Mariculture Committee

ICES CM 1997/F:6
Ref: Env

REPORT OF THE

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Rhode Island, USA
16–20 March 1997

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

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1 INTRODUCTION

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the Alton Jones Campus, University of Rhode Island with A H McVicar presiding as Chairman (Council Resolution 1996/2:29).

1.1 Opening and Structure of the Meeting

The meeting was opened at 0900 on Sunday 16 March 1997 with the Chairman welcoming participants, particularly those who have not previously attended WGPDMO. S MacLean, NOAA, welcomed the participants and gave a short resume of the function and history of NOAA.

A list of participants is appended in Annex 1.1.

Apologies were received from P van Banning (Netherlands), T Evelyn (Canada), V Kadakas (Estonia), T Bezgachina, (Russia), H Palm (Germany), S Mortensen (Norway), S Helgason (Iceland), F Perkins (USA) and T Renault (France).

It was indicated that the meeting would take the form of a series of plenary sessions with occasional specialist sub-groups being organised to consider some agenda items in detail before reporting conclusions back to the full WG for consideration and endorsement.

2 ICES ANNUAL SCIENCE CONFERENCE 1996: ITEMS OF RELEVANCE TO WGPDMO

Items of relevance to WGPDMO from the 84th ICES Annual Science Conference held in Reykjavik, Iceland were highlighted by the Chairman.

a) The Report of the Delegates Meeting:
   (i) noted that the new Study Group on the Statistical Analysis of Fish Disease Data in Marine Fish Stocks would be established only for one year with a very specific task arising from work done by the Sub-Group on Statistical Analysis of Fish Disease Data in Marine Stocks.
   (ii) stated that the recommendations originating from the Mariculture Committee were adopted by the Council.

b) The Publications Committee recommended that the Editor of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish be requested to accept a one year extension to enable a successor to be found. It was indicated that the Chairman of the Consultative Committee would consult with the Chairman of WGPDMO about the question of editorship of this series.

It was agreed by the WGPDMO that further discussion on this should take place in the appropriate part of the agenda when participants had had the opportunity to consider possible candidates for the position and that the Chairman would forward suggestions to the Chairman of the Consultative Committee.

c) MEQC proposed a Theme Session at the 1997 Annual Science Conference in Baltimore on the topic "Reproductive and endocrine disruption of marine species by anthropogenic factors" with, among others, D Vethaak being identified as a possible European contributor.

d) The Mariculture Committee:
   (iii) discussed the implications of the suggestion of incorporating WGPDMO and some other Working Groups and Special Topics Meetings into a new Marine Habitat Committee.
   (iv) The possible implications to WGPDMO were briefly discussed without conclusions being reached.
   (v) suggested that the Special Topic Session on "Hygiene in Mariculture" be included in the 1998 ASC in Portugal with possible involvement also of the WG on Marine Fish Culture.
   (vi) accepted the report of the 1996 meeting of WGPDMO and its recommendations.
   (vii) noted the forthcoming ICES/NASCO meeting on "Possible Farmed-Wild Salmon Interactions to be held in Bath, England in April 1997. Information on this meeting was made available to the WG participants.

(viii) noted the ICES Workshop on Interactions between Salmon Lice and Salmonids, held in Edinburgh, Scotland in November 1996.

e) The Baltic Salmon and Trout Assessment Working Group have again included in their Terms of Reference the requirement to "evaluate the available information on mortality caused by M-74 in Baltic salmon stocks and the relationship between M-74 and subsequent parr and smolt abundance".

As this item overlaps with one of the regular Terms of Reference of WGPDMO, continued co-ordination between the two groups is required to prevent duplication of effort and possible contradictions.

f) The full Terms of Reference of WGPDMO were agreed as C. Res. 1996/2:29.

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

3.1 Terms of Reference

The terms of reference published as C.Res 1996/2:29 were detailed (Annex 3.1). The repeated heavy agenda load was commented on. This had again necessitated extensive intersessional work by members of the WGPDMO selected by the Chairman; 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, indicating that these would be included in this Report as Annexes. Without the considerable effort made by these contributors, there would have been a danger of the consideration of some topics by the WGPDMO becoming superficial. It was agreed that there should be an attempt to reduce the size of future Terms of Reference through the restriction of topics to only the most pressing issues of concern in the subject area.

3.2 Adoption of Agenda

A draft agenda was circulated and agreed without alteration (Annex 3.2).

3.3 Selection of Rapporteurs

Rapporteurs were agreed as indicated in Annex 3.3.

4 OTHER RELEVANT REPORTS FOR INFORMATION

4.1 EU sponsored Workshop on *Gyrodactylus salaris*

WG members were made aware of the results of the EU sponsored "Workshop on Diagnosis of *Gyrodactylus salaris*" which was held in Oulu, Finland in April 1996. EU and national measures to protect areas where infection has been shown to be absent were discussed.

4.2 ICES Workshop on Interactions Between Salmon Lice and Salmonids

The draft report of this meeting was made available to the participants of the WG by the Chairman of the Workshop. This draft contained the warning that the document still had not been accepted by ICES. It was pointed out that only limited conclusions had been reached; the availability of good data was so limited that different interpretations could be made on the same information and polarisation of views among the participating scientists remained.

4.3 ASC Mini-Symposium on Identifying and Managing Diseases of Bivalve Shellfish

Participants were notified of information received from ICES that the proposal to hold this Mini-symposium during the 1997 ASC had been dropped.

4.4 1997 ASC

The list of proposed Theme Sessions was circulated for the information of the WG participants and the deadline for submission of abstracts noted.

5 WILD FISH INFECTIONS AND DISEASES

Lymphocystis: In North Sea dab (*Limanda limanda*) and flounder (*Platichthys flesus*), the prevalence of this disease has generally continued to decline, especially in the central and southern North Sea. In the south western Baltic Sea, the prevalence of lymphocystis in both flounder and dab showed no new trends.

Epidermal hyperplasia/papilloma: In North Sea dab, no new trends were reported.

VHS-like virus: This virus was isolated from herring (*Clupea harengus*) from the Baltic Sea and English Channel and from sprat (*Sprattus sprattus*), cod (*Gadus morhua*) and rockling (*Enchyleopus cimbrius*) from the Baltic Sea. The virus was also detected in Norway pout (*Trisopterus esmarkii*) from the North Sea. The isolate from the English Channel herring was indistinguishable from one previously obtained from cod. In contrast to previous years, the virus was not recovered from samples of cod, haddock (*Melanogrammus aeglefinus*) and herring exhibiting skin lesions from Scottish waters.

The North American strain of VHS-like virus was no longer detected in natural, unstressed populations of Prince William Sound herring, but was still found in "pound" herring. Disease outbreaks and mortality were associated with stress and particularly spawning. In addition, mortalities were associated with stocks concurrently infected with *Ichthyophonus*.

Other viruses: IPN virus was isolated from summer flounder (*Paralichthys dentatus*) and *Fundulus* sp. from Chesapeake Bay.

Skin ulcer disease: No major new trends were reported. The decreasing prevalence of ulceration in dab from Scottish waters continued. Recent North American studies have indicated that novel toxic dinoflagellates are responsible for producing some ulcerative skin lesions in fish. The prevalence of ulceration in Baltic cod, for the last two years was elevated in comparison to the long term trend data. The causes for this are uncertain but possibilities include the effects of harsh winter conditions and the result of discarded undersized fish which may have been injured and susceptible to secondary bacterial infections.

Hypermelanisation: The prevalence of this condition in North Sea dab seems to be increasing and some Dutch landings of affected fish were rejected for human consumption. The condition remains at low prevalence in Irish Sea dab. Hypermelanisation was also recorded in plaice (*Pleuronectes platessa*) and lemon sole (*Microstomus kitt*). The aetiology of this condition remains unknown.

Liver pathology: The decreasing trend in the prevalence of liver nodules/tumours in dab from the German Bight and Dogger Bank appears to be continuing. Similarly, a declining trend is present in flounder from Dutch coastal waters. This trend is less apparent in other areas and the overall prevalence in gross lesions remains at levels comparable to previous years. Dab from Irish Sea stations continue to show low prevalences of liver nodules and studies in Baltic flounder and dab confirm previous data that indicate liver nodules/tumours are rare in these fish stocks.

Studies investigating the occurrence of green discolouration of dab livers have revealed a possible association with the myxosporean parasite *Ceratomyxa drepanopsettae* which was found at high densities in the bile and bile ducts of affected fish.

Skeletal deformities: The prevalence of skeletal deformities remain low in dab and flounder from the North Sea and Baltic Sea. There appears to be an increasing prevalence of skeletal deformity in cod from the south-western Baltic.

***Vibrio alginolyticus*:** This bacterium was isolated from an epizootic associated with mortalities in wild tilapia species in the Salton Sea, California. Algal blooms, low dissolved oxygen, red tides and the presence of botulinum toxin were all possible contributory factors in the mortalities.

Rickettsia: An unidentified rickettsial pathogen was found associated with severe losses in both wild and farmed tilapia in Hawaii. The systemic granulomatous disease occurred in both estuarine and freshwater conditions.

***Lernaeocera branchialis*:** An increasing trend in the prevalence of this parasite has been noted in cod from Trinity Bay, Newfoundland.

***Kudoa* sp.:** Infections with *Kudoa* sp. in sea trout (*Salmo trutta*) in south-western France are a continuing problem, but no new trends were identified.

Monogenean parasites: The prevalence of the monogenean parasite *Ancyrocephalus paradoxus* in pike-perch (*Lucioperca lucioperca*) in Estonian waters was recorded at high levels (95%).

***Anguillicola crassa*:** For the third consecutive year eels (*Anguilla anguilla*) from the north-west coast of Spain (Galicia) were found to be free of the parasite.

Other parasites: A variety of parasites were recorded at high levels in eels from NW Spain. These included the protistan, *Myxidium* sp., digenean (*Deropristis inflata*) and acanthocephalan (*Acanthocephaloides propinquus*).

The prevalence of *Diplostomum* sp. and the microsporean parasites, *Glugea* and *Pleistophora* were markedly reduced in flounder from the western Baltic. An increasing trend in the prevalence of *Anisakis simplex* and *Pseudoterranova decipiens* in the cod population of the Barents Sea was noted.

5.1 Conclusions

i) There appears to be a general decrease in disease levels in the North Sea, although in certain areas, higher prevalences of gross disease persist.

ii) Hypermelanisation in dab is an increasing problem since this species is of increasing commercial importance and affected fish may be rejected for human consumption.

iii) The geographic and host range for VHS infections is increasing. The virus appears to have a widespread natural distribution.

6 ANALYSIS OF NATIONAL REPORTS OF NEW DISEASE TRENDS IN CULTURED FISH AND WILD AND CULTURED SHELLFISH

6.1 Finfish

6.1.1 Analysis by fish species

a) Atlantic salmon (*Salmo salar*)

Bacteria

Bacterial problems appear to be of decreasing importance to salmon farming in countries in the ICES area. The reason for this is believed to be the use of large scale vaccination programmes and improved management. Consequently. the use of antibiotics has been reduced to a low level. A rickettsia-like organism was diagnosed for the first time in one Atlantic salmon farm in Nova Scotia. Mortalities were low and a possible relationship to other known rickettsia is under investigation. In Scotland *Mycobacterium chelonae* was isolated from salmon at two farm sites. Mortalities were significant at one of these sites.

Infectious Salmon Anaemia (ISA)

In Norway, seven new outbreaks of ISA were diagnosed. At the end of 1996, 17 fish farms were under restriction. A diagnostic method based on antibodies has been developed. The method is being routinely used in diagnostics, but it is still not approved as the sole official diagnostic method.

Infectious hematopoietic necrosis (IHN)

The disease is of great concern to the aquaculture industry on the west coast of Canada. In contrast to previously recorded IHN outbreaks in wild and enhanced stocks of Pacific salmon, which have all occurred in fresh water, the outbreaks of IHN in 1996 have occurred in salt water. This is a new problem and only limited information was available to the WGPDMO.

Sea lice (*Lepeophtheirus salmonis*)

Sea lice infestations still remain the most important disease problem in Norway, Scotland and Eastern North America. The problem is increasing in Canada and USA, but is under control. In several countries, the use of oral treatment has increased.

Miscellaneous diseases

Jellyfish strikes affected several Scottish farm sites with significant mortalities.

Diseases of unknown aetiology

A new condition referred to as Haemorrhagic Kidney Syndrome, associated with significant mortality, has been reported from a large number of sites in New Brunswick, Canada, where it is affecting market size fish. The pathology is characterized by acute kidney tubule necrosis. The condition is believed to be infectious, but no causative agent has yet been identified. In Scotland, a condition known as Liver Haemorrhagic Syndrome affected salmon at three farm sites. The possible role of a new *Vibrio* sp. is being investigated.

b) **Other salmonids**

European lake trout rhabdovirus (ELTRV) was found for the first time in sea trout (*Salmo trutta*) in Sweden. The virus has previously been isolated only in two cases in Finland.

Proliferative kidney disease (PKD) was recorded from rainbow trout (*Oncorhynchus mykiss*) from Nova Scotia for the second time and was associated with mortality. The disease was also recorded for the first time in Estonia.

c) **Sea bass (*Dicentrarchus labrax*)**

Nodavirosis is recognized as a significant problem in sea bass in France, as well as in the other Mediterranean countries.

Fibrosis, mainly caused by *V. alginolyticus,* represents an increasing problem in larval and adult fish in all sea farming areas in Spain.

d) **Sea bream (*Sparus aurata*)**

A nodavirus-like case was reported from France. Symptoms are very similar to those previously reported from sea bass and virus was observed by electron microscopy. However, immunohistochemistry, as well as *in situ* hybridization for nodavirus, were negative.

In Spain pasteurellosis is still a problem in unvaccinated fish as well as infections with *Vibrio alginolyticus*. *Ichthyophonus* was recorded at high prevalence (60 %) but low intensities in sea bream in Spain.

e) **Turbot** (***Scophthalmus maximus***)

In turbot in Spain, *Flavobacterium maritimus* is an increasing problem, while *Enterococcus* is no longer a problem due to the high protection obtained by vaccination. Although limited to a small number of farms, Viral Erythrocytic Infection continues to be the most important viral disease in turbot culture in Spain. In France, the ciliate protist *Uronema* sp. was reported to cause high mortalities in two farms.

f) **Halibut** (***Hippoglossus hippoglossus***)

In Norway, several cases of nodavirosis were reported. An outbreak of IPN with close to 100% mortality was reported in juvenile halibut from Scotland.

g) **Sole** (***Solea solea)***

Vibriosis and flexibacteriosis constitute the main disease problems of Sole which is farmed on a small scale in Spain.

6.1.2 Conclusions

a) In salmon farming, the overall health situation has improved due to application of effective vaccination programmes and improved management procedures. Because of this, the use of antibiotics has been reduced to a low level.

b) A new condition referred to as Haemorrhagic Kidney Syndrome, associated with significant mortality, has been reported from a number of Atlantic salmon sites in New Brunswick, Canada.

c) Nodavirus infections are an emerging major disease problem in the farming of seabass and Atlantic halibut.

6.1.3 Recommendations

It is recommended that disease prophylaxis through improved management and vaccination programmes should be encouraged whenever possible.

6.2 Diseases and Parasites of Molluscs

In addition to the trends reported below, additional information contributed in the Country Reports has been summarised in Annex 6.

6.2.1 Analysis by disease or parasite

Bonamia ostreae of European oysters (*Ostrea edulis*) continues to decrease in Lake Grevelingen in The Netherlands, however, no equivalent decreases are reported from France, Spain or England. Some areas on the northwest Atlantic coast of Spain have been identified as being free of *B. ostreae* and this protistan has still not been observed in oysters from Denmark, Norway, Scotland and Atlantic and Pacific Canada.

Brown Ring Disease (BRD) was reported in Manila clams (*Tapes* (*=Ruditapes*) *philippinarum*) from Poole Harbour, England in 1995, the northernmost finding to date.

Marteiliasis

***Marteilia* sp.** (reported as *M. refringens*) prevalence in Pacific oysters (*Crassostrea gigas*) along the northern Mediterranean coast of Spain (Catalonia) has decreased from 80% in 1995 to 20% in 1996, however, the disease appears to be spreading northward along this coast.

***Marteilia* sp.** (reported as *M. refringens*) infections of the mussel *Mytilus galloprovincialis* continue to cause significant mortalities in Galicia, Spain, although prevalence has decreased.

MSX (*Haplosporidium nelsoni*) reported at epizootic levels in American oysters (*Crassostrea virginica*) from the Piscataqua River (southern Maine) in August 1995 (prevalences between 15 and 81% with associated mortalities) returned to low levels in 1996 when rainfall returned to normal levels. During this period, the prevalences in the Damariscotta River (in northern Maine where salinities did not increase in 1995) remained unchanged. Low salinity resulted in low prevalences of MSX in the upper portions of Chesapeake Bay.

Perkinsiosis:
Perkinsus marinus (Dermo) continues to be a significant problem in American oysters (*Crassostrea virginica*) in the Atlantic United States from Deleware Bay south to the Gulf of Mexico. Infections were found and confirmed in 1995 from Maine, continuing the range extension north from Cape Cod, Massachusetts (reported in 1990-92). All infections in Maine, to date, have been light, difficult to detect using standard diagnostic assays, and were not associated with mortalities.

Perkinsus atlanticus prevalences in clam *Tapes rhomboides* from along the northwest Atlantic coast of Spain continue to increase.

Summer Pacific Oyster Mortality (SPOM) decreased in Pacific oysters (*Crassostrea gigas*) in France in 1996 compared with 1995. Likewise, the mortalities reported in The Netherlands in 1994 and 1995 were not repeated in 1996 as no trace of SPOM was observed during specific surveys of *C. gigas* in June/July 1996.

Quahaug parasite X (QPX) of hard-shell (quahaug) clams (*Mercenaria mercenaria* and in the phenotypic variety *M. m.* var. *notata*) has been found in three locations in Massachusetts. At two of the locations (Provincetown and Duxbury) the prevalences were 36 to 60% with estimated mortalities of 20 to 60% and at the third location (Mitchell River, Cape Cod, where QPX associated mortality occurred in 1992) 2 of 40 clams had grossly clinical signs of infection. A new geographic record was reported from one location in Virginia (Chincoteague Bay, with prevalences of 8% (July) and 20% (August)) but with no associated mortalities. Although QPX was identified in New Jersey wild clams several years ago, it was not found there in 1996 surveys.

Juvenile Oyster Disease (JOD) continues to be a problem in the culture of American oysters (*Crassostrea virginica*) in the northeastern United States, but the prevalence and intensity of the disease have decreased.

Scallop Protistan X (SPX) of cultured Japanese scallops (*Patinopecten yessoensis*) in British Columbia, Canada caused an estimated 80% mortality among market-sized scallops being cultured on Denman Island in July 1996.

Gonadal neoplasms (germinoma) were found in soft-shell clams (*Mya arenaria*) from the Bay of Fundy and Gulf of St. Lawrence, in Canada, thus extending the geographic record for this disease. In the United States, higher prevalences, up to 42% (mean prevalence of 19.4% in 1994), were found in Maine (Washington County).

6.2.2 Conclusions

1. The *Marteilia* sp. infections in Pacific oysters in the Mediterranean seem to be spreading.

2. The decreasing trend of bonamiasis in the Netherlands continues but did not change in other countries enzootic for this disease. European oysters from Canada, Denmark, Norway and Scotland remain free of the disease.

3. MSX prevalence and pathogenicity continue to be governed by the salinity and temperature of the environment in enzootic areas.

4. The geographic range of *Perkinsus marinus* seems to be extending northward and to be less pathogenic at the northern limits of its range.

5. The apparent increases in QPX occurrences may be related to recent increased investigation efforts on this protistan as a consequence of associated mortalities that occurred in the early 1990's.

6.2.3 Recommendations

1. The *Bonamia*-negative European oysters from Denmark, Norway, Atlantic and Pacific Canada, suggest that low temperatures may affect bonamiasis. Thus, it is necessary to determine whether or not northern populations are sub-clinical carriers, resistant to bonamiasis, or are naïve and susceptible to the disease. Thus, it is recommended that ICES countries enzootic for *B. ostreae* assist the countries listed above by accommodating appropriate challenge experiments to confirm resistance/susceptibility of these oysters to infection.

2. The species of *Marteilia* infecting *Crassostrea gigas* along the Mediterranean coast of Spain should be confirmed before assigning a species name. It has been reported from Spain for the last two years as *Marteilia refringens,* based on the occurrence of presporulation stages only, and not on the spore stages which are considered necessary for identification. Also, the increasing geographic spread of this parasite in Spain is cause for concern and raises the question of alternate hosts. The *Marteilia* sp. that caused high mortalities (approaching 100%) in calico scallops (*Argopecten gibbus*) in Florida in the late 1980's also requires specific identification. Thus, it is recommended that member countries enzootic for *Marteilia* spp. investigate the specific identification of this parasite group and determine host specificity.

3. It is recommended that the United States continue to use the *Haplosporidium nelsoni*-specific DNA-probe for screening potential intermediate hosts and sub-clinical oyster carriers in order to elucidate the life-cycle and transmission route of this significant pathogen - especially as it pertains to cross-host species infection and reports of *Haplosporidium* infections in *C gigas* from other member countries.

4. It is recommended that countries that are developing DNA-probes for diseases of shellfish (i.e., MSX in the United States and *Herpes* in France) continue to evaluate and substantiate the specificity and sensitivity of the probes. It is also important that the protocols for each probe be clearly defined in detail such that application by non-specialist personnel produces consistent and reliable results.

5. To assist in determining geographic range, host specificity and screening stock for relocation, it is recommended that countries enzootic for significant pathogens, such as *Bonamia* spp., *Mikrocytos* spp., *Marteilia* spp. and *Perkinsus* spp. (as well as purifiable agents of uncertain taxonomic affinity), direct efforts towards the development of pathogen-specific, sensitive diagnostic tools.

6.3 Diseases and Parasites of Crustaceans

In addition to the trends reported below, additional information contributed in the Country Reports has been summarised in Annex 6.3

6.3.1 Analysis by disease or parasites

***Hematodinium* spp.**

Hematodinium perezi in blue crabs (*Callinectes sapidus*) reported from Texas, Georgia, South Carolina, North Carolina, Virginia, Maryland and Delaware, was most prevalent in juvenile crabs (15-25 mm carapace width) in December (95%) decreasing to 0% in March.

***Hematodinium* sp.**, the cause of bitter crab disease, was observed in *Chionoecetes opilio* off the coast of Newfoundland, Canada which constitutes a new geographic record.

Viruses in cultured penaeids are having a significant negative impact on the farmed shrimp industry in South Carolina. Taura virus outbreaks in 1995 have halved pondgate sales and a state hatchery encountered viruses that resembled white spot virus (WSV) and yellowhead virus (YHV).

Mesanophrys pugettensis, a systemic ciliate infection of Dungeness crabs (*Cancer magister*), was observed in a few moribund Dungeness crabs and red rock crabs (*Cancer productus*) near Nanaimo, British Columbia in June 1996. Although a new geographic record for this parasite, the high mortalities associated with this parasite in Puget Sound, Washington in spring of 1990 were not observed in Canada.

6.3.2 Conclusions

The number of different viruses being encountered during the culture of penaeids seem to be increasing.

6.3.3 Recommendations

It is recommended that the United States investigates the relationships between the WSV-like and YHV-like viruses in penaeid shrimp from South Carolina with similar pathogens reported from cultured penaeids in Asia in order to establish if these viruses are endemic to North America or if they have been accidentally introduced. If a new introduction has occurred it is vital to ascertain the risks and consequences now faced by cultured and wild shrimp stocks on the American continents.

6.4 Diseases and Parasites of Echinoderms

Paramoeba invadens infections of sea urchins (*Strongylocentrotus droebachiensis*) spread another 20 Km on the west coast of Nova Scotia in 1996, with an associate high mortalities of sea urchins. Another 60 Km of the western coast experienced partial mortalities

7 EVALUATE AND INTERPRET THE RESULTS OF THE STUDY GROUP ON THE STATISTICAL ANALYSIS OF FISH DISEASE DATA IN MARINE FISH STOCKS

7.1 Review of the Study Group Report

The chairman of the Study Group, A. D. Vethaak, presented the draft report of the Study Group which met in ICES Headquarters on 6–7 February 1997.

An overview was provided on the progress made with regard to the statistical analysis of fish disease prevalence data submitted intersessionally to the ICES Environmental Database. The new data submitted were restricted to diseases of dab (*Limanda limanda*) covering the period 1981-1996 and stations in the North Sea, English Channel and Irish Sea. However, it was emphasised that data submission was not complete and that, therefore, no benefit could be made of a full data set at the time of the meeting.

Four diseases of dab were used for statistical analysis: lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations, and liver nodules > 2 mm. The Study Group decided to perform a series of preliminary analyses on the data presented in several forms, by month, quarter, station, and ICES statistical rectangle, in order to discuss possible differences and propose a format for future analyses. The following decisions were made:

The dataset was restricted to the first 6 months of the year because 95 % of the data originated from that period. No significant differences were detected between stations and ICES rectangles and, therefore, it was decided that future analysis should be based solely on ICES statistical rectangles . Further justification was the fact that the use of ICES rectangles were more in line with the purpose of the analysis. Some countries submitting data did not use a specific station grouping. Guidelines were provided to designate stations to a particular ICES rectangle in the case of stations (e.g. hauls) that overlap two or more rectangles.

Temporal trends were presented only for statistical rectangles containing at least 8 years of data. Spatial patterns were presented using females of size group 2 (20–24 cm) for the period 1990–1996. Both temporal and spatial data in the form of graphs and maps were made available to the WGPDMO for evaluation and interpretation.

7.2 Discussion of the Report and Future Activities

The recommendations of the SG report were endorsed by WGPDMO.

The need for the incorporation of data obtained from studies on liver pathology and related biomarkers in the ICES Environmental Databank was recognised by the WG. This point is further dealt with under agenda item 8.

As a consequence of the experience obtained during the SG meeting concerning the delay in the submission of fish disease data, it was agreed that data should be submitted to the ICES Secretariat not later than 1 September 1997. Data submitted after this date will be excluded from the analysis. As a proposed schedule, the ICES Secretariat should compile the data and make them available on the ICES computer system for statistical analysis by 31 December 1997, sufficient time before the WG meeting. W. Wosniok will access the data and conduct by correspondence the statistical analysis with the following objective:

- to compile graphs of temporal trends and spatial distribution maps of the diseases: lymphocystis, epidermal hyperplasia/papilloma, skin ulcers and liver nodules for dab and flounder for the North Sea and other areas.

Intersessionally, the WGPDMO Chairman and designated members of the WG (S. des Clers, A. D. Vethaak, T. Lang) will assist with advice and decision making and compile a report for further evaluation, interpretation and finalization during the WGPDMO meeting.

