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Epidermal papilloma of North Sea dab (Limanda limanda);
histology, epidemiology and relation to dumping of
wastes from TiO_2 industry

Volkert Dethlefsen
Burkard Watermann

Bundforschungsanstalt für Fischerei
Institut für Küsten- und Binnenfischerei
Toxikologisches Laboratorium Cuxhaven
Niedersachsenstraße
2190 Cuxhaven
Federal Republic of Germany



ABSTRACT

A brief histological description of epidermal papilloma of dab (Limanda limanda) is given. Three different phenomena have so far been described under the heading of epidermal papilloma; hyperplasia, epidermal papilloma sensu strictu, and fibroma. The relation between these three lesions differs in different areas and from season to season.

Epidemiological data on occurrence and abundance of diseased dab in the German Bight are presented based on results obtained during 7 cruises (350 hours of bottom trawling) and the inspection of more than 100.000 dabs.

In the northern North Sea (north of $56^{\circ}00'N$) epidermal papilloma of dab were not found. They were present in the southern North Sea and have so far been found in German and Dutch coastal waters as well as in the Doggerbank area.

During 7 cruises the dumping area of wastes from TiO_2 production and its vicinity (active since 1969) was characterized by exceptionally high percentages of dab with epidermal papilloma and related lesions. Percentages comparable to these were encountered in no other area.

There exists some uncertainty as to the first occurrence of the lesions under consideration in the North Sea. While during two systematic fish disease surveys carried out in 1977 in the German Bight the disease was not present in any suspicious numbers, in May 1978 infection rates of up to 6 % per station were encountered.

The results are interpreted as an indication, that wastes from TiO_2 production which have accumulated considerably in the respective area possibly induce these lesions.

The German Bight, which is characterized as a sensitive area for a number of reasons (oxygen deficiency, reduction of species-numbers of macrobenthic organisms, obvious primary and secondary eutrophication effects), does not seem to be suited as dumping area for large quantities of domestical and industrial wastes.

INTRODUCTION

The fish

North Sea dab (Limanda limanda) is the most abundant flat fish of the North Sea (Bohl, 1957, and own data). Although not directly fished by the German fishing fleet its ecological importance cannot be overestimated. Dab is growing extremely slow; females reach 20 cm after 4 to 5 and males after 6 to 8 years (Bohl, 1957).

In the literature dab is considered to be a stationary fish, its seasonal on- and offshore migrations are not covering wide distances (Bohl, 1957).

The waste

Since 1969 650.000 t per annum of liquid acid wastes from production of titaniumdioxid are dumped into an area 12 sm NW of the island of Helgoland. In 1976 the quantity dumped was increased to 750.000 t per annum.

Chemical investigations on iron concentrations and pH alterations in the dumping area were carried out by Weichart (1975a and 1975b). Biological investigations were done by Rachor (1972) and Dethlefsen (1973). Results were reviewed by Rachor and Dethlefsen in 1975.

Conclusion by that time was, that no adverse effects of dumping of wastes from TiO_2 production occurred when certain dumping procedures were followed.

The disease

Epidermal papilloma of North Sea dab were first described in 1925 by Johnstone. Epithelial tumors were by that time found on 4 specimen of Limanda limanda. These tumors were not known to occur in the German Bight until May 1978. During earlier systematic fish disease surveys carried out by Dethlefsen in 1977 and Möller in spring 1977 (Möller, 1979) this disease was not present in the German Bight or the northern North Sea in any suspicious numbers. During May 1978 up to 6.6 % of North Sea dab in the German Bight was found to be afflicted with epithelial lesions. Histopathological investigation showed that these lesions were epidermal papilloma (Peters and Watermann, 1979).

MATERIAL AND METHODS

a) Sampling

7 cruises have been carried out to gather information on spatial and temporal distribution of dab (Limanda limanda) with epithelial tumors.

1. April/May 1978 "Victor Hensen"
2. January 1979 "Anton Dohrn"
3. July/August 1979 "Anton Dohrn"
4. January 1980 "Anton Dohrn"
5. February 1980 "Anton Dohrn"
6. June 1980 "Anton Dohrn"
7. June 1980 "Friedrich Heincke"

During these 7 cruises different sampling strategies were applied. While during the first cruise fishing was carried out in two dumping areas and a control area, during later cruises stations were localized on a grid almost symmetrically covering the area of the German Bight. During cruise 5 the sampling was carried out on a transect in a northerly direction off the German coast.

Trawling was done with a standard herring bottom trawl, trawling time was between 45 and 60 minutes. Either the whole catch or subsamples were analysed quantitatively, all dabs were length measured and diagnosed immediately after catching. After diagnosis samples were preserved for histology in 10 % formalin. In the first step of histological investigation 10 micron sections were stained with hematoxilin eosine. Later on preparations were made for thin and ultra thin sections with the appropriate methods described by Peters et al. (1978).

Presentation of data

Data on distribution of diseased dab are presented as % infection rate per square unit, numbers of fish investigated per square unit are given in Table 1. All results obtained for one square unit have been averaged for the respective cruise. All squares are given numbers (see Figure 1). Dumping is carried out at the lower right corner of square number 19.

Influence of the dumping is detectable mainly from square 19 in a northerly direction, but also in surrounding squares especially in numbers 11, 12, 18, 25, 20 and 27 (Figure 1). The distribution of the wastes is depending very much on hydrographic factors like currents and wave action but also on wind. An idealized picture of areas of increased concentrations of wastes drawn after published (Weichart, 1975a) and unpublished data (Albrecht, pers. comm.) is also given in Figure 1.

In the case of the cruise June 1980 additional data obtained by RV "Friedrich Heincke" during the same time are incorporated in the respective figure (thanks are due to Dr. Harald Rosenthal of Biologische Anstalt Helgoland for kindly offering this material).

RESULTS

Histology

Histological investigation revealed that the epithelial lesions on dab, sometimes occurring in groups, are epidermal tumors, often with short or long fingerlike extensions of the dermal connective tissue extending into the epidermal tumor tissue. While normal epidermis is composed of only a few (5 to 10 at most) layers of Malpighian cells these excrescences have ten to twenty times as many. The basal cells on the basal lamina are usually low and seldom of high prismatic shape. The spindle-shaped cells above are arranged in numerous layers which gradually become more polyhedral near the surface. The layer directly at the surface may be slightly flattened. Mostly the cells are packed tightly, small lymphocytes are scattered chiefly in the layers near the stroma. Mucus cells are almost completely absent. These changes in the skin can be considered epidermal hyperplasia or even papilloma if stroma folds are formed. In their flattened form, they greatly resemble those epithelioma that have been described as 'fish pox' especially in cyprinids (Schaeperclaus, 1979).

Neither in light microscopy nor in electronmicroscopy the presence of X-cells which are typical for epidermal papilloma of Pacific flatfishes were detectable. So far in none of the e.m. preparations, viruses or virus like particles could be identified.

Beside the described lesion another one, macroscopically very similar, mostly occurring on the fins was identified. Light-microscopy revealed that primarely dermis is involved in this lesion. The overlying epidermis is spongy or incomplete and infiltrated with leucocytes. It is containing large numbers of X-cells and leucocytes. The lesion is characterized by inflammatory, the X-cells found in these lesions are very similar to those in Pacific flatfish tumors. Since the discussion wether these X-cells are possibly of amoebic origin or degenerative tumor cells still is on the way nothing can be added to this discussion at the present stage. It is pointed out that in none of the epidermal papilloma or hyperplasia investigated histologically cells or cell types similar to those frequently being called X-cells were encountered.

Most of the hyperplasia and epidermal papilloma were found on the pigmented side of the infested fish (Table 2).

In the following text the term epidermal papilloma usually includes hyperplasia if not otherwise stated.

Epidemiology

1. April/May 1978

In the center of the area for dumping of wastes from titanium-dioxidproduction (dumping operation was started in May 1969) and in comparison areas (Figure 2) percentages of dab (Limanda limanda) with epidermal papilloma were higher than in a

comparison area 120 miles northwest of Cuxhaven and the sewage sludge dumping area and its vicinity more onshore. 2.0 to 6.6 % of the dab caught in the TiO_2 dumping area and its vicinity were afflicted with epidermal lesions while papilloma were found on 0.25 % of fishes from sewage sludge dumping area and 0.6 % of fishes from the northwestern comparison area (the number of fish investigated per square shown in Table 1) (Figure 2).

2. January 1979

During this investigation a grid of stations was fished covering the area of the German Bight almost symmetrically. No special emphasis was laid on fishing in the dumping areas. Dab caught in the vicinity of the dumping area for wastes from TiO_2 industry were most frequently found with epidermal papilloma.

4.6 was the percentage of infection near the dumping area while percentages of infestation decreased in the northerly direction from 1.5 to 0.9. During this cruise also fish from the outer Elbe estuary area were found to be affected with the epidermal tumors. On all other stations during this cruise lower percentages of infestation were encountered (Figure 3).

3. August 1979

The area with markedly increased infestation was larger than that found during the 2 preceding cruises. This time no infested dab were found in the outer Elbe estuary. Rates of infestation in the dumping area and its vicinity were near 0.6 to 2.5 % (Figure 4).

4. January 1980

During this cruise dab from the outer Elbe estuary were infested by 0.3 %, dabs from the dumping area and its vicinity by 1.15 to 2.63 %. Outside of this focus of infestation around the dumping area infestation rates between 0.2 and 0.6 % were found (Figure 5).

5. February 1980

Fishing was started west of Helgoland and a northern transect was performed through the dumping area of wastes from TiO_2 industry into Danish coastal waters (Figure 6). The percentage of infestation was high west of Helgoland reaching a maximum on the northern transect. In the dumping area for wastes from TiO_2 production percentage of infestation was 4.7. Infestation decreased in a northerly direction and was lower than 0.2 off the Danish coast.

6. and 7. June 1980 (Data from cruises "Anton Dohrn" and "Friedrich Heincke", both June 1980)

The pattern of distribution of diseased fish caught in June 1980 was very similar to that obtained for July/August 1979. Clearly the vicinity of the TiO_2 wastes dumping area again was characterized by high frequencies of diseased dab.

During these cruises at two stations in the southwestern part of the German Bight percentages of infestation of dab with epidermal papilloma was high. It should be noticed that altogether only 8 diseased specimens were captured in these two squares during these cruises (Figure 7).

Lengths of infested fish

During the winter cruises (January 1979 and January 1980) it could be shown that length distribution of healthy and diseased specimen was different. Most of the healthy fish were 14 cm long during these cruises, diseased fishes had their maxima in length distribution at 20 - 26 cm (Figure 8a and 8b). During the cruise in August 1979 (Figure 8c) the differences between length maxima of diseased and healthy dabs were not as marked as those encountered during the winter cruises.

