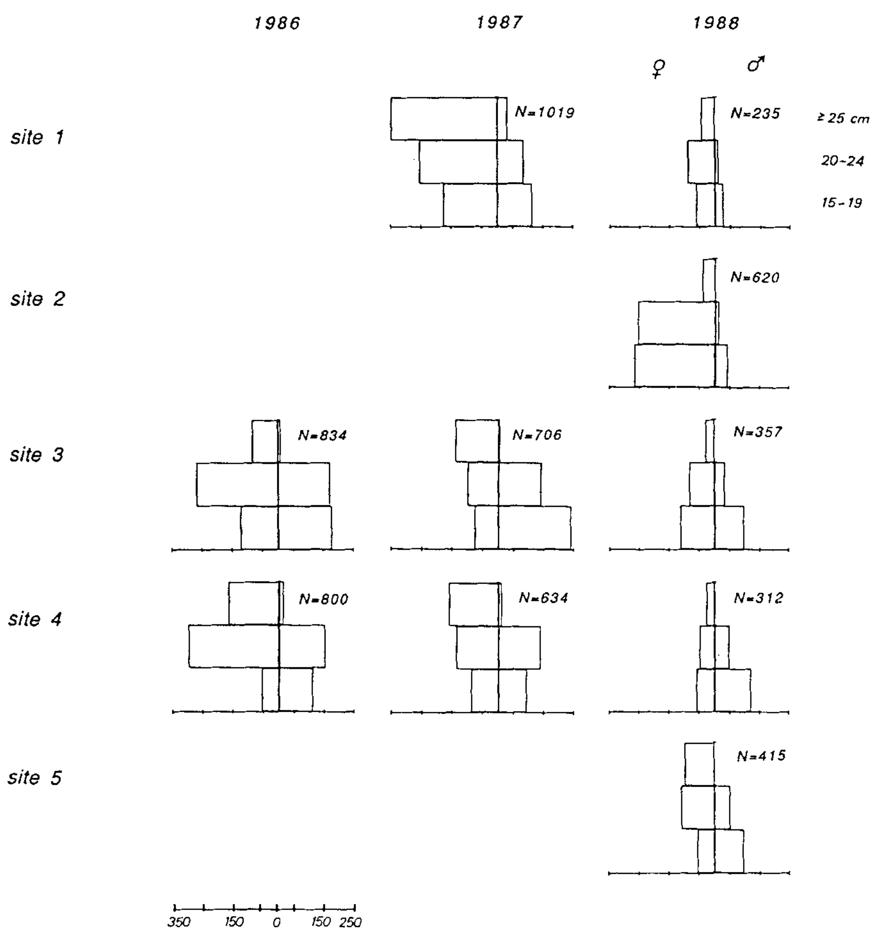


Figure 3 Sample composition pyramids of dab at each site showing the length distribution divided into three length classes with male and female fish indicated separately, in March/April 1986 to 1988 (based on length-stratified samples).

Table 3 Mean lengths and ages, condition factors and relative population densities of each length class of dab at each site in 1987, based on random samples.

	Length class (cm)	*Mean value*			
		Length	*Age*	*Condition factor*	*Number of fish captured/hour*
Site 1	15–19	17.4	2.2	0.86	28.5
	20–24	21.4	2.5	0.87	44.4
	>24	26.4	2.9	0.89	10.8
Site 2	15–19	16.8	2.0	0.85	7.7
	20–24	21.4	2.4	0.88	8.0
	>24	26.3	2.4	0.84	1.2
Site 3	15–19	17.1	2.3	0.76	87.5
	20–24	21.2	3.7	0.77	40.1
	>24	27.4	5.0	0.73	14.7
Site 4	15–19	17.0	2.2	0.74	161.5
	20–24	21.1	3.2	0.75	46.8
	>24	26.3	4.0	0.77	4.1
Site 5	15–19	17.2	2.1	0.73	147.3
	20–24	21.3	2.9	0.77	80.4
	>24	26.3	3.6	0.81	12.3

Histologically, confirmation of samples of epidermal hyperplasia/papilloma indicated that almost all cases could be classified as hyperplasia; only rarely were tumours with supporting connective tissue (papilloma) found. Subsamples of intestinal *Glugea* cysts were examined and most contained typical spore-like structures of the species *Glugea stephani.* However, the possibility cannot be excluded that other species—known to occur in Dutch dab—were present.