7.3 Evaluation and Interpretation of the Temporal and Spatial Disease Data

Temporal graphs and distribution maps were available for evaluation and interpretation of the following diseases of dab: lymphocystis, epidermal hyperplasia/papilloma and acute skin ulcerations. Data for liver nodules were too limited to allow spatial and temporal analyses..

Temporal trends (1981-96)

The predicted prevalence of lymphocystis showed an increasing trend from 1981 peaking in 1989 followed by a gradual decline to 1996. The predicted prevalence of epidermal hyperplasia/papilloma showed a pronounced increase from 1981 with a peak in 1986 and fluctuated at a relatively high level until 1996. The two data lines for the Skagerrak, covering the period 1984 to 1993, showed no trends for either lymphocystis or epidermal hyperplasia/papilloma originate No trend was detected for acute/healing skin ulcerations.

It should be emphasised that the data analysed for temporal trends were unbalanced as they consisted mainly of Danish and German data from the German Bight and west coast of Jutland. Both the dumping of titanium dioxide wastes in the German Bight area and oxygen deficiency in both the German Bight and along the Danish west coast are believed to be contributing factors to the observed temporal patterns.

Spatial patterns (1990-96)

Due to technical problems, data for 1993 were limited for spatial poattern analyses. Lymphocystis, epidermal hyperplasia/papilloma and acute skin ulcers are widespread in the North Sea. The areas with the highest predicted prevalences for lymphocystis are the east coast of the UK and the central North Sea. For epidermal hyperplasia/papilloma areas with highest predicted prevalences are the German Bight, Dogger Bank area and the east coast of the UK. Areas with highest predicted prevalences of skin ulcers are the Dogger Bank area and certain regions of both the east and west coasts of England. In general, areas with high disease prevalence include the central North Sea and the east coast of the UK. As it is recognised that a considerable bulk of data is still under preparation for submission to the ICES data bank, the WGPDMO considered it too premature to conduct a full interpretation in terms of biological significance of the findings.

7.4 Conclusions

The WGPDMO concluded that the tasks of the Study Group have been fulfilled and congratulated the Study group, as well as the ICES Secretariat, for their successful efforts. The completed fish disease databank, as well as the standardised methodologies developed for data submission and statistical analysis will facilitate future analyses combining fish disease data with environmental and fisheries data. It will also contribute to the OSPAR Quality Status Report 2000.

Future presentation of temporal trends will need to be separated to a number of specific geographic sub-areas, rather than covering the whole North Sea, to facilitate the interpretation of the results.

The data set is still not complete with respect to spatial coverage and species.

7.5 Recommendations

The WGPDMO recommended that:

- to encourage member countries to submit disease data on dab and other species from the North Sea and other areas for inclusion in the ICES Environmental Databank to the ICES Secretariat not later than 1 September 1997.

- in consultation with the WGPDMO Chairman, the ICES Secretariat compiles the submitted data and makes it available on the ICES computer system for subsequent statistical analysis by 31 December 1997

- Wosniok will get access to the data and conduct the statistical analysis by correspondence in sufficient time before the WG meeting.

- The WGPDMO Chairman and designated members of the WG (S. des Clers, A. D. Vethaak, T. Lang) will assist with advice and decision making and compile a report for further evaluation, interpretation and finalization during the 1998 WGPDMO meeting.

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

S. W. Feist presented an overview of the ICES Special Meeting (summary in Annex 8.1) which was held during 22-25 October 1996 in the MAFF, Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth laboratory, under the co-convenorship of S. W. Feist (UK) and T. Lang (Germany) and with the assistance of A. Köhler (Germany) as representative of the ICES Working Group on Biological Effects of Contaminants (WGBEC). Eighteen participants representing 9 ICES Member Countries attended the meeting. The Special Meeting addressed a variety of aspects of liver pathology including subcellular/cellular and biochemical biomarkers as well as macroscopic lesions (e.g. liver nodules) but particular emphasis was given to liver histopathology.

The WGPDMO endorsed the conclusions and recommendations of the meeting and made the following comments:

Results of studies on marine fish liver histopathology related to biological effects of contaminants, carried out by ICES Member Countries according to the guidelines provided in the report of the Special Meeting, should be reviewed annually as part of the regular work of the WGPDMO and the WGBEC. In order to facilitate co-ordination of work between the two Working Groups, it was encouraged that members of the WGBEC attend meetings of the

WGPDMO and vice versa as appropriate. An option discussed for the assessment of liver histopathological data was the organisation of a joint meeting by both Working Groups without a decision being reached on timing.

The WGPDMO endorsed the proposal of the Special Meeting participants to publish the results of the Special Meeting as follows:

(i) Technical aspects in the *ICES Techniques in Marine Environmental Sciences* (TIMES) series with A. Köhler, S. W. Feist and T. Lang as co-authors.
(ii) Proceedings of the meeting in the *ICES Journal of Marine Science* with all participants as co-authors.
(iii) A colour atlas on common histopathological liver changes in common European and North American flatfish species, in collaboration with North American colleagues (co-authors: contributors, possibly edited by S. MacLean (USA) and selected participants of the ICES Special Meeting).

The possibility to publish the colour atlas on CD ROM was discussed and there was general agreement that this was desirable but that the atlas should first be published conventionally. Options to publish the atlas still have to be investigated and contacts will be made with prospective publishers and potential sponsors.

The WGPDMO considered the possibilities to incorporate data on liver histopathology into the fish disease data section of the ICES Environmental Databank and it was felt that this will require only minimal adaptation of the ICES Fish Diseases Data Entry programme and the ICES Data Reporting Format. It was suggested that the co-convenors of the Special Meeting discuss with ICES the best approach to accomplish this.

The WGPDMO endorsed the recommendation of the Special Meeting that the CEFAS Weymouth Laboratory should act as the reference laboratory for the standardisation and intercalibration of liver histopathology diagnosis as part of quality assurance requirements. These are more fully considered in section 9 of the WGPMO report.

Comments on the results of the Special Meeting were received from the WGBEC:

(i) One comment concerned the title of the meeting and it was suggested by WGBEC to change the term "pathology" in the title to "histopathology". However, the WGPDMO did not agree with this proposal since the Special Meeting was not limited to histopathology and took account of other aspects of pathology including subcellular and biochemical biomarkers. Furthermore, since the meeting had been held and the report finalised, a change in the title was not possible.

(ii) Another comment from the WGBEC concerned a decision tree approach which was advocated in the report of the Special Meeting for future risk assessment but not developed further. Although the WGPDMO recognised the necessity to define criteria for this approach, as described by the WGBEC in its 1997 report, it agreed not to add this aspect to the already finalised report of the Special Meeting. The WGPDMO, however, endorsed the view of the WGBEC that links between the WGBEC and the WGPDMO should be promoted to help facilitate the development of expert systems or decision trees for the assessment of biological effects of contaminants. The WGPDMO appointed A. D. Vethaak as representative for an expert group addressing the above.

8.1 Conclusions

The WGPDMO appreciated the results of the Special Meeting and emphasised that it constituted an important step in the establishment of the use of liver pathology for international research/monitoring programmes on biological effects of contaminants. The outcome of the meeting is directly applicable to the WGPDMO Term of Reference relating to the development of a quality assurance plan for the incorporation of liver pathology in biological effects monitoring programmes (see section 10 of the report).

Recommendations

a) The WGPDMO recommended that the results of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants are published as follows:

(i) technical aspects in the ICES Techniques in Marine Environmental Science (TIMES) series with A. Köhler, S. W. Feist and T. Lang as co-authors
(ii) proceedings of the meeting in the ICES Journal of Marine Science with all participants as co-authors.

b) The WGPDMO recommended that the ICES Fish Disease Data Entry programme and Environmental Data Reporting Format is adapted to incorporate liver histopathology data. The co-convenors of the Special Meeting will liaise with the ICES secretariat in order to determine the best approach to accomplish this.

9 DEVELOPMENT OF A QUALITY ASSURANCE PLAN TO INCORPORATE LIVER PATHOLOGY AND FISH DISEASE MEASUREMENTS WITHIN THE BIOLOGICAL EFFECTS MONITORING COMPONENT OF THE JOINT ASSESSMENT AND MONITORING PROGRAMME OF OSPAR (OSPAR 1997/2.2)

T. Lang provided a written overview of the history of the OSPAR Joint Assessment Monitoring Programme (JAMP) and the draft guidelines for general and contaminant-specific biological effects monitoring to be included in this programme (Annex9.1). According to these guidelines, studies on externally visible fish diseases, macroscopic liver nodules, and liver histopathology of the common dab (*Limanda limanda*) or other target species beyond dab´s distribution limits are to be incorporated in the JAMP using the standard methodologies developed by ICES.

However, the WGPDMO noted that some of the components of the OSPAR draft guidelines for general biological effects monitoring relating to the above studies (see Annex 9.1, WGPDMO Appendix 2) did not accurately reflect the ICES standard methodologies and, therefore, required revision. A revised version of the guidelines was prepared and is included in this report (Annex 9.2).

The WGPDMO emphasised that quality assurance procedures for studies using externally visible diseases and macroscopic liver nodules of dab and flounder (*Platichthys flesus*) have been developed and assessed by ICES and are now fully established. These concern all steps in the process from sampling to data reporting and analysis. Therefore, the quality assurance plan for the incorporation of externally visible diseases and macroscopic liver nodules in the JAMP can be considered complete and operational.

The WGPDMO, however, recognised the need for a similar quality assurance plan for the incoporation of liver histopathology, an issue that was addressed earlier during the ICES Special Meeting on the "Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" (see section 8 and Annex 8.1). Quality assurance guidelines for sampling and processing have already been produced during that ICES Special Meeting. However, training and intercalibration for the diagnosis of histopathological liver lesions are still incomplete.

Therefore, the WGPDMO proposed that a training and intercalibration programme (TIP) for the diagnosis of relevant histopathologic liver lesions, as detailed in the report of the ICES Special Meeting, be conducted. This quality assurance plan should take account of the generic and specific quality assurance requirements detailed in the report of the ICES Special Meeting.

Considerations on the design and implementation of such a programme included the following:

i) A reference laboratory be established to take the lead in co-ordinating the training and intercalibration programme (TIP). The CEFAS Weymouth Laboratory, UK, has been proposed for this in the ICES Special Meeting report. This proposal was endorsed by the WGPDMO.

ii) Sets of reference slides with representative histopathologic liver lesions be prepared and circulated among participants of the TIP. In the first instance, these will be relevant participants of the ICES Special Meeting.

iii) For training purposes, this set should be accompanied by detailed comments and interpretations of lesions. For the future, CD-ROM representation of reference material might be useful as a supplement to the slides.

iv) Intercalibration exercises should be conducted on a regular basis, and intra-laboratory callibration standards should be established so that several individuals in one laboratory can discuss and review slides.

v) Reference material for the TIP not available through the reference laboratory will be provided from the Netherlands and the USA.

vi) The requirement for funding of the TIP was emphasised. It was suggested that the reference laboratory explore the possibility for financial support through the EU COST programme and/or ICES.

The WGPDMO agreed that S. MacLean, A. D. Vethaak, S. W. Feist and T. Lang would cooperate intersessionally to develop a proposal for the design of a TIP and for further actions. This proposal should be considered by WGPDMO at its 1998 meeting under the proposed Term of Reference on wild fish disease data.

9.1 Conclusions

The WGPDMO concluded that quality assurance requirements for the incorporation of externally visible diseases and liver nodules of dab and flounder in the OSPAR Joint Assessment and Monitoring Programme are met and that, therefore, these techniques are readily applicable. The appropriate steps for the establishment of a quality assurance plan for the incorporation of liver histopathology have been taken.

10 REVIEW OF THE RESULTS OF THE BMB/ICES SEA-GOING WORKSHOP FISH DISEASES AND PARASITES IN THE BALTIC SEA

T. Lang presented an overview of the results of the BMB/ICES workshop (Annex10.1) which was held onboard RV Walther Herwig in the period 25 November — 8 December 1994 under the co-convenorship of T. Lang (Germany) and S. Mellergaard (Denmark).

The scientific background data on diseases and parasites of fish species examined in the course of the workshop have been compiled in 10 scientific papers which will be submitted for publication to the ICES Journal of Marine Science.

The WGPDMO endorsed the view of the co-convenors that, due to the joint assessment and the practical exercises carried out during the workshop, methodologies used for fish disease studies in the Baltic Sea can be considered intercalibrated to a large extent and have reached a level comparable to that achieved for North Sea studies. However, it was emphasised that the Baltic Sea constitutes a unique environment characterised by strong gradients of abiotic factors, such as temperature, salinity and oxygen content, which have a marked impact on species abundance, diversity and physiological performance. Therefore, the ICES standard methodologies for fish diseases surveys developed mainly for the North Sea have to be adjusted to be applicable in the Baltic Sea. For the Baltic Sea, it is particularly crucial to incorporate the above abiotic factors into the statistical analysis of disease/parasite prevalence data. Furthermore, considerable spatial variation in the fish density and size spectrum occur in the Baltic Sea and therefore, the minimum requirements defined by ICES cannot always be met. This problem may be solved by using unstratified sampling as detailed in Annex 10.1. Differences in the time of main spawning activities and spawning migration patterns as well as pronounced spatial variation in growth characteristics have also been taken into account, particularly for disease examination in Baltic flounder.

The WGPDMO noted that co-operation between Baltic Sea countries conducting fish disease surveys has improved considerably during recent years, mainly due to BMB and ICES activities. The BMB/ICES workshop further strengthened these contacts.

10.1 Conclusion

The WGPDMO appreciated the results of the BMB/ICES workshop and considered its objectives fulfilled. Future fish disease research/monitoring programmes in the Baltic Sea will benefit from the scientific baseline data obtained during the workshop as well as from the achieved intercalibration and standardisation of methodologies.

11 OVERVIEW OF NEW AVAILABLE EVIDENCE ON THE CAUSE OF M-74, INFLUENCING FACTORS AND DISTRIBUTION

11.1 Current Information

M-74 was observed for the first time in Estonia in 1996. An intersessional request for information on the possible occurrence of M-74-like problems in native salmon (*Salmo salar*) stocks indicated that, with the additional exception of Finland and Sweden, none of the other WG-member countries had reported positive observations. Results from Finland and Sweden indicate that the disease has stabilised at a high level. The prevalence of female salmon giving offspring with the M-74 syndrome was 68% in 1996 in Sweden. It has been confirmed from both Finland and Sweden that M-74 also occurs in sea trout (*Salmo trutta*) populations, although with less severe consequences.

There has been no breakthrough concerning the elucidation of contributory factors to the M-74 problem. The research in this field has focused on the thiamine/thiaminase kinetics in fish and thiamine/thiaminase dynamics in the ecosystem. At present, M-74-induced mortality in salmon produced under aquaculture conditions is prevented effectively by thiamine treatment of brood fish, eggs or yolk-sac fry. Although a clear link between M-74 and environmental contaminants has not been confirmed, Finnish studies suggest an involvement of dioxin like organochlorines. These substances seem to disturb thyroid hormones, retinoid and thiamine metabolism and functions in mammals and birds. The contaminant level of these substances in female salmon, eggs and yolk-sac fry is under investigation. It is observed that yolk-sac fry dying from M-74 seem to be retarded in their development and have a lower oxygen consumption than healthy specimens. This indicates some impairment of the thyroid function. Additional facts that indicate the involvement of the thyroid metabolism are that: (i) incubation of yolk-sac fry in thyroid hormone solution seems to prevent the outbreak of M-74 and (ii) incubation of healthy yolk-sac fry in a solution of phenylthiocarbamate (an anti-thyroid drug) results in the appearance of M-74-like symptoms.

Research collaboration between Sweden, Finland and Latvia has been established to elucidate the occurrence and pathways in the ecosystem of thiamine in salmon caught on their feeding grounds.

The role of astaxanthin is currently being investigated but the results of these studies are not available.

11.2 Conclusions

The M-74 syndrome seems to have a complex background but there is, as yet, no clear view on the possible interactions between different factors of concern. At present, M-74-induced mortalities in salmon produced under aquaculture conditions are prevented effectively by thiamine treatment of brood fish, eggs or yolk-sac fry. However, this is a temporary solution for maintaining the smolt production programme, while the problem still persists in wild salmon and sea trout populations.

11.3 Recommendations

It is recommended that ICES Member countries actively continue to determine whether or not reproductive disorders similar to M-74 occur in their wild salmonid stocks. Information on how to detect and diagnose M-74 will be distributed among ICES member countries. E Lindesjoo (Sweden) agreed to intersessionally provide a short description of the disease to all members of the WGPDMO.

12 OVERVIEW OF NEW INFORMATION ON ICHTHYOPHONUS

12.1 Current Information

Working papers detailing results of sampling herring *Clupea herangus* for *Ichthyophonus* were submitted by Iceland, Scotland and Russia. Additional data were contained in the national reports on trends in marine fish diseases submitted by several countries.

The data from the Icelandic summer spawning stock indicated a low prevalence of infection which has remained unchanged since monitoring started in 1992 (Annex 12.1). During this time the prevalence of infection in the 25+ cm size group has been approximately 0.2% in a total of 1976 herring examined, considered to be probably a "normal" background level of the disease. From an area north and northeast of the Faroes in May to June 1996, an overall prevalence of infection with *Ichthyophonus* of 1.93% was determined in samples of 1350 Atlanto-Scandian herring. This represented a considerable reduction from the 1995 value of 3.85%. The prevalence of infection in the 1992 - 96 year classes did not change between 1995 and 1996 and the overall reduction was attributed to lower infection levels in younger herring.

A verbal report from Norway indicated the continued presence of low levels of infection in Norwegian spring spawning stocks in the Norwegian Sea. However, using the standard ICES method of gross examination of hearts, Russian studies showed a prevalence of *Ichthyophonus* in the Norwegian Sea ranging from 10.5% in February - March to 7.5% in June - July and 7.2% in September (732 herring) (Annex 12.2). A prevalence of 3.3% was detected in 60 Barents Sea herring.

Ichthyophonus could still be detected in northern North Sea herring stocks east of Orkney and Shetland but the prevalence levels were low, namely 0.03% in 10,863 herring from research vessel catches and 0.01% in 7,785 herring from commercial catches (Annex 12.3). These figures represent similar levels to that found in 1995. No *Ichthyophonus* has ever been detected in herring stocks west of Scotland.

In the Kattegat/Skagerrak area, a low level of *Ichthyophonus* was also reported in herring from the Swedish west coast (0.04% in 4,600 herring from commercial catches and 0.25% in 2,008 herring from research vessel catches). Although in earlier years *Ichthyophonus* had been found in herring in Estonian waters of the Baltic, there was no record of the disease there in 1996.

The USA Report on disease trends in marine fish stated that prevalences of *Ichthyophonus* in Pacific herring remained steady at around 25% during 1995 and 1996 in Prince William Sound and Sitka Sound, Alaska. Information was made available during the meeting on the method of examination used (histology of all major organs and tissues) and the prevalence of infection over the period 1990 to 1996 in Prince William Sound; 1990 decreasing from 15 to 5%, 1991 decreasing to 2%, 1993 less than 6%, 1994 increase to 29%, 1995 persistence at 29%. Also in 1995 a prevalence of 26% was found in Sitka Sound. In 1994, *Ichthyophonus* was considered to be the major pathogen responsible for herring declines for that year. The differences in detection methods from that being used in the eastern Atlantic area prevented direct comparison of data with that from European waters.

No reports on the prevalence of *Ichthyophonus* were presented by several countries who undertake routine herring population studies. As ACME has recommended the continuing monitoring of *Ichthyophonus* in herring stocks, these countries are encouraged by WGPDMO to integrate the examination of herring hearts for the presence of typical infection lesions into routine herring maturity studies, as limited additional effort is required to obtain valuable data.

12.2 Conclusions

Although *Ichthyophonus* infection persists in several European herring stocks, this was generally at a low level with no evidence of an epizootic. The higher levels of infection found by Russian studies and in USA Pacific herring require further evaluation.

12.3 Recommendations

It is recommended that ICES member countries who are undertaking routine herring stock assessment studies, integrate procedures for the examination of herring hearts for gross *Ichthyophonus* lesions into these studies, wherever this is not already occurring.

13. IMPACT OF ENVIRONMENTAL CONTAMINANTS ON FISH AND SHELLFISH IMMUNE MECHANISMS

13.1 Finfish Immune Mechanisms

During the last decade, comparative immunologists as well as fish pathologists, have realised that a broad spectrum of chemicals can modulate (suppress or stimulate) immune functions in fish. Numerous laboratory experiments have shown that environmental contaminants, like heavy metals, halogenated hydrocarbons, polyaromatic hydrocarbons, etc., exert immunotoxic effects in fish.

Until recently, the methods most frequently employed for these studies included: blood cell counts, hematocrit and leucocrit levels, lysosyme and ceruloplasm assays, total immunoglobulin levels, humoral immune response, macrophage functions (phagocytosis, chemotaxis, chemiluminiscence, etc.), size/distribution of melanomacrophage aggregates, adherence/NBT assays, phagocytic index and killing activity of neutrophils and monocytes, allograft rejection.

Recently, sophisticated molecular immunological methods developed in mammalian immunotoxicology, have also been used by fish immunologists. Of these, functional tests on lymphocytes, immunophenotyping of lymphocytes with flow cytometry, evaluation of killer cell activities, etc. are novel tools which promise rapid advancement in fish immunotoxicology in the near future.

Several immunological techniques used are not sufficiently validated for immunotoxicological work with fish in the field. Current knowledge highlights the need to employ a battery of tests rather than single parameters. In addition, the results obtained and the conclusions drawn from immunotoxicological studies with fish are often conflicting, even in tests using single chemicals. It seems evident that this is partly due to the lack of standardised methods. For example, diurnal and annual rhythms greatly influence the immune parameters in fish and should be taken into account. It is also important to recognise that data should not be extrapolated from one fish species to another. Additionally, extrapolation of test results from laboratory to field conditions is rarely attempted thus it is recognised that it is still premature to include immunological tests in large scale monitoring programmes. Results obtained from laboratory studies suggest that immunological methods can be useful aids for evaluating the impact of environmental contaminants on fish health.

13.2 Shellfish Immune Mechanisms

Few studies have concentrated specifically on the impact on immune mechanisms of shellfish, concentrating rather on the direct physiological effects of contaminant accumulation in the tissues (e.g., HPLC) or haematograms. Inconsistent results using these measures likely reflect misinterpretation when results are extrapolated to immunocompetence. Bivalves have highly variable normal haemocyte dynamics against which any abnormalities have to be assessed. For example, there are fixed phagocytes in the pericardial cavity as well as interstitial haemocytes which evade detection by haemolymph sampling. In addition, there are normal processes involving removal of haemocytes from the body (via diapedesis). This would appear to be a metabolic costly process, e.g., oysters which have a prolonged period of starvation under cold water temperatures are found with reduced populations of haemocytes prior to mortality. Low haemocyte counts are frequently extrapolated to immunocompetence, however, there are few studies which have attempted to quantify such a correlation. Studies are ongoing to isolate biomolecular parameters which may be more sensitive markers of pollutant effects. In addition to these, studies which challenge bivalves with known pathogen titres following exposure to varying concentrations of pollutants are beginning to yield results which will provide more detailed information to the question of contaminant effects on shellfish immune systems and disease susceptibility. As yet, these studies are too few to permit conclusive interpretation of results.

In addition to the haemolymph and lysosome parameters, another affect on haemolymph function is the development of haemic neoplasias. These effectively decode the haemocyte phagocytic function, rendering it useless in host defences, however, the correlation between neoplastic transformation and pollution is highly inconsistent. This most likely reflects a broad range of neoplastic conditions and inducers in bivalve molluscs (as well as other organisms).

The WGBEC submitted a review (WGBEC 97/8/1) for discussion on invertebrate immunology. This included an overview of the types of parameters analysed in relation to known chemical contaminants. The most consistent parameter measured to date was lysosomal membrane integrity since lysosome function is an essential process in the inverebrate's immunoprotection. Bivalve species were shown to have variable lysosomal tolerence to PAH, PCB and tetrafuran challenges, depending on the degree of environmental fluctuation in their natural habitat.

13.3 Conclusions

Taking the current information, as requested in the TOR, extensive discussion led to the following conclusions:

i) Numerous detailed reviews on this topic are available but the limited field studies conducted to date have not provided consistent results of a correlation between parameters measured and environmental contaminants.

ii) The WGPDMO recognises that studies of the immune system of fish and shellfish have the potential to be useful for two purposes, namely detecting variations in contaminant levels and correlating with variations in the prevalence of disease.

13.4 Recommendations

i) The WGPDMO encourages more baseline research into this area, especially relative to field investigations.

ii) Due to the burgeoning information on fish and shellfish immunotoxicology and the relationship to disease processes, the WGPDMO should keep abreast of this field.

14 REVIEW OF INFORMATION ON NODAVIRUS AND NODAVIRUS-LIKE AGENTS

14.1 Characterisation

A working paper from Spain, a review paper from France and two scientific papers, both in press, from Norway were presented to the WGPDMO (Annex 14.1-14.3).

Since 1985, a new disease which induces neurologic disorders has appeared in a wide range of species of cultured marine fish. The diseased fish commonly display vacuolating encephalopathy and retinopathy that frequently lead to death, especially in larval and juvenile stages of development. The lesions of the brain and the retina are commonly associated with clusters of unenveloped virus particles, clearly visible under the electron microscope as roughly spherical with a diameter of 25–30 nm. This disease was named *Viral Encephalopathy and Retinopathy* by the OIE (1995). The disease has been described in a growing number of fish species from Japan, Australia , Thailand, Tahiti, Martinique and Europe.

Biochemical studies performed on viruses isolated from striped jack *(Pseudocaranx dentex),* sea bass (*Dicentrarchus labrax*) and barramundi (*Latex calcarifer*) have allowed a more precise characterisation of the viral genome and the virion polypeptides. These studies have led to the conclusion that the virus belongs to the Nodavirus family.

From this knowledge, an *RT-PCR* assay has been developed using different sets of primers recognising the coat protein gene. At present the pair named F2-R3 (Nishizawa *et al.*, 1994 (Diseases of Aquatic Organisms 18: 103-107)) seems to be the most efficient since a 426 bp amplification product can be obtained from five different species of fishes. Other diagnostic tools have also been developed, namely immunohistochemistry using polyclonal antisera, cell culture and ELISA test for the detection of the virus of anti-nodavirus antibodies. However, these tools must be further developed and improved. This group of virus may be split in the near future into several genogroups (as occurred with the Aquareovirus), according to their pathogenic, immunogenic and molecular characteristics.

Studies should also seek to determine negative-positive threshold values for PCR and serological tests. The detection limit of the PCR method was shown to be between 1 and 100fg. The number of virus particles that these amounts of RNA represent is to be determined.

