Mariculture Committee

ICES CM1996/F:4

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

ICES Headquarters. Copenhagen, Denmark

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1 INTRODUCTION

The Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the ICES Headquarters, Copenhagen, Denmark from 21–26 March 1996 with Dr A H McVicar presiding as Chairman (Council Resolution 1995/2:29).

1.1 Opening of the meeting and structure of the meeting

The meeting was opened at 09.30 on Thursday 21 March 1996 with the Chairman welcoming participants, particularly those new to the WGPDMO. While a good proportion of ICES Member Countries was represented, the number which were not, was regretted. The functioning of the WG is dependant on the input from as wide an experience base as possible.

A List of participants is appended in Annex 1.

2 ICES STATUTORY MEETING 1995; ITEMS OF RELEVANCE TO THE WGPDMO

Items of relevance to the WGPDMO from the Report of the ICES Annual Science Conference held in Aalborg, Denmark in September 1995 were highlighted by the Chairman.

a) The Report of the Consultative Committee:

(i) indicated that Study Groups established as a supplement to Working Groups should have a limited life span (3 years or less). In this respect the decision C.M. 1996/F:3 (Recommendation 1) of the Sub-Group on Statistical Analysis of Fish Disease Prevalence Data in Marine Fish Stocks which had met on 19-20 March 1996 at ICES is particularly relevant:

"With the completion of the major parts of the Terms of Reference of the Sub-Group on Statistical Analysis of Fish Disease Prevalence Data in Marine Fish Stocks , the Sub-Group recommends that future work in this field be incorporated back into the Terms of Reference of WGPDMO".

(ii) indicated that there was confirmation that Dr R Elston (USA) was willing to convene a Mini-Symposium in 1997 on "Identifying and Managing Diseases of Bivalve Shellfish". No information on this proposed mini-Symposium was available to WGPDMO and a relevant member of the WGPDMO (S. McGladdery) will make direct contact with Dr Elston to obtain information on details of the meeting.

(iii) drew attention to the Intermediate Ministerial Meeting on Fisheries and the Environment of the North Sea which will be held in 1997 in Norway. This was indicated to WG members who may be involved in future input of data into preparation documents.

(iv) drew attention to the Workshop (to be chaired by Dr. A. H. McVicar (UK)) on "Interactions Between Salmon Lice and Salmonids", the Terms of Reference (C.Res.1995/2:57) for which were raised by the ANACAT. The meeting had not been referred to WGPDMO for comment but this was considered under Agenda Item 22.1.

(v) drew attention to the Special Meeting on "The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" to be chaired by Dr S. W. Feist (UK) and Dr T. Lang (Germany). Discussion on planning for this meeting is included under the WGPDMO Terms of Reference (C.Res.1995/2:29(m)).

b) The Mariculture Committee proposed a Special Topic session on "Hygiene in Mariculture" for the 1996 Annual Science Conference. Discussion on planning for this meeting is included in the WGPDMO Terms of Reference (C.Res.1995/2:29(o)).

c) The Mariculture Committee Report included reference to discussion on a proposal from the ANACAT Committee for a joint Theme Session on the subject "The effects of salmon lice on fish stocks" which could be planned for the 1996 Annual Science Conference. No recommendation was made on this proposal.

d) Reference was made to the paper (ICES C.M. 1995/F:15) on VHS virus isolation from North Sea cod presented to the Mariculture Committee.

e) Reference was made to the paper (ICES C.M. 1995/H:8, H:25) on *Ichthyophonus* from North Sea herring presented to the Pelagic Fish Committee.

f) Reference was made to the paper (ICES C.M. 1995/F:11) providing a preliminary report on the BMB/ICES Sea-going Workshop "Fish Diseases and Parasites in the Baltic Sea".

g) Reference was made to the current state of knowledge on M74 syndrome in Baltic salmon in the Report of the ANACAT Committee. Sea herring presented to the Pelagic Fish Committee. Discussion on further developments in this field is included in the WGPDMO Terms of Reference (C.Res.1995/2:29(k)).

h) Reference was made to the concern noted by the Publications Committee on the slowness of production of "The ICES Identification Leaflets on Diseases and Parasites of Fish and Shellfish" but that recent progress in the preparation of manuscripts had been indicated. The Committee agreed that the Leaflets were in demand.

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

The Terms of Reference as published in the ICES Annual Report as C.Res 1995/2:29 were detailed. The heavy agenda load necessitated extensive intersessional work by members of the WGPDMO identified by the Chairman as expert in specific areas. Several written discussion documents were produced for consideration at the WG meeting. The Chairman thanked these members for the reports they had provided and these are included in this Report as annexes.

3.1 Terms of Reference

The Terms of Reference were as published in the ICES Annual Report 1995, C.Res.1995/2:29 (Annex 3a).

3.2 Adoption of Agenda and selection of Rapporteurs

The draft agenda (Annex 3b) was approved without alteration by the WGPDMO. Rapporteurs were appointed for each of the agenda items, taking into consideration the range of expertise within the WG (Annex 3c).

4 CONSIDERATION OF RELEVANT REPORTS

a) ACME

T. Lang, as the WGPDMO 'shadow' with ACME, gave a brief resume of his role in acting as a link with ACME. This system facilitated the flow of relevant information from WGPDMO to ACME and also ensured that the WG was kept in close contact with ACME discussions, decisions and requests for services and advice. He noted that the work of WGPDMO was highly regarded by ACME.

b) Workshop on Biological Effects Monitoring Techniques held in Aberdeen, Scotland on 2–6 October 1995.

At the invitation of Dr R. Stagg, Chairman of the workshop , A. McVicar attended part of this meeting and gave a short resume of the role which fish disease monitoring could play as a tool in marine environmental monitoring and the part which WGPDMO had played in developing this concept. It is noted that this workshop recommended the use of:

(i) liver nodules as a biomarker of the general quality status of the environment and

(ii) externally visible fish disease as population / community "stress" indicators.

c) The WGPDMO noted a working document (Annex 4) prepared by T. Lang for the 1996 meeting of the WGBEC related to the development of a quality assurance programme for externally visible fish diseases. It was considered a useful basis for further discussion on the implementation of quality assurance measures in monitoring programmes on biological effects of contaminants.

d) Baltic Marine Biologists Working Group 25 on Fish Diseases and Parasites of the Baltic Sea.

The Chairman of this Working Group, Dr G. Bylund, who is also a member of the WGPDMO, gave an overview of information on this group relevant to WGPDMO. They had not met recently and decisions on their future activities were scheduled to be made in the near future.

e) The WGPDMO considered a draft document provided by T. Lang on available information on fish diseases/parasites in the Baltic Sea. This would to be incorporated in the report of the ICES ACME and ACFM on fisheries and fish stocks, including coastal species, for the HELCOM Third Periodic Assessment of the Baltic Sea. It was agreed that the Working Group members involved in fish diseases research/monitoring in the Baltic Sea would review the document intersessionally prior to its further consideration at the forthcoming 1996 meetings of the ACME and ACFM. Comments should be sent to T. Lang by 31 May 1996.

f) A series of meetings on oestrogenic compounds and their effect on the reproductive capacity of aquatic animals including fish had recently been held, particularly in USA, Denmark, UK and the Netherlands. The effects of these compounds on estuarine and marine fish and their role in inducing pathological abnormalities, particularly on the gonad, may warrant the future close attention of WPDMO.

g) The European Association of Fish Pathologists conference in Palma de Mallorca, Spain in September 1995 considered many topics in farmed fish which were of direct relevance to WGPDMO. Several members of WGPDMO were represented at that meeting and were able to provide first hand information relevant to current work of WGPDMO (e.g. Section 14 below).

h) For information, the WG members were appraised of forthcoming meetings pertinent to the work of WGPDMO:

- the EC sponsored Workshop on "The Diagnostics of *Gyrodactylus salaris*" organised by the National Veterinary and Food Research Institute, Oulu, Finland from 13 - 17 April 1996.
- the meeting of the National Shellfisheries Association to be held in Baltimore, USA on 17–19 April 1996.
- the International Symposium on "Fish Vaccinology" organised by the International Association of Biological Standardisation to be held in Oslo, Norway on 5–7 June 1996.
- the European Multicolloquium of Parasitology (EMOP) meeting to be held in Parma, Italy in September 1996 which includes a Theme Session on "Fish Parasites as Indicators of Environmental Quality".
- the ICES Special Meeting on "The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" to be held in Weymouth, UK on 22 – 25 October 1996. (see Section 17 below).

5 ANALYSIS OF NATIONAL REPORTS ON NEW DISEASES AND DISEASE TRENDS IN WILD FISH; CRUSTACEANS AND MOLLUSCS

5.1 Wild Fish Diseases

Lymphocystis: In North Sea dab (*Limanda limanda*) and flounder (*Platichthys flesus*) there seems to be a general decreasing trend of the prevalence. An exception is the German Bight were the prevalence in dab has been increasing during the past 3 years.

In the south-western Baltic Sea, the prevalence of lymphocystis in flounder seems to have increased since the beginning of the eighties. In Estonian waters, prevalences were highest during spawning season with males being more frequently affected than females. However, the prevalence was lower as compared to the south-western Baltic Sea.

Epidermal hyperplasia/papilloma: In North Sea dab, no new trends have been reported. In the Irish Sea (Liverpool Bay area), the prevalence appears to be increasing.

VHS-like virus: The virus was again isolated from skin papules in samples of cod during 1995 in the central northern North Sea. In the same area VHS-like virus was isolated from two haddock (*Melanogrammus aeglfinus)* showing evidence of petichial skin lesions. Serotyping studies have indicated that three serotypes of the virus have now been isolated in Scottish offshore waters in the last three years.

The North American VHS-strain has remained prevalent in the Prince Williams Sound herring population. The appearance of the virus was associated with the spawning stress of adults.

Other viruses: A paramyxovirus and a reovirus have been isolated from two different stocks of chinook salmon (*Oncorhynchus tshawytscha*) in Alaska. No mortalities were associated with these infections.

Skin ulcer disease: In dab from the central North Sea (Dogger Bank), prevalences in summer are still very high (up to 30 %). However, the increasing trend recorded in previous years did not continue.

The prevalence recorded in male flounder off the Estonian coast after spawning was considerably higher as compared to females.

Elevated prevalences were observed in Baltic cod (*Gadus morhua*) from stations off the German and Polish coast mainly affecting specimens of smaller size groups. There were several new reports of ulcerated cod from areas in the Bristol Channel.

Hypermelanisation: Prevalences in certain North Sea areas (Dogger Bank, Humber off Ground, Flamborough off Ground) remain high. The aetiology is still unclear.

Liver pathology: Prevalences of nodules/tumours in North Sea dab and flounder and flounder from the south-western Baltic Sea remain at a low level compared to previous years. Low levels also continue to be recorded from Irish Sea stations. It was emphasised again that histological conformation of macroscopic liver nodules is required in order to exclude non-neoplastic lesions from prevalence data. In studies carried out in the northern Baltic Sea, no correlations could be established so far between the prevalence of neoplastic liver lesions and levels of DNA adducts.

Swedish studies of perch (*Perca fluviatilis*) from Sundsvall Bay revealed reduced growth, significant degenerative liver changes and high levels of DNA adducts. These changes correlated well with high levels of PAHs along a gradient from an aluminium smeltery. Investigations of the deepwater species *Coryphaenoides rupestris* from a contaminated site in the Skagerrak showed a significant increase in hepatic melano-macrophage centers as well as induction of EROD in comparison to a reference site of the Faroe Islands.

Skeletal deformities: In winter flounder (*Pseudopleuronectes americanus*) collected from polluted areas in Boston Harbour high prevalences (38-40 %) of vertebral fusions were recorded by radiographic examination. American plaice (*Hippoglossaides platessoides*) were less affected.

***Posthodiplostomum cuticula*:** There is an increasing trend in the prevalence and intensity of this parasite originating from brackish water into freshwater bodies. Species affected were roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*).

Monogenean parasites: A clear declining trend in the infestations has been recorded in coastal brackish waters of Estonia.

***Kudoa* sp.:** From studies in sea trout (Salmo trutta) in south-western France there is indication of considerable annual fluctuations in the prevalence of *Kudoa* sp. with a maximum prevalence of 50 % in 1990-1991.

***Angillicola crassus*:** In south western France the prevalence of this parasite in eels (*Anguilla anguilla*) in estuarine conditions at 30 parts per thousand salinity is approaching 100%. In studies in north-western Spain the parasite has not been detected, for the second year.

Parasites of sea trout: Results of a survey of parasites of sea trout in Scotland identified a total of 43 species. Several were noted as potentially serious pathogens. These included the protistans *Chloromyxum truttae*, *Myxidium truttae*, the digenean *Diplostomum spathaceum*, the cestode *Diphyllobothrium ditremum*, the acanthocephalean *Echinorhynchus truttae* and the crustacean *Lepeophtheirus salmonis*.

5.2

Wild Shellfish, Crustaceans and Echinoderms are considered in Section 6.2–6.4.

6 ANALYSIS OF NATIONAL REPORTS OF NEW DISEASE TRENDS IN MARICULTURE.

6.1 Finfish

6.1.1 Analysis by fish species

a) Atlantic salmon (*Salmo salar*)

Bacterial problems

Furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*) which some years ago was a serious threat to the salmon industry, is now controlled in the main salmon farming countries. The reason for this is believed to be the use of large scale vaccination programmes and improved management. BKD (*Renibacterium salmoninarum*) has shown an increase at several farm sites in Atlantic Canada. In Norway, there have been some outbreaks of Cold Water Vibriosis (*Vibrio salmonicida*). Most of these cases are associated with inadequate vaccination practices. However, some cases in Northern Norway still remain unexplained. In the Shetlands, this disease was recorded at low level without major losses.

Pancreatic diseases

Infectious Pancreatic Necrosis (IPN) associated diseases in the immediate post smolt period are still recognised as a major disease problem in both Scotland and Norway. No distinct trends were reported. In Scotland, Pancreas Disease (PD) was still a significant disease on some salmon farms but generally showing a lower level of occurrence through the industry compared with previous years.

Infectious Salmon Anaemia (ISA)

The disease is still restricted to Norway. In 1995 only two new outbreaks were diagnosed. An antibody based diagnostic method has been developed and is now being evaluated for routine diagnostics.

Sea lice (*Lepeophtheirus salmonis*)

Sea lice infestations still remains the most important disease problem in Norway, Scotland and Eastern North America. The problem is increasing in Canada and USA.

b) Other salmonids

Furunculosis (*A. salmonicida* subsp. *salmonicida*) and vibriosis (*Vibrio anguillarum*) are currently serious disease problems in the marine farming of rainbow trout (*Oncorhynchus mykiss*). The severity of these diseases could possibly be reduced if adequate vaccination programs were applied. *Flavobacterium psychrophilium* infection is a problem of increasing concern in rainbow trout farming in the Baltic. Some of the drugs (quinolones) most commonly used in fish farming seem to have little effect in treating F. *psychrophilium* infections.

There are observations in France indicating that Pancreas Disease might occur in salmonid species other than Atlantic salmon, for example rainbow trout and brown trout (*Salmo trutta*).

In large brown trout, a problem characterised by rupture of the aortic bulb, was reported from France. The aetiology of this condition is unknown.

c) Sea bass (*Dicentrarchus labrax*)

Encephalopathy of sea bass caused by a nodavirus (Viral Nervous Necrosis Virus) is currently the main problem in production of sea bass. In 1995, outbreaks occurred throughout the Mediterranean, not only in larvae but also in juveniles and ongrowing fish (10-200 g).

d) Sea bream (*Sparus aurata*)

Pasteurellosis (*Pasteurella piscicida*) continues to be a major problem. Results of immunisation trials indicate that vaccination of juveniles can be useful in preventing this disease. High incidences of infections by *Flexibacter/Flavobacterium* were reported in the larval stage.

e) **Halibut (*Hippoglossus hippoglossus*)**

A nodavirus-like agent associated with vacuolating encephalomyelopathy and retinopathy was reported to cause mass mortality of larval and juvenile Atlantic halibut in Norway. This virus shows antigenic relationship to Japanese and French nodaviruses.

f) **Turbot (*Scophthalmus maximus*)**

A viral infection, assigned tentatively to the paramyxoviridae, caused significant problems in Spanish turbot farms. Infections due to *Streptococcus* sp. have decreased due to vaccination programmes. There has been an increase in the reported cases of *Flexibacter maritimus* infections. Attempts to develop an effective vaccine have not been successful.

g) **New technology in fish health management**

A patent has been issued in the USA to use ultrasound as the means of delivering various compounds to fish. This technology might have useful applications in the delivery of drugs and vaccines to fish.

6.1.2 Conclusions

a) In salmon farming the overall health situation has improved due to application of effective vaccination programmes and improved management procedures.

b) A nodavirus -like agent was isolated from Atlantic halibut .

c) The appearance of new viral diseases in the farming of marine fish is a matter of increasing concern and research should be encouraged.

6.1.3 Recommendations

It is recommended that the WGPDMO review available information on diseases in marine fish associated with nodavirus or nodavirus - like agents.

6.2 Molluscs

6.2.1 Analysis by disease

Quahaug parasite X (QPX) of hard-shell (quahaug) clams (*Mercenaria mercenaria*) in Canada showed no significant increase but is a consistent problem in hatchery broodstock, with high cumulative mortalities (90%). In the USA, however, significant mortalities of hard-shell clams occurred in two areas off Massachusetts. The reason for the sudden appearance of QPX in eastern US clams for the first time, and its taxonomic position, are currently under investigation.

Malpeque Disease (unidentified aetiology) appeared in susceptible American oysters (*Crassostrea virginica*) transplanted to two experimental sites in the southern Gulf of St. Lawrence, Canada. This disease has not been observed in local oysters since the last epizootic in the late 1950's, but still appears to be virulent. The prepatant period lasted over 16 months.

Denman Island Disease (*Mikrocytos mackini*) found in Pacific oysters (*Crassostrea gigas*) was shown to remain infective when in other oyster species (see National Report for 1994/95). Infected European oyster (*Ostrea edulis*), Olympia oyster (*Ostrea conchaphila*) and American oyster (*Crassostrea virginica*) all transmitted the disease to all these four oyster species and elicit the same lesions observed in wild infections.

Bonamia ostreae of European flat oysters (*Ostrea edulis*) was confirmed at low levels for the second year on the French Mediterranean coast and in Vendee (France). *Bonamia* was not detected this year in Normandy (France). There was no change in the distribution of *Bonamia* in Spain or England. In The Netherlands, *Bonamia* reached its lowest level (<10%) in five years. However, commercial-size *O. edulis* were also at their lowest density levels (< 5 specimens/100 m sq.). Norway and Scotland started a disease screening programme (under EC guidelines) and have not detected the parasite. Quarantined broodstock of two species of *Crassostrea* (*C. rivularis and C. sikamea*) imported from the USA and used for genetic experiments in France were found to be infected by a *Bonamia*-like parasite during the experiments

(Section 6.2.2 and Annex 12). This is the first observation of a *Bonamia*-like parasite in *Crassostrea* spp.. The infection was associated with mortalities.

Marteilia refringens was reported in Bouin (France), but not detected in Normandy or the Mediterranean coasts of France, in wild or cultivated stocks of *Ostrea edulis*. No change was reported in prevalence of *Marteilia* spp. off the northwest Atlantic coast of Spain. On the northern Mediterranean coasts of Spain (Catalonia) *Marteilia* spp. (reported as *M. refringens*) have increased in prevalence up to 80%, in both *O. edulis* and *C. gigas*. *Marteilia* spp. have still not been detected in oysters from The Netherlands, Scotland, England, Wales and Norway.

***Bucephallus* sp.** was present at high prevalences (45%) in the same oysters from the northern Mediterranean (Spain) as reported to be infected by *Marteilia* spp. (see above).

Steinhausia mytilovum was found in 35% of *Mytilus galloprovincialis* from the northern Mediterranean (Spain). This is the highest prevalence found for over 20 years.

Herpesvirus was reported from France for the fifth year in larval and juvenile oysters (*Crassostrea gigas* and *Ostrea edulis*) collected from hatchery, nursery and natural broodstock sources. No *Herpes* cases were found to transmit in the field, since no infections were found to spread to surrounding stocks (mostly juveniles examined). New diagnostic techniques have been developed. For example, PCR primers are available (IFREMER, La Tremblade, France) to detect infection in clinical cases. These will be used to examine a possible relationship between Summer Oyster Mortality (see below) and *Herpes* infections. Cross reactivity between the *Herpes* in *O. edulis* and *C. gigas* is under investigation at IFREMER, La Tremblade.

Summer Pacific Oyster Mortality (SPOM) continued to occur in France and showed an increase in the Netherlands from 20-40% to a level of 40-80%. Correlation to high temperatures (France and The Netherlands) and algae blooms (*Gymnodinium* sp.) (France) are under investigation, as well as *Herpes* virus (see above).

Brown Ring Disease (BRD) was confirmed as the cause of mortalities of Manila clams (*Ruditapes philippinarum*) in Poole Harbour, England. Spain and France reported no change in prevalence of infection.

Perkinsus marinus has spread to Maine and was reported for the first time at low levels in American oysters (*Crassostrea virginica*).

Perkinsus atlanticus was found during both histological and culture studies on 46 sites in the Galician Rias of Spain. An average prevalence of 63% was found. Infections in Rias showed localised distribution, a wide range of prevalences, and mortalities. Some areas, however, remained free of *Perkinsus*. The most susceptible species of clams were *Tapes decussatus* and *Ruditapes philippinarum*. *Venerupis pullastra* and *Tapes rhomboides* were less susceptible. In general, there appeared to be a decrease in clam infections. This was speculated to be due to i) heavily infected populations being wiped out and /or ii) heavy rainfall and decreased temperature inhibiting the parasite. Perkinsiosis continued to be the main disease concern of the clam industry in Spain.

Haplosporidium nelsoni (MSX) continued to cause sporadic mortalities in American oysters (*Crasssostrea virginica*) in Maine. (No changes in the level and distribution of MSX were reported from Maryland and Virginia).

Juvenile Oyster Disease (JOD) continued to be a significant disease problem in American oyster (*Crassostrea virginica*) hatchery production in Maine and New York. The cause of the disease is still under investigation.

Shell Abnormalities: In The Netherlands, *Polydora ciliata* showed a significant increase in prevalence and level of shell damage in *Crassostrea gigas,* maybe related to high summer temperatures. Canada also noted a significant increase in *Polydora* infection in *C. virginica* in certain sites in the Gulf of St. Lawrence (a similar increase has also been reported for the boring sponges *Cliona* spp.). Shell deformities in the form of blisters or chambers continue to cause problems in *C. gigas* and *O. edulis* oysters from Galicia (Spain).

Imposex (females developing male sexual organs) is reported for the first time from *Littorina littorea* (common periwinkle) by Germany. This problem has been strongly correlated to TBT pollution in coastal areas. Recent findings indicated that imposex in whelk species, including *Buccinum* and *Neptunea* spp, occurred over wider areas of the North Sea and Irish Sea and was not restricted to coastal areas and shipping lanes.

6.2.2 Summary and conclusions

i) The lack of a report on the disease status of shellfish in Ireland, Portugal and the USA (and Mediterranean countries), was regretted.

ii) A survey of Scandinavian shellfish is required to update the disease status of certain established oyster populations (e.g., Danish *Ostrea edulis*).

iii) In France, a *Bonamia*-like parasite was detected for the first time in two species of *Crassostrea* (*C. rivularis* and *C. sikamea*) originating from USA and held under experimental conditions. The origin of this parasite is under investigation as it was not detected in the pre-screened oysters.

iv) The demonstrated need for improved specificity of diagnostic tools for commercially important pathogens and the issue host-specificity (1995 Report of the WGPDMO - ICES C. M. 1995/F:3) continue to be of concern (e.g., *Mikrocytos mackini* in *Crassostrea virginica*, *Ostrea edulis* and *Ostrea conchaphila* ; *Haplosporidium nelsoni* in *C. gigas* ; *Marteilia refringens* in *Mytilus* spp. and *C. gigas*).

v) The 80% prevalence and stage of development of *Marteilia* sp., reported in the National Report from Spain as *M. refringens* in *Crassostrea gigas,* requires clarification since this is a significantly high level of infection for this oyster species.

6.3 Crustacean

***Haematodinium* sp.** in Norwegian lobster (*Nephrops norvegicus*) increased up to 10% prevalence in Botney Gut and Silver Pit areas of the North Sea. A *Haematodinium*-like parasite was also found in the prawn *Pandalus platyceros* of British Columbia, Canada. The decline noted in the WGPDMO report of 1994 continued.

Bacterial Shell Disease in Norwegian lobster (*Nephrops norvegicus*) showed no significant change in Botney Gut and Silver Pit areas of the North Sea.

6.4 Echinoderms

Paramoeba invadens of green sea-urchins (*Strongylocentrotus droebachiensis*) caused mass mortalities of up to 100 % at several locations along the Atlantic coast of Nova Scotia, Canada. The last epizootic of this disease occurred between 1980 and 1983.

7 EVALUATE THE DRAFT REPORT OF THE SUB-GROUP ON STATISTICAL ANALYSIS OF FISH DISEASE PRE-VALENCE DATA

The Chairman, A. D. Vethaak, presented the draft report of the Sub-group which met in Copenhagen for two days (19-20 March) prior to the meeting of the WGPDMO.

An overview was provided of the progress made with regard to the data submission to ICES and the statistical analysis of disease prevalence data submitted intersessionally. The WGPDMO greatly appreciated the new ICES Fish Disease Data Entry Program (FDE) developed during 1995, facilitating the incorporation of disease data into the ICES Environmental Databank, and congratulated ICES on the high quality of the final product.

The WGPDMO members who submitted data, as agreed during the 1995 meeting of the WGPDMO (see report of the WGPDMO, ICES C.M. 1995/F:3), considered the FDE a highly functional and user friendly computer program, meeting all requirements identified by the Sub-group for the submission and subsequent statistical analysis of data.

Statistical analysis (as detailed in the report of the Sub-group, ICES C.M. 1996/F:3) carried out by the Sub-group was based on a preliminary set of data submitted by 4 ICES Member Countries containing information from 11 sampling stations in the North Sea and Irish Sea over a period of 5 years.

The WGPDMO noted with satisfaction that the FDE programme, as well as the standardised statistical procedures developed by the Sub-group, should enable a comprehensive analysis of fish disease prevalence data. The WGPDMO emphasised that, in order to undertake a full analysis, incorporating contaminant and other environmental data available in the ICES Environmental Databank, ICES Member Countries should be encouraged to submit historic and current fish disease data according to guidelines given in the Sub-group report. It was considered that any future comparative analyses of fish disease and contaminant data should be carried out utilizing advice from specialists in these fields. In this context, the need for strengthening the contacts between relevant ICES Working Groups was highlighted by the WGPDMO.