In general it becomes obvious that tumors are occurring on older fish more frequently than on younger fish. Also the length distribution of dab with hyperplasia was different from that of dab with epidermal papilloma, both in summer and winter (Figure 9a and 9b). In Figure 9c it can be seen that this pattern was not valid for dab caught in Dutch coastal waters, here no differences in lengths of dab with hyperplasia and those with epidermal papilloma were detectable.

Length composition of dab from various stations of the German Bight did not differ much both during summer and winter and from season to season (Figure 10 and 11). This is in accordance with the results of Bohl (1957).

Condition factors

In his paper from 1979 Möller claims that nutrition could play an important role in governing the frequency of disease. With the example of lymphocystis Möller tried to correlate condition factors of dab from Dogger Bank and German Bight area with the intensity of infection. He found that there was a negative correlation between percentage of infection of dab with lymphocystis disease and the condition factor of apparently healthy fish from the respective stations. Möller does not include epidermal papilloma in this consideration. Only few own data are available to check this correlation for epidermal papilloma (Table 3).

From these data no such correlation can be deducted. There also does not seem to exist a difference in the condition factors of dab from the Dogger Bank and that from the German Bight.

Population density

Density of population of dab in the German Bight is varying with seasons.

During summer population density over the whole of the area under survey was rather uniform (Figure 12) while during winter the average catch per 30 minutes of trawling was low in deeper waters and high in onshore waters (Figure 13).

Dab seems to perform at least seasonal short distance migrations, into deeper waters in summer and into shallower waters during winter. This summer migration explains the fact that during both summer cruises (July/August 1979 and June 1980) the areas with increased percentages of infection are shifted slightly to the west and that infested fish in summer are in general more scattered over a wider area than during winter cruises.

For cruises in August 1979 and January 1980 it was tested whether a linear correlation between population density of dab and the percentage of infestation existed. The results were negative for the two cruises and the diseases epidermal papilloma, lymphocystis and ulcer disease. Correlation coefficients were always lower than 0.2.

Other diseases

Details on results of distribution and frequency of other diseases of dab will be subject of a separate paper. But for the considerations in this paper it is important to see the geographical distribution of other diseases of North Sea dab.

Two other major diseases are occurring on North Sea dab, lymphocystis and ulcers. Results on distribution of these diseases have been reported by Möller (1979) and Dethlefsen (1980). Also from newer results (Figure 14 to 17) it becomes obvious that these diseases are not accumulating in or near the dumping area for wastes from TiO_2 production.

DISCUSSION

Significance of the data

Although altogether more than 100.000 dabs were investigated for some stations especially during the cruises in January 1979 and January 1980 the number of dabs captured per hour was low. During these cruises for some of the stations the catches cannot be regarded as representative. So the significance of these results must be considered low. The quality of the data is also influenced by two other sources of error.

1. the natural variability within the sampled population,
2. the systematic error during sampling. Both errors can be kept small when numbers of fish investigated per station is high. Under the assumption that sampling of diseased fishes is done with an accuracy of ± 1 diseased fish per station the sampling error would be ± 1 % when 100 fishes are investigated per station and ± 0.1 % when 1000 fishes are investigated per station.

In Table 1 it can be seen that numbers of fish investigated per station were very different. Even when stations where less than 100 fish were investigated were left unregarded there are a number of stations where possible variation of percentages of infested fish are high.

It is the combination of all results obtained during 7 cruises that produces a more significant picture. When the results of 7 cruises are combined it is obvious that the dumping area for wastes from TiO_2 production and closely surrounded areas are characterized by a higher than average occurrence of dabs with epidermal papilloma. An area can be indicated where during all cruises increased rates of infection were encountered.

Infestation of dabs with epidermal papillomas in the northern North Sea was investigated by Möller (1979). He found that epidermal papilloma on dabs were absent in the northern North Sea. Infestation rate on the Dogger Bank was 0.6 %. In August 1979, when we fished on the Dogger Bank area, percentages of infestation were around 0.5 %.

Histological investigation of epidermal lesions revealed that at least 3 histologically different phenomena have been described as skin swellings (Möller, 1979). The first attempt to separate these three histologically different lesions by macroscopic investigation was carried out in August 1979. Subsequent histological investigation confirmed macroscopic diagnosis in most cases. During the cruise in August 1979 more than 90 % of the lesions encountered were classified as hyperplasia. On the Dogger Bank lesions of dab were frequently found to occur near or on the fins. Histological examination revealed that these lesions cannot be classified as epidermal papillomas but are characterized by X-cells, therefore probably not being neoplastic.

More than 50 % of the lesions found on dabs from the Dogger Bank area must be classified as X-cell lesions or early developmental stages of these. The percentages of dabs with these X-cell lesions in the German Bight was much lower and shown to be between 10 and 20 %. These results must be regarded as rather preliminary, they need further examination. But it becomes obvious that the infestation of dabs from Dogger Bank with epidermal papilloma must be lower than the 0.6 % given by Möller (1979) and 0.5 % found in the cruise of August 1979. Average infection of Dogger Bank dab with epidermal papilloma can be expected to be near 0.2 and 0.3 %.

The German Bight therefore seems to be the only area of the North Sea which is characterized by a mass occurrence of dab with epidermal papilloma. In no other area of the North Sea so far similarly high infestation rates of this disease were demonstrated. The center of the highest infestation rates during 7 cruises coincided to be the dumping area for wastes from TiO_2 production.

Chemical situation in the dumping area

The dumping operation was started in May 1969. By that time until 1976 650.000 t per annum of wastes were dumped into the area 12 sm northwest of Helgoland. After 1976 quantities dumped were increased to 750.000 t per annum. Significant chemical alterations of the water quality, which can be attributed to the dumping, have been demonstrated by different investigators.

1. Iron concentration in the dumping area is increased by the factor of 10 to 100 as compared to iron concentrations in areas more offshore (Weichart, 1975a; Schmidt, 1979; Albrecht, pers.comm.).
2. pH in the dumping area is constantly lower in a varying degree depending on spatial and temporal distance from the dumping place (Weichart, 1975b).
3. The CO₂ partial pressure is increased (a decrease of pH by 0.65 units means an increase of CO₂ partial pressure by four times) (Weichart, 1975b).
4. Alterations of pH could not be measured near the sea bottom (depth of water in the dumping area 25 m) (Weichart, 1975b).
5. In the dumping area and its vicinity residues of a quantity of more than some weeks of dumping operations is continually demonstrable (Weichart, 1975a).
6. No continuous accumulation of iron in the water column nor in the sediments could be measured (Weichart, 1975 a; Albrecht, pers.comm.).
7. At certain hydrographic conditions layers of iron hydroxide were found to cover the sediments of the dumping area (Weichart, 1975a).

Most of the results of chemical investigations were obtained in the period before 1976, whereafter the quantity dumped was increased by 100.000 t. It can be expected that the concentrations of the wastes in the dumping area today are higher than before 1976.

In the literature some examples exist where certain tumors of fishes are prevalent in polluted environments (Sindermann, et al., 1980). Epidermal papilloma in white crocker (Genyonimus lineatus) were found in sewage outfalls along the California coast. Other tumors like liver tumors in tomcod (Microgadus tomcod) were found in the Hudson River estuary in New York, in hag fish (Myxine glutinosa) from the Gullmar Fjord in Sweden and in English sole (Parophrys vetulus) from the Duwamish River estuary near Seattle.

Neoplasms have been induced experimentally in at least 9 species of fish with a variety of carcinogens (Sindermann, et al., 1980).

On the other hand Oishi et al. (1975) found epidermal papillomas on flatfish (Limanda schrenki) in coastal waters of Hokkaido/ Japan, in areas free of industrial and municipal discharges. Oishi therefore speculates that if skin papillomas of flatfish are induced by environmental factors at all also the involvement of naturally occurring contaminants as well as man made pollution must be considered in the etiology of these neoplasms.

Wellings (1969) in a review on environmental aspects of neoplasia in fishes summarizes the knowledge on flounder papilloma to date as follows; tumor genesis is related to as yet undefined environmental variables of a chemical, physical and biological nature. Although the etiology of the observed conditions remains obscure the conclusion can be drawn that environmental factors including man made pollution cannot be excluded to play an important role in the genesis of skin tumors of flatfishes. Since there has been no specific causative agent demonstrated the conclusion seems to be acceptable that epidermal papilloma in flat fish are the expression to a non-specific stimulus. A stimulus of this kind is present in the area under consideration.

The high prevalence of dab (Limanda limanda) with epidermal papilloma therefore is interpreted as circumstantial evidence for a possible correlation between the wastes and the disease.

For a number of reasons the German Bight is characterized to be a sensitive area. In the inner German Bight (area between the island of Helgoland and Cuxhaven) reduction of species numbers of macro benthos organisms is registered (Rachor, 1979). Wide areas of the German Bight are characterized by oxygen deficiencies (Rachor 1979, own data, unpublished). Primary and secondary eutrophication effects are obvious (Hickel, pers.comm). The area therefore does not seem to be suited as dumping area for large quantities of domestical and industrial wastes.

The combination of these factors,

1. that it cannot be excluded that epidermal papillomas of dab are influenced by wastes from TiO_2 production and
2. that the German Bight is considered to be highly polluted from a variety of other sources than dumping,

leads us to the conclusion that dumping of wastes from TiO_2 production should be terminated as soon as possible.

ACKNOWLEDGMENT

Thanks are due to Captains and crews of RVs "Victor Hensen", "Anton Dohrn" and "Friedrich Heincke" and to all good friends and colleagues, who helped investigating the enormous numbers of dabs. I hope that diseased dabs no longer occupy your dreams.

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Square	April 1978	January 1979	July/August 1979	January 1980	February 1980	June 1980	June 1980
1		15	178	73		126	
2		17	78	65		275	
3		12	356	35		77	
4		10		131		48	
5			151	212			203
6	43		873		866	530	726
7	658	535	243	332		2,424	1,310
8		28	829			217	
9		57	775	38		120	
10		14	641	74		194	
11			698	33		333	
12	2,039	29	418	173		235	
13					1,002		
14	973						
15		46	914	476		677	
16		27	1,526	128		572	
17		6	164	26		339	
18			7,837	59		609	101
19	1,599	29	6,924	780	464	2,833	644
20		173	569	610			
21						804	107
22			1,608			889	
23		53	978			720	
24		89	625			628	
25		49	59			533	
26	2,214	334	482		1,325		398
27							1,386
29		286	1,851	153		986	
30		285	1,276	206			
31		324		381			
32		54	862				
33		769	577	812			
34		1,728	1,382	323			508
39					17	502	
40			1,369			828	376
41			1,800			185	
42				9		405	
43			277	296		157	
44		189	612	35		434	
45		3,918	1,046		1,048		
46		4,951		403			243
47	629						
48	892						

Table 1 Numbers of dab (*Limanda limanda*) investigated per square during cruise 1 - 7.

		pigmented	blind	both
August 1978	n	29	19	-
	%	60,4	39,6	-
January 1980	n	20	12	2
	%	58,8	35,3	5,9
February 1980	n	79	26	2
	%	73,8	24,3	1,9

Table 2

Frequency of epidermal papilloma and hyperplasia on pigmented and blind side of dab (Limanda limanda).

