Mariculture Committee

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

La Tremblade, France

3–7 April 1995

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International Council for the Exploration of the Sea

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1 INTRODUCTION

The Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the IFREMER Laboratory, La Tremblade, France with Dr A H McVicar presiding as Chairman (Council Resolution 1994/2:23).

1.1 Opening of the Meeting and Structure of the Meeting

The meeting was opened at 09.00 on Monday 3 April 1995 with the Chairman welcoming participants, particularly those new to WGPDMO. Dr H Grizel, Director of the IFREMER Laboratory at La Tremblade welcomed the participants and gave a short resume of the function and history of the Laboratory.

A List of participants is appended in Annex 1a.

2 ICES 1994 ANNUAL SCIENCE CONFERENCE (82nd STATUTORY MEETING); ITEMS OF RELEVANCE TO WGPDMO

Items of relevance to WGPDMO from the ICES Annual Science Conference held in St John's, Newfoundland, Canada in 1994 were highlighted by the Chairman.

a) The Report of the Mariculture Committee particularly the proposed Theme Sessions for the 1995 Annual Science Conference, the proposed Special Topics for 1996 and the proposed joint Symposium with ANACAT for 1997, the presentation of the WGPDMO 1994 Report and the Report of the Sub Group on the Statistical Analysis of Fish Disease Data and the report of the Special Topics Session on "Parasites in Mariculture".

b) The MEQC Report and its references to the use of liver, gill and kidney histopathology in relation to biological effects available for monitoring, the M74 Study Group report and the reference on the paper on X-cell (1994\ E:12). With reference to the latter, the statement that "The paper considered whether these lesions may be related to the presence of anthropogenic contaminants "was considered to be misleading as this was not specifically considered by the paper.

c) Reference to the papers presented on *Hematodinium* in Scottish stocks of *Nephrops*.

d) Reference to the greater co-operation which would be worthwhile between the Study Groups of ANACAT and WGPDMO when fish disease matters were being considered.

e) Reference to Consultative Committee Report in which it was noted that the Mariculture Chairmen would be exploring ways of improving liaison with international aquaculture organisations (WAS, EIFAC) and ways in which ICES could respond in a fast and flexible way to disease issues in fish populations e.g., *Ichthyophonus* through the provision of advice from WGPDMO by the addition of a term of reference.

Items of outstanding business from the 1994 WGPDMO Report were briefly referred to and it was noted that most of these did appear for consideration and further development in the current year's terms of reference.

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

The terms of reference as published in C.Res 1993/2:23 were detailed. The heavy agenda load, with several items being added to the terms of reference during the Statutory Meeting in St John's, was commented on. This necessitated extensive intersessional work by members of the WGPDMO identified by the Chairman as expert in specific areas; these were requested to produce written discussion documents 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 Working Group on Pathology and Diseases of Marine Organisms (Chairman: Dr A. McVicar, UK) will meet in La Tremblade France, from 3-7 April 1995 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 Statistical Analysis of Fish Disease Data;

d) evaluate the results of the proposed intersessional study on antibiotics aimed at establishing correlations between zones of inhibition and minimal inhibitory concentrations (MIC) or minimal bactericidal concentration (MBC);

e) review the current research in sea lice treatment and control methods including: chemical, biological, immunological and management practices;

f) review the research on pathology and diseases in fish larvae reared in mariculture;

g) review the report of the BMB WG 25 "Fish Diseases and Parasites in the Baltic Sea" on the results of the "BMB/ICES Workshop on Diseases and Parasites of Flounder in the Baltic Sea" and the "BMB/ICES Sea-going Workshop on Fish Diseases and Parasites in the Baltic Sea" scheduled for late 1994 as soon as it becomes available, and report to ACME (HELCOM 6);

h) consider the role of flat oysters (*Ostrea edulis*), Olympia oysters (*Ostreola conchaphilia*), and American oysters (*C virginica*) as carriers of Denman Island Disease (*Mycrocytos mackini*) from Pacific oysters and evaluate the possibility that reciprocal transfers of *M mackini* may occur among the four oyster species;

i) consider disease data sets currently used in shellfish pathology laboratories and to recommend means of standardisation of disease records for use at the national level and by ICES;

j) review current monitoring procedures for detecting the prevalence of mollusc pathogens and to develop recommendations for appropriate protocols (sample size and techniques) for such surveys;

k) assess the usefulness of external fish diseases as a tool in the monitoring of biological effects of contaminants, and report to ACME;

l) review available information on fish diseases in the Baltic Sea and their possible impact on mortality of Baltic fish stocks and to prepare in co-operation with the Chairman of the Baltic Fish and the Baltic Marine Biologists (WG 25 "Fish Diseases and Parasites in the Baltic Sea") a report on this item to be included in the HELCOM Third Periodic Assessment (ACME and ACFM) (HELCOM 7);

m) maintain an overview of the M-74 syndrome and the *Ichthyophonus* issue as a part of its regular agenda and report to ACME if new information becomes available (HELCOM 12);

n) review, as a matter of urgency, the present status of *Ichthyophonus* on herring populations and to investigate problems with supplying data on its prevalence;

o) provide advice on methods to handle data in rapid response to disease issues in fish stocks.

3.2 Adoption of Agenda and Selection of Rapporteurs

An agenda was agreed upon (Annex 3a), expert discussion leaders were identified and rapporteurs were appointed (Annex 3b).

4 OTHER RELEVANT REPORTS FOR INFORMATION

Information relevant to the WGPDMO is presented at the following scientific meetings:

- Relevant parts of the September 1994 ACME Report were summarised for the WG. These included the references to M-74, its possible causative factors and proposed research; the occurrence of *Ichthyophonus* in herring studies on fish diseases in the Baltic Sea; types of studies for elucidation of possible relationships between fish diseases and pollution; statistical analysis of fish disease data. The extensive use made by ACME of the WGPDMO 1994 report was noted.

- International Workshop on Shell disease in Marine Invertebrates Environment-Host-Pathogen Interactions, 29–31 March 1995, Brest, France.

  A workshop detailing shell and exoskeleton disease characteristics was organised in response to two recently recognised molluscan diseases. The workshop concentrated on the mechanisms involved in producing conchiolin shell deposits laid down in response to Brown Ring Disease (BRD) in Manila clams (*Tapes philippinarum*) and Juvenile Oyster Disease (JOD) of American oysters (*Crassostrea virginica*). Additional shell deformities ("Pink mussel", *Mytilus galloprovincialis*, in the Adriatic Sea, disturbed mineralisation in pearl scallop shells, *Pinctada margaritifera*, and abnormal melanisation in *Pecten maximus*) were also discussed. Gross pathology and histopathology were compared between JOD, BRD and the other shell abnormalities. The problem of how to apply Koch's postulates to "syndrome" and opportunistic bacterial diseases was discussed in light of experimental transmission difficulties and the problem of diseases of undetermined aetiology. Haemolymph and haemocyte properties used for control of prokaryote infections and shell diseases were elucidated for the scallop *Pecten maximus*, the clams *T .philippinarum*, *T. decussatus*, the mussel *Mytilus galloprovincialis* and the oysters *Crassostrea gigas*. A possible host-defence role by haemocytes in the extrapallial fluid of clams was also presented. The specific host-parasite interaction between *Vibrio* P1 and clam tissues was discussed, including pathogen concentration via infection site selection, virulence factors associated with *Vibrio* P1 and the problems associated with development of an ELISA diagnostic kit for *Vibrio* P1. Development of molluscan disease (in relation to case histories for resistance against MSX and *Perkinsus marinus*) was also discussed as a probable protocol for disease management (even where the etiologic agent is unknown). The closing session concentrated on environment-host-pathogen.

interactions including: temperature on BRD, chemical pollutants (TBTM) and acid/alkaline conditions on *Anodonta cygnea* and modelling factors for determining long-term environmental changes on virulence of *Perkinsus marinus*. The effect of environmental degradation and intensive culture on shell disease in aquatic invertebrates were discussed and both were considered to have a significant role.

- The OIE International Conference on Preventing the Spread of Aquatic Animal Diseases Through International Trade to be held in Paris, France from 7–9 June 1995.

- The European Association of Fish Pathologists Seventh International Conference "Diseases of Fish and Shellfish" to be held in Palma de Mallorca, Spain from 10–15 September 1995.

All of these meetings contain items of direct relevance to WGPDMO, and WG members attending are encouraged to make known the views of WGPDMO, at the meetings.

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

Reports on new diseases and trends in diseases of wild and cultured marine fish, molluscs and crustaceans were received from Belgium, Canada (east and west coasts), Denmark, Estonia, Finland, France, Germany, Iceland, Netherlands, Norway, Portugal, Spain, UK England & Wales, UK Scotland, and USA (east and west coasts). Because of their bulk, these documents are not included in this Report but they may be obtained from WG members who attended the meeting. The main points highlighted in each national report are summarised below.

5.1 Wild Fish Diseases

Lymphocystis: In North Sea dab (*Limanda limanda*), no significant new trends in the disease prevalence were recorded.

In Chesapeake Bay striped bass (*Morone saxatilis*), the prevalence of lymphocystis appears to be increasing.
Epidermal hyperplasia/papilloma: In North Sea dab, no significant new trends in the prevalence were recorded.

Skin ulcerations: German and Scottish studies revealed an increasing prevalence in the central and northwestern North Sea since 1991.

Edwardsiella tarda: Epizootics have been observed in striped bass from Chesapeake Bay during summer associated with high water temperatures.

***Vibrio* sp.:** *Vibrio carchariae* and *V. vulnificus* were reported for the first time in striped bass of the Chesapeake Bay.

Abnormal skin pigmentations: Belgian studies on the occurrence of hypermelanisation in North Sea dab indicate that this condition may be epitheliocystis-like and caused by *Rickettsia* sp. However, more research is needed to clarify the aetiology. There is a general impression that the prevalence is increasing in the North Sea.

Liver nodules/tumours: German investigations have demonstrated that the decreasing trend in the prevalence of liver nodules >2 mm in dab from the German Bight previously recorded has continued in 1994.

A reduction of the prevalence of liver lesions in English sole (*Pleuronectes vetulus*) and brown bullhead catfish (*Ameirus nebulosus*) from US Pacific waters was observed following the closure of a creosote and a coking plant. Similar observations were made in a project to cap highly contaminated sediments with clean sediments.

Skeletal deformities: Belgian studies revealed an increasing prevalence of skeletal deformities in flatfish from the Belgian continental shelf compared to 1993.

VHS-like virus: The North American strain of VHS-like virus has been isolated from a fourth stock of herring (*Clupea harengus pallasi*) in British Columbia. Besides herring, the virus was isolated from tubesnouts (*Aulorhynchus flavidus*) and shiner perch (*Cymatogaster aggregata*) as well as from skin lesions of Pacific cod (*Gadus macrocephalus*), indicating that herring might not be the only fish that carry the virus.

Atlantic salmon (*Salmo salar*) proved entirely refractory to the virus in immersion challenges. However, after injection, classical VHS-signs appeared as well as mortalities.

Scottish virus isolates from cod (*Gadus morhua*) skin papules typical of the "Ulcus syndrome" sampled in 1993 were classified as VHS-virus. Work at the EU-reference laboratory suggests the cod isolates are very close to the European serotype I isolates from rainbow trout. Virulence tests with salmonids, turbot (*Psetta maximus*) and halibut (*Hippoglossus hippoglossus*) are under way.

IHN-virus: From experience with losses in farmed Atlantic salmon due to IHN-infections in British Columbia there is an indication that the IHN-virus may have a source in the wild marine fish fauna, because the smolts supplied to the marine fish farms originated from IHN-free systems. Young or adult sockeye salmon (*Oncorh-*

ynchus nerka) are also considered a potential source of the virus in penned Atlantic salmon.

Ichthyophonus **sp.**: Estonian investigations revealed the occurrence of granulomas caused by *Ichthyophonus* sp. in viviparous blenny (*Zoarces viviparus*) and in sea scorpion species (*Myoxocephalus quadricornis, M. scorpius*). A decrease in the level of this disease in North Sea herring was noted in several national reports (see also 18.1)

Eimeria sardinae: In Baltic herring and sprat (*Sprattus sprattus*) in the Gulf of Finland and the Gulf of Riga, infestations are well established at high prevalences (80% and 65%, respectively).

Pleistophora mirandellae: Finnish studies have demonstrated the occurrence of gonadal malformations and severely reproductive disorders in roach (*Rutilus rutilus*) from brackish waters possibly associated with an infestation with *Pleistophora mirandellae*.

Kudoa **sp.**: In the southwest of France, *Kudoa* infection, a previous problem in sea trout (*Salmo trutta*), was not observed in 1994.

A single case of *Kudoa* sp. infection was reported for the first time in cod from the English Channel.

Anisakis **sp.**: In France, high prevalences (30%) were recorded in small anchovies (*Engraulis encrassicolus*), causing cases of human anisakiasis.

5.2 Molluscs and Crustaceans

Because of the less clear distinction between disease problems of wild and cultured molluscs and crustaceans, compared with fish and the nature of the research currently being undertaken, this topic was linked with agenda item 6 (6.2, 6.3).

5.3 Conclusions

The Working Group noted a general trend to reduce national monitoring programmes on diseases of wild fish.

Members of the WGPDMO are encouraged to collate information from all their national laboratories known to conduct research/monitoring on diseases of wild fish and report these to the WG. There are indications that such data are not always reported, particularly these from university researchers.

The implication of *Rikettsia* sp. in hypermelanisation in North Sea dab should be clarified.

The identification of marine rhabdoviruses (VHS and VHS-like) requires clarification using genetic and other appropriate techniques since North American isolates have been shown not to be pathogenic to salmonids (see 22.1).

5.4 Recommendations

The WGPDMO recommends that ICES encourages its member countries not to reduce national research/monitoring activities on the occurrence of diseases/parasites in wild finfish and shellfish in order to obtain current information on biological changes in the marine environment.

The WGPDMO recommends clarification of identification of fish viruses by alternative methods.

6 ANALYSIS OF NATIONAL REPORTS ON NEW DISEASES AND DISEASE TRENDS IN MARICULTURE

6.1 Finfish

a) Atlantic salmon (*Salmo salar*)

Bacterial problems

The significance of bacterial problems, especially typical furunculosis (*Aeromonas salmincida* subsp. *salmonicida*), continues to decline. As a result, the use of antibacterial compounds has been reduced to a minimum.

Pancreatic problems

Infectious Pancreatic Necrosis (IPN) associated problems in the immediate post smolt period are recognised as a major disease problem in both Scotland and Norway. No distinct trends were reported. IPNV was isolated from fish held in sea water net pens off the east coast of Canada indicating that future monitoring for this agent in Atlantic Canada is required. A subunit vaccine against IPNV has been developed and initial pilot studies appear encouraging.

In Scotland, Pancreatic Disease (PD) is the second most important farmed salmon disease problem. Reports from France suggest that the virus associated with the so-called "sleeping disease" might be related to PD virus.

Infectious Salmon Anaemia (ISA)

The disease is still limited to Norway. In 1994 only one new outbreak of this disease was diagnosed. A long-term cell line (SHK-1) supporting the replication of the ISA virus has been established. The virus has been isolated, and work to develop a diagnostic method is in progress. There are indications that rainbow trout can serve as carriers of ISA without showing pathology.

Sea lice (*Lepeophtheirius salmonis*)

Sea lice infestations remain the most important disease problem in Norway and Scotland and, in 1994, was the most important disease for salmon culture on the east coast of Canada.

Diseases of unknown origin

In Norway, there have been several reported cases of skeletal deformities in Atlantic salmon during the last few years. These deformities are observed in fish at 6 or more months in the sea. There is no obvious mortality associated with this condition and it is regarded mainly as a quality problem.

b) Other salmonids

No new disease trends were reported.

c) Sea bream (*Sparus aurata*) and Sea bass (*Dicentrarchus labrax*)

Pasteurella piscicida is considered the most important disease problem is these fish species, especially in young fish. Further work to develop an effective vaccine is urgently needed. Viral encephalitis in larvae is of major concern is some French hatcheries.

d) Turbot (*Scophthalmus maximus*)

During the last few years, infections with *Enterococcus*-like bacteria have been of concern for the fish farming industry because of long-lasting low mortalities and growth retardation. Trials with a vaccine have shown promising results.

In France, *Pasteurella piscicida* was isolated from turbot for the first time.

Viral Haemorrhagic Septicaemia (VHS) was detected in turbot stocks in a "pump-shore" farm on an island on the west coast of Scotland. The virus was serologically similar to the European strains of VHS. Emergency procedures specified by the EC for VHS-free zones were immediately put into effect. The virus was not detected in salmonid farms in the surrounding area, nor in wild fish in the vicinity and elsewhere in Scottish waters. The source of the virus is still uncertain but is believed to be a wild marine source.

6.2 Molluscs

Sarcoma of soft-shell clams (*Mya arenaria*), combined with adverse environmental conditions has effectively wiped out the clam fishery in Chesapeake Bay, US (90% mortality annually). Investigation of the disease over the last 10-15 years has revealed that the disease can be transmitted. A Retrovirus has been isolated and is suspected to be the causative agent.

A **Herpes-like virus** was reported for the fourth year in France from larval and juvenile oysters (*Crassostrea gigas*) from hatchery and nursery batches. In 1994, two juvenile oyster batches showed > 80% mortality and were destroyed. No *Herpes* cases were found in juveniles growing around the infected batches. *Herpes* virus was detected for the first time in 3 years in *O. edulis* larvae and spat (first described in *O. edulis* spat in 1991).

Nocardia bacteria were found in a sample of Pacific oysters (*Crassostrea gigas*) suffering abnormally high mortalities (30%). Digestive gland degeneration, similar to that reported from Pacific oysters suffering 90% mortality in Washington State and California, was also noted. The exact aetiology of the digestive gland degeneration is unknown.

Bonamia ostreae in flat oyster (*Ostrea edulis*) was reported for the first time from the Mediterranean, Bouin and Normandy. It is believed that stocks were infected following uninspected importations from enzootic areas elsewhere in France. A similar scenario is reported from Portugal. As a result, of the appearance of *B. ostreae*, the production of *O. edulis* in Portugal was stopped. Increased mortalities (53-72% Yerseke Bank (autumn); 29-90% Lake Grevelingen (spring)) have significantly reduced flat oyster populations in the Netherlands and the prevalence of *B. ostreae* has subsequently decreased (16% Yerseke Bank and 27% Lake Grevelingen). *Bonamia* continues to spread within the Solent and tributaries of the River Crouch in England.

Denman Island Disease (*Mikrocytos mackini*) was reported from *Crassostrea gigas* (Pacific oyster), *Ostrea edulis* (flat oyster), *Ostrea conchaphila* (Olympia oyster), *Crassostrea virginica* (American oyster) and *Patinopecten yessoensis* (Japanese scallop) from British Columbia, Canada. Experimental infection of all these species was achieved by inoculation. In addition, the oyster species were infected in the field by natural proximity exposure. Manila clams (*Tapes philippinarum*) were not infected by inoculation and appear refractive. Seven monoclonal antibodies showed positive immunofluorescence against naturally and experimentally infected oysters (American, European, Pacific and Olympia). No cross reactivity was found with *B. ostreae* or *M. refringens* from European oysters or *Bonamia* sp. from New Zealand Dredge oysters (*Tiostrea chilensis*).

***Marteilia* spp.** and *Marteilia refringens* were found in *Mytilus galloprovincialis* and *Ostrea edulis*, respectively. In France, *Marteilia refringens* was found for the first time in flat oysters transferred from Brittany to the Mediterranean (see *Bonamia*). Low prevalences only were detected. Mussels from Portugal were found to be infected by a *Marteilidae* species (40–80% prevalence, Jan-Feb.), but this is not believed to be responsible for the 30% mortalities observed. *Marteilia* sp. was observed in American oysters (*Crassostrea virginica*) during field acclimatisation experiments in Marennes-Oleron, France. The infected batches were destroyed. This constitutes an important new field-transmission record between oyster species.

Perkinsus marinus: levels decreased in Chesapeake Bay, US, following an increase over the last few years. The disease has spread to Long Island and Massachusetts but is not causing significant problems. The decreased infection level is believed to be due to heavier rainfall and decreased salinity compared to previous years.

Perkinsus **sp.** infection of clams (*Tapes decussatus, T. semidecussatus* and *Venerupis pulastra*) showed a significant increase over the past year, along both coasts of Spain (Mediterranean and Atlantic).

Haplosporidium nelsoni: MSX increased in Virginia in Chesapeake Bay in 1994, but decreased drastically off Maryland (20 uninfected sites to 1 infected site). The cross-reactivity of a gene-probe for MSX and a *Haplosporidium* sp. from *C. gigas* requires further investigation to determine the exact relationship between the parasite in the two oyster species and, thus, the carrier potential of *C. gigas* for MSX.

Brown Ring Disease (BRD) in Manila clams (*Ruditapes philippinarum*) was detected for the first time in a population of manila clams from Poole Harbour, England. Mortalities could not be attributed to BRD since several species of shellfish were dying of anoxia in the same area. *Vibrio* P1 has not yet been isolated from clams showing "brown ring" signs.

Juvenile Oyster Disease (JOD) in *Crassostrea virginica* continues to be investigated. Transmission to naive oysters was achieved using flow-through exposure experiments and proximity exposures in recirculated water. The geographic distribution along the eastcoast of the United States has spread from Maine to Long Island Sound, N.Y. and continues to be associated with hatchery-reared stock. It can be carried by oysters as small as 1 mm in length. Development of oyster strains resistant to JOD and other management strategies show promise in industrial recovery from this problem.

Summer Pacific Oyster Mortality ("SPOM") increased in major *Crassostrea gigas* producing areas in the Netherlands (20–40% first-time observation), certain areas of France (but for the first time in Brittany), Spain and northern Portugal (90% mortality observed for the first time in certain batches). Temperatures > 20–22°C over a period of several weeks were associated with the mortalities. Oysters showing fast growth and maturation appear more susceptible than slow-growing, non-maturing individuals. Such mortalities occur regularly in areas of high turbidity, at high temperatures and where there is a lack of food. Similar summer mortalities may have occurred in Irish stocks of *C. gigas*, however, these observations could not be confirmed due to the lack of a country report from Ireland.

Withering Syndrome of Black Abalone (*Haliotis cracherodii*) from California demonstrated the presence of a *Pseudoklossia*-like coccidian and a rickettsia-like agent, however, these do not appear directly correlated with the Withering Syndrome. The syndrome has now appeared in several other abalone species from the same area.

Polydora **spp.**: The Netherlands reported an increase in prevalence and damage by these shell boring polychaetes (20–30% in *C. gigas* and 4–12% in *O. edulis*) which is attributed to abnormally high summer temperatures.

6.3 Crustaceans

Hematodinium spp. (Bitter Crab Disease) in tanner crabs *Chionoecetes bairdi* is increasing its range around southeastern Alaska reaching 90–100% in smaller embayments. The Bering Sea fishery has shown no changes from normal prevalences of 10–15%. Gross infection signs due to *Hematodinium*-like parasites in *Pandalus platyceros* decreased off British Columbia, Canada (1993: 2.1%; 1994: 0.7%) but sub-clinical infections were similar to previous surveys. *H. perezi* continued to be observed in blue crab (*Callinectes sapidus*) surveys in 1994 (15–20% in some sub-populations) in the Chesapeake Bay region.

Microsporidiosis showed a relatively high prevalence (10%) in *Pandalus platyceros* in British Columbia, Canada, for the first time. Other populations of shrimp from British Columbia showed continued low prevalences (< 1%).

Bacterial Shell Disease of lobsters (*Homarus americanus*) increased in lobster pounds in Nova Scotia, Canada and was thought to be due to a change in diet from shrimp to rock crab. Shell disease in the eastern US has been controlled using a Vitamin C supplemented diet and is no longer considered to be a disease concern.

6.4 SUMMARY/CONCLUSIONS

i) No significant new disease agents in fish, molluscs and crustaceans were detected in 1994.

ii) VHS was isolated from cultured turbot in Scotland with a suspected wild marine source.

iii) Expansion of the disease ranges of *Bonamia* and *Marteilia*, although slight, are still associated with significant mortalities.

iv) Of greatest interest is the issue of host-specificity and its relation to the issue shellfish as asymptomatic carriers or vectors:

a) *Crassostrea virginica* was found infected by a *Marteilia* species for the first time;

b) *Mikrocytos mackini* infects a number of different bivalves and,

c) *Perkinsus* sp.(p). can be found in a wide variety of different bivalves.

7 EVALUATION OF ACTIVITIES OF THE SUB-GROUP ON STATISTICAL ANALYSIS OF FISH DISEASE DATA

It was agreed with ICES not to hold the meeting of the Sub-Group (SG) on Statistical Analysis of Fish Disease Data in 1995. Intersessional work had taken place on the data entry program for the submission of fish disease data to be included in the ICES fish disease databank. This had only become available in a functional form shortly before the WG meeting. In addition, the chairman, Dr A.D. Vethaak, was unable to attend and chair the meeting due to health problems and several of the members of the SG were also not able to attend. Proposals for future actions of the Sub-group were circulated by A D Vethaak prior to the WGPDMO meeting (Annex 7a).

7.1 Discussions During the WGPDMO Meeting

These SG members attending the WGPDMO meeting took the opportunity to meet for an informal session to further the progress being made on the ongoing tasks of the SG.

The new data entry program for the fish disease data produced by ICES (Annex 7b) was demonstrated and it was agreed that it met the requirements initially specified. This will now enable the SG to progress the statistical analysis of the data for the next SG meeting. The importance of ensuring that the new fish disease databank is compatible with the other existing ICES environmental and biological databanks in order to include these data in the analysis was stressed.

The WG members agreed that next years data analysis should include spatial and temporal trend analysis of selected North Sea dab data utilising the new reporting format. This will also allow a preliminary assessment of possible problems involved in using the new format. For this analysis, participating countries will report data from the period 1990–1995 for two stations so that, in combination, large areas of the North Sea are covered. Dr A.D. Vethaak will co-ordinate this international data collection.

The WG discussed ongoing tasks of the SG and decided to continue this work by correspondence in 1995. Ongoing tasks of particular importance were identified:

- Histological confirmation of liver nodules from studies on fish disease ion different member countries (S. Feist, MAFF, Weymouth, has taken over this work from D. Bucke);

- Report on suitable statistical methods to analyse the ICES fish disease prevalence data using individual fish data and to correlate the results with sediment contamination data and other risk factors. (S. Des Clers);

- Collect new information and co-ordinate studies on age-length relationships in dab in connection to disease monitoring. (T. Lang & S. Mellergaard);

- To investigate the possible effect of fishing effort on spatial distribution of fish diseases. In order to meet this requirement, ICES is requested to provide the SG with effort data from demersal fishery (otter-trawl and beam-trawl) for the North Sea (T. Lang & S. Mellergaard);

- To collate available information on stock densities (CUE) of North Sea dab to assess the importance of this as a risk factor for fish diseases (S. Mellergaard & T. Lang).

7.2 Recommendations

The WGPDMO recommends:

a) that member countries participating in appropriate studies should report dab (*Limanda limanda*) disease data from two North Sea stations for the period 1990–1995 for statistical analyses by using the new data entry program;

b) that the Sub-group on Statistical Analysis of Fish Disease Data under the Chairmanship of Dr A D Vethaak meets in Copenhagen prior to the 1996 WGPDMO meeting in order to conduct a statistical analysis of the North Sea dab disease prevalence data submitted to the new ICES fish disease databank and report its conclusions to the 1996 meeting of WGPDMO;

c) that ICES ensures that the programs developed for the entry of data in the ICES environmental databank are compatible with both IBM and Mackintosh computers;

d) that an ICES Special Meeting on the use of liver pathology of flatfish for monitoring biological effects of contaminants be held in late 1996 at the MAFF Fish Diseases Laboratory, Weymouth, UK, under the convenorship of S.W. Feist and T. Lang.

8 EVALUATION OF THE RESULTS OF THE INTERSESSIONAL STUDY ON ANTIBIOTICS MIC AND MBC (SENSITIVITY TESTING OF ANTIMICROBIAL AGENTS)

8.1 Background

For some years, it has been suspected that the antibiotic sensitivity findings from different laboratories for a given fish pathogen were not comparable. In an attempt to resolve this question, the WGPDMO commissioned an investigation by various laboratories. The question was tackled using *Aeromonas salmonicida*, a bacterium that was causing unacceptably high losses in farmed salmon at the time. The initial investigation reported on in 1993, confirmed that antibiotic sensitivity findings obtained by different laboratories were often widely divergent. The study also concluded that a lack of standardisation of antibiotic sensitivity assays accounted for most of the divergence.

8.2 Current Studies

Follow-up studies in 1994 took two forms:

i) Two publications (listed below) dealing with the sensitivity of *A. salmonicida* to various antibiotics were considered. Results reported in these publications showed that, using specified techniques (which varied according to the study being considered !), it was possible to relate the size of the zone of growth inhibition to the minimum inhibitory concentration (MIC) of the antibiotic. This finding was important because it showed that the former parameter (which is conveniently measured) could be used to characterise the antibiotic sensitivity of a particular *A. salmonicida* isolate. The findings were also of considerable significance because they provided for the rational prescription of a drug for treating *A. salmonicida* infections if the levels of the drug attainable in vivo were sufficiently large relative to the MIC value.

ii) Unfortunately, limited laboratory trials (phase 2 of the work in 1994) (Annex 8a) to verify the relationship between the size of the zone of growth inhibition and MIC value did not always support the findings reported in the two publications mentioned above. In limited trials with oxolinic acid, for example, a given zone of inhibition yielded MIC values that were unexpectedly divergent. In addition, trials with a potentiated sulphur drug also yielded an unacceptably wide range of inhibition zone sizes as corresponding to a single MIC value. The reasons for these disquieting findings will have to be explored. It is possible that standardisation of the test inoculum is still a problem with an auto-agglutinating organism like *A. salmonicida*. It also seems probable that the amount of antibiotic present on discs sometimes varies more widely than one has come to expect.

Problems associated with minimal bactericidal concentration (MBC) were not investigated.

8.3 Conclusions

Final resolution of the antibiotic sensitivity testing problem will require additional laboratory studies. The direction that these studies should take should be a topic discussed during a workshop on this subject to be held at the up coming EAFP meeting in Mallorca, Spain, and the workshop participants will be informed by F Baudin-Laurencin that the antibiotic sensitivity issue has been one of particular concern to the ICES WGPDMO.

1) Hastings, T.S. and Mckay, A. (1987). Aquaculture Vol. 165.

2) Grant, A.N. and Laidler, L.A. (1993). Vet. Rec. 133: 389

9 REVIEW OF CURRENT RESEARCH IN SEA LICE TREATMENT AND CONTROL METHODS

Data obtained by request of WG members from industry, industry organisations, research institutes and official sources were received from Scotland (Annex 9a) and Norway (Annex 9b). Some data was also contained in the farmed fish disease reports from Canada (E) and Iceland. Unfortunately, no information was tabled from the two remaining major salmon farming areas namely Ireland and Faroes for comparison.

Caligus elongatus has previously caused significant problems in Iceland but has not required treatment since 1991 due to reduction in sea cage farming, fallowing of sites each year, location of sites in brackish water areas and low water temperatures.

Salmon louse *Lepeophtheirus salmonis* is recognised as the most serious disease of sea farmed salmon in Scotland, Norway and eastern Canada. The seriousness of the problem is reflected in the high research priority given to this condition in both Scotland and Norway. Additionally of major concern and further promoting the urgency of research in these countries, is the risk of withdrawal of permission for use of the main chemical currently in most common application, the organophosphate, dichlorvos.

There is a wide range of research currently in progress in all of the topic areas identified in the terms of reference. Much of the research is in early stages of development and under industrial confidentiality so that published or reportable conclusions for many studies are not yet available. Funding is provided by industry in Scotland, by the EC, research councils and official bodies to a range of research laboratories. There is also considerable research input directly from the fish farming industry and from chemical supply companies.