14.2 Epizootiology

There are several publications on epizootiology of this group. The possibility of horizontal transmission has been shown from diseased larvae to healthy ones, using bath or cohabitation. However transmission in juveniles succeeded only recently in grouper *Epinephelus* spp, sea bass and halibut *Hippoglossus hyppoglossus*. Evidence of vertical transmission is the detection of the virus at high frequency in the ovaries and fertilised eggs of striped jack and sea bass. The disease has been demonstrated to appear in larvae derived from nodavirus-positive spawners, but not from apparently negative ones. It has not been shown whether the virus is present on the surface or inside the egg. A considerable amount of work remains to be done to determine which factors influence infectivity, whether intrinsic (e.g. species, age, physiological status) or extrinsic (e.g. temperature, poor water quality, environmental stressors).

14.3 Immunity

It has been demonstrated that sea bass or striped jack brood stock may have anti-nodavirus antibodies. Consequently, fish can be expected to develop a specific immune response. Taking this into account, vaccine work should be encouraged (killed or live vaccine, synthetic peptides or recombinant proteins) for vaccination trials. Acceptable properties for a vaccine would include: efficacy and safety, capability of preventing the establishment of carrier stages, stability (for a live form , not to revert to the wild type) and the capacity to be identified and differentiated from the wild pathogen. Since early stages of fish lacking immunocompetence can be severely affected by nodavirus, alternative controls to vaccination should also be investigated.

14.4 Conclusions

Nodavirosis is of increasing concern in several species e.g. sea bass, turbot *Scophthalmus maximus* and halibut. Furthermore, nodavirus-like particles have been observed associated with pathological conditions in gilthead sea bream *Sparus aurata* and Atlantic salmon.

14.5 Recommendations

Cooperative work between concerned laboratories is necessary, since: (i) comparison must be done to characterise the isolated strains and (ii) because epizootiologic surveys are required for the different host species. The development of control measures, as well as vaccines also needs active collaboration.

15 REVIEW OF INFORMATION ON THE USE OF CORRELATIONS BETWEEN ENVIRONMENT AND PATHOLOGY (ECOPATHOLOGY) TO PROVIDE ADVICE ON POSSIBLE ALTERNATIVE MEASURES FOR DISEASE CONTROL IN FARMED FISH AND SHELLFISH

15.1 Overview of Ecopathology

F. Baudin-Laurencin (France) presented an overview of a developing scientific approach to assessment of the factors contributing to disease proliferation in farmed aquatic animals, termed "ecopathology"[1]

(Annex 15.1). This approach to disease-risk evaluation was assessed as a possible method of reducing the need for direct intervention (such as using antibiotics) to control disease losses in aquatic production systems. The examples presented were based on a mastitis study of farmed dairy cows and chronic respiratory disorders of pigs (see references in Annex 15.2) and how these might usefully be applied to aquaculture conditions. The ecopathology approach analyses a broad spectrum of factors, linked directly or indirectly to the affected farm, in an effort to (i) isolate the significant components contributing to disease losses; (ii) assess risk-effect factors and (iii) assess the economic feasibility of reducing exposure to major risk factors.

The Working Group discussion included comments on positive and negative aspects which would have to be taken into account if planning to apply this to aquaculture. Some experience was also presented with case-histories compiled by Norwegian, Canadian, Australian and French investigations.

Positive points:

- This approach takes into account more diverse parameters than would normally be included in routine pathological investigations (e.g., socio-economic factors).
- This approach provides a method by which qualitative environmental parameters can be included in a quantifiable disease-risk assessment.
- It may be applicable to general databases which include (or can be cross-referenced to) a range of parameters which can be linked directly or indirectly to the aquaculture system.

Negative points:

- This is a labour intensive approach which requires time for in-depth collection of data and evaluation of each disease case-history.
- It should not be used as a mechanism to negate other useful disease management tools, such as vaccines and selective husbandry.
- Each case requires a "blank sheet" approach to establish appropriate "descriptors" (10–20 factors selected as having an appropriate link to the disease problem under investigation).
- Accurate assessment of the significance of the different factors being evaluated, would necessitate the affected farm to forego immediate, traditional disease control measures which may be available (at least in certain pens, cages, tanks, etc.).

15.2 Conclusions

To date, the regular use of ecopathology has been limited to domestic animals, with positive results. It is noted, however, that comparable investigations have been, and are being applied, to aquaculture to varying degrees and with varying success. Successful examples of disease control measures with an ecopathology-like basis include:

(1) control of sea-lice (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar*) farms by fallowing sites.
(2) circumvention of Denman Island Disease (caused by *Mikrocytos mackini* in *Crassostrea gigas*), achieved by stocking beaches with seed following the transmission period, and harvesting 2yr old oysters low on the beach, before the onset of disease in March.

1

1. Defined by Toma *et al.* 1991(Glossaire d'Epidemiologiologie Animale. Le Point Vet. Maisons-Alfort, France) as a branch of epidemiology dealing with health in connection with environment: housing, feeding, microbial load, etc.

(3) the multifactorial assessment of mortalities of the pearl oyster *Pinctada margaritifera* following transportation from collection sites to pearl farms off north Western Australia.

It was noted, however, that a "farm classification" approach may not be as uniformly applicable to aquatic species, pathogens and culture systems, as in terrestrial systems at least in the near future. Which base-line environmental parameters interact with disease manifestation in the aquatic environment are not yet as well established as on land. Particular emphasis was placed on the fact that it requires in-depth experience of the system under investigation (host and pathogen interactions and dynamics) to compile effective data. Thus direct extrapolation of terrestrial protocols to the aquatic environment was not recommended.

15.3 Recommendation

It is recommended that ICES Member Countries consider ecopathological approaches to disease control assessments of aquaculture facilities, in order to develop control methods which reduce losses due to disease.

16 ASSESSMENT OF PROGRESS IN IDENTIFICATION OF ALTERNATIVE HOST SPECIES FOR THE TRANSMISSION OF BIVALVE PATHOGENS, INCLUDING THE DEVELOPMENT AND USE OF SENSITIVE DETECTION TOOLS

16.1 Update on Host-specificity Research in Europe and North America

MSX (*Haplosporidium nelsoni*) (information from S. E. Ford, Rutgers University, NJ, and E. M. Burreson, Virginia Institute of Marine Science): The search for a potential intermediate host is continuing using a DNA probe and PCR technology. The *H. nelsoni*-specific probes produced at VIMS have been rigorously cross-checked with a number of closely related protistan species in molluscs and crabs, and, to date, have reacted positively only with the previously unidentified haplosporidian in *C. gigas,* which has an almost identical base-pair matching. Field challenges of naive *C. gigas* in MSX-endemic waters have ceased due to a prohibition on the use of reproductively capable *C. gigas* in these areas. Earlier tests using *C. gigas*, which were sterilised by induced triploidy, demonstrated that the adults of the species did not become infected with *H. nelsoni.* The experiment needs to be repeated with juveniles, which may be a more suitable host, because the spore stage of *H. nelsoni* is found almost exclusively in young *C. virginica.* Despite the prohibition on field testing of *C. gigas*, this method of exposure remains advantageous because of the inability, to date, to transmit *Haplosporidium nelsoni* from one oyster to another in the laboratory.

Herpes-infection of *Ostrea edulis* (reported from France since 1994) showed cross-reactivity and DNA amplifications using several primers prepared for the *Herpes*-like virus from infected *Crassostrea gigas* (information from T. Renault, IFREMER, La Tremblade). Host-specificity studies, as outlined in the WGPDMO 1994/12.3, have not yet been conducted.

Denman Island Disease (information from S. M. Bower, Pacific Biological Station, Department of Fisheries and Oceans Canada, Nanaimo), caused by *Mikrocytos mackini* infections of *Crassostrea gigas,* is known to be infective and highly pathogenic to *Ostrea edulis*, *Ostrea conchaphila* and *Crassostrea virginica* under cold-water (< 12 C) conditions for at least three consecutive months. Temperatures above 15 C appear to completely inhibit progression of the disease. Seven monoclonal antibodies have been developed which are sensitive and appear specific to *Mikrocytos mackini.* These, along with traditional histopathological diagnostic techniques, were essential for establishing conclusive transmission to the alternative host species. Reciprocal infections between the alternate host species and *C. gigas* have also been completed (N.B. this is the first documented completion of the protocols recommended for assessing the significance of alternate species transmission of pathogens of shellfish - WGPDMO 1994/12.3). Investigations into development of a molecular probe to detect subclinical infections are currently being pursued.

Marteilia refringens from *Ostrea edulis,* as well as *Marteilia* sp. (reported as *M. refringens* or *M. maurini*) from *Crassostrea gigas*, *Ostrea edulis*, *Mytilus edulis, Argopecten irradians* and a number of other bivalve species still require investigation. No further information was available on the progress in development of a species-specific probe for *Marteilia refingens* reported last year (France).

16.2 Update on Techniques Applied to Detect Infections in Alternate Host Species

Stress testing is frequently used to enhance pathogen detection in sub-clinical hosts. However, this should only be used where there is well-determined knowledge of the physiological tolerance levels of the pathogen and the normal host-pathogen interactions. This is necessary since different infections react differently to the same stressors, e.g., *Mikrocytos mackini* disappears at temperatures > 15 C, whereas *Bonamia ostreae* may be inhibited by cold water conditions. Some parasites may even degenerate, rather than proliferate in stressed hosts, e.g., *Haplosporidium nelsoni*.

Research on development of sensitive pathogen-specific detection methods must be continued if progress in developing expertise in their use and in advancing shellfish epidemiology is to be achieved, including (but not limited to) the ability to detect alternate host species. Sensitive pathogen-specific diagnostic tools, such as DNA probes are still in the preliminary stages of development and should not be considered for routine diagnostics until they have been thoroughly tested and validated under typical conditions at more than one laboratory. . Such tools require (i) highly skilled personnel, sophisticated (and expensive) equipment; (ii) stringent specificity and sensitivity testing; (iii) stringent standardisation of methodology and (iv) quality assurance and quality control (QAQC) monitoring.

16.3 Conclusions

Cross-species transmission has been established conclusively for the first time. Field and laboratory experiments have shown reciprocal infectivity by *Mikrocytos mackini* between *Crassostrea gigas* and three other sympatric oyster species.

Cross-species transmission by *Haplosporidium nelsoni* has been examined using a highly sensitive and specific DNA probe. The specificity and sensitivity have been stringently tested, increasing assurance that the same organism can infect both *C. virginica* and *C. gigas*. Reciprocal infection studies have yet to be completed.

Multi-host specificity still has to be established for *Herpes* sp. infections of *C. gigas* and *O. edulis*, as well as *Marteilia refringens* of the same two species, and of *Mytilus edulis* and *M. galloprovincialis*. Definitive speciation of *Marteilia* infections continues to impede accurate assessment of the significance of infections by these parasites.

16.4 Recommendations

i) It is recommended that ICES Member countries, which are enzootic for *Herpes*-like viral infections of *Crassostrea gigas* and *Ostrea edulis,* continue development of diagnostic tools. Also tools which will be able to distinguish between different species of *Marteilia* spp. are required.

ii) It is recommended to all shellfish pathology laboratories that conduct stress testing to only use the test for diagnostics where there is well-determined knowledge of (i) the physiological tolerance levels of the pathogen and (ii) normal host-pathogen interactions. This is to avoid misinterpretation of negative results generated by inappropriate stressors.

iii) It is recommended to all shellfish pathology laboratories that pathogen-specific molecular or immunological tools should not be considered for routine diagnostics until they have been thoroughly tested and validated under typical conditions at more than one laboratory. This will avoid false expectations and false results occurring with newly developed tools at non-specialist laboratories.

iv) notwithstanding recommendation (iii), it is recommended that shellfish disease research laboratories with the requisite expertise continue to develop sensitive pathogen-specific detection methods. This is necessary to continue development of expertise in their use and advance shellfish epidemiology, including (but not limited to) the ability to detect alternate host species.

17 ASSESSMENT OF PROGRESS IN STUDIES ON THE POSSIBLE CAUSES OF BONAMIA OSTREA PERSISTENCE IN AREAS WITH LOW DENSITIES OF EUROPEAN OYSTERS AND THE POSSIBLE RELATIONSHIP TO DISEASE TOLERANCE STATUS

17.1 Overview of Bonamiasis Persistence in Europe and the US

The only intersessional report submitted on this topic was from The Netherlands (Annex 17). Results compiled from Lake Grevelingen between 1987 and 1996 showed an increase in mortality between 1988 (when the disease first appeared) and 1991, when it peaked at 84%. Following this mass mortality, oyster stocks were reduced from 300-500/100 ml (1987–1988) to 15/100 m2 (1993). This corresponded to an increase in mean prevalence2 of infection from 0% (1987) to 30% (1991). Prevalences of infection then dropped to a record low of < 1% in November 1996. Stocks have not recovered sufficiently to conclude that this is development of resistance rather than a reflection of low population densities (15-38/100 m2 between 1993-1996) or some other infection-inhibitory effect. The Netherlands intend to conduct a field challenge test by relaying oysters at high densities in Lake Grevelingen and monitor the prevalence of *Bonamia* in the different density stocks over a two year period.

The reports of resistance (tolerance) of *Bonamia ostreae* infections by *O. edulis* from the northeastern Pacific (WGPDMO Report, 1996, Section 6.2.3) have yet to be confirmed by laboratory challenge tests. These are necessary to confirm whether or not field observations of reduced prevalences of infection are due to oyster resistance or some other factor which inhibits *Bonamia* expression.

Continuing reports of no detection of *Bonamia ostreae* in European oysters from Canada and Scotland, along with new reports of similar findings in recent surveys conducted by Norway and Denmark, suggest that the absence of *Bonamia* may not necessarily reflect an absence of infections, but the possibility that northern water temperatures inhibit clinical proliferation of the parasite. Sub-clinical infections are difficult to detect using standard diagnostic tools. Stress-testing (warm water temperatures) have been conducted by both Atlantic and Pacific Canada and yielded negative results to date. However, these observations require further investigation, i.e., development of probes sufficiently sensitive to detect sub-clinical infections (see Section 6.2.3).

17.2 Conclusion

Decreasing prevalences of infection in Lake Grevelingen European oyster beds continue to occur in the presence of low densities of oysters. Based on the experience of the USA in developing and assessing resistance of oysters (*C. virginica*) to MSX, the field challenge experiments proposed by The Netherlands should ensure stringent controls to differentiate between disease resistance and some other effect on the prevalence of infection. Control oysters are required to accurately assess resistance, i.e., laboratory exposure of Lake Grevelingen oysters and known susceptibles. Uniform susceptible control stock for field trials at defined densities up to the 300–500/100 m^2 (pre-*Bonamia* levels) in the field might also be used. Ideally, both susceptible and potentially resistant Lake Grevelingen oysters should be spawned under identical conditions and the F1 generation from this spawning used for both field and laboratory-based challenge trials. The same type of trial is required to confirm the reports of *Bonamia ostreae* disease resistance in northeastern Pacific oysters.

17.3 Recommendations

1. It is recommended that ICES Member countries which have commercially important *O edulis* stocks consider the question of possible temperature inhibition of clinical infections. This is necessary to ensure that all possible factors responsible for inhibiting *Bonamia* infections are evaluated when assessing possible development of resistance.

2. ICES Member countries with enzootic infections by *Bonamia ostreae* are encouraged to test *Bonamia*-negative oysters from historically uninfected northern stocks, i.e., Canada, Scotland, Denmark and Norway.

2

2. Note the average of samplings does not reflect the cumulative mortality data, which is much greater.

3. ICES Member countries with areas endemic for bonamiasis are encouraged to collect: i) prevalence and mortality data from affected oyster beds; ii) oyster population density data; and iii) historic records of infections and stocking data (if available). This is necessary to enhance and reinforce the data base currently being compiled by The Netherlands on bonamiasis.

18 COMPILATION OF SPECIFIC REQUIREMENTS OF SHELLFISH PATHOLOGY LABORATORIES FOR RECORDING SHELLFISH DISEASE DATA AND EVALUATE THE APPLICABILITY OF THE ICES FISH DISEASE ENTRY PROGRAM (FDE) AND THE FISH DISEASE REPORTING FORMAT

Shellfish diagnostic laboratories that are government-based (e.g., IFREMER, DFO, NOAA-DNR) conduct various types of diagnostic activities and data compilation (see Annex 18). Country reports contain submissions from all these sources, however the only data likely to be available for submission to the ICES environmental databank will be from government surveys (there are some exceptions of university, thesis-based, surveys). There are few shellfish pathology surveys currently underway and several laboratories have reported cutbacks to survey funding.

Although the ICES environmental databank could be used as a databank for shellfish diseases, the data currently being collected by various laboratories are not directly applicable to the program because of the various formats in use. Also, each laboratory has different reasons and uses for the data they collect which often makes data collected by different laboratories incompatible.

The primary problem with investigating the applicability of shellfish disease data to the environmental assessment objective of ICES is selection of appropriate host and disease agents. Shellfish data may have to comprise a broader range of hosts and diseases than addressed by the cruise-based finfish collections. Preliminary attempts to compile base-line data, using a spreadsheet format have failed, due to the diversity of laboratory operations outlined in "Specific Requirements of Shellfish Pathology Laboratories" (see Annex 18).

Most laboratories may not be able to comply with the requirements of collecting data for the ICES environmental databank because of the added cost of examining shellfish (histological examination is required for most diseases as indicated in Annex 18). Also, many shellfish laboratories are independent and will find it difficult, if not impossible, to cover the additional costs.

Shellfish disease data collected by many laboratories tends to be restricted in scope. Thus, such data may be vulnerable to misinterpretations if applied directly to the analysis requirements of the ICES database.

18.1 Recommendations

i) The WGPDM members should compile a list of laboratories in their country that are conducting shellfish disease surveys and/or diagnosis indicating (a) the name and address (including mail, phone, FAX and E-mail) of the individual(s) responsible for the work conducted in the laboratory, (b) the type of shellfish disease work currently being conducted and (c) the format and availability of data records being held. ICES Member countries with shellfish disease survey data and/or ongoing disease surveys should consider submission of the data for compatibility examination with the ICES Environmental Databank.

ii) ICES Member countries are encouraged to consider establishing shellfish surveys for monitoring health status in relation to long-term natural and anthropogenic changes in the marine environment.

19 QUANTITATIVE INFORMATION ON FISH ESCAPES FROM MARICULTURE IN RELATION TO DISEASE TRANSFER TO WILD STOCKS

19.1 Review of Available Information

Intersessionally, all Member countries of the Working Group were requested to collate quantitative information on escapes of fish from aquaculture operations. A country by country review at the WG meeting indicated that very little concrete data were available on fish escapes and none of the information was quantified. However, there is an indication that considerable escapes occur. Data from the Faroe Islands presented in the draft report of the ICES Workshop on the Interaction between Salmon Lice and Salmonids indicated that 30 % of salmon caught by long line fishery conducted 200 nm north of the Faroes was salmon escaped from marine fish farms. Reports from Pacific Canada (BC) showed that during the period 1992 to 1996 the annual escapes of Atlantic salmon were approximately 42000 fish per year. Catches of Atlantic salmon escapees account for 0.004 % of the annual catch of Pacific salmon in 1996. Most escapes were associated with bad weather conditions, failure of equipment, or accidents.

The mechanisms to prevent disease introduction and transfer of exotic diseases from farmed or escaped fish and shellfish are listed in the OIE Aquatic Animal Health Code and the 1994 ICES Code of Practice on the Introductions and Transfers of Marine Organisms. Proper implementation of these codes should reduce the risk of spread of new diseases. Most diseases in farmed fish reflect the presence of disease in the surrounding natural environment. The highest risk appears to lie with local endemic diseases and the subsequent magnification of them through mariculture back into the wild population. A review in progress on disease implications of farmed wild salmonid interaction (ICES/NASCO Bath Symposium April 1997) (Annex 19) indicates that there is limited evidence of significant disease transfer from farmed to wild fish. A possible exception to this may be the sea lice situation, but the degree of interaction and consequences have not been quantified. No diseases in sea bass, sea bream or turbot are known to present a risk to locally occurring species. However, there is a potential for disease transfer to wild shrimp stocks by escapes of cultured shrimp. Unfortunately no information is available on this subject. One of the greatest difficulties in assessing the risk involved is the dearth of knowledge concerning diseases that occur in wild shrimp.

Escapes of molluscs from aquaculture production is not regarded as a problem in relation to spread of exotic diseases a) if the guidelines in the above mentioned Codes of Practice are followed and b) because of the limited mobility of these species. Motile spawning products have not been implicated in the transmission of any molluscan diseases to date.

19.2 Conclusions

Escapes of farmed fish have not been quantified due to the lack of defined data-gathering methods. Therefore, the possible risk of disease transfer from farmed to wild fish could not be determined. Exotic diseases are satisfactorily addressed by legislation codes of practice, but endemic diseases are of concern.

20 ICES DISEASE PUBLICATIONS — DIAGNOSTIC FICHES UPDATE

With the resignation of the current Editor of the *ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish* (Fiches) the need to identify a successor was intimated to the WGPDMO by the ICES General Secretary. The WGPDMO proposed S McGladdery (Canada) as the new Editor. As S McGladdery was prepared to accept the nomination, the WGPDMO Chairman agreed to forward her name to the Chairman of the Consultative Committee for consideration.

A list of new Fiches which are in the process of being published was provided to the WGPDMO. The value of the Fiches as practical reference guides was agreed by the participants. Suggestions for updated versions of some existing titles, where significant new information has become available since their publication, and of additional titles, should be forwarded to the series Editor by WG participants. To assist in identifying these the Editor will circulate all WG participants with the most recent list of Fiche titles and authors. It was recommended that the Editor of the Fiches select several members of the WGPDMO to assist in the editorial workload, both in technical aspects and in translation.

21 ANY OTHER BUSINESS

The notification from the 1996 ICES Annual Science Conference of the intention to hold a Theme Session on "Hygiene in Mariculture" at the 1998 ASC was discussed by the WGPDMO. The proposed outline programme on disease aspects contained in the 1996 WGPDMO Report was again considered acceptable. F Baudin- Laurencin (France) and S McGladdery (Canada) were appointed as organisers representing WGPDMO topics and they were requested to consult with the Chairman of the WG on Marine Fish Culture to jointly organise the Theme Session. WGPDMO members were requested to identify groups or individual scientists appropriate to approach for contributions and to pass this information to the above organisers.

22 ANALYSIS OF PROGRESS WITH TASKS

An analysis of progress of tasks in the Terms of Reference was conducted and presented in Annex 22. 1. All items had been dealt with in a comprehensive manner. Several intersessional tasks were identified during the meeting.

23 FUTURE ACTIVITIES OF THE WGPDMO

The future activities of the WGPDMO were discussed and it was agreed that there was sufficient work to require meeting in 1998. Proposals for topics to be considered, in the form of Terms of Reference for 1997 which could be recommended to Council, were compiled. (annex 23.1). An invitation was received from Professor Z Polanski to meet in the Sea Fisheries Institute, Gdynia, Poland, a proposal which met with unanimous approval by participants of the WG.

23.1 Justifications for Recommendations to Council

1. There are continued developments in the fields of pathology and diseases of wild and cultured marine organisms requiring specialist assessment and advice to ICES. To provide this, it is recommended that the WGPDMO should meet again during 1998. The proposal was made to meet in the Sea Fisheries Institute, Gdynia, Poland. Because of the need for information by other ICES groups for advice from the WGPDMO, it was proposed that the meeting should be held during March 1998.

1.a, 1.b. WGPDMO are required to maintain an overview of new trends in diseases of wild and cultured marine animals and to assess the significance of these to wild fisheries, environmental aspects and mariculture.

1.c. Data output from the ICES Environmental Database on fish diseases should be analysed and interpreted. Studies on fish liver histopathology are an important component of fish disease monitoring programmes carried out by ICES Member Countries and will be part of the biological effects component of the OSPAR Joint Assessment and Monitoring Programme and the Quality Status Report 2000 for the north-east Atlantic. Therefore, these data should be submitted to the ICES fish disease database on a regular basis according to a standard format and analysed together with other diseases.

1.d. A level of quality assurance is already achieved on the diagnosis of liver pathologies through the submission of material suspected to be tumours to one lab (CEFAS Weymouth). However, to take on board the recommendations of the ICES Special Meeting in Weymouth it became apparent that further intersessional development work is needed to take liver histopathology to the same level as external fish diseases. The WGPDMO therefore requested some of the members who are specialists in this area to undertake some intersessional work (by correspondence).

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

1.g. Research in this field is rapidly developing in many ICES Member countries. The biochemical and toxicological aspects are dealt with in other ICES WGs, but the evaluation of the pathological aspects and possible impact on the population level of marine and estuarine organisms within this field should be dealt with by the WGPDMO.

1.h. Training and intercalibration constitute an essential requirement for quality assurance of biological effects measurements within international monitoring programmes. Studies on fish liver histopathology are an important component of fish disease monitoring programmes carried out by ICES Member Countries and will be part of the biological effects component of the OSPAR Joint Assessment and Monitoring Programme and the Quality Status Report 2000 for the north-east Atlantic. Therefore these data should be submitted to the ICES Environmental Databank on a regular basis according to a standard format for subsequent analysis.

1.i. In-depth examination of the European oysters which are negative for *Bonamia* is necessary to ensure that all possible factors a responsible for inhibiting infection are evaluated when assessing possible development of resistance.

1.j. Long-term data on shellfish diseases is currently scattered within ICES Member Countries and the ICES Environmental Databank is encouraged to be used for monitoring health status in relation to long-term natural and anthropogenic changes in the marine environment.

1.k. Nodavirus is currently one of the most preoccupying problems in some farmed fish species. New nodavirus-disease seems to occur in species not previously associated with the disease. Diagnostic tools must be developed and validated to meet this new phenomenon. Epidemiological and immunological studies are encouraged as a basis for sanitary control.

24 APPROVAL OF RECOMMENDATIONS TO COUNCIL

The recommendations contained in this Report to ICES Council were discussed by the WGPDMO and approved.

25 APPROVAL OF THE DRAFT WGPDMO REPORT

A draft copy of the 1997 meeting of the WGPDMO was submitted to all WG members present before the end of the meeting and approved. These terms of reference where advice was specifically sought by other ICES bodies would be extracted with the relevant Annexes and sent separately to ICES.

26 APPOINTMENT OF A NEW WGPDMO CHAIRMAN

A formal election to appoint a new Chairman of the WGPDMO was conducted. S Mellergaard was proposed by A D Vethaak and seconded by T Lang. The appointment of S Mellergaard was approved unanimously by the WGPDMO members participating.

