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Exploration of the Sea

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

Moncton, New Brunswick, Canada, 21 - 26 March 1994

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*General Secretary
ICES
Palægade 2-4
DK-1261 Copenhagen K
DENMARK

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1 INTRODUCTION

The Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, New Brunswick, Canada, with Dr A.H. McVicar presiding as Chairman (C.Res.1993/2:25).

1.1 Opening of the Meeting

The meeting was opened at 09.30 hrs on Monday 21 March 1994 with the Chairman welcoming participants, particularly those new to WGPDMO. On behalf of DFO Gulf Fisheries Centre, Moncton, the participants were welcomed to the meeting by Mr A.O. Cormier, Director General, and by Dr S. McGladdery, Molluscan Section, DFO, Moncton.

The attendance of the Chairman of the Mariculture Committee, Dr R.H. Cook, at the meeting was particularly welcomed. Dr Cook welcomed members of WGPDMO on behalf of ICES and gave a brief overview of the relationship between WGPDMO and other ICES Working/Study Groups.

The strong participation from North America and by shellfish specialists was greatly appreciated. A list of participants is appended in Annex 1. Regrets were received from Sweden, Estonia, Ireland, Iceland, and Portugal.

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

Participants were reminded of the terms of reference as published in C.Res.1993/2:25. The heavy agenda load, due to several items added to the terms of reference during the Statutory Meeting in Dublin, was commented on. A direct consequence of this on the structure of the meeting was the need to spread the detailed discussion of specialist areas of the work into three separate expert subgroups, namely those dealing with wild marine fish, fish mariculture, and shellfish (both wild and cultured). The Working Group was scheduled to meet in plenary at regular intervals to receive progress reports from the expert subgroups and to deal with agenda items of common interest.

2.1 Terms of Reference

The terms of reference as listed in the Report of the 1993 ICES Statutory Meeting, C.Res.1993/2:25 were:

The Working Group on Pathology and Diseases of Marine Organisms (Chairman: Dr A. McVicar, UK) will meet in Moncton, New Brunswick, Canada from 21-26 March 1994 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 cultured fish and shellfish;*
- c) *evaluate the Sub-Group Report on the Analysis of Fish Disease Prevalence Data;*
- d) *assess the intersessional data presented on recent field trials and other relevant information on fish vaccines;*
- e) *compare antibiotic resistance profiles of *Aeromonas salmonicida* performed in selected laboratories;*
- f) *compare the European legislation, the OIE rules, and the regulations under consideration in North America regarding the transfer of molluscs between countries, and provide advice on the standardization of the control methods used to monitor mollusc disease, based on the information coming from the above comparison and from data on current mollusc research;*
- g) *evaluate the significance of additional and new data on Ichthyophonous infection in herring and consider the need for a third special joint meeting between pathology and stock assessment groups on Ichthyophonous;*
- h) *develop and coordinate plans for the Mariculture Committee special topic on "Parasites in Mariculture" at the 1994 Statutory Meeting;*
- j) *provide information to ACME on the types of studies that can elucidate possible relationships between fish diseases and pollution;*
- k) *prepare, in cooperation with the Baltic Marine Biologists, a preliminary report for ACME on fish diseases in the Baltic Sea, and provide plans for future studies of fish diseases in the Baltic Sea;*
- l) *compile and evaluate information with respect to the recently reported parasite in cod larvae from the Baltic and surrounding waters.*

2.2 Adoption of Agenda and Selection of Rapporteurs

An agenda was agreed upon (Annex 2), expert discussion leaders were identified, and rapporteurs were appointed (Annex 3).

3 REPORT ON THE ICES 1993 STATUTORY MEETING

Items of relevance to WGPDMO from the 81st ICES Statutory Meeting held in Dublin, Ireland, 23 September - 1 October 1993 were highlighted by the Chairman. These included (a) the progress report on the North Sea Task Force and the preparation of the Quality Status Report which WGPDMO had considerable technical input into and (b) the question raised in the Consultative Committee by the Mariculture Committee concerning the importance of ICES establishing a dialogue at a high level with the European Union regarding the implications of a recent directive permitting shipments within the EU of live aquatic organisms and the provision therein for preventing the transfer of disease agents.

It was noted that the Sub-Group on Statistical Analysis of Fish Disease Data was officially recognized as a sub-group of WGPDMO.

Reference was made to the MEQC comments on the overview of the BMB/ICES Workshop on board R/V *Walter Herwig III* (scheduled for November 1994) and, in particular, the recommendation, "if necessary, (for) a new standard methodology suitable for the Baltic Sea conditions to be adopted by Baltic Sea countries in present or future disease monitoring." Discussions were held during the WGPDMO meeting with the current BMB Chairman (G. Bylund) and it was agreed that regular communication between the two groups on this issue will be maintained and WGPDMO will thus be kept informed of developments.

The disease sections of the Mariculture Report were briefly reviewed and references to WGPDMO were highlighted. In particular, the report concluded that WGPDMO had confirmed its abilities to address both the wild and cultivated species issues within its current Working Group status. Comments on disease-related papers which had been presented at the ICES 1993 Statutory Meeting were also noted.

References to the *Ichthyophonus* epizootic in the report of the Pelagic Fish Committee and to discussions on the M-74 syndrome in Baltic salmon and *Lepcophtheirus salmonis* infections of sea trout in Ireland in the Anadromous and Catadromous (ANACAT) Committee Report were briefly noted.

The formation of the Study Group on Occurrence of M-74 in Fish Stocks and of the Study Group on Interactions of Wild, Ranched (Enhanced), and Reared Salmon, both of which had terms of reference overlapping interests of the WGPDMO, were discussed. Additional terms of reference for WGPDMO to accommodate these new Study Groups were included in the current terms of reference.

4 CONSIDERATION OF RELEVANT REPORTS

4.1 Baltic Marine Biologists (BMB)

G. Bylund, Chairman of the BMB, presented a brief overview of the formation and workings of the BMB Working Group stressing the involvement of participants from non-ICES countries. Two meetings scheduled for 1994, co-sponsored by ICES, are (a) a conference on diseases of flounder to be held in Finland, October 27-29, co-sponsored by the Nordic Governments (Chairman, G. Bylund) and (b) a Sea-going Workshop from 25 November - 6 December (Conveners, T. Lang and J. Thulin) on board the German research ship *Walther Herwig III*, designed to evaluate ICES disease monitoring procedures and to develop new standard methodologies for the Baltic. Based on the outcome of these meetings, the BMB will prepare reports on fish diseases in the Baltic and on plans for future studies on fish diseases in the Baltic (see C.Res.1993/2:25(k)).

4.2 M-74 Syndrome

A brief overview of the formation, structure, and content of the ICES Study Group on Occurrence of M-74 in Fish Stocks was presented by its Chairman, S. Møllergaard. As consideration of this syndrome was included in the terms of reference of the WGPDMO, highlights from the discussions regarding the conclusions of the SGM74 meeting are contained in Section 14 of this report.

4.3 Other Meetings

A brief review of the purpose, content, and main conclusions of the Sixth UK Workshop on Relationships of Pollutants and Diseases in Marine Fish held in England in November 1993 was presented by its convener, D. Bucke; a short report of the Nordic Council of Ministers-sponsored workshop on atypical *Aeromonas salmonicida* held in Finland was presented by G. Olivier; and an intimation of a forthcoming meeting of the Flatfish Toxicology Group in the Netherlands in April 1994 was given by A.D.Vethaak.

5 RECENT TRENDS IN DISEASES IN WILD FISH, CRUSTACEANS AND MOLLUSCS

5.1 Wild Fish Diseases

Summary/conclusions

Lymphocystis: In North Sea dab (*Limanda limanda*), the decreasing trend of lymphocystis continued in 1993. This trend is more pronounced in the southeastern region than in the western and the central North Sea. In Dutch mesocosm studies on the effects of contaminated harbour sediments on the disease status of flounder (*Platichthys flesus*), a possible contributing role of contaminants in the pathogenesis of lymphocystis was established.

Epidermal hyperplasia/papillomas: The decreasing trend observed since 1989 in North Sea dab continued during 1993.

Skin ulcers: Dutch mesocosm studies on the effects of contaminated harbour sediments on flounder demonstrated no detectable impact of contaminants on the development of skin ulcers.

Abnormal skin pigmentations: An increasing trend of hypermelanisation in North Sea dab has been observed in recent years. Hot spots are the Dogger Bank, an area off the Humber Estuary, and the German Bight. Belgian studies indicated that the causative agent was a chlamydia-like organism affecting the skin. This condition was accompanied by reduced haematocrit values.

Hypermelanisation has also been observed in flounder and solenette (*Buglossidium luteum*).

Pigment anomalies associated with atrophy of ocular tissues have been reported to occur in small numbers of angler fish (*Lophius* sp.) caught in the Celtic Sea.

X-cell gill lesion: This condition was observed in American plaice (*Pseudopleuronectes americanus*) in areas with heavy concentrations of organic contaminants in the St. Lawrence Estuary.

A steep increase from 1% to 12% was observed in dab at a localized area in the Skagerrak from 1992-1993.

Studies conducted in the USA indicate that the aetiological agent of X-cell lesions may be a myxosporean parasite instead of the previously suspected agent, an amoeba.

Liver nodules/tumours: The prevalence of liver nodules (> 2 mm in diameter) in North Sea dab showed a decreasing trend particularly in the German Bight. This trend seems to correspond with the overall decreasing

contamination level in this area. In studies on diseases in Icelandic dab, liver nodules were not observed.

Dutch mesocosm studies using flounder clearly demonstrated the capability of contaminants present in harbour sediments to induce/develop liver neoplasia and their precursors.

In dab and flounder in the western Baltic Sea, the prevalence of liver nodules/tumours was considerably lower than that reported for the North Sea.

Liver neoplasia were found for the first time in some specimens of large/old plaice (*Pleuronectes platessa*) of the southern North Sea.

A high prevalence of hepatic neoplasia was observed in mummichog (*Fundulus heteroclitus*) in American coastal waters at one site on the Atlantic coast heavily contaminated with PAHs (creosote).