9.1 Chemical Control

Organophosphates comprise the most widespread method of treatment of lice in sea farmed salmon although there is a trend towards reducing levels of use of dichlorvos and trichlorophon. Reasons for the reduction are multiple:

a) the substitution in Norway of Nuvan and Neguvon by Azametifos which requires substantially smaller quantities because of its greater activity;

b) the effectiveness of other chemicals such as hydrogen peroxide. However, the influence of this chemical will probably not be of major significance as there are significant concerns about cost and safety in its use partly restricting its application;

c) the effectiveness of various preventative measures in reducing the frequency of treatment.

There is a strong awareness of the delicate nature of the current position of the licence for use of dichlorvos because of its listing as a dangerous chemical and the need to find alternative chemicals for lice treatment or improved prevention or avoidance measures. In general, there is a presumption by the fish farming industry against the use of chemical treatment of lice and this is reflected in the strong emphasis towards preventative (e.g., vaccine) and management measures seen in the Scottish fish farming industry research funding and effort.

9.2 Biological Control

Wrasse cleaner fish, Labridae, are widely used as a lice control measure during the first year of salmon in sea cages and there is a general belief in their effectiveness. It is seldom that chemotherapy is necessary during the first sea year when wrasse are present. However, it is also recognised that cleaner fish are not a complete answer in themselves and they should be used in conjunction with other approaches. Recognised problems are associated with the long-term continuity of wrasse supply, the failure of wrasse to overwinter in sufficient numbers in salmon cages and the possibility of disease transmission by the wrasse into and between salmon farms.

Research is also currently in progress into the physiology and structure of lice in an effort to locate any "weak spot" in the natural defences of the lice or its life cycle strategies, into the development of lures to trap free living larval stages and into the biology of host finding by the lice. All of these studies are in relatively early stages of development and have not yet yielded results which are applicable to fish farm conditions.

9.3 Immunological Control

Based on the knowledge that lice have evolved a life cycle strategy which avoids natural stimulation of the host immune system, research is being conducted to identify "hidden antigens" associated with the gut of lice. The use of these in the stimulation of the salmon immune system is hoped to lead to the destruction of or interference with essential components in the structure or physiology of the louse gut whenever a blood meal is taken. Results from a joint Scottish-Irish study are still in the preliminary category but are encouraging in that lice on immunised fish developed short and deformed egg strings.

9.4 Management Approaches

Fallowing has been introduced on a wide scale in the Scottish fish farming industry as an integral part of a general disease management policy, particularly in association with furunculosis control, but this policy has had a major beneficial effect in breaking cycles of lice reinfection. There are indications that it is particularly effective when whole bodies of sea water where interaction between farms is possible is cleared and restocked with fish in a synchronised way. It is difficult to quantify the precise level of benefit, but industry are convinced about the advantages. No known structured research is currently in progress in this area.

9.5 Conclusions

There is extensive research currently in progress in Scotland and Norway on many aspects of sea lice biology, prevention and control, reflecting the primary position of the importance of this disease in the salmon farming industries in both countries.

Rather than a single method of prevention or control being used, there is evidence that a complex of different methods are being employed. Research is geared to further increasing the options available. There is concern about the future availability of the main chemical currently being used.

10 REVIEW OF RESEARCH ON PATHOLOGY AND DISEASES IN FISH LARVAE REARED IN MARICULTURE

10.1 Current state of Knowledge

A review of the current state of knowledge on pathology and diseases of fish larvae reared in mariculture was provided (Annex 10a).

10.2 Assessment

Until recently, management and nutrition have been considered the most important factors when it comes to growth and survival of marine larvae. However it has become obvious that micro-organisms also have a major impact. Primary pathogens or secondary pathogens may be considered in relation to particular environmental factors. It also appears that pathogens of larval fish might be different from pathogens of adults.

Except for a few well characterised disease agents (herpes virus of hirame, nodavirus /picornavirus causing encephalopathy, and other parasites), the micro-organisms involved are poorly characterised. Some bacteria for which identification has to be confirmed are recognised as possible causes of pathology. Most, however, are found in abundance in the intestine and cannot therefore be considered as true pathogens. Even for well defined pathogens, the routes of disease transmission (vertical and or horizontal) and reservoirs are not well known.

It must also be underlined, that the defence mechanisms in fish larvae are poorly understood. Because of the variability in larval size, defence mechanisms and pathogen interactions (which are different from those of adults), diagnosis is often difficult. This is especially true for bacteria.

10.3 Conclusions

Concerning control, the use in antibiotics or other solutions, for larval rearing, has to be considered. Probiotics could be a very satisfactory solution, but more research is required.

In summary, work, collaborative when necessary, involved with larval pathology, must be encouraged, especially concerning the following:

1. Identification of primary pathogenic bacteria;

2. Study of development of defence mechanisms and of the possibilities of transferring parental protection;

3. Epidemiology and control of viral diseases;

4. New approaches to control of bacterial diseases, using antibiotics, as well as hygienic control and probiotics.

11 REVIEW THE REPORTS OF THE BMB WG 25 "FISH DISEASES AND PARASITES IN THE BALTIC SEA" AND THE "BMB/ICES WORKSHOP ON DISEASES AND PARASITES IN THE BALTIC SEA"

The reports of the BMB WG 25 "Fish Diseases and Parasites in the Baltic Sea", "BMB/ICES Workshop on Diseases and Parasites of Flounder in the Baltic Sea" and the"BMB/ICES Sea-going Workshop on Fish Diseases and Parasites in the Baltic Sea" were summarised to the WG (Annex 11a and 11b).

11.1 Flounder Parasites

A BMB/ICES sponsored meeting on "Diseases and Parasites of Flounder in the Baltic Sea" was arranged at the Åbo Academy University, Finland, on 27–29 October 1995. In addition to 20 participants from countries around the Baltic Sea, several invited specialists from other countries participated. The aims of the meeting were:

- to bring together specialists actively engaged with work on flounder diseases to give presentations on the present state of knowledge concerning flounder diseases;

- to discuss and evaluate the significance of recent advances and new research methods;

- to discuss the direction for further investigations;

- to evaluate the use of certain target diseases and health parameters of flounders as biomarkers in biological effect monitoring programs and for hazard assessment.

The outcome of the meeting was published in a Proceedings Volume (The Baltic Marine Biologists, Publication No 15, 1994 (ISSN 0282-8839).

The presentations and discussions focused especially on the possibilities of employing new methodologies when utilising health parameters of flounder as biomarkers. The summary statements are appended as an Annex to this report (Annex 11c).

11.2 BMB/ICES Sea-going Workshop

A "BMB/ICES Sea-going Workshop on Fish Diseases and Parasites in the Baltic Sea" was held from 25 November–8 December on board the German RV "Walther Herwig III". The workshop was attended by 11 scientists representing all countries bordering the Baltic Sea, except Sweden. The results will be submitted for publication in Journal of Marine Science.

The purpose of the workshop was:

- to establish international co-operation between Baltic countries with regard to research and monitoring of fish diseases and parasites in the Baltic Sea.
- to provide scientific data on the prevalence and spatial distribution of fish diseases and parasites in the Baltic Sea to be used as a baseline information for further research and monitoring programmes.
- to evaluate the applicability of the ICES standard methodologies recommended for fish disease surveys (which have been developed based on experiences from the North Sea) and eventually bring up modifications to adapt these for the Baltic Sea.

The "BMB/ICES Sea-going Workshop on Fish Diseases and Parasites in the Baltic Sea" may be regarded as a milestone as it managed for the first time to assemble scientists from all the "old eastern countries" and to carry out fish disease investigations within the national waters of these countries.

11.3 Other Activities on Fish Disease Studies in the Baltic Sea

The WG noted, that fish disease monitoring programmes have recently been initiated by Estonia and Latvia and, therefore, recommend that these countries make use of the long-term experience of the ICES WGPDMO in this field.

11.4 Conclusions

The WGPDMO appreciated the organisation and performance of these two fish disease meetings focused on the Baltic Sea and considered the outcomes as a useful basis for future monitoring of fish diseases and parasites in this area.

12 EVALUATION OF THE POSSIBILITY THAT RECIPROCAL TRANSFERS OF *M MACKINI* MAY OCCUR AMONG FLAT OYSTERS, OLYMPIA OYSTERS, AND AMERICAN OYSTERS

Background information on current research was provided by Drs S.M. Bower and D. Hervio, Department of Fisheries & Oceans, Canada, Pacific Region. (Any specific questions on this research should be directed to Dr Susan Bower, Department of Fisheries & Oceans, Pacific Biological Station, Nanaimo, British Columbia, V9R 5K6, Canada).

12.1 Role

Mikrocytos mackini can infect flat (*O. edulis*), Olympia (*O. conchaphila*) and American (*C. Virginica*) oysters by proximal exposure as well as by inoculation. *M. mackini* can also infect Japanese scallops (*Patinopecten yessoensis*) by inoculation. Disease signs and mortality have been induced in the laboratory in all these hosts. Cross-species infections have been demonstrated for oysters under field conditions but not for *P. yessoensis*.

The only bivalve examined, so far, which has been refractive to *M. mackini* inoculation is the Manila clam, *Tapes philippinarum*.

12.2 Potential

The professional opinion of the scientists working with this parasite is that survivors of Denman Island Disease, irrespective of host species, should be considered as potential carriers of *M. mackini* until proven incapable of this role. Pacific oysters clearly transmit *M. mackini* to other Pacific oysters, as well as the species listed above. There is little reason, therefore, to assume the same parasite cannot be transmitted between other hosts. In order to address this question, Drs Bower and Hervio have started transmission experiments at DFO (Pacific Region) and results are expected later this year. Inoculation exposure will be used for initial investigation of transmission potential between infected oysters (flat and American) and uninfected oysters (flat, American and Pacific). Additional research is being conducted on warm-water temperature inhibition of *M. mackini* pathogenicity in each of these "new" host species.

12.3 Implications for Disease Management

In the light of increasing examples of putative and proven non-host-specificity by significant molluscan pathogens (a well-established fact for shrimp pathogens), bivalve and gastropod species living in proximity to hosts of significant pathogens should no longer be assumed free of the agent of the known host-species. Effective disease management therefore requires assessment of the risks associated with movement of shellfish species other than the normal host of a pathogen within its endemic range.

General considerations recommended for assessing the risk of a molluscan carrier species for a known, regulated, pathogenic agent.

1. Determination of the geographic range of Pathogen "A";
2. Determination of sympatric molluscan species and those likely to be considered for commercial movement from one location to another;

3. Survey examination of other "commercial" species for the presence of Pathogen "A" or a pathogen of unknown identity (possible morphologically distinct life-history stage);

4. Using all available diagnostic tools sensitive for detection of Pathogen A and statistically acceptable survey numbers:

   i) Pathogen detected: go to 5.
   ii) No pathogen ever detected: Low risk of vector-transmission.

5. Determine the nature of the pathogen within the alternate host, e.g.,:

   i) Are the agents necrotic or intact?
   ii) Are the agents within the tissues or loose in luminal spaces?
   iii) Is there evidence of parasite multiplication, or all development stages of the life-cycle within the alternate host tissues?
   iv) If available: Does a species-specific diagnostic probe confirm the identity of the agent as Pathogen A?

6. Determine the infectivity of Pathogen A to the "normal" host. In order for this to be of relevance to field, rather than laboratory conditions to following procedures are recommended:

   i) Laboratory-based proximity experiments, permitting initial observations under controlled conditions. Artificial manipulation should include stress-testing using extremes of ambient temperature or salinity conditions. "Proximity" is defined as contact between the normal and alternate hosts. Duration of the experiment will vary according to the knowledge of the life-cycle of Pathogen A in the normal host.
   ii) Field-based proximity experiments with infected normal hosts and sympatric alternate hosts. Manipulation to the extent that alternate hosts are in direct contact with infected hosts (or beds where individual pathogen-carrier status is unknown) is advised.

7.

   i) Confirmation of infection of alternate hosts under both laboratory and field exposure: go to 8.
   ii) Confirmation of accidental or "dead-end" infection of the alternate host species: low risk of vector transmission

8. Determination of infectivity of Pathogen A in the alternate host species to the normal host via laboratory-based proximity exposure experiments (necessary to avoid possibility of infection between normal hosts in the field, as well as requiring placement of infected alternate hosts at sites where the normal hosts are uninfected):

   i) Positive results: high risk of vector transmission
   ii) Non-infective to normal host species: low risk of vector transmission

Important note:

Inoculation-mediated transmission is not considered to be an effective tool for assessing the risk of cross-species transmission of specific pathogens under field conditions, thus, inoculation is not considered in the above guidelines. Inoculation infections are necessary, however, to precisely determine pathogenic infection levels, disease progression/remission, development of pathogen-specific diagnostic tools and other very important epidemiological factors.

12.4 Specific Examples

12.4.1 General examples

Denman Island Disease (*Mikrocytos mackini*) is the first example of a shellfish disease agent which can infect alternate hosts under both laboratory and field conditions, however, previous laboratory and circumstantial evidence points to many more non-specific pathogens, e.g.,:

i) *Perkinsus* spp. of Australian bivalves and gastropods (Goggin *et al.* 1989);
ii) Pacific Oyster Nocardiosis (PON) (Elston 1993);
iii) *Minchinia armoricana* of *Ostrea edulis* and *O. angasi;*
iv) *Marteilia refringens* between Pacific and American oysters.

12.4.2 Canadian examples

i) Transfer of hard-shell clams (*Mercenaria mercenaria*) from South Carolina to Atlantic Canada: The exporter was provided with a list of diseases, irrespective of host, for which the stock was to be examined. The clam source was certified free of all pathogens listed, including *P. marinus* (known to have a geographic range extending along the southeastern United States) before being introduced into quarantine in Atlantic Canada. We will examine 100% of the broodstock before release of the F1 generation.

ii) Transfer of European oysters (*Ostrea edulis*) from the west coast of Canada to Atlantic Canada was refused. The stocks originated from the Atlantic, were introduced to British Columbia via quarantine in 1990. Since their release, the oysters have been mixed in Denman Island Disease, Pacific Oyster Nocardiosis and *Bonamia ostreae* enzootic areas. Disease risk assessments based on this background information negated their return except via quarantine (which the proponent was unwilling to do).

12.4.3 European examples

The Pacific oyster, *C. gigas*, and mussel, *Mytilus galloprovincialis* has been found to be refractive to *Bonamia ostreae* and *Marteilia refringens* (EU Decision 93/169/CEE). The host-specificity of "brown ring disease" of Manila clams, Herpes infections of Pacific and flat oysters and *Perkinsus atlanticus* of Portuguese carpet clams (*Tapes decussatus, T. semidecussatus* and *Venerupis pullastra*) is currently being investigated. Since the EU Directive specifies screening *O.edulis* for *B. ostreae* and *M. refringens* to evaluate disease status of specified EU Zones and regulate transfers, it does not directly address spread of these other diseases either via normal or abnormal hosts (EU Decision 94/306/CE). Additional screening may be required by specific Countries within the EU (see 2:23j).

12.5 Recommendations

The WGPDMO recommends that molluscan shellfish destined for live transfer from areas endemic for a specific disease agent to areas free of the disease agent, be examined for the disease agent **even if they are not the normal host.** The WGPDMO also recommends that the host-specificity of known pathogens be examined, at least for mollusc species regularly transferred between endemic and non-endemic areas.

13 STANDARDISATION OF DISEASE RECORDS USED IN SHELLFISH PATHOLOGY LABORATORIES FOR USE AT NATIONAL LEVEL AND BY ICES

Computerised disease data sets are in use by a number of shellfish pathology laboratories. Although formats for compilation of observations from individual specimens vary greatly between laboratories, summary data sheets contain compatible information which can be adapted for a broadly applicable format. The most recent format for molluscan disease data was produced by IFREMER (February 1995) "Réseau REPAMO" (see attached flowchart for data compilation (Annex 13a)) which is compatible with DOS Windows and may be adaptable to other, widely used computer systems. Established data bases, such as Database 3, used by the Oxford Laboratory, Maryland, USA, can roll-up the information required for the summary reporting format outlined in Annex 13b. This will be assessed by ICES member countries and should be adapted to be consistent with the format currently used by ICES Headquarters for finfish disease data.

13.1 General Pathogen and Disease List (For Inclusion in Reports Submitted to ICES)

1. New or previously undescribed
2. *Haplosporidium nelsoni*
3. *Haplosporidium costale*
4. *Haplosporidium* spp.
5. *Perkinsus marinus*
6. *Perkinsus atlanticus*
7. *Perkinsus olseni*
8. *Perkinsus* spp.
9. *Bonamia ostreae*
10. *Bonamia* spp.
11. *Mikrocytos mackini*
12. *Mikrocytos roughleyi*
13. *Marteilia refringens*
14. *Marteilia sydneyi*
15. *Marteilia* spp.
16. *Marteiloides chumagensi*
17. Nocardiosis
18. Brown Ring Disease (BRD)
19. Juvenile Oyster Disease (JOD)
20. *Herpes* virus
21. Iridovirus Gill Disease
22. Oyster Velar Virus Disease (OVVD)
23. Nuclear Inclusion X (NIX)
24. Scallop Protozoan X (SPX)
25. Intracellular Bacteria Disease of Scallops
26. *Mya* sarcoma
27. *Mytilus* sarcoma
28. *Mya* gonadal neoplasm
29. Malpeque Bay Disease
30. Opportunistic Pathogens (hatchery, nursery or grow-out)

13.2 Recommendations

Standardisation of shellfish disease data recording is urgently required to accurately monitor disease trends and track emerging disease problems. The lack of a consistent disease data base for shellfish has meant that conclusive determination of sources of infection has been complicated and/or anecdotal. The WGPDMO recommends:

a) that ICES member countries use the proposed pathogen and disease list when submitting data to ICES and;

b) that compilation of shellfish disease data banks in ICES should be in a format consistent with that developed for finfish disease data.

14 PROCEDURES FOR DETECTING AND MONITORING THE PREVALENCE OF MOLLUSC PATHOGENS

14.1 Sample Sizes

Sample sizes for detecting specific pathogens are beginning to be defined (e.g., EU sample sizes for screening *O. edulis* for *B. ostreae* and *M. refringens*, n=150), however, other regulatory agencies use sample sizes ranging from n=30 to n=100). Sixty individuals are statistically required for detecting a single case present at 5% prevalence in a population of > 100,000. This standard is used for finfish health diagnostics and appears statistically acceptable for shellfish histology. Probability of detection is enhanced using more sensitive diagnostic tools. Although diagnostic protocols vary between laboratories, certain standards for disease-screening appear comparable. Accurate determination of prevalence, however, still appears to be problematic with techniques of variable sensitivity, such as histology. Until pathogen-specific sensitive tools are widely available, however, it appears appropriate to concentrate on presence/absence detection of known pathogens. Non-pathogen-specific survey screening continues to be limited to common parasites and diseases.

Screening for shellfish disease agents fall into three categories which each require different degrees of sampling intensity:

i) Screening shellfish for a regulated (commercially significant) disease agent which has not been detected previously:

This requires the highest intensity of disease-screening possible (within practical processing constraints) in order to ensure accurate determination of the disease status of the population and accurately assess risk of disease transfer with movement of the stock. The optimum protocols recommended for this degree of disease screening fall in-line with those required for determining the *Bonamia ostreae* and *Marteilia refringens* status of EU zones, as well as other internationally regulated disease (e.g., the Office International des Epizooties (OIE) disease list).

a) selection of optimum sampling schedules, based on known seasonal developmental cycles of the pathogen and peaks in prevalence in endemic areas;

b) selection of the optimum age/size of host known to be affected by the pathogen;

c) sample size of 150 specimens (95% probability of detection of a single infected specimen at a prevalence of 2% in a population of over 100,000);

d) selection of the optimum diagnostic tool for detection of the pathogen. If detection relies on histology, sections from two parts of the body are recommended (oblique dorso-ventral across the centre of the digestive gland and anterior gills plus dorso-ventral section across the pericardial sinus). Different bivalves and pathogenic agents may require alternate section levels to assure optimum pathogen detection under light microscopic examination;

e) additional tissues from sampled bivalves should be stored in a fixative appropriate for long-term storage and/or electron microscopy (if required for confirmation of light microscope observations).

ii) Screening the population for a specific pathogen known to occur from previous survey results:

This requires a lower degree of detection sensitivity than i) (with the exception of a population demonstrating < 5% prevalence, i.e., 1/150, of a regulated pathogen, which requires sampling at the same, or greater, intensity as i) to ensure accurate monitoring). "Moderate" sampling sensitivity is also appropriate for disease diagnosis of an open-water population for which the cause is undetermined or unconfirmed.

a) sampling is scheduled as for i) in the case of monitoring a regulated pathogenic agent, or during (not after) an undiagnosed disease outbreak;

b) sample sizes of at least 60 (90% probability of detection of a pathogen at 5% prevalence). Disease diagnostic submissions from smaller hatchery- or nursery-populations may require smaller sample numbers;

c) use of optimum technique for the pathogen being monitored, a broad-range diagnostic technique or series of techniques, where the pathogen is undetermined;

d) histology screening for a known pathogen may require one tissue block (rather than the two mentioned under i)) for light microscope preparations. Two tissue blocks are recommended for disease diagnosis;

e) additional tissues from disease diagnostic cases should be stored in a fixative appropriate for long-term storage and/or electron microscopy to permit confirmation of light microscope observations, where necessary.

iii) Bivalve surveys for diseases and parasites present at non-endemic levels require lower sampling intensity than disease diagnostics.

a) sample schedules can either be seasonal (4 times per year) or annual (repeating site location and time of year as closely as possible) to determine temporal fluctuations;

b) bivalve sizes should include juveniles and adults to determine age-related parasite and disease profiles;

c) broad-range screening techniques such as histology are appropriate, along with gill, digestive gland or other tissue smear to retrieve whole specimens of luminal and superficial parasites (and commensals);

d) sample sizes of >30 are appropriate for detecting common parasites within wild and cultured open-water populations.

14.2 Recommendations

The WGPDMO recommends:

a) that shellfish disease agents be carefully defined prior to starting any sampling programme and;

b) that the intensity of sampling the requirements outlined under points 14.1 (i–iii).

15 THE USEFULNESS OF EXTERNAL FISH DISEASES AS A TOOL IN THE MONITORING OF BIOLOGICAL EFFECTS OF CONTAMINANTS

15.1 Available Information

The WGPDMO was provided with and carried out extensive discussions on working documents provided on this topic (Annexes 15a and 15b).

15.2 Conclusions

The WG considers externally visible fish diseases to be a useful tool which should be incorporated into programmes for the monitoring of biological effects of contaminants. It is considered probable that external fish disease monitoring will be most beneficial as part of a long-term trend monitoring programme.

However, from a variety of field studies there is evidence that the occurrence of externally visible fish diseases is an **unspecific biomarker for environmental changes** of natural and/or anthropogenic origin affecting fish health rather than a specific biomarker for contaminant exposure.

Advantages of this approach are:

- it is a cost-effective means for biological effects monitoring not requiring expensive laboratory analyses;
- externally visible fish diseases represent an integrative endpoint of a chain of biochemical and physiological changes affecting the homeostasis of the fish;
- the measurement of significant changes in the prevalence of externally visible diseases can be used as an **ecologically relevant warning signal** of environmental changes.

However, the monitoring of fish diseases should be supplemented by the application of other biological effect techniques in order to establish more clearly possible cause-effect relationships between contaminant exposure and the occurrence of pathological changes.

15.3 Recommendation.

The WGPDMO recommends to ACME that monitoring of fish diseases should be incorporated into the ICES list of disciplines recommended for the monitoring of biological effects of contaminants.

16 OVERVIEW OF FISH DISEASES IN THE BALTIC SEA AND THEIR POSSIBLE IMPACT ON MORTALITY OF BALTIC FISH STOCKS.

16.1 Current Studies

There is increased interest focused on fish diseases in countries in the Baltic area. In national laboratories, considerable efforts are placed on specific diseases and disease aetiologies. Regular monitoring for fish diseases in the Baltic Sea has been performed on a large scale by German scientists since the eighties; monitoring activities on more restricted scale have been performed in Denmark, Finland and Sweden. Recently, fish disease monitoring programs have also been initiated in Estonia, Latvia and Poland. These monitoring activities are focused on a few target species (cod, herring flounder) and, in accordance with the rules proposed by ICES, on macroscopically visible disease signs e.g., skin ulcers, lymphocystis, liver nodules and fin lesions. The aim of most of this work is the monitoring of environmental quality, rather than investigating the effects of diseases on fish mortalities and fish stock sizes.

16.2 Mortality Effects

There is little conclusive information as regards the impact of diseases on mortalities in Baltic fish stocks. The methods employed for regular stock assessment of some species (i.e., herring and sprat) do not permit us to distinguish between mortalities induced by diseases and stock reduction due to fishing efforts. In a few cases, mortalities induced by specific diseases or specific environmental factors have been obvious, to justify the conclusions that these conditions have had a significant influence on stock size in Baltic fish. For example the supersaturation of water with gas in association with thermal discharge effluents from a nuclear power plant on the south coast of Sweden caused mortalities in garfish (*Belone belone*). It also seems reasonable, for example, to suggest that the recent *Ichthyophonus* epidemic had a significant effect on the herring population in the southern Baltic Sea

Data accumulated in the area during recent years clearly indicate that diseases especially have impact on early life stages of fish in the Baltic Sea. Most recently we have seen the drastic effect the M 74 syndrome induced on early fry of the Baltic stock of Atlantic salmon. Mortalities induced in early stages of perch appear associated with pulp mill effluents. Other works indicate that exudates from algae and spawning substratum algae have induced high mortality in eggs of Baltic herring.

Against this background, research projects have recently been initiated in several countries around the Baltic Sea (Denmark, Finland, Sweden) in order to evaluate the significance of diseases on reproduction and early stages of fish in this area.

16.3 Provision of Information to HELCOM

This statement was accepted by the WGPDMO and the chairman of the BMB Disease Working Group, also member of the WGPDMO, was appointed to transfer the information, in co-operation with the Chairman of the Baltic Fish WG, to be included in the HELCOM Third Periodic Assessment (ACME and ACFM) HELCOM 7).

17 OVERVIEW OF NEW INFORMATION ON THE M-74 SYNDROME AND *ICHTHYOPHONUS*

17.1 M-74 Syndrome

A summary of the main conclusions of the special topic Working Group on M-74 is appended (Annex 17a).

The M-74 syndrome continued to cause problems in 1994. Both in Finland and Sweden the mortality in the 1994 offspring was almost at the same level as in previous years i.e., 80-90%. In Sweden, a minor reduction was observed at some sites but it was probably due to a much harder selection of the brood stock.

Recent information indicates that the vitamin component is one of the key factors in the problem. In North America in the Great Lakes Basin, a M-74 like syndrome called the "Cayuga syndrome" has been observed in Atlantic salmon (*Salmo salar*), lake trout (*Salvelinus namaycush*) and coho salmon (*Oncorhynchus kisutch*). After having excluded PCB's, DDT's and other toxicants as main responsible factors, American scientists focused on vitamins, especially thiamin (vitamin-B1).

The main food item of these salmonids was alewife (*Alosa pseudoharengus*) which exhibited high thiaminase activity. The symptoms observed in the sac-fry suffering from the "Cayuga syndrome" were very similar to the symptoms observed in other animal species suffering from thiamin deficiency, namely neuromuscular dysfunction, tremor, convulsions and finally death. Thiamin treatment of the yolk-sac fry reduced the mortality to "background" levels.

Thiamin deficiency symptoms are very similar to the symptoms observed in M-74 syndrome. This combined with the fact that the preferred food items for Baltic salmon (sprat and herring) both have a high thiaminase activity indicated that thiamin deficiency might be involved in the M-74 syndrome. Experiments with thiamin treatment of yolk-sac fry have now been conducted in both Sweden and Finland with M-74 affected fry with the same positive results as found in the Great Lakes.

Thiamin treatment is very promising for the salmon stock enhancement programme in the Baltic Sea but the wild spawning salmon stocks are still seriously threatened.

There is some indications that a M-74 like problem occurs in the Baltic cod stocks but this requires further research.

17.2 *Ichthyophonus*

The WG decided that the *Ichthyophonus* question would be dealt with under agenda item 18.

18 REVIEW OF THE PRESENT STATUS OF ICHTHYOPHONUS IN HERRING POPULATIONS AND ON PROBLEMS WITH SUPPLYING DATA ON ITS PREVALENCE

The Working Group decided to discuss the *Ichthyophonus* component of Agenda item 17 (i.e., Term of Reference C. Res. 1994/2:23 (m) requesting (i) maintenance of an overview of *Ichthyophonus* and (ii) to report new information to ACME) together with agenda item 18 (TOR C.Res. 1994/2:23 (n) requesting, (i) as a matter of urgency, the present status of *Ichthyophonus* on herring populations and (ii) to investigate problems with supplying data on its prevalence).

18.1 Present Status of *Ichthyophonus* in Herring Stocks

18.1.1 Data available

In response to a specific request for current data on this topic, nine countries (Iceland, Sweden, England, Estonia, Germany, Netherlands, Norway, Scotland and Denmark) supplied information which is summarised in Annex 18a. In addition, Norway supplied some information from co-operative studies with PINRO, Murmansk, Russia on Norwegian Spring spawning herring stocks.

a) North Sea

Only low levels of *Ichthyophonus* were detected by research vessel surveys and in samples from commercial catches in the northern North Sea, particularly east and northeast of Shetland. Some surveys, even through that same area, did not reveal any evidence of infection. No infected fish were found by any survey in the central and southern North Sea.

b) Kattegat and Baltic

In the Kattegat the level of infection in 20+cm herring continued to show the progressive decline apparent since 1991. However, the prevalence level in small herring <20cm has remained roughly constant at around 1.5% since 1991. There is now no evidence of significant infection in samples of herring from Baltic herring stocks.

c) Norwegian Spring Spawning herring stocks

Infection has been shown to be still present in Norwegian Spring spawning herring stocks at appreciable levels, but accurate figures await the working up of survey results. Calibration of methods between Russian and Norwegian observers is required to assess the significance of the differences in the data sets of these two countries from Norwegian spring spawning stock.

d) Iceland

Only low levels of the infection, similar to previous years, were detected to the south east of Iceland. In this area, exceptionally high prevalence levels of *Ichthyophonus* can be found in common dab *Limanda limanda* and plaice *Pleuronectes platessa* but the significance of this observation to herring stocks in that area is not known. However, the lack of correlation between the infection level in these and in herring could suggest different strains or species of the parasite in the different host species.

18.1.2 Conclusions on Current Levels of Infection

There was a general indication of a marked decrease in the prevalence levels of the parasite in the North Sea in 1994 compared with 1991–1993. It is considered that the epidemic is probably over and that only background levels of infection are now present. Integration of disease data into herring stock assessment analytical techniques (Annexes 18b, 18c) indicated a focus of infection northeast of Shetland in the North Sea and evidence for an association between the epidemic and a high prevalence in a particular year class. The level of infection decreases within each year class with time suggesting a selective mortality of infected fish. This observation fits in with the conclusions of the Herring Assessment WG (South of 62°) 1994 based on stock analysis that a high mortality may occur in affected populations. These calculations also indicate the possibility that the disease may not show the highly acute nature considered possible in previous discussions (ICES Special Meeting on *Ichthyophonus*, Aberdeen, 1993) and that mortality in individual infected fish may occur over a much longer time span. It is disappointing that no ICES member country has provided resources for an experimental study of the time course of the disease in herring, as this information is considered critical for proper assessment of field data.

18.1.3 Recommendations for further study

a) Routine surveys for *Ichthyophonus* should continue, but integrated into stock assessment studies rather than as disease dedicated studies (see 18.2 below). Collection, combination and assessment of *Ichthyophonus* data in association with the Herring Assessment Working Group was recommended in the WGPDMO Report 1994 12.3.

b) The prevalence of *Ichthyophonus* in other species of fish should be measured and the possibility of these acting as carrier hosts for the disease in herring evaluated.

c) Experimental work to determine the time course of the disease in herring should be conducted.

d) The possibility of different species of *Ichthyophonus* being present in the North Sea and Kattegat Sea should be further investigated.

