Investigations of fish diseases in common dab (*Limanda limanda*) in Danish Waters

by

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Index

1 Preface 1

2 Summary 2

3 Summary in Danish 4

4 List of papers included 6

5 Introduction 7

6 Stress 7

6.1 Effect of stress 8

7 Biological stress factors 9

7.1 Predation 9

7.2 Pathogens 9

7.3 Nutrition 11

7.4 Algal toxins 13

7.5 Population density 14

7.6 Age 15

8 Physical-chemical stress factors 17

8.1 Temperature 17

8.2 Supersaturation 19

8.3 Salinity 20

8.4 Pollution 20

8.5 Oxygen deficiency 25

8.6 Fishing activity 27

9 The common dab 30

9.1 Habitat 30

9.2 Growth 30

9.3 Age composition 30

9.4 Spawning 31

9.5 Larval development 31

10 Diseases of importance for this study 32

10.1 Lymphocystis 32

10.1.1 Agent 32

10.1.2 Symptoms 32
10.1.3 Effect on host 32
10.1.4 Response to stress 34
10.2 Epidermal papilloma 34
10.2.1 Agent 35
10.2.2 Symptoms 35
10.2.3 Effect on host 35
10.2.4 Response to stress 36
10.3 Skin ulcers 37
10.3.1 Agent 37
10.3.2 Symptoms 37
10.3.3 Effect on host 38
10.3.4 Response to stress 38
10.4 X-cell gill disease 38
10.4.1 Agent 39
10.4.2 Symptoms 39
10.4.3 Effect on host 39
10.4.4 Response to stress 40
11 Final comments 42
12 Main conclusions 43
13 Literature cited 44
14 Paper 1. Adenovirus-like particles associated with epithelial hyperplasias in dab, *Limanda limanda* (L.) 53
15 Paper 2. Impact of oxygen deficiency on the disease status of common dab *Limanda limanda* 59
16 Paper 3. Epidemiology of X-cell gill disease in common dab *Limanda limanda* 75
17 Paper 4. The epidemiology of lymphocystis, epidermal papilloma and skin ulcers in common dab *Limanda limanda* along the west coast of Denmark 87

1. Preface

The present work was initiated to study the epidemiology of some fish diseases which have been used for monitoring the impact of environmental stress on the health condition of fish. Common dab and plaice were chosen as target species, and the results presented in this Ph.D.-thesis are focussed on common dab.

The study took place over a period of 11 years which made it possible to study the long-term fluctuations of the disease rates and by chance made it possible to study the impact of oxygen deficiency on the health condition of dab in the Kattegat and the North Sea.

The planning and implementation of the project were made in close collaboration with my good colleague and friend Else Nielsen who I wish to thank her for many constructive and enjoyable hours spent on planning and during our many "sea battles" onboard RV "DANA".

I am grateful for the excellent technical assistance carried out by Inger Hornum during the whole project. Furthermore, I wish to thank the large number of colleagues who have participated in the many cruises at sea, both for their assistance during the often long working hours and also for their very joyful company during our leisure time.

An important factor in a project like this has been the facility provided onboard RV "DANA". I wish to thank the crew for their interest in the project and for always doing their best to ensure that we got our samples under all weather conditions. I am also very grateful for their joyful company during the fish processing after a long day of work and during other leisure activities, for example by participating in my culinary experiments in the galley.

I also wish to express my gratitude to my dear chief Katherine Richardson and my supervisor professor Jens Laurits Larsen, the Royal Veterinary and Agricultural University, for encouraging me to write this Ph.D.-thesis and for commenting and making linguistic corrections to the manuscript.

Furthermore, I wish to thank the staff of the library at the "Castle" for fulfilling my continous demand of literature, and not forgetting my good colleagues at the Fish Disease Laboratory for listening to my complaints and problems during my work with the thesis.

The Danish Research Academy is acknowledged for funding the fee to the Royal Veterinary and Agricultural University for my one year PhD-study.

Finally I wish to thank my family and my cat for always expressing their pleasure when I arrived from my cruises at sea and for eating the fish I brought home with a minimum of complaints.

Herlev, August 1996
Stig Mellergaard

2. Summary

The objective of the presented investigation has been to study the epidemiology of some diseases which have been used for monitoring the impact of environmental stress on the health condition of common dab.

An introduction of some of the most important environmental stress factors that may trigger the outbreak of diseases in fish stocks is presented. The presentation is based on a series of examples from the literature with the addition of the contributions of the present study.

Investigation of the occurrence of the fish diseases, lymphocystis, epidermal hyperplasia /papilloma, skin ulcers and X-cell gill disease, in common dab has taken place in the German Bight, the eastern part of the North Sea, the Skagerrak and the Kattegat, throughout the period 1983 to 1993. Lymphocystis and epidermal hyperplasia/papilloma are viral associated diseases, skin ulcers are associated with bacterial infections, and X-cell gill disease is suggested being an amoebal infestation. The sampling was carried out annually in May onboard the RV "DANA".

One of the first results of this investigation was the demonstration of adenovirus-like particles in epidermal hyperplasia of dab. This was the first report suggesting a viral aetiology of epidermal hyperplasia and papilloma.

A part of the German Bight and the eastern North Sea were affected by oxygen deficiency during autumn in 1981 to 1983. In these areas, the prevalence of lymphocystis and epidermal hyperplasia/papilloma increased from 1983 to 1985. In the Kattegat, the prevalence of these two diseases displayed minimal variation from 1984 to 1986 subsequently followed by a significant increase the following two years. This increase coincided with the occurrence of oxygen deficiency in the area of investigation during late summer 1986 to 1988. The temporal trend of the development of the two virus-associated diseases, lymphocystis and epidermal hyperplasia/papilloma followed an almost identical pattern in the areas having suffered from oxygen deficiency. In contrast, the prevalence of skin ulcerations only displayed minor variation without any specific trend.

Skin ulcerations often originated primarily from traumatic skin lesions which might be secondarily infected by bacteria. The fact that the infectious matter is not the primary agent in skin ulcers may explain why these do not display the same response to oxygen deficiency as observed for lymphocystis and epidermal hyperplasia/papilloma.

In the Skagerrak, where oxygen deficiency was not observed, the prevalence of lymphocystis and epidermal hyperplasia/papilloma was low and only displayed minor variation. In contrast, the prevalence of skin ulcers showed an increase in 1989 and 1990. This may be attributed to an increasing fishing effort in the area an activity that may enhance the risk fish of getting skin lesions.

X-cell gill disease in dab seems to follow another pattern of development than observed for lymphocystis and epidermal hyperplasia/papilloma. This disease is widely distributed in very low prevalence and is not affected by oxygen deficiency. However, X-cell gill disease was observed in high rates in three distinct areas, two in the North Sea and one in the Skagerrak. On one occasion it was possible to separate the highly affected dab population from a significantly less affected population within a distance of 500 m. The gill lesion probably debilitates the fish to a degree where they are unable to travel over longer distances due to respiratory constraint. X-cell gill disease is probably lethal to dab.

This investigation has shown that oxygen deficiency may impair the resistance of affected dab stocks so that the prevalence of certain diseases of viral aetiology as lymphocystis and epidermal hyperplasia/papilloma increases. When the environmental stress ceases, the disease rate is gradually reduced to the background level. However, the outbreak of diseases of another aetiological background as skin ulcers and X-cell gill disease was not triggered by oxygen deficiency but may be attributed to other mechanisms of regulation.

3. Summary in Danish

Formålet med denne undersøgelse har været at få kendskab til epidemiologien af en række sygdomme, som har været benyttet til monitering af effekten af miljø-stress på helbredstilstanden hos ising.

Der gives indledningsvis en gennemgang af en række forskellige faktorer, som vides at kunne forårsage "miljø-stress", og dermed være i stand til at udløse forskellige sygdomstilstande hos fisk. Effekten af disse faktorer bliver belyst ved en række eksempler fra den foreliggende litteratur, herunder resultater opnået gennem dette projekt.

I perioden 1983-93 er der blevet gennemført undersøgelser af forekomsten af sygdommene lymphocystis, epitelhyperplasier/papillomer, sårdannelser og "X-cell gill disease" hos ising i området fra Tyske Bugt, langs den jyske vestkyst, samt i Skagerrak og Kattegat. Lymphocystis og epitelhyperplasier/papillomer er sygdomme af viral oprindelse, sårdannelser er ofte bakterielt associeret, og årsagen til "X-cell gill disease" er ukendt (muligvis forårsaget af en amøbe). Undersøgelserne blev gennemført med havundersøgelsesskibet DANA hvert år i maj måned.

Et af de første resultater af undersøgelserne var påvisning af adenovirus-lignende partikler i forbindelse med epitelhyperplasier og papillomer hos ising. Ætiologien af disse hudforandringer var ikke kendt tidligere.

En del af undersøgelsesområderne i Tyske Bugt og langs den jyske vestkyst havde været udsat for iltmangel i perioder i efteråret i 1981-1983. Undersøgelserne viste en stigning i prævalensen af sygdommene lymphocystis og epitelhyperplasier/papillomer i årene 1983-1985 i de områder, som havde været udsat for iltmangel. I Kattegat udviste prævalensen af disse to sygdomme minimale svingninger i perioden fra 1984-1986, hvorefter der blev registreret en kraftig stigning i de efterfølgende 2 år. Denne stigning faldt sammen med, at området blev udsat for iltmangel i sensomrene 1986-1988. Efter at perioderne med iltmangel ophørte, faldt forekomsten af disse sygdomme gradvist til niveauet observeret i 1984-86.

Det tidsmæssige udviklingsforløb for de to virus-inducerede sygdomme, lymphocystis og epitelhyperplasier/papillomer fulgte et næsten identisk mønster i alle de områder, der havde været udsat for iltmangel. Derimod udviste prævalensen af sårdannelser kun mindre svingninger uden nogen form for mønster.

Sårdannelser har for en stor dels vedkommende sin baggrund i primære traumatiske skader, som

sekundært bliver inficeret med bakterier. Det forhold, at smitstoffet ikke er det primære agens, forklarer sandsynligvis, at der ikke ses tilsvarende stigninger i forekomsten af sår, som ved lymphocystis og epitelhyperplasier/papillomer, i forbindelse med iltmangel.

I Skagerrak, hvor der ikke har været registreret iltmangel, var forekomsten af lymphocystis og epitelhyperplasier/papillomer lav og udviste kun mindre udsving i hele undersøgelsesperioden. Derimod blev der iagttaget en stigning i forekomsten af sårdannelser i 1989 og 1990, som muligvis kunne tilskrives et øget fiskeri i området, og dermed en øget risiko for at fiskene kunne få hudlæsioner.

"X-cell gill disease" hos ising synes at følge et anderledes epidemiologisk udviklingsmønster end lymphocystis og epitelhyperplasier/papillomer. Denne sygdom fandtes udbredt i hele undersøgelsesområdet med meget lav frekvens, og forekomsten syntes ikke påvirket af iltmangel.

"X-cell gill disease" blev observeret i høj frekvens på 3 lokaliteter, 2 områder langs den jyske vestkyst og 1 område i Skagerrak. I et af disse områder fandtes afgrænsningen mellem den syge og raske isingepopulation at ligge indenfor 500 m. Dette skyldes sandsynligvis, at sygdommen svækker de afficerede fisk i en sådan grad, at de ikke er i stand til at bevæge sig over større afstande. Sygdommen forløber sandsynligvis dødeligt hos ising.

Nærværende undersøgelse har vist, at iltmangel kan svække berørte isingebestande, således at modtageligheden og dermed forekomsten af sygdomme med viral baggrund, såsom lymphocystis og epitelhyperplasier/papillomer, øges. Når miljø-stressset ophører falder sygdomsfrekvensen gradvist til udgangsniveauet. Derimod synes sygdomme med anden ætiologisk baggrund, såsom sårdannelser og "X-cell gill disease" ikke at blive udløst af iltmangel, men må være underlagt andre regulationsmekanismer.

4. List of papers included

1. **Bloch, B., Mellergaard, S. & Nielsen, E.** 1986. Adenovirus-like particles associated with epithelial hyperplasias in dab, *Limanda limanda* (L.). Journal of Fish Diseases, 9; 281-285.

2. **Mellergaard, S. & Nielsen, E.** 1995. Impact of oxygen deficiency on the disease status of common dab, *Limanda limanda*. Diseases of Aquatic Organisms, 22; 101-114.

3. **Mellergaard, S. & Nielsen, E.** 1996. Epidemiology of X-cell gill disease in common dab *Limanda limanda*. Diseases of Aquatic Organisms, 25; 107-116.

4. **Mellergaard, S. & Nielsen, E.** The epidemiology of lymphocystis, epidermal papilloma and skin ulcers in common dab (*Limanda limanda*) along the west coast of Denmark. Diseases of Aquatic Organisms, *Submitted*

5. Introduction

The aquatic environment - probably the most suitable substrate for life - contains a broad spectrum of potential pathogenic organisms. Some of them are, under special circumstances, able to cause disease in fish. Fish will constantly be exposed to an infection pressure from these organisms. In order to meet this pressure fish have different protective measures such as a mucus layer, containing free fatty acids (Lewis, 1971), lysozymes (Fletcher & White, 1973), proteases (Hjelmeland *et al.* 1983) and, for some fish species, immunoglobulins (Fletcher & Grant, 1969), which form a protective film on the surface of their skin and an immunesystem which may catch and destroy "intruders".

The outbreak of diseases in fish populations, as in other animal populations, is not the mere result of the presence of an infectious agent. Rather, it is the result of a combination of a series of extrinsic factors which, in the presence of an infectious agent, may lead to disease. These extrinsic factors are also characterised as "environmental stress-factors".

Under normal circumstances there exists a stable ecological equilibrium between the fish, its environment and its pathogens and the fish remains healthy (Fig. 5.1). If this equilibrium comes out of balance either by changes in the environment or by changes in the virulence or in the abundance of the pathogen, the fish will become stressed and, in this way, be atrisk of developing disease.

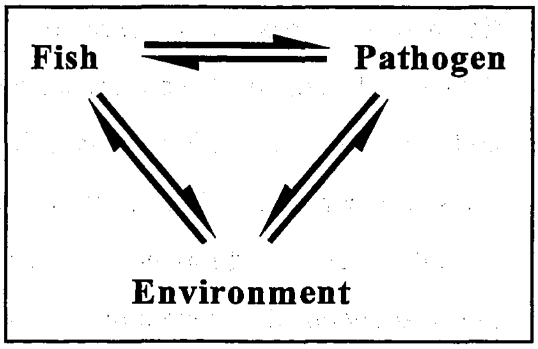

Fig. 5.1. Changes in this equilibrium may lead to disease.

6. Stress

Stress may be defined as the sum of all the physiological responses by which an organism tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force (Selye, 1950). The morphological, biochemical and physiological changes which occur as a result of stress constitute the General Adaptation Syndrome which is divided into 3 stages: **the stage of alarm - the stage of adaptation - the stage of exhaustion**.

6.1. Effect of stress

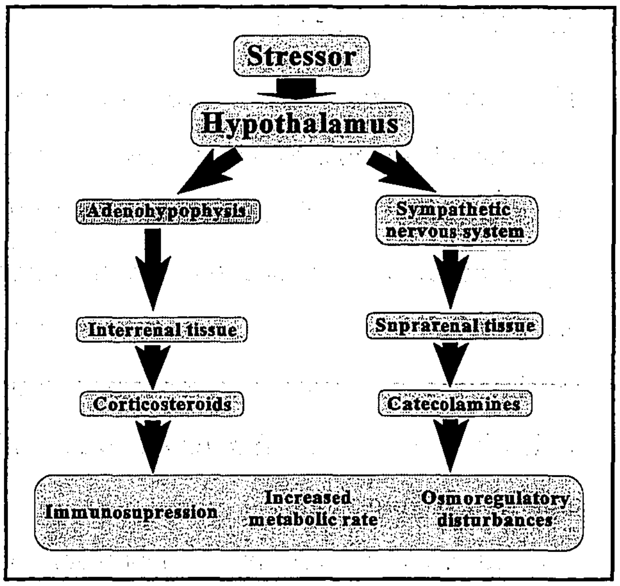

Fig 6.1. Pathways of the stress responce. Modified from Mazeaud et al.(1977) and Wedemeyer (1970)

The actual sequence of physiological and biochemical alterations initiated by stress is illustrated in Fig. 6.1. Via neurotrophic stimuli from the hypothalamus, the adenohypophysis releases adenocorticotrophic hormone (ACTH) which mediates the release of corticosteroids ("stress hormones") from the interrenal tissue (fish adrenal glands). At the same time, the hypothalamus stimulates the sympathetic nervous system to release adrenaline. The corticosteroids act on the immune-system by reducing the inflammatory response, the fagocytosis and the immunoglobulin synthesis. Corticosteroids in combination with adrenaline induce disturbances in the osmoregulation and an increase in the metabolic rate.

All these physiological and biochemical changes may facilitate the colonization, the penetration and spread of pathogenic organisms into the fish.

Stress is a consequence of variation from optimum conditions for any environmental stressor - natural as well as anthropogenic factors. Fig. 6.2 illustrates the principal factors, biological and physical-chemical, involved in environmental stress in aquatic organisms.

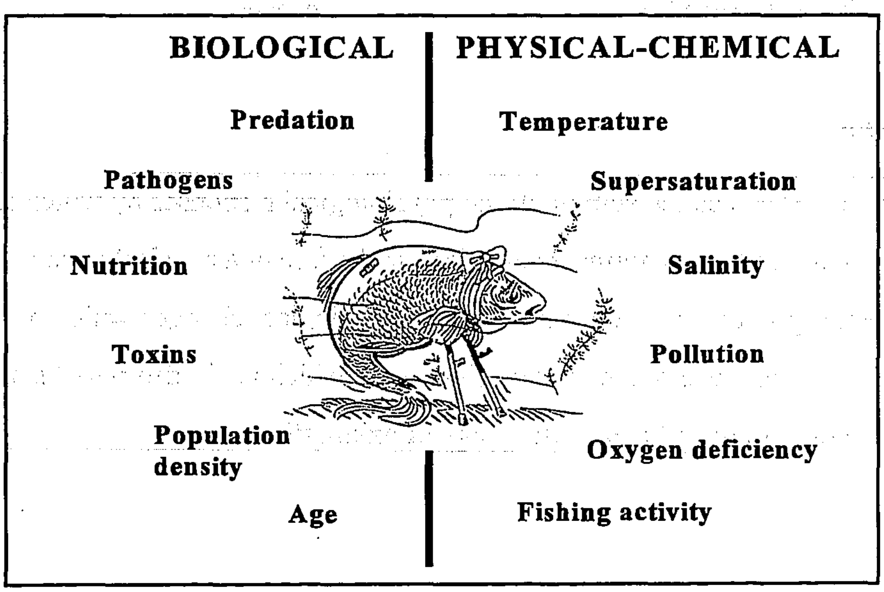

Fig. 6.2. The principal sources of environmental stress for marine organisms

7. Biological stress factors

7.1. Predation

A severe stress factor is introduced when a predator is released among prey fish. During the interval until the prey fish have escaped, are eaten or adapted to the presence of the predator by finding proper hiding-places, they will be more susceptible to pathogens because of the stress situation.

Sindermann (1990) described a case of mass mortality in juvenile herring (*Clupea harengus*) caused by predators. In this case, a number of seals had trapped a school of juvenile herring in a cove during ebbing tide and a combination of predator pressure, falling tide and oxygen depletion probably induced the high mortality among the herring.

7.2. Pathogens

Pathogens may act as stressors in different ways. They may act on the fish host by elicting a lethal effect resulting in high mortality in the fish population. The sub-lethal effect of a pathogen may be displayed by causing abnormal behaviour, reduced competence in escaping predators, increased visibility to predators, reduced reproductive potential or physiological dysfunctions. Alternatively, pathogens may increase the host's susceptibility to other pathogens or other environmental stressors.

An example of a pathogen having demonstrated lethal effect on wild fish stocks is the fungus *Ichthyophonus hoferi*. An *I. hoferi* epizootic in herring (*Clupea harengus*) in the Gulf of St. Lawrence caused severe mortalities during the period 1954-1956 (Sindermann, 1958). The epizootic resulted in a 50% reduction of the herring landings in this area the following years (Tibbo & Graham, 1963).

A similar epizootic was observed in the North Sea, the Skagerrak, the Kattegat and the Baltic Sea herring stocks during the period 1991-1993 (Mellergaard & Spanggaard, 1997). The effect of this epizootic combined with overfishing seemed to be that the North Sea herring spawning stock was reduced from 1 mill tons to 0.5 mill tons during the period 1990-1995.

Fish act as intermediate hosts for many digenean trematodes whose adult stages live in the digestive tract of marine mammals, birds or of predatory fish. The effect of the intermediate stages of some of these digeneans range from causing the death of the host to being more or less harmful depending on the infection pressure. One example of such a trematode is *Cryptocotyle lingua* causing the so-called "Black spot disease" in many fish species living in coastal areas. The adult *Cryptocotyle lingua* lives in the digestive tract of fish-eating birds like gulls. Eggs from the parasite are shed to the marine environment in droppings and eaten by a snail, the periwinkle (*Littorina littoria*), in which the development to rediae takes place in the digestive gland. Further development to cercariae takes place within the rediae and free swimming cercariae emerge from the snails and penetrate the skin of the fish where it encysts as metacercariae forming "black spots" in the skin (Fig. 7.1). The cycle is closed when the infested fish is eaten by a bird.

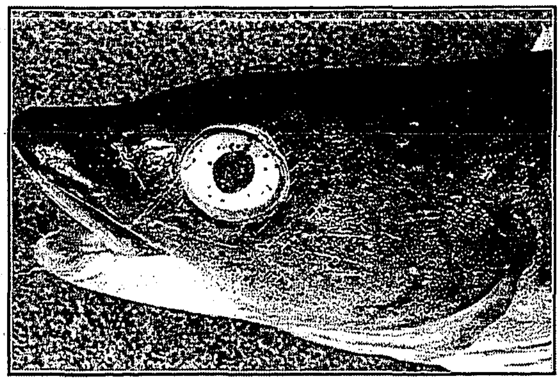

Fig. 7.1. *Cryptocotyle lingua* metacercaria in the skin of cod

In shallow water, which is the habitat of the snail, the shed of cercariae may be very intensive during summer time. As juvenile stages of many fish species stay in shallow water during this period, they may be exposed to an enormous infection pressure which may cause the death of the host. It has been demonstrated experimentally that massive cercarial invasion blind and kill juvenile herring (Sindermann & Rosenfield, 1954) and these authors postulated that similar infection pressure could be obtained in the inshore habitat of herring. A similar conclusion of *Cryptocotyle lingua* induced mortality in 0-group plaice (*Pleuronectes platessa*) was reported by MacKenzie (1968) and was confirmed experimentally by inducing mortality in 0-group plaice exposed to 100-1000 cercariae per fish (MacKenzie 1971 cited by Sindermann 1990). The cercariae also penetrate the cornea and heavily infestated fish may suffer from impaired vision resulting in the fish being in a poor nutritional state, a condition often observed in cod (*Gadus morhua*) and whiting (*Merlangius merlangus*) in Danish waters. Additionally, the impaired vision and nutritional state will increase the risk of the fish being eaten by a predator, mammal, bird or fish.

X-cell gill disease in dab (*Limanda limanda*) is another example of a pathogen that debilitates its host (Mellergaard & Nielsen, 1996)(Paper no. 3). The pathogen, probably a protozoan parasite, causes severe swelling of the gill lamellae. X-cell gill disease is endemic in the North Sea but occurs at epidemic levels in certain patches. The gill swellings probably impair the respiration of the fish making it unable to move across longer distances as discussed by Mellergaard & Nielsen (1996) who found significant different prevalences of X-cell gill disease in trawl tows taken 500 m apart. The respiratory problems made the fish unable to digest food resulting in severe emaciation (McVicar *et al.* 1987; Mellergaard & Nielsen, 1996). Furthermore, infected dab showed reduced reproductive potential by having a significantly decreased gonado-somatic index indicating lacking gonadal development (Knust & Dethlefsen, 1986). Many of the affected fish probably die from the infection. Although X-cell gill disease is an important stressor, it was not possible to demonstrate an increased disease rate of lymphocystis, epidermal papilloma or skin ulcers in the debilitated fish. This may be explained by the fact that X-cell gill disease primarily affect 2-3 years old fish while the other diseases primarily affect fish older than 3 years.

Infection by some pathogens may increase the host's susceptibility to other pathogens. Chum salmon (*Oncorhynchus keta*) suffering from Viral Erythrocytic Necrosis (VEN), a viral disease causing severe anemia but only low mortality (Evelyn & Traxler, 1978), appeared to have a threefold greater mortality rate from vibriosis, caused by the bacterium *Vibrio anguillarum*, compared to uninfected specimens (MacMillan & Mulcahy, 1980). Furthermore, the VEN infection reduced the resistance to other environmental stressors as infected fish showed a significantly decreased tolerance to oxygen depletion and a reduced ability to regulate serum sodium and potassium concentration when cultured in saltwater.

Another example where a pathogen stressor modified the resistance to other applied stressors was reported from Sockeye salmon (*Oncorhynchus nerka*) where smolt infested by the intestinal cestode *Eubothrium salvelini* proved to be significantly more susceptible to dissolved zink than were uninfected fish (Boyce & Yamada, 1977).

7.3. Nutrition

Insufficient food supply is a well known stressor in all organisms. Fish in poor nutritional state are often heavily infected with parasites which may aggravate the condition of the hosts. However, changes in the food composition of wild fish populations may also lead to shortage in essential nutrients like vitamins or minerals which may result in the development of deficiency diseases or reduced resistance to pathogenic organisms.

An example of a deficiency disease of current interest is the M-74 syndrome in the Baltic salmon (*Salmo salar*) stock. Increased mortality in salmon fry originating from ascending females was observed for the first time in 1974 (Norrgren *et al.* 1993). However, the problem aggravated seriously up through the 1990s where 80-90% of the fry died.
A similar problem was observed in Atlantic salmon in the Finger Lakes and in lake trout (*Salvelinus namaycush*) in the Great Lakes in North America where it was called the "Cayuga-syndrome" (Fisher *et al.* 1995).
Adult fish exhibiting M-74 have very pale muscles and reveal symptoms of neuromuscular disturbances (i.e. wiggling behaviour) while the fry have pale yellow yolk sacs, become ataxic, show haemorhages and precipitations in the yolk-sac and die within a few days at a stage where 2/3 of the yolk sac was absorbed. The symptoms of the adult fish, especially, showed some similarities with thiamine (vitamin B_1) deficiency in mammals and experimental bath treatment with thiamine cured the fry (Fisher *et al.* 1996). The thiamine deficiency may be linked to the high content of the enzyme thiaminase in the preferred prey fish, sprat (*Sprattus sprattus*) and herring for the Baltic salmon (Christensen & Larsson, 1979) and alewives (*Alosa pseudo-harengus*) in the Great Lake situation (Fisher *et al.* 1996). In the Finger Lakes and Great Lakes, the alewife is a non-indigeneous species that probably was introduced via balast water some 20 years ago but has been one of the preferred food items for salmonids in that area. In the Baltic Sea situation, there was a significant correlation with the spawning stock biomass of sprat and herring and the mortality rate of M-74 (Mellergaard, unpublished data). However, salmon have eaten sprat and herring for decades and, although the sprat and herring stocks are very large at present, high levels have been observed in earlier times without resulting in increased fry mortality. Therefore, it is likely that at least for the Baltic situation other factors may be involved. The possible implication of different PCBs is under evaluation and the lack of carotinoids which is responsible for the pink staining of the muscles in adult salmon and the dark yellow colour of the yolk is also a component of concern.

7.4. Algal toxins

Algal toxins have been responsible for severe stress and massive mortality in fish populations. The stress is induced when the toxin is present in sub-lethal levels. The occurrence of these toxins often elicits avoidance reactions in the fish trying to find layers in the water column free of algae and toxins. In the North Sea, the Skagerrak and the Kattegat, fish kills have been observed in association with blooms of *Chrysochromulina polylepis* and *Gyrodineum aureolum*. One of the most extensive fish kills caused by toxic algae in the northern Europe was observed during a *Chrysochromulina polylepis* bloom in 1988 in the North Sea, the Skagerrak and the Kattegat (Underdal *et al.* 1989). About 800 tons of rainbow trout, Atlantic salmon and cod was killed along the Norwegian and Swedish coast (Lindahl *et al.* 1990).The mortality mainly affected fish farms and this allowed for the possibility of giving an estimate of the mortality. It has not been possible to quantify the mortality in the wild fish stocks but in contrast to the farmed fish these have had the possibility to avoid the bloom by entering deeper strata of the water column which were free of algae. The algal toxins acted on the gill cells by increasing their permeability and the mortality was probably caused by osmoregulatory disturbances (Underdal *et al.* 1989; Dahl, 1988).

It appeared that the *Chrysochromulina polylepis* only produced toxic substances in salinities higher than 14‰ and, therefore, Danish fish farms only suffered limited losses when they were hit by the bloom. Mortality was only observed in one Danish farm while the fish populations in others was severely stressed but recovered when the bloom disappeared (Mellergaard, unpublished).

Non-toxic algae may also act as stressors. The marine diatoms *Chaetoceros concavicornis* and *C. convolutus* possesses setae with spinules. When these diatoms pass through the primary and secondary gill lamellae of fish, they may be retained in the interlamellar space. A study in Pacific salmon showed that the barbed setae of the diatoms severely irritated the goblet cells resulting in an overproduction of mucus which impaired the respiration of the fish (Albright *et al.* 1993). Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) cultured in sea-water net pens displayed increased mortality at a sub-lethal diatom concentration of 1-5 per ml. Under laboratory conditions, the addition of the bacterium *V. anguillarum* to the water greatly increased the mortality of coho salmon exposed to sub-lethal concentrations of *Chaetoceros* spp. This is a clear example of algae acting as a stressor in sub-lethal concentrations increasing the susceptibility of the fish to pathogens.

In Danish waters, blooms of the silicoflagellate *Dictyocha speculum* have induced mortality in rainbow trout (*O. mykiss*) cultured in marine fish farms. Some stages of this flagellate bear a spiny skeleton while others are naked. The dead trout exhibited gill damage, edema and cellular necrosis which indicate that the fish died of respiratory distress and osmoregulatory disturbances (Mellergaard, unpublished). As with the *Chaetocerus* spp., irritation from the spines might have been responsible for these damages. The stages observed in the marine environment during the fish kill appeared to be the naked form. The flagellate appeared not to produce toxins (Henriksen *et al.* 1993) so the mechanisms involved in this case of mortality in caged rainbow trout is still unknown. However, it has been suggested (Jenkinson, 1993) that some bloom-forming flagellates may alter the seawater characteristics through a production of extracellular organic material. The extracelular material increase the viscosity of the water surrounding the fish so that the energy expended in filtering water through the gills exceeds that which can be supported by the oxygen uptake. This phenomenon might have been involved in the kill of caged rainbow trout.

7.5. Population density

Population density is an important factor in the spread of diseases. The shorter the distance between susceptible hosts, the higher is the risk of becoming infected. Many parasites, like protozoans, monogeneans and digenean trematodes having a developmental life cycle where a free-swimming stage has to attach to a fish within a limited space of time, are extremely dependent on a high density of the host for having a successful infestation rate.

It is well known from aquaculture, that the mortality from Infectious Pancreatic Necrosis (IPN) in rainbow trout fry may be reduced by decreasing the population density e.g. by moving the fish to larger ponds. In this way, the infection pressure on each individual fish is reduced.

Density dependent disease problems in wild fish stocks have been observed in young saithe (*Pollachius virens*) along the west coast of Norway (Egidius *et al.* 1983). Young saithe gathered in dense schools in fjords and around aquaculture facilities suffered from vibriosis. Between 10%

and 100% of fish captured revealed skin lesions and large numbers of dead fish were observed in shallow areas. Around one fish farm, approximately 17,000 dead saithe were recorded over a few weeks. An experiment following the natural disease development in saithe kept in net cages in different stock densities demonstrated a clear density dependent mortality (Egidius *et al.* 1983).

Population density has also been a factor of importance in the spread of *I. hoferi*. The Norwegian Spring Spawning herring stock concentrate in extremely high densities in a few Norwegian fjords (Hjeltness & Skagen, 1992). The high stock density and resulting short distance for spread of the infection may be a factor of importance in explaining why the *Ichthyophonus*-infection has remained at a relative high level and for a longer period in this stock as compared with the North Sea stock.

