Mariculture Committee

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REPORT OF THE

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Bremen, Germany
29 February–4 March 2000

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 OPENING AND STRUCTURE OF THE MEETING

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the University of Bremen, Germany, with S. Mellergaard as Chair. The meeting was opened at 10.00 hrs on Tuesday, 29 February 2000, with the Chair welcoming the participants, particularly those who had not previously attended WGPDMO. The German host, W. Wosniok, welcomed the participants to the University of Bremen.

The list of participants is appended in Annex 1.

Apologies were received from P. van Banning (Netherlands), S. Ford and S. MacLean (USA), A. Helström (Sweden), S. McGladdery and S. Bower (Canada), T. Renault (France), F. Ruano (Portugal), T. Bezgachina (Russia), S. des Clers (UK), V. Kadakas (Estonia), and H. Palm who was replaced by R. Dobberstein during this meeting.

Three members of WGPDMO have retired in the past year: P. van Banning (Netherlands), will be replaced by Olga L.M. Haenen, the replacements for F. Baudin-Laurencin (France) and J. McArdle (Ireland) have not yet been announced. The Chair has been in contact with F. Geoghegan who has taken over some of J. McArdle's duties.

This year no shellfish specialists attended the meeting but contact will be needed during the meeting in order to treat the TORs, dealing with shellfish items properly and to deal with any recommendations which may be required for consideration at next year's meeting.

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

2 ICES ANNUAL SCIENCE CONFERENCE 1999: ITEMS OF RELEVANCE TO WGPDMO

Items of relevance to WGPDMO from the 1999 ICES Annual Science Conference held in Stockholm, Sweden were highlighted by the Chair:

a) The report of the Mariculture Committee:

   i) accepted the report of the 1999 meeting of WGPDMO without change and its recommendations.
   ii) the request to the Mariculture Committee to get an early release of WGPDMO report was with an endorsement forwarded to the Consultative Committee, which proposed that the report be made available to the Mariculture Committee as soon as it is complete, via restricted access website or e-mail and the committee given two weeks to review the report. The Chair of the Committee can collect any comments made and, at the end of the two weeks if no serious comments or objections have been received, the report can be made available to a wider audience.

b) Working Group on Biological Effects of Contaminants

   i) has on its agenda for the 2000 meeting a TOR b –"hear presentations, in collaboration with WGPDMO on the effects of contamination on invertebrate histopathology, and consider whether there is sufficient knowledge on this subject to support a recommendation that invertebrate histopathology could be used for biological effects monitoring in the marine environment" (information will be obtained from the Chairman of WGBEC during WGPDMO meeting).

c) Theme Sessions of interest at the 1999 ASC in Stockholm:

   i) M74 and similar reproductive disturbances in aquatic organisms were successfully discussed with contributions from Scandinavian and North American scientists.
   ii) Health and welfare in cultivated aquatic animals. The items dealt with did not fulfil what was expected to be included within the title of the session, especially in lack of ethical considerations.

d) The full Terms of Reference of WGPDMO were agreed as ICES C.Res. 1999/2:F:01.

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

3.1 Terms of Reference

WGPDMO took note of the Terms of Reference contained in ICES C.Res. 1999/2:F:01 (Annex 2). The heavy agenda once again demanded extensive intersessional work by the members of WGPDMO selected by the Chair. These persons were requested to produce written working/discussion documents, to be included in the Report as Annexes. As agreed at the 1998 WGPDMO meeting, all working documents were to be prepared 2 weeks before the meeting and distributed by e-mail. As a result, all national reports and a considerable part of the remaining working documents were distributed to the participants before the meeting. The Chair thanked the members for preparing these reports in advance, a work which ensures that the Terms of Reference can be treated efficiently.

3.2 Adoption of the Agenda

A draft agenda was circulated and accepted without alterations (Annex 3).

3.3 Selection of Rapporteurs

Rapporteurs were accepted as indicated in Annex 4.

4 OTHER RELEVANT INFORMATION

Information was given on a series of scientific conferences to be held in 2000:

1) Euroconference–Water and Life–Oceanography, Meteorology and Marine Resources, May 2000, Vigo, Spain
2) SETAC, May 2000, Brighton, UK
3) 100 Years of Science under ICES, August 2000, Helsinki, Finland
4) Theme Sessions at ICES Annual Science Conference, September 2000, Bruges, Belgium
   - Sustainable Aquaculture Development
   - New Trends in Feeding in Aquaculture
   - Temporal and Spatial Trends in the Distribution of Contaminants and their Biological Effects in the ICES Area
5) Risk Assessment Course, September–October 2000, VESO, Norway.

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

5.1 Wild Fish Stocks

Viruses. The host range for **VHS-like** viruses in wild fish is the focus of research in several countries in Europe and North America. VHSV was recorded with low prevalence (2 %) in Pacific herring *(Clupea harengus pallasi)* from Prince William Sound, western Canada. Of 27 fish species examined in a survey in the North Sea, Skagerrak and Kattegat, VHSV-positive pools were found only in herring (*Clupea harengus*) from the Skagerrak. This is in contrast to observations in 1997–1998 when VHS virus was recorded from a considerable number of wild fish species in the North Sea area and southern Baltic Sea.

ISAV has been isolated from wild sea trout (*Salmo trutta*) in a sea water location and a river in Scotland, from a single saithe (*Pollachius virens*), co-habiting with infected farmed salmon, in eel (*Anguilla anguilla)* and in farmed rainbow trout in fresh water. Laboratory results also indicate that the virus may be present in wild fish in fresh water (brown trout, salmon parr). Epizootic studies are in progress to determine the significance of these observations. In Canada, large-scale tests for ISAV in wild salmonids and non-salmonids have been negative. However, ISAV was detected in Atlantic salmon identified as "aquaculture escapees", as well as in two broodstocks collected from one river in southern New Brunswick (NB: these positive fish had been held in a land-based facility for up to six weeks before examination, hence, the source of infection is not clear). Finally, two wild Atlantic salmon collected from two rivers in Nova Scotia tested positive for ISAV using RT-PCR, but SHK culture was negative. There is still no clinical ISA in Nova Scotia.

Birnavirus II was isolated from 32 samples from different fish species, mainly flatfish, caught in the eastern part of the North Sea and Skagerrak. The isolations were not associated with pathological changes.

Viral Erythrocytic Necrosis (VEN) is widely distributed in herring *(Clupea harengus pallasi)* in Alaska with prevalences ranging between 25–50 %, at moderate intensities. However, it is a problem causing significant mortalities in young-of-the-year herring in this area.

Lymphocystis. In the SW Baltic (ICES Sub-division 24), lymphocystis increased in flounder compared to 1998. In other areas, significant trends were not reported. Suspected cases of lymphocystis were recorded from cod in the Bornholm Basin, SW Baltic Sea, but the diagnosis requires confirmation, since this disease has never been recorded from cod before. In Spain, lymphocystis was recorded from red seabream (*Pagellus bogaraveo)* in the vicinity of pulp mill effluents, but a cause/relationship has not yet been demonstrated.

Bacteria. *Yersinia ruckeri* was recorded from cod in Sweden.

Pfiesteria piscicida. The relationship between *Pfiesteria* and skin lesions in menhaden (*Brevoortia tyrannus)* in the Chesapeake Bay area remains debated. Recent studies show *Kudoa* infections to be associated with ulcerative skin lesions of menhaden, as well as *Mycobacterium, Aphanomyces* and other microbes. It is generally agreed that the lesions are not restricted solely to *Pfiesteria piscicida* exposure, but that *Pfiesteria* may play a role in initiating them. *Aphanomyces* infections are repeatedly identified in association with skin ulcerations in fish and increased attention should be focused on the potential role this pathogen may play in wild and farmed fish populations.

Parasites. The myxozoan parasite *Parvicapsula minibicornis* was associated with high mortalities in sockeye salmon (*Oncorhynchus nerka*) from the Fraser River system in western Canada.

The protozoan parasite *Glugea stephani* increased in dab in the Belgian area and reached the highest prevalences (10.9 %) since 1985.

A significant increase in infection with *Clavella adunca* in Arctic cod *(Boreogadus saida)* has been recorded in the southeastern part of the Barents Sea.

The salmon louse, *Lepeophtheirus salmonis*, has been found in high numbers with associated pathology in wild Atlantic salmon post-smolts in Norway.

Anisakis simplex shows an increasing trend in the spring-spawning herring population from the spawning areas of the southeastern Baltic Sea, with prevalences up to 63 % in the Gdansk Basin. The highest prevalence (< 100 %) of *Anisakis simplex* larva was recorded in cod (> 70 cm total length) from the same part of the Baltic Sea. Similar high prevalences were recorded from cod in the Barents Sea, as well as pink salmon *(Oncorhynchus gorbusha)* and keta *(Oncorhynchus keta)* from far eastern region of Russia.

Anguillicola crassus is spreading in eel populations and is now recorded from new areas in the eastern USA. This parasite was also recorded for the first time in eels from Spanish rivers (Atlantic coast).

Skin ulcers. The occurrence of acute/healing skin ulcers in cod in the SW Baltic Sea (ICES Sub-division 24) declined from the high prevalences reported in 1998 (30 % in Dec. 1998, 14 % in Dec. 1999). No changes were reported from other areas. Field observations from Poland indicate that skin ulcers in flounder and cod might be associated with lesions caused by lampreys.

Skeletal deformities. This condition persists at low prevalences in cod in some areas. Numerous factors have been associated with skeletal deformities in wild and farmed fish, including bacterial and parasite infections, contaminants, temperature shocks, etc. Skeletal deformities and skin ulcerations co-occur in the same areas and the same fish specimens in the southern Baltic Sea, which might indicate a common etiological background.

Hyperpigmentation. No significant changes in pigment anomalies in flatfish were reported.

Epidermal hyperplasia/papilloma. This condition was recorded for the fist time from flounder in the SW Baltic Sea.

Liver nodules. The decreasing trend in the North Sea dab continues, however, a slight increase in prevalence in flounder from the Belgian area was reported.

An association was again reported between the prevalences of toxicopathic liver changes in English sole *(Parophrys vetulus*) and the extent of urbanisation of water areas in Washington State, western USA.

Miscellaneous. An increasing trend in the prevalence of granulomas in visceral organs was recorded in cod caught in the southern part of the North Sea (especially in ICES statistical rectangle 34F3). Further characterisation and diagnosis of these lesions remains to be done.

5.1.1 Conclusions

1) Several pathogens/diseases were recorded for the first time. These included:
   - ISAV in eel (*Anguilla anguilla*), sea trout (*Salmo trutta*) and saithe (*Pollachius virens*) from Scotland,
   - lymphocystis in red seabream from the Atlantic coast of Spain,
   - *Yersinia ruckeri* in Baltic cod from the Swedish EEZ,
   - epidermal hyperplasia/papilloma in flounder from the SW Baltic Sea,
   - *Aphanomyces* from menhaden (*Brevoortia tyrannus*) from Chesapeake Bay, eastern USA.

2) VEN is causing anemia and mortality in young-of-the-year herring in Alaskan waters.
3) The occurrence of *Anisakis simplex* in the spring-spawning herring population shows an increasing trend in the southeastern part of the Baltic Sea.
4) Skeletal deformities and skin ulcerations co-occur in the same area and specimens of cod in the southern Baltic Sea, at slightly increased prevalences over previous years. This might indicate a similar etiologic background.

5.1.2 Recommendation

WGPDMO recognizes that there is an urgent need for clear definitions and guidelines concerning the meaning of the terms hypermelanization/hyperpigmentation/pigment anomalies and recommends that such guidelines should be prepared as an intersessional task.

5.2 Farmed Fish

Atlantic salmon (*Salmo salar*) - Viruses

Infectious Salmon Anaemia (ISA) No new trend was reported from Norway. The number of new infected sites and sites under restrictions remains similar to last year. In Scotland, by the end of 1999, the total number of farms found to be infected was eleven with a further 24 suspected of being infected. This represents only one new farm with clinical ISA detected in 1999 but an increase of nine suspected farms. This may indicate the level of success of the control programme carried out. In Canada, there are no new trends, although new positive farm sites were detected in several areas of the Bay of Fundy.

Further information on ISA is covered under a separate review (see Section 11, below).

Infectious Pancreatic Necrosis Virus (IPNV) In Norway and Scotland, infections with IPNV are still considered to be a major problem in the post-smolt phase. In Norway, there are apparent variations in the virulence within the predominant Sp serotype. Laboratory trials with vaccination are promising.

Salmon Pancreas Disease Virus (SPDV) No new trend was reported except from Norway, where two new cases with high mortalities were diagnosed.

Paramyxovirus In Norway, the importance of a paramyxo-like virus, detected in gills from fish suffering from epitheliocystis, remains unclear.

Togavirus In Eastern USA, a toga-like virus continues to be observed from approximately 50 % of marine cultured sites, compared to 46 % in 1998. No apparent pathology has been associated with this agent to date.

Salmon Swimbladder Sarcoma Virus (SSSV) In the USA, no further outbreaks have occurred.

Atlantic Salmon - Bacteria

Epitheliocystis-associated gill damage seems to be an increasing problem in some parts of Norway. The disease has been reported to affect salmon three to six months after transfer to sea. Mortalities can reach 10–30 % over a period of two to three months.

Piscirickettsia salmonis was detected in Norway (5 new cases) and Scotland with no significant levels of associated disease problems.

***Aeromonas salmonicida, Vibrio* spp.** Infections caused by *Aeromonas salmonicida* and *Vibrio* spp. are effectively controlled by vaccination. In general, outbreaks are only observed in unvaccinated fish stocks.

Atlantic Salmon - Parasites

***Paramoeba* sp**. gill infections caused severe problems in one farm in Northern Spain, forcing it to shut down.

Sea lice (*Lepeophtheirus salmonis*) Infestations with sea lice remain a major disease problem in Norway and Scotland. Recently, management-like synchronised treatments, etc., are being implemented to improve the disease control.

Other Salmonids

In Finland, **flavobacteriosis** continues to be a problem in rainbow trout (*Oncorhynchus mykiss*).

The myxozoan parasite, ***Parvicapsula minibicornis***, has been associated with renal pathology and pre-spawning losses in Canadian sockeye salmon (*Oncorhynchus nerka*). This constitutes a new host- and geographic-record for this parasite.

Viral Haemorrhagic Septicemia Virus (VHSV) was isolated during routine screening of rainbow trout from a Norwegian hatchery in 1998. Following stock eradication, no new cases have been reported.

Turbot (*Scophthalmus maximus*)

In Spain, ***Flexibacter maritimus*** continues to be a problem.

Halibut (*Hippoglossus hippoglossus*)

Nodavirus associated with vacuolating encephalopathy and retinopathy (VER) remains a problem in Norwegian Atlantic halibut fry. **Birnavirus II** and an **aquareovirus** were isolated from Icelandic halibut fry, without any apparent pathology. In Canada, an aquareovirus-like agent was isolated from cultured juveniles suffering mortality.

Sea bass (*Dicentrarchus labrax*), seabream (*Sparus aurata*)

Pasteurella piscicida (Photobacterium damsela) was the main disease problem for sea bass and seabream in Spain. Pasteurellosis was also suspected to have caused a massive mortality at one sea bass farm in Portugal.

Eel (*Anguilla anguilla*)

In Denmark, ***Herpesvirus anguillae*** infections cause occasionally problems, but a controlled viral exposure at an early larval stage seems to reduce the problem. In Spain, several disease outbreaks are caused by *Vibrio vulnificus*.

Amberjack (*Seriola dumerili*)

In Spain high mortalities have been experienced owing to a monogenean parasite belonging to the genus ***Zeuxapta***.

5.2.1 Conclusions

1) *Paramoeba* sp., a pathogen known to cause severe disease problems in Atlantic salmon culture in Tasmania, was recorded for the first time in Spain, causing high mortality on one farm. The disease has also been reported from New Zealand, USA, Chile, Ireland and France.
2) ISAV is still an important salmon pathogen in Norway, Scotland and Canada. Recent figures indicating a decrease of new clinical outbreaks may indicate success of control measures presently carried out in some areas.
3) Sea lice (*L. salmonis*) continue to be among the most important pathogens in salmon mariculture.
4) Nodavirus continues to cause problems in Norwegian halibut farming. Attention was also drawn to isolations of viruses previously unknown from halibut (Iceland and Canada), and note made of the implications that these infections might have on the future rearing of this fish species.

5.2.2 Recommendation

WGPDMO recommends that a report on the effectiveness of salmon farming management control methods, for the control of sea lice, should be prepared and evaluated at the WGPDMO meeting in 2001.

5.3 Wild and Farmed Shellfish and Crustaceans

5.3.1 Analysis by disease or parasite

Bonamia ostreae – Due to the high prevalence of *Bonamia* encountered in *Ostrea edulis* in the Spuikom, near Ostend in Belgium, efforts in cultivation of this species have now ceased and *C. gigas* are being cultured instead. In the Netherlands the prevalence of the disease during the spring increased significantly from 1998 (12 % to 28 %) but declined to < 1 % by the autumn. The mild winter 1998/1999 was thought to be a possible cause for the high spring level. In the rest of Europe, prevalence of the disease has not changed and there is no indication that it has spread. However, the disease persists in previously infected areas with some indication of declining prevalence in northwest Spain. No change was reported from the northeastern USA where the disease remains at low levels. The disease is still absent from Canada, Denmark, Norway, Sweden and Scotland.

***Marteilia refringens* and *Marteilia* sp.** – Despite increased surveillance, no evidence of *Marteilia refringens* has been found in *O. edulis* stocks from Belgium, Denmark, Ireland, Norway, Scotland, Sweden, England, Wales and North America. However, *M. refringens* remains prevalent in most coastal zones in France. In Spain, the parasite is still present in *O. edulis* but at a low prevalence at a single location. *Marteilia refringens* in *Mytilus edulis* was also recorded with a declining prevalence (less than 25 %) from the Atlantic coast of Spain and at low levels in mussels from France.

Summer mortality – During 1999, summer mortalities in *C. gigas* in France have been less severe than in previous years. The causes of these mortalities remain unknown.

Gill disease in the Portuguese oyster *Crassostrea angulata* was again reported from Portugal. The prevalence was up to 6 %, with affected animals showing characteristic gill lesions, often with associated necrosis and inflammation in other tissues.

***Perkinsus* spp.**

Perkinsus marinus was recorded in almost 100 % of *Crassostrea virginica* from the Gulf of Mexico to Massachusetts in the USA. The extraordinarily high prevalence is thought to be a consequence of a year-long drought in 1999, combined with higher than usual temperatures. Mortalities, due to the infection, exceeded 50 % in many areas during the year.

Perkinsus atlanticus infections in the clam *Ruditapes decussatus,* in Spain, were recorded at 30 % prevalence with no mortalities. In Portugal, infections of the same clam species were associated with mortalities close to 60 %. In France the same parasite was detected in *Ruditapes philippinarum.*

Nocardiosis was noted for the first time in British Columbia, and was associated with mortalities of approximately 10 % in suspension-grown *O. edulis*. Previous infections reported from *C. gigas* were not observed in 1999.

Digenean infections with *Prosorhynchus squamatus*-like larval trematodes were found in 93 % of a "blond" strain of *M. edulis* at a single locality on the Pacific coast of Canada. No change in the parasite distribution was seen on the Atlantic coast.

Haemic neoplasia in soft-shell clams (*Mya arenaria*) was associated with a serious mortality and complete loss of one cultured bed in Prince Edward Island. This is the first record of prevalences up to 95 % and mortalities associated with this disease in Atlantic Canada.

Neoplasia in cockles (*Cerastoderma edule*) in Galicia resembled that associated with epizootics in the 1980s in Ireland and France.

Haplosporidiosis – A *Haplosporidium*-like parasite was reported in cockles (*Cerastoderma edule*) from France. On the Atlantic coast of Spain, several cases of *Haplosporidium amoricanum* in *O. edulis* were also reported. In North America, *H. nelsoni* (MSX) in *C. virginica* spread up the estuary in Chesapeake Bay with associated mortalities of up to 50 %. The decline in prevalence of this disease in Long Island has continued with prevalence down to 12 % in 1999. However, high mortality was observed in susceptible seed. This finding indicates the potential for wild stocks to become resistant to the disease. A new outbreak of MSX was recorded in cultured stock from Barnstable harbour, on the north side of Cape Cod, Massachusetts.

Haplosporidium costale (SSO) was not detected in *C. virginica* between New Jersey and Massachusetts in 1999, where it was found at elevated prevalences in 1998.

Juvenile oyster disease (JOD) occurred for the first time in an upwelling nursery in Long Island Sound, and in a suspension-culture operation, where it caused approximately 60 % mortality. Elsewhere the disease persisted at low levels in 1999.

Paramoeba-associated mortalities of lobsters (*Homarus americanus*) were reported from commercial traps in western Long Island Sound, NY. The mortalities began during September when approximately 8 % of lobsters were found dead in the traps, with mortalities rising to 30 % after two days. Nervous system infection by an organism identified as a *Paramoeba* sp. is thought to be the causative agent.

Hematodinium has been found in blue crabs (*Callinectes sapidus*) in coastal embayments from New Jersey to Florida and in Texas. Prevalence was up to 100 %.

Other conditions – In *C. angulata* in Portugal, necrosis of the adductor muscle, referred to as "Foot disease" continues to be a problem, with approximately 20 % of the natural beds in the Sado Estuary being affected. The aetiology is unknown. Also in Portugal, infestation with "mud worms" in areas with high levels of suspended material has handicapped commercialisation of these oysters. In France, *Mytilicola intestinalis*, Rickettsia-like organisms and gregarines were detected in *M. edulis*. In addition, in Normandy, abnormal mortalities have been observed in juvenile and adult *Haliotis tuberculata*. A *Vibrio* sp. was detected in association with the mortalities.

5.3.2 Conclusions

1) No significant new trends were detected in the prevalence and distribution of *B. ostreae* and *M. refringens*.
2) Gill disease in Portuguese oyster was recorded for the second year associated with significant pathological changes. The aetiology has yet to be confirmed.
3) The *Haplosporidium*-like parasite in cockles from France appears to be a new record and its relationship to *H. nelsoni* needs to be clarified. *H. nelsoni* continues to spread in Chesapeake Bay and cause mortalities, particularly among juvenile oysters. However, the possibility of resistance in older wild oysters is encouraging and the nature of this apparent resistance should be studied further.
4) A *Paramoeba*-associated disease of lobsters (*Homarus americanus*) caused substantial mortalities in the western Long Island Sound, NY. Further information on the aetioligical background and effect on the stocks is needed.

5.3.3 Recommendation

WGPDMO recommends that a report on the progress of further investigations on the role of paramoebae and other factors in the mass mortality of lobsters in Long Island should be prepared and assessed by WGPDMO.

6 UPDATE INFORMATION ON THE DISEASES AND PARASITES OF BALTIC FISH, TO BE INCLUDED IN THE HELCOM FOURTH PERIODIC ASSESSMENT

T. Lang presented a report on fish diseases and parasites in the Baltic Sea that he compiled intersessionally with input from WGPDMO members from Baltic Sea countries (Annex 5). He explained that the report, once reviewed by WGPDMO, will be forwarded to the ICES ACME and ACFM for a further review and will then be submitted to the Helsinki Commission for inclusion in the Fourth Periodic Assessment, which will be published in the year 2001.

The report represents an update of a chapter published in the Third Periodic Assessment for the period 1989–1993 (HELCOM, 1996). A similar structure is being used for the new report, but it is focused on disease and parasite information for the period 1994-1998. The main fish species covered in the report are flounder, cod, herring and salmon. However, other species with significant diseases or parasites are also considered. The report further contains information on the impact of anthropogenic activities on the prevalence and geographical distribution of fish diseases and parasites and on the impact of fish diseases and parasites on Baltic fish stocks.

WGPDMO appreciated the outcome of the intersessional activity and regarded the report as a useful contribution to the Fourth HELCOM Periodic Assessment, providing a good overview on the status of fish diseases and parasites in the Baltic Sea.

In the discussion of the report, it was suggested that further information should be included covering the following issues:

- A figure providing information on temporal trends in the prevalence of the M74 syndrome based on Swedish and Finnish data;
- The spread of *Anguillicola crassus* in the Baltic Sea area (available information will be provided by all WGPDMO members from Baltic Sea countries);
- The occurrence of the Proliferative Kidney Disease (PKD) in sea trout;
- Reproductive disturbances in fish caused by the microsporidian parasite *Pleistophora mirandella*;
- Confirmation of the results on the occurrence of *Ichthyophonus hoferi* in heart tissue kept in culture medium for two months taken from herring without clinical symptoms of an infestation;
- More photographs showing diseases and more figures providing trend data (e.g., for the ulcer disease in Baltic cod);
- Baltic fish species found to be infected with Birnavirus II;
- Occurrence of *Pseudobacciger* sp. in Baltic herring.

It was further suggested that the report should contain information on disease trends in farmed fish from Baltic Sea mariculture facilities, because bacterial and viral diseases, which have occurred in previous years, are of significance and should, therefore, be included in an environmental report. G. Bylund volunteered to provide relevant information.

WGPDMO agreed that the report with the changes included during the meeting will be distributed electronically to WGPDMO members from Baltic Sea countries and to native English speakers from WGPDMO. Relevant information to be added and further comments should be sent to T. Lang within two weeks after the end of WGPDMO meeting. The revised report will then be distributed again for final approval by WGPDMO.

6.1 Conclusion

WGPDMO appreciated the report as a useful contribution to the HELCOM Fourth Periodic Assessment. Further information for inclusion in the report will be provided by WGPDMO members to T. Lang within three weeks after the end of WGPDMO meeting. The finalised report will be distributed thereafter for final approval by WGPDMO.

7 REVIEW PROGRESS IN THE DATA SUBMISSION TO THE ICES DATA BANKS AND CONTINUE THE STATISTICAL ANALYSIS OF ICES FISH DISEASE DATA IN RELATION TO ENVIRONMENTAL AND FISHERIES DATA

W. Wosniok and T. Lang presented a report (Annex 6) providing information on new data submissions to and on structural changes in the ICES Data Banks which have occurred in 1999, and on the results of a statistical analysis carried out intersessionally, using an updated set of data and improved statistical methods.

In 1999, five new sets of fish disease data for the period 1997–1999 were submitted to the ICES Environmental Data Centre. Four of these have been validated and incorporated in the Data Centre. There have been no new submissions for contaminants and biological effects data.

The ICES Oceanographic Data Centre has been modified considerably. Oceanographic data stored at ICES for the period 1980–1989 are now public domain and can be downloaded directly from the ICES website. More recent oceanographic data can be obtained from ICES on request.

Apart from new data derived from the ICES International Bottom Trawl Survey (IBTS) and from commercial catch data, no major changes occurred with regard to the ICES Fishery Data Banks. Data on fishing effort, originally planned to be included in the holistic analysis of the ICES fish disease data, are still not easily accessible.