Condition factor

June 1980 - German Bight

n	79	171	71	89	82
\bar{lx}	17,9	17,3	18,4	16,3	17,2
% ep.pap.	1,44	2,04	0,15	0,8	0,99
c.f.	1,07	1,08	0,92	1,06	0,96

June 1980 - Dogger Bank

n	90	72	82
\bar{lx}	15,0	17,8	18,2
% ep.pap.	0,47	0,57	1,06
c.f.	0,94	1,04	1,05

February 1980 - Dutch coastal waters

n	94	96	88
\bar{lx}	19,4	20,4	19,5
% ep.pap.	1,23	1,55	1,14
c.f.	1,07	1,06	1,03

February 1980 - German Bight

n	100	100
\bar{lx}	17,8	18,2
% ep.pap.	2,89	1,76
c.f.	1,04	1,03

Table 3

Condition factors of dab (Limanda limanda) in relation to frequency of infestation with epidermal papilloma in different areas of the North Sea.

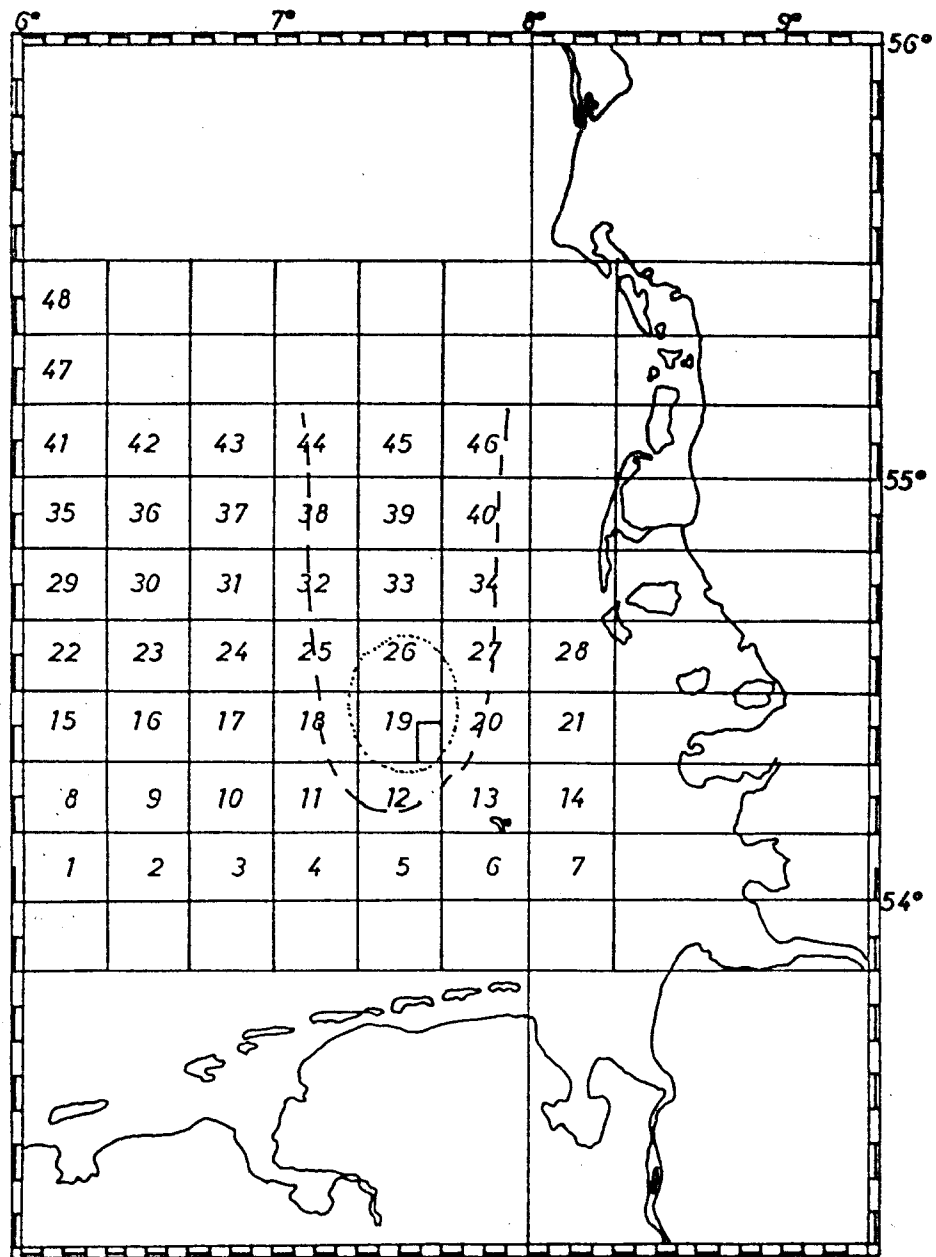


Figure 1

Squares, in which fishing was carried out during 7 cruises.
Areas of increased iron concentrations are indicated by dotted
and broken line.

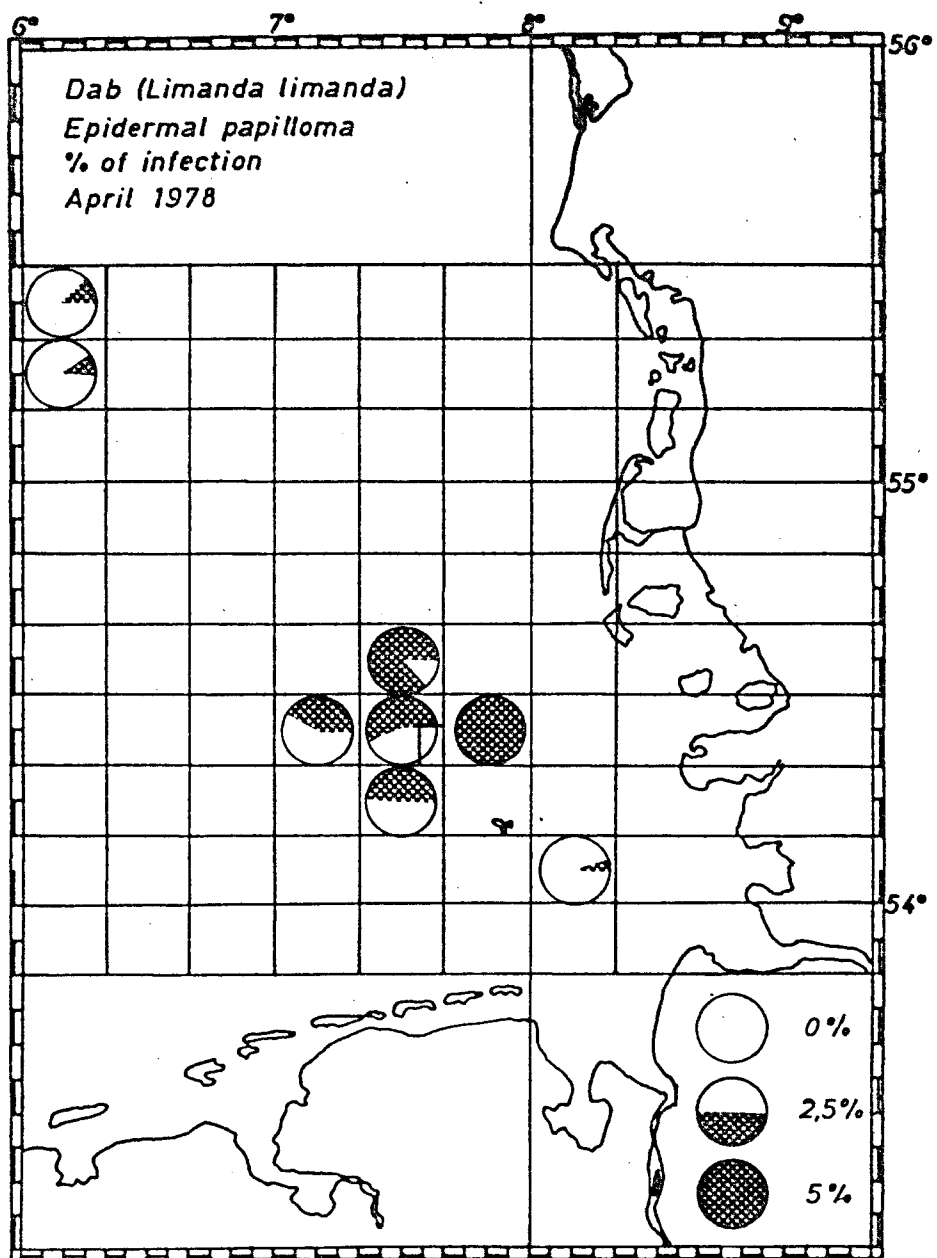


Figure 2

Percent of infection of North Sea dab with epidermal papilloma in April 1978.