VHS-like virus: A VHS-like virus has been isolated from Pacific herring (*Clupea herengus pallasii*) around Kodiak Island in Alaska southwards to Puget Sound in northern Washington state. The virus was also isolated from 3 of 5 herring samples taken along the coast of British Columbia. A VHS-like virus associated with skin lesions has also been reported in Pacific herring in coastal waters of the USA. The Pacific herring may be the reservoir host of the virus in Pacific North America.

Birnavirus: In Spain, a birnavirus (IPN-like) has been isolated from sandeel (*Ammodytes* sp.) and blue whiting (*Micromesistius poutassii*) used as feed for cultured turbot. A similar virus, associated with mortalities, has also been isolated from wild captured sole (*Solea senegalensis*) held in captivity.

Ichthyophonus sp.: High prevalence (>90%) of *Ichthyophonus* in dab was observed for the first time in southern waters of Iceland. This is the first recorded observation of the disease in the dab of this area.

5.2 Wild Shellfish Diseases

No reports of significant trends in shellfish diseases in wild populations were received.

6 RECENT TRENDS IN MARICULTURE DISEASES

6.1 Finfish

a) Atlantic salmon (*Salmo salar*)

Bacterial problems

Furunculosis: The significance of typical furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*) has been dramatically reduced in Scotland and Norway mainly due to the widespread use of improved vaccines and improved management techniques such as separating year classes, fallowing, and reduced stocking density. This has resulted in a marked reduction in the use of antibacterial compounds.

In Canada, furunculosis is an increasing problem on the west coast where the more susceptible Atlantic salmon is replacing Pacific salmon for farming purposes. This change in the salmon being farmed has resulted in a significant decrease in the importance of BKD and vibriosis.

Vibriosis: Cold Water Vibriosis (*Vibrio salmonicida*) has been causing significant losses on the northeastern USA and in northern Norway. On the east coast of Canada, cold water vibriosis is now the major disease problem. *Vibrio salmonicida* was recorded for the first time in Iceland.

Viral diseases

Pancreatic problems: Infectious Pancreatic Necrosis (IPN)-associated problems in the immediate post smolt period have been steadily increasing in significance during the last few years both in Scotland and in Norway. The same applies for Pancreatic Disease (PD), although this disease condition can also occur in older fish.

Infectious Salmon Anemia (ISA): This disease is still limited to Norway. Although many farms are still under restrictions due to ISA, the number of farms with clinical outbreaks of the disease has been dramatically reduced probably due to sanitary measures such as harvesting of infected fish and fallowing before restocking. At the end of 1993, all of the reported ISA infected fish were removed from the cages prior to restocking. There are indications that sea trout can serve as carriers of ISA without showing pathology.

Sea lice (*Lepeophtheirus salmonis*): Both in Scotland and Norway sea lice infestations are now considered to be the most important disease problem. Although there have been improvements in management practices for controlling the infestations including the use of wrasse cleaning fish and the use of new anti-sea lice compounds (e.g., H₂O₂), there is an urgent need for improvements in control methods and alternative treatments.

Gill Amoebiasis (amoeba-like): Gill amoebiasis was observed for the first time on all salmonid farms in

France. In Atlantic salmon the infection was associated with *Flexibacter* sp. and caused mortality.

Kudoa Infections (caused by *Kudoa* sp.): Flesh quality problems caused by *Kudoa* infections in Atlantic salmon are emerging as a cause for concern among salmon farmers on the coast of Canada. At a recent workshop on the topic held there, delegates were informed that the problem was also a cause for concern among Atlantic salmon farmers in Ireland.

b) Other Salmonids

In France gill amoebiasis (amoeba-like) and hyperplasia were observed in farmed trout (*Salmo trutta* and *Oncorhynchus mykiss*), but no mortalities were recorded.

Infections with *Cytophaga (Flexibacter) psychrophila* were recorded in rainbow trout from sea farms in Finland. The mortality was not high but the prevalence of fish with skeletal deformities was high in affected fish populations.

Flesh problems due to *Kudoa* infections (*Kudoa* sp.) have been reported from France where both seawater-cultured and wild sea-run brown trout are affected. It occurs at a prevalence of about 4% per year in farmed fish but the prevalence level has fluctuated in wild fish. The prevalence has declined over the last three years.

c) Non salmonid fish

Birnaviruses (IPNV-like) belonging to the serotypes Sp, Ab and VR-299 (WB) and strains which could not be typed, were isolated from turbot (*Scophthalmus maximus*) in Spain. The VR-299 serotype has not been previously isolated in Europe.

Paramyxovirus-infection in turbot associated with high mortalities was reported for the first time from Spain.

Epitheliocystis caused by a Chlamydia-like organism has been recorded for the first time in sea bass (*Dicentrarchus labrax*) on the Mediterranean coast of Spain

Infections caused by an *Enterococcus* sp. have become a serious problem in turbot farming in Spain and are associated with high morbidity and moderate mortality. The disease affects fish in size groups over 50 g. Chemotherapy has so far not been successful but vaccination trials are in progress and are promising.

Flexibacter maritimus, previously reported from France, is a new disease agent in turbot farming in Spain where it causes external as well as systemic infections. The prevalence, however, so far are low.

Vibrio vulnificus, biotype 2, which caused heavy losses in eel farms in Spain in past years, is no longer a problem because the farms started using fresh water instead of sea water.

The hybrid striped bass (*Morone saxatilis* x *M. chrysops*) industry in USA has experienced significant problems due to infections with *Mycobacterium fortuitum* and *M. marinum*. This has caused some hatcheries to close down.

Problems related to infections with the microsporidian *Tetramicra* sp. in turbot in Spain were reduced by culturing juvenile turbot in sand-filtered water.

The eel swimbladder nematode *Anguillicola crassus* was reported for the first time in Spain and Norway.

The eel gill monogenean *Pseudodactylogyrus anguillae* was reported for the first time in Spain.

Ichthyophonus was reported for the first time (with prevalence up to 100 %) on the Mediterranean coast of Spain in stocks of seabass and seabream (*Sparus aurata*).

Conclusions

- a) Sea lice infection currently poses the greatest threat to salmon farming in Norway and Scotland and there is an urgent need for improvements in control methods and alternative treatments.
- b) Virus-associated problems show an increase in some instances (IPN, IPN-like, PD) and a decrease in others (ISA).
- c) Bacterial diseases such as furunculosis and cold water vibriosis show a significant decrease in severity in some areas but the same disease may still cause problems in other areas.
- d) Protozoan parasitic diseases have featured more prominently as fish mariculture problems during the last year in several countries.

6.2 Molluscs

Bonamia ostreae of flat oyster *Ostrea edulis*: The severity of this disease in the Netherlands has increased over the last 3-4 years. Mortalities in the Yerseke Bank area and Lake Grevelingen reached over 80%. There has been a reduction in research support for developing disease resistant strains. High mortalities in conjunction with poor spatfall last year has significantly reduced commercial production of the Dutch oyster fishery. In the UK, under the EU regulations, both wild and cultured flat oyster populations were investigated for *Bonamia*. *Bonamia* has shown some spread over the last

year in both groups of oysters sampled in southern England. Stocks of flat oyster in Scotland were found to be free of this disease. In Galway Bay, Ireland, an increase in *Bonamia* infection was reported in OIE and EU Reports available to WGPDMO members.

Bonamia has not been reported from oyster stocks on the east coast of Canada. Stocks transferred to the west coast four years ago were introduced via broodstock quarantine and release of the F1 generation. A sample of the F3 generation did not show any sign of the parasite. Stocks of *O. edulis* growing beside the stocks introduced from eastern Canada are known to carry the disease along with its close relative "Denman Island Disease" (*Mikrocytos mackini*) (see below). *Bonamia* has been reported for the first time in flat oysters from a Maine population.

Denman Island Disease (*Mikrocytos mackini*): Susceptibility of flat oysters (*O. edulis*), American oysters (*C. virginica*), and Olympia oysters (*Ostreola conchaphila*) was confirmed in both the laboratory and the field from Vancouver Island off the west coast of Canada. Mortalities were greater in these three species than in the natural host, Pacific oyster (*C. gigas*). Field mortalities of Olympia oyster at one location on Vancouver Island have been tentatively linked to *Mikrocytos mackini* infections. There is no evidence of spread to *C. gigas* stocks 70 miles south of Vancouver Island.

***Marteilia* spp.:** In Spain *Marteilia* spp. were reported for the first time from Pacific oysters (*Crassostrea gigas*) in the Mediterranean. No information was reported on prevalence or life-history stage in the Pacific oysters examined. *Marteilia refringens* was found in flat oyster (*O. edulis*) populations only in Mediterranean Spain. The same parasite did not transmit to flat oysters on the Atlantic coast of Spain or from infected mussels (*Mytilus galloprovincialis*). Serious concern was raised regarding the lack of concrete information on the potential for mussels (*Mytilus* spp.) to be carriers of *M. refringens* and on taxonomic confusion over species differentiation (*M. refringens* and *M. maurini*). No *M. refringens* infections were found in flat oyster samples in England, Wales, Scotland, or the Netherlands. The causative agent of mass mortalities of calico scallop (*Argopecten gibbus*) (over 90% in 1988-90) off the east coast of Florida has been described as a *Marteilia* sp., but a similar epizootic has not been observed since.

Perkinsus atlanticus has shown an increase in prevalence in Spain in both *Ruditapes decussatus* (carpet clam) and *R. philippinarum* (Manila clam) to approximately 16%. Infections of *Perkinsus* sp. (probably *P. atlanticus*) have also been described in cockle species. Concern was raised over the specificity of these *Perkinsus* spp. and whether or not they are associated with clam mortalities occurring in the same areas.

Perkinsus marinus has shown high prevalence of infection in one cultured population of oyster (*Crassostrea virginica*) on the southern coast of Cape Cod in the USA. Reports of the disease in Maine have not been confirmed. This disease continues to have a significant impact on both wild and cultured American oyster (*C. virginica*) stocks from Delaware Bay to Texas. Reports of the presence of *P. marinus* in hardshell clam (*Mercenaria mercenaria*) populations in South Carolina have been supplemented by experimental demonstration of cross-transmission from infected American oysters (*C. virginica*) to uninfected clams. This raises questions about whether other bivalve molluscs serve as significant carrier hosts. Using the fluid thioglycollate method, *Perkinsus* spp. have now been found in most (19 of 23) species of bivalve molluscs examined from the state of Virginia in the USA. A monoclonal antibody test is also now being used in the detection of *P. marinus*. Such probes should become more available within the next few years.