For the statistical analysis carried out intersessionally, the updated oceanographic data were included. Apart from these data, the same data set as last year was used which was extracted from the ICES Environmental Data Centre and from the ICES Fishery Data Banks. As for last year's analysis, the analysis was restricted to an area in the southeastern North Sea (extended German Bight) because, for this area, the most comprehensive set of data for the period 1981–1997 was available. Both a univariate analysis, exploring the relationship between the prevalence of dab diseases and single potentially explanatory factors, and a multivariate analysis, considering all explanatory factors, were applied.

Based on problems identified during last year's analysis, a new statistical approach was developed for a more appropriate way to incorporate interpolated values. This method allows the statistical consideration of extra variances caused by the use of interpolated instead of measured values.

The results for the univariate analysis show that, with a few exceptions, the factors identified as having a significant relationship with the disease prevalence were identical to those identified in the previous analysis. However, significance levels were generally considerably weaker. The results of the multivariate analysis indicate that, even after applying stricter rules for the incorporation of interpolated values, significant relationships between explanatory factors and disease prevalences exist. However, in contrast to the previous analyses, fewer factors appeared as significant in the final models.

During the discussion of the report, WGPDMO stressed that there still is an apparent lack of data in the ICES Data Banks (e.g., for contaminants in water, sediments and biota, but also for fish disease data covering other areas and species), creating problems in the statistical analysis, particularly if the analysis is extended to cover larger regions in the ICES area. Since there is evidence that more relevant data are available in national data banks of ICES Member Countries, WGPDMO strongly emphasised the need to incorporate these data in the ICES Data Banks.

WGPDMO agreed that the analysis of the ICES data should be continued intersessionally and that a progress report should be presented and reviewed at the 2001 meeting of WGPDMO. For a more comprehensive use of available data, disease prevalence data used should not be restricted to female dab, size group 20–24 cm, but should also cover other size groups and data for male fish. As a strategy to accomplish this, it was suggested to adopt a method commonly applied in human epidemiology, which is based on the calculation of disease prevalences for a standard population (direct standardisation). Furthermore, based on the availability of data, other geographical areas within the ICES area should be identified which can be included in the analysis.

It was further suggested that a manuscript for submission to the *ICES Techniques in Marine Environmental Sciences* (TIMES) series be prepared, describing statistical methodologies developed so far for the statistical analysis of the ICES data in relation to fish diseases. This will not only be of use for the treatment of disease data, but also for the analysis of relationships within other types of regional and temporal data.

7.1 Conclusion

WGPDMO appreciated the results of the report and considered it as a progress in the long-term activities of WGPDMO regarding the holistic analysis of the ICES data in relation to fish diseases. The analysis should be continued, aiming at a more comprehensive use of data available in ICES Data Banks and of data known to exist in national data banks, which have not been submitted to ICES so far.

7.2 Recommendations

WGPDMO recommends that:

i) ICES Member Countries should be strongly encouraged to submit relevant new and historical data to the ICES Data Banks in order to enable a more comprehensive statistical analysis of ICES data in relation to the spatial and temporal occurrence of fish diseases. Major gaps identified concern data on contaminants in water, sediments, biota and data on the occurrence of diseases of wild fish outside the North Sea;

ii) the statistical analysis of the ICES data should be continued intersessionally, involving an updated data set and the use of improved statistical methods, and a progress report should be presented and reviewed at the 2001 meeting of WGPDMO;

iii) a draft manuscript for submission to the ICES TIMES series should be prepared for review at the 2001 WGPDMO meeting, describing the statistical methodologies developed for the analysis of the ICES data in relation to fish diseases (authors: W. Wosniok *et al*.).

8 MAINTAIN AN OVERVIEW OF THE SPREAD OF *ICHTHYOPHONUS* IN HERRING STOCKS AND THE DISTRIBUTION AND POSSIBLE CAUSE(S) OF THE M74 SYNDROME

8.1 Current Information on *Ichthyophonus*

Working papers dealing with the results of sampling herring, *Clupea harengus,* for *Ichthyophonus* submitted by Iceland, Norway, Russia, Scotland, England/Wales and Denmark were compiled and presented by A. McVicar (Annex 7). Data from Sweden are being collected and will be presented at a future meeting, while in Poland and Canada no *Ichthyophonus* monitoring took place in 1999.

In Icelandic waters, both summer-spawning herring and Atlanto-Scandic herring stocks were examined, using both a mid-water trawl and seine nets for catching. A total number of 2045 summer-spawning herring were examined and 3 infected fish (0.15 % mean prevalence) were found. In the Atlanto-Scandic stock, 12.7 % prevalence (61 of 479 examined) was detected among mid-water trawl catches, whereas 1.2 % prevalence (6 of 521 fish) was detected in seine net catches.

Norwegian data included examination of catches from Norwegian spring spawners, North Sea herring and Baltic spring spawners in the northeastern North Sea. Although the picture is somewhat confusing, very little *Ichthyophonus* was detected in seine net caches. Some catches by mid-water trawls demonstrated somewhat higher prevalences.

Russian data presented prevalence figures for herring in both the Norwegian Sea and the Barents Sea. Significant differences in prevalence figures were observed when two different methods of examination were used, i.e., the standard ICES method and the Russian method of “clinical analysis”, which is based on microscopic examination of stained sections from different organs. Norwegian Sea herring stocks demonstrated 3.4 % and 52.6 % infection prevalence using both methods, respectively. In the Barents Sea, the figures were 0 % and 3.3–14 %, respectively.

Scottish data from the North Sea, east of the Shetland Isles (area2, IVa), showed 0.11 % infection prevalence (2 out of 1876 fish) from seine net caches. Prevalence figures from mid-water trawl catches were 2.1 % (ICES Division IVa) and 0.2 % (ICES Division IVb), i.e., in 26 out of 1237 and 2 out of 967 fish, respectively.

Reports from England/Wales on the southern North Sea herring stated that no evidence of *Ichthyophonus* infection was found in 145 fish sampled in Flamborough and Celtic Deep areas.

No *Ichthyophonus* infection was detected by Estonian researchers from a total number of 1123 Baltic herring and 312 sprat sampled monthly from February to December. Nor was any *Ichthyophonus* infection detected in 32 European eelpout/viviparous blenny (*Zoarces viviparus*).

Data from stock assessment samples in Denmark indicate an infection prevalence of 0.1 % to 0.5 % from seine net catches. Preliminary results indicate that the prevalence rate is highly underestimated when using the standard ICES method; by the use of a special culture medium for tissue samples, the detection of sub-clinical infections may increase significantly.

From the USA there are no reports of obvious *Ichthyophonus* lesions from groundfish surveys in the Northwest Atlantic. Nor were any lesions found in Prince William Sound (Alaska) herring, in spite of a regular examination. The general data for 1999 are not yet complete, but it can be estimated that *Ichthyophonus* infection exists at a low prevalence. It was also indicated that *Ichthyophonus* infection may have been detected from ovarian fluids of several Pacific salmon species. The diagnosis was presumptive, based on the observation of morphological characteristics of the organism, growing in tissue culture.

8.2 Conclusions

1) *Ichthyophonus* infection continues to persist at a low prevalence in the herring stocks examined, i.e., in Icelandic waters, Kattegat, northern North Sea, Barents Sea and the Norwegian Sea, without any indication of an epizootic. WGPDMO recommends, however, that the situation should continue to be monitored.
2) Attention was again drawn, by two countries, to the difference observed in prevalence figures when using different catching methods, i.e., mid-water trawl and seine net. It is recommended that comparative analysis of data from the two catching methods should continue.
3) A further analysis on the suitability of a culture method (USA and Denmark) to detect sub-clinical infection is highly recommended, as it may increase the understanding of the behaviour of this infection in fish and the relationship to the appearance of clinical signs.

8.3 Current Information on M74

A review report was presented by G. Bylund (Annex 8).

M74 continues to threaten Baltic salmon. Thiamine treatment of broodfish, eggs or fry is now routinely used in most hatcheries in Finland and Sweden to control fry mortalities. After two years of declining prevalences, there was a steep increase in 1999 offspring. A prognosis based on the thiamine contents of the eggs suggests that the prevalence will remain high in year 2000 offspring. Although progress was made, there was no significant breakthrough in 1999 in research focusing on the aetiology of M74. The status of knowledge and available information was reviewed and discussed at two international meetings last year (the ICES ASC and the EAFP congress in Rhodes). Results presented indicate a positive correlation between M74 and the increasing sprat population in the Baltic Sea, but this does not explain the aetiology of the disease. It seems clear that low thiamine contents in forage fish of Baltic salmon is not a cause of the disease, as the thiamine contents in herring and sprat are above the level necessary for salmon. Recent studies show that the agent(s) responsible for the disease appear(s) to be present in most parts of the Baltic Sea, as salmon populations with feeding runs to the main Baltic Sea as well as those migrating only to the Gulf of Bothnia and the Gulf of Finland developed the M74 syndrome. So far, there are no reports of M74 in the Latvian salmon populations which have different patterns of feeding and spawning runs. Recent studies indicate that gut microflora might be associated with the thiamine/thiaminase kinetics of fish.

Several models for reproducing thiamine deficiency and M74 symptoms in fish, under laboratory conditions, have now been developed. These will facilitate research on the aetiology of the disease. Ongoing and recently initiated work is focusing more and more on lower levels in the food chain of Baltic fish, i.e., the effects of the eutrophication and accompanying algal blooms, changes in plankton fauna, etc., as potential factors in the thiamine/carotenoid status of fish.

8.4 Conclusions

1) It is evident that the aetiology behind the M74 syndrome is still unclear and that this disease syndrome remains a serious threat to wild salmon populations in the Baltic Sea. In light of the recent trend of steeply increasing disease prevalence, WGPDMO emphasises the urgent need for increased research efforts on this disease.
2) It is important that scientists working on this disease focus enough attention on the role of genetics in Baltic salmon populations, which might be a factor in the aetiology of M74 syndrome.

8.5 Recommendations

WGPDMO recommends that:

i) research activities on M74 should be encouraged in Baltic countries with spawning populations of wild Atlantic salmon;
ii) an overview of the geographical distribution of the M74 syndrome continue to be maintained and progress made with regard to the identification of possible causes.

9 INVESTIGATE GILL DISEASE IN *CRASSOSTREA ANGULATA* ADULTS, THE CAUSE OF SUMMER MORTALITIES OF *C. GIGAS* SPAT AND CLARIFY THE REPORT OF *M. REFRINGENS* IN *C. GIGAS* FROM SPAIN

A working document on these subjects was prepared by T. Renault (Annex 9).

Gill disease in *C. angulata* adults reported in the 1999 report of WGPDMO and known since the 1970s is currently under investigation in order to determine whether an iridovirus might be involved in the disease. It is planned that a survey of several oyster beds in different locations in Portugal will be undertaken during 2000. It is intended that the results from these investigations will be available for discussion at the next meeting of WGPDMO.

The possible role of a herpes-like virus in summer mortalities of *C. gigas* and two species of clam larvae (*Ruditapes decussatus* and *Ruditapes philippinarum*) is currently under investigation. It is now known that herpes-like viruses can be detected in many bivalve species and in some cases they have been associated with significant mortalities, particularly in larvae. The application of molecular probes specific for herpes-like virus, including *in situ* hybridisation techniques, are being applied at IFREMER (La Tremblade) on *C. gigas* samples from Portugal. Evidence of herpes-like virus in this material has not been found to date and it appears that the mortalities among oysters in Portugal have another aetiology.

It has still not been possible to obtain material to clarify the report of *M. refringens* in *C. gigas*. Molecular tools are available to detect a variety of life-cycle stages of this parasite and it is important that material is made available for analysis using these probes, so that the findings can be discussed at the next meeting of WGPDMO.

9.1 Conclusion

The questions behind this recommendation have not been answered, but it is encouraging to see progress in research planning between IFREMER and other affected countries for 2000 which should shed more light on the nature of the gill disease in *C. angulata*, the homogeneity of *Herpes*-like viruses affecting mollusc culture and the nature of *C. gigas* infection by *Marteilia refringens*. All these questions are significant for effective management of these mollusc infections/diseases under open-water culture conditions.

9.2 Recommendations

WGPDMO recommends that:

i) the agent of *Crassostrea angulata* gill disease be confirmed (work under way at IFREMER) and its infectivity to *Crassostrea gigas* and other oysters species be investigated and reported at the next WGPDMO meeting;

ii) continued attention be given to inter-specific transmission experiments with herpes-like virus;

iii) continued efforts be made by the EU/OIE Mollusc Disease Reference Laboratory to clarify reports of *M. refringens* in *C. gigas* from the Mediterranean coast of Spain.

10 COLLATE AND REVIEW AVAILABLE INFORMATION ON THE DISTRIBUTION AND EFFECT OF MARINE VHS-LIKE VIRUS ON CULTURED AND WILD FISH

S. Mellergaard presented an overview of the present knowledge on marine VHS-like virus (Annex 10).

In contrast to classic VHS, with its characteristic symptoms of disseminated haemorrhages, known from infections in rainbow trout (*Oncorhynchus mykiss*) in freshwater, the marine VHS-like virus infections occur with limited symptoms such as skin lesions or without gross lesions. Only turbot (*Scophthalmus maximus*) infected with marine VHS-like virus has shown symptoms similar to the classic symptoms described from rainbow trout.

In the North American Pacific area, marine VHS-like virus was observed for the first time in ascending chinook salmon (*O. tshawytscha*) and in coho salmon (*O. kisutch*) in 1988 and since then it has been isolated from 6 marine fish species.

In Europe, the awareness of VHS in marine fish species was raised in association with an outbreak of VHS in turbot in Scotland in 1994. Prior to this observation, VHS virus had been associated with outbreaks in rainbow trout in France (1980) and Denmark (1982) and in turbot in Germany (1991), but the source of these infections was regarded as being of freshwater origin. In the marine environment, VHS virus had been isolated from cod in 1979 and 1993 and from haddock in 1993 but these findings were regarded as obscure findings or a product of contamination.

Intensive monitoring on the geographic distribution and the host range has been initiated in many European countries. So far, these investigations have shown that marine VHS-like virus occurs in the British Channel, northern North Sea, North Atlantic north of Scotland, Skagerrak, Kattegat and the Baltic Sea, with highest prevalence in the Baltic Sea. So far, marine VHS-like viruses have been isolated from 14 marine fish species in Northern European waters.

Mass mortalities in several marine fish species associated with marine VHS-like virus have been reported since 1998 from the North American Pacific area.

The marine VHS-like isolates have shown low pathogenicity to salmonids where tested in Europe and North America. This has been in contrast to isolates from fresh water.

Genetic analysis of marine VHS-like viruses has so far revealed four different groups, namely a Pacific strain, a North Atlantic Scottish strain and two strains from the Baltic/Kattegat, one of which appears to be closely related to the freshwater isolates.

10.1 Conclusions

1) WGPDMO recognises that marine VHS-like virus may constitute a potential risk for culture of marine fish species because of the wide host range so far detected. There is also evidence accumulating that, in some circumstances, marine VHS-like virus infection may be associated with mass mortalities in wild marine fish populations.
2) During discussion, WGPDMO recognised that there were important gaps in knowledge, particularly in two areas:
    a) The infectivity and pathogenicity characteristics of the different marine isolates of VHS-like virus are emerging as particularly important features, useful in conjunction with genetic information, to distinguish different types or strains within this now demonstrably heterogeneous virus group. However, to date there has only been limited cross infectivity trials using different isolates and different types of non-salmonid marine fish species.
    b) WGPDMO notes the considerable evidence accumulating for different viral types being listed under the same name Viral Haemorrhagic Septicaemia. As previously noted by WGPDMO, the use of VHS as a name for marine rhabdoviruses is causing confusion. However, the justification for adopting different names for different strains is still doubtful, because of the current inability to differentiate the different isolates, particularly freshwater and marine. WGPDMO considered that no re-naming of these agents should take place until differential detection and diagnostic techniques have been developed, standardised, and validated.

11 COLLATE AND REVIEW AVAILABLE INFORMATION ON THE DISTRIBUTION, ORIGIN, HOST RANGE AND IMPACT ON SALMON CULTURE OF INFECTIOUS SALMON ANAEMIA (ISA)

A. McVicar presented information on the subject based on the past and current experience with the disease in affected areas (Annex 11).

Infectious Salmon Anaemia (ISA) is caused by an orthomyxovirus (ISAV) infection. The disease has only been recorded in farmed Atlantic salmon, *Salmo salar,* from Norway, eastern Canada and Scotland. Although initial occurrences were in freshwater salmon farms in Norway, it was demonstrated that this was associated with the use of untreated sea water in these farms, and all subsequent proven outbreaks of the disease, in all affected areas, have been in seawater salmon farms.

The origin of the infection in all affected areas remains unknown, but there is increasing evidence for a local natural presence being implicated. This is supported by evidence for differences in the structure of the viral genome in separate isolates of the virus. These variations are most extreme between infections in Nova Scotia compared with material from New Brunswick, Norway and Scotland. The latter three areas also show significant differences between virus isolates from the different countries and there is emerging evidence of the existence of genetic variations within countries. The extent and significance of these results, so far unpublished, remain to be confirmed. It was notable that all clinical outbreaks of ISA in Scotland were genetically identical in the parts of the genomes examined, and all such cases could be traced directly back to horizontal transfer of infection from a single farm point source

Clinical disease due to ISAV has only been recorded in Atlantic salmon. Evidence for persistence of the virus has been obtained in sea trout, *Salmo trutta,* eel, *Anguilla anguilla*, and rainbow trout, *Oncorhynchus mykiss.* Experimental challenges of herring, *Clupea harengus*, are preliminary, and virus replication has not been demonstrated. Evidence of resistance has been obtained for goldsinny, *Ctenolobrus rupestris*, turbot, *Scophthalmus maximus*, and sea bass, *Dicentrarchus labrax*. Preliminary studies on halibut, *Hippoglossus hippoglossus*, have so far indicated resistance to infection. Extensive studies of over 2000 wild marine fish species in Canada and Scotland have not revealed ISAV infection, with the one exception of virus isolation from a single saithe, *Pollachius virens*. As this fish had been co-habiting within a farm cage with salmon suffering clinical ISA and there was no evidence of viral persistence obtained, it is possible that this was due to temporary transfer of virus. Some laboratory results have indicated that the virus may also be present in fresh water in wild brown trout and salmon and in farmed rainbow trout in other areas distant from salmon farms.

The direct costs due to the high mortality levels in ISA-infected stocks have led to serious financial loss in all affected countries. Because this is compounded by a variety of factors affecting the fish farm (e.g., loss of growth potential, disinfection, fallowing requirements), additional costs to processors (e.g., disinfection of effluents) and loss of market for the final product, it is not possible to accurately estimate an actual total monetary loss.

11.1 Conclusion

ISA is a serious disease currently causing significant economic impact on salmon farming in three ICES Member Countries.

11.2 Recommendation

WGPDMO recommends that further research on ISA should be initiated. There is an urgent need for continued research on the detection, diagnosis, variation and pathogenicity of the virus as there are still many uncertainties and significant gaps in knowledge of ISAV. Research effort needs to be directed towards the validation of ISAV diagnostic and strain determinant methods, particularly RT-PCR techniques. Also, the significance of the range of variation of ISAV already apparent between different infected areas urgently needs to be evaluated.

12 REVIEW NEW INFORMATION ON THE STRUCTURE AND DIVERSITY OF NODAVIRUS, THE SPREAD, DIAGNOSIS AND EPIZOOTIOLOGY OF THE DISEASE, AND HOST IMMUNITY, TO PROVIDE EFFECTIVE ADVICE ON POSSIBLE CONTROL MEASURES

A report from the EAFP workshop on nodavirus (Baudin–Laurencin, F., and Richards, R. 1999. Bulletin of the European Association of Fish Pathology, 19(6): 284–285), was presented to WGPDMO by B. Hjeltnes (Annex 12). This report provides valuable information on the characterisation of the virus, diagnostic tools, epidemiology, transmission, immunology and vaccination. Furthermore, information on possible control measures: screening of brood stock; stocking of juvenile sea bass in the autumn at lower water temperatures; the importance of clean water source in the hatcheries; and surface disinfection of marine fish eggs with ozonated sea water, were briefly presented to WGPDMO.

WGPDMO is aware of an ongoing EU project covering different aspects of nodavirus. This project started in 1999 and, as a requirement of the project proposal, has to report progress to WGPDMO.

12.1 Conclusions

1) Infection caused by nodavirus still continues to be a significant disease problem in farming of halibut and sea bass.
2) Due to the limited participation at this meeting of WGPDMO of representatives from ICES Member Countries with nodavirus disease problems, it was felt that no advice could yet be given on possible control measures.

12.2 Recommendation

WGPDMO recommends that it review the next report from the ongoing EU project on nodavirus and other relevant information to provide advice on effective control measures.

13 INITIATE EXPERIMENTAL WORK TO DETERMINE WHETHER THE LACK OF *BONAMIA OSTREAE* INFECTIONS DETECTED IN FIELD OBSERVATIONS OF *OSTREA EDULIS* FROM COLD WATER CLIMATES REFLECTS PARASITE ACQUISITION WITH SUBSEQUENT LOSS OVER PROLONGED LOW WATER TEMPERATURES, OR SUPPRESSION OF INFECTIVITY OF THE PARASITE

The working document prepared on this topic by S. McGladdery and S. Bower, who were unfortunately unable to attend the meeting, was discussed (Annex 13). The report provided an update on the current situation on research efforts on this topic in Europe and North America. The main conclusion was that low water temperature appears to reduce the prevalence of the disease but the necessary experimental evidence is still lacking. However, a submission for funding to investigate the possible reasons for the absence of *Bonamia ostreae* in Atlantic and Pacific populations of *Ostrea edulis* has been prepared. In discussion, it was concluded that it will be extremely difficult to obtain conclusive proof that lower temperatures alone could reduce or eliminate *B. ostreae* infections and furthermore it was considered that "resistance" of northern stocks of oyster to bonamiasis was unlikely. In order to improve our understanding of the factors involved, it remains an important goal to identify the life-cycle requirements of the parasite.

13.1 Recommendations

WGPDMO recommends that the effect of temperature on *Bonamia* infection dynamics continue to be investigated and a report be submitted at the next WGPDMO meeting for discussion.

14 DEVELOP A PROPOSAL FOR INCORPORATION OF PARASITOLOGICAL STUDIES INTO EXISTING FISH DISEASE MONITORING PROGRAMMES

R. Dobberstein presented results of a study on the occurrence of *Echinorhynchus gadi* and *Hysterothylacium aduncum* in cod (*Gadus morhua*) and dab (*Limanda limanda*) from the Kiel Bight, Baltic Sea.

The prevalence of *E. gadi* in cod increased in the period 1995–2000 from approximately 50 % to 95 %, whereas the prevalence of *H. aduncum* decreased from 25 % to 8 % in the same period. Data for dab from the same area indicate an opposite trend, with the prevalence of *E. gadi* increasing from 0 % in 1995 and 1996 to 25 % and 7 % in the years 1999 and 2000, respectively. The prevalence of *H. aduncum* increased from 29 % in 1995 to 55 % in 2000.

Although causes for the observed changes have so far not been identified, an impact of hydrographic conditions (e.g., inflow of North Sea water) on the intermediate host fauna cannot be excluded. Interspecific competition could be a factor possibly explaining opposite trends in the prevalence of the parasites in the same host. Differences between the two host species might have been associated with differences in their biology and feeding preferences.

In the discussion of the report the following points were raised:

There are a number of examples from the literature where a link between changes in occurrence of parasites of wild marine fish species and environmental change, including anthropogenic effects, could be demonstrated (e.g., effects of acidification, sewage sludge dumping, oil contamination and eutrophication). Therefore, parasites are considered to have potential to be used as biological indicators in environmental monitoring programmes.

The identification of cause-effect relationships related to changes in spatial and temporal distribution patterns of parasites is complicated, because a wide range of biotic and abiotic factors, including the distribution and abundance of intermediate hosts, affect the occurrence of parasites. However, even if it is not immediately possible to identify causes for changes, parasites may be useful as "alarm bells", initiating further more in-depth studies.

Two basic strategies can be applied for studies using parasites as indicators of environmental change: a) to investigate alterations in the whole parasitofauna of a certain fish species, and b) to focus on specific parasites or groups of parasites known to be affected by changes in environmental parameters. A disadvantage of the first strategy in terms of its use for monitoring purposes is that it is labour-intensive and that only a few fish specimens can be examined per sample. However, a comprehensive analysis is considered useful as it provides relevant background information for selecting suitable parasite/host combinations. The application of the second strategy implies a proper knowledge of the biology of the parasites and their host, including information on their responsiveness to environmental change.

It was emphasised that the selection of parasites to be used as indicators for monitoring purposes depends on the objectives of the monitoring. For local short-term effects (e.g., in coastal areas), different species than for long-term effects in larger areas (including offshore areas) may be suitable.

14.1 Conclusions

WGPDMO considered parasites of wild marine fish to be of potential value as biological indicators of environmental change. However, before they can be recommended for monitoring purposes, a number of requirements have to be met, e.g., with regard to the following issues:

- the objectives of monitoring programmes involving parasites as biological indicators have to be clearly defined;
- parasites and their host species used for monitoring purposes have to be carefully selected based on knowledge of their life-cycles and their responsiveness to environmental change;
- there is a general need for more information on the biology of parasites and their hosts as well as their interactions;
- as a basis for a more comprehensive evaluation of the use of parasites as indicators, existing data sets on parasites of wild fish have to be compiled and analysed in order to obtain a better impression of the extent of local and temporal variation in their occurrence, and on the impact of environmental factors.

14.2 Recommendation

WGPDMO recommends that existing data on spatial and temporal trends in the occurrence of selected parasites of wild fish and potential environmental factors encountered be compiled and a report presented for review at the 2001 meeting of WGPDMO.

15 REVIEW PROGRESS MADE WITHIN THE BIOLOGICAL EFFECTS QUALITY ASSURANCE IN MONITORING PROGRAMMES (BEQUALM) WORK PROJECT ENTITLED "EXTERNAL FISH DISEASE AND LIVER HISTOPATHOLOGY"

S.W. Feist prepared a working document (Annex 14) and gave a brief description of the EU-funded BEQUALM project, started in 1998, which aims at establishing a European quality assurance framework for biological effects monitoring in the marine environment. He highlighted progress made with regard to Work Package 6 "External Diseases and Liver Histopathology", consisting of the following milestones:

- The preparation of laboratory reference materials for the diagnosis of flatfish liver histopathology;
- A first workshop was held at the CEFAS Weymouth Laboratory, 21–23 October 1999, under the co-convenership of S.W. Feist and T. Lang, with 12 scientists participating;
- Plans were developed for a ringtest for diagnosis of histopathological liver lesions, to be undertaken in 2000.

