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FISH DISEASE INVESTIGATIONS IN DANISH COASTAL WATERS WITH SPECIAL REFERENCE TO THE IMPACT OF OXYGEN DEFICIENCY.

by

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Abstract:

Long-term (1983-90) recordings of data on the prevalence of the externally visible fish diseases, lymphocystis, epidermal papillomas and skin ulcers in common dab (Limanda limanda L.) populations in the German Bight, the eastern North Sea, the Skagerrak and the Kattegat are presented.

The trends in the development of these diseases and the eventual background for the development are discussed. The data indicate that most skin ulcers probably develop from mecanical lesions caused by fishing gear.

The occurrence of oxygen deficiency, seems to trigger the spread and development of fish diseases such as lymphocystis and epidermal papillomas. The risk of getting a disease is significantly higher for fish in regions that have suffered from oxygen deficiency. However, an adaptation to recurring oxygen deficiency seems to take place.

Introduction.

Since the awareness of the close relationship between environmental stress and the outbreak of fish diseases was accepted in the early 1970ies (Snieszko, 1974), a series of investigations of the impact of pollution on fish diseases in natural fish stocks have been initiated. The first considerations and results of the work in this field were summarized by McIntyre & Pearce (1980).

In the following years, a series of studies in this field have been carried out in many countries especially the north European countries and the USA (Christensen, 1980; Dethlefsen, 1980, 1984; Möller, 1981; Mellergaard & Nielsen, 1984, 1985; Vethaak, 1985). However, much of the work done in this field has been concerned with short term investigations of relatively restricted areas e.g.(McArdle et al., 1982, Bucke et al., 1983, Möller, 1984, Bucke & Nicholson, 1987).

Long term investigations of the abundance and the dynamics of fish diseases covering larger sea areas have been reported by Dethlefsen et al. (1987) who covered most of the North Sea in the period 1979-86 and by van Banning (1987) who covered the south-east part of the North Sea in the period 1981-85.

This paper deals with the results of a long term fish disease study in the eastern North Sea, the Skagerrak and the Kattegat in the period 1983-1990. In addition, information on the effect of oxygen deficiency on the abundance and epidemiology of the fish diseases lymphocystis and epidermal papillomas is presented.

Material and methods.

Common dab (Limanda limanda) were sampled annually in May on board HS "Dana" using a standard fishing trawl, Nymplex fishing trawl, Star-model. The trawl was either rigged with 12" rubber discs or with 10" bobbins on the footrope - depending on bottom conditions in the area of research - and fitted with a footrope chain. The mesh size in the codend was 40 mm. The fishing took place at a number of stations where haul tracks had been available from commercial fishermen (Fig. 1.). Standard one hour hauls were taken with a speed of 3 knots. One or two hauls were taken at each station.

The total catch was sorted into species groups and the dabs were subjected to further investigation. A sample of 150-250 specimens corresponding to 15-20 kg per haul was examined. Subsamples were taken at random if the total weight of dabs exceeded 20 kg.

For all fish examined, the length, weight and sex were registered and otoliths removed for ageing later in the laboratory. The dabs were examined for lymphocystis, epidermal papillomas and skin ulcers using recommended procedures for detection (Dethlefsen er al., 1986). Epidermal hyperplasias were registered as epidermal papillomas and only unhealed skin ulcers were registered. The examination time was 5-10 seconds per fish.

To reduce variation of the results, the registered diseases were not differentiated into different stages, only their position (pigmented or unpigmented side) were noted. In addition, in order to obtain the highest degree of precision in the diagnostics of the diseases the same person carried out the registration during the whole registration period.

For presentation, the data from the trawl stations are compiled in geographic areas which are of interest for evaluating the results. The German Bight region includes data from stations 1, 2 and 3 (station 2 suffered from oxygen deficiency in 1982 and 83)(Fig. 1.), the North Sea (Horns Reef) region include stations 5 and 101 (area hit by oxygen deficiency in 1982 and 83), the North Sea (Dead area) region stations 10 and 11 (area suffered from oxygen deficiency in 1981 and 82), the North Sea (Coastal area) region stations 6, 8, 9 and 12, the Skagerrak region includes stations 16, 17, 18 and 19, Kattegat (North) region stations 120, 121, 123, 125 and 140 and Kattegat (South) region stations 126A, 126B, 127, 130 and 133 (different levels of oxygen deficiency recorded in summer periods since 1986).

Results.

The annual distribution of fish diseases within the selected areas is illustrated in fig. 2.

German Bight.

From 1983-85, the prevalence of lymphocystis increased from 8.9%-12.3%. In 1986-87, the prevalence was reduced to ca. 8%. However, in 1988, the prevalence increased to the highest level in the investigation period, 12.8%. Since then, the prevalence has gradually decreased to 5.1% in 1990. However, none of the changes in the prevalence of lymphocystis were statistically significant. For epidermal papillomas, the prevalence increased from 1.6% in 1983 to 8% in 1986. A chi-square test of the prevalence of 1986 against 1983 gives a chi-square value of 5.64 ($p<0.05$). After a decline in 1987, the prevalence increased to 9.7% in 1988; the highest level registered in the whole investigation area during the time of investigation. In 1989, the prevalence fell to 2.9% and remained at that level in 1990. The decrease of the prevalence from 1988-89 was statistically significant, chi-square=4.11 ($p<0.05$).