In response to queries regarding quality assurance of submitted fish disease data and data access restrictions, the WGPDMO confirmed that quality assurance is achieved by:

- the provision of supplementary information to ICES by the data originator with each data submission
- validation through ICES
- final cross-checking by return of a standardised summary table of submitted data from ICES to be confirmed by the data originator.

Clarifications of any data access restrictions will be provided by ICES.

The WGPDMO endorsed the view of the Sub-group that, since the data treatment procedures have been practically assessed and are now established, the statistical analysis of fish disease prevalence data should in the future form part of the regular work of the WGPDMO. Other tasks assigned to the Sub-group should also be transferred to the WGPDMO.

The WGPDMO congratulated the Chairman and members of the Sub-group on the successful outcome of their activities particularly taking into account the complexity of the tasks. The WGPDMO considered that a sound scientific basis for the collection and handling of fish disease data has now been firmly established.

7.1 Conclusions

The WGPDMO concluded that the introduction of the revised ICES Fish Disease Reporting Format, the Fish Disease Entry Program (FDE) and the standardised procedures developed for statistical analysis will allow the consistent input and analysis of fish disease prevalence data. Furthermore, it will allow correlation analyses with other data included in the ICES databanks, e.g. contaminants in sediments.

7.2 On-going tasks and recommendations

The WGPDMO endorsed the following on-going tasks and recommendations identified by the Sub-group and agreed to incorporate them in the future work of the WGPDMO.

Ongoing and New Tasks

1. Histological analysis of liver lesions collected during fish disease surveys (S. Feist).

2. Update information on age-length relationships for dab (T. Lang & S. Mellergaard).

3. Compile a list of relevant institutes and libraries which could be interested to receive information on ICES publications on fish diseases (S. Mellergaard).

4. Provide advice on statistical design for analysis of disease prevalence data, as well as on the choice of appropriate target species and disease conditions for an intended disease monitoring programme in the southern Gulf of St. Lawrence (A. D. Vethaak).

Recommendations

1. With the completion of the major parts of the Terms of Reference of the Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks, the Sub-Group recommends that future work in this field be incorporated back into the Terms of Reference of WGPDMO.

2. The Sub-Group recommends that ICES Member countries should submit current and historic fish disease data, using the ICES Fish Disease Entry Computer Program where possible, to ICES by December 31 1996 and annually thereafter at the end of each year.

3. The Sub-Group recommends to WGPDMO that an *ad hoc* two day intersessional meeting on Statistical Analysis of Fish Disease Data submitted to ICES to take place on 6 - 7 February 1997 to undertake analysis of the extended

fish disease data base and provide data suitable for interpretation by WGPDMO. S. Mellergaard, A. D. Vethaak, W. Wosniok and J. R. Larsen will participate.

4. The Sub-Group recommends that WGPDMO reviews the results of the ICES *Ad hoc* Meeting on "The use of liver pathology of flatfish for monitoring biological effects of contaminants" and assess progress on the implementation of standardised methodologies for the characterisation of liver pathology in disease monitoring programmes.

8 DISTINGUISHING BETWEEN DIFFERENT FINFISH VIRUSES

A full review of information on distinguishing between finfish viruses in order to evaluate the problem of possible mis-identification, was not available to the group because of lack of information. However, a useful summary statement outlining the nature of the problem was received (Annex 8).

The common naming of new viruses was discussed by the WGPDMO and it was recognised as a problem especially when the virus is named after a host or after disease symptoms, both of which may become inappropriate with developing knowledge.

The main problem is currently associated with the specific identification and use of the common name Viral Hemorrhagic Septicemia Virus (VHSV). However, a similar problem may also be encountered with other fish pathogenic viruses. VHSV isolates are anti-genetically indistinguishable, and to date, there are no genetic methods to distinguish freshwater isolates from North Sea isolates. However, the marine isolates do not cause hemorrhagic septicimia in either herring or cod. Methods based on nucleotide sequence analysis of the genomes are clearly needed to determine whether or not they are the same virus.

The WGPDMO is aware that this issue is currently being addressed by several specialised scientific groups and that more information should become available in 2-3 years time. As a consequence of this, and based on advice from expert groups, it was concluded that further work on this matter should not be undertaken by WGPDMO until sufficient new information becomes available.

8.1 Conclusions

Problems in identifying and assigning common names of fish viruses exist at two levels:

a) through the use of common names based on host or pathological features, which may change as scientific knowledge advances.

The WGPDMO emphasise that researchers naming viruses (and other infectious agents) should avoid using such common names and base the naming of the pathogen on a taxonomical stable feature of the organism. The formal naming of viruses should follow internationally agreed rules and the description of the disease should be used only for naming the disease.

b) through the inability of existing fish viral diagnostic technology to distinguish between strains of virus showing different infectivity, pathogenicity, distribution and genetic stability.

9 PROGRESS TO DATE ON DEVELOPMENT OF RESISTANCE IN OYSTERS TO COMMERCIALLY SIGNIFICANT DISEASES

The WGPDMO group discussed progress on the development of resistance to commercially significant diseases in American oysters (*Crassostrea virginica*), European oysters (*Ostrea edulis*) and Pacific oysters (*Crassostrea gigas*). It was concluded that resistance, in the classic sense (see definitions in Annex 9a), has not been demonstrated for any oyster disease to date, however, there are clear cases of the development of tolerance to significant infectious agents, e.g., *Haplosporidium nelsoni* and *Bonamia ostreae*. Only one case has demonstrated complete disappearance of clinical infections, namely, Malpeque disease of *C. virginica*. Information on oyster tolerance of commercially significant disease agents was investigated to determine which factors may be inhibiting or enhancing the development of tolerance to these agents (Annex 9a).

Conclusions

Naturally developed tolerance has been demonstrated by *C. virginica* to the causative agent of Malpeque disease (unknown aetiology), *H. nelsoni* (cause of MSX disease) and, possibly, *Perkinsus marinus* (causative agent of Dermo disease). There is also evidence of natural development of tolerance by *O. edulis* to *B. ostreae*. In the latter case, stocks from the Northeast Pacific showed 26% mortality compared with 99% in naive populations. In The Netherlands, outbreaks of bonamiasis in 1988 decimated over 90% of oysters in Lake Grevelingen. Surveys of surviving oysters demonstrated a parallel decline in prevalence of *B. ostreae* infection (Annex 9b). Since oyster populations are beginning to build up again, it is important to determine if the decline in *B. ostreae* infection is due to acquired tolerance by the oyster, or inhibition of parasite proliferation by low host densities.

Selective breeding is commonly used to accelerate production of oysters tolerant to specific infectious pathogens. This method of selection and/or over selection (see definition in Annex 9a) has led to inbreeding in certain stocks. This was particularly notable in *C. virginica* stock selected for tolerance to *H. nelsoni* infection, which showed enhanced susceptibility to *P. marinus*. The pathogen-specific tolerance trait may have been induced by inbreeding or by selection of a defence mechanism unsuited for control of a different pathogenic agent. Regular outbreeding with broodstock that have developed a natural tolerance to one or more infectious agents is, therefore, advisable. Areas within epizootic zones, which are closed to exploitation, allow natural tolerance to build up and can be used for outbreeding, as well as production of seedstock for local use.

Tolerant oysters frequently show no evidence of infection. Thus, they have the potential to be healthy carriers of infectious agents. Subclinical infections have been linked to the spread of *B. ostreae* throughout both coasts of the USA, as well as to the spread of MSX from Chesapeake Bay to Delaware and Maine on the east coast of the USA. Thus, selectively-bred stocks should be restricted to enzootic areas and not transferred to non-endemic areas.

Recommendations

The WGPDMO recommends:

a) that ICES member countries are encouraged to undertake studies to investigate persistence of *B. ostreae* in low densities of European oysters and refer their results to the WGPDMO for evaluation;

b) that assessment of the risk of disease transfer via movements of stocks which are tolerant to the agents responsible for significant diseases be reviewed by the WGITMO.

10 EVALUATION OF CROSS REACTIVITY BETWEEN A DNA PROBE AGAINST *HAPLOSPORIDIUM NELSONI* (MSX) AND A *HAPLOSPORIDIUM* SP. IN PACIFIC OYSTERS (*CRASSOSTREA GIGAS*)

Several reports cite a morphological similarity between *Haplosporidium nelsoni* (MSX) in the American oyster, *Crassostrea virginica*, and a *Haplosporidium* sp. found at low prevalences in the Pacific oyster, *C. gigas*, in Korea, Japan, the Pacific US and France. However, MSX infection has not been found in *C. gigas* during laboratory and field trials in enzootic waters. The similarity between the agent of MSX disease and the *Haplosporidium* sp. in Pacific oysters required clarification to:

i) prevent accidental transfer of *Haplosporidium nelsoni* with species other than *C. virginica* and
ii) determine if *C. gigas* could be the historic origin of MSX (*H. nelsoni*) in eastern US oysters

Recent development of DNA probes for *H. nelsoni* (Carolyn Friedman, Bodega Marine Laboratory, University of California at Davis and Nancy Stokes, Virginia Institute of Marine Science) has allowed detailed examination of the parasite in *C. gigas* described as *Haplopsoridium* sp. These probes cross-reacted with the *C. gigas* haplosporidian. PCR amplification, using primers derived from small subunit ribosomal DNA extracted from *H. nelsoni* plasmodia, showed no cross reactivity with *Haplosporidium louisiana* from mud crab (*Panopeus* spp.), *H costale* (SSO) from *C. virginica* or other haplosporidians and oyster parasites.

10.1 Conclusions:

a) There is evidence that *Haplosporidium* sp. reported in *Crassostrea gigas* is recognised as *Haplosoridium nelsoni* or a closely-related species.

b) Transmission experiments with infected *C. gigas* and healthy *C. virginica* are required to confirm that *C. gigas Haplosporidium* sp. is *H. nelsoni* and that *C. gigas* can carry infective *H. nelsoni.*

c) Although there is increasing evidence that *C. gigas* is infected by *H. nelsoni* or a closely-related haplosporidian, there is a continuing need to determine the potential carrier status of Pacific oyster for MSX disease.

10.2 Recommendation

The WGPDMO recommends that the protocol defined in the 1995 Report of the WGPDMO (ICES C.M.1995/F:3 - Section 12.3: Implication for Disease Management) should be used by appropriate laboratories to define the risk of transmission of *Haplosporidium nelsoni* to *C. virginica* by *C. gigas.*

11 GEOGRAPHIC DISTRIBUTION OF JUVENILE OYSTER DISEASE (JOD) AND STATUS OF *CRASSOSTREA VIRGINICA* STOCKS IN ICES MEMBER COUNTRIES

Since the appearance of Juvenile Oyster Disease (Syndrome) in 1988, in nursery-held *Crassostrea virginica* from Maine, the problem has spread to Virginia and Maryland. Concern has been raised over potential spread north to Canadian oyster stocks or elsewhere via healthy carriers (normal or abnormal hosts). This concern was reinforced last year by the report that *C. virginica* spat originating from broodstock imported to France from the UK demonstrated pathology similar to JOD (Annex 11).

Review of the information available on the current range of JOD revealed no extension since 1994. In addition, the causative agent(s) have still to be conclusively identified. Results from transmission experiments conducted to date are given in Section 12.

11.1 Conclusions

Until the causative agent is identified, an accurate geographic distribution of this disease or syndrome cannot be determined. Neither can identification of healthy carrier shellfish.

11.2 Recommendation

The WGPDMO recommends that ICES member countries are encouraged to pursue studies in order to define the cause(s) of the JOD syndrome.

12 DETERMINATION OF TRANSMISSION POTENTIAL OF *BONAMIA OSTREAE* TO AMERICAN OYSTERS (*CRASSOSTREA VIRGINICA*) AND JUVENILE OYSTER DISEASE (JOD) TO EUROPEAN AND PACIFIC OYSTERS (*OSTREA EDULIS* AND *CRASSOSTREA GIGAS* RESPECTIVELY) TO CLARIFY THE CARRIER POTENTIAL OF SPECIES RELATED TO THE PRIMARY HOSTS

The WGPDMO undertook an investigation of multi-host-specificity of significant pathogens of bivalves, due to reports demonstrating transmission of *Mikrocytos mackini* to several oyster species. The review concentrated on *Bonamia ostreae* and Juvenile Oyster Disease (JOD), but *Marteilia* spp. and herpesvirus studies were also included, since these are emerging as areas of concern with respect to identification and host-specificity.

Following 15 years of histological observations and transmission experiments, no evidence has been found which indicates that *Crassostrea gigas* can be infected by *B. ostreae*. In addition, movement of *C. gigas* throughout the EEC, since the ratification of EC directive #EEC/93/169, has given no evidence of spread of *B. ostreae* with unscreened *C. gigas*. No *Bonamia*-like parasites have been observed in American oyster (*C. virginica*) in areas enzootic for *B. ostreae* in the USA (Maine) or in France. However, *Bonamia*-like parasites have been detected in *C. rivularis* and *C. sikamea* held in quarantine (as per the ICES Code of Practice for the Introductions and Transfers of Aquatic Organisms) at IFREMER's La Tremblade laboratory, several months after introduction from the USA (Annex 12).

The transmission potential of *Marteilia* spp. to various bivalve species is also under investigation, due to the observation of *Marteilia* spp. in several molluscan species (*O. edulis*, *C. virginica*, *C. gigas*, *Mytilus edulis* and *M. galloprovincialis*) (Annex 12).

JOD has been successfully transmitted to healthy *C. virginica*, but no transmission experiments have yet been done with other oyster species (Annex 12).

In France, herpesvirus infections have been observed in both *C. gigas* and *O. edulis* and were associated with mortality. However, the viral aetiology of the spat mortalities has not yet been proven (Annex 12).

12.1 Conclusions

There is evidence that the Pacific oyster *C. gigas* is refractory to *Bonamia ostreae*. However, the detection of *Bonamia*-like parasites in *C. rivularis* and *C. sikamea* requires further investigation, especially, parasite identification and transmission experiments.

Marteilia spp. constitute a complicated transmission problem, since species identification (especially of early plasmodial stages) is not possible morphologically or ultrastructurally. The need for species-specific diagnostic tools is urgently needed to differentiate pathogenic from non-pathogenic species.

Transmission experiments with JOD have yet been conducted with other oyster species (*C. gigas*, *O. edulis*).

The occurrence of herpesvirus and associated mortalities in three of the most commercially important species of oysters (*C. gigas*, *O. edulis* and *C. virginica*), worldwide, urgently indicates a need for host-specificity investigation. The established assumption, that herpesvirus infections are husbandry-related, requires clarification in light of pathogenic infections in open-water oyster stocks. Moreover, the relationship between herpesvirus detection and mortality should be investigated using new sensitive biomolecular tools. Transmission from diseased to healthy oysters also needs to be performed to confirm the pathogenicity of the virus in spat.

12.2 Recommendation

The WGPDMO recommends that the protocol defined in the 1995 Report of the WGPDMO (ICES C.M. 1995/F:3–12.3 Implication for Disease Management) should be used by appropriate laboratories to define the risk of bivalve pathogen transmission by alternate hosts, including the development and the use of available pathogen-specific diagnostic tools.

13 VALUATE THE DRAFT FORMAT PRO-POSED FOR COMPILATION OF A REGISTRY OF MOLLUSCAN DISEASE DATA AND COMPILE THE COMMENTS OF ICES HEADQUARTERS AND MEMBER COUNTRIES

The WGPDMO members examined the ICES Fish Diseases Data Entry Program (FDE) and the draft format in Annex 13b of the 1995 Report of WGPDMO (ICES C.M. 1995/F:3) and believe that the FDE Program has the potential to be useful for the compilation of shellfish data. The Program has a format similar to that developed by IFREMER for its shellfish disease information. Unfortunately, a detailed examination of the FDE was not possible in the time available due to the complexity of the task.

With respect to annual reports of shellfish disease data, WGPDMO members felt it would be useful to have country maps attached to the draft form (or National Report Text) to indicate pathogen distribution (1 map for multiple pathogen reports, possibly 1 map per shellfish species).

13.1 Ongoing tasks

WGPDMO members circulated a modified draft form (see Annex 13) for use on reporting National Shellfish Information for the 96/97 meetings of the WGPDMO and will collect comments with respect to the format. The data collected using this form will be used to assess the capability of the FDE Program to meet the requirements of an international data bank for shellfish diseases data.

13.2 Conclusions:

Standardisation of shellfish disease data recording is urgently required to monitor disease trends.

However, it appears absolutely necessary to first:

i) determine the precise and specific needs and objectives of shellfish pathology laboratories concerning disease data recording;

ii) determine if these objectives are compatible with existing programmes developed for ICES FDE

iii) if there is compatibility, ensure that modifications required for specific shellfish disease agents could be made in the ICES FDE Program.

13.3 Recommendation

The WGPDMO recommends that ICES member countries take into account the WGPDMO conclusions in developing shellfish disease databanks, use the draft ICES Shellfish Disease Reporting form when submitting annual reports of shellfish disease data and submit pathogen distribution maps for their countries.

14 NEW INFORMATION ON ANTIBIOTIC SENSITIVITY OF FISH PATHOGENIC BACTERIA

At the 1995 WGPDMO meeting discrepancies were noted between the results of antibiotic sensitivity tests obtained during intersessional work. Since an *ad hoc* workshop was to be held at the 1995 EAFP International Conference, it was proposed to inform the EAFP Council of this concern. The results of discussions on this issue at the EAFP Conference would be reported back to the WGPDMO by members able to participate in the *ad hoc* workshop.

The EAFP workshop, organised by Dr. D. Alderman, was held on the 14th September, 1995, and established a working group (chaired by Dr. P. Smith) to continue necessary investigations with the following objectives (annex 14):

i) examine both disc diffusion and quantitative MIC tests,

ii) establish use of *Aeromonas salmonicida* and *A. hydrophila* as model organisms,

iii) use amoxycillin, oxytetracyclin, potentiated sulphonamide preparations and quinolones as chemotherapeutant models.

It was also considered that development of standard methodologies will require four stages:

Stage 1 Identification of significant parameters for each method.

Stage 2 Determination of suitable values for each parameter identified, leading to formulation of provisional standard methods.

Stage 3 Evaluation and validation of provisional standard methods.

Stage 4 Presentation of the proposed standard methods to EAFP.

Stage 1 is presently being achieved by gathering and comparing lists of significant parameters, e.g., media composition, incubation temperature, incubation time, etc..

Stage 2 must be completed at a meeting to be held in Weymouth in September 1996.

Stage 3 and 4 should be reached in time for the EAFP VII Conference, for September 1997.

14.1 Conclusions

The WGPDMO noted with satisfaction that the concern previously expressed have been taken up by the EAFP Working Group on the Sensitivity Testing of Fish Associated Bacteria and look forward to seeing results which will be presented at the next EAFP Conference in Edinburgh, 1997. With the application of the expertise available in the EAFP working group, WGPDMO members feel that no further action by WGPDMO is required in this area.

15 NEW INFORMATION ON M-74 AND ICHTHYOPHONUS

15.1 OVERVIEW OF NEW INFORMATION ON THE M-74 SYNDROME

15.1.1 Available data.

A clear explanation of the etiological background of the M-74 problem in Baltic salmon has not yet been given. The percentage of female salmon giving offspring with M-74 which peaked in 1993 at between 80 and 90% has shown a decreasing trend in 1994 and 1995 to a current level of approximately 55%. This may not reflect the true picture as there has been an increased selection on which brood fish are used by farmers.

In November 1995, a second Workshop on Reproductive Disturbances in Fish was held in Sweden where the following topics were dealt with:

- the Early Mortality Syndrome (EMS) and Swim-Up Syndrome (SUS) in North American salmonids.
- the M-74 Syndrome in Baltic salmon.
- recruitment problems in the Baltic cod stock.
- reproduction problems in other Baltic fish species.

The summary Report of this Workshop is included as Annex 15a. During the workshop it was recognised that the EMS and SUS in Pacific salmon, the Cayuga syndrome and M-74 in Atlantic salmon demonstrated similarities as all were:

- thiamine deficiency-related neurological syndromes
- female dependent
- responsive to thiamine treatment
- associated with food webs based on clupeoid fishes.

It was recognised that basic knowledge on the dynamics of thiamine in the aquatic ecosystem is very limited. Information from both Sweden and Finland suggested that M-74 like problems have now been observed in sea trout (Salmo trutta).

Data currently available from other Baltic fish species e.g. cod (*Gadus morhua*), roach (*Rutilus rutilus*) and eelpout (Zoarces viviparus) suffering from recruitment difficulties did not support the hypothesis that M-74 like problems were involved in these.

15.1.2 Conclusions

Dietary factors due to changes in the ecosystems or food webs seem to remain one of the most important factors behind several reproductive disturbances of fish (EMS, SUS, M-74, Cayuga syndrome). These syndromes may reflect the end point of a complex interaction between dietary components and environmental contaminants. Research in this field is still continuing in Sweden, Finland, USA and Canada.

It was recognised that M-74 like problems have recently been observed in Atlantic salmon in Galicia, Spain. From other countries, it was reported that wild salmon stocks have shown a decreasing trend in recent years and the possibility that similar reproductive disturbances to M-74 may be involved should not be excluded.

15.1.3 Recommendation

It was recommended that ICES Member countries should actively consider whether reproductive disorders similar to M-74 occur in their local wild salmon stocks.

15.2 Overview of new information on ichthyophonus

Working papers detailing results of sampling herring *Clupea harengus* for *Ichthyophonus* were submitted by Iceland, Norway and Scotland (Annexes 15b, 15c, 15d). Papers on this topic produced by Norway for the ICES Northern Pelagic WG of 1995 were also available for consultation. Additional information was contained in the national reports on trends in marine fish diseases submitted by several countries.

15.2.1 Distribution and prevalence

The declining trend in the prevalence of infection in the Norwegian spring spawning herring stock continued, with a sharp decrease between samples taken in December and January similar to that reported in previous years. *Ichthyophonus* infection has been mainly confined to the 1986-87 year classes with no indication of increasing prevalence of infection in younger year classes. Only low numbers of infected fish have been found during surveys. In North Sea stocks only a single diseased herring, caught south west of Norway (ICES Statistical Rectangle 43F6), was detected.

In Icelandic waters, very low levels of *Ichthyophonus* infection occurred in samples of Icelandic summer spawning herring stock. Higher levels of infection were found in Icelandic samples of Atlanto-Scandic herring with a total prevalence of 3.15% being considered to give a true reflection of the level of infection.

Low levels of infection were again detected in Scottish research vessel and commercial samples of herring from ICES areas IVa, IVb, particularly east of Shetland. Again no infection was found in samples taken west of Scotland (area VIa).In contrast to the Norwegian spring spawning stock, infection occurred in most size groups with 2 and 3 year old fish showing signs of infection.

German sampling for fish quality assessment studies also showed a low level of *Ichthyophonus* (1.1% prevalence) between Shetland and Norway and an observation for the first time of some infected fish (0.3% prevalence) in herring samples taken in the Celtic Sea / western English Channel. Dutch sampling in the southern North Sea continued to show no trace of infection in that area. The Estonian report noted the occurrence of *Ichthyophonus* infection in 3 large herring caught in the Gulf of Riga but there was no indication of infection in studies of smaller fish.

15.2.2 Diagnosis

The greatly different levels of infection found in the same herring stocks and a discrepancy in results obtained from similar samples of herring analysed by Russian and Norwegian scientists indicated a continued lack of compatibility of detection and diagnostic methods used by these two groups.

15.2.3 Conclusions

Ichthyophonus infection is still present in the following herring stocks: Iceland summer spawning, Norwegian Spring Spawning, Atlanto - Scandic between Iceland and Norway, Northern North Sea east of Shetland, Celtic Sea / western English Channel and in old fish in the Gulf of Riga. No infection was detected in the southern North Sea and west of Scotland. In all affected stocks the prevalence of infection was low, and there is no evidence of any epidemic. It was concluded that the disease is now present at only background levels.

16 EXAMINE FEASIBILITY OF, AND POTENTIAL CONTRIBUTIONS TO, AN ENVIRONMENTAL STATUS REPORT FOR THE ICES AREA ON AN ANNUAL BASIS, AND REPORT TO ACME BY THE END OF 1995

A brief report was given on the intention of the Environmental Status Report and the status of the discussion within ICES on its possible contents. According to its Term of Reference (ICES C. Res. 1995/2:29 l), the WGPDMO intersessionally considered the feasibility of the Environmental Status Report and the possible contributions of the WGPDMO to it. The conclusions and suggestions of the WGPDMO detailed in a letter from the Chairman to the ICES Environment Secretary (Annex 16) were endorsed by the WGPDMO.

17 REVIEW PROGRESS ON THE ICES SPECIAL MEETING ON THE USE OF LIVER PATHOLOGY OF FLATFISH FOR MONITORING BIOLOGICAL EFFECTS OF CONTAMINANTS

The co-convenors informed the WGPDMO on the preparations for the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants which will be held at the MAFF Fish Diseases Laboratory, Weymouth, UK, 22-25 October 1996 under the co-convenorship of S. W. Feist (UK) and T. Lang (Germany). Both the WGPDMO (represented by the co-convenors) and the WGBEC (represented by A. Köhler-Günther) are involved in the planning and organization of the meeting.

The co-convenors presented the preliminary programme (Annex 17) developed by the ICES-Sub-group on Statistical Analysis of Fish Disease Prevalence Data during their meeting prior to the WGPDMO meeting (Terms of Reference 2:30 d). The main objective of the Special Meeting is to bring together scientists involved in the field of biological effects monitoring using liver pathology, in order to intercalibrate methodologies in use, and to develop recommendations for the standardisation of sampling strategies as well as diagnosis and reporting of liver pathology of flatfish for monitoring purposes. It was pointed out that, although the main emphasis will be given to flatfish, other species will be considered as appropriate for comparative purposes.

The WGPDMO endorsed the proposals and the preliminary programme. It recognised that a close cooperation with WGBEC is highly desirable in order to make best use of expertise available in both WGs for a successful meeting. The WGPDMO appreciated the draft manuscript prepared by A. Köhler-Günther for the March meeting of the WGBEC. They considered it useful as basis for further discussion at the Special Meeting and for the development of final recommendations of methodologies for monitoring purposes to be established at the *ad hoc* meeting.

After noting the recommendations for quality assurance procedures for liver pathology contained within the draft WGBEC report (ICES, C.M1996/ENV:5), the WGPDMO drew attention to the established protocol already in place for the monitoring of fish diseases including gross liver pathology. It was considered that the Special Meeting will be the ideal forum to integrate these procedures and will, in addition, strengthen contact between the WGPDMO and WGBEC.

17.1 Conclusions

The WGPDMO is satisfied with the progress being made in the planning of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants and endorsed the steps already being taken by the co-convenors.