A detailed histological study of the liver nodules will be published separately.

Table 4 summarizes the overall frequency of disease in the seven selected categories. *Glugea* infection was the most prevalent disease, followed by lymphocystis, epidermal hyperplasia and liver nodules. The prevalence of other diseases, such as skin ulcers, fin rot and skeletal deformities (all cases concerned lordosis) were usually below 1%. The maximum prevalence of skin ulcers at any site was 1.7%, of fin rot 0.8%, and of skeletal deformities 1.3%. Since these diseases revealed no apparent regional variation, they were excluded from further consideration.

Table 4 Overall crude prevalences (%) of the different diseases for the whole study area during 1986 to 1988.

Disease category	*Prevalence (%)*
lymphocystis	5.1
epidermal hyperplasia	4.7
skin ulcers	0.6
fin rot	0.1
skeletal deformities	0.1
Glugea infections	9.0
liver nodules	2.2

Table 5 Total number of fish examined and fish affected by the 4 principal diseases at sites 1 (normal type), 3 (bold type) and 4 (italics) in 1987, broken down by length class and sex. Crude prevalences (%) are given in parentheses.

	Female			*Male*		
	15–19 cm	*20–24 cm*	*>24 cm*	*15–19 cm*	*20–24 cm*	*>24 cm*
Total number examined	177	255	347	116	89	35
	76	**102**	**138**	**243**	**141**	
	88	*138*	*161*	*94*	*140*	
lymphocystis	2 (11.0)	13 (5.1)	10 (2.9)	5 (4.2)	6 6.7)	3 (8.6)
	2 (2.6)	**11 (10.8)**	**15 (10.8)**	**24 (9.9)**	**24 (20.4)**	
	2 (2.3)	*7 (5.1)*	*10 (6.2)*	*3 (3.2)*	*7 (5.1)*	
epidermal hyperplasia	2 (1.1)	13 (5.1)	52 (14.9)	2 (1.7)	8 (9.0)	2 (5.7)
	0 (0.0)	**7 (6.9)**	**13 (9.4)**	**4 (1.6)**	**7 (4.9)**	
	1 (1.1)	*14 (10.1)*	*17 (10.6)*	*3 (3.2)*	*11 (7.9)*	
Glugea infection	20 (11.3)	28 (11.0)	48 (13.8)	14 (12.1)	11 (12.4)	5 (14.3)
	2 (2.6)	**10 (9.8)**	**6 (4.5)**	**17 (7.0)**	**8 (5.6)**	
	5 (5.7)	*14 (10.1)*	*11 (6.8)*	*8 (8.5)*	*16 (11.4)*	
liver nodules	0 (0.0)	1 (0.4)	26 (7.5)	0 (0.0)	4 (4.5)	5 (4.0)
	0 (0.0)	**4 (3.9)**	**7 (5.0)**	**1 (0.4)**	**2 (1.4)**	
	0 (0.0)	*3 (2.2)*	*16 (9.9)*	*0 (0.0)*	*3 (2.1)*	

Table 5 shows for 1987 the numbers of fish examined and affected and the prevalence of the four principal diseases, broken down by length class and sex. These figures are presented as an example, for 3 sites only, in order to allow other scientists to compare their findings with those of the present study.

Statistical Analysis of Disease Data

Length and sex effects. Prevalence of lymphocystis and liver nodules increased significantly with length and the prevalence of lymphocystis was significantly less in females than in males. The significant increase in epidermal hyperplasia with length, and the significantly lower prevalence of liver nodules in males differed among years as indicated by the significant two-way interactions (Table 6).

Spatial and temporal trends. There were significant differences between sites for epidermal hyperplasia, lymphocystis and *Glugea* infection, but not for liver nodules. Significant differences between years were found in the prevalence of all diseases except *Glugea*. Significant two-way interactions were found for epidermal hyperplasia (year · length) and for liver nodules (year · sex), indicating that

Table 6 Summary of the results of the logit analysis: a) significance tests with deviances, residual degrees of freedom (df) and associated probabilities (* = $p < 0.01$), and the models chosen in italics; b) parameter estimates of length and sex effects where significant. EP = epidemal hyperplasia; Ly = lymphocystis; LN = liver nodules and Gl = *Glugea* infection.