From 1983-90, the prevalence of skin ulcers varied from 0.7%-1.9%. The prevalence of ulcerations did not show the same peaks as observed for lymphocystis and epidermal papillomas.

North Sea (Horns Reef).

In this area, the prevalence of lymphocystis showed only a minor increasing trend from 1983-85 from 8.2% to 9.6%. During the following two years, the lymphocystis rate dropped to ca. 3% - a statistically significant drop - chi-square=4.12 ($p<0.05$). In 1988, a statistically significant increase to 18.4% was observed - chi-square=13.05 ($p<0.001$). However, this was followed by a decline to ca. 14% in 1990.

The prevalence of epidermal papillomas increased in the first four years from zero to 3.1%. A decline was observed in 1987 to 1.5%. As for lymphocystis, the prevalence of epidermal papillomas increased significantly in 1988 and reached a level of 7.4% - chi-square=4.09 ($p<0.05$). A reduction has been observed place during the last two years.

The prevalence of skin ulcers varied from zero to 1.3% without reflecting the variations as observed for lymphocystis and epidermal papillomas.

North Sea ("Dead area").

Also in this region, an increase in lymphocystis prevalence was observed during the first three years of the investigation from 10% to 14.5%. During 1986-87, it dropped significantly to 2.9% - chi-square=8.47 ($p<0.01$). However, this was followed by an increase to 12 % in 1988 - chi-square=6.0 ($p<0.05$). In 1989-90 the prevalence was reduced to ca. 9%.

During the first four years, the epidermal papilloma rate increased from 0.7% to 3%. A drop to 1.6% was observed in 1987 followed by an increase to 4.4% in 1988. During 1989-90 the prevalence rate decreased to ca. 2%. None of these changes were statistically significant.

For skin ulcers the prevalence rate was similar to the two previous stations varying from 0.5% to 1.5%.

North Sea (Coastal area).

The lymphocystis prevalence in this area increased from 1983-85 from 4.2% to 7.9% and decreased in 1986-87 to 2.1%. In 1988, the prevalence level reached 13.3% but was reduced to ca. 8% during 1989-90. Only the change of prevalence from 1987-88 was statistically significant - chi-square=8.82 ($p<0.01$).

An increase in the prevalence of epidermal papillomas from 1.2% to 4.3% was observed from 1983-85. After a decline to 1.2% during 1986-87, a significant increase to 7.5% was observed in 1988 - chi-square=5.35 ($p<0.05$) followed by a decrease ca. 2% the following two years.

In this region also, the skin ulcer rate fluctuated from 0.3%-1.9% without reflecting the trends of the other registered diseases.

Skagerrak.

During the whole period of investigation, the prevalence of lymphocystis and epidermal papillomas was low compared to the other areas. The prevalence of lymphocystis varied from 0.3%-1.7% and the epidermal papilloma rate differed from zero to 0.5%.

The main problem at this station was skin ulcers. In the period from 1984-88 the prevalence of skin ulcers was below 1% but in 1989 it increased; chi-square=3.64 ($0.1<p<0.05$) - to 5.3% with a decline to 3.4 % in 1990.
In the Skagerrak, none of the prevalences of the observed diseases followed the trends registered at the North Sea stations.

Kattegat (North).
During the first four years of investigation, the prevalence of lymphocystis varied from year to year from 1.4% as minimum to 3.9% as maximum level. However, from 1988, an increasing trend has been observed - 6.4% in 1988 and 9% in 1990.
In the period from 1984-89, the prevalence of epidermal papillomas fluctuated from 1%-2%. However, in 1990, an increase to 3.8% was observed.
From 1984-90, the prevalence of skin ulcers ranged from 0.2% to 0.9%. None of the changes in the disease levels in this region were statistically significant.

Kattegat (South).

From 1984-86, the prevalence of lymphocystis ranged from 3.3 to 4.3%. In 1987, an increasing trend in the prevalence rate began starting with 7% in 1987 and ending with 14.1% in 1989. The increase from 1986-88 was significantly significant - chi-square=6.12 ($p<0.05$). A decrease to 10.1% was observed in 1990.
The prevalence of epidermal papillomas varied from 0.6 in 1984 to 1.4% in 1986. In 1987 and 1988, the prevalence was approximately 2.5%. Since 1989, the prevalence has been 3.2%.
The prevalence of skin ulcers has remained relatively stable varying from zero to 0.5%.

Impact of oxygen deficiency on the development of diseases.

The Kattegat (South) area was exposed to oxygen deficiency in the middle of the investigation period, in late summer 1986. Since then, it has been an annually recurring problem, although, with different degrees of intensity. Thus, the data from this area will be used to elucidate a possible effect of oxygen deficiency on the spread of fish diseases.

The prevalence and incidence rates of lymphocystis in the period from 1984-1990 are illustrated in fig. 3. The prevalence rate started to rise in 1987 but the most prominent increase took place from 1987-88 i.e. two years after the occurrence of oxygen deficiency. After that period, the prevalence stabilized and decreased in 1990. The incidence rate reflect the changes in the prevalence.

Figure 4 illustrates the prevalence and incidence rates of epidermal papillomas. Although showing an increase from 1986-87, the prevalence differs from the trend for lymphocystis by stabilizing in 1988 and not showing a drop in 1990. The incidence curve reflects the changes in the prevalence.