18.2 Collection and Supply of Data on Ichthyophonus

18.2.1 Research effort.

The marked decline in the prevalence levels of *Ichthyophonus* in the North Sea-Baltic Sea areas will make substantial demands on resources if dedicated disease studies are undertaken. It was concluded that the results which may be obtained from such studies could not justify the effort expended. Several countries have already withdrawn from such studies. However, it was also concluded that it is necessary to maintain a low effort of monitoring of the levels (as requested in TOR C.Res. 1994/2:23 (m)) as background levels of the disease still exist in several herring populations and early warning information on potential repeat epidemics should be available to herring stock assessment specialists. The Norwegian participant has indicated a desire to maintain a higher level of disease monitoring in the Norwegian Spring Spawning herring stock and to maintain the current co-operative studies on these stocks with Russian scientists.

18.2.2 Proposed method of collection and supply of data

Several countries (Norway, Sweden, Netherlands, Scotland, England) have already integrated the collection of data on *Ichthyophonus* with herring stock assessment studies because of the considerable advantages of resource saving and the formation of a common data bank. It is recommended that this should be extended and formalised in the following way:

a) The gross appearance of the heart of each herring opened for biological/maturity/meristic studies by herring stock assessment researchers should be assessed and recorded in a separate column on stock assessment data recording sheets as "infected" or "uninfected". Guidance on the appearance of the two categories was provided by the ICES Special Meeting on *Ichthyophonus*, Lysekil 1991.

b) These data should be submitted to the relevant fish disease specialist in each country at the end of each calendar year and the quality and biological significance of the data assessed and any local trends considered.

c) These data should be submitted to the ICES data bank on fish disease. This is currently under development and should be expanded to accommodate the data from herring. Until this data bank is available, the data and disease interpretation of these should be submitted by each disease specialist identified in (b) to the relevant member of the Herring Stock Assessment WG.

d) WGPDMO should consider the data, on an annual basis, evaluate these and report conclusions to ICES and to relevant ICES WGs (if these are meeting prior to the compilation of the WGPDMO Report).

e) The relevant herring Working Groups should evaluate the data in stock assessment models for evidence of, and impact on, herring populations. If impact is suspected the magnitude of the effect should be assessed.

18.2.3 Recommendation

It is recommended that a standardised data collection and reporting scheme for *Ichthyophonus* prevalence, integrated with herring stock assessment studies, and validated and assessed by fish disease specialists, be implemented as soon as possible.

19 ADVICE ON METHODS TO HANDLE DATA IN RAPID RESPONSE TO DISEASE ISSUES IN FISH STOCKS

This agenda item (Term of Reference C.Res. 1994/2:23 (o)) originated from the ICES Consultative Committee Report 1994. It was stated that failures of communication had occurred especially with regard to who (herring stock assessment scientists or disease scientists) was responsible for the compilation of relevant prevalence data (on *Ichthyophonus*) and requested advice on how ICES could respond in a fast and flexible way in providing advice in issues of this nature. As proposed procedures on this topic specifically relating to *Ichthyophonus* were dealt with above (18.2.2, 18.2.3), the WGPDMO attempted to discuss this term of reference in a wider context. It proved difficult to respond positively because of ambiguity in the precise question being raised by the TOR. After several possible interpretations were considered, it was decided to base the WGs response on two discussion documents (Annex 19a and 19b) presented at the meeting.

19.1 Disease Induced Mortality

The first document (19a) provides information on problems associated with the calculation of mortality rates in North Sea herring stocks affected by the *Ichthyophonus* epidemic. The key conclusion was that despite the high prevalences of the disease in 1991–1993, no clearly defined serious impact on the stock size was demonstrated from stock assessment/modelling studies. Since the prevalence of the disease has decreased markedly since 1993, it is now doubtful whether any effect on the North Sea herring stock will be detected. Non disease-associated variations and sampling difficulties significantly contribute to difficulties in this area. The difficulties in interpretation of "one momentum" data (i.e., prevalence of infection) were also referred to and the need to obtain indices of rates of infection/mortality (i.e., incidence of infection) stressed. Reference was made to the calculation of mortality rates in a plaice population infected with *Ichthyophonus* (McVicar, ICES CM 1981/G:49) based on calculated incidence of the disease in that species. The regrettable failure of any ICES member country to resource research in this area for herring was referred to above (18.1.2).

19.2 Purpose of Request for Disease Information

The second discussion document (19b) drew attention to the broader aspects of rapid response to disease issues in fish stocks. The importance of being provided with well-defined requests relating to diagnosis and impact of diseases, on individuals or stocks or as indicators for environmental changes was highlighted. Different data sets will be required for each of these different purposes. Because of the disparate nature of data required, it is not possible to provide a "catch all" advisory statement. Each request should be considered on its own merits, with the individual circumstances being taking into account. Logically this should be done with reference to disease specialists and ideally co-ordinated through WGPDMO.

19.3 Recommendation

When disease issues are of concern to other ICES groups or outside organisations, requests should be channelled through the WGPDMO Chairman to relevant WG specialist members, either intersessionally or at the WG meeting, in order to facilitate a rapid and informed response.

20 ICES DISEASE PUBLICATIONS

20.1 Diagnostic Fiches

With the inability of the Editor of the Diagnostic Fiches to attend the 1995 meeting of WGPDMO, it was not possible to make progress on the current status of Fiches which had been submitted for publication but have not yet appeared in print. Members of the WG were reminded of the need to update existing fiches where significant new data on a disease is known and were encouraged to submit new titles and manuscripts to the Editor. A list of proposed new titles is presented (Annex 20a).

As previously requested, ICES should further publicise the existence and availability of the disease fiches. The Editor should provide information for publication in the EAFP Bulletin and AFS (Fish Health Section) News Letter.

20.2 Training Guide

Members of the WG who had participated in the development of the Training Guide indicated that it had progressed beyond the final draft stage and is now with ICES for publication.

20.3 Recommendation

Further publicity of the ICES Disease Fiches should be promoted by the Editor and by ICES.

21 ANALYSIS OF PROGRESS WITH TASKS

An analysis of progress of tasks as outlined in the Terms of Reference C. Res. 1994/2:23 is presented in Annex 21a and an indication given of intersessional work required. Agreement had been received from ICES to cancel TOR 2:23:1 and not to require the Sub-Group on Statistical Analysis of Fish Disease Data meet prior to the WGPDMO meeting.

22 ANY OTHER BUSINESS

22.1 Virus Common Names

The 1994 Report by the WGPDMO pointed out the need for an expert panel to examine the appropriateness of giving common disease names to viral isolates from fish and shellfish without evidence that the isolates are capable of causing disease (see CM 1994/E:5, item 18.2). The report pointed out the potential economic consequences of this practice and highlighted two groups of viruses where the practice has had, or could have had, negative impacts for the regions affected.

The recent isolation of a virus from cultured turbot in Scotland and from wild cod in Scottish waters underlines the need to urgently deal with this issue. The viral isolates were identified as VHS virus, largely on the basis of serology and in the absence of any evidence of their ability to cause disease in salmonids. As a result, the status of the affected fish farming area was changed from VHS-negative to VHS-positive.

The call for an expert panel is therefore again being reiterated, and the panel is being asked to look into methods for improving the precision with which viruses are identified. Also, because virulence testing in fish is a cumbersome procedure, the panel should consider whether it would be possible to identify genomic markers of virulence in viral isolates.

22.1.1 Recommendation

The WGPDMO recommends that ICES convenes a panel of experts to look into methods for improving the precision with which finfish viruses are identified and to consider the possibilities of identifying genomic markers of virulence in viral isolates.

22.2 North Pacific Marine Science Organisation (PICES)

For the information of WGPDMO members, an outline of the structure and organisation of PICES was presented (Annex 22a). It appears that, currently, there is no significant activity in the disease field by PICES. However, as valuable information on diseases of marine organisms is regularly provided from the Pacific coasts of Canada and USA by WGPDMO members in these countries, future activities in this field by PICES would be of direct interest to WGPDMO.

22.2.1 Recommendation

It is recommended that, through formal contacts between ICES and PICES, information on diseases of marine organisms be exchanged between WGPDMO and any specialists who may become involved in this field in PICES.

23 FUTURE ACTIVITIES OF WGPDMO

The possible future activities of WGPDMO were discussed and it was agreed that there was sufficient work to require a meeting again in 1996. Proposals for topics to be considered, in the form of proposed terms of reference for 1996 which could be recommended to Council, were compiled (Annex 23a).

23.1 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, WGPDMO should meet again during 1996. In order to have close contact with ICES staff involved in the compilation of the fish disease data base the proposal was made to meet in ICES Headquarters during March 1996, the earlier timing of the meeting reflecting the frequent need for WGPDMO to supply advice to other ICES groups meeting in the early part of the year.

1a, 1b. 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.

1c, 2. With the completion of the data entry programme for fish disease data by ICES and the intersessional provision of data into this by participating countries, problems in the practical use of the programme should be considered and a spatial and temporal trend assessment analysis of this data should be undertaken.

1d. Significant practical and legal problems were identified in the current practices of identifying and naming fish viruses. The extent of the problem and possible ways of resolving this should be discussed as a basis for an ICES convened future meeting of experts on this topic.

1e. Several research programmes are in existence to develop pathogen resistant strains of cultivated oysters and assessment of data available is required to ensure availability of appropriate information to growers in endemic areas with these diseases.

1f. As indicated in section 6.2 of this Report, possible problems of cross-reactivity of a gene probe for the shellfish disease MSX and a *Haplosporidium* sp. from *C gigas*. There is need for intersessional investigation of this problem and an evaluation of the results to determine the potential vector (carrier) status of Pacific oyster for MSX disease.

1g. As indicated in section 6.2 of this Report there are indications the Juvenile Oyster Disease (JOD) is showing an increase in its geographic range. A wider assessment of this potential problem is necessary to determine its full significance.

1h. *Bonamia ostrae* continues to show an expansion of its range and the risk of transmission of this serious disease by different molluscs should be urgently assessed.

1i. Progress should be maintained on the development of an international (ICES) data bank on shellfish diseases.

1j. Serious problems were detected in data produced intersessionally on methods used to determine antibiotic sensitivity of fish bacteria which required further investigation. Results of further intersessional studies should be assessed.

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

24 APPROVAL OF RECOMMENDATIONS TO COUNCIL

The Recommendations to Council from the WGPDMO 1995 meeting were approved.

25 APPROVAL OF DRAFT WGPDMO REPORT

The draft of the 1995 meeting of WGPDMO was submitted to all WG members present and approved. The Chairman drew attention to the need to send the final version to ICES with a minimum of delay because of the need of other ICES bodies for information contained. The Chairman indicated that the WG response to the Terms of Reference set by ACME, ACFM , HELCOM in the draft Report would be extracted with the relevant appendices and separately sent to ICES.

26 CLOSING OF THE MEETING

The meeting was closed by the Chairman at 12:00 on 7 April 1995. Dr H Grizel, Director of the IFREMER La Tremblade Laboratory, was thanked for the excellent facilities provided, the efficient organisation which had ensured the WG had functioned smoothly and the warm hospitality which he and his staff at La Tremblade extended to all of the WG members. Full appreciation of the WG members was also extended to the secretarial staff and others at La Tremblade for the way in which they cheerfully accepted the disruption and hard work associated with hosting the WG.

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ANNEX 1a

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ANNEX 3a

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

La Tremblade 3-7 April 1995

AGENDA

1) Opening of the Meeting. Structure of the meeting.

2) ICES Statutory Meeting 1994; 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 the results of the proposed intersessional study on antibiotics (MIC) or (MBC).

9) Review the current research in sea lice treatment and control methods.

10) Review the research on pathology and diseases in fish larvae reared in mariculture.

11) Review the reports of the BMB WG 25 "Fish Diseases and Parasites in the Baltic Sea", the "BMB/ICES Workshop on Diseases and Parasites of Flounder in the Baltic Sea" and the "BMB/ICES Sea-going Workshop on Fish Diseases and Parasites in the Baltic Sea".

12) Evaluate the possibility that reciprocal transfers of *M mackini* may occur among flat oysters (*Ostrea edulis*), Olympia oysters (*Ostreola conchaphilia*), and American oysters (*C virginica*).

13) Recommend means of standardisation of disease records used in shellfish pathology laboratories for use at the national level and by ICES.

14) Recommend appropriate protocols for monitoring procedures for detecting the prevalence of mollusc pathogens.

15) Report on the usefulness of external fish diseases as a tool in the monitoring of biological effects of contaminants.

16) Prepare an overview report on fish diseases in the Baltic Sea and their possible impact on mortality of Baltic fish stocks.

17) Overview report of new information on the M-74 syndrome and *Ichthyophonus*.

18) Review the present status of *Ichthyophonus* on herring populations and report on investigations on problems with supplying data on its prevalence.

19) Advice on methods to handle data in rapid response to disease issues in fish stocks.

20) ICES Disease Publications. Diagnostic fiches - update.

continued

AGENDA (continued)

21) Analysis of progress with tasks.

22) Future activity of WGPDMO.

23) Any other business.

24) Approval of recommendations.

25) Approval of draft WGPDMO report.

26) Closing of the meeting.

ANNEX 3b

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

La Tremblade, France, 3-7 April 1995

RAPPORTEURS

Agenda items	Rapporteurs
1-4	A. McVicar
5 (fish)	T. Lang, S. Mellergaard, D. Declerck
5 (shellfish/crustaceans)	S. McGladdery, P. van Banning
6 (fish)	B. Hjeltnes, G. Bylund, S. Feist
6 (shellfish/crustaceans)	S. McGladdery, P. van Banning
7	T. Lang, S. Mellergaard
8	T. Evelyn, F. Baudin-Laurencin
9	A. McVicar, B. Hjeltnes
10	F. Baudin-Laurencin, J. Barja
11	G. Bylund, T. Lang
12–14	S. McGladdery, P. van Banning, A. Farley
15	T. Lang, S. Feist
16–17	S. Mellergaard, G. Bylund
18	A. McVicar, B. Hjeltnes
19	P. van Banning, T. Lang
20–22	A. McVicar
23	S. Feist

ANNEX 7a

Draft proposals for future action of the Sub-group on Statistical Analysis of Fish Disease Data (Proposals from A D Vethaak).

Working Group on Pathology and Diseases of Marine Organisms, 1995.

Plan of future action of the SG (concept)

Completed tasks

1) Completion of new data format (S. Mellergaard and A.D. Vethaak)

2) Completion of ICES training guide (S. Mellergaard)

3) Compile a list of addresses of submitting members for ICES headquaters (A.D. Vethaak)

Ongoing tasks

1) Determination of contaminant maps (A.D. Vethaak)

2) Histological confirmation of liver nodules (S. Feist?)

3) Report on suitable statistical methods to analyse the ICES fish disease prevalence data using individual fish data and to correlate results with sediment contamination data and other relevant factors (S. Des Clers).

4) Update new data and coordinate future studies on age/length relationships in dab (T. Lang and S. Mellergaard)

5) Review and report on available data on:

   a. fishing effort in the North Sea and Baltic Sea;
   b. stock identity and population densities of cod, dab and flounder in the same areas (T. Lang, S. Mellergaard, A.D. Vethaak)

6) Explore possibilities of data submission by Canadian and America (C. Couillard) and nonsubmitting European member countries (A.D. Vethaak)

7) Compile a mailing list of scientists interested in studies in marine fish diseases (S. Feist?)

New tasks

1) To assist ICES with the possibilities for conversion of existing fish disease data into new ICES format (S. Mellergaard/ A.D. Vethaak)

2) To undertake a preliminary analysis using (converted) disease data sets of selected areas submitted by some members in summer 1995. This will also allow a preliminary assessment of possible problems involved using the new format (S. Mellergaard and A.D. Vethaak);

Recommendation

1) To recommend to the WGPDMO that the Subgroup should meet in Copenhagen HQ during Spring 1996 in order to make a fullblown analysis of all data submitted.

ANNEX 7b

ICES Fish disease data entry format.

Working Group on Pathology and Diseases of Marine Organisms, 1995.

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ÝÝÝÝ° Sampling Date..: 06-02-91               Sampling Time..:             °ÝÝÝÝ
ÝÝÝÝ° Specie ........: PLAT FLE               Water Depth ...:             °ÝÝÝÝ
ÝÝÝÝ° ShipCode ......: 99                                                  °ÝÝÝÝ
ÝÝÝÝ° Cruise ........: 9999                                                °ÝÝÝÝ
ÝÝÝÝ°                                                                      °ÝÝÝÝ
ÝÝÝÝ° Latitude ......:     563600 North                                    °ÝÝÝÝ
ÝÝÝÝ° Longitude .....:     752000 East        JMP Area ......:             °ÝÝÝÝ
ÝÝÝÝ°                                         Other Area ....:             °ÝÝÝÝ
ÝÝÝÝ° Purp.Of Mon. ..: [*]   ___B_                                         °ÝÝÝÝ
ÝÝÝÝ° Organizations .: [*]   I____                                         °ÝÝÝÝ
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ÝÝÝÝ° VESSL .........: Research                                            °ÝÝÝÝ
ÝÝÝÝ° Station Type ..: Coast                                               °ÝÝÝÝ
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Level 2

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ÝÝÝÝÝÝÝÝÝÕá[Sample Info]áááááááááááááááááááááááááááááááááááááááááá¢ÝÝÝÝÝÝÝ
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ÝÝÝÝÝÝÝÝÝ° ShipCode : 99         Cruise ..: 99       Specie ..: PLAT FLE°ÝÝÝÝÝÝÝ
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ÝÝÝÝÝÝÝÝÕá[Disease Haul Information]áááááááááááááááááááááááááááááá¢ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ°                                                            °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ° Damaged .........: No            Scientist ...: SORENSEN   °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ° Cod End Mesh Size:  50           Observer  ...: SORENSEN   °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ°                                                            °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ° Duration ........:                                         °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ°                                                            °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ° Net Opening .....:               Stratified ..: Random     °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ° Speed ...........:   30          Other Haul ..:            °ÝÝÝÝÝÝ
ÝÝÝÝÝÝÝÝ° Inspection time .:   50                                    °ÝÝÝÝÝÝ
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Level 1 and 2 are to be filled once for each haul.

Level 3.

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ÝÝÝÝÝÝÝÝÝÝÖá[Sample Info]áááááááááááááááááááááááááááááááááááááááááááá¢ÝÝÝÝÝÝÝÝ
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ÝÝÝÝÝÝÝÝÝÝ° ShipCode : 99         Cruise ..: 99        Specie ..: PLAT FLE°ÝÝÝÝÝÝÝÝ
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ÝÝÖá[Disease Speci]áááááááááááááááááááááááááááááá¢Öá[Diseases]áááááááá¢ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °°                     °ÝÝÝÝÝÝÝÝ
ÝÝ° Ind/Bul/Hom ..: Individual                   °° Chk Aff  Disease    °ÝÝÝÝÝÝÝÝ
ÝÝ° SexCode ......: Male                         °°                     °ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °° [ ] [ ]  CRYP COT   °ÝÝÝÝÝÝÝÝ
ÝÝ° No Examined ..:      1                       °° [ ] [ ]  EPID PAP   °ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °° [ ] [ ]  GLUG STE   °ÝÝÝÝÝÝÝÝ
ÝÝ° Min Length ..:           Min Age ..:         °° [ ] [ ]  ICHT SPP   °ÝÝÝÝÝÝÝÝ
ÝÝ° Max Length ..:           Max Age ..:         °° [ ] [ ]  LIVE NOD   °ÝÝÝÝÝÝÝÝ
ÝÝ° Mean Length .:   220     Mean Age .:         °° [*] [*]  LYMP CYS   °ÝÝÝÝÝÝÝÝ
ÝÝ° Std. Dev. ...:           Age Detec.: Otolith °° [ ] [ ]  PSEU TUM   °ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °° [ ] [ ]  SKEL DEF   °ÝÝÝÝÝÝÝÝ
ÝÝ° Size Code....:                               °° [*] [*]  SKIN ULC   °ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °° [ ] [ ]  XGIL LES   °ÝÝÝÝÝÝÝÝ
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ÝÝÝÝÝÝÝÝÝÝ° ShipCode : 99         Cruise ..: 99        Specie ..: PLAT FLE°ÝÝÝÝÝÝÝÝ
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ÝÝÖá[Disease Speci]áááááááááááááááááááááááááááááá¢Öá[Diseases]áááááááá¢ÝÝÝÝÝÝÝÝ
ÝÝ°                ôááááááááááááá¢               °°                     °ÝÝÝÝÝÝÝÝ
ÝÝ° Ind/Bul/Hom ..° Individual    °              °° Chk Aff  Disease    °ÝÝÝÝÝÝÝÝ
ÝÝ° SexCode ......° Bulked        °              °°                     °ÝÝÝÝÝÝÝÝ
ÝÝ°               ° Homogenized   °              °° [ ] [ ]  CRYP COT   °ÝÝÝÝÝÝÝÝ
ÝÝ° No Examined ..âááááááááááááááì               °° [ ] [ ]  EPID PAP   °ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °° [ ] [ ]  GLUG STE   °ÝÝÝÝÝÝÝÝ
ÝÝ° Min Length ..:           Min Age ..:         °° [ ] [ ]  ICHT SPP   °ÝÝÝÝÝÝÝÝ
ÝÝ° Max Length ..:           Max Age ..:         °° [ ] [ ]  LIVE NOD   °ÝÝÝÝÝÝÝÝ
ÝÝ° Mean Length .:   220     Mean Age .:         °° [*] [*]  LYMP CYS   °ÝÝÝÝÝÝÝÝ
ÝÝ° Std. Dev. ...:           Age Detec.: Otolith °° [ ] [ ]  PSEU TUM   °ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °° [ ] [ ]  SKEL DEF   °ÝÝÝÝÝÝÝÝ
ÝÝ° Size Code....:                               °° [*] [*]  SKIN ULC   °ÝÝÝÝÝÝÝÝ
ÝÝ°                                              °° [ ] [ ]  XGIL LES   °ÝÝÝÝÝÝÝÝ
ÝÝ°             Diseases É     Haul É            °°                     °ÝÝÝÝÝÝÝÝ
ÝÝ°             ôôôôôôôôôôô    ôôôôôô            °°       Specie É      °ÝÝÝÝÝÝÝÝ
ÝÝâááááááááááááááááááááááEditáááááááááááááááááááááááì°   ôôôôôôôô      °ÝÝÝÝÝÝÝÝ
                                                   âááááááááááááááááááááááì
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At level 3 it is possible to order data on individual fish basis or as bulked or homogenized data

The column "Chk" indicates by asterisks which diseases the fish are checked for. The column "Aff" indicates which diseases are observed on the individual fish.

ICES working group on Pathology and Diseases of Marine Organisms

Correlation between MIC and zone diameters for strains of A. salmonicida.

For this experiment two antibiotics were used, potentiated sulfonamides (we have used co-trimoxazole a combination of trimethoprim and sulphamethoxazole 1:20) and oxytetracyline.

For MIC we have a used a plate assay (Iso Sensitest Agar) containing from 0.125 to 256 µg/ml of co-trimoxazole or oxytetracycline. The inoculum for the MIC test was prepared from fresh cultures grown on Iso Sensitest Agar and plates were inoculated with 20 µl drops calibrated to contain 1 X 10^4 cfu. MIC were read after 48 hr incubation at 22°C. Antibiograms were performed on Iso Sensitivity test agar with discs of 25 µg of co-trimoxazole and 30 µg of oxytetracycline (discs from Oxoid), zones were read after 48 hr incubation at 22°C.

Results obtained are presented in Table 1. With oxytetracycline there was good agreement between MIC and zone diameters for susceptible and resistant strains of A. salmonicida with no intermediate strains detected (Fig 1). The results with co-trimoxazole are interesting since there was a larger range of MICs found (Fig 2).

Comments on the exercise

Following the exercise of 1993 were strains were compared in different laboratories, the major finding was that there was no standardization of technique to determine antibiograms with strains of A. salmonicida.
For several antibiotics results were in agreement but large variations were found for oxolinic acid (quinolones) and potentiated sulfanomides. A small experiment was performed this year on strains of A. salmonicida where the MIC was correlated with the zones of inhibition for oxolinic acid, oxytetracycline and co-trimoxazole (potentiated sulfonamide) figs 1, 2 and 3.
Results obtained for oxolinic acid where the MIC and zones of inhibition were compared (see comments by Trevor Hastings) indicate some of the problems associated with this work.

First, as with the antibiogram technique, MIC can be performed using a variety of techniques, some of the important factors include:
- MIC determined in liquid media or agar media
- Type of media used
- Inoculum standardization
- Concentration of bacteria used

Some of Trevor's concerns include the methodology used to determine MIC, his data indicate that liquid and agar media do not seem to provide the same MIC (media were also different). To confirm the above the same strains would have to be run at the same time using both techniques. His statement that some strains gave zones of inhibition of 20 to 24 mm and yet had an MIC of greater than 5 µg/ml is very puzzling and disturbing since the reason for using an antibiogram technique (when it is correlated with MIC values) is to give an indication of the level of resistance of a strain to a specific antibiotic. For the strains described by Trevor the interesting question is are those strains resistant or of intermediate resistance to oxolinic acid?. In other words we have to determine sensitive-resistant breakpoints. This task is the difficult part and can only be done if an acceptable MIC method is approved and if we have information on pharmakokinetics of the particular antibiotic in fish. There is information on pharmakokinetics of drugs in the literature but we need to summarize the data and compare to the MIC values obtained. I suggest that this is a task that could be done by some of us but only for the important therapeutic antibiotics.

Results obtained using oxytetracycline are presented in fig 1 there seems to be a good separation of resistant and susceptible strains and maybe some type of breakpoint could be achieved with this antibiotic.

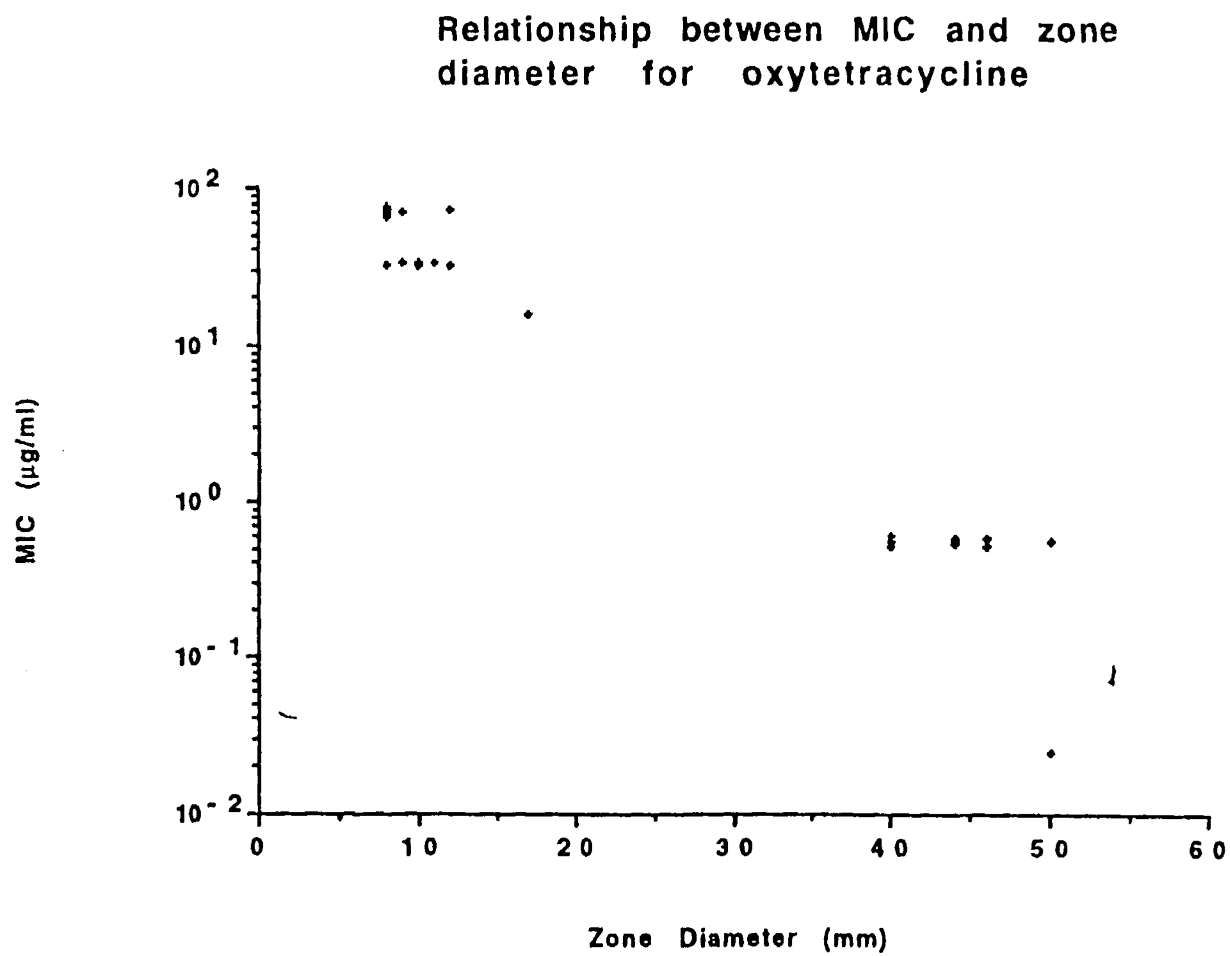
The results with co-trimoxazole are similar to those obtained with oxolinic acid, there seems to be a good separation between susceptible strains (MIC < 2 µg/ml) and resistant strains (all > 256 µg/ml). Some strains were of the "intermediate" type (MIC of 4,8 and 16 µg/ml) would these latter strains represent true intermediate isolates ?. As with oxolinic acid the levels of the drugs in vivo needs to be evaluated from the existing literature and compared to MIC obtained, only then will MIC values be validated.

This is what we have achieved so far, I think this is an interesting topic to pursue and the results should be made available to somebody for the upcoming EAFP meeting in September Since a special session is suppose to be held on standardization of antibiogram determination. I could talk to Pete Smith who will be chairing the session since Trevor Hastings and I will probably not attend.

SUMMARY TABLE MIC

TABLE 1									
			Oxytetracycline				Potentiated Sulfanomides		
			MIC	Zone diameter			MIC	Zone diameter	
strain		#	µg/ml	mm			µg/ml	mm	
86049-19	Spain	1	0.5	40			2	38	
ae-or	NB	2	32	10			4	33	
ns4	NS	3	32	12			>256	8	
94438	NB	4	32	8			>256	8	
harris-2	NS	5	32	8			>256	8	
cyp-93	BC	6	64	8			>256	8	
BJ	NB	7	0.5	46			2	46	
BC-11	BC	8	64	8			1	38	
BC-7	BC	9	64	8			>256	8	
95063	NB	10	0.5	44			1	42	
94402	NB	11	0.5	44			1	40	
878	Que	12	32	11			16	20	
f-1 cla	Que	13	32	10			2	34	
289-4	BC	14	64	8			>256	8	
gre -93	BC	15	64	9			>256	8	
837	Que	16	32	9			16	20	
289-9	BC	17	64	8			1	40	
F	NB	18	0.5	40			1	34	
M17-k	BC	19	64	8			>256	8	
m-17-s	BC	20	64	8			>256	8	
oka 2	BC	21	32	10			1	34	
ds otcr	BC	22	64	8			2	25	
450sk	FRA	23	0.025	50			1	44	
94319-55	BC	24	0.5	46			1	40	
94390	NB	25	0.5	44			1	42	
289-4	BC	26	64	8			>256	8	
810	Que	27	32	10			16	14	
818	Que	28	16	17			2	26	
65-R	FRA	29	32	11			2	29	
93442-16	BC	30	64	8			1	42	
94292	BC	31	32	12			>256	8	
MTOO4	SCO	32	0.5	40			0.5	44	
NG 10	NB	33	0.5	44			1	42	
OAR	NB	34	64	12			2	31	
als	NB	35	0.5	50			0.5	40	
lll	FRA	36	32	10			4	31	

FIG. 1.



Relationship between MIC and zone diameter for oxytetracycline
MIC (µg/ml)
10^2
10^1
10^0
10^{-1}
10^{-2}
0
10
20
30
40
50
60
Zone Diameter (mm)

03/31/95 FRI 14:38 FAX 902 426 1862 DFO BF&A HFX. NS

FIG 2.