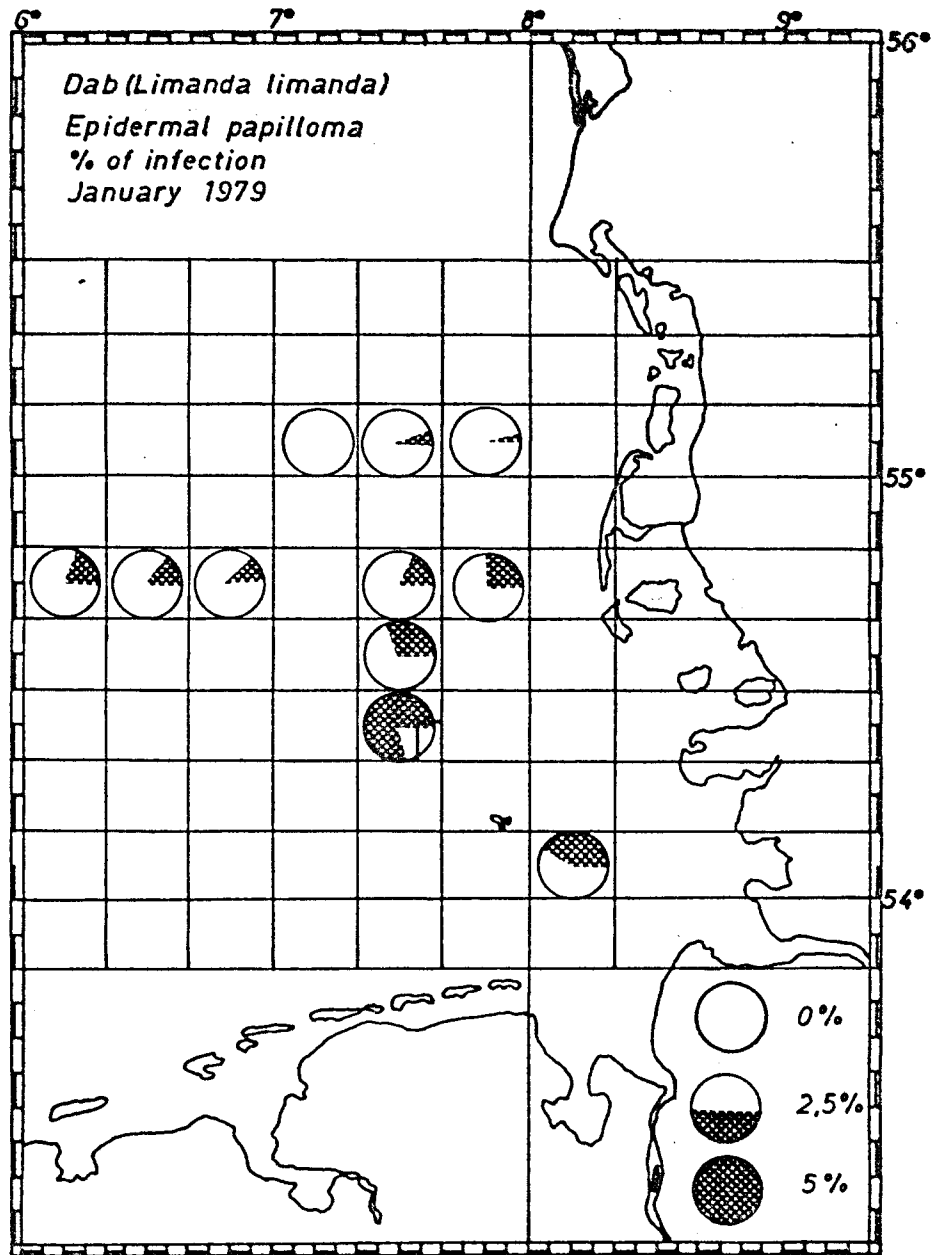


Figure 3

Percent of infection of North Sea dab with epidermal papilloma in January 1979.

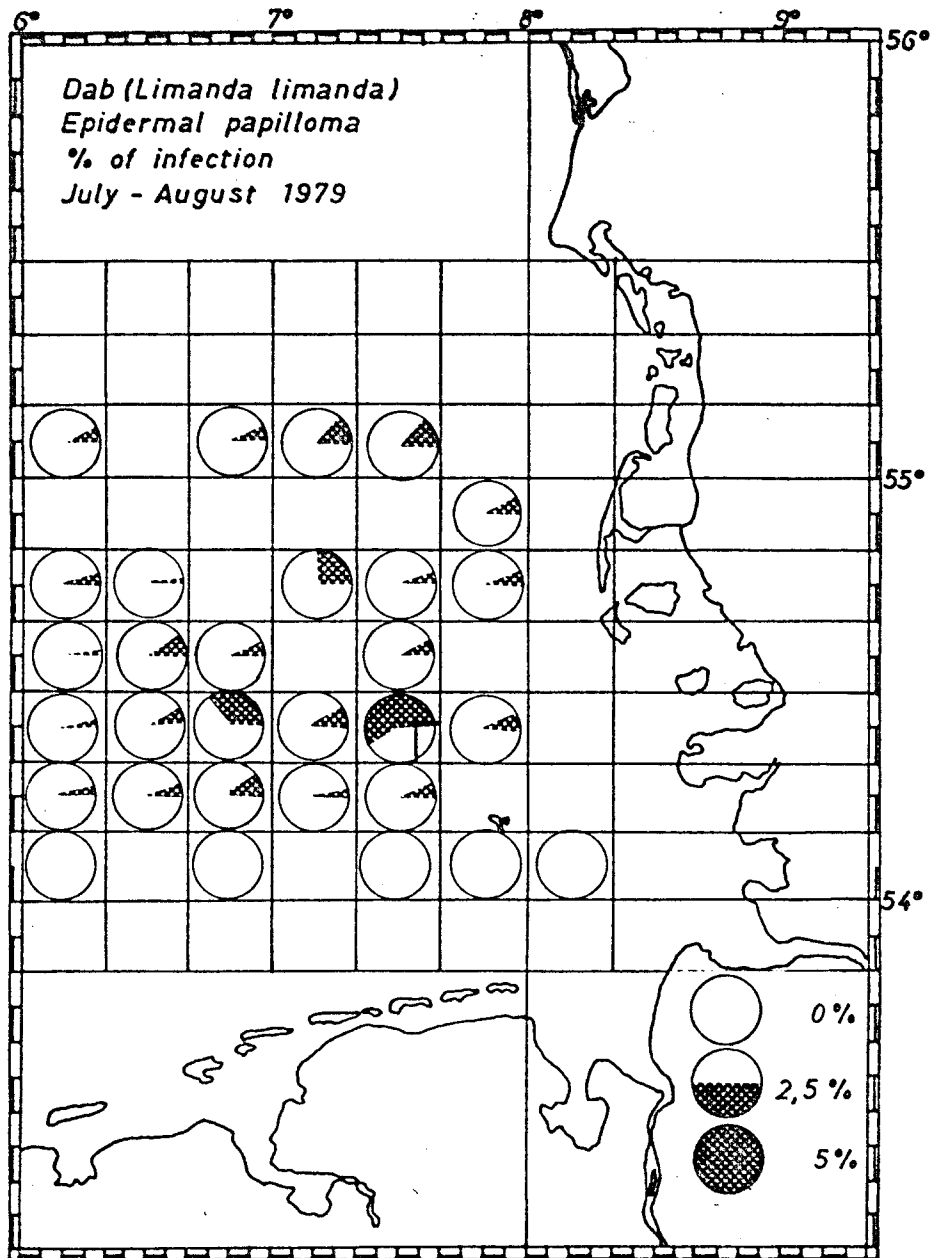


Figure 4

Percent of infection of North Sea dab with epidermal papilloma in August 1979.

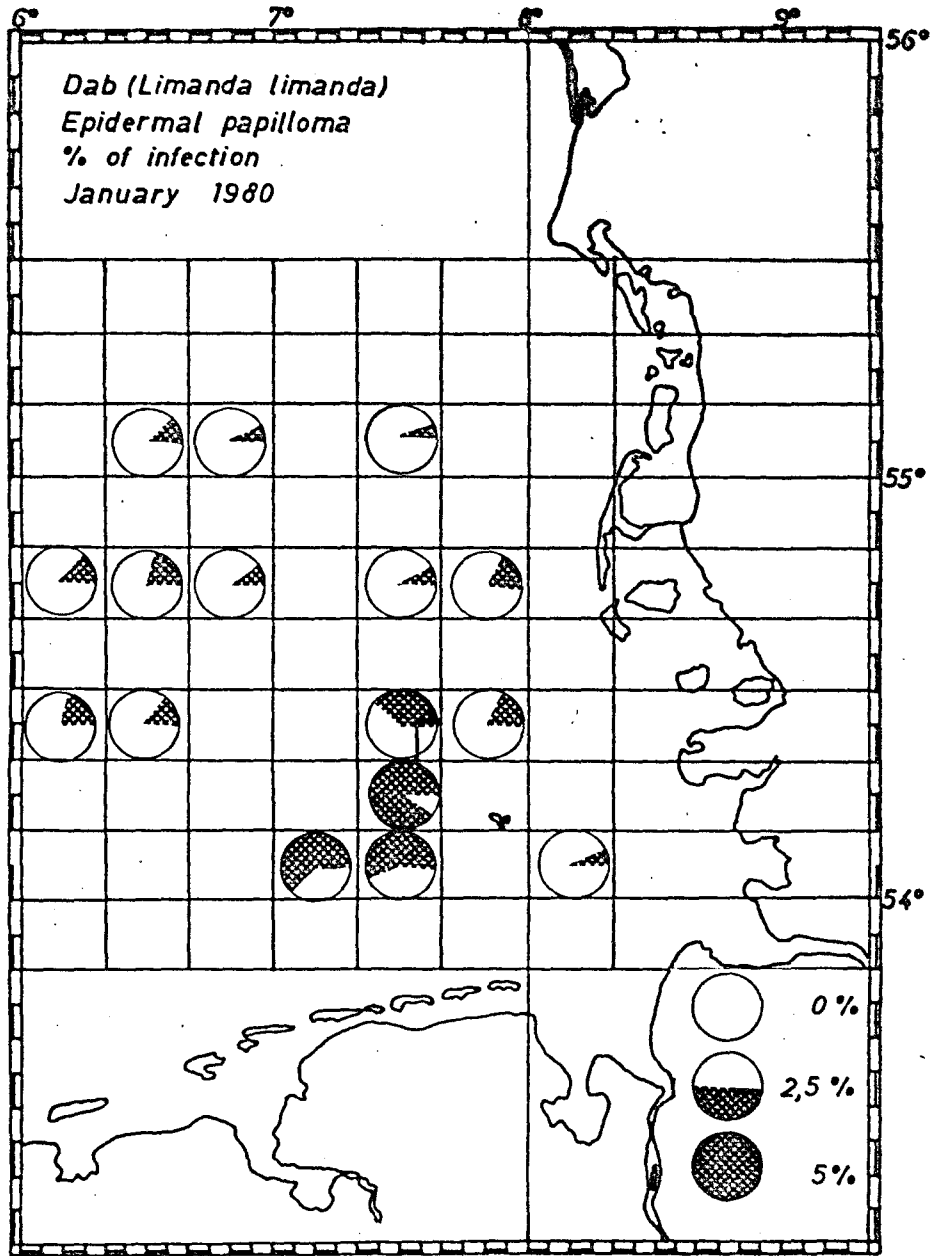


Figure 5

Percent of infection of North Sea dab with epidermal papilloma in January 1980.