The dab population in this area may be divided into groups as follows:

	Disease		
	Present	Absent	Total
Exposed to oxygen def.	a	b	a+b
Not exposed	c	d	c+d
Total	a+c	b+d	

The ratio of incidence rates or relative risk would be:

$$\frac{a/(a+b)}{c/(c+d)}$$

If this ratio is ≤1, no association exists between the assumed factor and disease; the higher the figure - the closer association.

In the following calculations, c is given as the mean value of the data from 1984-86 i.e. before the oxygen deficiency.

<u>Relative risk:</u>

	84-86/87	87/88	84-86/88
All diseases*	1.9	1.8	3.4
Lymphocystis	1.9	2.0	3.8
Epidermal papillomas	2.8	0.9	2.6

* Combination of lymphocystis, epidermal papillomas and skin ulcers.

Testing the data for eventual association between oxygen deficiency and fish diseases gives the following chi-square values.

Chi-square:

	84-86/87	87/88	84-86/88
All diseases	1.44	2.37	7.01** ($p<0.01$)
Lymphocystis	1.07	2.74	6.27* ($p<0.05$)
Epidermal papillomas	1.8	0.01	0.64

Chi= 3.84 ($p<0.05$); Chi=6.61 ($p<0.01$)

Using these chriteria, there seems to be a statistically significant association between oxygen deficiency and the presence of fish diseases (i.e., when comparing the disease rates before and two years after the oxygen deficiency took place ($p<0.01$) and also between the occurrence of oxygen deficiency and lymphocystis ($p<0.05$)).

In fig. 5, the annual age distribution of lymphocystis infected fish is presented from 1984-89. In order to eliminate an eventual year to year variation in the age composition of the total dab population, the figures presented represent the mean age composition during the period 1984-89. Furthermore, the lymphocystis and epidermal papilloma data 1984-86 represent the mean composition in this period.

The age composition of lymphocystis infected fish in 1984-86 represents the composition of an "unstressed" population where the main proportion of diseased fish is 4-5 years old. The effect of oxygen deficiency reflected in the 1987 data is a serious spread of lymphocystis to the younger year classes. In the following years (1988 and 1989), the age of the main proportion of infected fish is 3+ and 4+.

Similar results for epidermal papillomas are presented in fig. 6. Although the results for epidermal papillomas are based on a more restricted number of data compared with the lymphocystis figures, it reflects the age distribution pattern of lymphocystis. Also here, the epidermal papillomas have spread to the young age groups in 1987 and a high proportion of young infected fish is seen in 1988. The age distribution for 1989 tends to approach the normal condition.

Discussion.

In addition to the Kattegat (South) region, oxygen deficiency has occurred in other of the areas involved in this investigation (Mellergaard & Nielsen, 1987). Oxygen deficiency has been reported in the North Sea (Dead area) in 1981 (Dyer et al., 1983), in the German Bight and North Sea (Horns Reef) in 1982 and 1983 (Dethlefsen & Westernhagen, 1983; Westernhagen et al., 1986).

The results from these areas reveal common features. The prevalence of lymphocystis increased until 1985 after which it decreased in the following two years during which it - for the North Sea (Horns Reef) and the North Sea (Dead area) region - almost reached the "background" level as observed in the Kattegat before the occurrence of oxygen deficiency. For the epidermal papillomas, the increase of the prevalence continued until 1986 before a decrease was seen. Thus the regression of epidermal papillomas seems to react more slowly than lymphocystis, when the environmental conditions have stabilized. Until 1989, the prevalence of epidermal papillomas remained at a much higher level in the German Bight than in the other areas of the North Sea. Hence, the spread of this disease in the German Bight seems to be potentiated by other factors - possibly substances within the pollutants dumped in the area - besides oxygen deficiency.

The North Sea (Coastal area) region has not suffered from recorded oxygen deficiency. However, its location close to the afflicted areas makes it a kind of "drainage" area for escaping fish. This may be the explanation for the increase in the prevalence of both lymphocystis and epidermal papillomas observed until 1985. In contrast to the other North Sea areas, the prevalence of both diseases decreased in the following two years. In 1988, an increase in the prevalence of both lymphocystis and epidermal papillomas took place in all North Sea areas including the German Bight. For most regions, the increase - up to five times the levels registered the previous two years - was statistically significant. At present, there is no explanation of this phenomenon as an eventual occurrence of oxygen deficiency has not been reported in these areas. Furthermore, an increasing trend in the disease rates as observed following oxygen deficiency has not been seen.

Hydrographically, the Skagerrak differs from the North Sea and Kattegat stations in that the waters found there originate, as a rule, from "offshore" areas and are thus, less influenced by river inputs and other near-coast activities. In this respect, the Skagerrak can be regarded as an "unspoiled" area - a fact which is reflected in the prevalence of fish diseases. The frequency of both lymphocystis and epidermal papillomas is very low.

However, in the Skagerrak the prevalence of skin ulcers was at the same level as observed at the North Sea stations until 1988 after which an appearent increase has taken place. At the North Sea stations, the presence of skin ulcers did not show the fluctuations observed for lymphocystis and epidermal papillomas. This fact indicates that skin ulcers are more or less unaffected by stressors such as oxygen deficiency. The only common factor that might cause skin damage in these areas seems to be the fishery activity. Furthermore, most of the skin ulcers observed during these investigations appear to be excoriations with loss if the superficial dermal layers. Thus we regard skin ulcers in dab in the open sea areas as primarily being the result of mechanical skin damage caused by fishing gears followed by an infection with potential pathogenic bacteria. The increase in skin ulcers observed in the last two years in the Skagerrak may be related to an increase in fishing effort in this area.

