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REPORT OF THE WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Copenhagen, 2 - 5 March 1992

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REPORT OF THE WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

ICES, Copenhagen, 2-5 March 1992

1 INTRODUCTION

The Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at ICES, Copenhagen, with Dr B.J. Hill as Chairman (C.Res.1991/2:41). The participants were welcomed by ICES General Secretary, Dr Emory Anderson.

1.1 Opening of the Meeting

The meeting was opened at 09.00 on Monday 2 March with the Chairman, as last year, expressing disappointment at the low attendance rate of the Working Group's members and, particularly, the absence of any participant from North America. This would inevitably mean that some items could not be dealt with as effectively as they might have been. Apologies had been received from some Working Group members, who explained that their non-attendance was due to budgetary restrictions on overseas travel. The list of participants is at Annex 1.

2 TERMS OF REFERENCE, ADOPTION OF AGENDA, SELECTION OF RAPPORTEURS

Participants were referred to the Terms of Reference (TOR) for the meeting given to the Working Group by the ICES Council through C.Res.1991/2:41. There had been amendments made by ICES to the TOR recommended by the Working Group in its 1991 report. In particular, the task of statistical analysis of the compiled national data on disease prevalence rates in marine fish had been allocated to a Sub-Group of the Working Group formed especially for this and which had met for 3 days at ICES Headquarters on 27-29 February. Their report was to be considered by the full Working Group at this meeting. Two additional tasks had been given to the Working Group; these were TOR e) and f).

TERMS OF REFERENCE

a) Review the results of the statistical analysis of disease prevalence data conducted by the Sub-Group indicated below and report to the 1992 ACMP meeting;

b) Compile and analyse national reports on recent disease trends in mariculture, provide advice on preventive and control measures, and report to the 1992 ACMP meeting;

c) Review current fish disease control measures in order to complete the updating of the relevant ICES/EIFAC guidelines on the "Code of Practice in Introductions and Transfers" in close collaboration with the Working Group on Introductions and Transfers of Marine Organisms;

d) Analyse the compiled information from ICES Member Countries on cases of disease interactions between farmed and wild populations, evaluate for any evidence of detrimental impact of disease on wild fish in marine fish farms, and report to the 1992 ACMP meeting;

e) Prepare, in response to a request from NASCO for its West Greenland and North-East Atlantic Commission areas, an inventory of parasites and diseases of wild and reared salmon by country, and report to the Working Group on North Atlantic Salmon;

f) Evaluate the impact of *Ichthyophonus* spp. on affected herring stocks, estimate the extent of mortality on these stocks from this disease, and report to the 1992 meetings of the Herring Assessment Working Group for the Area South of 62°N, the Working Group on Assessment of Pelagic Stocks in the Baltic, and the Atlanto-Scandian Herring and Capelin Working Group.

A Sub-Group consisting of members of the Working Group on Pathology and Diseases of Marine Organisms will meet under the chairmanship of Dr J. Thulin (Sweden) at ICES Headquarters from 27-29 February 1992 to:

a) Statistically analyse the compiled national data on disease prevalence rates in marine fish stocks which have been submitted to ICES;

b) Analyse, evaluate, and assess any disease data submitted to ICES for the purposes of the North Sea Task Force and the Baltic Marine Biologists, taking into account the residence time of dab and flounder in the areas under study;

c) Develop plans for assessing the relationship between disease prevalence rates in marine fish and contaminant concentrations in sediments, which will utilise the results of the 1990/1991 Baseline Study of Contaminants in Sediments in the OSPARCOM area;

d) Report to the Working Group on Pathology and Disease of Marine Organisms at its 1992 meeting.

An Agenda was agreed (Annex 2) and rapporteurs selected for individual tasks (Annex 3).

3 REPORT ON THE 1991 ICES STATUTORY MEETING

The Chairman drew attention to items of relevance to WGPDMO arising in reports at the 79th Statutory Meeting held in La Rochelle, France, 26 September - 4 October 1991. One item was the TOR (c) given to the Study Group on Pollution Affecting Shellfish in Aquaculture and Natural Populations, which was due to meet from 1-3 July 1992. This was "in conjunction with the WGBEC identify relationships between pollutants and disease susceptibility in shellfish". The Chairman was unable to explain why the WGPDMO, with all its experience of dealing with this topic in connection with disease in fish, had not been given the task or asked to collaborate with the Study Group. The Working Group agreed that its concern about this matter should be passed on to ICES.

4 RECENT TRENDS IN MARICULTURE DISEASES

Written reports were received from ten ICES member countries. The main trends and developments identified were as follows:

A. FISH

1. Atlantic Salmon

Infectious pancreatic necrosis (IPN). Its importance is recognized in most European countries and is reported for the first time in the Faroe Islands. Strict control of the disease status of individual broodfish used to supply eggs to the salmon farming industry is considered to be effective in preventing the disease. There is a trend towards increasing pathogenicity in farmed salmon in sea water, with associated mortalities, in Scotland, Norway, and the Faroes.

Pancreas disease (PD). In Ireland, Scotland and Norway the severity of the disease was less than in 1990. Research is being carried out in Scotland, Ireland, Norway and France to identify the suspected virus, using culture, and experimentally infecting fish. There is evidence that stopping feeding immediately when PD is suspected prevents the destruction of the exocrine pancreas, thus enabling rapid recovery yet still permitting resistance to develop.

Infectious Salmon Anaemia (ISA). The disease remains confined to Norway, where the number of infected farms has increased. There are signs that the severity of the disease may have decreased due to improved management methods.

Other viral diseases. The report from Canada noted that there is strong evidence to suggest that the "marine anaemia" on the west coast of Canada is associated with a retrovirus.

Furunculosis (typical). The disease was diagnosed for the first time in the Faroe Islands with losses of up to 5%. The isolated strains were multi-resistant to available licensed antibiotics. A further spread was seen on the east coast of Canada and in Norway. The increasing problems of antibiotic resistance reported last year have spread further (Norway, Scotland, Ireland and the Faroe Islands). Improved management has been shown, in both Norway and Scotland, to affect the severity and occurrence of furunculosis. An outbreak of furunculosis was observed in fish imported from Sweden to Denmark. The resistance patterns of isolated strains were different from those of the strains usually found in Denmark.

Bacterial kidney disease (BKD). An increase in mortalities in seacage culture of Atlantic salmon was reported from the east coast of Canada and a third case of the disease was reported from Finland.

Vibriosis. Although still a problem on the east coast of Canada, vibriosis seems to be less important in other reporting countries in 1991, possibly because of the increased use of vaccination.

Sealice (*L. salmonis*) remains an important disease problem in several countries. Experimental work is now in progress towards the development of a vaccine in Scotland and Ireland. In the field new control methods are increasingly being assessed (wrasses, pyrethrins, hydrogen peroxide, Ivermectin, azamethiphos) but dichlorvos remains the most commonly used treatment. It is to be emphasized that cleaning of the nets can reduce re-infection and fallowing of the area can be very effective in managing the problem.

Other conditions recorded

Systemic *Hexamita salmonis* infection continues to spread in a localised part of northern Norway with associated mortality.

Diseases reported for the first time

In France, from September to December, a high mortality (20%) was associated with signs of meningo-encephalitis, the aetiology of which is unknown.

2. Other salmonids

Rainbow Trout

Viral haemorrhagic septicemia (VHS) still remains a major disease problem for marine-reared rainbow trout in Denmark.

Furunculosis (typical) was diagnosed for the first time in the Faroe Islands. Further spread of the disease has occurred in Finland, with increasing numbers of antibiotic-resistant strains being isolated.

Vibriosis. An increased number of outbreaks occurred in Finland despite the fact that the fish were vaccinated (by dipping). A re-appraisal of the vaccination procedure being used in Finland would seem to be called for.

Brown Trout

In France, the main problem in this species in marine farms was muscular infestation with *Kudoa* sp. (*Myxosporidia*), associated with muscular post-mortem liquefaction (10 - 20% of fish at commercial size).

3. Non-salmonids

Infectious pancreatic necrosis (IPN) was diagnosed in Atlantic cod fry, reared for experimental purposes on salmon farms in the Faroe Islands. In Spain, the virus was detected but not associated with mortality in turbot.

A Picorna-like virus causes encephalitis in sea bass larvae in hatcheries in France. This important disease, first reported in 1990, shows no sign of abatement.

Vibriosis has been a serious disease in juveniles of turbot in Spain, Denmark and Norway and of cod in Norway, Canada and Denmark, indicating that when cultivating new marine species outbreaks of vibriosis can be expected.

A microsporidian, *Tetramicra brevifilum*, causes mortality and poor growth in turbot (Spain) and is increasing in importance.

Diseases reported for the first time

Flexibacteriosis (systemic) was observed for the first time and has been a significant problem in sea bream in France (Mediterranean).

Iridovirus was observed in Denmark, in turbot fry skin, and is associated with high mortality, but its role in the mortality is unknown.

Conclusions:

a) There is a trend towards increasing problems with IPN-associated mortalities in sea-farmed Atlantic salmon in several ICES member countries.

b) Management techniques are proving to be effective in reducing some disease problems in fish farming. These include reduction in stocking density, prevention of overlap of different year classes, and temporary removal of stocks from sites or larger areas (fallowing), and such methods should be encouraged wherever possible as an adjunct to chemotherapy.

c) The increasing resistance of *Aeromonas salmonicida* to the limited range of licensed antibiotics available to fish farming is a matter for concern as it is inevitably leading to increasing problems in controlling furunculosis.

d) Until the susceptibility of a new species of farmed fish to diseases present in existing stocks on a farm is known, caution should be exercised. Whenever possible, the different species should be segregated.

e) It should not be assumed that it is safe to transfer fish from one infected area to another with the same disease. There is the possibility of pathogen strains with different properties, including antibiotic resistance profiles, serotypes and degree of virulence, being translocated in this way.

Recommended Actions

1. In view of the trend towards increasing pathogenicity of IPN in sea-farmed Atlantic salmon, noted in several ICES member countries, it is recommended that research be encouraged to obtain further information on the source of this disease in the marine environment and the reduction of its effect.

2. WGPDMO encourages research into management techniques which may reduce disease problems in marine fish farming.

3. In view of the increased number of reports of furunculosis in wild marine salmonid populations, WGPDMO urges ICES member countries to exercise particular caution about the potential risk of spreading this disease during sea ranching/stock enhancement programmes.

B. SHELLFISH (Molluscs)

A. *Ostrea edulis*

Bonamiosis (*B. ostreae*)

Recrudescence of bonamiosis has occurred in France in the St. Philibert River (prevalence 44%) in Brittany without any apparent reason. In the Netherlands, the stock of 3-5 year-old oysters in Lake Grevelingen has been almost exterminated because of the disease. However, the prevalence of the parasite in the surviving stock has decreased sharply to below <10%. Furthermore, there has been a remarkable survival of young stock which is also affected by a low (<2%) prevalence of infection. In Ireland (Galway Bay), bonamiosis has spread in the wild oyster beds. No significant change has occurred in Spain and the UK.

B. *Crassostrea gigas*

No significant disease was reported from the different countries for this species.

B. ostreae has never been observed in the tissues of *C. gigas* and field and experimental observations have not provided any evidence of a possible role for *C. gigas* as a carrier of *B. ostreae*.

In the USA, members of the Atlantic States Marine Fisheries Commission have opposed the introduction of *C. gigas* to the Atlantic coast to restore the oyster populations, depleted by disease, until social, economic and political concerns are addressed.

C. *Crassostrea virginica*

An organism, similar to *Bonamia* in size but not affecting haemocytes, has been newly discovered in association with an acute mortality of hatchery reared juvenile oysters, *Crassostrea virginica*, on Long Island, NY. Losses of 80-90% in oysters measuring less than 31 mm occurred during peak high water temperature in summer. The disease, also found in wild populations in Long Island Sound, is characterised grossly by a heavy brown/yellow line of conchiolin in the shell to which the developing muscle does not attach. Histopathological lesions include ulceration, inflammation and haemorrhage in the mantle epithelium and mantle epithelial cells contain the small, round organism. The disease also has been found in slightly larger juvenile oysters (29-45 mm) in the Damariscotta River, Maine, where significant but less severe mortalities occurred. Infected oysters maintained in cold water tend to survive infection, although the organism remains present; survivors continue growing. It is suspected that the parasite is retained in the brood stock and that unregulated movement of seed oysters contributed to the spread of this disease from one geographic location to another.

D. *Tapes philippinarum*

Brown ring disease of farmed and wild clams has declined in many areas in France. Production levels have recovered to the level (600 t) prevailing before the disease appeared. Certain sanitary measures (eradication, low density, initial screening of the spat) and possibly higher water temperatures could be the reason for this reduction of impact of the disease.

E. Mussels

The unidentified *Marteilia* sp. continues to be present in stocks in Spain and France, but without associated mortalities. Its relationship, if any, to *M. refringens*, the serious pathogen of flat oyster, remains unclear.

Conclusions

The Working Group concludes that the extensive field observations combined with the detailed laboratory studies conducted in France provide sufficient evidence that *C. gigas* does not act as a vector for *Bonamia ostreae* and that transfers of this oyster species from Bonamiosis-infected areas to uninfected areas carry no discernible risks of spreading the disease.

Recommended Actions

1. France and Spain should increase research effort to resolve the question of the identity of the *Marteilia* sp. found in mussels and to determine its relationship to *M. refringens*.

2. Studies should be conducted similar to those with *C. gigas* to determine whether other bivalve molluscs (such as mussels, clams and scallops) are capable of being vectors for the transfer of *B. ostreae* from infected to non-infected areas.

5 ANALYSIS OF CASES OF DISEASE INTERACTIONS BETWEEN FARMED AND WILD POPULATIONS OF FISH

Because of the growing concern being publicly expressed that increasing levels of parasites and diseases could be spilling over from marine salmon farms into wild salmonid populations with detrimental effects on stock levels, the Working Group reviewed available information and case studies relating to this issue. The principal means of disease interaction between farmed and wild stocks were discussed by the Working Group.

a) <u>The introduction of diseases new to an area by introduction of farmed or wild fish stocks from other areas</u>

The movement of live fish without adequate disease screening constitutes the most important potential means of disease spread to both wild and farmed fish stocks. The introduction of furunculosis into farmed salmon in Norway through imported live smolts provided an example of such an occurrence. This disease subsequently spread between farms and thence also to wild stocks. Several studies are underway to evaluate the effect of the disease on wild salmon, but conclusive evidence of an adverse impact on such stocks has yet to be established. However, there is historical evidence that the introduction of furunculosis into wild salmon populations in Britain, earlier this century, had a serious impact on these stocks. The subsequent spread of the disease to farmed salmon, with the advent of salmon farming, has had a major economic impact. In the Baltic Sea area, the current spread of furunculosis in farmed salmonid stock has been attributed to the presence of the disease in wild stocks. The release of salmon for sea ranching activities may be an important contributory factor in this regard.

Diseases of freshwater origin have a similar potential for spread between farmed and wild stocks. The infestation of wild Norwegian salmon with the monogenetic trematode *Gyrodactylus salaris* has had a major impact on wild salmon and may have resulted from transfers of infected stock for farming and enhancement purposes. Similarly, the parasite *Anguillicola crassus* is known to have originated in eels (*Anguilla japonica*) imported into Europe from Asia. However, whether this parasite has adversely affected European eel stocks, e.g., by damaging their ability to migrate or in other ways, has not been conclusively demonstrated.

b) <u>The amplification of indigenous disease problems due to the special situation on fish farms</u>

The basis of this possible effect is that fish farms, by their nature, contain large populations of fish confined together at relatively high stocking densities, creating a much greater potential for the production of high levels of pathogenic organisms and parasites. The presence of relatively high numbers of wild fish in the vicinity of fish farms tends to maximise the possibility of spread to such fish due to the shedding of pathogens and/or parasites from such farms.

It is this rationale which has led to an opinion that farmed salmon could be the cause of high levels of sea lice on wild salmonids, in Ireland and Norway. However, caution is needed on this issue, as a recent scientific report from Ireland (Sea Trout Working Group Report, December 1991) stated that it was not possible to demonstrate a significant correlation between the production of large numbers of sea lice from sea cage sites and subsequent infestation of sea trout in specific bays in Ireland. Furthermore, an appraisal of the sea trout problem in Ireland carried out by the WGPDMO in 1991 concluded that "the hypothesis that in all probability sea lice derived from fish farms was a major factor in the collapse of sea trout stocks in the region (West of Ireland) could not be justified in the light of available scientific evidence".

It must also be stated that several significant diseases of marine fish in mariculture may well have their origin in wild marine fish which act as reservoirs for the pathogens and thus sources of diseases for farmed fish. It is likely that the organisms responsible for diseases such as Infectious Salmon Anaemia (ISA) and Pancreas Disease (PD) originate in wild fish or other organisms living or migrating in the vicinity of fish cages. Also, known viral diseases such as Infectious Pancreatic Necrosis (IPN), whose prevalence and significance in some ICES member countries has increased recently in farmed salmon, may originate in wild marine stocks of fish or shellfish in which the virus is known to occur.

This scenario may well apply to many other diseases, although such information is currently lacking and further studies are needed.

Conclusions

1. From a disease point of view, the interaction between farmed and wild fish is a complex two-way process with opportunities for spread in both directions, but on the evidence available it would seem that, if anything, wild fish pose a greater disease threat to farmed fish than vice versa.