a) *Significance tests*

	df	*deviance*			
		EP	*Ly*	*LN*	*Gl*
null model	57	258.6	211.2	276.6	122.5
all (4) main effects	48	52.8	76.0	47.8	56.9
without site	52	71.8*	112.7*	60.0	103.2*
without year	50	67.5*	92.4*	53.2	57.3
without sex	49	52.8	89.2*	52.4	58.0
without length	50	132.4*	103.6*	166.5*	58.4
with site · year	45	51.3	*48.8**	44.7	48.7
with site · sex	44	48.5	73.9	42.6	46.9
with site · length	40	42.4	71.8	42.6	47.4
with year · sex	46	49.3	73.1	36.3*	56.1
with year · length	44	37.1*	66.3	44.0	55.5
with sex · length	46	49.2	72.5	47.2	56.4
site	53				*59.3*
site + year + length + year · length	45	*37.2*			
year + sex + length + year · sex	50			*47.4*	

b) Parameter estimates

	estimates			
	EP	*Ly*	*LN*	*Gl*
length 15–19 cm	0	0	0	—
length 20–24 cm	0.40	0.70	2.48	—
length >24 cm	0.83	0.85	4.13	—
female	—	0	0	—
male	—	0.48	−2.61	—

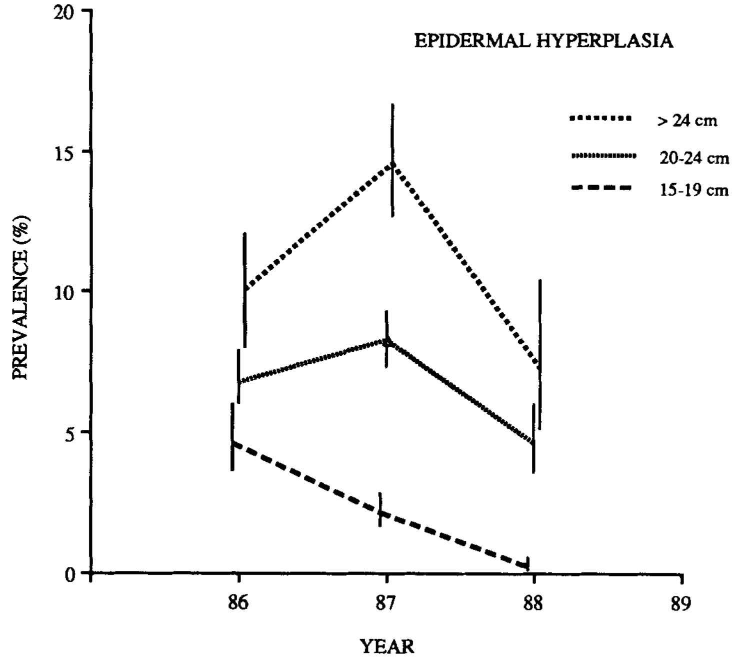


Figure 4 Adjusted prevalences (±SE) of epidermal hyperplasia for different length classes of female dab plotted against years at the TDW dumping site (Site 4).

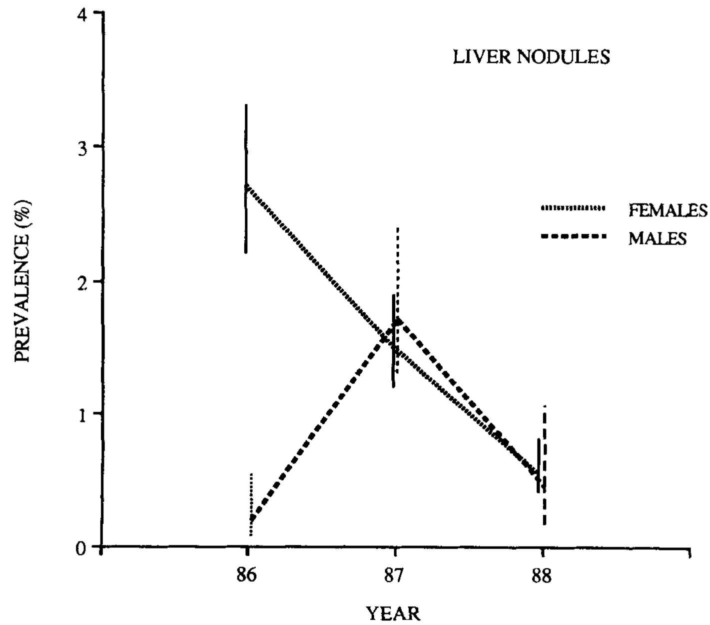


Figure 5 Adjusted prevalences (±SE) of liver nodules for male and female dab 20–24 cm in length plotted against year.

for these diseases the differences between years were not consistent, but depended on the length class or sex respectively (Table 6; Figure 4 and 5). Adjusted prevalence of epidermal hyperplasia showed a significant increase in 1987, and dropped sharply afterwards, except for small fish where prevalences showed a consistent decrease over the study period. Adjusted prevalences of liver nodules increased in male fish in 1987 and fell afterwards; in female fish they showed a steady decrease during the study period.