In the Kattegat, the disease rate was relatively stable during the first three years of the investigation. After the occurrence of oxygen deficiency in the southern Kattegat in 1986, an increase of the frequency of lymphocystis and epidermal papillomas was observed in this area from 1987 until this year. In the region Kattegat (North) an increase in the disease rate - at a lower level than in the Kattegat (South) - was observed from 1988. This may be due to the same "drainage" effect as hypothesized in the North Sea (Coastal) area.

The impact of oxygen deficiency results in an increase in the prevalence of lymphocystis. This increase was especially pronounced the second year after the first onset of oxygen deficiency. This observation coincides with the fact that there is a significantly higher risk of getting lymphocystis when

exposed to oxygen deficiency.
The first time oxygen deficiency occurs in an area it seems especially to trigger the outbreak of diseases in young fish. This is observed for both lymphocystis and epidermal papillomas. However, as oxygen deficiency has been an annually recurring event in the southern Kattegat since 1986 - although with varying degree of intensity from year to year - an adaptation seems to take place. The intensity of the oxygen deficiency in 1988 was greater than in 1986 but the age distribution of diseased fish recorded in 1989 seems to approach normal conditions. This may also explain the decrease in the disease rate observed in 1990. The observed variations in the age composition of diseased fish were not due to significant variations in the age distribution of the total fish stock the years in question.
When talking about oxygen deficiency as a factor triggering the outbreak of fish diseases, it has to be borne in mind that oxygen deficiency, in addition to the direct impact on the fish, also affects the bottom fauna. This may result in changes in the species composition or direct suffocation of the bottom fauna which, in the end, may result in starvation of the fish and in that way cause additional stress.
The general trends of the development of lymphocystis, epidermal papillomas and skin ulcers in the German Bight and the eastern North Sea correspond to the findings reported by Dethlefsen et al. (1987). The observations of van Banning (1987) generally showed lower prevalence of lymphocystis than those found in the present investigation. The Dutch disease data which is collected from February-April ought to be comparable with data collected in May (Wolthaus, 1984). Therefore, these differences may be due to different criteria in the choice of stations. Van Banning (1987) only observed increasing trends for epidermal papillomas. The duration of the present study and the choice of station pattern made it possible to observe clear trends - both increasing and decreasing - of lymphocystis and epidermal papillomas. Although not always statistically significant, these trends give important information about the disease situation in the different regions investigated eapecially when the observed trends are common for many regions.
Long term disease recordings like the present study make it

possible to establish a base line level and to investigate the impact of different environmental stress factors on the dynamics of fish diseases.

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Fig. 1. Map showing the position of the trawl stations.