18 POSSIBLE WGPDMO CONTRIBUTION TO ICES/NASCO SYMPOSIUM ON POSSIBLE FARMED-WILD SALMON INTERACTION

The proposed ICES/NASCO Symposium on "Interactions between Salmon Culture and Wild Stocks of Atlantic Salmon: The Scientific and Management Issues" due to be held in Bath, UK on 17–22 April 1997. Mr A. F. Youngson, one of the Co-Convenors, approached A. H. McVicar as Chairman of WGPDMO to map out a session area on "Disease and Parasite Interactions" with a view to establishing what sort of material might be received and how the session might be given direction (Annex 18a). Due to the short response time required, it was not possible to obtain a co-ordinated response from WGPDMO Members, so a personal response by the Chairman was submitted. This was tabled to WGPDMO for discussion (Annex 18b).

The topic was relatively recently reviewed by WGPDMO (see WGPDMO 1994 Report) but it was considered that the continued concern remaining about possible interactions between wild and farmed salmon, together with new information continually being tabled, fully justified such a session on disease and parasite interactions. Although there are examples of farmed species other than Atlantic salmon possibly interacting with local wild fish (especially in the Mediterranean), the WGPDMO considered that the title of the proposed session restricted contributions to infectious diseases of Atlantic salmon. A recent paper by (T. Hastein & T. Lindstad (1991), (Aquaculture 98, 272–282) gave an overview of much of the topic area and may provide a useful guide to areas where the session could be particularly focused. The results of the Workshop on the Interactions between Salmon Lice and Salmonids to be held in Edinburgh, Scotland on 11 -15 November

1996 (Chairman A. H. McVicar) will be relevant to the ICES/NASCO Symposium and will have to be taken into account in the final planning of the latter.

Members of WGPDMO endorsed the comments made by A. H. McVicar to A. F. Youngson. Possible subject topics and an outline programme were considered. Potential contributors would be identified by A. H. McVicar and B. Hjeltnes and forwarded to A. Youngson on his request.

18.1 Recommendations

WGPDMO recommends to ICES ANACAT Committee that the proposed session on "Disease and Parasite Interactions" at the planned ICES/NASCO Symposium on "Interactions between Salmon Culture and Wild Stocks of Atlantic Salmon: The Scientific and Management Issues" should be arranged as follows:

1. Risk of introduction of new diseases.
2. Farmed fish as reservoirs of existing disease for wild fish and/or disease amplification in farms.
3. Wild fish as reservoirs of disease to farmed fish.
4. Disease risk reduction by control strategies in farms.

19 ICES SPECIAL TOPIC ON HYGIENE IN MARICULTURE

A plan for a Special Topic Committee Session entitled Hygiene in Mariculture for the 1996 Annual Science Conference was discussed by the WGPDMO. As noted in the Mariculture Committee (F) 1995 ICES Annual Report, this topic should be regarded as a proactive initiative towards improvements of husbandry measures in mariculture, which may, in the long term diminish negative environmental effects. The WGPDMO felt it would be extremely difficult to identify contributors, and for these to meet the ICES deadline of April 15th 1996 for submission of abstracts. It was therefore considered not possible to give adequate attention to this topic at present.

The following tentative structure for the Special Topic session was proposed:

a) Hygiene relating to husbandry of fish and shellfish for control of diseases within rearing facilities.

Topics which would be dealt under this heading could include:

* prevention of disease introduction;
* fallowing of sites;
* reduction in stressors to the stock;
* management techniques and disinfection of effluent water to reduce infection impacts.

b) Hygiene relating to the possible dissemination of disease arising from activities associated with farming in locations distant to the farm.

Topics which would be dealt under this heading could include:

* prevention of possible introduction and dissemination of new species and diseases in open water arising from fish and shellfish;
* prevention of spread of pathogens during slaughtering and processing;
* disposal of dead animals (which may be diseased);
* release of chemotherapeutants
* control of effluent from processing plants;
* well boat disinfection.

c) Hygiene relating to the possible introduction and dissemination of new species and diseases in open waters, arising from fish and shellfish movements.

The WGPDMO agreed that in the discussion of hygiene in finfish and shellfish culture, the aspect of risk to human health should be excluded.

Recommendations:

The WGPDMO recommends:

That this session should be postponed to the 1997 Annual Science Conference to be held in the USA and that S. McGladdery, Canada, H. Rosenthal, Germany and B. Hjeltnes, Norway, act as coordinators.

20 LEGISLATIVE CONTROL STATUS OF *GYRODACTYLUS SALARIS*

20.1 Notifiable status of *Gyrodactylus salaris*

The Term of Reference C.Res.1995:2:29 was raised, with input from NASCO, at the ICES Annual Science Conference in Aalborg, through the ANACAT Committee, as a consequence of concern about the spread and pathogenicity of *G. salaris* in wild Atlantic salmon (*Salmo sala*) in western Norwegian rivers. Particular emphasis was placed on the possibility of spread of the parasite to UK and areas where the parasite may not be present.

20.2 The current situation

The current status of *G. salaris* was summarised in working documents tabled by WGPDMO members (Annexes 20 a, 20 b). In EC legislation, *G. salaris* is included as a List III Disease, which currently permits the use of national control regulations. To date, there is no OIE status for *G. salaris*, however, members of the WGPDMO are aware that both organisations are actively reconsidering the list status of the parasite. Since these discussions are ongoing and have significant political implications, discussion was limited to the scientific aspects of the disease and its consequences.

There is scientific evidence for:

- the severity of the disease in wild Atlantic salmon in western Norway.
- the pathogenicity of the disease in other (Scottish) wild Atlantic salmon stocks
- areas of Europe (e.g. UK) where the disease may be absent
- the possible spread of the parasite with movement of farmed fish.

Evidence was also discussed on other salmonid fish species which may be infected, some with no apparent disease signs. Survival of the parasite for short periods on several non-salmonid fish, which may act as vectors also appears possible. Furthermore, it was suggested that no species of freshwater fish could be ruled out as vectors. Difficulties currently being encountered in the development of legislative control measures on the spread of *G. salaris* concern the lack of knowledge of the epidemiology of the disease, practical diagnosis of the species and the absence of data on the distribution of the parasite in many parts of Europe. The WGPDMO noted information on the forthcoming meeting on diagnosis of *G. salaris* to be held in Oulu, Finland in April 1996.

20.3 Conclusions

a) WGPDMO recognises the risk and the potentially serious consequences arising from the translocation of *G. salaris* to susceptible wild salmon stocks where the parasite may be absent.
b) Until a survey is completed on the actual distribution of the parasite in the natural range area of Atlantic salmon, WGPDMO recommends to ACFM that such measures as are available should be used to limit the risk of spread of the parasite into areas where there is currently some evidence of its absence.

21 ICES DISEASE PUBLICATIONS. DIAGNOSTIC FICHES UPDATE

The "ICES Training Guide" will be published in the ICES TIMES Series as no. 19 entitled "Common diseases and parasites in the North Atlantic: training guide for identification" and will be released during April 1996.

For the distribution of information on ICES publications on fish diseases each WG-member should compile a list of relevant institutes and libraries within their countries which might be interested in receiving such material and send it to S. Mellergaard before the end of April.

New proposed titles of ICES Disease Diagnostic Fiches (WGPDMO 1996)

Viral encephalopathy and retinopathy in cultured marine fish	F. Baudin-Laurencin & B. Hjeltnes
Monogeneans of Mediterranean farmed fish	F. Baudin-Lauencin
Turbot paramyxovirus	Juan Barja
Turbot paramijxo virus	Juan Barja

To facilitate the work of the editor of the fiches, Dr. G. Olivier, draft fiches should be submitted both as a hard copy and on a diskette (IBM or Mackintosh).

22 OTHER BUSINESS

22.1 ICES C. Res. 1995/2:57

The resolution on the proposed workshop on the Interactions between Salmon Lice and Salmonids was not included in the Terms of Reference for the 1996 WGPDMO. However, since the subject clearly falls within the topic area normally covered, this item was raised in this part of the agenda:

A Workshop on the Interactions between Salmon Lice and Salmonids (Chairman Dr A. McVicar, UK) will be held in Edinburgh, UK, 11 - 15 November 1996 to:

a) summarise available information on the interactions between salmon lice and salmonids;
b) assess the effects of salmon lice on salmonid stocks;
c) identify data deficiencies, research needs, and cooperative programmes required;
d) report to the ICES ANACAT Fish and Mariculture Committees.

Taking these objectives into account the WGPDMO suggested the following topics for inclusion in the Workshop:

1. A review of the problem
2. An assessment of the pathological impact on the host
3. Reservoirs of infection in wild and cultured stocks
4. Transmission of infection to new hosts

The number of participants at the Workshop would be limited in order to maintain the workshop ethos of the meeting. The Chairman indicated that he will contact expert members in relevant countries who might be able to participate.

22.2 Environmental factors

The role of environmental factors influencing disease outbreaks in intensive culture systems was discussed by the WGPDMO. It was felt that a review and evaluation of published studies is required for ICES to provide sound advice on potential problems/risks associated with the development of mariculture systems.

22.3 Risk Assessment

The WGPDMO discussed possible contributions to the debate on Risk Assessment regarding fish and shellfish diseases. It was stated that since the Office International des Epizooties (OIE) has a well established structure and protocols already in place for risk assessments, a detailed consideration on this aspect was not warranted. However, assessment of risks associated with individual diseases, such as included in this report, will continue to be considered by the WGPDMO.

23 ANALYSIS OF PROGRESS WITH TASKS

An analysis of progress of tasks as outlined in the Terms of Reference C. Res. 1995/2:29 is presented in Annex 23a and an indication given of intersessional work required (Annex 23b).

24 FUTURE ACTIVITIES OF THE WGPDMO

The future activities of the WGPDMO were discussed and it was agreed that there was sufficient work to require a meeting again in 1997. Proposals for topics to be considered, in the form of proposed terms of reference for 1997 which could be recommended to Council, were compiled (Annex 24).

24.1 Justification for Recommendations to Council

1. There are continued developments in the field of pathology and diseases of wild and cultured marine organisms, requiring specialist assessment and advice to ICES. To provide this, it is recommended that the WGPDMO should meet again during 1997. The proposal was made to meet in Rhode Island (USA). It was recognised that groups within ICES, meeting early in the year, require advice from the WGPDMO, it was therefore proposed that the meeting should be held during March 1997.

1.a.,1.b A watching brief should be maintained on new disease trends in wild and cultured marine animals which may have implications to wild fisheries, environmental assessment and mariculture.

1.c. Intersessional work on the selection and use of methods to analyse the newly extended ICES Fish Disease Database has been undertaken by a Study Group. The Results of this activity need to be interpreted, particularly in relation to temporal and spatial distributions of fish diseases.

1.d The Report and recommendations of the ICES Special Meeting on the "The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" should be reviewed and further action as appropriate will be considered.

1.e The objectives (listed below) of the Sea-going Workshop held in November/December 1994 were clearly related to long-term issues considered by the WGPDMO, and the results of the Workshop should, therefore, be reviewed by the WGPDMO:

* to provide scientific background data on the prevalence and spatial distribution of fish diseases and parasites in the Baltic Sea

* to intercalibrate methodologies used for sampling, diagnosis, reporting and analysis of disease data

* to assess, in the Baltic Sea, the applicability of the standardised methodologies recommended by ICES for fish disease surveys

* to enhance cooperation between Baltic Sea countries with regard to research and monitoring of fish diseases and parasites.

1.f ICES C. Res. 1993/2:23 m requested that the WGPDMO maintain an overview of the M-74 syndrome and the *Ichthyophonus* issue as part of its regular agenda.

1.g It is currently well known that a variety of contaminants, rather than inducing specific clinical disease symptoms, suppress the defence mechanisms of organisms rendering them more susceptible to disease. Sensitive immunological methods have been developed for monitoring effects of contaminants on specific and non-specific immune mechanisms. The possibility for applying these methods for detecting early changes in fish health parameters should be reviewed and assessed in relation to pathology and diseases of marine organisms.

1.h. A nodavirus-like agent associated with pathology was reported to cause mass mortality of larval and juvenile Atlantic halibut in Norway. This virus shows antigenic relationship to nodavirus reported from Japan and France. There is indication that the virus may have serious implications for farmed marine fish and it, therefore, deserves further attention.

1.i For several diseases, there is a lack of understanding of basic causal factors and, thereby, a lack of effective control measures. For some diseases the aetiology and pathogenesis are well established; however, alternative control measures are needed which do not have an impact on the environment.

1.j. In the light of the increasing evidence for the occurrence of non-host-specificity of important molluscan pathogens, there is, in an effective disease management, a need for an assessment of the risks associated with movements of shellfish species other than the normal host of a pathogen within its endemic range. For this purpose the protocol detailed in the 1995 report of the WGPDMO (ICES C.M. 1995/F:3) should be used by appropriated laboratories to assess the risk of bivalve pathogen transmission by alternative hosts, including the development and use of specific and sensitive detection techniques.

1.k. There is evidence for a decrease in the prevalence of *Bonamia ostreae* in some populations located in enzootic areas and, therefore, studies should be undertaken to determine whether reduced prevalences indicate tolerance or inhibition of parasite dissemination due to low host densities.

1.l. Standardisation of shellfish disease data recording is urgently required to accurately monitor disease trends. However, the first step is to identify specific needs of shellfish pathology laboratories recording disease data.
An assessment should then follow focusing on the compatibility of the shellfish disease data structure with the structure and format of the ICES fish disease databank.

1.m The Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks has completed its main task in developing the structure of an ICES Fish Disease Database and its has been recommended that the Sub-Group should be disbanded. However, due to the newness of the extended database, significant intersessional preparatory work is still required for the selection and sue of appropriate analytical methods. Because of the size and complexity of this work, it is proposed that a Study Group be established to meet for one year only. This meeting between selected WGPDMO members and ICES staff should take place prior to the next WG meeting to undertake analysis of the extended fish disease database and provide data suitable for interpretation by the WGPDMO.

25 APPROVAL OF RECOMMENDATIONS TO COUNCIL

The recommendations to ICES Council were discussed by the WGPDMO and approved.

26 APPROVAL OF DRAFT WGPDMO REPORT

A draft copy of the Report of the 1996 meeting of the WGPDMO was submitted to all WG members present before the end of the meeting and approved. These terms of reference where advice was specifically sought by other ICES bodies would be extracted with the relevant appendices and sent separately to ICES.

27 CLOSING OF THE MEETING

The meeting was closed by the Chairman at 1340 on 26 March 1996. Full appreciation was extended to the commitment and strong team spirit shown by the WG members in successfully completing such a heavy programme of work and at the same time, in maintaining such a high quality of critical discussion and expert evaluation. The Chairman also formally extended his appreciation, and that of the whole WGPDMO, to the ICES Secretariat for their major assistance in many ways during the course of the WG and particularly for the friendly and cheerful way they carried this out.

ANNEX 1

WHEN COMPLETE, PLEASE RETURN THIS LIST TO THE ICES SECRETARIAT

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

ICES Headquarters, 21 -26 March 1996

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ANNEX 3a

2:29 The **Working Group on Pathology and Diseases of Marine Organisms** (Chairman: Dr A. McVicar, UK) will meet at ICES Headquarters from 21-26 March 1996 to:

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

b) analyse national reports on new disease trends in mariculture for fish and shellfish;

c) evaluate the Sub-Group report on the analysis of wild fish disease prevalence data;

d) assemble current information on distinguishing between different finfish viruses in order to evaluate the problem of possible misidentification;

e) determine the progress to date on development of resistance in oysters to commercially significant diseases;

f) evaluate cross reactivity between a DNA probe against *Haplosporidium nelsoni* (MSX) and a *Haplosporidium sp.* in Pacific oysters (*Crassostrea gigas*);

g) determine the geographic distribution of Juvenile Oyster Disease (JOD) status of *C. virginica* stocks in various ICES Member Countries;

h) undertake or investigate ongoing transmission experiments for infectivity of *Bonamia ostreae* to American oysters (*Crassostrea virginica*) and JOD to Pacific and flat oysters, in order to clarify the carrier potential of these oyster species for these diseases;

i) evaluate the draft format proposed for compilation of a registry of molluscan disease data and compile the comments of ICES headquarters and member countries;

j) review progress made intersessionally with respect to antibiotic sensitivity on fish pathogenic bacteria;

k) maintain an overview of the M-74 syndrome and the *Ichthyophonus* issue as a part of its regular agenda and report to the Advisory Committee on Fishery Management and the Advisory Committee on the Marine Environment if new information becomes available (HELCOM 12);

l) examine the feasibility of, and potential contributions to, an Environmental Status Report for the ICES Area on an annual basis, and report to the Advisory Committee on the Marine Environment by the end of 1995;

m) evaluate progress in the organisation of the ICES Special Meeting on "The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants";

n) consider potential contributions to the 1997 ICES/NASCO Symposium on the "Interactions of Salmon Culture and Wild Stocks of Atlantic Salmon: The Scientific and Management Issues";

o) plan a Special Topic Committee Session entitled "Hygiene in Mariculture" for the 1996 Annual Science Conference;

p) advise on the Organisation Internationale Epizootic (OIE) and EU listing on the status of the parasite *Gyrodactylus salaris* and report to the Advisory Committee on Fishery Management.

ANNEX 3b

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Copenhagen 21 - 26 March 1996

AGENDA

1) Opening of the Meeting. Structure of the meeting.
2) ICES Statutory Meeting 1995; items of relevance to WGPDMO.
3) Terms of reference, adoption of agenda, selection of rapporteurs.
4) Other relevant reports for information.
5) Analysis of national reports on new diseases and disease trends in wild fish, crustaceans and molluscs.
6) Analysis of national reports on new diseases and disease trends in mariculture and provide advice on preventative control measures.
7) Evaluate the Sub-Group Report on the Statistical Analysis of Fish Disease Data.
8) Evaluate information on distinguishing between different finfish viruses.
9) Review data on disease resistance in oysters.
10) Review information on cross reactivity of a DNA probe against MSX and *Haplosporidium sp.*
11) Review information on the geographic distribution of JOD in *C virginica.*
12) Review of experimental data on transmission of *Bonamia* and JOD.
13) Evaluate a draft format for a registry of molluscan disease data.
14) Review new information on antibiotic sensitivity of fish pathogenic bacteria.
15) Overview new information on the M-74 syndrome and *Ichthyophonus*.
16) Possible WGPDMO contribution to an Environmental Status Report.
17) Review progress on ICES Special Meeting on flatfish liver pathology.
18) Possible WGPDMO contribution to ICES/NASCO Symposium on possible farmed-wild salmon interaction.
19) ICES ASC Special Topic on Hygiene in Mariculture.
20) Legislative control status of *Gyrodactylus salaris.*
21) ICES Disease Publications. Diagnostic fiches update.
22) Any other business.
23) Analysis of progress with tasks.
24) Future activity of WGPDMO.
25) Approval of recommendations.
26) Approval of draft WGPDMO report.
27) Closing of the meeting.

ANNEX 3c

RAPPORTEURS

Agenda items		Rapporteurs
1-4	Introd. etc	A McVicar
5	Wild fish disease trends Wild shellfish/crustaceans disease trends	T Lang, S Feist S McGladdery, P van Banning
6	Farmed fish disease trends Farmed shellfish/crustaceans disease trends	B Hjeltnes, G Bylund S McGladdery, P van Banning
7	Sub Group report	T Lang, S Mellergaard, S. Feist
8	Finfish virus naming	B Hjeltnes, I. Dalsgaard
9-13	Shellfish topics	S McGladdery, T Renault , + others
14	Antibiotic sensitivity	F Baudin-Laurencin, B Hjeltness
15	M74 *Ichthyophonus*	G Bylund, S Mellergaard B Hjeltnes, A McVicar
16	Environmental Status Report	A McVicar, T Lang
17	Liver Pathology Meeting	T Lang, S Feist
18	ICES/NASCO Symposium	B Hjeltnes, A McVicar
19	ASC Hygiene Topic - Fish Shellfish	B Hjeltnes, F Baudin-Laurencin T Renault, J Barja
20	*G salaris*	F Baudin-Laurencin, A McVicar
21	Publications	S Mellergaard
22	Other business	S Feist, S McGladdery
23	Progress with tasks	A McVicar
24	Future activity / recommendations	S Feist, S McGladdery
25-27	Approvals and closing	A McVicar

ANNEX 4

ICES Working Group on Biological Effects of Contaminants

Ostende, Belgium, 4-7 March 1996

Agenda Item 7
Review the Report of the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques and address the following issues that arise out of this:
ii) ***Develop a quality assurance programme for each of the following methods:***
Externally visible fish diseases (Thomas Lang)

State of the art of fish disease monitoring

Epidemiological studies on diseases and parasites of wild marine fish have a long tradition in ICES. For example, intensive regular North Sea monitoring programmes focussing on externally visible diseases of the common dab (*Limanda limanda*) already started at the end of the seventies. At the same time, first systematic investigations were initiated in the Baltic Sea with cod (*Gadus morhua*) and flounder (*Platichthys flesus*) as major target species. At present, the majority of countries bordering the North and Baltic Seas are carrying out regular fish disease surveys. However, some countries reduced or even stopped their programmes which has been regretted repeatedly by ICES.

When results of the early studies were discussed within ICES-bodies, it became apparent that there was a lack of intercalibration and standardization of methodologies applied and, therefore, results reported seemed not to be comparable. In the beginning of the eighties, the *ICES Working Group on Patholoy and Diseases of Marine Organisms* (WGPDMO) started first attempts to solve this problem. Since that time, three sea-going workshops (1984 North Sea, 1988 Kattegat, 1994 Baltic Sea) were held under the auspices of ICES in order to intercalibrate and standardize methodologies for fish disease surveys and to establish practical guidelines for an intergrated international programme to determine long-term trends in fish disease prevalence levels. The guidelines provided included recommendations on minimum sampling requirements, target fish species, types of diseases to be monitored and cut-off points thereof, sampling stations and areas and additional measurements (Dethlefsen *et al.* 1986, Anon. 1989, Lang *et al.* 1995). Most of the existing regular fish disease monitoring programmes are designed according to these guidelines.

From the beginning of wild fish disease studies, ICES member countries have been requested to submit their results to ICES on an annual basis. At first, paper formats were used for this purpose, now a (recently revised) fish disease format as well as a specific data entry programme are available facilitating compatibility with the other environmental data (for example contaminants in biota and sediments, hydrography) stored in the ICES Environmental Databank. The fish disease prevalence data submitted to ICES partly reach back until 1981 and represent an unique set of long-term data on biological community/population responses of marine organisms to environmental changes. The statistical analysis of these data in conjunction with the other environmental ICES-data is a tasks addressed by the *ICES Subgroup on Statistical Analysis of Fish Disease Prevalence Data in Marine*

Fish Stocks. At its forthcoming meeting (19-20 March 1996, Copenhagen), an evaluation of the revised disease data format and a preliminary analysis of the data are part of the agenda.

Other ICES-activities coordinated by the WGPDMO related to externally visible fish diseases were the ICES/IOC Bremerhaven Workshop on Biological Effects of Contaminants in The North Sea (Vethaak *et al.* 1992) and the publication of the "Training Guide for the Identification of Common Diseases and Parasites of Fish in the North Atlantic" (Bucke *et al.*, in press)

Apart from these official ICES-activities, scientists involved in fish diseases monitoring programmes in the North Sea always have been working closely together and studies carried out have been coordinated to a large extent. Due to the political changes in the Baltic Sea area and the activities of the Baltic Marine Biologists (BMB) Working Group 25 "Fish Diseases and Parasites in the Baltic Sea", the cooperation between scientists involved in fish diseases studies in the Baltic Sea has improved considerably during past years, as well.

In conclusion, it can be stated that methodologies used for studies on externally visible diseases of wild fish in the Oslo/Paris- and Helsinki-Convention areas are coordinated and standardized to a large extent on an international level. Standard operating procedures including sampling design, disease diagnosis and standard protocols for recording and reporting of fish diseases data have been developed and applied successfully. All methodologies have now been practically tested for a considerable period of time. Due to activities (e.g. sea-going workshops) organized by WGPDMO and BMB WG 25, inter-laboratory performance testing exercises regarding disease diagnosis have been carried out successfully. Therefore, disease data submitted to the ICES Environmental Databank by institutes actively participating in the above quality assurance procedures can be regarded to be of consistently high quality.

Objectives of fish disease monitoring

From the beginning of systematic surveys on the spatial distribution of diseases in wild marine fish, one of the main objectives of these studies has been to test whether changes in the prevalences of certain diseases beyond normal background levels could be used as biological indicator for an impact of anthropogenic pollutants released into the marine environment. Consequently, first North Sea studies mainly carried out by German and U.K. scientists were related to investigate effects of dumping activities (sewage sludge, industrial wastes) on the health status of fishes.

After long-term experience there is now a general consensus that in most cases a direct cause/effect-relationship between the exposure to contaminants and the occurrence of elevated disease prevalences is only hard to establish *in situ* due to the fact that most common diseases have a multifactorial instead of a mono-causal aetiology involving the impact of anthropogenic and/or natural variations of host, pathogen and environment characteristics. However, in some cases an impact of anthropogenic pollutants on prevalences of non-infectious externally visible disease could be demonstrated (for example effects of pulp mill effluents and other industrial discharges in the Baltic Sea) and there is increasing evidence that environmental changes such as oxygen deficiency may significantly affect the prevalence of infectious externally visible diseases.

Due to their responsiveness to environmental changes and their high ecological relevance (fish diseases may have an impact on growth, reproduction and survival in affected fish populations), it has been recommended repeatedly to include externally visible fish diseases as bioindicator in monitoring programmes on biological effects of contaminants. Studies on spatial and temporal aspects can provide generic information on population/community responses to environmental stressors including exposure to contaminants.

Since the outbreak of externally visible fish diseases (as well as liver tumours which are also included in most monitoring programmes) represents an endpoint of numerous biochemical and physiological changes affecting the homoeostasis of affected fish, they can be a useful bioindicator for chronic rather than for acute environmental stress and may, therefore, be a more appropriate integrative indicator for complex changes typically occurring under field conditions as compared to biomarkers for subtle early changes on subcellular or cellular level (EROD-activity, lysosomal stability etc.). The latter can be considered of higher toxicological value than externally visible fish diseases and may, due to their more rapid response, be more suitable for the monitoring of acute effects of point source contamination with environmental chemicals known to affect the biomarkers.

Further advantages of studies on fish diseases as bioindicators are:

- target diseases are, with a certain degree of training, easy to recognise
- a large number of fish can be screened within a short time, thus enabling sound statistical data analysis
- it is a cost-effective means for monitoring not requiring expensive and time-consuming laboratory analyses.

Ideally, for the monitoring of biological effects of contaminants on a larger scale, bioindicators measuring biological responses on different levels of organization (subcellular, cellular, tissue level, individual, population/community) should be combined in order to obtain a more comprehensive overview on anthropogenic effects in the marine environment. In order to obtain information on possible cause-effect relationships, chemical monitoring should be applied at the same time, preferably using samples obtained from the same individuals when contamination in biota is to be analysed.