27 CLOSING OF THE MEETING

On behalf of the participants at the 1997 WPDMO meeting, the Chairman expressed appreciation to Sharon MacLean, NOAA for the excellent organisation of the meeting and for her major assistance, happily and cheerfully given in so many ways which enabled the WG to function in such a productive and pleasant way. He also thanked the staff of the Centre who made the participants stay such a memorable one.

As outgoing Chairman, he expressed his thanks for the exceptional commitment and strong team spirit which the WG participants had consistently shown throughout his five years as Chairman, particularly in view of the heavy programme of work allocated to each meeting. He expressed his good wishes and full support to the incoming Chairman. The meeting was closed at 1230 on 20 March 1997.

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS
University of Rhode Island, USA, 16 - 20 March 1997.

Annex 1.1 LIST OF PARTICIPANTS

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ANNEX 3.1. TERMS OF REFERENCE FOR THE 1997 WGPDMO MEETING

C.Res. 1996/ 2:29 The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chairman: Dr A.H. McVicar, UK) will meet at a venue in Rhode Island, USA from 16-20 March 1997 to:

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

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

c) review the Report of the Study Group on Statistical Analysis of Fish Disease Data and interpret the data provided;

d) review the results of the Special Meeting on "The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" and assess progress on the implementation of standardised methodologies for the characterisation of liver pathology in disease monitoring programmes, in consultation with the Working Group on Biological Effects of Comtaminants;

e) develop a quality assurance plan to incorporate liver pathology and fish disease measurements within the biological effects monitoring component of the Joint Assessment and Monitoring Programme of OSPAR [OSPAR 1997/2.2];

f) review the results of the BMB/ICES Seagoing Workshop on "Fish Diseases and Parasites in the Baltic Sea" in the light of their possible implications for future research/monitoring programmes in the Baltic Sea;

g) compile available evidence on the causes of the M-74 syndrome in Baltic salmon and provide a summary of the progress in understanding the relevant environmental factors influencing the occurrence of M-74 along with an account of the geographical extent of its distribution [HELCOM 1996/8];

h) maintain an overview of the *lchthyophonus* issue as part of its regular agenda and report to the Advisory Committee on Fishery Management and the Advisory Committee on the Marine Environment if new information becomes available;

i) review current information on the impact of environmental contaminants on the immune mechanisms of marine finfish and shellfish in collaboration with the Working Group on Biological Effects of Contaminants;

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

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

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

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

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

o) provide quantitative information on the escape of fish from mariculture operations in the context of the transfer of disease to wild stocks and advise on the means by which this impact could be controlled and report to ACME before its June 1997 meeting [OSPAR 1997/4.1].

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Rhode Island, USA, 16 - 20 March 1997.

ANNEX 3.2. AGENDA

1. Opening of the meeting.
2. ICES ASC 1996; items of relevance to WGPDMO.
3. Terms of Reference, adoption of the 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 (TOR a).
6. Analysis of national reports on new diseases and disease trends in cultured fish, crustaceans and molluscs (TOR b).
7. Evaluate and interpret the Study Group Report on the Statistical Analysis of Fish Disease Data (TOR c).
8. Evaluate the Report of the Special Meeting on Flatfish Liver Pathology (TOR d).
9. Develop a quality assurance plan on liver pathology analysis (TOR e).
10. Review results of the BMB/ICES Sea Going Workshop (TOR f).
11. Compile available evidence on causes of M-74, influencing factors and distribution (TOR g).
12. Overview new information on *Ichthyophonus* (TOR h).
13. Review information on contaminant effects on the immune mechanisms of (i) fish and (ii) shellfish (TOR i).
14. Review information on nodavirus/nodavirus-like agents (TOR j).
15. Review information on environment - pathology correlations (ecopathology) as a basis for alternative disease control measures (TOR k).
16. Alternative host species for the transmission of bivalve pathogens (TOR l).
17. Causes of persistence of *Bonamia ostreae* and relationship to disease tolerance (TOR m).
18. Requirements for shellfish disease data recording and compatibility with the ICES Fish Disease Data format (TOR n).
19. Quantitative information on fish escapes from mariculture in relation to disease transfer to wild stocks (TOR o).
20. ICES Disease publications. Diagnostic Fiches update,
21. Any other business.
    ICES 1998 ASC Special Topic on Hygiene in Mariculture.
22. Analysis of progress with tasks.
23. Future activity of WGPDMO.
24. Approval of recommendations.
25. Approval of draft WGPDMO Report.
26. Appointment of new WGPDMO Chairman.
27. Closing of the meeting.

ANNEX 3.3. RAPPORTEURS

Session(s)	Rapporteurs
1-4.Introductory sessions	McVicar
5. Wild fish Wild shellfish	Feist, Karasev, DeClerc McGladdery, Bowers
6. Farmed fish Farmed shellfish	Hjeltnes, Bylund McGladdery, Bowers
7. Disease data	Vethaak, Lang
8. Liver pathology	Feist, Lang
9. QA	MacLean, Feist, Lang
10.BMB/ICES Workshop	Lang, Mellergaard
11.M74	Mellergaard, Bylund
12. *Ichthyophonus*	Hjeltnes, McVicar
13.Contaminants/immunity	Vethaak, Bylund, MacLean
14. Nodavirus	Baudin-Laurencin, Hjeltnes
15.Ecopathology	Baudin-Laurencin, McGladdery
16-18. Alternative hosts, tolerance,	McGladdery, Bowers
19.Escapes	Mellergaard, Lindesjoo
20. Publications	McVicar
21-24. AOB, progress, recommendations	Lindesjoo, MacLean, McVicar
25-27. Approval, Chairman appointment , closing	McVicar

ANNEX 6.1 NEW INFORMATION ON DISEASES AND PARASITES OF MOLLUSCS

Malpeque disease of American oysters (*Crassostrea virginica*) remains under investigation. There have been no new developments since the 1995 report from Canada and the causative agent is still under investigation.

Bonamia ostreae of European oysters (*Ostrea edulis*) was surveyed for the first time in recent years in Norway and showed no *Bonamia* (or *Marteilia*) infections. In Denmark a survey of oysters in the Limfjord revealed no *Bonamia.* Historically, oysters imported in the early 1980s from France, to replenish overfished stocks, were found to be infected by *Bonamia.* Affected oyster beds were dredged and the Limfjord population did not recover until 1994/5. The re-establishment of the fishery led to re-examination of their disease status in 1996. The disappearance of *Bonamia* may reflect the cold-water climate which correlates to those of Pacific and Atlantic Canada, Norway and Scotland, which have also yet to detect established *Bonamia* infections in their European oysters.

Brown Ring Disease (BRD) of Manila clams (*Tapes (=Ruditapes) philippinarum*) is no longer studied in the Mediterranean Sea due to the decreased interest in growing Manila clams in these waters. There is no reported change in the disease status of BRD in France and there were no cases reported in northwestern Spain. The etiological agent of BRD, previously referred to as *Vibrio* P1, has now been classified as *Vibrio tapetis.*

Marteiliasis

***Marteilia* sp.(p).** in Pacific oysters (*Crassostrea gigas*) along the northern Mediterranean coast of Spain (Catalonia) requires specific identification (currently based on spore morphology and formation), because only presporulation stages have been observed in *C. gigas*.

Marteilia refringens was not observed in European oysters (*Ostrea edulis*) from Norway or Denmark.

MSX (*Haplosporidium nelsoni* of American oysters (*Crassostrea virginica*)) DNA probe used in preliminary test yielded positive results from water, sediment and zooplankton samples from the lower Chesapeake Bay (known to have high infection pressure). Concurrent sampling in Delaware Bay has yielded negative results, so far, possibly due to a recent decrease in prevalence of MSX in local oysters. An important initial finding with the use of this probe is the need to ensure rapid processing of samples. Long-term storage posed some problems with respect to DNA quality, especially for sampling with potentially low quantities of MSX DNA.

Perkinsiosis:

Perkinsus marinus (Dermo) in selectively bred American oysters, which survive Dermo infections, show improved survival and delayed onset of disease and mortality.

Perkinsus atlanticus infections in Spain are stable, but continue to be a significant concern for clam culture (*Tapes decussatus, T. semidecussatus, T. philippinarum* and *Venerupis pullastra*). Infections were also reported from *T. decussatus* and *R. philippinarum* from France with no changes in the prevalence and distribution.

***Herpes*-like virus**

Crassostrea gigas (Pacific oyster) infections were diagnosed in 1996 using two diagnostic tools (PCR and southern blot) recently developed at the IFREMER laboratory, La Tremblade, France. *Herpes*-PCR detected positive *C. gigas* infections during the summer months in larvae and juveniles from hatchery, nursery and natural broodstock sources. Due to broad deployment of hatchery seed throughout France, the exact foci of infection are not possible to detect. Detection of *Herpes*-DNA is still being analysed in relation with mortality data associated with Summer Pacific Oyster Mortality (SPOM).

Ostrea edulis (European oyster) infections can be detected using the *Herpes*-PCR technique, however, *O. edulis* mortalities decreased in hatcheries and nurseries in France in 1996, thus, few specimens of this species were screened for *Herpes* using the DNA probe.

Denman Island Disease (*Mikrocytos mackini*) of Pacific oysters (*Crassostrea gigas*) on the west coast of Canada, although pathogenic for all species of oyster tested (*Crassostrea virginica, Ostrea edulis* and *Ostrea conchaphila*), did not develop in any of the oysters held at 15 °C or warmer temperatures. No change observed in geographic distribution, prevalence and intensity from previous years.

Summer Pacific Oyster Mortality (SPOM) occurrence in Pacific oysters (*Crassostrea gigas*) in The

Netherlands may be correlated to temperature. This disease was evident during the hot summers of 1994 and 1995 but was absent during the normal "cool" summer of 1996. This observation corroborates the French suggestion that SPOM is probably a "high temperature stress" mortality (probably related to infection with secondary, opportunistic agents in the weakened oysters).

Quahaug parasite X (QPX) of hard-shell (quahaug) clams (*Mercenaria mercenaria* and in the phenotypic variety *M. m.* (var.) *notata*) in Canada continues to cause mortalities in hatchery-held broodstock, but wild quahaugs have not yet been found with systemic infections (although focal infections are occasionally observed). Field exposure experiments in Massachusetts indicate that QPX infection spreads slowly.

Juvenile Oyster Disease (JOD) decreases in the cultured American oysters (*Crassostrea virginica*) were attributed to the implementation of appropriate management methods (i.e., deployment of larger seed (>25 mm shell height) into culture containers before the onset of disease with warm summer temperatures and use of resistant stocks (survivors of past epizootics)). The cause of the disease is still under investigation.

Scallop Protistan X (SPX) reported to cause one incidence of high mortalities in market sized, cultured Japanese scallops (*Patinopecten yessoensis*) in British Columbia, Canada is not likely to recur because hybrid scallops (Japanese scallops, that survived an SPX epizootic, crossed with native weathervane scallops) were proven to be resistant to SPX in laboratory challenge experiments and field trials. SPX did not develop in two native species of scallop (*Chlamys hastata* and *Chlamys rubida*) and was not infective to Pacific oysters (*Crassostrea gigas*). SPX was described as a new species of *Perkinsus* (manuscript submitted to Canadian Journal of Zoology in March 1997). Although morphologically similar to other species of *Perkinsus*, unique characteristics include the occurrence of all life stages (including biflagellated zoospores) in the scallop tissues, development and pathogenicity at cool temperatures (about 10 °C), lack of positive reaction in the Ray's test (no iodine staining of hypnospores following "culture" in thyoglycollate medium), and occurrence of about 40% divergence in the ITS1 and ITS2 regions of the ribosomal RNA of SPX in comparison to two other species of *Perkinsus* (divergence between the other species was 13% or less). These characteristics alter some parameters used to identify this group of shellfish pathogens.

Gonadal neoplasms (germinoma) does not seem to compromise the survival of the soft-shell clam (*Mya arenaria*). However, the majority of the affected clams were female which could produce only one third as many oocytes as healthy clams, thereby impacting on reproductive potential. No etiological agent has been identified but a viral cause and association with toxic algal blooms have been proposed.

Imposex and Intersex investigations in Germany have revealed a strong correlation between severity of female gonadal alteration in the periwinkle *Littorina littorea* and concentration of TBT. Investigation of the deep-water gastropods (*Buccinum* and *Neptunea* spp.) reinforced last years observation that the phenomenon was not restricted to shipping lanes or inshore areas. Similar findings are beginning to emerge from TBT-gastropod investigations of *L. littorea* and the dogwhelk *Nucella lapillus* in Atlantic Canada.

ANNEX 6.2 NEW INFORMATION ON DISEASES AND PARASITES OF CRUSTACEANS

***Hematodinium* spp.**

Hematodinium perezi in juvenile blue crabs (*Callinectes sapidus*) showed a significant reduction in the prevalence of infection from December to March (95% to 0%). However, it is unknown if infected crabs die or rid themselves of the infection. During preliminary experiments, there was a reduction in infection intensity in crabs held at 9 °C. This parasite was also found in green crabs and mud crabs from Maryland Bays, but not in amphipods.

***Hematodinium* sp.**, the cause of bitter crab disease in *Chionoecetes* spp. is still present in *C. bairdi* in southeastern Alaska, USA, and remained patchey in *C. opilio* in the Bering Sea.

***Hematodinium* sp.**, in the Norway lobster (*Nephrops norvegicus*) continued to occur in the Botney Gut - Silver Pit areas of the North Sea with highest prevalences (10%) in the small lobsters.

Hematodinium - like protistan in spot prawns (*Pandalus platyceros*) in British Columbia was not encountered during 1996.

Viruses in cultured penaeids (native white shrimp and specific pathogen free Pacific white shrimp from Hawaii) from a state hatchery in South Carolina were initially identified as white spot virus (WSV) and yellowhead virus (YHV) that cause significant losses of cultured penaeids in Asia. However, PCR testing using various primers gave conflicting results leading to the speculation that WSV and YHV "like" viruses may be enzootic to indigenous wild shrimp which tested positive with one of the probes. This possibility is currently under investigation.

ANNEX 8.1 Summary of the ICES Special Meeting on 'The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants', 22-25 October 1996, Weymouth, UK

The meeting was held at the Weymouth Laboratory of the MAFF Centre for Environment, Fisheries and Aquaculture Science (CEFAS) under the co-convenorship of S.W. Feist (UK) and T. Lang (Germany) with the assistance of A. Köhler (Germany) as representative of the ICES Working Group on Biological Effects of Contaminants (WGBEC). 18 scientists from 9 ICES Member Countries attended the meeting.

The main objective of the meeting was to bring together specialists from ICES Member Countries involved in research/monitoring programmes on contaminant-associated liver pathology of flatfish in order to

- *provide an overview of on-going activities in ICES Member Countries*
- *discuss and evaluate the suitability and applicability of different techniques used to measure liver pathology of fishes for monitoring purposes*
- *elaborate standardized guidelines and quality assurance procedures for field sampling, processing and interpretation of liver pathology for monitoring programmes*

Detailed information on the programme of the meeting is given in the attached Agenda. Basically, the meeting was split into two sections. The first consisting of the presentation of key note talks and reports of national activities as well as discussions on the formulation of recommendations on appropriate biological effects techniques and quality assurance procedures. Secondly, practical sessions were devoted to the demonstration of different techniques by selected participants and assessments on the classification and intercalibration of histopathological liver lesions using sets of prepared microscope slides submitted by the participants.
Biological effects techniques for measurement of fish liver pathology for monitoring purposes recommended by the participants are given in Table 1. The full report contains guidelines for methodologies to be used for liver histopathological studies, cellular/subcellular and biochemical biomarker studies and for quality assurance procedures. Including are recommendations for the distribution of reference material and on the identification of a possible lead laboratory for liver histopathology of marine fish. Detailed information is also provided on sampling strategies and classification criteria for histopathology diagnosis.
The main conclusions and recommendations of the ICES Special Meeting are given below.

General Conclusions

- The participants of the ICES Special Meeting on the Use of Liver Pathology for Monitoring Biological Effects of Contaminants agreed that the meeting constituted an important step forward in the identification and standardisation of suitable techniques for monitoring biological effects of contaminants.
- Biological effect monitoring programmes should be designed according to accepted epidemiological principles and methods and should include histological and cellular/subcellular biomarkers. If a logical decision tree approach is used, these biomarkers have high potential for risk assessment.
- Future research should focus on developing more sensitive biomarkers of contaminant exposure and effects in order to elucidate the underlying mechanisms involved in liver pathogenesis/carcinogenesis.
- Future joint studies between ICES Member Countries and other countries (such as PICES Member Countries) working in the area of contaminant-associated liver pathology of marine fish species should be encouraged by organising symposia, workshops and joint research activities.

Recommendations

Based on the results of the discussions and practical workshops, the participants of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects recommended that

- ICES Member Countries should be encouraged to incorporate histological liver lesions and biomarkers, particularly those recommended in the report of the ICES Special Meeting, in national monitoring programmes on biological effects of contaminants. Methodologies should be according to procedures and diagnostic criteria detailed in the report.
- monitoring should be combined with research activities in order to evaluate and, as appropriate, implement new promising biological effects techniques and to elucidate underlying mechanisms involved in the development of pathological liver changes.
- the ICES Working Group on Pathology and Diseases (WGPDMO) and the ICES Working Group on Biological Effects of Contaminants (WGBEC) review the results of these studies on a regular basis, possibly at joint special topics meetings.

- data obtained from studies on liver pathology and related biomarkers are submitted to ICES and that the ICES WGPDMO together with the ICES secretariat explore ways to incorporate the data into the ICES Environmental Databank for subsequent statistical analysis.
  the MAFF CEFAS, Weymouth, UK acts as reference laboratory for liver histopathology quality assurance procedures involving the preparation and distribution of reference materials and the organisation of intercalibration exercises and workshops as appropriate.
- the technical aspects detailed in the report are published in the ICES TIMES Series under the co-authorship of A. Köhler-Günther, S.W. Feist and T. Lang taking into consideration a draft manuscript already prepared by A. Köhler-Günther.
- the proceedings of the ICES Special Meeting are published in the ICES Journal of Marine Science with all participants as co-authors in order to guarantee a more widespread distribution of the findings and conclusions of the meeting in the scientific community
- a colour atlas on liver histopathological changes of selected marine flatfish species from European and US waters is published, partly based on the liver lesions classified at the ICES Special Meeting
- international environmental monitoring organisations consider the results of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants and incorporate recommended biological effects techniques and related quality assurance procedures into monitoring programmes as appropriate.

Table 1: Biological effects techniques for measurement of fish liver pathology recommended for monitoring purposes

Effects measure	Technique	Status	Contaminant response
macroscopic liver changes			
• nodules > 2 mm in diameter	macroscopic, subsequent histological confirmation, paraffin sections, H&E	**A**	neoplasia: probably specific, carcinogens (1) non-neoplastic: probably unspecific
liver histopathology			
• general necrotic/degenerative changes	paraffin sections, H&E staining	**B**	probably unspecific (2)
• unique degenerative changes	same	**B**	specific, PAHs, PCBs, DDTs (1)
• storage conditions	same	**B**	probably unspecific (3)
• inflammatory changes	same	**B**	probably unspecific (3)
• non-neoplastic proliferative lesions	same	**B**	probably unspecific (3)
• vascular abnormalities	same	**B**	probably unspecific (3)
• foci of cellular alteration	same	**B**	probably specific (1)
• benign tumours	same	**B**	probably specific (1)
• malignant tumours	same	**B**	probably specific (1)
liver histochemistry			
• lysosomal membrane stability	cryo sections, neutral red	**B**	general toxicity
• enzyme-altered foci (G6PDH)	cryo sections,	**B**	specific
liver immuno-histochemistry			
• proliferating cell nuclear antigen (PCNA)	paraffin sections, antibodies	**B**	regeneration, proliferation (3)
• CYP1A	paraffin sections, antibodies	**B**	polar contaminants, PAHs, PCBs
liver biochemistry			
• CYP1A (EROD)	photometrically	**B**	polar contaminants, PAHs, PCBs
• DNA adducts	^{32}P-postlabelling	**B**	genotoxicity (PAHs, others)

Status A: Quality assurance in place **B**: development and intercalibration needed **(1-3)** : relative importance as biomarker for exposure/toxicity, 1 highest importance

ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants
Agenda

1. Opening of the meeting and general introduction
2. Adoption of the agenda
3. Appointment of rapporteurs
4. Current status of studies on liver pathology
4.1 Histological, cellular/subcellular and biochemical techniques and their field application
4.2 European epidemiological studies
4.3 US perspective
4.4 Reports on national activities in ICES Member Countries
4.5 Experimental studies on hepatic carcinogenesis in fish
4.6 Summary review of the current status of studies on flatfish liver pathology.
5. Assessment of techniques for the determination of liver pathology
5.1 Histopathology and electron microscopy
5.2 Cellular/subcellular biomarkers
5.3 Biochemical biomarkers
5.4 Integration of chemical and biological effects monitoring
6. Practical workshop: demonstrations of techniques
6.1 Histopathology
6.2 Glucose-6-phosphatase dehydrogenase
6.3 Proliferating cell nuclear antigen
6.4 Immunohistochemical demonstration of cytochrome P4501A1
6.5 Lysosomal membrane stability
7. Recommendation of techniques for monitoring purposes
8. Standardisation of monitoring techniques
8.1 Field sampling
8.2 Processing
8.3 Diagnostic criteria for histopathology
8.4 Quality assurance requirements
9. Any other business
10. General conclusions
11. Recommendations
12. Approval of the report and closing of the meeting

ANNEX 9.1

Development of a quality assurance plan to incorporate liver pathology and fish disease measurements within the biological effects monitoring component of the Joint Assessment and Monitoring Programme of OSPAR (OSPAR 1997/2.2)

Introduction to the OSPAR Joint Assessment and Monitoring Programme (JAMP)

In 1994, the Oslo and Paris Commissions (OSPARCOM) agreed to develop a new joint monitoring programme for the maritime area of the Oslo and Paris Conventions, to update and take over from the OSPAR Joint Monitoring Programme (JMP) and the Monitoring Master Plan (MMP) of the North Sea Task Force (NSTF). The new joint monitoring programme, the Joint Assessment and Monitoring Programme (JAMP), was adopted by the Commissions in 1995. The JAMP will form the basis of five regional Quality Status Reports (QSRs) (Arctic Waters, the Greater North Sea, The Celtic Seas, the Bay of Biscay and Iberian Coast, and the Wider Atlantic) to be completed by mid-1999 the results of which will be synthesised in the QSR 2000 for the whole convention area which will be produced by 31 December 1999.

As major improvement, the JAMP will include a strong component focusing on biological effects of contaminants, which will be fully integrated with the use of diagnostic chemical analysis, and the broad objectives of which will be to

- identify where contaminants are causing biological effects
- predict the highest organisational level at which these effects occur (i.e. cell, individual, community or ecosystem)
- to determine whether these effects result in harm to living resources and/or marine ecosystems, or otherwise interfere with other legitimate uses of the sea

The definition of the biological effects monitoring and assessment component of the JAMP, including the identification of suitable biological effects techniques, was addressed at the OSPAR/ICES Workshop on Biological Effects Techniques, Aberdeen, Scotland, 2-6 October 1995. Based on the outcome of this expert workshop, the OSPARCOM Ad Hoc Working Group on Monitoring (MON) elaborated draft guidelines for biological effects monitoring at its Stockholm meeting, 4-8 November 1996.

In these guidelines, studies on external(ly visible) fish diseases, liver nodules and liver (histo)pathology are amongst the suite of techniques recommended for use in the following tiers of the OSPAR JAMP:

General biological effects monitoring

- to monitor the general status of the marine environment
- to assess areas with known or suspected environmental impact from contaminants

Contaminants-specific biological effects monitoring

- to establish the spatial distribution and extent of biological effects of specific contaminants in marine organisms
- to establish temporal trends in biological effects in order to estimate the magnitude of changes in biological effects with time

Studies on liver (histo)pathology, liver nodules and external(ly visible) fish diseases were recommended for general biological effects monitoring (the first two were classified as biomarkers for early changes and the latter as indicator for population/community responses). For contaminant-specific monitoring (PAHs), only liver (histo)pathology was recommended besides other techniques.

In the guidelines, information is provided on target organs/organisms for the above studies, effects measured, means of interpretation, methodologies, and the establishment of quality assurance/control procedures. Details can be found in extracts from the Annexes of the OSPARCOM Summary Record of the meeting of MON 1996 which are attached as WGPDMO Appendices 1,2 and 3:

- ANNEX 12: **Draft guidelines on contaminant-specific biological effects monitoring** (Technical Annex 2)

- ANNEX 14: **Draft guidelines for general biological effects monitoring** (Technical Annex 1).

- ANNEX 13: **Taking QA Forward in Biological Effects Monitoring for the JAMP**

State-of-the-art quality assurance of studies on externally visible fish diseases, liver nodules and liver histopathology

Since studies on the prevalence and spatial distribution of diseases/parasites of wild marine fish have constituted an important component of national monitoring programmes implemented in many ICES Member Countries to assess biological effects of anthropogenic activities in the marine environment for the last 1-2 decades, and since ICES bodies such as the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) have always been involved in the planning and co-ordination of such studies, methodologies applied have been intercalibrated and standardised to a large extent. Recommendations derived from discussions and practical exercises carried out during two sea-going workshops (1984 in the North Sea, 1988 in the Kattegat) have been published (Dethlefsen *et al.* 1986, Anonymous 1989, Anonymous 1996, Bucke *et al.* 1996) and form the basis of most of the on-going programmes in the ICES area. For the Baltic Sea (which is not part of the OSPAR Convention area), intercalibration and standardisation of methodologies have been achieved due to activities of the Baltic Marine Biologists (BMB) Working Group 25 'Fish Diseases and Fish Parasites in the Baltic' and the joint BMB/ICES Sea-going Workshop 'Fish Diseases and Parasites in the Baltic Sea' held in 1994 (Lang *et al.* 1996) (see separate section in the WGPDMO report).

Within the ICES Environmental Databank there is a section with fish disease prevalence data which are annually submitted to ICES by ICES Member Countries according to well-defined quality assurance procedures. These procedures have been developed by the WGPDMO and the ICES Sub-group on Statistical Analysis of Fish Disease Data in Marine Stocks. Furthermore, the Sub-group and its successor, the ICES Study Group on Statistical Analysis of Fish Disease Data in Marine Stocks, developed standardised methods for statistical analysis of fish disease data hold by ICES (see separate section in the WGPDMO report).

In conclusion, quality assurance guidelines exist for the following aspects of fish disease monitoring:

- sampling strategies
- suitable fish species
- externally visible diseases/parasites suitable for monitoring purposes
- monitoring of liver nodules
- diagnostic techniques, including grading systems, of externally visible diseases
- histological confirmation of liver nodules by a reference laboratory
- data reporting
- data analysis

Therefore, quality assurance requirements can be considered fulfilled and, therefore, studies on externally visible fish diseases and liver nodules according to the ICES guidelines are readily applicable in international monitoring programmes such as the OSPAR JAMP.