Under culture conditions, elvers (juvenile eel, *Anguilla anguilla*) revealed morphological changes in the gastro-intestinal tract when kept in high stocking density. This stress effect was reflected in degenerative changes in the gastro-intestinal mucosa, a condition which was reduced by decreasing the stock density and provision of material providing hiding places for the fish (Willemse *et al.* 1984). Similar symptoms were observed in eel kept in tanks where the hierarchic structure was disturbed (Peters, 1982)

7.6. Age

As in man, fish have diseases which break out at different ages. In rainbow trout farming, diseases like IPN, hexamitiasis and Fry Mortality Syndrome are typical "children's diseases".

The investigations on X-cell gill disease (Mellergaard & Nielsen, 1996) showed a characteristic age profile of infected fish (Fig. 7.2) compared with fish infected with lymphocystis (Fig. 7.3). X-cell gill disease was most prevalent in 2 and 3 years old dab while lymphocystis mainly affected 3 to 5 year old dab. One explanation for this difference might be that the incubation time of X-cell gill disease is shorter than for lymphocystis, or alternatively, that the difference in the age composition reflects variations in the infection pattern of the two infectious agents.
Maturation and spawning may be regarded as a kind of stress, especially in female fish. During maturation, the major part of the energy uptake is used for gonadal development. This results in

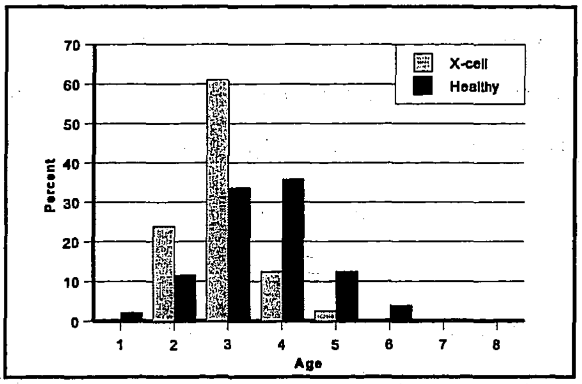

Fig.7.2. Age composition of X-cell gill disease affected and healthy dab

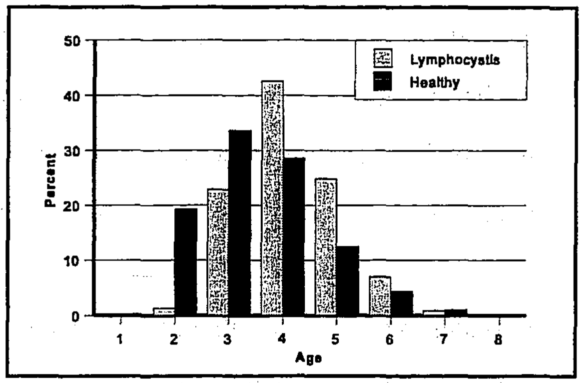

Fig. 7.3. Age composition of lymphocystis affected and healthy dab

the female fish being in a very poor nutritional condition after spawning. This has been reflected in a high infection rate of lymphocystis in Baltic and Elbe flounder in the period following the spawning season (Vitinsh & Baranova, 1976; Anders, 1984).

In aquaculture, rainbow trout are very sensitive to infection after spawning. Outbreaks of bacterial diseases like furunculosis caused by the bacterium *Aeromonas salmonicida* and fungal infections by *Saprolegnia* sp. are very common during this period.

8. Physical-chemical stress factors

8.1. Temperature

Temperature is a very important stressor as it affects the rate of metabolism, immunological response, reproduction, amount of oxygen dissolved in water, toxicity of pollutants and growth of fish pathogens and parasites.

Most fish species have their specific temperature ranges where the function of their immune system is optimal. Outside thise ranges, the function is impaired. For rainbow trout, the optimal temperature is 12-18°C, and the production of humoral antibodies is limited or lacking at temperatures below 5°C. At temperatures above 18°C, the risk of contracting diseases is increased. Stable temperature conditions tend to maintain the equilibrium illustrated in Fig. 8.1 but a sudden change in temperature is a severe stressor (Larsen & Mellergaard, 1981).

Rapid temperature changes severely increase the plasma corticoid ("stress-hormone") concentrations in juvenile chinook salmon (Strange *et al.* 1977) and this observation is probably valid for most fish species. Most examples illustrating the effect of variations in temperature originate from aquaculture. A study of disease outbreaks in a Danish marine fish farm showed that most outbreaks of bacterial infections were a consequence of a rapid increase in the water temperature (approx. 5°C) prior to the disease outbreak (Larsen & Mellergaard, 1981).

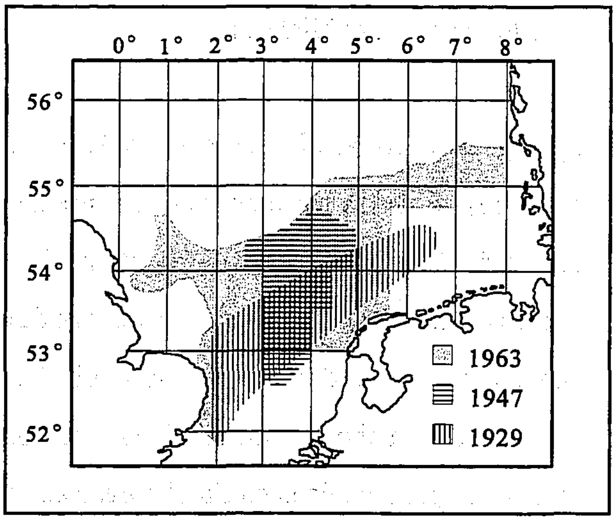

Fig. 8.1. Areas in the North Sea where fish mortality were observed during the cold winters of 1929, 1947 and 1963 (after Woodhead 1964b)

Low water temperatures during cold winters have seriously affected the fish stocks of the North Sea. Catches of dead fish, plaice (*Pleuronectes platessa*), dab *(Limanda limanda)*, sole (*Solea*

solea) and turbot (*Scophthalmus maximus*) have been reported after cold winters in 1928-29 (Dannevig, 1930; Johansen, 1929; Lumby & Atkinson, 1929), 1946-47 (Simpson, 1953) and during the winter 1962-63 (Woodhead, 1964) (Fig. 8.1). Woodhead (1964) found that the mortality in the fish probably was caused by severe osmoregulatory disturbances as he registered significantly increased serum sodium concentrations in cod and sole caught at water temperatures between 0°C and 3°C compared to fish caught at 12°C-14°C. The sole appeared to respond to these low temperatures by being in a semi-torpid condition and its normal diurnal rythm - buried in the sea-bed during day time and active during night time- apparently had disappeared (Woodhead, 1964). Additionally, many of the sole revealed ulcerations, starting as white patches, skin necrosis, and developed further to open ulcers penetrating deeply into the muscular tissue (Woodhead, 1964). The infection occurred throughout the southern North Sea. A similar observation was made in the Kattegat after the cold winter 1985-86. In March and April, many of the sole caught in the commercial fishery had ulcerations as described from the North Sea (Mellergaard, unpublished).

Spring ulcer disease in eel is a problem where temperature also plays an important role. In a Danish fjord with a large waste water input, skin ulcers in eel were observed in high prevalences (up to 80%) from March to May after which they disappeared during June (Dalsgaard, 1981; Jensen *et al.* 1983). The immune system of the eel is hardly active at temperatures between 0°C and 4°C as observed in March and April. During winter time, eel lie in an inactive, hibernating state buried in the sediment. If the sediment has a high organic load, the concentration of facultative pathogenic bacteria like *Aeromonas*, *Pseudomonas* and *Vibrio* sp. may result in a high infection pressure. With temperatures a few degrees above the freezing point, bacteria are usually present in fish tissue (Bisset, 1947) and their metabolic rate will probably increase more rapidly with increasing temperature than the activation of the immune system of the eel and, hence, be able to cause the skin inflammation. When the immune system of the eel is activated during May with temperatures of between 10°C and 13°C, the fish will actively combat the infection and this together with the mortality of severely ulcerated eel, may explain the decreasing prevalence of the disease observed during June.

The spread of parasites is often temperature dependent. Snails infected with *Cryptocotyle lingua* (the periwinkle (*Littorina littoria*) and the pond snail (*Lymnaea* spp.)) infected with the eye fluke (*Diplostomum spataceum*) live in shallow waters which may reach high temperatures during summer. The number of cercaria of *Diplostomum spataceum* shed by pond snails increases with increasing temperature (Lyholt & Buchmann, 1996). At high temperature, shed of cercaria from these snails may be so intensive that the water becomes cloudy and it is in such cases where the infection pressure may cause severe stress to the fish host as described earlier under the section 7.2 Pathogens.

8.2. Supersaturation

Supersaturation of water with gas (primarily nitrogen) combined with changes in atmospheric pressure and temperature often results in gas embolisms called gas bubble disease in fish (Rucker, 1972). A nitrogen saturation of 110-120% in the water is sufficient to induce gas bubble disease. Fish characteristically developed air bubbles under the skin, in the fins, tail and mouth, behind the eyeballs and in the vascular system causing gas embolisms and death (Fig. 8.2). Supersaturation with nitrogen occurs in natural waters in association with keeping fish in fresh well water where the water comes up from high pressure in the underground to normal atmospheric pressure and in association with fish living in ice-covered lakes due to a freeze-out of nitrogen (Mathias & Barica, 1985) . However, supersaturation may also be an environmental problem seen in connection with hydroelectric power plants and with "thermal" pollution from industrial facilities or power plants. In hydroelectric power plants, water is directed under high pressure through the turbines and the effluent water will release gases causing supersaturation in the effluent area. The ability of dissolving gases in water decreases with increasing water temperature. Therefore, when the temperature in cooling water is increased without any equilibration with the atmosphere (i.e. during its passage through the condenser system of the plant) the water becomes supersaturated. Fish often concentrate in effluent areas and will be at risk of getting gas bubble disease when they remain in the area either because they are prevented from further migration up in river systems

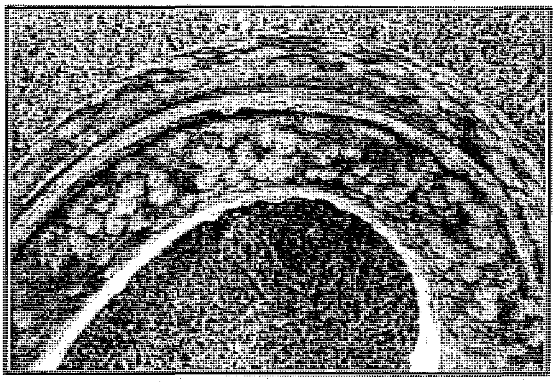

Fig. 8.2. Gas bubble disease in eel

(for example due to blockage by a dam) or because heated effluent areas contain increased food resources. Not all fish die from the lesions. However, in survivors the skin covering the gas bubbles can become necrotic and may, thus, act as the port of entrance for facultative pathogenic microorganisms from the environment.

Gas bubble disease is well known in aquaculture facilities especially in association with leaks in gaskets of water pumps where air is forced into the water under pressure and produces a supersaturated condition when released to atmospheric pressure. It has commonly been observed in Danish eel farming systems but has only caused mortality in one case.

8.3. Salinity

Changes in salinity due to tidal fluctuations are observed in estuarine areas. To adapt to these variations of salinity the fish have to change their osmoregulation twice a day. The impact of this stress factor may be reflected in increased disease rates in fish staying in such areas. Flounder living in the tidal zone in the Weser estuary (Möller, 1981) and in the Elbe estaury (Möller, 1984) showed higher prevalences of lymphocystis and skin ulcers compared to fish living up-and downstream of the tidal zone. This increased prevalence may be the result of a combination of the fluctuations in salinity and a shortage of food as the biomass of invertebrates was very low in the tidal zone (Möller, 1990). Dutch studies of diseases in flounder showed a significantly higher prevalence of skin ulcers from fish in an area close to the dranage sluices of the Lake Ijssel where frequent changes in salinity took place compared to other localities in the Dutch Wadden Sea. No significant difference was observed in the prevalence of lymphocystis between the two areas (Vethaak, 1992).

8.4. Pollution

The Advisory Committee of Marine Pollution of the International Council for the Exploration of the Sea has defined **pollution** *as the introduction by Man, directly or indirectly, of substances or energy into the marine environment resulting in such deleterious effects as harm to living resources, hazzard to human health, hinderance of marine activities including fishing, impairment of quality for use of sea water and reduction of amenities*, and **contamination** *as the situation which exists where either the concentration of a natural substance (e.g. a metal) is clearly above normal, or the concentration of a purely man-made substance (e.g. DDT) is readily* *detectable, but where no judgement is passed to the existence of pollution (i.e. adverse effects).* As it appears there exists a gradual transition between these two definitions (a grey zone) where it depends of personal judgement to define whether a possible source of environmental stress is pollution or contamination. Therefore, in the following the term pollution includes both definitions.

For most people, environmental stress is equated with pollution by toxic substances. However, pollution involves all kind of human discharges that may add to environmental stress. These include a large spectrum of substances ranging from nutrients like nitrate and phosphate which mainly originates from the runoff of surface water from agriculture, to the discharge of organic matter from urban or industrial sewage water, to thermal pollution from power plants to the release of environmental contaminants like heavy metals, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) from industrial discharges. Pollution stress may either act directly on the fish by affecting specific organs or enzyme systems or indirectly by causing environmental changes which may induce stress to the fish.

The relationship between pollution and diseases is illustrated in Fig 8.3. The disease rate may be unaffected by the pollutant as indicated in line A or there is a negative correlation, the prevalence decreases towards the source, curve B, sometimes observed in association with parasitic infections. Curve C illustrates a positive correlation where a disease occurring in endemic levels increases proportionally to the distance of the pollution source, the most common situation, and curve D shows an example of a pollution induced disease e.g. neoplasms.

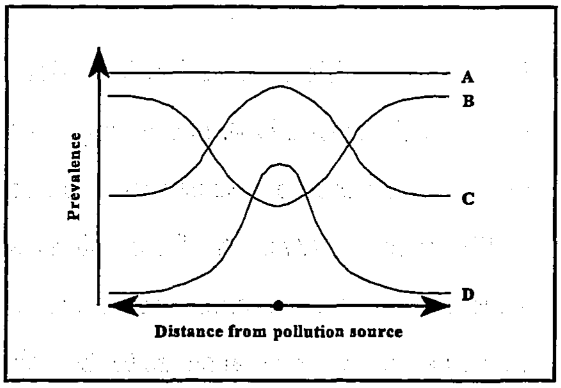

Fig. 8.3. Four hypothetical relationships between disease prevalence and a source of anthropogenic pollution (after Patton & Couch 1984)

In the following, a series of examples of studies illustrating the effect of different pollutants will be presented.

An example illustrating an "improved" condition caused by pollution has been reported from roach where the frequencies of the gill parasites *Dactylogyrus* sp. and *Paradiplozoon* sp. decreased with decreasing distance from the outlet of effluent water from a bleached kraft mill (Thulin *et al.* 1988).

In many parts of the world, it is common to dump municipal sewage water at sea. In addition to its enormous organic load, sewage often contains heavy metals and other contaminants. In the North Sea, dumping of municipal sewage water still takes place along the British coast line. In Scotland, the effect of sewage sludge dumping on the fish disease status of dab in two dumping areas compared with adjacent control areas has been examined. The disease levels in dab were found to be significantly lower at the dump sites than at the reference areas (McVicar *et al.* 1988). This finding may be explained by the "fertilizing" effect at the dump site. The dump sites were situated in an area with a strong coastal current that dispersed the dumped material over a large area. This fertilization may increase the biomass of invertebrates and, thus, the food resources available for the fish populations in these areas.

In contrast to this observation investigations in the New York Bight and along the coast of the southern California have shown significantly increased prevalence of fin erosions in areas with dumping of municipal waste water compared to non-polluted areas (Mahoney *et al.* 1973; Mearns & Sherwood, 1977). The fin erosions were associated with bacterial infections of the genera *Aeromonas*, *Pseudomonas* and *Vibrio* and it was hypothesized that dense bacterial populations as well as environmental stress to the fish due to the high organic load in the dumping areas were responsible for the high prevalence of fin erosions (Mahoney *et al.* 1973).

Fin erosions in perch (*Perca fluviatilis*) and ruffe (*Gymnocephalus ceruna*) have been observed as a direct effect of bleached kraft mill effluents (Lindesjöö & Thulin, 1990). Up to 80% of the fish in the vincinity of the outlet showed fin erosions and the prevalence decreased with increasing distance from the contaminant source.

Skeletal deformities have also been observed in association with pulp mill effluents. Severe deformations of the jaws (a distinct upwards bending) have been reported in northern pike (*Esox *lucius*) caught in the effluent area of a pulp mill. In some areas, the deformities were observed in up to 40% of the fish. Laboratory experiments where fourhorn sculpin (*Myoxocephalus quadricornis*) were exposed to pulp mill effluent water resulted in the development of vertebral deformities (Bengtsson *et al.* 1988).

In flounder (*Platichtys flesus*) chronically exposed to bleached pulp mill effluents cellular changes in gill and liver tissue have been recorded (Lethinen *et al.* 1984). Fish exposed to effluents from mills using chlorine and oxygen in the bleaching process showed a high degree of infestation of a trichodinid ciliate. This appeared likely to be the result of cellular damage in the gills which improved the nutritional conditions for the parasites which live on cellular debris.

The four examples presented above illustrate the effect of the effluent water from pulp mills. This effluent is a mixture of water with a high content of organic matter and a number of chemicals (different chlorine compounds) used in the bleaching process. In these investigations there has not been any attempts to elucidate whether the observed effects are caused by a single component in the effluent water or the mixture of all the substances .

"Haemorrhagic syndrome" in Atlantic salmon is an example of a disease having its background in a complex interaction between two contaminant sources. When ascending salmon reached a certain level of the River Don in Scotland, they were hit by a fatal disease of the blood and liver characterised by hyperbilirubinaemia, haemolytic anaemia and structural changes of the liver making the fish bloody red all over the surface (George *et al.* 1992). It was not possible to associate any infectious agent to the syndrome and, therefore, focus was turned to contaminants or pollutants. A number of paper mills discharged effluent water into the river but exposure to this did not affect the fish. At the level of the river where the problem occurred a minor pipe discharging effluents from industrial drains was found. Fish exposed to this industrial effluent showed elevated mixed-function oxidase (MFO) activity in the liver but no external symptoms. However, when effluent from the industrial drain was added to the paper mill effluent, the syndrome developed.

Chlorine has also been identified as the main factor in the development of papilloma in the oral cavity of black bullhead (*Ictalurus melas*) living in the final oxidation pond of a waste water treatment plant where chlorine was used for disinfection of the water. The prevalence of the papillomas decreased from 73% to 23% associated with a reduction of the chlorine concentration in the effluent water (Grizzle *et al.* 1984). No evidence of the involvement of virus-particles in the pathogenesis of the papilloma was found.

The basic toxic principles involved in most of the problems observed in fish in association with heavy metals are poorly understood. Some heavy metals may cause problems by competative inhibition of the different enzyme systems of the fish. Sublethal concentrations of cadmium provoked neuromuscular disturbances such as hyperexitability, spasms and tetanic body contractions in flounder (Larsson *et al.* 1981). These reactions seemed to be related to disturbances in the ion balance - in particular, a reduction in the plasma concentration of calcium and potassium. One third of minnows (*Phoxinus phoxinus*) exposed to cadmium for 70 days developed vertebral fractures probably because of vigorous contractions of the large lateral muscle groups due to cadmium-induced neuromuscular disturbances (Bengtsson *et al.* 1975). Monitoring of vertebral defects in fourhorn sculpin (*Myoxocephalus quadricornis*) has been used to trace heavy metal pollution from a metal ore smeltery emitting heavy metals and arsenic. Approximately 40% of the fish in the vincinity of the smeltery revealed skeletal deformities (Bengtsson *et al.* 1988).

Eel kept in copper-contaminated fresh water developed vibriosis in spite of *Vibrio anguillarum* being a halophilic bacteria. It has been suggested that *V. anguillarum* is a common inhabitant of eel and copper may disturb the commensal association between the fish and the bacterium to one of pathogenecity (Rødsæther *et al.* 1977).

Organochlorine substances like DDTs and PCBs may interfere with the reproduction of fish in two ways: 1) The development of the embryo may be disturbed by contaminants entering the egg already during maturation in the female gonads or 2) contaminants may interfere with the development after the release of the spawning products into the surrounding water before or after fertilisation. The observation of developmental defects in pelagic embryos of several flatfish species in the North Sea has been related to contaminants although it cannot be excluded that other environmental stressors like low water temperature may add to the problem (Cameron *et al.* 1992). Experiments with Baltic flounder and herring showed that the viable hatch of eggs was significantly affected at ovary PCB levels above 120 ng/ g wet weight (Westernhagen *et al.* 1981; Hansen *et al.* 1985).

Other organochlorines, such as the pesticide Kepone have been shown to induce scoliosis in sheepshead minnow (*Cyprinodon variegatus*). The effect appears to be a dose-dependent and time related phenomenon. The vertebral curvature is apparently caused by long-term rigorous contractions of the skeletal musculature in one side of the body causing fracture of the central part of the vertebrae (Couch *et al.* 1977).

Field studies in Puget Sound in western U.S.A. of the etiological relationship between the prevalences of hepatic neoplasms in bottom dwelling marine fishes, mainly English sole (*Parophrys vetulus*), and the concentration of toxic chemicals has shown a significant correlation between the sediment concentration of aromatic hydrocarbons (PAHs) and the prevalence of hepatic neoplasms (Malins *et al.* 1988). These observations have been confirmed by laboratory studies.

8.5. Oxygen deficiency

Oxygen is necessary for all life. Therefore, it is evident that reduced oxygen concentration in the water may induce stress in affected organisms. Oxygen tolerance varies with different species. The tolerance seems to be lower for pelagic fish species than for demersal species (Scholz & Waller, 1992). The lethal oxygen level for plaice and flounder is within the range 0.4 to 1.3 ml/l (Muus, 1967; Bagge, 1970; Scholz & Waller, 1992; Weber, 1993).

The oxygen content of water decreases with increasing temperature and salinity. This means that oxygen deficiency may occur in areas with stagnant shallow water during summer time or in areas receiving thermal effluent from e.g. power plants.

The most severe cases of oxygen deficiency in marine waters are primarily caused by eutrophication due to enrichment of the water with nutrients like nitrogen and phosphorous. These fertilizers enhance the growth of phytoplankton, which under normal conditions will be grazed down by zooplankton. If the algae production exceed the grazing capacity of the zooplankton, the surplus will sediment. The biodegradation of the sedimented phytoplankton is an oxygen demanding process and, if the water body over the sediment is stagnant or stratified, oxygen deficiency may occur.

Incidents of intensive oxygen deficiency in the open sea have been described from the open continental shelf of the east coast of the United States in 1976 where an area of 12000 km^2 was found more or less anoxic (Sindermann, 1984). This event caused severe mortalities in fish, shellfish and in the benthic fauna. However, the impact on the disease status of the affected fish populations was not investigated.

For decades, oxygen deficiency has been a common phenomenon in many Danish fjords during late summer. In the North Sea, more precisely along the westcoast of Denmark and in the German Bight, oxygen deficiency covering areas of up to 8000 km^2 took place in 1981, 1982 and 1983 and in an area in the southern Kattegat a similar event took place during late summer 1986 to 1988 (Fig. 8.4). The background for the above mentioned events has been a combination of nutrient enrichment and special climatic conditions, such as warm summers and calm weather.

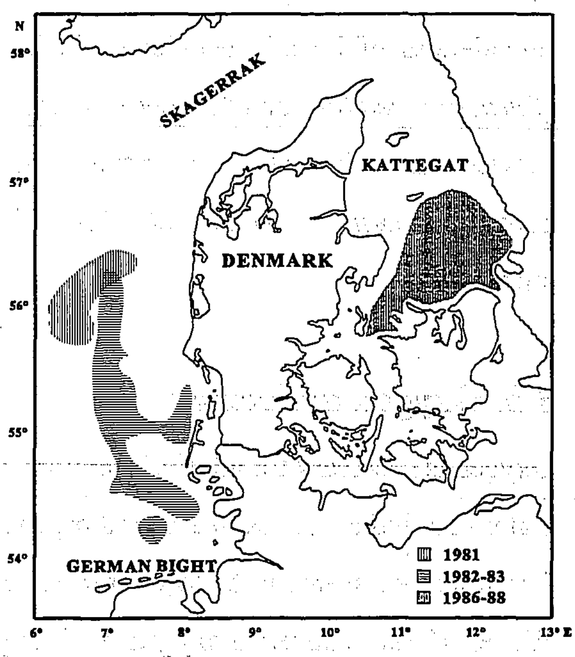

Fig. 8.4. Areas affected by oxygen deficiency in the North Sea and the Kattegat (from Dyer et al. 1983, Westernhagen et al. 1986 and Kronvang et al. 1993)

From aquaculture, it is well known that oxygen deficiency may trigger the outbreak of diseases in fish (Wedemeyer, 1981) but only a few examples exist from wild fish stocks.

An *Aeromonas liquefaciens* epizootic observed in threadfin shad (*Dorosoma petenense*) and

American shad (*Alosa sapidissima*) in the San Joaquin River, California was probably triggered by very low oxygen content in the water due to organic pollution from a tomato and peach cannery (Haley *et al.* 1967).

The most intensive study to date of the impact of oxygen deficiency on the disease status of wild fish was done in the Kattegat (Mellergaard & Nielsen, 1995) (Paper no. 2). Here, information on the disease status of common dab was avaliable before the severe oxygen deficiency events took place. The prevalence of the two viral diseases lymphocystis and epidermal papilloma increased significantly the years following the oxygen deficiency. In spite of a lack of disease data before the oxygen deficiency occurred in the North Sea, the temporal disease pattern of lymphocystis and epidermal papilloma observed in the affected areas revealed distinct similarities with the pattern observed in the Kattegat (Paper no. 4). This observation strongly indicate that oxygen deficiency also has played an important role in provoking the outbreak of lymphocystis and epidermal papilloma in the North Sea.

8.6. Fishing activity

Fishing activity can stress fish in a number of different ways. Contact and escape from fishing gear can cause skin lesions which depending on size, may cause osmoregulatory disturbances and may be the port for entry of facultative pathogenic bacteria. Fish caught in gill nets may be attacked by snails which may rasp small circular holes in the skin. When fishermen clean their nets, undersized fish are often voilently removed from the net resulting in severe damage to the fins. Fish caught in bottom trawls are often towed for several hours. The fish are then damaged during the trawl movements over the bottom, especially when the codend is packed with fish. In addition, some fish escape through the net meshes and may be injured during this process.

Most of the skin ulcerations observed in dab that are described in Papers 2 and 4 probably originated from traumatic skin damages caused by fishing gears (Mellergaard & Nielsen, 1995). This may also explain why skin ulcers appear to be independent of the stress effect caused by oxygen deficiency in contrast to lymphocystis and epidermal papilloma.

Fishery-induced skin injuries have been described from dab caught as by-catch in the shimp fishery in the Wadden Sea (Lüdemann, 1993). The described damages were mainly fissures and haemorrhages in the skin. Recently, severe excoriations affecting 20% to 50% of the pigmented side of plaice have been observed in purse-seine catches from the Skagerrak. As the purse-seine fishery is probably the gentlest method of catching fish, the damaged fish probably originated from discards from beam trawlers conducting an intensive fishery in the area (Mellergaard, unpublished).

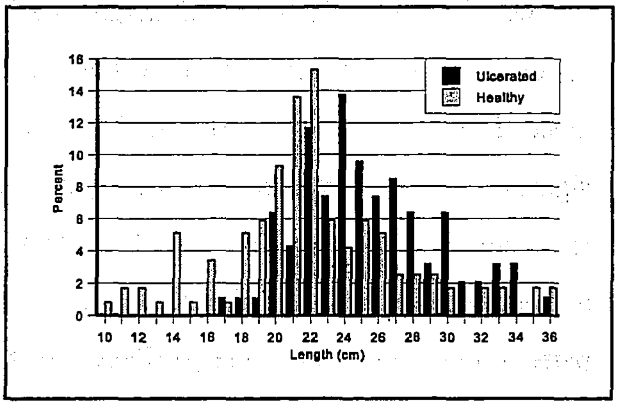

Fig. 8.5. Length distribution of healthy and ulcerated cod

A severe case of fishery-induced ulcerations was observed in cod in the Baltic Sea, in 1982. During that year, the cod stock biomass was high and an intensive fishery especially around Bornholm was carried out. During a stock assessment cruise in March, approximately 10% (n=1445) of the cod were found to exhibit ulcerations and, during a cruise in May, the prevalence of ulcerated cod had increased to approximately 40%. The length distribution of ulcerated and healthy cod is illustrated in Fig. 8.5. Most of the ulcerated fish were below 30 cm which was the marketable size. The commercial trawls were rigged with a mesh size which would allow at least 50% of 28.5 cm long cod to escape the meshes.

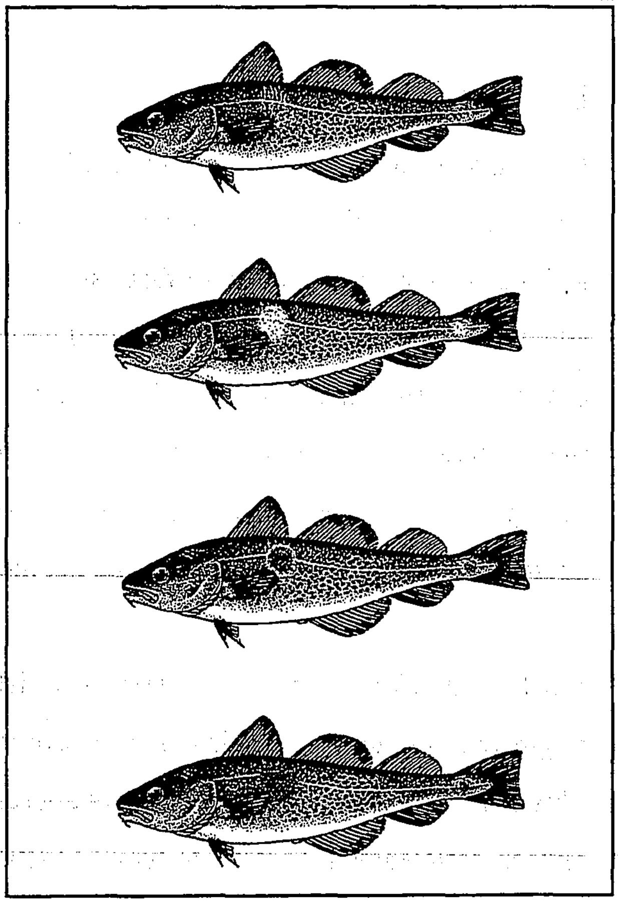
Fig. 8.6. Sequential development of fishery induced ulcerations in cod

From the appearance of the skin damages (Fig. 8.6) it seemed likely that the skin lesions were caused by the fishing gear. The sequential development of the ulcerations started with excoriations primarily situated below the first dorsal fin sometimes associated with minor damages in the tail region. Edema in the affected area caused raising of the scales and a progressive skin necrosis developed resulting in a white necrotic spot, 2-3 cm in diameter. The central part of the necrosis was sloughed off exposing a haemorrhagic ulcer which developed further.Ulcerations could also be seen at the tail root.

One characteristic of the phenomenon was that many of the ulcerations occurred bilaterally at the thickest part of the fish. It seems likely that many of the ulcerated cod had been trapped during their passage through the net meshes and the bilaterally occuring ulcers might have been caused by the severe pressure from the knots of the trawl. The damaged skin was then open for infection by facultative pathogenic bacteria which probably resulted in the death of most of the affected fish. This meant that more than 40% of the undersize fish were likely to die as a result of skin lesions induced by fishing activity.

9. The common dab (*Limanda limanda*)

The common dab, in Danish "ising", is a flatfish. One of its characteristics is that the lateral line creates a semi-circle formed bow at a position opposite the pectoral fin (Fig. 9.1). The colour of the pigmented side varies from beige through different shades of brown to reddish brown. It has a rough surface, especially on the pigmented side, because of the position of the scales. The colour of the fish reflects the colour of the bottom sediment.

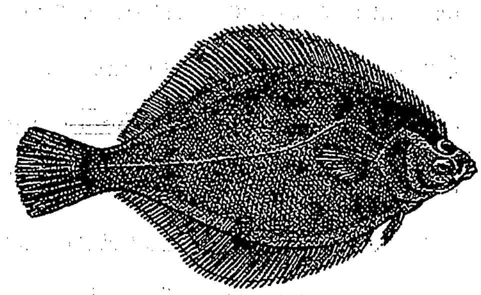

Fig. 9.1. Common dab (*Limanda limanda*)

9.1. Habitat

The dab are widely distributed in Danish waters at water depths from 5 to 100 m. They prefer soft sandy bottom conditions which is the habitat for their main food items, small molluscs, worms, brittle stars, small sea urchins and hermit crabs.