QUALITY ASSURANCE PROGRAMME

Monitoring of externally visible fish diseases in the Oslo/Paris-Convention area should be carried out according to guidelines elaborated by the *ICES Working Group on Pathology and Diseases of Marine Organisms* (WGPDMO) and the *ICES Sub-group on Statistical Analysis of Fish Diseases Prevalence Data in Marine Fish Stocks*. For the Helsinki-Convention area (Baltic Sea), deliberations of the *Baltic Marine Biologist (BMB) Working Group 25 „Fish Diseases and Parasites in the Baltic Sea"* should be utilized in addition.

A. Approved methods

Methods applied in present monitoring programmes on externally visible fish disease and liver nodules/tumours carried out in the European ICES-area are established for a long period and are to a large extent according to guidelines given by ICES (Dethlefsen et al. 1986, Anon. 1989, Bucke et al. 1996). Since they have been standardized and intercalibrated repeatedly on an international level they can be considered as approved methods and standard operating procedures..

A summary of approved methods is given in the following. Some suggestions for modifications and additional standard procedures are included which are solely based on personal ideas of T. Lang and other individuals and need to be endorsed by the ICES WGPDMO before inclusion in any official programmes/publications. For identification, these suggestions are typed in italics.

During 22-25 October 1996, an ICES *Ad Hoc* Meeting on „The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" will be held in the MAFF Fish Diseases Laboratory, Weymouth, UK the major aim of which is to intercalibrate and standardise methodologies used and to provide guidelines for methods suitable for monitoring purposes. This meeting can be regarded as continuation of the ICES activities for quality assurance of fish diseases studies.

1. Fish Species

The fish species chosen for monitoring purposes should be selected because it is benthic, fairly static (outside the spawning period), abundant, and exhibits high prevalences of diseases which are easily recognised.

For the shallow waters of the North Sea (< 100 m) North Sea, the common dab (*Limanda limanda*) fulfils those specifities. For estuaries and coastal regions as well as the Baltic Sea, the flounder (*Platichthys flesus*) is suitable. The cod (*Gadus morhua*) was chosen as an additional species for disease monitoring. *However, this species is rare in the North Sea at present and, therefore can only be recommended for spatial and temporal fish disease monitoring in the western Baltic Sea where it is more abundant. If disease monitoring in the North Sea includes studies on prevalences and intensities of parasitic infestation, the whiting (Merlangius merlangus) may be suitable due to its availability and the presence of conspicuous externally visible parasites.*

2. Sampling strategy

2.1 Sampling gear

Sampling on a long-term basis should preferably be conducted using identical equipment (ship, gear etc.) to minimise sampling variability. *The use of identical fishing gears is also recommended since the type of gear used may influence the prevalence of diseases in a sample due to selective catching because of differences in the behaviour and catchability of diseased and healthy fish. For sampling of demersal fish in the open North Sea, the GOV-trawl (equipped with codend, mesh size 50 mm) is recommended, since it is a standard gear also used for internationally coordinated stock assessment programmes.*

2.2 Sampling sites

Selection of sampling sites should be done according to fish species availability, disease occurrence, and knowledge on pollution and fish stock movements (migration). *In addition, information on size and age distribution in the populations of the target fish species should be available (age/length* *keys). It should be avoided that sampling sites are mixing areas of different stocks of a certain species, since these stocks may differ in their genetic and behavioural features and their susceptibility to environmental stressors. Changes in the proportion of each stock represented in the samples may, thus, affect the disease prevalence (Lang et al. 1995).*

Sampling should be accurately positioned on a nominated latitude and longitude with all repeat hauls being within clearly defined limits, e.g. radius of 2-4 nautical miles. Sampling on a station should be based on multiple hauls, even in the presence of large numbers of fish. This is necessary to reduce sampling variation, i.e. haul-to-haul variation, the problem of patchiness, etc. Therefore, at least 2 hauls, but preferably 5 hauls per station should be aimed for.

2.3 Sampling frequency and season

Sampling should be conducted on a long-term basis, once a year within the same narrow time window (2 weeks to one month) or, if possible, at two periods to provide separate data from the summer and winter periods. The non-spawning period is recommended, since the spawning may be associated with considerable migrations of the fish between their feeding and spawning grounds (for example in the North Sea dab, *Limanda limanda*, see Damm et al. 1991, Rijnsdorp et al. 1992). Sampling during spawning time may, thus, not reflect the spatial distribution patterns of the diseases typical for most of the year. *Furthermore, sampling during the spawning period should be avoided, since the adverse effects of spawning stress on the health status may exceed the effects of any other environmental stressors the effects of which are to be monitored (Lang et al. 1995).*

2.4 Sample size and statistical analysis of fish diseases prevalence data

The minimum sample size of fish to be examined for diseases should be based on the statistical requirements of the specific monitoring program and might differ for spatial and temporal monitoring.

According to ICES-guidelines, it is recommended to examine 250 specimens per haul which allows the detection of a disease prevalence of at least 1.5 % with 95 % confidence limits. These specimens should be sorted out of total catches or subsamples and should belong to 3 size groups:

Dab (*Limanda limanda*)	15-19 cm	20-24 cm	≥ 25 cm	size group
	100	100	50	specimens
Flounder (*Platichthys flesus*)	20-24 cm	25-29 cm	≥ 30 cm	size group
	100	100	50	specimens
Cod (*Gadus morhua*)	< 29 cm	30-44 cm	≥ 45 cm	size group
	100	100	50	specimens

However, in many cases it will not be possible to sample a sufficient number of specimens per size group either due to low catches or due to a particular length-frequency distribution with small or large fish dominating. Furthermore, due to size stratification, the prevalences recorded do not necessarily represent the prevalence in the population and, therefore, data have to be interpolated.

Another disadvantage of the examination based only on length stratification is that the age of the fish, which may have a severe effect on the presence or absence of diseases, is not taken into account. Since the growth patterns of fish may vary considerably between different sampling sites it might be that fish of the same size from different sites differ significantly in age and, therefore, the probability for the occurrence of diseases may be different. This may possibly lead to misinterpretation of disease prevalence data. For this reason, information about the age structure of fish species monitored is essential for the assessment of results on spatial trends of disease prevalences.

It is felt that, for spatial and temporal monitoring purposes, the ICES-guidelines for sample design and sample size need to be reviewed and, if necessary, revised. This task should be addressed by the ICES Sub-group on Statistical Analysis of Fish Disease Prevalence Data in Marine Fish Stocks.

3. Diseases

3.1 Disease examination procedures

Diseases examination for spatial and temporal monitoring purposes should be carried out by trained experts following a strict protocol detailed below. If new staff has to be trained this should be done only by experts and results have to intercalibrated internally.

After each haul, fish species to be examined should be sorted, either from the total catch, or from representative subsamples. The sample weight should be measured and the length-frequency distribution should be recorded (total length to the nearest cm below) separately, for females and males. Measured fish should either be completely examined for diseases, or be sorted according to length into different size categories prior to examination.

The fish species selected for examination should be examined whilst fresh, e.g. shortly after they have been landed on the ship or taken from nets (not frozen or refrigerated). An area for working should be cleared, preferably a bench or table at standing height, with good lighting and running water.

Two people at least are needed for examining large number of fish. One person makes the examination and the other records (in pencil) onto special paper forms (to be developed) or directly onto a computer keyboard if direct data entry programmes are used. The positions should be interchangeable, so that both workers know what is seen and transcribed.

1. Take the fish, with bare hands (or wearing thin gloves), rinse it in clean water and, under a good light, examine it externally, and whether or not an anomaly is observed, give the total measurement of the fish and sex. Externally visible diseases (including those affecting the gills) quantified for monitoring purposes should be recorded according to the guidelines on type and severity of diseases given below.

2. For internal examination of flatfish for liver nodules, place the fish underside downwards, make an incision on the upper side with a sharp blade from the pectoral fin to the outer edge of the abdominal cavity. With the finger, pull out the intestine, and the liver will be clearly visible. Carefully dissect with a blade around any adhesions, and the liver will come free. Examine the liver on both sides. Any nodules > 2 mm in diameter should be recorded. *It is advised that all nodules should be examined histological in order to confirm macroscopic findings.* For confirmation of liver

nodules, the affected area, including some normal, *adjacent* tissue should be carefully dissected (up to 5 mm thick pieces only) and placed in a jar of 10 % neutral buffered formalin or Bouin's fluid for preservation and subsequent histological examination.

Note 1: It is advised to make the sample from the first haul a practise run, and not to count the results on the final reporting form. This should sort out any problems which may arise, especially for those not working at sea regularly. Eventually make an intercalibration of this sample if more than one person is to be involved in the disease diagnosis.

Note 2: It is advisable to complete the examination of each trawl haul before the next haul is landed. With hauls coming in near together, or of short duration, timing is critical. Additionally, most research ships work to strict timing, including meal breaks, therefore planning between the scientists and crew is necessary to keep the harmony sweet.

3.2 Diseases useful for monitoring purposes

Diseases used for spatial and temporal monitoring should be ones that

- occur commonly in the selected fish species
- are easy to recognise
- have a possible response to surrounding environmental conditions
- the response can be expressed in significant prevalence values.

For the host species given in 1.4, the following diseases and minimum requirements are recommended for monitoring purposes on an international level :

Host species	Disease	Minimum requirement for international reporting
Dab (*Limanda limanda*)	Lymphocystis Epidermal hyperplasia/papilloma Skin ulcer disease X-cell gill lesion Liver nodules	More than one surface nodule Lesions larger than 2 mm Open lesions (including acute and healing stages) one ore more filaments affected Larger than 2 mm in diameter
Flounder (*Platichthys flesus*)	Lymphocystis Skin ulcer disease Liver nodules Skeletal deformities *	see above see above see above Grossly visible
Cod (*Gadus morhua*)	Skin ulcer disease Skeletal deformities Pseudobranchial swelling (X-cell disease) *Cryptocotyle lingua* *Lernaeocera branchialis* *	see above Grossly visible or by filleting Grossly visible one or more cysts in the skin one or more parasite in the gill cavity

* not included in the former ICES-recommendations

4. Reporting and statistical analysis of disease data

Fish disease prevalence data obtained according to the ICES guidelines should be submitted to ICES on an annual basis using the ICES Reporting Format for Fish Disease Data (Version 2.2) which has recently been revised. The fish disease data are part of the ICES Environmental Databank which, at present, contains data on contaminants in seawater, sediments and biota and on fish disease prevalences. The structure of the environmental data facilitates compatibility of data and, therefore, joint statistical analysis.

For the submission of fish disease prevalence data, ICES provides on request the new ICES Fish Disease Data Entry Program (FDE 2.0) which allows the transformation of data into the ICES Reporting Format for Fish Disease Data.

Evaluation and statistical analysis of fish disease data submitted to the ICES Environmental Databank are carried out by the ICES Sub-group on Statistical Analysis of Fish Disease Prevalence Data in Marine Fish Stocks. For monitoring purposes, the Sub-group should provide guidelines on appropriate methods of statistical analysis of spatial and temporal diseases prevalence data as well as on factors with potential impact on diseases prevalences which should be included in the data analysis (e.g. age, length, sex etc.).

B. Nomination of lead laboratories

There is a number of laboratories in the European ICES area with a long-term experience in monitoring of diseases and parasites in wild marine fish according to methodologies elaborated by ICES. For the North Sea, these are for example

- *Federal Research Centre for Fisheries, Institute of Fishery Ecology, Cuxhaven, Germany*
- *MAFF Fish Diseases Laboratory, Weymouth, UK*
- *SOAFD Marine Laboratory, Aberdeen, UK*
- *Danish Inst. for Fisheries Research, Fish Disease Laboratory, Frederiksberg C, Denmark*
- *National Institute for Coastal and Marine Management, Middelburg, The Netherlands.*

If possible, these institutes should be involved in disease monitoring programmes and if international QA programmes will have to be established.

C. Inter-laboratory performance assessment

Inter-laboratory performance assessment for diagnosis of externally visible fish diseases has been carried out repeatedly during 4 sea-going workshops (North Sea 1984, 1990 Kattegat 1988, Baltic Sea 1991, 1994) organized by ICES and other co-sponsors (IOC, BMB). Therefore, a new assessment would only be necessary if institutes which had not been involved in earlier activities will be involved in future monitoring programmes.

The diagnosis of pathological liver changes will be intercalibrated between laboratories during the ICES *Ad Hoc* Meeting on „The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants", October 1996.

D. Conclusions

Except liver pathology, methodologies used for fish diseases surveys in the Oslo/Paris-Convention area are approved and have been intercalibrated between laboratories involved repeatedly. Data derived from fish diseases studies have already been reported to the ICES Environment Databank for a considerable time using standardized reporting formats. Standardized methods for analysis of disease prevalence data are in the process of being developed by the ICES Sub-group on Statistical Analysis of Fish Disease Prevalence Data in Marine Fish Stocks.

Therefore, fish disease studies according to the above guidelines are ready for application within the OSPAR Joint Monitoring and Assessment Programme (JAMP).

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ANNEX 8

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Identification and classification of fish viruses

Among the agenda items for the 1996 WGPDMO meeting is listed the task: "assemble current information on distinguishing between finfish viruses in order to evaluate the problem of possible misidentification.". The background for this is that practical and legal problems have been identified in current practices of identifying and naming fish viruses. Such problems are currently associated with viral hemorrhagic septicemia virus (VHSV) in the North Sea.

There are currently seven European marine isolates of VHSV from wild or farmed marine fish, including turbot and cod. Preliminary studies have indicated that these isolates are non-virulent, or of low pathogenicity for rainbow trout. There are also five marine VHSV isolates from Pacific fish species in North America. The Pacific VHSV isolates are also non-virulent for rainbow trout. The problem arises in whether the freshwater VHSV and the marine VHSV should be considered the same virus. The marine VHSV and the freshwater isolates are antigenically indistuingishable, and there are so far no genetic methods to specifically identify freshwater isolates or North Sea isolates of VHSV.

In serological tests the North American and European VHSV isolates are also indistinguishable. Based on nucleotide sequence analysis of parts of the genomes of the North American VHSV and the European VHSV it has been shown that the viruses share an about 80% sequence similarity. Based on genomic regions which are diverged between these viruses, it has been possible to develop a DNA probe assay (Batts et al., 1993), and a polymerase chain reaction (PCR) assay (Einer-Jensen et al., 1995) which both are able to distinguish between North American and European VHSV isolates.

Similar methods are clearly needed in order to distinguish between the European marine and freshwater VHSV isolates. Such methods cannot, however, be developed without enough knowlegde of the virus genomes on the nucleotide sequence level. Therefore, the genetic variation among the marine and freshwater VHSV isolates must be studied on the nucleotide sequence level in order to be able to detect genomic regions which are diverged between the marine and freshwater isolates. Once such diverged regions are identified, these can be used in developing specific

DNA probe, PCR, or monoclonal antibody based assays which are able to distinguish between marine and freshwater isolates of the virus.

Similar problems are also encountered with other fish pathogenic rhabdoviruses, such as spring viremia of carp virus (SVCV). So far there is no knowledge of the genetic variation of SVCV, and there are no methods for distinguishing between different SVCV isolates. This has caused some legal problems in theUK when the authorities have tried to find out the origin of the virus infection in certain fish stocks.

In Finland there has been a programme for testing freshwater and marine cultured salmon and rainbow trout extending more than ten years with no record of VHSV, IHNV or SVCV. A rhabdovirus was isolated from lake trout (Salmo trutta lacustris) in 1987 in Finland (Koski et al., 1992). Serological and biophysical characterization showed that the virus was a new, previously undescribed fish rhabdovirus and was named European lake trout rhabdovirus (ELTV) (Björklund et al.,1994). The same virus was isolated again from lake trout in 1995. We are currently working on cloning and sequencing parts of the viral genome in order to establish the relationship of ELTV with other fish rhabdoviruses, and in order to be able to develop molecular diagnostic tools for specifically identifying the virus.

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ANNEX 9a

PROGRESS TO DATE ON DEVELOPMENT OF RESISTANCE IN OYSTERS TO COMMERCIALLY SIGNIFICANT DISEASES

Definition

Resistance *- the classical definition states that the pathogen does not gain entry into a resistant host.*
Tolerance *- (often referred to as "resistance") is the ability to restrict infections and tolerate parasitism for prolonged periods.*
Overselection *- the selection of animals which have survived a natural infection as well as subsequent inoculation with concentrated pathogen.*

DISEASE CASE HISTORIES

A. *OSTREA EDULIS* TOLERANCE TO *BONAMIA OSTREAE*

a) Natural selection of disease tolerance

European oysters from a population exposed to *B. ostreae* since 1963 (20 years), off the west coast of the USA, showed 26% mortality compared with 99% mortality in a naive oyster stock. The surviving tolerant oysters had lesions indicative of chronic infection and possible degradation of the parasite within the tissues. This, along with variations in susceptibility to bonamiasis, provides strong evidence of inherited tolerance (Elston et al. 1987, Elston and Wilkinson 1988).

In The Netherlands a four year study (1991-1995) (attached document, Report prepared for WGPDMO by Paul van Banning) has shown a strong decrease in prevalence of *Bonamia ostreae*, oyster population density, and a change in oyster sex ratios, since the onset of epizootics in 1988 in Lake Grevelingen. The first strong year class of oysters since the *B. ostreae* outbreak was observed in 1995, and will be monitored this year to determine whether or not reduced prevalences of *B. ostreae* indicate "real" tolerance or low-host-density suppression of parasite proliferation.

b) Selective breeding

A programme of selection was developed at IFREMER's Laboratory at La Tremblade. This programme was based on selection of survivors and inoculation of purified *B. ostreae* into the pericardial cavity of successive generations (Hervio et al. 1995). Two strains from these breeding challenges were selected in 1985 and 1989. Significant improvements were observed in the first generation, which showed higher survival than naive oysters. In 1989, field observations over a 7 month period showed that F1 oysters produced from overselected broodstock had higher survival than susceptible controls (72-94% compared with 48-66%, respectively) but levels of infection in overselected F1 oysters were similar or greater than the controls, thus the net gain in tolerance was 49% in overselected oysters, compared with 32% in controls (Martin et al. 1993). A decrease in survival performance, partly attributed to inbreeding, was noted in the second generation. The third generation recovered, however, and a further improvement of the tolerance is linked to successful outbreeding (genetic load clearing). A manuscript is being prepared for publication of these results (Naciri-Graven et al., in preparation).

c) Other biomolecular studies

Significant differences were found in haemograms from selectively bred tolerant oysters and susceptible stocks at IFREMER, La Tremblade (Cochennec et al. 1995). The different haemocyte counts seem to correlate with tolerance. Increased agranular cell concentrations correlate with increased susceptibility to bonamiasis. This haemocyte research will be continued to confirm these observations and compare *O. edulis* from The Netherlands and Norway (and possibly Canada). Research on antibacterial peptides (including cecropins) at IFREMER-DRIM-Universite de Montpellier II, indicates lethality to *B. ostreae in vitro* (Morvan et al. 1995). *In vivo* results are not yet available.

B. *CRASSOSTREA VIRGINICA* TOLERANCE TO *HAPLOSPORIDIUM NELSONI* (MSX)

a) Natural selection of disease tolerance

Initial observations of oysters surviving MSX epizootics in the early 1980's suggested a natural development of disease tolerance. Sublethal infections, however, restrict gametogenesis in proportion to level of infection. Remission of infections prior to the next peak of infection, however, allowed some oysters to mature and spawn, thereby ensuring inheritance of the factors conferring MSX tolerance (Ford and Figueras 1988). MSX occurs in tolerant stocks but mortality is lower than in susceptible oysters. Naturally tolerant stocks were, therefore, selected for the accelerated breeding programmes (described below) as well as for restocking MSX epizootic areas (Matthiessen et al. 1990).

MSX takes a month longer to appear in tolerant oysters than susceptibles (August rather than July) and mortality in the latter reached 80% by September, but only 3% in tolerant oysters (Barber et al. 1991). Littlewood et al. (1992) found that survival of *C. virginica* in MSX enzootic areas was directly correlated to air exposure, however, intensity and prevalence of infection showed no distinct correlation.

b) Selective breeding

Preliminary success with selective breeding resulted in a 2-9 fold increase in survival of oysters. This led to wide use of selectively bred oysters on oyster grounds. Initial enthusiasm also suggested that disease-tolerant oysters would help the oyster industry (rapid growth, high meat yields and uniform quality) "whether or not a disease is present" (Haskin and Ford 1987, Ford and Haskin 1988). In addition, Vrijenhoek and Ford (1988) found no decrease in heterozygosity during preliminary selection work (5-6 generations), however, by the early 1990's Allen (1993) found that intense selection pressure and population "bottlenecks" had significantly reduced genetic variability. He also concluded that the lack of tolerance by the same oysters to Dermo (*Perkinus marinus*) may be due to genetic homogeneity compromising alternate defence mechanisms necessary to control alternate pathogens. Certainly, there was too little genetic variability to sub-select for multiple-disease tolerance (Allen 1994).

Haemolymph sampling over a 1-year period showed no evidence of a defensive role by serum lectins (which had been linked, tentatively, to MSX tolerance) against either MSX or *P. marinus* and differences in total protein were related to pathology rather than disease tolerance (i.e., no use for screening potentially tolerant individuals) (Chintala and Fisher 1989, Chintala et al. 1994).

c) Other biomolecular studies

Investigation of MSX tolerance in diploid and triploid oysters showed that triploids suffered less mortality than diploids. Condition index was also greater in triploids, even though prevalence of infection was greater than in diploids. The reason for this discrepancy between level of infection and mortality is not understood (Matthiessen 1992).

C. *CRASSOSTREA VIRGINICA* TOLERANCE TO *PERKINSUS MARINUS*

a) Natural selection of disease tolerance

Two groups of oysters produced from North Carolina broodstock during two different years performed better than several native Chesapeake Bay oyster stocks in high salinity, disease-prevalent areas. Infected North Carolinan oysters grew more vigorously and longer into the winter season than local Chesapeake oysters. The genetic or physiological traits of the North Carolina stock are being investigated to determine their potential for use in enhancing oyster production in the Chesapeake Bay region (Paynter 1994).

b) Selective breeding

See Section B.b) and Allen (1993, 1994).

c) Other biomolecular studies

Oysters infected with *P. marinus* showed increased reactive oxygen intermediate (ROI) production (NADPH oxidase and myeloperoxidase). The role of these biomolecular reactions in innate tolerance is still under investigation (Austin and Paynter 1994). Investigation of ROI activity in various bivalves by Anderson et al. (1994) showed that standard ROI-eliciting procedures were negative in soft-shell (*Mya arenaria*) and hard-shell clams (*Mercenaria mercenaria*) and weak in *C. virginica* and the mussel *Geukensia demissa.*

D. *CRASSOSTREA GIGAS* TOLERANCE TO *HAPLOSPORIDIUM NELSONI* AND *PERKINSUS MARINUS*

a) Laboratory trials

Flow through exposure of *C. gigas* to *P. marinus* resulted in 80% infection but only a single heavy infection. Growth of infected *C. gigas* was faster than that of control *C. virginica.* However, *C. gigas* suffered 76% mortality which was not attributed to *P. marinus* infection (Barber and Mann 1993). Lower Chesapeake Bay salinities can drop to 10.5-18.0 ppt., known to cause 90-99% mortality in *C. gigas* (Farley et al. 1991).

b) Field trials

Crassostrea virginica demonstrated 84-92% infection by MSX and 96-100% infection by *P. marinus*. *Crassostrea gigas* showed 0% MSX and 24% *P. marinus* (all of which were light compared with infection of *C. virginica*). Total mortalities of *C. gigas* (25%) were attributed to *Polydora* shell damage and environmental parameters, rather than the protistan infections (Burreson et al. 1994). These results concur with those obtained under laboratory conditions.

c) **Triploidy trials**

Triploidy provided no increased tolerance to *P. marinus* in either *C. gigas* or *C. virginica* controls. Both triploid and diploid *C. gigas* were more tolerant than *C. virginica* (Meyers et al. 1991). Attempts to boost disease tolerance via hybridisation of oysters was unsuccessful. All crosses between *C. gigas, C. virginica* and *C. rivularis* (diploid and triploid larvae) died after 8-10 days without showing any growth (Allen et al. 1993).

d) **Other biomolecular investigations**

Sami et al. (1991) compared host defense mechanisms in diploid and triploid *C. gigas* by comparing Concanavalin-A (Con-A) binding sites (surface receptors that indicate the extent of cell defence activation). Triploid oyster haemocytes have significantly more Con-A binding sites than diploid oysters (59.4 +/- 20.8% compared with 25.3 +/- 11.5%). This suggests a greater defensive capacity of haemocytes in triploids, but has yet to be demonstrated in response to MSX or Dermo pathogens.

E. *CRASSOSTREA VIRGINICA* TOLERANCE TO JUVENILE OYSTER DISEASE (JOD)

a) **Natural selection of disease tolerance**

There is no evidence, to date, of development of tolerance in wild populations of oysters. This may be due to the predominant use of hatchery-produced *C. virginica* seed for affected growing grounds, where there are negligible wild seed sources.

b) **Selective breeding**

Farley et al. (1996) found that F1 and F2 progeny, produced from JOD survivor broodstock, showed consistently higher survival (1-15% mortality compared to 43-72%) and lighter conchiolin deposits than naive oysters (5% compared to 40%) grown at the same sites. This seed is being used at endemic nursery sites.

F. *CRASSOSTREA VIRGINICA* TOLERANCE TO MALPEQUE DISEASE

a) **Natural selection of disease tolerance**

Following the initial epizootic of Malpeque disease in 1915, which wiped out 99% of *C. virginica* in Malpeque Bay, Prince Edward Island (PEI) (Canada), the bay was closed to fishing (stocks were too low to exploit commercially). By the early 1930's, the stocks had recovered and appeared healthy. They were moved around PEI and caused a second epizootic. This led to the conclusion that mortalities were due to a contagious disease agent (Needler and Logie 1947). By the mid-1950's, PEI oyster stocks had recovered and illegal transfers of apparently healthy animals, for processing in New Brunswick, led to the spread of the disease to the mainland. The New Brunswick oyster industry and government agencies chose to replace the devastated oyster stocks with tolerant PEI oysters and, thus, spread the disease to the remaining susceptible oysters in the southern Gulf of St. Lawrence. Since the 1960's there have been no outbreaks of Malpeque disease and all oysters are assumed to be tolerant (Drinnan and Medcof 1961).

Transfer experiments demonstrated that the F1 generation of oysters surviving the introduction of Malpeque disease showed disease tolerance. As the F1 generation matured and spawned, local and introduced tolerant stock bred to produce the homogeneous stocks now present in the southern Gulf.

Recent transfer experiments have demonstrated that Malpeque disease can still be transmitted from tolerant to susceptible oysters (surface cleaned prior to transfer from historically uninfected Cape Breton sites), despite no clinical signs of the disease since the early 1960's. This indicates that the agent responsible for the disease has remained viable in the tolerant oysters for over 25 years (McGladdery, unpublished data).