The above mentioned quality assurance activities focused mainly on externally visible diseases/parasites and on macroscopic liver nodules but did not cover the use of liver histopathology as indicator of biological effects of contaminants. This issue was addressed recently at the ICES Special Meeting on the 'Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants', Weymouth, 22-25 October 1996 (see separate section in the WGPDMO report). In the report of the Special Meeting, detailed guidelines are provided on

- suitable techniques for monitoring purposes
- sampling strategies for monitoring of liver histopathology/histochemistry
- processing of fixed samples
- diagnosis of and classification criteria for liver histopathology

- quality assurance/control requirements, e.g. intercalibration of diagnosis, identification of a lead laboratory etc.

Extracts from the report have already been incorporated into the OSPAR MON guidelines on contaminant-specific biological effects monitoring (attached to this Annex as Appendix 1).

A description of methodologies for monitoring of liver histopathology/histochemistry is in preparation for publication in the ICES Techniques in Marine Environmental Sciences. Furthermore, it is planned to produce a colour atlas on liver histopathological changes in major flatfish species monitored in the European and North American ICES area. These publications could form the basis for further intercalibration and standardisation required for incorporation into the JAMP.

In contrast to externally visible fish diseases and liver nodules, the diagnosis of liver histopathological changes has not yet been fully intercalibrated between laboratories. However, it is envisaged that this should occur in the near future. The WGPDMO should be responsible for the planning and co-ordination of intercalibration exercises.

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ANNEX 9.2

Proposal for revision of the draft OSPAR guidelines for general biological effects monitoring under JAMP

1.2.3 Liver Histopathology

Target organ/organism:	Liver in dab (or other target species beyond dab´s distribution limits
Effect measured:	• Unique degenerative changes (hepatocellular and nuclear polymorphism, megalocytic hepatosis/hepatic megalocytosis, hydropic vacuolisation of biliary epithelial cells and/or hepatocytes) • Foci of cellular alteration • Benign tumours • Malignant tumours
Means of interpretation:	contaminant-related, particularly carcinogens
Methodology:	An ICES TIMES report should be available in 1997
Quality assurance/control:	initiated due to the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants
Suitability for application: B. Procedures for diagnosis of histopathology still need further quality assurance	

1.2.4 Macroscopic Liver Nodules

Target organ/organism:	Liver in dab (or other target species beyond dab´s distributional limits)
Effect measured:	Sampling of macroscopic liver nodules > 2 mm in diameter and subsequent quantification of histologically identified liver neoplasms
Means of interpretation:	Specifically related to liver carcinogenesis
Methodology:	ICES has published a standard protocol in TIMES No. 19 (Bucke *et al.* 1996)
Quality assurance/control:	Procedures have been established within ICES
Suitability for application: A.	

1.3.1 Externally Visible Fish Diseases

Target organ/organism:	Dab (or other species beyond dab´s distibutional limits)
Effect measured:	• lymphocystis • epidermal hyperplasia/papilloma • skin ulcerations • x-cell gill disease
Means of interpretation:	Unspecific indicator of environmental stress. Data are of value for general biological effects monitoring and for subsequent temporal trend monitoring.
Methodology:	ICES has published a standard protocol in ICES TIMES No. 19 (Bucke *et al.* 1996). Data are stored in the ICES Environmental Databank. Standardised methodologies for data submission and statistical analysis have been developed by ICES.
Quality assurance/control:	Procedures are well-established and include sea-going workshops, inter-laboratory calibration exercises and quality control of data submitted to the ICES Environmental Databank.
Suitability for application: A.	

ANNEX 10.1

Review of the the results of the BMB/ICES Sea-going Workshop "Fish Diseases and Parasites in the Baltic Sea" in the light of their possible implications for future research/monitoring programmes in the Baltic Sea.

Introduction

Studies on the prevalence and spatial distribution of diseases/parasites of wild marine fish constitute an important component of national monitoring programmes implemented in many ICES Member Countries to assess biological effects of anthropogenic activities in the marine environment.

Mainly due to activities of the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) methodologies applied in fish disease/parasite surveys have been intercalibrated and standardised to a large extent and recommendations derived from discussions and practical exercises carried out during two sea-going workshops (1984 in the North Sea, 1988 in the Kattegat) regarding suitable fish species, diseases/parasites and diagnostic techniques etc. have been published (Dethlefsen *et al.* 1986, Anonymous 1989, Anonymous 1996, Bucke *et al.* 1996). However, these recommendations were mainly based on the experience from the workshops and other studies in the North Sea, where most disease monitoring programmes have been carried out in the past two decades, and their applicability in other seas such as the Baltic Sea was never assessed thoroughly.

Although a number of countries bordering the Baltic Sea have been carrying out fish disease/parasite surveys for a considerably long time, there has always been a lack of information on prevalences and spatial distribution of fish diseases/parasites as compared to the North Sea. This is partly due to the fact that most programmes have been restricted regionally and/or temporally and/or that many results thereof have not been published in the open literature in the past. In addition, the eastern Baltic Sea countries have been underrepresented in the ICES WGPDMO for a long time and, therefore, did not actively take part in the discussion and exercises regarding the application of standardised methodologies and the attempts to combine national fish disease/parasite data for a joint statistical analysis.

In the past years, this situation has improved mainly due to activities of the Baltic Marine Biologists (BMB) Working Group 25 'Fish Diseases and Fish Parasites in the Baltic' which was established in 1989 and since then organised a number of meetings attended by scientist from Baltic Sea countries for exchange of information and co-ordination of research/monitoring. Joint activities were organised such as the 1991 BMB Sea-going Workshop 'Fish Diseases and Fish Parasites in the Baltic' (the results of which have not been published) and the 1994 BMB Symposium 'Diseases and Parasites of Flounder (*Platichthys flesus*) in the Baltic Sea' held in Turku, Finland (Bylund and Lönnström 1994).

In order to further increase the knowledge on fish diseases/parasites in the Baltic Sea and to develop standardised techniques as well as to strengthen contacts between BMB and ICES, both organisations agreed to hold a joint international sea-going workshop similar to the ones carried out by ICES before in the North Sea and the Kattegat. The formal decision was taken during the 1993 ICES Annual Science Conference in Dublin, Ireland, according to ICES Council Resolution/1993 3:9.

The workshop was held onboard the German RV "Walther Herwig III" under the co-convenorship of T. Lang (Germany) and S. Mellergaard (Denmark) with 12 scientists attending representing 8 of the 9 Baltic Sea countries. The workshop started and ended at Kiel, Germany

The objectives of the workshop were as follows:

- *to provide scientific background 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,*
- *to intercalibrate methodologies used for fish sampling, disease/parasite diagnosis, reporting and analysis of disease data,*
- *to assess the applicability of the standard methodologies recommended by ICES for fish disease surveys, and, if necessary, to recommend modifications to be followed in fish disease/parasite studies in the Baltic Sea, and*
- *to enhance international co-operation and co-ordination between Baltic Sea countries with regard to research*

and monitoring of fish diseases/parasites in the Baltic Sea.

Practical work was carried out in 11 sampling areas along a transect from the Mecklenburg Bight in the south-western Baltic Sea to the Gulf of Finland in the north-eastern Baltic Sea. The region covered represented the largest area of the Baltic Sea ever being studied for the occurrence of fish diseases/parasites during a narrow time-window and by using identical, intercalibrated methods. In total, 36 fishery hauls were performed using a standard bottom trawl. Besides fishery, hydrographic measurements (bottom water temperature, salinity and oxygen contents) were carried out.

As main target species for disease/parasite studies, the flounder (*Platichthys flesus*) was selected due to its abundance and wide distribution in the Baltic Sea and since the flounder has been recommended before by ICES as indicator species for fish disease surveys in the Baltic Sea (Anonymous 1989). Besides flounder, other abundant fish species were examined as well. These were cod (*Gadus morhua*) which has also been recommended by ICES as target species, herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). In addition, samples of turbot (*Scophthalmus maximus*) and 4-bearded-rockling (*Enchyleopus cimbrius*) were taken for bacteriological tests.

I. Scientific background data on the prevalence and spatial distribution of fish diseases and parasites in the Baltic Sea

The following publications providing scientific background data and guidelines for fish disease studies in the Baltic Sea have been prepared on the basis of the workshop results and will (probably) be submitted to the ICES Journal of Marine Science. From the titles of the papers it can be seen what kind of scientific background data were obtained during the workshop. Some preliminary results and conclusions have already been published by Lang *et al.* 1996 for the 1996 ICES Annual Science Conference.

Lang, T., Mellergaard, S. Introduction to the BMB/ICES Sea-Going Workshop 'Fish Diseases and Parasites in the Baltic Sea'.

Lang, T. Mellergaard, S., Wosniok, W., Damm, U. Kadakas, V., Neumann, K. Externally visible diseases and liver nodules in Baltic flounder (*Platichthys flesus* L.) - results of the BMB/ICES Sea-Going Workshop 'Fish Diseases and Parasites in the Baltic Sea'.

Bogovski, S., Lang, T., Mellergaard, S. Histopathological examinations of liver nodules in flounder (*Platichthys flesus*) from the Baltic Sea.

Køie. M. Metazoan parasites of flounder (*Platichthys flesus* (L.)) along a transect from the south-eastern to the north-western Baltic Sea.

Drevs, T., Kadakas, V., Lang, T., Mellergaard, S. Age/length relationship in flounder (*Platichthys flesus*) along a transect from the south-western to the north-eastern Baltic Sea.

Bogovski, S., Sergeyev, B., Muzyka, V., Karlova, S. Correlations between cytochrome P450, heme synthesis enzymes, and aromatic compounds in flounder (*Platichthys flesus*) from the Baltic Sea.

Mellergaard, S., Lang, T. Disease problems in Baltic cod (*Gadus morhua* L.) from the Mecklenburg Bight to the Estonian coast.

Grygiel, W. Externally visible pathological changes in herring (*Clupea harengus membras* (L., 1761)) and sprat (*Sprattus sprattus balticus* (Schneider, 1904)) on a transect from the south-western to the north-eastern Baltic Sea.

Wiklund, T., Tabolina, I., Bezgachina, T.V. Bacteriological investigations of fish with skin ulcerations along a transect from the south-western to the north-eastern Baltic Sea.

Lang,T., Mellergaard, S., Bezgachina, T., Bogovski, S., Grygiel, W., Kadakas, V., Køie, M., Nagel, G., Neumann, K., Paukste, A, Tabolina, I., Wiklund, T. BMB/ICES Sea-going Workshop 'Fish Diseases and Parasites in the Baltic Sea' - conclusions and recommendations for future fish disease surveys in the Baltic Sea.

II. Intercalibration of methodologies used for sampling, diagnosis, reporting and analysis of disease data.

A general prerequisite for quality assurance and comparability of data obtained by different observers is the intercalibration and standardisation of all methodologies applied in the process from sampling to data analysis. This is of course also true for fish disease studies. Since the 1980´s, ICES has, therefore, undertaken several activities aiming at intercalibration and standardisation of methodologies used for fish diseases surveys (see above).

Since the BMB/ICES sea-going workshop brought together scientists actively involved in fish disease monitoring in the Baltic Sea who jointly carried out all activities regarding sampling, disease diagnosis and data reporting etc., methodologies can now be considered intercalibrated on a level comparable to North Sea studies.

For fish disease data reporting and statistical analysis, guidelines have been developed by the ICES Subgroup on Statistical Analysis of Fish Disease Data in Marine Fish Stocks in collaboration with the ICES secretariat which should be followed for processing of Baltic Sea fish disease data. These include annual submission of disease data to the ICES Environmental Databank by either using the ICES Fish Disease Entry Programme or, directly, the Fish Disease Reporting Format (both are available from ICES) and the application of statistical models recently being developed by ICES for standardised analysis of spatial and temporal trends in disease prevalence data submitted to ICES .

In conclusion, methodologies used for fish disease studies in the Baltic Sea can be considered to fulfil quality assurance requirements.

III. Assessment, in the Baltic Sea, of the application of quality assurance activities to the standardised methodologies recommended by ICES for fish disease surveys

Basically, the participants of the BMB/ICES workshop agreed that the ICES guidelines for fish disease surveys already published (see above) can be applied in the Baltic Sea. However, since the Baltic Sea, due to its characteristics, constitutes an unique marine environment quite different from other seas such as the North Sea, specific factors have to be considered during fish disease monitoring programmes in the Baltic Sea some of which are outlined below:

- There are strong gradients of abiotic factors such as salinity, temperature, oxygen contents which have a severe impact on the species abundance, diversity and physiological performance. These gradient are much stronger as for example in the North Sea. Since it is known that these factors might influence the occurrence of fish diseases they have to be considered for disease data analysis.

- The number of fish species suitable as target organisms for disease monitoring in the Baltic Sea is limited. Most promising fish species are flounder (*Platichthys flesus*) and cod (*Gadus morhua*) and, possibly, herring (*Clupea harengus*). However, for screening of grossly visible diseases, particularly as indicators of biological effects of contaminants, only the first two species are suitable as they are demersal species and, therefore, directly exposed to sediment contaminants. Herring is a pelagic species and, therefore, normally less influenced by contaminants. Furthermore, externally visible diseases are rare in herring.

- Since the number of specimens is low in certain Baltic Sea areas and since the size spectrum differs considerably between areas, the minimum requirements defined by ICES regarding the number of fish to be examined for diseases per size group (e.g. for flounder, size group 20-24 cm: 100 specimens; size group 25-29 cm: 100 specimens; size group _30 cm: 50 specimens) frequently cannot be met, even after several hauls at the same locality. Therefore, it is advisable to take unstratified samples representative for the population structure as large as possible (regarding the number of specimens) or at least according to minimum statistical requirements for detecting significant differences between areas, even if the prevalence of a disease is low. For example, a sample of 300 or 150 specimens out of a population size of > 100.000 individuals is required to detect a disease prevalence of 1 % or 2 %, respectively, with a confidence level of 95 %. If the size distribution differs between areas, an appropriate statistical model should be used which accounts for these differences.

- The mentioned fish species have pronounced migration patterns related to feeding and spawning activities which differ between areas. For example, the flounder in the western Baltic Sea spawns in deep waters during winter/early spring whilst the flounder in the north-eastern Baltic Sea spawns in shallow coastal waters in spring/summer. Since spawning activities are an important factor for disease predisposition and transmission (and for availability of samples), these patterns have to be known.

- Growth patterns of fish from the south-western and north-eastern Baltic Sea differ considerably. Therefore, it is not sufficient to calculate disease prevalence data only according to size-groups (as originally recommended by ICES) for comparison purposes. In addition, age should be measured, although the method is more time-consuming. In order to obtain single fish data, otoliths should preferably be taken individually and not be pooled according to cm classes.

IV. Enhancement of co-operation between Baltic Sea countries with regard to research and monitoring of fish diseases and parasites.

Co-operation between Baltic Sea countries and co-ordination of studies on fish diseases involving the eastern countries have improved considerably during previous years, mainly due to activities of the BMB Working Group 25 'Fish Diseases and Fish Parasites in the Baltic'. With the joint BMB/ICES Sea-going Workshop these contacts have further been strengthened and there is now a lively exchange of information between all countries bordering the Baltic Sea. Furthermore, with its participation, ICES has given another strong signal showing its interest in fish disease studies in the Baltic Sea.

In this context, it is noteworthy that the ICES WGPDMO has been invited to hold its 1998 meeting in the Sea Fisheries Institute, Gdynia, Poland.

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ICES WG on Pathology and Diseases
of Marine Organisms
Rhode Island, 16-20 March 1997

Working Document

A report on herring (*Clupea harengus*) from the Icelandic summer spawning stock and the Atlanto-Scandian stock examined for *Ichthyophonus* in Iceland in 1996.

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Introduction.

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

Material and Methods.

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

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

All measurements, sex determination and aging of the herring was done for the purpose of stock assessment studies. All samples were aged.

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

Results.

From a total of 1976 herring 25 cm and larger from the Icelandic summer spawning stock, only four were found to be infected (Fig. 1, Table 1). The overall prevalence of infection being 0,2%. The infected herrings were six and seven years old (Table 2).

A total of 1350 herrings of the Atlanto-Scandian herring stock were examined, all of them in the 25+ cm size group (Fig. 2, Table 3). The prevalence of infection in a sample ranged from 0,0 to 6,0%. The total prevalence of infection was 1,93% (2,34% in males, 1,55 in females).

There was some variation in the prevalence of infection in different age classes (Table 4). Comparing the combined data for year classes from 1982-1986 (10-14 years old in 1996) to the year classes from 1987-1991 (5-9 years old in 1996) indicate that the older herring is carrying the bulk of the infection (Table 5).

Broken down by fishing gear, the total prevalence of infection in MWT was 2,21% (21 of 988 herrings) and in purse seine 1,38% (5 of 362 herrings)(Table 3).

Discussion.

The prevalence of *Ichthyophonus* in the Icelandic summer spawning herring is very low, and has been low since this monitoring started in 1992. During this time the prevalence of infection in the 25+ cm size group has been approximately 0,2%. This is probably a "normal" background level of the disease.

The prevalence of 1,93% in the samples of Atlanto-Scandian herring is a considerable reduction from the 1995 value of 3,85%. As pointed out by D.W. Skagen in the 1996 Report of the WGPDMO (ICES CM/F:4, annex 15c), the infection seem to be mainly confined to certain year classes. The results presented here indicate that the prevalence of infection in the year classes of 1982-1986 did not change from 1995 to 1996. This might suggest that the disease is not significantly affecting the mortality of those older herrings still carrying *Ichthyophonus*. The overall reduction of the prevalence of infection is due to much lower levels in younger herring, none of the 5-years-old was infected.

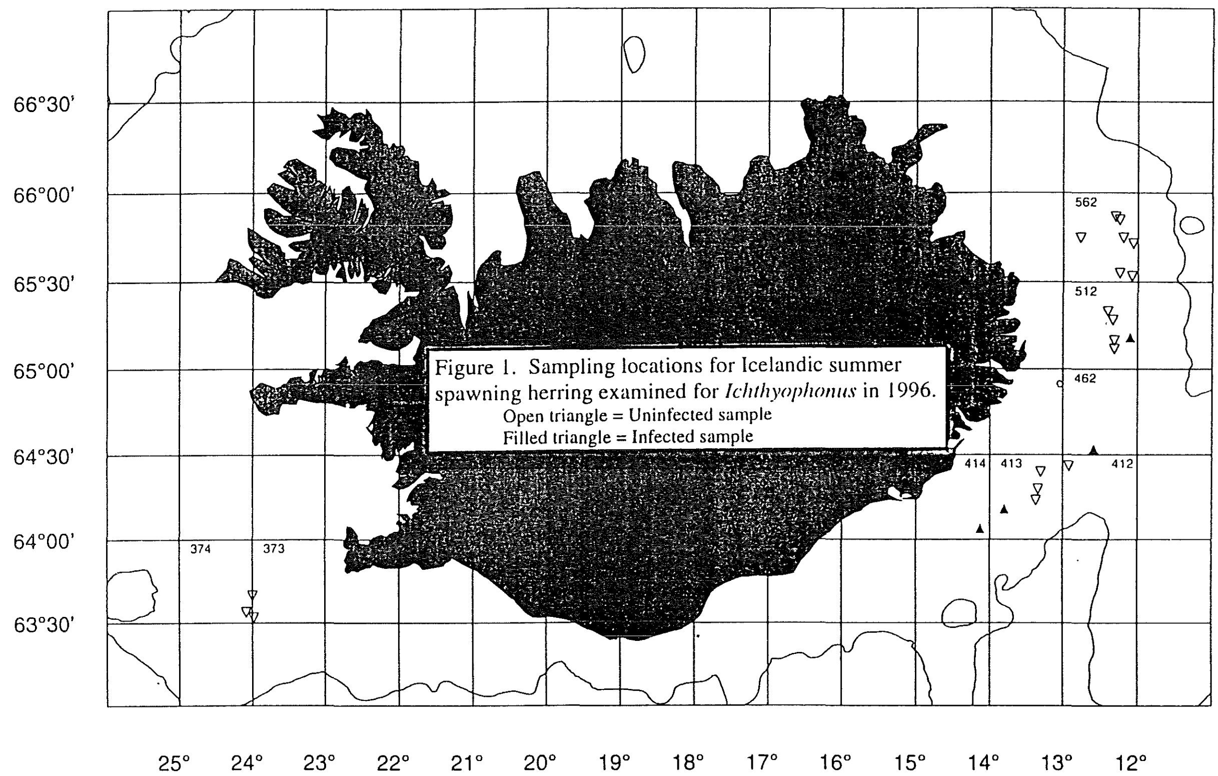

Figure 1. Sampling locations for Icelandic summer spawning herring examined for *Ichthyophonus* in 1996.
Open triangle = Uninfected sample
Filled triangle = Infected sample

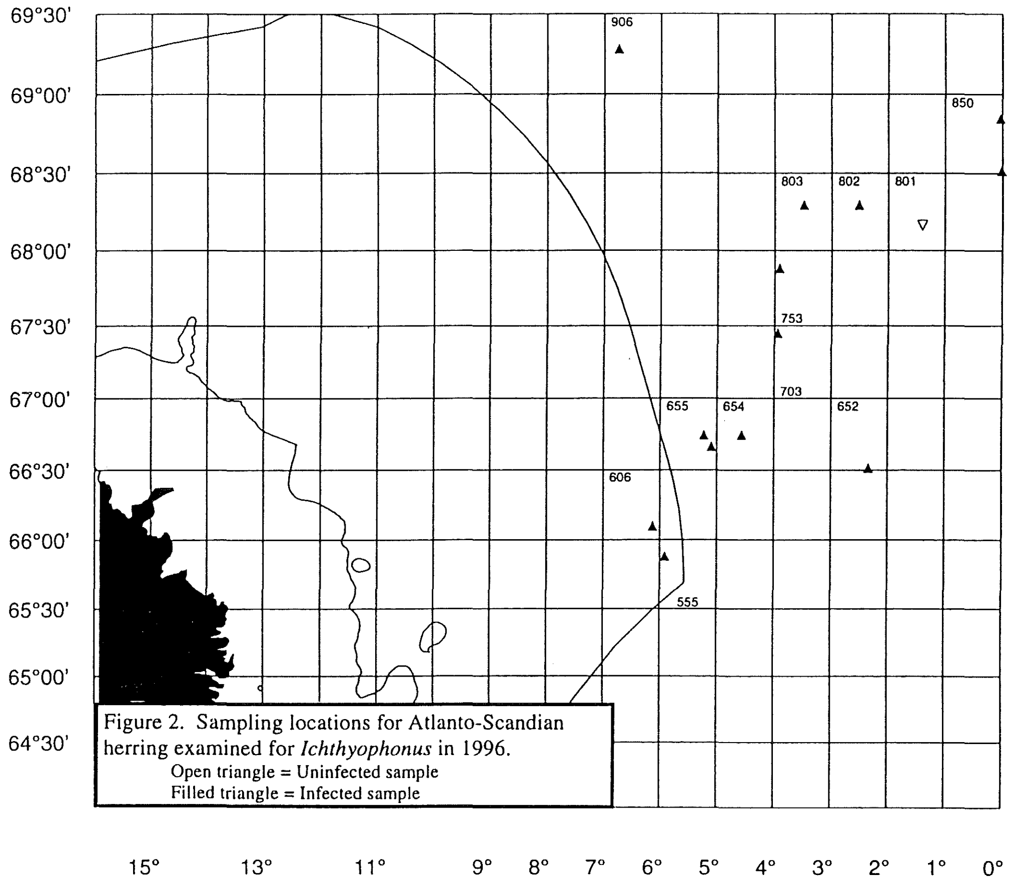

Figure 2. Sampling locations for Atlanto-Scandian herring examined for *Ichthyophonus* in 1996.
Open triangle = Uninfected sample
Filled triangle = Infected sample

Table 1. Infection of Ichthyophonus in size group 25 cm and larger of summer spawning herring from Icelandic waters in 1996. (MWT = Mid Water Trawl, PS = Purse Seine)

Statistical rectangle	Date	Station Number	Fishing gear	Mean length cm	S.D.	Total no. examined Males	Females	No. affected M	F
373	21.1.96	B2-96-31	MWT	28.9	2.9	43	29		
374	19.1.96	B2-96-27	MWT	28.5	2.7	33	28		
374	19.1.96	B2-96-28	MWT	26.2	1.8	11	10		
412	5.10.96	Fish.vessel	PS	33.1	2.4	45	55		
413	30.9.96	Fish.vessel	PS	33	1.3	37	40		
413	1.10.96	Fish.vessel	PS	33.1	1.5	39	46		
413	2.10.96	Fish.vessel	PS	33.3	1.4	46	46		1
413	16.10.96	Fish.vessel	PS	29.3	2.6	41	55		
414	12.10.96	Fish.vessel	PS	30.7	2.8	50	47	1	
462	18.10.96	Fish.vessel	PS	31.6	2.1	45	44	1	
512	20.11.96	A15-96-979	MWT	29.8	2.9	47	49		
512	20.11.97	A15-96-981	MWT	31.1	2.9	47	51		
512	21.11.96	A15-96-984	MWT	31.4	2.7	50	49		
512	19.11.96	Fish.vessel	PS	29.3	2.4	46	51		
512	24.11.96	Fish.vessel	PS	29.3	2.5	41	56	1	
562	19.11.96	A15-96-973	MWT	32.8	2.4	52	48		
562	19.11.97	A15-96-977	MWT	31.4	2.7	52	48		
562	3.11.96	Fish.vessel	PS	31.9	2.6	46	54		
562	5.11.96	Fish.vessel	PS	33.3	2.3	50	50		
562	7.11.96	Fish.vessel	PS	33.3	2.0	46	54		
562	12.11.96	Fish.vessel	PS	32.7	2.0	45	55		
562	21.11.96	Fish.vessel	PS	31.2	2.5	39	60		
			Total in MWT			335	312	0	0
			Total in Purse Seine			616	713	3	1
				Total		951	1025	3	1
				Total males and females			1976		
				Prevalence (%)			0.20		

Table 2. Age distribution of Icelandic summer spawning herring examined for Ichthyophonus infection in Iceland in 1996, number infected and prevalence of infection in each age class.