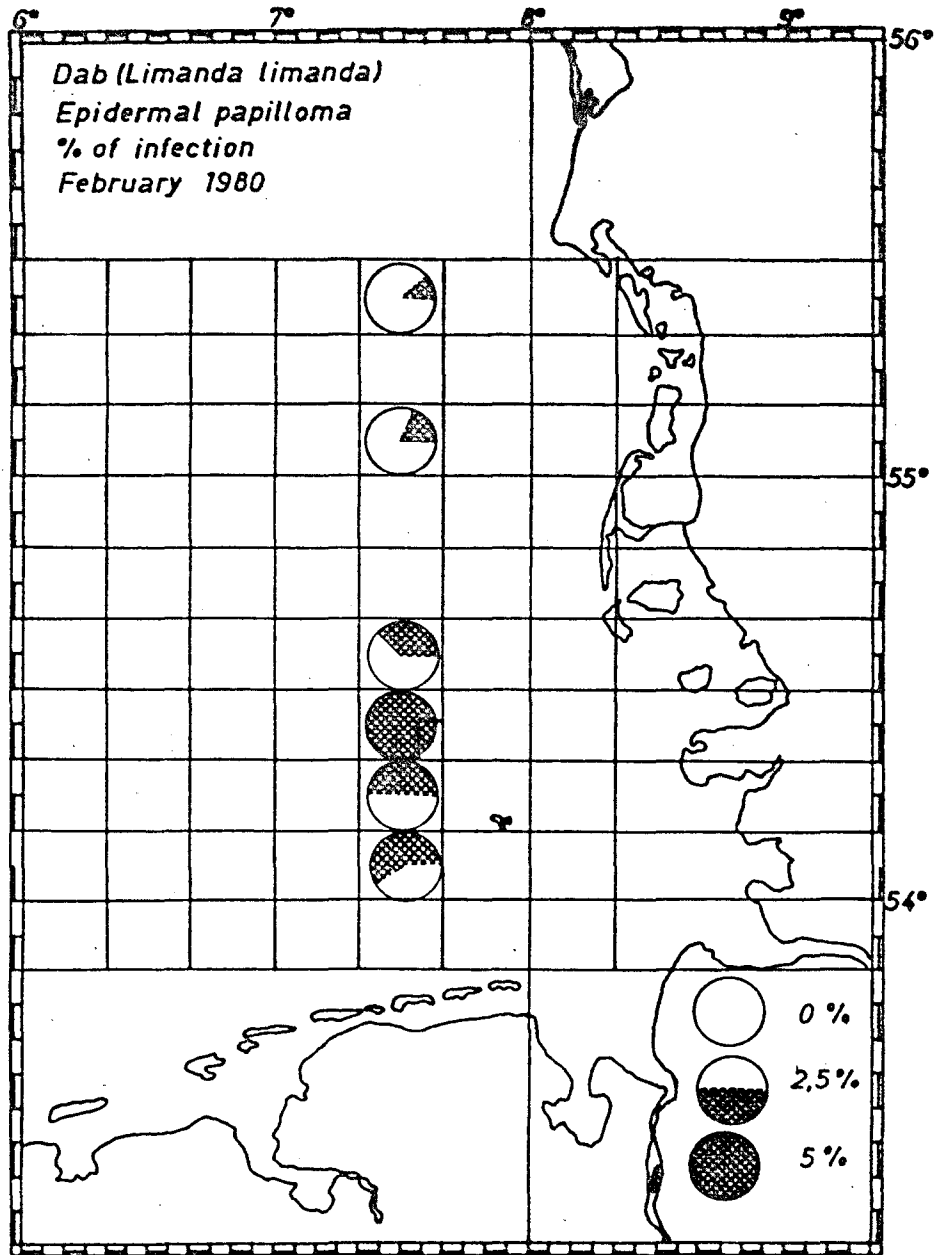


Figure 6

Percent of infection of North Sea dab with epidermal papilloma in February 1980.

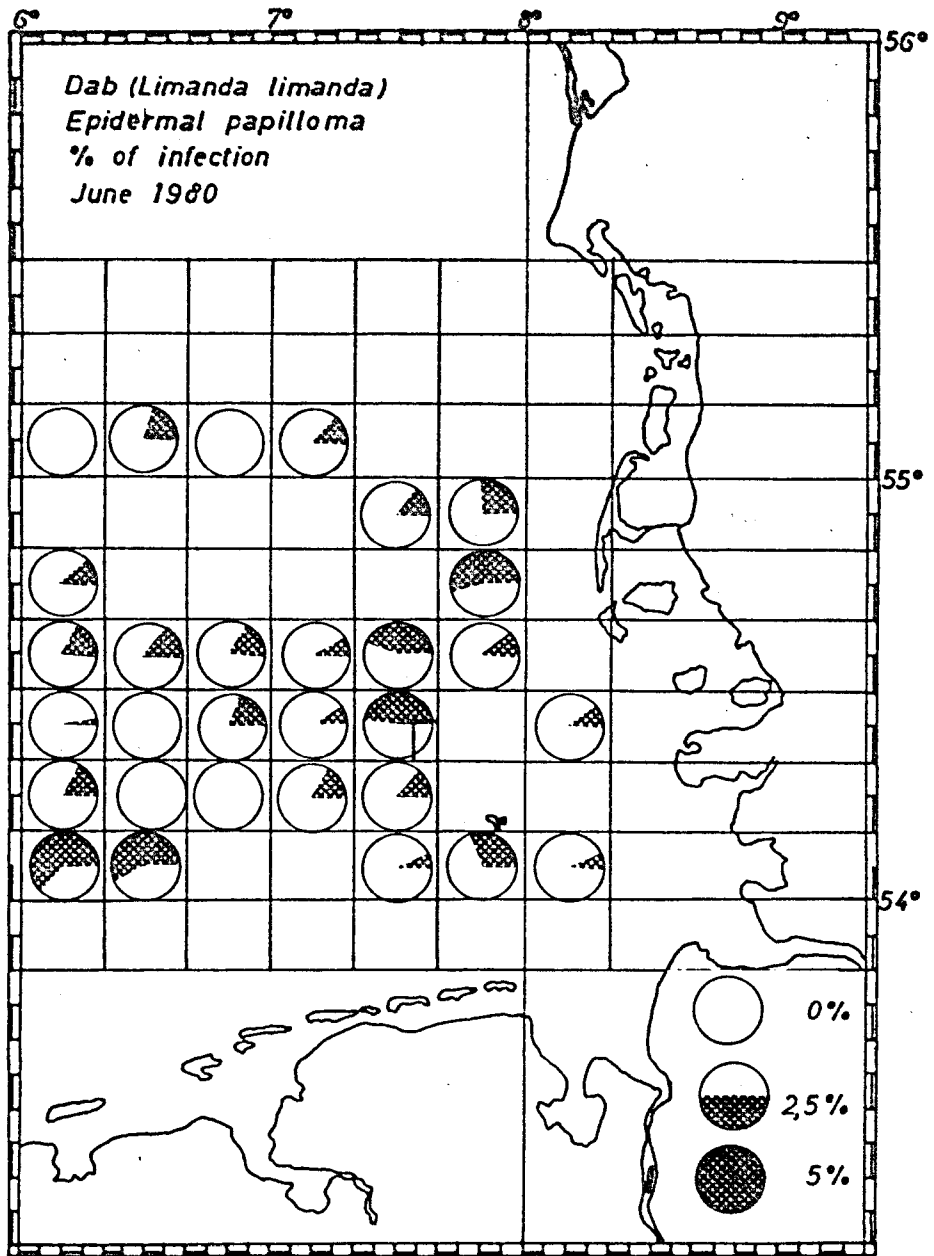


Figure 7

Percent of infection of North Sea dab with epidermal papilloma in June 1980.

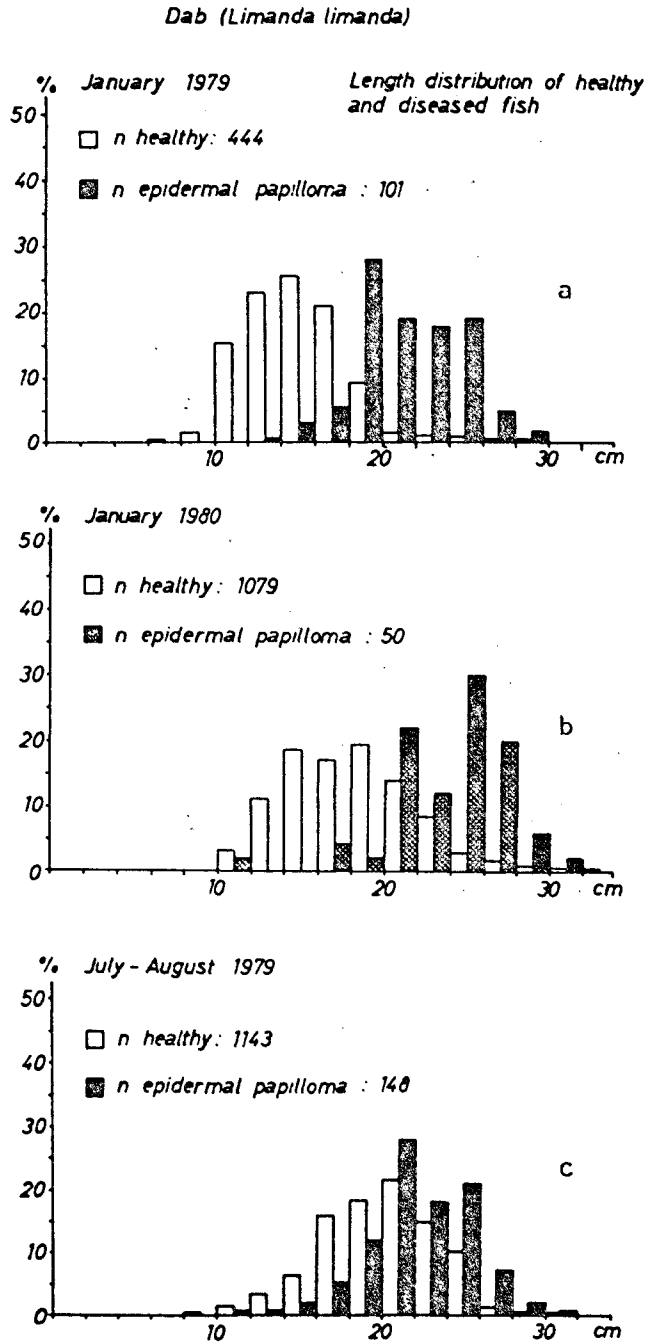


Figure 8

Length of healthy and diseased dab (*Limanda limanda*).