S.W. Feist informed WGPDMO that a full report of the BEQUALM workshop, information on the activities of the other Work Packages and information on the status of the BEQUALM database will be available on the CEFAS BEQUALM website (http://www.cefas.co.uk/bequalm).

Reference was made to a request from ICES to the BEQUALM workshop to consider changes needed in the ICES Data Reporting Formats, in order to incorporate data on the occurrence of histopathological liver lesions of flatfish as part of the ICES fish disease data bank. At the workshop, a list of specific lesion categories was elaborated for which data should be reported to ICES (see Annex 14).

WGPDMO reviewed and endorsed the list of lesions and agreed with the participants of the workshop that the ICES Reporting Formats should be modified accordingly and that these lesions should be added to the already existing list of diseases for dab and flounder. However, the revision of the Reporting Formats should not be made before the quality assurance procedures for the diagnosis of histopathological liver lesions, developed within the BEQUALM project, have been established successfully. It cannot be excluded that the experience made in the course of the project might lead to a need for a further revision of the list of lesions.

15.1 Conclusion

WGPDMO acknowledged the progress made within the BEQUALM Work Package "External Fish Diseases and Liver Histopathology" and endorsed the plans for the ring test.

15.2 Recommendations

WGPDMO recommends that:

i) the ICES Data Reporting Formats for fish diseases should be modified according to the recommendations made at the BEQUALM Workshop, in order to incorporate data on histopathological liver lesions. However, this should be done only after the BEQUALM project has been finalised, and quality assurance procedures have been developed and implemented in participating ICES Member Countries, for the diagnosis of histopathological liver lesions;

ii) a report on progress made in the BEQUALM Work Package "External Fish Diseases and Liver Histopathology" should be presented and reviewed at the 2001 WGPDMO meeting.

16 PROVIDE A REPORT WITH ADVICE ON NEW TECHNIQUES IN PATHOLOGY AND OTHER METHODS FOR THE DETECTION OF ENDOCRINE DISRUPTING CHEMICALS IN MARINE AND ESTUARINE ORGANISMS AND APPROPRIATE NEW TARGET SPECIES REPRESENTING THE MAIN ECOLOGICAL LEVELS OF THE MARINE ECOSYSTEM

S. Feist prepared the following written contribution on this topic after WGPDMO meeting. The text has been circulated to WGPDMO members for approval.

The topic of endocrine disruption in marine and estuarine species continues to be of high priority and there remains the need to develop robust techniques for the detection of endocrine disrupting chemicals and their effects in a variety of organisms. Although most attention has been given to the effects of oestrogen mimics, there is increasing effort to the development of methods for testing for androgens and thyroid-acting substances. Almost all research has been devoted to responses in fish hosts and (within ICES Member Countries) in particular in the European flounder (*Platichthys flesus*). This species has been shown to be susceptible to endocrine disruption and exhibits elevated levels of vitellogenin (VTG) in certain estuaries. In addition, the induction of intersex condition in male flounder has been shown to occur in contaminated estuaries. VTG induction in male fish is now generally regarded as a very sensitive biomarker of oestrogen induction and is now in use in several laboratories. However, it is apparent that some fish species are relatively insensitive to VTG induction. The histological endpoint is valuable in demonstrating the induction of intersex in otherwise male fish but the relationship between the physical condition and elevated VTG levels still needs to be clarified since it is not clear that intersex fish also show elevated VTG levels. However, the use of histology requires a high level of technical skill and access to an experienced pathologist familiar with the species under study. Additional endpoints currently in use are effects on gonado-somatic index and effects on gross morphology, particularly secondary sexual characteristics. These are currently being studied in goby species. Higher level effects on sex ratios and fecundity also need to be assessed, however measures of effects on reproductive success will involve significant method development.

Similar techniques are also being applied to investigate effects on various crustaceans, including *Carcinus, Chaetogammarus* and *Crangon* species, with emphasis on vitellin measurements and effects on breeding success. Histological endpoints are also likely to be of value, but until sufficient baseline data have been accumulated, it is difficult to be clear on the specific histological parameters which may be used. New approaches using immuno-histochemical methods are also beginning to be used for marine species. These methods will be valuable in enabling detection and localisation of VTG or vitellin in various fish and crustacean species. The development of such techniques will prove essential in efforts to understand physiological and physical changes associated with exposure to endocrine disruptors and could provide a valuable tool for detection of early effects.

A biomarker of androgen exposure in sticklebacks, *Gasterosteus aculeatus*, has been developed based on the protein spiggin which is produced in male sticklebacks, secreted from the kidneys, and used in nest-building activities. A histological method measuring renal tubule cell hypertrophy has also been developed. It is possible that similar techniques could be used in other nest-building marine fish that utilise spiggin or a similar protein.

As briefly indicated, there are a number of marine or estuarine fish species that are used as target species for endocrine disruption studies and for these a suite of methods is being applied to detect biological responses. However, several are still under development. The same is true for crustacean species although there is more developmental work still to be undertaken. Molluscan species have not attracted much attention as susceptible species to endocrine disruption apart from a large volume of work that has been done on TBT-related imposex in gastropods. Although it is clear that molluscs form a very important component in the marine ecosystem, the current lack of methods to detect endocrine disruption in this group provides a barrier to their use.

16.1 Conclusion

1) Using histolopathology in biological effects studies of endocrine disrupting chemicals requires a high level of technical skill and access to an experienced pathologist familiar with the species under study.
2) Although there exist some pathological tools to be used in biological effects studies of endocrine disrupting chemicals, research is still needed to refine these methods and to develop new techniques before histopathology can be fully applied in such studies.

17 DEVELOP PROPOSALS FOR THE INCLUSION OF MAPS OF THE DISTRIBUTION OF FISH AND SHELLFISH DISEASES OF CONCERN FOR MARICULTURE AND TEMPORAL TRENDS OF WILD FISH DISEASES OF CONCERN FOR MARINE ENVIRONMENTAL MONITORING PROGRAMMES

S. Mellergaard presented an outline of a proposed mapping system with illustrative examples of the known spatial distribution patterns of several fish and shellfish diseases which could be included in a web-based ICES Environmental Status Report (Annex 15). Comparison was made with a similar system developed by the WG on Harmful Algal Bloom Dynamics, already used by ICES. The proposal was considered to be most appropriate for presence/absence information for both wild and farmed fish and shellfish.

T. Lang and W. Wosniok presented an outline of a slightly different proposal (Annex 15). This focused on diseases in wild fish of importance in environmental monitoring, particularly in common dab, and on achieving integration between the ICES fish disease database as part of the ICES Environmental Data Centre, the ICES Environmental Status Report and the ICES website. It was considered essential that the data presented should be informative to a wide range of scientists and non-scientists and be as illustrative as possible. In contrast to maps on farmed fish and wild and farmed shellfish, maps would be based on ICES statistical rectangles. A series of maps giving an indication of the level of sampling, and trends in the prevalence of important diseases over 5 years were considered important aspects of the proposal. Safeguards would need to be included to prevent misinterpretation of data when presented in a simplified way.

WGPDMO identified areas where additional information and decisions were required before the proposals on mapping systems could be further developed. These included the need to clarify the extent to which the ICES Secretariat would be involved in the development of the website, if a format proposed by WGPDMO could be acceptable to ICES or if it would be preferable to use an existing format. Copyright difficulties would have to be overcome and the responsibility for regular updating (annually) of maps, all required clarification. It was evident that not all disease conditions would be appropriate for display in a map format.

WGPDMO considered that development of fish and shellfish disease data maps should be continued. It was considered appropriate that disease conditions on which extensive data were available would be an appropriate starting point and that within three weeks of the WGPDMO meeting, suggestions for appropriate diseases of wild and farms stocks and distribution maps should be sent to the Chairman.

17.1 Conclusion

The desirability for and feasibility of producing maps of marine fish and shellfish diseases for the ICES Environmental Status Report was recognised by WGPDMO. Data on appropriate diseases to be considered should be sent to the Chairman within three weeks of the completion of the 2000 meeting of the WGPDMO. The development of the fish and shellfish disease maps should be continued intersessionally, led by T. Lang and W. Wosniok for diseases of environmental monitoring interest and by S. Mellergaard for farmed and farm-associated diseases.

17.2 Recommendation

WGPDMO recommends that the development of maps of marine fish and shellfish diseases should be continued intersessionally and progress should be evaluated by WGPDMO at its next meeting.

18 DEVELOPMENT OF THE ICES DATA CENTRE

A new database (based on Microsoft ACCESS) will be formed in the near future for storing fish disease data. Suggested amendments to the data format, such as including liver pathology, are recommended to be added in connection with the planned move to ACCESS. It is proposed that suggested changes to the format be forwarded to J.N. Jensen, Environmental Data Scientist at the ICES Environmental Data Centre, by WGPDMO. The suggestion for changes in the liver pathology reporting format is covered in Section 17 and Annex 14 of the present report.

The Fish Disease Data Entry program (FDE) has been unstable in the NT environment. To overcome this problem, ICES has indicated they will develop a conversion program to be used to convert existing FDE data as well as 2.2 data into the new ACCESS database.

18.1 Recommendations

WGPDMO recommends that:

i) submission status of fish disease data should be made available on the ICES website, in order to complete the inventory for data stored in the ICES Environmental Data Centre. Furthermore, this will be of relevance for the planned contribution of WGPDMO on trends in wild fish diseases to the ICES Environmental Status Report (see Section 17, above).;

ii) The following priorities were made by WGPDMO for the development of the ICES Environmental Data Centre:

   a) to include the fish disease inventory on the ICES website;

   b) to include fish disease submission status on the website;

   c) submission of liver pathology data to the database can wait until collected samples have been analysed;

   d) no economic resources should be spent on a replacement of the FDE.

19 ICES DISEASE PUBLICATIONS, DIAGNOSTIC FICHES UPDATE

S. Mellergaard read out the report prepared by S. McGladdery on the current status of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. WGPDMO appreciated the efforts of S. McGladdery involved in providing this report and in the development of the Disease and Parasite Fiche Series in general.

It was suggested that S. McGladdery should again contact individuals already identified as either existing or proposed authors of fiches and stress that updated fiches need to be completed by stated deadlines. In cases where authors are not able to complete fiches, alternative authorship will be sought.

Several new titles for fiches have been identified as indicated in the report (Annex 16) and a few are already in preparation. In addition, S. Ford has agreed to draft a fiche on juvenile oyster disease (JOD) and the fiche on M74 will be co-authored by S. Mellergaard and G. Bylund. It is anticipated that completed fiches will be placed on the ICES website for viewing but not printing. Printed versions will however be available and should be purchased directly from ICES.

19.1 Conclusion

It was recognised by WGPDMO that more effort in the production of diagnostic fiches was required since demand for them is likely to increase as they become more widely available via the Internet. To assist in this, members of WGPDMO should encourage relevant colleagues to complete fiches without delay.

20 ANY OTHER BUSINESS

Presentation of results within the responsibilities of WGPDMO

It is of high importance that the work undertaken within the responsibilities of WGPDMO reaches a wider audience. Thus, WGPDMO encourages its members and cooperation partners to present the activities of the working group at scientific meetings and other fora. Forthcoming relevant meetings are given in this report under the theme Other Relevant Information.

Collaboration with international organisations

WGPDMO stressed the need for further development of cooperation with other international organisations working within fields of similar responsibilities as WGPDMO, such as OIE, PICES and FAO. Currently, there are several cases of diseases that are spreading over wide geographical areas, some becoming global disease problems. Thus, preventive and control measures for the prevention of further spread of these diseases, as well as a harmonisation of legislation between different countries, should be discussed at the international level.

Election of Chair

The Chair had been acting for a three-year period and WGPDMO recommended unanimously that he should be appointed for another three-year period.

20.1 Recommendations

WGPDMO recommends that:

i) the results from the work within the responsibilities of WGPDMO should be presented at scientific meetings and other relevant fora;

ii) the Chair be encouraged to continue international contacts between WGPDMO and corresponding working groups at OIE, PICES, and FAO.

21 ANALYSIS OF PROGRESS WITH TASKS

An analysis of the progress of tasks in the terms of reference was conducted and presented in Annex 17. All items had been dealt with in a comprehensive manner. Several intersessional tasks were identified during the meeting.

22 FUTURE ACTIVITY OF WGPDMO

Since there are several issues of importance in the field of pathology and diseases of marine organisms requiring further consideration, it was agreed that a further meeting of WGPDMO is required in 2001 to consider the results of intersessional work and to discuss outstanding items. It was agreed that the invitation to host the meeting from Dr J. Barja, University of Santiago, Santiago de Compostella, Spain, be accepted. The proposed dates are 13–17 March 2001.

23 APPROVAL OF RECOMMENDATIONS

The recommendations contained in this report to the ICES Council were discussed by WGPDMO and approved. The recommendations and justifications for recommendations to the Council are added in Annex 18.

24 APPROVAL OF THE DRAFT WGPDMO REPORT

The report of the 2000 meeting was approved before the end of the meeting and the draft report was circulated to the participants on 6 March. The conclusions on the Terms of Reference and associated Annexes where advice was specifically sought by other ICES bodies would be extracted and sent separately to ICES.

25 CLOSING OF THE MEETING

On behalf of the participants of the 2000 meeting of WGPDMO, the Chair expressed appreciation to W. Wosniok for his hospitality and the excellent facilities provided for WGPDMO in the University of Bremen, and for his considerable organisation and support before and during the meeting.

The meeting was closed at 13.00 on 4 March 2000.

ANNEX 1: LIST OF PARTICIPANTS

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ANNEX 2: TERMS OF REFERENCE

ICES C.Res. 1999/2:F:01

The Working Group on Pathology and Diseases of Marine Organisms [WGPDMO] (Chair: Dr S. Mellergaard, Denmark) will meet in Bremen, Germany from 29 February to 4 March 2000 to:

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

b) update information on the diseases and parasites of Baltic fish, to be included in the HELCOM Fourth Periodic Assessment (this is being produced intersessionally for review at the 2000 meeting of WGPDMO, and for subsequent consideration by the ACME) [HELCOM 2000/3];

c) review progress in data submissions to the ICES Data Banks and continue the statistical analysis of ICES fish disease data in relation to environmental and fisheries data intersessionally, in order to extend the analysis to enlarged areas and time windows, and to develop and optimise suitable models and statistical methods;

d) maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome;

e) investigate gill disease in *Crassostrea angulata* adults, the cause of summer mortalities of *C. gigas* spat, and clarify the report of *M. refringens* in *C. gigas* from Spain;

f) collate and review available information on the distribution and effect of marine VHS-like virus on cultured and wild fish stocks;

g) collate and review available information on the distribution, origin, host range and impact on salmon culture of Infectious Salmon Anaemia (ISA);

h) review new information on the structure and diversity of nodavirus(es), the spread, diagnosis and epizootiology of the disease, and host immunity, to provide effective advice on possible control measures;

i) initiate experimental work to determine whether the lack of *Bonamia ostreae* infections detected in field observations of *Ostrea edulis* from cold water climates reflects parasite acquisition with subsequent loss over prolonged low water temperatures, or suppression of infectivity of the parasite;

j) develop a proposal for incorporation of parasitological studies into existing disease monitoring programmes. For this purpose, it was considered useful to compile and evaluate long-term data sets already existing in ICES Member Countries;

k) review progress made within the Biological Effects Quality Assurance in Monitoring Programme (BEQUALM) work project titled 'Fish disease and liver pathology';

l) provide a report with advice on new techniques in pathology and other methods for the detection of endocrine-disrupting chemicals in marine and estuarine organisms and appropriate new target species representing the main ecological levels of the marine ecosystem;

m) develop proposals for the inclusion of maps of the distribution of fish and shellfish diseases of concern for mariculture and temporal trends of wild fish diseases of concern for marine environmental monitoring programmes.

n) answer request on priorities for the development of the ICES Data Centre.

ANNEX 3: AGENDA

1) ICES ASC 1999; items of relevance to WGPDMO.
2) Terms of Reference, adoptation of the agenda, selection of Rapporteurs.
3) Opening of the meeting 10.00
4) Other Relevant reports for information.
5) analyse national reports on new disease trends in wild and cultured fish, molluscs and crustaceans;
6) update information on the diseases and parasites of Baltic fish, to be included in the HELCOM Fourth Periodic Assessment (this is being produced intersessionally for review at the 2000 meeting of WGPDMO, and for subsequent consideration by the ACME) [HELCOM 2000/3];
7) review progress in data submissions to the ICES Data Banks and continue the statistical analysis of ICES fish disease data in relation to environmental and fisheries data intersessionally, in order to extend the analysis to enlarged areas and time windows, and to develop and optimise suitable models and statistical methods;
8) maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome;
9) investigate gill disease in *Crassostrea angulata* adults, the cause of summer mortalities of *C. gigas* spat, and clarify the report of *M. refringens* in *C. gigas* from Spain;
10) collate and review available information on the distribution and effect of marine VHS-like virus on cultured and wild fish stocks;
11) collate and review available information on the distribution, origin, host range and impact on salmon culture of Infectious Salmon Anaemia (ISA);
12) review new information on the structure and diversity of nodavirus(es), the spread, diagnosis and epizootiology of the disease, and host immunity, to provide effective advice on possible control measures;
13) initiate experimental work to determine whether the lack of *Bonamia ostreae* infections detected in field observations of *Ostrea edulis* from cold water climates reflects parasite acquisition with subsequent loss over prolonged low water temperatures, or suppression of infectivity of the parasite;
14) develop a proposal for incorporation of parasitological studies into existing disease monitoring programmes. For this purpose, it was considered useful to compile and evaluate long-term data sets already existing in ICES Member Countries;
15) review progress made within the Biological Effects Quality Assurance in Monitoring Programme (BEQUALM) work project titled 'Fish disease and liver pathology';
16) provide a report with advice on new techniques in pathology and other methods for the detection of endocrine-disrupting chemicals in marine and estuarine organisms and appropriate new target species representing the main ecological levels of the marine ecosystem;
17) develop proposals for the inclusion of maps of the distribution of fish and shellfish diseases of concern for mariculture and temporal trends of wild fish diseases of concern for marine environmental monitoring programmes.
18) answer request on priorities for the development of the ICES Data Centre.
19) ICES Disease publications. Diagnostic Fiches update.
20) Any other business.
21) Analysis of progress with tasks.
22) Future activity of WGPDMO.
23) Approval of recommendations.
24) Approval of draft WGPDMO Report.
25) Closing of the meeting, Saturday 15.00.

ANNEX 4: RAPPORTEURS

Session (s)	**Rapporteurs**
1–4. Introductory sessions	S.Mellergaard
5. Wild fish Farmed fish Wild shellfish Farmed shellfish	W. Grygiel, G. Bylund S. Helgason, B. Hjeltnes D. Declerck,, S. Feist
6. Update information on the diseases and parasites of Baltic fish, to be included in the HELCOM Fourth Periodic Assessment	A. Karasev, T. Lang
7. *Ichthyophonus* in herring stocks and the M74 syndrome	G. Bylund, S. Helgason
8. Gill disease in *Crassostrea angulata* adults, the cause of summer mortalities of *C. gigas* spat, and clarify the report of *M. refringens* in *C. gigas* from Spain	J. Baja, S. Feist
9. Marine VHS-like virus	S. Mellergaard, A. McVicar, J. Baja
10. Infectious Salmon Anaemia (ISA)	B. Hjeltnes, A. McVicar
11. Nodavirus	B. Hjeltnes, S. Helgason
12. Lack of *Bonamia ostreae* infections in *Ostrea edulis* from cold water climates	S. Feist, A. Karasev
13. Proposal for incorporation of parasitological studies into existing disease monitoring programmes	R. Dobberstein, T. Lang
14. Progress in data submissions to the ICES Data Bank	W. Wosniok, T. Lang, D. Declerck
15. Considerations for the development of the ICES Data Centre	W. Wosniok, E. Lindesjöö
16. Progress made within the BEQUALM	T. Lang, W. Grygiel
17. New techniques in pathology and other methods for the detection of endocrine disrupting chemicals	E. Lindesjöö
18. Maps of the distribution of fish and shellfish diseases	A. McVicar
19. ICES Disease publications. Diagnostic Fiches update	D. Vethaak
20. AOB, Progress, Recommendations	S. Feist, E. Lindesjöö
21–25. Approval of report, closing	S. Mellergaard

ANNEX 5: HELCOM FOURTH PERIODIC ASSESSMENT OF THE STATE OF THE MARINE ENVIRONMENT OF THE BALTIC SEA, 1994–1998

Draft prepared by T. Lang

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DISEASES AND PARASITES OF BALTIC FISH

Introduction

The occurrence of diseases and parasites of fish in the Baltic Sea has interested scientists for many decades. However, apart from more or less anecdotal records of conspicuous phenomena such as skeletal deformities, skin lesions and grossly visible parasites, it was not before the 1970s that more systematic epidemiological studies into the prevalence, geographical distribution, and causes of diseases and parasitic infestations commenced. These studies were mainly linked to two major concerns: to a possible impact of diseases the fishery and human consumers, and to a possible impact of anthropogenic contaminants on the health of marine fish.

At present, most Baltic Sea countries are carrying out fish disease monitoring programmes which are, however, still variable in terms of their objectives, the regional and temporal coverage as well as the target species and diseases/parasites studied (Lang and Dethlefsen, 1996). Furthermore, it is almost characteristic for monitoring programmes that data generated are often either unpublished or scattered throughout various reports and, therefore, not readily accessible. Consequently, it is not easy to obtain a comprehensive overview of the health status of Baltic Sea fish.

This chapter will, however, attempt to provide a brief description, focused on the period 1994–1998, of the prevalence and geographical distribution of selected fish diseases and parasites characteristic for the major wild Baltic Sea fish species on which most research and monitoring activities have been conducted: flounder (*Platichthys flesus*), cod (*Gadus morhua*), herring (*Clupea harengus*) and, recently, Atlantic salmon (*Salmo salar*). In addition, other significantly affected species are also considered. Besides grossly visible fish diseases/parasites recommended by ICES for fish disease surveys (ICES, 1989; Bucke *et al*., 1996) and widely used for biological effects monitoring purposes, additional diseases/parasites are highlighted that have received attention due to their possible link to marine contamination or their suspected impact on mortality and fish stock size. As a new section, brief information on major new trends with regard to diseases of farmed marine fish in the Baltic Sea is provided.

The information presented constitutes an updated version of a contribution to the HELCOM Third Periodic Assessment for the period 1989–1993 (HELCOM, 1996) and was compiled by T. Lang (Germany). The text has been reviewed by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), the ICES Advisory Committee on the Marine Environment (ACME) and the ICES Advisory Committee on Fishery Management (ACFM).

Available Data

Wild fish

The information provided is largely based on recent annual reports of the ICES WGPDMO, the ICES ACME, results from the 1994 BMB/ICES Sea-going Workshop ‘Fish Diseases and Parasites in the Baltic Sea’, and further published or as yet unpublished information provided by Baltic Sea countries.

One of the most consistent data sets on the prevalence and spatial distribution of diseases/parasites of flounder, cod, and herring was obtained during the 1994 BMB/ICES Workshop. Practical work was conducted on board R/V ‘Walther Herwig III’ on a transect from the Mecklenburg Bight to the western Gulf of Finland, representing the largest area in the Baltic Sea ever studied for this purpose in a narrow time-window. Scientists from eight of the nine Baltic Sea countries attended the workshop (Lang and Mellergaard, 1999).

Flounder (*Platichthys flesus*)

Besides cod, flounder is certainly the best-studied Baltic fish species with regard to its diseases and parasites and a large body of relevant reports is available (HELCOM, 1996; Lang *et al*., 1999).

The lymphocystis disease (Plate A5.1.1), caused by Iridoviridae, continued to be the most prevalent externally visible disease. The prevalences observed in single sampling areas during the 1994 BMB/ICES Workshop were in the range of 4.0 % to 11.2 % (off the Estonian coast) to 22.2 % to 38.1 % (off the German coast), indicating a decreasing trend from the western to the eastern areas (Lang *et al*., 1999). Figure A5.1 (upper figure) provides information on temporal changes in prevalence for the period 1994–1999 recorded in ICES Sub-divisions 22, 24, 25, and 26, covering the southwestern Baltic Sea. A continuation of the increasing trend in prevalence reported previously for the western Baltic Sea in the period 1986–1993 (Lang and Dethlefsen, 1994) was not detected. At least for ICES Sub-division 24, the data for the period 1994 –1997 indicate an opposite trend (Dethlefsen and Lang, 2000, unpublished data).

A multivariate statistical analysis of the 1994 workshop data revealed a significant relationship between length, age, and gender of the flounder and the lymphocystis prevalence recorded. Furthermore, it could be shown that differences in these demographic factors between sampling areas to a large extent explained the differences in observed prevalence between areas (Lang *et al*., 1999). It is, therefore, important to consider these factors in any regional assessment.

The lowest prevalences of the skin ulcer disease of flounder (Plate A5.1.2) recorded in the course of the 1994 workshop were detected in the two western-most areas off the German coast (0.0 %). The maximum prevalence (11.6 %) occurred off the Latvian coast. The data indicated a clear increase in prevalence from the western to the eastern sampling sites (Lang *et al*., 1999). This increase is also reflected in Figure A5.1 (lower figure) which illustrates changes in prevalence over the period 1994–1998 in ICES Sub-divisions 22, 24, 25, and 26. While the data indicate an increase in prevalence for the years 1994–1996, particularly in ICES Sub-division 26, the prevalence dropped again thereafter (Dethlefsen and Lang 2000, unpublished data).

Kosior *et al*. (1997) observed a slight increase in prevalence from 1.05 % (1994), 1.20 % (1995) to 1.97 % (1996) at stations within the Polish Exclusive Economic Zone. These values are low compared to other data recorded in adjacent areas in the same period (Lang *et al*., 1999; Dethlefsen and Lang 2000, unpublished data).