For lymphocystis the two-way interaction between site and year was significant, meaning that for this disease the differences between the sites were not consistent, but depended on the year of sampling (Table 6; Figure 6). Adjusted prevalence of lymphocystis showed, in general, a similar temporal trend to epidermal hyperplasia, except at the TDW-dumping site, where prevalence decreased from 1986 onwards.

As an example, adjusted prevalence of epidermal hyperplasia and *Glugea* infection are plotted against sites in 1988 (Figure 7). Highest levels of epidermal hyperplasia occurred at the offshore TDW-dumping ground (Site 4) and at the site near the Rhine estuary (Site 1). Prevalences of *Glugea* were highest in coastal areas (Sites 1 and 2) (Figure 7). This is in contrast to lymphocystis where, except in 1987, prevalences in offshore areas, including the TDW dumping ground (Sites 3, 4 and 5) were higher than at the coastal sites (Figure 6).

Age as a confounding variable. In the statistical analysis, length was used as a co-variate. However, if age not length is the true determining factor, differences in growth rates between sites will restrict the applicability of the co-variate length

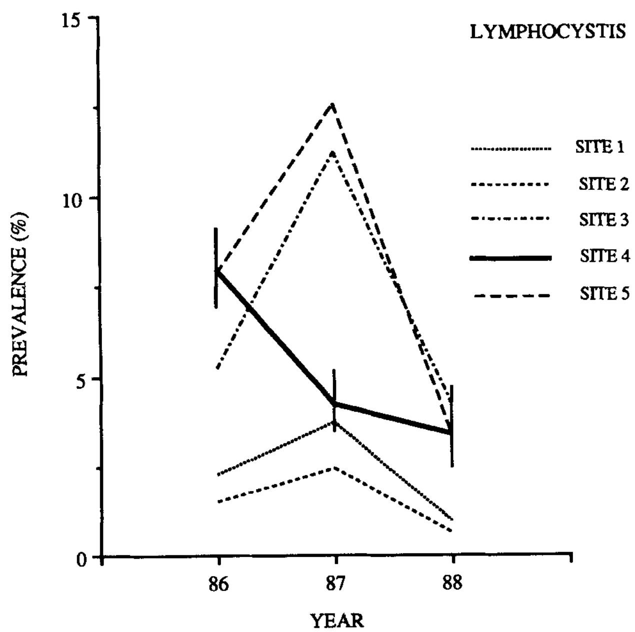
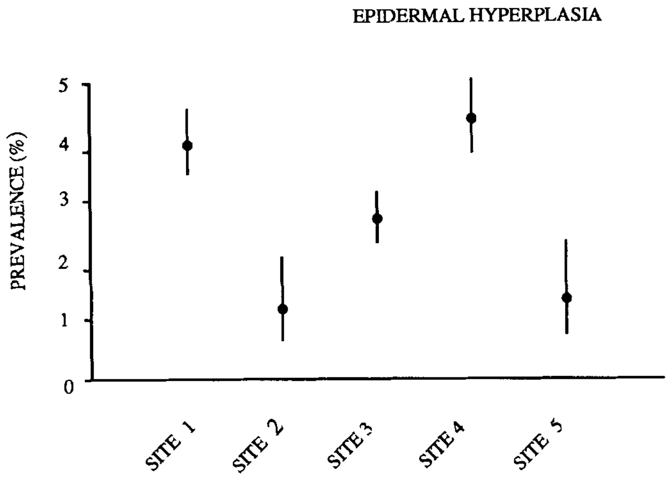


Figure 6 Adjusted prevalences (±SE) of lymphocystis for female dab 20–24 cm in length plotted for each site in 1988.