Perkinsus spp. There are now axenic cultures of *Perkinsus marinus* and presumptive *P. atlanticus* (from *Macoma balthica*) available at several institutions which can be used as reference materials. RNA sequencing has been used to differentiate all the *Perkinsus* species described to date. Species-specific probes are in the process of being developed in the USA and Canada (in collaboration with Australia).

Haplosporidium nelsoni (MSX) does not appear to be showing any change in its geographical range. It has been reported as far north as Massachusetts with one population on the south shore of Cape Cod having high infection prevalence. There have been no new reports of MSX from Maine since 1990. In 1993 MSX was of secondary importance (after *P. marinus*) as an agent of oyster (*C. virginica*) mortalities in the Chesapeake and Delaware bays, following a trend observed in recent years.

Brown ring disease has been detected for the first time in Manila clams in Galicia, northern Spain, at variable prevalence rates. The disease appears to be spreading with increasing mortalities (5-60%) around the southern coast of Spain.

Juvenile Oyster Disease (JOD) has been affecting hatchery-spawned *C. virginica* since 1989, but declined in 1993. The causative agent is unknown, but the syndrome has characteristics similar to brown ring disease. Decreased mortalities are thought to be associated, at least in part, with changes in husbandry techniques such as:

- i) increased water flow rate over the oysters;

- ii) decreased tray stocking density;
- iii) earlier oyster set;
- iv) earlier transplant from trays to estuarine floor.

Herpes-like virus was found in two Pacific oyster hatcheries in France during May and June. Until now, the effects are limited and in 1993 the two hatcheries have restarted oyster production after disinfection and changing the broodstock without reoccurrence of the disease. The Herpes-like virus kills the larvae around 6th day post-spawning. Five cases of mortality associated with the presence of these viruses have been reported in France in juvenile Pacific oyster transferred to open water sites from hatcheries. The mortalities occurred during July among small batches and seemed to be related with stress and high temperature. No further mortality has been reported and no new cases of Herpes viruses infections have been observed in neighbouring populations since July 1993.

Withering Syndrome has been reported from red abalone *Haliotis rufescens* from California, USA, and appears similar to previous reports from black abalone (*H. cracherodii*). It is characterized by atrophy of the foot, weakness, and emaciation followed by death. The aetiology is currently under investigation with focus on two parasites found in affected abalone (a kidney coccidian and an intestinal rickettsia-like prokaryote).

Chlamydial Gill Lesions in Pacific oyster (*C. gigas*) were reported for the first time from an overcrowded grow-out site at Marennes-Oleron in France. Stocks originating from Marennes-Oleron consistently showed a significant decrease of the gill lesions when regularly transferred to Brittany, Normandy, and the Mediterranean. No mortalities have been associated with these gill lesions.

Urastoma cyprinae (gill turbellarian) has shown a rapid increase in prevalence to 100% in some mussel (*Mytilus galloprovincialis*) grow-out sites in Galicia, Spain. No mortalities have been associated with this parasite to date.

6.3 Crustaceans

Hematodinium: There have been no new reports of this parasitic dinoflagellate in *Nephrops norvegicus* from the North Sea. A species of *Hematodinium* (probably *H. perezii*) has been observed again in recent years in blue crab (*Callinectes sapidus*) from the states of Maryland and Virginia in the USA. It is more prevalent in smaller crabs and maximum prevalence occurs in autumn.

"Bumper car disease", caused by a systemic ciliate (*Mugardia* sp.), has become a significant problem in

certain lobster (*Homarus americanus*) impoundments in Maine, resulting in significant losses (mortalities and unsaleable weak lobsters) in late winter/early spring.

Conclusions

- a) The geographic range and mortalities due to *Bonamia ostreae* in the flat oyster populations of Europe is increasing.
- b) The lack of knowledge on the taxonomy and transmission of molluscan *Marteilia* spp. is a serious impediment to effective disease regulation for both mussel and oyster industries in Europe.
- c) In recent years *Perkinsus marinus* has expressed itself more strongly in northern regions of its geographic range along the east coast of the USA.
- d) Concern was raised over the host specificity of *Perkinsus* spp. and whether or not they are associated with clam mortalities occurring in Atlantic Spain.

6.4 Recommendations

The WGPDMO recommends that further research be conducted on species of the genera *Marteilia*, *Haplosporidium*, *Bonamia*, *Mikrocytos*, and *Perkinsus* with respect to:

- a) investigations on host-specificity and the role of other bivalve species as carriers;
- b) improvement of diagnostic tools for their detection;
- c) taxonomic characterization of the different species;
- d) determination of the life-cycles of *Haplosporidium nelsoni*, *Marteilia refringens* and *Bonamia ostreae*.

7 CONSIDERATION OF THE REPORT OF THE SUB-GROUP ON ANALYSIS OF WILD MARINE FISH DISEASE PREVALENCE

A.D. Vethaak, Chairman of the Sub-Group on Statistical Analysis of Fish Disease Data, presented a progress report. The Sub-Group was given its own terms of reference (C.Res.1993/2:25:1). Communication during 1993-1994 was carried out by correspondence. However, as some of the members attended the WGPDMO meeting, part of the Sub-Group's tasks were dealt with during the WGPDMO meeting, and a brief progress report was submitted. The complete report of the Sub-Group is published as C.M.1994/F:7, Ref.E. The Chairman of the Sub-Group was congratulated on the comprehen-

siveness of the progress report, the contents and conclusions of which were accepted without change by the WGPDMO.

The Sub-Group evaluated the new ICES format for reporting fish disease data. The new format structure is designed as a general format with the potential for including additional/new diseases of fish as well as shellfish if necessary. It also allows pooled sample data to be submitted. To facilitate data entry, the WGPDMO strongly recommends that ICES provide participating countries with a special entry programme for fish disease data.

The Sub-Group also discussed a working document prepared by T. Lang and S. Mellergaard on age/length relationships in North Sea dab and concluded that age data should be included in future fish disease investigations.

ICES had addressed the question as to whether *Ichthyophonus* data should be submitted in the same reporting format as other marine fish diseases to the WGPDMO. The WG recommends that *Ichthyophonus* data be included in the ICES fish disease format as indicated in the Section 12 (below). It is recommended by WGPDMO that the assessment of the data should be made by the Herring Assessment Working Group. The WGPDMO should be kept informed of data results.

Because of the amount of work still outstanding on the analysis of data on marine fish diseases, the WGPDMO concluded that the Sub-group should continue working until 1996.

7.1 Recommended Actions

- 1) that the Sub-Group on Statistical Analysis of Fish Disease Data work by correspondence in 1994 and meet for two days prior to the WGPDMO meeting in 1995 and report to WGPDMO at that meeting. Fish disease data submitted to the new ICES data base should be analysed prior to the WGPDMO meeting in 1996,
- 2) that the ICES Secretariat provide participating member countries with a data entry program for the Fish Disease Data Format as soon as possible after the 1994 Statutory Meeting,
- 3) that this format should include provision for more information on age/length relationships in dab and flounder to be collected by ICES member countries conducting fish disease monitoring programmes, to allow a more accurate analysis of disease prevalence data. All fish disease data should be submitted to ICES using the Fish Disease Data Format Data Entry Program.

8 ADVICE TO ACME ON FISH DISEASE STUDIES IN RELATION TO POLLUTION

C.Res.1993/2:25(j) requested that the WGPDMO provide information to ACME on the types of studies that can elucidate possible relationships between fish diseases and pollution.

8.1 Methodology

Three types of approaches have been used to study relationships between fish diseases and pollution: field studies; mesocosm and controlled field studies; and laboratory studies.

Field studies

Two main purposes can be identified for field studies:

- a) to measure the significance of the direct effects of pollutants. [Examples where diseases have been associated with pollution include (i) studies on the correlation between gradients of bleached kraft pulp mill effluents and the occurrence of fin erosion and skeletal malformations (ii) on the effects of PAHs in sediments on the formation of liver neoplasia in benthic fish species, particularly in river estuaries and (iii) on the relationship between salmon haemolytic anaemia and a combination of resin acid and hydrocarbon pollution.];
- b) to monitor trends of naturally-occurring diseases in fish sampled from broad areas where increases or decreases in long-term changes in disease prevalence can reflect even subtle changes caused by environmental stressors, including pollutants. [Examples where fish diseases have been thought to respond to environmental changes are associated with localised oxygen deficiency leading to eutrophication and possibly to large-scale changes in water movement.]

In several cases field studies have provided circumstantial evidence for a cause-effect relationship between pollution and fish diseases. Field studies are also important because they can identify infectious and non-infectious diseases which may be related to pollution. It must be realized that field studies are only meaningful when they are conducted in a standardized way in order to compare spatial and temporal trends of diseases in relation to physical, chemical, and biological data.

Mesocosm and controlled field studies

Mesocosm studies can closely resemble natural conditions and have led to increased understanding and the prediction of interactions between pollution and disease by allowing manipulation and control of both biological and environmental factors with a possible impact on

diseases. They are also a very good tool for the validation of results derived from laboratory studies or for the validation of developed biomarkers to be applied in the field. A further approach worthy of application would be to enclose fish in net cages in selected polluted and non-polluted areas in the marine environment and to measure their health status.

Laboratory studies

Laboratory studies are important because they provide definitive demonstration of cause-effect relationships in highly controlled environments. They are the ideal tool for research and development of specific techniques such as molecular/cellular biomarkers relevant to disease induction. They can be used to document the multiple steps involved in the development of fish diseases following exposure to toxic substances. For example, it is possible to either expose short-lived surrogate fish species, or conduct *in vitro* tests, and apply a pluralist approach of cellular biomarkers of exposure which can indicate cellular injury and pathology.

8.2 Conclusions and Recommendations to ACME

It is recommended that the best strategy to elucidate relationships between pollution and diseases should comprise an integrated approach combining field, mesocosm, and laboratory studies (Figure 1). Due to the large resources and efforts necessary to meet this recommendation, standardized research in this field should be encouraged and collaborative efforts between institutes established.