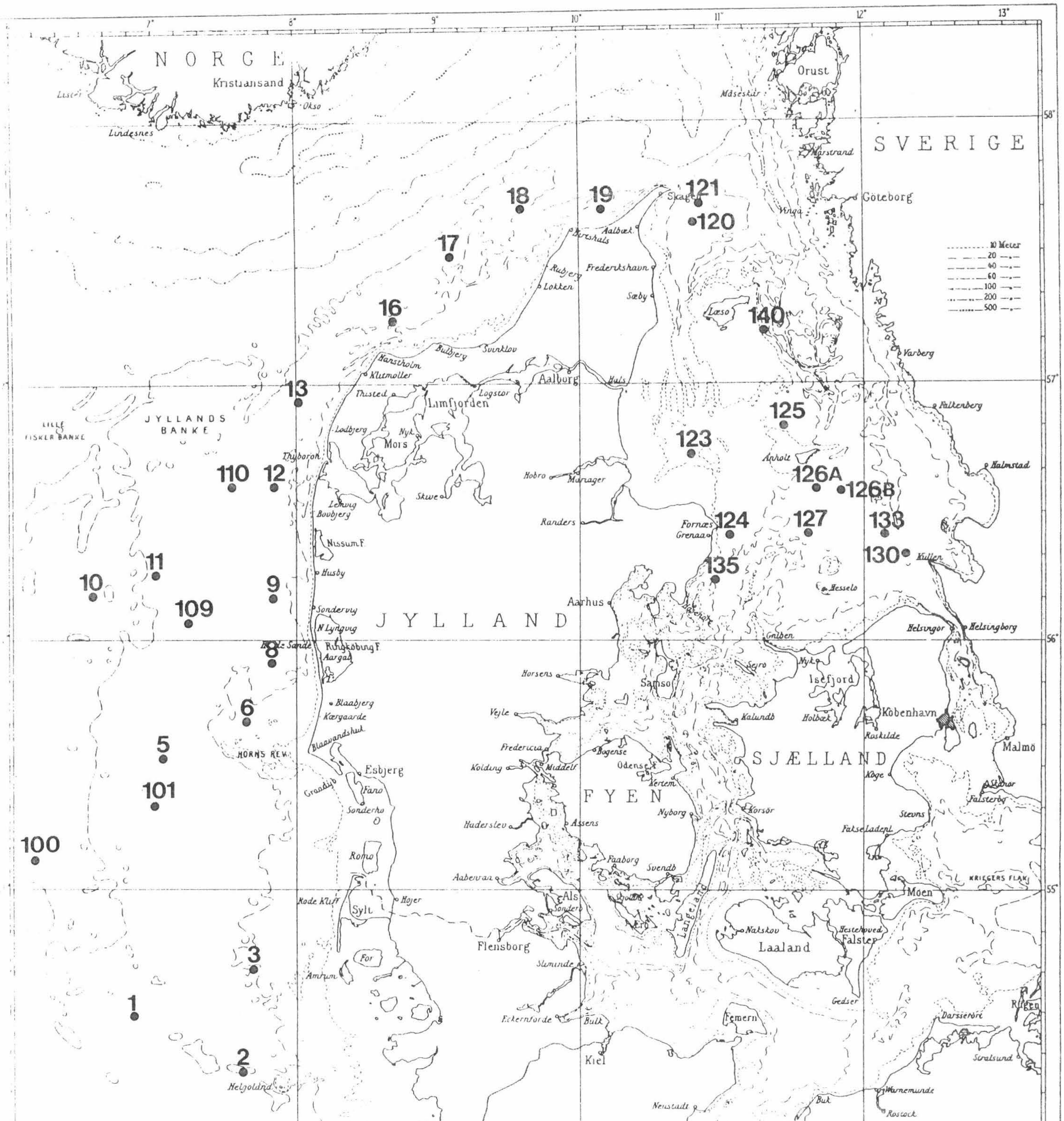
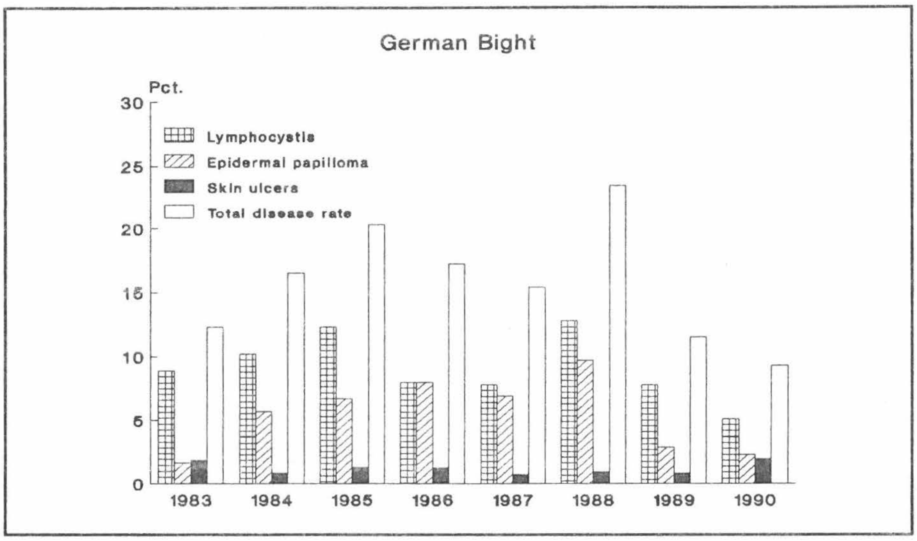


Fig. 2. Disease rates at different areas of research.