2. From a review of the published data available, WGPDMO did not find evidence of a significant detrimental effect on wild fish stocks as a consequence of the occurrence of disease in mariculture. This situation needs to be kept under review, as studies in this area are currently in progress in Ireland, Scotland and Norway.

Recommended Actions

In view of the increasing concern with this question, the WGPDMO feels that more definitive data need to be obtained and recommends that appropriate ICES member countries encourage detailed systematic research on disease interactions between farmed and wild fish in the marine environment.

6 REVIEW OF CURRENT FISH DISEASE CONTROL MEASURES FOR UPDATE OF ICES/EIFAC GUIDELINES ON THE "CODE OF PRACTICE ON INTRODUCTIONS AND TRANSFERS"

H. Grizel (who is also a member of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO)) gave a preliminary reaction of the WGITMO to the revisions for molluscs recommended by the WGPDMO. Many of the proposals were considered to be useful and they could be incorporated into the technical appendices of the Code. However, for a number of reasons, some of the changes proposed caused problems for the WGITMO sub-group dealing with this topic, and could not be adopted. It was felt that the suggestions of WGPDMO are more relevant to transfers of fish and shellfish which form part of current commercial practice rather than to new introductions. The sub-group also felt that the use of diagnostic kits for disease diagnosis would not be feasible for most of the molluscan diseases and that more classical methods (histology and smears) must be retained until more reliable rapid tests become available.

Both working groups recognize the need to adapt the technical annexes of the Code of Practice relating to transfers forming part of current commercial practice, to take account of the diagnostic methods currently being drafted by expert groups within the EC and OIE (Office International des Epizooties). As more than half of the ICES member countries are also in the EC, this is an important consideration for these countries, which will in the future be required to use the methodologies laid down by the EC Scientific Veterinary Committee.

In relation to changes to the fish disease sections contained in the ICES/EIFAC Code of Practice, the Working Group feels that it would not be appropriate to propose major changes at this time, because of pending EC and OIE methodologies in preparation.

Recommended Actions

The Working Group recommends that:

1. The ICES Secretariat should obtain copies of the EC and OIE method documents and make them available to both the WGPDMO and WGITMO at an early date.

2. A one-day joint meeting be held between WGPDMO and WGITMO during their 1993 meetings to discuss these and other appropriate fish and shellfish disease methodologies in order to complete the revision of the disease guidelines in the Code of Practice on Introductions and Transfers.

7 INVENTORY OF PARASITES AND DISEASES OF WILD AND REARED SALMON IN COUNTRIES AT THE WEST GREENLAND AND N.E. ATLANTIC COMMISSIONS AREAS OF NASCO

This term of reference was discussed at length by the WGPDMO. Additional comments were given on the background to this item by Dr R. Grainger of the ICES Secretariat, who had attended some of the NASCO discussions. Considerable problems with compiling and presenting these kinds of data were identified.

The inventory (Annex 4) was prepared using data submitted by WGPDMO members, despite considerable concern being expressed that this would be both incomplete and potentially misleading. It is strongly emphasized by the WGPDMO that this information has severe limitations and should only be used with full awareness of the following constraints (not a definitive list):

a) The information more likely reflects the amount of research activities in this field in Member Countries rather than the actual situation. The recent increase in salmon farming in several countries has resulted in strong emphasis on disease research in farmed salmon in comparison with the extensive older information on the occurrence of, in particular, parasites in wild salmon;

b) Many reports of diseases/parasites are of single observations often in individual fish and equal significance cannot be assigned to all examples listed;

c) Information was not available from some relevant member countries;

d) The record of a disease/parasite does not necessarily mean that it is still present. It is known that some of the records are of accidental or temporary infections;

e) Unless a disease/parasite has been specifically looked for, its absence from the list for a particular country, or in farmed or wild fish cannot be taken as evidence of its absence;

f) The different data provided had not been compiled on the same basis, e.g., some data were based on old records, whereas others were for the last 5 years only; and

g) There is controversy regarding the specific identification of some pathogens/parasites and, consequently, records of a particular example from different countries may in reality refer to different infections.

Recommended Actions

NASCO should take note of the limitations of this inventory and its vulnerability to misinterpretation, and the WGPDMO urges caution in its use. For example, the circumstances of pathogen/parasite detection and diagnosis must be taken into account in assessing the relative degrees of significance of the listed examples.

8 EVALUATION OF THE IMPACT OF *ICHTHYOPHONUS* SPP. ON AFFECTED HERRING STOCKS, INCLUDING EXTENT OF MORTALITY

During the past year there has been an increasing concern about the occurrence of the parasitic fungus *Ichthyophonus* spp. in herring stocks within parts of the ICES area and the relationship of this disease to possible mortalities.

Initial sampling showed the occurrence of the disease in parts of the North Sea, Eastern North Atlantic and the Baltic Sea. Differences in sampling and diagnostic features led to an attempt to standardisation at the ICES Special Meeting on the *Ichthyophonus* Problem in the European Herring held at Lysekil, Sweden, on 7 November 1991. The problem was referred for consideration by the WGPDMO at its 1992 meeting to evaluate the impact of *Ichthyophonus* on affected herring stocks, to estimate the extent of mortality on these stocks, and to report to the 1992 meetings of the Herring Assessment Working Group for the Area South of 62°N, the Working Group on Assessment of Pelagic Stocks in the Baltic, and the Atlanto-Scandian Herring and Capelin Working Group.

Results of the analysis of data obtained between June 1991 and February 1992 are summarised in Figure 1. Absence and prevalence below approximately 5% and above 5% are denoted with different symbols in the figure. Due to various confounding factors with impact on the results, it was considered to be misleading to give information on actual disease prevalences at present and preferable to give an impression of the spatial distribution of the disease and a rough index on its abundance within ICES statistical rectangles. Confounding factors identified include differences in sampling methods (e.g., samples from commercial catches show lower prevalence compared to samples from research vessels), differences in diagnostic procedures (e.g., microscopic examination of squash preparations of heart tissue reveal higher prevalence levels than macroscopic observation), differences in the length composition of the catch (the prevalence of infection increases with length), and possible migratory and behavioural differences. To facilitate greater standardisation, the Working Group, therefore, designed a reporting format as shown in Figure 2.

From Figure 1, two areas with high levels of infection can be identified, namely, the Kattegat/Western Baltic Sea area and an area north of 60°N between Shetland and Norway. Uninfected areas are recognised within the Central/Eastern Baltic Sea, the Bothnian Bay, and to the west of Scotland and Ireland. Although low levels have been found off the Dutch/Belgian coasts, the limited sampling so far in the southern North Sea indicates no major problems at present. Information on elevated infection levels in the Norwegian spring spawning stock has not been included on the map.

Data submitted by letter to WGPDMO also indicated the occurrence of *Ichthyophonus* in Estonian waters and in stocks of the Pacific herring off the Canadian coast, whereas *Ichthyophonus* was absent from the limited sampling of Icelandic herring stocks.

The terms of reference of the WGPDMO required an estimate of the extent of mortality of herring stocks due to *Ichthyophonus* infection. However, the lack of precise information on the prevalence of the disease within separate herring stocks, as well as the lack of knowledge on its temporal dynamics derived from experiments, made it impossible to establish reliable mortality rates to be used for stock assessment purposes. An analysis of disease dynamics and prevalence has previously been applied on an *Ichthyophonus*-infected plaice stock to establish a mortality rate in a natural population. Based on this approach, examples of annual mortality rates within different scenarios involving the prevalence and the survival time of the fish were calculated (see Table 1). This was considered justified because available information indicates that this disease is considered lethal for both plaice and herring.

Table 1 Annual mortality rates (%) calculated from theoretical prevalence levels and average maximum survival (100/200 days) of infected individual fish.

Prevalence (%)	Mortality rate (%)	
	100 days survival	200 days survival
5	18.5	9.1
10	36.5	18.3
15	54.7	27.4

The mortality rates given in the table represent the additional contribution possible from *Ichthyophonus* infection above other forms of natural mortality and fishing mortality.

Studies are currently in progress to determine more accurate prevalence levels and mortality rates.

It was concluded that historical herring stock assessment data should be re-evaluated within the relevant ICES Working Groups in order to identify certain unpredicted recruitment failures which may have been due to former and current *Ichthyophonus* epidemics. It was realised that it would be useful to include historical data on the possible impact of *Ichthyophonus* epidemics on the north-east Atlantic herring stock during the 1950s.

Studies recently performed have shown many other fish species to be infected in areas with high levels of infection in herring.

Recommended Actions

a) Sampling and examination procedures for herring should be standardised according to the methods recommended in the report from the ICES Special Meeting on the *Ichthyophonus* Problem in the European Herring held at Lysekil, Sweden, on 7 November 1991. This means the conduct of macroscopic and microscopic examinations of heart tissue. Data obtained should be reported to the ICES Secretariat using the standard reporting format (Figure 2).

b) Historical herring stock assessment data should be re-evaluated within the relevant ICES Working Groups in order to identify certain unpredicted recruitment failures which may have been due to former *Ichthyophonus* epidemics.

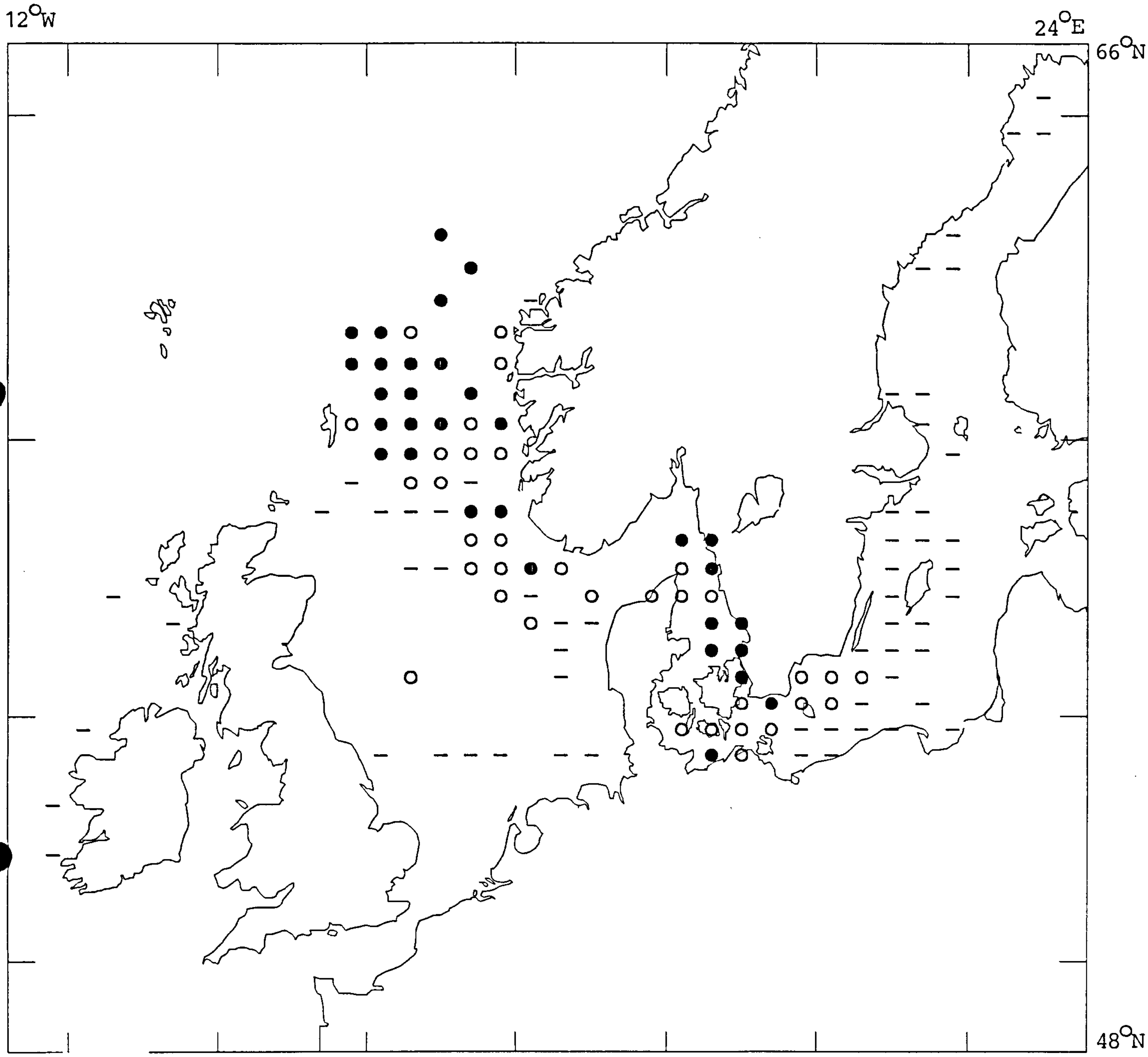
c) ICES should encourage stock assessment specialists from the USA and Canada to provide historical data on the possible impact of known *Ichthyophonus* epidemics on the north-east Atlantic herring stock from the 1950s onwards.

d) Other ICES Working Groups concerned with wild as well as farmed fish species should be informed by ICES that these species might also be affected by this epidemic situation, but that not all species may suffer significant disease-related mortalities.

e) It is recommended that a second ICES Special Meeting on *Ichthyophonus*, involving stock assessment and disease specialists, should be held in autumn 1992, in order to:

   - produce reliable information on the prevalence of *Ichthyophonus* in different herring stocks based on results obtained using the standardised methods recommended by the WGPDMO; and

   - estimate the extent of mortality in herring stocks utilising data obtained from experimental work and field observations.



- No infection
o Low (<5%) infection
● High (>5%) infection

Figure 1. Distribution of *Ichthyophonus* spp. in herring (based on ICES statistical rectangles).

ICES ICHTHYOPHONUS REPORTING FORMAT

Completed forms to be sent to ICES and to be considered by WGPDMO

COUNTRY:
DAY/MONTH/YEAR:
STATION LATITUDE:
STATION LONGITUDE:
ICES RECTANGLE NO:
NO. OF HAULS:

OBSERVER:
*STRATIFIED SAMPLING: YES/NO
COMMERCIAL HAUL/RESEARCH HAUL:
TYPE OF GEAR:
REMARKS:

FISH SPECIES: Herring (Clupea harengus)

SIZE GROUP: 15-19 cm

MEAN LENGTH ±SD:

(recommended 100 fish)

TOTAL NO. EXAMINED		
Male	Female	Unknown

	TOTAL NO. AFFECTED			PREVAL.
	Male	Female	Unknown	(%)
MACROSC. EXAM.				
**MICROSC. EXAM.				
MIC. + MAC. EXAM.				

SIZE GROUP: 20-24 cm

MEAN LENGTH ±SD:

(recommended 100 fish)

TOTAL NO. EXAMINED		
Male	Female	Unknown

	TOTAL NO. AFFECTED			PREVAL.
	Male	Female	Unknown	(%)
MACROSC. EXAM.				
**MICROSC. EXAM.				
MIC. + MAC. EXAM.				

SIZE GROUP: ≥25 cm

MEAN LENGTH ±SD:

(recommended 100 fish)

TOTAL NO. EXAMINED		
Male	Female	Unknown

	TOTAL NO. AFFECTED			PREVAL.
	Male	Female	Unknown	(%)
MACROSC. EXAM.				
**MICROSC. EXAM.				
MIC. + MAC. EXAM.				

*Stratified sampling: YES: when there has been a selective sampling of length groups or year classes from the catch. NO: when there has been a total or random sampling.

**MICROSC. EXAM.: Based on squash preparation, should include all specimens which have been regarded as negative in the MACROSC. EXAM of the hearts.

The method of observation and analysis should be as detailed in the report of the ICES-Special Meeting on the Ichthyophonus problem in the European herring held at Lysekil, Sweden, November 7 1991.

Figure 2

9 REVIEW OF THE RESULTS OF THE SUB-GROUP ANALYSIS OF DISEASE PREVALENCE DATA FOR MARINE FISH STOCKS

The Working Group was informed about the meeting of the Sub-Group on Analysis of Disease Prevalence Data for marine fish stocks which met from 27 to 29 February to:

a) statistically analyse the compiled national data on disease prevalence rates in marine fish stocks which have been submitted to ICES;

b) analyse, evaluate and assess any disease data submitted to ICES for the purposes of the NSTF, and the Baltic Marine Biologists (BMB), taking into account the residence time of dab and flounder in the areas under study;

c) develop plans for assessing the relationship between disease prevalence rates in marine fish and contaminant concentrations in sediment, which will utilise the results of the 1990/1991 Baseline Study of the Contaminants in Sediments in the OSPARCOM area;

d) report to WGPDMO at its 1992 meeting.

J. Thulin, who chaired the sub-group meeting, presented the report of the sub-group, summarising the background for the meeting. The report of the sub-group is attached as Annex 5.

The meeting was specifically convened to conduct a first evaluation of fish disease monitoring data collected for the North Sea Task Force (NSTF) as part of the contributions to the 1993 Quality Status Report (QSR) on the North Sea. The meeting had focussed on the overall sampling methodology and statistical methods for evaluation of the data and the preparation of products which could be forwarded to the NSTF. The evaluations dealt only with data on dab (*Limanda limanda*) for the following diseases: lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations, and liver nodules/tumours. Dab was the main species of interest to the NSTF and few data were available on other species.

The meeting had based its work on a discussion paper prepared immediately prior to the meeting which reviewed possible statistical approaches for dealing with the data and raised a number of problematic issues in relation to the assessment of fish disease monitoring data. The meeting concurred with the proposal that logistic regression analysis (LRA) provided an appropriate analytical tool; however, this was only possible on sub-sets of the data due to the limitations of the data available. LRA was used, therefore, in the study of test data sets in order to evaluate as far as possible the main sources of variation in the fish disease data.