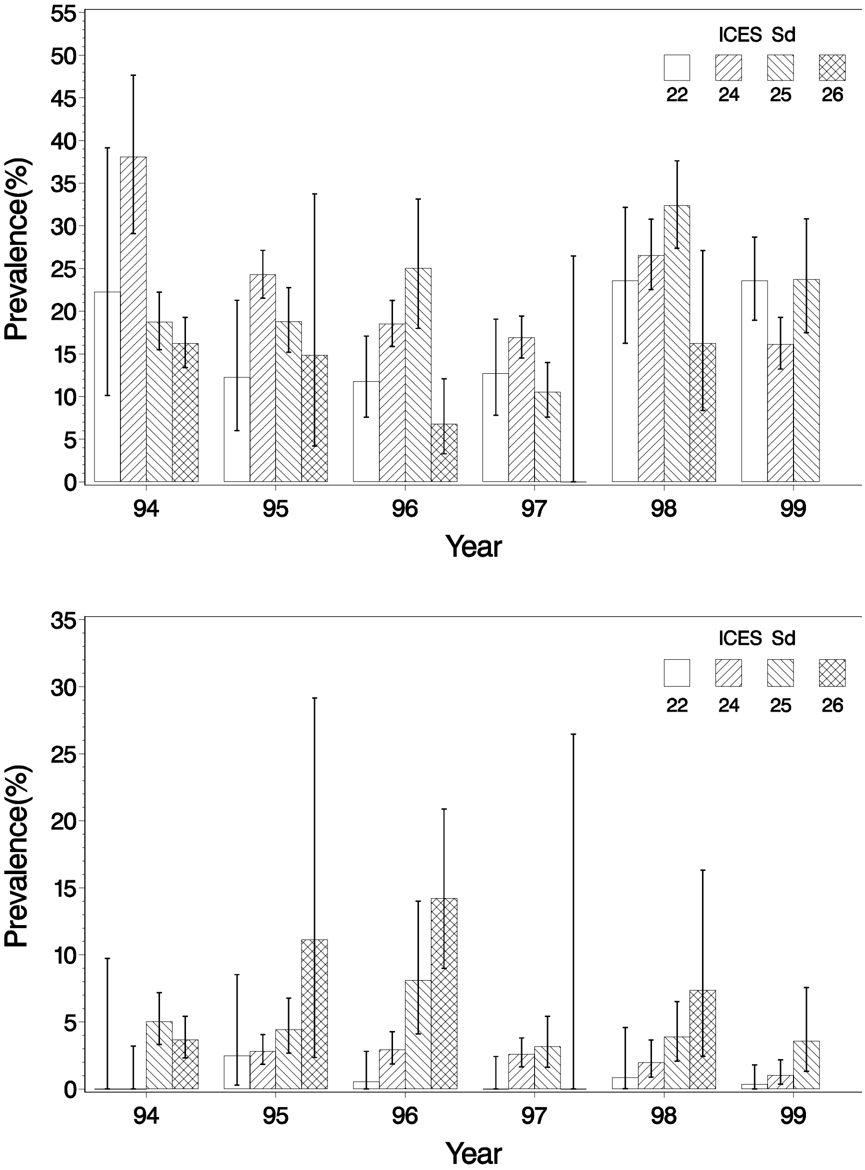
From bacteriological studies there is evidence that the skin ulcer disease in flounder is associated with one, well-defined biotype of atypical *Aeromonas salmonicida*, which has also been recovered repeatedly from other Baltic fish species with acute skin ulcerations (Wiklund *et al*., 1999).

Statistical analyses have shown that skin ulcers are significantly more prevalent in male as compared to female flounder and that large fish are more often affected than small ones (Lang *et al*., 1999; Wiklund and Bylund, 1993). Furthermore, the analysis of the 1994 data strongly indicated that the disease is associated with water salinity because a negative correlation was found between the prevalence and the salinity (Lang *et al*., 1999).

The occurrence of higher prevalences of liver tumours in wild flatfish is regarded as an indicator of exposure to carcinogenic anthropogenic contaminants. Therefore, relevant studies have been incorporated in most fish disease monitoring programmes, using methodological guidelines provided by ICES (ICES, 1989; Bucke *et al*., 1996). In Baltic flounder, histologically confirmed cases of liver tumours and their putative precursors (foci of cellular alteration, FCA) have only rarely been recorded. At sampling sites in the southwestern Baltic Sea, prevalences were in the range of 0.0 % – 3.0 % in the period 1994–1999 (Lang, 2000, unpublished data). Slightly increased prevalences were identified in the eastern Baltic Sea off the Estonian coast (Lang *et al*., 1999, Bogovski *et al*., 1999) and at certain locations at the southwestern coast of Finland (Wiklund and Bylund, 1994b). There is no evidence for temporal trends in any of these areas.

Other gross flounder diseases, such as acute/healing fin rot/erosion and skeletal deformities, have occurred only occasionally at generally low prevalences (< 1 %), without revealing any clear spatial or temporal trends (Lang *et al*., 1999; Dethlefsen and Lang, unpublished data).

Figure A5.1. Prevalence (with 95 % confidence intervals) of lymphocystis (upper figure) and acute/healing skin ulcerations (lower figure) in Baltic flounder (*Platichthys flesus*) from ICES Sub-divisions 22, 24, 25 and 26 in the period 1994–1998 (after Lang *et al.*, 1999, and Dethlefsen and Lang, 2000, unpublished data).



The parasitic fauna of Baltic flounder is considerably diverse, largely reflecting the changes in salinity in a northeasterly direction. A comprehensive synopsis was provided by Køie (1999) as part of the results obtained during the 1994 BMB/ICES Workshop. Typically, marine metazoan parasites (*Podocotyle atomon, Brachyphallus crenatus, Anisakis simplex, Holobomolochus confusus, Lernaeocera branchialis*) dominated in the western-most sampling areas, whereas parasites adapted to a low salinity (*Diplostomum spathaceum*, *Cotylurus* sp., *Corynosoma* sp., *Rhaphidascaris acus*, *Contracaecum osculatum*) were most common towards the east and north. Small encapsulated larvae of bird parasites (*Contracaecum* sp., *Paracuaria* sp., *Cosmocephalus* sp.) were recorded in all areas. The study confirmed earlier sporadic findings of juvenile stages of the copepod *Lernaeocera branchialis* in flounder gills, suggesting in contrast to previous reports that the whole life cycle of this parasite, the adult females of which parasitize mainly cod and other gadoids, can be completed in the Baltic Sea.

Further comprehensive information on parasites of Baltic flounder has been provided by Fagerlund and Køie (1994), Turovski (1994), Palm and Dobberstein (1999), and Palm *et al.* (1999). However, although the usefulness of parasites as indicators of environmental change (including effects of contaminants or eutrophication) has been suggested repeatedly, none of the reports available covering the period 1994–1998 provide information on temporal or spatial changes in the occurrence of flounder parasites or on possible links with anthropogenic impacts. However, Turovski

(1994) reported a steady increase in infestation of flounder from Estonian waters with fresh water and brackish water parasites in the period 1984–1993, possibly reflecting long-term changes in salinity.

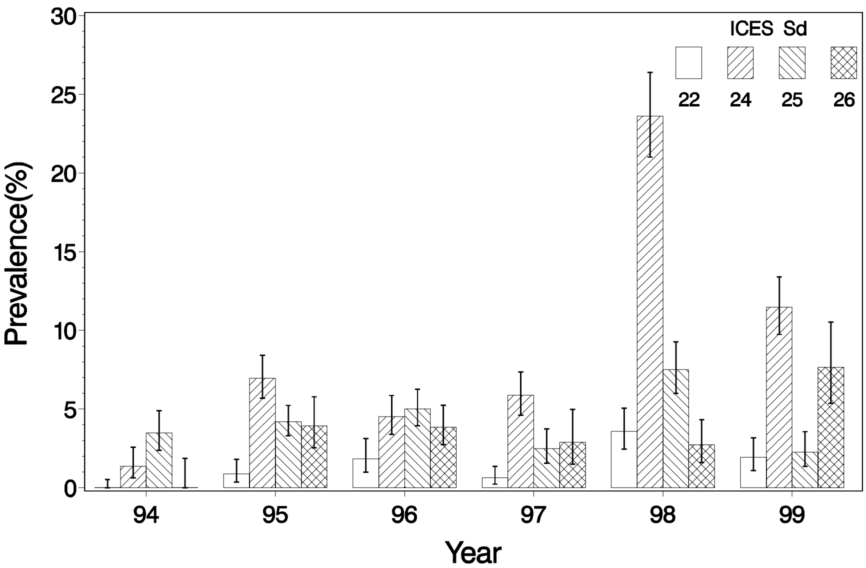
Cod (*Gadus morhua*)

Due to its commercial significance, Baltic cod has been a subject of a large variety of studies on spatial/temporal trends of externally visible diseases and parasites (see HELCOM, 1996). The most prevalent externally visible disease of Baltic cod is the infectious bacterial skin ulcer disease (Plate A5.1.3), often called ulcus-syndrome (Jensen and Larsen, 1979).

Although there is consensus that the conspicuous disease signs (open, red ulcers affecting the skin and, occasionally, tending into the musculature) are caused by bacterial infections (e.g., *Vibrio anguillarum*, *Flavobacterium* sp., *Aeromonas* sp., *Pseudomonas* sp., often as mixed infections), the primary cause may be different. For example, Danish studies on ulcerated cod occurring at high prevalences (26 %–48 % in May) around Bornholm in 1982 revealed that these lesions were most likely caused primarily by a mechanical damage of the skin of specimens which were discarded or escaped from fishing nets (Mellergaard and Bagge, 1998) and that, subsequently, the lesions were invaded by pathogenic bacteria leading to a secondary infection. However, there is also evidence from the literature that pollution might have been involved in the aetiology of the disease in cod from the Danish Belt Sea in the late 1970s (Christensen, 1980; Jensen, 1983).

Prevalences recorded during the 1994 BMB/ICES Workshop were in the range of 0.0 %–3.8 % in relation to single sampling sites, with highest prevalences off the Polish coast. Affected cod belonged to the size groups 22–35 cm > 35 cm, corresponding to age groups 2 and 3, respectively, whereas smaller cod were not affected (Mellergaard and Lang, 1999). As shown in Figure A5.2, prevalences recorded in December of the years 1995–1997 in ICES Sub-divisions 22, 24, 25, and 26 were below 7 %. However, a steep increase was observed in December 1998 in ICES Sub-division 24, with a maximum prevalence of 23.4 %. A slight increase was also noted in Sub-division 25. In December 1999, the prevalence was lower, but still remained on an elevated level in ICES Sub-division 24 (Dethlefsen and Lang, 2000, unpublished data).

Figure A5.2. Prevalence (with 95 % confidence intervals) of acute/healing skin ulcerations in Baltic cod (*Gadus morhua*) from ICES Sub-divisions 22, 24, 25, and 26 in the period 1994–1998 (after Mellergaard and Lang, 1999, and Dethlefsen and Lang, 2000, unpublished data).



In the Belt Sea, elevated prevalences were also found in May 1998 (27.6 %) and September 1999 (16.1 %) (Dethlefsen and Lang, 2000, unpublished data) at a station in the Mecklenburg Bight. From the size distribution of the affected specimens there is evidence that the majority of diseased fish belonged to one strong year class, and it can be speculated that stock density effects might have triggered the spread of the disease.

High prevalences (maximum values at certain stations > 40 %) of skin ulcers in cod in Danish and German waters were also observed in a Danish study in spring 1998. Interestingly, subsequent virological, bacteriological, mycological, and histological studies failed to provide conclusive information on the causes of the epizootic. However, it could be confirmed that the ulcers were not caused by VHS-like virus, a speculation that had been made before, based on findings from North Sea cod (Mellergaard 2000, pers. comm.). Data from sampling sites in the Polish Exclusive Economic Zone revealed a slight increase in prevalence from 0.40 % (1994) to 1.37 % (1995) and 2.76 % (1996) (Kosior *et al.*, 1997)

Skeletal deformities (compression of vertebrae, lordosis/scoliosis, deformation of the head skeleton) (Plate A5.1.4) have been known for a long time to affect Baltic cod and may have multifactorial natural or anthropogenic causes (HELCOM, 1996) which most likely originate during early life stages. An impact of contaminants, in particular metals, has been discussed repeatedly. However, no clear cause-effect relationships have been established (Lang and Dethlefsen, 1987; HELCOM, 1996; Mellergaard and Lang, 1999).

From historic data, skeletal deformities in cod may occur at very high prevalences, i.e., > 50 % in the Sound and ≤ 75 % in the Gulf of Riga (Lundbeck, 1928; Berzins, 1943). During the BMB/ICES Workshop, prevalences of skeletal deformities were in the range of 0.0 % – 3.8 %, according to sampling area, with the highest value in the Gulf of Gdansk. As with the skin ulcer disease, larger fish were more frequently affected than smaller ones. More recent data for the period 1995–1999 from sampling sites in the southern Baltic Proper and the Belt Sea revealed slightly higher values, with a maximum of 8.7 % in December 1998 at a station northeast of Rügen (Dethlefsen and Lang, unpublished data). Interestingly, areas with the highest prevalences were almost identical with those characterized by elevated prevalences of skin ulcers. Prevalences recorded in Polish waters in the period 1994–1996 ranged from 0.09 % – 0.39 % (Kosior *et al.*, 1997).

Another disease of cod recommended for monitoring purposes (ICES, 1989; Bucke *et al.*, 1996) is the X-cell disease, which leads to swelling of the pseudobranch (pseudobranchial pseudotumours) and sometimes also affects adjacent tissues (Plate A5.1.5). Its aetiology is still unclear, however, it has been suggested repeatedly that it is caused by parasitic protozoans (HELCOM, 1996).

During the 1994 BMB/ICES Workshop, the X-cell disease was only recorded at the two western-most stations, with a prevalence of 0.7 % and 0.2 %, respectively (Mellergaard and Lang, 1999). The authors speculate that salinity may play a role in the distribution of the disease. Monitoring data from subsequent years confirm that the disease is rare in the Baltic Sea and that it is regularly recorded only in the western Baltic Sea. However, the disease was also occasionally found in more eastern waters, e.g., outside the Gulf of Gdansk (Dethlefsen and Lang 2000, unpublished data).

Data for the period 1994–1998 confirm earlier findings that the infestation of Baltic cod with the copepod *Lernaeocera branchialis* (Plate A5.1.6) in the gill chamber and encysted metacercariae of the digenean trematode *Cryptocotyle lingua* (Plate A5.1.7) in the skin is restricted to the southwestern part of the Baltic Sea (HELCOM, 1996). During the BMB/ICES Workshop, maximum prevalences in relation to sampling sites were 1.0 % and 21.9 %, respectively (Mellergaard and Lang, 1999). According to data from the German fish disease monitoring programme, maximum prevalences of *Lernaeocera branchialis* in the years 1995–1998 occurred in the Kiel Bight and Mecklenburg Bight and were in the range of 1.2 % to 3.6 %. For metacercariae of *Cryptocotyle lingua*, maximum values were recorded in the Kiel Bight, with prevalences ranging from 38.6 % to 62.4 % (Dethlefsen and Lang, 2000, unpublished data). Mellergaard and Lang (1999) suggested that the pronounced spatial patterns for these parasites are determined by salinity. For *Lernaeocera*, it seems as if the parasite itself is restricted to saline waters, whereas for *Cryptocotyle*, it is likely that salinity affects the distribution of its intermediate host, the periwinkle (*Littorina littorea*).

A comprehensive checklist of parasites of Baltic cod from German waters is presented in Palm *et al.* (1999). Based on a study of ciliates in Baltic cod and flounder from the Kiel Fjord, Palm and Dobberstein (1999) discussed the usefulness of trichodinid ciliates as biological indicators of eutrophication in brackish water environments. A currently unclassified protistan endoparasite has been found in cod eggs from the Gotland Deep. Prevalences recorded were in the range of 0.0 % to 75.0 % in eggs from different female fish. There was indication from incubation experiments that the infections affected the larval viability but not the embryo viability or the embryo development (Kjørsvik *et al.*, 1999). Information on other studies on cod parasites has been provided in HELCOM (1996).

Herring (*Clupea harengus*)

In the Third Periodic Assessment, a description was provided of the biology, prevalence, and distribution of the two herring parasites *Anisakis simplex* and *Ichthyophonus hoferi* for the period before 1994. Both of these parasites mainly affect the migratory spring-spawning herring that invades the southern Baltic Sea in autumn/winter for spawning, coming from their feeding areas in the Kattegat, Skagerrak and North Sea (HELCOM, 1996).

Since that time, no major change in the overall prevalence of *Anisakis simplex* has been recorded. Larval nematodes can still be found at high prevalences and intensities in the body cavity and, very rarely, in the musculature of adult herring. In the past, the highest prevalences occurred in the southwestern Baltic Sea in the major spawning areas, and they decreased towards more eastern coastal areas off the Polish coast. However, the results of recent studies indicate that the prevalence in eastern waters has increased over the past years (ICES 1998, 2000a; Podolska, 2000, pers. comm.). During the BMB/ICES Workshop in December 1994, Grygiel (1999) detected a maximum prevalence of 39.5 % in herring from the Mecklenburg Bight and noted that only specimens larger than 20 cm were affected and that the prevalence increased with increasing body size. These findings are well in accordance with earlier findings from the same area (Lang *et al*., 1990).

The 1991 *Ichthyophonus hoferi* epizootic in herring (Plate A5.1.8), which caused high mortalities in the Kattegat and southwestern Baltic Sea, has declined since then and affected specimens have only rarely been found, e.g., in the Kattegat, where prevalences of 1.1 % and 0.5 % were detected in 1993 and 1998, respectively (Mellergaard and Spanggaard, 1997; ICES, 1999a). However, there is evidence that the infection is enzootic to the western spring-spawning stock and that further epizootics are likely to occur. Due to the possibility of new epizootics, ICES (1996b) has recommended that Member Countries should continue to monitor the infection as part of the national stock assessment surveys in order to identify regions with potential future epizootics as early as possible and to provide useful background data for comparison purposes.

Danish studies have demonstrated the occurrence of VHS-like virus in clinically healthy herring and sprat (*Sprattus sprattus*) from the western Baltic Sea. Viral Haemorrhagic Septicaemia (VHS) is one of the most important viral diseases in farmed salmonids in Europe and, originally, it was assumed that the virus was restricted to fresh water. However, over the past ten years it has been isolated from fourteen marine fish species in the North Sea and five species in the Baltic Sea (herring, sprat, cod, turbot (*Scophthalmus maximus*), and four-bearded rockling (*Enchyleopus cimbrius*)), and has also been identified in marine species in Pacific North America. Based on these findings and genetic studies, it has recently been suggested that the virus originates from the marine environment and had been introduced decades ago to the fresh water via aquaculture activities (Meyers and Winton, 1995; Dixon, 1999). Marine and freshwater viruses differ in terms of their genotype and their pathogenicity to salmonids, with the marine virus being less pathogenic. Prevalences calculated for five sampling areas visited in 1998 covering a region from the Kattegat to Bornholm were in the range of 0.0 % – 4.0 % and 0.0 % – 9.3 % for herring and sprat, respectively (Mellergaard, 2000, pers. comm.).

Other significant diseases of Baltic herring include lymphocystis, the prevalence of which recorded during the BMB/ICES Workshop ranged from 0.0 % – 7.4 % depending on sampling area and, rarely, cases of skeletal deformities and epidermal hyperplasia (Grygiel, 1999). Infection with the bacteria *Pseudomonas anguilliseptica*, associated with haemorrhagic eye lesions (Lönnström *et al*., 1994) and possibly involved in the transmission of the infection between trout mariculture facilities in Finnish waters, was still prevalent in the period 1994 - 1998 (Bylund, 2000, pers. comm.).

Studies of the parasitofauna in the Gulf of Finland and Gulf of Riga revealed a high prevalence of *Eimeria sardinae* in herring and sprat gonads, with maximum values of 80 % and 65 %, respectively, and a recently decreasing trend (Turovski *et al*., 1993; ICES, 1995a; Kadakas, 2000, pers. comm.). There is, however, no indication of negative effects of the parasites on affected fish.

Atlantic salmon (*Salmo salar*)

The most spectacular health problem affecting Baltic Sea fish species is the M74 syndrome of Atlantic salmon, which continued to cause high mortalities during 1994–1998 in yolk-sac fry obtained from wild salmon and salmon artificially reared for re-stocking purposes within Swedish and Finnish compensatory stock enhancement programmes. The syndrome has been known since 1974, but massive fry mortalities have only occurred since the beginning of the 1990s (Bengtsson *et al*., 1999). Table A5.1 summarizes data recorded on salmon from different Swedish and Finnish river systems in the period 1985–1999. The highest mean prevalence (percentage of female salmon producing offspring affected by M74) recorded in female broodfish from different Swedish and Finnish rivers was 75.1 % in 1993 and fry mortalities reached levels of 90 %. After 1996, the prevalence dropped considerably, to a value of 13 % in 1998. More recent data for 1999, however, indicate another increase to 37 % (ICES, 2000a).

A change in the selection process of female broodfish applied as a measure to reduce M74 problems in offspring was discussed as one of the reasons for the sharp decline from 1996 to 1998. Since the occurrence of M74 can be predicted with a high degree of certainty from the condition of the adult females (behaviour, colour characteristics), apparently healthy females were selected and stripped in order to avoid fry mortality, thus leading to the observed decline in prevalence.

In Europe, M74 seems to be restricted to the Baltic Sea, where it affects salmon spawning in Swedish, Finnish, Estonian and Russian rivers, but not in Latvian rivers. There is evidence that the syndrome also affects wild Baltic sea trout (*Salmo trutta*) (Soivio, 1996; Landergren *et al.*, 1999). A condition very similar to M74 is known from salmonid species in the North American Great Lakes and Finger Lakes, where the syndrome has been called Early Mortality Syndrome (EMS) or Cayuga Syndrome, respectively (Fitzsimons *et al.*, 1999).

Current information from Finnish, Swedish, and North American studies provides evidence that the M74/EMS syndrome is associated with a maternally transmitted vitamin deficiency (Vitamin B_1, thiamine) (Bylund *et al.*, 1995; Bengtsson *et al.*, 1999). Measurements in adults, eggs, and larvae as well as the fact that thiamine treatment of early life stages is protective against the development of M74 and EMS symptoms have provided evidence for this assumption. So far, there is no conclusive evidence on the causes of the deficiency or on the role of other potential factors involved, but dietary factors, such as changes in the abundance of the main prey species sprat and herring and their importance as food items have repeatedly been discussed as causes for M74. Furthermore, correlations have been determined between the occurrence of M74 and concentrations of dioxin-like organic contaminants which are suspected to influence the metabolism of thiamine and carotenoids. Other factors considered are eutrophication and associated algal blooms, genetic factors related to the compensatory stock enhancement programme which may lead to a genetic 'bottle-neck' effect, and differences between Baltic salmon stocks in terms of migration patterns and timing of the spawning run which may explain observed regional differences in the occurrence of the syndrome (Bengtsson *et al.*, 1999; Bylund, 2000, pers. comm.).

Other Diseases in Oher Fish Species

Compared to information for the period 1989–1993 (HELCOM, 1996), no major new disease outbreaks or parasitic infestations have been recorded in the Baltic Sea. Most of the diseases and parasites continued to occur without causing severe problems. However, apart from diseases mentioned above, there are some new findings that should be followed in the future.

1) IPN-like Birnavirus II has been isolated from dab, plaice (*Pleuronectes platessa*), cod, lemon sole (*Microstomus kitt*), and long rough dab (*Hippoglossoides platessoides*) from the Skagerrak area (Mellergaard, 2000, pers. comm.). However, the infections were not associated with clinical disease signs.
2) Larvae of *Anisakis* sp. were detected in garfish (*Belone belone*) from the Gulf of Finland (ICES, 2000a).
3) In 10 % – 20 % of pikeperch (*Stizostedion lucioperca*) from the Gulf of Riga, an eye pathology was observed which leads to blindness (ICES, 1999a).
4) In 1995, Proliferative Kidney Disease (PKD) was recorded for the first time in wild sea trout in one of the Estonian spawning rivers. Since the disease occurred at a high prevalence (> 50 %) and was associated with clinical disease symptoms, there was concern that it might be one of the major factors leading to mortality. Since then, the causative agent of PKD, the PKX organism, has also been found in sea trout from two other rivers. From recent data, there is indication that salmon parr might also be infected (Kadakas, Viilmann and Kangur, 2000, unpublished data).
5) A new digenean endoparasite, *Pseudobacciger harengulae*, was recorded in herring from the west coast of Sweden in the years 1994 to 1996 (Rahimian, 1998a). This parasite has been observed before to affect different clupeid species in tropical and temperate regions of the Atlantic, Pacific and Indian Oceans.
6) *Ichthyophonus hoferi* was detected in sprat and flounder from the Skagerrak and Kattegat area (Rahimian, 1998b) and in herring from the Gulf of Riga in the northeastern Baltic Sea (ICES, 1996a).
7) A marked reduction in the prevalence of the parasites *Diplostomum* sp., *Glugea* sp. and *Pleistophora* sp. in flounder from the western Baltic Sea was observed in 1996 (ICES, 1997).
8) A declining trend in the occurrence of monogenean fish parasites has been observed in Estonian waters (ICES, 1996a).
9) Histopathological lesions consisting of focal hepatocellular degeneration were recorded in perch (*Perca fluviatilis*) along a polycyclic aromatic hydrocarbon gradient at the Swedish east coast (Ericson *et al.*, 1998).

Some of the other significant diseases or parasites of Baltic Sea fish species described in HELCOM (1996) continued to occur:

- High prevalences (> 50 %) of skin ulcer disease continued to occur in four-bearded rockling (*Enchelyopus cimbrius*) in deep-water regions off the Polish coast (Dethlefsen and Lang, 2000, unpublished data).
- Baltic cod embryos and yolk-sac larvae were found to be infested with a yet unknown protistan endoparasite (Kjørsvik *et al.*, 1999; Pedersen *et al.*, 1993).

- The swimbladder nematode (*Anguillicola crassus*) of the European eel (*Anguilla anguilla*), introduced to the Baltic Sea in the 1980s, continued to be widespread. However, there is indication that prevalences are lower than in pure freshwater environments and that both prevalences and intensities have tended to stabilize or even decline, possibly due to an adaptation of the host to the parasite (Svedäng, 1996). Factors limiting the distribution of the parasite seem to be high salinity and low temperature (Svedäng, 1996; Nielsen, 1997; Knopf *et al*., 1998).
- Reproductive disorders recorded in roach (*Rutilus rutilus*) in brackish waters at the Finnish coast (Wiklund and Bylund, 1994a; Wiklund *et al*., 1996) which were associated with a microsporidian infection (*Pleistophora mirandellae*) continue to be observed. This parasite has recently also been recorded in roach from Swedish brackish-water environments (Luksiene *et al*., 2000).

Impact of Athropogenic Activities on the Prevalence and Geographical Distribution of Fish Diseases and Parasites

It is now generally accepted that the identification of causes leading to changes in temporal and spatial distribution patterns of wild fish diseases is difficult. This is largely due to the fact that the aetiology of the majority of diseases is of a multifactorial nature, involving a wide range of endogenous and exogenous natural and anthropogenic stressors (Vethaak and ap Rheinallt, 1992; ICES, 1995b; Lang and Dethlefsen, 1996). However, the HELCOM Third Periodic Assessment highlighted a number of examples from the Baltic Sea for which there has been a strong indication for links between anthropogenic environmental changes and the occurrence of elevated prevalences of certain fish diseases and parasites in certain areas (HELCOM, 1996). These included effects of pulp mill effluents and discharges from other industries, temperature changes associated with discharges of heated effluents, oxygen deficiency likely to be related to eutrophication, and the introduction of non-indigenous species.