G. OTHER EXAMPLES

Kent et al. (1989) found that mussels (*Mytilus edulis*), suffering from haemic neoplasia, had significantly reduced phagocytic capability compared to healthy mussels. De-differentiation of haemocyte function, therefore, reduces the general defence (and feeding) capability of affected mussels.

Coustau et al. (1991) found that mussels with a predominantly *edulis* genome are more heavily infected by *Prosorhynchus squamatus* in parental and intergressed *M. edulis/galloprovincialis* mussel populations. This may be a source of natural selection against *M. edulis* (due to parasitic castration) and may favour extension of the range of *M. galloprovincialis*. In hybrid zones, recombinant genotypes are more susceptible than either parental genotype, thus it appears that parental genotype determines susceptibility.

II. GENETIC ENGINEERING - SHELLFISH

Cytolysins, antibacterial and other foreign-body recognition proteins have been identified in mussels, oysters and penaeid shrimps. The possibility for enhancement of production of these protein factors, as a way to reinforce disease tolerance, is being investigated via genetically engineered immunostimulation. Expression of reporter genes under the control of the HSP-70 promoter from *Drosophila* has been obtained, *in vivo*, in young embryos and, *in vitro*, in dissociated cells of *C. gigas*. A preliminary transfection has been achieved using microinjection, lipofection, electroporation and particle bombardment (Cadoret et al. 1994).

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ANNEX 9B

LONG-TERM CHARACTERISTICS OF THE OYSTER DISEASE BONAMIASIS IN THE EUROPEAN FLAT OYSTER (*OSTREA EDULIS*) POPULATION OF LAKE GREVELINGEN, THE NETHERLANDS.

Data for information and discussion on the topic "development of resistance in oysters" at the 1996 ICES WG PDMO meeting in Copenhagen, March 21-26.

Introduction.

An important part of the wild stock of the European oyster *Ostrea edulis* in the Netherlands is located in the salt water Lake Grevelingen. This lake is situated in the south of the Netherlands, and formed by the enclosure of the Grevelingen estuarium in 1971 by dikes. After this enclosure the composition of flora and fauna has been changed, but suprisingly, the European flat oyster *Ostrea edulis* showed to be able to maintain itself. Successful reproduction resulted even in an important stock for the Dutch oyster fishery.

In this isolated oyster population no oyster pathogens were present, as was concluded on the results of yearly histological monitoring of oyster diseases since 1980. The only observed infection problem is the shell disease by the fungus *Ostracoblabe implexa*, but this is considered as mainly a problem of the shell and not as a real pathogen for the oyster itself.

In 1988 the situation changed with the first presence of the protozoan oyster pathogen *Bonamia ostreae* in *O. edulis* in Lake Grevelingen. The pathogen was probably introduced in Lake Grevelingen with material transported with oyster fishing ships coming from the Oosterschelde estuarium, an other Dutch oyster area. This area , known as Yerseke Bank, was already bonamiasis infected since 1980 with oyster importations from France. Soon after the first observation of bonamiasis in the *O. edulis* stock of lake Grevelingen, the disease spread through the oyster population and high mortalities developed (prevalence levels up to 80 %). This resulted in a serious reduction of the stock and with this in a sharp decrease of oyster fishery landings (fig.1).

The situation of lake Grevelingen with an isolated and selfsustaining oyster stock under "pressure" of bonamiasis offered good possibilities for field studies on development of resistance to bonamiasis. Such resistance can be concluded out of changing epizootic characteristics of bonamiasis after several generations of oyster reproduced by the "survivor" oysters. However, such research needs long-term studies.

Research for bonamiasis resistance can be accelerated by using hatchery produced material (more generations within shorter time) and more defined genetical material (better oyster selection), as is carried out in France at the moment. Unfortunately, such research programme is not carried out in the Netherlands.

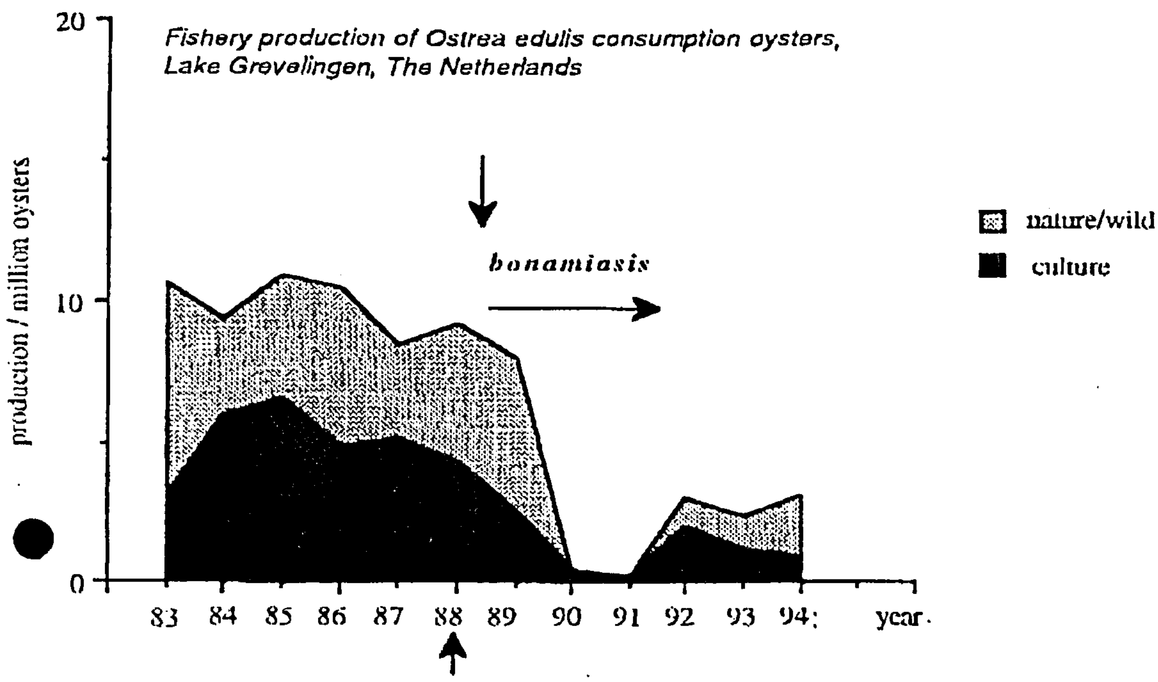
However, the unique situation of lake Grevelingen with an isolated *O. edulis* stock under bonamiasis circumstances without interference of other oyster pathogens, offers an interesting opportunity to study the development of any change (e.g. resistance) to bonamiasis under wild circumstances. For this purpose data on bonamiasis are collected over de period 1991-1995.

Materials and methods.

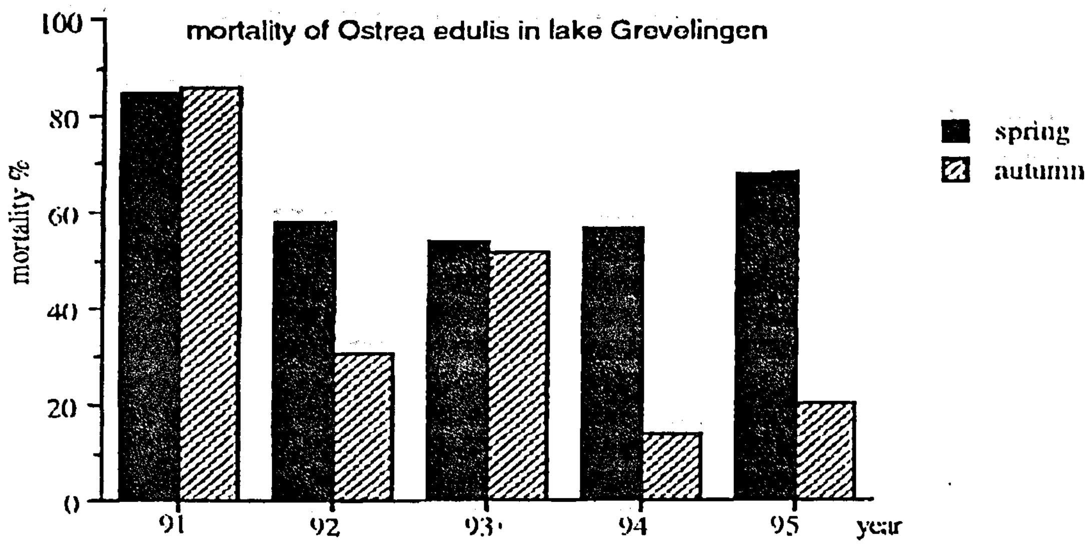
With the long-term (5 year) programme several sites (5-7) of the lake Grevelingen are sampled for oysters in spring (May) and autumn (September) season. With each season sampling (n: 83-149 oysters) the bonamiasis characteristics are estimated by histology in oysters older than 2 years. For the general biological information data are collected on mortality, sex ratio and presence of infiltration of neoplastic blood cells in the connective tissue of the oyster (as sign of infection/stress). The histological data on bonamiasis are used to express prevalence and the intensity of infection.

RESULTS

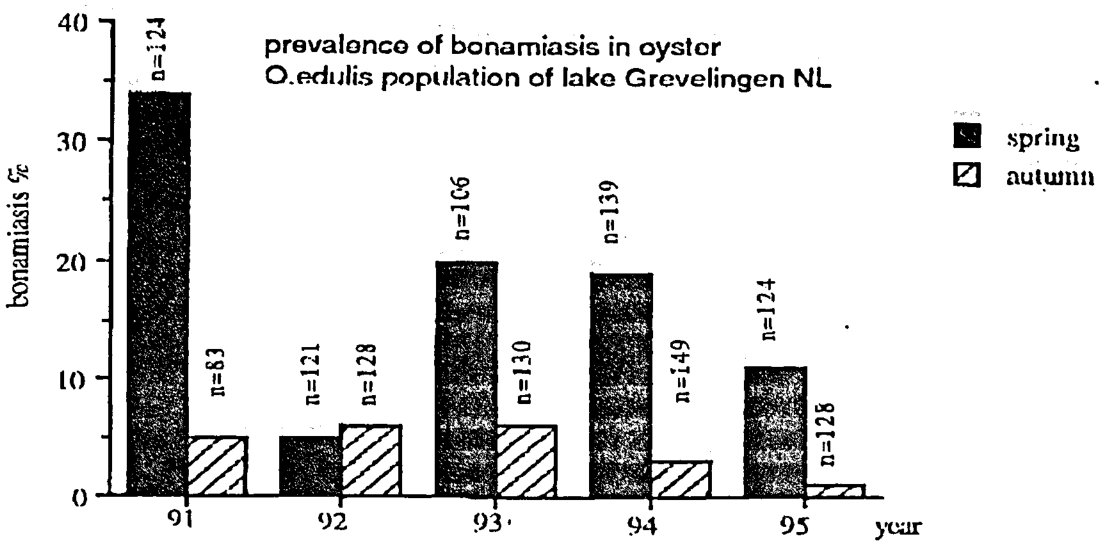
1. Impact of bonamiasis on *O. edulis* oyster fishery production, 1984-1994



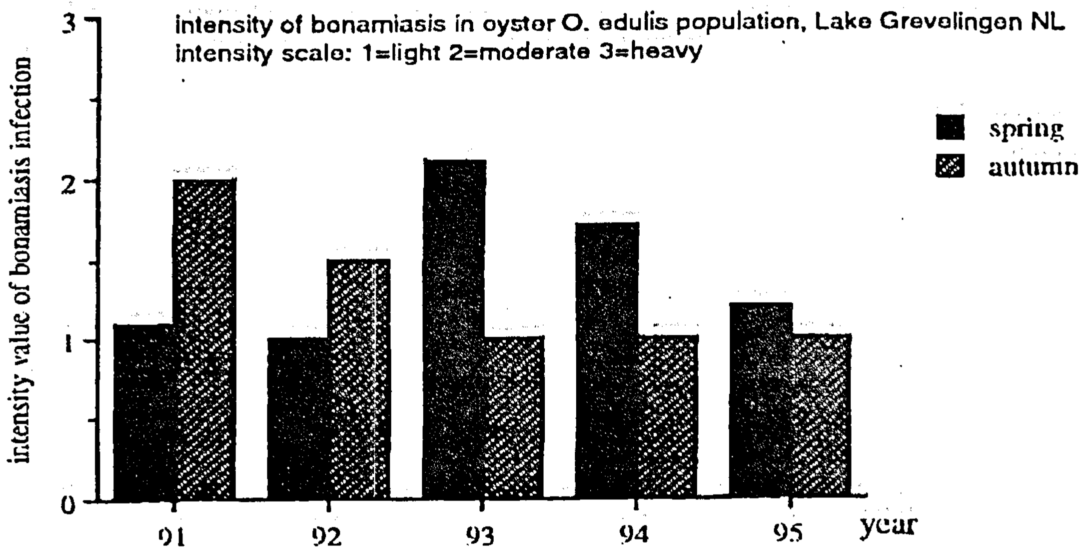
2. Mortality of *O. edulis*, lake Grevelingen, 1991-1995



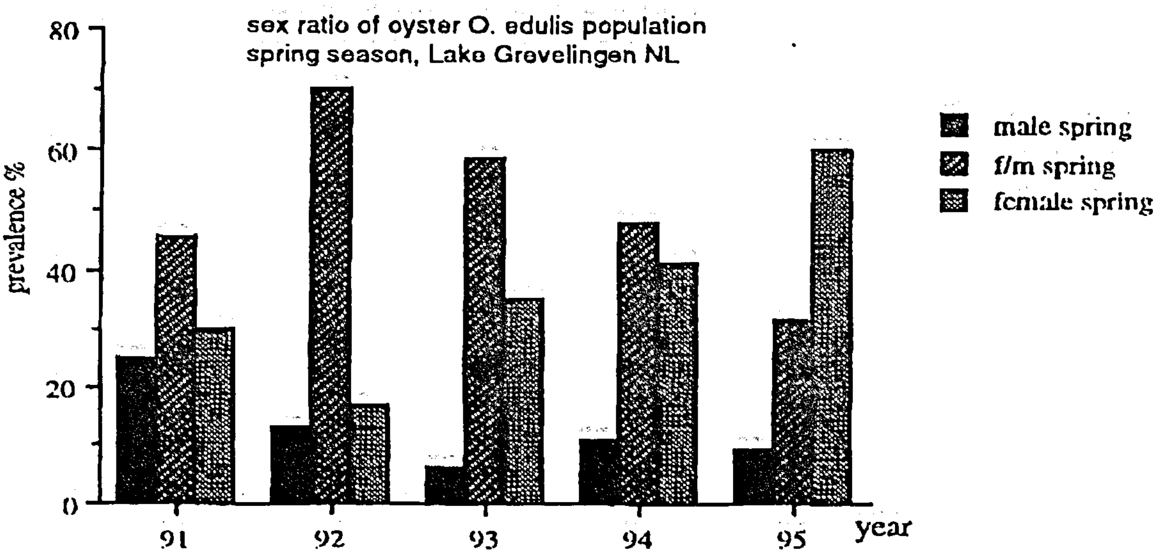
3. Prevalence of bonamiasis in *O. edulis* population, lake Grevelingen, 1991-1995

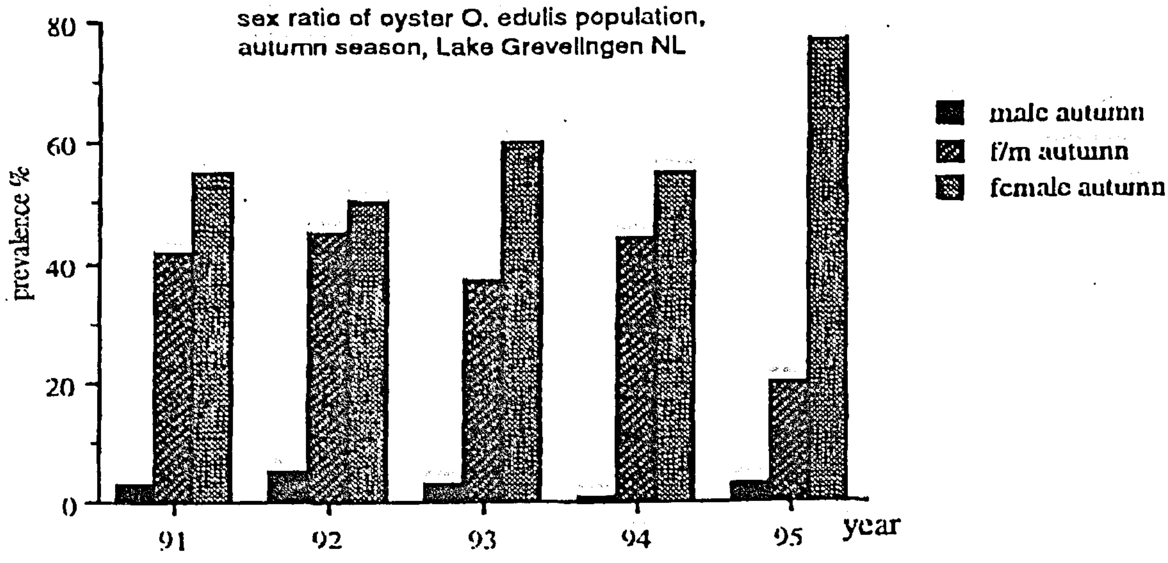


4. Intensity of bonamiasis infection in *O. edulis* population, lake Grevelingen, 1991-1995

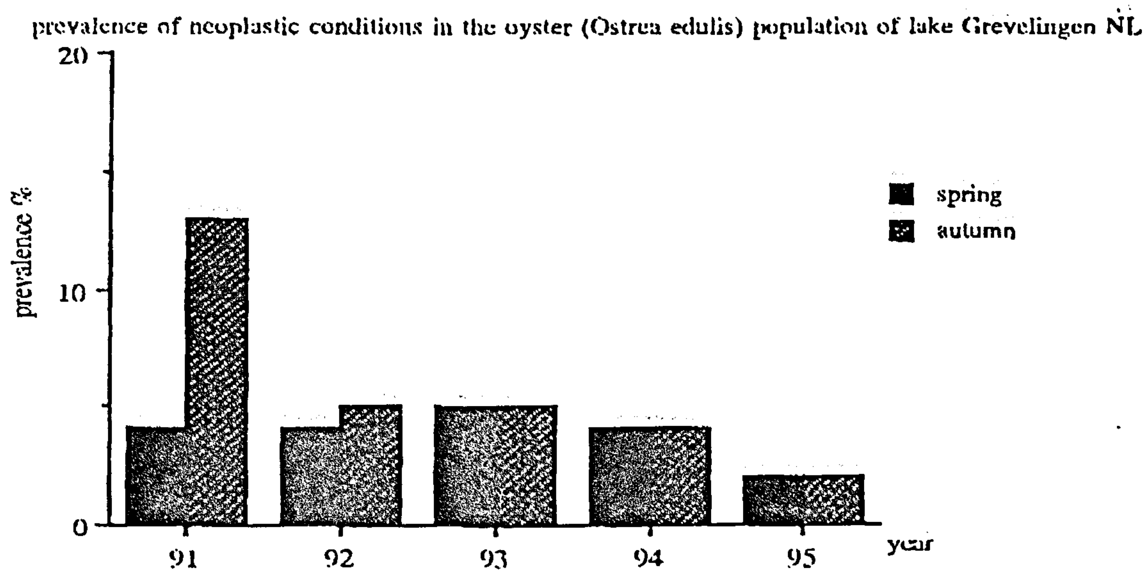


5a/b Sex ratio in *O. edulis* population, spring/autumn. lake Grevelingen, 1991-1995





6. Prevalence of infiltration of neoplastic blood cells in connective tissue of *O. edulis*, lake Grevelingen, 1991-1995



CONCLUSIONS

1. Bonamiasis resulted in a nearly total destruction of the stock of *O. edulis* in lake Grevelingen, but after the first epizootic period a reduced stock remained since 1991, based on survivors of the epizootic and their natural reproduction.

2. The mortality of the *O. edulis* population in lake Grevelingen decreased over the period 1991-1995: slightly in the spring season, strongly for the autumn season.

3. The prevalence of bonamiasis decreased in the *O. edulis* population in lake Grevelingen over the period 1991-1995.

4. The intensity of bonamiasis infection in *O. edulis* of lake Grevelingen shows a decreasing tendency to a more "light" scale over the period 1991-1995.

5a/b. The sex ratio of the *O. edulis* population in lake Grevelingen has changed to the presence of more females, both for spring and autumn season of the period 1991-1995.

6.The prevalence of caracteristic neoplastic blood cell infiltration in connective tissue as sign of (infection) stress shows an decreasing trend over the period 1991-1995.

Discussion

The isolated *O. edulis* population of lake Grevelingen can be considered to be build up by survivors and by new generations of oysters, living under bonamiasis circumstances. The results of the 5 year data on mortality (2), bonamiasis prevalence (3) and bonamiasis intensity (4) show a decreasing tendency. It seems therefore, that bonamiasis gets a less important impact on the *O. edulis* stock of lake Grevelingen. With this, thoughts can be given to a possible building up of resistance by natural selection. These thoughts can be seen as supported by the observation of the decreasing trend in the infection/stress indication, based on the decreasing presence of connective tissue infiltration by neoplastic blood cells (6). Also the change to more female status (5a/b) of the Grevelingen oyster population is interesting and might be considered as a positive reproduction sign as result of decreased bonamiasis pressure, although the link between sex en bonamiasis development is not considered as proven.
However, the thoughts of a "possible first development of resistance" must be considered very carefully. The most important lack of knowledge is the impact of the reduced population density (as result of the high mortality over years) on the survival and spread of *B. ostreae*. It might be that the low population density has resulted in the observed decreasing trends of the bonamiasis characteristics, and that in fact no real resistance has been build up by natural selection.
Stock assessment at the end of 1995 showed the presence of an important stock of young oysters (2 year age), probably developing at the end of 1996 to a high density population. The 1996 and 1997 bonamiasis characteristics of this population will provide us with better signs on the real development of bonamiasis resistance of the *O. edulis* population of lake Grevelingen.

PvB
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ANNEX 11

GEOGRAPHIC DISTRIBUTION OF JUVENILE OYSTER DISEASE (JOD) AND STATUS OF AMERICAN OYSTERS *Crassostrea virginica* STOCKS IN ICES MEMBER COUNTRIES

USA (Atlantic)

Juvenile Oyster Disease was first recognised as a serious problem in hatchery-reared, tray cultured spat in 1988. Since then, mortalities of 40-90% have been reported in the Damariscotta River, Maine, several sites in Massachusetts and in Oyster Bay and Fishers Island, New York (Davis and Barber 1994, Relyea 1994). Connecticut oysters are, as yet, unaffected. The cause of the disease is still under investigation, however, induction of clinical signs, indicating transmission, has been achieved under experimental conditions (Lewis et al. 1995, 1996). Salinities > 18 ppt. were correlated with transmission, however, exposed oysters held for 7 months at 5 ppt., demonstrated a reappearance of clinical signs when salinity was raised to 26 ppt. Mortalities can be reduced by holding susceptible oysters at low salinities (Lewis et al. 1996) and by transplanting larger spat from the hatchery to nursery trays (Davis and Barber 1994).

CANADA (Atlantic)

Sharon McGladdery - No clinical signs of JOD have been detected to date in *Crassostrea virginica* in Atlantic Canada.

FRANCE

Tristan Renault - In 1993, some clinical signs similar to those of JOD were observed on *C. virginica* spat originating from broodstock imported from Wales to the IFERMER's laboratory at Ronce-les-Bains, France. Broodstock and spat were separated immediately after spawning. Broodstock were destroyed, post-spawning, as per ICES Introduction and Transfer Guidelines, and examined for disease agents. None were found (including shell deformities). In juveniles, clinicals signs were restricted to animals maintained in the IFREMER's nusery. The batch was destroyed.

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ANNEX 12

DETERMINATION OF TRANSMISSION POTENTIAL OF *BONAMIA OSTREAE* TO AMERICAN OYSTERS (*CRASSOSTREA VIRGINICA*) AND JUVENILE OYSTER DISEASE (JOD) TO EUROPEAN AND PACIFIC OYSTERS (*OSTREA EDULIS* AND *CRASSOSTREA GIGAS* RESPECTIVELY) TO CLARIFY THE CARRIER POTENTIAL OF SPECIES RELATED TO THE PRIMARY HOSTS

Introduction

The WGPDMO undertook an investigation of examples of non-host-specificity in molluscs due to the reports at the WGPDMO Meeting in 1995 and at the Statutory Meeting at St. John's, Newfoundland in 1994 of multi-host transmission by *Mikrocytos mackini* from *Crassostrea gigas*. This investigation concentrated on *Bonamia ostreae* and JOD, but *Marteilia* spp. and Herpesvirus studies are also reported.

Transmission Potential of *Bonamia* spp. to various oyster species (*Crassostrea virginica, C. gigas, C. rivularis* and *C. sikamea*)

H. Grizel, G. Tige and T. Renault - ICES Statutory abstract (not presented) - No *Bonamia* sp. has been shown to infect *Crassostrea gigas*. Oysters surveyed histologically, and challenged with experimental infections over a six month period, showed no evidence of *Bonamia* in heart and gill smears or in histological sections (Renault et al. 1995a). (Early stages of *Marteilia* spp. have been found at negligible levels in *C. gigas* from time to time).

It was not possible to detect *B. ostreae* in American oysters *Crassostrea virginica* deployed at enzootic field locations (Marennes-Oleron) but, a *Marteilia*-like parasite was found in the American oysters in 1993 (Renault et al. 1995b). *Bonamia*-like parasites have been detected, however, in *C. rivularis* and *C. sikamea*, introduced from California and reared in quarantine at IFREMER's La Tremblade laboratory (see Section 6.2 and 6.5). Concerning these parasites, transmission experiments are in progress (T. Renault, personal communication).

Dr. B.J. Barber, University of Maine at Orono (pers. comm.) indicated that there was no evidence of *Bonamia ostreae* transmission to American oysters (*Crassostrea virginica*) in Maine. It should be noted, however, that there are few wild-spawning *C. virginica* in Maine. Most oysters are produced from hatchery broodstock, thus, exposure to infected European oysters is minimal.

Transmission Potential of *Marteilia* spp. to American oysters and between Mussel and Scallop species.

Work is ongoing to develop nucleic acid (DNA) probes to distinguish between *Marteilia* spp. which are beginning to emerge in a broad range of bivalves, some with serious consequences, e.g., in Calico scallop *Argopecten gibbus* from Florida (Moyer et al. 1993). Other species and hosts include: *Marteilia refringens* in *Ostrea edulis* (Grizel et al. 1974), *Crassostrea gigas* (Cahour 1979), *Mytilus edulis* (Tige and Rabouin 1976), *Mytilus galloprovincialis* (Villalba et al. 1993), *Ostrea chilensis* (Grizel et al. 1993) and *Ostrea angasi* (Bougrier et al. 1986); *Marteilia maurini* in *Mytilus galloprovincialis* (Comps et al. 1982), *M. edulis* (Auffret and Poder 1985) and *Marteilia* sp. in *M. edulis* (Comps et al. 1975).