Research should focus on the following issues:

- 1) a rationalized approach to field and mesocosm studies;
- 2) the impact of toxicants on the immuno-competence of fish as it relates to disease;
- 3) causal mechanisms involved in the formation of liver tumours and other pollution-associated diseases including reproductive disorders in fish;
- 4) the development and application of biomarkers for a) immunotoxicology in fish and b) early stages of liver tumours.

9 ANALYSIS OF INTERSESSIONAL EXERCISE ON A COMPARISON OF THE DETERMINATION OF BACTERIAL ANTIBIOTIC RESISTANCE PROFILES

As part of an intersessional study, 10 *Aeromonas salmonicida* isolates were tested for their antibiotic sensi-

tivities in six laboratories, each using their own methodology. A summary of the methods used is presented in Annex 5. As a first observation, the methodology used in the various laboratories was not standardized and varied considerably. Once the antibiograms were performed the participants were asked to categorize the strains as susceptible (S), intermediate (I), or resistant (R). Results were tabulated and are presented in Annex 5. Notwithstanding the variability in methodology and considering the small sample of strains tested, results for certain antibiotics (chloramphenicol, oxytetracycline, amoxicillin, penicillin) were consistent between the different laboratories. With streptomycin, one laboratory found all strains resistant while the other two laboratories found them susceptible. This discrepancy is likely due to the zone of inhibition used to determine susceptibility of resistance. Furans, potentiated sulfonamides and the quinolones (oxolinic acid and flumequine) gave contradictory results.

There are several possible explanation for these results: the difference in methodologies; the large variation in the concentration of antibiotic in the disks used; and the lack of standard zones of inhibition to establish resistance or susceptibility. As the results were considered problematic, there should be an effort to standardize the procedure of antibiogram determination. This procedure would include:

- a) start with a fresh culture of the isolate on TSA (48 hour culture);
- b) take a sample (several colonies) from this culture and prepare a suspension in peptone-saline (saline containing 0.1% peptone) then adjust to a standard turbidity;
- c) inoculate Mueller Hinton plates with the suspension by either swab or flooding, the end result being to obtain semi-confluent growth and a dry plate surface on which to deposit the appropriate disks;
- d) incubate for 48 hours at 20-22°C.

Conclusions

From the results obtained in this intersessional study, it was agreed that the methods to determine antibiotic resistance in the various laboratories need to be standardized. Two groups of antibiotics were found to be particularly problematic, the quinolones and the potentiated sulfonamides. These findings suggest that the data presented in the literature on drug resistance or susceptibility of various strains of *A. salmonicida* are not readily comparable.

Additional research is needed to establish the relationship between inhibition zone diameters and minimal inhibi-

tory concentration (MIC) or minimal bactericidal concentration (MBC) with serum levels of a particular antibiotic in the host.

Future work

One of the reasons for the differences obtained in the various laboratories in categorizing the strains of *Aeromonas salmonicida* as susceptible, intermediate, or resistant is the fact that there is no standardized correlation between zones of inhibition and MIC or MBC. In order to address this deficiency, it is recommended that some laboratories be identified to compare zones of inhibition and MIC or MBC. It is suggested that each laboratory focus on a single antibiotic or family of antibiotics and that a sample of at least 30 isolates be tested. Isolates tested should include susceptible, intermediate, and resistant ones.

Recommendations

The WGPDMO recommends that a standardized method to determine antibiotic resistance of *A. salmonicida* be followed. The Working Group also recommends that further intersessional studies be initiated on the correlation between zones of inhibition and MIC or MBC. For this task national representatives should evaluate the feasibility of using national or other laboratories to participate in this exercise.

10 ANALYSIS OF INFORMATION ON FIELD TRIALS OF FISH VACCINES

This term of reference has now been largely superseded by events in the fish farming industries as several vaccines have recently proved to be an effective tool for preventing diseases. Field trials of these have been completed, so the importance of evaluating the efficacy of such field trials is not as critical as was previously thought.

By vaccinating fish the use of chemotherapeutics has been significantly reduced. Successful vaccines have been available for some time for *Vibrio*-related diseases. Although furunculosis has been a major concern, until recently there has been a lack of effective anti-furunculosis vaccines, but during the last two years this situation has significantly changed. Several effective vaccines against furunculosis are now available and, due to the strong protection provided by the vaccines, most of the Atlantic salmon in the major salmon producing countries such as Scotland and Norway are now vaccinated. Consequently, data from field trials comparing vaccinated and unvaccinated fish are not readily available.

However, there is still an obvious need for new approaches including vaccines for the control of important diseases in mariculture. Vaccination trials with a recombinant IPN vaccine are being carried out in Scotland, Norway, and USA. So far, the results are promising. Sea lice infestations are now the principal cause of concern in salmon farming in both Scotland and Norway, partly due to the small number and the nature of the treatment methods. There is an obvious need for new approaches such as vaccination to combat these infestations.

Marine fin-fish culture is expanding rapidly and although vaccines are available for vibriosis in turbot and pasteurellosis in sea bass and sea bream there is a need for further research in disease control methods. Since these species will require protection from disease at a very early age (first feeding), there is an identified need for new approaches (including vaccines) to control infection at this early life stage. WGPDMO recommends that available information in this area be collected and further research efforts supported.

11 EVALUATION OF INTERNATIONAL DISEASE REGULATIONS ASSOCIATED WITH MOLLUSC TRANSFER, AND OF METHODS FOR MOLLUSC DISEASE MONITORING AND CONTROL REGULATIONS ON MOLLUSC TRANSFER

The European Union (EU), Canada, and the USA all show fundamental differences with respect to the regulation of transfers of mollusc diseases. As a base reference, these are compared with the Disease Code of the "Office International des Epizooties" (OIE):

The Office International des Epizooties "Animal Disease Code for Fish, Molluscs and Crustacea".

Information on National health status is collected for molluscs and their larvae for listed disease agents (*Bonamia ostreae*, *Bonamia* sp., *Mikrocytos mackini*, *M. roughleyi*, *Haplosporidium nelsoni*, *Marteilia refringens*, *Marteilia sydneyi*, *Perkinsus marinus*, *P. olseni*, *P. atlanticus* and Iridoviruses, including OVVD). Countries, zones and establishments are declared free of these organisms when there have been no recorded cases for at least the previous two years. Infected zones, etc., can be reclassified as free of one or more of these disease agents if they are not detected for a period of two years following a surveillance scheme described in the OIE Manual of Recommended Diagnostic Techniques. This information is maintained for recommendation purposes.

European Union - "laying down the sampling plans and diagnostic methods for the detection and confirmation of certain mollusc diseases".

Within the EU, zones and establishments are predefined and then classified based on *Bonamia ostreae* and *Marteilia refringens* of flat oyster (*Ostrea edulis*). A two-year examination period is required to define the pathogen-free status of each country, zone or establishment and annual monitoring is required to maintain the pathogen-free status as defined. The certification is made for each country, zone and establishment.

Flat oysters may only be moved into a pathogen-free zone or establishment from a pathogen-free zone or establishment. Flat oysters from a zone or establishment defined as having one or both pathogens may only be moved to locations with an identical or greater pathogen status. *Crassostrea gigas* has been accepted as a non-carrier species and can move freely between zones and establishments.

In cases of abnormal mortality the country concerned is required to undertake the research necessary to elucidate the aetiology and to inform Brussels (EU Headquarters).

For species introductions or transfers from outside the EU, pathogen and host species falling under regulation conditions are considered but not defined.

North America - A number of different regulations and recommended procedures are applied to different USA Atlantic and Pacific States, as well as Canadian Provinces

Transfers of live molluscs for relay in open water (not direct consumption) from one state or province to another is often prohibited unless permission is obtained from the appropriate state or Canadian federal authority.

United States:

Permission to transfer shellfish from one state to another is predicated on

- i) there being no detectable pathogens in a representative sample of shellfish which is evaluated histologically and cytologically; and
- ii) often on whether the proposed shipment of shellfish comes from an area which is within the geographic range of an infectious pathogenic organism. If the states share an estuary, movements of shellfish within that body of water may occur with fewer or no restrictions. Disease control methods and policies are in the process of being reviewed and refined in a number of states with consideration being given to regulation of commercial ventures

and, in some cases use of shellfish in scientific investigations. Disease examinations of samples from shellfish stocks proposed for transfer are carried out by experienced shellfish pathologists. The final decision concerning a proposed shipment is made by the responsible managerial agency of the recipient State.

Canada:

In Canada disease risks associated with transfers within and between Provinces are considered case by case by Introduction and Transfers Committees composed of appropriate Federal and Provincial representatives. Lists of disease agents have been drafted on the following basis:

- List 1 - Agents of infectious diseases of concern not present in Canada.
- List 2 - Agents of infectious diseases which occur in parts of Canada and are subject to regional control.
- List 3 - Agents of infectious diseases of species which do not presently occur in Canadian waters.
- List 4 - Agents of infectious diseases with negligible or questionable significance in Canada since i) they cause no significant pathology or, ii) the pathological condition is related to husbandry.

These lists cover bivalve molluscs, echinoderms, and crustaceans. All disease agents will be reportable but only Lists 1 and 2 will fall under regulated control. In addition to the shellfish portion of the Fish Health Protection Regulations (FHPR) there is the Fisheries (General) Act of Canada which specifies (where "fish" includes shellfish) that "no person shall, unless authorised to do so under a licence, a) release live fish into any fish habitat; or b) transfer any live fish to any rearing facility." Criteria for meeting licence requirements include "the fish do not have any disease or disease agent that may be harmful to the protection and conservation of fish...".

Protocols for establishing and maintaining the disease-agent status for Provinces, zones and establishments are in the draft stage. Movements will be regulated similar to EU regulations but will include more host species and some zones may vary for different species groups (e.g., molluscs and crustaceans).

STANDARDIZATION OF MOLLUSC DISEASE MONITORING

EU and OIE monitoring is based on annual or biannual samples depending on the best time for detection of the

pathogen of concern. Sample size is 150 specimens and a minimum of three samples, spread over two years, is required per zone to acquire pathogen-free status. Thereafter similar samples are required to maintain the pathogen-free status.