	Mean length (cm)
German Bight	17.9
Offshore central area	17.4
Coastal central area	17.1
Skagerrak	17.6
Kattegat	18.8

Table 9.1. Mean length of dab in the different areas investigated during this study

9.2. Growth

Most dab are below 30 cm in length. Only 1 per thousand was found to be above 30 cm during the present study and 50% of the fish were within the range of 15 to 20 cm. The length varies with the habitat areas as shown in table 9.1. Similar differences may be observed in other areas of the North Sea. In general, males are shorter than females at the same age. The growth of the dab is more slow than that of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) and varies considerably depending on the presence of food items and the stock density.

9.3. Age composition

The age composition of the dab in the different areas investigated is shown in Fig. 9.2. In all areas except the Skagerrak, 3 year old fish were the most prevalent. In the Skagerrak, the fish were younger and exhibited faster growth (reflected in the mean length) than that of the eastern North Sea population. The Kattegat dab had the highest mean length but were a little older than the dab populations in other areas (Fig. 9.2). At the age of 4 years, the dab reach the size of interest for commercial fishing (25 cm).

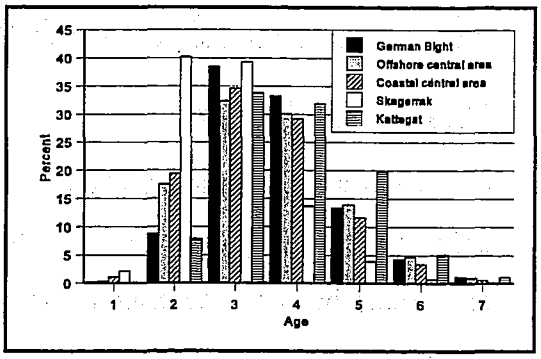

Fig. 9.2. The age composition of the dab in the different areas investigated.

9.4. Maturity and spawning

Common dab reach maturity at the age of 2 years. The spawning period in the North Sea is from January to June with the highest intensity during March and April. During this period, the fish becomes migratory and are able to migrate several hundred kilometres. However, when spawning is completed the fish return to their feeding grounds where they remain relatively stationary until the next spawning season (Rijnsdorp *et al.* 1992).

9.5. Larval development

During the first months of development, dab larvae live in the free water masses (pelagic stage). During this stage, the metamorphosis (the change from round- to flatfish) is initiated and when this development reaches a certain level, the larvae settle on the bottom. In contrast to the juvenile stages of plaice and flounder which live in the shallow coastal water, the newly settled and the juvenile dab live in the deeper parts of the sea (8-12 m).

The common dab fulfils the requirements for a fish species to be used in studies of the impact of environmental stressors on fish disease: it is bentic (bottom dwelling), fairly static (outside the spawning period), abundant and exhibits diesases which are easily recognized (Bucke *et al.* 1996).

10. Diseases of importance for this study.

10.1. Lymphocystis

Lymphocystis is a common, chronic, and benign infection caused by an iridovirus that results in uniquely hypertrophied connective tissue cells. It is reported from most parts of the world and the disease has been described from more than 125 fish species from fresh, brackish and sea water.

10.1.1. Agent

Lymphocystis lesions were described from plaice and flounder in the North Sea as early as 1874 (Westernhagen *et al.* 1981). At that time the disease was supposed to be caused an unknown sporozoan parasite but already in 1914 Weissenberg (1914) concluded that lymphocystis might be of viral origin. This hypothesis was confirmed by Weissenberg (1951). The size of the iridovirus range from 130 to 300 nm depending on fish species. In flounder and dab, the size of the virions are between 200 and 250 nm (Anders, 1984).

10.1.2. Symptoms

In flatfish, the lymphocystis lesions appear on the skin surface as elevated nodular clusters. The colour is white to greyish but, if the lesions occur on the pigmented side, they may be normally pigmented. Vascularity may give the nodular masses a reddish appearance. In light cases, only a few single nodules may be found, primarily on the fins. As the infection progress, the whole body surface may become affected (Figs. 10.1 & 10.2, page 41). In plaice and flounder, the nodular clusters are often more elevated than in dab where the clusters often form a more uniform layer in the skin. Each nodule is a single hyperthrophied virus filled connective tissue cell that, in plaice and flounder, may reach a size of 2 mm while they seldom reach more than 1 mm in dab (an increase in cell size of more than 100,000 times).

10.1.3. Effect on host

The impact of lymphocystis on flatfish seem to be negligible. Witt (1957) found that white crappies (*Pomoxis annularis*) infected with lymphocystis exhibited a weight reduction of 3 to

5% compared to uninfected specimens of the same length. However, Petty & Magnuson (1974) found that lymphocystis infected bluegill (*Lepomis macrochirus*) weighed slightly more than uninfected specimens even after removal of the nodular lesions. The present study showed that the mean weight of lymphocystis infected dab was significantly higher ($p<0.001$) than that of uninfected fish in the North Sea area even if the data were restricted to the age groups having the highest prevalence of the disease (3 to 5 year old fish). In general, the condition factor [100 x weight (g) x length^{-3} (cm)] of lymphocystis affected dab is higher than for unaffected specimens although the difference is only significant in the German Bight (Area 1) (Table 10.1).

	Unaffected	Lymphocystis
German Bight	0.9346	0.9432*
Offshore central area	0.9692	0.9738
Coastal central area	0.9583	0.9590
Skagerrak	1.0214	1.0189
Kattegat	0.9770	0.9830

(* p<0.05)

Table 10.1. The condition factor for unaffected and lymphocystis affected dab in different areas (partly extracted from table 4, Paper 4)

The age distribution of lymphocystis affected dab is shown in Fig 10.3. Approximately 90% of the lymphocystis affected fish are between 3 and 5 years.

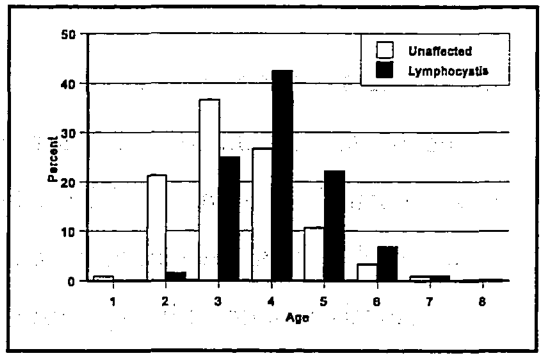

Fig. 10.3. The age distribution of unaffected and lymphocystis affected dab

Rejection of lymphocystis lesions has been observed under experimental conditions (Petty & Magnuson, 1974; Roberts, 1976). An experiment where lymphocystis infected flounder were tagged and photographed before release showed that even severely affected fish had rejected all nodular processes when recaptured. (Banning, pers. comm.). This phenomenon is probably due to an immunological reaction (Roberts, 1976). It is not yet clarified whether this rejection of the lymphocystis lesions cause a total recovery from the disease or whether it represents a shift in an immunological equilibrium where the fish is still latently infected by the lymphocystis virus. The rejection of the lymphocystis nodules might explain the observed seasonal variation of the lymphocystis infection rate with the highest rates being observed in winter and spring (Wolthaus, 1984).

In a tagging experiment with lymphocystis affected and unaffected flounder, Vitinsh & Baranova (1976) registered a lower return rate of affected than unaffected flounder over a 3 years period. They concluded that lymphocystis may have a lethal effect on flounder. In contrast, Ryder (1961) found a higher return rate in tagged lymphocystis infected walleye (*Stizostedion vitreum*) than in healthy specimens. This difference was assigned to a higher catchability of infected fish because the nodular processes were caught in the net meshes. The results obtained during the present study do not give any indications of a lethal effect in dab caused by lymphocystis. Fig. 10.4 illustrates the prevalence of lymphocystis in age groups of dab in the North Sea (based on the data used in Paper 4). The lymphocystis infection seems to take place in 2, 3 and 4 year old fish and then remain at a relatively constant level in the 5 to 8 year old dab. There is no sudden drop in the infection level in any of the older year classes that might indicate increased mortality.

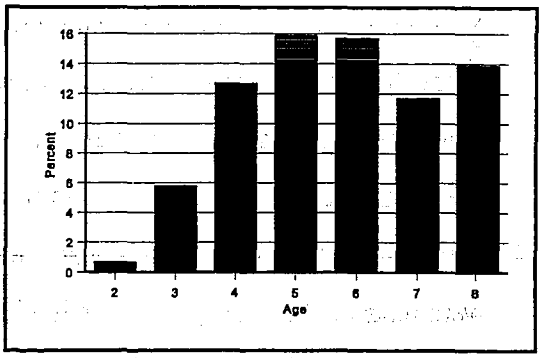

Fig.10.4. Prevalence of lymphocystis per age group for dab in the North Sea

10.1.4. Response to stress

Lymphocystis is one of the diseases recommended for use in monitoring the effect of environmental stress on wild fish stocks (Sindermann, 1980; Bucke *et al.* 1996). Environmental factors such as tidal fluctuation have been proven to be important in triggering the outbreak of lymphocystis in flounder living in estuarine areas (Möller, 1981; 1984; 1990). Oxygen deficiency caused by eutrophication has been shown to be an important factor provoking the outbreak of lymphocystis in dab in both the Kattegat and the North Sea (Mellergaard & Nielsen, 1995, Mellergaard & Nielsen, submitted) (Papers 2 & 4). In addition, high lymphocystis rates have been observed during spawning in walleye (Amin, 1979), flounder (Vitinsh & Baranova, 1976; Anders, 1984) and dab (Wolthaus, 1984).

10.2. Epidermal papilloma

Epidermal hyperplasia and epidermal papilloma is a common, benign infection probably caused by an adenovirus. The disease has been described from dab in North Sea and the Kattegat. The precise differentiation between epidermal hyperplasia and epidermal papilloma can only be made histologically and, therefore, both lesions will be designated epidermal papilloma in the following.

10.2.1. Agent.

The first description of epidermal papilloma in North Sea dab originates from 1925 where it was described as "cutaneous warts" (Johnstone, 1925). It was "rediscovered" in the 1970s when monitoring programmes on the impact of pollution on fish diseases were initiated in the North Sea (Dethlefsen, 1980). Epidermal papilloma was found in elevated prevalences in the German Bight especially in an area where dumping of titanium dioxide waste occurred. The lesions were expected to be closely associated with the titanium dioxide waste (Dethlefsen *et al.* 1987). During the first years of the present investigation, it appeared that the development of epidermal papilloma closely resembled the one observed for lymphocystis. Therefore, it was considered likely that these lesions had an infectious background. Electron microscopy of samples from epidermal hyperplasia revealed the presence of icosahedral adenovirus-like particles in the nuclei of normal-looking cells situated near the surface of the epithelium (Bloch *et al.* 1986)(Paper 1). Later, EM-examination of samples from true epidermal papilloma gave similar results (Bloch, unpublished). The adenovirus-like particles had an average diameter of 70 nm. Attempts to cultivate the virus has failed, so far.

10.2.2. Symptoms

Epithelial hyperplasia are found on the skin surface as small, rounded, half-transparent, creamy white slightly raised patches with a smooth surface measuring 2 to 10 mm in diameter (Fig. 10.5, page 41) . They may appear as single or multiple lesions which, in some cases, may fuse together forming a confluent patch. There seems to be a gradual transition from epithelial hyperplasia to epithelial papilloma. The epidermal papilloma becomes more raised, opaque, irregular and get a rough (papillomateous) surface structure measuring 10 to 20 mm in diameter with red cords representing blood vessels. The papillomas may also fuse together and form large patches (Fig. 10.6, page 41).

10.2.3. Effect on host

The effect of the epidermal papillomas on dab seem to be insignificant. The mean weight of epidermal hyperplasia/papilloma affected dab was significantly higher ($p<0.001$) than that of unaffected specimens in the North Sea. This significant difference still was evident when the data were restricted to 3 to 5 year old dab in which epidermal papillomas were most prevalent. In general, the condition factor of epidermal papilloma infected dab was higher thanfor unaffected fish. However, this difference was not statistically significant (Table 10.2).

	Unaffected	Epid. Papilloma
German Bight	0.9352	0.9383
Offshore central area	0.9693	0.9815
Coastal central area	0.9580	0.9662
Skagerrak	1.0213	1.0296
Kattegat	0.9830	0.9953

Table 10.2. The condition factor for unaffected and epidermal papilloma affected dab in different areas (extract from Paper 4, table 4)

The age distribution of epidermal papilloma affected and unaffected fish is illustrated in Fig. 10.7. As for lymphocystis, more than 90% of the epidermal papilloma infected dab were between 3 and 5 years old.

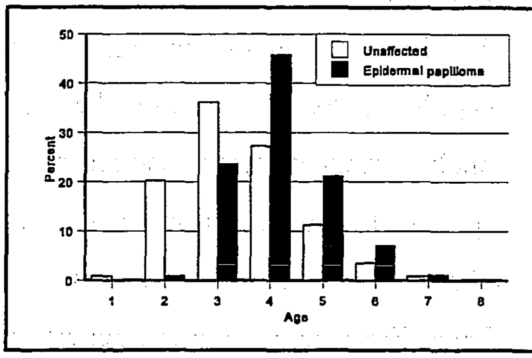

Fig. 10.7. The age distribution of unaffected and epidermal papilloma affected dab

The course of this infection is probably not lethal. As illustrated in Fig. 10.8, the prevalence of epidermal papilloma per age group (based on data from Paper 4) seems to increase in 2 to 5 year old fish and reaches a relatively constant level in 6 to 8 year old fish. As for lymphocystis, epidermal papillomas seem to display seasonal variations with the highest infection rate being observed during winter and spring (Wolthaus, 1984). This might indicate the presence of similar regulation mechanisms for epidermal papilloma as for lymphocystis and the chance of the dab being latently infected with epidermal papilloma.

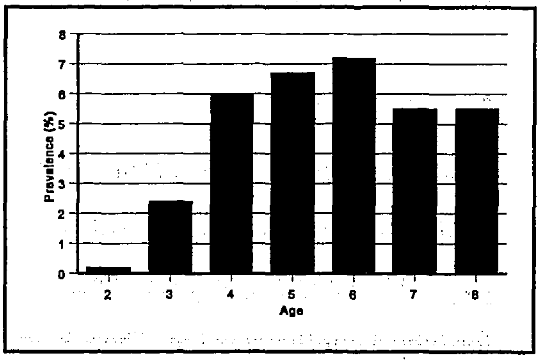

Fig. 10.8. Prevalence of epidermal papilloma per age group for dab in the North Sea.

10.2.4. Response to stress

Epidermal papilloma is one of the diseases recommended for use in environmental monitoring programmes (Bucke *et al.* 1996). Environmental stress caused by eutrophication has been shown to trigger the outbreak of epidermal papilloma both in the Kattegat and in the North Sea (Mellergaard & Nielsen, 1995; Mellergaard & Nielsen, submitted) (Papers 2 & 4). The disease has also been reported in elevated frequencies in an area in the German Bight where dumping of titanium dioxide waste took place (Dethlefsen *et al.* 1987). However, the titanium dioxide dump site coincide with an area which was affected by oxygen deficiency in 1982-1983 so the reported high prevalences may be due to an additive effect of the two stressors.

10.3. Skin ulcers

Skin ulcers are a break in the skin or mucous membrane with loss of surface tissue, disintegration and necrosis.

10.3.1. Agent

Skin ulcerations have been attributed to bacterial infections but can arise after primary traumatic injury, scale loss, parasitic infestations (for example copepods) or debilitation caused by adverse environmental factors. A number of bacterial species have been associated with skin ulcers. Under marine conditions these have mainly been *Vibrio anguillarum* (Haastein & Holt, 1972), "atypical" *Aeromonas salmonicida* (Wiklund & Dalsgaard, 1995), *Aeromonas hydrophila* and *Pseudomonas* sp. (Vethaak, 1992).

10.3.2. Symptoms

Skin ulcers may, in some instances, start with small necrotic (white) patches often surrounded by a haemorrhagic zone. The necrotic center may be sloughed off and the ulcer appears red often with a white margin of necrotic tissue (Wiklund & Dalsgaard, 1995). In other cases skin ulcers originate from traumatic lesions in the skin (e.g. due to contact with fishing gear) with the typical appearance as excoriations where epidermis, scales and subcutis are lost, thus exposing the underlying muscular tissue (Lüdemann, 1993; Mellergaard & Nielsen, 1995; Mellergaard & Nielsen, submitted) (Papers 2 & 4) (Fig. 10.9, page 41). Acute traumatic skin lesions have the colour of the exposed tissue. If the lesions are infected by bacteria they appear read due to the inflammatory processes.

Healing ulcerations are partly open with white to brown pigmentations in the scar tissue. Healed ulcers show complete closure of the lesions. If scales are lost these will not regenerate and damaged scales will often be deformed during the healing process. Scars will appear lighter than the surrounding tissue often surrounded by a zone with darker pigmentation. In cases where scales are lost, the surface of the scars will be smooth.

10.3.3. Effect on host

The effect of skin ulcers on the affected fish depend on the size of the lesions. However, disintegration of the cutaneous surface may impair the osmotic barrier to the surroundings and may lead to osmotic disturbances. Bacterial infections may aggravate this situation and if the fish are unable to eliminate this infection immunologically it may die due to a septicaemia. The present investigation has shown that the condition factor of ulcerated dab is reduced compared to unaffected specimens (Table 10.3). In the coastal area of the eastern North Sea and the Skagerrak this difference is statistically different.

	Unaffected	Skin ulcer
German Bight	0.9356	0.9225
Offshore central area	0.9699	0.9520
Coastal central area	0.9589	0.8929**
Skagerrak	1.0218	0.9835*
Kattegat	0.9820	0.9852

(* $p<0.05$)(** $p<0.01$)

Table 10.3. The condition factor of unaffected and skin ulcer affected dab in different areas (partly extracted from Paper 4, table 4)

As for lymphocystis and epidermal papilloma it is mainly the 3 to 5 year old fish which are affected by skin ulcers (Fig. 10.10). These fish have the size where they are caught in commercial fishing gear and may get their primary skin lesions either by escaping the gear (trawl and gillnet) or by being discarded as undersized fish.

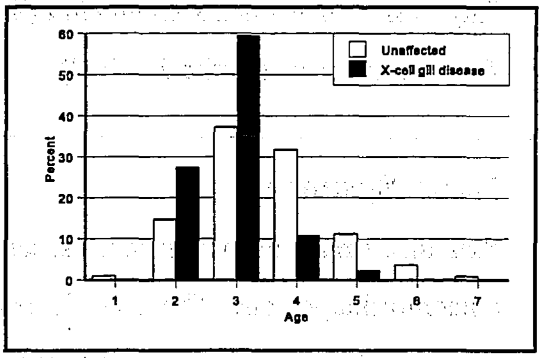

Fig. 10.10. The age distribution of unaffected and skin ulcer affected dab

10.3.4. Response to stress

Skin ulceration is one of the diseases recommended for use in environmental monitoring programmes (Bucke *et al.* 1996). Environmental stress caused by fluctuations in the salinity has been proven to trigger the outbreak of skin ulcerations (Vethaak, 1992). However, the present investigation did not show any effect of environmental stress caused by oxygen deficiency on the prevalence of skin ulcers.

10.4. X-cell gill disease

X-cell gill disease is a disease affecting dab which causes an accumulation of masses of large cells which completely fill the area between the secondary gill lamellae. This cell accumulation results in an enlargement of the gill lamellae which impairs the respiratory capacity of the fish thus resulting in severe debilitation.

10.4.1. Agent

The precise aetiology of X-cell gill disease is unknown. However, some studies have suggested that the large cells (the so-called X-cells) are protozoan parasites (Dawe, 1981; Watermann & Dethlefsen, 1982; Harshbarger, 1984) The disease is widely distributed in the North Sea and the Kattegat where it occurs in low prevalence (<1%) except in distinct patches where it occurs at high frequencies (up to 38%).

10.4.2. Symptoms

Dab affected by X-cell gill disease are generally emaciated with slightly elevated operculum. The gills are pale compared to healthy specimens (Fig. 10.11, page 41). The gill lamellae are swollen and often protrude from the elevated gill cover. The lamellae appear light pink in moderate cases ande creamy white in severe cases (Fig. 10.12, page 41).

10.4.3. Effect on host

X-cell gill disease significantly reduces the growth and condition factor of the affected fish (Tables 5, 6 & 7; Mellergaard & Nielsen, 1996) (Paper 3). It is mainly the young dab (2 to 3 years old) that are affected (Fig. 10.13) Almost 90 % of the affected fish are within this age interval. This is further elucidated in Fig. 8, Paper 3 which clearly demonstrates that the infection mainly affects the 2 and 3 year old fish in each cohort examined.

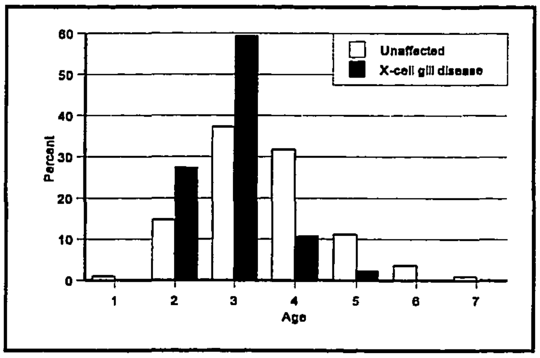

Fig. 10.13. The age distribution of unaffected and X-cell gill disease affected dab

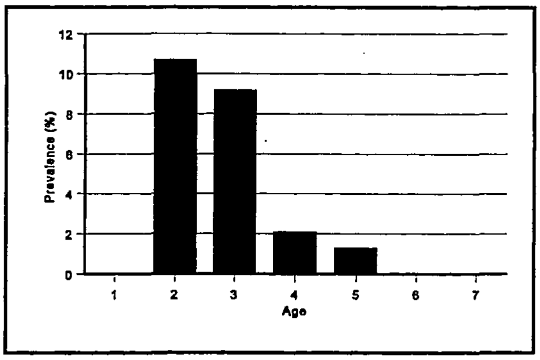

Fig. 10.14. Prevalence of X-cell gill disease per age group

The disease seems to affect the normal physiology of the fish as affected dab did not show any gonadal development (Knust & Dethlefsen, 1986). X-cell gill disease is probably fatal to the host as suggested based on the general impression of the condition of affected fish and Fig. 9 in Paper 3 where the slope of affected fish and Fig. 9 in Paper 3 where the slope of the regression line of the abundance per age group is steeper for the affected than for the healthy population. In addition, the prevalence is approximately 10% for age group 2 and 3 followed by a rapid decline in the group of 4 and 5 year old fish (Fig. 10.14).

Recovery has been observed in a few X-cell gill disease affected dab held in tanks under laboratory conditions (Diamant & McVicar, 1987) but most fish are expected to die from the infection.

10.4.4. Response to stress

In contrast to observations on lymphocystis and epidermal papilloma there is no indication that the frequency of X-cell gill disease is affected by environmental stress. The mechanisms involved in the patchy distribution of the disease are unknown.

Fig. 10.1. Lymphocystis lesions, single nodules and large clusters, affecting the fin of dab

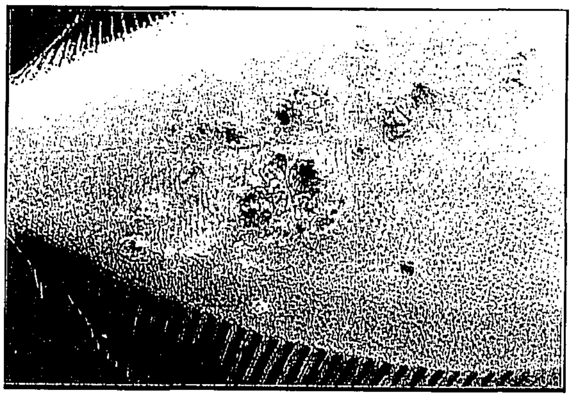

Fig. 10.2. Lymphocystis nodules on the body surface of dab

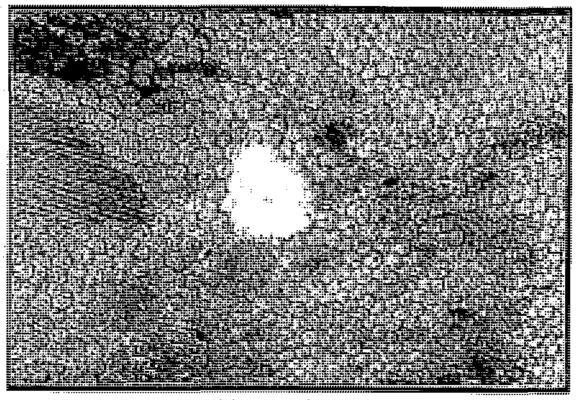

Fig. 10.5. Epidermal hyperplasia on the body surface of dab.

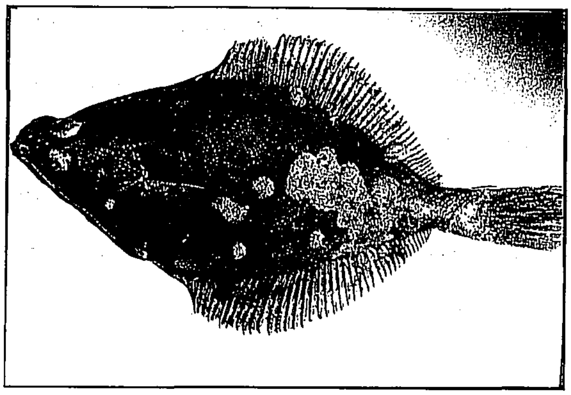

Fig. 10.6. Epidermal papillomas, single and fused, on the body surface of dab

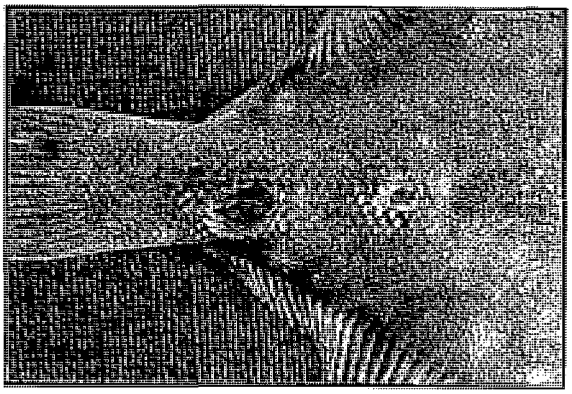

Fig. 10.9. Infected skin ulcers which originate from traumatic lesion

Fig. 10.11. X-cell gill disease. Note the bright red colour of normal the gill (above) and the creamy white colour of the X-cell gill disease affected dab (below)

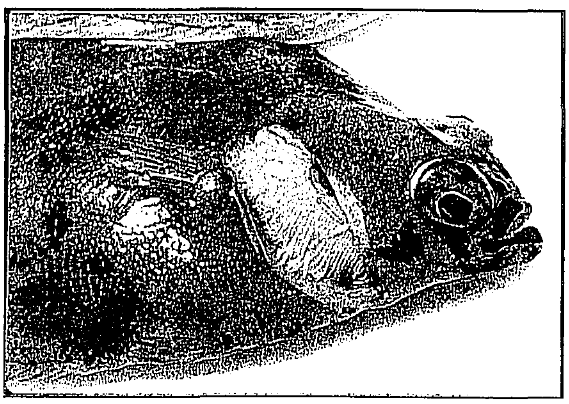

Fig. 10.12. X-cell gill disease affected dab with severely swollen and pale gill lamellae

11. Final comments

Before starting field studies of fish diseases, it is important to have a proper knowledge of the biology of the target fish e.g. migration pattern. There is, for example, no sense in using a migratory fish in an attempt to elucidate the effect of a point source on the disease status of this specific species as it may be influenced by many other, even stronger, stressors during its migration. Relatively stationary demersal fish like dab and flounder are to be preferred for this purpose. Lack of knowledge of the migratory behaviour of cod in the Elbe estuary might have led to a misinterpretation of the observation of a high prevalence (50%) of skeletal deformities during the months of June and July (Möller, 1984). It appeared that the stock density was extremely low in June and July and the high prevalence of deformed fish was likely to be due to the abnormal migratory behaviour of these fish. Thus, these fish were probably not able to migrate together with the normal population.

Care should be taken when considering skeletal deformities. Many of these lesions may have been induced during the larval stage and may, therefore, not reflect the environmental status of the habitat of the adult fish. Furthermore, electroshock caused, for example, by lightening may also induce vertebral damage like fractures due to extreme contractions of the musculature because of the electric stimulus (Spencer, 1967).

Environmental stress plays an important role in the pathogenesis of diseases in fish. However, in most cases, the environmental stress has a multifactorial background making it difficult to estimate the proportion that each factor contributes to the final outcome. Many attempts have been made to prove direct cause-effect relationships between environmental factors (such as contamination by various metals and organic substances) and the occurrence of fish diseases in field studies. However, in most cases, it has only been possible to establish a circumstantial relationship. Direct evidence can only be established in laboratory studies.

12. Main conclusions

The present study has demonstrated adenovirus-like particles in association with epithelial hyperplasia and papilloma in dab. It is likely that adenovirus are responsible for the development of these skin lesions as this disease responds in the same way as lymphocystis (caused by iridovirus) to environmental stressors (e.g. oxygen deficiency).

The results obtained in the Kattegat showed a correlation between oxygen deficiency and the viral diseases, lymphocystis and epidermal hyperplasia/papilloma. Oxygen deficiency triggered outbreaks of these diseases and the prevalence increased during the years with impaired oxygen conditions. Subsequently, the prevalence decreased to the background level. The prevalence of skin ulcerations only showed minimal year to year variation and did not follow any trend as observed for the other diseases.

Similar observations were made in the German Bight and the eastern North Sea. Although base line data from the period prior to the occurrence of the oxygen deficiency were not present, the trend in the prevalence of lymphocystis and epidermal hyperplasia/papilloma was similar to the one observed in the Kattegat in association with oxygen deficiency. Furthermore, lymphocystis and epidermal hyperplasia/papilloma acted similarly to the so far "unknown" factor causing the increase in the prevalence rate of these diseases in 1988. As observed in the Kattegat, the prevalence of skin ulcerations was not affected by environmental stress caused by oxygen deficiency or by the above mentioned "unknown factor". This may be due to the fact that the aetiology of most of the skin ulcers observed during this study probably was a primary mechanical trauma which was secondarily infected by bacteria.

X-cell gill disease did not seem to be affected by environmental stressors as observed for lymphocystis and epidermal hyperplasia/papilloma. The disease was observed in three "hot-spot" areas from which the affected dab probably were unable to move due to respiratory constraint caused by the severe gill lesions. In contrast to the other diseases dealt with in this study X-cell gill disease is probably lethal to the affected fish.

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14. Paper 1

Bloch, B., Mellergaard, S. & Nielsen, E. 1986. Adenovirus-like particles associated with epithelial hyperplasias in dab, *Limanda limanda* (L.). Journal of Fish Diseases, 9; 281-285.

Journal of Fish Diseases 1986, **9**, 281–285

SHORT COMMUNICATION

Adenovirus-like particles associated with epithelial hyperplasias in dab, *Limanda limanda* (L.)

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During a combined fish stock assessment and fish disease survey in the eastern North Sea and Skagerrak in May 1983 and 1984 a number of dabs, *Limanda limanda* (L.), bearing epithelial hyperplasias or papillomas were observed in certain areas, especially in one area approximately 30 miles west of Thyboroen in which oxygen deficiency had been reported in the summer periods since 1981 (Pope & Portmann 1982).

The prevalence of epithelial hyperplasias or papillomas among dabs in this area had showed an increasing tendency from 1983 to 1984; 1·5% (n=401) in May 1983 (Mellergaard & Nielsen 1984) to 2·3% (n=2415) in May 1984. The mean age of the affected fish was 4 years and none of the fish were less than 3 years old.

The epithelial hyperplasias or papillomas were mainly seen as small, rounded, half-transparent, creamy white patches with a smooth surface measuring 2–10 mm in diameter (Fig. 1). Some larger more irregular patches with a diameter of 5–15 mm, some of which were fused together, were also observed, but less frequently. These patches had a rougher surface through which red cords representing blood vessels were evident, indicating that these were true epithelial papillomas.

During the 1984 cruise some of the small patches were collected for histological examination and fixed in 10% phosphate buffered formaldehyde. The specimens were embedded in paraffin wax and 4-μm-thick sections were stained with Mayer's haematoxylin and PAS-haematoxylin.