Transmission Potential of Juvenile Oyster Disease (JOD) to European oysters (and other bivalve species)

Austin Farley and Bruce Barber (personal communications) - JOD has been transmitted under experimental conditions between batches of *C. virginica* oysters, but no similar experiments have been tried with other oyster species. Anecdotal field observations indicate that JOD is oyster specific, since there has been no evidence of clinical signs in sympatric European oysters (*Ostrea edulis*), mussels (*Mytilus edulis, M. trossulus*) or clams (*Mercenaria mercenaria, Mya arenaria, Spisula solidissima*).

Transmission Potential of Herpesvirus between *Crassostrea* and *Ostrea* spp. of oysters

Work is ongoing on a PCR technique for diagnosing Herpesvirus infections in different species of oysters. This needs to be pursued and reference material compiled from different countries and oyster species. This material can be used to determine the host-specificity of Herpesvirus-like infections, as well as the problem of sub-clinical carriers (see Section 6.2 and 6.5).

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ANNEX 13

ICES SHELLFISH DISEASE REPORTING FORM - Draft

Shellfish Species **YEAR**

Country

Geographic Location (Attach Map)

[1]Pathogen Type	Identification	Hatchery Nursery/Open-water	Research/Survey/ Diagnostics	Size-group	# samples (# individuals)	Mortalities (est. % range)	Peak Problem (Month)	Diagnostic technique
e.g., no pathogen detected	NA	Open water	Survey	Market-size	10 (250)	0-100%	Jul-Sep	Histology
e.g., VIRUS	*Herpesvirus*	Hatchery	Diagnostics	Spat	10 (300)	0-100%	Jul-Sep	Histology

Comments:

[1] See attached sheet for entry information

Data Entry Information for ICES Shellfish Disease Reporting Form

Pathogen Type	Virus	
	Bacteria	
	Fungus	
	Protista	
	Digenea	
	Turbellaria	
	Cestoda	
	Copepoda	
	Decapoda	
	Polychaete	
	Nemertean	
	Sponge	
	Other	
Identification	Name or published/recognised description of the pathogen, disease or syndrome	
Hatchery/Nursery/Open water	Hatchery	Tables
	Nursery	Racks / Trays
	Open-water	Bouchot (Pilons)
		Bottom
		Bags, Baskets
		Lines
	Other	
Research / Survey / Diagnostics	Were the samples collected primarily for research or as part of a survey or as a diagnostic examination submitted due to abnormal growth or mortality	
Size Group	Larvae	
	Spat (post-metamorphosis)	
	Juvenile-adult (market-size, pre-market-size, etc.)	
# Samples (# individuals)	Number of samples and total number of individuals examined	
Mortalities (estimated % range)	No or Yes (+ estimated range of prevalences)	
Peak Problem Month(s)	Months during which mortalities or other abnormalities are most severe	
Diagnostic Technique(s)	Gross Observation	
	Tissue smears	
	Histology	
	Electron Microscopy	
	Bacteriology	
	Cell Monolayer	
	Immunodiagnostics	
	Minimum Essential Medium (MEM)	
	Thioglycollate	
	Biomolecular techniques	
	Other.	

ANNEX 14

WORKING GROUP ON THE SENSITIVITY TESTING OF FISH ASSOCIATED BACTERIA

I must first thank you all for agreeing to take part in this working group and then apologies to you all for my delay in getting the show on the road.

According to the notes that Dave Alderman made of our meeting in Mallorca we agreed that our objectives should initially be;

1. To work on both disc diffusion and quantitative MIC methods.

2. To use *Aeromonas salmonicida* and *Aeromonas hydrophila* as prototype organisms.

3. To include amoxycillin, oxytetracycline, potentiated sulpha preparations and the quinolones as the prototype agents.

GENERAL OUTLINE OF THE PROCEDURE OF THE WORKING GROUP.

It is considered that to move towards agreed standard methods we must proceed through four stages

STAGE 1

Identification of significant parameters of each method.

STAGE 2

Determination of suitable values for each parameter identified leading to formulation of provisional standard methods.

STAGE 3

Evaluation and validation (and modification) of provisional standard methods.

STAGE 4

Presentation of the proposed standard methods to the EAFP.

GENERAL TIMETABLE OF THE WORKING GROUP

The general strategy is that Stage 4 should be reached in time for the EAFP meeting in Edinburgh in 1997.

It is therefore proposed that we aim to complete Stage 2 at a meeting coinciding with the UK EAFP meeting in Weymouth in September 1996.

DETAILED STRATEGY FOR STAGE 1

In this stage our aim is to identify all the individual significant parameters that must be specified in the two standard methods we are aiming to produce. In this sense a significant is any aspect of a method that influences the quantitative result obtained from that test. Obvious examples of such significant parameters would be, for example, media composition, incubation temperature, incubation time.

It is hoped that the members of the working group will have, from their own experience, knowledge of many of the important parameters. In my own research a student is just completing a study of 17 parameters influencing the quantitative results obtained from disc diffusion tests. All had some effect!

My suggestion is that each member compiles a list of all of the parameters that they know to have importance. At this stage these lists need not necessarily be accompanied with any data justifying the inclusion of any particular item. The list should be inclusive in that they should include every parameter we can think of. (It will be easier to eliminate parameters shown to have little relevance than to try and introduce new parameters at a late stage.)

These lists should be sent to me and the resultant information will be distributed to all members.

It is important to note that at this time we are not attempting to reach consensus on the values that should be placed on any parameter. For example in Stage 1 it will be sufficient to establish that incubation temperature is a significant parameter, but reaching consensus as to which temperature is most appropriate in any situation, is a Stage 2 activity.

STAGE 1 PRODUCT

The product of Stage 1 will be two agreed lists of significant parameters. One relating to disc diffusion the other to quantitative MIC determinations.

STAGE 1 TIME SCALE.

The product of Stage 1 should be produced by the end of March 1996. The stage 1 process will involve;

i) Sending of list from participants to me.

ii) Collation and redistribution of lists to participants by me.

ii) Sending of comments on collated list from participants to me.

iv) Distribution of final lists of significant parameters to all participants by me at the end of March 1996.

This should give everybody an idea of the speed with which they should address the compiling of their lists. (In general it can be said that they should not be as slow as I was in issuing this first letter)

STAGE 2

It is intended that Stage 2 will proceed in two phases.

i) In the first phase participants will be asked to suggest the values they would prefer to see adopted for each of the significant parameters identified in Stage 1. Equally information will be collected on the values that participants would not be prepared to accept.

ii) These preferences will be circulated, by me, through the working group for comment.

iii) A discussion document will be drawn up for presentation to the working group at the UK EAFP meeting Sept 1996. This document will present the extent of consensus and conflict in the group. It will be used as a basis for the discussions at that meeting

iv) The meeting in September will attempt to resolve the conflicts within the group. It will also set the agenda for the activity of the working group for 1996-1997.

Pete Smith

WGPDMO 1996 Copenhagen

Working document on M 74

Summary of the Second Workshop on Reproduction Disturbances in Fish

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Background

On 20-23 November 1995, the Swedish Environmental Protection Agency (SEPA) hosted the Second Workshop on Reproduction Disturbances in Fish. The workshop was financed jointly by SEPA, the Swedish National Board of Fisheries, the Swedish Council for Forestry and Agricultural Research, the World Wide Fund for Nature, the Swedish Power Board and the Nordic Council of Ministers. The workshop featured presentations by invited speakers, plenary lectures, posters and group discussions including the following areas:

- The Early Mortality Syndrome (EMS) and Swim-Up Syndrome (SUS) in North American salmonids
- The M74 syndrome in Baltic salmon (*Salmo salar*)
- Recruitment problems in the Baltic cod (*Gadus morhua*)
- Reproduction problems in other Baltic fish species

The workshop was attended by over 70 scientists from 10 countries, representing expertise in disciplines such as environmental chemistry and toxicology, molecular biology, population genetics, fisheries ecology, fish physiology, pathology and parasitology, aquaculture and environmental protection.

M74/EMS in salmonids

Early Mortality Syndrome may be regarded as the collective name for the reproductive disturbances observed during the last 20 years in salmonids in the Baltic Sea and the North American Great Lakes. Accordingly, the M74 syndrome in Baltic salmon and the Cayuga syndrome in Atlantic salmon (*Salmo salar*) should be considered as special cases of EMS. This was further demonstrated at the workshop by several oral presentations and by the two videofilms that documented the symptoms of the M74 syndrome (Per-Åke Hägerroth) and EMS (Susan Marcquenski).

In the North American Great Lakes, recent outbreaks of the Early Mortality Syndrome affect a large number of salmonid species: coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tsawytscha*), steelhead salmon (*Oncorhynchus mykiss*), lake trout (*Salvelinus namaycush*) and brown trout (*Salmo trutta*), with differences in the stage of fry affected and the morphological and behavioural symptoms (Susan Marcquenski). The main factors thought to lie behind EMS are changes in ecosystems (*e.g.* introduction of non-indigenous fish species) and nutritional factors.

The Cayuga syndrome in Atlantic salmon from the North American Finger Lakes demonstrates similarities with M74 in Baltic salmon (*i.e.* both are thiamine-related neurological syndromes) and offers particularly good oppportunities for parallel research and comparison with M74 (Jeffrey Fisher, Jenny Lundström).

There are a number of ecological similarities between the reproductive disturbances in the North American salmonids and the M74 syndrome in Baltic salmon, *e.g.* the syndromes have a rapid onset, development of the syndromes is female-dependent, affected fry are responsive to thiamine treatment, and food webs are based on clupeiod fish (Report from working group 3).

The M74 syndrome is still raging at high levels in the Swedish salmon hatcheries, where reproduction is based on feral broodstock. During the past four years (1992-1995), 57 to 87% of the female spawners produced offspring that were affected by M74 (Bengt-Erik Bengtsson). Wild salmon spawners can be assumed to suffer from similar losses, as fry hatched from eggs brought in from the wild also experience high mortality from M74, and results of electrofishing in rivers show a paucity of parr compared to the number of adults that migrated upriver to spawn in previous years.

Data from Sweden and Finland suggest that sea trout (*Salmo trutta*) develop a disease similar to M74 (Antti Soivio). Finnish scientists have also been able to demonstrate that thiamine treatment is effective for curing affected fry of sea trout (Perttu Koski). From these observations we may assume that M74 now also occurs in sea trout from the Baltic, and that current reproductive disturbances in salmonids should be monitored carefully all around the Baltic Sea.

Since the Uppsala workshop on reproduction disturbances in fish was held in October 1993 (Norrgren 1994) there has been a considerable increase in research efforts in both the Baltic and Great Lakes regions. The Uppsala workshop launched the "carotenoid/astaxanthin hypothesis"(presented by Åke Lignell and co-workers, Lignell 1994) as the explanation for the M74 syndrome (salmon fry with low levels of carotenoids showed a high incidence of M74).

If we were to try and identify a "take-home message" from the Lidingö workshop, it is probably that both M74 in Baltic salmon and EMS/SUS in Great Lakes salmonids can be treated with thiamine. Positive results of such treatment were reported from Canada (John Fitzsimons), the United States (Jeffrey Fisher, Michael Hornung), Sweden (Patric Amcoff, Joakim Larsson) and Finland (Perttu Koski). This points to a similar origin for these syndromes and considerably narrows the focus of research on the mechanisms involved.

However, the role of astaxanthin and lipophilic toxic contaminants has not yet been resolved, and interesting results were presented which indicate that these hypotheses can not yet be ruled out (Annette Pettersson, Michael Hornung, Christoffer Rappe, Pekka Vuorinen). Contaminants that are receptor-active and "new pollutants" that are known or suspected to be increasing in biota are particularly interesting to study (Report from working group 1).

There is still a lack of knowledge about the role of thiamine (and other vitamins) in fish metabolism and of the dynamics of thiamine in aquatic ecosystems. We also lack information about the long-term effects of the thiamine-treatment practices carried out in the hatcheries today (Report from working group 1).

Genetic bottlenecks have occurred both in stocks of Baltic salmon and in chinook and coho salmon in the Great Lakes. There is no direct evidence that genetic factors are involved in the occurrence of M74 and EMS, but their role deserves further studies (Torbjörn von Schantz, Åsa Langefors, Per-Erik Olsson, Report from working group 2).

Dietary factors, which may result from changes in ecosystems or food webs, seem to remain one of the most important factors behind reproductive disturbances in feral salmonids (Lars

Karlsson, Erkki Ikonen, Susan Marcquenski). The presence/dynamics of clupeiod fish species that are eaten by the affected salmonids also seem to be important. In the Baltic, abiotic factors such as high winter water temperatures may also be important for the incidence of M74 (Report from working group 3).

If we were able to identify the reasons for M74 within the next few years, and if there were a cure that could be applied instantly, the wild-spawning Baltic salmon populations would still have to be protected. This means reducing coastal and offshore fisheries on mixed populations, and reducing releases of reared smolt (Reports from working groups 2 and 3). To alleviate the effects of M74 on wild-spawning populations, different measures (*e.g.* genebanks, finclipping to identify hatchery stocks) must be developed to preserve wild stocks (Lars-Ove Eriksson, Jorma Piironen).

Other species

Baltic cod populations are suffering from recruitment difficulties (Stig Mellergaard, Lennart Balk), but present data do not support the existence of a syndrome similar to M74 in salmon. Rather, the major prerequisites for successful spawning in Baltic cod are adequate salinity and oxygen conditions in the spawning areas (Lars Vallin, Anders Nissling).

There are indications of reproductive disturbances in other species of fish in the Baltic, but they are different from the M74 syndrome and EMS. However, there have so far been very limited efforts to search for reproductive disturbances in a wide range of fish species. Two contributions on species other than salmonids and cod were presented at the meeting: a Finnish study on the ability of parasites (microsporidians) to cause reproductive disorders in roach (*Rutilus rutilus*) (Göran Bylund); and an Estonian study on polycyclic aromatic hydrocarbons in eelpout (*Zoarces viviparus*) (Risto Tanner).

Conclusions

The positive effects of the coordination of research around the Baltic Sea and cooperation with researchers in the United States and Canada in this field are very evident, and it was further emphasized by the participants that cooperation must continue. This process will be served by coordination of research programmes, exchange of materials and methods, and financial support to attend workshops and other meetings where ideas and results can be exchanged. The participants encouraged each other to utilize established and new networks, communication via Email/Internet and participation in workshops/symposia for this purpose.

References:

Lignell, Å. (1994). Astaxanthin in yolk-sac fry from feral Baltic salmon. In: Norrgren, L. (ed.) (1994). report from the Uppsala workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency Report 4346, pp. 94-96.

Norrgren, L. (ed.) (1994). Report from the Uppsala workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency Report 4346.

ANNEX 15b

I.C.E.S., meeting of WGPDMO
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A report on herring (*Clupea harengus*) from the Icelandic summer spawning stock and the Atlanto-Scandian stock examined for *Ichthyophonus* in Iceland in 1995.

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Introduction.

In the Northeast Atlantic prevalence of *Ichthyophonus* infection in herring has been monitored for some time, in Iceland since 1992. This monitoring was recommended by ICES when it became clear that this fungal disease was reaching epozootic levels in at least some of the herring stocks in the northeast Atlantic.

Material and Methods.

Samples from the Icelandic summer spawning stock were taken from research vessels using midwater trawl during stock assessment. Routine measurements, maturity studies and scale sampling for aging of random samples were made aboard the research vessels, the heart was then dissected from each herring and frozen individually along with station number and serial number of fish. Trays with 100 small compartments (12x29x15 mm) were used to freeze the hearts individually. Additional samples from fishing vessels operating with purse seines were routinely measured at the Marine Research Institute and treated in the same way as described above.

Samples from the Atlanto-Scandian herring were taken from research vessels using midwater trawl during research cruises north and northeast of the Faroe Islands, but most of the samples were teken from commercial purse seine catches landed in Iceland. These samples were treated as described above.

All measurements, sex determination and aging of the herring was done for the purpose of stock assessment studies. All samples were aged, except a sample from Icelandic summer spawning herring on station A2-95-50 (statistical rectangle 462).

After thawing, each heart was carefully examined with a dissecting microscope for possible *Ichthyophonus* "cysts". Squash preparations were made from suspicious hearts to ensure *Ichthyophonus* identification.

Results.

From a total of 2051 herring from the Icelandic summer spawning stock, only two were found to be infected (Fig. 1, Table 1). Most of the examined herring was in the 25+ cm size group, 805 males and 945 females, a total of 1750 fishes (Table 2).

Both the infected herrings were in this length group, the total prevalence of infection being 0,11%.

Broken down by fishing gear, the number of herring of size group 25+ cm in MWT was 1253, two of which were infected (prevalence of infection 0,16%). None of the 497 herrings in this size group from purse seine were infected.

In size group 20-24 cm 275 herring were examined, but only 26 in size group 15-19 cm, none of these were infected (Table 1).

A total of 1589 herrings of the Atlanto-Scandian herring stock were examined, all of them in the 25+ cm size group (Fig. 2, Table 4). The prevalence of infection in a sample ranged from 0,0 to 5,5%. The total prevalence of infection was 3,15% (3,36% in males, 2,94 in females).

There was some variation in the prevalence of infection in different age classes (Table 5). In those age classes represented by 100 fishes or more, a prevalence of 8,89% in 11 years old herring is somewhat outstanding.

The prevalence of infection in united samples from each 10 days period in May does not indicate changes in the prevalence during that time (Table 6).

Broken down by fishing gear, the total prevalence of infection in MWT was 3,02% (16 of 530 herrings) and in purse seine 3,21% (34 of 1058 herrings)(Table 7).

Two of the statistical rectangles, no. 353 and 553, were represented by more than 200 herrings examined, the total prevalence was 3,13% and 2,99% respectively (Table 7).

Discussion.

The prevalence of *Ichthyophonus* in the Icelandic summer spawning herring is very low, and has been low since this monitoring started in 1992. In the 25+ cm size group the prevalence has been approximately 0,2%, the 1995 prevalence of 0,11% (0,16% in MWT) indicates unchanged situation. It is possible that prevalence of this magnitude is a "normal" situation in this stock.

The prevalence of 3,15% in the samples of Atlanto-Scandian herring is about 30 fold what was found in the Icelandic summer spawners. Variation in prevalence between samples when samplesize is only 30 to 100 fish is to be expected, pooling the data in various ways is then nessesary to examine the estimate of the total prevalence. Combining the data from MWT and purse seine is believed to be justifiable here since there is no obvious difference in the observed prevalence of infection in herring taken in these two fishing gears.

During the time of sampling, the Atlanto-Scandian herring was on a rapid migration through the area north of the Faroe Islands, thus, prevalence of infection in a statistical rectangle or area has no meaning. Doing so, however, for two statistical rectangles that were represented by more than 200 fishes indicates only a small variation.

Examining the various pooling of data presented in Tables 6 and 7 reveals that the variability of the prevalence only ranges between 2,86 and 3,58%, indicating that the total prevalence of 3,15% is a fair description of the infection at this time and place.

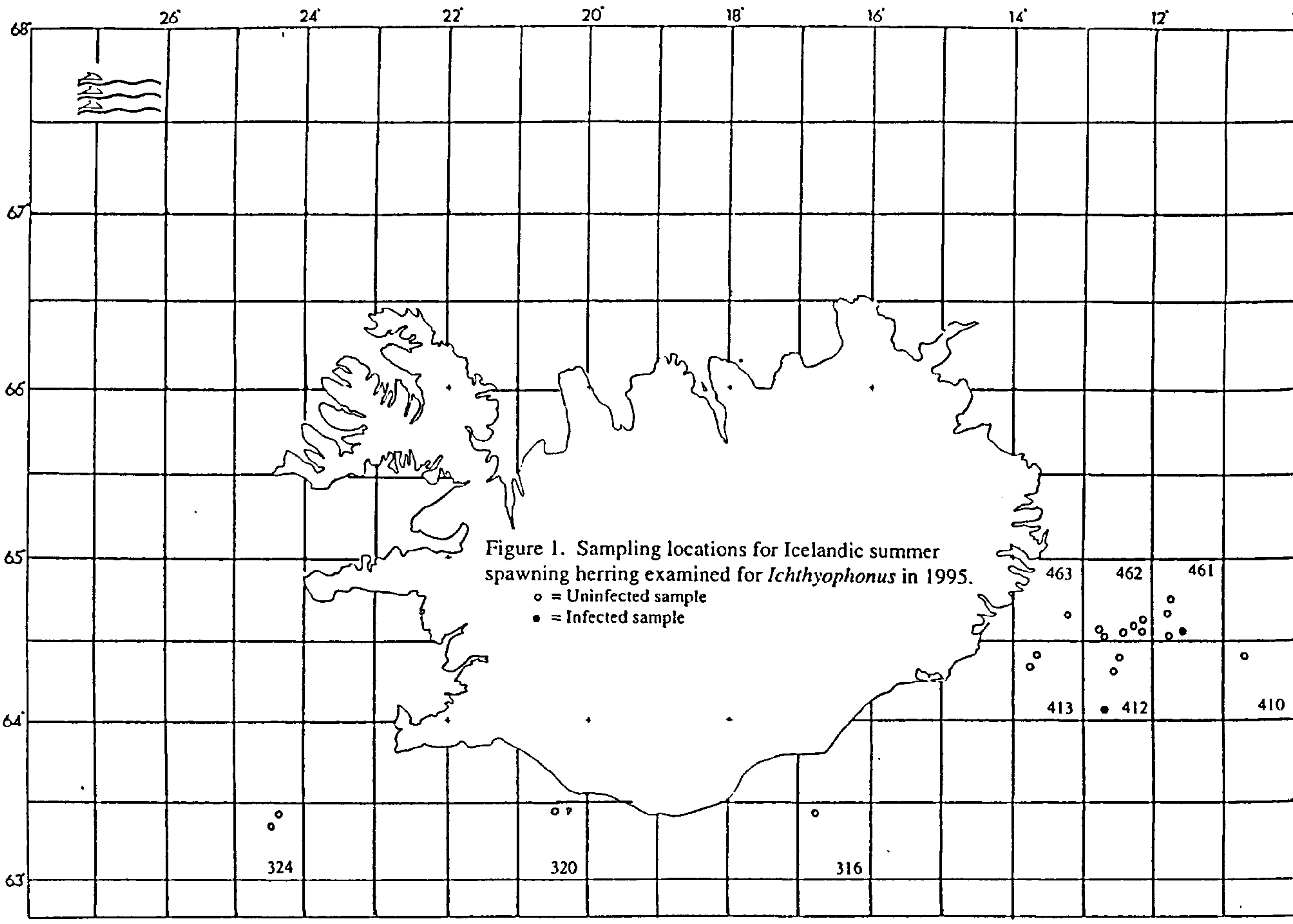

Figure 1. Sampling locations for Icelandic summer spawning herring examined for *Ichthyophonus* in 1995.
o = Uninfected sample
• = Infected sample

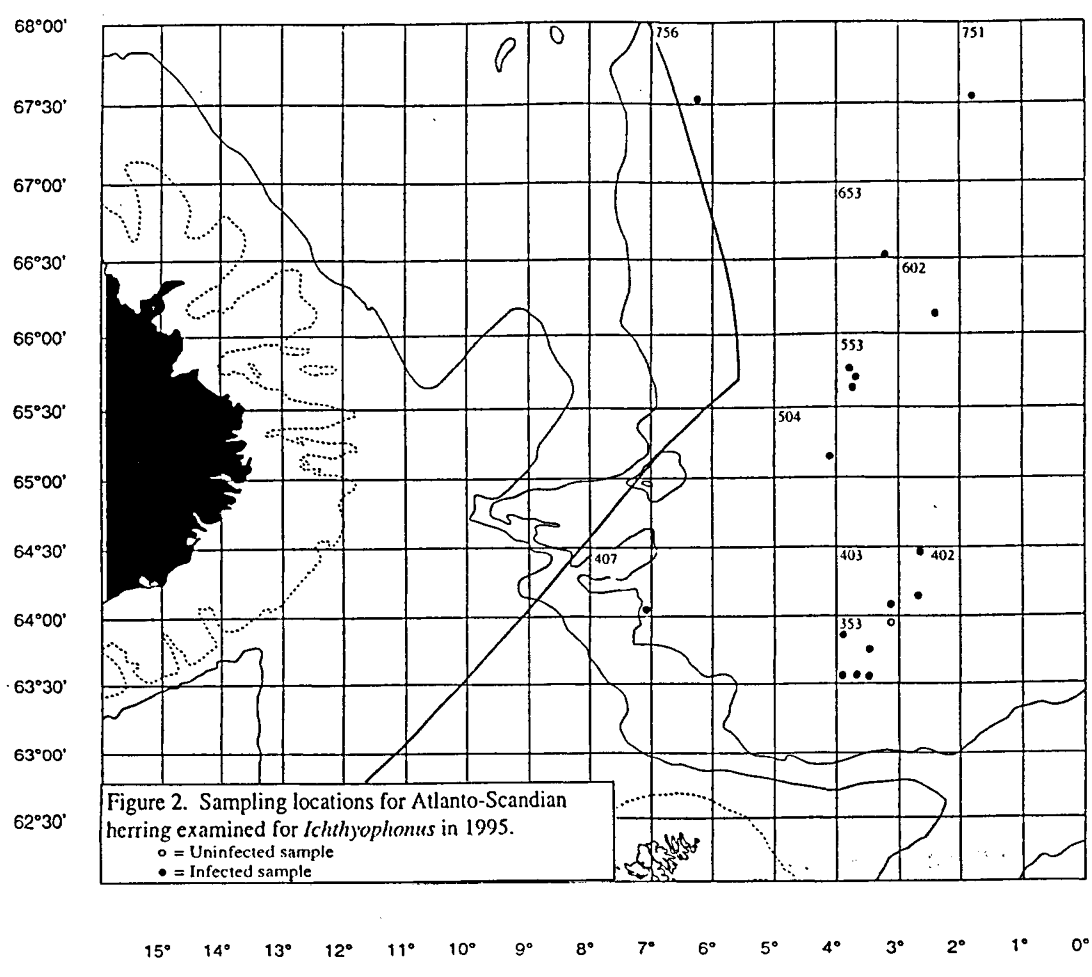

Figure 2. Sampling locations for Atlanto-Scandian herring examined for *Ichthyophonus* in 1995.
o = Uninfected sample
• = Infected sample

Table 1. Herring (Clupea harengus) from Iceland
Samples examined for Ichthyophonus in 1995 (MWT = Mid Water Trawl, PS = Purse Seine)
M=Males, F=Females