For the full data sets, the sub-group prepared presence/absence maps for the diseases lymphocystis, epidermal hyperplasia/papilloma, and acute/healing skin ulcerations; liver nodules/tumours were excluded because not all data reported had been confirmed by histological examination. Additional prevalence maps showing a breakdown of the data by season and sex are included in the sub-group report (Annex 5).

The sub-group was concerned that the maps provided to NSTF could only be used with a full appreciation of the limitations of the data sets and the procedures employed in their production. In relation to this, the sub-group recommended that only the presence/absence maps are suitable for forwarding to the NSTF and emphasised the requirement that a warning statement be associated with each map to avoid possible mis-use of the maps. The sub-group further recommended that the section of the QSR dealing with fish diseases should be reviewed by WGPDMO. The following members were designated to conduct this review: Vethaak, Lang, Mellergaard, M^c^Vicar, Bucke, and Thulin.

Additional conclusions and recommendations were developed during the sub-group evaluations of the available data, including the need for future re-evaluation of the data and improvements in the guidelines for fish disease monitoring, including the formats for reporting data to the ICES Secretariat for inclusion in the fish disease data bank.

For future assessments, this first evaluation process had proven a valuable exercise in identifying improvements required and the need for more detailed and complete information to be collected and reported to ICES. In many cases, relevant information is available and will be submitted to ICES for incorporation in the data bank already established at the ICES Secretariat.

Whilst the assessment had considered the influence of a number of biological factors in general terms, information allowing more detailed investigation of these and other factors (haul effects, appropriate information on migration habits, age-length relationships, etc.) is still lacking. No assessment of the fish disease data in relation to environmental factors had been possible as the data on sediment contamination will not be available until the end of April 1992.

Assessment of fish disease data for the Baltic region was not possible as these data will not be available until April 1992.

In connection with the proposals for future data evaluation, the sub-group recognized the need to evaluate data on cod and flounder; few data on these species are available for the North Sea area. These species are, however, target species for disease surveys in the Baltic Sea where dab are not available.

In relation to a comparison of fish disease and environmental data, the sub-group considered that this could only be achieved by a group involving multi-disciplinary specialists when all relevant data are available, particularly in those cases where abnormal patterns or trends in disease data are detected. The data sets collected in connection with the NSTF programme are in general being assessed separately and the WGPDMO considered that there is considerable potential value in a re-evaluation of the NSTF data in a multi-disciplinary context at some point in the future. Recognising that the time constraints imposed by the need to compile the QSR in 1993 would mean that such assessments would take place too late for incorporation in the QSR, the sub-group did however support the recommendation that a further meeting to evaluate the fish disease data be convened in 1993.

The assessments had confirmed the need for improved information on migration characteristics, stock identification, and age-length relationships for the species used in fish disease work, as requested by WGPDMO in 1991. Information had been provided in papers on dab migration and stock identification using parasite prevalences for the Dutch coastal areas and German Bight, however, no information had been available for the other areas of the North Sea. The WGPDMO proposed that further information might be obtained by requesting the ICES O-Group Flatfish WG and the International Bottom Trawl Survey WG (formerly IYFS WG) to extend their surveys to cover dab in more detail (by developing age-length keys for dab, for example). Also, further attempts at locating information already existing should be made.

The sub-group had not been able to consider the liver nodule/tumour data in detail, for the reasons described above. However, it was noted that, due to its nature, this disease appeared to provide the best possibilities for detecting a direct influence of a contaminant or group of contaminants in inducing disease. The need for histological confirmation of liver nodules/tumours by a small number of experts was stressed, and the WGPDMO was informed that a number of workers had agreed to forward material already collected to D. Bucke for examination. The results thus obtained would be incorporated in the data already reported to ICES to permit the inclusion of these data in a future evaluation.

The WGPDMO endorsed the conclusions and recommendations of the sub-group and agreed to incorporate them in an appropriate manner in the WGPDMO recommendations.

Recommended Actions

To ACMP:

1) That only the presence/absence maps of fish disease, suitably annotated with a statement concerning the limitations on the use of these maps, should be released to NSTF for possible inclusion in the QSR.

2) That the section of the QSR dealing with fish disease data should be refereed by members of WGPDMO.

To Council:

3) That the Training Guide for Marine Fish Disease Surveys be published as soon as possible.

4) That a further 3-day meeting to assess fish disease data be convened in conjunction with the 1993 WGPDMO to:

   a) evaluate data on species other than dab, including the data for the Baltic area which will be available after April 1992;

   b) re-evaluate the data sets already submitted to ICES but supplemented with the additional information to be requested from the data originators;

   c) extend the analysis of the ICES data sets using logistic regression analysis;

   d) evaluate data on liver nodules/tumours in dab in the light of the results of histological confirmation of existing fixed material; and

   e) if practicable, compare fish disease prevalence data with the results arising from the (ICES/NSTF/JMP) environmental data assessment meetings being conducted in 1992.

   In this connection, individual institutes should analyse their existing data banks and report additional information to ICES prior to the next WGPDMO meeting to facilitate such analyses.

5) That ICES Working Groups responsible for fish disease surveys (International Bottom Trawl Survey Working Group, O-Group Flatfish Working Group) be requested to obtain data suitable for the development of age-length keys, for dab (*Limanda limanda*) in particular.

10 ANY OTHER BUSINESS

10.1 Publications

a) Training Guide for Marine Fish Disease Surveys

Last year the WGPDMO agreed that the Chairman would send the Training Guide as soon as it was completed to ACMP in June for publication. The document reached ACMP too late for consideration at their annual meeting. The Working Group confirmed that the need for the Guide still exists and ICES should be encouraged to publish it as soon as possible.

b) Preparation of a Video to complete the Training Guide

The Working Group agreed to wait for the outcome of the Training Guide and then reconsider the need for a video.

c) Diagnostic fiches

Ten further diagnostic fiches (Nos. 41-50) have recently been published. The Editor (G. Olivier) wrote to the various authors for the next 10: of these, 6 have been received, corrected and sent back to the authors. The Working Group agreed to recommend that the ICES Secretariat take more positive action in giving the fiches greater publicity, for example, by sending a complete list of all the published fiches and information on how to obtain them to the newsletters and bulletins of the major fish pathology and biology associations. The Working Group also agreed about the need to update the older fiches and complete the outstanding ones.

d) Glossary of Terms used in Pathology

The ICES Secretariat informed the Working Group that the glossary will be ready for publication later this year as an ICES document.

10.2 Diseases in Wild Fish and Shellfish

After the presentation of the report on *Ichthyophonus* in herring stocks, some members of the Working Group felt the need for further discussion of examples of diseases in wild stocks, in order to evaluate new observations and trends which could be of significance to stock survival.

a) Furunculosis in Sea Trout

In 1991, the WGPDMO recommended that "additional studies be carried out in sea trout in fresh water and sea water, particularly in thin fish from seriously affected river systems". Sea trout from the lower reaches of a number of river catchments in Ireland which had experienced collapses of sea trout were sampled for evidence of disease. Most of the samples examined by a number of different laboratories yielded no evidence of disease. However, in one study, a small number of sea trout sampled were found to be infected with furunculosis. As the number of cases was small, it is not possible to say at this stage what role, if any, furunculosis has to play in the overall decline of sea trout stocks in Ireland.

b. Parasites in herring

Estonian researchers informed the WGPDMO by letter that both the prevalence and intensity of parasites of herring in their waters have increased during recent years, associated with emaciated herring. Further studies will be performed in cooperation with other scientists from the Baltic Marine Biologists (BMB).

c. Haematodinium-like parasites in *Nephrops* populations

Norwegian lobster (*Nephrops norvegicus*) caught along the west coast of Sweden have been found infected with a Haematodinium-like parasite. Externally visible signs of infection include yellowish colouration of the shell and whitish haemolymph. *Nephrops* populations in the Clyde estuary (West Scotland) also show high levels of infection with Haematodinium with similar effects. A low level of infection was also noted in the Botney Gut-Silver Pit areas off Belgium. Studies are currently in progress to evaluate the biology of this disease and its impact on the populations of *Nephrops*, in several areas.

d. Shell disease in *Nephrops* populations

The Belgian research on *Nephrops norvegicus* stocks in the Botney Gut-Silver Pit areas has also revealed the presence of shell disease at prevalence rates up to 40% in 1991. The bacterial agents isolated were *Vibrio* spp.

11 ANALYSIS OF TASKS

An analysis of progress in the tasks of the Working Group is attached at Annex 6.

12 FUTURE ACTIVITIES

The future activities of the WGPDMO were discussed and members agreed on the proposed new terms of reference, as stated in the recommendations (Annex 7).

The term of office for the chairman, Dr B. Hill, is now completed. Dr A. McVicar was proposed as the new chairman and it was unanimously agreed that this should be the recommendation to the Mariculture Committee.

13 APPROVAL OF REPORT

The Working Group approved the draft report of the meeting.

14 CLOSING OF MEETING

On behalf of the Group, A. McVicar thanked the outgoing Chairman, B. Hill, for his guidance of the meeting and for his work as Chairman of the Group over the past three years.

The meeting was closed at 18.30 hrs on 5 March 1992.

ANNEX 1

LIST OF PARTICIPANTS IN THE WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Name	Address	Telephone	FAX
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I. Dalsgaard	Fish Disease Lab. D.F.& H. Bülowsvej 13 1870 Frederiksberg C Denmark	(45)31352767	(45)35282079
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H. Grizel	IFREMER B.P. 133 17390 La Tremblade France	(33)46363007	(33)3346361847
B.J. Hill **Chairman**	Fish Disease Laboratory 14, Albany Road Granby Estate, Weymouth Dorset DT4 9TH, UK	(44)305772137	(44)305770955
B. Hjeltnes	Institute of Marine Research Dept. of Aquaculture P.B. 1870, N-5024 Bergen Norway	(47)5-238303	(47)5-238333
J. Höglund	The Veterinary Institute Box 7073 S-75007 Uppsala, Sweden	(46)18647156	(46)18309162
T. Lang	Bundesforschungsanstalt f. Fischerei Deichstrasse 12 D-2190 Cuxhaven Germany	(49)4721/38034	(49)4721/38035

Name	Address	Telephone	FAX
J. McArdle	Fisheries Research Centre Abbotstown, Castleknock Dublin 15, Ireland	(353)1-210111	(353)1-205078
A.H. McVicar	SOAFD Marine Laboratory P.O.Box 101, Victoria Road Aberdeen AB9 8DB, UK	(44)224 876544	(44)224295511
S. Mellergaard	Fish Disease Laboratory D.F. & H. Bülowsvej 13 1870 Frederiksberg C Denmark	(45)31352767	(45)31628536 (45)35282079
A. Strøm	Fiskasjúkudeildin c/o Royndarstoðin FR-410 Kollafjordur Faroe Islands	(298)21311	(298)21579
J. Thulin	Institute of Marine Research P.O.Box 4, S-45321 Lysekil Sweden	(46)523-14180	(46)523-13977
S. Wilson	ICES Palægade 2-4 DK-1261 Copenhagen K Denmark	(45)33154225	(45)33934215

ANNEX 2

ICES WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Copenhagen, 2-5 March 1992

A G E N D A

1. Opening of meeting.

2. Terms of reference, adoption of Agenda, selection of rapporteurs.

3. ICES Statutory Meting 1991: points of relevance to WGPDMO.

4. Analysis of national reports on recent trends in mariculture diseases and formulation of advice onprevention and control methods.

5. Analysis of cases of disease interactions between farmed and wild populations.

6. Review of current fish disease control measures for update of ICES/EIFAC guidelines on the "Code of Practice on Introductions and Transfers".

7. Prepare inventory of parasites and diseases of wild and reared salmon in West Greenland and North-East Atlantic Commission areas of NASCO.

8. Evaluate the impact of *Ichthyophonus* spp. on affected herring stocks, including extent of mortality.

9. Review the results of the sub-group's statistical analysis of disease prevalence data for marine fish stocks.

10. Any other business:

    10.1 Publications:

    - training guide and video for marine fish disease surveys
    - diagnostic fiches.

    10.2 New developments in diseases of wild populations of fish and shellfish.

11. Analysis of progress with tasks.

12. Future Activity of WGPDMO.

13. Approval of draft Working Group Report.

14. Closing of the meeting.

ANNEX 3

RAPPORTEURS

Agenda item	Rapporteurs
4.	F. Baudin-Laurencin and I. Dalsgaard
5.	J. McArdle, A. McVicar and B. Hjeltnes
6.	J. McArdle and H. Grizel
7.	J. Höglund and G. Bylund
8.	T. Lang and S. Mellergaard
9.	J. Thulin and S. Wilson
10.	A. Strøm and D. DeClerck

ANNEX 4

Inventory of Parasites and Diseases of Wild and Reared Salmon in Countries at the West Greenland and N.E. Atlantic Commissions Areas of NASCO

Diseases	BELGIUM	CIS	DENMARK	ENGLAND	FAROES	FINLAND	FRANCE	GREENLAND	ICELAND	IRELAND	NETHERLANDS	NORWAY	SCOTLAND	SPAIN	SWEDEN
A. Viral Diseases															
VHS					0		0		0	0		0	0		
IHN					0		0		0	0		0	0		
IPN					F		F		0	F		FW	FW		
Viral papilloma										FW		FW	FW		
VEN/EIBS					F		0			F		FW	F		
Swim bladder tumor										0			F		
B. Diseases of unknown etiology															
Pancreas Disease					0		F			F		F	F		
ISA							0					F	0		
Epitheliocystis									F			F			

F = Found in farmed fish.
W = Found in wild fish.
0 = Looked for but not found.
Blank = No records.

Diseases	BELGIUM	CIS	DENMARK	ENGLAND	FAROES	FINLAND	FRANCE	GREENLAND	ICELAND	IRELAND	NETHERLANDS	NORWAY	SCOTLAND	SPAIN	SWEDEN
C. <u>Bacterial diseases</u>															
Aeromonas salmonicida					FW		F		F	FW		FW	FW		
Aeromonas sp. (motile)					F		F		F	FW		FW	FW		
Renibacterium salmoninarum					F		0		FW	0		FW	FW		
Yersinia ruckeri					0		0		F	F		F	F		
Vibrio sp.					F		F		F	FW		FW	FW		
V. anguillarum					F		F		F	FW		FW			
Vibrio salmonicida					F		0		0	0		F	F		
Flexibacter columnaris							F			F		FW	FW		
Flexibacter sp.					F							F			
Pseudomonas sp.					F				F	FW		F	F		
Serratia sp.										0			F		
Lactobacillus sp.									F						
Mycobacterium sp.									F			FW			

F = Found in farmed fish.
W = Found in wild fish.
0 = Looked for but not found.
Blank = No records.

Diseases	BELGIUM	CIS	DENMARK	ENGLAND	FAROES	FINLAND	FRANCE	GREENLAND	ICELAND	IRELAND	NETHERLANDS	NORWAY	SCOTLAND	SPAIN	SWEDEN
D. <u>Fungal infections</u>															
Ichthyophonus hoferi							0			W		FW	W		
Exophiala salmonis							F		F	F		F	F		
Phoma herbarum							0			F		F	F		
Saprolegnia parasitica										FW		FW			
Saprolegnia sp.							FW			FW		FW	FW		
Saprolegnia diclina										0			F		
Dermocystidium sp.												FW	F		
Paecilomyces farinosus										0		F	F		
Phialophora sp.										F			F		

F = Found in farmed fish.
W = Found in wild fish.
0 = Looked for but not found.
Blank = No records.

Diseases	BELGIUM	CIS	DENMARK	ENGLAND	FAROES	FINLAND	FRANCE	GREENLAND	ICELAND	IRELAND	NETHERLANDS	NORWAY	SCOTLAND	SPAIN	SWEDEN
E. <u>Protozoan infections</u>															
Myxobolus neurobius												F	W		
Myxidium truttae													W		
Myxidium oviforme												W	W		
PKX organism (probable myxosporid)										F		FW	F		
Ichthyobodo (Costia) necatrix					FW		F		F	F		F	FW		
Ichthyophthirius multifiliis							F			F		FW	FW		
Hexamita sp.										F		F	F		
Trichodina sp.					F		F			FW		F	FW		
Leptotheca sp.												FW	FW		
Epistylis sp.												F	F		
Apiosoma sp.												FW	F		
Scyphidia sp.					F					F		FW	FW		
Chilodonella cyprini												FW	FW		
Trichophyra sp.										F		FW	F		
F. <u>*Monogeneans*</u>															
Gyrodactylus derjavini												FW	FW		
Gyrodactylus truttae												FW	FW		
Gyrodactylus salaris												FW	0		
Discocotyle sagittata												W	W		

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W = Found in wild fish.
0 = Looked for but not found.
Blank = No records.