Relationship between MIC and zone diameter for potentiated sulfanomides

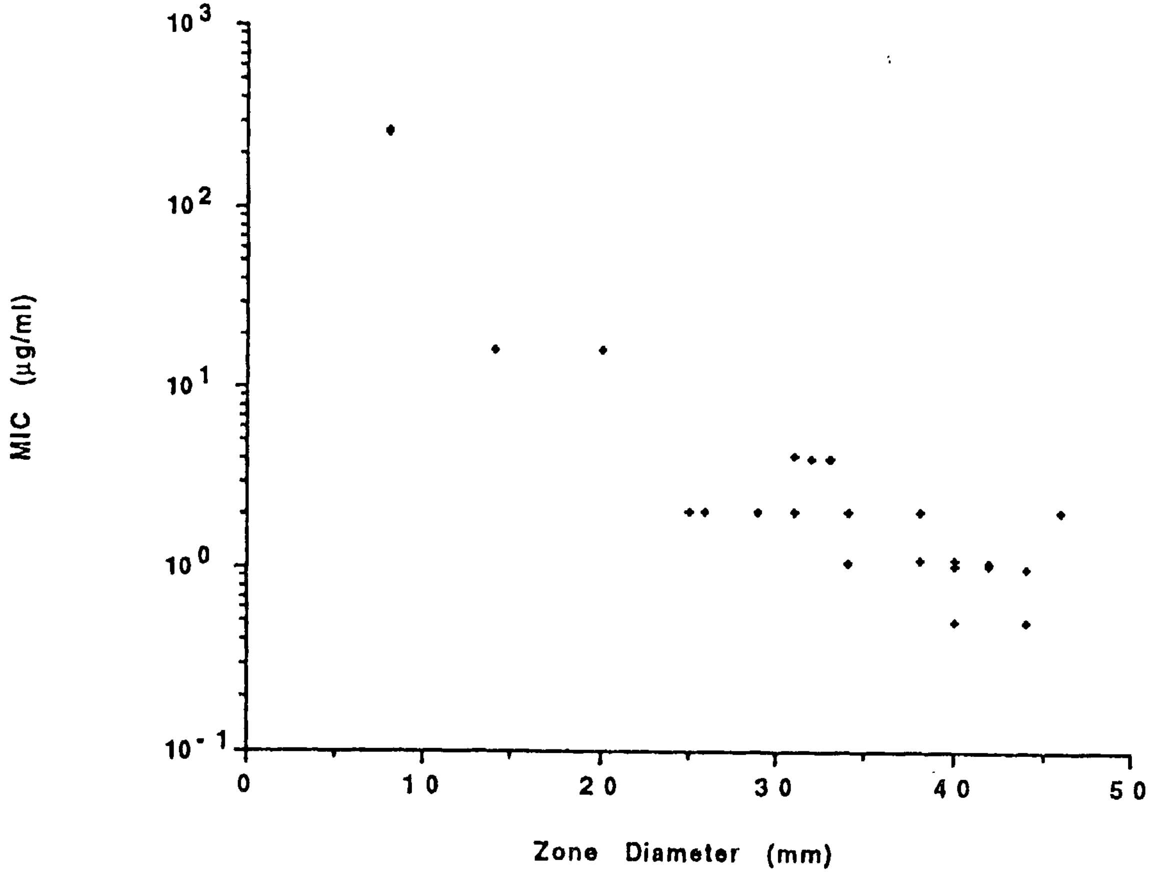
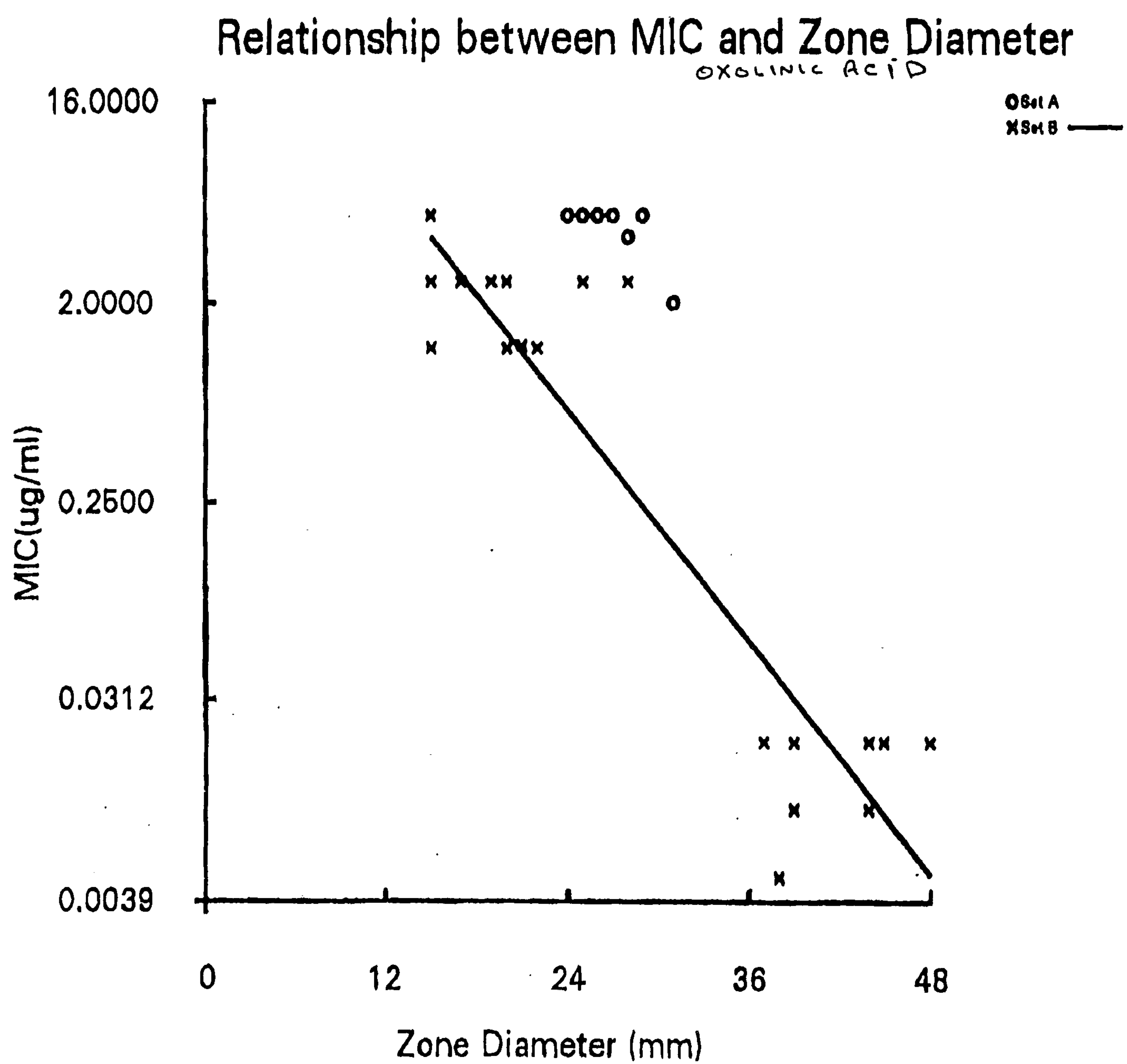


FIG. 3.


Relationship between MIC and Zone Diameter
OXOLINIC ACID
O Set A
X Set B
16.0000
2.0000
0.2500
0.0312
0.0039
MIC(ug/ml)
0
12
24
36
48
Zone Diameter (mm)

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

La Tremblade, April 1995.

Sea lice treatment and control in Scotland

A H McVicar

1. Introduction

This paper is produced in response to the ICES Resolution C.Res. 1994/2:23.e:

"Review the current research in sea lice treatment and control methods including: chemical, biological, immunological and management practices."

Sea lice (particularly *Lepeophtheirus salmonis*) are presently considered to be the greatest single disease problem in Scottish salmon farming. Losses of up to 20% of total salmon production have been attributed to lice infection. The seriousness of the problem is reflected by the continued number one priority status given by industry and their representative bodies to research effort in this area (CFRD Working Group on Fish Diseases in Aquaculture, November 1994). Currently the Scottish Salmon Growers Association devote 66% of their scientific research budget to lice related projects and many individual fish farming companies co-operate directly in projects through providing access to salmon stocks for sampling and tests. The main focus of research is aimed at finding non-chemical methods of control, with particular emphasis in vaccine development. Research is currently also in progress on various aspects of lice biology particularly of larval stages eg host finding and in the development of alternative chemotherapeutants. Alternative novel methods are being investigated in association with a number of commercial companies.

Major research projects on these topics are currently in progress in the SOAFD Marine Lab Aberdeen, Stirling University Institute of Aquaculture, Zoology Department University of Aberdeen and in co-operation with the University of Cork. Funding for these projects is derived from Government, Research Council, European Community, Crown Estate Commission and industry sources and is in excess of approximately £3m annually.

Infection with *L salmonis* in Scottish waters is endemic as there is a naturally occurring level of lice even in areas well separated from fish farms (up to 54 lice on a sea trout caught at Montrose on the east of Scotland). As total avoidance of infection is not a realistic option, control and management have to be based on the maintenance of low levels of infection on farms and the removal of infection by treatments. It is noteworthy that pump-ashore salmon farms do not experience sea lice problems.

2. Chemical Control.

Aquagard and hydrogen peroxide are the mainstay of chemotherapy of lice infection in Scotland and although the latter is widely used, it is not yet fully licenced.

A wide range of alternative chemicals are currently under test and as these are subject to guarantees of confidentiality to the manufacturers it is not possible to list these. It is possible that an improved organophosphorus compound and a pyrethroid derivative will be subject to a licensing procedure during 1995. A related study is investigating the basis of resistance to chemotherapeutants in populations of sea lice on wild and cultured salmonids. There are major concerns about the possibility of withdrawl of the licence for Aquagard before efficacious alternative lice control methods are available.

3. Biological control.
Wrasse, particularly Goldsinny (*Ctenolabrus rupestris*) and Rockcook (*C. exoletus*) have been widely used during the first summer of salmon in sea cages, with some of the larger companies stocking all post smolt cages at a level of one wrasse to 50 smolts. There is a general belief in the effectiveness of cleaner fish during the year of smolt input into sea cages but it is, as a whole, not considered to be the complete answer and some farmers have now ceased using them. Many post smolt cages stocked with wrasse required no treatments for *L salmonis* during the first year but it is difficult to separate the effect of wrasse from other measures carried out simultaneously on farm sites (eg fallowing of the site prior to smolt input). Negligible numbers of wrasse survive through the winter period and it is not believed that these remaining have any effect on lice numbers during the second year. There is a question about the sustainability of exploitation levels of the wild populations of wrasse.
Research is being published on the biology of wrasse, on their naturally occurring diseases and on their susceptibility and carrier status with respect to salmon diseases. Research is also in progress on ways of improving the overwintering capability of wrasse.
Chemicals linked to louse pheromonal activity associated with males finding females and any chemical (semiochemical) basis for host location are being investigated to evaluate the possibility of using such mechanisms to develop a "lure" to trap lice.
Studies are in progress on the structure and physiology of motile and non motile stages of *L. salmonis* in an effort to identify factors contributing to the difference in the susceptibility of these different life cycle stages to chemotherapeutants. No significant differences have so far been detected in either the cuticle or gut.

4. Immunological control.
Salmon are not known to develop a natural immunity to sea lice, probably as a consequence of lice developing a life cycle strategy of not transferring any important antigenic material to the host fish during attachment and feeding. Therefore the development of a louse vaccine may be dependent on the identification of antigens normally hidden and which may confer protection eg associated with the louse gut. Salmon immunised with total lice extract, although responding against some proteins, did not show protection against infection probably because of antigenic competition. Two refinement approaches are currently being pursued:
a) separation of proteins by chromatography and the selection of these associated with the louse gut is currently being pursued in Plymouth University.
b) using monoclonal antibodies to proteins expressed from louse DNA libraries, protein components of the louse gut were identified in Aberdeen by immunohistochemical screening of sectioned lice. The antibody response of salmon immunised with selected clones expressed as fusion proteins was monitored by ELISA and responding fish experimentally challenged with sea lice copepodites. The development of experimental challenges with lice was used as an indicator of protection. In preliminary trials , lice on salmon immunised with three of the antigens developed short and deformed egg strings. Additional studies are in progress to further investigate the worth of these three antigens and their potential as a basis of a vaccine will be assessed. Up to the present point, results have been encouraging. This is a major study financed by the EC and industry involving the Marine Lab Aberdeen, University of Aberdeen, IOA Stirling University and University College Cork, Ireland.

5. Management control.
A major strategy in the control of furunculosis introduced by the Scottish salmon farming industry has been the fallowing of sites prior to the introduction of smolts. It is not typical for a company to introduce such strategies on some sites but not others and the opportunity for direct comparison of the value of fallowing in lice management is thus difficult to determine. Considering Scotland as a whole, it is unusual for a site which has been fallowed prior to introduction of smolts to require significant chemotherapy for lice during the first year, although there is some variation apparent between sites, probably associated with the proximity of other cage sites and of river estuaries with infected populations of wild salmonids. This is in marked contrast to previous experiences in many sites with overlapping year-classes of salmon where there was clearly transfer of lice from older to younger fish and a need for frequent treatments from shortly after smolt introduction. Such sites often showed a progressively increasing lice problem over a period of years. Area management plans, where fallowing is co-ordinated over identifiable sea areas and involving a multiplicity of farm sites, is considered by industry to enhance the beneficial effects of individual site fallowing.
Although there is no known specific research identified in Scotland on the role of fallowing in sea lice management and no easily quantifiable beneficial effects which can be separated from other control strategies, it is the general opinion in the salmon farming industry that the use of fallowing to break the life-cycle of lice on farms is a strong contributory factor in sea lice control and certainly affords newly transferred smolts some degree of protection.

6. Conclusions.
Research on *L salmonis* occupies a major role in the total research effort on fish diseases in Scotland. Funding is broadly based involving government, Research council and industry (both production and supply) sectors and a wide front of specialist expertise is being applied. The urgent need to rapidly supplement the availability of licenced chemotherapeutants is driving a significant level of effort in this area. There is a major emphasis now being directed at non-chemical methods of lice control, particularly towards the development of a vaccine.

Latest weapons in the war on lice

Dr CHRISTINA SOMMERVILLE, Institute of Aquaculture, University of Stirling, looks at treatments and the need for an integrated pest management strategy

UNTIL recently, there were no options for sea lice control. Only one treatment compound was available, Dichlorvos (DDVP) which had been under threat of withdrawal for a long period of time while the licensing authority considered giving it a licence. Even when this compound became more secure, its use was problematical, largely as a result of the passion stirred up by the green lobby.

When the first evidence of resistance was reported five years ago (Jones, Sommerville & Wootten 1990), it was clear that alternatives were urgent. Thanks to the farsightedness of some commercial companies, fortified by injections of cash and subtle pressure from the SSGA and ourselves, a number of alternatives have been tested. Some 30+ drugs have now been screened by Stirling alone, mainly in collaboration with the pharmaceutical and chemical companies supported by the SSGA and the Crown Estates.

During the last few years several promising compounds have emerged both for bath and oral (in feed) treatments. Though the choice is currently limited, within the next few years we anticipate a further 4 – 5 products to be added to the list.

Aquagard, the product name for dichlorvos, is now well established as a louse treatment. It was fully licensed in 1989 with the proviso that its use be reduced by 50 per cent despite the fact that it was the only licensed compound available. Since then it has been monitored annually and the current usage has gone down to less than 10 per cent of the 1989/90 level. Such reduction has been achieved largely by restrictions on discharge licences permitted by the river boards.

Resistance was tested four years ago and half of the sites sampled (seven out of 14) were showing some degree of reduced sensitivity. It is possible that this proportion has increased since but there has never been a nationwide survey. Stirling University has just started a study which will, among other things, try to find out the mechanism for resistance.

The future of dichlorvos is still open to debate and this is not unconnected with the public fears regarding sheep dip. Despite many years of use of this compound for fish, there has not been any documented human or ecological disaster. Problems with the use of dichlorvos in pest control are associated with its application and the fish farming industry has been very responsible in this regard.

Paramove and Salartect are both hydrogen peroxide and are not yet fully licensed. Animal Test Exemptions (ATX) were granted after submission for licence by two companies independently, Solvay Interox and Brenntag, enabling a large number of treatment trials under a wide range of conditions. The licence submissions are being considered separately by the licensing authority who require further information before licence will be granted. However the potential for full licence is strong and is expected very soon.

There is now a good deal of experience in using hydrogen peroxide, which has been shown to be very effective. Some caution is felt to be necessary in warmer temperatures, however.

Both products are now available at 35 and 50 per cent concentrations. The 50 per cent product has advantages in transport and handling and there do not appear to be any real disadvantages although it is slightly more hazardous. This concentration is being used in the Faroes and in Norway.

BATH TREATMENT

Although it is expensive, hydrogen peroxide does not affect appetite and there are no residues; also, unlike other compounds, it has a very clean image. The use of hydrogen peroxide as a water treatment has shown that it has beneficial effects on the bacterial load in the environment and this suggestion of other advantages means that it has had no problem with discharge consents. In the US, hydrogen peroxide has been "given the nod" for use as a fungicide and there would appear to be good reason to explore the potential of this compound further.

Salmosan, from Ciba Geigy, is the organophosphate azimethophos with the potency of one tenth the dose of dichlorvos. It has the added advantage of breaking down very quickly in the environment with a half life estimated at 10-11 days at 20 C pH7. When licensed, the product will be supplied in water soluble sachets so that there will be no weighing out, thus its handling will be rendered as safe as possible. Unlike dichlorvos, its toxicity to humans is apparently low and on a par with the pyrethroids.

This organophosphate was submitted for a UK licence as long ago as 1988 and is still under consideration. The process of obtaining a licence for it has been a learning experience for all involved, especially regarding the time it takes to undergo all the various processes. It is now in the third and final stage of assessment for a licence. All that remains is some further toxicity data on the effect on sentinel species in the field i.e. stage 4 lobster larvae.

This has to be done to GLP (good laboratory practice – a sort of "kite mark" for the standard of the experimentation). Because of the difficulties of doing this in the field and because lobster larvae are seasonal, it takes some time. Possibly computer generated models will accelerate this process in the future.

The MRL's (Maximum Residue Levels) need to be set and this has yet to be discussed by the EC, but research has shown that it may not be necessary. There are no tissue residues and it is therefore hoped that it will go into the Annex II category, where it is considered to be so safe that no figures are needed. No EQS's (Environmental Quality Standards) have yet been set and this is the responsibility of the river boards. Such action will not hold up the licence, but it could hold up the usage, depending on their attitudes. The expected launch date for licence is early 1995.

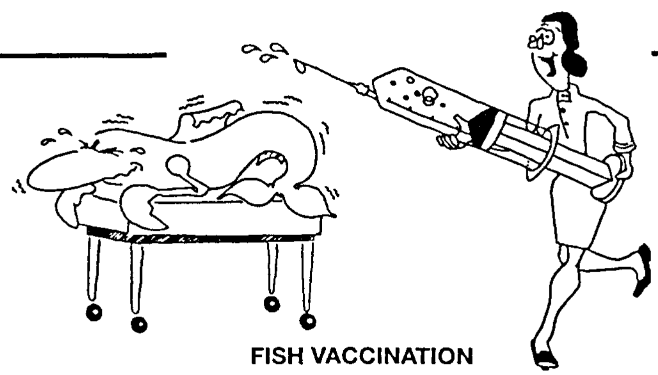
Salmosan is in use currently in Norway under the Norwegian equivalent of an ATC. According to Ciba Geigy, 90 per cent of the market there is using it. It is much less expensive there than hydrogen peroxide and only marginally more expensive than Aquagard. It appears to act very rapidly and is particularly efficacious at the higher temperatures when the minimum effective dose can be reduced.

Compound X

This enigmatically titled compound is a cypermethrin produced by Grampian pharmaceuticals. It appears to control chalimus as well as adults, is apparently safe to humans and is effective at very low doses – less than 100 ppb. The treatment volume is 500ml of a 1 per cent solution and is therefore considered to be safer to handle. It adsorbs to solids of all kinds including sediments and is then rendered toxicologically inactive.

I am told that Ecotox data on 12 different marine species has been c lected and, on 11, eg starl mussels, *Corophium*, thei are safety margins of 1,000 fold. Though it requires a continuous feeding re there is a short withdrawal of up to 24 hours and the applied for is 50ppb. An a tion for an ATC has been ted in the UK and the hopeful that this will be available soon.

The use of Pysal was powered by the Norwegians and it has not been progressed in the UK. The product is pyrethrum which shows good efficacy, but there are problems associated with its application. These relate to its rapid decomposition in light; the fact that it is not very water soluble and that pyrethrum is a natural product. As such its purity is difficult to define for the purpose of a licence.



FISH VACCINATION

Oral treatments

Ivermectin is the best known oral treatment at the present time but it is not licensed for fish. It can only be used as a POM (Prescription Only Medicine). Consequently, it can only be prescribed by a vet when there is nothing else available or suitable which will treat the particular problem on a particular farm in a particular situation. It is used under a "cross species" code of practice and would normally be restricted to a small number of animals (bearing in mind that the legislation was originally designed for mammalian husbandry systems). It might therefore be prescribed, for example, where there was resistance to all other treatments.

Because it is to be used in feed, only a product which has been licensed for use in feed must be used. Thus the product Ivomec, a premix for pigs, should be selected. The vet will only prescribe this if there is a discharge consent from the river board which the vet must see. The river board for their part must see Ecotox data before they can consider issuing a discharge consent and in the case of Ivomec this does not exist.

The SSGA are attempting to acquire this data so that EQS's can be set. In the absence of a licence, no MRL's have been set. However, work published by Roth, Rae and McGrath on tissue residues has led to the suggested guide of 1200 degree days.

Despite the widespread use of the product in Ireland on an experimental basis, there will never be a licence for its use in fish since the producers, Merck Sharp and Dohme, will not progress it, as they disapprove of its use in fish. On the other hand, the family of compounds to which it belongs, the ivermectins and the milbemycins, have considerable potential.

A further five in-feed compounds are being developed and their chemical nature has not been revealed, though they are very promising.

BP have been testing in-feed compounds with low or no environmental impact at their Norwegian research centre (ARC). ARC's sea lice project has been running for almost three years, and, after an extensive screening programme, a suitable candidate compound for an oral treatment was identified which is said to be highly effective against all stages of lice and having a wide therapeutic window. It is also safe to the handler. Trials are currently being carried out and extensive ecotoxicity and environmental sampling programmes are now running. They hope to complete studies within six months.

Ewos also has a product which prevents the parasite from becoming mature. This is the only infeed compound which is specifically targeted against juveniles, i.e. chalimus and pre-adults. It has been tested in Norway since last May using a 2-week continuous feed of a low dose and Ewos report very encouraging results. A total of 19 trials have been carried out thus far and the data is still being analysed. Because it will not kill adult lice, a two week treatment may need to be repeated.

Ewos are satisfied that it is safe for humans, fish and the environment and are currently in discussion with the river boards on discharge.

News is also good regarding the withdrawal time, which they anticipate will be short. An application for an ATC was made in the UK at the same time as Norway but there is no information about that as yet. More work has still to be done on this compound but they hope that it will get an ATC early next year.

The practice of stocking wrasse into sea cages is now well established and despite the variability in the reported success, there does seem to be a place for them at some sites and in some situations. Their use requires careful management but the reduced need to treat has been frequently reported. Unfortunately, although they have a value, they are clearly not the complete answer to lice problems.

The advantages of fallowing are now indisputable and practice has proven the benefits. To be useful, a fallowing should be as long as possible but if we take into account the longevity of the female louse when not attached to a host, add to that time taken from the hatching of an egg through to nauplius stages up to the infectious copepodite stage and the duration of that copepodite stage, we reach a figure of approximately 10 weeks. However, the longevity of the louse off the host and her ability to produce viable eggs throughout that period is not known in the wild situation and may well be shorter.

Similarly, despite the fact that the copepodite stage may survive up to two weeks in the lab, it does not mean that it is infective for that period. Indeed it has a finite energy source of lipid and we can predict that the infective period is rather shorter than that. Thus we have suggested a fallow of not less than six weeks.

For fallowing to be really effective, it must be carried out synchronously within a given area. We can't be precise about the size of the area at the present time because we don't know the geographic distribution of the lice populations – this is something we hope to determine shortly.

Because *Caligus* has a wide host range, fallowing will probably have less impact on *Caligus* populations but will serve to prevent amplification of their overall population size by reducing the number of hosts available.

Two other possible tools for the future are light lures and vaccines. The former is based on the fact that the infectious stage, the copepodite is phototactic. If you shine a light into a tank full of them, they will tend to aggregate in that direction. Again, this is lab-generated data. What happens to the light under water and how attractive it is remains open to question as the quality, direction and other parameters change even within short depths. The behaviour of these parasites is very complex and is designed to find a host, which they do superbly well. Is it possible to fool them? Pilot lures are under test.

A search for a vaccine has been under way for some years by a consortium of labs consisting of Stirling, Aberdeen, Cork and SOAFD, who are also the co-ordinators. It is largely funded by the EU, with support from the SSGA, MAFF and SOAFD. The broad approach is one which was shown to be successful in producing a vaccine against ticks in cattle in Australia.

The Australians took 12 years to find an effective product and it is unlikely that we will produce a vaccine in less than this period, bearing in mind that they were dealing with a mammal with a sophisticated immune system and a parasite which is a specialised blood feeder.

The immune response in fish is not yet so clearly understood as in mammals, especially its defences against macro parasites. In addition, lice are mixed tissue and fluid feeders and, by the time they are feeding on blood, they have already severely compromised the fish. Nevertheless we are pursuing the sea lice vaccine with cautious optimism.

I've tried to summarise the options which are currently available and those which will be available within the next year or two. There is, however, no possibility of totally eliminating lice from salmon farms. It is unrealistic to imagine that somewhere there is a single drug, chemical or other treatment which, if we could only find it, will wipe out lice for good – no more than there is for mosquitoes or flies or other arthropod pests.

Though chemical treatments have a vital role in lice management, we cannot afford to rely on a single compound, nor yet drug treatments alone, because the development of resistance seems inevitable and can occur surprisingly rapidly.

A set of resistance management principles were set by UK workers in the field of pest management in 1993. Though they were considered in relation to insect pests, they are very applicable and are suitably modified as follows:

Resistance Mananagement Principles

- Always include efficient cultural/biological controls i.e. husbandry and biology as well as chemicals.
- Time the application of the insecticides against the most susceptible life stages based on local pest thresholds (monitoring and applying when the population structure is appropriate).
- Do not rely on a single pesticide class (we are entering into a period of having the luxury of choice).
- Use insecticides at recommended dose rates and spray intervals (this is probably the way resistance has developed against dichlorvos).
- When there is more than one generation of the pest insects, use different classes of insecticides in alternation (this is self evident – eg. organophosphate, hydrogen peroxide, cypermethrin).
- In the event of a control failure due to resistance, do not respray an insecticide of the same class.

Special considerations for salmon cages

- Don't treat piecemeal; do the whole site, otherwise adjacent cages will experience the dilute chemical;
- Use full tarpaulins whenever possible
- Synchronise treatments with adjacent sites
- Where possible assess treatment efficacy accurately by direct counting of lice 24 hours after treatment

It is important to ensure that no one product is used so frequently as to promote resistance. There is time to take a proactive approach to minimise resistance development in the battle against lice and this will be best achieved by using an "integrated pest management strategy".

The salmon farming industry already has some options, with more to come, and can now design and adopt integrated pest management strategies for the control of sea lice infestation.

Prevention – using current knowledge and applying it, for example, to site selection, stock management, hygiene, fallowing, single year class stocking, stress minimisation and so on.

Monitoring is a must. We have been advocating this for many years and it has, for many, been incorporated into normal routine. It forms the basis for decisions on prophylaxis and therapy.

All compounds have advantages and disadvantages and many of these will come to light with experience of their use, enabling complementary use of each compound and providing a basis for a decision on when and how to treat. These considerations should override economic or convenience ones where avoidance of resistance development is concerned.

Recognising this, it is likely that the manufacturers will co-operate and advise on how new products can be integrated with others to avoid the development of resistance.

An integrated management strategy will achieve, over all the cycle, a reduced number of treatment episodes, a minimisation of resistance development and greater safety to fish, humans and the environment.

ICES WG on Pathology and Diseases in Marine Organisms
3-7 April 1995, La tremblade, France.

Sea lice treatment.

Brit Hjeltnes, Institute of Marine Research, Bergen, Norway.

Wrasse

Wrasse as cleaningfish are frequently used in Norwegian salmon farming. In 1994 more than 100 fish farms used 1.6 mill wrasse against sea lice infestations. Most of the fish are caught locally but there is established at least one company collecting fish at the Southern coast of Norway (Skagerak) and distributing them to fish farmers.

Wrasse are used almost exclusively with small fish the first year in sea. Some of the wrasse populations tend to be carriers of atypical furunculosis and there have been several outbreaks of clinical disease.

Syncroniced treatment

This seems to be effective when used.

Chemical treatment

Dichlorvos (Nuvan) and trichlorfon (Neguvon) are still the most common drugs used for treatment. Approximately 90 % of the sea lice infections are treated with these compounds. Some fish farmers have claimed that these chemicals tend to be less effective in controlling the infection because of an increasing resistance problem. However, the use of large cages have made it more difficult to calculate the right amount of drug to be used. Operator safety and environmental impact are major concerns with chlorvos compounds.

The amount Nuvan and Neguvon are being substituted by a new dichlorvos compound Salmosan (azametifos). Salmosan can be used in a much lower concentration than Nuvan and it has a short with-holding period. This drug has no effect on the calamus stages and the effect seems to be temperature independent in the range of 7 - 16°C.

Pyrethrum has a very limited use due to its toxicity limits and to problems in the administration of the drug. To day, the component is only used when fish are revaccinated in sea water. This compound kills all stages of sea lice.

The use of oxygenperoxid (H_2O_2) has decreased probably due to the hazard in handling associated with this compound.

Formalin is being used in some regions in Northern Norway.

There are no reported use of Ivermectin in Norway.

New chemical treatment.
Pharmaceutic companies are trying out many compounds. Several of these are very effective in laboratory trials. No trials have so far been carried out on the environmental impact of these new compounds.

Compound	1991	1992	1993	1994
Neguvon	2144	1946	1779	1227
Nuvan	3588	3115	2470	1147
Azametifos				389
Pyrethrum				31.5

Registered sale of compounds (k) used against sea lice infection in Norwegian aquaculture (NMD 08.02.1995).

Compound	1993	1994
Hydrogenperoksid	710 tonns	290 tonns

Registered sale of hydrogenperoxid (tons) used against sea lice infections in Norwegian aquaculture (NMD 08.02 1995)

RESEARCH ON PATHOLOGY AND DISEASES IN FISH LARVAE REARED IN MARICULTURE

F. Baudin Laurencin : Draft report

In 1993, about 300 millions of marine fish fry were produced in the world, mainly in Japon (200 millions), the most important species being : yellowtail *Seriola quinqueradiata*, red seabream *Pagrus major* and hirame (= japaneese flounder) *Paralichtys olivaceus*. One hundred million was produced in Europe, essentially seabass *Dicentrarchus labrax*, gillthead seabream *Sparus aurata* and turbot *Scophthalmus maximus*. More recently new species were also considered for mariculture : halibut *Hypoglossus hypoglossus*, cod *Gadus morhua*, wolffish *Anarhichas lupus*, umbrina *Umbrina cirrosa*, yellowtail *S. dumerili*, sole *Solea solea*, etc...

From the seventies, people have first condidered zootechnical and nutritional problems and they have been more speaking in terms of survival and growth than in terms of mortality and pathology. However the negative effects of inappropriate feed and rearing conditions have been reported while the quality of these parameters were increased. More recently it has became more obvious that, even if abiotic factors can be at the origine of mortalities, bad growings, malformations, biotic parameters can be also involved : alone, as true pathogens, or as secondary pathogens, in relation with particular environmental factors. A lot of research on the etiology and control of pathology and diseases has already been carried out and the main part of this report will be an unexhaustive relation of these works.

It must also be reminded of the present existence of special groups and meetings devoted to fish larvae rearing, thus in some extend, concerned by pathological problems.