Statistical rectangle	Date	Number examined in each size group 15-19 cm M	15-19 cm F	20-24 cm M	20-24 cm F	25 + cm M	25 + cm F	Total in sample N	Affected Num.	%	Station Number	Fishing gear
316	16.2.95	1	0	9	16	39	35	100	0		A2-95-81	MWT
320	29.11.95	0	2	36	48	7	7	100	0		A16-95-842	MWT
324	25.11.95	7	7	7	34	16	29	100	0		A16-95-830	MWT
324	26.11.95	1	0	3	1	40	55	100	0		A16-95-831	MWT
Total in 324		8	7	10	35	56	84	200	0			
410	6.2.95	0	0	8	6	31	36	81	0		A2-95-55	MWT
412	4.1.95	5	2	13	20	25	35	100	0		B1-95-2	MWT
412	14.2.95	0	0	10	10	21	29	70	0		B2-95-80	MWT
412	15.2.95	0	0	2	2	45	51	100	1		A2-95-76	MWT
Total in 412		5	2	25	32	91	115	270	1	0.37		
413	20.9.95					42	58	100	0		Fish.vessel	PS
413	21.9.95					49	51	100	0		Fish.vessel	PS
Total in 413						91	109	200	0			
461	30.11.95			9	13	41	37	100	0		A16-95-843	MWT
461	30.11.95					42	58	100	0		A16-95-844	MWT
461	30.11.95					39	61	100	1		A16-95-845	MWT
461	1.12.95					45	55	100	0		A16-95-849	MWT
Total in 461				9	13	167	211	400	1	0.25		
462	6.2.95	0	0	6	5	39	50	100	0		B2-95-52	MWT
462	2.2.95	0	1	3	11	33	52	100	0		A2-95-50	MWT
462	1.12.95					57	43	100	0		A16-95-846	MWT
462	1.2.95	0	0	1	2	47	50	100	0		Fish.vessel	PS
462	10.10.95					49	51	100	0		Fish.vessel	PS
462	14.10.95					47	53	100	0		Fish.vessel	PS
Total in 462		0	1	10	18	272	299	600	0			
463	14.2.95					51	49	100	0		B2-95-82	MWT
Total examined		14	12	107	168	805	945	2051	2	0.10		

Table 2. Infection of Ichthyophonus in size group 25 cm and larger of herring from Icelandic waters in 1995. (MWT = Mid Water Trawl, PS = Purse Seine)

Statistical rectangle	Date	Station Number	Fishing gear	Mean length cm	S.D.	Total no. examined Males	Females	No. affected M	F
316	16.2.95	A2-95-81	MWT	28,0	2,7	39	35	0	0
320	29.11.95	A16-95-842	MWT	25,8	2,1	7	7	0	0
324	25.11.95	A16-95-830	MWT	29,6	2,9	16	29	0	0
324	26.11.95	A16-95-831	MWT	29,8	2,4	40	55	0	0
410	6.2.95	A2-95-55	MWT	27,1	1,9	31	36	0	0
412	4.1.95	B1-95-2	MWT	28,1	2,5	25	35	0	0
412	14.2.95	B2-95-80	MWT	28,1	2,6	21	29	0	0
412	15.2.95	A2-95-76	MWT	31,5	2,8	45	51	1	0
413	20.9.95	Fish.vessel	PS	32,7	1,7	42	58	0	0
413	21.9.95	Fish.vessel	PS	32,8	1,8	49	51	0	0
461	30.11.95	A16-95-843	MWT	28,7	2,9	41	37	0	0
461	30.11.95	A16-95-844	MWT	31,8	2,4	42	58	0	0
461	30.11.95	A16-95-845	MWT	31,3	2,3	39	61	0	1
461	1.12.95	A16-95-849	MWT	32,3	2,1	45	55	0	0
462	6.2.95	B2-95-52	MWT	28,8	2,5	39	50	0	0
462	2.2.95	A2-95-50	MWT	31,0	2,7	33	52	0	0
462	1.12.95	A16-95-846	MWT	32,1	2,7	57	43	0	0
462	1.2.95	Fish.vessel	PS	30,9	2,8	47	50	0	0
462	10.10.95	Fish.vessel	PS	31,7	2,3	49	51	0	0
462	14.10.95	Fish.vessel	PS	32,4	2,1	47	53	0	0
463	14.2.95	B2-95-82	MWT	32,6	2,0	51	49	0	0
			Total in MWT			571	682	1	1
			Total in Purse Seine			234	263	0	0
				Total		805	945	1	1
				Total males and females			1750		
				Prevalence (%)			0.11		

Table 3. Age distribution of Icelandic summer spawning herring examined for Ichthyophonus infection in Iceland in 1995, number infected and prevalence of infection in each age class.

Age Class	Number Aged	Number Infected	Prevalence (%)
1	120		0.00
2	123		0.00
3	489		0.00
4	312		0.00
5	369		0.00
6	214	1	0.47
7	67		0.00
8	59	1	1.69
9	42		0.00
10	34		0.00
11	29		0.00
12	8		0.00
13	2		0.00
14	1		0.00
Uncertain	82		0.00
Total	1951	2	0.10

Table 4. Infection of Ichthyophonus in size group 25 cm and larger of Atlanto-Scandian herring caught by Icelandic research and fishing vessels in April - June 1995. (MWT = Mid Water Trawl, PS = Purse Seine)

Statistical rectangle	Date	Station Number	Fishing gear	Mean length cm	S.D.	Total no. examined Males	Females	No. affected M	F
353	8.5.95	Fis.vessel	PS	35,6	1,8	51	49	0	0
353	10.5.95	Fis.vessel	PS	33,8	2,3	53	46	2	1
353	10.5.95	Fis.vessel	PS	36,0	1,2	42	49	2	3
353	10.5.95	Fis.vessel	PS	35,2	1,9	49	40	1	1
353	17.5.95	Fis.vessel	PS	34,9	2,3	47	48	2	1
353	20.5.95	A8-95-261	MWT	33,7	2,6	42	58	2	3
402	16.5.95	A8-95-242	MWT	33,2	2,1	51	49	2	2
402	7.5.95	Fis.vessel	PS	35,4	1,9	23	17	1	1
403	15.5.95	Fis.vessel	PS	32,4	2,5	39	39	2	2
407	29.5.95	Fis.vessel	PS	34,9	2,4	44	56	3	2
504	19.5.95	Fis.vessel	PS	34,8	2,0	46	52	1	1
553	20.5.95	Fis.vessel	PS	34,8	2,0	53	47	2	0
553	20.5.95	Fis.vessel	PS	34,7	1,7	31	37	2	0
553	23.5.95	Fis.vessel	PS	33,1	2,4	44	56	2	2
602	26.4.95	B6-95-229	MWT	33,6	2,4	32	68	0	2
653	24.5.95	A8-95-295	MWT	34,8	2,1	67	33	2	1
751	27.4.95	B6-95-236	MWT	32,0	2,4	44	56	0	1
756	21.6.95	A9-95-358	MWT	36,0	1,4	15	15	0	1
			Total in MWT			251	279	6	10
			Total in Purse Seine			522	536	20	14
				Total		773	815	26	24
				Total males and females			1588		
				Prevalence (%)			3.15		

Table 5. Age distribution of Atlanto-Scandian herring examined for Ichthyophonus infection in Iceland in 1995, number infected and prevalence of infection in each age class.

Age Class	Number Aged	Number Infected	Prevalence (%)
4	25		0.00
5	173	2	1.16
6	398	9	2.26
7	156	1	0.64
8	16		0.00
9	13	3	23.08
10	73	4	5.48
11	135	12	8.89
12	536	17	3.17
13	9		0.00
16	1		0.00
Uncertain	53	2	3.77
Total	1588	50	3.15

Table 6. Prevalence of Ichthyophonus in samples of Atlanto-Scandian herring collected in 10 days periods in May 1995.

Dates	Number Examined	Number Infected	Prevalence (%)
1.-10. May	419	12	2,86
11.-20. May	639	22	3,44
21.-30. May	400	13	3,25
Total	1458	47	3.22

Table 7. Atlanto-Scandian herring examined for Ichthyophonus in 1995. Various pooling of data on prevalence of infection.

	Number Examined	Number Infected	Prevalence (%)
Total in MWT	530	16	3,02
Total in Purse Seine	1058	34	3,21
Total in stat. rect. 353	574	18	3,13
Total in stat. rect. 553	268	8	2,99
Stat. rect. 353 on May 10	279	10	3,58

ANNEX 15c

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Ichthyophonus hoferi disease in herring in samples from Norwegian surveys 1995-96.

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Herring samples taken by trawling during surveys have been examined routinely for macroscopic signs of *I. hoferi* disease. This is done in samples taken for recording age, length, weight etc. in individual herring. The examination for *I. hoferi* disease is restricted to looking for macroscopic lesions in the heart and for externally visible changes in the skin. The sample size is 50-100 herring per haul. The results here are reported as unweighted percentages.

Norwegian spring spawning herring

The wintering areas in Ofoten and Tysfjord were covered by acoustic surveys in December 1995 and in January 1996, conducted in a similar way as in previous years. The results are shown in Table 1. A drop in prevalence from December to January was seen also in similar data from the winter 1994-95, when the overall prevalence dropped from 2.4 to 1.3.

The spawning area was covered by an acoustic survey in February - March. The results from this survey are shown together with preliminary acoustic estimates of the stock abundance in Table 2, and the area distribution of the samples is shown in Figure 1.

North Sea.

In the acoustic survey for herring in June - July 1995 a single diseased herring was found out of 1643 examined. In the IBTS survey in October - November none of the 878 herring examined had signs of the disease. The area distribution of the samples from these two surveys are shown in Figures 2 and 3, and the age distributions in Figures 4 and 5, respectively.

Comment.

In previous years, there has been a declining trend in the prevalence of *I. hoferi* disease in both the stocks considered here (Skagen, 1995 a,b). The disease has mainly been confined to the yearclasses born in 1986-87 and older. The present results indicates that the prevalence remains at a low level. In particular, there are no signs of increasing prevalence in the younger year classes which hitherto have been only modestly affected by the disease.

References:

Skagen, DW (1995a)
Trends in Prevalence of *Ichthyophonus* disease in Norwegian Soring Spawning Herring in the Ofoten area in 1992-95
Work. Doc. Northern pelagic WG.

Skagen, DW (1995b)
Prevalence at age of *Ichthyophonus hoferi* disease in the North Sea 1991-94. Norwegian data from surveys and commercieal catches.

Table 1.
Prevalence (unweighted) of *I. hoferi* disease in Norwegian spring spawning herring in the wintering area i Ofoten-Tysfjord
December 1995 - January 1996

	December 1995			January 1996		
Year class	**Diseased**	**Examined**	**% diseased**	**Diseased**	**Examined**	**% diseased**
1993-95	4	52	7.7	0	43	0
1990-92	21	1439	1.5	6	1712	0.4
1989-89	7	245	2.9	0	99	0
1984-87	1	26	3.8	1	26	3.8
1983 +	7	163	4.3	1	60	1.7
Total	**40**	**1925**	**2.1**	**8**	**1939**	**0.4**

Table 2.
Prevalence of *I. hoferi* disease in Norwegian spring spawning herring in the spawning areas February-March 1996.
For explanation of areas see map.
* indicates preliminary figures.

	Area I		Area II		Area III		Area IV		Area V	
Year-class	Diseased	Exam.	Diseased	Exam.	Diseased	Exam.	Diseased	Exam.	Diseased	Exam.
1993-95	0	8	0	2	0	0	0	11	0	2
1990-92	1	233	2	162	0	113	0	808	0	157
1989-89	0	2	0	15	0	24	1	70	0	15
1984-87	0	0	0	1	0	5	0	6	0	4
1983 +	0	2	0	30	0	42	1	97	0	18
Total	1	245	2	210	0	184	2	992	0	196
Biomass*		1373		652		529		2089		160
Sp. biom*		661		598		493		2017		151

Totals:

Diseased:	5
Examined:	1970
Percent diseased:	0.25

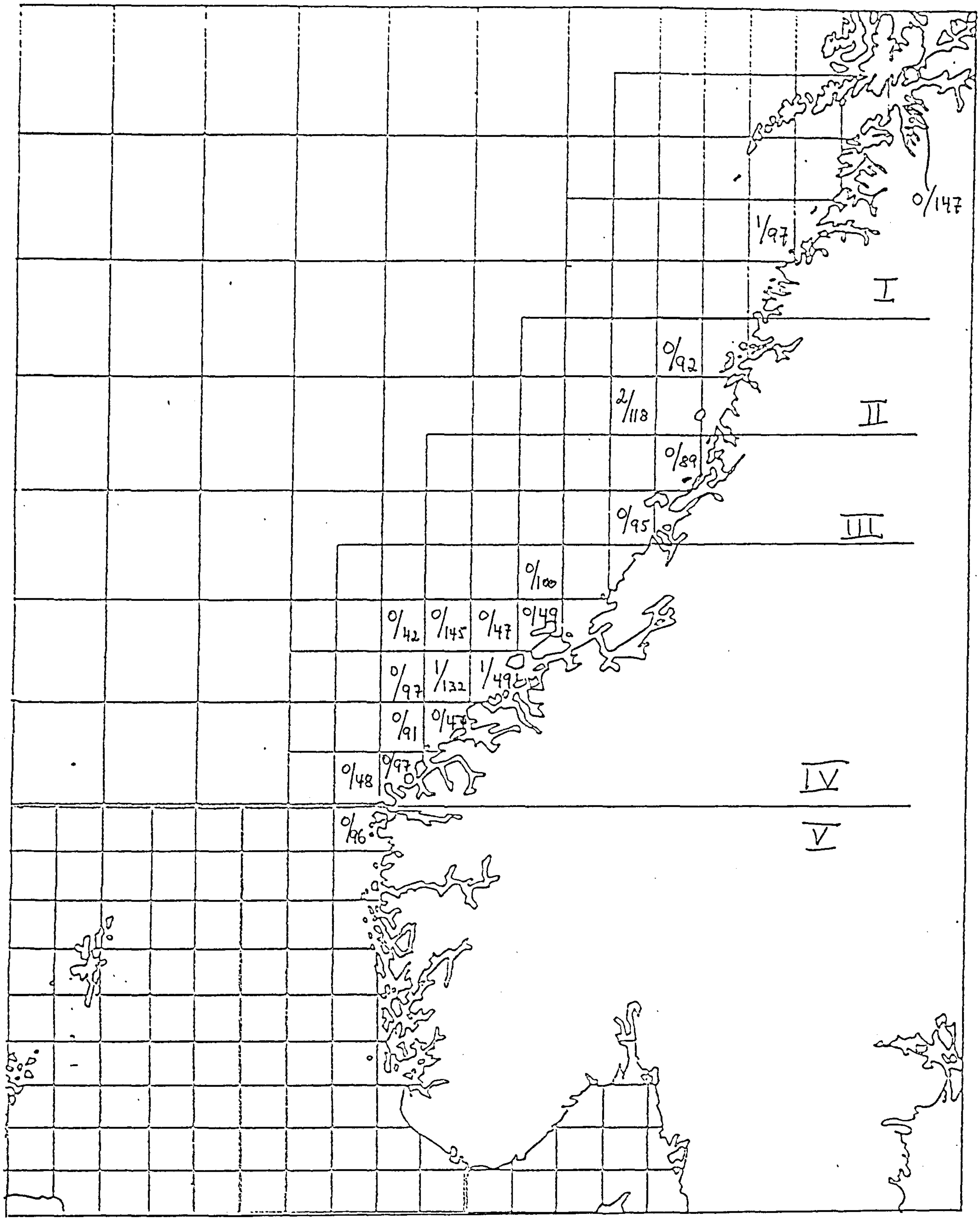


Figure 1
Prevalence (No. diseased/No. examined) of *I. hoferi* disease in Norwegian spring spawning herring. R/V 'Michael Sars', February-March 1996.

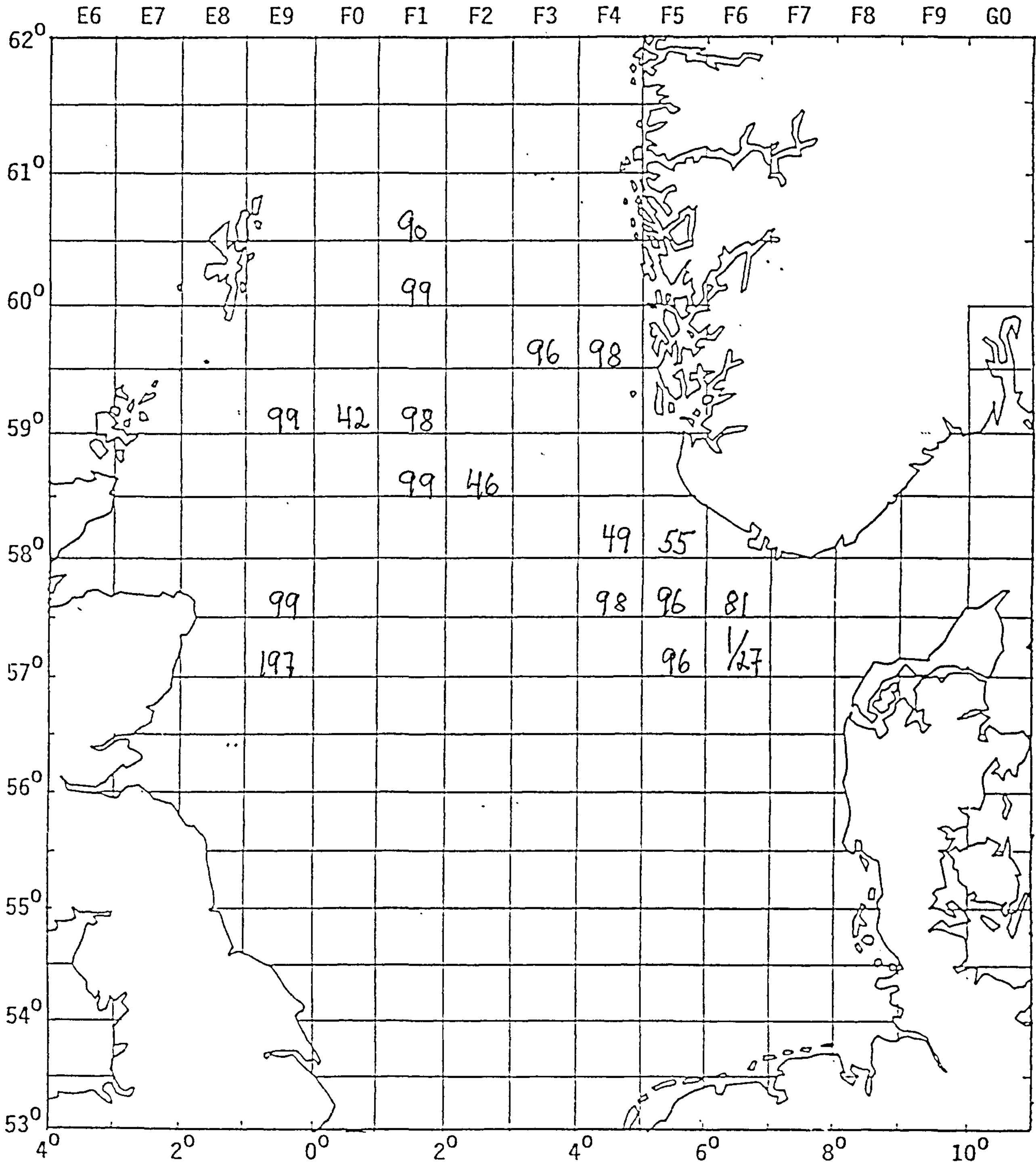


Figure 2
No. examined for *I. hoferi* disease in North Sea herring.
R/V 'G.O. Sars', June-July 1995
Disease was only found in one herring, in square 43F6.

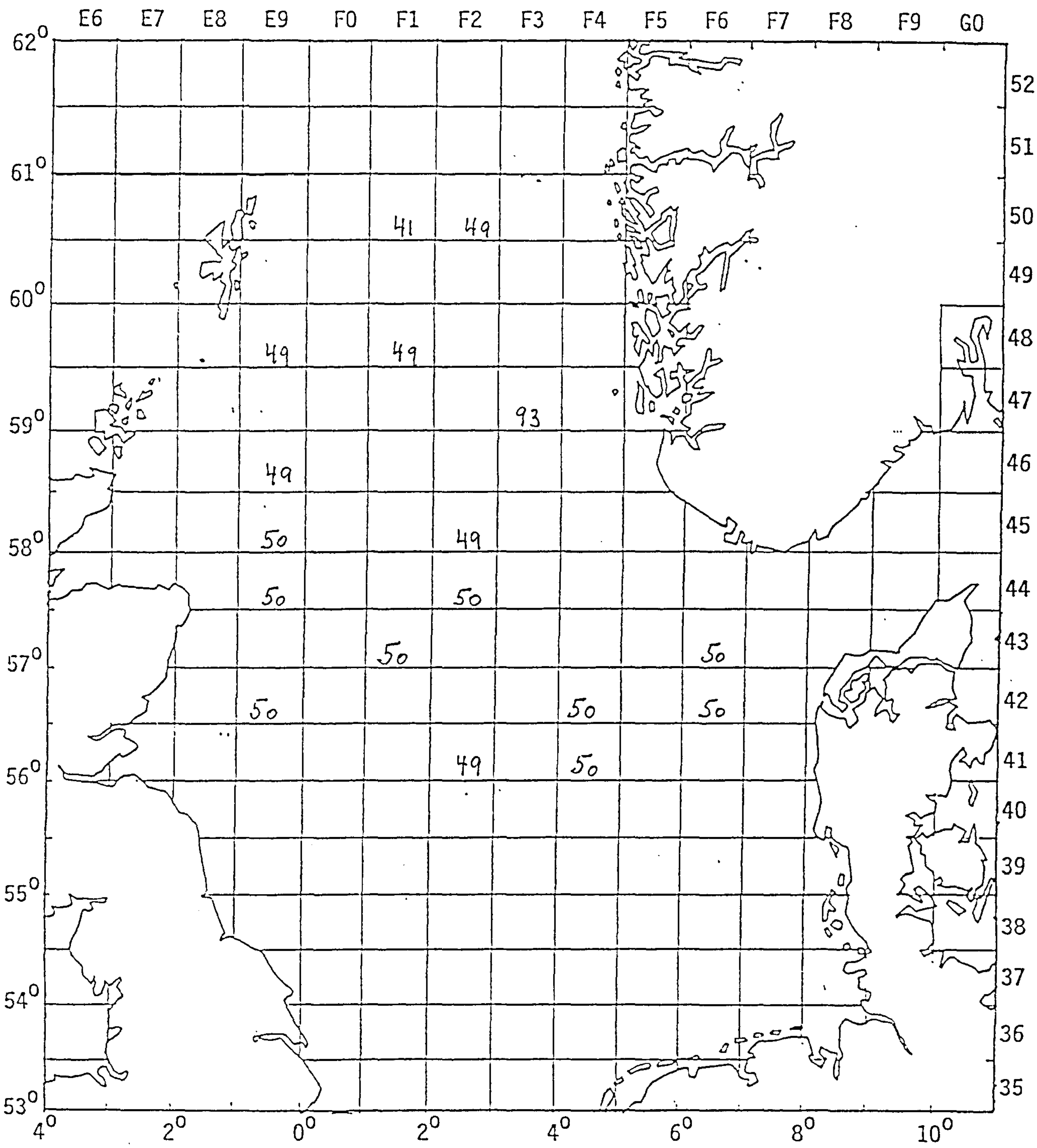


Figure 3
No. examined for *I. hoferi* disease in North Sea herring.
R/V 'G.O. Sars', October-November 1995
No diseased herring was found.

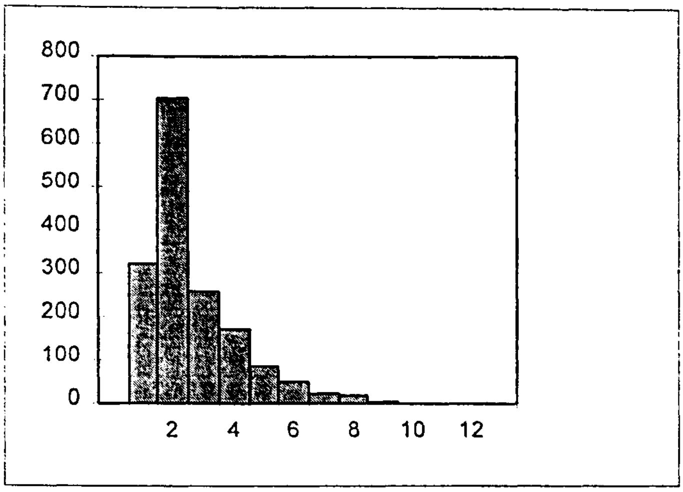


Figure 4.
Age distribution (winter rings) of herring examined for *I. hoferi* disease
R/V 'G.O. Sars', June-July 1995
Disease was only found in one herring, aged 2.

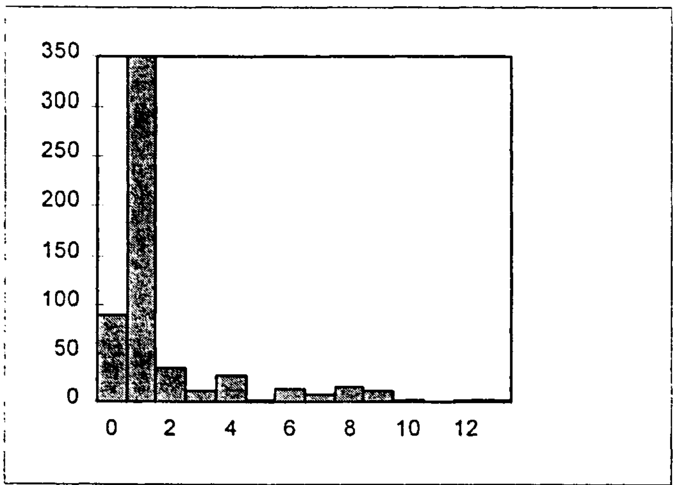


Figure 5.
Age distribution (winter rings) of herring examined for *I. hoferi* disease
R/V 'G.O. Sars', October-November 1995

ANNEX 15d

ICES TERM OF REFERENCE 95:29.K

Prevalence of *Ichthyophonus* in Scottish waters during 1995.