Diseases	BELGIUM	CIS	DENMARK	ENGLAND	FAROES	FINLAND	FRANCE	GREENLAND	ICELAND	IRELAND	NETHERLANDS	NORWAY	SCOTLAND	SPAIN	SWEDEN
G. <u>Trematodes</u>															
Crepidostomum farionis										W		W	W		
Diplostomum spathaceum										W		W	W		
Diplostomum sp.										FW		W	W		
Apatemon sp.													W		
Phyllodistomum simile													W		
Hemiurus sp.												W	W		
Derogenes sp.												FW	W		
Lecithaster sp.													W		
Brachyphallus sp.													W		
Tetracotyle sp.												W	W		
H. <u>Cestode infections</u>															
Cyathocephalus truncatus												W	W		
Diphyllobothrium ditremum larvae										W		W	FW		
Diphyllobothrium dendriticum larvae										FW		W	FW		
Diphyllobothrium sp. larvae										W			W		
Eubothrium crassum										FW		FW	FW		
Hepatoxylon sp. larvae													W		

F = Found in farmed fish.
W = Found in wild fish.
0 = Looked for but not found.
Blank = No records.

Diseases	BELGIUM	CIS	DENMARK	ENGLAND	FAROES	FINLAND	FRANCE	GREENLAND	ICELAND	IRELAND	NETHERLANDS	NORWAY	SCOTLAND	SPAIN	SWEDEN
I. Nematode infections															
Anisakis sp. larvae										W		FW	W		
Hysterothylaceum sp. (larvae and adults)												FW	W		
Capillaria salvelini													W		
Capillaria sp.													W		
Metabronema sp.												W	W		
Rhabdochona salvelini													W		
Rhabdochona sp.												W	W		
Cystidicola farionis													W		
Cystidicoloides sp.													W		
J. *Acanthocephalan* infections															
Neoechinorhynchus rutili													W		
Echinorhynchus truttae													W		
Pomphorhynchus laevis													W		
Acanthocephalus lucii													W		
K. Crustacean infections															
Lepeophtheirus salmonis					FW		F		F	FW		FW	FW		
Caligus elongatus					FW		F		F	FW		FW	FW		
Salmincola salmonea												FW	W		

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W = Found in wild fish.
0 = Looked for but not found.
Blank = No records.

Diseases	BELGIUM	CIS	DENMARK	ENGLAND	FAROES	FINLAND	FRANCE	GREENLAND	ICELAND	IRELAND	NETHERLANDS	NORWAY	SCOTLAND	SPAIN	SWEDEN
L. <u>Molluscan infections</u>															
Margaritifera margaritifera (glochidia)										F		FW	F		
Mytilus edulis												FW	F		
M. <u>Leech infections</u>															
Hemiclepsis marginata													W		

F = Found in farmed fish.
W = Found in wild fish.
0 = Looked for but not found.
Blank = No records.

ANNEX 5

REPORT OF THE ICES WGPDMO SUB-GROUP ON ANALYSIS OF DISEASE PREVALENCE IN MARINE FISH STOCKS

(Copenhagen, 27-29 February 1992)

1. OPENING OF THE MEETING AND BACKGROUND INFORMATION

The sub-group met at ICES headquarters in Copenhagen under the Chairmanship of Dr Jan Thulin, Sweden.

The meeting was opened at 09.00 hrs on Thursday, 27 February, by the Chairman. The participants are listed in Appendix 1. The meeting was convened in response to the request from the North Sea Task Force (NSTF) to provide an overview of the current status of fish disease in the North Sea and specifically for the sub-group to consider data collected in connection with the NSTF Monitoring Master Plan. A main goal was, therefore, to provide information for incorporation into the North Sea Quality Status Report (QSR) to be published in 1993. It was agreed that, in particular, the meeting should focus on methodology and statistical treatment of the data.

2. SELECTION OF RAPPORTEURS

Rapporteurs were selected as follows:

Thursday, 27 February	- McVicar/Hoglund
Friday, 28 February	- Lang/des Clers
Saturday, 29 February	- Bucke/Von Landwuest.

3. TERMS OF REFERENCE, ADOPTION OF THE AGENDA

The terms of reference provided were for the group to:

a) statistically analyse the compiled national data on disease prevalence rates in marine fish stocks which have been submitted to ICES;

b) analyse, evaluate and assess any disease data submitted to ICES for the purposes of the NSTF, and the Baltic Marine Biologists (BMB), taking into account the residence time of dab and flounder in the areas under study;

c) develop plans for assessing the relationship between disease prevalence rates in marine fish and contaminant concentrations in sediment, which will utilise the results of the 1990/1991 Baseline Study of the Contaminants in Sediments in the OSPARCOM area; and

d) report to WGPDMO at its 1992 meeting.

The agenda (Appendix 2) was adopted without change.

4. PRESENTATION OF DISEASE AND OTHER DATA AVAILABLE FOR ANALYSIS

The meeting was informed that the following data sets had been provided to ICES:

Belgium	1990-91	dab
Denmark	1983-91	dab
Germany	1988-91	dab and cod
Netherlands	1984-91	dab
Sweden	1990-91	cod and flounder
UK-England	1988-91	dab
UK-Scotland	1988-91	dab

Only the Dutch (1991 data only) and the Danish scientists had validated their data in response to the request by ICES to check the print-outs of the database (ASCII-coded) by the time of the assessment meeting.

In consideration of the available data, information was only extracted from the database for the prevalence of diseases in dab (the main species of interest in the NSTF monitoring programme), specifically, lymphocystis, hyperplasia/epidermal papilloma, acute/healing ulcerations, and liver nodules >2 mm, identified according to ICES agreed standards (ICES Cooperative Research Report No. 166 (Anon., 1989)). Data were available from about 800 sampling locations distributed throughout the North Sea area. Because the data were collected throughout the year, from many different localities, with different sizes of dab, and because many samples contained fewer than the recommended numbers of fish, especially for the larger size category (≥ 25 cm), the sub-group had to recommend how the data should be aggregated for analysis. It was agreed that data on diseases of cod and flounder should be evaluated in future years, when more data would be available.

Data sets and distribution maps on contaminant levels in sediments will not be available until after the

ICES/NSTF sediment data assessment meeting scheduled for late April 1992 and, consequently, this sub-group could not make any comparison between the two data sets (*cf.* term of reference c). Also, data from the BMB Sea-Going Workshop held in October 1991 will not be available until after April 1992 (*cf.* term of reference b).

It was agreed that statistical validation guidelines should be established by the sub-group to be incorporated into ICES data handling routines.

5. PRELIMINARY DATA ANALYSIS AND INTERPRETATION ISSUES

A discussion paper produced by Vethaak, Carr, Wilson, Mellergaard and Thulin, entitled "Problems related to statistical analysis of fish disease prevalence data submitted to ICES/NSTF: suggestions for a plan of strategy" (attached as Appendix 3) was presented by Vethaak and Carr. Experience gained from the ICES/IOC Bremerhaven Workshop provided the background to the consideration of the problems on data collection and interpretation. Points highlighted included the need for quality assurance of the data, and information on confounding factors (*e.g.*, age, sex, region, migration, stocks, etc.) in order to permit a sound statistical analysis and interpretation of the data. Several possible methods of statistical analysis of the data were presented (*e.g.*, pooled sample summaries, stratified data comparisons, direct and indirect standardisation, Mantel-Haenzel test for homogeneity, logistic regression analysis). It was recommended that the logistic regression analysis would be the most appropriate for the type of data available, in combination with other methods if necessary, and that a specialist discussion within the *ad hoc* group would produce a final decision. The authors emphasised that the paper was intended for further discussion and approval by the sub-group.

The discussion of the proposals in the paper covered the following points, and it was agreed that:

a) The production of raw field data without interpretation of significance could be misleading to non-specialists. Data maps of disease prevalence of fish in the North Sea produced for NSTF and/or ICES should include a statement on the need for fish disease specialist interpretation. Prevalence maps should also show where no fish, or inadequate numbers, were sampled and, consequently, where no fish disease data are available.

b) Stringent guidelines need to be further established by the sub-group concerning sampling for fish diseases, to ensure that only verified data are submitted to and used by ICES. Data from different size-classes should not be grouped in a different way from that recommended in the ICES Cooperative Research Report No. 166 (Anon., 1989).

c) Because of the different age-length relationships in different regions, age-length keys are required from, *e.g.*, stock assessment groups for use in disease data analysis.

d) When stratified sampling is used, fish disease data do not represent the whole population.

e) In order to evaluate possible pollution effects on fish disease, it is important to control other sources of variability (seasonal, spatial, biological, etc.) as far as possible in the sampling procedures.

f) It was recognised that in the Quality Status Report (QSR) the fish disease data evaluation will be published in a summary form for non-specialists. The section of the QSR describing the fish disease data should be reviewed by ICES-WGPDMO prior to its publication.

g) The sub-group regretted that the 'Training Guide for Marine Fish Disease Surveys' has not been finalised for publication.

Specifically, the discussions led to the following comments and recommendations:

Sampling issues

Time of the year

Surveys should be carried out each year during the same time windows in order to obtain comparable data and to allow for the evaluation of long-term trends.

Surveys should be made during two seasonal periods, during spawning time on the spawning grounds when fish from various locations may congregate, and outside the spawning season on the feeding grounds when local fish are present. Data for these two periods may not be comparable. Therefore, it is up to each institute to state which period the data refer to; the reporting forms should be modified to facilitate this.

Site

Two types of sampling a selected site are being employed:

a) in a 'fixed-point sample', several hauls are collected from a single trawling track;

b) in surveys of a general area, several hauls are made covering the area.

Better information should be obtained on the variability of data within a given area, as it may not be possible to compare prevalences from different locations, especially if the areas are covered using different sampling strategies.

The sub-group noted that not all NSTF stations are appropriate for fish disease studies due to the insufficient numbers of fish available. It is, therefore, recommended that disease specialists are consulted to assist in selecting sites appropriate to fish disease studies in any future development of the monitoring programmes.

Fish size-intervals

It is not possible to obtain reliable prevalence values by pooling across different size groups. It is, therefore, important to collect enough fish for each size group to reach the minimum sample-sizes recommended by ICES.

The acute lack of data on age-length relationships in diseased fish was noted. In addition to the need to obtain such information, it is also recommended that the effect of disease on growth be evaluated and, whenever possible, that otoliths of diseased fish be collected during fish disease surveys for comparison with age-length keys from healthy fish.

Sex

A better knowledge of differences in age-at-length between the two sexes is necessary to help in the interpretation of disease data.

Liver nodules

Liver nodules are considered as potentially important indicators of a direct pollution effect. There is, however, at present a lack of information to allow a reliable interpretation of existing liver nodule data. It has been demonstrated, for example, that not all nodules larger than 2 mm are neoplastic lesions. Therefore, it is recommended that, in the future, visual diagnosis be confirmed by histological examination. To facilitate the interpretation of existing data, the WGPDMO recommends that all Working Group members send their existing material for histological confirmation to D. Bucke at the Fish Disease Laboratory in Weymouth. ICES will contact individual institutes to supplement the data collected so far by codes specifying whether the nodules have been confirmed as neoplastic or not. For future data collection, this will be specified on the standard reporting forms.

A review of the current knowledge on dab liver nodules will be presented in a paper at the 1993 WGPDMO meeting by D. Bucke, A.D. Vethaak, and T. Lang. A protocol outlining standardised methods for the sampling and fixation of dab liver samples with nodules will be circulated by D. Bucke as soon as possible.

It is recommended that the otoliths of diseased fish be collected for age determination, as liver nodules are present mainly in the largest size group which is characterized by a large variation in fish age-at-length.

Experimental work

Whenever variations in fish disease above natural levels are detected and are considered to be significant by fish disease specialists, the cause-effect relationship should be investigated using a multidisciplinary approach and should include experimental studies.

Recommendations for future data analyses

The sub-group discussed the use of different statistical approaches and recommended methods for data analyses to be used by ICES in the future.

Fish disease prevalence was presented by ICES statistical rectangle, for two seasons, two sexes and by length group. From this exercise, it was clear that the variability resulting from the pooling of data from several hauls, sites, months, and countries needs to be quantified beforehand.

It is recommended that members of the WGPDMO present results of statistical analyses of these different sources of variability in national data at the next meeting, using logistic models.

6. EVALUATION OF THE DATA SETS

6.1 Detailed Analysis of Test Data Sets

Logistic regression analysis was used to evaluate the effect of confounding factors such as host sex, site, country, season and year on disease prevalence. Data on prevalence of epidermal hyperplasia/papilloma in dab in 1990 and 1991 were selected as a test set, considering the data from the six ICES rectangles with the largest number of observations. Preliminary results of the analysis revealed that, for epidermal hyperplasia/papilloma the minimum number of fish in the mid-size group recommended by ICES is sufficient. This, however, may not be the case for rarer diseases such as acute/healing skin ulcerations.

Pooling of data between countries may introduce problems; relatively large differences in prevalence rates were found between German and UK data in two ICES rectangles sampled by both of these countries. However, these apparent differences may be artificial, as other confounding factors such as season, sex, and inter-haul variability were not taken into account.

6.2 Data Presentations

On the basis of the exploratory analyses described in Section 6.1, the following data evaluation-presentation procedure was adopted.

6.2.1 Data sets

Data for dab (*Limanda limanda*) reported by Belgium, Denmark, Germany, Netherlands, UK-England and UK-Scotland were evaluated.

Data for the years 1989, 1990 and 1991 were included.

Data on lymphocystis and skin ulcers supplied by Germany were excluded.

6.2.2 Diseases

Evaluations were conducted for the following diseases in dab (*Limanda limanda*):

1) Epidermal hyperplasia/papilloma;
2) Lymphocystis; and
3) Acute/healing skin ulcerations.

Data on liver nodules/tumours were not evaluated; fewer data for this disease were reported, because generally observations involve only the larger size-class ($\geq$ 25 cm). Also, for the data reported on liver nodules, they did not concern histologically confirmed nodules in all cases.

6.2.3 Pooling of data

Data were pooled in order to obtain a suitable level of information for both analytical and presentation purposes, by area and by season. The justification for the pooling approach employed, and comments on the consequences and limitations of such pooling, are described below.

a) Area aggregation of data:

The ICES statistical rectangle (0.5 degree latitude x 1 degree longitude) was selected as the unit for pooling data. This unit provided numbers of fish examined in the pooled data sets which were considered appropriate in relation to disease identification in most areas. Using smaller units, the requirements concerning the minimum numbers of fish (i.e., < 100 fish in a given size-class) were frequently not met. The main disadvantage noted in basing the evaluation on areas as large as the ICES rectangles was the considerable local variations in disease prevalence observed by some investigators in areas as small as a few nautical miles distant from each other; it was considered that the precision of the disease estimates obtained could be poor and result in misleading pictures of the distributions of disease prevalence. However, for the objective of producing a first evaluation of the disease prevalence data on a North Sea-wide scale, it was accepted that the area aggregation employed represented a pragmatic solution which could be used, provided that the results obtained were not taken out of the context of an appreciation of this and other limitations.

b) Seasonal aggregation of data:

On the basis of the exploratory analyses, and information available on dab migration, it was decided to employ a 2-season breakdown in the final evaluations, involving a nominal 'migratory' season (December-May) and a 'stationary' season (June-November, but preferably concentrating on July, August, September). Clearly, the use of a cut-off based on sampling month across the entire North Sea is not an ideal approach and recommendations relating to future evaluations need to address this issue. However, the general season categories employed were considered appropriate as a basis for gaining some insight into the possible influence of fish migration and seasonal differences noted in several previous studies on diseases in dab, whilst again leading to pooled data sets of an appropriate size in terms of numbers of fish examined. Data for all years (1989-1991) were pooled, as the possible year effect was considered to be relatively unimportant in relation to other effects (age-length, sex, season and site, etc.).

c) Observer effects:

Pooling of data by area and season involved combining data reported by different observers. The exploratory analyses identified this as a possible major source of variation. However, the final evaluations provided some indication that this may have been due to the inclusion of samples not meeting the requirements of minimum sample size (numbers of fish), where a sample with, for example, low disease prevalence by one country was being compared with a sample of a few fish obtained by another country. Again, awareness of this possible source of variation was noted as a major point to be taken into account when considering the maps produced, and as a point to be addressed in the recommendations resulting from the evaluation experience. Most countries had reported data for relatively few locations; data from the UK-England and Germany, however, covered large areas of the North Sea. The pooling of data in areas sampled by these two countries involved mainly the evaluation of the epidermal papilloma data; German data on the other two diseases evaluated were excluded from the assessment for other reasons.

d) Pooling procedure:

For each ICES statistical rectangle and each season ('stationary' or 'migratory'), the data on the numbers of fish examined for each size-class were added. If this resulted in a total of at least 100 fish having been examined, the data sets were pooled together, and disease prevalence rates were computed for that area/size-class/season.

6.2.4 Factors considered in the evaluations

A number of factors to be taken into account in the initial interpretation of the data (but excluding consideration of factors such as contaminant gradients, etc.) were raised. The main factors proposed were inter-observer variablity, sample size, inter-haul variability, site, year, season, age (or length), and sex.

The consequences of pooling by area and season, and possible observer effects, are noted above. Of the remaining factors mentioned, it was not possible to address inter-haul variation from the data sets available.

In relation to the age-length effect, in the absence of age-length keys for dab in the North Sea, it was not possible to investigate the age effect in any detail. Length is used, therefore, as a surrogate parameter for age, but is not entirely suitable due to differences in growth rates between male and female dab and differences in growth rates in different parts of the North Sea. For the purposes of evaluating and presenting the data, it was decided to utilize data on the middle size class (20-24 cm fish); this represented a compromise. Using the smaller size-class (15-19 cm), disease prevalence rates are lower; given the large number of confounding factors which could only be considered at a relatively superficial level, the ability to detect prevalence using larger size fish was preferable. In the largest size-class (≥ 25 cm), however, many samples were excluded as not having met the requirements concerning minimum numbers of fish.