While normal dab epithelium consists of five to ten cell layers, these excrescences consisted of up to 50 layers (Fig. 2). The surface of the epithelium was smooth and appeared normal. The small patches appeared to be epidermal hyperplasias. In a few specimens an incipient folding of the epithelium covering small extensions of dermal connective tissue was seen. This observation indicates that the epidermal hyperplasias might be the initial stage in the development of epithelial papillomas. The basal cells, which were mainly vertically arranged, were low prismatic to cuboidal. The central part of the epidermal cells was more horizontally arranged and polyhedral in shape. These cells were larger than the basal cells. The superficial cell layers consisted of horizontally arranged, elongated and flattened cells. The hyperplasia contained eosinophilic granu-

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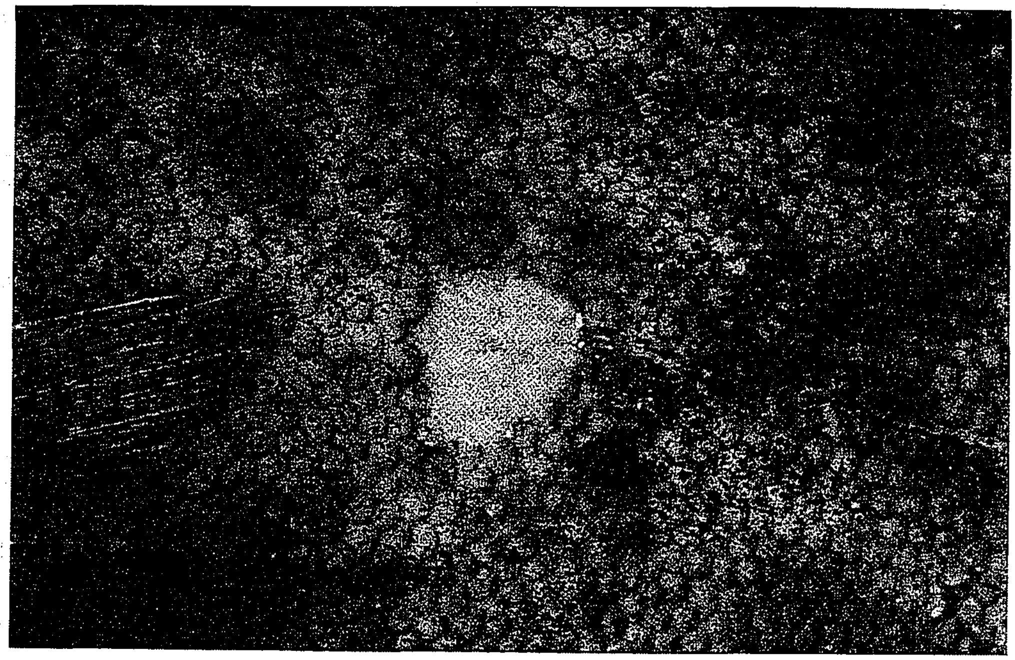

Figure 1. Half-transparent epithelial hyperplasia on the pigmented side of dab (×3).

Figure 2. Section showing the transitional zone between the epithelial hyperplasia (left) and the normal skin (right) (×200).

lated cells, but mucous cells were almost absent. Below the hyperplasia the pigment cells, which normally form a continuous cell layer, were irregularly dispersed.

In order to investigate the possible presence of virus or virus-like particles in the hyperplasias, four of the formaldehyde-fixed specimens representing four different fish were examined electron microscopically. The specimens were post-fixed in 1% osmium tetroxide in 0·13 M phosphate buffer and embedded in Vestopal-W (Serva). Sections stained with uranyl acetate and lead citrate were examined in a JEM 100 B electron microscope.

In sections of all four tumours examined inclusions of virus-like particles were found in nuclei of otherwise normal-looking cells usually situated near the surface of the skin (Figs 3 & 4). In a few cases the particles were also found in cellular debris attached to the surface. In one tumour only a few cells containing a few virus-like particles were found. In another tumour the virus-containing cells were numerous and could be seen until approximately 20 cell layers from the surface, i.e. the outer third of the epithelium. The number of virus-like particles varied in this and the two other cases between a few and 200 particles per section of nucleus. In a few cells bundles of fibrillar material were found close to the inclusions of the virus-like particles, but apart from these no other inclusions of material were observed. As both hexagonally and pentagonally outlined particles were observed the particles were considered to be icosahedrons. They measured approximately

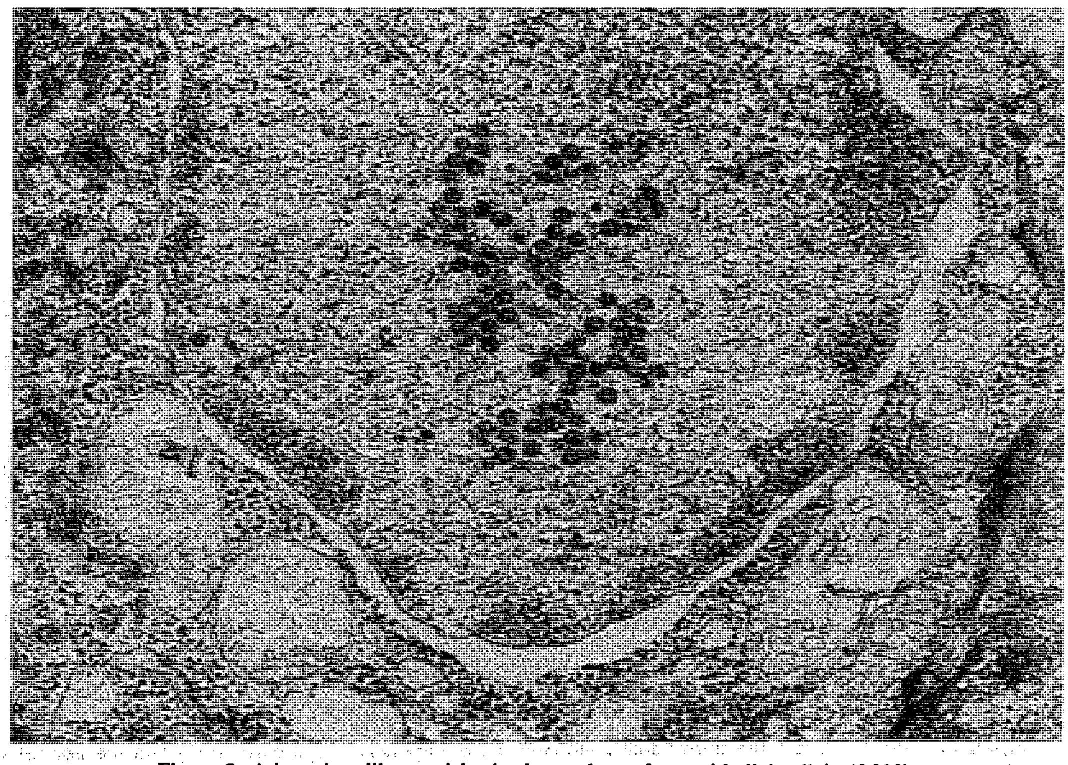

Figure 3. Adenovirus-like particles in the nucleus of an epithelial cell (×40 000).

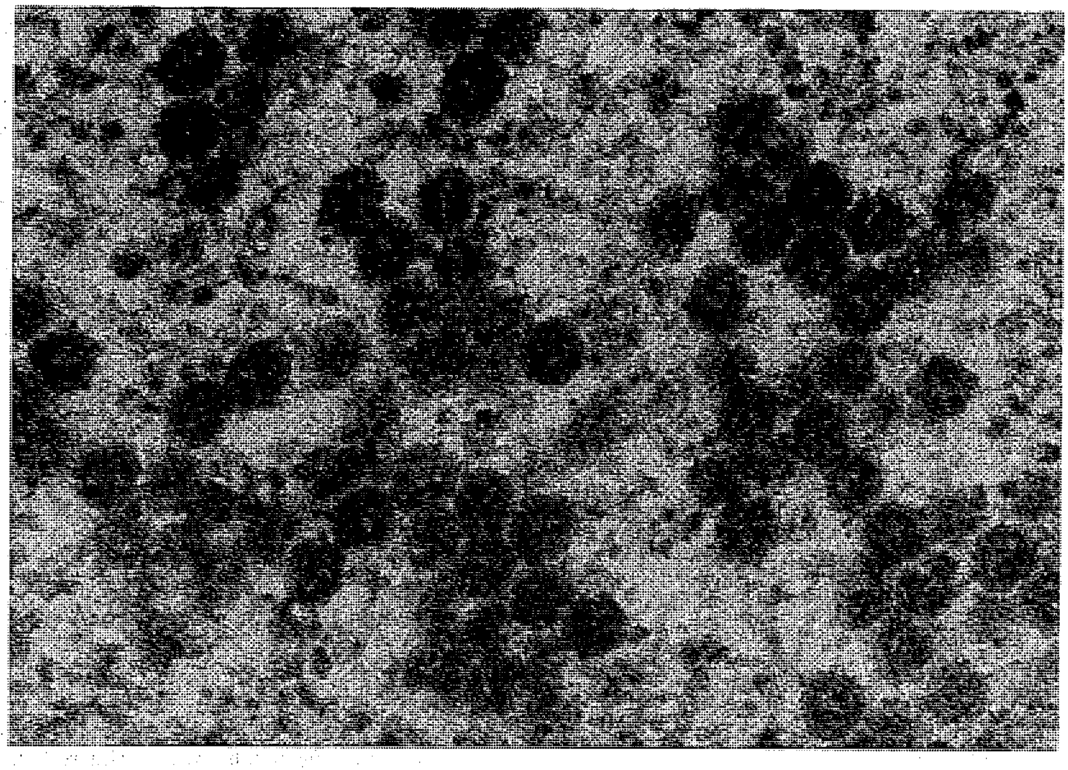

Figure 4. The same group of adenovirus-like particles as shown in Fig. 3. Note the pentagonally outlined particle at the left side of the picture (×135 000).

80 nm from vertex to vertex. The average diameter was approximately 70 nm measured in the least compressed direction. The non-homogeneously stained core was surrounded by a capsid approximately 7 nm thick.

Based on the intranuclear localization of the particles, their size and morphology it seems likely that the particles are adenovirions or adenovirus-like virions. The particles differ from human adenovirus by containing a more loosely packed core which results in low contrast between the capsid and the core. The particles share these features with the adenovirus-like particles associated with epithelial hyperplasias in cod, *Gadus morhua* L., (Jensen & Bloch 1980) and the average diameter of the present particles is approximately the same diameter as the cod virions.

Both the vertex to vertex as well as the face to face dimensions of the present particles equal the corresponding values of adenovirus type 5 determined on freeze-dried virions by Nermut (1975). However, differences in methodology make a comparison somewhat uncertain. The adenovirus-like particles were not observed in the adjacent normal epithelium.

The presence of epithelial tumours in Pleuronectidae has previously been reported by Johnstone (1925), McArn, Chuinard, Miller, Brooks & Wellings (1968), McCain, Gronlund, Myers & Wellings (1979), Dethlefsen (1980) and Möller (1981), but the presence of virus-like particles associated with epidermal hyperplasias in dab has not previously been reported.

However, hexagonal and pentagonal virus particles have been described from an angioepithelial polyp in starry flounder, *Platichthys stellatus* (Pallas), (McArn *et al.* 1968) but these particles were only half the size of the present adenovirus-like particles.

The increasing trend of the prevalence of epithelial hyperplasias and papillomas in an area with seasonal oxygen deficiency might be due to the impact of environmental stress on the fish. Because of the stress condition the fish will be less resistant to infections, e.g. viral infections.

Attempts at cultivating the virus-like particles in fish cell cultures for further characterization, as well as investigations on the distribution of adenovirus-associated epidermal hyperplasias and papillomas in dabs from the eastern North Sea, Skagerrak and Kattegat will be initiated in the near future.

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15. Paper 2

Mellergaard, S. & Nielsen, E. 1995. Impact of oxygen deficiency on the disease status of common dab, *Limanda limanda*. Diseases of Aquatic Organisms, 22; 101-114.

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Impact of oxygen deficiency on the disease status of common dab *Limanda limanda*

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ABSTRACT: An epidemiological survey of the fish diseases lymphocystis, epidermal papilloma and skin ulcers in common dab *Limanda limanda* L. was conducted in the southern Kattegat each year in May from 1984 to 1993. During the period of investigation, severe oxygen depletion occurred in late summer 1986 and 1988. After the oxygen deficiency in 1986, the occurrence of lymphocystis and epidermal papilloma increased and peaked in 1989 with prevalences of 14.7 and 3.3%, respectively. The prevalence of skin ulcers never exceeded 0.6%. The relative risk of contracting lymphocystis increased significantly from 1987 to 1991 compared with 1984 to 1986, the period prior to the severe oxygen depletion. A significant increase in the relative risk of contracting epidermal papilloma was observed from 1987 to 1990. Females were 3 times more likely to contract this disease than males. The relative risk of skin ulcers did not change significantly during the investigation period. The prevalence of lymphocystis and epidermal papilloma was negatively correlated with the minimum oxygen levels measured in August and September the previous year; this negative correlation was significant ($p < 0.05$) for lymphocystis in September, while not for epidermal papilloma ($p < 0.1$). The prevalence of lymphocystis and epidermal papilloma was significantly correlated ($p < 0.01$). No significant correlation was observed between stock density (expressed as catch per unit effort) and the diseases in question. It is probably the stress caused by the oxygen deficiency — especially the sublethal levels — that triggered the outbreak of the 2 viral diseases lymphocystis and epidermal papilloma.

KEY WORDS: Oxygen deficiency · Dab · Lymphocystis · Epidermal hyperplasia/papilloma · Skin ulceration · Epidemiology

INTRODUCTION

The occurrence of diseases in wild fish stocks is not a new phenomenon. Most of the lesions observed in flatfish today, during fish disease surveys, had already been described at the beginning of this century (Johnstone 1905, 1925). However, the prevalence of the diseases was not registered at that time.

The close relationship between environmental stress and the outbreak of fish diseases has been widely accepted since the early 1970s (Wedemeyer 1970, Snieszko 1974), and since then a series of investigations into the impact of pollution on diseases in natural fish stocks have been conducted. The first considerations and results of work on the monitoring of the biological effects of marine pollution were summarized by McIntyre & Pearce (1980).

In the following years, a series of studies were carried out in many countries, especially in those bordering the North Sea and in the USA (Christensen 1980, Dethlefsen 1980, 1984, Möller 1981, Despres-Patanjo et al. 1982, Dethlefsen & Watermann 1982, Mellergaard & Nielsen 1984, 1985, Vethaak 1991, 1992, 1993). However, most of the work done in this field has been concerned with short-term investigations in relatively restricted areas (McArdle et al. 1982, Bucke et al. 1983, Möller 1984, Bucke & Nicholson 1987).

Long-term disease investigations are needed to assess the impact of temporal and spatial variations of environmental parameters on the disease status of fish stocks. Such investigations of the abundance and the dynamics of fish diseases covering large sea areas have been reported by Dethlefsen et al. (1987) and Dethlefsen (1990), who covered most of the North Sea in the periods 1979 to 1986 and 1979 to 1989, respectively, by Banning (1987), who concentrated on the southeastern part of the North Sea in the period 1981 to 1985 and by Mellergaard & Nielsen (1990), who dealt with the Danish coastal zones.

The present work describes a long-term study of the epidemiology of selected externally visible skin lesions on dab in an area of the southern Kattegat, and the possible association of the prevalence of the lesions with oxygen deficiency during the investigation period. Other parameters that might be of importance as stress factors are also included.

MATERIAL AND METHODS

Fish sampling. Common dab *Limanda limanda* L. were sampled annually in May from 1984 to 1993 on board the RV 'Dana' using a standard fishing trawl, a Nymplex fishing trawl, Star model. The trawl was either rigged with 12" (30 cm) rubber discs or with 10" (25 cm) bobbins on the foot rope, depending on the bottom conditions in the area of research, and fitted with a foot rope chain. The stretched mesh size in the cod end was 40 mm. Fishing took place at a number of sites where trawl tracks had been available from commercial fishermen. The present work deals with data obtained from 4 sites which were severely affected by oxygen deficiency in autumn 1986 and 1988 (Fig. 1). Standard 1 h hauls were taken at a speed of 3 knots. One or two hauls were taken at each station.

Handling of the sample. The total catch was sorted into species and the dab were subjected to further investigation. A sample of 150 to 250 specimens (a sample size required for the detection of a prevalence of at least 2% with 95% confidence; Martin et al. 1987) was examined, corresponding to 15 to 20 kg per haul. Subsamples were taken at random if the total weight of dab exceeded 20 kg. For all fish examined, the length, weight, sex and health status were registered. After recording the disease, the otoliths from the first 96 fish were removed for ageing later in the laboratory.

Selection of gross lesions. The dab were examined for the presence of the diseases lymphocystis, epidermal hyperplasia/papilloma and skin ulcers using recommended detection procedures (Anon 1989). These diseases have been well described in literature (Möller & Anders 1983, Bucke et al. 1995) in terms of their gross appearance and aetiology.

Lymphocystis (Fig. 2): A viral disease, recognized by the presence of creamy white, occasionally pigmented nodules up to 1 mm in diameter situated in the skin (dermis) of the fins and the body. The criterion for recording was the presence of more than 1 surface nodule.

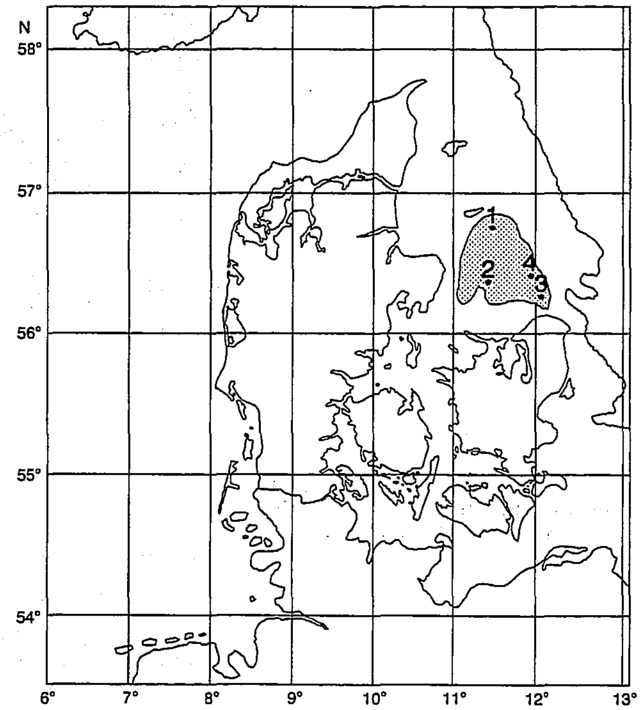

Fig. 1. Location of the 4 sites within the area (dotted) affected by oxygen deficiency in the southern Kattegat

Epidermal hyperplasia/papilloma (Fig. 3): Hyperplasias were recognized as slightly elevated, smooth, opalescent to creamy white areas in contrast to the papillomas which were more elevated, gray to creamy white areas with irregular surface often showing haemorrhages. In some cases, they appeared darkly pigmented. In the present work, epidermal hyperplasias and papillomas are both designated 'epidermal papillomas'. The aetiology is probably viral (Bloch et al. 1986). The criterion for recording was 1 or more lesions larger than 2 mm in diameter.

Skin ulcerations (Fig. 4): Usually irregular rounded lesions which, if infected by bacteria, appear haemorrhagic. In the process of healing, the periphery becomes pigmented. The aetiology is probably primary traumatic skin damage followed by a secondary infection by bacteria (Mellergaard & Nielsen 1990). The criterion for recording was 1 or more open lesions.

Macroscopic inspection. Prior to external inspection the fish were rinsed with sea water. Both sides of the fish were carefully examined visually and by palpation and the fins were spread and lifted. The examination took place under a strong light source.

In order to obtain the highest degree of precision in diagnosing the diseases, only 1 person conducted the registration during the whole period of investigation. The otoliths were read by 2 skilled persons.

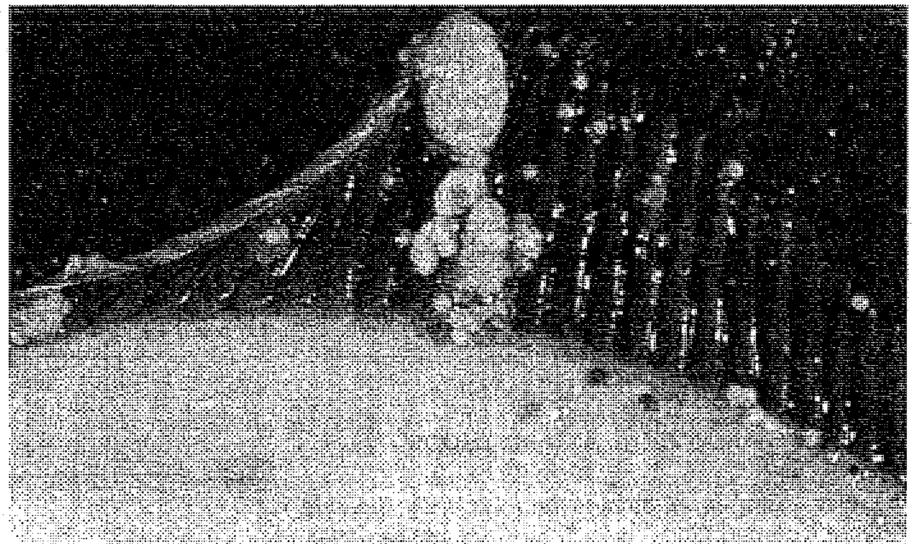

Fig. 2. *Limanda limanda*. Lymphocystis. Clusters of nodules on the fins typical of lymphocystis

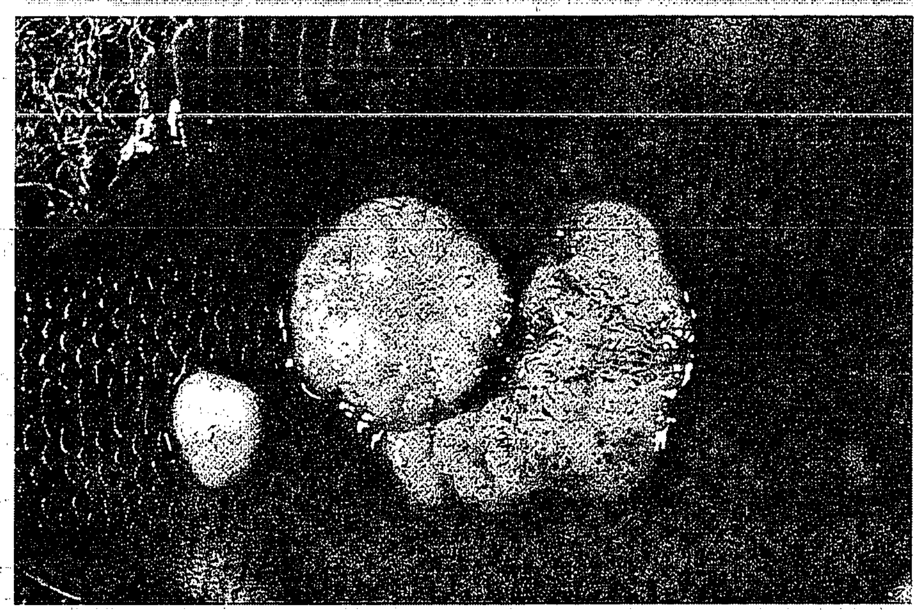

Fig. 3. *Limanda limanda*. Epidermal hyperplasia/papilloma. Multiple epidermal papilloma on the pigmented side of a dab

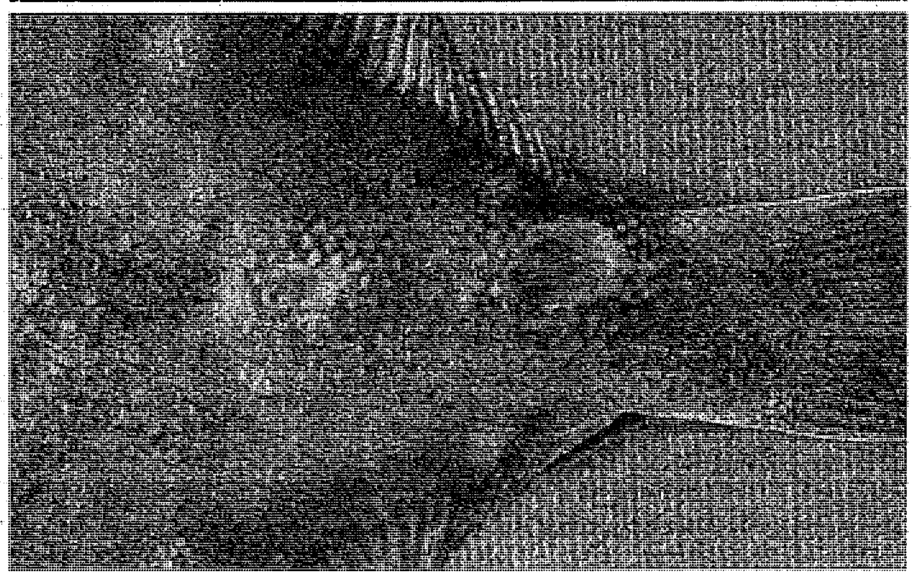

Fig. 4. *Limanda limanda*. Skin ulcer. Typical abrasion-like ulceration

Data analysis. Most of the data analysis was carried out using the public domain software 'Epi-Info' version 5.0 (Dean et al. 1990). The strength of association between a factor and a disease is known as the relative risk (*RR*). The *RR* is calculated as the ratio between the disease rate in an 'exposed' group and the disease rate in an 'unexposed' group. All *RR* estimations are presented with 95% confidence intervals. Stratified analysis was carried out for the factors site, sex and age (age 3 to 6 yr, as these were the predominant groups). The Mantel-Haenszel summary *RR*, which is a weighted average of the separate *RR*, was calculated for all strata to control confounding. The proportion of the disease in the 'exposed group' due to a certain exposure, e.g. oxygen deficiency, is called the attributable fraction and was calculated as $(RR-1)/RR$ (Martin et al. 1987).

Data on disease rates are not normally distributed. Therefore, the nonparametric Wilcoxon signed rank test was used for testing differences of the mean between paired data, Kruskal-Wallis 1-way ANOVA for testing differences of the mean of several groups and Spearman rank correlation test for testing correlation between different variables.

As severe oxygen deficiency first occurred during late summer and early autumn 1986, the disease data from the period 1984 to 1986 should be unaffected by the oxygen depletion, since these were sampled in May. As the *RR* of lymphocystis and epidermal papilloma in 1984 to 1986 did not demonstrate any statistical difference between years, all data from this period were used for characterising the 'unexposed' group (i.e. reference group) of lymphocystis and epidermal papilloma, respectively. As the data on skin ulcers were limited, only selected statistical analysis was conducted for this lesion.

The condition factor [100 × weight (g) × length^{-3} (cm)] was calculated based on the total weight of individual fish (in contrast to ungutted weight as recommended in Anon 1989).

Oxygen data. Data on the oxygen concentrations in the area in August and September were obtained from the ICES Hydrographic Service, ICES Headquarters, Copenhagen, Denmark.

RESULTS

Catch data

All data presented were sampled at 4 stations situated within the oxygen-depleted area (Fig. 1). A total of 11 298 dab were examined during this investigation. Table 1 lists the number of dab inspected each year for externally visible diseases according to sex, site and

Table 1. *Limanda limanda.* Number of dab inspected for externally visible diseases according to sex, site and age

Year	Sex		Site				Age[a]			
	Male	Female	1	2	3	4	3	4	5	6
1984	338	264	218	384	–	–	146	196	117	22
1985	602	650	256	376	406	214	221	207	134	46
1986	550	490	341	213	326	160	95	115	94	34
1987	440	387	263	309	156	99	320	102	20	1
1988	415	404	158	398	206	57	156	139	42	8
1989	475	509	234	411	215	124	150	180	94	17
1990	526	481	269	458	186	94	134	149	71	12
1991	693	644	475	346	246	270	157	164	104	24
1992	741	689	383	564	215	268	144	156	48	8
1993	1101	900	749	764	386	102	207	237	68	25
Total	5887	5418	3346	4223	2342	1388	1730	1645	792	197

[a]The number of aged fish is less than the total number as only a subsample of the fish from each site was aged

Table 2. *Limanda limanda.* Total number of fish examined and fish affected by the 3 diseases investigated

	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Total number examined	602	1252	1040	827	819	984	1007	1337	1429	2001
Lymphocystis	24	38	45	56	117	145	112	86	62	114
Epidermal papilloma	7	11	15	22	23	32	27	16	21	16
Skin ulcers	0	3	4	2	0	6	2	0	2	0

age. In some cases, it was not possible to obtain the minimum sample size but as the data in most of the calculations were pooled for all stations within the affected area, these results were included. The number of aged fish is less than the total number examined because otoliths were only removed from a subsample of 96 dab from each haul. In Table 2, the total number of fish examined and the number of fish affected by each of the 3 diseases are listed for each year.

Disease examination

Lymphocystis

From 1984 to 1986 the prevalence of lymphocystis was 3 to 4% (Fig. 5). In 1987, this increased to 6.8%. This trend continued and peaked in 1989 with a prevalence of 14.7%. From 1990, the prevalence started to decrease and reached 4 to 6% in 1992–93.

In the period 1984 to 1986, the *RR* for contracting lymphocystis should be 1.0, as this was chosen as the 'unexposed' group, not having been exposed to oxygen deficiency. However, to demonstrate the annual variation within this period, the data from 1984 to 1986 are presented in the same way as the rest of the data set (Fig. 6). If the lowest value of the confidence level is more than 1.0, the result is significantly ($p < 0.05$) higher than the reference (unexposed) group. The *RR* in 1984 to 1986 was approximately 1. From 1987, a significant increase of *RR* to between 1.5 and 4 was observed, and this increase continued for the following 2 years, peaking in 1989 with an *RR* of contracting lymphocystis of 4 compared to the period 1984 to 1986. In 1990, a decline started but both the 1990 and 1991 figures were significantly higher than in the 'unexposed' period 1984 to 1986. The 1992 data reached the same level as the reference period while the *RR* in 1993 was just above the significance level.

Analysis of the *RR* of lymphocystis stratified by sites (Fig. 7) showed that for the years 1988 to 1990, the *RR* was significantly higher than in the 'unexposed' period for Sites 1 to 3 while for Site 4, the *RR* became significantly higher from 1987 to 1991. In addition, Site 1 demonstrated a significant increase in 1993. The Mantel-Haenszel weighted *RR* adjusted for sites demonstrated similar values as for the crude *RR*. Hence, the sites do not act as a confounding factor. Kruskal-Wallis 1-way ANOVA did not show significant difference in the annual variations of the *RR* among sites.

Analysis of the *RR* of lymphocystis stratified by sex (Fig. 8) demonstrated that the *RR* in male dab became significantly higher in 1987 than in 1984 to 1986 and remained significant until 1991, and became significant again in 1993. In contrast, the *RR* in female dab

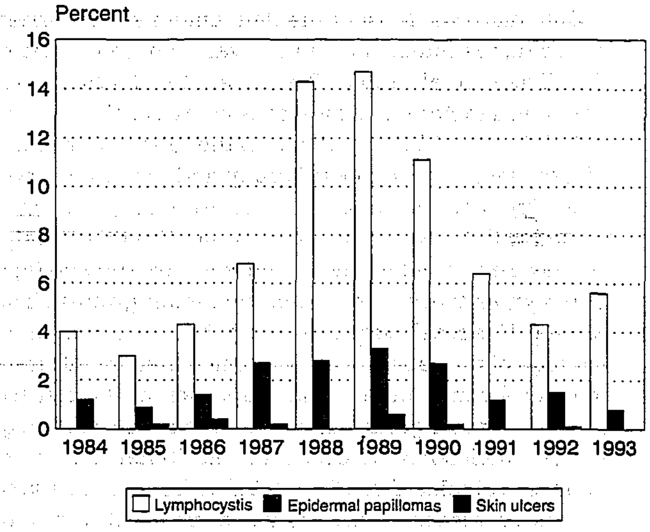

Fig. 5. *Limanda limanda*. Annual prevalence of lymphocystis, epidermal papilloma and skin ulcers

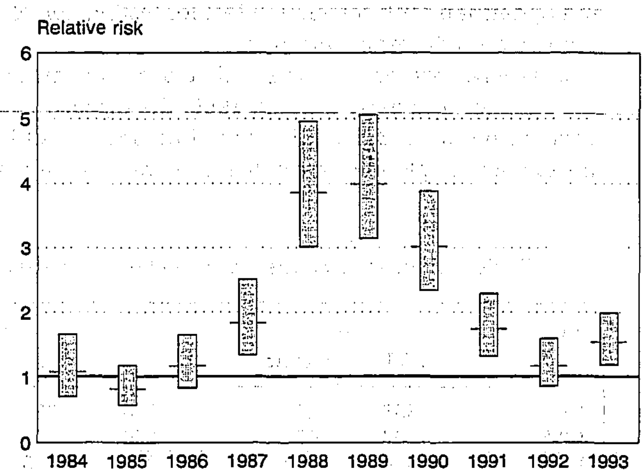

Fig. 6. *Limanda limanda*. Crude relative risk for lymphocystis presented with 95% confidence intervals (bar). Where bars are above 1 (bold line) the figures are significantly different from the 1984 to 1986 figures

did not reach significantly higher levels than those in 1984–86 until 1988, and only remained significant until 1990. The Mantel-Haenszel weighted *RR* adjusted for sex revealed similar values as for the crude *RR*, indicating that sex does not act as a confounding factor for lymphocystis. The *RR* for females contracting lymphocystis compared with males was 1.04. Wilcoxon signed rank test did not demonstrate a significant difference in the *RR* between males and females.