- Just for memory, the ICES working group "on mass rearing of juvenile marine fish". They meet every two years and in 1991 already they identified quality criteria for eggs and larvae such as deformities, survival, growth, disease resistance... Hygiene strategies were also recommended, and the need for research on larval associated bacterial populations, pathogens and probiotics, development of immune system in early life stages was pointed out.
- A symposium on fish and crustacean larviculture "Larvi91" occured at Ghent (Belgium) in 1991. Besides nutrient manipulation and requirements, formulated feed and rearing techniques, it was also treated of stress effects, vertebral malformations, pathology and diseases which references are reported here after. A new "Larvi95" will be held again this year, and again it will be reported of microbiology and disease control.

- On going AIR programmes are also dealing with fish larval diseases, meeting together several scientists in Europe.

The following classified bibliography is of course not exhaustive but gives different aspects and results on the present topic. The subsequent conclusions should offer the opportunity to identify the remaining problems and to recommand actions for their control.

Following parts : Lists of references are presented at the end of each part. Only the numbers of the references are given in the text.

1. Environmental and nutritional pathology (33 ref.)
2. Parasitic pathology (12 ref.)
3. Bacterial pathology (50 ref.)
4. Viral pathology (16 ref.)

1. Environmental and nutritional pathology

1.1. Environement as an occasional or favorable factor for infectious diseases. E.g., Ref. 24 - 26.

1.2. Environment as a main factor for mortality, growing or malformations. See ref. in Table 1

Effect / Cause	Mortality	Bad growing	Malformations Skeletal anomalies	Other anomalies
Temperature	23	23		
Salinity	12 - 23	23		12
Light photoperiod	2	2 - 16		
Environmental stressors			30	4
Natural pollution (Amonia & nitrites : high concentrations of nitrites can be tolerated)	17	17		
Artificial pollution (heavy metals, pesticides)	15		3 - 9 - 13 - 27 - 31 - 33	12

Table 1

1.3. Nutritional pathology - See table 2

Effects / Causes	Survival and bad growing	Histopathology aspects	Skeletal anomalies
Starvation	14	5 - 10 - 11 -20 - 22 - 32	
Feed quality	19 - 21		13 - 29

Table 2 - The above ref. concern : plaice, yellowtail, ayu, turbot, seabass, seabream

1.4. Anomalies in the development of the swimbladder

- *Underdevelopment of the swimbladder (seabass, seabream)*

Factors : rearing techniques

Species : seabass, gillthead, seabream

Consequences : low mortality, slow growing, subsequent vertebral anomalies

Ref. 6, 7, 8, 28

- *Excessive inflation : swimbladder stress syndrom*

Factors : environmental stressors

Species : seabass, G. seabream, *Mugil cephalus*, *Lates calcarifer*

Ref. 1 - 18 - 25

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2. Parasitic pathology

1. Protozoa

1.1. Endoparasites of embryos and larvae of cods and turbots

- Taxonomy : unidentified flagellate(s)
- Host species : cod, turbot
- Age : embryos and yolk-sac larvae
- Prevalence : 20-40 % in larvae issued from wild
- Incidence : high mortality (till 80 %) brood stock
- Origin : fish gametes
- Hanging problems : taxonomy, diagnosis in brood stock, control
- References : 1 - 7 - 8 - 9

1.2. Dinoflagellates in **Sardina pilchardus** *larvae.*

- Taxonomy : *Ichthyodinium chabelardi* (dinoflagellate)
  NB - in wild fish, but some relationship with 1.1.
- References : 2 - 3

1.3. Other flagellates or ciliates

- Taxonomy : Amyloodinium, Ichtyobodo, Cryptocaryon (no accurate data)
- Host species - all
- Age : old larvae or young juveniles
- Incidence : high mortality
- Origin : plurifactorial
- Control : sanitary measures
- Hanging problems : accurate diagnosis, control
- References : private indications (J.C. Raymond)

2. Metacercaria

- Taxonomy : metacercaria, larval digenetic trematodes, several species, e.g. Acanthostonum, Cryptocotyle
- Host species : red seabream, hirame, gillthead seabream, etc...
- Incidence : high mortality
- Prevalence : high in extensive mariculture in particular conditions (intermediate hosts, e.g. Littorina sp.)
- Control : molluscocids, intensive farming
- Hanging problems :
- References : 4 - 5 - 6 - 10 - 11

3. Parasitic crustaceans

- Taxonomy : copepods, several species, e.g. *Ergasilus*
- Host species, e.g. : red seabream (14-20 days larvae)
- Incidence : weak
- References : 5 - 12

NB - Copepodites of *Lernocaera sp* were observed to attack and kill herring larvae **(Rosenthal, 1967** *in :* 5)

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3. Bacterial pathology

3.1. Life bacteria associated to larval rearing

3.1.1. Life feed associated bacteria

The abundance of bacteria (mainly *Vibrio* sp, *Aeromonas* sp.) can be very high in life feeds (algae, *Brachionus*, Artemia). In some cases, the development of some species has been associated to fish larvae mortalities. It has sometimes been pointed out that fish larvae refused to ingest preys containing large quantities of bacteria.

Ref. 10 - 34 - 35 - 43 - 44 - 49

3.1.2. Bacteria in fish larvae

The numbers of bacteria (most of them vibrionaceae and *Pseudomonas*) in fish larvae can be high. They have mainly been observed in the intestinal lumen and have sometimes been observed penetrating the mucosa. The passage from a diversified to a monospecific microflora has been associated to mortality. The role of toxins has been considered.

Ref. 1 - 3 - 4 - 17 - 18 - 23 - 24 - 29 - 30 - 31 - 43 - 46 - 48

In some cases, specific strains observed in the gut were considered as realy pathogen, e.g. as a cause of intestinal necrosis.

Ref. 2 - 6 - 8 - 12 - 16 - 19 - 22 - 25 - 26 - 28 - 32 - 38 - 39

It also was considered that some bacterial populations in fish larvae intestine could be of interest for establishment of an indigenous microflora and of an immunological importance, by exposing antigenic determinants.

Ref. 18 - 20 - 30 - 36

3.1.3. Trials for controling bacterial populations in fish larvae

Use of antibiotics or chemical compounds

Ref. 1 - 20 - 33 - 40 - 43 - 44 -45

Use of probiotics (Vibrionaceae, lactic bacteria, yeasts)

Ref. 11 - 13 - 14 - 15 - 37 - 47 - 50

Immunological aspects, immunostimulants and vaccines

Ref. 5 - 21 - 41 - 42

Techniques : bioencapsulation, rinsing of the food organisms prior to feeding them to larvae

Ref. 7 - 9 - 23 - 27 - 33

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4. Viral Pathology

Herpes virosis of Paralychtis olivaceus

Incidence : 80-90 % mortality
NB - specificity, temperature relationship
Hanging problems : epidemiology, control

References : 8 - 9 - 10

Nodavirosis : Nervous necrosis or viral encephalopathy

Taxonomy : Nodavirus (firstly described as picornavirus like)
Host species : Barramundi *Lates calcarifer*, parrotfish *Oplegnathus fasciatus*, seabass, redspotted grouper *Epinephelus akaara*, turbot, striped jack *Pseudocaranx dentex*.
Origin : from parents
Prevalence : depending on brood stock
Incidence : very high mortality

References : 1 - 5 - 6 - 12 - 13 - 14 - 15 - 16

Hanging problems : taxonomy (one or several similar virus ? different serotype ?) epidemiology, control.

Birnavirosis

Host species : Seabass, gillthead seabream, turbot
Prevalence : variable
Incidence : not proved in marine species - some strains were found pathogen for Rainbow trout fry

References : 2 - 3 -4 - 7

Paramyxovirosis of Black seabream

Taxonomy : paramyxovirus *like*
Host species : Black seabream *Acanthopagrus schlegeli*
Incidence : 100 % mortality in natural outbreaks

Reference : 11

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Conclusions

a. Etiological factors

Except for a few well characterized factors (herpes virus of hirame, nodavirus of viral encephalopathy, metacercaria, parasitic crustaceans, may be cod endoparasites) the pathological factors are badly identified. Some bacteria which taxonomy has generally to be precised have been recognized as possible cause of pathology, but most of them, even found in abundance in the intestine cannot *a priori* be considered as real pathogens.

In the same way, environment and nutrition are usually secondary factors which the roles are badly known in cases of mortality, bad growing, etc...

b. Epidemiology

Even for the well defined pathogens, the conditions of disease transmission are not well known : vertical and/or horizontal transmission of the virus, origin of pathogenic bacteria,...

The future (in adults) of some parasites and virus was also to be studied : e.g. for nodavirosis.

c. Diagnosis methodology

Except for Herpes virus, Nodavirus and some parasites usually recognized in histology... Except when a pathogenic bacteria can be isolated... it is difficult to ascertain a diagnosis.

d. Control

- *Environmental and nutritional factors*

They are today better known for some fish species, and a future control can be envisaged.

- *Parasites and virus*

Prevention is easy for metacercaria but more difficult when the origin of the parasite or of the virus remains unknown.

For herpes virus or nodavirus, do we have to recommand a sanitary control similar to the rhabdovirosis reglementation ?

- *Bacteria*

The interest of antibiotics in larval rearing has to be precised. Ecological aspects have also to be discussed.

Probiotics could be a very satisfactory solution, but a lot of research has to be done about.

Hygienic solutions must be considered.

At the whole, research concerning larval pathology must be encouraged, and, as a priority, in 2 directions :

1. Identification of actual pathogenic bacteria in relation with research on probiotics.
2. Epidemiology and control of viral diseases.

ANNEX 11a

DISEASES AND PARASITES OF FLOUNDER (Platichthys flesus) IN THE BALTIC SEA

A BMB/ICES sponsored meeting on "Diseases and Parasites of Flounder in the Baltic Sea" was arranged at the Åbo Akademi University, Finland, on October 27 - 29 1995. In addition to 20 participants from countries around the Baltic Sea several invited specialists from other countries participated.

The presentations and discussions were mainly grouped around the following topics: Lymphocystis-disease, skin ulcer disease, liver neoplasias and new methodologies when utilizing health parameters of flounder as biomarkers. The discussions ended up in the following summarizing statements:

Lymphocystis-disease

-the results of the contributions presented at the meeting clearly revealed that lymphocystis is the most prevalent externally visible disease of the Baltic flounder occurring in the entire Baltic Sea without showing a clear spatial trend. In the south-western Baltic Sea, a clear increase in the prevalence has been recorded since 1986. The reasons for this have not yet been identified.
-in contrast to other abundant diseases such as bacterial skin ulcerations and fin rot, there is no doubt about the etiology of the disease. The infectious agent is a virus belonging to the Iridoviridae-group. However, the existencxe of different virus strains/species possibly beeing responsible for variations in the prevalence and spatial distribution of the disease cannot be excluded.
-the lymphocystis disease is clearly linked with sex and age of the flounder. Male flounders are more frequently affected as compared to females and the disease occurs at a higher prevalence in intermediate age groups than in young or older fish. These observations have to be considered when interpreting observed crude prevalences as well as spatial and temporal trends.
-taking into account the multifactorial etiology of many fish diseases and the difficulties to quantify the importance of single components in their etiology, the relation-ship between the occurrence of Lymphoystis-disease and the degree of marine pollution - although repeatedly beeing suggested in the past - is hard to establish. However, there is indication from the mesocosm studies carried out in the Netherlands and from field studies in some sea areas that increased prevalence of Lymphocystis-disease might be an un-specific biological indicator for any environmental change (including pollution) leading to physiological stress and associated effects on the immune defence mechanisms of fish.

Skin ulcers

-flounder from the Baltic Sea and from the North Sea show distinct differences in disease patterns and epidemiology of skin ulcer disease which may reflect differences in the biology of flounder in these areas and/or disease etiology.
- the etiology of skin ulcer disease appears to be complex. It is clearly multifactorial including bacterial infection. Studies of the Baltic flounder suggest one particular

pathogen to be involved (an atypical Aeromonas salmonicida) whereas those of the North Sea flounder indicate merely secondary infections by a variety of opportunistic bacteria.
-skin ulcers and fin rot are probably expressions of the same disease syndrome. Healed fin rot should not be confused with fin erosion, the latter described i.a. in fish from the Baltic Sea exposed to pulp mill effluents.
-skin ulcer in flounder is a non-specific disease which may be considered as a general indicator of environmental stress. So far a specific contaminant-related cause is not established.
-future studies of skin ulcer disease should concentrate on refined bacterial methods and immunological studies including also the effects of stressors on the mucus production and composition of the skin.

Liver neoplasias

-in the southern Baltic Sea liver neoplasias in flounder have been recorded at low prevalences, with no specific spatial or temporal trends. In the northern Baltic Sea especially flounders of the largest size classes show high prevalences of liver changes including preneoplastic and neoplastic lesions and cases of carcinomas. The etiology of these lesions has not been resolved.
-field and laboratory studies carried out in North America indicated high prevalences of liver changes in benthic fish species sampled from polluted waters.
-in these livers sequential stages of histopathological changes leading to neoplasia were considered to be associated with organic contaminants, especially with aromatic hydrocarbons.
-multidiciplinary studies, involving pathologists, chemists, biochemists and others, have provided results which harden the evidence that certain organic contaminants have directly induced the liver changes.
-large scale mesocosm studies in the Netherlands provided convincing evidence that liver neoplasms and related lesions similar to those occurring in wild flounder, developed when flounders were exposed to a complex mixture of anthropogenic contaminants including polycyclic aromatic hydrocarbons. This is the first study in which liver neoplasms and related lesions were induced under simulated field conditions at pollution levels similar to those prevailing in a natural environment.

New methodologies

-numerous field studies on bottomfish in European as well as in North American marine waters have established strong and consistent statistical associations between the prevalence and risk of occurrence of hepatic neoplasms and other lesions involved in the histogenesis of hepatic neoplasia, and potential exposure to environmental contaminants such as PAHs, PCBs, and DDTs. However, these associations established in epizootiological studies in fish are not sufficient evidence to support a causal relationship, and can be strengthened considerably in similar field studies by inclusion of newer methodologies that can quantify actual exposure to xenobiotic chemicals such as PAHs, as well as measuring specific biochemical and molecular responses to contaminant exposure. These newer diagnostic and predictive biomarkers of contaminant exposure and effects include measures of levels of particular metabolites of aromatic hydrocarbons present in bile that fluoresce at unique wavelengths corresponding to particular parent compounds such as benzo(a)pyrene, naphthalene, and phenanthrene. These metabolites are often expressed as fluorescent aromatic compounds (FACs), and are a reliable estimate of recent actual exposure to both high and low molecular weight aromatic hydrocarbons, which are readily metabolized in fish. Because these metabolites indicate actual exposure to PAHs, they provide more meaningful evidence of exposure than do sediment levels of these contaminants. Moreover, bile samples can easily be collected from individual specimens in parallel with liver samples collected for histopathology and other biomarkers to be discussed below.

-other biomarkers of contaminant exposure and effect that has proven quite useful in field studies investigating potential links between fish diseases (e.g., hepatic lesions) and xenobiotic chemical exposure are various measures of induction of xenobiotic metabolizing enzymes, such as those in the cytochrome P450 (CYP) mixed function oxidase system, specifically as measures of a unique isoenzyme (CYP1A) involved in metabolism and activation of potential toxicants and carcinogens to their toxic/carcinogenic intermediates. The CYP1A isoenzyme is induced by binding of compounds such as PAHs, PCBs, and dioxins to the cellular Ah receptor, followed by translocation of this receptor complex to the nucleus, and subsequent transciption of the CYP1A mRNA to the ribosome complex in the rough endoplasmic reticulum, and translation of the active enzyme (CPY1A). Induction of this enzyme can be measured as catalytic activity of aryl hydrocarbon hydroxylase (AHH) or 7-ethoxyresorufin O-deethylase (EROD), or by immunologic methods detecting expression of the mRNA transcript (Western blotting) or the mature enzyme (enzyme linked immunosorbent assay, ELISA). Polyclonal and monoclonal antibodies to the CYP1A protein isoenzyme from several fish species are now available and have a broad cross reactivity to CYP1A isoenzymes in other species. Expression of hepatic CYP1A can therefore be readily quantified in the same livers of individual specimens collected for histopathology, using either AHH, EROD, or the immunologic ELISA method. Moreover, CYP1A expression can be localized by immunohistochemical methods in various target cells in liver (e.g., hepatocytes, endothelial cells, biliary epithelial cells) and other tissues, providing significant insight into the biochemical mechanisms underlying any observed pathologic responses in tissue sections. Use of these immunohistochemical localization procedures, has for example, helped to provide a biochemical basis for the substantial differences in neoplasm and preneoplastic lesion prevalences in two sympatric flatfish species in Puget Sound, Washington, USA, namely the starry flounder (resistant) and English sole (highly susceptible).

-a measure of genotoxic damage resulting from exposure to PAHs that can also be of great use in field studies, and which has been highly validated in laboratory exposure studies is the 32P-postlabelling method of identifying xenobiotic-DNA adducts in liver tissue. Such adducts are considered to represent the initial molecular lesions indicating genotoxicity, some of which are responsible for the mutations in replicating DNA that lead to initiation of the carcinogenic process. Levels of xenobiotic-DNA adducts in liver are regarded as a molecular dosimeter of exposure to genotoxic PAHs over the life of the fish, since many of these adducts are quite persistent over time. As with the biliary FACs, and CYP1A assays, xenobiotic-DNA adducts can be collected and measured in liver of specimens examined also for histopathology. In fact, studies in subadult English sole from Puget Sound in Washington State, U.S.A., have statistically linked levels of these earlier biomarkers of contaminant exposure and effects to the later occurring, more chronic effects represented by prevalences of hepatic lesions involved in the earlier steps of hepatic neoplasia. Demonstration of such linkages among measures of actual exposure, early biochemical responses specific to such exposure, and more chronic effects experimentally validated as known pathologic responses to contaminant exposure (e.g., liver lesions) considerably strenghthens and provides a mechanistic, toxicologic basis for the use of hepatic lesions and perhaps other diseases as indicators of environmental contaminants.

-inclusion of these measures of early biochemical and molecular responses in field studies also monitoring more chronic responses to contaminant exposure such as certain disease symptoms (e.g. liver tumors and related lesions, skin ulcers, fin erosion) will greatly increase our ability to demonstrate toxicologically meaningful links between fish diseases and pollution exposure in wild fish populations, and therefore should be initiated as soon as is practical in such studies in the Baltic Sea as well as in other sea areas. All of these methods are readily available and are already being used to great advantage in similar studies in many parts of the world. In short, concurrent and integrated application of these biochemical markers along with histopathology will enhance our ability to make reasonable decisions as to whether or not certain fish diseases are toxicologically plausible as field indicators of the chronic effects of contaminant exposure.

The papers were published in a Proceedings volume: Diseases and Parasites of Flounder in the Baltic Sea, The Baltic Marine Biologists, Publication No 15, 1994 (147 pp), ISSN 0282-8839.

MEETING OF THE ICES - WGPDMO
3-7 APRIL, 1995, LA TREMBLADE, FRANCE

ICES Council Resolution 1994/2:23g requested that the WGPDMO should

..... review the ..... results of the BMB/ICES Sea-going Workshop "Fish Diseases and Parasites in the Baltic Sea" ..... and report to ACME.

The BMB/ICES Sea-going Workshop **"Fish Diseases and Parasites in the Baltic Sea"** was held from 25 November - 8 December on board the German RV "WALTHER HERWIG III" under the convenorship of T. Lang (GER) and S. Mellergaard (DEN). It was attended by 12 participants (11 scientist + 1 technical assistant) representing 8 of the 9 countries bordering the Baltic Sea. Only Sweden was missing on the list of participating countries.

Practical work was carried out at 11 stations (36 fishery hauls) on a transect from the western (Belt Sea, Mecklenburg Bight) to the eastern (Gulf of Finland, Estonian waters) Baltic Sea. The region covered represents the largest area in the Baltic Sea ever studied for the occurrence of fish disesases and parasites using identical methodologies during a narrow time-window.

Due to its abundance and wide distribution in the Baltic Sea, the main target species was the **flounder** (*Platichthys flesus*). However, other abundant fish species such as **cod** (*Gadus morhua*), **herring** (*Clupea harengus*) and **sprat** (*Sprattus sprattus*) were examined as well.

The following **parameters** were investigated:

- grossly (mainly externally) visible diseases and parasites of flounder, cod, herring, sprat
- liver nodules of flounder
- histopathology of liver nodules
- MFO
- external/internal parasites of flounder
- bacteriology of ulcer disease in flounder and other species

In addition, otoliths were taken from almost all flounder examined for diseases in order to obtain age/length-keys for the analysis of a possible relationship between age and prevalences of certain diseases. Furthermore, the age/length-keys should be used to identify distinct flounder stocks in different areas.

The main reasons for holding the workshop were:

- Despite the fact that studies on diseases and parasites of wild marine fish in many ICES-countries constitute an important component of national monitoring programmes on biological effects of contaminants, there is still a striking lack of information for the Baltic Sea. The results of the Workshop should, therefore, provide scientific data on the prevalence and spatial distribution of fish diseases and parasites in the Baltic Sea to be used as baseline information for further research and monitoring programmes.

- In contrast to the North Sea, there have been only few attempts to intercalibrate and standardize methodologies used by different countries for fish disesase/parsite surveys in the Baltic Sea. In 1991, a BMB-Workshop was held on the German RV "ALKOR", the results of which, however, have not been published yet due to certain data limitations. The BMB/ICES-Workshop should, therefore, help to achieve a similar status quo for the Baltic Sea as already developed for the North Sea. For this purpose, one objective of the workshop was to evaluate the applicability of the ICES standard methodologies recommended for fish diseases surveys which were mainly developed according to the conditions in the North Sea but also contain some recommendations for Baltic Sea surveys. If considered neceassary, new recommendations for the particular conditions in the Baltic Sea should be developed.

- Due to the former political situation in eastern Europe, the contacts between scientists and institutes around the Baltic Sea involved in fish pathology and parasitology are still less developed than in western Europe. The workshop should, therefore, enhance these contacts in order to establish further international cooperations between Baltic countries with regard to resarch and monitoring into/of fish diseases and parasites in the Baltic Sea

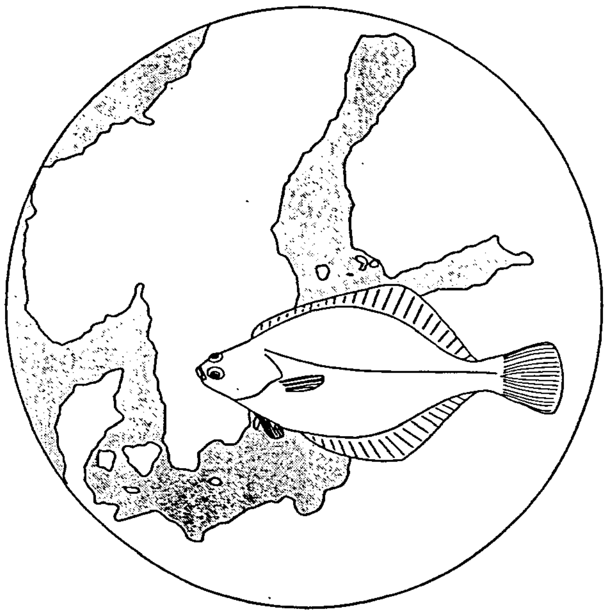
The **preliminary results** of the workshop revealed some relatively clear spatial trends for externally visible diseases of flounder and cod and for liver nodules in flounder.
In **flounder**, the prevalence of lymphocystis decreased from the western to the eastern part of the survey area, and, in contrast, the prevalences of acute skin ulcerations and liver nodules were increased in the eastern part.
In Baltic **cod**, conspicuously externally visible parasites (*Cryptocotyle lingua, Lernaeocera branchialis*) used for monitoring purposes in the North Sea were restricted to the westernmost stations (Belt Sea, western Arkona Sea) . Skin ulceration were also more prevalent in the western part (Arkona Sea, Bornholm Sea), and skeletal deformities occurred most frequently in the Eastern Gotland Sea (Gdansk Bay).
Any speculations about reasons for these spatial characteristics would be too premature since the analysis of the workshop data has yet not been completed.

From the experience of the practical work, the workshop-participants considered it necessary to elaborate some **new recommendations** for fish disease surveys in the Baltic Sea providing guidelines for sampling strategies, disease diagnosis and grading (including parasitological and bacteriological methodologies), statistical analyis, and incorporation of additional measurements of risk factors for the occurrence of diseases to be followed in current and future monitoring programmes. The formulation of these guidelines is at present under way and it is intended to publish them together with all results of the practical work as a series of separate papers probably in a special volume of the *ICES Journal of Marine Science.*

ANNEX 11c

THE
BALTIC MARINE BIOLOGISTS
PUBLICATION No. 15
1994

DISEASES AND PARASITES OF FLOUNDER (*Platichthys flesus*) IN THE BALTIC SEA



ÅBO 1994
ISSN 0282-8839

SUMMARIZING STATEMENTS

The presentations and discussions were mainly grouped around the following topics: *Lymphocystis-disease, skin ulcer disease, liver neoplasias and new methodologies when utilizing health parameters offlounder as biomarkers.* The discussions ended up in the following summarizing statements:

Lymphocystis-disease

-the results of the contributions presented at the meeting clearly revealed that lymphocystis is the most prevalent externally visible disease of the Baltic flounder occurring in the entire Baltic Sea without showing a clear spatial trend. In the south-western Baltic Sea, a clear increase in the prevalence has been recorded since 1986. The reasons for this have not yet been identified.

-in contrast to other abundant diseases such as bacterial skin ulcerations and fin rot, there is no doubt about the etiology of the disease. The infectious agent is a virus belonging to the Iridoviridae-group. However, the existencxe of different virus strains/ species possibly beeing responsible for variations in the prevalence and spatial distribution of the disease cannot be excluded.

-the lymphocystis disease is clearly linked with sex and age of the flounder. Male flounders are more frequently affected as compared to females and the disease occurs at a higher prevalence in intermediate age groups than in young or older fish. These observations have to be considered when interpreting observed crude prevalences as well as spatial and temporal trends.

-taking into account the multifactorial etiology of many fish diseases and the difficulties to quantify the importance of single components in their etiology, the relationship between the occurrence of Lymphoystis-disease and the degree of marine pollution - although repeatedly beeing suggested in the past - is hard to establish. However, there is indication from the mesocosm studies carried out in the Netherlands and from field studies in some sea areas that increased prevalence of Lymphocystis-disease might be an unspecific biological indicator for any environmental change (including pollution) leading to physiological stress and associated effects on the immune defence mechanisms of fish.

Skin ulcers

-flounder from the Baltic Sea and from the North Sea show distinct differences in disease patterns and epidemiology of skin ulcer disease which may reflect differences in the biology of flounder in these areas and/or disease etiology.

- the etiology of skin ulcer disease appears to be complex. It is clearly multifactorial including bacterial infection. Studies of the Baltic flounder suggest one particular pathogen to be involved (an atypical *Aeromonas salmonicida*) whereas those of the North Sea flounder indicate merely secondary infections by a variety of opportunistic bacteria.

-skin ulcers and fin rot are probably expressions of the same disease syndrome. Healed fin rot should not be confused with fin erosion, the latter described i.a. in fish from the Baltic Sea exposed to pulp mill effluents.

-skin ulcer in flounder is a non-specific disease which may be considered as a general indicator of environmental stress. So far a specific contaminant-related cause is not established.

-future studies of skin ulcer disease should concentrate on refined bacterial methods and immunological studies including also the effects of stressors on the mucus production and composition of the skin.

Liver neoplasias

-in the southern Baltic Sea liver neoplasias in flounder have been recorded at low prevalences, with no specific spatial or temporal trends. In the northern Baltic Sea especially flounders of the largest size classes show high prevalences of liver changes including preneoplastic and neoplastic lesions and cases of carcinomas. The etiology of these lesions has not been resolved.

-field and laboratory studies carried out in North America indicated high prevalences of liver changes in benthic fish species sampled from polluted waters.

-in these livers sequential stages of histopathological changes leading to neoplasia were considered to be associated with organic contaminants, especially with aromatic hydrocarbons.

-multidiciplinary studies, involving pathologists, chemists, biochemists and others, have provided results which harden the evidence that certain organic contaminants have directly induced the liver changes.

-large scale mesocosm studies in the Netherlands provided convincing evidence that liver neoplasms and related lesions similar to those occurring in wild flounder, developed when flounders were exposed to a complex mixture of anthropogenic contaminants including polycyclic aromatic hydrocarbons. This is the first study in which liver neoplasms and related lesions were induced under simulated field conditions at pollution levels similar to those prevailing in a natural environment.

New methodologies

-numerous field studies on bottomfish in European as well as in North American marine waters have established strong and consistent statistical associations between the prevalence and risk of occurrence of hepatic neoplasms and other lesions involved in the histogenesis of hepatic neoplasia, and potential exposure to environmental contaminants such as PAHs, PCBs, and DDTs. However, these associations established in epizootiological studies in fish are not sufficient evidence to support a causal relationship, and can be strengthened considerably in similar field studies by inclusion of newer methodologies that can quantify actual exposure to xenobiotic chemicals such as PAHs, as well as measuring specific biochemical and molecular responses to contaminant exposure. These newer diagnostic and predictive biomarkers of contaminant exposure and effects include measures of levels of particular metabolites of aromatic hydrocarbons present in bile that fluoresce at unique wavelengths corresponding to particular parent compounds such as benzo(a)pyrene, naphthalene, and phenanthrene. These metabolites are often expressed as *fluorescent aromatic compounds* (FACs), and are a reliable estimate of recent actual exposure to both high and low molecular weight aromatic hydrocarbons, which are readily metabolized in fish. Because these metabolites indicate actual exposure to PAHs, they provide more meaningful evidence of exposure than do sediment levels of these contaminants. Moreover, bile samples can easily be collected from individual specimens in parallel with liver samples collected for histopathology and other biomarkers to be discussed below.

-other biomarkers of contaminant exposure and effect that has proven quite useful in field studies investigating potential links between fish diseases (e.g., hepatic lesions) and xenobiotic chemical exposure are various *measures of induction of xenobiotic metabolizing enzymes*, such as those in the cytochrome P450 (CYP) mixed function oxidase system, specifically as measures of a unique isoenzyme (CYP1A) involved in metabolism and activation of potential toxicants and carcinogens to their toxic/carcinogenic intermediates. The CYP1A isoenzyme is induced by binding of compounds such as PAHs, PCBs, and dioxins to the cellular Ah receptor, followed by translocation of this receptor complex to the nucleus, and subsequent transciption of the CYP1A mRNA to the ribosome complex in the rough endoplasmic reticulum, and translation of the active enzyme (CPY1A). Induction of this enzyme can be measured as catalytic activity of aryl hydrocarbon hydroxylase (AHH) or 7-ethoxyresorufin O-deethylase (EROD), or by immunologic methods detecting expression of the mRNA transcript (Western blotting) or the mature enzyme (enzyme linked immunosorbent assay, ELISA). Polyclonal and monoclonal antibodies to the CYP1A protein isoenzyme from several fish species are now available and have a broad cross reactivity to CYP1A isoenzymes in other species. Expression of hepatic CYP1A can therefore be readily quantified in the same livers of individual specimens collected for histopathology, using either AHH, EROD, or the immunologic ELISA method. Moreover, CYP1A expression can be localized by immunohistochemical methods in various target cells in liver (e.g., hepatocytes, endothelial cells, biliary epithelial cells) and other tissues, providing significant insight into the biochemical mechanisms underlying any observed pathologic responses in tissue sections. Use of these immunohistochemical localization procedures, has for example, helped to provide a biochemical basis for the substantial differences in neoplasm and preneoplastic lesion prevalences in two sympatric flatfish species in Puget Sound, Washington, USA, namely the starry flounder (resistant) and English sole (highly susceptible).