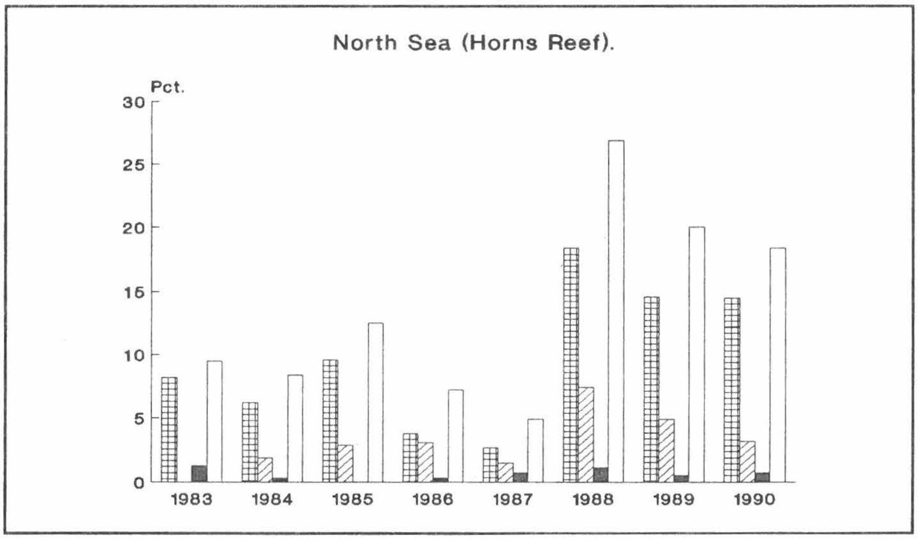
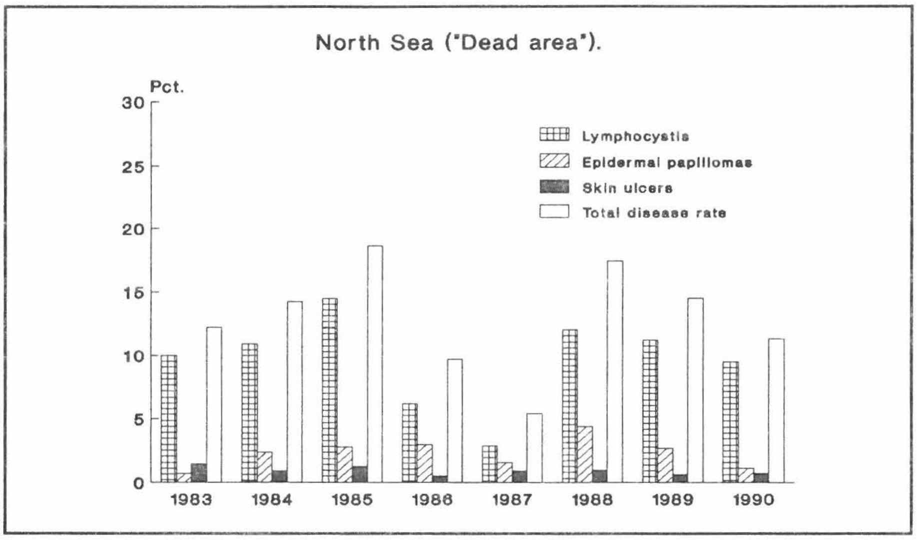
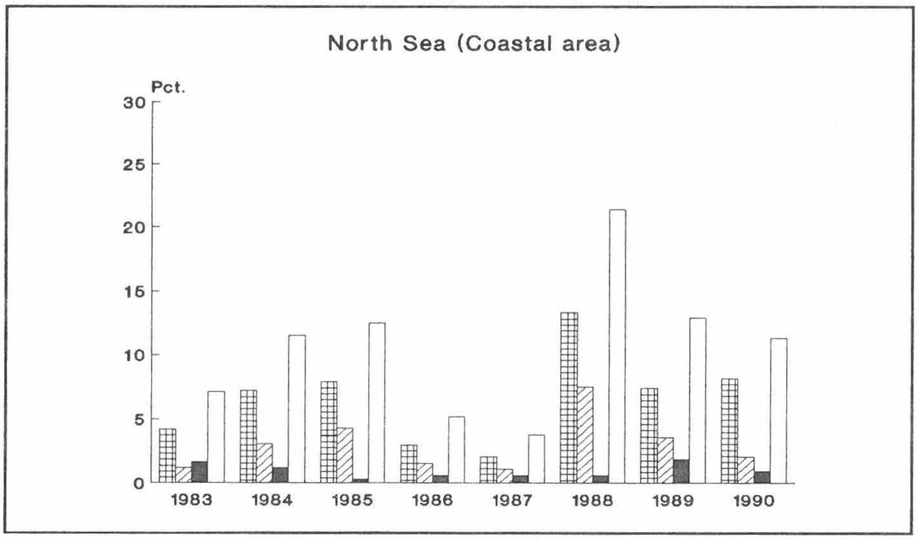


Fig. 2. (continued).





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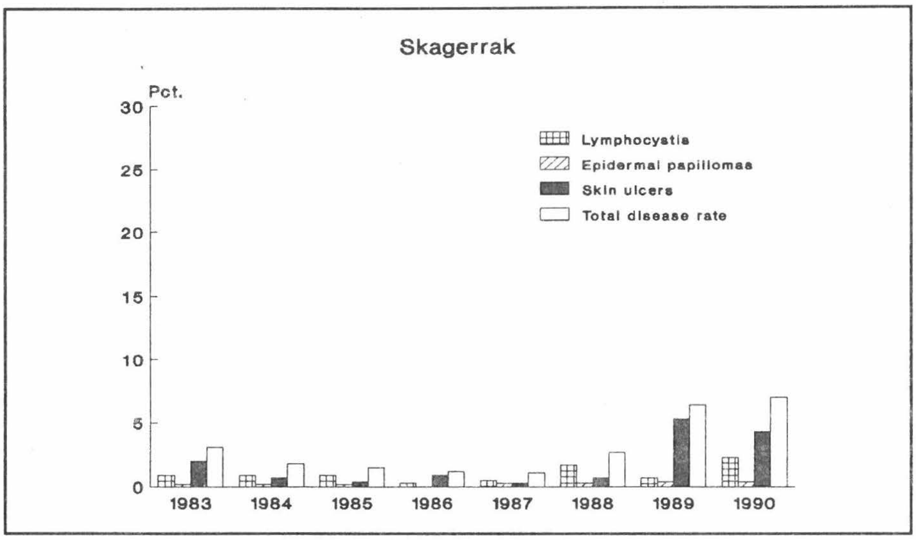