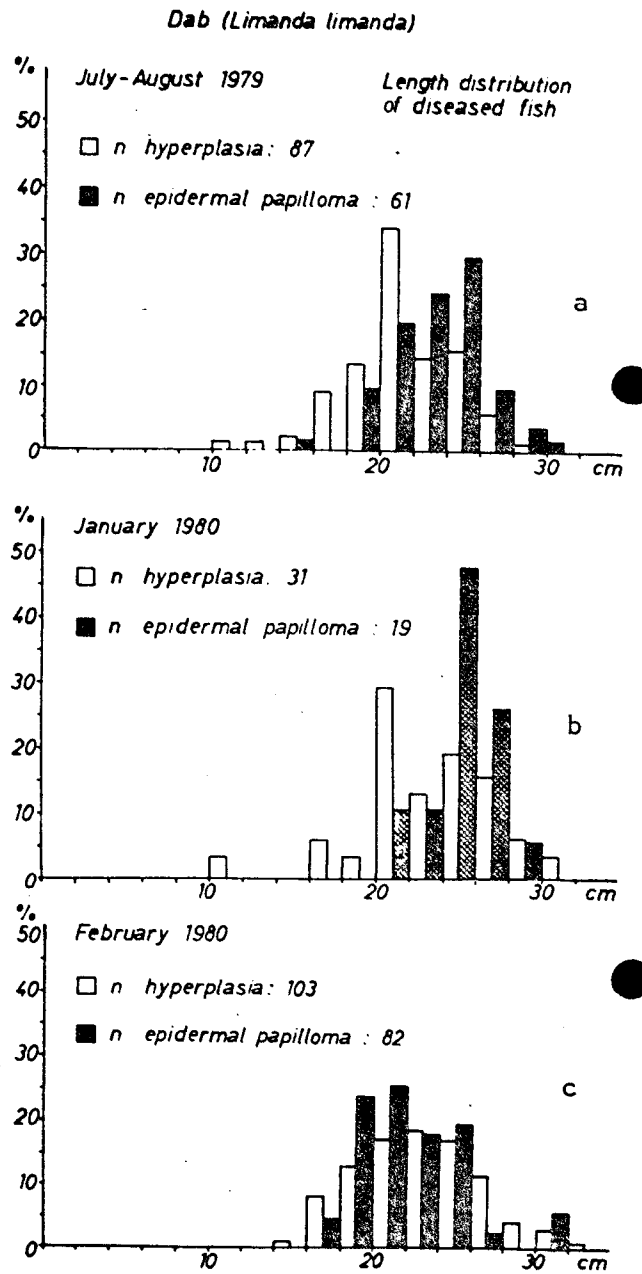


Figure 9

Length of dab (*Limanda limanda*) with hyperplasia and epidermal papilloma.

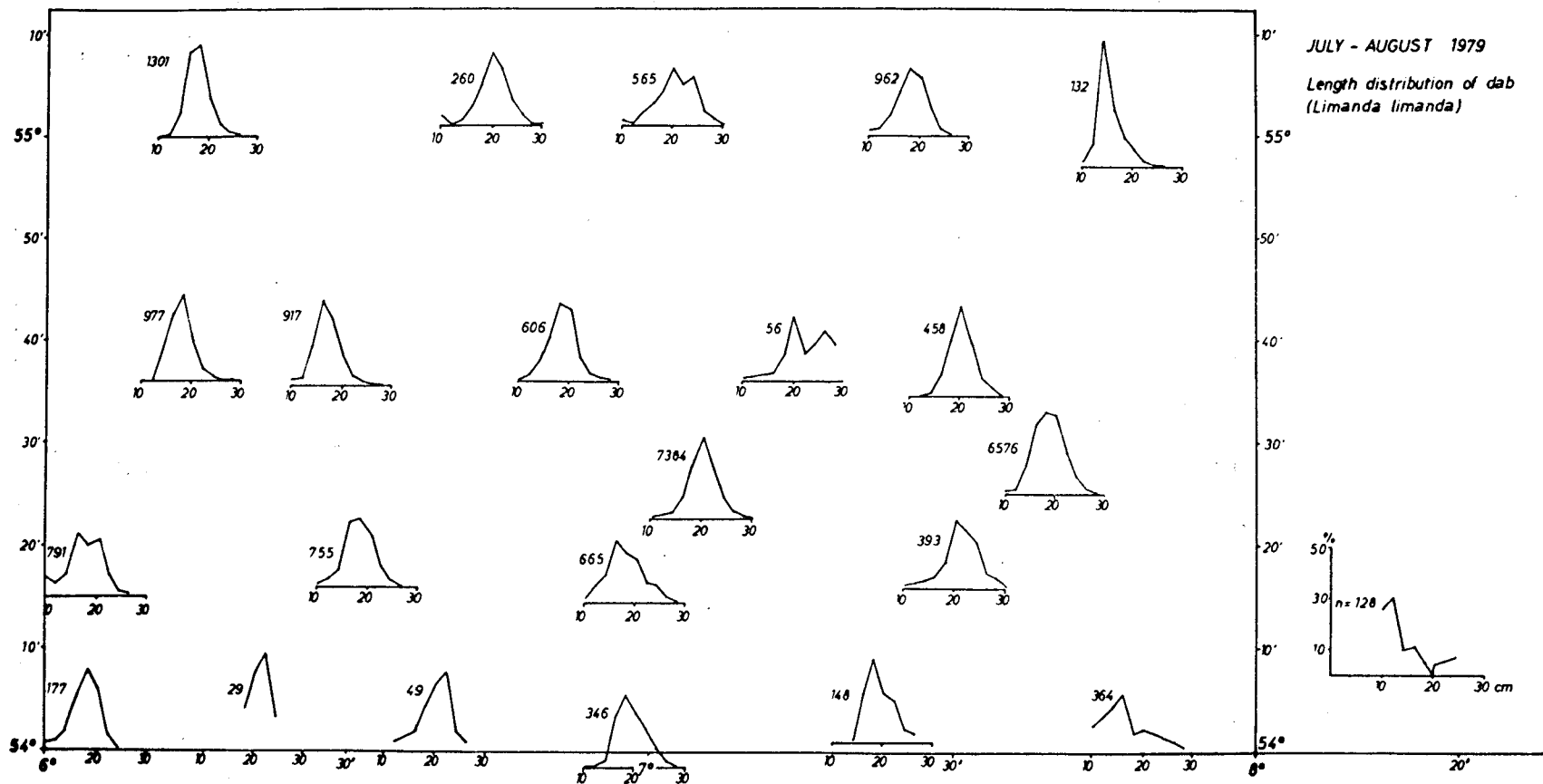


Figure 10

Length distribution of dab (*Limanda limanda*) in the German Bight (July/August 1979).

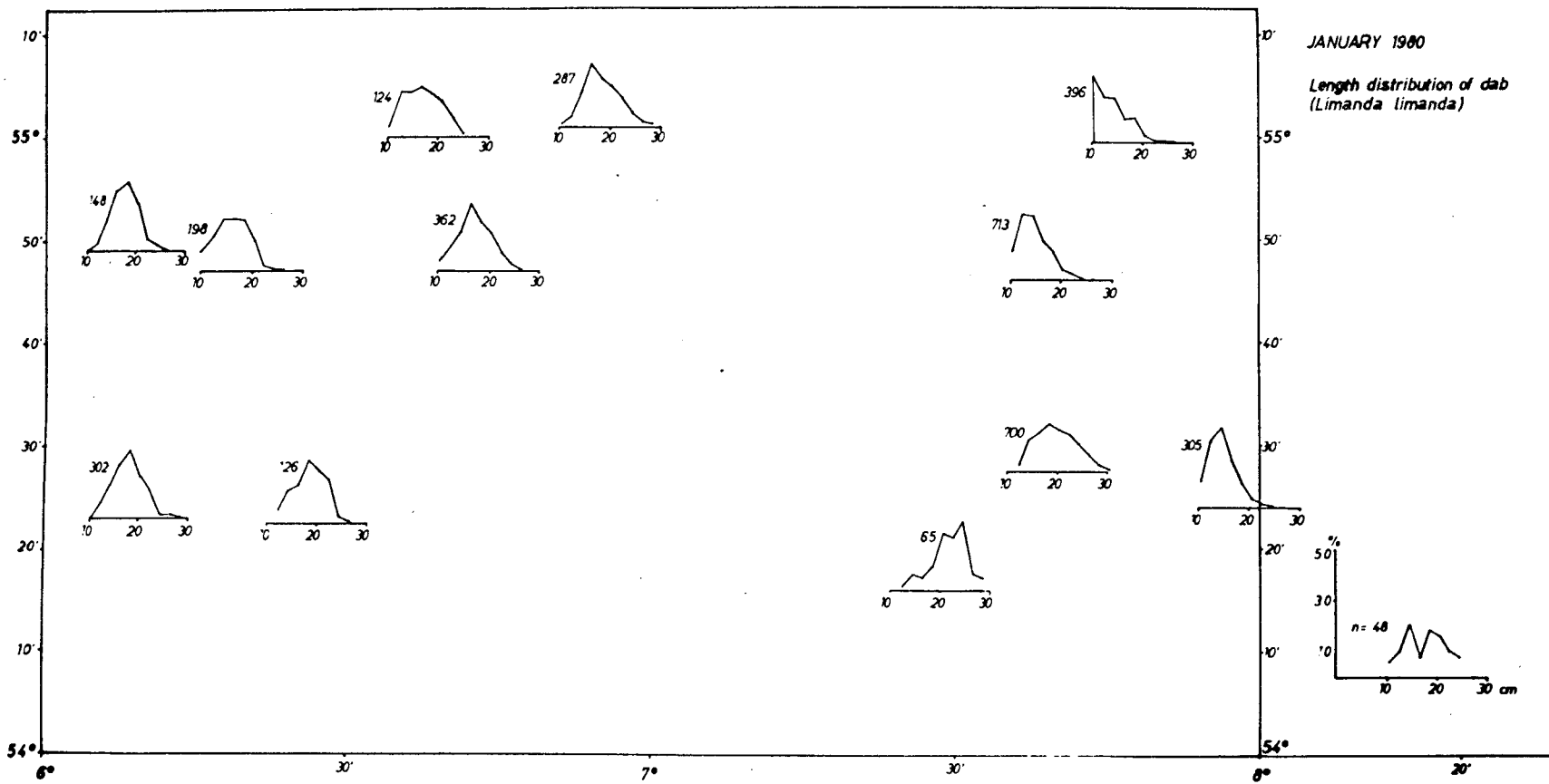


Figure 11

Length distribution of dab (*Limanda limanda*) in the German Bight (January 1980).