Despite improvements over the past years with regard to anthropogenic impact on the marine environmental quality of the Baltic Sea, there still exist disease problems affecting Baltic fish species which very likely are attributable to human activities:

1) The M74 syndrome continues to occur in Baltic salmon and trout and threatens the survival of the few still naturally reproducing salmon stocks in the Baltic Sea.
2) From Swedish studies there is indication that the prevalence of parasitic nematodes in perch and eelpout (*Zoarces viviparus*) might be increased in polluted areas (ICES, 2000a).
3) Palm *et al*. (1999) demonstrated local differences in the occurrence of skin parasites (ciliates) of cod and flounder as indicators of eutrophication.
4) The parasitic nematode *Anguillicola crassus* introduced to Europe from Asia still persists in eel (*Anguilla anguilla*) from the Baltic Sea.
5) A new herring parasite (digenean trematode *Pseudobacciger harengulae*) has been observed at the Swedish west coast, possibly introduced via ballast water discharges (Rahimian, 1998a).
6) An elevated prevalence of liver lesions has been found in perch along a PAH gradient in Swedish coastal waters (Ericson *et al*., 1998).
7) Reproductive disorders indicating reduced reproductive capacities were observed in female perch, roach, and pike (*Esox lucius*) from coastal Swedish and Lithuanian brackish-water areas affected by thermal effluents (Luksiene *et al*., 2000).
8) The prevalence of liver neoplasms in flounder from areas in the Gulf of Finland continues to be high (Lang *et al*., 1999; Bogovski *et al*., 1999; Bylund, pers. comm.) and this has been attributed to contaminants.

For other contaminant-associated diseases mentioned in the HELCOM Third Periodic Assessment (HELCOM, 1996), there is evidence that a reduction in industrial discharges has led to a decrease in disease prevalence. Examples are skeletal deformities in perch and pike and fin erosion in perch and ruffe (*Gymnocephalus cernua*) in Swedish coastal areas affected by pulp-mill effluents (Lindesjöö and Thulin, 1990, 1992; Lindesjöö *et al*., 1994; Lindesjöö, 2000, pers. comm.).

Impact of Fish Diseases/Parasites on Baltic Sea Fish Stocks

There is little conclusive information regarding the impact of fish diseases or parasites on Baltic Sea fish stocks. In only a few cases have mortalities, induced by specific diseases or environmental factors, been so obvious that it seems justified to assume that these conditions might have had a significant effect on stock size in the affected fish species (HELCOM, 1996). The *Ichthyophonus* epizootic in the beginning of the 1990s was an example of such a case. However, since it ceased shortly thereafter, it is currently not regarded as a problem for the herring stocks in the Baltic Sea. As stated above, future epizootics with possible stock effect cannot be excluded.

The M74 syndrome of Baltic salmon remains a significant problem. Although thiamine treatment of early stages of salmon and a more careful selection of adult females for breeding purposes have improved the situation in hatcheries, the syndrome undoubtedly continues to threaten the survival of the few remaining local stocks reproducing under natural conditions and, unfortunately, there is no clear indication so far of a significant and longer-lasting decrease in occurrence of the syndrome in wild salmon.

The high prevalences of acute skin ulcerations in Baltic cod observed during the past few years is causing concern that this disease might affect the stocks by significantly increasing the mortality and possibly also by reducing the fitness and reproductive potential of affected but surviving cod. Mellergaard and Bagge (1998) suggested that the ulcers they considered to be caused by mechanical damage associated with the fishery might be lethal for the fish due to the large area of the body affected. A high prevalence of skin ulcers not only potentially affects the stock due to increased mortality but also the fishery, since affected fish in most cases are not marketable due to the occurrence of conspicuous disease signs on the body surface.

There is no indication that flounder stocks are affected by the most prevalent diseases, lymphocystis and skin ulcerations. For both diseases, it is well known that skin lesions may heal and that the diseases do not affect the condition of the host in a way that it may die from the disease. Therefore, it seems unlikely that these diseases contribute significantly to natural mortality. However, again gross disease signs may affect the marketability of fish caught in a commercial fishery.

As already pointed out in HELCOM (1996), it has to be emphasized that attempts to quantify disease-induced mortalities in general suffer from a number of confounding factors and methodological problems and that, therefore, it has so far not been possible to develop and validate realistic epidemiological models for wild fish diseases, estimating disease-induced mortality rates with a high degree of probability.

Farmed Fish

The sea-farming activities in the Baltic Sea are mainly focused on rainbow trout (*Oncorhynchus mykiss*), with the main part of the production concentrated in the northern-most parts of the Baltic Sea Proper, i.e., the coastal areas of Finland and Sweden. The production volume remained at a rather constant level during the 1990s, with a yearly production of 18 000 to 22 000 t. During recent years, several new fish species have been included in the sea-farming programmes, so far more or less on a pilot scale. The most promising of these candidate species appears to be whitefish (*Coregonus lavaretus*) and char (*Salvelinus alpinus*).

Of the viral diseases, IPN (Infectious Pancreatic Necrosis) appears to be widespread in the Baltic Sea. However, rainbow trout is rather resistant to this disease and the virus is mostly recorded from fish without disease symptoms which may act as carriers. A new rhabdovirus was isolated from brown trout (*Salmo trutta*) in northern Finland in 1987 (Koski *et al*., 1992). The virus caused low mortality in brown trout fry and was characterized as European Lake Trout Virus (ELTV) (Björklund *et al*., 1994). In 1995, the virus was again isolated in brown trout without clinical symptoms in northern Finland and, in 1996, a very similar virus was isolated from brown trout in the archipelago of Stockholm. The latter fish were kept as broodfish in net cages and showed disease symptoms and low mortality. It appears that brown trout may carry the virus and remain without disease symptoms, unless stressed by handling or other rearing procedures.

Since first recorded in the Gulf of Bothnia in 1985, furunculosis caused by *Aeromonas salmonicida salm.* rapidly spread over large areas in the Baltic Sea and the Baltic Sea is now considered an endemic area for this disease. It is frequently recorded in farmed rainbow trout as well as in wild Atlantic salmon caught as broodfish. Furunculosis and vibriosis, the latter caused by *Vibrio anguillarum*, constituted the main disease threats to the Baltic sea-farming industry during the past decade. However, the significance of these two diseases has rapidly been decreasing due to the introduction of efficient vaccines and the application of large-scale vaccination programmes.

Bacterial Kidney Disease (BKD) was recorded for the first time in 1989 in the Åland Islands. Today, the disease is frequently recorded in rainbow trout sea farms in Finland and Sweden. Reports from Estonian studies in spawning rivers located in the Gulf of Finland indicate that this disease also occurs in wild sea trout (Kadakas, Viilmann and Kangur, 2000, unpublished data). Farmed rainbow trout are not very sensitive to this disease and, consequently, it does not cause significant economic losses. However, in order to prevent the spread of the disease to inland waters, firm restrictions are applied in Finland and Sweden concerning movements of living fish and fish products from the Baltic Sea to fresh waters.

Flavobacteriosis caused by *Flavobacterium psychrophilum* (= *Flexibacter psychrophilus*) is another infectious disease which emerged as a serious problem in fish farms in the northern Baltic Sea during the 1990s. Although originally known as a disease in fish fry and yearlings in freshwater farms (Rainbow Trout Fry Syndrome, RTFS), infections have now frequently been recorded in rainbow trout in sea farms and appear to be an important component in the winter mortality syndrome of rainbow trout in northern fish farms. Control of the disease is difficult since drug treatment has not been efficient and vaccines are not available so far (Bylund, 2000, pers. comm.).

Conclusions

The present compilation of available information shows that only a few new trends regarding the occurrence of diseases and parasites in wild fish from the Baltic Sea have been recorded in the period 1994–1998. However, in certain cases, significant changes in the prevalence occurred, e.g., an increase in prevalence of skin ulcerations of cod from areas in the southwestern Baltic Sea at the end of the 1990s. A decrease in the prevalence of the M74 syndrome was observed recently. However, more current data indicate an increase in 1999 and, thus, there is still concern that M74 continues to be a major threat to the survival of the few remaining naturally reproducing salmon stocks in the Baltic Sea.

As a major new finding, the occurrence of viral infections originally thought to be restricted to farmed fish (VHS-like rhabdovirus and IPN-like Birnavirus) has been observed in wild fish. Another new finding is the occurrence of *Pseudobacciger harengulae*, a new parasite of herring from the Swedish west coast, possibly introduced via ballast water.

For a number of diseases and parasites, the impact of human activities on their prevalence and distribution remains likely. Other diseases show a clear decrease in prevalence, reflecting an improvement in the state of the environment linked to a reduction in industrial discharges. Both the negative and positive findings clearly indicate that changes in the prevalence or intensity of diseases and parasites in wild fish can be used as an indicator of biological effects of anthropogenic activities. It should, therefore, be emphasized that studies of fish diseases/parasites in wild fish in the Baltic Sea should be continued by Baltic countries and be included in future international Baltic marine monitoring and assessment programmes. Intercalibrated and standardized methodologies for fish disease surveys have been developed and established successfully, and are largely applied by national laboratories (ICES, 1989; Bucke *et al.*, 1996; Lang and Mellergaard, 1999).

Regarding farmed fish, the introduction of efficient vaccines seems to largely eliminate the problems caused by the most important bacterial diseases, furunculosis and vibriosis. However, there is concern that new disease problems might emerge in the fish farming industry due to increased trade and movements of live fish and fish products between European countries and due to the introduction of new fish species in the fish farming industry.

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Table A5.1. Prevalence* of the M74 syndrome in female Atlantic salmon (*Salmo salar*) used as broodfish from different Swedish and Finnish rivers (values in bold italics: calculation of prevalence based on less than 20 specimens) (Source of information: ICES, 2000a.)

River system	**ICES Sub-division**	**1985**	**1986**	**1987**	**1988**	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**
Simojoki	31						***12***	0	53	74	*53*	92	***86***	86	31	38
Torne älv	31								70	74	85	66			25	56
Lule älv	31								58	66	57	48	61	38	6	34
Skellefetälven	31								40	49	69	49	77	16	4	42
Ume/Vindel älven	30	40	20	25	19	16	31	45	77	88	85	74	78	37	9	53
Ångermanälven	30								50	77	64	45	63	21	3	28
Indalsälven	30	4	7	8	7	3	8	7	45	72	65	52	64	22	1	20
Ljungan	30								***64***	***97***	***50***	56	28	*29*	10	25
Ljusnan	30							17	33	59	86	52	72	22	6	41
Dalälven	30	28	8	9	20	11	9	21	79	85	53	55	57	38	9	33
Mörrumsån	25	47	49	65	46	58	72	65	55	90	***80***	63	***56***	23		
Neva/Åland	29									70	50					
Neva/Kymi	32								45	60–70		57	***40***	79	42	
Mean total		29.8	21.0	26.8	23.0	22.0	26.4	25.8	55.8	75.1	66.4	59.1	62.0	37.4	13.3	37.0

*Prevalence: Percentage of female broodfish giving offspring with M74 symptoms.

Figure A5.1. Common diseases and parasites in Baltic fish species.

Plate A5.1.1. Lymphocystis in flounder.

Plate A5.1.2. Acute skin ulceration in flounder.

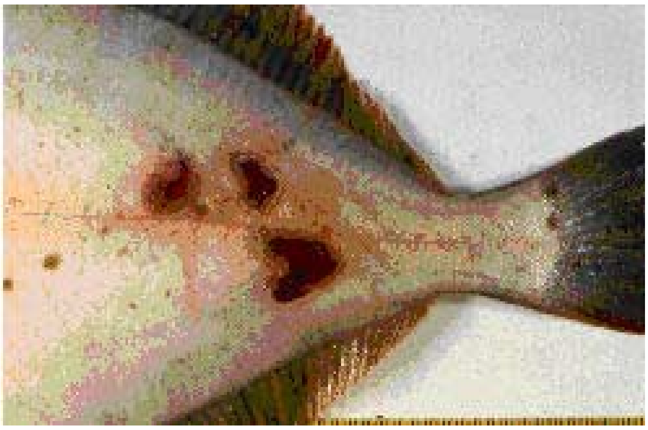

Plate A5.1.3. Acute skin ulcerations in cod.

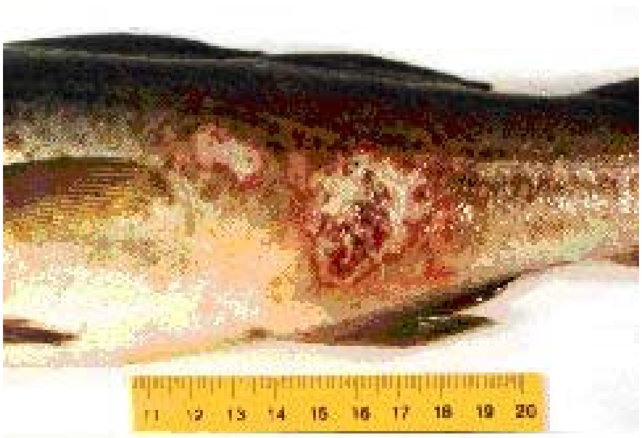

Plate A5.1.4. Skeletal deformity in cod.

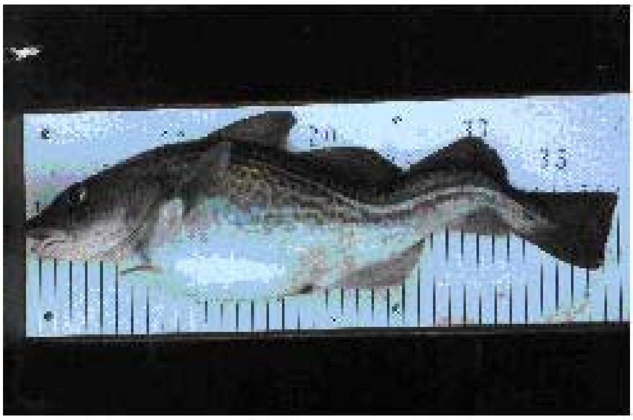

Plate A5.1.5. X-cell disease leading to swelling in the pseudobranch in cod.

Plate A5.1.6. *Lernaeocera branchialis* in the gill chamber of cod.

Plate A5.1.7. Encysted metacercariae (black spots) of *Cryptocotyle lingua* in the skin of cod.

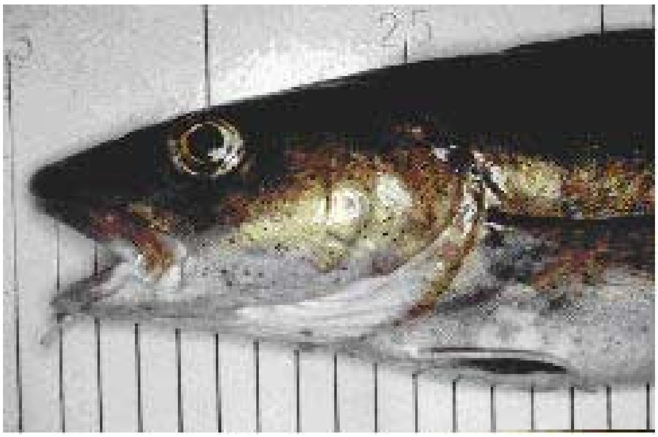

Plate A5.1.8. Granulomas (white nodules) in the heart tissue of herring caused by *Ichthyophonus* sp.

ANNEX 6: REVIEW PROGRESS IN DATA SUBMISSIONS TO THE ICES DATA BANKS AND CONTINUE THE STATISTICAL ANALYSIS OF ICES FISH DISEASE DATA IN RELATION TO ENVIRONMENTAL AND FISHERIES DATA

Working document prepared by W. Wosniok and T. Lang

1 Introduction

A major part of WGPDMO's work during the past years was dedicated to the statistical analysis of fish disease data submitted by Member Countries to the ICES Environmental Data Centre. For the 1998 meeting, a report was produced providing information on spatial and temporal trends in the prevalence of externally visible diseases of dab (*Limanda limanda*) and flounder (*Platichthys flesus*) from the North Sea and western Baltic Sea (Wosniok *et al*., 1999). Although this report contains useful information and helped identifying areas of concern with regard to changes in disease prevalences, it did not focus on potential causes of the observed disease trends.

As a consequence, the next step was an attempt to combine the fish disease data with other data stored in the ICES Data Banks for a more holistic analysis, aiming at the identification of factors involved in the changes in prevalence observed. At the 1999 WGPDMO meeting, a report was presented providing an overview of relevant ICES data (contaminants, oceanographic and fisheries-related data) available for three North Sea areas (extended German Bight, extended Dogger Bank and extended Firth of Forth) for the period since 1981. The report further contained the results of a case study for which a subset of these data for the extended German Bight extracted from the ICES Data Banks was statistically analysed by means of logistic regression analysis (Lang and Wosniok, 2000).

The results of this analysis were considered promising as a close relationship with variation in the disease prevalence of a number of parameters were identified that were worth studying in greater detail. However, a striking lack of data in the ICES Data Banks was identified, in particular for contaminants in biota and sediments, leading to considerable gaps in temporal data and creating problems in the analysis. A major problem was the need to apply temporal data interpolations to fill these gaps, a procedure that might have affected the results of the analysis and their interpretation.

Therefore, WGPDMO decided in 1999 to continue with the efforts to improve the data basis and the analytical procedures and to revisit this topic at its 2000 meeting, with the aim of reviewing progress in data submissions and in the development of optimised statistical models taking into account the shortcoming identified earlier.

2 New Data Submissions and Updates

2.1 ICES Environmental Data Centre

The ICES Environmental Data Centre was extended in 1999 to include part of the work programme for OSPAR and HELCOM in order to store data on quality assurance of biological and chemical measurements within four new data banks. Since this was done without any increase in staff members, not much time was left for further activities.

According to the inventory (updated 2 February 2000), there were no new data submissions for contaminants in biota, sediments, or sea water. Thus, the gaps in information relevant for a holistic analysis of the fish disease data identified last year still exist.

No biological effects data are expected to be submitted before the EU BEQUALM programme is completed towards the end of 2000.

Five new data sets related to wild fish diseases (dab and flounder) were submitted in 1999. These were derived from UK (data for 1998, 1999), Germany (data for 1998) and Dutch (data for 1997, 1998) studies. Four of these have been validated and incorporated in the ICES Data Centre. The complete ICES holding of fish disease data by submitting laboratory and year as of 23 February 2000 is given in Table A6.1.

2.2 ICES Oceanographic Data Centre

The ICES Oceanographic Data Centre has been updated considerably during the past year. All historic oceanographic data available for the period before 1990 are now public domain and are available on the web. Data can be downloaded as *.zip files for single Marsden squares (5° latitude and longitude each) which can be selected on a map provided in the data inventory. After decompressing, *.cvs files are available for a ten-year period each. Parameters included are temperature, salinity, dissolved oxygen, pH, nutrients, hydrogen sulphide, alkalinity, chlorophyll *a*, and these are presented by detailed geographical coordinates where measurements were made. The inventory also contains data distribution maps illustrating the number of available data, and mean, maximum and minimum data values averaged over the period 1980–1989 for the above-mentioned parameters.

The ICES Oceanographic Data Centre further provides new information on surface (underway) temperature/salinity data for the period 1990–1999 measured in the whole Atlantic, including the North Sea. These can also be downloaded.

2.3 ICES Fishery Data Banks

Apart from regular updates in the ICES Fishery Data Banks (IBTS and commercial catch data), no major changes have occurred. It still seems to be a problem to get easy access to fishing effort data (STCF data), because the database consists of about 60 different fleets, which are difficult to aggregate.

3 Continuation of the Statistical Analysis

Since, except for the oceanographic data, no major additional historical data sets were available from the ICES data banks, no significant new results could be expected from a further statistical analysis of the fish disease data in combination with other ICES data. However, due to the availability of more oceanographic data and because the statistical procedures were modified in order to improve the treatment of interpolated data, a further analysis largely based on the strategy applied last year seemed to be justified.

3.1 Improvement of statistical procedures

The statistical analysis of relations between fish diseases and potential explaining parameters as presented in the 1999 WGPDMO report used interpolated values to fill gaps in the time series of explaining parameters. As pointed out in that report, the uncritical use of interpolated values in a statistical analysis, particularly simply treating them like regularly measured values, can lead to misleading conclusions. Therefore, a statistical approach was developed to incorporate interpolated values in a more appropriate way.

An interpolated value is an estimate for a missing measurement. This estimate cannot be expected to be identical with the measurement, had the latter been made, instead a random difference between both must be assumed. The distributional characteristics of this difference can be approximated by a normal distribution with expectation zero and standard deviation s, where s is inferred from the interpolation process. With this information, the impact of using interpolated values to analyse the relation between fish disease data and a fixed set of explaining parameters can be assessed by the following procedure:

1) Estimate a basic set of logistic model parameters (coefficients), using interpolated values for the potential explaining quantities, where necessary.
2) Replace each interpolated value by (interpolated value + random error), where the random error is generated from a Normal (0,s) distribution.
3) For each fish disease data point, generate a random replicate from a binomial distribution, using the number of fish examined and the empirical prevalence as binomial parameters.
4) Estimate a new set of model coefficients for the data set generated in steps 2 and 3 and record the estimates.
5) Repeat steps 2 to 4 “sufficiently often” (experience has shown that 1000 replicates were sufficient).
6) The recorded sets of estimates establish an empirical distribution for the estimates of each model coefficient, accounting as well for the usual binomial variation as for the uncertainty introduced by using interpolated values. Derive p values for the hypothesis “coefficient = 0” from this distribution.

3.2 Use of updated data sets

As for the last year’s analysis, only data for the area extended German Bight were used due to the best data coverage compared to the other areas. Only disease data for dab were included in the analysis. Fish disease data submitted in 1999 were not considered since they either did not cover this area or were not in a format to be used directly. Updated oceanographic data were used, which improved to data coverage (e.g., for water temperature and salinity) for the period 1981–1989 considerably.

3.3 Results of Analysis

3.3.1 Univariate analysis

As a first step, a univariate analysis was carried out, considering relationships between single potentially explanatory factors with the prevalence of lymphocystis, epidermal hyperplasia/papilloma and acute/healing skin ulcerations of dab. The procedure applied was identical to the one used last year. However, data were corrected for the use of interpolated values as explained above.

The results are given in Table A6.2. For comparison purposes, the results from the last year's analysis are also included in the table.

The data show that, with only a few exceptions, the factors identified as having a significant relationship with the disease prevalence were identical to those identified previously. However, as expected, the p-values calculated using the correction were much higher in most cases, indicating a less significant relationship.

3.3.2 Multivariate analysis

For the multivariate analysis, the models developed for last year's analysis were used, i.e., a long-term model including observations for almost the whole range 1981–1997, a medium-term model with observation covering approximately one half, and a short-term model covering one third of the period. A multivariate analysis comprising all potentially explanatory parameters available simultaneously was considered inappropriate as it would have required either massive extrapolations or would have been restricted to a very narrow time span.

Those parameters identified last year to be significantly related to the disease prevalence were tested again, calculating corrected p values, which account for the effects of interpolation. The results are provided in Table A6.3 for the three models by disease. The table also provides information on correlations between single parameters. This indicates possible alternative relationships between the disease prevalence and explanatory factors, because significant parameters can be replaced by those which are highly correlated.

The table shows that only a few significant parameters were left. For lymphocystis, salinity was a significant factor in the long-term and medium-term models. Salinity was correlated with P04 in the long-term model, and with S63 Hg in the medium-term model. Epidermal hyperplasia/papilloma was significantly related to N03 and S63 CB153 (in all cases significantly correlated with P04) only in the medium-term model, but also shows a relationship with N03 in the other models. For acute/healing skin ulcerations, only WB Cd was significant in the long-term and short-term models. A weak relationship was found with CPUE in the long-term and medium-term models. CPUE was correlated with WB Hg in all three models.

The results clearly indicate that even after applying stronger rules for the assessment of interpolated data, significant relationships between potentially explanatory factors and the disease prevalence can be demonstrated. However, there are no single factors that uniquely appear in all situations investigated. This is obviously largely due to the high correlations that exist between the explanatory quantities. The results also reveal the complex multifactorial nature of disease aetiology.

4 Perspective for Future Activities

All analyses so far used only a restricted subset of the available data (females, 20–24 cm) in order to keep heterogeneity due to length and gender effects as small as possibly. A more comprehensive use of the existing data requires the inclusion of more length classes and both sexes. Simply extending the previous analysis to the whole data set would introduce inappropriate heterogeneity due to variations in population structure over time and space. The approach of direct standardisation is a way to eliminate the effect of variation in population structure. Here, disease rates are first calculated for each length class, gender, location and time point. From these, an expected prevalence is calculated for a standard population having a fixed representative length-sex structure. These expected prevalences can directly be compared over space or time without interfering with population structure effects.

Another restriction still is that only for few regions of the North Sea is the data density sufficiently high to allow the analysis between fish diseases and explaining factors. Submission of additional data would allow statements about larger geographical areas and/or extended time ranges, which at present are not considered possible. Therefore, there is a need for further submissions of data, known to be available in national data banks, to the ICES Data Banks. Member Countries should be strongly (!) encouraged to provide these data.

It is intended that future activities related to a holistic analysis of ICES fish disease data involve areas other than the North Sea. However, most disease data submitted are from the North Sea and the majority of data generated in existing fish disease monitoring programmes covering other areas (e.g., the Baltic Sea, Barents Sea, Atlantic North America) have not been submitted yet. Therefore, ICES Member Countries should be encouraged to submit these data using the standardised data submitting formats, provided that the data have been collected according to the ICES standard guidelines for fish disease surveys.

Literature cited

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Table A6.1. ICES Holding of Fish Disease Data as of 23 February 2000.