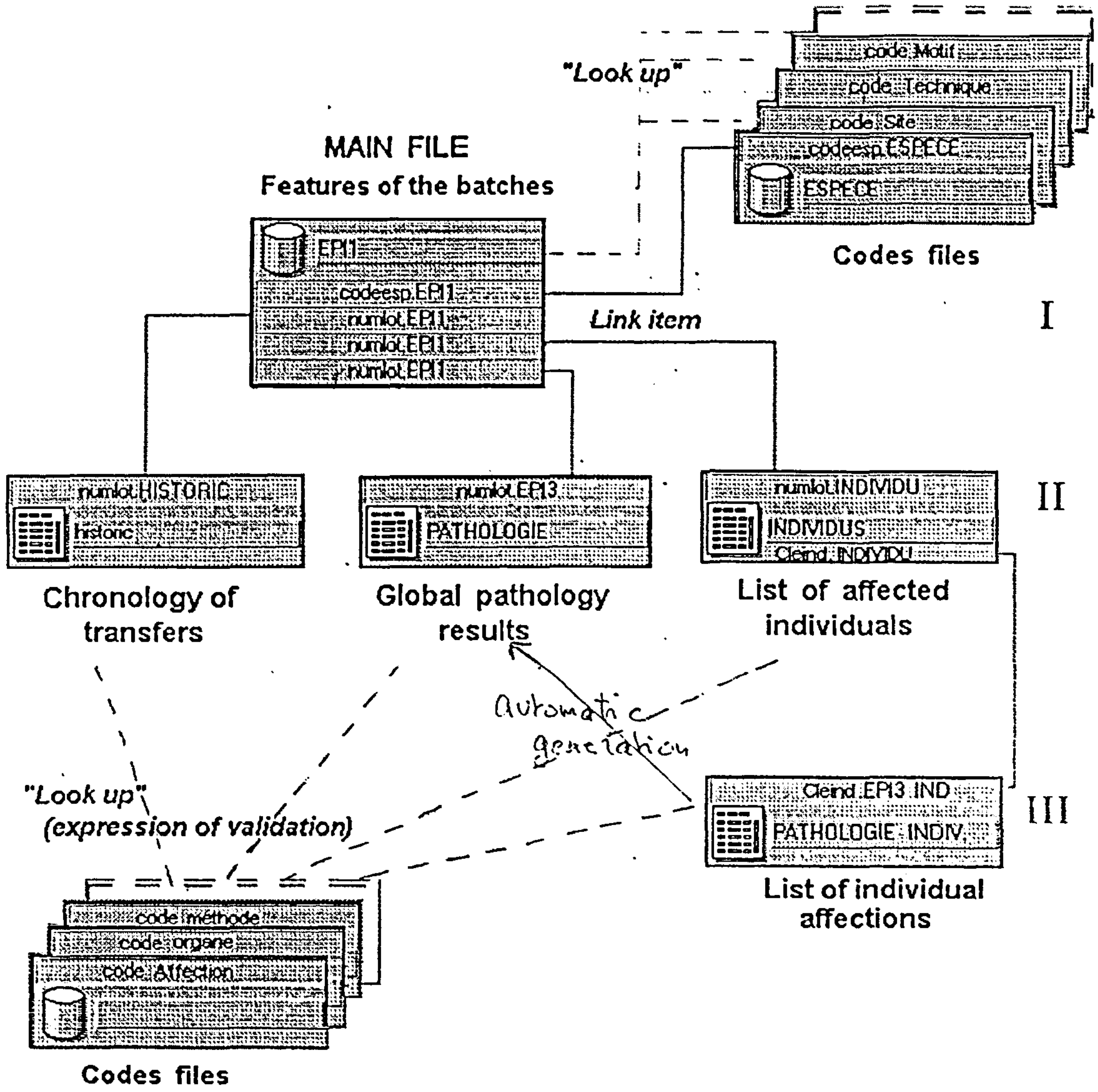
-a measure of genotoxic damage resulting from exposure to PAHs that can also be of great use in field studies, and which has been highly validated in laboratory exposure studies is the ^{32}P-postlabelling method of *identifying xenobiotic-DNA adducts* in liver tissue. Such adducts are considered to represent the initial molecular lesions indicating genotoxicity, some of which are responsible for the mutations in replicating DNA that lead to initiation of the carcinogenic process. Levels of xenobiotic-DNA adducts in liver are regarded as a molecular dosimeter of exposure to genotoxic PAHs over the life of the fish, since many of these adducts are quite persistent over time. As with the biliary FACs, and CYP1A assays, xenobiotic-DNA adducts can be collected and measured in liver of specimens examined also for histopathology. In fact, studies in subadult English sole from Puget Sound in Washington State, U.S.A., have statistically linked levels of these earlier biomarkers of contaminant exposure and effects to the later occurring, more chronic effects represented by prevalences of hepatic lesions involved in the earlier steps of hepatic neoplasia. Demonstration of such linkages among measures of actual exposure, early biochemical responses specific to such exposure, and more chronic effects experimentally validated as known pathologic responses to contaminant exposure (e.g., liver lesions) considerably strenghthens and provides a mechanistic, toxicologic basis for the use of hepatic lesions and perhaps other diseases as indicators of environmental contaminants.

-inclusion of these measures of early biochemical and molecular responses in field studies also monitoring more chronic responses to contaminant exposure such as certain disease symptoms (e.g. liver tumors and related lesions, skin ulcers, fin erosion) will greatly increase our ability to demonstrate toxicologically meaningful links between fish diseases and pollution exposure in wild fish populations, and therefore should be initiated as soon as is practical in such studies in the Baltic Sea as well as in other sea areas. All of these methods are readily available and are already being used to great advantage in similar studies in many parts of the world. In short, concurrent and integrated application of these biochemical markers along with histopathology will enhance our ability to make reasonable decisions as to whether or not certain fish diseases are toxicologically plausible as field indicators of the chronic effects of contaminant exposure.

Flow chart for shellfish disease data compilation (IFREMER 1995).

Working Group on Pathology and Diseases of Marine Organisms, 1995.

DATABASE REPAMO



Conception :
Francis DELAPORTE
IFREMER SISMER
with cooperation of
IFREMER LABORATORY

ANNEX 13b

Shellfish disease data entry form (Database 3, as used by Oxford Laboratory, USA).

Working Group on Pathology and Diseases of Marine Organisms, 1995.

PATHOGENIC AGENT or DISEASE:	YEAR:

COUNTRY ZONE/LOCATION (place name or coordinates)

HOST SPECIES (common name + scientific name) AGE SIZE (mean length in mm across longest axis)

SOURCE	WILD	
	CULTURED	Hatchery Nursery Bottom Suspension
SPAT ORIGIN		

DATE(S) OF COLLECTION	
TOTAL No. SAMPLES	
TOTAL No. INDIVIDUALS EXAMINED	TOTAL No. POSITIVE

% MORTALITY	MEAN "BOX" LENGTH

DIAGNOSTIC METHOD(S)

ENVIRONMENTAL OBSERVATIONS	Salinity Temperature Turbidity Dissolved Oxygen pH Plankton Profile Substrate Type Other

OTHER COMMERCIAL IMPACTS

ANNEX 15a

External fish diseases as a tool in the monitoring of biological effects of contaminants(Discussion paper by T Lang).

ICES WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

3-7 April 1995, La Tremblade, France

AGENDA ITEM: 15 (k) **VERSION: 1**

ICES Council Resolution 1994/2:23 k requested that the WGPDMO should

..... *assess the usefulness of external fish diseases as a tool in the monitoring of biological effects of contaminants, and report to ACME.*

Studies on the prevalence and spatial distribution of externally visible diseases in wild marine fish constitute an important component of current monitoring programmes on biological effects of contaminants in many ICES member countries. Most of these national programmes have been confined to the North Sea, but other seas such as the Irish and the Baltic Sea, and Atlantic and Pacific coastal waters of North America are monitored as well. Results of most of these programmes are submitted annually to ICES and are incorporated into the ICES Environmental Databank. In the databank, fish disease data since 1982 are included. At present, a new fish disease databank is being developed based upon a new format for data submission. A full analysis of the data will be performed by the **ICES Sub-group on Statistical Analysis of Fish Disease Data** after conversion of all data according to the new databank structure.

The **major advantages of externally visible diseases as biomarkers** are: they are easily detectable, with a certain degree of training and according to published ICES-guidelines the examination of fish can be done even during stock assessment surveys and even by non-specialists, a large number of fish can be examined in a short time thus enabeling sound statistics, cost implications (except ship time) are relatively low, they represent an integrative endpoint of a chain of biochemical and physiological changes affecting the homoeostasis of the fish and, thus, reflect chronic rather than acute stress. Moreover, diseases may have implications on the population level since they may affect both survival and reproduction. Hence, fish diseases are often considered an environmentally more relevant biomarker as compared to subtle changes on lower organisational levels (e.g. molecular, cellular), the significance and ecological relevance of which is often unresolved.

Although the existence of **causal links between pollution and the occurrence of externally visible diseases** has been postulated repeatedly, it is, however, generally accepted nowadays that these links are only hard to establish *in situ*. This is due to the increasing evidence that the majority of diseases reported to occur in wild fish, including the "classical" externally visible diseases used for monitoring purposes (in flatfish mainly *lymphocystis, skin ulcerations, fin rot, epidermal hyperplasia/papilloma*; in roundfish mainly *skin ulcerations, skeletal deformities, x-cell-disease, epidermal hyperplasia*) have a complex instead of a mono-causal aetiology. The occurrence of diseases in most cases is not a specific response to contaminant exposure but an unspecific reaction to multifactorial biotic and abiotic conditions affecting both the presence and the virulence of pathogens (which are involved in the aetiology of most diseases) as well as the susceptibility of the fish to the disease. The latter is associated with what has been defined as "stress", an unspecific condition which - besides other multiple biochemical and physiological alterations - is characterized by a supression of the specific and unspecific immune system increasing the vulnerability of organisms to diseases.

In order to obtain estimates of the **impact of factors others than contaminant exposure** contributing to the aetiology and pathogenesis of diseases, it is essential to measure additional parameters known or suspected to act as risk factors (e.g. length/age, sex, population density, seasonal effects due to migration and spawning activities, endocrinological state, behavioural characteristics, condition factor etc.; hydrographic parameters such as water temperature, salinity, oxygen-saturation, depth; general climatic conditions; direct and indirect impact of fishing activities; artificial habitat changes etc.). Furthermore, it is essential to chemically quantify **tissue burdens of contaminants and/or their metabolites** and desirable to obtain information on **environmental concentrations of contaminants** to which the fish are exposed. Although this appears to be self-evident, it has to be noted that in many present biological effects monitoring programmes there is a striking lack of data on the contamination of the target species.
In this context it should be emphasized that there is a **need for more studies** (both experimental and field studies) elucidating the links between natural as well as anthropogenic factors and the aetiology and pathogenesis of diseases used for monitoring purposes. Furthermore, increased effort should be dedicated to studies on the *in situ*-dynamics of these diseases including investigations into immunological and behavioural aspects as well as into effects on the population level (e.g. mortality, reproduction).
Another prerequisite for a reasonable use of externally visible fish diseases as biomarkers is **continuity**. Only from long-term studies, information about temporal trends as well as natural fluctuations of disease prevalences can be obtained.

In conclusion, externally visible fish diseases should be considered an **unspecific biomarker for environmental changes** of natural and/or anthropogenic origin affecting fish health rather than a specific biomarker for contaminant exposure (it should be noted that this is also true for other biomarkers at present discussed as being more specific and useful for monitoring purposes as compared to externally visible fish disesases).
When changes in the prevalence in a given area are beyond a certain degree of natural variability the extent of which can be deduced from long-term studies, this is an indication for an environmentally induced disturbance of the equilibrium between environment and fish health exceeding the normal range of physiological capability for adaptation. Besides other factors with potential impact, one cause for this disturbance might be changes in the level of contaminant exposure.

It is, therefore, recommended to **include studies on the occurrence of externally visible fish diseases in monitoring programmes on biological effects of contaminants** in the marine environment. However, this should be done following a rationalized approach (see 1994 Report of the ICES Working Group on Pathology and Diseases of Marine Organiusms) taking into account the non-specifity of externally visible diseases as biomarkers for contaminant exposure and the need for additional parameters to be measured in combination.
The measurement of significant changes in the prevalence of externally visible diseases can be used as **ecologically relevant warning signal** indication environmental stress. However, the monitoring of fish diseases should be supplemented by the application of other biological effect techniques in order to establish more clearly possible cause-effect relationships between contaminant exposure and the occurrence of pathological changes.

External fish diseases as a tool in the monitoring of biological effects of contaminants (Discussion paper by S Feist).

ICES WGPDMO MEETING

LA TREMBLADE, FRANCE

3-7 APRIL 1995

DISCUSSION DOCUMENT

Term of reference K.

To assess the usefulness of external fish diseases as a tool in the monitoring of biological effects of contaminants.

External diseases in marine fish have been used as indicators of the general health of fish stocks for almost 2 decades as a tool for the monitoring of biological effects of contaminants. Early surveys examined many species and included a wide variety of conditions, including parasites. The effort required to provide sufficient data on the health status of several species rapidly surpassed the resources available. This resulted in international collaboration and the rationalisation of marine fish disease investigations. Monitoring then focused on selected species and a clearly defined set of 'diseases' which were readily identifiable (in most cases). This more targeted approach has become widely adopted and large amounts of data are submitted annually to ICES and to various national monitoring programmes.

The basis for using external diseases as an indicator or biomarker of pollutant-related effects rests with the assumption that environmental contaminants, either directly or indirectly, affect disease susceptibility. This may occur in several ways, for instance, by a reduction in immunocompetance or direct stress effects. Observable macroscopic diseases offer a very attractive method to screen large numbers of individuals for pathological change and, generally speaking, do not involve further laboratory investigation for confirmatory diagnosis. Thus, from this approach has evolved the standard methodology. There are, however, several problems associated with using these diseases. Firstly, the aetiology of several are uncertain or unknown, consequently factors involved in their manifestation in individuals or populations are also poorly understood. Secondly, it is clear that some conditions (eg lymphocystis) are infectious and that for these the effects of changes in population structure are likely to outweigh any affects of chronic exposure to low levels of anthropogenic contaminants. Equally, diet and physical factors, such as temperature, dissolved oxygen, salinity and others are also likely to be important.

However, with these limitations in mind, manifest disease can still be regarded as an end-point in an unequal struggle between the fish and environmental and/or biological stressors. The relevance of a disease is obvious at the individual level and may directly result in mortality or indirectly by predation if the fish is debilitated. In the context of biological effects monitoring the usefulness of recording overt disease is primarily in assessing effects at the population level where large-scale and possibly long-term changes in disease prevalence are sought. Whether or not external diseases continue to be used for biological effects monitoring will, to a large part, depend on the outcome of the analysis of these data which form part of the ICES databank and which will be assessed by the ICES Sub-group on the Statistical Analysis of Fish Disease Data in the near future.

For many years, the use of additional laboratory investigations have been recommended, particularly for the characterisation of hepatic pathology. In addition, other 'target' organs have been suggested for examination. These include skin and gills because of their direct contact with the environment, as well as components of the CNS and endocrine systems. However, these organs are not generally sampled for histopathological investigation during fish disease surveys. Since there is increasingly strong evidence that liver changes can occur as a result of exposure to marine xenobiotics, this organ is examined routinely in larger/older fish and livers with macroscopic changes are fixed for confirmatory histopathological diagnosis.

There is a clear distinction between pathological effects directly caused by pollutants and the induction of disease which, in most cases, is likely to result from a complex aetiology. The use of detailed histopathology for the accurate diagnosis of putative toxic-induced changes in the liver has thus become established. However, only those livers showing gross changes are examined histologically. Recent research has demonstrated that there are many categories of contaminant-induced lesions, most of which have been recorded in dab and flounder from the North Sea. Of these, certain pre-neoplastic lesions or foci are of particular importance and show potential for use as biomarkers. Furthermore, the use of immunohistochemical markers to detect these early changes may significantly increase the detection rate and reveal foci not apparent to the naked eye resulting in greater sensitivity.

These techniques, together with certain cell function tests, offer great potential for the detection of early contaminant-induced pathology at the cellular and sub-cellular levels. However, because of the laboratory effort required for these investigations, only limited numbers of samples can be adequately examined. In addition, more research is required in order to clearly understand the step-wise progression (and possibly regression) of hepatic pathologies in marine flatfish before they can be used with confidence as specific biomarkers of toxic effect in the marine environment. It must be noted that the use of any molecular probe is dependant on the clear demonstration of its effectiveness based on laboratory experimentation, as well as from applications using field material.

In conclusion, taking into account the logistical problems inherent in a histological/cytological approach, it is recommended that, in addition to standard disease recording according to ICES guidelines, histological investigations continue to be included in disease monitoring programmes for use in confirmatory diagnoses of hepatic pathology and, more importantly, broadened to include studies on limited numbers of livers (and possibly other organs) using routine histopathology and currently available immunohistochemical probes to detect early changes.

(Review statement by S Mellergaard).

International Council for the Exploration of the Sea
Working Group on Parasites and Diseases of Marine Organisms
3-7 April 1995
La Tremblade
France

Agenda item m

Overview of latest information on the M-74 syndrome in Atlantic salmon in the Baltic Sea

The M-74 syndrome continued causing problems in 1994. Both in Finland and Sweden the mortality in the 1994 offspring was almost at the same level as in the previous years i.e. 80-90%. In Sweden, a minor reduction was observed at some sites but it was probably due to a much harder selection of the brood stock.

The newest information indicates that the vitamin component is one of the key factors in the problem. In the Great Lakes Basin, a M-74 like syndrome called the "Cayuga syndrome" has been observed in Atlantic salmon (*Salmo salar*),in lake trout (*Salvelinus namaycush*) and in coho salmon (*Oncorhynchus kisutch*). After having excluded PCB's, DDT's and other toxicants as main responsible factors, American scientists focused on the vitamins especially thiamin (vitamin-B_1).

The main food item of these salmonids was alewife (*Alosa pseudoharengus*), a non-indigenous fish of the herring family (probably imported via ballast water) which exhibited high thiaminase activity. The symptoms observed in the sac-fry suffering from the "Cayuga syndrome" - uncoordinated swimming movements, lethargy and weak avoidance reactions - were very similar to the symptoms in other animal species suffering from thiamin deficiency - neuromuscular incoordination, tremor, convulsions and finally death.

Analysis of the whole body thiamin content revealed concentration up to 124 µg/g in syndrome-afflicted sac-fry while the lowest level measured in healthy sac-fry was 234 µg/g.

Thiamin treatment experiments demonstrated significantly higher survival in fry treated at 150-190 TU°C post hatch with 300 ppm thiamin (or more) for one hour - survival in treated groups more than 80 % and in untreated groups less than 20%.

Similar experiments have also been conducted in both Sweden and Finland with M-74 affected fry with the same positive results. The Finnish experiments demonstrated a reduction in the mortality from 60% in the untreated group to 1.5% in the treated group.

In Sweden, thiamin measurements in salmon sac-fry revealed levels in healthy specimens of 1500 µg/g compared to 40 µg/g in M-74 affected fry. However, other vitamins - the antioxidant group - vitamin C and E and the carotenoids also seem to be factors that have to be considered.

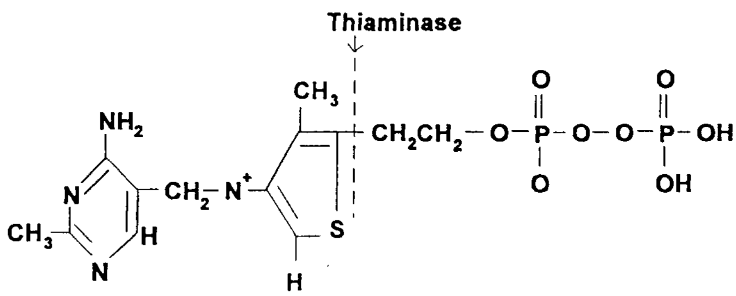
The thiamin treatment may be regarded as a symptomatic treatment that is very promising for the stock enhancement program in the Baltic but the wild salmon stocks are still seriously threatened by the M-74 syndrome. There is still many open questions in this problem. Why has the M-74 not occurred at an earlier time? Sprat and herring have been the main food item for Baltic salmon for decades. It is likely that we once again deals with a multifactorial problem.

In Sweden a series of public and private organisations have allocated funds to a research programme "Reproductive disturbances in Baltic fish". This programme is running from 1994-1999, and deals with the investigation of many of the different components that may be involved in the problem.

Additionally, the Nordic Council of Ministers has funded a research programme also dealing with reproductive disturbances in fish with participation of laboratories from the Nordic countries.

Thiaminase

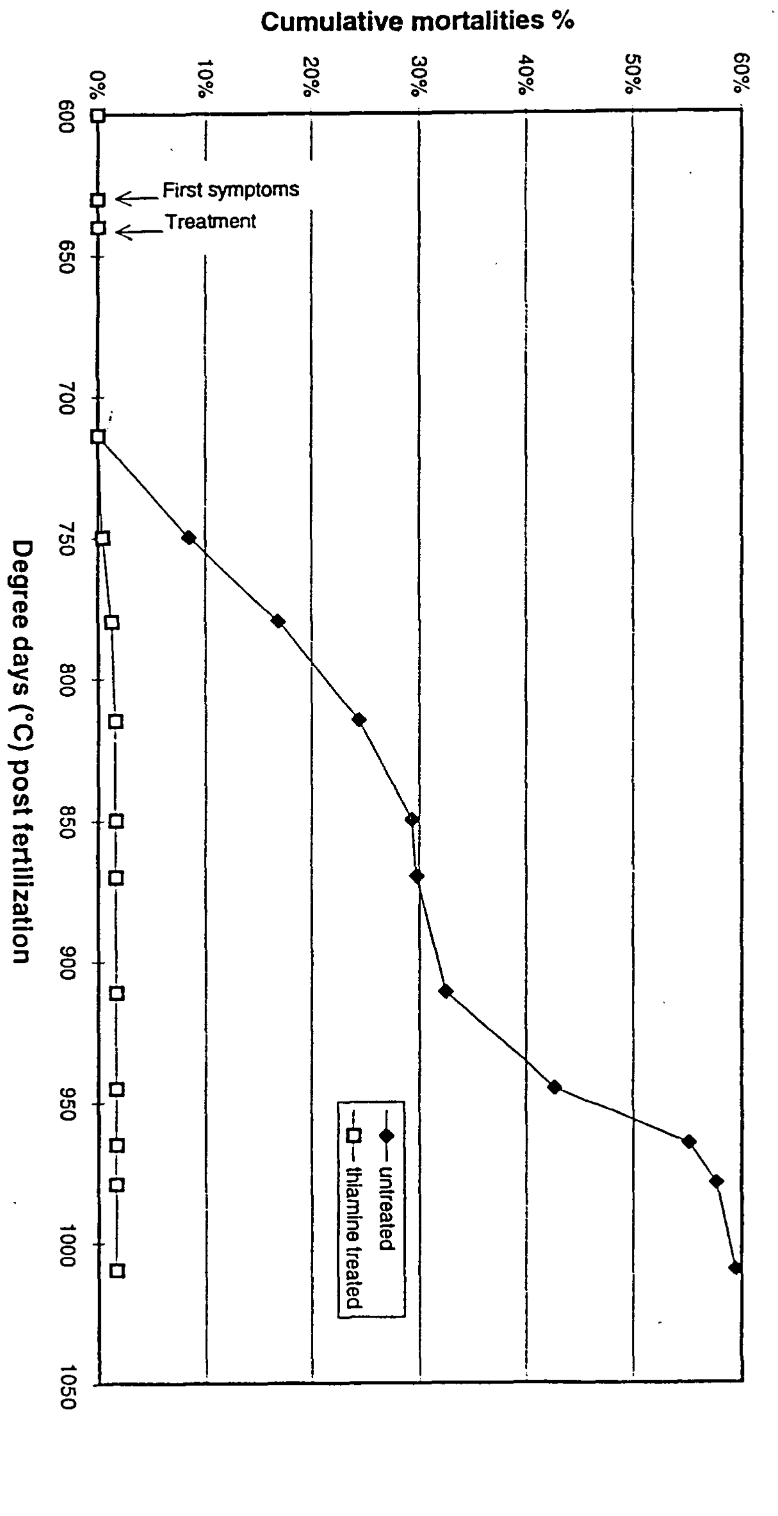
Many freshwater and saltwater fish species contain thiaminase. The greatest antithiamin activity is in spleen, liver, intestine and heart. The specific component appears to be haemin, a partially degraded metabolite of haemoglobin. It acts by splitting thiamin at its methylene bridge.



ANNEX 17b

Results of a Finnish experiment on treating Baltic salmon yolk-sac fry with thiamine (G Bylund).

Working Group on Pathology and Diseases of Marine Organisms, 1995.



ANNEX 18a

Ichthyophonus. **Summary of status in the North East Atlantic/Baltic in 1994.**

A. H. McVicar

Working Group on Pathology and Diseases of Marine Organisms, 1995.

1. Iceland

a) Research vessel (mid water trawl) catches.
4/1489 herring examined from south east of Iceland visibly infected with *Ichthyophonus* and confirmed by squash preparations made up as follows:
3/1278 25cm + herring.
1/186 20 - 24cm herring.
0/25 15 - 19 cm herring.

J Palsson, Reykjavik observes that the prevalence of *Ichthyophonus* in Icelandic herring is very low, as it has been since this monitoring started in 1992. The occurrence of infection in the 20–24 cm size group is the first record in this size group.

2. Sweden

a) Research vessel catches from standard Swedish sampling stations from the west coast of Sweden.
48/2208 herring examined were visibly infected with *Ichthyophonus* and were made up as follows:

0/99 30 cm + herring.
8/574 25–29cm herring.
13/1047 20–24cm herring.
27/2208 19 cm and < herring.

b) Commercial catches from commonly used fishing banks off the west coast of Sweden.
1/153 30 cm + herring.
1/1077 25–29cm herring.
2/1989 20–24cm herring.
0/860 19 cm and < herring.

H Rahimian, Göteborg, noted the new increase in the level of infection in the small size groups of herring and the higher level of infection in research as opposed to commercial catches from the same area.

3. England

a) Research vessel trawl catches from throughout the North Sea in August 1994 revealed only one grossly in fected herring in each of statistical rectangles 45F1, 45F2 (total hearts examined = 1403) and no evidence of infection in 110 herring examined in the Irish Sea. S. Feist, Weymouth noted that the disease had practically disappeared from the North Sea.

4. Estonia

In Estonian waters, A Turovski noted that from a prevalence of just under 3% in herring (3400) and 1.5% in sprat (900) in 1991 the prevalence of *Ichthyophonus* decreased in 1992–93 to 0.2% (1460 herring, 670 sprat) and was not detected in 1994 surveys (960 herring, 310 sprat). Two herring (7 and 9 years old) were found with *Ichthyophonus* granuloma in 1994 and the proportion of bottom fish (several species) increased from 2–3% in 1992 to 5–17% in 1994 (mean 6.5%). It was suggested that the clupeid infection with *Ichthyophonus* was a peripheral wave of a distant epizootic which took place in the southern and western Baltic in 1990 with some evidence of an earlier event around 1978.

5. Germany

a) Research vessel catches.

Cessation of systematic surveys because, according to Lang, Cuxhaven, due to the decline of the epidemic in the Baltic and the North Seas. However, a stock assessment survey in February 1995 in the northern North Sea gave evidence that *Ichthyophonus* was still present in the area and to the southwest of the Norwegian coast a considerable number of infected herring (number not recorded) was found among larger size groups, particularly in areas with low numbers of herring in catches. No evidence of infection was detected in "some hundreds of herring" examined in November–December 1994 from a transect taken from the western Baltic (Mecklenburg Bight) to the Gulf of Finland.

6. Netherlands

a) Research vessel catches.

Samples from the English Channel, SW North Sea and the northern North Sea west of 0° (total 2967 herring) did not reveal any infected fish. P van Banning, IJmuiden noted that the sharp decrease in the prevalence of *Ichthyophonus* observed in 1993 had continued in 1994 and raised the question whether ICES need to continue or stop the official request to monitor the level of this disease.

7. Norway

a) Research vessel catches.

Ichthyophonus infection was detected in 19/ 1363 herring examined as part of the Norwegian international acoustic survey in June–July 1994 in the north eastern North Sea and in 11/856 in the October–November 1994 bottom trawl survey in the northern North Sea north of approximately 56°N.

b) Commercial catches.

Ichthyophonus was detected in 3/2919 herring commercially caught mainly caught by purse seiners.

D. Skagen, Bergen, noted that high prevalence are connected with certain year classes and suggested that herring is infected at a young stage in certain years and that the manifest disease appears after an incubation period of variable duration. Trawl catches had a higher level of infection. The Norwegian data showed that the prevalence levels decline within individual year classes. The Norwegian data also suggest that the disease rate in the North Sea has declined in recent years and that the disease at present does not induce any appreciable additional mortality in the North Sea herring stock.

The Norwegian report also gave some information on data from Russia (PINRO, Murmansk) which has still to be verified. This data showed exceptionally high levels (up to 100%) of *Ichthyophonus* in Norwegian spring spawning stocks. Until intercalibration between the Russian and ICES recommended methods is achieved it is not possible to directly compare these results with others from the same area.

8. Scotland

a) Research vessel catches.

0/1353 herring examined from east of Scotland to east of Shetland in May 1994 revealed *Ichthyophonus* infection, 0/621 and 0/409 from ICES Division IVb in August 1994 and February 1995, 3/469 and 3/274 from ICES Division IVa in August 1994 and February 1995 respectively were infected with *Ichthyophonus*. J. Simmonds, Aberdeen reported data from acoustic surveys conducted in 1992–94 which provided estimates of infection rates in the survey area east of 6'E. Almost all the infected fish were found in ICES area IVa. The percentage of herring infected dropped from 5.2 (of 532) in 1992 to 3.6 (of 1480 in 1993 to 1.2 (of 45) in 1994. The infection rate was shown to show some reduction in older fish.

b) Commercial samples.

3/3918 herring examined from the general North Sea area (ICES squares 37F0 to 51E9) were infected with *Ichthyophonus* in the 3rd quarter of 1994, while 4/1391 were infected in the fourth quarter between 46F0 and 49 F1. No *Ichthyophonus* infection was detected in 1958 herring from Area IVa during 1994. There was a low level of *Ichthyophonus* infection in the northern North Sea east of Shetland in 1994.

9. Denmark

No data was available from Denmark because of the belief that the level of *Ichthyophonus* infection had fallen to low levels where the effort in monitoring did not justify the result.

10. General conclusions

There is a general perception of a significant decrease in the prevalence levels of *Ichthyophonus* in the North Sea in 1994 although there is evidence of a steady level of infection in the younger year classes (<20cm) in the Kattegat area. The Baltic adult herring stocks showed no appreciable level of infection. However, low levels of the disease still persists in many areas but it is not known if these represent background levels (as opposed to epidemic proportions). Clarification is required on data from the common Norwegian/Russian herring stocks.

Working document
Herring Assessment WG for the Area South of 62oN 1995
WG on Pathology and Diseases of Marine Organisms 1995

Prevalence at age of *Ichthyophonus hoferi* disease in the North Sea 1991-94 Norwegian data from surveys and commercial catches

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This working document is a summary of the prevalence data from two surveys in the North Sea where we now have data covering 4 consecutive years, and in addition data from the commercial fishery in 1994. The development of the prevalence at age over these years in the survey data show systematic trends, which have led us to speculate if this disease infects certain year classes at an early age and give rise to manifest disease later in life. Some consequences of this hypothesis are discussed.

Surveys:

Summer survey: This is the Norwegian part of the international acoustic surveys for herring, which take place in June-July. Our surveys cover the North-eastern part of the North Sea. Samples are mainly taken with pelagic trawl, and most often on acoustic registrations.

Autumn survey: This is the Norwegian part of the International Bottom Trawl Surveys (IBTS) in the 4th quarter. It takes place in October-November and covers the North Sea north of approx. 56°N, exept the Norwegian trench and the areas to the west of Shetland-Orkneys. One bottom trawl haul (GOV-trawl) is taken in each ICES rectangle in fixed positions. In addition, occasional pelagic hauls are taken on acoustic registrations.

Commercial catces:

The sampling of commercial catches improved substanstially in 1994. The majority of the samples were from purse seiners fishing primarily for herring, two samples were from bycatches in the industrial small meshed trawl fishery.