Histological examination (and/or heart smears in the case of *Bonamia*) for all parasites remains the standard diagnostic protocol. Other methodologies can be accepted following approval by the appropriate authorities in the OIE, EU, USA, and Canada. In the case of abnormal mortalities, tissue-section histology is required.

The standard diagnostic technique in Canada involves observations of histological tissue-sections. Thioglycollate culture is also used on sub-samples ($n < 10$) on the east coast for screening for *Perkinsus* spp. parasites in new transfer proposals. Sample sizes vary according to source, species and stage of bivalve being examined. Maximum sample sizes are currently 50 specimens, one to three sections per specimen. Blocks and wet tissues are stored for a minimum of one year for back-referencing. East and west coast laboratories use different fixatives and sectioning techniques, however, the histological slides produced have been accepted as compatible. Health reports are produced case by case and copied to the shellfish supplier and appropriate regulatory agency (east coast: Introduction and Transfer Committees; west coast: Transplant Committee). Canada's Fish Health Protection Regulations are currently being revised and will include for the first time all commercially important shellfish species when passed through Legislation. The protocols for compliance to the FHPR will be different for shellfish and finfish. Sampling protocols, techniques, etc., are in a draft form due for review.

Histological examination in the USA is usually the technique of choice except for *Perkinsus* spp., where organ samples (rectum, mantle, digestive gland, or gill), and sometimes hemolymph, are incubated in fluid thioglycollate medium to induce enlargement of the cells of the parasite.

CONCLUSIONS

- a) Histological examination is a standard survey tool used in most shellfish pathology laboratories.
- b) Sample sizes for disease-screening vary significantly from country to country.
- c) The lists of parasites and host species falling under regulated control in the EU, Canada, and the USA appear to vary significantly.

RECOMMENDATIONS

The WGPDMO recommends that:

- a) appropriate sample sizes and protocols for examination of shellfish should be determined, as well as for other diagnostic protocols;
- b) the disease lists of the OIE be the minimum basis for establishing regulated disease controls;
- c) review the disease lists (point b above) on a yearly basis taking into account the annual country reports to ICES;
- d) a shellfish disease data table which can be used by all ICES member countries for listing diseases of significance be established.

12 EVALUATION OF ADDITIONAL AND NEW DATA ON *ICHTHYOPHONUS*

12.1 Additional Data on *Ichthyophonus*

Ichthyophonus infection in spring-spawning herring stock in the western Baltic decreased significantly and in 1993 only a low level was detected. A low level was also observed from herring in Estonian waters.

In the Kattegat and Skagerrak area, there was a decrease in infection prevalence in the larger size class of herring. A notable change in the disease pattern was that in the smaller size class of herring (1+ group) an increasing prevalence was recorded (up to 30%).

Ichthyophonus infection persisted in herring of the area east of the Shetland Islands (ICES division IVa) at levels similar to that previously found. Herring of the central and southern part of the North Sea remained uninfected. No new data were available from Norwegian spring-spawning stocks. Overall, there was no apparent decreasing or increasing trend in *Ichthyophonus* prevalence level in the North Sea.

Herring in Icelandic waters again showed *Ichthyophonus* infection, but at very low prevalence.

Based on very limited data, no *Ichthyophonus* infection was found in herring from the Atlantic coast of North America. However, in cod from Nova Scotia, presumptive *Ichthyophonus* was found in gonads sampled for research and in fillets from commercial catches.

Dab (*Limanda limanda*) in Icelandic waters exhibited high levels of infection (more than 90% prevalence). In the infected fish, a strong cellular reaction was observed.

12.2 New data on *Ichthyophonus*

Danish research showed that continuous culture of *Ichthyophonus* could be achieved by changing the pH of the culture medium during growth of the fungus. No pathological changes were observed after injecting and feeding mice with *Ichthyophonus*, possibly suggesting no harmful effects in mammals.

There are preliminary indications from Canadian research for the presence of at least two morphologically different types of *Ichthyophonus*.

12.3 Conclusions and Recommendations

To assess any possible impact on the herring stocks, further collection of data on the prevalence of *Ichthyophonus* is needed. However, only macroscopic methods of assessment should be considered until information on the rate of disease progression in herring is available from experimental studies. Data should be collected and combined with data from herring stock assessments both in European and North American waters, and analysed by the Herring Assessment Working Group. The WGPDMO should be kept informed of biological conclusions derived from these data.

It was emphasized that due to possible consequences of the disease on herring and other fish populations, work on experimental infection with *Ichthyophonus* is urgently needed to model the impact of the infection on fish stocks.

The amount of additional and new disease data of *Ichthyophonus* was considered insufficient for analyses at a third special ICES meeting in 1994, and the WGPDMO does not recommend a further meeting at present.

13 PARASITES OF BALTIC COD LARVAE

13.1 Compilation and evaluation of data on a parasite of Baltic cod

In two papers recently published, a protistan parasite has been described in the yolk sac in both egg and young larvae of Baltic cod. Morphologically, the parasite appears to be an endoparasitic dinoflagellate.

In cod, it occurred in relatively small numbers in the yolk. The prevalence varied from 0-30% in different batches of cod larvae and appeared to cause some mortality.

A similar parasite has been observed in turbot eggs and yolk-sac larvae from a hatchery in the western Baltic.

The parasite was detected at the early gastrula stage. Infested embryos often contained a few large parasites as well as larvae at several smaller stages. The prevalence ranged from 0-40%. As in cod, the parasitic infestation in turbot seemed to disappear after some time. No associated mortality was observed due to the infestation.

13.2 Conclusion

Due to limited information, the WG concluded that an evaluation of the impact of this parasite on either Baltic cod or turbot was not possible at present.

14 M-74 DISEASE IN BALTIC SALMON

14.1 S. Møllergaard, Chairman of the ICES Study Group on the Occurrence of M-74 in Fish Stocks, presented its report to WGPDMO and the conclusions were noted.

M-74 is a syndrome of yet unknown aetiology causing high mortality in Baltic salmon yolk-sac fry.

The following points were raised in discussion:

- a) to elucidate the possible impact of food items taken up by adult salmon on the viability of the yolk-sac larvae, it might be useful to focus on food items taken up 2-3 months prior to spawning. As revealed by Canadian studies, most of the transfer of nutritional components from the adult fish into the eggs takes place within this period;
- b) the possible role of algal toxins should be considered.

14.2 Conclusions

The WG concluded from the information available that nutritional and chemical components may be the main factors involved in the syndrome. However, due to the lack of specific disease studies, pathogens cannot yet be excluded as possible aetiological factors.

The WGPDMO congratulated the Chairman of SGM74 on the comprehensive nature of the report.

15 ICES STATUTORY MEETING 1994 SPECIAL TOPIC SESSION ON "PARASITES IN MARICULTURE"

15.1 Special session on "Parasites in Mariculture"

As indicated in C.Res.1993/2:25(i) a special session at the 1994 ICES Statutory Meeting to be held in St. John's, Newfoundland will be devoted to the topic

"Parasites in Mariculture". For the purposes of the session, "parasites" will be taken to include fungi, protozoa, and metazoa (but not viruses and bacteria). Papers for the session are being solicited on two themes under the above topic: 1) Parasites in shellfish: the carrier problem and 2) Problem parasites in finfish mariculture. Drs A. McVicar and S. McGladdery will be convenors/organizers of the special session, and paper titles and abstracts should be submitted to them via the ICES Secretariat (deadline for submission of titles and abstracts: 30 April 1994). Time allotted to the special session may only permit for the presentation of ten orally presented papers. Submissions in the form of posters will also be welcome.

15.1 Mariculture/Environment Assessment

Discussion on Dr J. Stewart's document on "Criteria for Mariculture Environmental Impact Assessment" indicated that the assessment approach had merit. However, it appears to need refining to take into account that diseases in farmed fish may not always be useful/reliable indicators of environmental deterioration on farm sites. The following refinements are proposed:

- 1) A change in species being cultured could result in a change in the frequency/severity of diseases occurring due to innate differences in disease susceptibility or resistance of the new species rather than due to environmental pollution.
- 2) The indicator may act too slowly to be of practical use to the individual fish farmer or the environmentalist.
- 3) Using fish disease as an indicator may prove misleading if the disease agent is introduced via smolts (i.e., from an exogenous source).
- 4) The farming operation may enrich the infectious load (and thus the occurrence of disease) of the water without causing environmental deterioration via organic overloading.

The WG believes that the document will be of considerable interest to the Working Group on Environmental Interactions of Mariculture (WGEIM) and could be considered during its special topic session on "Mariculture and Coastal Zone Management".

16 ADVISORY COMMENTS TO ICES STUDY GROUP ON INTERACTIONS OF WILD, RANCHED (ENHANCED) AND REARED SALMON, REYKJAVIK, APRIL 1994

Communication was received from A. Youngson, Chairman of the Study Group on Interactions of Wild,

Ranched (Enhanced), and Reared Salmon raising the following points for consideration by WGPDMO:

- a) a general assessment of the scope and value of the published literature on the role of farmed salmon in propagating bacterial, viral or parasitic diseases among wild salmon;
- b) an assessment of the scope and value of research known to be being performed now;
- c) an assessment of the likely time-scale of these developments;
- d) following from (a-c) above, an opinion as to whether disease interactions might merit inclusion in any ANACAT/Mariculture Committee Joint Meeting at the 1995 Statutory Meeting as suggested in C.Res.1993/2:62(b).

The WG discussed this extra task and have addressed the points as tabled:

- 1) The literature on interactions of disease between wild and reared salmon is scarce. What is available, contains mostly circumstantial evidence for interaction of disease between wild and farmed fish. (For example the Reports of the Furunculosis Committee, UK, 1930, 1933.)
- 2) A number of research projects are being conducted in ICES member countries:
 - in Scotland, a project on the association between sea-lice, IPN, PD and furunculosis in sea trout is in progress,
 - also in Scotland, a project on diseases in wrasse (Labridae) (cleaner fish) and cultivated salmon to identify whether either species act as a reservoir of infection for the other,
 - in England, a project on epizootics of wild freshwater fish (including salmonids),
 - in Norway, projects on the interaction of sea-lice in cultivated salmon and wild salmon and sea trout,
 - in Norway, a project on the interaction of typical furunculosis in wild and cultivated salmon,
 - especially in Norway, and in some other countries, there are a number of studies into the spread of *Gyrodactylus salaris* in wild salmonid stocks,
 - in Ireland, investigations on mortalities associate with disease, particularly lice, in sea trout (see:

Report of Sea Trout 1993, The Department of the Marine, Dublin, Ireland),

- in the USA, it has been reported that significant losses of Pacific salmon were associated with the Erythrocyte Inclusion Body Syndrome (EIBS) virus,
- EU countries with wild and farmed salmonid stocks routinely conduct monitoring programmes for diseases listed in EC Directive 91/67.