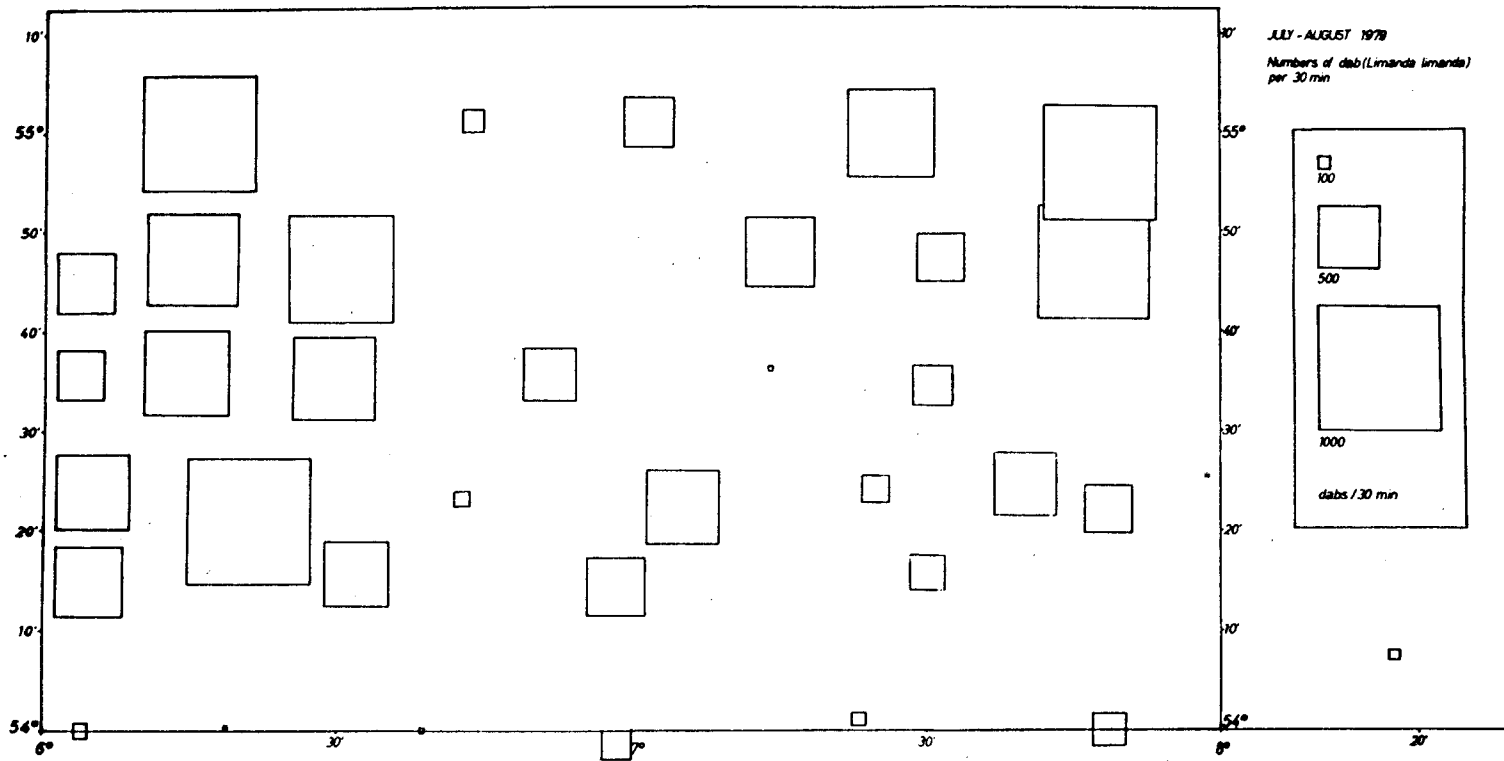


Figure 12

Numbers of dab (*Limanda limanda*) caught per station in 30 minutes (July/August 1979).

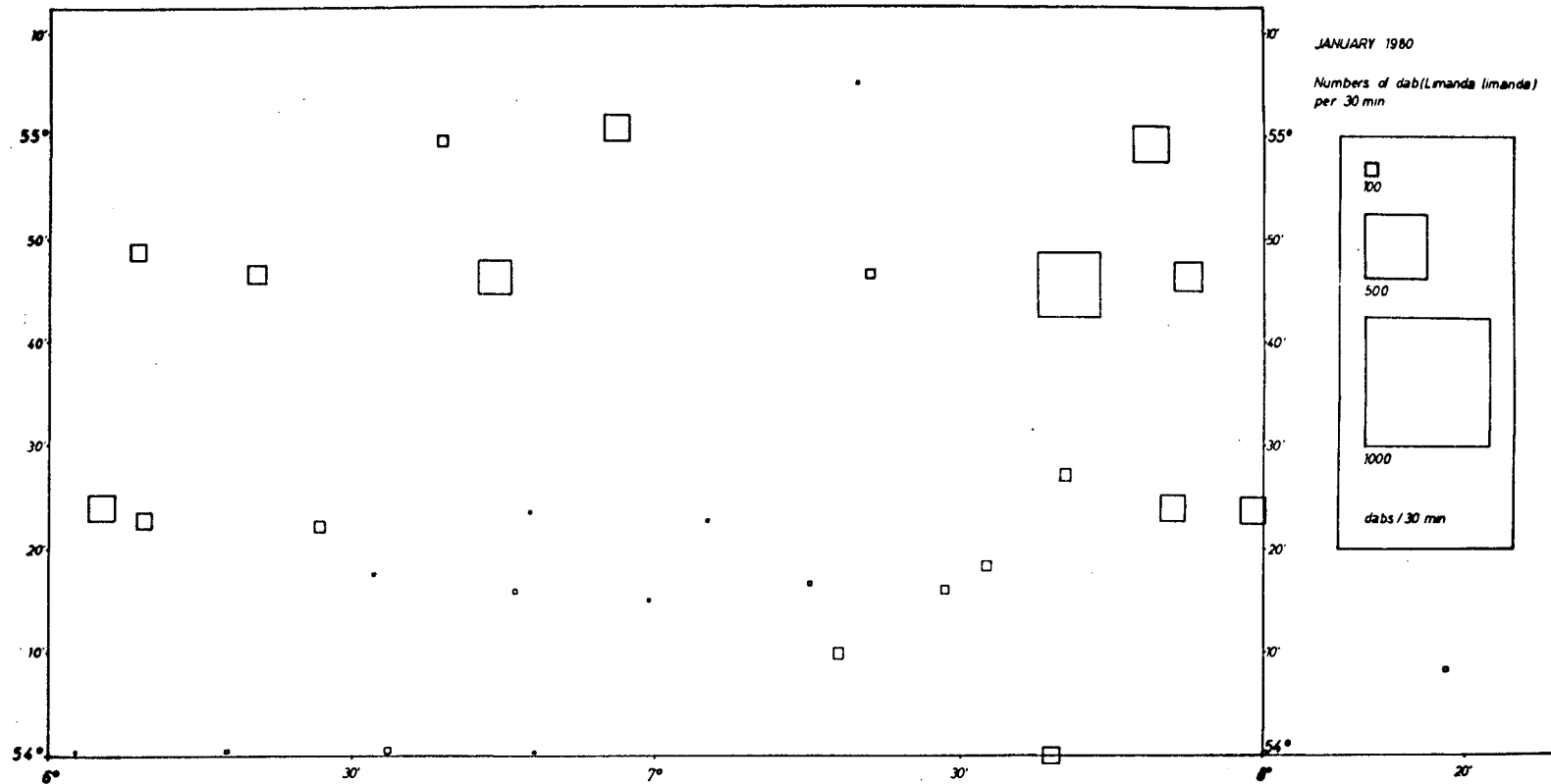


Figure 13

Numbers of dab (*Limanda limanda*) caught per station in 30 minutes (January 1980).

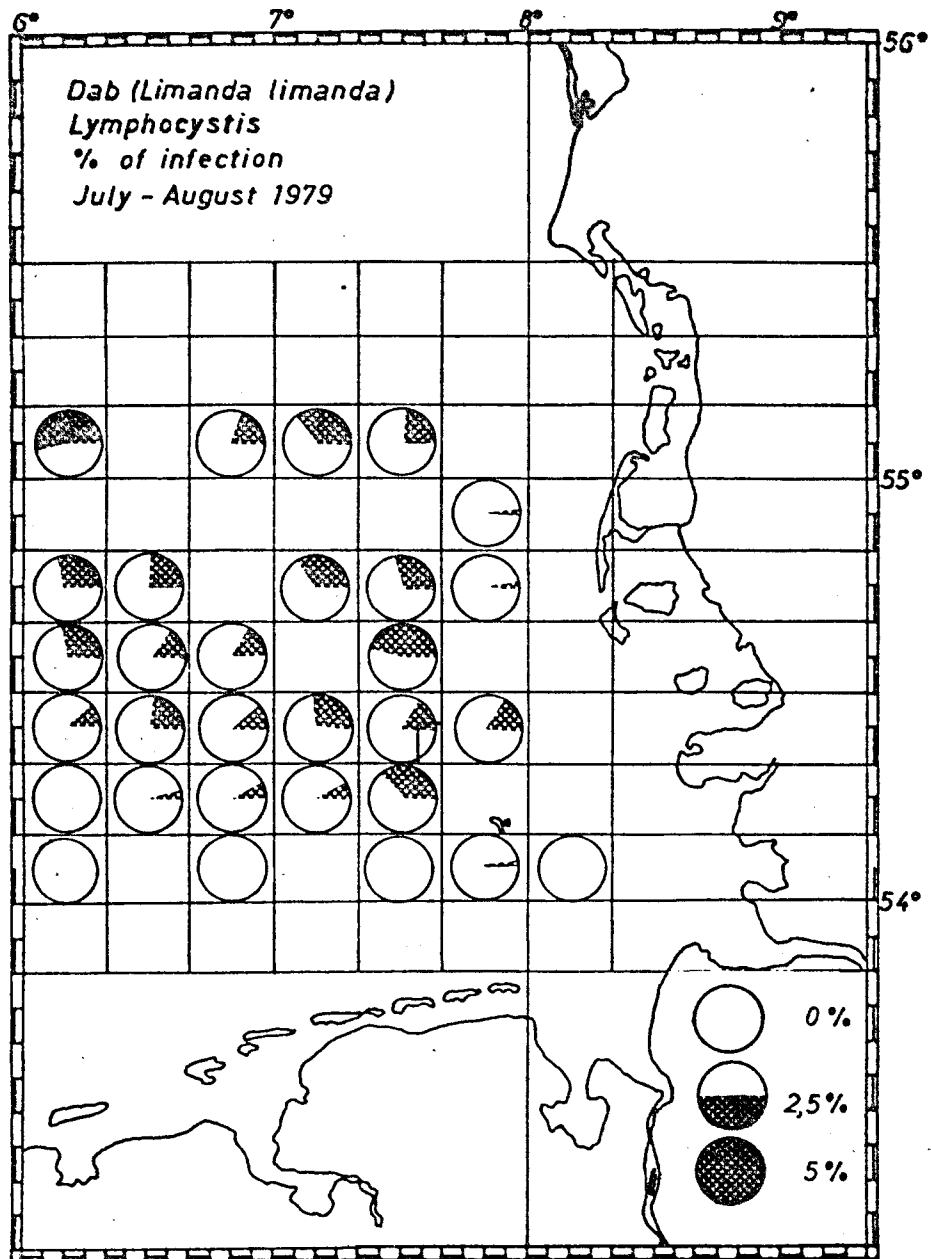


Figure 14

Percent of infection of North Sea dab with lymphocystis in July/August 1979.