Analysis of the *RR* of lymphocystis stratified by age (Fig. 9) showed that the RR for 3 yr old dab increased significantly in 1987 compared to 1984 to 1986, and

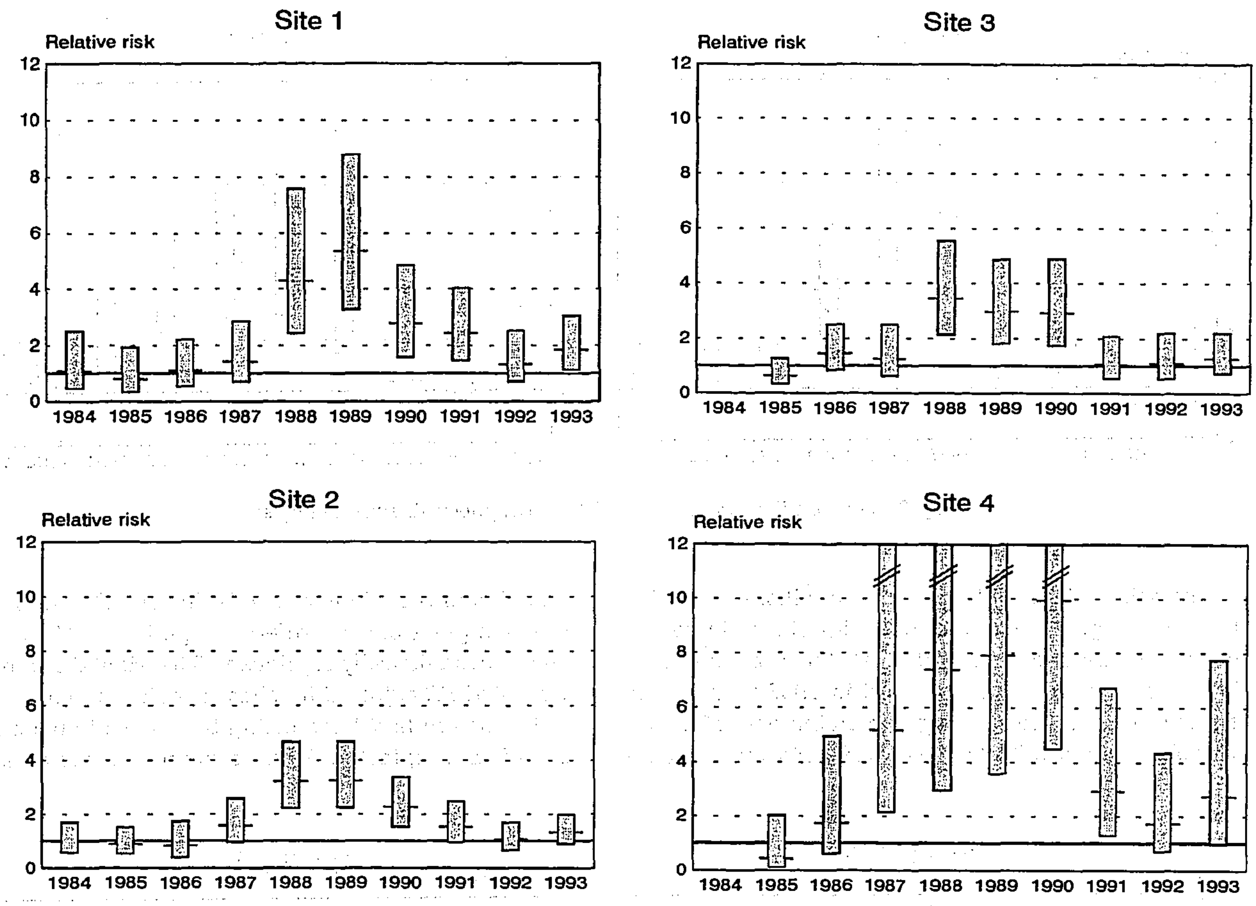

Fig. 7. *Limanda limanda*. Relative risk for lymphocystis stratified by sites

remained significant until 1992. The *RR* for lymphocystis for 4 yr old dab became significantly higher in the period 1988 to 1990 and for the 5 yr old age group levels were only significant in 1989 and 1990. Six yr old fish showed significant levels in the period 1988 to 1990. The Mantel-Haenszel weighted *RR* adjusted for age revealed similar values as for the crude *RR*, indicating that age is not a confounding factor for lymphocystis. Kruskal-Wallis 1-way ANOVA demonstrated no statistically significant difference in the annual variation of the *RR* among the age groups.

As demonstrated in Table 3, the attributable fraction of lymphocystis, which was the estimated increase in the disease rate due to oxygen deficiency, amounted to 45% in 1987, increasing to 75% in 1989. After this, a decline was observed.

Epidermal papilloma

The prevalence of epidermal papilloma varied from 0.9 to 1.4% in the period 1984 to 1986 (Fig. 5). In 1987, an increase was observed and this increase peaked in 1989 with a prevalence of 3.3%. From 1990, a decline began and the starting level was reached in 1991. In the period 1991 to 1993 the prevalence varied between 0.8 and 1.5%.

The *RR* data for epidermal papilloma are presented in the same way as for lymphocystis (Fig. 10). The *RR* for 1984 to 1986 was approximately 1. A significant increase in the *RR* to a level between 2 and 3 was observed from 1987 to 1990, with a peak of 3.3 in 1989. However, the *RR* for the period 1991 to 1993 corresponded to the initial level.

Table 3. *Limanda limanda*. Attributable fraction for lymphocystis and epidermal papilloma for the period 1987 to 1993

	1987	1988	1989	1990	1991	1992	1993
Lymphocystis	0.454	0.741	0.749	0.668	0.425	0.146	0.351
Epidermal papilloma	0.571	0.594	0.649	0.575	0.047	0.224	−0.426

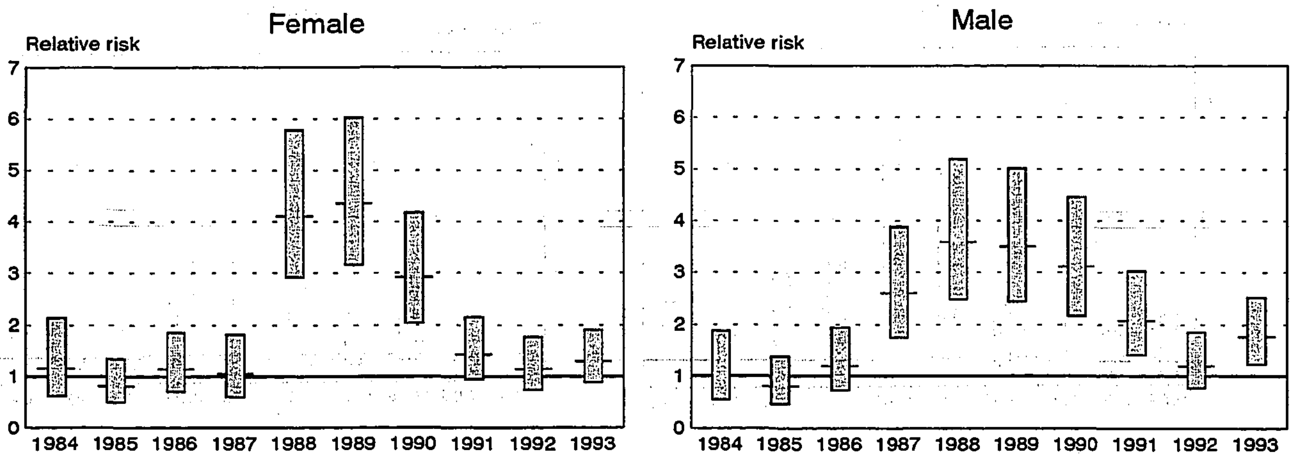

Fig. 8. *Limanda limanda*. Relative risk for lymphocystis stratified by sex

Analysis of the *RR* of epidermal papilloma stratified by sites (Fig. 11) showed that significant increases occurred only at Sites 1 and 3. At Site 1, a significant increase in the *RR* was observed in 1987 to 1990 and again in 1992. At Site 3, a significant increase of the *RR* was observed in 1987, 1989 and 1990. However, Sites 2 and 4 showed similar trends in the *RR*, although the changes from the reference period 1984 to 1986 were not statistically significant. The Mantel-Haenszel weighted *RR* adjusted for sites revealed similar values as for the crude *RR*, indicating that sites did not act as a confounding factor. Because the number of affected

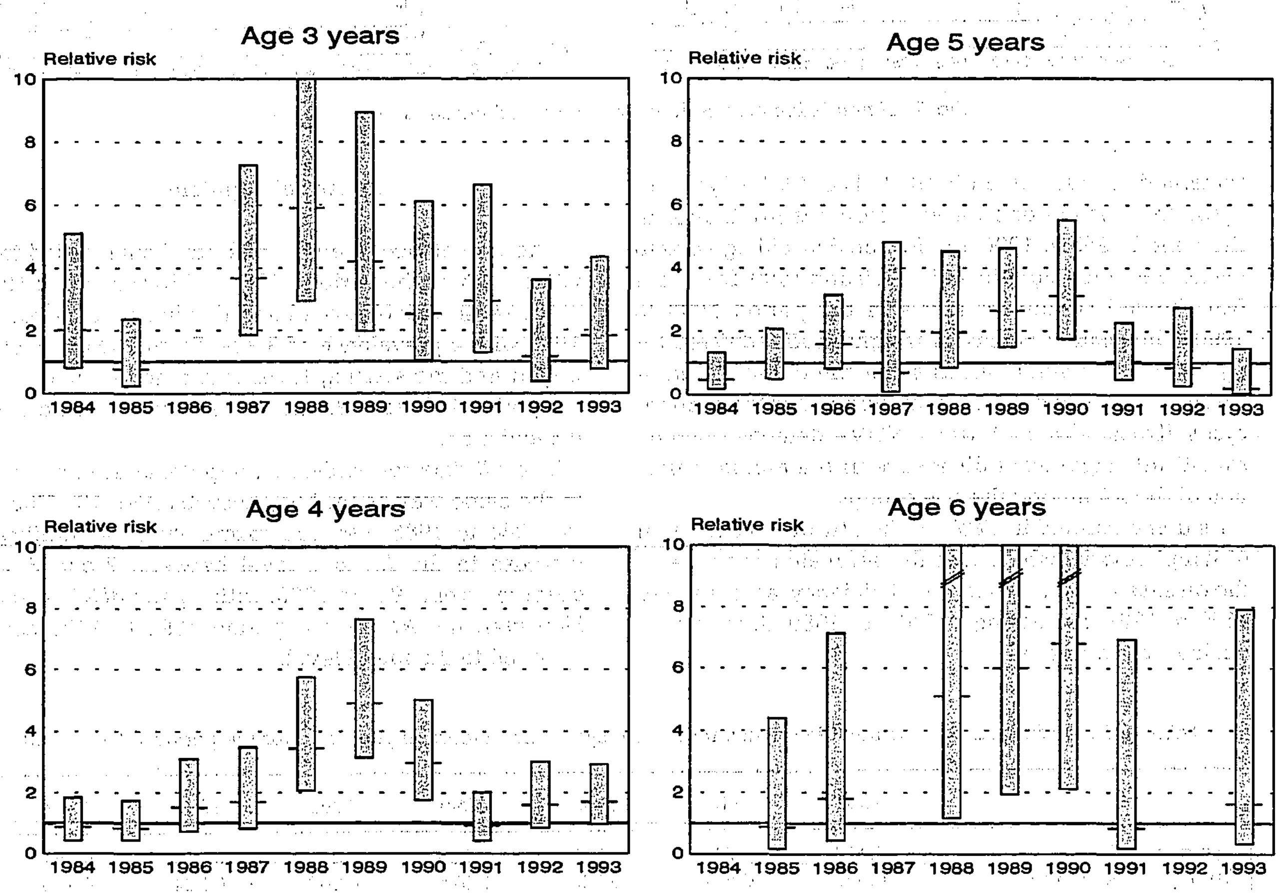

Fig. 9. *Limanda limanda*. Relative risk for lymphocystis stratified by age

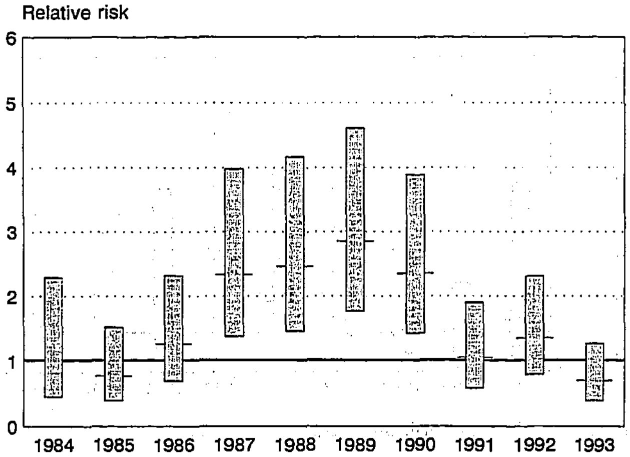

Fig. 10. *Limanda limanda*. Crude relative risk for epidermal papilloma presented with 95% confidence intervals (bar). Where bars are above 1 (bold line) the figures are significantly different from the 1984 to 1986 figures

fish was limited, the confidence level of some of the values was very broad. Kruskal-Wallis 1-way ANOVA did not show a significant difference in the annual variations of the *RR* among sites.

The *RR* of epidermal papilloma stratified into females and males (Fig. 12) showed that the *RR* in females became statistically significant from 1987 to 1990, after which it reached the original level. In contrast to the females, the *RR* of the males became significantly higher than in the 'unexposed' period only in 1989. However, the crude *RR* did not differ from the Mantel-Haenszel weighted *RR* adjusted for sex, indicating that this factor is not to be regarded as a confounder. For females the *RR* of contracting epidermal papilloma was significantly higher ($p < 0.001$) than for males, namely 2.8. The Wilcoxon signed rank test did not demonstrate a significant difference in the annual variation of the *RR* between males and females.

Stratification of the *RR* into age groups (Fig. 13) demonstrated significantly elevated *RR*s only for 3 and 5 yr old fish. For the 3 yr age group, the *RR* in 1987 and 1988 was significantly higher than for the 'unexposed' period while for the 5 yr age group only the *RR* for 1989 became significant. Also in this case, the confidence levels were broad for some of the values because of the limited number of affected dab. The Mantel-Haenszel weighted *RR* adjusted for age revealed similar values as for the crude *RR*, indicating that age is not a con-

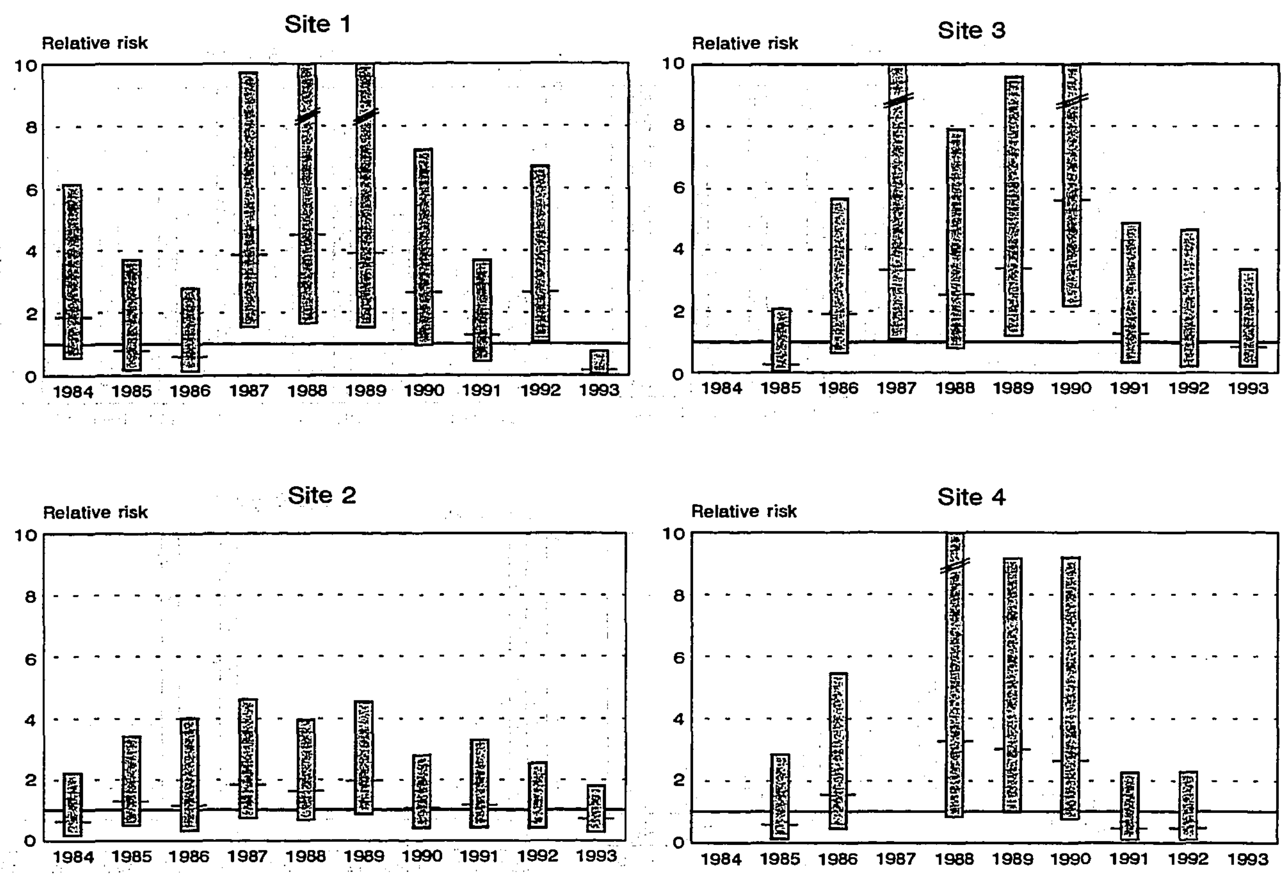

Fig. 11. *Limanda limanda*. Relative risk for epidermal papilloma stratified by sites

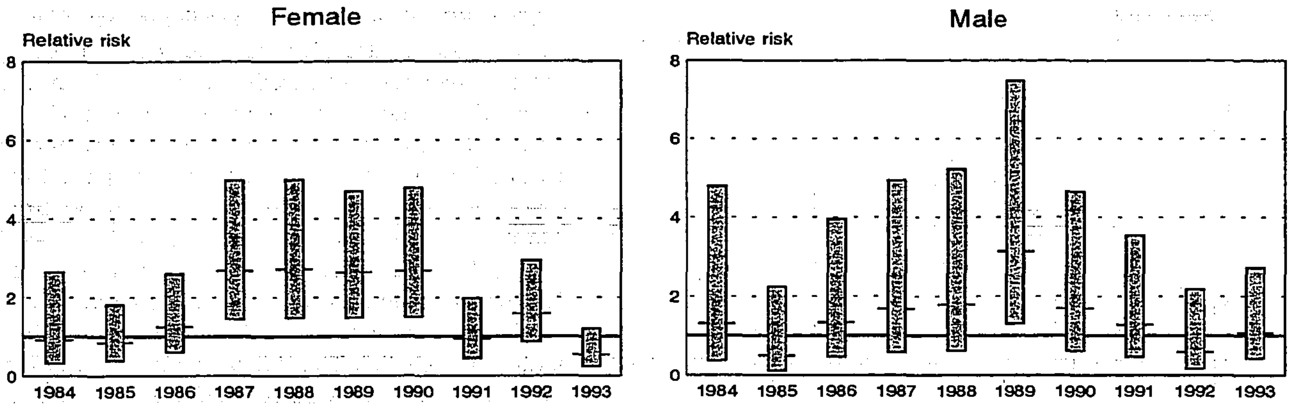

Fig. 12. *Limanda limanda*. Relative risk for epidermal papilloma stratified by sex

founding factor for epidermal papilloma. Kruskal-Wallis 1-way ANOVA demonstrated no statistically significant difference of the annual variation of the *RR* among age groups.

As shown in Table 3, the attributable fraction of epidermal papilloma amounted to 57% in 1987, increasing to 65% in 1989. From 1990, a decline was observed.

Skin ulcers

The prevalence of skin ulcers was low, not exceeding 0.6%. It varied during the whole investigation period without any definite trend (Fig. 5).

The *RR* demonstrated similar fluctuations (Fig. 14), without any significant variations between the periods with and without oxygen deficiency.

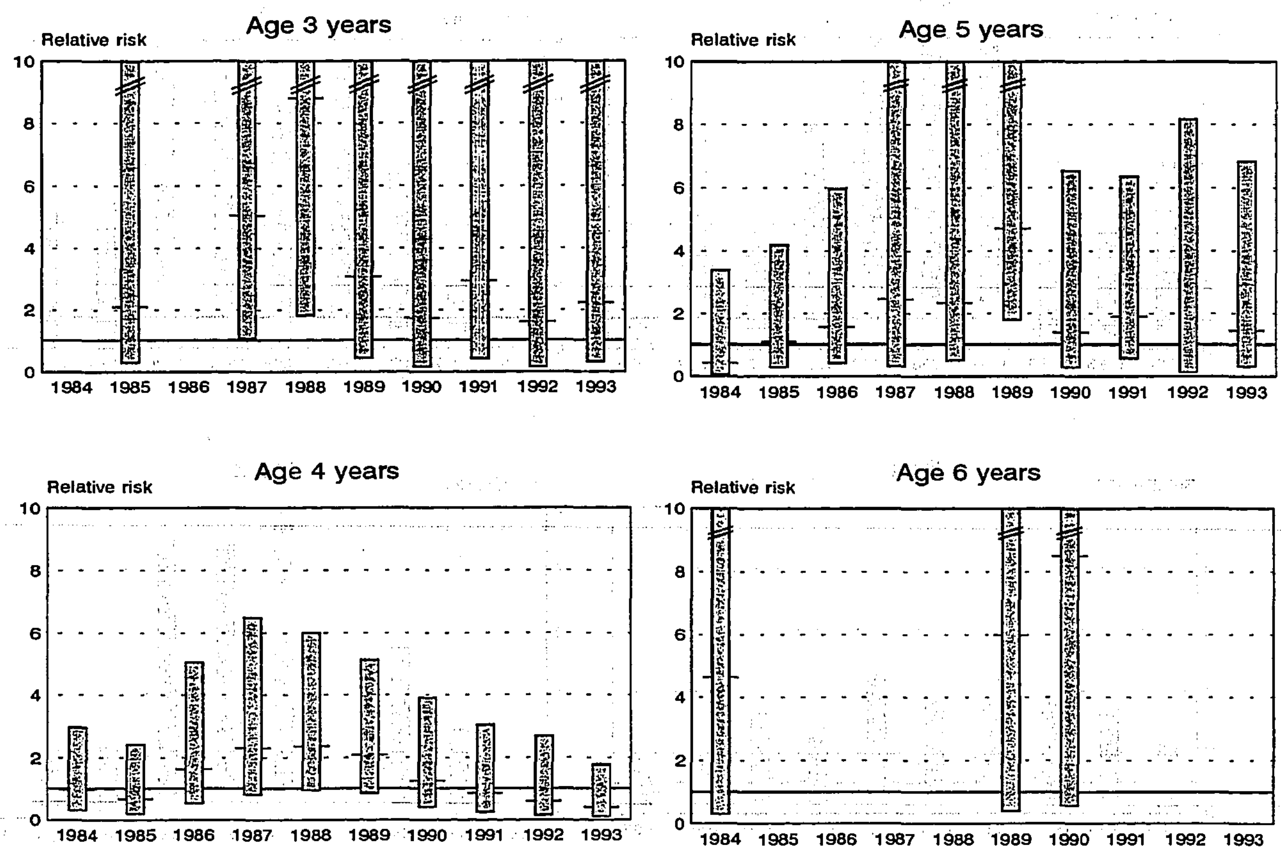

Fig. 13. *Limanda limanda*. Relative risk for epidermal papilloma stratified by age

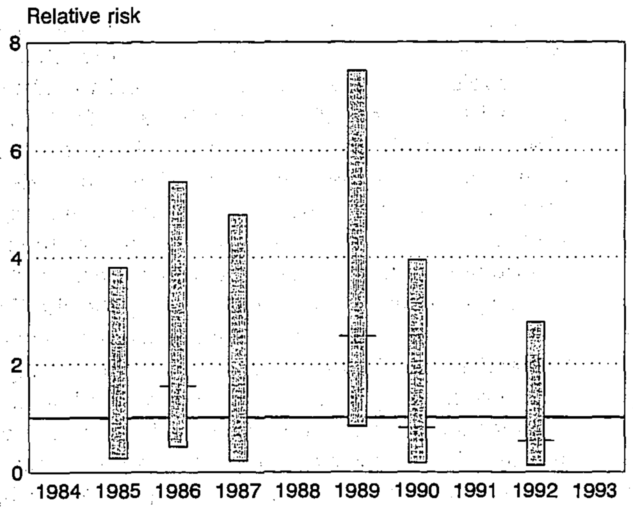

Fig. 14. *Limanda limanda.* Crude relative risk for skin ulcers

Skin ulcers occurred more frequently in female than in male dab. The *RR* of females contracting skin ulcers compared to males was 3.74, a statistically significant difference ($p < 0.01$).

Correlations

Diseases

The Spearman rank correlation test demonstrated a statistically significant positive correlation ($p < 0.01$) between the prevalences of lymphocystis and epidermal papilloma for the period 1984 to 1993, while skin ulcers correlated significantly neither with lymphocystis nor epidermal papilloma.

Diseases and stock density

The catch per unit effort (CPUE), i.e. the number of dab per hour of trawl time for the area, is illustrated in Table 4. The stock density was calculated on the basis of the approximate area fished during 1 h of trawl tow and was within the range 2 to 21 dabs per 1000 m^2. The Spearman rank correlation test did not show a statistically significant correlation between the prevalence of any of the diseases and the stock density in the period 1984 to 1993.

Diseases and condition factor

Evaluation of an eventual correlation between the mean condition factor of the dab population (Table 4) and the prevalence of the different diseases was conducted using the Spearman rank correlation test, and did not show any significant correlation.

Diseases and oxygen deficiency

The test for correlations between diseases and oxygen deficiency was conducted by comparing the prevalence of the different diseases in a year with the mean and minimum oxygen levels measured in August and September the previous year (Table 5), as it was expected that low oxygen levels in the autumn might affect the disease level the following year. The Spearman rank correlation test demonstrated a negative correlation between the different diseases and oxygen level. A statistically significant correlation ($p < 0.05$) was demonstrated for lymphocystis and the minimum oxygen levels in September, while the signifi-

Table 4. *Limanda limanda.* Catch per unit effort (fish h^{-1}) and mean condition factor for dab for the period 1984 to 1993

	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Catch per unit effort	778	343	370	797	1619	2443	421	3263	2614	2200
Mean condition factor	0.973	0.992	0.976	0.986	0.959	0.992	0.984	0.995	0.969	0.953

Table 5. Mean and minimum oxygen levels (ml l^{-1}) for August and September in the oxygen-depleted area of the Kattegat for the period 1983 to 1992

		1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
August	Mean	2.75	3.44	3.86	3.26	3.66	3.03	2.72	2.79	2.79	3.68
	Minimum	2.46	3.36	3.54	3.16	3.15	1.79	2.50	2.39	2.42	2.91
September	Mean	2.93	2.71	3.76	2.15	3.03	1.29	2.85	1.72	2.27	2.47
	Minimum	2.55	2.55	2.13	0.95	1.81	0.46	1.67	1.35	1.69	1.78

cance levels for the similar correlation for epidermal papilloma and for ulcers were $p < 0.1$ and $p < 0.5$ respectively.

DISCUSSION

The present study demonstrated that oxygen deficiency may trigger the outbreak of endemic fish diseases such as lymphocystis and epidermal papilloma while the prevalence of skin ulcers was found to be unaffected. The occurrence of oxygen deficiency in the southern Kattegat has been caused by increasing eutrophication problems up through the 1970s and the early 1980s (Kronvang et al. 1993). Physical (density stratification) and chemical (increasing nutrient levels) conditions in the water body resulted in the occurrence of severe oxygen deficiency in the bottom water in August and September from 1986 to 1988 (Hansen et al. 1995).

The importance of environmental stress in the dynamics of fish diseases is well known (Fry 1969, Wedemeyer 1970, Snieszko 1974). In aquaculture, outbreaks of bacterial diseases in fish species induced by oxygen deficiency have been described previously (Wedemeyer 1981). However, disease outbreaks in wild fish stocks have been associated only in a very few cases with oxygen deficiency. From fresh water, Haley et al. (1967) described an *Aeromonas liquefaciens* epizootic event in American shad *Alosa sapidissima* and threadfin shad *Dorosoma petenense*, probably triggered by the very low dissolved oxygen content in the water of a river basin due to organic pollution. Mellergaard & Nielsen (1987) reported on the correlation between oxygen deficiency in certain areas of the eastern North Sea and increased disease prevalence in dab. These observation were confirmed by Dethlefsen (1990). The present work is the first study in which the long-term effects of oxygen depletion on the health status of fish have been studied.

Flatfish such as plaice *Pleuronectes platessa*, flounder *Platichthys flesus* and common dab have demonstrated the ability to adapt to low oxygen levels (Steffensen et al. 1981, Weber 1993). In the Kattegat, the duration of the periods with oxygen depletion (2 to 3 ml O_2 l^{-1}) in the bottom water was several weeks. So, the fish probably adapted to the low oxygen condition. However, in some instances the oxygen levels were further reduced to lower than 2 ml O_2 l^{-1} (Table 5). Under laboratory conditions dab showed avoidance reactions at oxygen levels of 0.9 to 1.1 ml l^{-1} (Weber 1993) and such reactions probably took place on some occasions in the sea. However, as the oxygen deficiency covered large areas, many fish were probably unable to escape and, while remaining in the area during the extreme oxygen deficiency conditions, they were exposed to concentrations close to the lethal oxygen levels which are reported to be 0.4 to 1.3 ml l^{-1} (Muus 1967, Bagge 1970, Scholz & Waller 1992, Weber 1993). It is probably the sum of all the stress factors occurring during the oxygen deficiency periods that triggers the disease outbreaks. Of these factors, the sublethal oxygen levels are probably one of the main contributors to the outbreak of lymphocystis and epidermal papilloma, which is indicated by the significant correlation between the minimum oxygen levels in September and lymphocystis and the almost significant correlation ($p < 0.1$) for epidermal papilloma.

Under aquaculture conditions, reduction of growth associated with low oxygen levels has been observed in common carp *Cyprinus carpio* (Chiba 1965), in largemouth bass *Micropteris salmonides* (Stewart et al. 1967) and in coho salmon *Oncorhynchus kisutch* as well as chum salmon *Oncorhynchus nerka* (Brett & Blackburn 1981). These fish species demonstrated severe reduction in growth at oxygen levels between 2 and 3 ml l^{-1}. This would probably be the case for dab, too. Therefore, it might be expected that long periods of oxygen deficiency might reduce the condition factor of the fish and hence could increase their susceptibility to diseases. However, no correlation between the condition factor and the diseases in question was observed. This finding corresponds with that of Dethlefsen et al. (1987), who did not observe any correlation between the prevalence of lymphocystis and the nutritional status of dab in the southern North Sea. This finding is in contrast to Möller (1981), who found a significant positive correlation between the condition factor and lymphocystis in dab in the southeastern North Sea, and to Vethaak et al. (1992), who found a high prevalence of lymphocystis associated with low condition factor. In the present study, no difference in the condition factor was observed between the period before the severe oxygen deficiency, 1984 to 1986, and the period after, 1987 to 1993. A possible explanation for this is that the benthic fauna are rapidly re-established, as their pelagic larval stages are unaffected by the oxygen depletion at the bottom and they will settle when the oxygen conditions are normalised.