These projects are ongoing or starting as funding becomes available.

- 3) A joint meeting could be useful between the ANACAT and Mariculture Committees if only to spell out what the current situation is on this problem.

Conclusion

The WGPDMO concluded that most of these projects were under-resourced and, with additional funding, could be fruitfully expanded. Furthermore, the WG would like to draw the attention to its 1992 report, C.M.:1992/F:2, ref. Session O, item 5: "Analysis of cases of disease interactions between farmed and wild populations of fish". The conclusions in the 1992 Report are still valid at this time as the situation has not significantly changed since then. The WG's opinion is that most disease organisms present in farmed fish were also present in wild fish stocks. Furthermore, it was considered that wild fish were more likely to act as reservoirs of disease for farmed fish than vice versa.

17 ICES DISEASE PUBLICATIONS

17.1 Diagnostic Fiches

The translation of a group of five fiches is almost finished and this group of fiches should be published in the coming year. Members of the Working Group indicated several areas of interest where they will submit fiches in the near future. The desirability of seeking fiches from other disease specialists not belonging to the WGPDMO was stressed.

As initiated at the last WGPDMO meeting, and since no additional fiches were submitted, it was felt that some of the older fiches should be updated. Input for this task was sought from the group and a positive response obtained.

17.2 *Ichthyophonus* Workshop

The publication associated with the special *Ichthyophonus* workshop is in an advanced stage of preparation and its publication is currently being discussed with ICES.

18 OTHER BUSINESS

18.1 Interactions with Baltic Countries

It was evident from discussions that there is an extensive marine research effort in countries bordering the Baltic Sea, including studies on diseases, which is not presently readily available to WGPDMO. Although the Baltic Marine Biologists Working Group has established contact with some of the researchers working on fish diseases in these countries, it was considered that further encouragement for the participation of other Baltic nations would be desirable.

Recommendation:

The WGPDMO urges ICES to encourage member countries around the Baltic Sea so far not represented in the WGPDMO to appoint and send representatives to the WG meetings.

18.2 Problems in the Use of Virus Common Names

The WGPDMO points out the need for an expert panel to examine the appropriateness of giving disease names to viral isolates from finfish and marine bivalves without evidence that the isolates are capable of causing these diseases. (The disease names are often applied simply on the basis of morphological and serological similarities that the isolates share in common with a known viral fish pathogen). The use of a disease name in connection with such isolates can have serious negative impacts on the fisheries interests of a region or country that may be unwarranted. A number of isolates should be examined in this context, including birnavirus isolates (often referred to as Infectious Pancreatic Necrosis (IPN) virus or "IPN-like" viruses). In this regard, it is particularly important that the use of the name Viral Haemorrhagic Septicaemia Virus for the rhabdovirus recently isolated from certain Pacific salmon, Pacific herring, and cod in the Pacific Northwest of North America (henceforth, "the Pacific Region") be reviewed. The review should take into account the points listed below.

- 1) The virus is apparently not new to the Pacific region as it is widely distributed in Pacific herring, which may be its natural host. (In sampling to date, it has been found in Pacific herring from Kodiak Island in Alaska southwards to Puget Sound in the state of Washington.)

- 2) Despite years of intensive salmonid culture and fish health research in the Pacific Region, the virus has never been found associated with a case of disease in salmonids. Indeed, the limited number of feral Pacific salmon spawners that proved positive for the virus were all healthy fish; also, in laboratory tests to date, the virus has shown little or no pathogenicity for salmon and trout.

- 3) The virus occurs only rarely in salmonids. Of the approximately fifty thousand salmonids examined virologically thus far in British Columbia, not one was positive for the virus. Of the hundreds of thousands of salmonids tested virologically to date in the Pacific coast states of the USA, all were negative for the virus except for the few mentioned in item 2, above.

- 4) Despite morphological, serological, and other similarities with VHS virus, the new rhabdovirus can be readily distinguished from it by use of a nucleic acid probe.

Without a review of the above facts, North America will inevitably remain labelled as a VHS-positive zone. The economic consequences of this would likely be measured in terms of reduced trade in fish (especially live fish) and in the additional costs of running unnecessary health certification programs for VHS virus.

Recommendation:

WGPDMO recommends that ICES bring to the attention of relevant international disease organizations, such as OIE, the problem of common names being assigned to viruses without full scientific basis and recommend that a code of practice be established.

18.3 Parasites as early warnings of environmental change

Dr D. Marcogliese an observer from the Institut Maurice-Lamontagne, DFO, Québec, gave a presentation on research he conducted on the potential for parasites to be used as "early warning" signs of environmental change. He showed a distinct correlation between acidified streams in Nova Scotia and parasite diversity in eels. His work was noted as being a valuable addition to the understanding of parasite-environment related research and that this type of data collection should be continued.

19 ANALYSIS OF PROGRESS WITH TASKS AND FUTURE ACTIVITIES RECOMMENDED TO COUNCIL

An analysis on the progress of tasks as outlined in the Terms of Reference C.Res.1993/2:25 is presented in Annex 4 and an indication given of intersessional work required. The future activities of the WGPDMO were discussed and members agreed on the proposed terms of reference for 1995 as stated in the recommendations (Annex 6).

19.1 Justification for recommendations to Council (Annex 6)

1. There are extensive 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 1995. An invitation to use facilities in La Tremblade has been received from H. Grizel, Laboratory Director. This is an appropriate venue because of the strong basis of shellfish pathology in this laboratory.
- 1a,b. A watching brief should be maintained on new disease trends in wild and cultured marine animals which may have implications to wild fisheries, environmental assessment, and mariculture.
- 1c,2. ICES Secretariat staff are currently working on the compilation of a data bank on marine fish disease. The WGPDMO specialist Sub-Group on this topic should work intersessionally in cooperation with ICES and should meet as a Study Group prior to the WG meeting with the objective of carrying out a full analysis of the data bank.
- 1d. As indicated in Section 9 of this report, the need for international standardization of methods used for the determination of antibiotic resistance of fish bacteria has been acknowledged and, as a first step, information on the biological implications of inhibition zone sizes should be assembled and assessed.
- 1e. Sea lice infection is now recognized as the disease of greatest significance to salmon farming industries in several ICES member countries but there are limited options available for control. Developments in control methods should be reviewed.
- 1f. The culture of marine fish species is assuming increasing importance in ICES member countries and it is therefore timely to review and assess available knowledge on disease problems, particularly in the early stages of fish development.
- 1g. As indicated in Section 18.1 of this report, it is necessary to maintain close coordination between studies being undertaken in the Baltic (BMB-associated studies) with these in other sea areas.
- 1h. Available information on the transfer of Denman Island Disease with various oyster species should be reviewed and assessed and advice provided on risks associated with introductions of these species.
- 1i. As a first step towards establishing an international (ICES) data bank on mollusc diseases it is necessary to review and standardize existing national data.
- 1j. Sampling sizes used for disease monitoring have been developed separately for different animal groups (fish, molluscs) and types of studies (wild, mariculture). Differences in sampling strategy and the implications of these differences should be assessed with particular reference to molluscs.

ANNEX 1

LIST OF PARTICIPANTS

Working Group on Pathology and Diseases of Marine Organisms

Name	Address	Telephone	FAX	E-mail
Dr A.H. McVicar (Chairman)	SOAFD Marine Laboratory P.O. Box 101 Victoria Road Aberdeen AB9 8DB United Kingdom	(224) 29-5605	(224) 29-5620	
Paul van Banning	Netherlands Institute for Fisheries Investigations P B 60 1970 AB Ymuich The Netherlands	02 550 64732	02 550 64644	
Juan L. Barja	University of Santiago de Compostela Dept. Microbiologia y Parasitologia Fac. Biologia 15706 Santiago de Compostela Spain	34 81 563100	34 81 596904	
David Bucke	MAFF Fish Diseases Laboratory 14 Albany Road Grand Industrial Estate Dorset, DT4 9TH United Kingdom	0305 772137	0305 770955	
Goran Bylund	Institute of Parasitology Abo Akademi University Artillerigatan 6 SI - Finland	358 21 654301	358 21 654748	GBYLUND@Fi nnabo.ABO.FL
Robert Cook (Mariculture Chairman)	Dept. of Fisheries and Oceans Scotia-Fundy Region P.O. Box 550 Halifax NS B3J 2S7 Canada	(902) 426-9068	(902) 426-2706	
Catherine Couillard	Institut Maurice-Lamontagne DFO, Quebec Mont-Joli Canada	418 775 0681		
Trevor P.T. Evelyn	Pacific Biological Station Nanaimo BC V9R 5K6 Canada	(604) 756-7066	(604) 756-7053	
Henri Grizel	IFREMER BP 133 17390 La Tremblade France	33 46 363007	33 46 361847	

Name	Address	Telephone	FAX	E-mail
Brit Hjeltne	Institute of Marine Research Department of Aquaculture P.O. Box 1870 5024 Bergen Norway	47 55 238303	47 55 238333	
Thomas Lang	BFA für Fischerei Institut für Fischereiökologie Deichstrasse 12 27472 Cuxhaven Germany	04 721 38034	04 721 53583	
Felix Baundin-Laur- encin	CNEVA BP 70 29280 Plouzane France	(33) 98 224462	(33) 98 055165	
Sharon McGladdery	DFO Gulf Fisheries Centre P.O. Box 5030 Moncton, NB E1C 9B6 Canada	(506) 851-2018	(506) 851-6671	
Stig Møllergaard	Danish Institute for Fisheries and Marine Research Fish Disease Laboratory Bülowsvej 13 DK-1870 Frederiksberg C, Denmark	45 31 352767	45 35 282711	
Carol Morrison (Observer)	DFO Benthic Fisheries and Aquaculture Division P.O. Box 550 Halifax, NS B3J 2S7 Canada	(902) 426 6206		
Gilles Olivier	DFO P.O. Box 550 Halifax, NS Canada B3J 2S7	(902) 426-7588	(902) 426-1862	G_OLIVIER@ BIONET.BIO. NS.CA
Frank O. Perkins	Virginia Institute of Marine Science College of William and Mary Gloucester Pt., VA, 23062, USA	(804) 642-7017	(804) 642-7097	
Dick Vethaak	National Institute for Coastal and Marine Management Ecotoxicology Section P.O. Box 207 9750 AE Haren Netherlands	31 50 331358	31 50 340772	