Age Class	Number Aged	Number Infected	Prevalence (%)
1	7		0.00
2	208		0.00
3	265		0.00
4	474		0.00
5	330		0.00
6	482	3	0.62
7	178	1	0.56
8	66		0.00
9	34		0.00
10	21		0.00
11	6		0.00
12	23		0.00
13	10		0.00
Uncertain	40		0.00
Total	2144	4	0.19

Table 3. Infection of Ichthyophonus in size group 25 cm and larger of Atlanto-Scandian herring caught by Icelandic research and fishing vessels in May - June 1996.
(MWT = Mid Water Trawl, PS = Purse Seine)

Statistical rectangle	Date	Station Number	Fishing gear	Mean length cm	S.D.	Total no. examined Males	Females	No. affected M	F
555	8.5.96	A6-96-257	MWT	35.7	2.4	31	68	1	3
606	8.5.96	A6-96-259	MWT	34.7	3.0	50	50	2	0
652	10.5.92	A6-96-278	MWT	32.5	2.6	43	57	0	1
654	10.5.96	A6-96-274	MWT	35.2	2.2	48	52	1	0
655	10.5.96	A6-96-272	MWT	34.9	2.5	48	52	4	2
655	22.5.96	Fis.vessel	PS	30.8	1.8	52	48	1	0
703	26.5.96	Fis.vessel	PS	36.5	1.1	36	26	1	0
753	17.5.96	Fis.vessel	PS	34.9	2.0	52	48	1	0
801	15.5.96	A6-96-308	MWT	31.5	1.7	43	57	0	0
802	15.5.96	A6-96-307	MWT	33.8	2.5	48	52	0	1
803	14.5.96	A6-96-303	MWT	35.2	2.3	56	44	1	2
850	13.5.96	A6-96-294	MWT	31.6	1.8	37	52	1	1
850	13.5.96	A6-96-298	MWT	31.8	2.2	41	59	0	1
906	6.6.96	Fis.vessel	PS	33.9	23.4	56	44	2	0
			Total in MWT			445	543	10	11
			Total in Purse Seine			196	166	5	0
				Total		641	709	15	11
				Total males and females			1350		
				Prevalence (%)			1.93		

Table 4. Age distribution of Atlanto-Scandian herring examined for Ichthyophonus infection in Iceland in 1996, number infected and prevalence of infection in each age class.

Age Class	Number Aged	Number Infected	Prevalence (%)
4	15		0.00
5	227		0.00
6	373	2	0.54
7	225	2	0.89
8	53	2	3.77
9	14		0.00
10	20		0.00
11	48	2	4.17
12	80	5	6.25
13	265	12	4.53
14	9		0.00
Uncertain	22	1	4.55
Total	1351	26	1.92

Table 5. Comparison on prevalence of Ichthyophonus in two groups of year classes of Atlanto-Scandian herring examined in 1995 and 1996.

	Year Examined	Number Examined	Number Infected	Prevalence (%)
Year classes 1982-1986				
	1995	766	36	4.70
	1996	422	19	4.50
Year classes 1987-1991				
	1995	768	12	1.56
	1996	892	6	0.67

ANNEX 12.2

ICES Working Group on Pathology
and Diseases of Marine Organisms

MORBIDITY AND DEVELOPMENT OF ICHTHYOPHONOSIS IN NORWEGIAN SPRING-SPAWNING HERRING IN 1996

by

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INTRODUCTION

Mycosis of Atlanto-Scandinavian herring caused by *Ichthyophonus hoferi* fungus have been studied by specialists of PINRO since 1992. In the period of 1992-1996, combined investigations have been carried out annually to estimate the rate of the epizooty and to form a theoretical basis for evaluation of negative influence of the disease on the organism and population of herring. The investigations included mycological, clinical and pathologohystological analyses.

Material on sickness rate of spring-spawning herring is presented, and main features of development of ichthyophonosis in 1996 are shown.

MATERIAL AND METHODS

Material was collected in February-September, 1996, in the spawning grounds and fattening areas of spring-spawning herring (Fig. 1).

Herring for analysis were taken from the trawl scientific catches (February-March and June-July in the Norwegian Sea, and May-June in the Barents Sea) and commercial catches (September, the Norwegian Sea).

50 specimens were taken randomly from big catches for analyses, and all fish were analyzed if a catch was small.

In the process of diagnosis, specialists weighed and measured herring, took otoliths to read age, revealed symptoms caused by *Ichthyophonus hoferi*, studied fresh and PAS-coloured preparations of squashed fish tissues and took tissues samples for hystological investigation.

Ichthyophonosis was diagnosticated if herring had necrotic changes of skin and muscles, as well as ulcers, nodules on the skin, in the muscles and internal organs including heart (a method of clinical analysis).

Morbidity of herring, as a statistics reflecting a share or percentage of sick specimens in a population, was estimated by results of clinical analysis proved by microscoping of smears from fish tissues in the cases of doubt.

To compare the obtained data with ICES data, fish with nodules on the heart were separated from a sample, and percentage of them was calculated (ICES method).

Statistical analysis was carried out in accordance with laws of alternative distribution.

RESULTS

It was revealed that the number of herring from the Norwegian Sea which had clinical (macroscopic) signs of ichthyophonosis increased from February to June. Maximum number of diseased fish (89.2%) was registered in the open sea during fattening period (June-July). Later on, this morbidity was the same to September, includingly (Table 1).

At the same time, a portion of fish with nodules on the heart was the same during the whole period of investigations. The number of such specimens among the analysed fish constituted 7.2-10.5%.

Table 1 shows that herring in the Barents Sea (1991-1995 year-classes), were infestated with *Ichthyophonus* less than in the Norwegian Sea. Due to data of a clinical analysis, morbidity of young 1993 year-class constituted 14.3 %, whereas that of 1994-1995 year-classes - 20.0 %. Nodules on the heart of these year-classes were not revealed. 14.3% of herring of 1994-1995 year-classes had neither any symptoms of ichthyophonosis, nor even spores of fungus.

Among herring of 1991-1992 year-classes, caught in June in the western part of the Barents Sea, 44.4% of diseased specimens were revealed, and 11.1% of fish had nodules on the heart.

Analysis of age composition of herring from ichthyopathological samples have shown that all analysed diseased fish can be divided by three groups only (Tables 2 and 3). Each group included fish of 3-6 year-classes.

The reliable differences between morbidity by age within each group were not revealed.

Due to hystological data, 16 % of analysed fish had strongly infestated all internal organs, muscles and gills. 10-60 resting *Ichthyophonus* spores of 50-60 μM were seen in one field of vision of microscope at magnification of 9 x (Fig. 2).

In skeleton musculature around spores, thin fibre capsules have being formed. Fungus developed inside of capsules (Fig. 3 and 4).

On the sections of the heart and parenchymatous organs, the infiltration of tissues with young cells of connective tissue (polyblasts) were observed, that justified on incapsulation. But in spite of it, there were no capsules around spores of *Ichthyophonus* located in the heart, liver, spleen and kidneys, or they were so weakly developed that could not prevent the development of fungus. In these organs, more often in the liver, the germination of *Ichthyophonus* hyphas through capsules of the connective tissue was revealed.

There were no tissues with active granulomas containing phagocyting cells. Correspondingly, no lysis of fungus spores was observed. Besides the proliferation of the connective tissue elements, the necrobiotic events predominated in tissues of diseased herring during the whole period of investigations, such as dystrophy and necrosis of vessels walls, muscle fibres, parenchyma and fibre structures (Fig. 5).

CONCLUSIONS

The results of investigations have shown that in 1996 compared to previous years the morbidity of herring in the Norwegian Sea increased in average to 75.6 %. This is a sufficient increase, since in 1994-1995 the percentage of fish with symptoms of *Ichthyophonosis* constituted 21.2-20.4 %, correspondingly.

Specimens with nodules on the heart appeared in samples more often. If in 1995 less than 1 % of fish with hearts infestated by *Ichthyophonus* were revealed, than in 1996 - 9.0 %.

At the same time, the epizootic situation in the Barents Sea have possibly improved slightly. Specimens with symptoms of ichthyophonosis occurred among 1993-1995 year classes more seldom than among mature herring, the disease was slighter, and a part of immature fish was not infestated.

Sufficient increase of a morbidity was peculiar for herring of 1991-1992 year classes migrated in June from the Barents Sea to the Norwegian Sea. Later on, the differences in both morbidity and in the measure of development of ichthyophonosis symptoms increased with the increase of fish age. Correspondingly, the higher percentage of fish with necrosis and ulcers was revealed for herring of 1983 year class. It constituted 26 % in February-March and 40 % - in September, 1996.

Development and symptoms of the disease are determined by several reasonable factors, including specific features of inflammation provocated by a pathogene, as well as by the depth of damaged tissues.

Due to etiology, the inflammation in herring caused by *I. hoferi* is a specific one. Therefore, ichthyophonosis is characterized by events, peculiar for the majority of such type diseases, namely, the consequent change of various morphological inflammations with predomination of proliferation, the formation of granulomas and long wavy course with periods of aggravation and damping. In such periods, a clinical picture of the disease can change in dependence with the immune status of an organism.

Due to our data, the period of ichthyophonosis acutation was in 1993-1995. In this period, in spite of the severe infestation of fish with *Ichthyophonus*, symptoms of the disease were absent or developed very slowly. It was caused by the predomination of not the proliferative, but necrobiotic processes as a consequence of a highetened sensibility of herring to *Ichthyophonus*. In spite of all this, not incapsulated spores of fungus developed freely in herring tissues.

Reactiveness of herring organism has been apparently changing till the end of 1995; therefore during 1996, fish have restored their cell immunity, spores have incapsulated, and specific clinical features have correspondingly developed.

Table 1. Number of herring infestated with *Ichthyophonus hoferi* in the Norwegian and Barents Seas in February-September, 1996

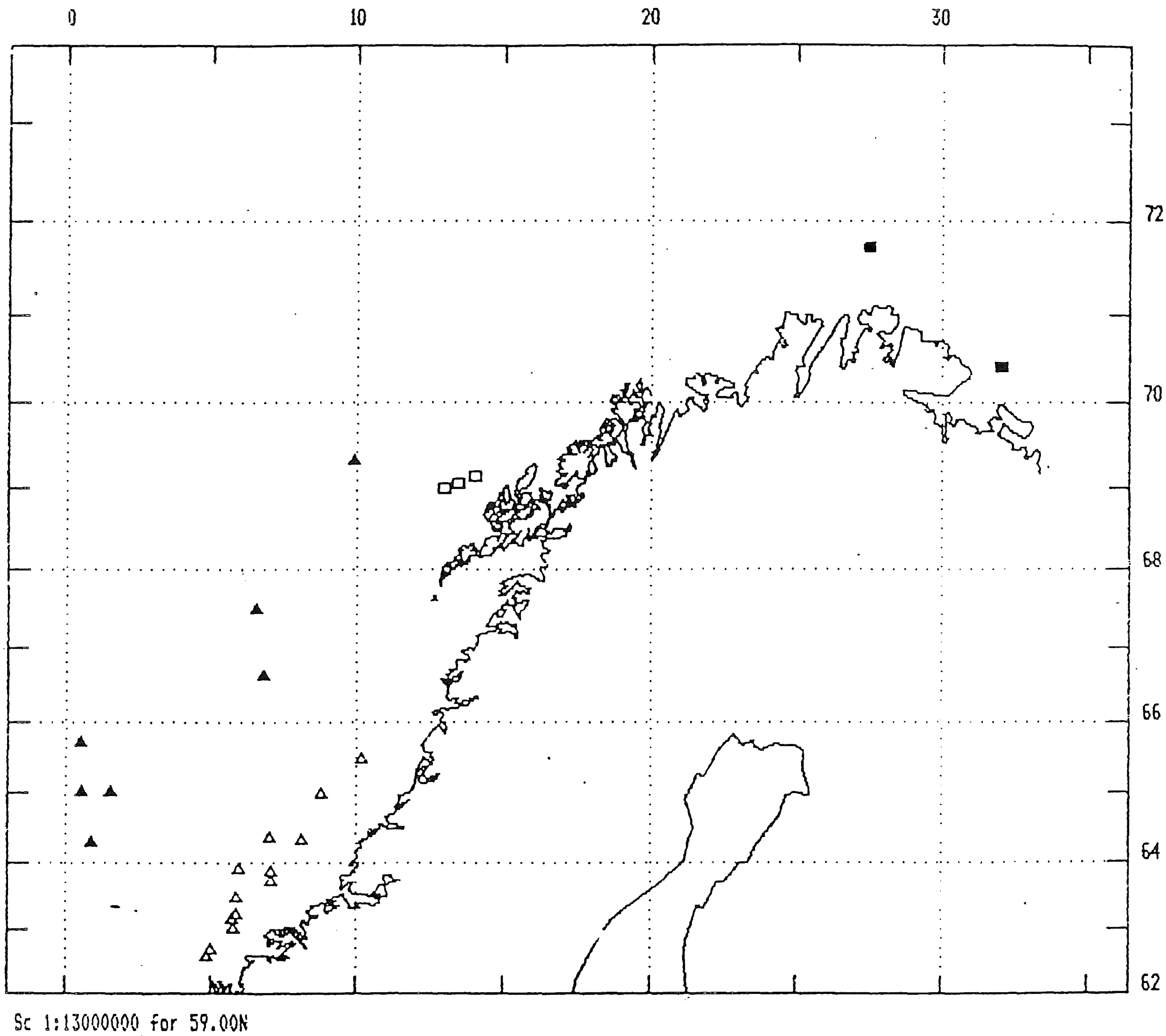
Period of investigations	Volume of a sample, spec.	Number of diseased fish	
		ICES method	Clinical analysis
The Norwegian Sea			
February-March	390	10.5 ± 1.6	64.9 ± 2.4
June-July	120	7.5 ± 2.4	89.2 ± 2.8
September	222	7.2 ± 1.7	86.9 ± 3.1
The Barents Sea			
May-June	60	3.3 ± 2.3	26.7 ± 5.7

Table 2. Number of fish infestated with *Ichthyophonus* by age groups in the Norwegian and Barents Seas in February-September, 1996.

Age	Volume of a sample, spec.	Number of diseased fish, %	
		ICES method	Clinical analysis
1-3	62	1.6 ± 1.6	28.3 ± 6.2
4	127	4.7 ± 1.9	67.7 ± 4.9
5	222	7.2 ± 1.7	60.3 ± 3.5
6	102	3.9 ± 1.9	70.0 ± 4.8
7	37	13.5 ± 5.6	66.7 ± 8.2
8-12	25	16.0 ± 7.3	86.4 ± 7.3
13	95	27.4 ± 4.6	90.6 ± 3.2

Table 3. Mean length and weight of three age groups of herring which differ from each other by sickness rate in February-September, 1996.

Age	No. of spec	Total length, cm	Weight, g	Number of diseased fish	
				ICES method	Clinic.analys.
1- 3	62	19.8 ± 0.4	78.0 ± 5.4	1.6 ± 1.6	28.3 ± 6.2
4- 7	488	28.9 ± 0.1	205.8 ± 2.6	6.4 ± 1.1	64.6 ± 2.4
8-13	120	36.0 ± 0.1	359.9 ± 4.9	25.0 ± 3.9	89.7 ± 2.9



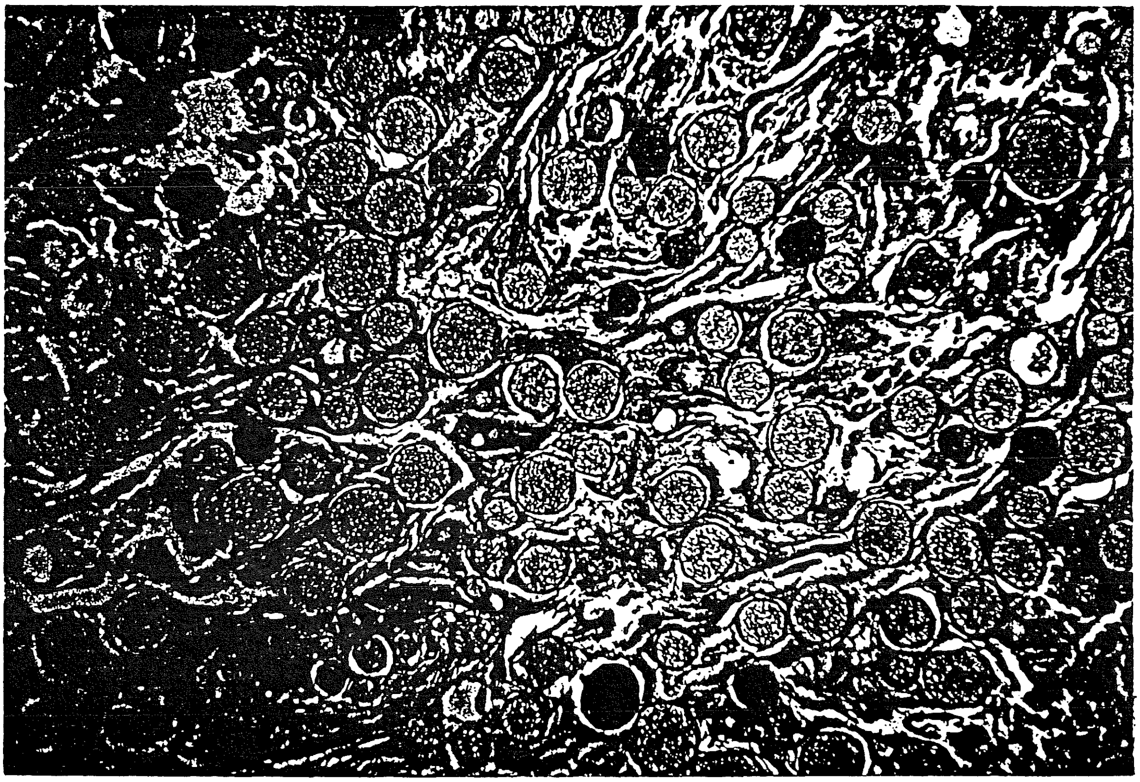
Δ _ February-March

■ - May-June

▲ - June-July

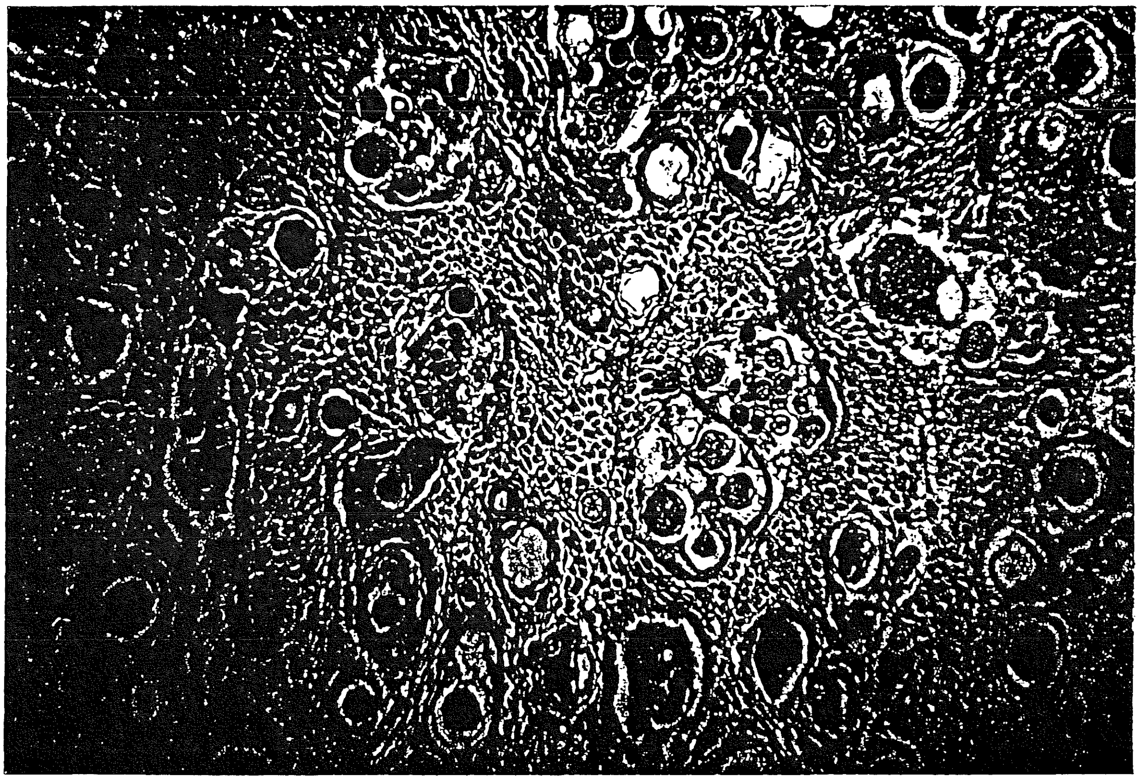
□ - September

Fig. 1. Areas of herring sampling in the Norwegian and Barents Seas in February-September, 1996.



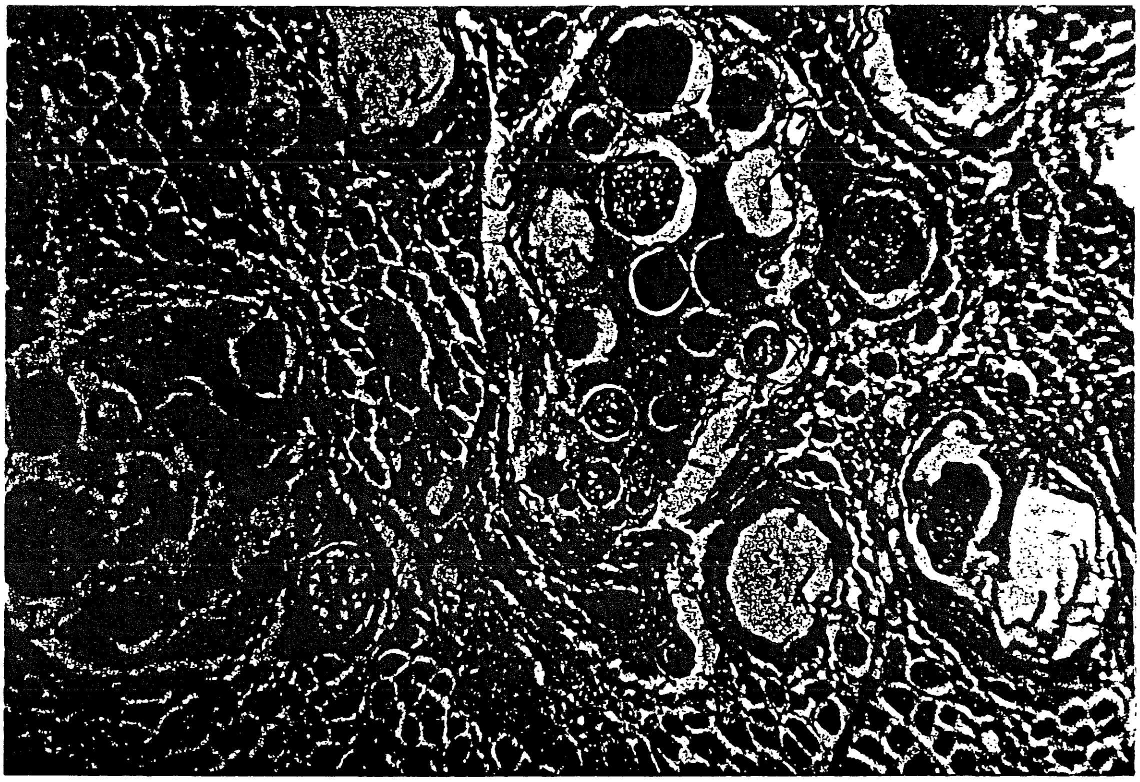
___ - 50 μM

Fig. 2. Spores of *Ichthyophonus* in the heart of hering. July, 1996. Colouring with hematoxylin-eosine.



___ - 50 μM

Fig. 3. Incapsulated spores of *Ichthyophonus* in the skeleton musculature of herring, July, 1996. Colouring with hematoxylin-eosine.



_______ - 50 μM

Fig. 4. Development of *Ichthyophonus* spores in incapsulated breeding grounds of herring skeleton musculature, July, 1996. Colouring with hematoxylin-eosine.

___ - 50 μM

Fig. 5. Necrobiosis of muscle and connective tissues of herring under ichthyophonosis, March, 1996. Colouring with hematoxyline-eosine.

ANNEX 12.3

ICES WGPDMO
Rhode Island, USA. 16-20 March 1997

Working Document

Prevalence of *Ichthyophonus* in herring (*Clupea harengus*) in Scottish waters during 1996 determined from research vessel and commercial catches.

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1. Introduction.
The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) has been requested, as part of its regular tasks, to monitor the levels of the parasitic infection *Ichthyophonus* in herring stocks in the ICES area, with the objective of being able to advise ICES of early changes in prevalence, possibly leading to a recurrence of the epizootic which occurred in the early 1990s. Council resolution 1996/2:29(h) refers to the current request by ICES.

2. Materials and Methods.
Samples of hearts were examined during routine herring stock assessment studies on board research vessels and in commercial landings from Scottish waters and any hearts showing gross nodular lesions typical of *Ichthyophonus* infection were recorded as positive.

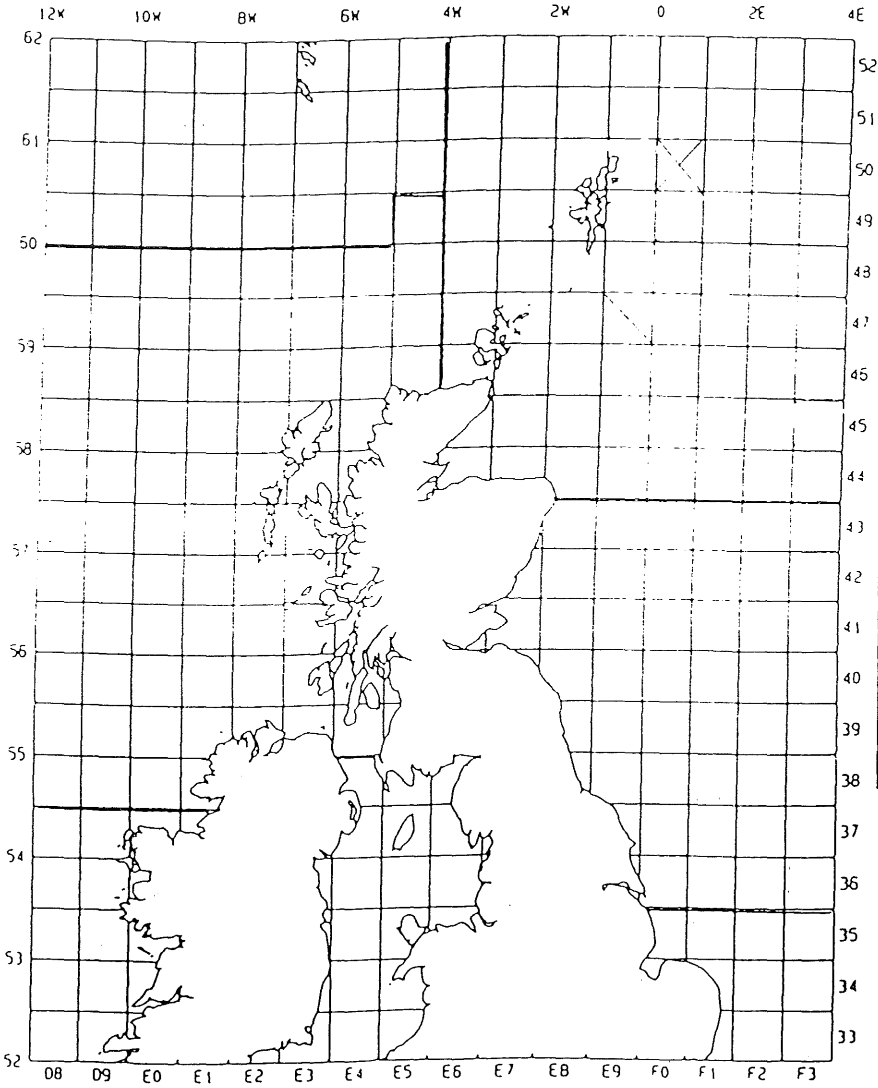
3. Results
From a total of 10,863 herring from research vessel catches *Ichthyophonus* infected 3 herring were detected all from Area IVa east of Orkney and Shetland in length groups 20.0-24.5 and 25-29 5 cm. Similarly from a total of 7,785 herring caught commercially, only one fish caught in Area VIa showed evidence of infection (29.9cm , 3 years old).