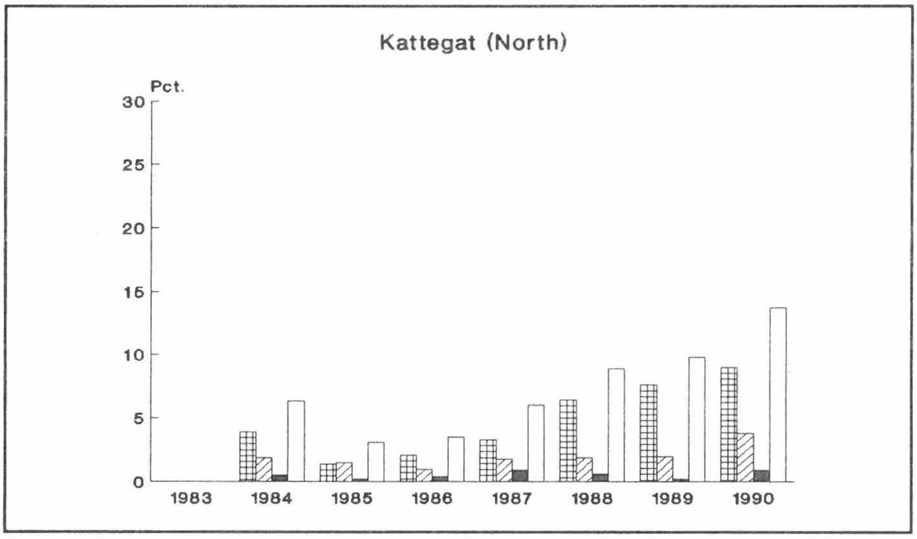

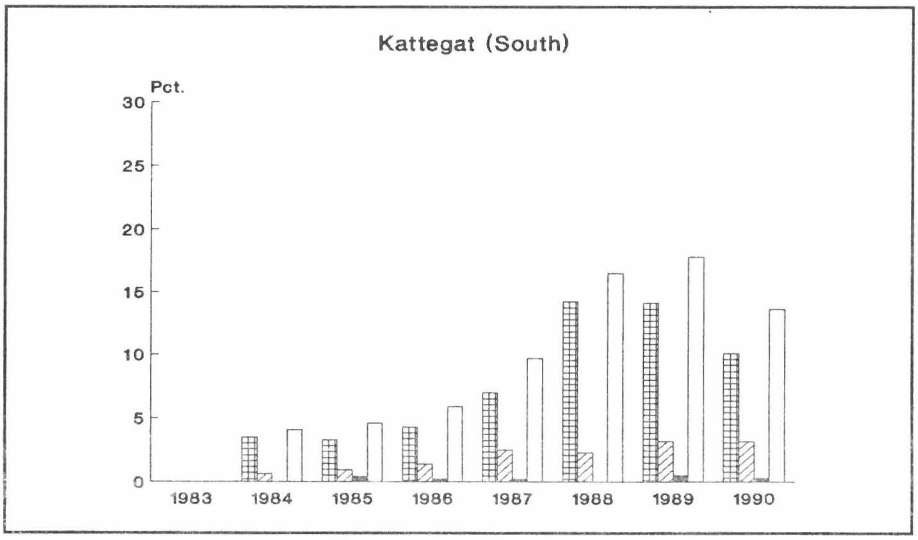

Fig. 3. Prevalence and incidence rates of lymphocystis in Kattegat (South).

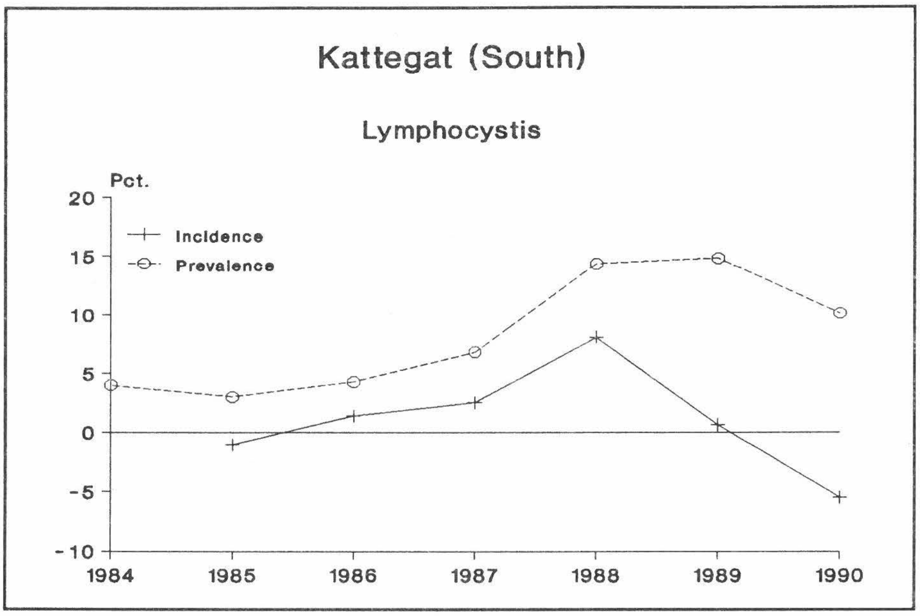

Fig. 4. Prevalence and incidence rates of epidermal papillomas in Kattegat (South).