The data presentations agreed for inclusion in this report are intended to provide some insight into the remaining factors (sex and season).

6.2.5 Maps

For each of the three diseases evaluated, the results are summarised in two pages of maps: Figures 1 and 2 present the data for epidermal hyperplasia/papilloma, Figures 3 and 4 present the data for lymphocystis, and Figures 5 and 6 present the data for acute/healing skin ulcerations.

Figures 1, 3 and 5 show maps coded for presence (+) or absence (-) of the disease in the 20-24 cm size-class in a given ICES rectangle for both sexes and both seasons pooled. Points plotted as "." indicate areas where samples were collected, but even after pooling did not meet the minimum requirement of 100 fish examined.

Figures 2, 4 and 6 each show four maps of the disease prevalences obtained for the 20-24 cm size-class for each season and each sex separately. The symbols (circles) plotted are size proportional to the prevalence value.

The sub-group recommended that the maps be annotated with a warning about the potential for mis-use of these figures due to the many factors not taken into account in these presentations. Ignoring some of these factors may be justified on the basis of experience gained by the sub-group in considering the aggregated data. Problems remain, however, in relation to the influence of sample size on disease prevalence estimates, among others. It was shown that when prevalences were less than 3-6 % it may be important to increase sample sizes above the minimum recommended, in order to achieve a desirable precision of estimates. The following information on this subject was presented by S. des Clers as a proposal for future consideration for fish disease surveys.

Influence of sample size on the detection ability and the estimation of prevalence

a) Minimum detection level at each site/station (S hauls) calculated from the binomial distribution with a = 0.05 (a = (1 - preval./n) (prev. = prevalence)

Number of fish collected (n)	100	100	50
Minimum detectable prevalence (%)	3	3	6

b) Standard error (%) on each side of the disease prevalence (%) in a sample according to a normal approximation for the binomial distribution

$$(SE = \frac{\sqrt{1.96 \text{ prev.} - (1 - \text{prev.})}}{n})$$

	No. of fish collected	
	100	50
Disease prevalence	Standard error (± %)	
10%	6	8
5%	4	6
1%	2	3

c) Minimum significant differences between prevalences in different samples (or ICES rectangles) according to prevalence (%) and sample size (a = 0.05, b = 0.90):

The minimum numbers needed in each statistical rectangle to detect a prevalence of X% according to the arcsine formula to approximate the binomial distribution.

Disease prevalence	No. of fish collected	Minimum significant difference
10%	100	20%
10%	300	10%
5%	200	10%
5%	500	5%

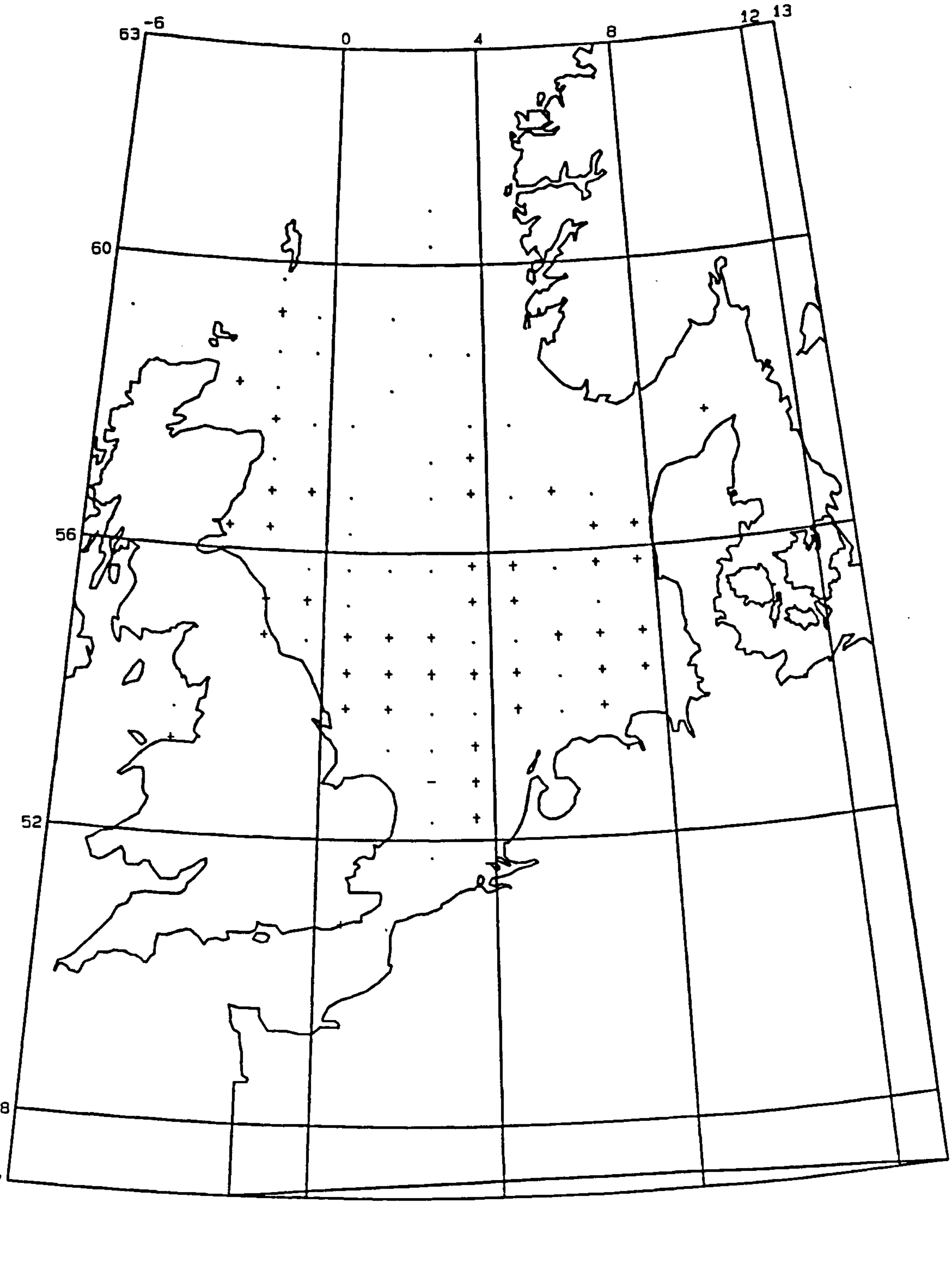

Figure 1 Epidermal papilloma/hyperplasia in dab (*Limanda limanda*) in the size class 20-24 cm.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

Dec–
May

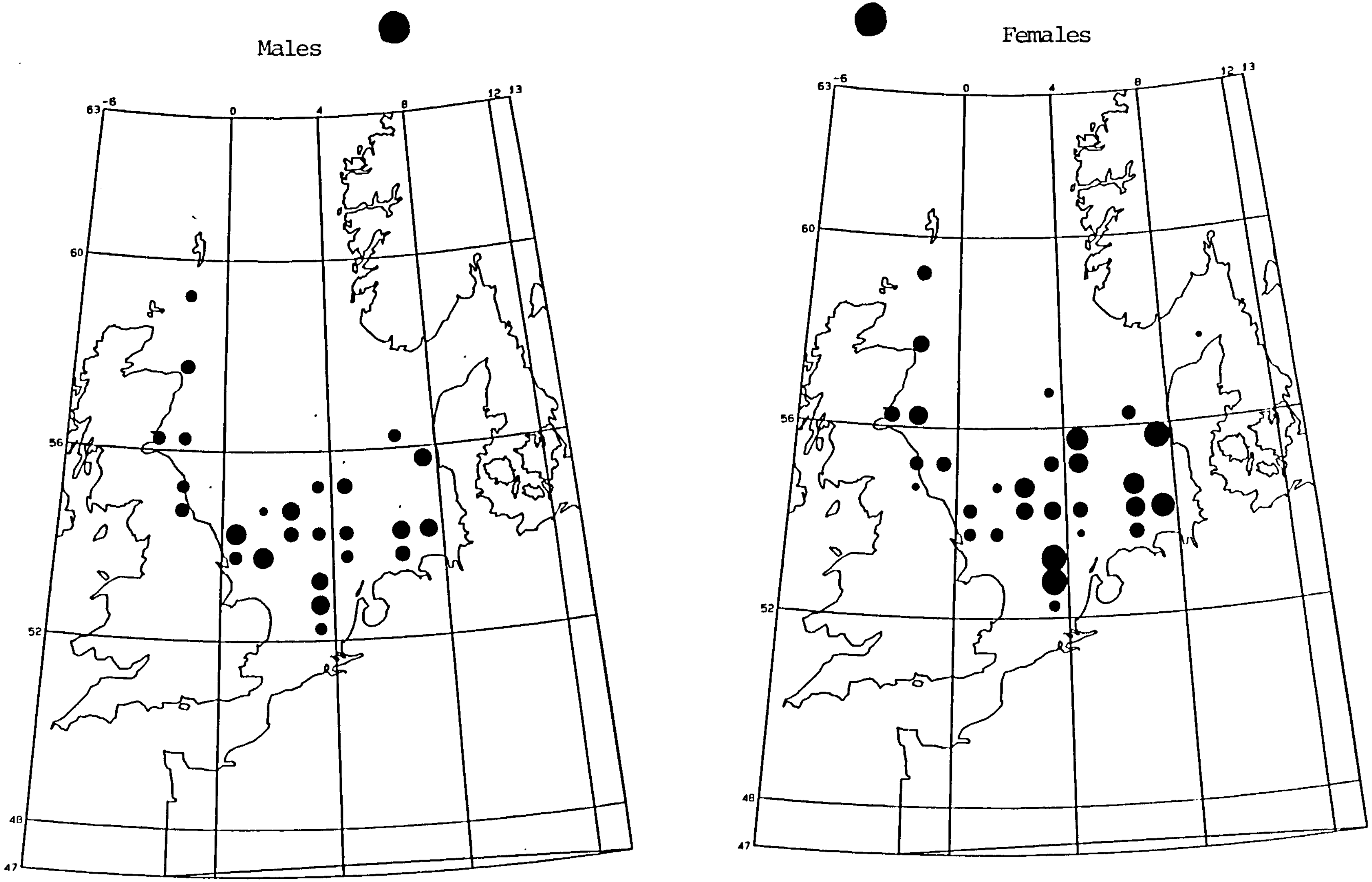

Figure 2A Epidermal papilloma/hyperplasia in dab (*Limanda limanda)* in the size class 20-24 cm by sex and season.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

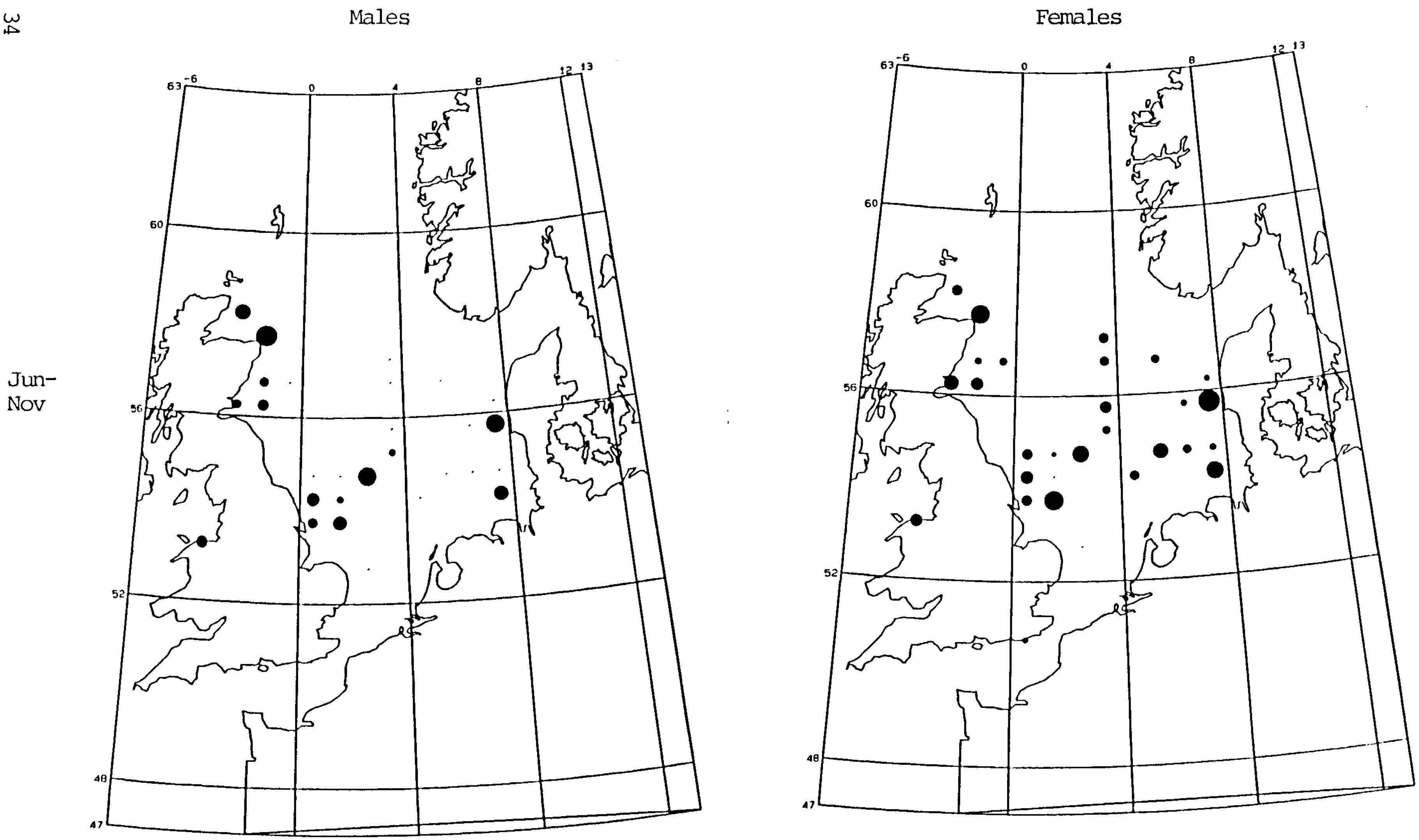

Figure 2B Epidermal papilloma/hyperplasia in dab (*Limanda limanda)* in the size class 20-24 cm by sex and season.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

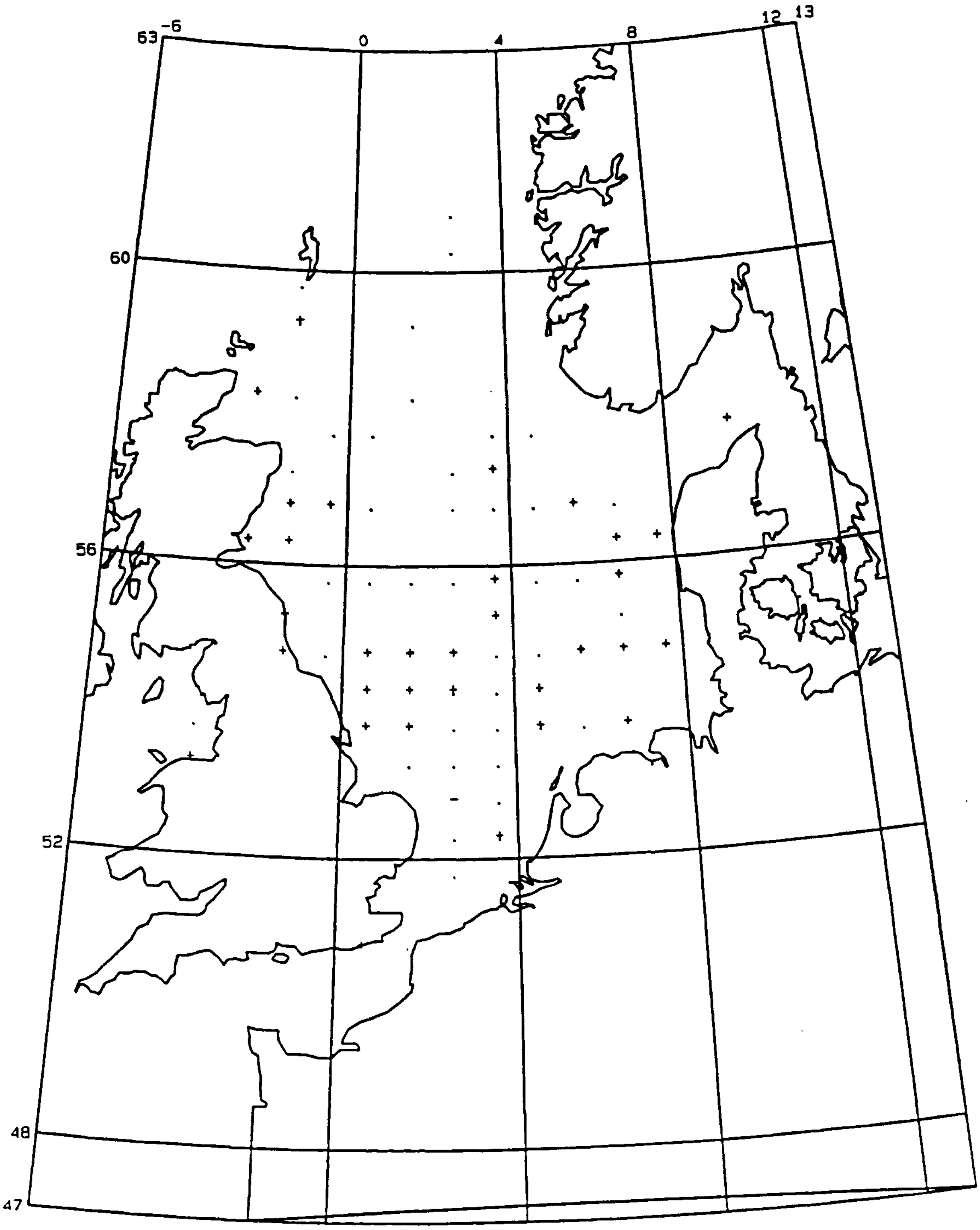

Figure 3 Lymphocystis in dab *(Limanda limanda)* in the size class 20-24 cm.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