ANNEX 2

AGENDA

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Moncton, New Brunswick, Canada, 21 - 25 March 1994

1. Opening of the Meeting. Structure of the meeting.
2. ICES 1993 Statutory Meeting; items of relevance to WGPDMO.
3. Terms of reference, adoption of agenda, selection of rapporteurs.
4. Other relevant reports for information
 - a) The BMB Working Group and BMB/ICES Workshop on flounders - Chairman, G. Bylund.
 - b) SGM74 - review of report - Chairman, S. Møllergaard.
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.
 - a) Evaluation of the intersessional work of the WGPDMO Sub-Group on the analysis of wild marine fish disease prevalence data - Chairman, A.D. Vethaak.
 - b) Provide information to ACME on the types of fish disease studies that can elucidate possible relationships between fish diseases and pollution.
8. Analysis of results of intersessional international comparison of antibiotic resistance profiles of Aeromonas salmonicida.
9. Analysis of intersessional data on field trials and other relevant information on fish vaccines.
10. Evaluation of the international regulations on mollusc transfer and of the standardization of mollusc disease monitoring and control methods.
11. Evaluation of the significance of new data on Ichthyophonus and advice on the need for a third species ICES meeting on this topic.
12. Compile and evaluate data on a parasite of Baltic cod larvae. Evaluate data on M-74 syndrome in Baltic salmon.
13.
 - a) Develop and coordinate plans for a special topic session of "Parasites in Mariculture" at the ICES 1994 Statutory Meeting.
 - b) Discussion of document on Criteria for Mariculture Environmental Impact Assessment (Stewart).
 - c) Provide advice on topics requested to ICES Study Group on Interactions of Wild, Ranches (Enhanced), and Reared Salmon due to meet in Reykjavik, Iceland, 5-6 April 1994 (Chairman: A. Youngson).

...Cont'd

14. ICES Disease Publications.
 - a) Diagnostic fiches - update.
 - b) Report of Special Meeting on Ichthyophonus
15. Analysis of progress with tasks.
16. Future activities of WGPDMO.
17. Any other business.
18. Approval of recommendations.
19. Approval of draft WGPDMO report.
20. Closing of the meeting.

ANNEX 3

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Moncton, New Brunswick, Canada, 21-26 March 1994

RAPPORTEURS

<u>Agenda items</u>	<u>Rapporteurs</u>
1-4	A. McVicar
5 (fish)	S. Møllergaard, T. Lang
5 (shellfish/crustaceans)	S. McGladdery, P. van Banning
6 (fish)	B. Hjeltneess, G. Bylund
6 (shellfish/crustaceans)	S. McGladdery, P. van Banning
7	A.D. Vethaak
8	G. Olivier
9	G. Olivier
10	H. Grizel, S. McGladdery
11	G. Olivier, P. van Banning
12	S. Møllergaard, T. Lang
13	D. Bucke, T. Evelyn
14	G. Olivier, F. Baudin-Laurencin

ANNEX 4

PROGRESS WITH TASKS AND ACTION LIST

1 Tasks completed

- i) Analysis of trends of diseases in wild marine animals.

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

Reports of new diseases, new geographic distributions and trends in diseases in mariculture were evaluated from national reports and conclusions and advice presented as appropriate.
- iii) Review of the Report of the Sub-Group on Statistical Analysis of Fish Disease Data.

The Sub-Group report was considered and endorsed by WGPDMO.
- iv) Assess results of an intersessional review of field trials of fish vaccines.

The successful introduction of a range of new vaccines superseded the need for such a review.
- v) Assess results of an intersessional comparison of antibiotic resistance profiles of *Aeromonas salmonicida*.

A report was prepared (Annex 5), results were analysed, and advice offered.
- vi) Analysis of disease aspects of mollusc transfer regulations.

The review of the different regulations revealed substantial differences some of which could have significant implications to disease transfer.
- vii) Evaluate additional and new data on *Ichthyophonus*.

Additional and new data submitted directly and in national reports was not consider sufficient to justify a Third Special Meeting on this disease.
- viii) Review the Report of the Study Group on M-74 syndrome.

The conclusions of the Report were noted. Until additional specific disease studies are performed the possibility of an infectious disease involvement should not be excluded.
- ix) Develop and coordinate plans for a special topic session on "Parasites in Mariculture" at the ICES 1994 Statutory Meeting.

Theme topics were selected and coordinators appointed.
- x) Provide ACME with information on disease studies used for pollution monitoring.

An advisory statement to ACME is included in this report.
- xi) Compile and evaluate information on a parasite of Baltic cod larvae.

Published papers on the parasite were considered and it was concluded that there was insufficient information available to make an assessment on the impact of this parasite on wild fish stocks.

2 Tasks to be continued

- i) Prepare in cooperation with the BMB a preliminary report for ACME on fish diseases in the Baltic and outline plans for future studies on fish diseases in the Baltic (C.Res.1993/2:25(k)).

The BMB are currently at an early stage of assessment of fish diseases in the Baltic and as indicated in Section 4.1 of this report, are scheduled to convene a conference and a sea-going workshop during 1994. The BMB Chairman (G. Bylund) has indicated that, following their meetings, the Group will prepare a report for submission to ACME and that WGPDMO will receive copies for information.

3 Action list

The Working Group on Pathology and Diseases of Marine Organisms:

- a) urges ICES to encourage the Baltic countries, not yet members of ICES, to apply for membership and to appoint members for the WGPDMO;
- b) recommends that ICES bring to the attention of relevant international disease agencies, such as the OIE, the problem of common names being assigned to viruses without adequate scientific basis and recommend that a code of practice be established;
- c) recommends that ICES provide participating member countries with a data entry program for marine fish disease data as soon as possible after the 1994 Statutory Meeting [It should be noted that until this is available there can be no further submission of marine fish disease data to ICES.];
- d) recommends that more information on age/length relationships in dab and flounder be collected by ICES member countries conducting fish disease monitoring programmes to allow a more accurate analysis of disease prevalence data;
- e) recommends that further research should be conducted on species of the genera *Marteilia*, *Haplosporidium*, *Bonamia*, *Mikrocytos*, and *Perkinsus* with respect to:
 - i) investigation on host-specificity and the role of other bivalve species as carriers;
 - ii) improvement of diagnostic tools for their detection;
 - iii) taxonomic characterization of the different species;
 - iv) determination of the life-cycles of *Haplosporidium nelsoni*, *Marteilia refringens* and *Bonamia ostreae*.
- f) recognizes that there is an urgent need for further research into improvements in control methods and alternative treatments of sea lice in salmon farming;
- g) recommends that a standardized method to determine antibiotic resistance of *A. salmonicida* be followed [The Working Group also recommends that further intersessional studies be initiated on the correlation between zones of inhibition and to establish the relationship between bacterial inhibition zone diameters and minimal inhibitory concentration (MIC) or minimal bactericidal concentration (MBC) with serum levels of a particular antibiotic in the host. For this task national representatives should evaluate the feasibility of using national and/or other laboratories to participate in the exercise.];
- h) recommends that appropriate sample sizes and protocols for examination of shellfish should be determined, as well as for other diagnostic protocols, that the disease lists of the OIE be the minimum basis for establishing regulated

disease controls (reviewed annually,) and that a shellfish disease data table which can be used by all ICES member countries for listing diseases of significance be established;

- i) recommends that data on the prevalence of *Ichthyophonus* is still needed using only macroscopic methods of assessment [Data should be collected and combined with data from herring stock assessments both in European and North American waters, and analysed by the Herring Assessment Working Group. The WGPDMO should be kept informed of biological conclusions derived from these data.];
- j) appoint Drs A. McVicar and S. McGladdery as conveners/organizers of the special topic session on "Parasites in Mariculture" at the 1994 Statutory Meeting. Paper titles and abstracts should be submitted to the conveners via the ICES Secretariat (deadline for submission of titles and abstracts is 30 April 1994) and all WG members were asked to encourage papers from their respective countries.

Intersessional comparison of results of the determination of bacterial antibiotic resistance profiles

Table 1. SUMMARY OF LABORATORY PROCEDURES USED TO ASSESS ANTIMICROBIAL SUSCEPTIBILITY

Laboratory	CA	FR	DE	NO	SC	I R
Media to grow inoculum	BHI 24 h	TSA plate	VIB broth	TSA plate	TSA plate 48 h	TSA plate
Standardization of inoculum	none	none	1/100 dilution	MacF. # 2	OD of 1.0 at 540 nm 1/100 dilution	none
Method of inoculation	swab excess liquid squeezed out	flooding remove excess liquid	pasteur pipette (4drops) spread	flooding remove excess liquid	200 µl spread	?
Media for antibiogram	TSA	MH	MH	TSA	TSA for all except (TMP+SZ) on DSTA	TSA
Incubation time	48h	24-48h	48 h	24-48h	TSA, 22° 24h DSTA, 22° 48h	48h
Temperature	22°	20°	20°	20°		22°