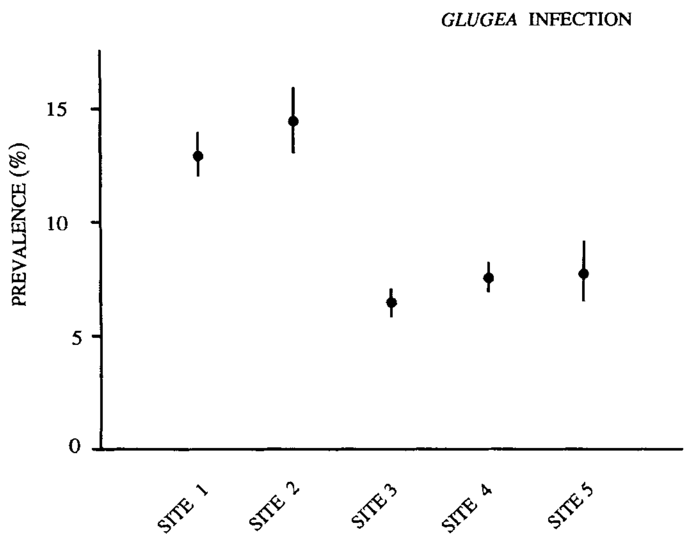


Figure 7 Adjusted prevalences (±SE) of epidermal hyperplasia and *Glugea* infection for dab 20–24 cm in length plotted for each site in 1988.

to correct for the age effect. Indeed, growth rates of dab seemed to be considerably higher in coastal areas (Sites 1 and 2), resulting in a lower mean age in all three size groups. In contrast, dab from the offshore TDW dumping site and the two other offshore sites (particularly Site 3) exhibited much lower growth rates (Table 3). This means that differences in prevalence levels of *Glugea* infection between the coastal and the offshore sites would have been accentuated had the same age groups been compared, whereas differences in prevalence levels of lymphocystis would have been reduced. However, the observed differences in prevalence of epidermal hyperplasia between the offshore TDW-dumping site and the two other offshore sites (Figure 7) cannot be explained by differences in age composition.

Intensity of Disease

Table 7 gives the degrees of severity of the four principal diseases at each site. No statistical analysis was attempted on these data because of the low numbers of fish in some of the cells. But marked differences between sites can be seen only for *Glugea*. Severe cases (grade III) of epidermal hyperplasia occurred only in affected fish from the dumping ground (Site 4) and in the site near the Rhine estuary (Site 1). However, the numbers of affected fish were so low at two of the sites that the validity of this finding is doubtful. As regards *Glugea* infection there appeared to be a tendency for infections to be more severe (grade II and III) at Site 1 than at the other sites.

Associations between Disease Conditions

It was found that 5.4% of the fish affected by epidermal hyperplasia and 6.2% of those affected by lymphocystis also had liver nodules, whereas this percentage was only 2.0% in fish not affected with epidermal hyperplasia or lymphocystis. In order to test whether the observed diseases are associated with one another a chi-square test of independence was performed. From the test results, which include a coefficient of concurrence (Table 8), it can be concluded that both of the external diseases (epidermal hyperplasia and lymphocystis) are significantly more common in dab with liver nodules than in those without liver nodules ($p < 0.001$), although there was no significant association between the external diseases ($p = 0.45$). *Glugea* infections were not significantly associated with any of the other three diseases ($p \geq 0.40$).

Correlations of Disease Occurrence with Dumping Substances and other Relevant Factors

The data on heavy metals were used to search for correlations with spatial trends in disease prevalence. Levels of chromium appeared somewhat higher at the TDW dumping site (Site 4) and at the site near the Rhine estuary (Site 1). However, the standard errors are large compared to the differences among sites (Figure 8). Similar data for hermit crabs are presented in Figure 9, but only for the offshore sites as none were captured inshore. Highest levels of iron, chromium, and titanium were found at the offshore TDW dumping site (Site 4), and in one of the reference sites (Site 5), but again the standard errors are large compared to the differences among sites.

Correlations of adjusted disease prevalence at each site with relative population density and fish condition factor (Table 3), fishing activity, water depth and sediment composition (Table 2) were calculated for 1988 (Table 9). Three of these factors may be associated with regional differences in disease prevalence (Spearman's $r > 0.8$): absence of fisheries activity with *Glugea* infection (−0.9) and low condition factor (−0.8) and increased water depth (0.8) with lymphocystis. None of them, however, appeared to show an association with epidermal hyperplasia.

Table 7 Degrees of severity of the four principal diseases. The values refer to the percentage of affected fish having grade I, II and III of the disease. N is the total number of fish affected.