Stock density expressed as CPUE was not found to be significantly correlated with any of the diseases investigated. The low CPUE found in 1990 is probably not a natural phenomenon but is probably a reflection of bad weather conditions during fishery that year. Even if the CPUE for 1990 was elevated to the 1989 level, the correlation between the CPUE and the different diseases did not become statistically significant. However, despite the fact that a statistically significant correlation between stock density and the diseases in question could not be established, high stock density probably adds to the stress caused by the oxygen deficiency, as the risk of contracting a disease increases with decreasing distance between individuals (Möller 1985).

The attributable fraction or aetiological fraction represents a quantitative expression of the proportion of disease in the fish stock due to the environmental parameters. It appears that up to 75% of the lymphocystis cases and 65% of the epidermal papilloma cases are due to environmental stress factors, in this case mainly oxygen deficiency.

Most of the 19 observed cases of skin ulcers looked like skin abrasions, indicating that the primary cause may be a traumatic injury from fishing gear. If skin ulcers had an infectious, e.g. bacterial, background they would be expected to respond to the impact of stress (Haley et al. 1967). However, conclusions concerning the origin of the ulcers cannot be made based on 19 cases. The fact that the occurrence of lymphocystis and epidermal papilloma was significantly correlated and showed an identical response to oxygen deficiency indicates that both may be of similar aetiological origin, probably viral (Weissenberg 1951, Bloch et al. 1986). Dethlefsen et al. (1987) also found significant positive correlation between the 2 diseases on some occasions, especially during winter.

Spatial distributions of diseases

The spatial distribution of diseases seems to differ in that Site 4 demonstrated a significant increase in *RR* for lymphocystis 1 yr earlier than the other sites, while Site 1 had a significant increase in *RR* for epidermal papilloma 1 yr prior to the other stations. However, when considering the Mantel-Haenszel weighted *RR* adjusted for sites, the effect of oxygen deficiency is reflected in a significant increase in the *RR* for both lymphocystis and epidermal papilloma already the year after the first severe oxygen depletion took place. Additionally, no statistically significant difference in the annual variations of the *RR* among sites was observed.

Sexual difference in susceptibility to diseases

Lymphocystis appeared to be evenly distributed among males and females. However, the *RR* of lymphocystis in females remained at the 1984 to 1986 level 1 yr longer than observed for males, after which it increased to the 'male level'. There are no clear indications on the background for this observation. There was no difference among the sex ratios in 1986, 1987 and 1988 that might explain the delayed response observed in the females.

Vitinsh & Baranova (1976) observed a higher prevalence of lymphocystis in male than in female flounder. Calculations based on their presented data demonstrate an *RR* for females having the disease compared with males ranging from 0.26 to 0.58. The difference was only statistically significant at 1 out of 7 sites. Similarly, Vethaak (1992) found a significantly higher prevalence of lymphocystis in male than in female flounders along the Dutch coast.

Based on figures on dab presented in Dethlefsen et al. (1987), investigations in the German Bight area in January 1981 demonstrated an *RR* of 1.22 for females being infected by lymphocystis compared to males. This observation corresponds with our findings of an even *RR* for both sexes. An even proportion of males and females infected by lymphocystis was also reported in yellowfin sole *Limanda aspera* Pallas (McCain et al. 1979). The observed difference between dab and flounder indicates that there might be a difference in the infection pattern in different fish species.

In contrast to lymphocystis, epidermal papilloma revealed a significant increase in the *RR* for females the year after the occurrence of oxygen deficiency, while a significant response in males was observed only in 1989. A possible explanation for this observation is that the females have a significantly higher risk of contracting epidermal papilloma than males, i.e. 2.8 times. Similar observations were made in the German Bight area in January 1981 (Dethlefsen et al. 1987). Calculation of the *RR* based on their presented data demonstrated a significantly higher risk for females of contracting epidermal papilloma than for males, i.e. 2.3 times. Most female dab had spawned in May in the Kattegat, and this might explain the sexual difference in the susceptibility to epidermal papilloma as female fish in the postspawning period are often in a weak condition. However, if this was the case one would also expect female dab to have a higher risk of getting lymphocystis than males, which is not true. So, it is not clear why females are more susceptible to epidermal papilloma than males.

As for epidermal papilloma, the *RR* of getting skin ulcers was significantly higher (3.7 times) in females than in males. Of the 19 dab with skin ulcers, 13 were between 20 and 25 cm, and within this length group, females made up 67% of the whole population. The mean length of ulcerated female dab (21.5 cm) was not significantly different from the mean length of ulcerated males (20.3 cm). Therefore, the higher risk for ulcerations in female fish is probably because females are longer than males. This fits in with our assumption that most of the skin ulcers observed during this study were primarily due to traumatic skin damage caused by fishing gear, as large fish are more likely to get stuck in the meshes of these gears.

Field investigations are seldom able to present clear-cut proof of the association between specific environmental parameters and the outbreak of fish diseases, as the initial stressors often have a multifactorial background. An attempt to establish a cause-effect relationship is even more difficult when the environmental impact gradually builds up and has a relatively long duration, which gives the fish the opportunity to adapt to the system. However, the present study clearly indicates that oxygen deficiency, especially sublethal oxygen levels, is a very important stress factor for viral diseases such as lymphocystis and epidermal papilloma. Laboratory experiments need to be established to further clarify the implications of different oxygen levels on the outbreak of fish diseases.

The strong association between oxygen deficiency and lymphocystis and epidermal papilloma demonstrated in the present work is apparently not a phenomenon solely localised to the Kattegat region. There are also indications that oxygen depletion is involved in the dynamics of fish diseases in the North Sea (Mellergaard & Nielsen 1987).

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16. Paper 3

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Epidemiology of X-cell gill disease in common dab *Limanda limanda*

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ABSTRACT: An epidemiological study of X-cell gill disease in common dab *Limanda limanda* L. was conducted at a series of stations in the eastern North Sea, the Skagerrak and the Kattegat during the period 1986 to 1993. At most of the stations the prevalence of X-cell gill disease varied from 0 to 1% throughout the whole period of investigation, except at 3 stations where high prevalences, up to 38%, were observed. Spatial and temporal variations of prevalence were observed. On one occasion it was possible to separate a highly affected dab population from a significantly less affected population within a distance of 500 m. This significant spatial variation was not found for the diseases lymphocystis, epidermal papilloma and skin ulcers. At one station the relative risk of contracting X-cell gill disease was significantly higher in female dab compared to males while the opposite was the case at the 2 other stations. X-cell gill disease mainly affected 2 and 3 yr old dab and had a serious effect on the condition of the fish. The condition factor of affected fish was significantly lower compared to healthy specimens. It was not possible to prove an increased mortality in infected fish, although their general condition was extremely poor.

KEY WORDS: X-cell gill disease · Dab *Limanda limanda* · Epidemiology · Low condition factor · Mortality

INTRODUCTION

For the last 15 yr fish disease investigations have regularly been carried out in the North Sea (Möller 1981, Bucke et al. 1983, Dethlefsen 1984, Mellergaard & Nielsen 1984, Banning 1987, McVicar et al. 1988). These investigations have dealt mainly with externally visible diseases such as lymphocystis, epidermal papilloma and ulcerations, but some authors (Dethlefsen 1984, McVicar et al. 1988) also reported observations of severely swollen, pale gills in dab—a condition called X-cell gill disease, based on the presence of so-called X-cells in the gill tissue (McVicar et al. 1987). A more generalized condition of this disease has been described by Diamant & McVicar (1987). Additionally, X-cells have been observed associated with epidermal tumours in flatfish (Brooks et al. 1969, Peters et al. 1978, Peters & Watermann 1979) and pseudobranchial tumours in cod (Alpers et al. 1977, Morrison et al. 1982, Watermann & Dethlefsen 1982).

Reports on the spatial distribution of X-cell gill disease in dab in the North Sea have focussed on the central and southern parts (Knust & Dethlefsen 1986, McVicar et al. 1987, Diamant & McVicar 1989), and the results of these studies demonstrate a certain patchiness of the distribution of the disease.

The endemic occurrence of X-cell disease in most of the North Sea combined with certain epidemic situations indicates that the disease has an infectious background, although the exact etiology has not yet been identified. Some studies suggest that X-cells are protozoan parasites (Dawe et al. 1979, Watermann & Dethlefsen 1982, Harshbarger 1984).

The present work describes the epidemiology of X-cell gill disease in dab in the eastern part of the North Sea and the Skagerrak, and concentrates on 3 areas where the disease occurred in high prevalences.

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MATERIALS AND METHODS

Fish sampling. Common dab *Limanda limanda* L. were sampled annually in May from 1986 to 1993 on board the RV 'Dana' using a standard Nymplex fishing trawl, 'Star' model. The trawl was rigged either with 12" (30 cm) rubber discs or with 10" (25 cm) bobbins on the foot rope—depending on the bottom conditions in the area of research—and fitted with a foot rope chain. The stretched mesh size in the cod end was 40 mm. Fishing took place at a number of sites where trawl tracks were available from commercial fishermen (Fig. 1). Hauls of 1 h were taken at a speed of 3 knots. Normally 1 or 2 hauls were taken at each station, except on special occasions when fishing took place in a grid pattern as described in 'Results: Spatial variations'.

Handling of the sample. The total catch was sorted into species and the dabs were subjected to further investigation. A sample of 150 to 250 specimens [a sample size required for the detection of a prevalence of at least 2 % with 95 % confidence (Martin et al. 1987)] corresponding to 15 to 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, sex and health status were registered. The otoliths were removed from the first 96 fish for ageing.

Selection of gross lesions. The dabs were examined for the presence of the diseases lymphocystis, epidermal hyperplasia/papilloma, skin ulcers and X-cell gill disease using recommended procedures for detection (Anonymous 1989). These diseases have been well described in the literature (Möller & Anders 1983, Bucke et al. 1995) in terms of their gross appearance and etiology.

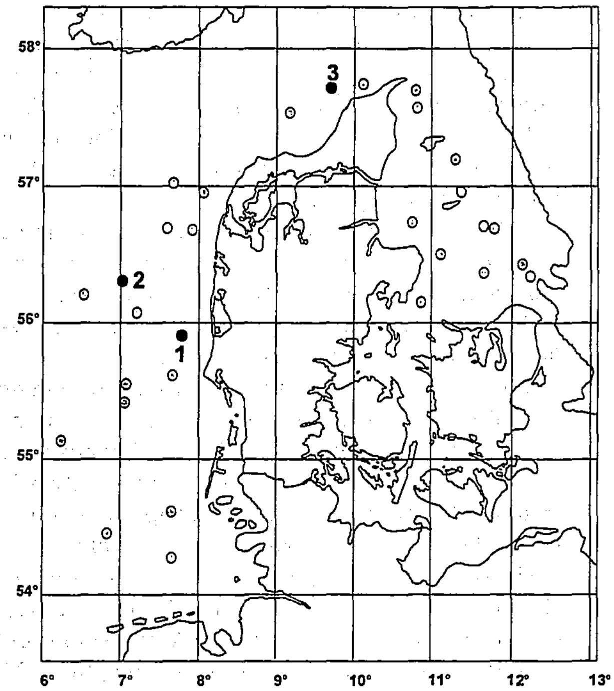

Fig. 1. Stations around Denmark visited during this fish disease survey. The black and numbered localities represent the 3 stations with high prevalence of X-cell gill disease in dab

Fish affected by X-cell disease are generally emaciated with slightly elevated operculum. The gills are pale. The gill lamellae are swollen and appear light pink in moderate cases while creamy white in severe cases (Fig. 2). X-cell gill disease was registered from 1986 onwards.

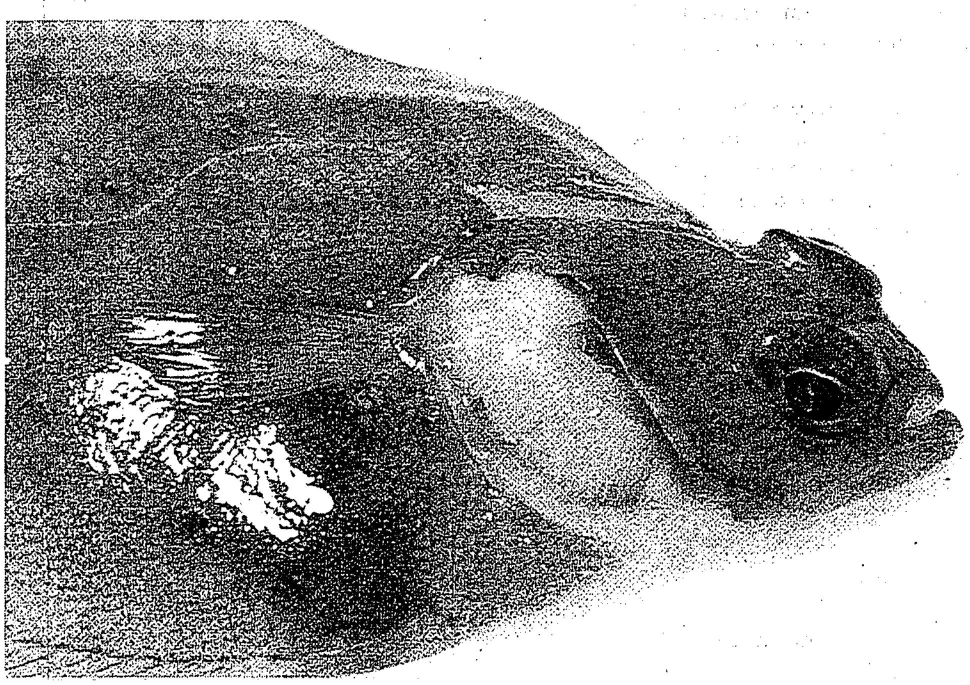

Fig. 2. *Limanda limanda*. X-cell gill disease affected dab. Notice the swollen and pale gill lamellae

Macroscopic inspection. The macroscopic inspection was carried out according to the recommendations in Anonymous (1989). In order to obtain the highest degree of precision in the diagnostics of the diseases, only 1 person conducted the registration during the whole period of the investigation. The otoliths were read by 2 skilled peoples.

Data analysis. Most of the data analyses were carried out using the public domain software 'Epi Info' version 5.0 (Dean et al. 1990) and Statistix, version 4.1 (Analytical Software). The strength of association between a 'factor' and a disease is known as the relative risk (RR). The RR is the ratio between the disease rate in an 'exposed' group and the disease rate in an 'unexposed' group and is presented with 95 % confidence intervals. The RR in an 'exposed' group is significantly different from the RR in an 'unexposed' group if the confidence interval for RR does not encompass the value 1.

The statistical tests applied for the data treatment were chi-square test, 2-sample *t*-test and Mann-Whitney rank test. To test for difference between the slopes of 2 regression lines a modified *t*-test was used (Zar 1984).

The condition factor [100 × weight (g) × length^{-3} (cm)] was calculated based on the total weight of individual fish (in contrast to gutted weight as recommended in Anonymous 1989).

RESULTS

The intensity of the gill lesions ranged from a few swollen lamellae on 1 gill arc to extreme conditions with all gill lamellae affected to a degree where the tips of the lamellae protuded as a creamy white band outside the opercular edge, with the latter being the predominant finding.

The prevalence of X-cell gill disease showed considerable spatial and temporal variation. At most of the stations the prevalence varied from 0 to 1 % throughout the whole period of investigation, except at the 3 stations marked in Fig. 1 where prevalences up to 38 % were observed.

The total number of dab and X-cell gill disease affected fish separated by sex and year and the mean prevalence of the disease for each hot-spot station are presented in Table 1. With regard to the total number of fish examined, an even sexual distribution was observed at Stn 1 while at Stns 2 and 3, a significantly higher number of females ($p < 0.001$) was present in the catches.

Temporal variations

At Stn 1 the prevalence ranged from 2.6 to 36.6 % while the range for Stn 2 was from 0.0 to 10.4 %. For both

Table 1. *Limanda limanda*. Total number of dab examined, number affected by X-cell gill disease separated by sex and year, and mean prevalence (%)

Year	Station 1							Station 2							Station 3						
	Total examined			X-cell disease			Prev.	Total examined			X-cell disease			Prev.	Total examined			X-cell disease			Prev.
	n	♂	♀	n	♂	♀	%	n	♂	♀	n	♂	♀	%	n	♂	♀	n	♂	♀	%
1986	411	173	238	14	8	6	3.4	249	98	151	0	0	0	0.0	321	18	303	0	0	0	0.0
1987	314	114	200	115	48	67	36.6	346	130	256	40	22	18	10.4	116	10	106	0	0	0	0.0
1988	406	190	216	30	13	17	7.7	433	211	255	33	12	21	7.1	187	31	156	1	1	0	0.5
1989	1144	648	496	136	46	90	11.9	421	191	233	3	1	2	0.7	11	1	10	0	0	0	0.0
1990	820	419	401	33	19	14	4.0	301	130	173	2	2	0	0.7	160	27	133	0	0	0	0.0
1991	459	229	230	12	6	6	2.6	439	261	229	51	43	8	10.4	419	84	335	2	2	0	0.5
1992	1351	623	728	43	18	25	3.5	908	380	561	33	17	16	3.5	535	41	494	5	2	3	0.9
1993	–	–	–	–	–	–	–	565	231	348	14	10	4	2.4	590	70	520	73	26	47	12.4
Total	4905	2396	2509	383	158	225		3838	1632	2206	176	107	69		1902	254	1648	81	29	52	

these stations large temporal variations were observed. In contrast to Stns 1 and 2, the prevalence at Stn 3 was at a low level, from 0.0 to 0.9%, in the period from 1986 to 1992, while it increased to more than 12% in 1993.

The relative risk (RR) of contracting X-cell gill disease at the 3 stations is illustrated in Fig. 3. For Stn 1, the RR relative to 1986 (taken here as the 'unexposed' group) was significantly higher from 1987 to 1989 while no significant difference was observed in the period 1990 to 1992. For Stn 2, the RR was calculated based on the 1987 figures as the prevalence of X-cell gill disease in 1986 was zero. The risk in 1988 and 1991 was not significantly different from 1987 while it was significantly lower in 1989 and 1990, and in 1992 and 1993. At Stn 3, the disease was not observed until 1988, hence this year was applied as baseline year. The RR in 1989 and 1990 was zero due to no observed cases and in 1991 and 1992 the risk was not significantly different from 1988. In 1993, the RR was significantly higher than in 1988.

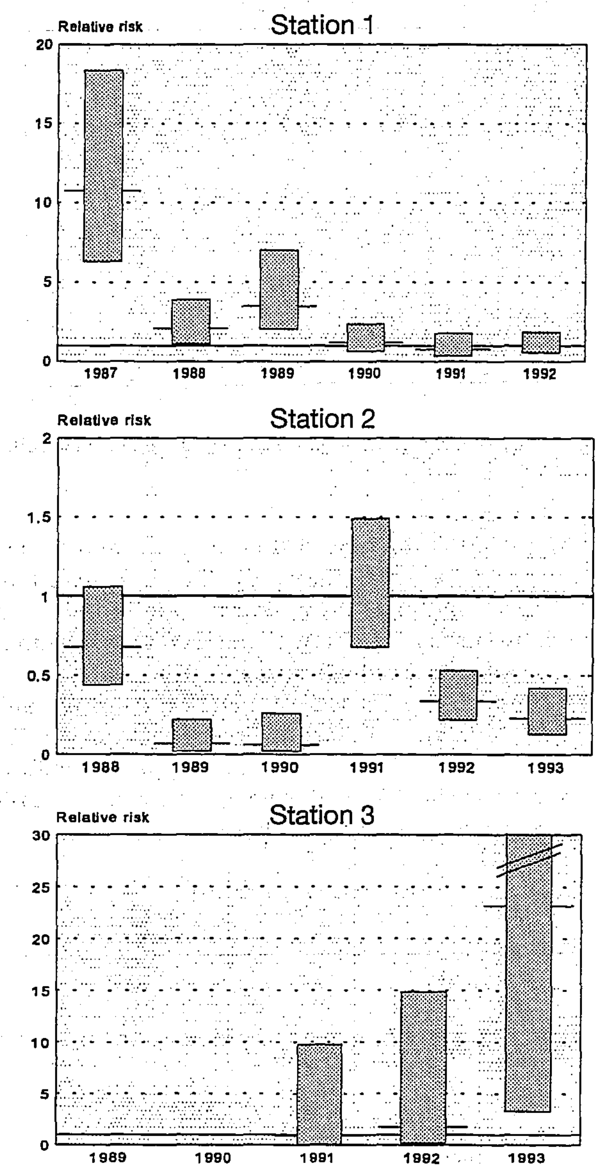

Fig. 3. *Limanda limanda*. Relative risk of X-cell gill disease in dab at the 3 hot-spot stations; the tick mark indicates the relative risk and the bar the confidence interval

Table 2. *Limanda limanda*. Prevalence (%) of X-cell gill disease in different hauls at Stn 1

Year	Haul A	B	C	D	E	F
1986	0.0	6.7				
1987	35.6	38.0				
1988	3.1	10.8				
1989	15.9	5.3	11.2	1.8	23.3	2.5
1990	3.5	4.7	3.5			
1991	3.5	1.5				
1992	1.7	2.8	5.1	5.3	3.9	

Spatial variations

Besides the observed temporal variations in the prevalence of X-cell gill disease at Stn 1, large haul-to-haul variations also occurred in some years, e.g. 1988 (Table 2). In order to investigate this variation, the

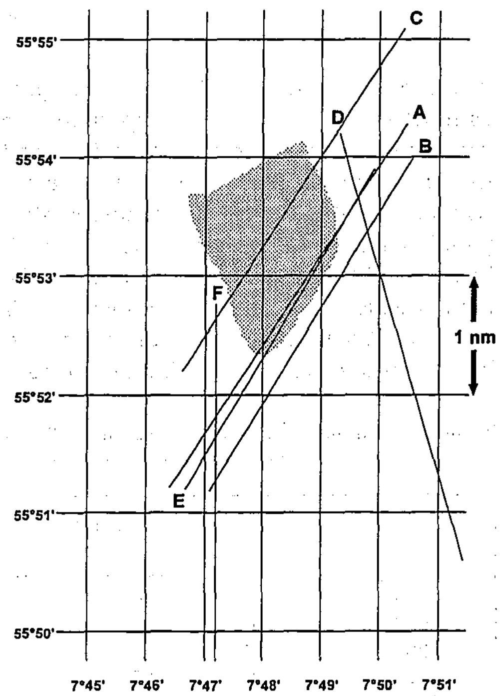

Fig. 4. Trawl tracks used in 1989 to investigate the spatial distribution of X-cell gill disease in dab at Stn 1. Dotted area indicates the possible distribution of a severely affected dab population

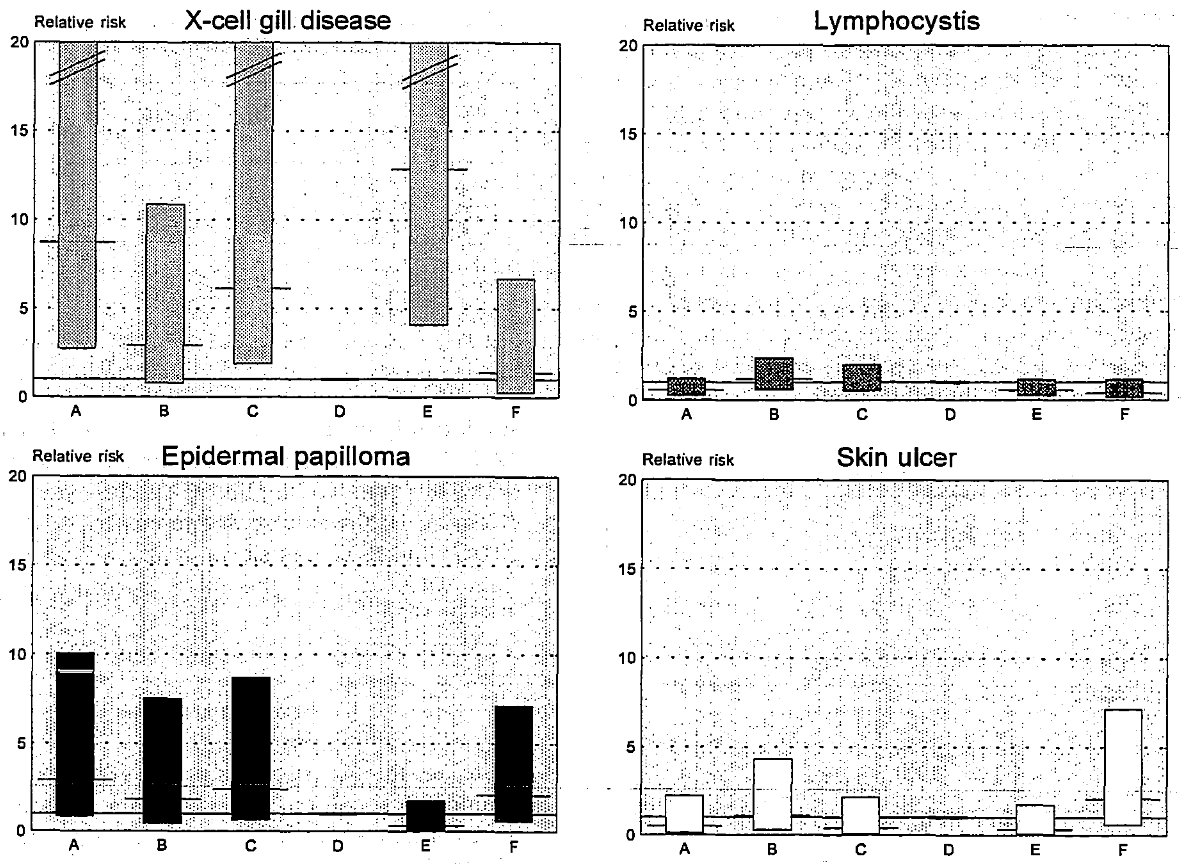

Fig. 5. *Limanda limanda*. Relative risk of X-cell gill disease, lymphocystis, epidermal papilloma and skin ulcers for dab collected along 6 trawl tracks at Stn 1 in 1989

trawling was carried out in a grid-like pattern in 1989 as illustrated in Fig. 4. This experiment showed high prevalence of X-cell gill disease in 3 of the 6 trawl tracks as listed in Table 2. Fig. 5 illustrates the relative risk of X-cell gill disease, lymphocystis, epidermal papilloma and skin ulcers observed in the different hauls in 1989. All calculations were made relative to haul D which had the lowest prevalence. The relative risk of contracting X-cell gill disease was significantly higher on tracks A, C and E, ranging from 6.1 to 12.8 ($p < 0.001$), while the risk on tracks B and F did not differ significantly from track D. The other investigated diseases did not show similar significantly higher relative risks in tracks A, C and E compared with D as observed for X-cell gill disease.

In contrast to Stn 1, Stn 2 revealed minimal haul-to-haul variations (Table 3). The prevalence of X-cell disease at Stn 3 was low until it appeared with high prevalence in 1993. In that year, a marked haul-to-haul variation was observed (Table 4).

Table 3. *Limanda limanda*. Prevalence (%) of X-cell gill disease in different hauls at Stn 2

Year	Haul			
	A	B	C	D
1986	0.0	0.0		
1987	10.5	10.3		
1988	7.3	6.8		
1989	0.9	0.5		
1990	0.7			
1991	10.0	10.8		
1992	3.9	1.6	4.1	4.7
1993	2.3	2.6		

Table 4. *Limanda limanda*. Prevalence (%) of X-cell gill disease in different hauls at Stn 3

Year	Haul	
	A	B
1986	0.0	0.0
1987	0.0	0.0
1988	1.9	0.0
1989	0.0	
1990	0.0	
1991	0.9	0.0
1992	1.5	0.4
1993	7.6	16.2

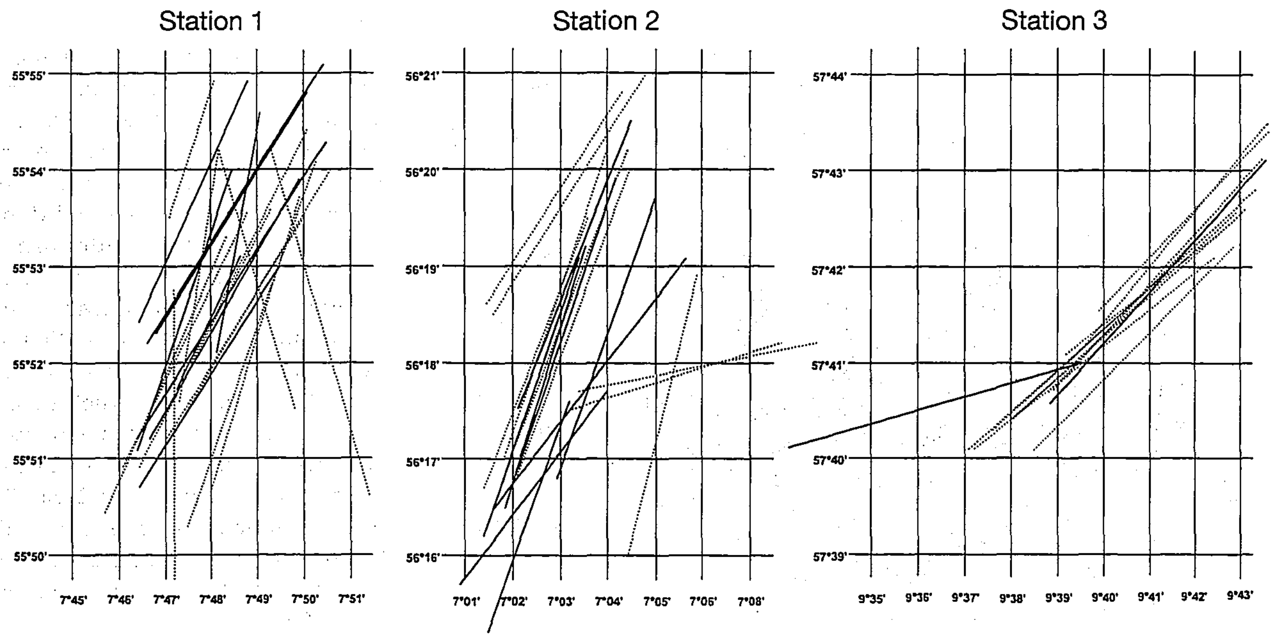

Fig. 6. Trawl tracks taken throughout the period 1986 to 1993 at the 3 hot-spot stations. Solid line indicates a prevalence of X-cell gill disease above 4% and dotted line a prevalence below 4%

Fig. 6 illustrates the trawl tracks taken at each station throughout the period of the investigation. All tracks were taken within an area of approximately 4 × 3 nautical miles (7.5 × 5.5 km). Full lines indicate that samples caught in these hauls had a prevalence of X-cell gill disease above 4% while dotted lines indicate a prevalence below this level. At Stns 1 and 2, there exists no clear trend in the patchy distribution of high prevalences (>4%) of X-cell gill disease, although there may be a tendency towards concentration in the central parts of those areas. For Stn 3, the prevalence of X-cell gill disease was below 4% until 1993. In that year, the haul with the highest prevalence (16.2%) was the one that diverged from the main track area.