	A	B	C	D	E	F	G	H	I	J
1	Antibiotic resistance of <i>A. salmonicida</i> isolates									
2	Strains were susceptible (S), intermediate (I) or resistant (R)									
3										
4	Antibiotic	Penicillin			Amoxicillin			Ampicillin		
5	Dosage	62.5	10		?	2		10	25	
6	Country	DE	CA		DE	SCO		CA	IRE	
7	Strain#									
8	65R	S	S		S	S		S	S	
9	91730	S	S		S	I		S	nd	
10	D-819	S	S		S	S		S	S	
11	BJ	S	S		S	S		S	S	
12	A450	S	S		S	S		S	S	
13	B-93	S	S		S	S		S	S	
14	F	S	S		S	S		S	S	
15	416	S	S		S	S		R	S	
16	818	S	S		S	nd		S	S	
17	1545-R1	S	S		S	I		S	S	
18										
19	Antibiotic	Oxolinic acid					Oxolinic acid			Flumequine
20	Dosage	2	2	2	2		10	10	10	30
21	Country	CA	NO	IRE	SCO		FR	DE	NO	FR
22	Strain#									
23	65R	R		I	I		S	S	24	S
24	91730	S	S	ND	S		S	S	S	S
25	D-819	R	nd	R	R		S	S	nd	S
26	BJ	S	S	S	S		S	S	S	S
27	A450	S	S	S	S		S	S	S	S
28	B-93	R		R	R		S	S	18	S
29	F	S	S	S	S		S	S	S	S
30	416	R		R	I		S	S	22	S
31	818	R		R	ND		I	S	12	S
32	1545-R1	R		R	R		S	S	22	S
33										
34	Antibiotic	Potentiated sulfonamide								
35	Dosage									
36	OMP/TMP	5.2	2.5		?	2.5	1.25			
37	SULFA	240	200		100	50	25			
38	Country	DE	SCO	NO	IRE	FR	CA			
39	Strain#									
40	65R	S	S	MS	R	S	S			
41	91730	S	I	R	nd	S	R			
42	D-819	S	S	ND	S	S	S			
43	BJ	S	S	MS	S	S	S			
44	A450	S	S	MS	S	S	S			
45	B-93	S	S	MR	R	S	S			
46	F	S	S	MS	S	S	S			
47	416	S	S	MR	S	S	S			
48	818	S	ND	R	R	R	R			
49	1545-R1	S	I	R	R	R	I			
50										
51	Antibiotic	Tetracyclines								
52	Dosage									
53	Tetracycline	80				10				
54	xytetracycline		30	?	30		25			
55	Country	DE	CA	NO	FR	IRE	SCO			
56	Strain#									
57	65R	R	R	R	R	R	R			
58	91730	R	R	R	R	nd	R			
59	D-819	S	S	nd	S	S	S			
60	BJ	S	S	S	S	S	S			
61	A450	S	S	S	I	S	S			

	A	B	C	D	E	F	G	H	I	J
62	B-93	R	R	R	R	R	R			
63	F	S	S	S	S	S	S			
64	416	S	S	S	S	S	S			
65	818	R	R	R	R	R	nd			
66	1545-R1	R	R	R	R	R	R			
67										
68	Antibiotic	Streptomycine								
69	Dosage	100	10	10						
70	Country	DE	CA	IRE						
71	Strain#									
72	65R	S	S	R						
73	91730	R	R	nd						
74	D-819	S	S	R						
75	BJ	S	R	R						
76	A450	S	S	R						
77	B-93	S	S	R						
78	F	S	S	R						
79	416	S	S	R						
80	818	S	S	R						
81	1545-R1	S	S	R						
82										
83	Antibiotic	Furanaces								
84	Dosage	?	260	300	300	25	200			
85	Country	NO	DE	FR	CA	IRE				
86	Strain#						SCO			
87	65R	MR	S	S	S	R	I			
88	91730	MR	S	S	S	nd	I			
89	D-819	nd	S	S	S	R	I			
90	BJ	MR	I	S	S	R	I			
91	A450	MR	S	S	S	R	I			
92	B-93	S	S	S	S	S	S			
93	F	S	S	S	S	S	S			
94	416	MR	S	S	S	R	I			
95	818	S	S	S	S	S	nd			
96	1545-R1	S	S	S	S	S	S			
97										
98	Antibiotic	Cloramphenicol								
99	Dosage	30	30	25	60					
100	Country	FR	CA	IRE	DE					
101	Strain#									
102	65R	R	R	R	R					
103	91730	S	S	nd	S					
104	D-819	S	S	S	S					
105	BJ	S	S	S	S					
106	A450	S	S	S	S					
107	B-93	S	S	S	S					
108	F	S	S	S	S					
109	416	S	S	S	S					
110	818	S	S	S	S					
111	1545-R1	S	S	S	S					
112										

	A	B	C	D	E	F	G	H	I	J	K	L
1	Summary of zones of inhibition (mm)											
2												
3												
4	Antibiotic	Penicillin			Amoxicillin							
5	Dosage	62.5	10		?	2						
6	Country	DE	CA		DE	SCO						
7	Strain #											
8	65R	46	26		48	36						
9	91730	42	22		48	27						
10	D-819	40	28		44	30						
11	BJ	38	18		32	34						
12	A450	40	22		46	31						
13	B-93	40	28		44	36						
14	F	36	24		42	33						
15	416	40	18		44	30						
16	818	40	27		38	nd						
17	1545-R1	40	26		38	26						
18												
19	Antibiotic	Oxolinic acid					Oxolinic acid			Flumequine		
20	Dosage	2	2	2	2		10	10	10		?	30
21	Country	CA	NO	IRE	SCO		FR	DE	NO		NO	FR
22	Strain #											
23	65R	20	17	16	25		25	32	24		20	30
24	91730	48	32	nd	46		>40	48	38		30	>40
25	D-819	20	nd	10	22		28	30	nd			30
26	BJ	46	30	>40	50		>40	38	35		28	>40
27	A450	46	34	33	42		>40	50	40		33	>40
28	B-93	10	12	8	17		30	22	18		13	>40
29	F	40	33	>40	48		>40	46	38		33	>40
30	416	22	15	14	24		26	30	22		16	30
31	818	14	10	10	nd		15	27	12		12	25
32	1545-R1	24	14	12	16		26	29	22		17	34
33												
34	Antibiotic	Potentiated sulfonamide										
35	Dosage											
36	OMP/TMP	5.2	2.5		?	2.5	1.25					
37	SULFA	240	200		100	50	25					
38	Country	DE	SCO	NO	IRE	FR	CA					
39	Strain #											
40	65R	44	53	22	10	25	26					
41	91730	34	33	8	nd	40	8					
42	D-819	44	57	nd	32	36	20					
43	BJ	35	55	20	30	40	30					
44	A450	50	53	24	32	40	30					
45	B-93	48	44	18	9	40	22					
46	F	56	64	23	40	40	24					
47	416	48	62	19	28	36	25					
48	818	36	nd	8	10	8	8					
49	1545-R1	38	27	12	10	8	15					
50												
51	Antibiotic	Tetracyclines										
52	Dosage											
53	tetracyclin	80				10						
54	ytetracycline		30	?	30		25					
55	Country	DE	CA	NO	FR	IRE	SCO					
56	Strain #											
57	65R	15	8	8	8	10	8					
58	91730	16	8	8	8	nd	8					
59	D-819	40	36		30	>40	35					
60	BJ	28	12	24	>40	>40	38					
61	A450	44	12	29	20	>40	32					

	A	B	C	D	E	F	G	H	I	J	K	L
62	B-93	14	8	8	10	10	8					
63	F	44	38	29	32	>40	35					
64	416	36	35	26	42	20	34					
65	818	18	8	8	8	10	nd					
66	1545-R1	18	8	8	8	10	8					
67												
68												
69	Antibiotic	Streptomycine										
70	Dosage	100	10	10								
71	Country	DE	CA	IRE								
72	Strain #											
73	65R	36	14	10								
74	91730	14	R									
75	D-819	40	14	10								
76	BJ	34	R	12								
77	A450	36	14	12								
78	B-93	38	18	10								
79	F	38	14	12								
80	416	38	14	11								
81	818	36	14	12								
82	1545-R1	40	17	10								
83												
84	Antibiotic	Furanaces										
85	Dosage	?	260	300	300	25	200					
86	Country	NO	DE	FR	CA	IRE						
87	Strain#						SCO					
88	65R	15	28	28	30	10	20					
89	91730	17	36	>40	28	nd	20					
90	D-819	nd	30	36	24	10	19					
91	BJ	15	22	>40	28	10	21					
92	A450	13	28	32	28	0	15					
93	B-93	25	48	>40	32	32	39					
94	F	22	42	36	28	>40	37					
95	416	15	26	36	24	11	20					
96	818	26	44	36	40	22	nd					
97	1545-R1	25	42	40	30	>40	38					
98												
99	Antibiotic	Cloramphenicol										
100	Dosage	30	30	25	60							
101	Country	FR	CA	IRE	DE							
102	Strain#											
103	65R	R	R	10	22							
104	91730	>40	48	nd	48							
105	D-819	40	38	>40	50							
106	BJ	>40	46	>40	30							
107	A450	>40	46	>40	54							
108	B-93	>40	40	>40	52							
109	F	>40	44	>40	52							
110	416	48	38	30	48							
111	818	40	48	35	59							
112	1545-R1	46	38	>40	46							

ANNEX 6

Recommendations to Council

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Moncton, New Brunswick, Canada, 21-26 March 1994

Recommendations

1. The WGPDMO recommends that it meet in La Tremblade, France, from 3-7 April 1995:
 - 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 WGPDMO Sub-Group report on intersessional work on the analysis of wild fish disease prevalence data;
 - d) to 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) to review the current research in sea lice treatment and control methods including: chemical, biological, immunological, and management practices;
 - f) to review the research on pathology and diseases in fish larvae reared in mariculture;
 - g) to consider the implications to the studies of the WGPDMO on the results from the workshops arranged in 1994 by the BMB Fish Disease Working Group;
 - h) to consider the role of flat oysters (*Ostrea edulis*), Olympia oysters (*Ostreola conchaphila*), and American oysters (*C. virginica*) as carriers of Denman Island Disease (*Mikrocytos mackini*) from Pacific oysters and evaluate the possibility that reciprocal transfers of *M. mackini* may occur among the four oyster species;
 - i) to consider disease data sets currently used in shellfish pathology laboratories and to recommend means of standardization of disease records for use at the national level and by ICES;
 - j) to 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;
2. The WGPDMO further recommends that the Sub-Group on Statistical Analysis of Fish Disease Data in marine fish stocks work by correspondence in 1994 and meet for two days prior to the WGPDMO meeting in 1995 to continue preparations for the analysis of fish disease data submissions to the new ICES database with a view for full analysis prior to the WGPDMO meeting in 1996.