	Lymphocystis				*Epidermal hyperplasia*				*Glugea infection*				*Liver nodules*			
	I	*II*	*III*	*N*	*I*	*II*	*III*	*N*	*I*	*II*	*III*	*N*	*I*	*II*	*III*	*N*
Site 1	84	9	7	*43*	78	15	7	*86*	55	20	25	*161*	61	31	8	*33*
Site 2	100	0	0	*3*	100	0	0	*5*	67	17	16	*89*	0	100	0	*1*
Site 3	89	9	2	*137*	76	24	0	*70*	78	12	10	*121*	58	36	6	*32*
Site 4	84	14	2	*108*	80	11	9	*111*	60	24	16	*131*	63	32	5	*59*
Site 5	93	7	0	*14*	100	0	0	*6*	69	25	6	*32*	100	0	0	*4*

Table 8 Association between the four principal diseases, abbreviated as in Table 6: cells on the lower left and the main diagonal contain numbers of fish showing each combination of having (+) or not having (−) 2 diseases. Cells on the upper right contain Cohen's kappa (k) with asymptotic standard error and the probability of the likelihood ratio chi-square test of independence.

	+ Ly	− Ly	+ EP	− EP	+ LN	− LN	+ GI	− GI
+ Ly	305	–	$k = 0.01 \pm 0.014$		$k = 0.06 \pm 0.019$		$k = 0.01 \pm 0.012$	
− Ly	–	5637	$p = 0.45$		$p < 0.001$		$p = 0.48$	
+ EP	17	261	278	–	$k = 0.05 \pm 0.018$		$k = 0.01 \pm 0.013$	
− EP	288	5376	–	5664	$p = 0.001$		$p = 0.40$	
+ LN	19	110	15	114	129	–	$k = 0.01 \pm 0.009$	
− LN	286	5527	263	5550	–	5813	$p = 0.40$	
+ GI	24	510	29	505	9	525	534	–
− GI	281	5127	249	5159	120	5288	–	5408

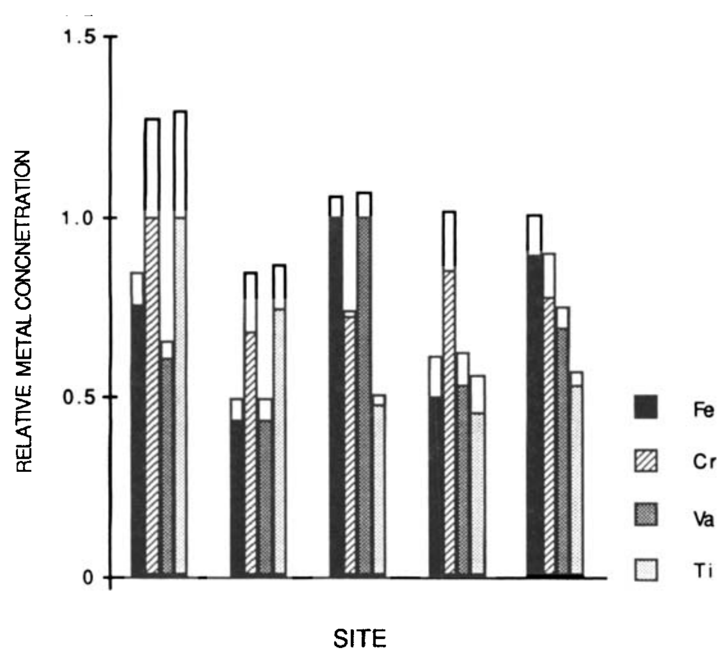


Figure 8 Concentrations of Fe, Cr, Va and Ti in the fine sediment fraction (<63 μm) at each site: mean of 1988 and 1989 values with SD represented by the unshaded portion of each bar. For clarity, the *Y*-axis gives relative concentrations: maximum observed concentrations (mg/kg) of each metal (represented by 1.0) were 29176 (Fe), 110.4 (Cr), 108 (Va) and 5503 (Ti).