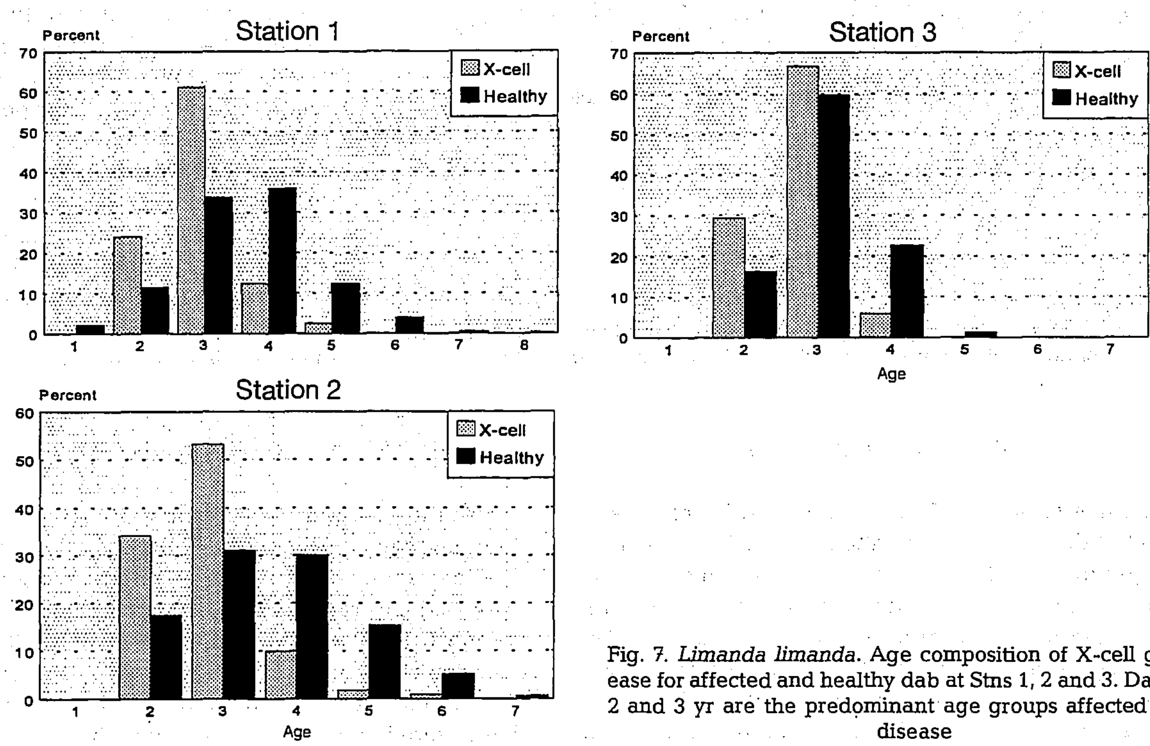

Fig. 7. *Limanda limanda*. Age composition of X-cell gill disease for affected and healthy dab at Stns 1, 2 and 3. Dab aged 2 and 3 yr are the predominant age groups affected by the disease

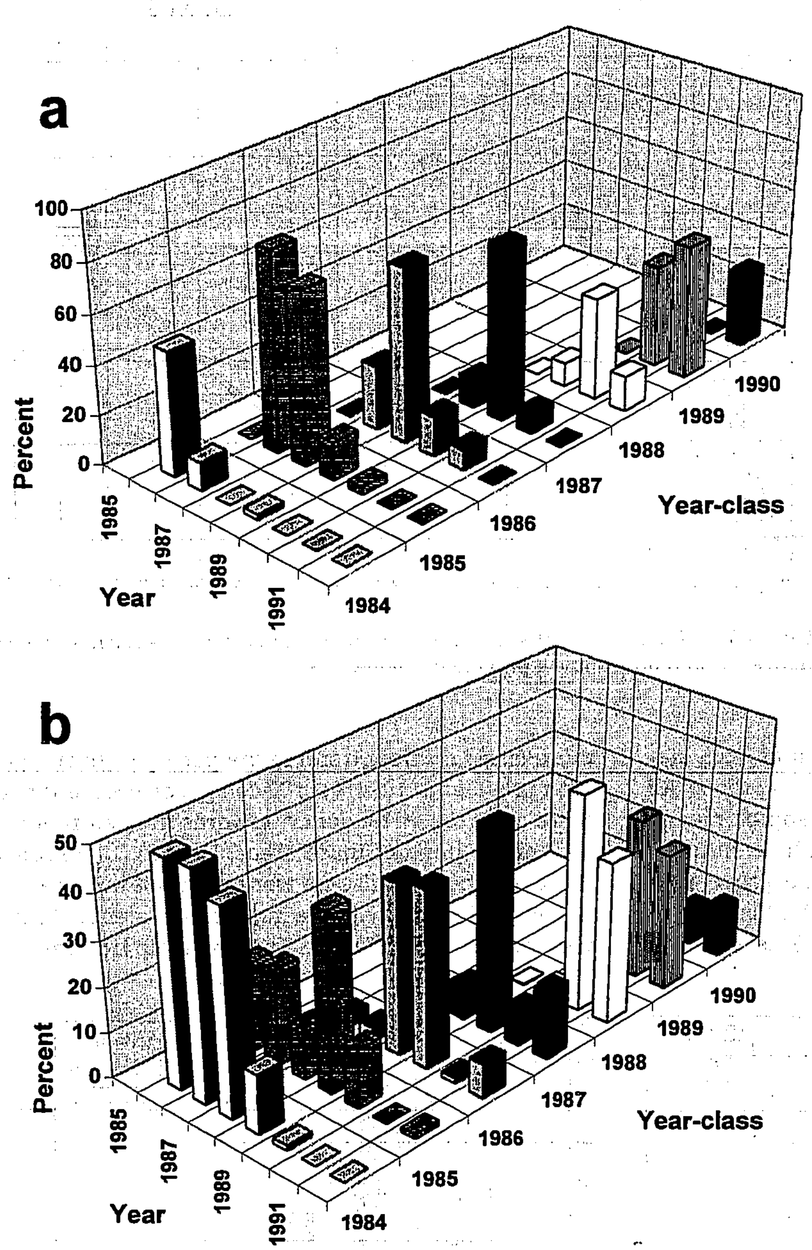

Fig. 8. *Limanda limanda*. Age composition of the different year-classes (cohorts) of (a) X-cell gill disease affected, and (b) healthy dab at Stn 1

At Stn 2, the age distribution of both the affected and healthy fish was very similar to that observed at Stn 1, with the most severely affected age groups being the 2 and 3 yr old fish. The age range for the X-cell affected dab was 2 to 6 yr while the healthy fish were within the age groups 1 to 7 yr. The healthy fish had a significantly higher mean age (3.6 yr) ($p < 0.001$) compared to the X-cell affected fish (2.8 yr).

At Stn 3, the age structure of the healthy dab was more narrowly concentrated around the 3 yr old fish in comparison to Stns 1 and 2. Similar to those 2 stations, the mean age of the healthy group was significantly higher (3.1 yr) ($p \leq 0.05$) than for the affected fish (2.8 yr).

The age structure of each year-class (cohort) is illustrated in Fig. 8 for the diseased and healthy dab at Stn 1. It displayed a similar structure from year to year with a peak for affected fish at an age of 2 to 3 yr while 3 and 4 yr old fish were predominant in the healthy group.

The regression lines for the abundance [(ln+1) transformed] relative to age of the affected and unaffected dab were calculated (Fig. 9) to assess whether X-cell gill disease caused increased mortality in the affected stock compared with the healthy population. The slope of the regression line for the affected fish was steeper than for the unaffected stock, suggesting an increased mortality due to the disease. However, a test for equality of the slopes of the 2 lines did not show a statistically significant difference between the age-related decline of the 2 groups.

Effect on age structure

The age structure of the healthy and X-cell disease affected dab stock is illustrated in Fig. 7. At Stn 1, X-cell gill disease was observed in age groups 2 to 5 yr, mainly affecting the 2 and 3 yr old fish, while the age of the healthy group ranged from 1 to 8 yr. The mean age was significantly higher (3.6 yr) ($p < 0.001$) in the healthy population compared to the X-cell disease affected stock (2.9 yr) (Mann-Whitney rank test).

Effect on growth

Healthy fish were significantly longer ($p < 0.0001$) than affected specimens at both Stns 1 and 2 (Mann-Whitney rank test). The mean length of diseased dab was 13.6 cm (range 8 to 23 cm) compared to 16.8 cm (range 6 to 40 cm) for unaffected fish at Stn 1, and 13.7 cm (range 9 to 26 cm) compared to 17.0 cm (range 7 to 33 cm) at Stn 2. In contrast, the affected fish at Stn 3 were longer, with a mean length of 18 cm (range 12 to 25 cm) compared to 17.4 cm (range 8 to 29 cm),

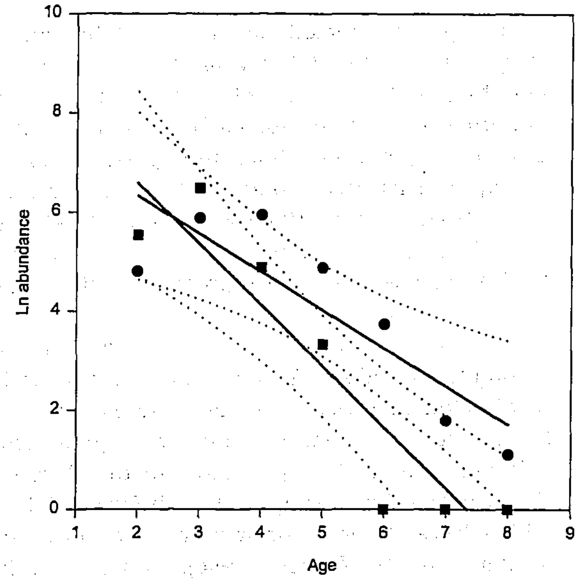

Fig. 9. Ln abundance per age group of healthy (●; upper regression line with 95% confidence interval) and X-cell affected dab (■; lower regression line with 95% confidence interval). The regression line for the X-cell affected population has a steeper slope than the one for the healthy population, suggesting an increased mortality in the affected dab

Table 5. *Limanda limanda*. Mean length (cm) for X-cell gill disease affected and healthy dab separated by age. *p < 0.005

Age (yr)		Stn 1	Stn 2	Stn 3
2	Healthy	12.71	12.82	14.79
	X-cell	12.50	12.41	15.90
3	Healthy	15.86 *	16.06 *	17.80
	X-cell	13.88	14.72	17.36
4	Healthy	19.35 *	19.26	20.49
	X-cell	15.39	16.33	14.50
5	Healthy	22.00 *	21.35	
	X-cell	19.14	19.0	

Table 6. *Limanda limanda*. Mean condition factor for X-cell gill disease affected and healthy dab separated by sex. *p < 0.001

Sex		Stn 1	Stn 2	Stn 3
♂	X-cell	0.846 *	0.890 *	0.920 *
	Healthy	0.934	0.973	0.992
♀	X-cell	0.874 *	0.872 *	0.913 *
	Healthy	0.963	1.000	1.016
Total	X-cell	0.859 *	0.883 *	0.915 *
	Healthy	0.951	0.990	1.013

for the unaffected fish, but these figures were not significantly different.

Table 5 presents the length data stratified by age. At Stn 1, the healthy 3, 4 and 5 yr old fish were significantly longer than the X-cell affected ones (p < 0.005) while no significant difference was observed in the 2 yr old fish. At Stn 2, only the 3 yr old healthy fish were significantly longer (p < 0.005) than the X-cell affected ones. No significant differences between the mean lengths of any of the age groups was observed at Stn 3.

Effect on condition factor

A common feature of all 3 stations was that the condition factor of affected dab was significantly lower (p < 0.001) than that of unaffected fish (*t*-test) (Table 6). The condition factor of healthy and diseased fish stratified by sex was significantly different (p < 0.001) for both males and females.

As the X-cell disease seemed to have an effect on the growth of the fish, subsamples of affected and unaffected fish of approximately equal length (13 cm) were analysed in order to assess the effect of the disease on age structure, weight and condition factor. The key values appear in Table 7. Differences were observed between the mean weight and the mean condition factor of the 2 groups. However, only the mean condition factor was significantly different (p < 0.002, *t*-test).

Table 7. *Limanda limanda*. Mean length, weight, condition factor and age for a subsample of X-cell gill disease affected and healthy dabs

	n	Mean length (cm)	Mean weight (g)	Mean condition factor	Mean age (yr)
X-cell	26	12.56	15.60	0.7341	2.59
Healthy	22	12.94	19.23	0.8282	2.64

Sex related differences

At Stn 1 female dab had a significantly higher risk of contracting X-cell disease than males (RR = 1.36; p < 0.002). However, the opposite situation was observed at Stns 2 and 3 where female dab had a significantly lower risk of contracting X-cell disease compared to males (RR = 0.48 and RR = 0.28, respectively) (p < 0.00001).

DISCUSSION

In Danish coastal areas, X-cell gill disease in common dab occurred at low prevalence except at 3 hotspot stations (Fig. 1). At these stations, considerable variation of the prevalence—spatial as well as temporal—ranging from a few percent up to 38% was observed (Table 1, 2 & 3). Similar patchiness in the distribution of X-cell gill disease has been reported from other areas of the North Sea: the Dogger Bank and the Humber area (Knust & Dethlefsen 1986, McVicar et al. 1987) and in certain areas along the Scottish east coast (Diamant & McVicar 1989). However, prevalences higher than 10% have only been observed in Danish coastal areas and in the southern Moray Firth (Diamant & McVicar 1989). The present work deals with disease investigations over a period of 7 to 8 yr while formerly published work on X-cell gill disease (Knust & Dethlefsen 1986, Diamant & McVicar 1989) dealt with observations over a period of 3 yr. Knust & Dethlefsen (1986) demonstrated distinct temporal and spatial variations in the prevalence of X-cell gill disease in the North Sea. Although they observed hot-spot sites in both the Dogger and Humber regions, these were not at the same positions at each time of year. Diamant & McVicar (1989) visited the same stations each year from 1985 to 1987 and found a very high prevalence (more than 60%) at one station in 2 consecutive years. These authors also observed considerable spatial variations even within relatively short distances just as observed in the present work.

The scenario from Stn 1 in 1989 (Fig. 4) showed that within a distance of about ¼ nautical mile (500 m), the distance from haul A and E to haul B, the prevalence varied significantly from approximately 20 to 5% (Table 2). Based on the 6 hauls taken there in 1989, it was possible to frame a high prevalence area (Fig. 4, dotted area) within very narrow limits. The relative risk of lymphocystis, epidermal papilloma and skin ulcers within the different hauls did not differ significantly. However, the observation of a seriously X-cell affected population being separated from a significantly less affected population within a distance of 500 m gives an impression of the severity of the disease. The gill lesions probably debilitate the fish to a degree where they are unable to travel over longer distances due to respiratory constraint. The impact of the disease was also reflected in the significantly lower condition factor of affected dab compared to that of unaffected fish, whether considering all fish examined, fish separated by sex, or fish within the same length group. This clear difference in the condition factor between affected and unaffected fish was only partly reflected in the observations of Knust & Dethlefsen (1986). In general, these authors found that the condition factor of affected fish was reduced compared to healthy specimens and that this difference was found to be statistically significant regarding the males but only sporadically significant for the females.

The present study showed that mainly the young age groups became infected with X-cell gill disease. The mean age of affected dab at the 3 hot-spot areas ranged from 2.8 to 2.9 yr while the mean age for the healthy population was between 3.1 and 3.6 yr. This pattern was repeated for each year-class (cohort) at Stn 1 throughout the whole study period as illustrated in Fig. 8. This corresponds with the observations of Knust & Dethlefsen (1986) who found X-cell affected fish within the age range of 2 to 5 yr in contrast to the healthy fish which were represented within the age group 3 to 7 yr.

The length distribution of affected fish differed among the 3 stations. At Stns 1 and 2, the mean length of affected fish was approximately 14 cm while at Stn 3 it was 18 cm, although identical age groups were affected. A possible explanation for this difference is that we may be dealing with 2 different dab stocks which have different growth rates, as proposed by Mellergaard & Nielsen (1984). Differences in the length distribution of X-cell affected fish between the present study and others (Knust & Dethlefsen 1986, Diamant & McVicar 1989) may be attributed to different growth rates of the affected dab stocks.

The observed difference in the relative risk of X-cell gill disease between males and females, with a significantly higher risk for females contracting the disease compared to males at Stn 1, while the opposite situation was true at Stns 2 and 3, has no obvious explanation. Knust & Dethlefsen (1986), McVicar et al. (1987) and Diamant & McVicar (1989) did not mention such a phenomenon. It is unlikely that it is due to statistical variability ($p < 0.005$), but from a biological point of view mere variability is a more plausible explanation.

The impact of the disease on growth became apparent in the 3 yr old age group at Stns 1 and 2 (Table 5), indicating that the disease has to be established in the fish for a certain period before growth is seriously reduced. This is supported by the fact that no adverse effect on growth was observed at Stn 3 where the disease had reached epidemic proportions within the previous year. The observation that the same age groups (2 and 3 yr old fish) are affected by X-cell gill disease in each cohort suggests that the duration of the disease is short, which may be due to either high recovery or high mortality rate. The latter is the most likely explanation. Affected fish have a severely reduced capacity to withstand the stress of capture. Most of these fish die shortly after being brought on deck, probably severely debilitated by respiratory problems as indicated by Diamant & McVicar (1987). One impact of the disease was a significantly lower condition factor for affected fish than for healthy specimens. Furthermore, there is a significant decline in the prevalence of the disease from age 3 to 4 yr (Fig. 7) and, although not statistically significantly different, the plots of the regression lines for the abundance at age of healthy and diseased dab

(Fig. 8) showed a steeper slope for the X-cell affected fish, indicating higher mortality in this group than in the healthy population. Taking these factors in consideration, it is likely that X-cell gill disease is fatal for affected dab, although Diamant & McVicar (1987) recorded that recovery may occur in some cases. Mortality in fish due to conditions where X-cells have been involved has previously been described in starry flounder *Platichthys stellatus* with skin tumours (Campana 1982).

The epidemiological data on X-cell gill disease presented in this study showing temporal and spatial fluctuations clearly add to previous suggestions (McVicar et al. 1987, Diamant & McVicar 1989) that the disease has an infectious etiology. The background for the shifts in the epidemiological status of X-cell gill disease in certain areas e.g. from endemic to epidemic situations can only be explained by either changes in the virulence of the possible infectious agent or changes in the immunological status of the local dab populations.

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17. Paper 4

Mellergaard, S. & Nielsen, E. The epidemiology of lymphocystis, epidermal papilloma and skin ulcers in common dab (*Limanda limanda*) along the west coast of Denmark. Diseases of Aquatic Organisms, *Submitted*

Submitted to:

DISEASES OF AQUATIC ORGANISMS

Dis Aquat Org

The epidemiology of lymphocystis, epidermal papilloma and skin ulcers in common dab (*Limanda limanda*) along the west-coast of Denmark.

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ABSTRACT: A survey of fish diseases in the common dab *Limanda limanda* L. was conducted in 4 areas, the German Bight, 2 areas along the west coast of Denmark and the Skagerrak in May during the years 1983 to 1993. A total of 53302 dab were examined for the presence of the diseases lymphocystis, epidermal papilloma and skin ulcers. The present study describes long-term variations in the prevalence of these three diseases. The German Bight and the 2 areas along the west coast of Denmark showed significant similarities in the temporal trend of the diseases investigated with peaks in 1985 and 1988 for both lymphocystis and epidermal papilloma. A similar trend was not observed in the Skagerrak. Skin ulcerations did not reveal any temporal trends in any of the 4 areas. The highest prevalence of lymphocystis was observed in the most offshore area along the Danish west coast with a peak value of 14.9% in 1988, for epidermal papilloma in the German Bight with a peak value of 9.4% in 1988 and for skin ulcers in the Skagerrak in 1989 with 4.4%.
Areas in the German Bight and along the west coast of Denmark had suffered from oxygen deficiency in late summer during the years 1981 to 1983. These events may have been an important factor triggering the outbreak of lymphocystis and epidermal papilloma as the prevalence of these diseases increased from 1983 until 1985 followed by a decline until 1988. Although, it was not possible to establish significant correlations between the oxygen levels at the bottom and the disease prevalence in the present case, the disease pattern showed evident similarities with formerly described oxygen deficiency-induced outbreaks of lymphocystis and epidermal papilloma in dab in the Kattegat. In 1988, the prevalence of lymphocystis and epidermal papilloma increased significantly without any apparent reason.
The disease pattern observed in the Skagerrak differed significantly from the other 3 areas with skin ulcerations being the most prevalent disease. A part of the explanation for this may be that the Skagerrak has not suffered from the same impaired environmental conditions as the other areas.

KEY WORDS: Lymphocystis, Epidermal papilloma, Skin ulcers, Dab, Oxygen deficiency, Epidemiology

INTRODUCTION

The occurrence of diseases in wild fish stocks was already well described at the beginning of this century (Johnstone 1905, Johnstone 1925), although the prevalence and geographic distribution of the diseases had not yet been determined. However, since the early 1970s, when the close relationship between environmental stress and the outbreak of fish diseases was accepted (Wedemeyer 1970, Snieszko 1974), a series of investigations has been conducted into the impact of pollution on disease in natural fish stocks. The first considerations and results of this work on monitoring of biological effects of marine pollution were summarized by McIntyre & Pearce (1980).
During the late 1970s and early 1980s, studies were carried out in many countries especially those bordering the North

Sea and in the USA (Christensen 1980, Dethlefsen 1980, Dethlefsen 1984, Dethlefsen & Watermann 1982, Möller 1981, Despres-Patanjo et al. 1982, Mellergaard & Nielsen 1984, 1985, Vethaak 1991, 1992, 1993, Lindesjöö et al. 1994, Mellergaard & Nielsen 1995, 1996). However, most of this work has concerned short term investigations in relatively restricted areas (McArdle et al. 1982, Bucke et al. 1983, Möller 1984, Bucke & Nicholson 1987, Vethaak et al. 1992).

In order to get sufficient data for a more overall assessment of the impact of temporal and spatial variations of environmental parameters on the disease status of fish stocks, long-term disease investigations are needed. Some long-term investigations including the prevalence and the dynamics of fish diseases over wider sea areas have, however, been reported by Dethlefsen et al. (1987) and Dethlefsen (1990), who covering most of the North Sea, by Banning (1987) in the south-eastern North Sea and by Mellergaard & Nielsen (1990, 1995, 1996) in Danish coastal zones.

The present work deals with a long-term study of the epidemiology of selected externally visible skin lesions of dab in an area stretching from the German Bight to the Skagerrak. Possible association has been sought between observed temporal and spatial variations in disease rates and environmental parameters that might be of importance as stress factors.

MATERIAL AND METHODS

Fish sampling. Common dab (*Limanda limanda* L.) were sampled annually in May from 1984 to 1993 on board the RV "Dana" using a Nymplex standard fishing trawl, Star model. The trawl was rigged either with 12" (30 cm) rubber discs or with 10" (25 cm) bobbins on the foot-rope, depending on the bottom conditions, and fitted with a foot rope chain. The stretched mesh size in the codend was 40 mm. Fishing took place at a number of sites where trawl tracks had been available from commercial fishermen. Standard 1 h hauls were taken with a speed of 3 knots. One or two hauls were taken at each station. The present work deals with data obtained from a set of stations (Fig. 1) which are classified into 4 areas based on geographical conditions and length and age parameters of the fish.

Handling of the sample. The total catch was sorted into species and the dab were subjected to further investigation. A sample of 150 to 250 specimens (a sample size required for the detection of a prevalence of at least 2% with 95% confidence (Martin et al. 1987) corresponding to 15 to 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, sex and health status were registered. After the disease recording, the otoliths from the first 96 fish were removed for ageing later in the laboratory.

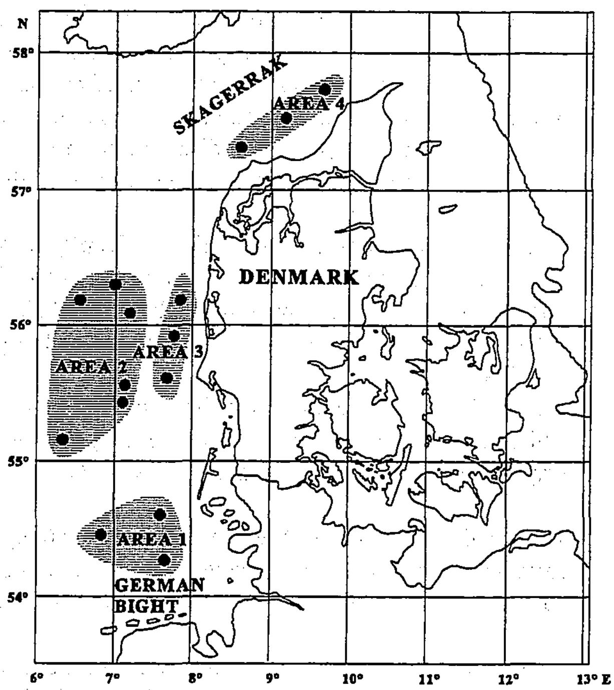


Fig. 1. *Limanda limanda.* Station pattern within the 4 areas investigated

Selection of gross lesions. The dab were examined for the presence of the diseases lymphocystis, epidermal hyperplasia/papilloma and skin ulcers using recommended procedures for detection (Anon. 1989). These diseases have been well described in literature (Möller & Anders 1983, Bucke et al. 1996) in terms of their gross appearance and aetiology.

Lymphocystis: The criterion for recording was the presence of more than one surface nodule.

Epidermal hyperplasia/papilloma: The criterion for recording was one or more lesions larger than 2 mm in diameter. In the present work, epidermal hyperplasia and papilloma are both designated "epidermal papilloma".

Skin ulcerations: The criterion for recording was one or more open lesions.

Macroscopic inspection. Prior to external inspection the fish were rinsed with sea water. Both sides of the fish were carefully examined visually and by palpation, and the fins were spread and lifted. The examination took place under a strong light source.

In order to obtain the highest degree of precision in the diagnostics of the diseases, only one person conducted the registration during the whole period of the investigation.

The otoliths were read by two skilled persons.

Analysis of the data. Most of the data analysis were carried out using the public domain software "Epi-Info" version 6.01 (Dean et al. 1990) and Statistix®, version 4.1 (Analytical Software). The strength of association between a factor and a disease is known as the relative risk (RR). The RR is calculated as the ratio between the disease rate in an "exposed" group and the disease rate in an "unexposed" group. All RR estimations are presented with 95% confidence intervals.
The RR in an "exposed" group is significantly different from the RR in an "unexposed" group if the confidence interval does not encompass the value 1 ($p<0.05$). All calculations of RR were done relative to the 1983 figures for all areas.
The statistical tests applied for the data treatment were: Chi-square, 2-sample t-test, Mann-Whitney rank test, 1-way ANOVA and Kruscal Wallis 1-way ANOVA.

The condition factor (100 * weight (g) * length^{-3} (cm)) was calculated based on the total weight of individual fish (in contrast to gutted weight as recommended in Anon. 1989).

Oxygen data: Data on the oxygen concentrations in the areas were obtained from the ICES Hydrographic Service.

RESULTS

All data presented were sampled at 15 stations distributed along the Danish west coast (Fig. 1). These stations were separated into 4 areas, based on geographical conditions as well as length, age and condition factor characteristics of the examined dab. A southern zone was designated Area 1, representing the German Bight, as well as 2 central zones, one offshore, Area 2 and one coastal, Area 3 and a northern zone, Area 4, representing the Skagerrak. The mean length, mean age and mean condition factor for the The

Table 1. *Limanda limanda*. Characteristics used for separation of the 4 areas

Area	Mean age (yrs)	Mean length (cm)	Mean condition factor
1	3.7	17.9	0.9380
2	3.6	17.4	0.9821
3	3.4	17.1	0.9840
4	2.8	17.6	1.0335

Table 2. *Limanda limanda*. Total number of dab examined and number of fish affected with lymphocystis, epidermal papilloma and skin ulcers separated by area and year

Year	Area 1				Area 2				Area 3				Area 4			
	n	Lympho-cystis	Epidermal papilloma	Skin ulcer	n	Lympho-cystis	Epidermal papilloma	Skin ulcer	n	Lympho-cystis	Epidermal papilloma	Skin ulcer	n	Lympho-cystis	Epidermal papilloma	Skin ulcer
1983	907	80	13	17	657	61	6	7	460	14	4	10	693	5	1	13
1984	2925	294	170	27	5054	452	120	33	698	50	17	8	408	4	1	3
1985	1628	205	111	16	1529	210	55	16	727	47	22	4	981	9	3	2
1986	1185	95	98	13	2038	131	69	12	831	23	21	3	759	3	0	2
1987	850	66	56	7	1947	89	34	17	504	16	8	2	487	1	1	1
1988	913	117	86	7	1612	240	96	16	734	100	58	5	785	9	1	5
1989	1075	83	36	11	2238	277	74	19	1554	115	60	27	159	0	2	7
1990	1159	63	29	22	1757	194	35	11	1239	102	28	11	368	11	2	14
1991	1184	87	58	8	2078	159	53	10	891	59	27	10	931	9	2	7
1992	1329	60	73	7	2639	161	61	15	1748	57	42	7	1006	14	4	4
1993	-	-	-	-	1461	103	37	23	-	-	-	-	1187	5	2	22
Total	13153	1150	730	134	23002	2076	640	179	9384	583	287	87	7763	70	19	80

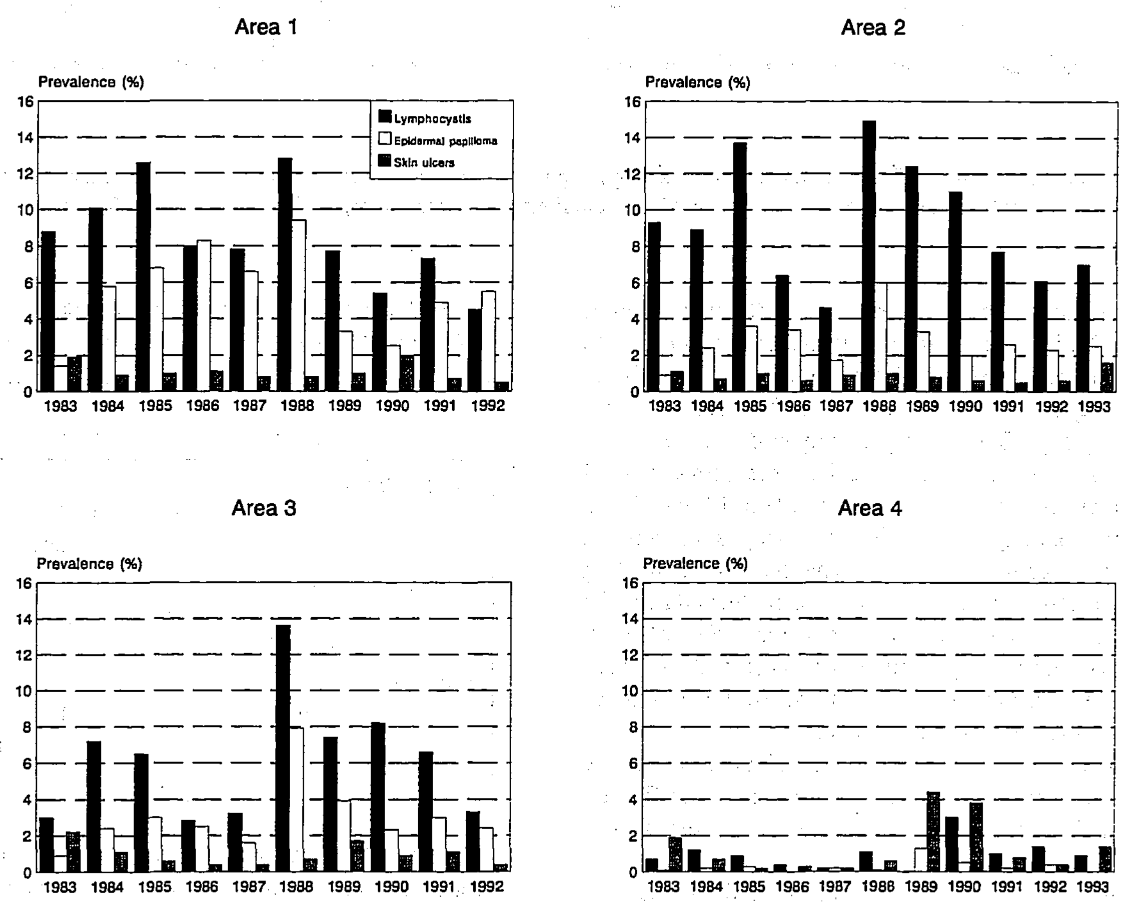
mean length of the dab in the 4 areas differed significantly (1-way ANOVA) (p>0.0001) and a comparison of means (Tukey) showed that the 4 areas were different from each other. The mean age and the mean condition factor of the dabs differed significantly (Kruscal-Wallis) (p<0.0001) between the 4 areas and a comparison of means (modified LSD) showed that all 4 areas differed from each other. In 1993 the sampling was restricted to the areas 2 and 4. During the 11 years of investigation a total of 53302 dab were examined. The distribution of the number of examined dab and dabs affected with lymphocystis, epidermal papillomas and skin ulcers, respectively, on the different areas appears from Table 2.