Examination of herring:

In large catches, a sample of 50 - 100 herring was examined. In smaller catches, all the herring was included. The standard examination included age determination (by counting otolith winter rings) and examination for I. hoferi disease. The diagnostic criterium used here was macroscopically visible lesions in the heart.

The prevalences are presented by age in two forms:
1. As percentage diseased of the fish actually examined.
2. By weighting by the numbers caught in each haul.

Results:

The results from each survey are shown in Tables 1 and 2. Since the size of the catches vary considerably, and it is likely that diseased herring is over-represented in small catches with trawl, the estimated percentage after weighting by the number caught, is probably the more realistic of the two prevalence estimates. For the commercial purse seine fishery, this should not be a problem, and only the unweighted prevalences are reported (Table 3). For the commercial trawl fishery, the data are very sparse (Table 4).

Tables 5 and 6 show summaries of the survey data. In the summer surveys, the large prevalences appear to be connected to the year classes 1986 (4-ringers in 1991) and older. The younger year classes never show prevalences above 5%. The picture in the autumn surveys is generally the same, although less striking. It also appears that within each of these year classes, the prevalence is reduced as time passes. These data are also shown in Figures 1 and 2. The remarkable feature here is that for most year classes, the decline in prevalence over time is near linear, except when the prevalence becomes very low, and the slope seems to be the same for all year classes. Again, this is clearest for the summer survey data. The prevalences in the autumn surveys are lower and less regular.

Comments:

The results indicate that the high prevalences mostly are connected to certain year classes, and less so to specific years or ages. Using a general linear model procedure, it was found that the percentage diseased in the catch was better explained by a model with yearclass and age as explanatory variables than by one with year and age, both for the summer surveys ($R^2 = 0.92$ and 0.52 respectively) and the autumn surveys ($R^2 = 0.48$ and 0.20 respectively). This has lead us to a working hypothesis suggesting that herring is infected at a young age in certain years, and that the manifest disease appears after an incubation period of variable duration. The following is an attempt to see if the time course of the prevalences found here can be compatible with this hypothesis.

According to the working hypothesis, each year class can be divided into two parts, one uninfected with stock abundance N_h and mortality Z and one infected with stock abundance N_d and mortality $Z + M_d$, where M_d is the additional mortality associated with the risk of developing manifest disease. Since the infected subpopulation presumably has higher mortality and therefore disappears more rapidly, one would expect that the overall prevalence decreases as time passes.This can be modelled as follows:

Assume that it takes the time Δt from a fish develops visible signs of the disease until it dies. The number with manifest disease at time t will then equal the number $D(t)$ that will die in the time interval $(t, t+\Delta t)$, which is $D = M_d * \overline{N}_d * \Delta t$ where $\overline{N}_d$ is the average over the period Δt.To simplify the formulas, we may write $D(t) = \phi * N(t)$, where ϕ becomes

$$\phi = M_d*(1-\exp(-(M_d + Z)*\Delta t))/(M_d+Z)$$

There is a good deal experience suggesting that the catchability by trawl is increased for diseased herring. To account for this, we introduce a catchability factor ρ which is the ratio between catchabilities for diseased and healthy fish. The prevalence in the catches can now be expressed as:

$P(t) = D(t)*\rho/(D(t)*\rho + N_D(t) - D(t) + N_H(t))$.

To simplify the expressions further we introduce $q(t) = N_d/(N_d+N_h)$., for the infected fraction of the population, i.e. the fraction being at risk of developing disease. With some manipulation we arrive at:

$$q(t) = 1/(1 + (1/q(0)-1)* \exp(-M_d*t))$$

and

$$P(t) = \rho\phi q(t)/(\rho\phi q(t)-\phi q(t)+1)$$

An example of the function $P(t)$ is given in fig. 3. Here, the parametes have been chosen to give a decline in $P(t)$ similar to that in the summer surveys data. This curve indicates that it is possible, under the present working hypothesis, to obtain a prevalence at age pattern over the years similar to the one observed, using parameters which are not too unrealistic.

In conclusion therefore, the decline in disease prevalence as it appears in the surveys, the summer survey in particular, appears to be compatible with the hypothesis that the fungus infects parts of a year class at a fairly early stage in life, and induces an additional mortality to the infected part over a long period of time.

This conclusion does of course not exclude other explanations for the trends in the disease prevalence. A morphological correlate to the proposed incubation stage is not known at present. The present findings do indicate, however, that systematic investigaions of the early stages of the disease may be a useful direction for future studies.

Apart from this, the present data, including the low prevalences in the commercial purse seine catches, strongly suggest that the disease rate in the North Sea has declined in the later years, and that the disease at present does not induce any appreciable additional mortality in the North Sea herring stock.

Table 1:

Survey	Age =>	0	1	2	3	4	5	6	7	8	9+
Summer 1991											
	No caught	0	207	946	556	583	931	228	106	25	48
	No. exam.		78	128	111	115	131	32	11	6	3
	% weighted		4.4	1.4	3.4	11.6	15.1	18.0	15.0	0	0
	% of exam.		3.8	4.7	6.3	23.5	30.5	53.1	54.6	0	0
Summer 1992											
	No caught	0	1242	1490	1279	1331	1627	1490	623	136	211
	No. exam.		73	125	115	137	136	169	49	17	25
	% weighted		0	1.0	1.6	0.9	7.6	11.5	15.3	9.4	1.9
	% of exam.		0	4.0	10.4	5.8	28.7	31.4	32.7	52.9	16.0
Summer 1993											
	No caught	0	29042	6774	2590	1345	951	439	324	100	88
	No. exam.		901	393	284	218	156	81	66	35	25
	% weighted		0.0	0.1	0	1.3	0.4	3.9	6.3	16.5	21.1
	% of exam.		0.1	0.8	0	2.3	0.6	12.4	13.6	14.3	20.0
Summer 1994											
	No caught	0	4271	8556	7230	1973	848	613	327	176	36
	No. exam.		226	506	268	142	82	75	27	37	14
	% weighted		0	0	0	1.0	0	0.3	0.9	3.4	14.3
	% of exam.		0	0	0	2.1	0	2.7	11.1	16.2	35.7

Table 2:

Survey	Age =>	0	1	2	3	4	5	6	7	8	9+
Autumn 1991											
	No caught	0	0	52	156	164	550	439	249	61	32
	No. exam.			18	31	62	44	25	12	2	2
	% weighted			0	1.7	10.6	1.7	7.2	0.9	0	0
	% of exam.			0	6.5	24.2	18.2	8.0	16.7	0	0
Autumn 1992											
	No caught	415	1866	1143	595	614	1165	779	225	106	21
	No. exam.	78	168	146	119	100	196	172	45	21	6
	% weighted	0	0	0	3.4	5.0	4.3	5.7	3.4	1.1	27.0
	% of exam.	0	0	0	3.4	5.0	7.1	8.7	8.9	4.8	33.3
Autumn 1993											
	No caught	5943	5994	1761	252	465	415	491	309	136	30
	No. exam.	338	368	190	62	97	70	75	86	26	19
	% weighted	0	0	0.9	0	0.4	0.9	0.9	6.9	0	3.4
	% of exam.	0	0	0.5	0	2.1	4.3	6.7	7.0	0	5.3
Autumn 1994											
	No caught	533	4815	2117	1763	478	287	85	121	141	81
	No. exam.	21	246	236	146	45	31	30	41	35	25
	% weighted	0	0	0.6	0.2	0.6	0	1.7	1.2	0	0
	% of exam.	0	0	1.3	2.1	6.7	0	3.3	2.4	0	0

Table 3: Commercial purse seine catches 1994

Age =>	0	1	2	3	4	5	6	7	8	9+
No. exam.	0	0	1819	459	150	170	116	73	85	47
No. diseased			0	1	0	0	0	2	0	0

Table 4: Commercial trawl catches, april 1994

Age =>	0	1	2	3	4	5	6	7	8	9+
No. exam.	0	0	22	6	6	9	5	9	2	1
No. diseased			1	0	1	3	2	5	1	1

Table 5: Percent (weighted) with disease - Summer surveys

Age =>	0	1	2	3	4	5	6	7	8	9+
1991	-	4.4	1.4	3.4	11.6	15.1	18.0	15.0	0	0
1992	-	0	1.0	1.6	0.9	7.6	11.5	15.3	9.4	1.9
1993	-	0	0.1	0	1.3	0.4	3.9	6.3	16.5	21.1
1994	-	0	0.6	0.2	0.6	0	1.7	1.2	0	0

Table 6: Percent (weighted) with disease - Autumn surveys

Age =>	0	1	2	3	4	5	6	7	8	9+
1991	-	-	0	1.7	10.6	1.7	7.2	0.9	0	0
1992	0	0	0	3.7	5.0	4.3	5.7	3.4	1.1	27.0
1993	0	0	0.9	0	0.4	0.9	0.9	6.9	0	3.4
1994	0	0	0.6	0.2	0.6	0	1.7	1.2	0	0

Fig. 1

Prevalence in catch in summer surveys by year class

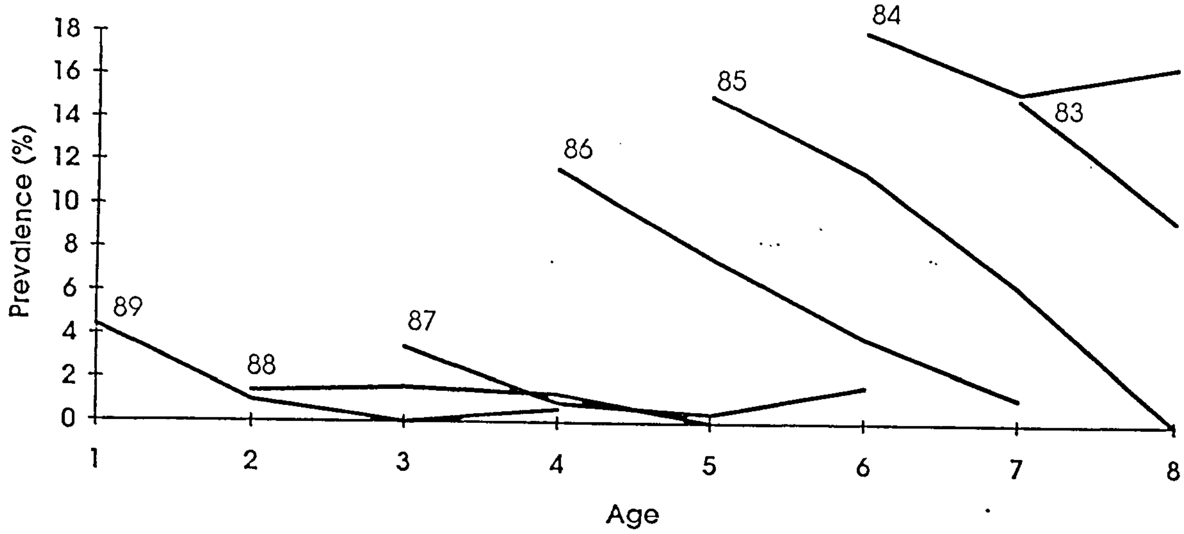


Fig. 2

Prevalence in catch in autumn surveys by year class

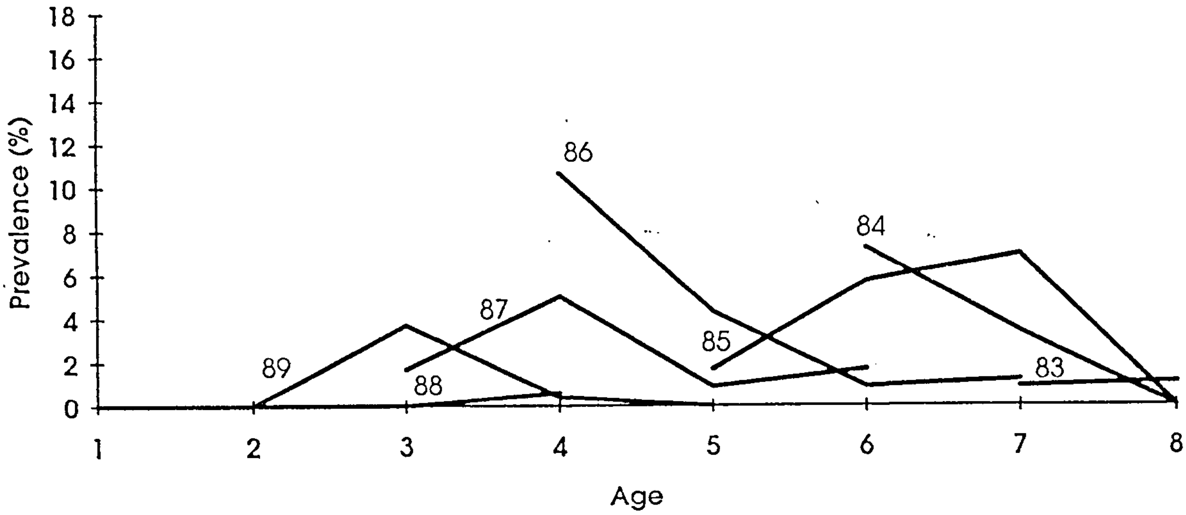
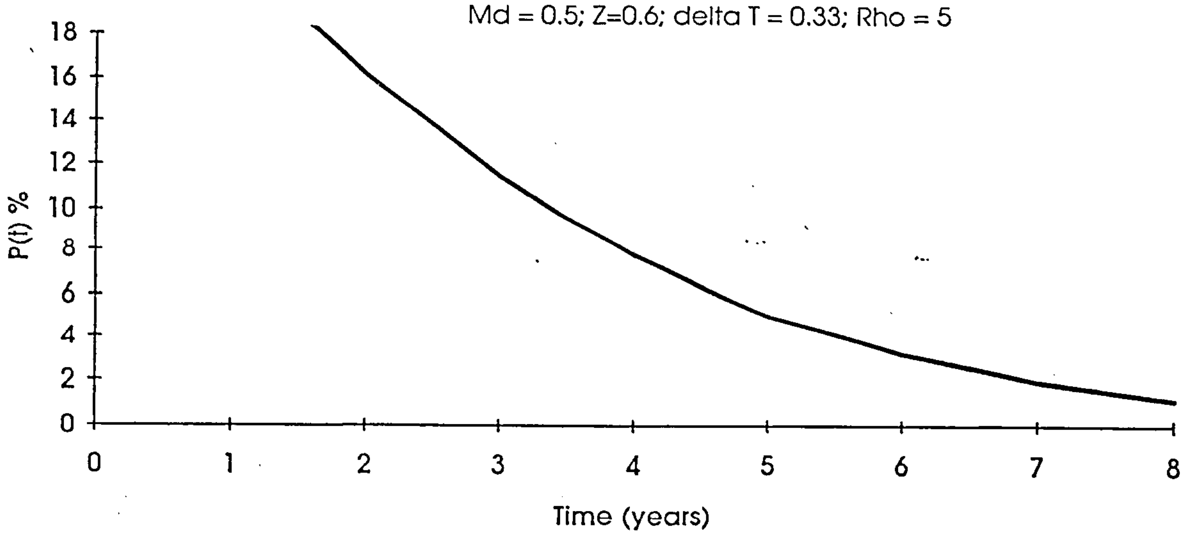


Fig. 3

Modelled prevalence

Md = 0.5; Z=0.6; delta T = 0.33; Rho = 5



Working Group on Pathology and Diseases of Marine Organisms, 1995.

Ichthyophonus Infection in Herring stocks East of 6°E and South of 62°N in the years 1992-94 estimated using trawl samples and abundance data from ICES coordinated acoustic surveys.

By
John Simmonds, Marine Laboratory, Aberdeen, Scotland

Introduction.

Acoustic surveys on herring conducted in 1992-94 provided herring abundance data. An integrated sampling program for ichthyophonus infection provided estimates of infection rates throughout the survey area.

This data was analyzed to give infection rates over the whole survey area east of 6°E. Data was analyzed for each ICES statistical rectangle for those with trawl sample data the infection rate was calculated directly from the samples. For rectangles without trawl samples the infection rate was estimated from the surrounding rectangles by linear interpolation. Equal weight was given to each trawl sample other weighting methods were tried but the results were not significantly different. The numbers of infected fish were calculated from the total abundance and the infection rate in each ICES statistical rectangle.

In order to provide estimates of the age distribution of ichthyophonus infection the numbers data from the trawl hauls from the SCOTIA surveys in the western North Sea were used to obtain a single age ichthyophonus infection key. This was used to raise the numbers at age over the whole of survey area provide estimates of infection at age in the population for 1992 and 1993. The data in 1994 was too sparse for age estimation.

Results

Figures 1 to 3 show the prevalence found in trawl samples which were taken during all the surveys in 1992 93 and 94 respectively using the same scales. The distribution of infection rates is shown in Figures 4 and 6 for 1992 and 93 respectively. The data for 1994 have not been mapped in this way but the infection is in the same area, north east of Shetland and west of Bergen. The infection rate near Shetland would not be visible on the scales used for 1992 and 93, there would be a small but unreliable peak due to a single gaul with 16 out of 21 fish found infected off Bergen.

The distribution of estimated total numbers of infected herring are shown in Figures 5 and 7 for 1992 and 1993 respectively. In this study the Baltic spring spawning herring are included with the North Sea autumn spawning fish as there was no information on the proportions of infected fish from each population.

Almost all the infected fish were found in ICES area IVa. The numbers and proportion of infected fish by year for ICES area IVa are given in Table 1.

Table 1 Numbers and percentage of Infected herring in ICES Area IVa in 1992 93 and 94 estimated from acoustic surveys.

Year	1992	1993	1994
Number	532	148	45
% Infected	5.29	3.6	1.2

The age breakdown was estimated using the infected fish found on the SCOTIA survey for 1992 and 1993. Samples were assumed to have equal weight and a single age infection length key was determined by combining all the samples. A single length key was obtained for same area by combining the individual sub area length keys and weighting them by the abundance for these areas. The infection rate by age for the Orkney Shetland area is given in Table 2. Also included in this table is the number of otoliths sampled and found infected to give an indication of the quality of the estimates at age. It should be bourn in mind that the infection rate in table 2 is not derived directly from numbers sampled but using a global age length key. Also the assumption that the age at length within the area is homogenous is questionable. However, the biggest changes in length at age are for 1 and 2 ring fish which show effectively zero prevalence of infection so this problem is not important. The for 1992 and 1993 the numbers infected are sensibly zero for 3 years and younger with a rapid rise through 4 year olds and a peak at 5 years. The infection rate shows some reduction for older fish but the rise shown for 8 and 9+ is unlikely to be real. The infection rate at age in 1994 has not been calculated due to the small number of infected fish in the SCOTIA samples. This data has been included to indicate the comparative levels.

Table 2. Numbers of fish otolithed and examined for ichthyophonus infection for 1092-94 and percentage infection rate by age class for SCOTIA Surveys in 1992 and 1993. The percentage infection rate is derived a using global length stratified age length infection key, and a global length key derived from the complete SCOTIA survey.

	SCOTIA 1992 Survey			SCOTIA 1993 Survey			SCOTIA 1994 Survey	
Age Class	Total Number of otoliths taken by age	Numbers of infected fish by age.	Percentage infection rate.	Total Number of otoliths taken by age	Numbers of infected fish by age.	Percentage infection rate.	Total Number of otoliths taken by age	Numbers of infected fish by age.
1	82	0	0.0	78	0	0.0	101	
2	260	0	0.0	689	1	0.1	950	
3	115	1	0.7	663	5	0.6	450	1
4	125	3	5.0	348	13	3.4	166	
5	187	11	12.3	360	27	8.3	157	
6	162	11	14.2	558	26	5.0	178	3
7	54	0	0.0	345	10	3.0	262	
8	25	2	11.3	113	6	4.8	172	
9	19	0	0.0	90	7	6.9	131	
Total	1029	28	5.6	3249	95	2.8	2567	4

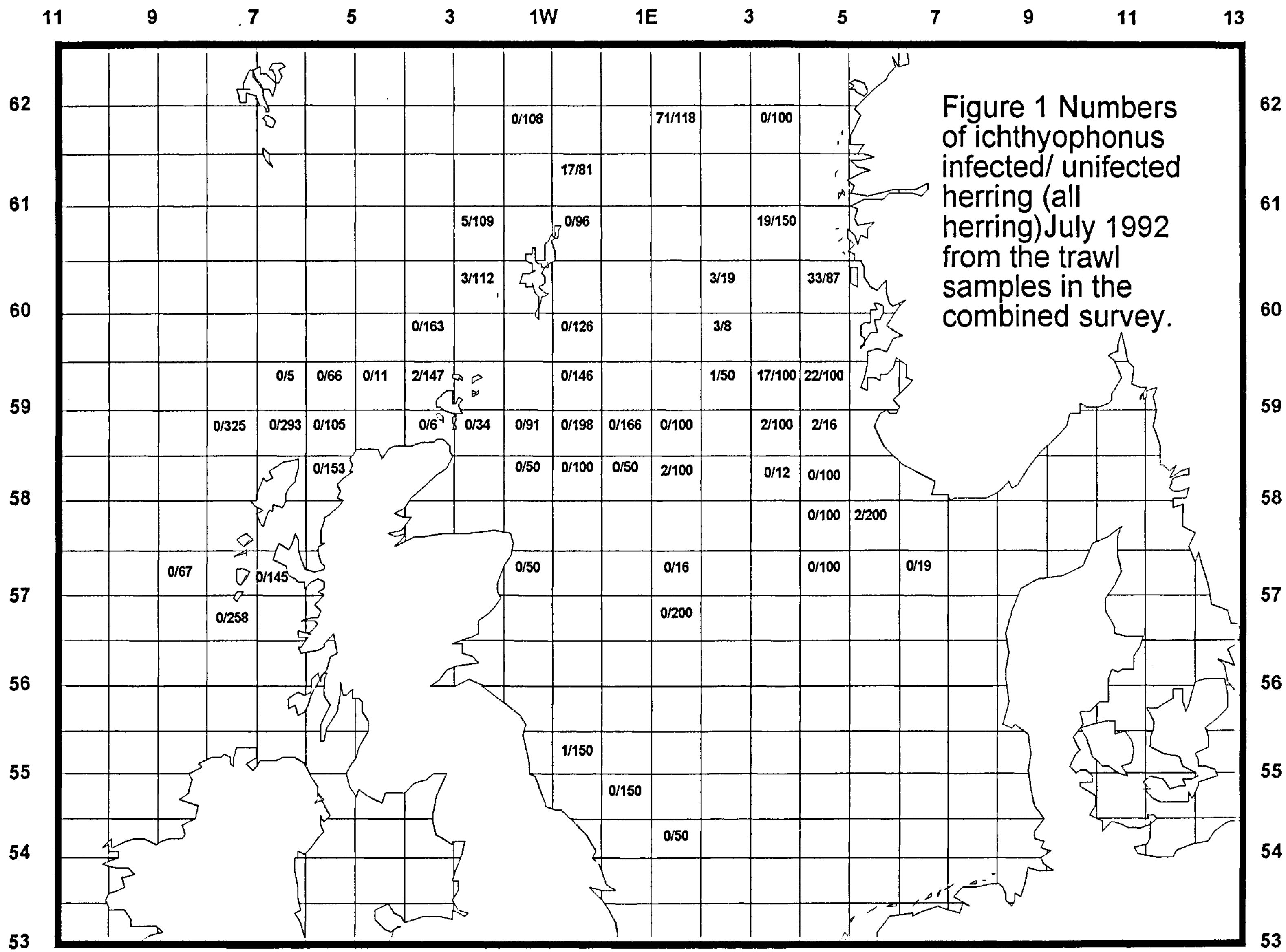


Figure 1 Numbers of ichthyophonus infected/ unifected herring (all herring)July 1992 from the trawl samples in the combined survey.

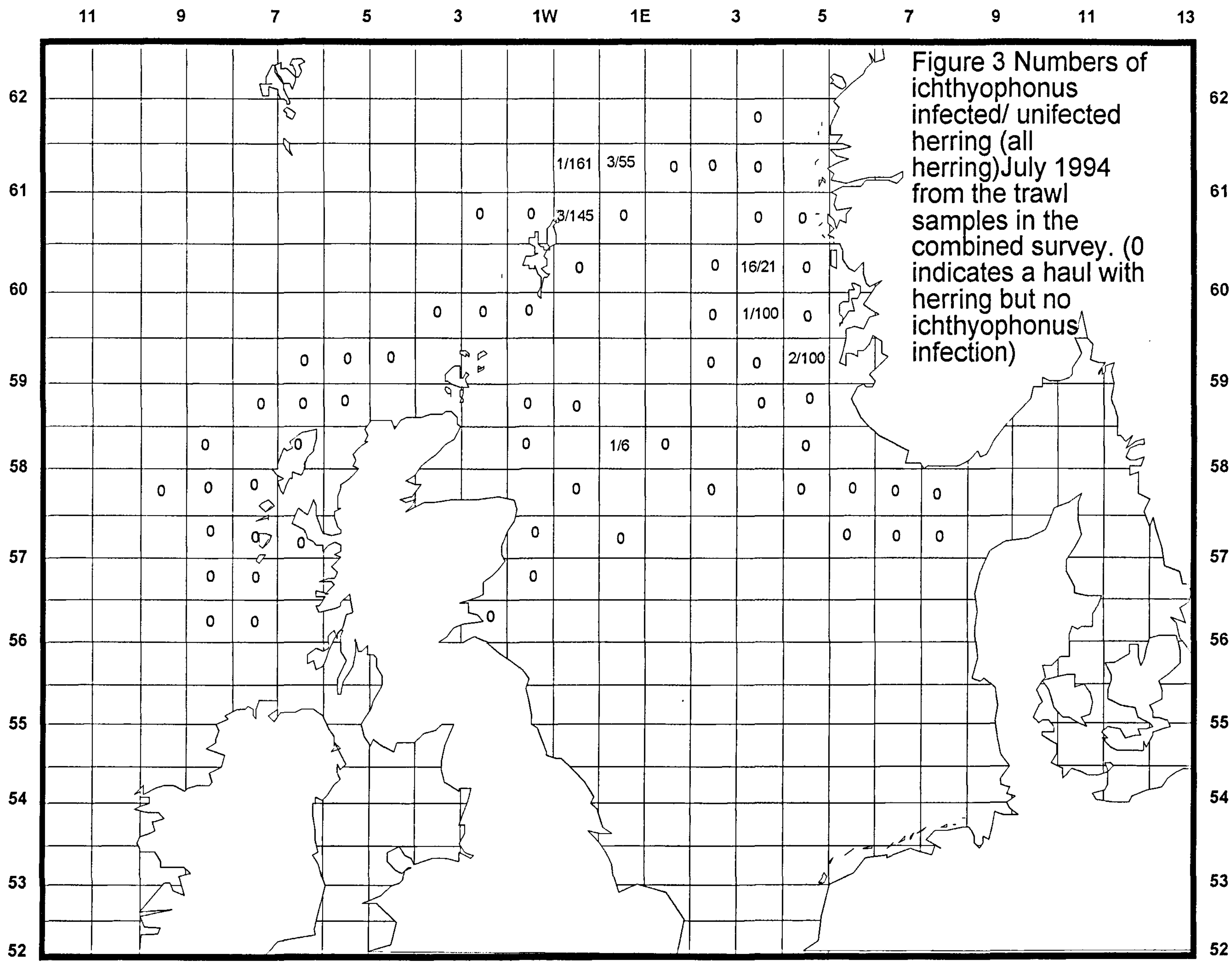


Figure 3 Numbers of ichthyophonus infected/ unifected herring (all herring)July 1994 from the trawl samples in the combined survey. (0 indicates a haul with herring but no ichthyophonus infection)

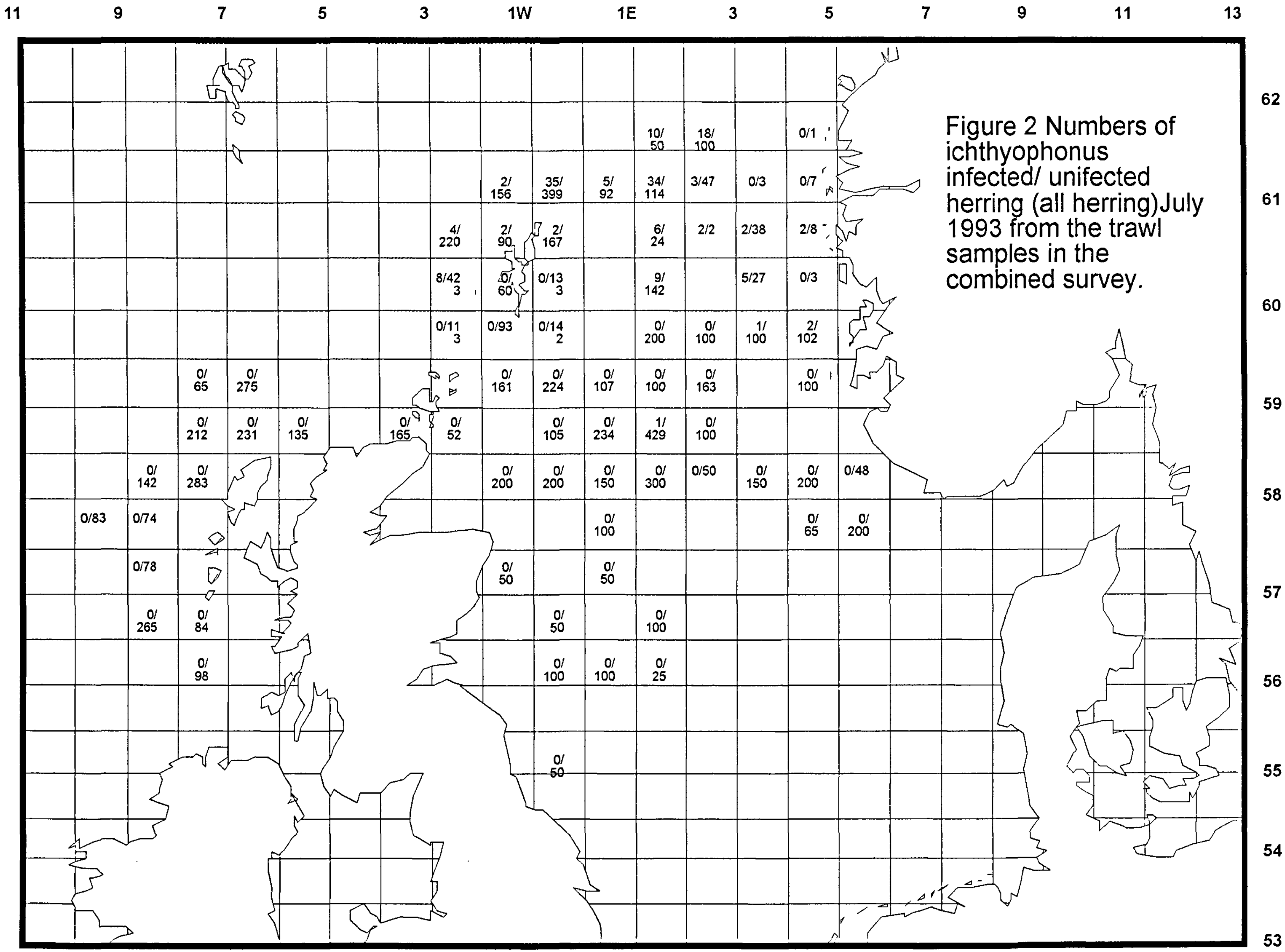


Figure 2 Numbers of ichthyophonus infected/ unifected herring (all herring)July 1993 from the trawl samples in the combined survey.