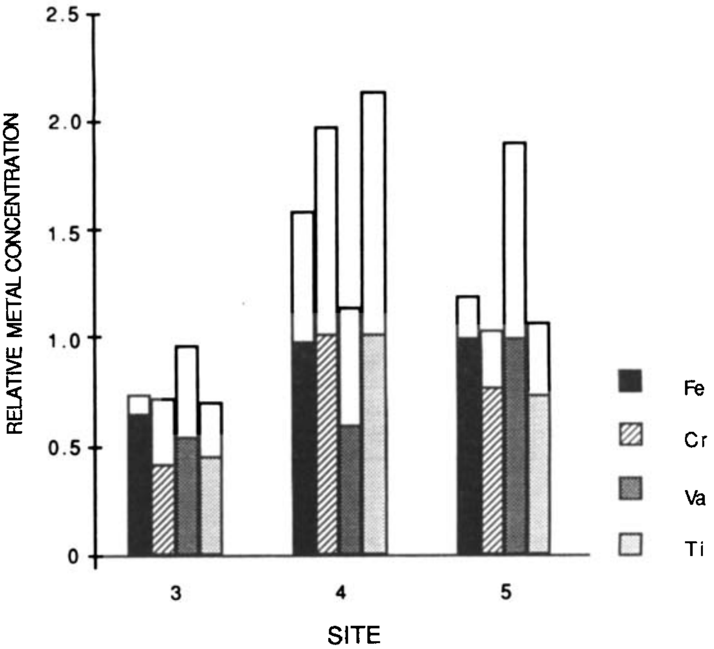


Figure 9 Concentrations of Fe, Cr, Va and Ti in hermit crab abdomens at the 3 offshore sites: mean of 1986, 1987 and 1988 values with SD represented by the unshaded portion of each bar. *Y*-axis as in Figure 8: maximum observed concentrations (mg/kg) of each metal (represented by 1.0) were 176 (Fe), 0.45 (Cr), 0.53 (Va) and 16.73 (Ti).

DISCUSSION

The data show that a clear correspondence exists between prevalence of epidermal hyperplasia in dab, and the discharge of large quantities of dilute acids of TDW to two sites. These sites differ from each other in that the dilute acids are a major source of pollution at the offshore dumping site whereas they represent just one of the many sources of pollution at the Rhine estuary site. The similarity between the disease prevalence in spite of these differences suggests the possibility of a causal link between epidermal hyperplasia and the TDW wastes.

Table 9 Spearman rank correlations between adjusted prevalences of disease at each site in 1988 and other factors (* $= p < 0.05$).

	Lymphocystis	*Epidermal hyperplasia*	*Glugea infection*
Rel. population density	0.7	0.4	−0.3
Condition factor	−0.8*	−0.5	0.7
Fishing activity	0.6	0.7	−0.9*
Water depth	0.8*	0.0	−0.5
Sediment <63 μm (%)	0.3	0.6	−0.7

However, at neither site is there clear evidence of elevated waste constituents in sediments and biota.

The possibility of a causal relationship between TDW and fish diseases should be considered against the background of the natural occurrence of disease in fish populations, and the recognised multifactorial aetiology of disease (Vethaak and ap Rheinallt, 1990). Many fish diseases including epidermal hyperplasia/papilloma are known from at least the turn of the century (Heron *et al.*, 1988), and pollution is unlikely to be their sole cause. Information on the natural background levels of selected diseases including epidermal hyperplasia in the south-eastern part of the North Sea for the period 1981 to 1985 has been published by Banning (1987), who showed that epidermal hyperplasia in dab occurs over the whole area investigated, with hot spots in the German Bight and offshore Dogger Bank area.

There is only limited knowledge of the aetiology of the diseases included in this study. The contrasting spatial patterns of lymphocystis, a viral disease (Anders, 1988), and of epidermal hyperplasia, could reflect their differing aetiologies. This explanation is further supported by the fact that lymphocystis and epidermal hyperplasia were not significantly associated with each other in individual dab (Table 6). On the other hand, the strong association of both epidermal hyperplasia and lymphocystis with liver nodules, suspected to be caused by chemical agents (Malins *et al.*, 1988), indicates that a general mechanism such as immunosuppression or nutritional deficiency may underlie the observed disease prevalence.

Dabs spawn along the Dutch coast between March and June. The relatively high disease prevalence observed at this season could therefore be associated with lower resistance during spawning, resulting in the relatively lower condition factor. However, neither the condition factor, nor any of the other factors considered in this study, seemed to be obviously related to the observed regional pattern of epidermal hyperplasia. It is possible that the impact of pollution stress, resulting in immunosuppression (Vos *et al.* 1989), could have acted synergistically as a further cause, increasing disease prevalence: further research is needed to clarify the aetiology of this disease.