4. Discussion and Conclusions.
Although *Ichthyophonus* is still present in North Sea herring stocks in Scottish waters, in 1996 this was restricted to Area IVa east of Orkney and Shetland in contrast to its occurrence also in Area IVb in previous years. The prevalence levels were low , 0.03% in research vessel catches and 0.01% in commercial catches. It is concluded that the disease persists at non-epidemic levels in herring stocks in Scottish in the northern North Sea. *Ichthyophonus* has not been detected in herring to the west of Scotland (Areas VIa, VIIa) and in the Irish Sea (Area VIIa).

5. Acknowledgements.
Members of the Marine Laboratory Fish Stock Monitoring Section undertook all observations of herring hearts for *Ichthyophonus.*

Prevalence of *Ichthyophonus* in herring hearts in research vessel catches in Scottish waters in 1996

(X = statistical rectangle with infection detected)



Area VIa

Month	Infected
03	0/1704
11	0/560

Area VIIb

Month	Infected
11	0/128

Area IVa

Month	Infected
02	0/327
03	0/319
05	1/424
07	2/3970
08	0/664

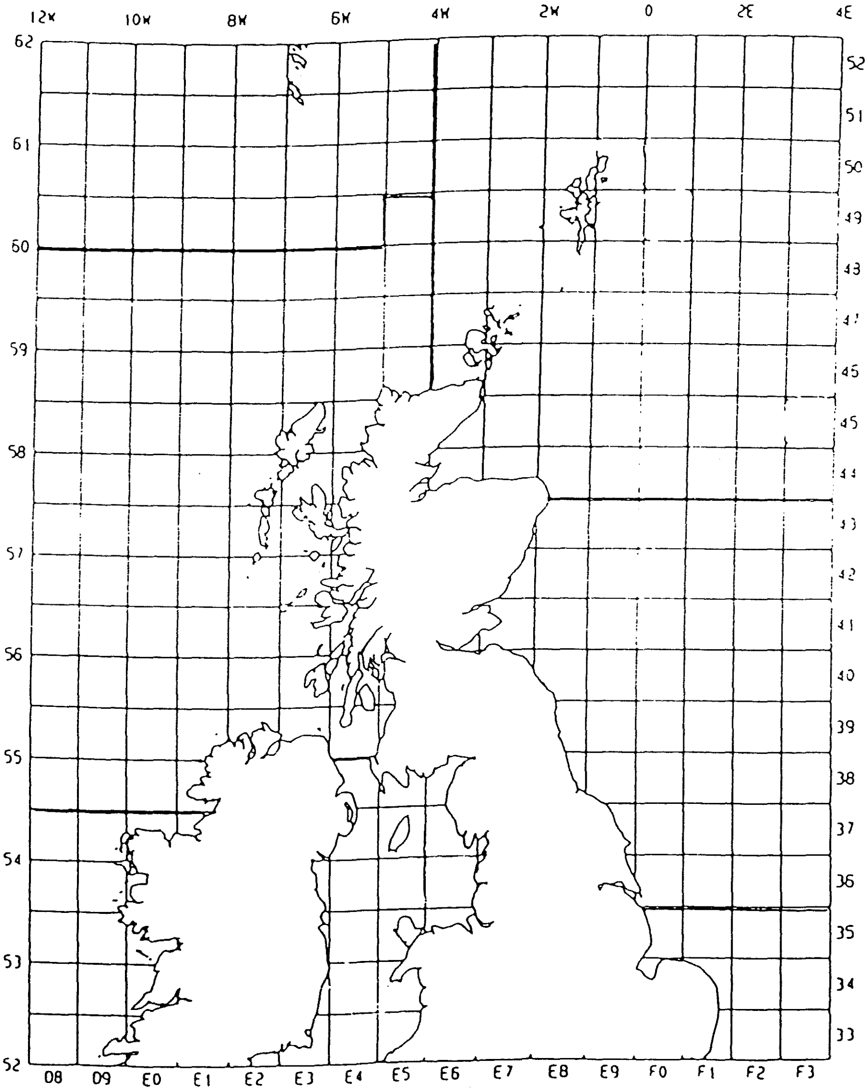
Area IVb

Month	Infected
02	0/461
05	0/1381
08	0/646

Area VIIa

Month	Infected
03	0/279

Prevalence of *Ichthyophonus* in herring hearts in commercial vessel catches in Scottish waters in 1996



Area VIa

Month	Infected
1-3	0/246
4-6	0/199
7-9	0/1517
10-12	0/1087

Area IVa

Month	Infected
1-3	-
4-6	0/649
7-9	1/3106
10-12	-

Area IVb

Month	Infected
1-3	-
4-6	0/411
7-9	0/570
10-12	-

ANNEX 14.1

Nodavirus in Marine Fishes. News & Trends.

Draft to ICES 1997 (Rhode Island, USA) by

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From the papers published during the last years, we can extract some conclusions:

1.- The growth of the sea bass nodavirus in a cell culture system, namely the SSN-1 cells from *Channa striatus* (Frerichs et al. 1996) open a number of possibilities for the research on that virus in different species of fishes, even if the cells are carrier of a retrovirus.

2.- The set of primers described in the early work of Nishizawa et al.(1994) as F2-R3 that amplifies de T4 region of the virus genom (specifically, the nucleotids no. 604-1030 in the RNA2 segment) seems to be at present the more efficient one for the RT-PCR procedure. Products identical to that obtained from SJNNV were amplified from other diseased fish, as the redspotted grouper and japanese parrotfish (Nishizawa et al. 1994).

4- The detection limit of the PCR method was shown to be 100 fg of viral genom (Nishizawa et al. 1994) after 25 cycles. By increasing the number of cycles to 35, it was possible to detect a smaller quantity of SJNNV RNA in the range of 1-0.1 fg.

However, the in vivo limit of the method when applied for detection of the virus in the gonads is not known until now, because of the lack of available methods to enumerate virus in the samples. May be in the near future, the SJNNV and other related virus can be propagated in the SSN-1 cell line and solve this point.

It´s necessary to establish the correlation between an amount of RNA (in fg or in pg) and the number of viral particles.

5.- Some cases of failure of the PCR methodology to detect de SJNNV in selected striped jack spawners, have been demonstrated recently (Nishizawa T., Muroga,K. & Arimoto, M., 1996).

Anyway, In some virus-negative spawners, after the first 11-14 days it is possible to detect it, but not steadly(consistently).

However, in the virus-positive spawners, the virus can be detected on 3-4 days old larvae.

6.- The studies on the tissue distribution of SJNNV in aparently healthy adult fish subjected to spawning, demonstrated that the virus was present in gonads, liver, kidney, stomach & intestins, but not in Brain, Spinal cord & retina, the target organs of the virus in the diseased larvae. Never appears in muscles, heart, spleen and gills.

However, in fish (larvae or adults) showing clinical signs of disease, the virus was found in brain and retina.

Good correlation between FAT an the PCR procedures was demonstrated.

Now, the differences are clear on tissue distribution on Carrier fish and in Diseased ones. So, the virus originates in various organs of the striped jack spawners and further is shed from the intestins and gonads to contaminate the eggs.

7.- New species of fish has been recently added to the list of them suffering from the same disease caused for this type of agent:

a)Sevenband Groupper *Epinephelus septemfasciatus* (Fukuda et al. 1996) in Japan

b) *Epinephelus malabaricus* (Boonyaratpalin et al. 1996) in Thailand, this as a picorna-like.

c) Gilthead Seabream *Sparus aurata* (Comps & Raymond. 1996).

From all these facts, is clear the necessity of studies on:

1.- Patogenicity studies to clarify the host specificity of that group of viruses.
2.- Diagnostic protocols validated for a number of fish species.
3.- Epizootiology for the important species. Studies on the target tissues in the asymptomatic individuals.
4.- Prophylactic measures to reduce the incidence of the disease and avoid the spread of the agents.

ANNEX 14.2

NODAVIRUS : FUTURE RESEARCH WORK

F. Baudin Laurencin

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Introduction

First accounts of a vacuolating encephalopathy and retinopathy relate to outbreaks causing heavy losses in larval and juvenile sea bass in Martinique (Caribbeans) and were described as a "Sea bass Viral Encephalitis" (SVE) (**Bellance & Gallet de Saint-Aurin, 1988**). Numerous tiny virus particles were observed in nervous cell cytoplasms obviously appearing as the causal agent, and named "Sea bass Encephalitis Virus". A "Viral Nervous Necrosis" (VNN) was then described in larvae and juveniles of reared Japanese parrotfish, *Oplegnathus fasciatus* (**Yoshikoshi & Inoue, 1990**). Similar clinical conditions have also been recorded for the larval and/or juvenile form of barramundi *Lates calcarifer* in Australia (**Glazebrook et al., 1990, Munday et al., 1991**) and in Tahiti (**Renault et al., 1991**), turbot *Scophthalmus maximus* in Norway (**Bloch et al., 1991**) and a number of other marine fish species in Japan (**Muroga, 1995**).
Several authors have provisionally suggested that the causative virus found in barramundi, European sea bass and turbot could be classified as picorna-like virus, from its size, replication site and nucleic acid (**Glazebrook et al., 1990** ; **Breuil et al., 1991** ; **Renault et al., 1991** ; **Bloch et al., 1991**).
Mori et al. (1992) biochemically characterized VNN virus isolated from striped jack *Pseudocaranx dentex* (SJNNV) and proposed it as a member of the *Nodaviridae* family. More recently, the etiological agents of barramundi and sea bass encephalitis (Fish viral Encephalitis, FVE) have been proved to be antigenically related (**Munday et al., 1994**) and to have the same features of nucleic acid and coat protein as SJNNV. This could be interpreted as a new group of the Nodaviridae family, probably distinct from the viruses currently described in insects (**Comps et al., 1994**). Considering some discrepancy between the coat protein gene of VNN agents in Japanese fish species (including SKNNV) and insect nodaviruses, **Nishizawa et al. (1995b)** proposed to classify fish nodaviruses into a new group of Nodaviridae. Thus today, although more research work has to be done in order to achieve a more precise characterization of the possibly different viral strains related to the different fish species concerned, we can tentatively consider that the aetiological agent of the syndrome described in more than 20 fish species such as SVE, VNN, SJVNN, FVE and Viral Encephalopathy and Retinopathy (VER : **OIE, 1995a**) belong to the Nodaviridae family. In the present paper, for simplification sake, it is called Nodavirus and the subsequent disease fish Nodavirosis or VER.

A lot of research work has already been carried out on the virus and the disease, but more work will be necessary to meet the real demands of fish farmers : what should we do to stop or limit the disease or its effects ? In fact theoretical answers exist as for the other fish viral diseases, and since no treatment is available today, the ways of control are : 1) Sanitary measures, to prevent the fish from having any contact with the virus and to act on secondary or favouring factors of the disease ; 2) Measures favouring host resistance to the viral infection : vaccination and immunostimulation.

However in order to apply these measures, it is necessary to have sufficient knowledge in the subsequent areas :

- Characterization and identification techniques of the aetiological agent (or agents if several strains are involved) : molecular structure, physico-chemical and biological properties (including pathogenecity, antigenicity and immunogenicity, sensitivity to heating, drying, chemical disinfectant treatment... and diagnostic methods.
- Epizootiology : virus reservoirs, transmission modes, receptivity factors.
- Immunology : response of fish to nodavirus infection and vaccination.

Hereafter follows a report about what is known and the research work to be done in these different areas. To conclude, these is an attempt to examine the conditions and ways necessary to carry out on such work and subsequently any control method.

I. Characterization of the virus(es) and identification techniques (diagnostic methods)

As mentioned above, similar Nodaviruses were partially characterized from 5 Japanese fish species (**Nishizawa et al., 1995b**) and from barramundi and European sea bass (**Comps et al., 1994**). The virus particles, unenveloped, spherical and 25-30 nm in diameter have been visualised (using E.M.), mainly in the cytoplasm of the nerve cells and in the extracellular spaces. The virions form crystalline arrays or are packed in membraneous structures. Infected material has been purified by sucrose and CsCl gradient centrifugations. The genome consists of two pieces of ssRNA with molecular weights of respectively 1.056×10^6 and 0.495×10^6 Da. Characterization of the virion's structural polypeptides on SDS PAGE resolved two polypeptides P1 and P2 with molecular weight of 42 and 36 to 40 KDa. **Nishizawa et al. (1995a)** succeeded in generating monoclonal antibodies against SJNNV, which recognize the coat protein (42 KDa) and have a neutralizing activity. Some data suggest the existence of several viral strains : variable weight of P2 polypeptide, failure of ELISA using anti-SJNNV rabbit serum to detect Nodavirus from other species (**Arimoto et al., 1992, 1993**), temperature of infection which, for instance, appears lower in halibut than in Mediterranean fish species.
The presumptive diagnosis can usually be made on the basis of the light microscopic appearance, electron microscopy being a confirmatory technique. However these methods are not sufficient to detect carriers showing no clinical signs and even to appreciate the significance of a poorly developed lesional pattern. Immunohistochemistry has been initiated by IFREMER Palavas and is now the most common technique used in Europe. Fixed, paraffin embedded sections of tissues are treated with the anti-nodavirus serum and the specific Ag-Ab complexes visualised by the peroxydase staining method as described by **Le Breton et al. (1996)**. There is evidence of tiny antigenic foci even when the lesions are not easy to observe. Other diagnostic procedures were first developed in Japan and are reported by **OIE (1995b)** : indirect fluorescent antibody technique (FAT) and ELISA using polyclonal antibody sera, a Polymerase Chain Reaction (PCR) amplification based on the sequence data of SJNNV coat protein gene (RNA2) (**Nishizawa et al., 1994**). The latter method was shown to be successful in detecting the virus at stages where antigens were not detected by the ELISA test and it is also available for the diagnosis of nodavirus in a wide range of species. It was also shown (**Mushiake et al., 1992 ; Breuil**, pers. comm.) that antinodavirus Ab can be detected in the serum of adult fish (SJ and sea bass) allowing the selection of spawners based upon serological examination.

Most diagnostic techniques (e.g. PCR) have also been recently developed in European countries (France, Greece) and thus the first goal, as regards to diagnosis is to facilitate laboratory techniques in order to use diagnostic tools for the purpose of epidemiology. Studies should also seek to determine negative-positive threshold values for serological tests, sensitivity in detecting nodavirus or virus products in tissues, specificity in relation to other non-related viruses which may be isolated from marine fish and ability to detect different strains of nodavirus. An *in situ* PCR should also be performed on tissue sections in order to locate even minute amount of virus, e.g. in asymptomatic carriers and/or in tissues which, up to now, have not been considered as infected.
However, as regards the characterization of the virus and of different viral strains it is necessary to culture them in a range of fish tissue cell cultures. Today only a snakehead *Channa striatus* retrovirus infected cell line has been found suitable for the nodavirus culture (**Frerichs et al., 1996**). The growth characterization of the nodavirus in all cultures should be evaluated to define *in vitro* growth parameters and the sensitivity of the virus to heat, pH, salinity, UV light and chemical disinfectant treatments. Long-term storage stability should also be examined. Novel cell lines should be developed, characterised and tested for susceptibility to nodavirus infection as there would be clear advantages in using cells lines other than the retrovirus-infected snakehead cell line for *in vitro* isolation, propagation and assay of the nodavirus. Monoclonal antibodies should also be prepared to establish a panel of products for the antigenic characterization of strains from different hosts and geographical areas.

II. Epizootiology

As for other fish viruses it is likely that only fish are sources of fish Nodaviruses, which is confirmed by their relative specificity. These viruses are presently known only because of their effects on farmed fish. However it is obvious that the contamination of farmed fish all over the world has no doubt originated from wildlife. Whether wild fish can be affected (with clinical signs and mortality) or simply act as healthy virus carriers is not known. The risks of reciprocal contamination between farmed and wild fish depend on the transmission mode(s). A risk assessment would be of interest both for fisheries and for fish farming. The presence of a nodavirus epizootic in a farm should result in the release of very high levels of viruses but how long these viruses can remain infective in environmental conditions is not known.

Transmission : **Glazebrook et al. (1990)** demonstrated experimentally the possibility of horizontal transmission from diseased barramundi larvae to healthy ones. **Mori et al. (1991)**, then **Arimoto et al. (1993)** transmitted the disease respectively to redspotted grouper *Epinephelus akaara* and striped jack larvae exposed to a bath of filtrates of homogenates from diseased fish. **Arimoto et al. (1993)** was also able to transmit the disease to healthy larvae (1 day old) by cohabitation with infected striped jack larvae. However the exposure of juvenile (78 mm) SJ to the same type of bath challenge as larvae failed to reproduce the disease. Also, in the same conditions, the larvae of other marine fish, i.e. red sea bream *Pagrus major*, yellowtail *Seriola quinqueradiata* and goldstriped amberjack *Seriola lalandi* could not be contaminated. More recently, **Breuil** (pers. comm.) transmitted the disease to sea bass larvae by bath in experimentally contaminated water.
Nguyen et al. (1996) reported that a virus multiplication was observed in the epidermal cells of SJ larvae which could act in favour of horizontal transmission, but the authors still consider that the role of skin as a portal of entry remains unclear even if the virus could enter the spinal cord via sensory and/or motor nerve cells linked to the epithelium.
Arimoto et al. (1992) detected the virus at high frequency from the ovaries of SJ spawners and from fertilized eggs but it has not been shown whether the virus is present on the surface

or inside the eggs. Using PCR **Nakai et al. (1996)**, detected the virus in the ovarian tissue of some SJ spawners when no virus was detected in other SJ spawners. Subsequently the disease occurred in larvae obtained from the first spawner group and not in larvae obtained from the virus-negative fish.
Using PCR as well, **Comps** (pers. comm.), detected viral material in samples of eggs and the ovarian liquid of female sea bass.
Therefore, even if vertical transmission has not been demonstrated in other fish than SJ, it is seemingly a transmission mode in other susceptible species, including Mediterranean sea bass.

Factors of receptivity :

Species : the disease has been observed in about 20 farmed species. It is worth noting that some species do not seem to be susceptible. For instance, the disease has never been identified in gilthead seabram *Sparus aurata* which is often reared in close proximity to sea bass in the Mediterranean, nor in red seabream and yellowtail which constitute a big part of the aquaculture production in Japan.

Age : Whatever the fish species, larvae always seem the most susceptible. In S.J., mortalities (usually high) are most frequently encountered in 2 to 10 day old larvae. In Mediterranean sea bass the disease appears from d10 to d45 with a higher case number between d15 and d30 and an acute evolution. Mortalities can be very high (up to 100 %), but there are substantial variations even for subsequent breedings in the same hatchery, with a mean mortality of 10 to 30 % during a 3-4 day period of time.
Juveniles appear more resistant. **Arimoto et al. (1993)** failed to transmit the disease to juvenile S.J., when larvae were infested in the same challenge conditions.
Nodavirosis was only recognized as a cause of large losses in juvenile sea bass in 1995, even though the disease has been detected in 2 to 250 g fish in 1985-86 in Martinique, in 1990 on the French Mediterranean coast and, for several years, in Tunisia. In these juveniles, daily mortalities are usually low, (the bigger the fish, the lower the mortalities), but after several weeks the total losses can exceed 50 %.

Temperature : In hatcheries, the temperature is adapted to favour the best growth of the larvae and thus usually at an optimally high level (about 20° C for sea bass, turbot or S.J., but only 15° C for halibut). In juveniles and in on-growing sea bass, the outbreaks of Nodaviroses appear only in warm water ($\geq$ 20° C), and are more prevalent in the Mediterranean areas where the summer temperatures are usually high.

Other factors (from observations by **J.C. Raymond**, pers. comm.)

- Poor renewal of water in the farming area (which could lead to high concentrations of the virus).
- Environmental stressors (transport, handling...).
- Poor water quality.

Even if it is likely that both vertical and horizontal transmissions of Nodavirosis exist in sea bass and may be in other European susceptible species as well, and even if some factors of receptivity emerge from field observations, these data are today insufficient to undertake a sanitary control of the disease, e.g. in the Mediterranean area. Complementary data have to be collected from laboratory experiments or from field observations.

1. Transmission trials (in laboratory) : Firstly they could concern sea bass.
   Vertical transmission should be studied by incubating newly hatched larvae derived from previously identified infected and non-infected parents and then looking for the disease in larvae.

Horizontal transmission has only been demonstrated in larvae where the origin of the disease in hatcheries is more likely to stem from vertical transmission. The conditions of an eventual horizontal transmission in juveniles and in on growing fish have to be determined. Factors such as species, population, age, temperature and environmental conditions usually affecting disease susceptibility, should be examined. Serum antibody levels should be researched after positive or negative transmission trials as well as an eventual acquired protection after a first contact with the virus.

2. Field observations : Epizootiological investigations into the occurrence and spread of nodavirus infections

Epizootiological studies should be done in order to establish the extent and economic impact of nodavirus in the Mediterranean aquaculture industry. A first step has been taken with the short-term investigation "An Epidemiological Inquiry into Nodavirus in Sea bass" supported by the EU Commission.

Participants from Mediterranean countries should undertake the analysis and comparison of data from separate geographical areas where different environmental and management parameters may modify the patterns and influence of the disease. Sampling and visits to mariculture sites could be made through the good offices of the Federation of the European Aquaculture Producers and the adherent National Aquaculture Associations in the Mediterranean Region in order to obtain and develop information on :

- the prevalence of the disease
- the species affected
- the course and severity of the clinical condition
- the climatic and other environmental factors
- the management practice employed
- the sanitary treatments and other disease control measures
- the socio-economic impact of the disease.

This approach should be supported by comprehensive sampling and the examination of both wild and cultured fish stocks through the range of developmental stages from egg to adult. Inapparent and overt disease status should be assessed by a clinical and histopathological examination complemented by laboratory diagnostic procedures : serum neutralization (SN) in cell culture, enzyme -linked immunosorbent assays (ELISA) to quantify serum antibody levels, and *in situ* hybridisation following polymerase chain reaction (PCR) amplification, to identify the presence of nodavirus in fish tissue samples. These techniques will generate additionnally data to the antigenic links between nodavirus strains from different sources.

Such data collection could be recorded and complemented in the Pan-European Aquaculture Database that has been developed specifically by the FEAP for the benefit of individual producers and the National Aquaculture Associations.

This facility will be helpful in keeping records of the topics cited above and in geomapping disease presence and spread.

III. Immunology - Fish response to nodavirus infection and vaccination

An ELISA procedure for antibody detection was developed by **Mushiake et al. (1992 - 1993)** for anti-nodavirus Ab detection from S.J. serum and these authors reported that S.J. brood stocks reared at various facilities had antibodies against SJNNV at high frequency (65 %). In the same way **G. Breuil** (pers. comm.) detected anti-nodavirus Ab in the serum of adult sea bass. Consequently, we can expect fish to develop a specific immune protection after a first contact with the virus. From **J.C. Raymond** (pers. comm.) only one clinical

outbreak of the disease has been observed during the breeding time of any batch of sea bass. Of course, if a protective immune response exists,.the immunized fish could thus become asymptomatic carriers.
In fact, vaccination trials have been carried out against most fish viral diseases currently known and, at least in laboratory conditions, a protective immune response has nearly always been obtained.
Groups of vaccines can be prepared including several types : 1) killed vaccines which include formerly pathogenic viral particles "simply" inactivated by physico-chemical techniques and vaccines obtained by the so called new biotechnologies : subunit vaccines, purified immunogenic but non-pathogenic fractions of the virus, and chemically synthesized vaccines, i.e. synthetic peptides of defined antigenic determinants, 2) live vaccines which also include two categories : viral particles whose pathogenicity is "simply" attenuated by physico-chemical techniques (they can multiply in the fish, but their pathogenicity is weak although their antigenicity is preserved) and vaccines obtained from genetic engineering, such as gene-deleted vaccines (non-essential virulence genes are deleted), reassortants (a type of hybrid where only genes of the pathogen code for antigenic proteins), vector based vaccines (containing one or more genes from a heterologous microorganism).
All these types of vaccines present advantages and disavantages. Killed vaccines do not multiply in fish and large quantities must be applied to individuals, usually using the injection method : today the only commercialised viral vaccine against Infectious Pancreatic Necrosis is an injectable vaccine containing a recombinant IPN virus protein included in an adjuvanted multivalent bacterial vaccine (**Frost et al., 1996**). Live attenuated vaccines multiply in fish and are thus suitable for bath vaccination but they must prove to be stable (i.e. they must not revert to a wild type or pathogenic organism) and to be incapable of interfering with diagnostic tests so that one can clearly differenciate between vaccinated animals and those carrying the wild pathogen. **Benmansour et al. (1996)** reported on their selection of a mutant strain of the Viral Haemorrhagic Septicaemia virus which may be completely non virulent, and present antigenic and genetic markers allowing its rapid identification.

Considering all these data, the assessment of antinodavirus Ab in farmed fish correlated with clinical observations should allow the establishment of an epidemiological surveillance scheme to be used in the future control of the disease. Complementary laboratory studies should be carried out to assess the humoral and non-specific immune response of fish surviving experimental infection in order to facilitate the understanding of field results.
The immune response of fish to different types of vaccines should also be examined, firstly in the laboratory, serologically and according to fish resistance to challenge infection. The effectiveness of several types of vaccines should be closely monitored, using and comparing the different routes of vaccine administration : injection, which could be of interest in on-growing fish and spawners and which constitutes a reference method for the assessment of the intrinsic protective quality of a vaccine ; immersion which could be applied to very young fish ; oral pathway, the best method to prevent stress effects and limit labour costs, but which has until now been known for its weak or not repeatable efficacy. As a guide for choosing the best type of vaccines for preparation and assessment, the properties of current acceptable vaccines (**Janssen, 1992**) should be borne in mind :

- inocuity and absence of side effects
- capability of preventing the establishment of carrier stages
- stability : a live vaccine must not revert to a wild type organism
- capacity to be identified in the fish and differenciated from the wild pathogen
- cheap to prepare, easy to administer.