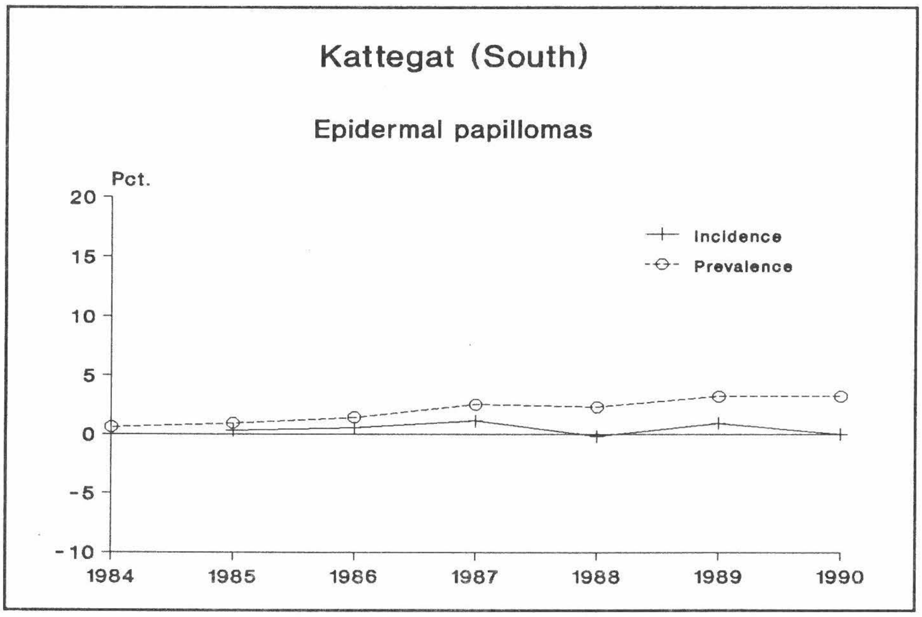

Fig. 5. Age distribution of lymphocystis infected fish in Kattegat (South).

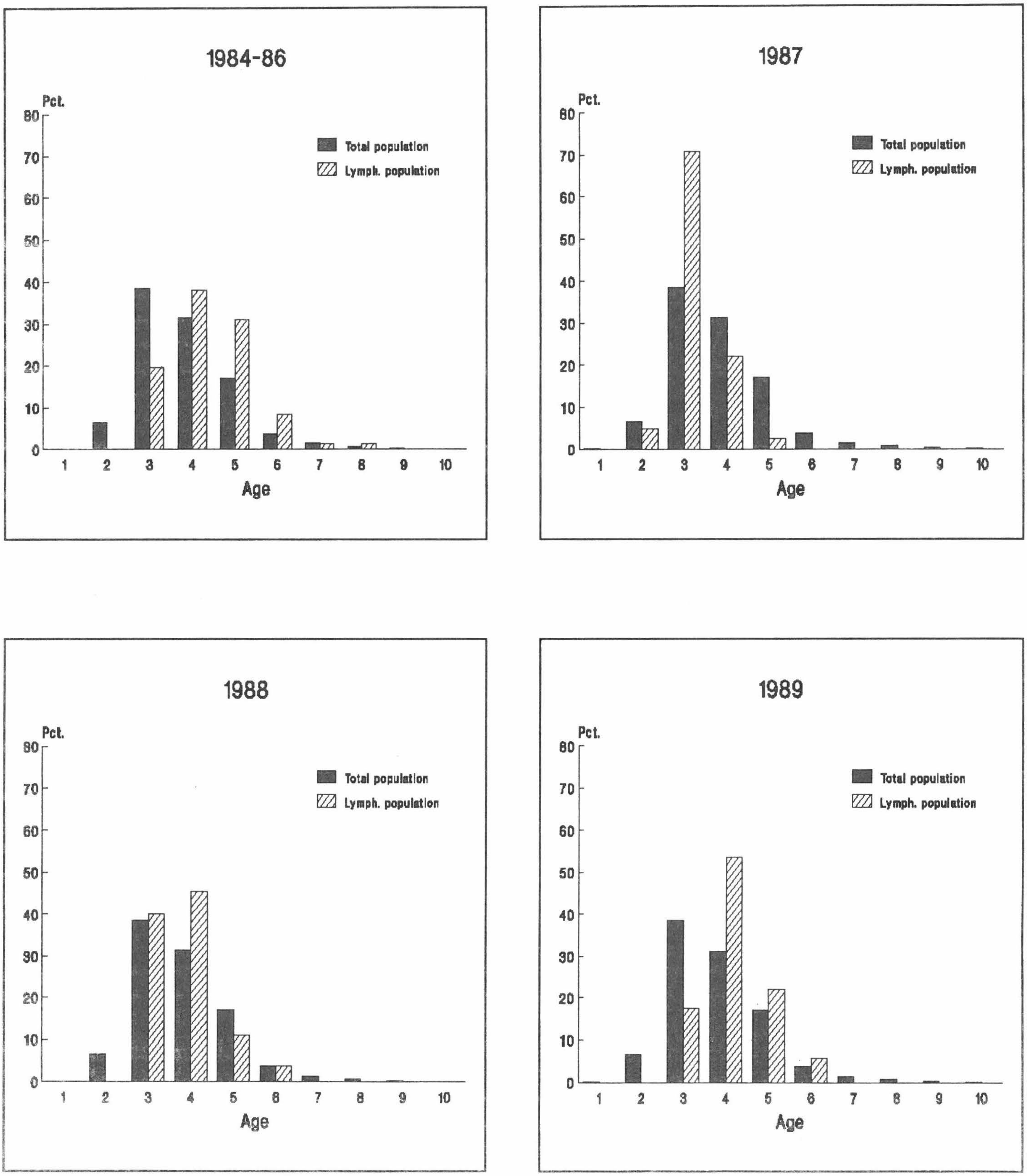

Fig. 6. Age distribution of epidermal papilloma infected fish in Kattegat (South).