FDE submitted data	**Format 2.2 ASCII files**
DFHU84DF.FDB	ALUK91DF.UK
DFHU85DF.FDB	ALUK92DF.UK
DFHU86DF.FDB	ALUK93DF.UK
DFHU87DF.FDB	ALUK94DF.UK
DFHU88DF.FDB	ALUK95DF.UK
DFHU89DF.FDB	ALUK96DF.UK
DFHU90DF.FDB	ALUK97DF.UK
DFHU91DF.FDB	ALUK99DF.UK
DFHU92DF.FDB	BFCG81DF.GE
DFHU93DF.FDB	BFCG82DF.GE
DGWN91DF.FDB	BFCG83DF.GE
DGWN93DF.FDB	BFCG84DF.GE
DGWN94DF.FDB	BFCG85DF.GE
DGWN95DF.FDB	BFCG86DF.GE
DGWN96DF.FDB	BFCG87DF.GE
DOUK91DF.FD1	BFCG88DF.GE
DOUK91DF.FD2	BFCG89DF.GE
DOUK92DF.FDB	BFCG90DF.GE
DOUK93DF.FDB	BFCG91DF.GE
DOUK94DF.FDB	BFCG92DF.GE
DOUK95DF.FDB	BFCG93DF.GE
DOUK96DF.FDB	BFCG94DF.GE
DOUK97DF.FDB	BFCG95DF.GE
DOUK98DF.FD1	BFCG96DF.GE
DOUK98DF.FD2	BFCG97DF.GE
RIVO83DF.FDP	BFCG98DF.GE
RIVO84DF.FDL	
RIVO84DF.FDP	
RIVO85DF.FDP	
RIVO86DF.FDL	
RIVO86DF.FDP	
RIVO87DF.FDL	
RIVO87DF.FDP	
RIVO88DF.FDL	
RIVO88DF.FDP	
RIVO89DF.FDL	
RIVO89DF.FDP	
RIVO90DF.FDL	
RIVO91DF.FDP	
RIVO92DF.FDP	
RIVO93DF.FDP	
RIVO94DF.FDP	
RIVO95DF.FDP	
RIVO96DF.FDP	
RIVO97DF.FDB	
RIVO98DF.FDB	

Table A6.2. Results of the univariate analyses, comparing the results obtained ***with and without*** correction for the use of interpolated values.

			LYM				EPI				ULC			
	n_o	n_i	dir_u	p_u	dir_c	p_c	dir_u	p_u	dir_u	p_c	dir_u	p_u	dir_c	p_c
Temp.	261	98	+	0.0001	+	0.00	+	0.0001	+	0.00	+	0.0001	+	0.00
Sal.	239	98	+	0.0001	+	0.00	+	0.0001	+	0.00	-	0.8911	-	0.95
PO_4	48	71	+	0.1688	+	0.21	-	0.0638	-	0.13	-	0.0418	-	0.12
NO_3	48	71	+	0.0362	+	0.09	-	0.7437	-	0.76	-	0.0832	-	0.17
O_2	20	29	+	0.0013	+	0.09	+	0.1358	+	0.16	-	0.0733	-	0.13
WB CB153	120	43	+	0.2149	+	0.29	-	0.7491	-	0.78	+	0.9132	+	0.98
WB HCB	114	43	-	0.6210	-	0.73	-	0.7535	-	0.87	-	0.0001	-	0.01
WB Cd	185	52	-	0.5397	-	0.60	+	0.2417	+	0.27	-	0.0001	-	0.00
WB Hg	186	52	-	0.0066	-	0.04	-	0.0051	-	0.02	-	0.0555	-	0.16
S63 CB153	5	42	+	0.5098	+	0.50	-	0.0098	-	0.01	+	0.2030	+	0.17
S63 HCB	3	27	-	0.0006	-	0.01	-	0.0534	-	0.13	+	0.0317	+	0.10
S63 Cd	7	34	+	0.2290	+	0.29	+	0.7014	+	0.72	+	0.9162	-	0.97
S63 Hg	8	42	+	0.0114	+	0.05	-	0.4048	-	0.57	+	0.8843	+	0.91
My CB153	36	96	+	0.0001	+	0.00	+	0.0001	+	0.00	+	0.4567	+	0.51
My HCB	33	39	-	0.0183	-	0.01	-	0.0544	-	0.03	-	0.0128	-	0.00
My Cd	50	96	+	0.1833	+	0.18	+	0.1119	+	0.12	-	0.0238	-	0.03
My Hg	51	96	+	0.0001	+	0.00	+	0.0001	+	0.00	-	0.1370	-	0.13
LimL CB153	4	13	+	0.0192	+	0.02	-	0.4267	-	0.47	+	0.9582	-	0.99
LimL HCB	3	5	+	0.1218	+	0.27	-	0.7319	-	0.84	+	0.6986	+	0.77
LimL Cd	5	28	+	0.0235	+	0.03	+	0.8689	+	0.82	-	0.0115	-	0.01
LimM Hg	5	28	+	0.0019	+	0.01	-	0.8375	-	0.91	-	0.0064	-	0.01
CPUE	295	97	+	0.0001	+	0.00	+	0.0001	+	0.00	-	0.0060	-	0.06

dir_u, p_u: results obtained **without** correction for the use of interpolated values
dir_c, p_c: results obtained **with** correction for the use of interpolated values

Table A6.3. Results of the multivariate analysis with and without correction for the use of interpolated values.

Long-term model	Variables considered: CPUE, T, S, PO4, N03, WB CB 153, WB HCB, WB Cd, WB Hg, My CB153, My Cd, My Hg		
	Significant parameters **without correction**	**corrected p values**	**correlated parameters**
LYM	Sal (-, p < 0.0001) My Cd (-, p < 0.0001)	Sal (+, p = 0.00) My Cd (-, p = 0.28)	P04 My Hg
EPI	NO3 (+, p = 0.0003) WB Cd (+, p < 0.0001) My CB153 (-, p < 0.0001) My Cd (-, p = 0.0011)	NO3 (+, p = 0.07) WB Cd (+, p = 0.84) My CB153 (-, p =0.94) My Cd (-, p = 0.89)	P04 Temp Temp My Hg
ULC	CPUE (-, p = 0.0163) WB Cd (-, p = 0.0008)	CPUE (-, p = 0.07) WB Cd (-, p = 0.00)	WB Hg Temp
Medium-term model	Variables considered: CPUE, T, S, PO4, N03, WB CB 153, WB HCB, WB Cd, WB Hg, My CB153, My Cd, My Hg, S63 CB153, S63 Hg, My HCB		
	Significant parameters **without correction**	**corrected p values**	**correlated parameters**
LYM	CPUE (-, p = 0.0213) Sal (-, p < 0.0001) My Cd (-, p < 0.0001)	CPUE (-, p = 0.49) Sal (+, p = 0.00) My Cd (-, p =0.37)	WB Hg S63 Hg My Hg
EPI	NO3 (+, p < 0.0001) WB CB153 (-, p = 0.0089) My CB153 (+, p = 0.0004) S63 CB153 (-, p < 0.0001)	NO3 (+, p = 0.00) WB CB153 (-, p = 0.78) My CB153 (+, p = 0.95) S63 CB153 (-, p = 0.00)	P04 WB HCB S63 Hg My CB153
ULC	CPUE (-, p = 0.0016)	CPUE (-, p = 0.06)	WB Hg
Short-term model	Parameters considered: CPUE, T, S, PO4, N03, WB CB 153, WB HCB, WB Cd, WB Hg, My CB153, My Cd, My Hg, O2, S63 CB153, S63 Hg, My HCB, LimM Hg, LimL Cd		
	Significant parameters **without correction**	**corrected p values**	**correlated parameters**
LYM	NO3 (+, p = 0.0093) My CB153 (+, p < 0.0001) LimL Cd (-, p = 0.0070)	NO3 (+, p = 0.11) My CB153 (+, p = 0.64) LimL Cd (-, p = 0.48)	P04 LM Hg LM Hg
EPI	NO3 (+, p = 0.0005) WB Cd (+, p = 0.0288)	NO3 (+, p = 0.11) WB Cd (+, p = 0.79)	P04 LM Hg
ULC	WB Cd (-, p = 0.0070)	WB Cd (-, p = 0.00)	LM Hg

ANNEX 7: OVERVIEW OF NEW INFORMATION ON *ICHTHYOPHONUS*

Working document prepared by A. McVicar

Current Information

The following is a summary of information on data obtained during 1999 which was requested intersessionally from WGPDMO participants.

1 Canada

No surveys were conducted on the Canadian east coast in 1999.

2 Iceland

Since monitoring of *Ichthyophonus* in Icelandic summer-spawning herring started in 1992, there has been little change in the prevalence of this parasite in the size group 25 cm and larger; it has ranged from 0 to 0.24 % each year (Table A7.1). A total of almost 11 000 herring have been examined these 8 years, the average prevalence of *Ichthyophonus* being 0.15 %. In 1998 no infection was observed, but as the prevalence in this herring stock is low, the probability of finding infected herring in a small sample size is low.

The occurrence of *Ichthyophonus* in Icelandic summer-spawning herring is sporadic, only one infected fish has been found in positive samples and those samples are distributed over the distribution range of this herring stock.

Samples of Atlanto-Scandian herring have been examined for *Ichthyophonus* since 1995; most of the samples were taken in mid-water trawl by research vessels, but some were taken from commercial catches (Table A7.2). The prevalences of *Ichthyophonus* in samples taken in 1995–1997 were similar; the average each year was 1.93–3.15 %. Infected herrings were found in almost all samples; the prevalence in individual positive samples was 1–6 %. No difference was found between samples taken in mid-water trawl and samples from commercial catch caught in purse seine.

In 1998, all samples were taken by research vessels in mid-water trawl. In half of those samples, the prevalence of *Ichthyophonus* ranged from 1 % to 5 %, indicating a similar level of infection as in the previous three years. The other half was quite different, as the prevalence of *Ichthyophonus* in each sample ranged from 11 % to 38 %. All the samples examined were taken during the period from 11 to 17 May, those with low prevalence in an area close to 64°50′N to 66°15′N, but those with high prevalence close to 66°15′N to 67°20′N. The sampling locations for these two groups of samples are, thus, geographically slightly different.

In 1999, five samples were taken by research vessel in an area close to 67°30′N to 69°00′N. All these samples were positive for *Ichthyophonus*. The prevalence of *Ichthyophonus* in four of these samples ranged from 1 % to 5 %, being comparable to what was observed in 1995–1997 and in the “low prevalence group” of 1998. In one sample, however, the prevalence was 49 % (100 herrings examined).

Samples from the commercial catch examined in 1999 were all caught north of 68°N, most of them north of 71°N. Of twelve samples examined (average sample size 43 herrings), 4 were found positive (prevalence 2–5 %).

All the samples with prevalence over 10 % were caught by research vessels in mid-water trawl. It is reasonable to assume that swimming capability of infected herring is affected by *Ichthyophonus* infection. Infected herrings could be more vulnerable to fishing gear like mid-water trawl, not being able to keep up with the main school. It is possible that the samples with high prevalence of *Ichthyophonus* observed 1998 and 1999 do not indicate any real changes, but are due to circumstantial events during collection of samples.

Table A71. Infection of *Ichthyophonus* in size group 25 cm and larger of summer-spawning herring from Icelandic waters in 1992–1999, commercial and research vessels.

Year	Commercial vessels		Research vessels		Total number		
	Examined	Infected	Examined	Infected	Examined	Infected	%
1992	0		576	1	576	1	0.17
1993	0		1268	3	1268	3	0.24
1994	0		1278	3	1278	3	0.23
1995	497	0	1253	2	1750	2	0.11
1996	1329	4	647	0	1976	4	0.20
1997	685	1	679	0	1364	1	0.07
1998	500	0	199	0	699	0	0
1999	1846	2	199	1	2045	3	0.15
Total					10956	17	0.16

Table A7.2. Infection of *Ichthyophonus* in size group 25 cm and larger of Atlanto-Scandian herring caught by Icelandic commercial and research vessels in 1995–1999.

Year	Commercial vessels		Research vessels		Total number		
	Examined	Infected	Examined	Infected	Examined	Infected	%
1995	1058	34	530	16	1588	50	3.15
1996	362	5	988	21	1350	26	1.93
1997	0		1300	31	1300	31	2.38
1998	0		1200	169	1200	169	14.08
1999	521	6	479	61	1000	67	6.70
Total					6438	343	5.33

3 Norway

Summary of the statement submitted by Norway together with individual catch data:

Macroscopic examination (skin and heart) has been part of the routine examination of herring samples since the last epizootic. Some of these fish have been aged and all are length measured, so some kind of allocation to, e.g., year classes should be possible.

The herring were Norwegian spring spawners, North Sea herring, and perhaps occasionally Baltic spring spawners in the northeastern North Sea in the summer. As a general rule, herring caught north of 62°N would be Norwegian spring spawners.

The general impression is that the picture is a bit confusing. In the commercial catches, there is very little disease. In the surveys, the prevalence is quite variable, but there are at least some surveys where the prevalences were worth noticing. The contrast between surveys and commercial catches is perhaps once again a question of catchability, i.e., the sick herring are more prone to be caught in a situation where a large part of the herring escapes the gear.

4 Russia

Ichthyophonus hoferi of herring in the Barents and Norwegian Seas in 1999.

Month	**Area**	**Length of analysed fish** minimum–maximum	**Number of fish examined** individuals	**Number of infected fish %** ICES method	Russian method
March	Barents Sea	8.0–13.5	50	0.0	14.0±4.9
May	Barents Sea	8.0–14.5	153	0.0	3.3±1.4
June/July	Norwegian Sea	14.0–38.7	116	3.4±1.7	52.6±4.6

5 Scotland

From the **northern North Sea** east of Shetland, Scottish data showed *Ichthyophonus* infection in 2 of 1876 herring from commercial catches (0.11 % prevalence) in area 2 (IVa), i.e., in the Shetland area in 1999. A total of 6142 herring from commercial catches was examined from areas around Scotland. From research vessel catches, a total of 5379 herring was examined from Scottish waters with 26 of 1237 (2.1 %) from ICES Division IVa being grossly infected and 2 of 967 (0.21 %) infected in Division IVb.

6 England

In the **southern North Sea**, English data showed no evidence of *Ichthyophonus* in 145 herring sampled in the Flamborough and Celtic Deep areas.

7 Estonia

In 1999 monthly from February to December in total 1123 Baltic herring *(Clupea harengus membras)* and 312 sprat *(Sprattus sprattus balticus)* were examined on the presence of *Ichthyophonus hoferi* but this parasite was not found. In addition, 32 viviparous blenny *(Zoarces viviparus)* were examined and also no *Ichthyophonus* was found.

Earlier, in the late 1970s and in the 1980s, resting spores of *Ichthyophonus* were found in viviparous blenny, in four-horned sculpin *(Myoxocephalus quadricornis),* and in cod *(Gadus morhua*). Investigations on the presence of *Ichthyophonus* in Baltic herring and sprat started only in 1991, and also in these fishes the parasite was found. The last findings of *Ichthyophonus* in Estonian waters were in 1995. Since then this parasite has not been found. (Data of Aleksei Turovski, parasitologist, Estonian Marine Institute).

8 Denmark

In total, 485 herring were examined for the presence of *Ichthyophonus* and 4 fish had affected hearts. In the Kattegat, heart samples from 100 apparently healthy herring were transferred to Leibowitz culture medium, After 2 months the samples were investigated under microscope and *Ichthyophonus hoferi*-like spores were observed in 63 of the samples. This observation has to be confirmed by immunohistochemical staining,

Data from stock assessment samples in Denmark indicate an infection level of 0.1–0.5 % in commercial catches.

9 Sweden

Ichthyophonus data are being collected on a regular basis by Sweden and will be presented to ICES at a future meeting when collated.

10 USA

No obvious *Ichthyophonus* lesions were reported from groundfish surveys in the NW Atlantic. In Alaska, the Prince William Sound herring are examined on a regular basis and no obvious lesions were found. The data for 1999 isn't complete yet, but the general statement is that *Ichthyophonus* exists at a low prevalence. Regarding new information, T. Meyers may have cultured *I. hoferi* from ovarian fluids of several salmon species during standard virological assays. This is a presumptive diagnosis based on observation of morphological characteristics of the organism that grew in tissue culture. This observation was not confirmed with other methods. The ovarian fluids were submitted remotely, so there is no information regarding overt lesions. This is the first time observed in 15 years of such sampling. The relevant data are:

- Southeast Alaska 4 of 5 pooled ovarian fluid samples coho (*O. kisutch*)
- 7 of 92 ovarian fluid samples sockeye (*O. nerka*)
- Ketchican 2 of 3 pooled ovarian fluid samples pink salmon (*O. gorbuscha*)
- Juneau 1 of 152 ovarian fluid samples coho (*O. kisutch*)

More information, can be obtained from T. Meyers directly via e-mail (fishpath@fishgame.state.ak.us).

11 Poland

No monitoring for *Ichthyophonus* was conducted in 1999.

12 Conclusions

Ichthyophonus infection persists in herring stocks in Icelandic waters, particularly in Atlanto-Scandian stocks, in the Kattegat, northern North Sea, Barents Sea and Norwegian Sea. Currently there is no evidence of an epizootic in any of these areas, but the situation should continued to be monitored. Attention was again drawn by two countries to the possibility that certain types of fishing gear selectively caught infected fish, indicating that data from such catches may not give a reliable index of the actual prevalence levels of *Ichthyophonus* in the herring population.

ANNEX 8: OVERVIEW OF THE DISTRIBUTION AND POSSIBLE CAUSES OF THE M74 SYNDROME

Working document prepared by G. Bylund

Present state

The positive trend of decreasing prevalences reported for the two previous years has changed and a steep increase in M74 prevalences was again recorded in Finnish and Swedish river systems in 1999 (Table A8.1). Having been as high as 75.1 % (1993) the mean prevalence was down to 13.3 % in 1998. In 1999 the mean prevalence was 37.0 %. A Finnish prognosis for this spring (2000) is based on the thiamine content of salmon eggs in the autumn 1999 and indicates that the M74 prevalence will remain on a high level this year, too.

Recent results

Two international meetings/workshops dealing with M74 and EMS (Early Mortality Syndrome) were arranged in 1999. In connection with the ICES Annual Science Conference in Stockholm (29 September to 2 October 1999) a Theme Session was arranged on “M74 Syndrome and Similar Reproductive Disturbances in Marine Animals”. In connection with the Ninth International Conference “Diseases of Fish and Shellfish” arranged by the European Association of Fish Pathologists in Rhodes (19–24 September 1999) a Round Table discussion was held on “The early mortality syndrome (M74) in Atlantic salmon”. In addition to abstracts from these meetings two more comprehensive reports with results from research projects on M74 and EMS were published in 1999 (Ambio XXVII, Nr. 1, 1999 and TemaNord 1999:530; see detailed references below). Some of the most significant results presented are summarized below:

1) A strong positive correlation was previously demonstrated between the M74 prevalence and the increasing sprat population in the Baltic Sea. Comparisons of stomach contents in salmon collected 1959–1962 and 1994–1997 indicate that herring and to some extent the three-spined stickleback has increased in the diet of salmon. As sprat constituted a smaller part of the diet in 1994–1997 it is difficult to explain M74 with its positive correlation to the sprat population alone.
2) it was previously suggested that the disposition for M74 develops in salmon in its feeding areas in the southern part of the Baltic Sea Proper. Recent investigations show that the disease is found also in salmon populations with feeding migrations limited to the Gulf of Bothnia (the highest incidence of all parts) or the Gulf of Finland. This indicates that the agent(s) causing M74 is present in all these areas. Latvian salmon populations feeding primarily in the eastern part of the Baltic Proper are not affected by M74. These populations have a later spawning run and feed later in the summer than other populations examined.
3) The thiamine levels in muscles and gonads of salmon from the Gulf of Bothnia decreased in the course of the spawning run, but this was not the case with the Latvian salmon.
4) The supply of thiamine for Baltic Sea salmon from its main prey species, herring and sprat, seems to be adequate and exceeded the levels recommended for salmon growth. It is thus unlikely that low thiamine content of the prey is the cause of the low egg thiamine levels associated with M74. A similar situation was demonstrated in the Great Lakes area in salmon populations developing the Early Mortality Syndrome.
5) There were seasonal, temporal and age-related differences in the thiaminolytic activity of forage fish species in the Great Lakes. The amount of thiamine-degrading activity in two exotic fish species (alewife and smelt) was up to a hundred times the activity observed in a native species (bloater).
6) There is a positive correlation between M74 and the occurrence of organochlorines (OCs), especially dioxin-like compounds, in female muscle samples from Baltic salmon. The concentrations of total PCBs and PCDDs increased in herring and sprat with age and the levels of planar PCDs and PCDFs were two to three times higher in sprat than in herring. In salmon females the concentrations were two to nine times higher than in the forage fish species. Nevertheless, firm conclusions cannot so far be drawn concerning the role of organochlorines in the M74 aetiology.
7) There is information available indicating that the intestinal microflora of fish might play an important role in the thiamine/thiaminase levels of the fish (i.e., thiaminolytic and/or thiamine-producing bacteria).
8) Several models have recently been developed for reproduction of the M74/EMS in laboratory. This will facilitate further characterization of the syndrome and the clarification of the aetiology

Ongoing and initiated research

Ongoing research continues to focus on, inter alia, the role environmental pollutants and oxidative stress may play in the uptake, deposition and metabolism of thiamine and carotenoids in salmon. Recent efforts focus more and more on lower levels in the food chain of salmon and salmon prey species, inter alia, on long-term changes in the plankton fauna of the Baltic Sea and on the role the increased eutrophication with accompanying cyanobacteria and algal blooms may play in the aetiology of the disease.

Due to reorganization of research resources the research activities on M74 in Sweden are presently slowing down. However, an EU project with participants from several countries has been initiated.

Recently published reports on M74/EMS

McDonald, G., Fitzsimons, J.D., and Honeyfield, D.C. (Eds.) 1998. Early life stage mortality syndrome in fishes of the Great Lakes and the Baltic Sea. American Fisheries Society, Symposium 21. Bethesda, Maryland. 177 pp. ISBN 1-888569-08.

Bengtsson, B.-E., Nellbring, S., and Hill, C. (Eds.) 1999. Reproductive disturbances in Baltic Sea fish: An international perspective. AMBIO, 28 (Special Issue): 1.

Bengtsson, B.-E., Nellbring, S., and Hill, C. (Eds.) 1999. Nordic research cooperation on reproductive disturbances in fish. Report from the Redfish Project. TemaNord 1999:530. 166 pp.

ICES. 2000. Theme Session on the M74 Syndrome and Similar Reproductive Disturbances in Marine Animals. *In* ICES Annual Report for 1998/1999, pp. 211–214.

Table A8.1. M74 prevalence (%) in female broodfish from different rivers in Sweden and Finland during 1985–1999. (Prevalences in bold italics are based on less than 20 females.)

River system	Sub-div.	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Simojoki	31						***12***	0	53	74	***53***	92	***86***	86	31	38
Torne älv	31								70	74	85	66			25	56
Lule älv	31								58	66	57	48	61	38	6	34
Skellefteälven	31								40	49	69	49	77	16	4	42
Ume/Vindelälven	30	40	20	25	19	16	31	45	77	88	85	74	78	37	9	53
Ångermanälven	30								50	77	64	45	63	21	3	28
Indalsälven	30	4	7	8	7	3	8	7	45	72	65	52	64	22	1	20
Ljungan	30								***64***	***97***	***50***	56	28	***29***	10	25
Ljusnan	30							17	33	59	86	52	72	22	6	41
Dalälven	30	28	8	9	20	11	9	21	79	85	53	55	57	38	9	33
Mörrumsån	25	47	49	65	46	58	72	65	55	90	***80***	63	***56***	23		
Neva/Åland	29									70	50					
Neva/Kymi	32								45	60–70		57	***40***	***79***	42	
Mean total		29.8	21.0	26.8	23.0	22.0	26.4	25.8	55.8	75.1	66.4	59.1	62.0	37.4	13.3	37.0

ANNEX 9: INVESTIGATE SELECTED DISEASES IN PORTUGAL AND SPAIN

Working document prepared by T. Renault

INVESTIGATE THE CAUSATIVE AGENT OF GILL DISEASE IN *CRASSOSTREA ANGULATA* IN PORTUGAL (I)

Gill disease of *Crassostrea angulata* was reported for the first time to WGPDMO, occurring in low prevalences in Portugal. Since, at the light microscopic level, these resemble the lesions associated with mass mortalities of the same oyster species in the 1970s, it is important to confirm the aetiologic agent.

Thirty oysters originating from Sado River (sent by Dr F. Ruano, IPIMAR Lisbon) were prepared at the IFREMER's laboratory in La Tremblade (France) in order to perform analyses by transmission electron microscopy. Examinations will be done in 2000 and will allow determination of the cause of gill lesions in *Crassostrea angulata*.

A research project concerning this question was proposed in November 1999 to the European Commission for funding. The project aims to assess the zoosanitary status of some wild *Crassostrea angulata* populations (Portugal and Spain). As no proper data concerning diseases are yet available for *C. angulata*, this will bring valuable information on pathogens in this species. Zoosanitary analyses will have the objective of determining the presence of pathogens (metazoans, protozoans, bacteria and <u>viruses</u>) in natural *C. angulata*.

The main objective is to provide a survey and continuous monitoring of the incidence and infection rates of pathogens, particularly <u>iridoviruses</u>, on natural beds of the Portuguese oyster, *Crassostrea angulata*. Oysters originating from two different geographical sites, an estuarine polluted area (e.g., Sado Estuary) and one in an aquacultural non-polluted area (e.g., Faro area) will be subjected to histological examination. Thirty individuals will be collected monthly for each site and analysed. Coincidentally with animal sampling, most important water parameters (temperature, salinity, DO, and pH) will be checked. In case of doubt on the histological diagnosis or for a complementary lesional study, material will be analysed by transmission electron microscopy.

CLARIFY THE REPORT OF *MARTEILIA REFRINGENS* IN *CRASSOSTREA GIGAS* FROM SPAIN (II)

No material has been obtained from Spain at this time in order to clarify the report of *Marteilia refringens* in Japanese oysters. Molecular tools are available now to perform this type of analysis.

INVESTIGATE THE ROLE OF HERPES-LIKE VIRUS IN THE SUMMER MORTALITIES OF *CRASSOSTREA GIGAS* SPAT REPORTED IN PORTUGAL (III)

Reports of mortalities in *Crassostrea gigas* seed imported to Portugal from France, which showed signs of a possible viral infection, required further investigation

The first description of a herpes-like virus was reported in adult Eastern oyters, *Crassostrea virginica*. More recently, in 1991, viruses interpreted as belonging to the *Herpesviridae* were associated with high mortality rates of hatchery-reared larval *Crassostrea gigas* in France. Since 1991 sporadic high mortalities of larval *C. gigas* have been regularly observed in some private French hatcheries, occurring each year during the summer period in association with a herpes-like virus. The pathogenicity of the virus has been demonstrated by experimental transmission of the infection to axenic *C. gigas* larvae. Additionally, some mortalities reported among *Ostrea edulis* spat (since 1991) and *C. gigas* spat (since 1993) in France have also been associated with detection of a herpes-like virus. More recently, replication of herpes-like viruses has been observed in *Ostrea angasi* adults in Australia and in larval *Tiostrea chilensis* in New Zealand. Herpes-like virus infections in oysters belonging to the genera *Ostrea* and *Crassostrea* seem to be ubiquitous and are associated with substantial mortalities. In addition, concomitant high mortalities were reported in 1997 and 1998 among *Crassostrea gigas*, *Ruditapes decussatus* and *Ruditapes philppinarum* larvae in private French hatcheries. These concomitant mortalities were associated with the detection of a herpes-like virus in both species by PCR analysis. This result also indicated a possible interspecific transmission of herpes-like virus infections in bivalve molluscs and underlines the need of studying diversity among herpes-like viruses.