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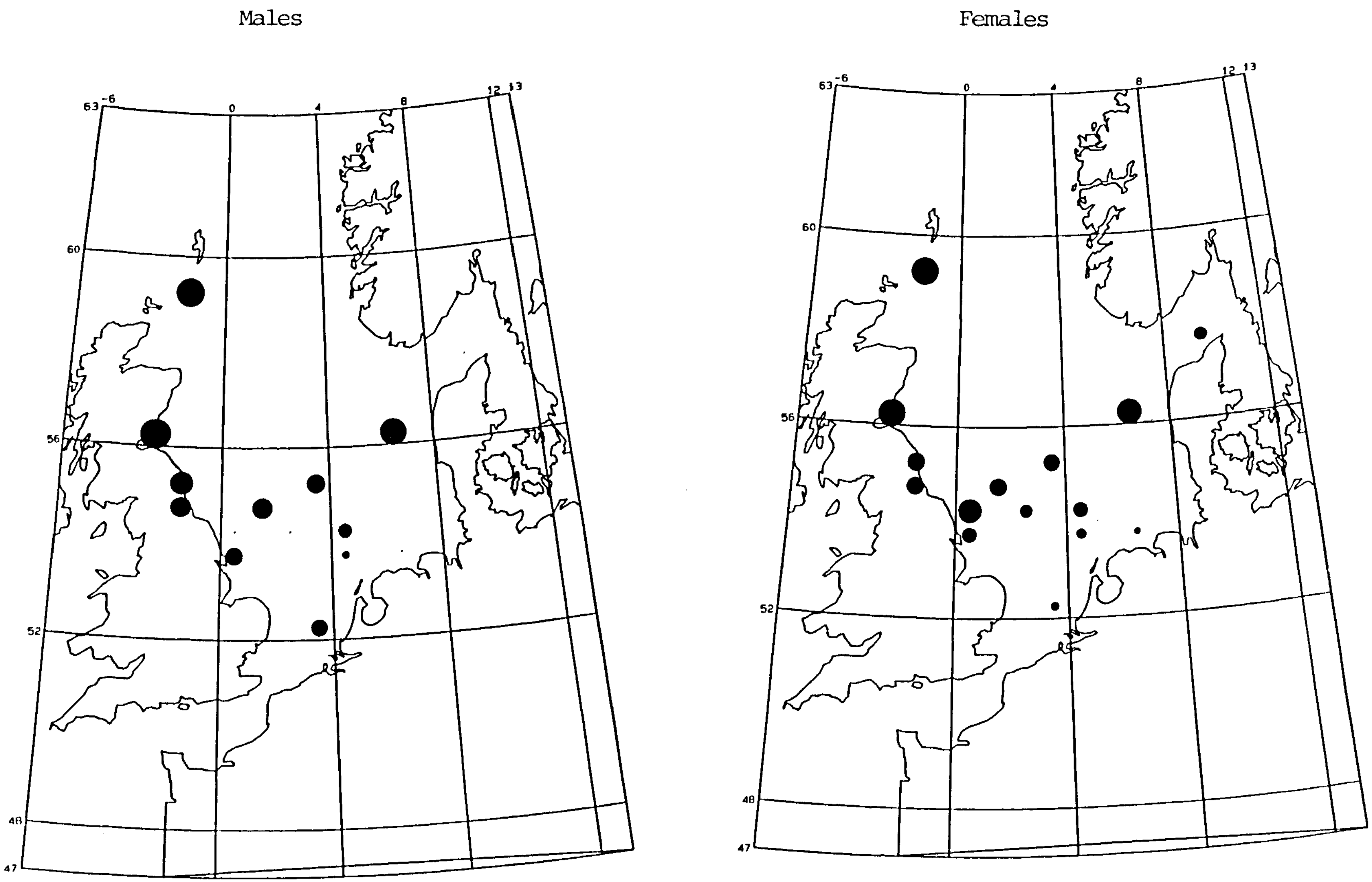

Figure 4A Lymphocystis in dab (*Limanda limanda)* in the size class 20-24 cm by sex and season.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

Jun-
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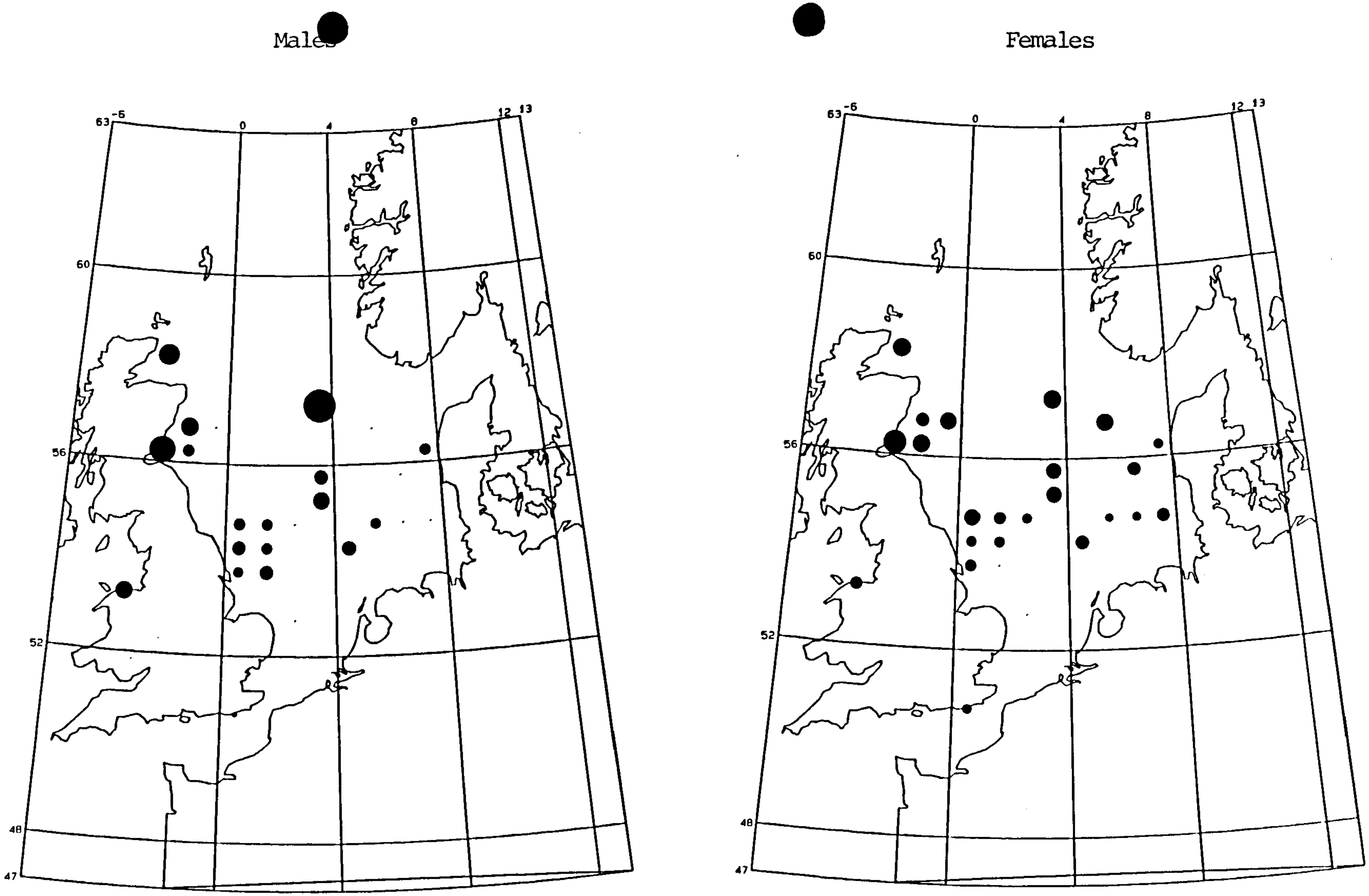

Figure 4B Lymphocystis in dab (*Limanda limanda)* in the size class 20-24 cm by sex and season.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

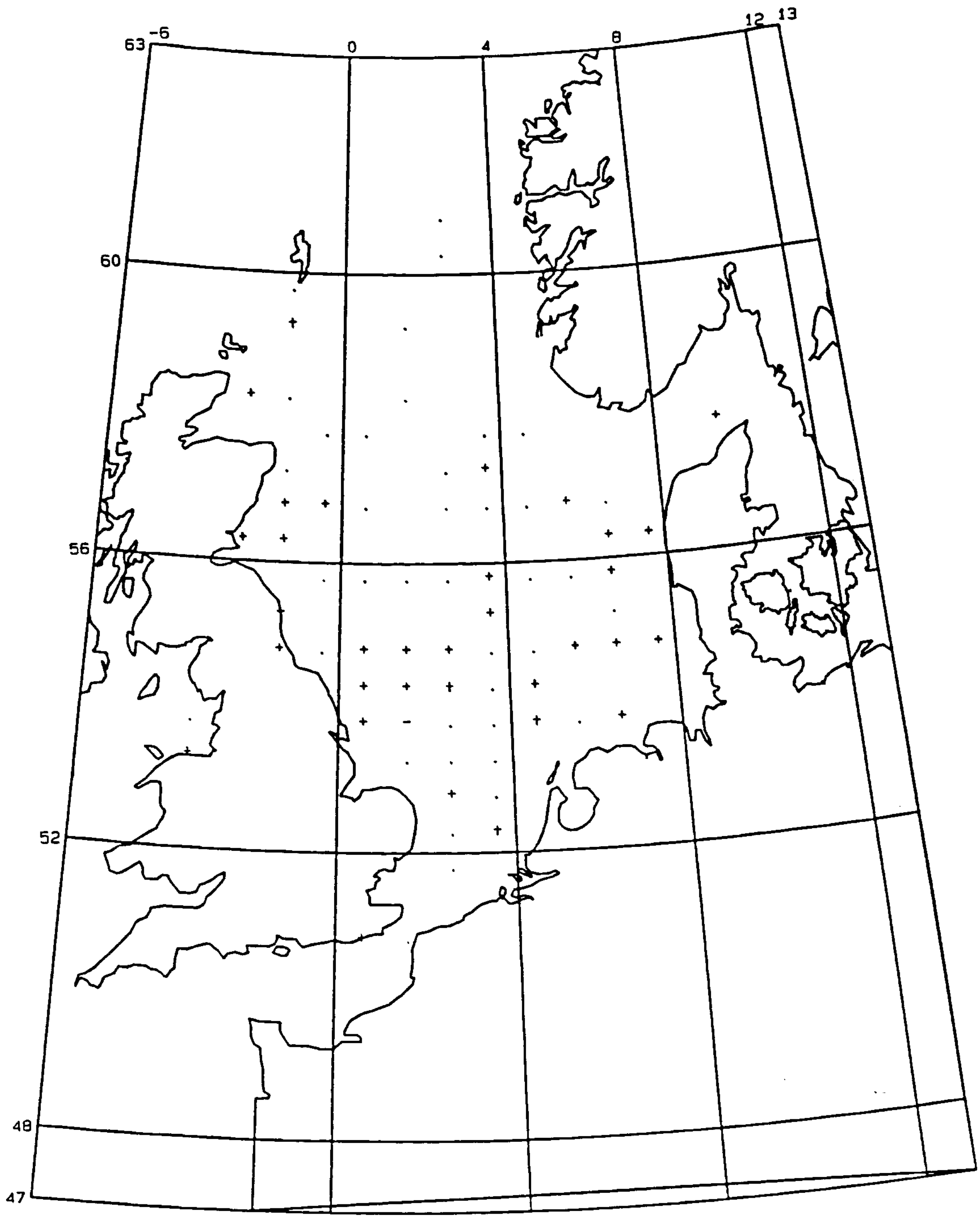

Figure 5 Skin ulcers in dab *(Limanda limanda)* in the size class 20-24 cm.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

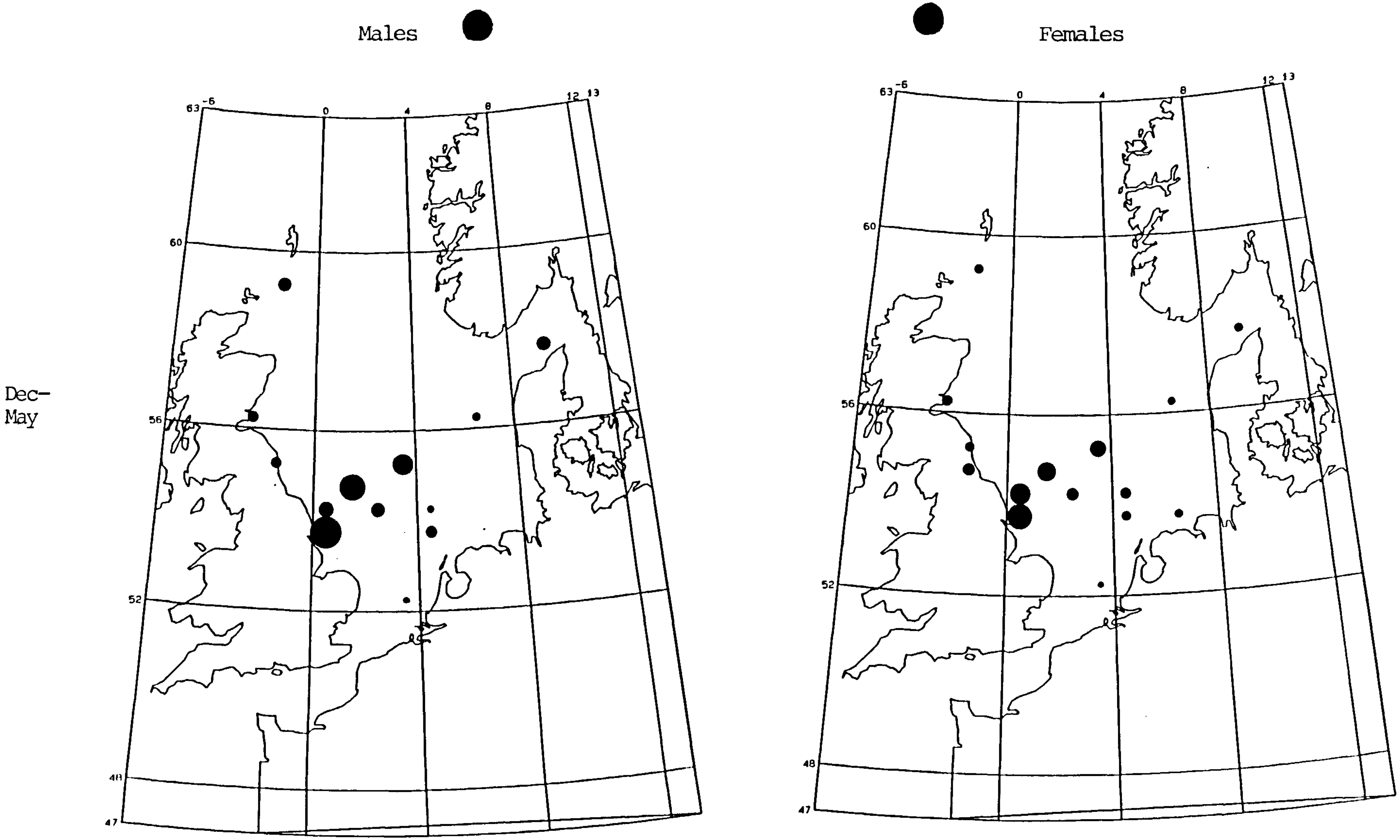

Figure 6A. Skin ulcers in dab (*Limanda limanda)* in the size class 20-24 cm by sex and season.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