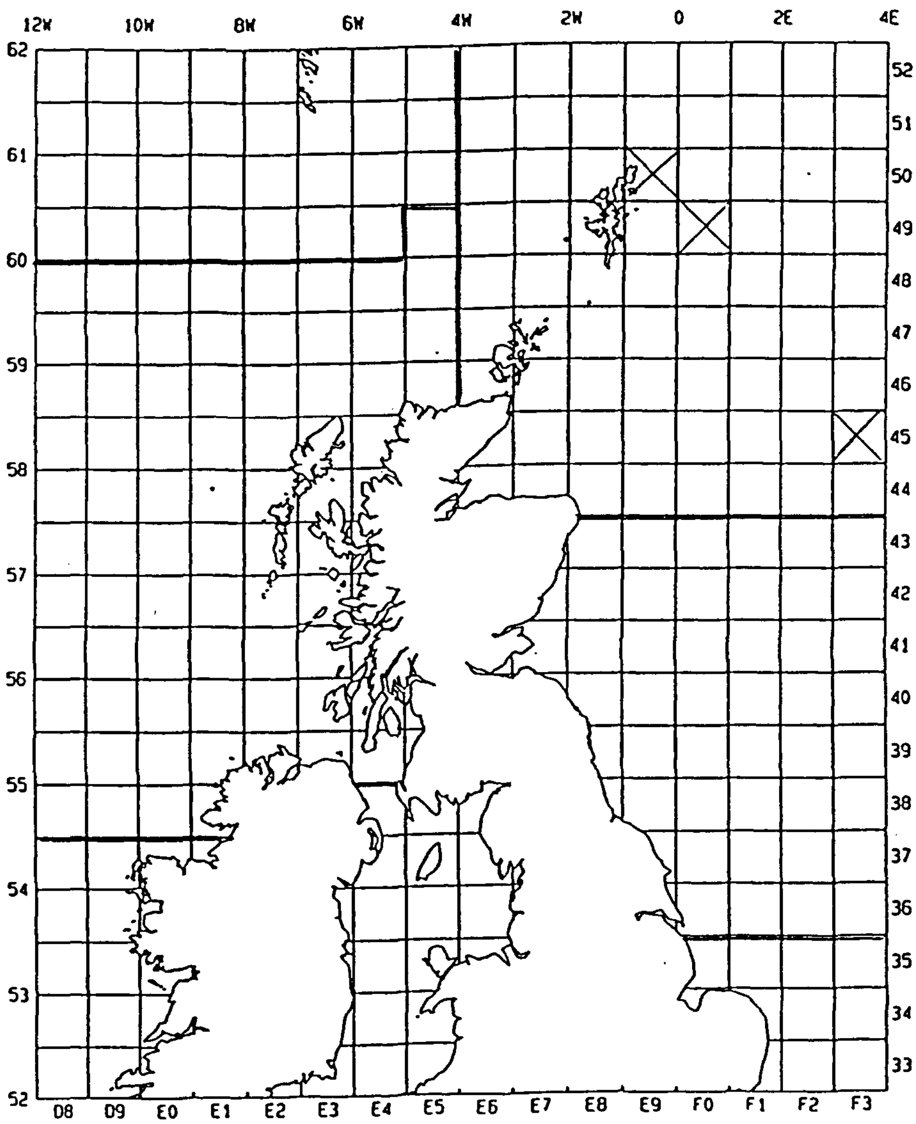
A H McVicar
SOAEFD Marine Laboratory, Aberdeen.

RESEARCH VESSEL CATCHES - MACROSCOPIC EXAMINATION OF HEARTS

(X = statistical rectangle with infection)

area IV a

month	infected
02	0/274
03	0/245
05	5/538
07	6/2721
08	0/527



area VI a

month	infected
03	0/1272
07	0/1884

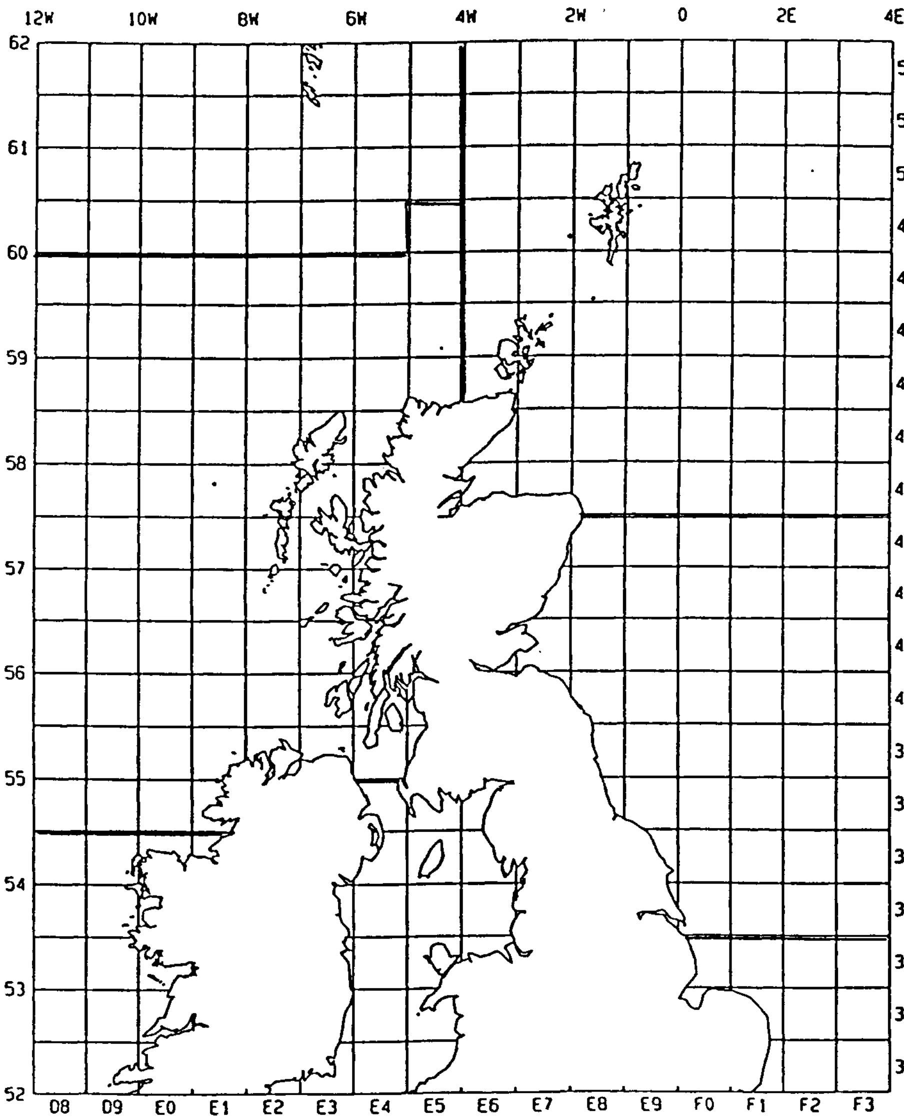
area IV b

month	infected
02	0/409
05	0/1066
08	0/729

COMMERCIAL VESSEL CATCHES - MACROSCOPIC EXAMINATION OF HEARTS

area VI a

month	infected
01 - 03	0/163
04 - 06	0/206
07 - 09	0/783
10 - 12	0/304



area IV a

month	infected
01 - 03	-
04 - 06	0/639
07 - 09	4/2566
10 -12	0/1006

area IV b

month	infected
01 - 03	-
04 - 06	0/147
07 - 09	4/963
10 - 12	-

CONCLUSIONS

Ichthyophonus was still present in the herring population in Scottish waters in 1995, but again limited to Areas IVa and IVb. Prevalence levels were low (<1%) in both research vessel and commercial catches. There was no evidence of an epidemic situation and it was concluded that the disease was present at only background levels.

Dr J Pawlak
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Demnark

144/11

Dear Janet

PROPOSED ICES QUALITY STATUS REPORT ON THE MARINE ENVIRONMENT

1. The following is an agreed statement from WGPDMO on the above topic:

"WGPDMO, having considered ICES C.Res.1995/2:29 (l) by correspondence, recommends
(a) that an ICES QSR would be a useful opportunity for a regular interdisciplinary overview of changes in the state of the marine environment and
(b) that fish disease data should be included.
Relevant disease data are available and these to be used should be closely linked to a definitive statement of the objectives of the QSR. The frequency of the Report should be considered in relation to the availability of resources and the scope and quality of the Report."

2. In summary, the following main points were raised in discussion:

a) Feasibility and participation by WGPDMO.
The opportunity for a regular interdisciplinary overview of changes of the state of the marine environment to be used by ICES customers and others involved in marine research and utilisation was considered useful. There was general agreement that fish disease data should be included in any proposed Environmental Review Reports. There is a preference that this should be collated through ICES (WGPDMO) and not through individual country submissions.
b) Nature of disease data.
The potential for incorporating fish disease data into environmental assessment studies has been considered by WGPDMO on several occasions in the recent past and the recommendation that such data are useful included in the WG Reports. Comments on the nature of disease data which could be used in an ICES QSR included:

- annotated presence-absence maps,
- written reports on new trends particularly WGPDMO assessment of trends and patterns using the ICES disease data base
- Changes in the distribution and occurrence of parasites with complex life cycles reflecting variations in sensitivities shown by different parasite species, parasite stages, intermediate hosts and final hosts to various environmental parameters.

Strong emphasis was placed on the need for a clear definition of the objectives of any Status Report to enable the most appropriate selection or targeting of disease data.
c) Integration of data.
Without information on the nature and detail of data being submitted from other fields it is not clear how disease data may be integrated into a Status Report. Close co-operation between WG PDMO, ACME (and ACFM if fishery data is to be included) and ICES staff will be necessary during the planning and compilation stages.
d) Frequency of Report.
Concern was expressed that an annual Report would be over-ambitious and that the resource demands would detract from the quality of the product; a well planned report every second or

third year was considered preferable. The lessons learned during the production of the NSTF QSR should be carefully evaluated. The scope of the Status Report should be closely linked to objectives which are clearly defined at the outset; concern was expressed at the dangers of such projects expanding through inadequate control after their initiation.

Yours sincerely

Alasdair H McVicar

ANNEX 17

1996 Report of the ICES Working Group on Pathology and Diseases of Marine Organisms

ICES *ad hoc* Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants

22-25 October 1996, Weymouth, U.K.

Preliminary Programme

22 October - "State of the Art"

am:
1. Welcome address and general introduction
2. Overview of available histological, cytological and molecular techniques and their field applications
3. Overview of European epidemiological studies
4. Overview - US perspective

pm:
5. Reports on national activities
6. Summary of current status

23 October - " Techniques, Pros and Cons"

am:
1. Techniques for the preparation of tissue for histopathology and electron microscopy - advantages, disadvantages and limitations
2. Cytological and molecular techniques advantages, disadvantages and limitations
3. Practical demonstrations

pm:
4. Practical workshop - evaluation of submitted material
5. General discussion - identification of problem areas

24 October - "International Calibration and Standardization"

am:
1. Discussion on standardization of methodologies, formulation of procedures to be adopted, including quality assurance requirements
2. Discussion on standardization of diagnostic criteria for histopathology, procedures to fulfil quality assurance requirements

pm:
3. Practical assessment and intercalibration of recommended procedures using sets of standard material
4. Preparation of the meeting report

25 October - "Conclusions and Recommendations"

am:
1. Conclusions and recommendations for future research and monitoring programmes
2. Finalisation and adoption of the report
3. Closing of the meeting

ANNEX 20a

Report on

the issues involved with *Gyrodactylus salaris*

There is today an abundant documentation on *G. salaris* and however no definite answer is given to the subsequent mean present problem which is :

"Taking into account the *G. salaris* story in Norway (Johnsen & Jensen, 1991) and the fact that the wild populations of salmon parr have been severely reduced after the introduction of the parasite in this country,
what should be done for preventing similar losses in countries (and particularly in the Bristish Isles), in the rivers of which there is abundant susceptible (?) wild salmon populations (McKenzie, 1995) and where *G. salaris* is today possibly absent in wild or farmed salmon or other salmonid species ? "

According to the OIE International Aquatic Animal Health Code (1995),

Article 1.5.5.1.

1. Any *importing country* should only accept into its territory, live *aquatic animals* which have been subjected to examination by a member of the *Personnel of the Competent Authority* of the *exporting country* or a *certifying official* approved by the importing country, and which are accompanied by an *international aquatic animal health certificate*.

3. Any importing country may prohibit the introduction into its territory of aquatic animals when the exporting country is considered to harbour or contain a *disease/disease agent* of concern to the importing country and capable of being transmitted to its own stock of aquatic animals, unless the aquatic animals are derived from an *aquaculture establishment/zone* with equal or better disease status for the disease in question than the zone they will be introduced to

According to the U.E. regulations, the Council directive 91/67, the most important diseases of aquatic animals are **classified (decreasing importance)** in 3 lists : I, II, III

Article 7

1. The placing on the market of live fish belonging to the susceptible species referred to in Annex A, column 2 of lists I and II, their eggs or gametes, shall be subject to the following additional guarantees:

(a) where they are to be introduced into an approved zone, they must, in accordance with Article 11, be accompanied bv a movement document corresponding to the model set out in Annex E, Chapter 1 or 2, certifying that they come from an approved zone or an approved farm. Pending the outcome of the review provided for in Article 28, additional guarantees to be met for the introduction into an approved zone of fish coming from an approved farm situated in a non-approved zone shall be fixed in accordance with the procedure laid down in Article 26. Pending that decision, national rules shall continue to apply subject to compliance with the general provisions of the Treaty;

Article 14

1. Without prejudice to the requirements for diseases referred to in Annex A, column 1 of list III, established in accordance with the procedure laid down in Articles 12 and 13, the placing on the market of live farmed fish (molluscs or crustaceans) not belonging to the susceptible species referred to in Annex A, column 2 of lists I and II as well as their eggs and gametes shall be subject to the following additional requirements:

(a) where they are to be introduced into an approved zone, they must be accompanied in accordance with Article 11 by a movement document corresponding to the model to be drawn up in accordance with the procedure laid down in Article 26, certifying that they come from a zone of the same health status, from an approved farm in a non-approved zone or from a farm which may be situated in a non-approved zone on condition that such a farm contains no fish, molluscs or crustaceans belonging to the susceptible species referred to in Annex A, column 2 of lists I and II and is not connected with a watercourse or with coastal or estuarial waters.

However, Gyrodactylosis due to *G. salaris* does not appear today in the O.I.E. notifiable or significant diseases lists or in the E.U. lists. Presently, these 2 organisms are considering the possibility for including *G. salaris* in these lists. The question for WGPDMO thus is to give its advice about.

A number of points must be taken into account :

- Apart some countries where the presence of the parasite has been proved, the repartition of the parasite in Europ is badly known. Also it is not to day sure that *G. salaris* is absent in certain countries or zones, e.g; in British Isles.

- The identification of the parasite is still difficult and essentially based on morphology, even if new methods will perhaps be available in the next years. Consequently, the demonstration of a free status needs presently a huge effort while the benefit of it, in most European countries where Atlantic salmon is only present in small quantities and does not appear (neither other salmonids) to suffer of the parasite, is reduced.

- If the transmission of the parasite and its pathogenic effects are known for some large populations of salmon and other salmonids, it is also well known that there are genetic adaptations and that populations can be more or less susceptible. Extrinsec factors could also intervene.

- If not susceptible fish species (carpids, eels, etc...) and also eggs or genital products can be potential vectors for the parasite, the related risk is badly known, and we cannot to day say how an eventual regulation can take it into account.

For all these reasons but also considering the risk for eventually uninfested British Isles (or a part of them) the WGPDMO advises that :

- a preliminary 1 or 2 years survey should be made in all European countries for a better understanding of the geographic repartition of the parasite.

- a transient status (2 years) of an *a priori* free zone should be given for British Isles (or a part of them) prohibiting the introduction of living salmonids (and potential carrier fish species) from *a priori* infested other European countries. During this transitory period, a survey should be done for trying to prove that the *a priori* free zone is actually free of *G. salaris*.

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ANNEX 20b

WGPDMO 1996 Agenda Item 20

Notifiable status of *Gyrodactylus salaris*

Alasdair H McVicar, SOAEFD MLA, Aberdeen.

1. Term of Reference.

ICES Council Resolution 1995:2:29.p stated:
"Advise on the Organisation Internationale Epizootic (OIE) and EU listing on the status of the parasite *Gyrodactylus salaris* and report to the Advisory Committee on Fishery Management."

2. Current listing.

a) OIE.

The Report of the Meeting of the Fish Diseases Commission of the OIE in Paris on 20 - 22 November 1995, Section 2 is published as follows:

" 2. International Aquatic Animal Health Code and Diagnostic Manual for Aquatic Animal Diseases.

> 2.1 Modifications to the International Aquatic Animal Animal Health Code - Possible deletions and additions to the listed diseases.
>
> Gyrodactylosis and white spot disease were considered for addition to the International Aquatic Animal Animal Health Code (the Code). Both diseases are serious. It was proposed to place them on the list of other significant diseases and to prepare relevant texts and chapters in the Code and Diagnostic Manual for Aquatic Animal Diseases (the Manual). No deletions of diseases were proposed."

Draft texts for Chapter 3.1.9 for the OIE Code on "Gyrodactylosis" and Chapter XX for the OIE Manual on "Gyrodactylosis of Atlantic Salmon" were included in the Report of the meeting. Relevant pages of the OIE Meeting Report are appended.

b) EU.

In Council Directive 91/67/EEC "concerning the animal health conditions governing the placing on the market of aquaculture animals and products" *Gyrodactylus salaris* is included as a List III disease of fish. Susceptible species are still to be specified under the terms of Articles of the Directive. In addition, the EU regulations providing measures to prevent the introduction and spread of the disease need to be formulated and until they are, national control regulations are followed.
During 1995 and 1996 extensive discussion have taken place at the EU Private Experts Meeting (Scientific Veterinary Committee: Fish Disease Sub-group) who are now extensively considering the listed status of *G salaris* and whether additional controls may be necessary to prevent spread of the disease.

3. Distribution.

Records exist in the literature for the occurrence of *G salaris* in the following EC countries: Sweden, Finland, Denmark, Germany and Spain and the parasite has also been recently recorded from Portugal. *G salaris* is absent from the UK. Various salmonids are susceptible to infection and it is probable that the movement of live rainbow trout associated with aquaculture has contributed to the spread of the parasite. The parasite is restricted to freshwater but because of the severe effects on Atlantic salmon populations as seen in western Norway after introduction of the disease, there are serious implications to the abundance of all stages of salmon including sea caught fish. NASCO has raised this as an issue of concern with OIE, EC and ICES.

4. Diagnosis.

Experts agree that *G salaris* is a separate species which can be identified through features of attachment hook morphology. Closely related species may show closely similar characteristics which without proper care could be confused. The molecular genetic approach is being developed and is now showing considerable potential as a definitive and practical specific diagnostic too (Cunningham *et al* (1995) J Fish Disease, **18** , 539 - 544). A meeting of invited participants has been organised by the EC to familiarise experts in the Community with these methods in the species identification of *G salaris* in Oulu, Finland from 13 - 17 April 1996.

5. Pathogenicity.

Experiences with wild and experimental populations of Norwegian Atlantic salmon and with experimental populations of Scottish salmon have demonstrated the serious pathogenicity which *G salaris* shows to salmon (Bakke & MacKenzie (1993) Fish. Res. **17**, 69-86). Other species of salmonid can become infected and transmit the disease but the pathogenicity is lower in these.

ANNEX 23a

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS 1996: PROGRESS WITH TASKS

(i) Analysis of national reports on new diseases and disease trends in wild fish, crustaceans and molluscs. Reports of new diseases, new geographic distribution and trends in diseases in wild marine organisms were evaluated from national reports and conclusions presented.

(ii) Analysis of national reports on new diseases and disease trends in mariculture and provide advice on preventative control measures. Reports of new diseases, new geographic distribution and trends in diseases in mariculture animals were evaluated from national reports and conclusions presented.

(iii) Evaluate the Sub-Group Report on the Statistical Analysis of Fish Disease Data. The draft Report of the Sub-Group (ICES CM 1996 F:3) was discussed and the conclusions noted. With the completion of the main Terms of Reference of the Sub-Group, the recommendation for the transfer of the work in that area back into the Terms of Reference of WGPDMO was noted and the identified intersessional tasks accepted by WGPDMO for action.

(iv) Evaluate information on distinguishing between different finfish viruses. A full review of data on this topic was not completed but useful summary statements of existing problems were received and are included as Annexes. Advice was given on the the designation of common names to fish viruses.

(v) Review data on disease resistance in oysters. The data contained in a prepared review paper included here as Annex 9 were evaluated and advice given on problems with tolerance of disease and the transfer of disease in bivalve molluscs. It was recommended that WGITMO consider risks of disease transfer in view of the conclusions reached.

(vi) Review information on cross reactivity of a DNA probe against MSX and *Haplosporidium* sp. Data available were considered and advice presented on the use of appropriate protocols to enhance clarification in this area.

(vii) Review information on the geographic distribution of JOD in *C virginica.* With the current problems in identification of the causitive agent of the disease, the accurate geographic distribution of the disease can not be determined.

viii) Review of experimental data on transmission of *Bonamia* and JOD. Through assessment of available data on the multi host specificity of significant pathogens of bivalve molluscs, the WG gave advice on which pathogens pose significant risk of transmission with shellfish stocks and advocated use of established Diseases Management protocols.

ix) Evaluate a draft format for a registry of molluscan disease data. Draft data entry sheets were developed and WGPDMO members asked to evaluate their use in the field before the next WG meeting.

x) Review of new information on antibiotic sensitivity of fish pathogenic bacteria.
Following the highlighting of this problem by the WGPDMO the European Association of Fish Pathologists have taken an initiative and, as an organisation, are actively undertaking an international exercise to co-ordinate methodology in this area. It was concluded that no further action is currently required by the WGPDMO in this area.

xi) Overview of new information on the M-74 syndrome and *Ichthyophonus*.
New information on the M74 syndrome did not yet offer a clear explanation for the condition and advice was given on the need to determine if there was any occurrence in European waters outside the Baltic. *Ichthyophonus* was still being reported in several stocks of herring but it was advised the prevalence levels were considered to be at background levels.

xii) Possible WGPDMO contribution to an Environmental Status Report.
A Report was submitted intersessionally to ICES to meet the required deadline.

xiii) Review progress on ICES Special Meeting on flatfish liver pathology.
Progress on the planning of this meeting was satisfactory and an outline programme proposed.

xiv) Possible WGPDMO contribution to ICES/NASCO Symposium on possible farmed-wild salmon interaction.
The Chairman of WGPDMO had submitted a paper on possible disease-related topics intersessionally to one of the convenors and the contents of this were endorsed by the WG. An outline programme was tabled.

xv) ICES Annual Science Conference, Special Topic on Hygiene in Mariculture.
A tentative agenda for the Special Topic Session was proposed. It was recommended that the Session be prosponed until the 1977 Annual Science Conference.

xvi) Legislative control status of *Gyrodactylus salaris.*
Advice was given in the Report on the risks and potentially serious consequences of the spread of this parasite to areas currently free of infection and that appropriate controls to prevent spread should be used.

ANNEX 23b

ACTION LIST

The Working Group on Pathology and Diseases of Marine Organisms:

a) urges members to encourage research on new viral diseases, particularly noda-like viruses.

b) urges ICES to encourage member countries to submit reports on fish and shellfish disease status and trends to WGPDMO even when no representative attends.

c) urges members to update the disease status of Scandinavian oysters

d) urges ICES to encourage improvements in the specificity of disease diagnosis in farmed bivalve shellfish.

e) urges ICES to recommend that Member countries submit current and historic fish disease data using the ICES Fish Disease Entry Programme, where possible, to ICES by 31 December 1996 and annually thereafter at the end of each year.

f) recommends that an *ad hoc* meeting between nominated members of WGPDMO and ICES staff should take place to analyse the extended fish disease data base before the next WGPDMO meeting.

g) urges ICES to recommend to Member countries that the formal naming of viruses should follow international rules of nomenclature while the description of the disease should be used only for the disease.

h) urges ICES to encourage Member countries to undertake studies on *Bonamia* persistence in low oyster densities.

i) urges WGITMO to assess the risk of shellfish disease transfer with stocks tolerant to these diseases.

j) urges members to undertake MSX transmission experiments with infected *C. gigas* and healthy *C. virginica* and to determine the potential carrier status of *C. gigas*.

k) urges ICES to recommend to Member countries that standardised protocols are used to define the risk of transmission of MSX and JOD.

l) urges ICES to recommend continued investigation into the cause(s) of JOD in *C. virginica*.

m) urges members to collect data on shellfish diseases using the draft ICES Shellfish Disease Reporting Form and to include pathogen distribution maps.

m) urges ICES to undertake appropriate studies to determine if the M74 syndrome exists outside the Baltic area.

n) urges ICES to recommend to Member countries that such measures as are available should be used to limit the risk of spread of *Gyrodactylus salaris* into areas where there is currently some evidence of its absence.

o) requests a relevant member (S McGladdery) to obtain information on details of the proposed mini-Symposium on "Identifying and Managing Diseases of Bivalve Shellfish" and to circulate this information to WGPDMO.

p) urges the Chairman to request ICES to provide clarification on restrictions in access to, and protection of, data held in the fish disease databank.

ANNEX 24

RECOMMENDATIONS TO COUNCIL

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

ICES Headquarters, Copenhagen, Denmark, 21–26 March 1996

Recommendations

1 The ICES WGPDMO recommends that it meet at Rhode Island (USA) under the Chairmanship of A. H. McVicar from 18–22 March 1997:

a) to analyze national reports on new disease trends in wild fish, crustaceans and molluscs;

b) to analyze national reports on new disease trends in mariculture for fish and shellfish;

c) intersessional work on the selection and use of methods to analyze the newly extended ICES Fish Disease Database has been undertaken by a Study Group. The results of this activity need to be interpreted, particularly in relation to temporal and spatial distributions of fish diseases.

d) The Report and recommendations of the ICES Special Meeting on the "The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" should be reviewed and further action as appropriate will be considered.

e) to review the results of the BMB/ICES Sea-going Workshop "Fish Diseases and Parasites in the Baltic Sea" in the light of their possible implications for future research/monitoring programmes in the Baltic Sea;

f) to maintain an overview of the M-74 syndrome and the *Ichthyophonus* issue as part of the regular agenda of the WGPDMO;

g) to review current information on the impact of environmental contaminants on the immune mechanisms of marine finfish and shellfish,

h) to review available information on diseases in marine fish associated with nodavirus or nodavirus-like agents to provide a basis for recommendations for future research.;

i) to review information on the use of correlation between environment and pathology (ecopathology) to provide advice of possible alternative measures for disease control in farmed fish and shellfish;

j) to assess the progress in the identification of alternate host species for the transmission of bivalve pathogens, including the development and use of sensitive detection tools;

k) to assess the progress in studies on the possible causes of *Bonamia ostreae* persistence in areas with low densities of European oysters and the possible relationship to disease tolerance status;

l) to compile specific requirements of shellfish pathology laboratories for recording shellfish disease data and evaluate the applicability of the ICES Fish Disease Data Entry Program (FDE) and the Fish Disease Reporting Format.

2 The WGPDMO recommends that a Study Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks will be established under the chairmanship of Dr A. D. Vethaak, and including S. Mellergaard (Denmark), and W. Wosniok (Germany) and will meet at ICES Headquarters from 6–7 February 1997 to select and use methods to analyze the newly extended ICES Fish Disease Database to provide data particularly on temporal and spatial distributions of fish diseases.