Work concerning the potential causative role of herpes-like viruses infecting bivalves in summer mortalities observed in *Crassostrea gigas* in Portugal has been done at the IFREMER's laboratory in La Tremblade using molecular probes. Oysters sampled in 1994 (5 individuals) and in 1998 (2 individuals) have been analysed using *in situ* hybridisation on histological sections with a specific probe for herpes-like virus DNA. No obvious specific labelling was detected on slides. No abnormal cell was observed on the same slides. These observations may indicate that mortalities among oysters in Portugal could be due to another cause.

ANNEX 10: COLLATE AND REVIEW AVAILABLE INFORMATION ON THE DISTRIBUTION AND EFFECTS OF MARINE VHS-LIKE VIRUS ON CULTURED AND WILD FISH STOCKS

Working document prepared by S. Mellergaard

Introduction

Viral Haemorrhagic Septicaemia (VHS) is caused by a Novirhabdovirus belonging to the rhabdovirus family. VHS is the most important viral disease of farmed salmonid fishes in Europe (Olesen, 1998). VHS is widely distributed in the continental part of Europe having intensive production of rainbow trout in freshwater aquaculture. Finland, Sweden, Norway, UK, and Ireland are considered free of VHS. In Spain the virus has not been diagnosed for more than ten years and it has never been diagnosed in Portugal or Greece (Olesen, 1998).

The first record of disease symptoms which later on was regarded as VHS dates back to Schäperclaus (1938) where a syndrome called "Nierenschwellung" (kidney swelling) was described in rainbow trout (*Oncorhynchus mykiss*). Pliszka (1946) described a similar problem occurring in the southern Poland. In 1953, the syndrome was observed in Denmark (Schäperclaus, 1954) and in 1955 in France (Besse, 1955). In the middle of the 1950s it became apparent that the syndrome had an infectious background but the viral aetiology was not confirmed until Jensen (1963) made the first isolation of virus on trout cell cultures.

Marine isolations of VHS

VHS was regarded as a freshwater trout disease until 1988 when VHS-virus (VHSV) was isolated for the first time in North America from returning chinook salmon (*Oncorhynchus tshawytscha*) (Hopper, 1989) and coho salmon (*O. kisutch*) (Brunson *et al*., 1989). However, on two earlier occasions outbreaks of VHS in rainbow trout reared in sea water had been described from France in 1980 and Denmark in 1982 (Castric and de Kinkelin, 1980; Hørlyck, 1984). The sources of infection were not identified with certainty but it was suspected that the virus had been introduced from the freshwater environment.

The first isolation of a marine VHSV was in fact done in a cod (*Gadus morhua*) caught in Danish waters. Jensen *et al*. (1979) reported on a rhabdovirus isolated from cod. Comparison with other rhabdoviruses was not done until 1987 where Jørgensen and Olesen (1987) demonstrated that it was a VHSV similar to the isolates from fresh water. Therefore, Jørgensen and Olesen (1987) suggested that the source of the virus was probably due to contamination rather than the cod being a new host. However, recent studies on the infectivity of the virus to rainbow trout indicated that the cod rhabdovirus was a true marine VHS isolate (Olesen, pers. comm.) and this has further been confirmed by genetic evidence (Snow *et al*., 1999).

Subsequently, VHSV was isolated from adult coho salmon returning to hatcheries in Washington State on the American Pacific coast in 1989, 1991, and 1994 (Meyers and Winton, 1995).

In North American Pacific waters, VHSV has been isolated from a series of different fish species. Meyers *et al.* (1991, 1992) reported on the isolation of VHSV from Pacific cod (*Gadus macrocephalus*). Associated with an epizootic in Pacific herring (*Clupea harengus pallasi*) VHSV was isolated from affected herring and one Pacific cod (Meyers *et al.,* 1994). Associated with epizootic mortality in Pacific hake (*Merluccius productus*) and walley pollock (*Theragra chalcogramma*) the host spectrum was further broadened to these species (Meyers *et al*., 1999). An event of mass mortality in a number of different fish species in British Columbia further added pilchard (*Sardinops sagax*) and blackcod (*Anaplopoma fimbria*) as new hosts for the marine VHSV (Traxler *et al.,* 1999).

In 1991, VHSV was isolated from turbot kept in culture facilities along the German Baltic coast (Schlotfeldt *et al*., 1991). However, with reference to formerly conducted experiments where Castric and de Kinkelin (1984) had demonstrated the susceptibility of both turbot and sea bass (*Dicentrarchus labrax*) to VHSV originally isolated from from rainbow trout, this case was suspected to originate from a freshwater source. VHSV was isolated associated with mortality in farmed turbot (*Scophthalmus maximus*) in Scotland, in 1994 (Ross *et al*., 1994; Munro, 1996). This observation could not be referred to as originating from fresh water as it was the first record of VHSV in the British Isles. A VHSV outbreak in turbot has been reported from Ireland (McArdle, pers. comm.)

In the North Sea area, research in the host spectrum and the spatial distribution of marine VHS-virus has been conducted in a collaboration between Danish, Norwegian and Scottish institutes (funded by EU-FAIR-programme). The Danish part of the research programme has covered the eastern part of the North Sea, the Skagerrak, the Kattegat and the Baltic Sea. More than 17 000 fish belonging to 30 different fish species and 3 non-fish species (2 crustaceans and 1 squid) have been examined virologically for the presence of VHSV (Mortensen *et al.*, 1999a, 1999b). So far, these investigations have resulted in approximately 100 isolates of VHSV from 11 different fish species. The Norwegian investigations have resulted in two isolates (pers. comm.). During the Scottish investigations approximately 19 000 fish originating from 22 different host species have revealed 21 VHSV isolates (King *et al.,* 1999).

One isolate from herring has been made in the English Channel (Dixon *et al.,* 1997). However, already in 1993 VHSV was isolated from two cod caught east of the Shetland Islands (Smail, 1995) and from one haddock (*Melanogrammus aeglefinus*) but these were regarded as an obscure finding until the VHS outbreak in a Scottish turbot farm occurred in 1994 (Ross *et al.,* 1994).

Host spectrum

The present host spectrum of marine VHSV is listed below. So far VHSV has been isolated from 8 different species in the North American Pacific area and 14 species in the North Sea area but the number of host species still seems to be increasing.

Fish species	**Year**	**Reference**
North American Pacific area:		
Chinook salmon (*Oncorhynchus tshawytscha*)	1988	Hopper, 1989
Coho salmon (*O. kisutch*)	1988	Brunson *et al.,* 1989
Pacific cod (*Gadus macrocephalus*)	1991	Meyers *et al.,* 1991, 1992
Pacific herring (*Clupea harengus pallasi*)	1993	Meyers *et al.,* 1994
Pacific hake (*Merluccius productus*)	1998	Meyers *et al*., 1999
Walley pollock (*Theragra chalcogramma*)	1998	Meyers *et al*., 1999
Pilchard (*Sardinops sagax*)	1998/1999	Traxler *et al*., 1999
Blackcod (*Anaplopoma fimbria*)	1998/1999	Traxler *et al*., 1999
North Sea area:		
Herring (*Clupea harengus*)	1996 1996/1997	Dixon *et al*., 1997 Mortensen *et al*., 1999a
Sprat (*Sprattus sprattus*)	1996/1997	Mortensen *et al*., 1999a
Cod (*Gadus morhua*)	1979 1993 1996/1997	Jensen *et al*., 1979; Jørgensen and Olesen, 1987 Smail, 1995 Mortensen *et al*., 1999a
Whiting (*Merlangius merlangus*)	1996/1997	Mortensen *et al.,* 1999a
Haddock (*Melanogrammus aeglefinus*)	1993	McVicar, pers. comm.
Blue whiting (*Micromesistius poutassou*)	1996/1997	Mortensen *et al*., 1999a
Norway pout (*Trisopterus esmarki*)	1996/1997	Mortensen *et al*., 1999a
Poor cod (*Trisopterus minutus*)	1998/1999	King *et al*., 1999
Lesser argentine (*Argentina sphyriaena*)	1996/1997	Mortensen *et al*., 1999a
Rockling (*Rhinonemus cimbrius*)	1996/1997	Mortensen *et al*., 1999a
Turbot (*Scophthalmus maximus*)	1991	Schlotfeldt *et al.,*1991; Ross *et al.,* 1994
Plaice (*Pleuronectes platessa*)	1998/1999	Mortensen *et al*., 1999b
Dab (*Limanda limanda*)	1998/1999	Mortensen *et al*., 1999b
Flounder (*Platichthys flesus*)	1998/1999	Mortensen *et al.,* 1999b

Marine VHSV-associated pathology

The first isolate of marine VHSV was made from one pool of 7 cod displaying stage I of the Ulcus Syndrome (the papulo-vesicular stage (Jensen and Larsen, 1979)). The samples taken for cultivation were papules with underlying tissue (Jensen *et al*., 1979). The following years numerous attempts to isolate the rhabdovirus from similar skin lesions failed. Hence, the virus is more likely to be regarded as an accidental finding, than being directly associated with the skin lesion.

In the cases of isolation of VHSV in sea water-reared rainbow trout in France and Denmark (Castric and de Kinkelin, 1980; Hørlyck *et al.,* 1984), the affected fish revealed all the classical gross symptoms of VHS: exopthalmus, haemorrhages in skin, eyes, gills, muscles, swimbladder, liver, gonads, and abdominal adipose tissue and high mortality. As these cases probably originated from fresh water, the observed lesions were directly associated with the VHSV.

After experimental infection of sea bass and turbot both fish species displayed classical gross symptoms of VHS except for the turbot which did not show haemorrhages in the muscles (Castric and de Kinkelin, 1984). The histopathological findings for both species were necrosis of the spleen and the interstitial tissue of the kidney and haemorrhages in the different intestinal organs.

In the North American cases in chinook and coho salmon, the isolation of VHSV was done in connection with routine examinations of returning brood fish and the fish revealed neither pathological lesions nor mortality (Hopper, 1989; Brunson *et al.,* 1989).

Associated with the isolation of VHSV from farmed turbot (Schlotfeldt *et al.,* 1991; Ross *et al*., 1994, Munro, 1996), pathology such as exophthalmos, haemorrhages in the skin, eyes, musculature and on the viscera, resembling the classical symptoms in rainbow trout, were observed as well as high mortality. These changes were regarded as being a product of the viral infection.

The VHSV isolated from Pacific cod was made from ulcerative skin tissue resembling those described from the Ulcus Syndrome (Meyers *et al.,* 1992). Attempts to isolate the virus from kidney and spleen tissue from the same fish failed suggesting that a generalised infection is out of question. The association between the virus and the skin lesions was unclarified.

Meyers *et al*. (1994) isolated in 1993 VHSV from Pacific herring displaying skin ulcers or subdermal haemorrhages without being able to detect other pathogens in the affected fish. This finding was reproduced during these authors' study in 1994 reported in Marty *et al.* (1998). Besides the gross lesions the histopathological investigation showed varying degrees of congestion of the liver with diffuse single hepatocyte necrosis. Some of the kidneys of affected herring were congested and showed varying degrees of degeneration and pyknosis of the tubular epithelium. The spleen was also congested some with haemorrhages and in general active RE foci were found in liver, kidney and spleen. Lymphocytic infiltration in the meninges was prevalent.

Meyers and Winton (1995) reported that marine VHSV infection caused acute mortality in juvenile herring due to acute necrosis of liver and kidney while skin lesions were more prominent in older fish, maybe due to greater host resistance. However, the skin lesions may become life threatening for the herring resulting in osmoregulatory shock.

In the Danish investigations attempts to correlate skin lesions in both herring and cod with the isolation of marine VHSV was unsuccessful. Most isolates originate from apparently healthy fish.

Mass mortalities in marine fish stocks associated with marine VHS

Mass mortalities in marine fishes have recently been reported from the North American Pacific area.

In August 1998, mass mortalities in Pacific herring, Pacific hake and in walleye pollock were reported in Lisianski Inlet, Alaska. Marine VHSV could be isolated from samples of dead fish of all three fish species (Meyers *et al.,* 1999). None of the affected fish showed any gross external or internal lesions and the histology displayed unspecific changes of which some could be referred to as autolytic changes.

During the period November 1998 to February 1999 another mass mortality occurred a little further south in Queen Charlotte Strait, on the northeast coast of Vancouver Island. In particular pilchard but also Pacific herring, blackcod, ratfish (*Hydrolagus colliei*) and shinner perch (*Cytomaster aggregata*) were reported floating on the surface moribund.

Epizootiology

The epizootiology of marine VHSV in Pacific herring appears to be like an opportunistic pathogen triggered by stress (Meyers *et al.,* 1994). The VHS epizootics in Pacific herring have involved a series of stressors like exceptionally strong year classes of fish, infection by other diseases such as ENV, the rigour of spawning in shallow water, pursuit and capture in a pound net fishery, harassment by predators, colder than normal sea water temperatures, nutritional deprivation, and acute exposure to pollutants (hydrocarbons). Most of these stressors were abundant in the Prince William Sound area (Meyers *et al*., 1994). For the pilchard and herring mortality in Queen Charlotte Strait, low water temperatures have been suggested as the triggering factor for the VHS infection (Traxler *et al.,*1999).

Stress may increase the prevalence of VHSV and enhance the spread of the disease, especially if the stock density is high. Such a case was observed in the spawn-on-kelp fishery in Prince William Sound where mature herring is caught and kept in net pens in order to collect their eggs on kelp (*Microcystis* blades). Under these circumstances the prevalence of VHSV increased significantly in the herring in the enclosures compared to the free-living stock and the release of virus reached levels where it could be isolated from the water (Hershberger *et al.,* 1999).

Many of the other species from which VHS-virus have been isolated e.g., salmonid species, Pacific cod and Pacific hake, are fish species which predate on herring. The VHSV seems to be enzootic in the North American Pacific herring stock and the infection is probably transferred to other species through predation.

Pathogenicity of marine VHSV to different fish species

Unlike the classical VHSV, the North American salmonid isolates appeared to be relatively avirulent for rainbow trout.

Meyers and Winton (1995) conducted infection trials with the Orcas Island and Makah VHSV isolates on coho salmon and rainbow trout. Waterborne virus challenges of rainbow trout and coho salmon fingerlings (2–3 g) resulted in mortality below 10 %. This level has to be compared to a range of mortality of 70–100 %, which normally is observed in rainbow trout during disease outbreaks with the classical Egtved virus.

Infection trials with the VHSV isolated from Pacific cod were carried out on coho, chinook, sockeye (*O. nerka*) and pink salmon (*O. gorbuscha*) and rainbow trout. The experiments resulted in a cumulative mortality of 12 % above the control group for the rainbow trout whereas the other salmonid species were refractory to the marine VHSV (Follet *et al.,* 1997).

The Canadian herring isolates of VHSV were used in susceptibility studies of Pacific herring, shiner perch and Atlantic salmon (*Salmo salar*). Both waterborne exposure and IP injection resulted in a significant mortality in both herring (27 %) and shiner perch (38 %) while the Atlantic salmon was unaffected (Traxler and Keiser, unpubl.; Meyer and Winton, 1995).

The pathogenicity of the most recent North American VHSV isolates originating from mass mortalities of pilchard and Pacific herring were tested by injection and bath exposure on Atlantic, chinook and sockeye salmon. The injection trial caused only mortality in the Atlantic salmon (40 %) while chinook and sockeye salmon were refractory to the infection. The waterborne trial gave no mortality (Traxler *et al*., 1999).

Attempts to infect rainbow trout fry with 32 different marine VHSV isolates failed to show any pathogenicity (Mortensen *et al.,* 1999c). However, infection trials by IP injection of a selection of Danish marine VHS isolates on rainbow trout resulted in mortalities ranging from 0–65 %. This has to be related to 99 % mortality in a positive control group infected with a freshwater VHS type (Mortensen *et al.,* 1999c).

Kocan *et al.* (1997) fulfilled the postulate of Koch by infecting specific-pathogen-free (SPF) reared herring with North American VHSV isolates by bath challenge. Mortality began 4–6 days after exposure and moribund fish displayed petechial haemorrhages on the lower jaw, mouth and eyes.

A similar experiment was conducted by Snow and Smail (1999) which infected SPF turbot with VHSV originating from an outbreak in cultured turbot (Ross *et al.,* 1994; Munro, 1996). The fish were infected by three different routes: IP injection, co-habitation and immersion. The mortality for the injected turbot was 100 %, for the co-habitation experiment 60 % and for the bath infection 71 %.

Stressors like exposure to toxicants seem to be able to activate subclinical VHSV infection in Pacific herring. Adult Pacific herring with unknown VHSV status were exposed to weathered crude oil for 16–18 days and the prevalence of VHSV and total polynuclear aromatic hydrocarbon load in the fish were significantly correlated (Carls *et al*., 1998).

Differences between North American and European freshwater VHSV

Serological analyses were not able to distinguish the North American VHSV isolates from the typical European reference strains (Winton *et al*., 1989). However, the North American VHSV isolates were found to be less pathogenic to rainbow trout than the European reference strains (Winton *et al*., 1991).

DNA hybridisation techniques made it possible to find a nucleotide sequence of the N gene of the Makah VHSV isolate (Brunson *et al*., 1989) that was absent in the European VHSV isolate (07-71). By using a DNA-probe complementary to this it was possible to distinguish the European freshwater isolates from the North American marine VHSV isolates (Batts *et al*., 1993).

Based on the knowledge that the North American Makah VHSV isolate appeared to have a unique 20 nucleotide sequence in close proximity to the N gene which was not present in the European VHSV isolates a polymerase chain reaction (PCR) technique was developed. By using a specific primer set for the amplification of this specific nucleotide fragment it was possible to separate the North American and European VHSV isolates within half a day (Einer-Jensen *et al.,* 1995)

Genetic comparison of four North American VHSV isolates recovered from chinook and coho salmon and one from Pacific cod and four European isolates including one cod isolate (Jensen *et al.,* 1979) was done by using T1 ribonuclease fingerprinting. The result revealed two distinct groups comprising the North American and the European isolates, respectively (Oshima *et al.,* 1993).

The result of the three latter studies indicate that the North American isolates of VHSV are not of European origin and that the virus may be enzootic within the marine environment.

Stone *et al*. (1997) applied RT-PCR methods to the detection and sequencing of the glucoprotein gene of a number of North American and European VHSV isolates from marine and fresh water. A phylogenetic analysis of a 360 nt region of the glucoprotein gene revealed 3 different genotypes, one type comprising the Scottish turbot VHSV isolate (Ross *et al.,* 1994) and two cod isolates found off the Shetland Islands (Snow, 1995). The German turbot isolate (Schlotfeldt *et al*., 1991) and a number of freshwater isolates from rainbow trout comprised another group and the North American isolates created a third group.

In order to detect nucleotide sequence variation within the nucleoprotein gene of 39 VHSV isolates of European marine origin by applying a ribonuclease protection assay it was possible to define ten different groups. Nucleotide sequencing and phylogenetic analysis demonstrated three distinct genotypes (Snow *et al*., 1999), one type comprising the Scottish turbot VHSV isolate (Ross *et al*., 1994) and isolates from wild marine fishes in the adjacent sea areas and two other types including isolates from other areas of European waters. One of these latter groups comprised VHSV isolated from wild marine fishes mainly from the Baltic Sea, the German turbot isolate (Schlotfeldt *et al*., 1991) as well as isolates causing mortality in rainbow trout from continental Europe, suggesting a relationship between these types. This observation is coincident with the findings of Stone *et al*. (1997). The North American Pacific herring VHSV isolates comprised a fourth distinct group.

Origin of VHSV

Both Meyers and Winton (1995) and recently Dixon (1999) have discussed the origin of the VHSV. They conclude that is likely that the VHSV has its origin in the marine environment and may constitute a potential risk for mariculture. It may have been transferred to the freshwater environment by marine fish species, e.g., herring, sprat, sandeel (*Amodytes marinum*) which formerly have been used for trout feed. After the VHSV had been introduced to the freshwater environment the virus may through mutations have reached the high level of virulence against rainbow trout which is characteristic for the freshwater types of VHSV. There seems to be a lot of circumstantial evidence that may support this hypothesis, e.g., the genotyping of Stone *et al*. (1997) and Snow *et al*. (1999) but with the existing methodologies it will be hard to come up with firm proof.

Conclusions

- Marine VHSV has low pathogenicity to salmonids.
- North American VHSV isolates differ genetically from European isolates and can be differentiated by PCR.
- North American VHSV isolates are not of European origin.
- The host spectrum of marine VHSV is broad; so far 8 in the North American Pacific area and 14 in the North Sea area.
- Marine VHSV may constitute a potential risk for mariculture as well as for marine fish stocks, e.g., herring and sprat in the Baltic area.

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ANNEX 11: COLLATE AND REVIEW AVAILABLE INFORMATION ON THE DISTRIBUTION, ORIGIN, HOST RANGE, AND IMPACT OF INFECTIOUS SALMON ANAEMIA (ISA) ON SALMON CULTURE

Working document prepared by B. Hjeltnes and A. McVicar

1 Distribution of ISA

To date ISA has only been recorded from three countries, namely Norway, Canada and Scotland.

a) Norway

ISA was first diagnosed as a disease in November 1984 in the southwest of Norway in farmed Atlantic salmon parr in freshwater hatcheries using untreated sea water. By spring 1985, approximately 80 % of the parr in the affected hatchery had died (Hastein, 1997). The subsequent spread of the disease to marine farms was associated initially with the transfer of fish from these affected smolt producers and subsequently with movements of fish, harvest and killing processes and the proximity of farms to previously infected farms. During 1986–1987, disease outbreaks occurred in post-smolts and adult salmon in sea water and over a wider area, with an exponential build-up of farms showing the disease during the late 1980s. From 1987, ISA spread rapidly within Norway to the entire country and appeared as zootic in many regions (Hastein, 1997). Up to 80 new outbreaks of ISA per year were occurring by 1990. Subsequently the serious impact of the disease was extensively brought under control through the use of a variety of disease control and management techniques. Since the early 1990s, new outbreaks have occurred each year, but the number per year has been less than five with the exception of 1998 and 1999 when over ten new cases were recorded in both years. Although a single case of ISA was reported in fresh water in Norway in 1998 (Nylund *et al*., 1999) the authors could not conclude that there was any evidence of vertical transmission of the virus nor could they exclude a seawater source. Farms in all parts of western Norway have been affected.

b) Canada

During 1996, a disease condition caused significant kidney pathology and high levels of mortality in Atlantic salmon in New Brunswick in eastern Canada. The condition was at that time termed haemorrhagic kidney syndrome (HKS) and had an unknown aetiology, but in 1997 was shown to be due to ISA. The disease had spread by late 1999 to affect most of the salmon farms in New Brunswick. Control programmes have been implemented and some farms have been successfully restocked without recurrence of the disease. All outbreaks have been confined to sea water.

In 1999, evidence of ISA was found in an Atlantic salmon broodstock population in a marine farm in Nova Scotia. Positive test results for the presence of virus were also obtained from post-smolts which had co-habited with the affected population and subsequently were transferred to two farms on the east and southwest of Nova Scotia. No clinical signs or mortalities were associated with the findings in any case.

c) Scotland

ISA was first diagnosed in one farm in western Scotland in May 1998 and by late 1998 further cases were confirmed in other farms in western Scotland and in Shetland. A total of 10 farms were confirmed as being infected with ISA by the end of 1999, with a further 15 suspected of being infected on the basis of positive laboratory tests. In 1999 one additional farm in Shetland was confirmed as being infected, with a further 9 under official suspicion of carrying infection. As required by EU legislation, all fish on farms where ISA had been confirmed were immediately removed and the affected farm disinfected. Only seawater farms were affected by ISA. Strict disease containment measures were enforced on all farms where there was a suspicion of the infection, and on all other farms in the zones surrounding infected and suspect farms.

All farms where ISA was confirmed were directly associated with the first infected farm through movement of live fish prior to the detection of the disease, common use of contaminated equipment or proximity to untreated processing effluent or other affected farms. The finding of evidence of infection without disease in (i) farms with no direct connection with the primary source of the disease, (ii) in wild salmonid and non-salmonid fish in both fresh and sea water, and (iii) in freshwater farmed rainbow trout has led to a re-evaluation of the evidence available and to review of the eradication policy for ISA in the UK.

2 Origin of ISA

The origin of the ISA virus is unknown in all areas where the disease has occurred. Risk factor analysis in Norway (Vaagsholm *et al*., 1994), Canada (Washburn and Gillis, 1997) and Scotland (Anon., 2000) have all concluded that ISA is a disease which is transmitted from infected salmonids to new hosts through sea water, particularly via blood, mucus, faeces and urine. To date, no connections have been made between the outbreaks in the three affected countries nor between the two outbreaks within Canada. This apparently independent emergence of the disease in these different salmon farming areas may indicate, as has also been suggested by Nylund (1997), that there may be local natural reservoirs of infection.

A survey of possible origins of the primary source of ISA in Scotland involved an examination of the possibilities of an illegal importation of infected fish, accidental introduction of infection with contaminated equipment from an infected area and possible natural local sources. No final conclusions have been reached. The occurrence of infection in fish species other than salmon (eel, sea trout, and saithe) in Scotland close to where outbreaks of ISA occurred in salmon farms could either indicate a local source of infection, or a transfer of infection from the farms into the local stocks. These data do not permit a distinction to be made between these alternatives. Similar uncertainties surround the findings of ISAV in escaped and wild salmon returning to a river in New Brunswick, Canada where it has not been possible to determine if infection occurred naturally in the wild fish or was obtained through co-habitation with infected farmed fish.

Genetic evidence indicates similarities between Norwegian, Nova Scotian and Scottish strains of infection, but also significant differences. All outbreaks of confirmed ISA in Scotland were identical (in the nucleic acid sequences so far examined), providing supportive evidence for dissemination of the disease from a point source by the actions of fish farmers. However, one sample of ISAV from a Scottish farm not showing clinical disease revealed variations in one segment of the genome from other Scottish strains. However, the extent of genetic variations occurring in the different infected areas and rate of change is currently unknown, and it is therefore not possible to evaluate the significance of such differences.

Laboratory results (RT-PCR) in Scotland have indicated that the virus may also be present in other areas distant from salmon farms, namely in wild Atlantic salmon parr and in farmed rainbow trout. Similar results have been obtained from two wild fish from two rivers in Nova Scotia. In all of these cases virological tests were negative and the significance of these results is currently unknown.