Males Females

Jun-
Nov

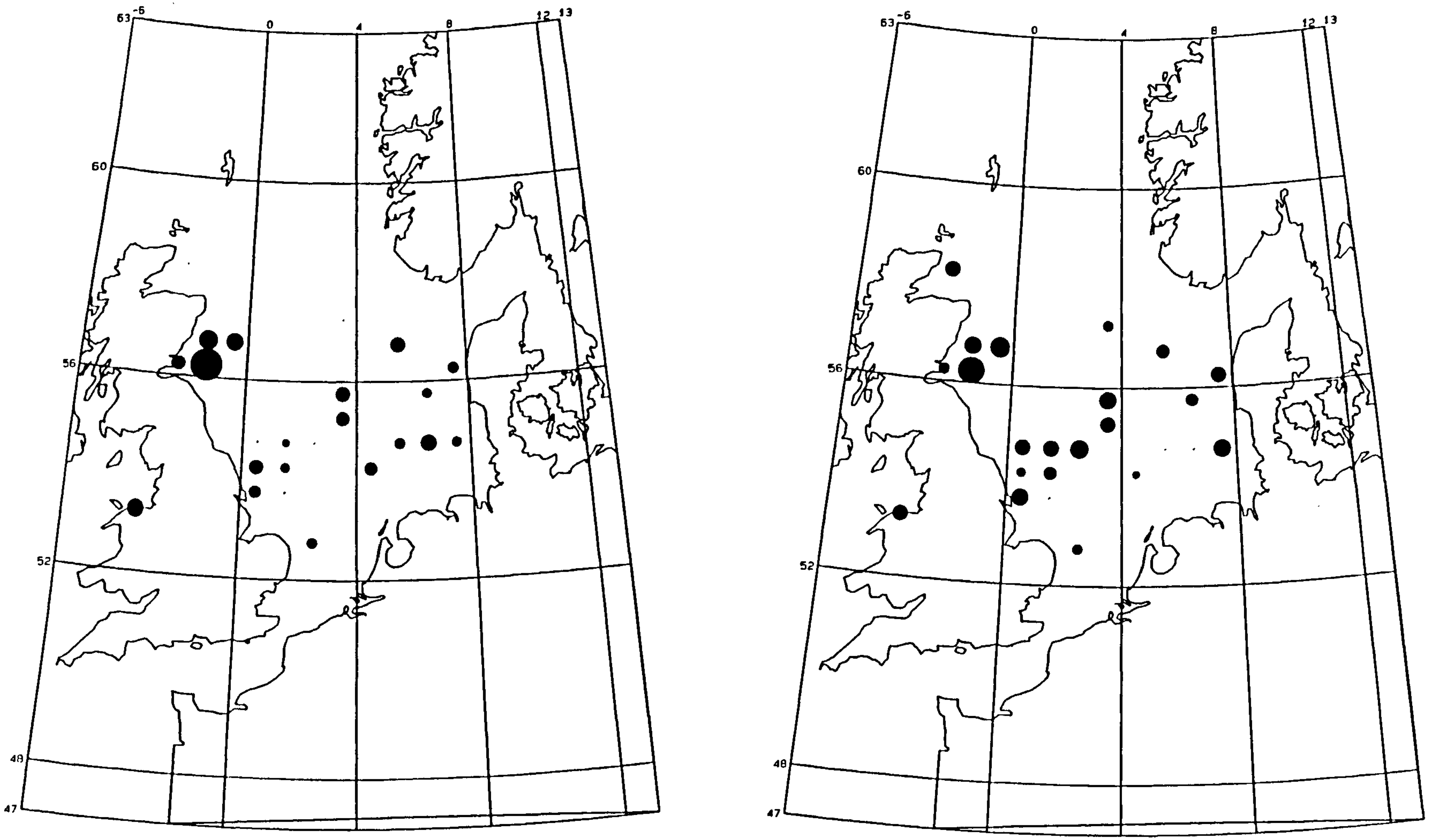

Figure 6B. Skin ulcers in dab (*Limanda limanda*) in the size class 20-24 cm by sex and season.

NOTE: THIS FIGURE SHOULD NOT BE REPRODUCED. IT IS IMPORTANT THAT IT BE CONSIDERED IN THE CONTEXT OF THE REPORT.

7. CONCLUSIONS/RECOMMENDATIONS

a) Fish disease temporal trend data are considered to be potentially a very useful tool for use in detecting abnormal changes or variations in prevalence or the severity of a particular disease, in association with factors such as contamination. Stringent control of known sources of variability in sampling should be exercised.

b) Conclusions from fish disease prevalence data and prevalence maps should only be made by fish disease specialists with full awareness of the limitations of the data and the problems with their statistical significance.

c) Age-length keys for dab (*Limanda limanda*) are required from fish stock assessment groups or from disease studies for use in fish disease data analysis. Data from diseased fish should be obtained by disease specialists to quantify the effect of disease on growth.

d) Only the presence/absence maps of fish disease should be released to NSTF for possible inclusion in the QSR. Because of the problems of high variability in disease prevalence data noted above, the disease prevalence maps produced by this sub-group should not be included in a report (*e.g.*, the QSR) to be made publicly available. The section of the QSR dealing with fish disease data should be refereed by members of WGPDMO.

e) ICES should be encouraged to publish the Training Guide for Marine Fish Disease Surveys as soon as possible.

f) There is a requirement for an analysis of the variability produced by grouping data from different hauls at point sampling locations, within limited areas, and within larger areas such as ICES statistical rectangles. Individual institutes should analyse their existing data sets and report additional information to ICES prior to the next WGPDMO meeting to facilitate such analyses.

g) Whenever variations in the prevalence or severity of fish diseases above natural levels are detected, and are considered to be significant by fish disease specialists, the cause-effect relationship should be investigated using a multidisciplinary approach, which should include experimental studies.

h) It is recommended that logistic models be used to compare fish disease data. Only data satisfying the minimum requirements of the ICES recommended guidelines (Anon., 1989) should be included in the analysis.

i) Each institute collecting fish disease data should inform ICES when submitting these data as to whether samples were collected during the spawning period, outside the spawning time, or whether this is unknown. Historical data should be similarly qualified. ICES will update the existing database accordingly and D. Bucke will modify the reporting forms to take this into account.

j) Liver nodules in dab are considered to be potentially important indicators of direct pollution effects, but there was a lack of information available for reliable interpretation of existing data. It was recommended that, where existing fixed material was available, this should be reappraised, together with additional samples, at the Fish Disease Laboratory, Weymouth, UK. A review of current knowledge on dab liver nodules/tumours will be presented at the 1993 WGPDMO meeting by Bucke, Vethaak and Lang.

k) For statistical validation of the fish disease data submitted to ICES, the following minimum criteria must be met:

   1) Collection of the minimum numbers of fish for each size group, per sample, as recommended by the 1988 ICES sea-going workshop (Coop. Res. Rep. No. 166, 1989).

   2) Data should be submitted on the basis of single hauls. A sample is collected from a small area and comprises one or several hauls. In the case of several hauls, the date and station coordinates are averaged over all hauls and repeated on each reporting form for each haul, hauls representing sub-samples.

   3) Grossly visible liver nodules/tumours >2 mm must be histologically confirmed as neoplasia.

Acknowledgements:

The sub-group wish to acknowledge the particular efforts of D. Vethaak and M. Carr in the preparation of the discussion document for the meeting, and M. Sørensen and S. Wilson of the ICES Secretariat in the compilation of data supplied to ICES and the preparation of assessment products for the meeting.

REFERENCE

Anon. 1989. Methodology of fish disease surveys. Coop. Res. Rep. No. 166.

APPENDIX 1

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APPENDIX 2

ICES WGPDMO Sub-Group on Analysis of Disease Prevalence in Marine Fish Stocks

AGENDA

1. Opening of the meeting. Background information.
2. Selection of rapporteurs.
3. Terms of reference.
4. Presentation of disease and other data available for analysis.
5. Statistical methods to be applied.
6. Data treatment within sub-groups.
7. General evaluation and discussion of results obtained.
8. Any further remarks.
9. Conclusions and recommendations.
10. Approval of the draft Sub-Group-Report.
11. Closing of the meeting.

APPENDIX 3

PROBLEMS RELATED TO THE STATISTICAL ANALYSIS OF FISH DISEASE PREVALENCE DATA SUBMITTED TO ICES/NSTF: SUGGESTIONS FOR 'A PLAN OF STRATEGY'

Discussion paper

Prepared for the ICES Sub-Group WGPDMO, 27-29 February 1992, Copenhagen

by

A.D. Vethaak, M. Carr, S. Wilson, S. Mellergaard, and J. Thulin

1. INTRODUCTION

This discussion paper was prepared one day prior to the meeting of the Sub-Group of WGPDMO with the goal to discuss problems related to the statistical analysis of fish disease prevalence data (in the framework of the ICES recommendations on the methodology of fish disease surveys (Anon., 1989)). Based on data taken from the ICES/NSTF data set (1990-91), as well as data from the Bremerhaven Workshop (1990), recommendations are given for a general strategy for the statistical analysis of the data submitted to ICES/NSTF. Recommendations for the future are also discussed.

The following data sets are used as examples in this paper. They all are related to diseases of dab and have been obtained following the recommendations and guidelines of ICES (Anon., 1989).

- data (March/April, 1990-91) from the Netherlands and Denmark submitted to ICES/NSTF; and
- data from the Bremerhaven Workshop (March, 1990) (Vethaak, Bucke, Lang, Wester, Jol and Carr, unpublished data).

2. STEPS TO FOLLOW IN THE ANALYSIS AND INTERPRETATION OF THE DATA

- Quality assurance of the data (e.g., confounding effects in the data, such as are liver nodules histologically confirmed or not?.).
- Statistical analysis of the data with the objective of assessing the effects of potential confounding factors (length, age, sex, season, sampling year) and subsequently plotting representative disease prevalence values on ICES maps.
- Interpretation of the disease patterns in the light of available knowledge of residence time, migration patterns and stock-related factors of the fish species in question. Further interpretation to establish correlations with potential environmental factors, especially sediment contaminant distribution, but also fishery intensity, benthos composition, population density, etc. It must be clear that the establishment of correlations between disease patterns and environmental or host-related factors contains many uncertainties, and therefore should be approached with a practical awareness of the problems.
- Suspected cause-and-effect relationships should be elucidated by laboratory experiments

3. STATUS OF DATA SUBMITTED TO ICES AND DATA STORAGE

3.1. Data availability

Data were supplied to ICES by the following countries:

Belgium	(1990-1991)	dab
Denmark	(1984-1991)	dab
Germany	(1988-1991)	dab, cod
Netherlands	(1984-1991)	dab
Sweden	(1990-1991)	cod, flounder
UK England	(1988-1991)	dab
UK Scotland	(1988-1991)	dab

3.2. Data status

All data were entered into the ICES computer and internally checked for consistency with paper form submissions.

Listing were returned to all data originators for validation, but due to time pressures confirmations were only received from the Netherlands (1991 data) and Denmark (1984-1991 data). Whilst this means that non-finalized data sets were included in the assessments, most originators responded to questions raised by ICES and checked for errors in those parameters of importance to the assessment analyses. Final validation is not expected to substantially change any of the results of the assessments.

From the available data, all data on dab (*Limanda limanda*) for the diseases lymphocystis, epidermal hyperplasia/papilloma, acute and healing skin ulcers, and liver nodules/tumours according to the size classes 15-19 cm, 20-24 cm, and 25+ cm were extracted for assessment, and prepared as SAS data sets for further analysis.

Data for the period 1989-91 were utilized in the primary data sets; data prior to 1989 were excluded from the primary data set as these data pre-dated the Workshop on the Methodology of Fish Disease Surveys (Anon., 1989), but some were used in exploratory analyses.

Data on lymphocystis and acute/healing skin ulcers in dab were excluded from the assessments on the basis of information brought to the meeting.

4. POTENTIAL PROBLEMS IN THE DATA

The ICES data have been collected at different times (year/season), for different sites, using different ships; and different observers have measured disease prevalence. Each sample taken (a sample being pooled hauls) will have different proportions of males and females, and different proportions in the different size categories. One cannot simply ignore these sources of variation in an analysis (for example by pooling over all sex, size, year and season categories) without first examining their importance.

There is considerable variation between different observers in the recognition of disease signs (Anon., 1989). Clear cut-off points and criteria for relevant diseases were defined in 1988 and, therefore, data obtained before 1989 may not be compatible. A major problem concerns the identification of liver nodules/-tumours. They have not been consistently confirmed (by all observers) by histological investigations and, therefore, certainly include a large bias of non-relevant lesions.

Concerning the effects of inter-ship/gear variation, there is, at present, no possibility for investigating this source of variation.

Seasonal and annual variation can be important (e.g., Dethlefsen *et al.*, 1987; Vethaak and Meer, 1991; Vethaak and Rheinallt, 1992) and should be taken into account in any analysis of disease prevalence.

Inter-haul variation is important. The Bremerhaven Workshop data suggested that disease prevalence varied between hauls. Representative numbers of fish for a certain sampling site can often not be realised by single hauls. It is suggested that at least 3 hauls should be taken; in the data reported, this is not always the case.

Disease prevalence is low (of the order of 5-10% for the most common disease). Also, the pattern of disease is generally agreed to be patchy (or as statisticians say, there exists "overdispersion in the data"). There are effectively two separate factors influencing sample size. The first tells us that we will need, e.g., 50 fish to detect a disease prevalence of 10%. The second tells us that even if we manage to collect 50 fish on the first haul, due to overdispersion we need to have replicate hauls.

The length distribution for males and females is not the same. For example, very few males are found in the ≥24 cm size category. Information on age-length relationships for healthy as well as diseased dab in the North Sea is very limited and not broadly available; this is considered an important subject for future study.

5. METHODS OF STATISTICAL ANALYSIS

There are a number of statistical procedures commonly applied in the analysis of fish disease data. On a simple level, one can present crude data pooled over different categories, for example, size, sex or season, but this ignores variation due to sex, size, etc. Another method involves comparison of the crude strata, for example, males of size 20-24 cm, etc. But this is time-consuming and does not make use of all the data. Direct or indirect standardisation can be used. However, a single index can mask important differences between strata.

There are various ways of analysing two-by-two tables given in the epidemiological literature, for example, the Mantel-Haenzel test for homogeneity across strata. However, such an approach does not allow tests of interaction between the various factors. It also assumes that the data are "structured".

An approach which has been accepted by many scientists in many fields, including epidemiology, is logistic regression analysis. This approach allows one to carry out a test of homogeneity across strata (as mentioned above), and also to test whether or not interactions exist in the data. It can also be used to analyse unbalanced data. The software to carry out such an analysis is widely available, for example GLIM, BMDP, SAS, SYSTAT. All of these packages are available on a PC. Though data can be unbalanced to a certain degree, conclusions can be misleading if the data are very unbalanced.

Some combination of these techniques will probably be required. For example, an analysis of each variable is useful to gain a general picture of the data. We would recommend, given the variety of techniques available, that a statistician be involved at this stage.

6. ICES/NSTF EXAMPLE

The data from the Netherlands and Denmark (1990-91; sampling time = spring) were pooled over the two size categories 20-24 cm and ≥24 cm for lymphocystis. The results are shown below. For the position of the sampling sites, see Figure A.

First, we looked at the effects of single factors:

Year	No. affected	No. examined	Proportion
1990	75	2,539	0.030
1991	80	2,551	0.031
Size			
15-19	84	3,110	0.027
20-24	56	1,606	0.035
25+	15	374	0.040
Sex			
male	76	2,283	0.033
female	79	2,807	0.028
Site			
Site 1	14	986	0.014
Site 2	16	1,090	0.015
Site 3	16	552	0.029
Site 4	17	711	0.024
Site 5	36	89	0.004
Site 6	76	673	0.113
Site 7	13	389	0.033

Data set used for logistic regression analysis (with r = number of fish affected, n = number of fish examined):

Year	Site	Sex	Size	r	n
1	1	1	1	1	200
1	1	1	2	1	9
1	1	2	1	1	118
1	1	2	2	0	122
1	1	2	3	0	25
2	1	1	1	3	218
2	1	1	2	4	76
2	1	1	3	0	1
2	1	2	1	2	63
2	1	2	2	1	81
2	1	2	3	1	63
1	2	1	1	3	227
1	2	1	2	0	42
1	2	2	1	0	115
1	2	2	2	2	170
1	2	2	3	0	34
2	2	1	1	3	218
2	2	1	2	4	76
2	2	1	3	0	1
2	2	2	1	2	63
2	2	2	2	1	81
2	2	2	3	1	63
1	3	1	1	2	76
1	3	1	2	0	1
1	3	2	1	2	78
1	3	2	2	2	36
2	3	1	1	7	180
2	3	1	2	0	45
2	3	2	1	2	73
2	3	2	2	1	58
2	3	2	3	0	5
1	4	1	1	0	66
1	4	1	2	0	15
1	4	1	3	0	2
1	4	2	1	1	85
1	4	2	2	3	135
1	4	2	3	4	62
2	4	1	1	4	105
2	4	1	2	2	31
2	4	1	3	0	1
2	4	2	1	2	125
2	4	2	2	1	78
2	4	2	3	0	6
1	5	1	1	0	177
1	5	1	2	0	5
1	5	2	1	0	152
1	5	2	2	0	68
1	5	2	3	0	16
2	5	1	1	0	119
2	5	1	2	0	15
2	5	2	1	2	62
2	5	2	2	1	68
2	5	2	3	0	7
1	6	1	1	18	125
1	6	2	1	10	111
1	6	1	2	4	27

Year	Site	Sex	Size	r	n
1	6	2	2	10	82
1	6	1	3	1	1
1	6	2	3	3	6
2	6	1	1	12	115
2	6	2	1	5	83
2	6	1	2	7	47
2	6	2	2	6	68
2	6	1	3	0	2
2	6	2	3	0	6
1	7	1	1	0	2
1	7	2	1	1	43
1	7	1	2	0	2
1	7	2	2	3	64
1	7	1	3	0	1
1	7	2	3	3	29
2	7	1	1	0	14
2	7	2	1	1	97
2	7	1	2	0	18
2	7	2	2	3	76
2	7	1	3	0	13
2	7	2	3	2	30

The results of the logistic regression are shown in the table below (CI = confidence interval). From this it can be seen that the estimated odds ratio for females (compared to males) is 0.71, meaning that females for each stratum defined by size, year and site, have a 71% chance of lymphocystis compared to males (Note: males are 100%).