Disease examination

Lymphocystis

In Area 1, the prevalence of lymphocystis varied from 12.8% as peak values to 4.5% as the lowest level. The temporal pattern showed an increase in the prevalence from 1983 to 1985 followed by a decrease the following two years then a sudden decrease in 1988 followed by a further decline the succeeding years (Fig. 2).
The RR of contracting lymphocystis was significantly higher in 1985 and 1988 compared to 1983. In 1984, 1986, 1987, 1989 and 1991 the RR was at the same level as in 1983 while it was significantly lover in 1990 and 1992 (Fig. 3).
In Area 2, the prevalence of lymphocystis ranged from 4.6% to 14.9% (Fig. 2). It showed a similar pattern in the annual variation of the prevalence as observed in Area 1 with increasing prevalence from 1983 to 1985 followed by a decrease during 1986 and 1987, a three fold increase from 4.6% to 14.9% from 1987 to 1988 with a gradual decline the following years. The RR was significantly increased in 1985 and 1988 to 1989 while significantly reduced in 1986 to 1987 and in 1992 (Fig. 3). The RR in 1990 to 1991 and in 1993 was at the same level as in 1983.
Area 3 revealed an increase in the prevalence from 3.0% in 1983 to 7.2% in 1984 followed by a decrease to 3.2% in 1987 (Fig. 2). In 1988, an increase to 13.6% was observed

Fig. 2. *Limanda limanda*. The prevalence rate of lymphocystis, epidermal papilloma and skin ulcers at the 4 areas investigated



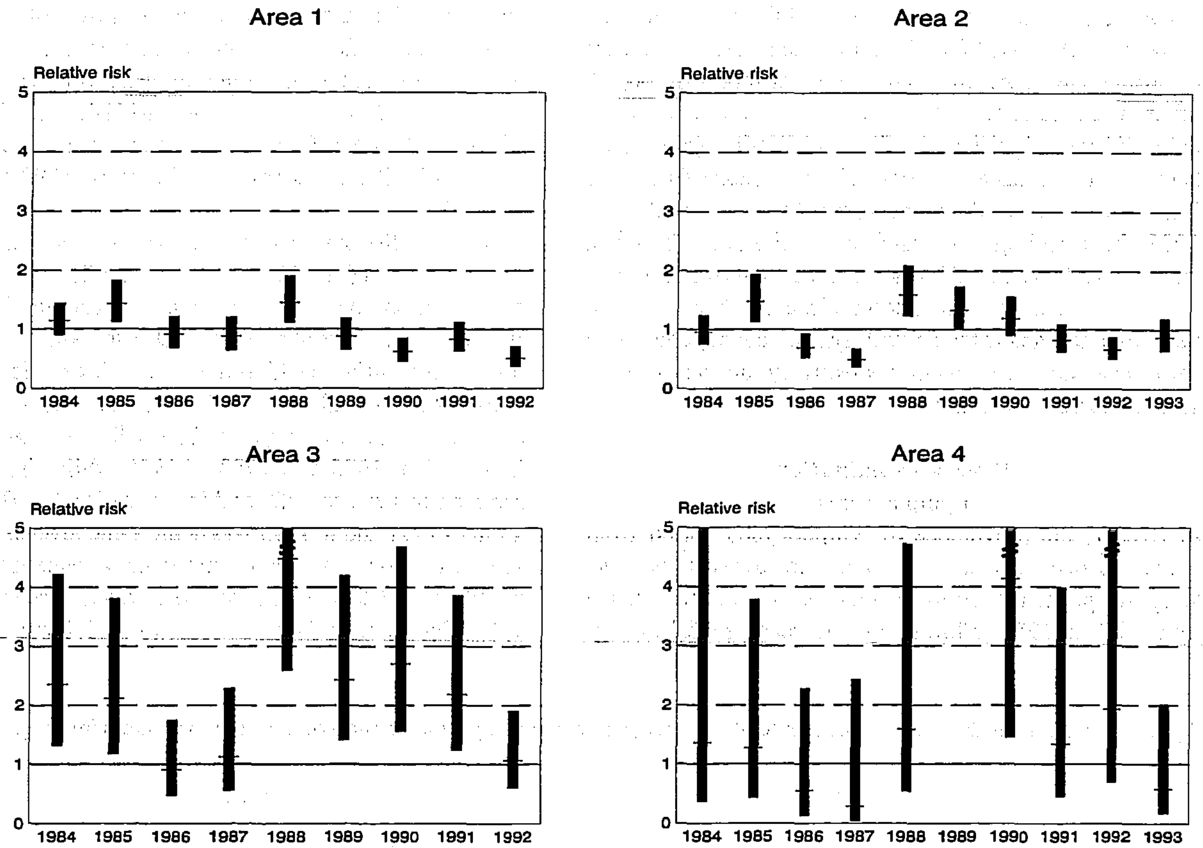


Fig. 3. *Limanda limanda*. The relative risk of contracting lymphocystis at the 4 areas presented with 95% confidence intervals (bar). Where bars do not encounter 1 (bold line) the figures are significantly different from the 1983 figures.

followed by a decline as observed in the two previous areas. The RR was significantly increased from 1984 to 1985 and during the period from 1988 to 1991 relative to 1983 (Fig. 3). In 1986, 1987 and 1992, the RR did not show any significant changes compared to 1983.

In contrast to Areas 1, 2 and 3, the prevalence of lymphocystis in Area 4 varied from 0% in 1989 to 3% in 1990 without any trends (Fig. 2). Most years it was at a level of approximately 1%. The RR of contracting lymphocystis in Area 4 was at the same level during the 11 years of investigation except for 1990 where it increased significantly relative to 1983 (Fig. 3). Because the number of affected fish was limited the confidence levels of the RR were very broad.

Lymphocystis appeared evenly distributed among the sexes except in Area 3 where females had a significantly lower risk of contracting the disease (0.71 times) than males (Table 3).

Lymphocystis did not affect the mean condition factor of the dab in the different areas except for Area 1 where the condition factor of lymphocystis-affected fish was

Table 3. *Limanda limanda*. Sexual difference in the risk of contracting the diseases in the 4 areas (female relative to male)

	Area 1	Area 2	Area 3	Area 4
Lymphocystis				
	0.93	1.0	0.71 $p < 0.001$	0.88
Epidermal papilloma				
	1.47 $p < 0.001$	1.76 $p < 0.001$	1.22	1.54
Skin ulcer				
	0.98	1.86 $p < 0.001$	0.8	2.24 $P < 0.05$

significantly higher compared to the unaffected fish (Table 4). In general, the condition factor of lymphocystis-affected fish was higher than for the unaffected. The mean age was found significantly higher for lymphocystis-affected than for unaffected fish in all 4 areas investigated (Table 4).

Epidermal papilloma

In Area 1, the prevalence of epidermal papilloma ranged from 1.4% to 9.4% (Fig. 2). It increased from 1.4% in 1983 to 8.3% in 1986 with a minor decrease in 1987. The prevalence increased to 9.4% in 1988 followed by a rapid decline from 1989 to 1990 to 2.5%. In 1991 and 1992 the prevalence increased to approximately 5%. Compared to 1983, the RR was significantly increased during the whole period except for 1990 (Fig. 4).

In Area 2 the prevalence was at its lowest level 0.9% in 1983 peaking at 6% in 1988 (Fig. 2). An increase was observed from 1983 to 1985 followed by a decrease to 1.7% in 1987. After having peaked with a prevalence of 6% in 1988 a gradual decrease to approximately 2% was observed the following years. With the exceptions of 1987 and 1990, the RR was significantly increased during the period from 1984 to 1993 (Fig. 4). In 1988, the RR was 6.5 times higher than in 1983. In 1987 and 1990 the RR did not differ significantly from 1983.

The temporal variation pattern of the prevalence of epidermal papilloma in Area 3 was very similar to Area 2 (Fig. 2). The prevalence was 0.9% in 1983 with an increase until 1986. A decrease to 1.6% was observed in 1987 followed by a increase to 7.9% in 1988. From 1989 the prevalence showed a decline to about 2%. The RR was increased relative to 1983 during the whole period peaking with 9.8 in 1988 (Fig. 4). The increase of the RR was statistically significant in 1985,1988, 1989 and 1991.In Area 4, the prevalence of epidermal papilloma was within the range of 0% to 1.3% and did not show any significant pattern (Fig. 2). The RR of contracting epidermal papilloma was at the same level during the whole period of investigation but revealed broard confidence levels due to the restricted number of infected specimens (Fig. 4).

In Areas 1 and 2 females had a significantly higher risk of getting epidermal papilloma than males while in Areas

Table 4. *Limanda limanda*. Mean condition factor and age for unaffected and lymphocystis, epidermal papilloma and skin ulcer affected dab for the 4 areas. (p-values in case of significant difference (Kruscal-Wallis))

Lymphocystis		**Area 1**	**Area 2**	**Area 3**	**Area 4**
Condition factor	Unaffected	0.9346	0.9692	0.9583	1.0214
	Lymphocystis	0.9432	0.9738	0.9590	1.0189
		(p<0.05)			
Age	Unaffected	3.66	3.52	3.38	2.79
	Lymphocystis	4.19	4.17	4.12	3.42
		(p<0.0001)	(p<0.0001)	(p<0.0001)	(p<0.0005)
Epidermal papilloma					
Condition factor	Unaffected	0.9352	0.9693	0.9580	1.0213
	Epid.papilloma	0.9383	0.9815	0.9662	1.0296
Age	Unaffected	3.69	3.57	3.41	2.80
	Epid.papilloma	4.07	4.27	4.10	3.83
		(p<0.0001)	(p<0.0001)	(p<0.0001)	(p<0.001)
Skin ulcers					
Condition factor	Unaffected	0.9356	0.9699	0.9589	1.0218
	Skin ulcer	0.9225	0.9520	0.8929	0.9835
			(p=0.0008)	(p=0.0177)	
Age	Unaffected	3.71	3.58	3.43	2.79
	Skin ulcer	4.18	4.18	4.00	3.28
		(p<0.0001)	(p<0.0001)	(p<0.01)	(p<0.0005)

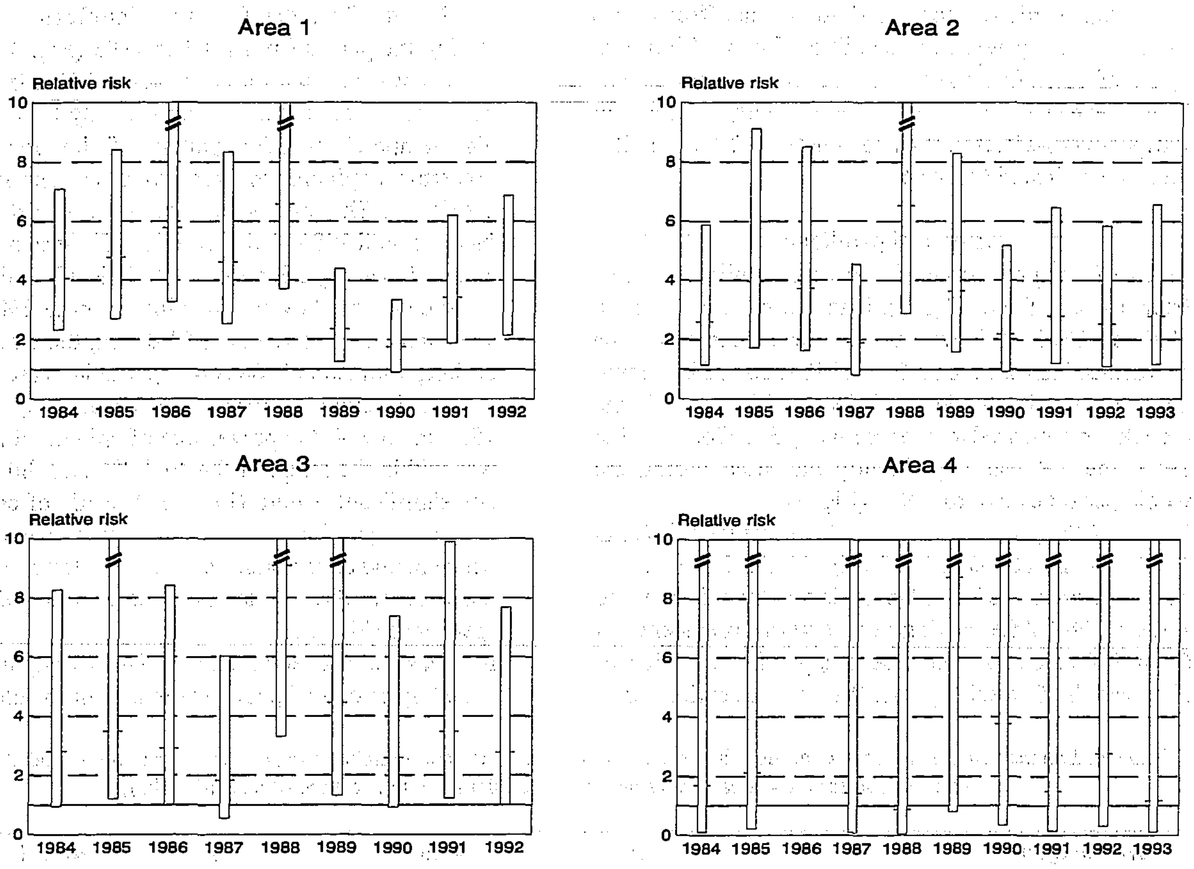


Fig. 4. *Limanda limanda.* Relative risk of contracting epidermal papilloma at the 4 areas presented with 95% confidence intervals (bar). Where bars do not encounter 1 (bold line) the figures are significantly different from the 1983 figures

3 and 4 the disease was evenly distributed among the sexes (Table 3).

In general, epidermal papilloma-affected fish had a higher condition factor than unaffected fish but the difference was not statistically significant (Table 4).

As for lymphocystis the mean age of epidermal papilloma-affected fish was significantly higher compared to the unaffected population in all areas (Table 4).

Skin ulcerations

The prevalence of skin ulcers in Area 1 varied from 0.5% as the lowest to 1.9% as the peak value with a mean level of approximately 1% (Fig. 2). It did not show any special trends. The RR did not differ significantly between the years relative to 1983 (Fig. 5).

In Area 2, the prevalence was around the same level as in Area 1, 0.5% to 1.6%, with a mean level just below 1% (Fig. 2). The RR did not differ significantly compared to 1983 (Fig. 5).

Area 3 reflected the prevalence observed in the Areas 1 and 2 being within the range of 0.4% to 2.2% with minor annual variations without any pattern. The RR was lower relative to 1983 but did not differ significantly during the period of investigation.

In contrast to the previous 3 areas, skin ulcerations seemed to be the most prevalent disease in Area 4. Apart from 1983 with a prevalence of 1.9% the level was within the range of 0.2% to 0.7% in the period 1984 to 1988 (Fig. 2). The prevalence increased to 4.4% in 1989 and 3.8% in 1990 followed by a decrease to a level of approximately 1% in the period 1991 to 1993. The RR did not differ significantly relative to 1983 (Fig. 5).

Female dab had a significantly increased risk at contracting skin ulcers in Areas 2 and 4 (1.9 and 2.2 times, respectively) while the risk was reduced in Area 1 and 3 (Table 3).

Skin ulcerations had a negative effect on the condition of the fish. The difference in the condition factor for the

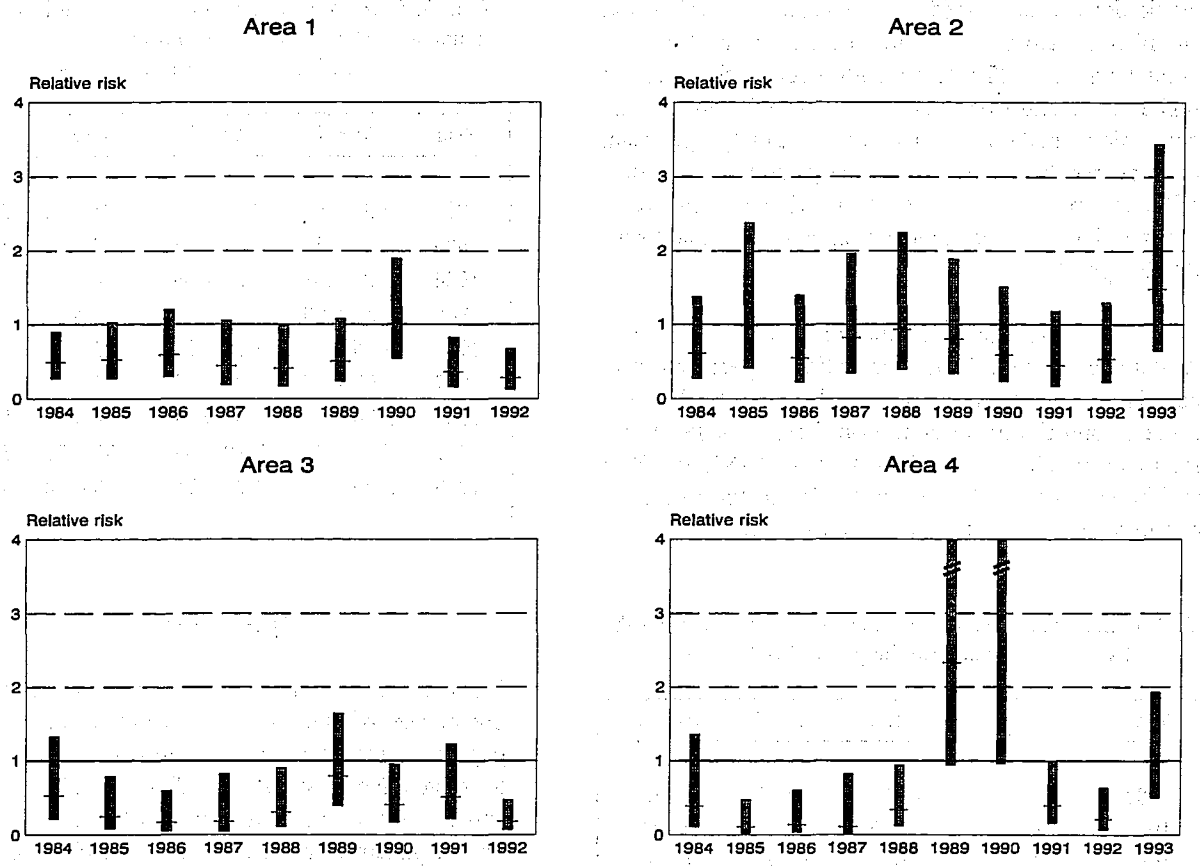


Fig. 5. *Limanda limanda*. The relative risk of contracting skin ulcers at the 4 areas presented with 95% confidence intervals (bar). Where bars do not encounter 1 (bold line) the figures are significantly different from the 1983 figures

ulcerated dab was statistically significant from the unaffected fish in Areas 3 and 4 (Table 4).
The mean age of ulcerated dab differed significantly from unaffected fish in all areas (Table 4).

Correlations

Diseases

The prevalence of lymphocystis and epidermal papilloma showed a positive but not statistically significant correlation ($0.49 < r < 0.56$, $0.12 < p < 0.09$) (Spearman rank correlation test) for Areas 1, 2 and 3 for the whole period of investigation. Similar correlation patterns were observed for the other combinations of diseases in the different areas.

Diseases and stock density

The mean stock density calculated on the basis of the area fished during 1 h of trawl tow was 19, 7, 6 and 2 dabper 1000 m^2 in Areas 1, 2, 3 and 4, respectively. The

Table 5. *Limanda limanda*. The catch per unit effort (no/h) for the 4 areas investigated during the period 1983 to 1993

Year	Area 1	Area 2	Area 3	Area 4
1983	226	379	987	139
1984	7327	681	380	73
1985	1403	917	242	164
1986	609	1334	280	132
1987	1007	1132	1082	84
1988	464	410	1007	160
1989	5753	1794	1636	34
1990	2903	1014	531	145
1991	3326	1119	1283	617
1992	2370	987	709	704
1993	-	658	-	553
Mean	2539	948	814	255

stock density expressed as the catch per unit effort (number of dab per hr of trawl time) showed marked annual variation (Table 5) with Area 1 showing the highest trend and Area 4 the lowest. The Spearman rank correlation test did not show any statistically significant correlation between the stock density and the prevalence of the different diseases except for skin ulcers in Area 2 where a significant negative correlation was found ($r = -0.6207$, $p<0.05$).

Diseases and condition factor

A statistically significant correlation (Spearman rank correlation test) between the prevalence of the different diseases and the mean condition factor (Table 6) was found for lymphocystis in Area 3 ($r= -0.8788$, $p<0.001$) and in Area 4 ($r = -0.6119$, $p<0.05$) and for epidermal papilloma in Area 3 ($r = -0.6525$, $p<0,05$).

Table 6. *Limanda limanda*. The mean condition factor for the 4 areas investigated for the period 1983 to 1993

Year	Area 1	Area 2	Area 3	Area 4
1983	0.939	1.018	1.015	1.126
1984	0.928	0.990	0.959	0.990
1985	0.926	1.001	0.971	1.035
1986	0.973	0.997	1.031	0.991
1987	0.964	0.980	1.008	1.041
1988	0.921	0.872	0.952	1.034
1989	0.926	0.895	0.889	1.075
1990	0.959	1.034	0.990	0.980
1991	0.934	0.944	1.000	1.031
1992	0.914	0.970	1.006	0.990
1993		1.001		1.020

Diseases and oxygen deficiency

Test for correlations between the diseases and oxygen deficiency was conducted by comparing the prevalence of different diseases in one year with the mean and minimum oxygen levels measured in August and September the previous year, as it was hypothesised that low oxygen levels in the autumn might affect the disease level the following year. The Spearman rank correlation test did not reveal any clear correlation pattern between the prevalence of the diseases and the mean and minimum oxygen levels measured in August and September the previous year.

Table 7. *Limanda limanda*.Oxygen level (ml/l) (mean/minimum) at the bottom in August for the 4 areas investigated during the period 1983 to 1990

Year	Area 1	Area 2	Area 3	Area 4
1982	3.39/1.66	3.15/2.14	2.91/1.22	5.13/5.00
1983	2.34/1.39	2.47/0.43	3.44/1.13	4.95/3.88
1984	3.88/3.41	3.81/2.86	5.20/4.45	5.81/5.31
1985	5.54/5.00	5.43/4.44	5.61/5.22	5.38/4.61
1986	4.75/3.33	3.78/1.37	4.95/4.13	5.38/4.94
1987	5.07/4.35	4.96/3.85	5.91/5.59	5.44/4.87
1988	5.24/4.77	5.41/3.09	5.18/4.91	5.46/4.16
1989	5.46/4.92	3.90/2.08	5.85/5.26	5.28/4.56
1990	5.29/5.08	4.73/4.35	5.60/4.98	6.26/5.05

DISCUSSION

The present study describes long-term variations in the prevalence of the diseases, lymphocystis, epidermal papilloma and skin ulcers in dab in 4 different Areas in the eastern North Sea and the Skagerrak. The Areas 1, 2 and 3 showed significant similarities in the temporal disease pattern with peaks in 1985 and 1988 for lymphocystis and epidermal papilloma, although, based on different disease levels. A similar pattern was not observed in Area 4, the Skagerrak. Skin ulcerations did not reveal any kind of temporal trends in any of the 4 areas. The highest prevalences for lymphocystis and epidermal papilloma were observed in Area 1 (German Bight) and in Area 2 (off the west coast of Denmark) and for skin ulcers in Area 4 (Skagerrak).

Similar temporal parallelisms between the diseases lymphocystis and epidermal papilloma have been described before from the German Bight and the Dogger Bank (Dethlefsen 1990) and from the Kattegat (Mellergaard & Nielsen 1995) where statistically significant correlation was found between the 2 diseases. In the present study, these diseases were also found positively correlated in the Areas 1, 2 and 3 but this correlation was not statistically significant.

The westernmost part of Area 2 suffered seriously from oxygen deficiency in September 1981 (Dyer et al. 1983) and in August and September 1982 and 1983 most of Areas 1, 2 and 3 were hit by oxygen depletion (Dethlefsen & Westernhagen 1983). It is likely that oxygen deficiency may be one of the provoking factors for fish diseases in these areas. The hypothesis that oxygen depletion might be involved as a stress factor in the outbreak of lymphocystis and epidermal papilloma in the eastern North Sea was put forward by Mellergaard & Nielsen (1987), whose observations confirmed that in the Kattegat, oxygen deficiency was an important factor in provoking the outbreak of these diseases (Mellergaard & Nielsen 1995). Dethlefsen (1990) found that low oxygen levels may be one of the stress factors contributing to the outbreak of lymphocystis and epidermal papilloma in the German Bight. It has not been possible to establish significant correlations between lymphocystis and epidermal papilloma in the present material as demonstrated for the Kattegat (Mellergaard & Nielsen 1995) probably due to the lack of baseline prevalence for the areas (i.e. before the oxygen deficiency occurred). However, comparing the development of the prevalence pattern of lymphocystis and epidermal papilloma in the period 1983 to 1987 for the Areas 1 to 3 with the observations from the Kattegat (Mellergaard & Nielsen 1995) there are evident similarities that may suggest that oxygen deficiency may be involved in the disease outbreak.

The significant increase in the relative risk of contracting both lymphocystis and epidermal papilloma observed in 1988 can neither be explained by oxygen deficiency nor by significant changes in the water temperature. Furthermore, it is unlikely that changes in virulence of the infectious agents of both diseases should take place at the same time. However, there must have been one factor or another to have increased the dab' susceptibility to these two viral diseases in the eastern part of the North Sea. The phenomenon seemed to be localized to this area, as Dethlefsen (1990) demonstrated an increase in the prevalence in 1988 of lymphocystis and epidermal papilloma in the German Bight but not in the Dogger Bank area, while our data from the Skagerrak (Area 4) similary did not reveal significant changes in the disease rates that particular year.

For Area 1 (German Bight) the dumping of waste from titanium dioxide production may be an additional factor to be considered, which might elevate the prevalence of lymphocystis and epidermal papilloma. Larsson et al. (1980) showed that titanium dioxide industrial effluent caused significant disturbances in the electrolyte balance, in carbohydrate metabolism and changes in the blood parameters of flounder (*Platichthys flesus*). Such changes may be stressful and lead to increased susceptibility to diseases. Dethlefsen et al. (1987) demonstrated elevated rates of lymphocystis and epidermal papilloma in the dumping areas within the German Bight and similar observations have been made in titanium dioxide waste dumping areas along the Dutch coast (Vethaak 1991).

A common feature for the Areas 1, 2 and 3 was that skin ulcerations did not show similar temporal fluctuations as observed for lymphocystis and epidermal papilloma. Similar observationswere made in the Kattegat (Mellergaard & Nielsen 1995) where the relative risks of contracting both lymphocystis and epidermal papilloma were affected by oxygen deficiency while skin ulcerations seemed unaffected.

In Area 4 (Skagerrak) the 3 diseases investigated did not display similar temporal variations in the prevalence as observed in Areas 1, 2 and 3. The significant increase in the relative risk of contracting lymphocystis in 1990 might be due to accidental occurrence. The increase in the prevalence of skin ulcers in 1989 and 1990 might be due to increased fishing intensity in the area as a part of the fishing fleet operating in the Kattegat area turned to the Skagerrak because the environmental conditions had caused serious reduction in the abundance of fish species of commercial interest.

The stock density was highest in Area 1 (German Bight) with mean catches of 2539 dab per hr trawling and lowest in Area 4 (Skagerrak) with 255 dab per hr. It was not possible to establish any clear correlation between the prevalence of the diseases and the stock density except for skin ulcers in Area 2 which were found significantly negatively correlated with stock density. Such a correlation is unlikely to be general as the transmission of diseases should be facilitated by higher stock density. Although, positive correlations between disease prevalence and stock density could not be established it is possible that the differences in stock density between the Areas 1, 2 and 3 and Area 4 is a factor of importance when considering the differences in disease levels.

Lymphocystis was found to be significantly negatively correlated to the mean condition factor of the dab in Areas 3 and 4 and similarly for epidermal papilloma in Area 3. As the condition factor reflects the nutritional status of the fish this may be a factor contributing to outbreaks of diseases in some areas. A negative correlation between lymphocystis, epidermal papilloma and skin ulcers was observed at different locations along the German and Danish coastal waters (Möller 1981) and for lymphocystis in the German Bight (Vethaak et al. 1992). In general, the condition factor for lymphocystis- and epidermal papilloma-affected fish was higher than for the unaffected but this difference was only significant for lymphocystis in Area 1. In contrast, skin ulcerations showed a negative effect on the fish reflected in a decreased condition factor compared to the unaffected fish. The difference in the condition factor between ulcerated and healthy fish was significantly different in Areas 3 and 4. This negative effect may probably be assigned to osmoregulatory problems because of the damaged epithelial lining.

Lymphocystis was found evenly distributed among males and females except in Area 3 where females had a significantly reduced risk of contracting the disease compared to males. Mellergaard & Nielsen (1995) found an even relative risk for both sexes in the Kattegat. The observation in Area 3 may be a coincidence.

Epidermal papilloma revealed a significant increase in the relative risk for females in Areas 1 and 2. Although the relative risk was increased for the females no statistical difference was observed in Areas 3 and 4. A similar pattern has been observed in the Kattegat (Mellergaard & Nielsen 1995) and in the German Bight (Dethlefsen et al. 1987). There is no obvious reasons that females should be more susceptible to epidermal papilloma than males. At the time of year when the present investigations took place, the fish were in the post-spawning period where their condition often are poor and this might explain the difference. However, in that case one should also expect an elevated risk of contracting lymphocystis, which was not observed.

The relative risk of contracting skin ulcerations was found significantly increased only for females in Areas 2 and 4 while slightly reduced in the other areas. In the Kattegat region female dab had a significantly higher risk than males of getting skin ulcers (Mellergaard & Nielsen 1995). Many of the skin ulcers observed during this study looked like skin abrasions indicating that they may originate from traumatic skin damage probably caused by fishing gear. Most ulcerated fish were within the length range 18-23 cm a size which enables some of them to escape the net meshes of commercial trawls or they might have been discarded if caught. Fishery induced skin injuries have been shown correlated with the length of the fish (Lüdemann 1993). In general, female dab are longer than males and this may explain the higher risk observed for females in Area 2 and 4. There is no obvious explanation on why females had a reduced risk in the other areas.

The mean age of diseased fish was significantly higher compared to unaffected fish for all 3 diseases examined indicating that it is mainly older fish that become diseased.

The present study demonstrated marked geographical differences in the disease levels in dab. Areas such as the German Bight and the west coast of Denmark having suffered from oxygen deficiency showed significantly higher prevalence of lymphocystis and epidermal papilloma compared to an unaffected area like the Skagerrak. Although, environmental stress often has a multifactorial background, the impact of oxygen depletion has probably been underestimated (Sindermann 1984). Therefore, there is a risk that the increased eutrophication of estuarine and coastal waters may be the most important danger to the health status of wild fish populations.

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DFU-rapporter

nr. 5 En undersøgelse af maveindholdet af Østersølaks i 1994-1995
Ole Christensen

nr. 6 Udsætningsforsøg med Østersølaks
Heine Glüsing, Gorm Rasmussen

nr. 7 Kampen om Limfjorden - Livsformer, miljøværdier og reguleringsformer
Kirsten Monrad Hansen

nr. 8 Tangetrappen 1994-95
Anders Koed, Gorm Rasmussen, Gert Holdensgård, Christian Pedersen

nr. 9 Status over bundgarnsfiskeriet i Danmark 1994
Anders Koed, Michael Ingemann Pedersen

nr. 10 Måling af kvalitet med funktionelle analyser og protein med nærinfrarød refleksion (NIR) på frosne torskeblokke
Niels Bøknæs

nr. 11 Acoustic monitoring of herring
J. Rasmus Nielsen

nr. 12 Blåmuslingers vækst og dødelighed i Limfjorden
Per Dolmer

nr. 13 Mærkningsforsøg med ørred og regnbueørred i Århus Bugt og Isefjorden
Heine Glüsing, Gorm Rasmussen

nr. 14 Jomfruhummerfiskeriet og bestandene i de danske farvande
Mette Bertelsen

nr. 15 Bærekapaciteet for havørred (*Salmo trutta* L.) i Limfjorden
Kaare Manniche Ebert

nr. 16 Sild og brisling i Limfjorden
Jens Pedersen

nr. 17 Produktionskæden fra frysetrawler via optøning til dobbeltfrossen torskefilet. Optøningsrapport (del 1).
Niels Bøknæs

nr. 18 Produktionskæden fra frysetrawler via optøning til dobbeltfrossen torskefilet. Optøningsrapport (del 2)
Niels Bøknæs

nr. 19 Automatisk inspektion og sortering af sildefileter
Stella Jónsdóttir, Magnus T. Ásmundsson, Leif Kraus

nr. 20 Udsætning af helt, *Coregonus lavaretus* L., i Ring Sø ved Brædstrup
Thomas Plesner, Søren Berg

nr. 21 Udsætningsforsøg med ørred, (*Salmo trutta* L.) i jyske og sjællandske vandløb
Heine Glüsing, Gorm Rasmussen

nr. 22 Kvalitetsstyring og målemetoder i den danske fiskeindustri. Resultater fra en spørgebrevsundersøgelse
Stella Jónsdóttir

nr. 23 Quality og chilled, vacuum packed cold-smoked salmon
Lisbeth Truelstrup Hansen (Ph.D. thesis)

nr. 24 Investigations of fish diseases in common dab (*Limanda limanda)* in Danish Waters
Stig Mellergaard (Ph.D. thesis)