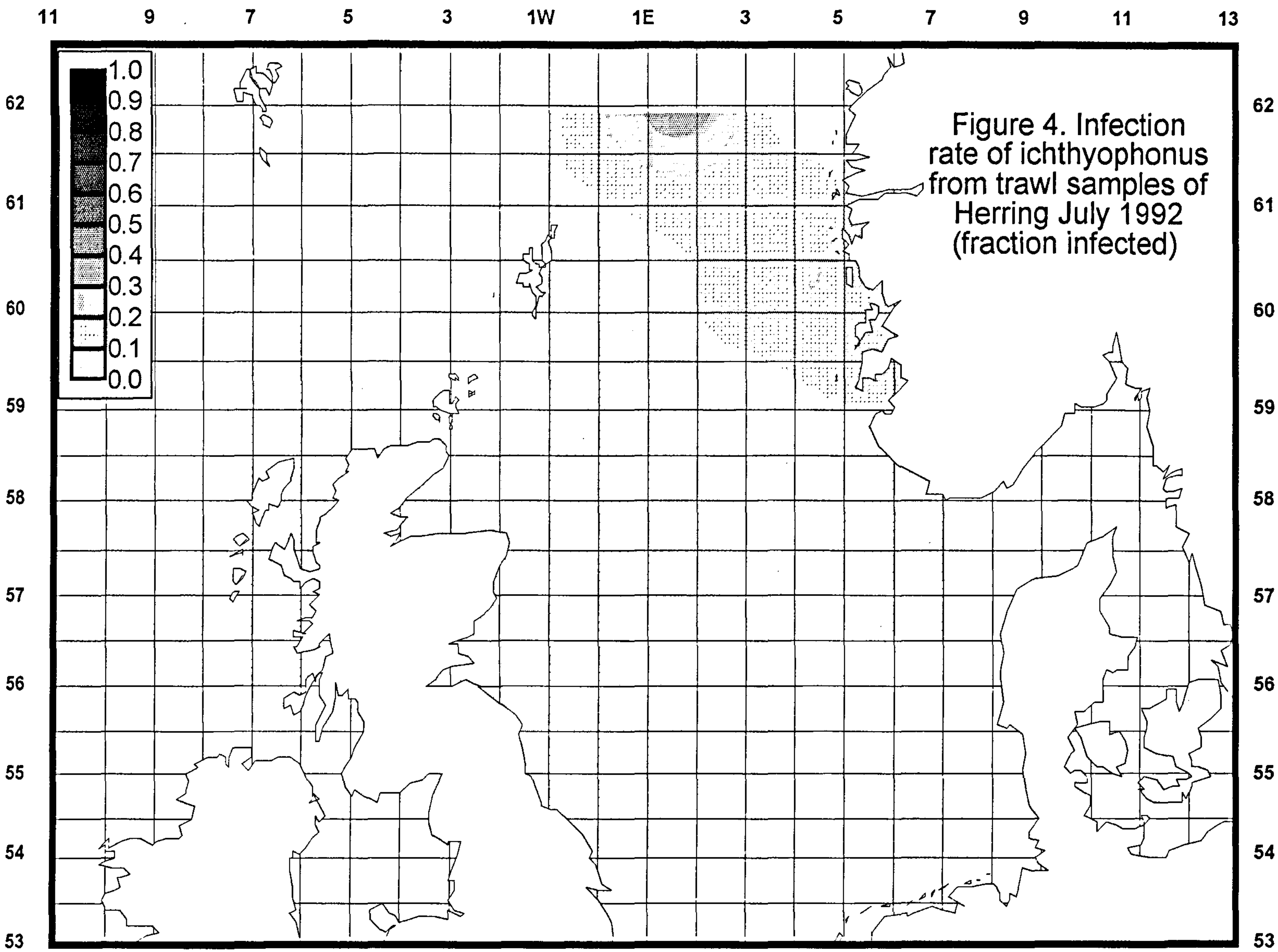


Figure 4. Infection rate of ichthyophonus from trawl samples of Herring July 1992 (fraction infected)

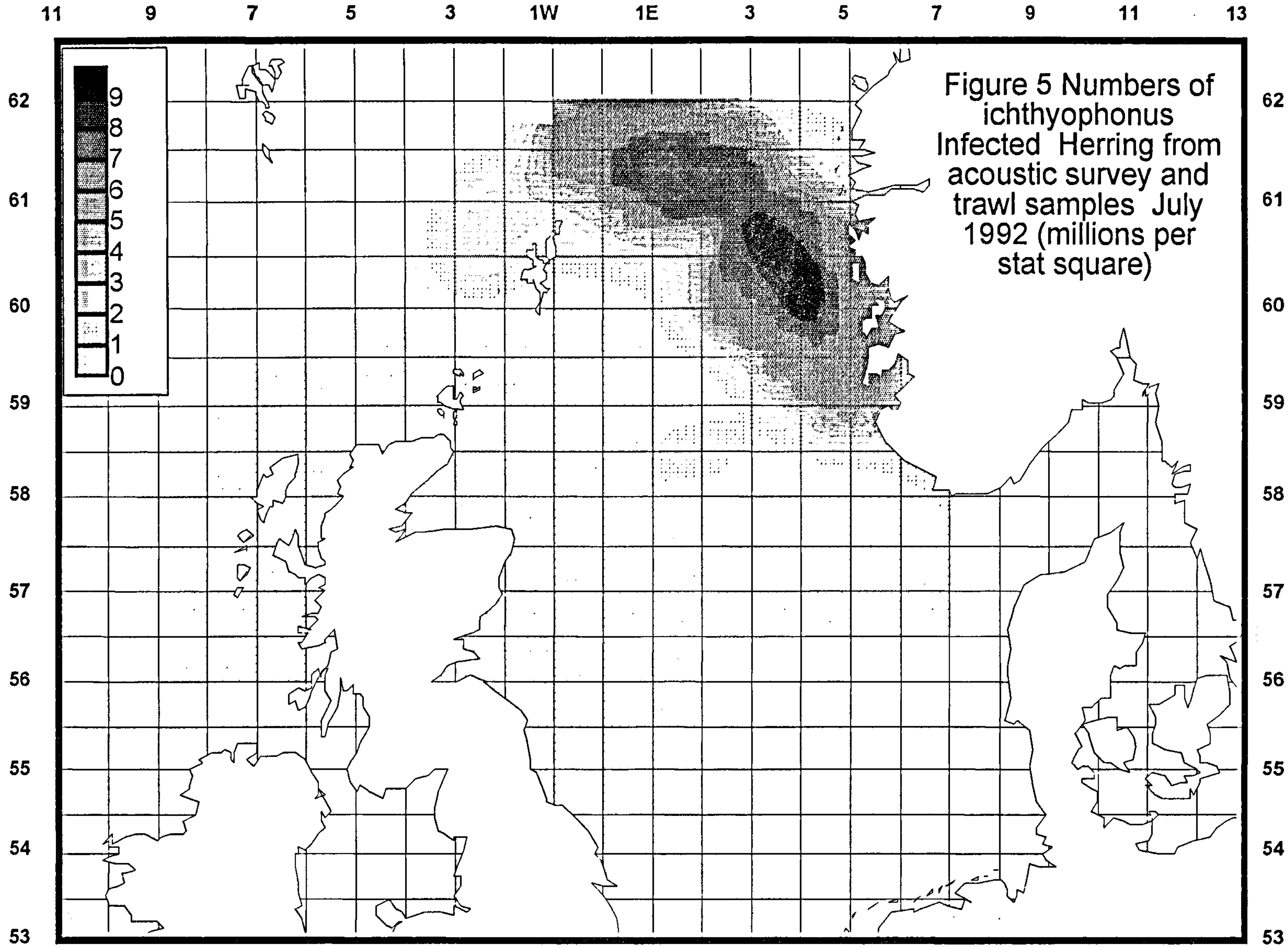


Figure 5 Numbers of ichthyophonus Infected Herring from acoustic survey and trawl samples July 1992 (millions per stat square)

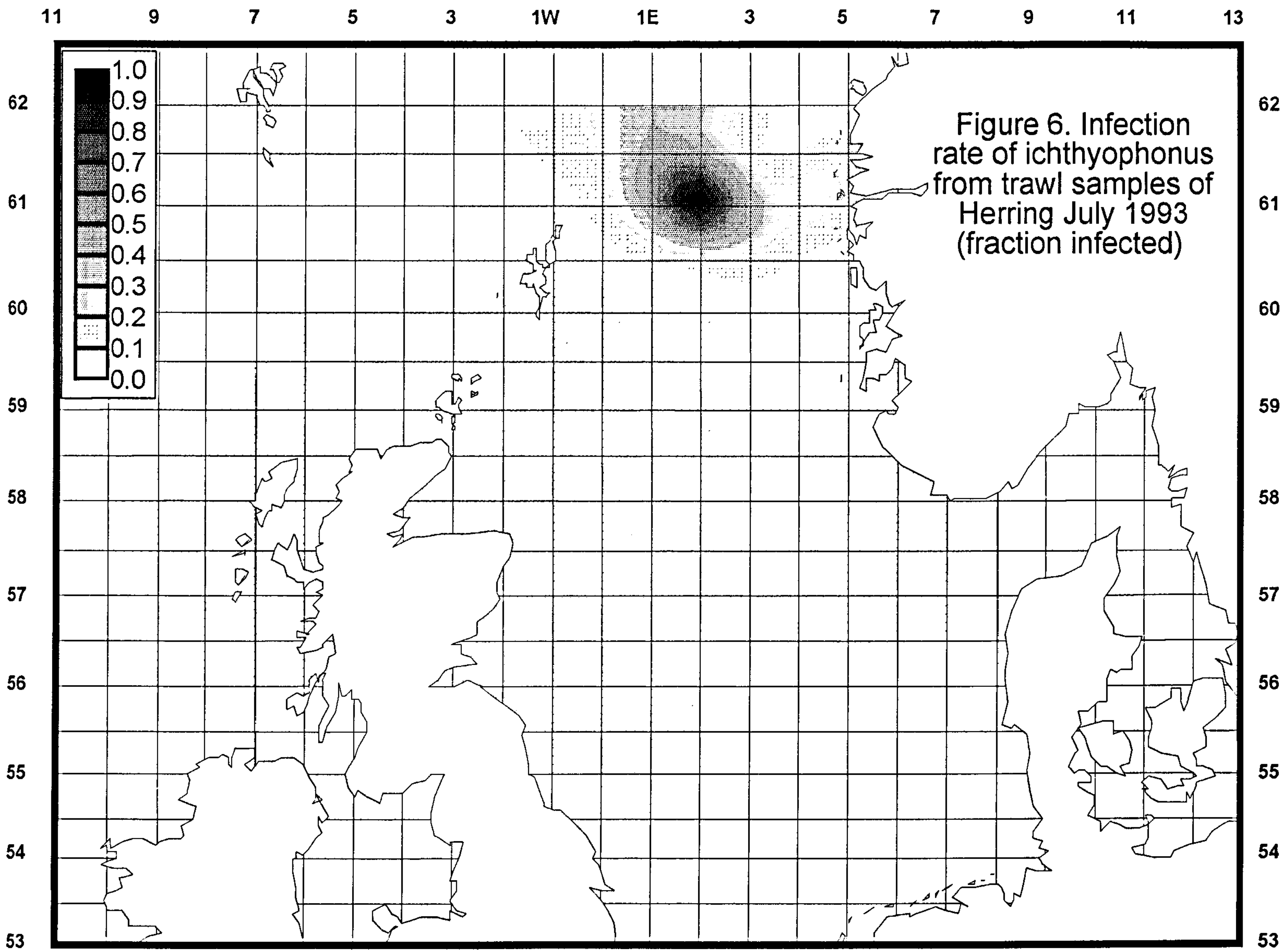


Figure 6. Infection rate of ichthyophonus from trawl samples of Herring July 1993 (fraction infected)

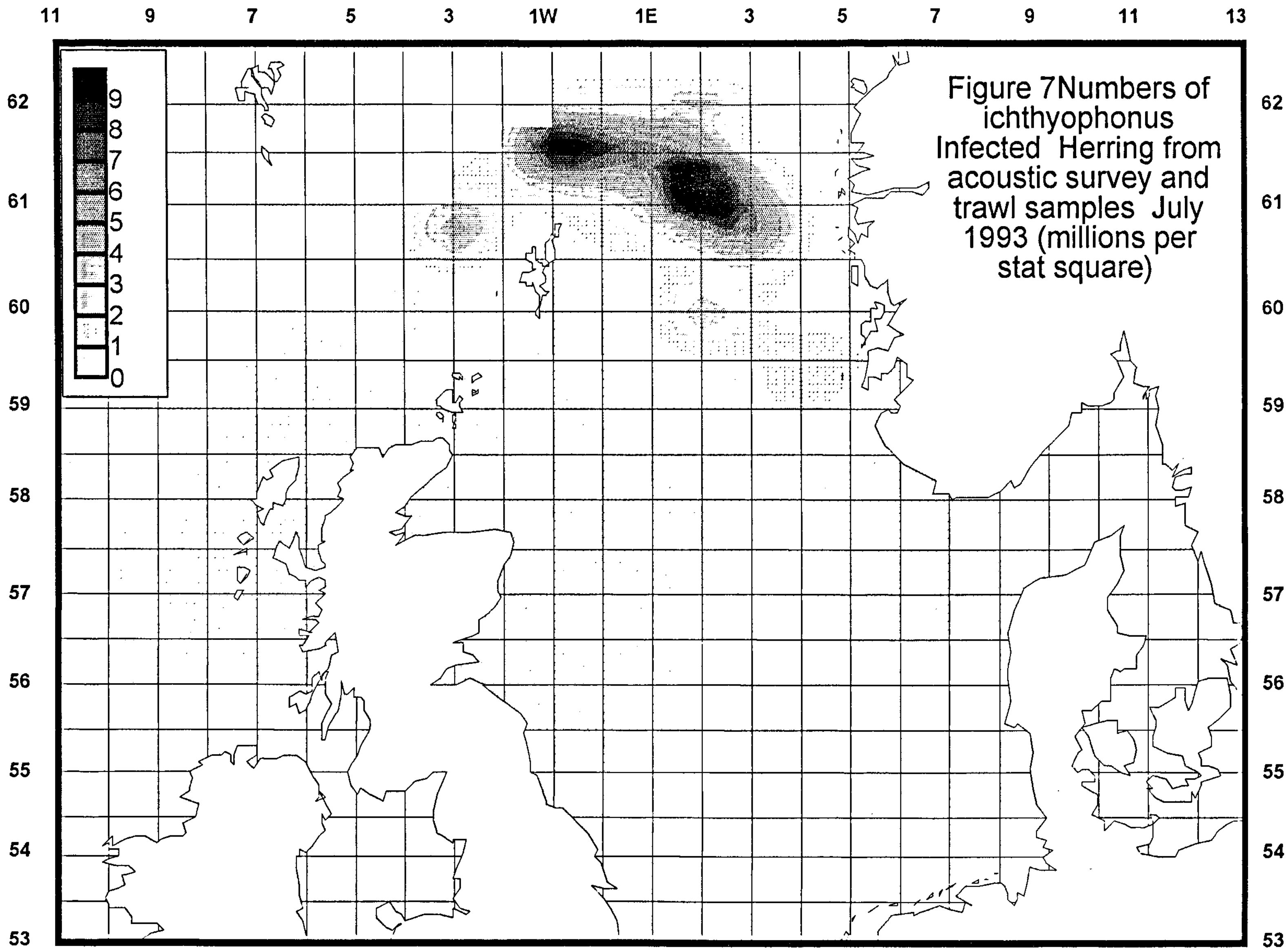


Figure 7Numbers of ichthyophonus Infected Herring from acoustic survey and trawl samples July 1993 (millions per stat square)

DISCUSSION PAPER ON SIGNIFICANCE OF DISEASE DATA IN RELATION TO EFFECTS ON FISH STOCKS.

DISCUSSION PAPER FOR WG PDMO, meeting 3-7 April 1995, La Tremblade, France.

Paul van Banning / Netherlands Institute for Fisheries Research

General thoughts.

The variability in the abundance of aquatic populations does not allow for a simple concept to select and express the impact of certain parameters. In marine populations, fluctuations in population number often seems to be the rule, reflecting a mixture of natural causes (e.g. levels of larval productions, predation, diseases) and human influences (e.g. fishery, aquaculture). Changes of one parameter can be overshaded by other more dominantly acting parameters present within the natural variability. Models to express impacts of a selected parameter in a natural marine environment by means of traditional monitoring and statistical analyses are limited by the difficulties to reach the precise data needed. In general, effect studies in marine populations are carried out by collecting the data of the selected mechanisms (probably) causing the variabilities. This means a data collection by monitoring of the populations concerned, followed by statistical analyses and , if possible, a modelling based on the data.

The results of the mathematical modelling are not always clear: while simple facts can show obvious increased levels of changes in fish populations (e.g. high disease prevalences), the expected impacts of such facts on fish stocks seems not present, or can not be demonstrated with the stock assessment calculations. May be overshading by other more important population regulating parameters play a role and suppress or flatten the expected level of the effect.

Most problems with studies of the effects in the marine populations are raised by the difficulties to get a sufficient data collection. Problems with time series and sampling techniques are well known difficulties. The estimation of a prevalence of a disease, on which mortality calculations are based, is in fact an 'one momentum' information: there is no information of the past, important data are already lost (the died part of the stock has disappeared and stays out of the observations). Further, data for the coming period are not stable (unknown which specimens become also infected). Such data need a long-term research to express the natural variability of an epizootic. Even with sufficient disease data over a long period, it remains questionable if the methods used for estimation of the marine (fish) populations are able to demonstrate clearly an impact of a disease on a population.

The *Ichthyophonus* epizootic of herring stocks of the east Atlantic can be considered as best example of a study carried out with disease data to estimate an effect on fish stocks. To get an impression on the status of the effect studies of *Ichthyophonus* on herring stocks, results of some recently performed studies on this topic are herewith discussed.

Evaluation of the effects of *Ichthyophonus.*

The fungal disease *Ichtyophonus* of herring in the east Atlantic can be considered as the best studied epizootic topic of wild marine fish since 1991. Several ICES member countries paid attention to this topic and provided important data on prevalences per area, per herring stock, series of long-term data over 3-4 years, and effects of sampling (fishing) methods. Mortalities are considered to be high, based on the sensitivity of herring for *Ichthyophonus* and the observed high prevalences (reaching ranges between 50 - 100 % for some herring stocks). The expectation was (and still is) that such high mortalities must have probably declining effects on the herring stocks concerned, especially in the period just after the 1991 peak of the epizootic. The first signs of such effects are expected with the results of the calculations of the ICES Herring Stock Assessment Working Groups. In their reports following is recorded concerning the effects of *Ichthyophonus*:

Herring Assessment Working Group For The Area South Of 62° N /C.M. 1994 / Assess:13/ March 1994:

> *"It was concluded from the very preliminary analysis that the stock may have suffered considerable mortality from the diseases in 1991"*
>
> *"The analysis suggested that the present low levels of infection rate are rather unimportant for stock dynamics"*
>
> *" In conclusion, there is no evidence from presently available information that Ichthyophonus-induced herring mortality is significant at present, although there are substantial indications that high mortalities occurred in 1991".*

North Sea Autumn-Spawning Herring Stock Assessment Update / Doc. ACFM / K.R. Patterson / November 1994: separate work on modelling the effect of the *Ichthyophonus* outbreak in this stock:

> *"does not support the hypothesis that additional natural mortality occurred in 1991".*

The Atlanto-Scandian Herring and Capelin Working Group C.M. 1995 / Assess:9 / October 1994: concerning the Norwegian Spring-Spawning Herring :

> *"precise estimates of the infection* rate *could not be estimated from the available data. However, the prevalence of this disease does not seem to be decreasing, and the Working Group therefore decided to apply the present high natural mortality of M=0.23 in the prognosis".*

concerning the Icelandic Summer-Spawning Herring (*Ichthyophonus* infection ?):

> *"this herring stock have remained relatively stable over a wide range of stock size and fluctuations in environmental conditions in Icelandic waters ".*

A Method for Assessing the Impact of Parasite-Induced Mortality in Exploited Populations / K.R. Patterson, WD. February 1994:

> *"...final prevalences are rather low, of the order of 1 to 3 % in the catches and in the surveys. Nevertheless and even though fishing mortality remains constant over the time period, stock numbers fall by some 20 % over the period of the simulation. This numerical experiment also illustrates that even quite low levels of observed prevalence, the consequences of a disease such as Ichthyophonus with a high and rapid mortality of infected fish can be to cause significant declines in stock size".*

Estimation of the consequences of *Ichthyophonus*-induced mortality on the herring stocks in the North Sea and Division IIIa-SW Baltic / O. Hagström & K.R. Patterson / WD., November 1993:

> *"This analysis suggest a 'worst case' impact of the Ichthyophonus outbreak on total stock size of the North Sea herring to be some 20% reduction in the case of unchanged fishing mortality" ......"a more reasonable estimate of the impact of the disease is that a reduction in stock size of less than 10 to 15 % could be expected"*
>
> *"In the case of the spring spawning stock in Division IIIa-Sw Baltic the analysis suggest a smaller reduction of the total stock in 1992 ...the most pessimistic assumption...gives a reduction in stock size of about 10 % due to the Ichthyophonus outbreak"*

For discussion

In conclusion, the 1991 *Ichthyophonus* epizootic has activated further developments of mathematical models to express the mortality effects on herring stocks of the E-Atlantic.

Despite the high prevalences of the disease observed, the modellings could not show so far serious impacts on the stock sizes (as expected), only indications (assumptions) of low level impacts (10 - 15 % mortality levels) were demonstrated.

With the decreasing trend since 1993 of prevalences of *Ichthyophonus* in the North Sea and adjacent areas, it is questionable if any impact can be shown on the present herring stocks. May be additional views can be provided with the special studies which are still going on with severely *Ichthyophonus* infected local herring stocks of northern Norway.

However, as general point for discussion, the question remains about the significance of disease data as a tool to express an impact on the population dynamics of wild marine fish.

ANNEX 19b

Fish disease data in relation to effects on fish stocks.

Working Group on Pathology and Diseases of Marine Organisms, 1995.

A. H. McVicar

Points for discussion on Agenda Item 19, TOR (o).
(Provide advice on methods to handle data in rapid response to disease issues in fish stocks).

1. Identification of the question being asked.

a) **Diagnosis** of the condition

- immediate reference to the specialist national lab for advice on the nature and quality of the material to be sampled;
- specialist involvement at the earliest possible stage to avoid mis-diagnosis;
- instruction in observation of the condition i.e., a negative does not mean a negative when it has not been searched for.

b) **Pathological/mortality** effects on fish

- investigate prevalence taking into account the possibility of bias in sampling due to catching method, distribution of host population in catch etc. Some factors will be more important than others - see G Begg thesis;
- obtain evidence for loss of condition or other impairment of body function;
- in cases of fish/shellfish kills obtain samples of apparently healthy, moribund and dead animals with material stored to meet the criteria in (a);
- integrate sampling and data with stock data.

c) Diseases as indicators

- occurrence of unusual disease conditions may give the first signs of a change in host, environment or disease characteristics;
- separate as far as possible each of these three factors using other local data by integrating sampling;
- use ICES standardised methods of screening, diagnosis and data recording;
- for fish stock separation use recommended criteria for selection of condition to study.

2. Establish the rapidity of response required.

3. Data handling.

- is the quality of the data sufficiently good;
- is the quantity meeting minimum standards;
- is the format appropriate for the ICES data bank currently being established;
- does the data integrate with other data being collected;
- delineation of geographic/ spatial, temporal and biological limits.

ANNEX 20a

Working Group on Pathology and Diseases of Marine Organisms, 1995.

Proposed new titles for ICES Disease Diagnostic Fiches.

Title	**Proposed author(s)**
a) Brown Ring Disease in Manila Clams	C. Paillard & P. Maes
b) Denman Island Disease	S. Bower
c) *Herpes*-like virus in *Crassostrea gigas* and *Ostrea edulis*	T. Renault *et al.*
d) QPX (Chytrid-like disease of hard-shell clams)	S. McGladdery
e) Infectious Salmon Anaemia (ISA)	B. Hjeltnes
f) M-74 Syndrome	G. Bylund
g) Cold water vibriosis (*Vibrio salmonicida*)	B. Hjeltnes
h) *Epitheliocystis*-like pigmentation abnormalities	S. Mellergaard
i) *Pasteurella piscicida* in sea bream and sea bass	J. Barja
j) *Enterococcus* in turbot	J Barja
k) *Perkinsus atlanticus* in *Tapes decussatus*	F. Perkins and/or C. Azevedo
l) Pancreas Disease of Atlantic salmon	A. McVicar
m) Mycobacteriosis in mackerel	S. Feist

ANNEX 21a

PROGRESS WITH TASKS AND ACTION LIST

1. Progress with tasks

i) Analysis of trends of diseases in wild marine animals. Reports of new diseases, new geographic distribution and trends in diseases were evaluated from national reports and conclusions presented.

ii) Analysis of trends of diseases in mariculture. Reports of new diseases, new geographic distribution and trends in diseases in mariculture were evaluated from national reports and conclusions presented.

iii) Analysis of the activities of the Sub-group on Statistical Analysis of Fish Diseases. Due to the cancellation of the meeting of the SG discussion was limited to the identification of intersessional tasks to be undertaken prior to the proposed next meeting of the SG in 1996.

iv) Assessment of intersessional study on sensitivity testing of antimicrobial agents. A report was prepared (Annex 8a) and serious procedural problems were identified, requiring further research.

v) Review of sea lice research. Reviews of current research on sea lice in Scotland and Norway were presented (Annexes 9a, 9b) and an analysis included in this Report.

vi) Review of research on pathology and diseases in fish larvae in mariculture. Reviews of literature and current research on diseases in mariculture fish larvae are presented (Annexes 10a, 10b) and an analysis presented.

vii)Review of ICES and BMB activities in the field of fish diseases in the Baltic Sea and provide a report to ACME. Summaries of recent meetings and workshops on fish diseases in the Baltic Sea are presented (Annexes 11a, 11b, 11c) and a report to ACME included in this Report.

viii)Evaluation of the possibility of reciprocal transfer of *Mikrocytos mackini* between various shellfish species. The risks of survivors of infection of Denman Island Disease acting as carriers of the disease were assessed and procedures to reduce the risk from this disease recommended.

ix) Recommendation for standardisation of shellfish disease records. The extent of the need for standardisation was assessed and recommendations made for alleviation of the problems.

x) Review of procedures for detecting and monitoring shellfish disease prevalences. Recommendations for sampling requirements to meet different purposes from screening shellfish diseases are presented.

xi) Assessment for ACME of the usefulness of external fish disease as a tool in monitoring biological effects of contaminants. An evaluation of the contents of a review paper and discussion document presented (Annexes 15a, 15b) and of discussion by the WG is given, with a recommendation made to ACME.

xii)Assessment for ACME and ACFM of fish diseases in the Baltic and their possible impact on mortality of fish stocks. An advisory statement was agreed with the Chairman of BMB to be used in discussions by him with the Chairman of the Baltic Fish WG in the formulation of a section in the HELCOM Third Periodic Assessment.

xiii)Report to ACME on new information on the M-74 Syndrome and *Ichthyophonus*. A summary of current knowledge of M-74 is included (Annexes 17a, 17b) and an advisory statement to ACME included in this Report. New information on *Ichthyophonus* is included in section 18 of the Report.

xiv)Review of the present status of *Ichthyophonus* in herring populations and an investigation of problems in supplying prevalence data. A review of data available is presented (Annex 18a), working documents using herring stock assessment techniques included (Annexes 18b, 18c) and an advisory statement provided in the report. A recommendation is made to ICES on the continued collection and assessment of data on *Ichthyophonus*.

xv) Advice on methods to rapidly handle fish disease data. Advice is provided in the Report on a mechanism to rapidly handle fish disease data.

2. Action list

The Working Group on Pathology and Diseases of Marine Organisms:

a) Urges members of the WG attending international conferences to make known the views of WGPDMO when appropriate.

b) Urges ICES to encourage its member countries not to reduce national research/monitoring activities on the occurrence of diseases/parasites in wild finfish and shellfish.

c) Urges appropriate WG members to submit fish disease data from two North Sea sampling station for the period 1990–95 to ICES using the new data entry programme.

d) Urges ICES to ensure that the fish disease data programme is fully compatible with ICES environmental databanks and with IBM and Mackintosh computers.

e) Urges further intersessional research by WG members on problems associated with sensitivity testing of antimicrobial agents.

f) Recommends that additional precautions be included to prevent spread of disease with live transfer of molluscs from disease endemic areas to clean areas and that additional research be undertaken into host specificity of known mollusc pathogens.

g) Urges ICES member countries to use the proposed shellfish pathogen and disease list when submitting data to ICES and for ICES to develop a format consistent with that developed for finfish disease data.

h) Urges ICES member countries to undertake properly structured sampling for shellfish diseases.

i) Recommends to ACME that monitoring of fish diseases should be incorporated into ICES disciplines recommended for the monitoring of biological effects of contaminants.

j) Urges ICES member countries to incorporate monitoring of the prevalence of *Ichthyophonus* into herring stock assessment studies.

k) Urges ICES member countries to undertake experimental work on the time course of *Ichthyophonus* disease in herring and to investigate the possibility of there being different species of *Ichthyophonus* in different fish species or that these different fish may act as carrier reservoirs of the disease.

l) Urges ICES member countries to use a standardised data collection and reporting scheme for *Ichthyophonus*.

m) Recommends that disease questions from ICES groups or outside organisations be channelled through the WGPDMO Chairman to facilitate a rapid and informed response.

n) Recommends that further publicity of ICES Disease Fiches be promoted by the Editor and by ICES.

o) Recommends that ICES convenes a panel of experts to evaluate fish virus identification.

p) Recommends that co-operation between ICES and PICES on fish and shellfish disease matters be encouraged.

ANNEX 22a

General information on PICES (Summary provided by T Evelyn).

Working Group on Pathology and Diseases of Marine Organisms, 1995.

General Information on PICES

The North Pacific Science Organization (perhaps better known by the acronym PICES!) came into being in 1992 to a) promote and coordinate marine scientific research on the chemical, physical, and biological processes in the north Pacific Ocean and to b) promote the collection and exchange of data stemming from that research.

At the moment, four countries are members of the organization (Canada, China, Japan, and the USA) but two other countries (Republic of Korea and Russia) have made application for membership and may by now be members. The Organization has a Governing Council consisting of a Chairman, Vice-Chairman, and two delegates from each member country. There is also a Finance and Administration Committee, a Secretariat, a Scientific Board (consisting of a Chairman and the chairmen of the Organization's four Scientific Committees), and four scientific committees: 1) the Biological Oceanography Committee, 2) the Fishery Science Committee, 3) the Marine Environmental Quality Committee, and 4) the Physical Oceanography and Climate Committee. A number of "Working Groups" have also been established to work on particular projects, the groups being disbanded as their tasks are completed. Unlike ICES, however, a working group on disease in marine fish has not been formed.

Currently, PICES research will emphasize an international program to investigate "Climate Change and Carrying Capacity" in the temperate and subarctic regions of the North Pacific.

PICES sponsors scientific workshops at each of its annual meetings and publishes accounts of its activities in its Annual Reports (three published to date) and in a semi-annual newsletter entitled "Pices Press". Anyone interested in PICES' activities would be well advised to read these publications. Both the Annual Reports and the newsletters are published by the Secretariat, and enquiries about their availability should be addressed to: PICES Secretariat, c/o Institute of Ocean Sciences, P.O. Box 6000, Sidney, B.C., Canada V8L 4B2. (Phone: (1-604)-363-6366; Fax: (1-604)-363-6827; Omnet: PICES.SEC; Internet: pices@ios.bc.ca).

ANNEX 23a

RECOMMENDATIONS TO COUNCIL

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

IFREMER, La Tremblade, France 3–7 April 1995.

Recommendations

1. The ICES WGPDMO recommends that it meet at ICES Headquarters in Copenhagen under the Chairman ship of Dr A. H. McVicar from 18–22 March 1996:

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

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

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

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

e) to 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 ACME if new information becomes available (HELCOM 12).

2. The WGPDMO further recommends that the Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks, under the Chairmanship of Dr A. D. Vethaak, work by correspondence in 1995 and meet at ICES Copenhagen for two days prior to the WGPDMO meeting in 1996:

a) to undertake analyses of data submitted to the ICES Disease databank;

b) to undertake analysis of data collected in ongoing tasks intersessionally by SG members;

c) to analyse the conclusions of the SG on the data intersessionally submitted by member countries on dab (*Limanda limanda*) disease data from two North Sea stations for the period 1990–1995;

d) to finalise plans for an ICES Special Meeting on the use of liver pathology of flatfish for monitoring biological effects of contaminants; and report conclusions to WGPDMO.