Effect	Odd-ratio	95% CI
1991 *vs.* 1990	0.98	0.83 - 1.16
Large *vs.* small	1.52	1.27 - 1.82
F *vs.* M	0.71	0.59 - 0.85
Site 2 *vs.* Site 1	1.02	0.70 - 1.47
Site 3 *vs.* Site 1	2.17	1.50 - 3.15
Site 4 *vs.* Site 1	1.77	1.23 - 2.55
Site 5 *vs.* Site 1	0.33	0.17 - 0.62
Site 6 *vs.* Site 1	9.12	6.78 - 12.25
Site 7 *vs.* Site 1	2.51	1.68 - 3.73

7. PROBLEMS OF INTERPRETATION

7.1. Length as a measure of age

Many studies (e.g., the Bremerhaven Workshop data set) have pin-pointed the fact that length as a variable of disease prevalence is not truly valid; ideally, age should be considered. There are considerable differences in growth rates, both between males and females, and also regionally across the North Sea. At present, length/age keys for the NSTF sampling sites are not available.

7.2. Liver nodules/tumours

As long as liver nodules/tumours are not confirmed histologically, their value in fish disease studies will be limited. Therefore, it is strongly recommended that histological techniques be included in order to confirm the identity of grossly visible liver lesions. At present, the majority of the data available are for unconfirmed lesions. The data set submitted by the Netherlands, however, includes only histologically confirmed cases.

7.3. Seasonal variations in disease prevalence and migratory habits

When trying to interpret fish disease data with regard to, e.g., contamination status of the sediment, it is important to consider the migratory behaviour (e.g., residence time) of the dab in a given area. Damn *et al.* (1991) and Rijnsdorp *et al.* (1992) have demonstrated by tagging experiments that dab exhibit migratory behaviour in January through April associated with spawning activities. Dab tagged in the Southern Bight were recaptured at locations to the south-west of the release site. However, dab released in the German Bight did not show a clear direction preference; part of the dab showed a tendency to a northwestern migration and another part to a western migration. The distance of the migration was up to 200 nm. After the spawning period, the fish seem to return to their feeding grounds and become relatively stationary until late autumn.

From the above, it is clear that the use of dab as an indicator species has limitations, especially during the main spawning period of the species (January-April). Comparisons of disease prevalences should be done at the same time of the year, not solely from the viewpoint of potential seasonal variation in disease prevalence, but also from the apparent seasonal migratory habits of the fish species. Moreover, it is advisable that disease studies should concentrate on the non-migratory period (July, August, September), and preferably be conducted in the middle or at the end of that period to increase the representativity of the disease data collected. There is a dilemma here. Ideally, the fish disease surveys would coincide with the period when disease prevalence is greatest. In general, dab seems to have higher disease levels in spring than in autumn (at least in the southern part of the North Sea), the spring season being its migratory period.

7.4 Stock-related factors influencing disease prevalence

Attempts have been made to separate the eastern North Sea dab stocks based on different parasitic infestations and growth patterns of the fish. The parasites used for this task have been *Myxobolus aeglefini*, *Stephanostomum baccatum* and *Glugea stephani*.

Based on preliminary Dutch data (van Banning, unpublished data) and some Danish data, it is possible to split the eastern North Sea dab stocks into five different stocks: a Skagerrak stock, a Danish west coast stock, a German Bight/Wadden Sea stock, a Dutch Wadden Sea stock, and a central North Sea stock (see Figure B).

The Skagerrak stock is characterised by high infestation rates of *Myxobolus* and a relatively fast growth rate. The Danish west coast stock is characterised by a lower *Myxobolus* rate and a lower growth rate than the Skagerrak stock. The German Bight/Wadden Sea stock has no infection with *Myxobolus* and is relatively slow growing compared to the more northern stocks. The Dutch Wadden Sea stock has a very high prevalence of *Glugea* compared with other areas and the central North Sea stock has a high prevalence of *Stephanostomum*.

In conclusion, the available knowledge of stock separation of dab should be taken into account in the interpretation of the disease patterns.

7.5. Comparison of local/regional disease prevalences

Comparing disease patterns in an area as large as the southern/central North Sea is problematic, since regional differences may be due to stock-related factors (as explained in point 6.4), which may be different for different areas or migratory habits (e.g., possible clustering effects of older, thus more diseased, fish) (as explained in point 6.3). Knowledge of these factors is still largely lacking, which severely limits the possibilities for reliable interpretation/evaluation of the disease data.

8. SOME PROPOSALS FOR DISCUSSION/ACTION

- Sampling design? Does it need change or not?

- Age/length keys. These should be developed and included in the analysis.

- Histological confirmation of grossly visible liver nodules should be a prerequisite and included in the analysis.

- Inter-observer variation. This problem can be aided by accelerating the publication by ICES of the 'Training Guide for Marine Fish Disease Surveys' (Bucke *et al.*), and making it widely available as soon as possible.

- ICES should provide data (maps) of relevant potential factors influencing disease, other than sediment contaminant distributions, in order to allow a more reliable and "balanced" interpretation of the possible relationships between fish disease and pollution.

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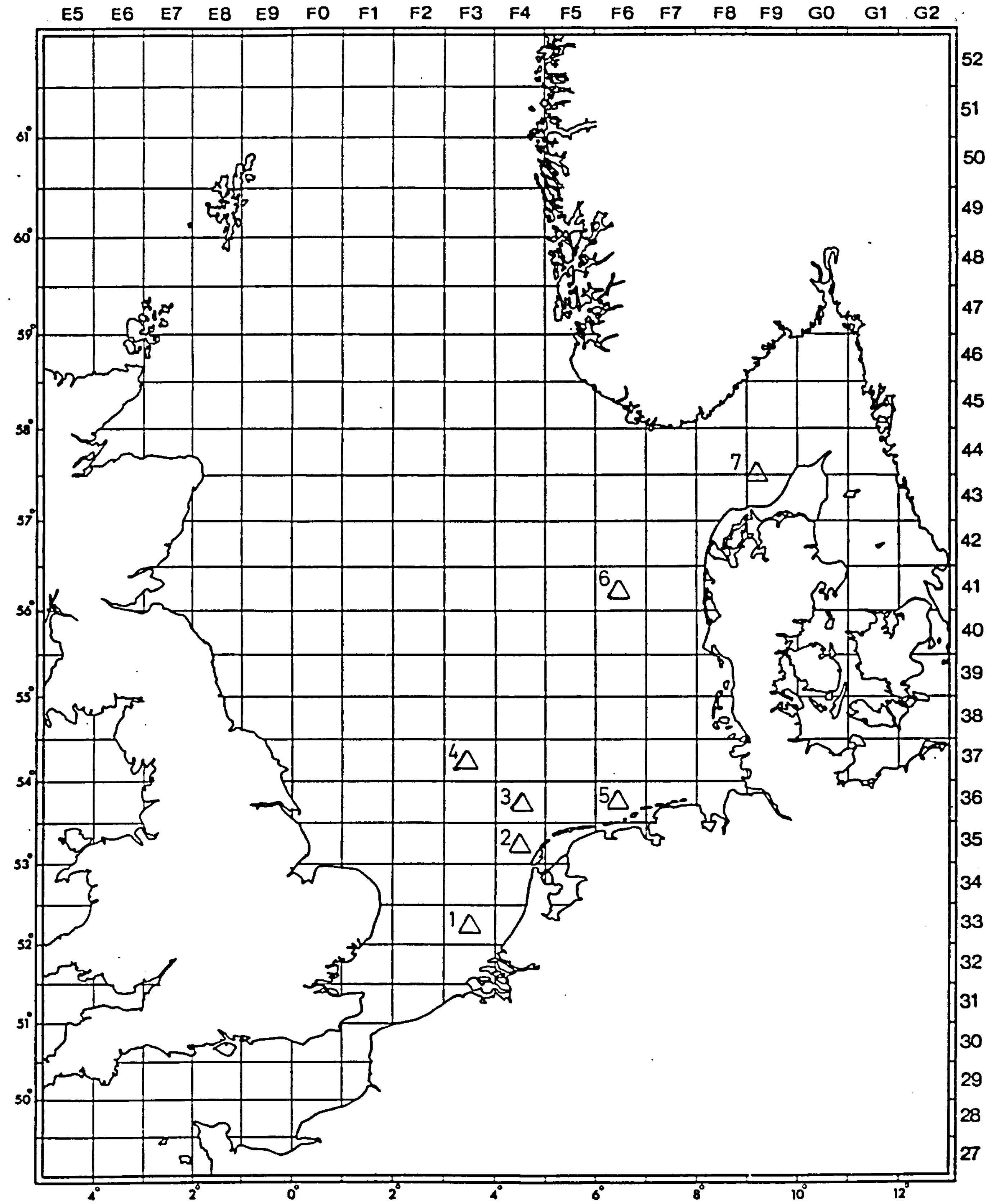

Figure A. Positions of sampling sites for fish diseases under the North Sea Task Force Monitoring Master Plan (Netherlands and Denmark).

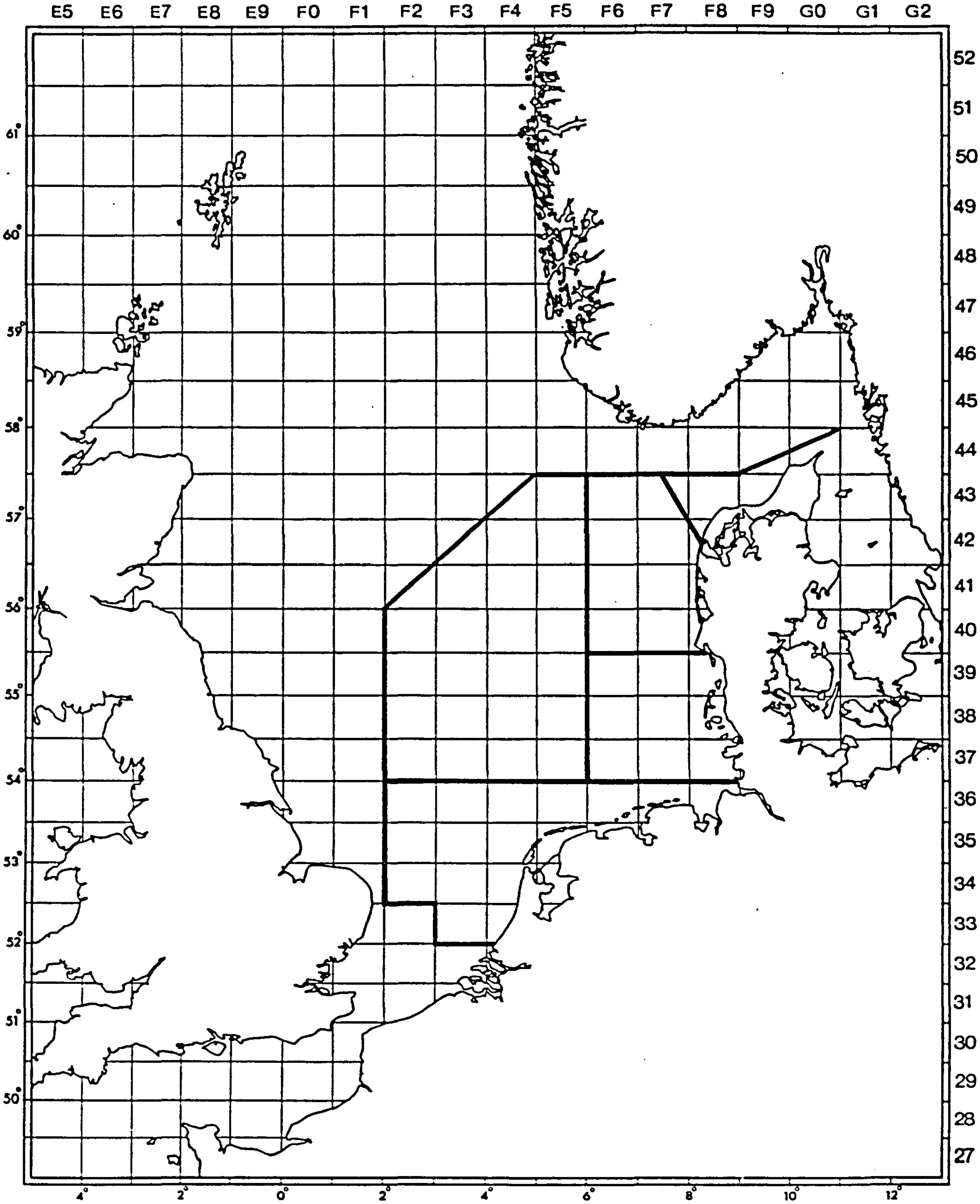

Figure B. Separation of dab stocks on the basis of fish disease surveys based on van Banning, unpubl. data).

ANNEX 6

ANALYSIS OF PROGRESS WITH TASKS

1. Tasks completed.

   i) Inventory of parasites and diseases of wild and reared salmon.

   Available data were tabulated and sent by fax to WGNAS in Dublin on 5 March 1992.

   ii) Review the results of the sub-group analysis of disease prevalence data in marine fish stocks and report to the 1992 ACMP meeting.

   The sub-group report was considered and endorsed by WGPDMO.

   iii) Evaluate the impact of *Ichthyophonus* on affected herring stocks.

   Data collected subsequent to the Lysekil ICES special meeting were assessed and a report prepared for circulation to the relevant working groups indicated in the terms of reference.

   iv) Analysis of cases of disease interactions between farmed and wild populations of fish.

   Known and possible cases of disease interactions between farmed and wild fish were evaluated and conclusions are presented in this report.

   v) Analyse national reports on recent disease trends in mariculture.

   Available data were appraised, trends identified, and advice on prevention and control offered in this report.

   vi) Training guide for fish disease surveys.

   The final draft was submitted to ACMP in 1991.

2. Tasks to be continued.

   i) Review of disease control methods relevant to the "Code of Practice in Introductions and Transfers".

   A one-day joint meeting should be held between WGPDMO and WGITMO during their 1993 meetings to discuss appropriate methodologies and allow completion of the revision.

   ii) Diagnostic fiches.

   Diagnostic fiches in the sequence 51-60 which have not yet been published should be finalised and submitted for publication. Earlier fiches should be considered for revision by the editor in consultation with the original authors wherever possible.

3. Tasks on which progress has not been made.

   None.

4. New tasks.

   i) Evaluation of disease prevalence data in marine fish stocks.

   Using newly standardised and validated data made available to ICES intersessionally by WGPDMO members, analyse the current and historical information available for trends.

   ii) Evaluation of the impact of *Ichthyophonus* on herring.

   Consider the conclusions of the proposed intersessional sub-group meeting for analysis of results from studies on *Ichthyophonus* and evaluate any additional new data available.

   iii) Mollusc disease studies.

   Evaluate current research on mollusc diseases (e.g., epidemiological surveys, experimental pathology, diagnostic methods) in ICES member countries in order to standardise approaches.

ANNEX 7

RECOMMENDATIONS

Recommendation 1

The Working Group on Pathology and Diseases of Marine Organisms recommends that a second ICES Special Meeting on *Ichthyophonus*, involving stock assessment and disease specialists, should be held in September 1992, Aberdeen (Convener: Dr A. McVicar) in order to:

- produce reliable information on the prevalence of *Ichthyophonus* in different herring stocks based on results obtained using the standardised methods recommended by the WGPDMO; and

- estimate the extent of mortality in herring stocks utilising data obtained from experimental work and field observations.

Recommendation 2

The Working Group on Pathology and Diseases of Marine Organisms recommends that the Training Guide for Marine Fish Disease Surveys be published as soon as possible, preferably in the Techniques In Marine Environmental Sciences series.

Recommendation 3

The Working Group on Pathology and Diseases of Marine Organisms recommends that a 3-day meeting to assess fish disease data be convened under Dr J. Thulin in conjunction with the 1993 WGPDMO to:

a) evaluate disease prevalence data on species other than dab, including the data for the Baltic area which will be available after April 1992;

b) re-evaluate the disease prevalence data sets already submitted to ICES but supplemented with the additional information to be requested from the data originators;

c) extend the analysis of the ICES disease prevalence data sets using logistic regression analysis;

d) evaluate data on liver nodules/tumours in dab in the light of the results of histological confirmation of existing fixed material;

e) if practicable, compare fish disease prevalence data with the results arising from the assessment of data on contaminants in sediments conducted in 1992.

In this connection, individual institutes should analyse their existing data banks and report additional information to ICES well in advance of the next WGPDMO meeting to facilitate such analysis.

Recommendation 4

The Working Group on the Pathology and Diseases of Marine Organisms recommends that it meet at ICES Headquarters for four days during March 1993 to:

a) evaluate disease prevalence data in marine fish stocks using the methodological changes recommended by the 1992 Copenhagen Sub-group meeting and compare fish disease prevalence data with the results arising from the assessment of data on contaminants in sediments conducted in 1992;

b) update and analyse information on prevalence and impact of *Ichthyophonus* on different herring as well as other fish stocks and consider the conclusions of the proposed intersessional sub-group meeting;

c) analyse national reports on new disease trends in mariculture, and provide advice on preventive and control measures;

d) analyse national reports on new disease trends in wild fish, crustacean and mollusc populations;

e) analyse and update information from studies in progress on disease interactions between farmed and wild fish populations;

f) evaluate current research on mollusc diseases (e.g., epidemiological surveys, experimental pathology, diagnostic methods) in ICES member countries in order to standardise approaches.

Recomendation 5

A one-day joint meeting between WGPDMO and WGITMO should be held during their 1993 meetings to discuss relevant methodologies for the diagnosis of diseases of fish and shellfish, in order to complete the revision of the ICES/EIFAC Code of Practice on Introductions and Transfers.