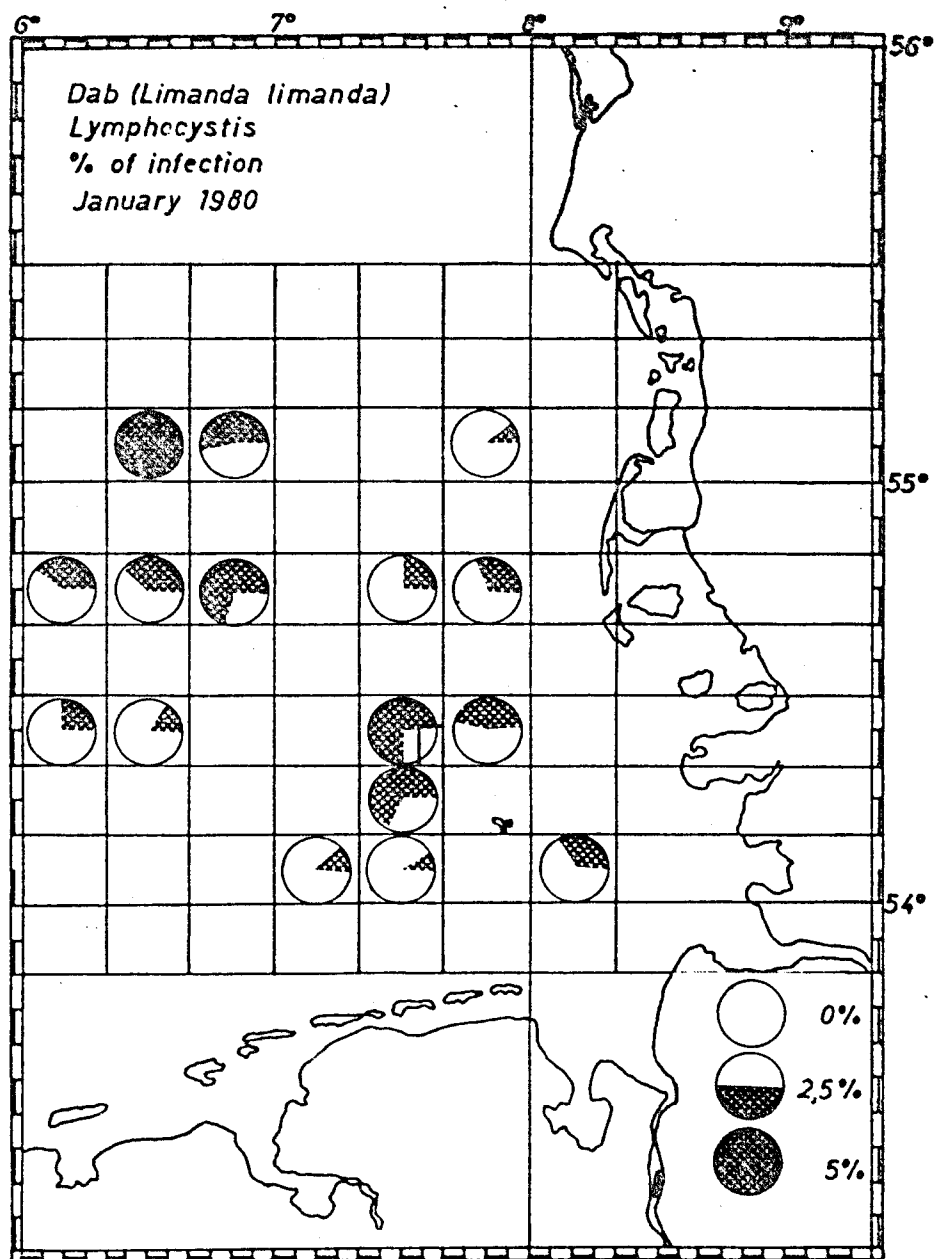


Figure 15

Percent of infection of North Sea dab with lymphocystis in January 1980.

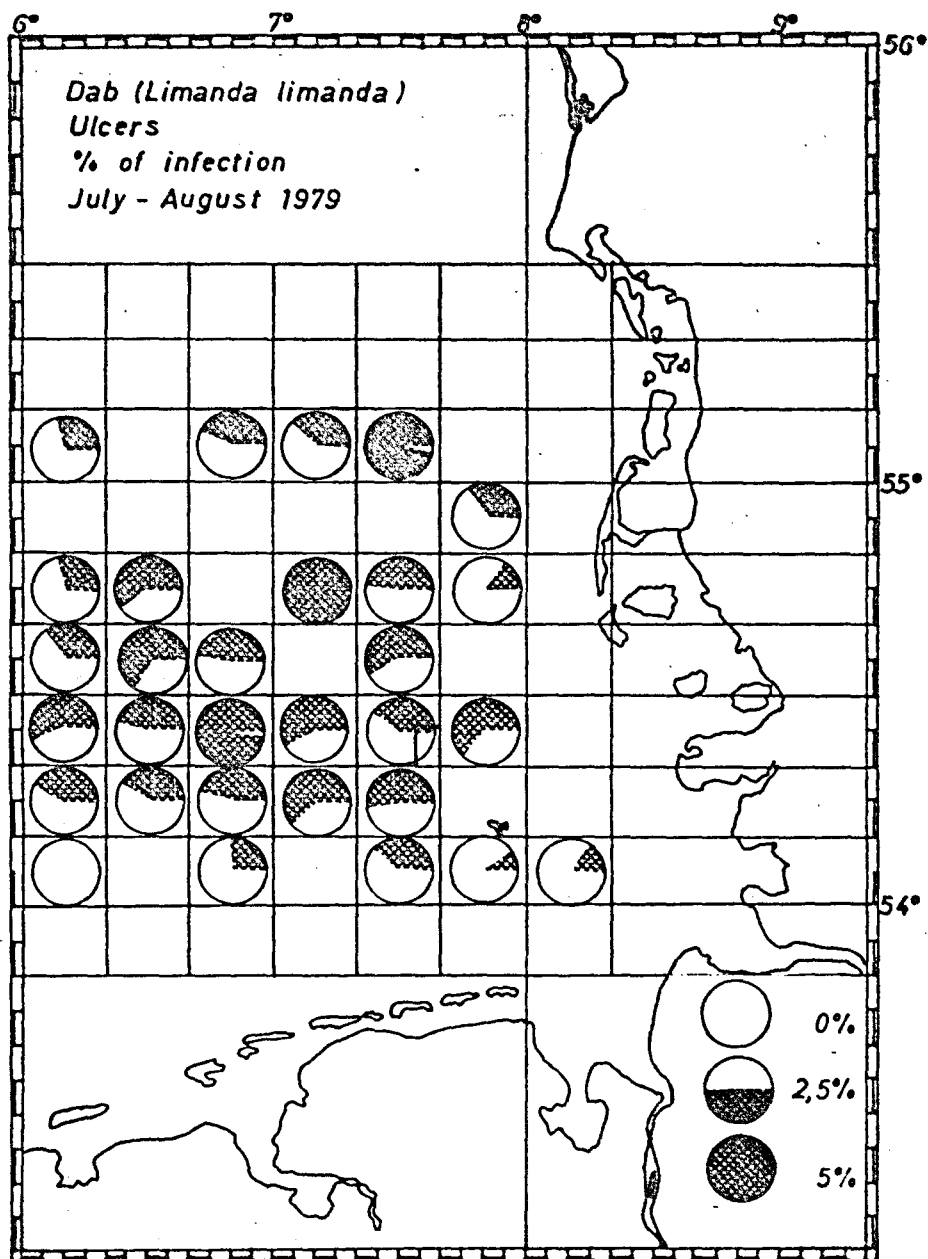


Figure 16

Percent of infection of North Sea dab with ulcers in July/August 1979.

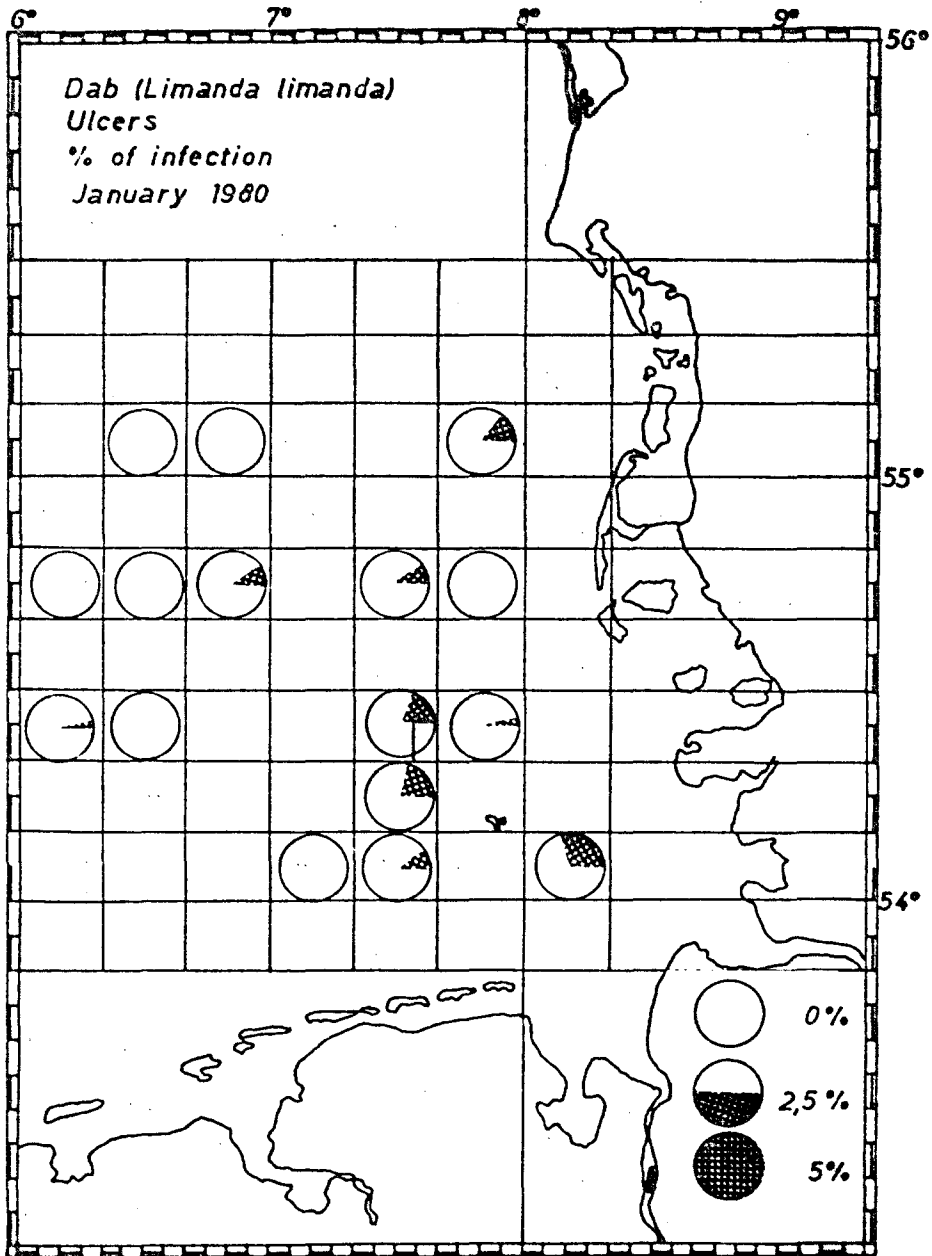


Figure 17

Percent of infection of North Sea dab with ulcers
in January 1980.