The above findings may be compared with German investigations which reported higher levels of epidermal hyperplasia/papilloma in dab in a TDW area and implicated chromium and iron in the aetiology of the disease (Dethlefsen *et al.*, 1987).

There are, however, some notable differences between the Dutch and German dumping areas. The Dutch area has higher rates of water exchange and large current velocities, resulting in a less stable bottom. The metal content of the water phase is mainly influenced by natural water currents and is not indicative of effects of dumping (Bos, pers. comm.), and it is likely that effects in the water column (from precipitation of ferric iron hydroxide contained in the waste) are of minor importance to demersal fish such as dab. The Dutch dumping area has a mainly coarse sand sediment. In the German area, fine depositional sediments accumulate water-borne contaminants so that levels of contamination are considerably higher there than in the Dutch area. In the German Bight concentrations of iron, chromium and vanadium in the $<20\,\mu m$ fraction were found to be 1.5 to 4 times higher than in adjacent areas (Deutsches Hydrographisches Institut, 1984). Although in the present study some indication was found of a relationship between TDW dumping and the chromium content of surface sediments (Figure 8), the increase in concentration was much less than at the German site. Furthermore, most of the pollutants introduced by the rivers Rhine and Meuse, and by offshore dumping in the Belgian and Dutch continental shelf are dispersed in a plume running south-north (Ruijter, 1985) and thus probably influencing sites to the north of them, in particular Site 3 (Figure 2). In this way other contributing sources, perhaps distant, may mask the effects of specific dumping-related materials. Contamination of the NW offshore reference site (Site 3) can be attributed to both coastal and offshore sources, owing to northward transport of fine particles.

Furthermore, in this study the various sites differ not only by their exposure to wastes from dumping but also with respect to hydrological conditions, sediment composition, and perhaps bioavailability of contaminants and biological factors too. These other differences reduce the validity of explaining spatial differences in diseases in terms of dumping wastes alone. Because these differences are not amenable to manipulation as in a multifactorial experiment (the ideal situation), long-term monitoring aimed at detecting temporal trends may in fact be a more valid approach than searching for spatial differences in disease prevalence.

Another important point of discussion concerns the value of the dab as an indicator fish species for environmental pollution. When this study was undertaken, the general view expressed in the literature was that dab migration probably occurs only over relatively short distances, with spawning and feeding largely in the same areas (Bohl, 1957). However, there are indications that mature dab tagged off the Belgian coast migrate in a north-easterly direction during the second half of the year and return the next spring season, the mean distance covered by individual dab being 80 miles in the study of DeClerck (1984). Fish could therefore migrate to or from any of the sites included in this study, thus obscuring the link between exposure and disease. The possibility of an onshore migration between the dumping area (Site 4) and the Rhine estuary (Site 1), makes it impossible to discriminate between the specific effects of TDW and effects of the general pollution load of the river Rhine. Neither, on the other hand, is the possibility eliminated that high levels of epidermal hyperplasia observed in the Rhine estuary are in fact caused by exposure to TDW. More information is required on the biology and population dynamics of dab in this part of the North Sea before definite conclusions can be drawn.

CONCLUSIONS

1. There is a consistently higher prevalence of epidermal hyperplasia/papilloma in dab in Dutch areas of the North Sea where discharges of dilute wastes of titanium dioxide acids take place, when compared to surrounding sites. A similar spatial pattern has not been found for other diseases, though there is a significant association between the occurrence of epidermal hyperplasia/papilloma and lymphocystis and of the occurrence of liver nodules in individual fish.

2. This supports findings obtained by the German investigations in their dumping area and therefore could suggest further evidence for a link between the waste discharges and the prevalence of disease.

3. Interpretation of a causal link is complicated by the interference of other inputs from river water with long-distance dispersion of the dumped substances to other parts of the continental shelf, by local hydrographic conditions, and by possible local migration of dab.

4. However, on the basis of the present results, the possibility that discharges of titanium dioxide wastes contribute to the occurrence of epidermal hyperplasia/papilloma in dab cannot be proven or discounted.

5. For the above-mentioned reasons dab disease studies are of limited use for monitoring the effects of Dutch TDW dumping, even though the prevalence of epidermal hyperplasia/papilloma could reflect pollution in more general terms.

6. In spite of the limitations, the fact that dumping of TDW in the Dutch part of the North Sea has ceased provides an unique opportunity for future studies that can monitor changes within the sites over longer periods of time.

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