The immune response to the inactivated virus should be examined first of all. A recombinant capside coat protein could also be prepared (**Nakai et al., 1996**) but, as mentioned above, such material usually has to be injected to make it effective. Finally if vaccination is retained as a necessary way to control the disease, the possibility of preparing and using live vaccine should be explored.
Finally, the effects of non-specific immunostimulation by different products (levamisol, glucans, peptides...) could also be assessed, mainly in hatcheries, when the immune system is still immature, leaving the fish highly susceptible to infection factors (**Skjermo et al., 1995**).

Conclusion

The ownership of diagnostic tools determines the capability of carrying out research work in the other areas. However, today or in the near future, three main techniques can be used as regards research into epidemiology and immunology : immunohistochemistry for diagnosis in clinically diseased fish, PCR (at least the technique described in OIE manual) for diagnosis in asymptomatic carriers, ELISA for detection of Ab levels in fish sera added to PCR for the determination of the disease status in geographical zones but also as an indication of immunization level in vaccination trials.
At the present time the snakehead cell line can already be used for the initial characterizations of the virus and the comparison of different strains. Of course, further research concerning other diagnostic techniques should be undertaken. This is necessary in order to acquire a better understanding of the disease and, in the future, to put these techniques in practice, according to their suitability.
The achievement of the whole research work necessitates cooperative work between qualified laboratories and professionals. If we consider the Mediterranean sea bass problem (without dismissing the other European farmed fish concerned by the disease), a widespread cooperation within the whole of Europe is necessary. It was well understood by the FEAP and the National Aquaculture Associations which organised in Rome (January, 1996) a meeting between most representatives of European laboratories involved in research on Nodavirosis. A project involving concerted research was initiated, then drawn up and proposed within the framework of the FAIR programme of the European Community. It was not accepted, for mainly debatable reasons, the first being that Nodavirus could not be demonstrated as the actual cause of the disease ! The research work proposed in that project was more or less what is suggested in this present paper. Are there any other lines of research which will stop and limit the effects of the disease ?
Thus, today, the work could be done in laboratories involved in the project (and may be others), and also in the field, with producer organizations so that immediate measures for sanitary control could be carried out, either to prevent vertical transmission (**Nakai et al., 1996, Mushiake et al., 1994**), or to protect zones which could be considered as free of the disease. The crux of the problem is that this work can only continue with strong financial support. If a new proposal of the reviewed project is proposed to the European Community, and is finally accepted, no money will be available before the end of 1997. At present it is important to decide whether or not the protection of fish farming is worth the cost of the necessary research work.

Aknowledgments

To Dr G. Breuil (IFREMER, France) and Dr J.C. Raymond (SAVU-CNPEM, France) for personnal communications of their observations.

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ANNEX 14.3

Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*

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ABSTRACT: Reared larval and juvenile Atlantic halibut *Hippoglossus hippoglossus* collected during incidences of mass mortality in two hatcheries were studied by light and electron microscopy. A vacuolating encephalopathy and retinopathy as well as endocardial lesions were observed. By transmission electron microscopy isometric, spherical, unenveloped virus particles with mean capsid diameters of approximately 25 nm were detected. The virions were found intracytoplasmically in semicrystalline arrays or as membrane-associated aggregates and single particles. Infection could be observed in neurons, astrocytes, oligodendrocytes, microglia/macrophages, lymphocyte-like cells, vascular endothelium, endocardial endothelium, myocardial myocytes and in the mesothelium of the epicardium. Specific immunolabeling was obtained in affected tissues on immunohistochemistry applying primary antisera against nodaviruses isolated from the striped jack *Pseudocaranx dentex* and the sea bass *Dicentrarchus labrax*. We suggest that the mortality was caused by a nodavirus-like agent and that this disease of the Atlantic halibut is closely related to diseases termed viral nervous necrosis (VNN), viral encephalopathy and retinopathy and fish encephalitis, which have been associated with mass mortality in numerous cultured marine teleost species. In the Atlantic halibut, viral endocardial lesions were a constant finding, suggesting that viremia may be an important factor in the pathogenesis of the infection.

KEY WORDS: Atlantic halibut * fish * immunohistochemistry * electron microscopy * nodavirus * viral encephalopathy and retinopathy * neuronal vacuolation

Detection of a nodavirus-like agent in heart tissue from reared Atlantic salmon *Salmo salar* suffering from cardiac myopathy syndrome (CMS)

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ABSTRACT: The present study shows that a nodavirus-like agent is associated with the lesions of cardiac myopathy syndrome (CMS), a disease of unknown etiology which affects reared adult Atlantic salmon *Salmo salar*. In archive paraffin embedded heart tissue from Atlantic salmon diagnosed as suffering from CMS, a distinct immunohistochemical reaction was observed when using a primary antibody against striped jack nervous necrosis virus (Nodaviridae). Immunolabeling was detected in the mesothelium and hypercellular lesions of the epicardium and in endothelial cells and myocytes within mild multifocal lesions of the atrial and ventricular trabeculae. Transmission electron microscopy, performed on deparaffinized samples of the same tissue blocks, revealed substantial amounts of virus-like particles (VLP) in the cytoplasm of endocardial endothelium, in myocytes and in mesothelial cells of the epicardium. The VLP were isometric, spherical and unenveloped, with mean capsid diameters of approximately 25 nm, resembling a virus belonging to the Nodaviridae.

KEY WORDS: Cardiac myopathy syndrome (CMS) • fish • Atlantic salmon • nodavirus • immunohistochemistry

ANNEX 15.1

I.C.E.S. WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS, West Greenwich, RI (USA) 16-20 march 1997

Report on

Ecopathology approach as an alternative disease control

F. Baudin Laurencin

The interrelationship between animal, infectious agents and the environment is an "old" well known concept, and for fish it has been well illustrated by the diagram of Sniezko. It confers on a farm the complex properties of an ecosystem, and the state of health (equilibrium) results from the "harmonious" functioning of the system.

However, in the facts, veterinary medicine (and fish pathology) is today dominated by the explanatory scheme below :

pathogen ==> disease

==> immunity

This scheme leads to an **increasing use of drugs and vaccines** which can give good results for epizootics, even if in this way elimination of a strong pathogen often leaves the space to another one. However, more enzootic diseases are less susceptible to medicines and it has been proved that the application of another scheme can be useful for stopping or limiting the effects of such diseases :

environmental characteristics $\longleftrightarrow$ **farmed population characteristics**

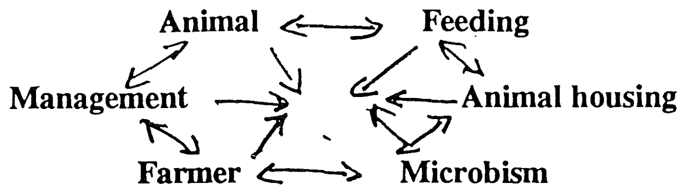
state of disease

The reductionist approach to analysis of elementary relationships cannot take into account the complexity of the system. By contrast, a global approach will provide a way of describing the behaviour of factors and proposing methods for intervention. Such an approach has been named ecological pathology or ecopathology : "The ecopathology studies the set of factors which are interrelated in the economical, human, physical, biological environment of the animals and which can be responsible for a pathological status and affect the productivity" (free translation from Ganiere *et al., 1991).*

From the epistemological point of view, ecopathology is characterized by three choices (Faye, 1995).

- <u>choice of complexity</u>

It takes into account in the same time all the characteristics of the breeding system (Tillon, 1987) which can be presented as an hexagone.



- <u>Choice of globality</u>

It places to the same distance the zootechnical performances and the sanitary status. The point is less for the disease to be eradicated, but just limited considering the economical and social objectives of the farming.

- <u>Choice of on the field study</u>

The farm is not only the place, but the material of the study. Of course, ecopathology can be considered as a branch of epidemiology but in the same time descriptive, analytic and operational, and adding ecology (a scientific discipline) to the epidemiological approach.

The scale of observation, the farmed population on a site, is specific to ecopathology which aim is to dissect the links between how the farm is working (functioning) and its health status.

Methodology

"There is a significant difference between conventional epidemiology and ecopathology. The former attempting to reconstitute the properties of a system by identifying the properties of its elements, applies a deductive procedure. The latter uses an inductive procedure in which an examination of the whole system at once precedes and provides orientation for analytical investigations" (Fourichon, 1991).

The aim of ecopathological investigations is finally the determination of risk factors and farm "classification" in relationship with these risk factors.

Risk factors are defined as characteristics of the individual farmed population and of environment under human control ; when present and capable of expressing themselves within a breeding system, they increase the probability of the appearance and development of a disease.

Starting from a more extensive field of investigation, ecopathology seeks to establish a limited number of risk indicators from a large number of variables. These risk indicators which can be modified are considered in planning intervention programmes, for the farm.

The overall strategy used to follow for ecopathological studies (aiming and ~~at~~ finding out risk-factors comprises four stages.

1. Protocol development phase.
Preliminary works in order to select the measurements relevant to undertake. Must be taken into account : literature data; meetings with practitioners (vets, farmers), field case studies, experimentation.

The aim is to select adequate forms for inquiries. Of the relevance and accuracy of the recorded data will depend on the final findings.

2. Data collection phase
The recruitment is made on voluntary basis within an homogeneous animal production system.

3. Data processing
The objective of the statistical analysis will be to select the most relevant combination of explicative variables (risk factors) associated to the different levels of the disorders. Multivariate methods are extensively used and especially the correspondence analysis and the hierarchical cluster analysis.

Results :

1. Farm typology :
In the factorial plans, can be identified several groups of farms each of them related with different levels of their health status (defined by health descriptors).

2. Associated risk factors
They appear in the same way in the factorial plans, as related to the health status.

Application :

a) Each farm can be identified in a group defined by health status and risk factos.

b) Forecasts are obtainable by modelling and simulating interventions on risk factors.

c) On the basis of these models, it is possible to propose intervention programmes for a farm, allowing corrections of unfavourable situations and the prevention of diseases.

Conclusions

Even if the term "ecopathology" was for the first time proposed in the context of fish production systems (Tufféry, 1971) the method was never really used in fish pathology, but on the contrary it has given interesting results in cow, sheep, goat, pig, poultry farming.

Two papers are annexed to this report, illustrating application of ecopathological method to the "udder infection" complex of dairy cows and to the control of the chronic respiratory disorders of pigs.

The same methodology could be used in fish farming, mainly for enzootic diseases or syndroms and in case of chronic disorders even if a pathogen has been identified as a determining factor (beside occasional or favorizing other factors...)

It could be discussed forward if some fish pathologies such as IPN, Pancreas Disease, sea lice, fry mortality syndrom, Pasteurellosis, sea bream winter disease, etc... would not be susceptible to a sort of control by ecopathological method.

Joined papers : *Madec F., 1995*
Faye B. et al, *1994*

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ANNEX 15.2

Literature citations presented for C. Res.1996/2:29 k.

Faye, B., Dorr,. N., Lescourret, F., Barnouin, J. and Chassagne, M. (1994) Farming practices associated with the "udder infection" complex. *Veterinary Research* 25: 213-218.

Summary - A total of 4129 dairy cows from 47 dairy farms were submitted each year after calving to a milk sampling for bacteriological examination. Six criteria defined the udder infection complex: frequency of minor, major and rare pathogens; rate of clinical and subclinical mastitis; and index of gravity of mastitis. Forty-seven variables described practices and their associations with the type of farms (defined by the above 6 criteria) were studied. The associated variables were dipping practices, covering of the milking parlour, disinfection practices, housing cleanliness and milk production.

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Annex 18. Specific Requirements of Shellfish Pathology Laboratories - Data Availability

A preliminary survey of shellfish pathology laboratories revealed a significant difference in types of diagnostic activities and data compilation. These can be generalised as:
Research laboratories working on specific diseases or disease agents.
Diagnostic laboratories screening specific hosts for specific diseases only (e.g., under the EC Directive)
Diagnostic laboratories screening for all infectious agents (e.g., to establish pathogen status zones or for introduction and transfer risk assessment) in a select number of host species.
Diagnostic laboratories screening for all infectious agents in a wide range of shellfish species.

Reasons for ICES to Store Disease Data

Data-storage is designed for two general objectives: i) accumulation of specific data applicable to defined multi-parameter research analyses, and ii) non-specific data archiving for undefined future reference and research.

The primary objective for an international disease database at ICES is to provide a multi-parameter database upon which changes in the ocean environment can be monitored and their significance assessed. Diseases form one of four data-bases selected to meet this objective: (i) bio-physical characteristics, biological effects measurements and tissue contaminant measurements from fish, invertebrates and mammals; (ii) general hydrographic parameters and contaminants of sea water; (iii) physio-chemical characteristics of the sediment, grain size distribution and contaminants in the sediment and (iv) fish disease data collected from standardised surveys.

Obvious constraints to disease data, such as (i) the amount of comparable data which can be compiled by member countries; (ii) consistency of sample collection, and (iii) consistency of data interpretation and storage; were addressed by the WGPDMO Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks Report (ICES CM1996/F:3) and a training guide was prepared for fish disease survey data collection (ICES Techniques in Marine Environmental Sciences, No.19. ISSN 0903-2606, 1996). Since this protocol addresses the collection of finfish disease data only, it was decided to assess the potential of shellfish disease data to augment the scope of the disease data base, using the protocols designed for finfish.

Species Suitable for Environmental Monitoring

Criteria used for fish include: (i) benthic; (ii) sedentary (relative to the geographic zone the report covers); (iii) abundant (or readily available for collection) and (iv) exhibit diseases which are easily recognised. Although there may be a fundamental difference with proximity to shore (based on comparative collection methods) shellfish can meet these criteria as follows:

(i) most bivalves and crustaceans are benthic; even infaunal bivalves have epibenthic contact via siphons.

(ii) many bivalves are sedentary and even motile species, such as scallops, show limited migratory range compared with many marine vertebrates and crustaceans. Cultured bivalves are, by necessity, static for prolonged periods of their lives (often years). Caged bivalves are currently being used to monitor pollutants in well-defined gradients or plumes.

(iii) all commercially important shellfish are abundant, however, few have an international distribution, especially between North America, Europe and Scandinavia (a notable exception is mussels, *Mytilus* spp.). Cultured species, such as *Crassostrea gigas, Ostrea edulis, Patinopecten yessoensis* and *Tapes (=Ruditapes) philippinarum*, have an international distribution, with a few exceptions (e.g., *C. gigas, P. yessoensis* and *T. philippinarum* do not occur in Atlantic Canada).

(iv) There are few grossly diagnosable diseases which are pathogen-specific for shellfish, thus, gross diagnosis of disease is open to question. Exceptions would be shell disease caused by *Ostracoblabe implexa* of European oysters; clionid sponge shell damage; *Polydora* spp. shell boring polychaetes; "Maladie des branchies" of Portuguese oysters. Abscess lesions, gill deformities, etc., all require microscopic examination and/or biomolecular diagnosis (where available).

The most obvious bivalve species for international monitoring is the blue mussel. Many attempts to use this species as an environmental sentinel in the past have met with varying success. Few, however, have addressed parasites and diseases. Atlantic Canada has a mussel-watch histopathology collection from the late 1980's which has yet to be examined. Plans are underway to have this material appended to a Master's project, starting in 1997, with duplicate sampling in 1997-98. Histopathology results will be compared to oceanographic data collected from the overlapping areas to assess any possible correlations.

Another group with a strong potential as environmental sentinels are clams. Although mortalities are more difficult to monitor in infaunal species, their link to sediment and sea water quality is evident. The amount of base-line data on clam diseases is growing as different species are brought into culture systems. This data should be assessed to determine whether or not clams can be considered as a multi-species data group.

Sampling procedures

Methodology for finfish and shellfish will inevitably vary due the reduced hit-miss risk with sedentary shellfish. The selection of set stations, however, can be duplicated by choice of consistent sampling sites within a defined environmental (e.g., contaminated gradient) or zonal framework. Sampling frequency can also fall within a spawning / non-spawning season selection. Therefore, exact timing will vary with the species being examined.

The suggested minimum of a 5 year survey negates many historical shellfish surveys' data. Most fall into the 2-4 year range, thus, continuity is a problem. However, the zonation concept behind the EC Directive for establishing pathogen status profiles, which comprises a two year survey with a minimum of 4 collections covering peak pathogen transmission seasons, followed by annual maintenance checks, may produce data of use for an FDE-like system. A similar zonation concept is being considered by Canada. These initiatives may also provide data of use for regression back to historic shellfish disease surveys.

The question of sample size will constitute a fundamental difference for most diseases of shellfish collected to date. Base-line shellfish survey numbers range from 60-150 individuals per sample depending on the prevalence of infection accepted for detection within a given probability. For finfish the minimum recommended sample size is 250 (for external lesions) and 50 (internal). This reflects the time and effort required for gross examination and preservation of "suspect" tissues for histology compared with diagnosis primarily by histology.

Disease Examination Procedures

As mentioned above, the requirement for histological confirmation is common to both data sources, however, useful shellfish diseases (e.g., neoplastic changes) rarely result in grossly visible lesions - thus the actual screening requires histology or histocytology, depending on the type of neoplasia. Some disease agents are beginning to fall within the realm of pathogen-specific biomolecular diagnosis, but the tools required are not yet readily available to all shellfish disease laboratories. As mentioned under "Specific Requirements of Shellfish Pathology Laboratories", different pathology goals require different examination procedures. Most surveys for non-specified pathogens, however, use histology. Precise protocols may vary between laboratories (fixative, tissue section selection, embedding medium, etc.), however, the end results are usually comparable - especially if a common stain is used (e.g., Haematoxylin and eosin) and samples are processed live and within accepted time-limits (for most species within 12-24 h, depending on ambient conditions) to prevent / reduce demersion tissue changes.

Diseases Useful for Monitoring Purposes

Criteria listed for finfish diseases:
(i) occur commonly in some or all selected fish species;
(ii) are easy to recognise;
(iii) have a possible response to surrounding environmental conditions
(iv) have a response that can be expressed in significant prevalence values.

Possible applicable shellfish diseases include: *Ostracoblabe implexa*, *Rickettsia*-like organisms in the gills and/or digestive gland, haemic neoplasia, ciliates on the gills or in the digestive gland and anomalous conchiolin deposition.

Disease Diagnosis

Most diseases of finfish are recorded either as present/absent or within a 3-stage qualitative scale. The scale levels are clearly defined. This is applicable to most shellfish diseases, with the caveat that some diseases can escape detection using standard histology diagnosis. Such diseases may, therefore, be considered useless for an FDE program (e.g., MSX, Dermo and Bonamiasis) unless monitored by more sensitive diagnostic tools. There are no historic data-bases using such tools, therefore, this must be taken into consideration for establishing shellfish disease data-bases . Changing diagnostic tools which enhance sensitivity and speed of diagnosis will disrupt the comparability and continuity of the data.

Data Treatment and Submission - FDE Data Format

Logistic regression analysis is the preferred statistical tool for epidemiological data. This can be accomplished using a number of PC programs or via the ICES Environmental Data Reporting Format for Fish Disease Data (FDE). This program was released in November 1994 and information on it can be accessed from the ICES Secretariat. Note that this program has extensions for less common diseases to facilitate cross-comparison with common disease agents. This is a good mechanism for retaining "peripheral" observations made during examinations for "standard" diseases or disease agents. However, shellfish data formats are not yet available.

The record format is as follows:

(i) *Sample Master Record* = general information on a sample of a single species collected within in limited time period. This may comprise repeat samples within a specified zone (e.g., for shellfish, from bottom, mid-water and surface sections of a long-line culture situation). Wild and cultured sources may require additional definition.

(ii) *Haul Information Record* = details on the method of collection. This is cruise-based and applies to field diagnostics only. There is negligible applicability to shellfish sample collection (e.g., sexing on site), thus, significant adaptations would have to be made to address shellfish collection criteria such as hand-picking, tonging, dredging, fixation on site, fixation post-air-storage, etc.

(iii) *Fish Disease Specimen/Sub-sample Record* = records number of specimens examined, different size-groups, sex-ratios, age-classes (sub-samples), number of diseases looked for. Although designed for field-based data entry, this could be adapted to laboratory data collected sequentially from the initial processing and completed from the histological examination.

(iv) *Fish Disease Data Record* = records the number of individuals affected by a particular disease within a sample (and sub-sample). This is compatible with any disease-data entry (finfish or shellfish). However, the program does not appear to record body site / tissue infected or the intensity (grade) of infection. These factors are noted in the collection of Training Guide for Identification of Fish Diseases and are important components of any disease data.

Overall the FDE Program could be compatible for shellfish disease data. The sections for cruise and haul, however, are not appropriate and could be by-passed.

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Annex 22 Analysis of progress with tasks

a. Analysis of national reports on new disease trends in wild fish, crustaceans and molluscs.Reports on new diseases and trends on diseases were evaluated from national reports presented at the meeting and conclusions were presented.

b. Analysis of national reports on new disease trends in mariculture for fish and shellfish. Reports on new diseases and trends on diseases were evaluated from national reports presented at the meeting and concluysions were presented.

c. Review of the Report of the Study Group on Statistical Analysis of Fish Disease Data and interpretation of the data provided. The draft report of the Study Group was evaluated and biological interpretations made of the data provided.

d. Review of the results of the Special Meeting on "The Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants" and assessment of progress on the implementation of standardised methodologies for the characterisation of liver pathology in disease monitoring programmes, in consultation with the Working Group on Biological Effects of Comtaminants. The conclusions of the Special Meeting were endorsed and the data presented in the Report used as basis for Agenda item (e)..

e. Development of a quality assurance plan to incorporate liver pathology and fish disease measurements within the biological effects monitoring component of the Joint Assessment and Monitoring Programme of OSPAR [OSPAR 1997/2.2]. Mechanisms to implement a QA plan for liver pathology studies were developed.

f. Review of the results of the BMB/ICES Seagoing Workshop on "Fish Diseases and Parasites in the Baltic Sea" in the light of their possible implications for future research/monitoring programmes in the Baltic Sea. The objectives of the Seagoing Workshop had been fully met, closer co-operation between research groups has been established and the fish disease monitoring programmes are using the same protocols as studies in the North Sea permitting integration of Baltic data into the ICES Fish Disease Databank.

g. Compilation of available evidence on the causes of the M-74 syndrome in Baltic salmon and provision of a summary of the progress in understanding the relevant environmental factors influencing the occurrence of M-74 along with an account of the geographical extent of its distribution [HELCOM 1996/8]. A summary of the current state of knowledge of the possible causes of M-74 was compiled. Direct questioning of salmon stock biologists and disease specialists did not indicate the occurrence of M-74 outside the Baltic in 1996.

h. Maintainance of an overview of the *lchthyophonus* issue as part of the regular agenda. The current status of *lchthyophonus* in eastern North Atlantic herring was assessed and a report compiled for presentation to ACME.

i. Review of current information on the impact of environmental contaminants on the immune mechanisms of marine finfish and shellfish in collaboration with the Working Group on Biological Effects of Contaminants.A summary of the current state of knowledge, including information from WGBEC was compiled and recommendations made on research needed.

j. Review of available information on diseases in marine fish associated with nodavirus or nodavirus-like agents to provide a basis for recommendations for future research. Current knowledge on Nodavirus was assessed and a summary provided as an Annex to this Report with recommendations for future research.

k. Review of information on the use of correlations between environment and pathology (ecopathology) as a basis for possible alternative measures for disease control in farmed fish and shellfish. The current state of knowledge and application of ecopathology in disease control in mammals was reviewed and its potential application to mariculture assessed.

l. Assessment of the progress in the identification of alternate host species for the transmission of bivalve pathogens, including the development and use of sensitive detection tools. Evidence for cross host species transmission has been established for *Microcystis mackini* and *Haplosporidium nelsoni* was reviewed. Research to address the lack of evidence for other pathogens was recommended.

m. Assessment of the progress in studies on the possible causes of *Bonamia ostreae* persistence in areas with low densities of European oysters and the possible relationship to disease tolerance status. An overview of *Bonamia* persistence in Europe is presented and research to differentiate disease resistance from other effects proposed.

n. Compilation of specific requirements of shellfish pathology laboratories for recording shellfish disease data with an evaluation of the applicability of the ICES Fish Disease Entry Program (FDE) and the Fish Disease Reporting Format. The difficulties of shellfish laboratories complying with the requirements of collecting shellfish data for the ICES Environmental Database were discussed and proposals were made on action which may be taken.

o. Provide of quantitative information on the escape of fish from mariculture operations in the context of the transfer of disease to wild stocks and advise on the means by which this impact could be controlled. A report was prepared for sending to ACME before its June 1997 meeting [OSPAR 1997/4.1].

Annex 23.1 RECOMMENDATIONS TO COUNCIL

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS
Rhode Island, USA 16 - 20 March 1997

Recommendations

The Working Group on Pathology and Diseases of Marine Organisms [WGPDMO] recommends that it meet at the Sea Fisheries Institute, Gdynia, Poland on 3-7 March 1998 under the Chairmanship of S Mellergaard to:

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

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

c) review and analyse the data extracted from the ICES Fish Disease Databank intersessionally and at the WG meeting;

d) review proposals for the development of a training and intercalibration programme for the diagnosis of histopathological liver lesions as part of a quality assurance scheme;

e) compile available evidence on the causes of the M-74 syndrome in Baltic salmon and provide a summary of the progress in understanding the relevant environmental factors influencing the occurrence of M-74 along with an account of the geographical extent of its distribution [HELCOM 1996/8];

f) maintain an overview of the *lchthyophonus* issue as part of its regular agenda and report to the Advisory Committee on Fishery Management and the Advisory Committee on the Marine Environment if new information becomes available;

g) review available information on pathological aspects of endocrine disrupting chemicals in estuarine and marine organisms.

h) review intersessional progress in the development and implementation of a quality assurance plan for the integration of flatfish liver histopathology data into the OSPAR Joint Assessment and Monitoring Programme and in the adaptation of the ICES Fish Disease Data Entry Programme and Fish Disease Reporting Format for the incorporation of liver histopathology data into the ICES Environmental Databank.

i) assess the progress in studies on the possible causes of *Bonamia ostrea* persistance in areas with low densities of oysters and the possible relationship to disease resistance and water temperature inhibition of infection.

j) to prepare an information package on the ICES Environmental Databank and Disease Data Reporting Format for submission to laboratories in ICES member countries identified as conducting shellfish disease surveys and / or diagnostics.

k) to review new progress in diagnostics, epidemiology and immunology of nodavirus and new nodavirus-like associated diseases.