3 Host range of ISA

Nylund (1997) reviewed the host list of ISAV and indicated that in addition to Atlantic salmon, the virus has been shown to replicate in *Salmo trutta* and *Onchorhynchus mykiss* and possibly in other species of *Onchorhynchus* and *Salvalinus*. No replication of the virus was found to occur in goldsinny, *Ctenolabrus rupestris*, turbot, *Scophthalmus maximus,* or sea bass, *Dicentrarchus labrax.* Hastings *et al.* (1999) also recorded herring, *Clupea harengus,* as a susceptible species but indicated that the experiments involving this species were preliminary and that virus replication had not been demonstrated. These reports were from experimental challenges and there are no records in the scientific literature of surveys for the natural occurrences of ISAV in wild fish populations.

As part of the epidemiological survey required by the EU legislation to determine possible sources and reservoirs of infection an extensive sampling programme was initiated in Scottish waters in 1998 and is still in progress. A wide range of marine fish species were tested (1444 fish) and found to be negative for ISAV, except for a single saithe (*Pollachius virens*) caught within a cage of a farm suffering from a clinical outbreak of ISA. ISAV was also isolated from non-diseased sea trout (*Salmo trutta*) in coastal waters in west Scotland and Shetland and from eel, *Anguilla anguilla,* showing signs of disease in a freshwater loch in west Scotland.

In Canada the virus has not been detected in 648 marine non-salmonid fish nor in a limited number of trout species in the wild.

4 Impact of ISA on salmon culture

The monetary value of the impact of ISA in affected countries is difficult to assess, as there are many consequential factors, which have to be taken into account beyond the direct loss of stock due to mortality. Loss of growth potential through early harvest of infected and associated stocks, removal of part or the whole of farms from production for extended periods, resources required for disinfection, loss of revenue in primary and secondary processing, distribution, etc., loss of markets to smolt producers due to the inability to restock fallowed farms are only some of the many direct and indirect sources of financial loss which may have to be considered. Consequently, it has not been possible to estimate an actual monetary value for losses.

The mortality level in ISA-infected farms varies considerably from insignificant to moderate to severe. On an individual farm in Scotland mortalities of up to 0.25–0.5 % per week occurred for the whole farm, with a peak of 30 % mortality in a single cage being found. In each affected country, removal of stocks suffering significant disease levels is obligatory although there are differences in the extent and speed of removal required. In the EU immediate removal of all stocks from an infected farm is required. In Canada a cage-by-cage eradication programme is followed where ISA mortalities exceed 0.05 % per day, and in Norway a similar eradication programme is implemented where early harvest is required starting with cages where mortality of 0.5 fish per thousand per day occur. Containment controls and fallowing requirements are imposed on all surrounding farms (based on zoning criteria as recommended by OIE). A compensation programme has been developed in Canada where cages have been removed which has cushioned the impact to some extent. Similar schemes are not available in Norway and Scotland.

5 References

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ANNEX 12: REVIEW NEW INFORMATION ON THE STRUCTURE AND DIVERSITY OF NODAVIRUS(ES), THE SPREAD, DIAGNOSIS, AND EPIZOOTIOLOGY OF THE DISEASE, AND HOST IMMUNITY, TO PROVIDE EFFECTIVE ADVICE ON POSSIBLE CONTROL MEASURES

Minutes from the EAFP NODAVIRUS WORKSHOP

Working document prepared by F. Baudin-Laurencin and R. Richards

Abstract

The aims of this workshop were to compare the most recent data obtained in different parts of the world on nodavirus and nodavirosis in fish species and, consequently, to derive a better general understanding of the virus and the associated disease. Eighteen papers were presented during the three sessions.

Characterisation of the virus and diagnostic tools

The session was opened by R. Thiéry, who summarised current knowledge on nodavirus biology and molecular biology, highlighting differences between nodaviruses infecting insects and fish. Recent data were also reported on the phylogeny of 2 isolates from Mediterranean and Atlantic sea bass. W. Starkey then presented a study on nucleotide sequence and phylogenetic analysis of nodaviruses isolated from Europe, Asia, and Japan. The majority of nodaviruses isolated from Sea Bass, and also isolated from Barramundi, Rock Porgy, Shi Drum, Brownspotted Grouper, Redspotted Grouper, and Japanese flounder, were relatively conserved (nucleotide sequence homologies greater than 91 %), and clustered together in dendrograms constructed using the neighbour joining method. One sea bass nodavirus, isolated from the French Atlantic coast was more divergent, and clustered as an independent lineage. Nodaviruses isolated from Barfin flounder and halibut, Striped Jack, and Tiger Puffer, were most divergent and clustered into three further lineages. B. Munday presented a study of the genomic variation of nodaviruses infecting Barramundi, which suggested that considerable genomic variation occurs in nodaviruses infecting Barramundi from different geographic regions. This is probably a consequence of the great distances separating some populations of Barramundi, which have different spawning seasons related to latitude, and thus are biologically distinct. These findings have important consequences for the design of molecular diagnostic reagents. The next three presentations were concerned with nodavirus isolated from Halibut. B. Hjeltness described the characterisation of a Norwegian nodavirus isolate from this species. The halibut nodavirus RNA 2 nucleotide sequence was 80 % homologous to the RNA 2 of Striped Jack Nervous Necrosis Virus (SJNNV). However, the Halibut nodavirus RNA 2 ORF lacked six nucleotides compared to SJNNV, comprising one single deletion and a five nucleotide deletion separated by four nucleotides, and the 3′ non-coding region contained a 21 nucleotide insert and a three nucleotide deletion. Phylogenetic analysis based on a 421-nucleotide fragment of RNA 2 of the Atlantic Halibut and other fish nodaviruses suggested a close relationship between nodaviruses infecting Halibut, Barfin flounder, and Pacific cod. The nucleotide sequence of the Halibut nodavirus was used to design primers for use in diagnostic PCR. A. Nylund presented a study on the phylogenetic relationships of a nodavirus isolate from Atlantic halibut. This represented the first report on the presence of viral nervous necrosis in adult and mature Halibut. Phylogenetic comparison of the halibut nervous necrosis virus nucleotide sequence with the corresponding sequences from other species of marine finfish indicated that the nodavirus isolates studied could be separated into six clusters, with sequence identities greater than 95 % within clusters. The Halibut nodavirus isolate was closely related to isolates from two other cold-water species, Barfin flounder and Pacific cod. These results suggested that transportation of nodavirus isolates between Europe and the Pacific might have occurred. B. Dannevig then described the isolation of nodavirus from farmed Atlantic halibut in Norway suffering from viral encephalopathy and retinopathy. Tissue homogenates from moribund halibut larvae were inoculated into SSN-1 cells grown in Leibovitz L-15 medium containing 5 % serum and antibiotics. Cytopathic effects were evident in primary cultures by 5–6 days post infection (p.i.) and in new cultures following serial passage. Infected cells were reactive against antiserum raised against sea bass and striped jack nodaviruses in indirect immunofluorescence tests. Viral nucleic acid was detected by RT-PCR using primers corresponding to striped jack nodavirus. Cell culture passaged virus retained virulence for halibut in bath-challenge experiments; mortality was observed at 6 days p.i. and nodavirus antigen was detected by immunohistochemistry in CNS and retina. This was the first report on cell culture isolation of nodavirus from Norwegian Atlantic halibut. S. Péducasse presented findings on the physical and chemical inactivation of sea bass nodavirus on the SSN-1 cell line. No reduction in infectivity occurred following exposure of the virus to −80 ˚C, −18 ˚C, and 4 ˚C. Complete inactivation was observed within 5 days at 37 ˚C. Reduction in virus titre within 24 hours, and complete inactivation at 48 hours occurred at pH 11. Infectivity was stable at pH 5 to pH 7 for at least three months, whereas inactivation was obtained at pH 2, pH 8, and pH 9 at this time. No significant loss of infectivity was recorded after three months in fresh water, whereas complete inactivation was obtained after two months in sea water and half-strength water. The session was concluded by T. Nakai, who presented a study entitled *in vitro* and *in vivo* infectivity of fish nodavirus. Seventeen nodavirus isolates from thirteen marine fish species were assayed for infectivity on the SSN-1 cell line. The isolates were divided into four groups on the basis of SSN-1 infectivity, and there was close correlation between the infectivity groups and the coat protein gene genotype of isolates. In pathogenicity tests with larvae of three marine fish species, only a SJNNV genotype was highly virulent to larval striped jack and Japanese flounder. Conversely, only a BFNNV genotype from Atlantic halibut was virulent to larval Atlantic halibut. These data suggest that different host-specificity or infection mechanisms are present among piscine nodavirus isolates.

Epidemiology and transmission of the virus

The first report of a viral nervous pathology date back to the 1980s in the French Indies on *Dicentrarchus labrax.* Since then, Nodavirus infections have been documented in most marine finfish-producing countries worldwide. Over 20 different marine species have been described as sensitive to these viruses. Cross-infectivity using Barramundi Nodavirus strains was achieved in a variety of species including freshwater species such as the Australian catfish, *Tandanus tandanus* and the Silver perch*, Bidyanus bidyanus* (Munday). This may be related to the fact that these viruses were still detectable in fresh water after six months (Thiéry). Similar tests run with *Sparidae/Pagrus auratus* and *Salmonidae*/*Oncorhynchus mykiss* and *Salmo trutta,* were not successful (Munday).

Most of the authors have reported evidence of a close but not identical antigenic structure of the Nodaviruses, identifying different viral strains (Le Breton). Furthermore, PCR analysis has confirmed the presence of at least two strains of nodavirus affecting Barramundi, *Lates calcarifer,* in Australia (Glazebrook, Munday).

Apart from the species sensitivity, the main epidemiological factors influencing the course of the disease have been identified as the water temperature, the age and the size of the fish and immuno-depressive factors such as stress and interfering pathologies (Le Breton, Sommer). Experimental infections of spotted wolffish, *Anarhicas minor,* by i.m. or i.p. (Sonuner) and turbot, *Scophthalmus maximum,* by i.m. (Husgård) were successful and confirmed the importance of these epidemiological factors. Mortality on 3 g challenged turbot fry occurs after 2–3 days post injection and rises to 55 %–66 % within 18 days while on 10 g and 15 g fish, mortality was observed after 4 days with a maximum of 44 %–50 % after 18 days (HusgAr6). Cumulative mortality of 10 g experimentally infected spotted wolffish reached 20 % at 7 ˚C and 40 % at 12 ˚C (Sommer). This confirms observations made on redspotted grouper (Tanaka, 1998). Pathogenicity of the virus increases and incubation period decreases with an elevation of the temperature.

Natural infection occurs in a wide range of temperature, from 4 °C to 30 °C depending on the species. The temperature range reported for natural infection of *Dicentrarchus labrax* is 17 °C–28 °C. However, using IHC analysis, the presence of the virus was detected in 3 g. European sea bass exhibiting no clinical symptoms and kept at 15 °C–16 °C (Galeotti). In this case and in a case of natural outbreak on 15–25 g fish of the same species reared at 26 °C–27 °C, histological analysis of brain and retina proved negative while IHC analysis was positive. This underlined the eventual adaptation of Nodaviruses to lower temperatures in European sea bass (Galeotti), which seams to be confirmed by recent field epidemiological observations (Le Breton). Furthermore a Mediterranean strain of Nodavirus was still detectable at 14 ˚C for up to six months in sea water (Thiéry). A possible carrier stage and/or convalescent stage as reported in Japan (Fukuda, 1996) can also be considered. On the other hand, an increasing importance of stress factors in the induction of natural outbreaks has been observed (Bleie, Le Breton).

The vertical and the horizontal transmission of the disease have been described in Japan on Striped jack, *Pseudocaranx dentex,* and in Europe on *Dicentrarchus labrax*. On *Hippoglossus hippoglossus,* different vectors of the virus, i.e., offspring, foodstuff, fish carriers, zooplankton are suspected and under investigation (Bleie). However, the portal of entry in natural infection is not known. Experimentally, cohabitation challenge tests with juveniles of this species and of the spotted wolffish were not successful (Sonuner).

Different portals of entry have been tested experimentally on 35 g *Dicentrarchus labrax* using i.m. challenge, caudal immersion, branchial impregnation, oral and anal intubation (Péducasse). In all cases, transmission of the virus was achieved. Regardless of the mode of entry, the virus seems to reach the central nervous system through the cranial and/or efferent nerve (Péducasse). This suggests that natural infection can take place through the intestinal and branchial epithelium as well as through the integument and/or the lateral line.

Immunology and vaccination

Information concerning these two areas is very limited.

Peptide or recombinant vaccines for the fish industry are not well developed and preliminary studies with IHNV and HSV in salmonid fish have failed. In the case of nodavirus, two experimental vaccines (peptide and recombinant) have been tested in France and in Japan, respectively. No result was reported but the development of such vaccines against the nodavirus could also be used for studying the fish immune system (Coeurdacier).

Preliminary studies of the fish immune response to the nodavirus have revealed specific antibodies in the serum of the sea bass (Breuil) and the stripped jack (Nakai). Preliminary epidemiological analysis in the sea bass revealed no significant differences between wild (17 % seropositive fish) and farmed reared (18 % seropositive) adult fish in Palavas (France). Furthermore a higher proportion of seropositive fish was found in the females (18 %) compared to the males (3 %). Serological study of 30 females for two years revealed a lower serum antibody level during winter. This observation suggests that the screening of the broodstock by ELISA detection of the nodavirus antibodies should be made during summer prior to the maturation period of the females. However data on neutralising antibodies to nodavirus in these species are very limited. These aspects should be further developed by the use of *in vitro* titration of the neutralising antibodies on fish cell lines.

Conclusions

Most of the data presented were new and interesting, and would have necessitated comprehensive discussion. Unhappily, the abundance of presentations finally left too little time. Consequently, it has been decided to extend the workshop to a forum on the Internet, allowing permanent exchanges and discussions of data and hypotheses.

Acknowledgements

Report compiled by William Starkey, Alain Le Breton, and Gilles Breuil (rapporteurs for the Sessions 1, 2, and 3, respectively), and Felix Baudin-Laurencin (coordinator).

Speakers and presentations

Hogne Bleie:	Factors contributing to overt Nodavirus disease of halibut fry.
Gilles Breuil:	Nodavirosis: Immunology and vaccination against nodavirus: the state of the knowledge.
Gilles Breuil:	Antibody level against nodavirus in the serum of sea bass spawners.
Jean-Luc Coeurdacier:	Preliminary studies of immunogenicity and protection against nodavirus.
Birgit Dannevig:	Isolation of nodavirus from farmed Atlantic halibut in Norway, suffering from viral encephalopathy and retinopathy.
Marco Galeotti:	IHC appearance of nodaviral infection in juvenile and larval stocks of sea bass.
Brit Hieitness:	Characterisation of a Norwegian nodavirus isolated from Atlantic halibut.
Susanna Husgård:	Experimental challenge of juvenile turbot *Scophthalmus maximum* with a nodavirus.
Alain Le Breton:	The epidemiology and the transmission of nodavirus: the state of the knowledge.
Barry Munday:	A review of information on genomic variation of nodaviruses infecting barramundi, *Lates calcarifer*.
Barry Munday:	A review of information on infectivity of barramundi nodaviruses for other fish species.
Toshihiro Nakai:	*In vitro and in vivo* infectivity of fish nodavirus.
Are Nylund:	The phylogenetic relationship of nervous necrosis virus from halibut *(Hippoglossus hippoglossus).*
Sonia Péducasse:	Chemical and physical inactivation of sea bass nodavirus on SSN I cell-line.
Sonia Péducasse:	Study of portals of entry and progression for nodavirus in juvenile sea bass.
Arm Inger Sommer:	Susceptibility of nodavirus infection in marine fish and factors, which affect the viral nervous necrosis (VNN) mortality.
Bill Starkey:	Nucleotide sequence and phylogenetic analysis of the coat protein gene of piscine nodavirus.
Richard Thiéry:	Characterisation of nodavirus and diagnostic tools: the state of the knowledge.

ANNEX 13: INITIATE EXPERIMENTAL WORK TO DETERMINE WHETHER THE LACK OF *BONAMIA OSTREAE* INFECTIONS DETECTED IN FIELD OBSERVATIONS OF *OSTREA EDULIS* FROM COLD WATER CLIMATES REFLECTS PARASITE ACQUISITION WITH SUBSEQUENT LOSS OVER PROLONGED LOW WATER TEMPERATURES OR SUPPRESSION OF INFECTIVITY OF THE PARASITE

Working document prepared by S.E. McGladdery and S. Bower (DFO, Canada) with input from P. van Banning (RIVO, The Netherlands)

The experimental work to examine field observations was not undertaken this year, as planned. The results from uninfected Canadian *Ostrea edulis,* sent to IFREMER, La Tremblade, in 1998, indicated that these oysters are susceptible to challenge infections. Thorough pre-challenge screening indicates that the infections were acquired from the challenge, rather than emergence of sub-clinical (suppressed) infections. More detailed results may be available directly from Dr Tristan Renault, who is conducting the experiments in France.

Europe

Most field information has been compiled by Paul van Banning. In Dutch waters, temperatures < 5 °C appear to inhibit *Bonamia ostreae* survival and/or proliferation. Long cold winters have consistently been associated with low prevalences in spring and vice versa (Lake Grevelingen, 1988–1999). In winters 1993/1994, 1995/1996, and 1996/1997 temperatures < 5 °C persisted for over 10 weeks (15, 17, and 11, respectively). Spring prevalences in these years showed a decrease from over 20 % to approximately 17.5 %, 6 %, and 3.5 %, respectively. In 1994/1995 and 1997/1998, temperatures < 5 °C persisted for only 4 weeks. Spring prevalences in these years were 12.5 % with the 1997/1998 level showing a three-fold increase. 1980s temperature profiles submitted by Dr S. Mellergaard from Vilsund Bridge, Denmark, where *B. ostreae* was detected in introduced oysters, but has subsequently disappeared, show duration of < 5 °C water temperatures exceeding 20 weeks.

North America

There is still no evidence of *B. ostreae* infection in Canadian *O. edulis*. April samples of Atlantic coast broodstock (including a sample conditioned for spawning at 20 °C and re-laid in 1 °C which appeared to be “stressed”) and a sample of brooding mature oysters collected in July (15–18 °C) all proved negative using histology and heart smears.

Low prevalences exist in Gulf of Maine stocks of *O. edulis*, where cold water temperatures in the mid-1990s persisted for 16–20 weeks. This is similar to Nova Scotia (just north of Maine) where no *B. ostreae* has yet been detected. European oyster culture in the Gulf of St. Lawrence has been sporadic. Despite strong summer growth, cold winter temperatures persist longer and low salinities preclude open-water survival during the spring melt of winter ice.

On the west coast, *B. ostreae* continues to show no evidence of spread to populations north of the San Juan Islands, Washington, immediately adjacent to the B.C./USA boarder. Dr Jean-Pierre Joly, visiting scientist from IFREMER at PBS, surveyed *O. edulis* from one grow-out site in B.C. for *B. ostreae* by histology and tissue imprints after collection in October. All were negative. An additional sample of oysters from the same stock was held in the lab at about 19 °C for an additional 65 days, to increase the chances of detection. In addition to microscopic examination of histological sections and tissue imprints, these oysters were assayed by PCR using a probe against *B. ostreae* (that will soon be published by Franck Berthe *et al.,* IFREMER, La Tremblade) and found free of infection.

Observations from both coasts reinforce the hypothesis that *Bonamia ostreae* distribution is attributable to water temperature (just as *M. Mackini* has not made it south into Washington State).

Conclusions

A submission for funding, which includes investigation of the absence of *Bonamia ostreae* in Atlantic and Pacific populations of *Ostrea edulis*, has been approved and it is hoped that experiments can be conducted in collaboration with US colleagues working in *Bonamia ostreae* endemic areas (e.g., Damariscotta, Maine where preliminary trials were held). It may also be possible to conduct challenges using quarantine facilities at the Atlantic Veterinary College as part of a Post-Doctoral research project.

ANNEX 14: REVIEW PROGRESS MADE IN BIOLOGICAL EFFECTS QUALITY ASSURANCE IN THE BEQUALM MONITORING PROGRAMME PROJECT ENTITLED "FISH LIVER HISTOPATHOLOGY, LIVER NODULES, AND EXTERNAL FISH DISEASE MEASUREMENT"

Working document prepared by S. Feist and T. Lang

The EU-funded BEQUALM programme has now been in existence for over a year and for most components significant progress has been made in the establishment of networks of participants for each work package (WP) and there have been workshops organised for most WPs during the last year. The external fish disease and liver histopathology component (WP6) was no exception and efforts in this WP culminated in a workshop which was held at CEFAS Weymouth Laboratory in October 1999. The following report provides a summary of the key findings and recommendations of the meeting and includes sections of report of specific relevance to the activities of the ICES WGPDMO.

For BEQUALM WP6, the first year milestones consisted of:

a) the preparation of laboratory reference materials (LRMs), including processed tissues, micrographs of relevant lesions and associated documentation;
b) undertaking a workshop for the establishment of protocols, practical exercises on diagnostic criteria including assessment of prepared slides from different flatfish species and setting in place agreed limits of acceptable variation in diagnostic reporting;
c) establishing and implementing an intercalibration programme for liver pathology diagnosis using material supplied by the lead laboratory.

As planned, the Workshop was held at the CEFAS Weymouth Laboratory on 21–23 October 1999 with S.W. Feist and T. Lang as co-conveners. A total of 12 scientists participated and apologies were received from a further three (see below for details). The meeting agenda is reproduced below.

Agenda

21 October

- Opening the Workshop
- Adoption of the agenda and schedule of the workshop
- Appointment of rapporteurs
- Brief presentation of national activities with regard to fish disease monitoring, including studies on liver histopathology, with special emphasis on sampling strategies, protocols used for histology, new techniques
- Discussion on the preparation of protocols for the identification of externally visible diseases and macroscopic lesions, dissection and sampling of tissues, fixation, histological processing, sectioning, staining and criteria for disease diagnosis
- Distribution of reference materials and initial training in diagnostic criteria

22 October

- Training and intercalibration of diagnostic criteria and performance limits
- Assessment of material collected from national monitoring programmes with regard to quality and types of lesions

23 October

- Practical exercises as required to complete the assessment
- Preparation of protocols for the identification of externally visible diseases and macroscopic lesions, dissection and sampling of tissues, fixation, histological processing, sectioning, staining and criteria for disease diagnosis (cont.)
- Discussion of the amendments to the biological effects component of the ICES Environmental Data Centre required for incorporation of data on fish liver histopathology
- Planning of ringtests to be undertaken in the year 2000
- Discussion of publications
- Finalisation of draft workshop report

Results of the Workshop held at CEFAS Weymouth Laboratory, 21–23 October 1999

Several key points and recommendations arose from the workshop. These are summarised below.

- It was noted with concern that some of the long-term disease monitoring programmes carried out in the North Sea and Baltic Sea have been reduced considerably or even terminated in recent years mainly due to reduction in project budgets.
- Where there was a reduction of programmes, it was reported that it was not always possible to adhere to internationally standardised sampling strategies developed mainly by ICES. These may not be related as the same sampling effort is resulting in a decline in catch numbers.
- Existing fish disease monitoring programmes are increasingly integrated with other biomarker studies and analytical measurements.
- In addition, a number of successful research projects involving laboratory studies into the use of biochemical, molecular and cellular biomarkers of contaminant effects have been carried out.
- The BEQUALM workshop developed detailed protocols for fixation, histological processing and staining of livers for general diagnosis to be used in monitoring programmes.
- Categories of histological liver lesions applicable in monitoring studies were established and a set of diagnostic criteria to be used for histological assessment was prepared during the BEQUALM workshop.
- It was agreed that there was a need for continued development of techniques of potential value for liver pathology assessment.
- It was recommended that for quality assurance purposes a CD-ROM providing practical guidelines for sampling, processing, disease diagnosis (including external diseases and liver pathology) and accompanying measures should be developed as a training guide for field studies.
- This CD-ROM would also incorporate the proposed “Atlas of flatfish liver histopathology”.
- It was recommended that a BEQUALM ring test for standardisation of interpretation of liver pathology be organised by CEFAS Weymouth for the year 2000. Participating laboratories will receive a set of histological slides with a wide range of histopathological liver changes representing the categories detailed in the present report.
- It was recommended that outside experts should be engaged as impartial judges in the event of disagreements of interpretation.

In addition, the main aims of the meeting were achieved.

The full report of the meeting is nearing completion and will be available shortly. Other points considered during the meeting included the requirements for the BEQUALM database, the nature and scope of publications and modifications needed to the ICES Environmental Data Centre/Environmental Data Reporting Formats for the incorporation of liver histopathology data. Relevant sections are provided below.

Modification of the ICES Environmental Data Centre/Environmental Data Reporting Formats for the incorporation of fish liver histopathology data

On request by ICES, the participants of the BEQUALM Weymouth Workshop discussed changes needed in the ICES Data Reporting Formats in order to incorporate data on the occurrence of histopathological liver lesions of flatfish as part of the fish disease data bank. This request was based on the fact that ICES itself was requested as part of the work programme funded by OSPAR to change its Environmental Data Reporting Formats in order to incorporate data generated within the biological effects component of the OSPAR Joint Assessment and Monitoring Programme (JAMP).

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) had already considered changes needed at its 1998 meeting. WGPDMO recommended at that time to add a number of new disease categories to the already existing ICES Fish Disease Data Reporting Formats, which were selected on the basis of their importance to indicate contaminant exposure:

- early non-neoplastic toxicopathic lesions;
- foci of cellular alteration;
- benign neoplasms;
- malignant neoplasms;
- non-specific lesions.

Each category comprises a number of lesions defined at the 1996 ICES Special Meeting on the use of Flatfish Liver Pathology for Monitoring Biological Effects of Contaminants (ICES, 1997). The main reason for introducing only five categories combining different lesion types instead of using a category for each single type of lesion is that categories used for monitoring purposes should be robust and should take into account the difficulties in differentiation of some of the tumour types and putative pre-neoplastic lesions recorded.

However, the participants of the BEQUALM Weymouth Workshop felt that it would be advantageous to use more than five categories based on specific lesions since that would add considerably more information to the ICES data enabling a more detailed statistical analysis, e.g., with the view to establish time-scales and predictions for the development of neoplastic lesions on a regional basis as part of a risk assessment. A grouping of data according to the above five categories could still be done if considered appropriate. The following categories were ultimately recommended for reporting to ICES:

- hepatocellular and nuclear polymorphism;
- hydropic vacuolisation of biliary epithelial cells and/or hepatocytes;
- phospholipidosis;
- fibrillar inclusions;
- spongiosis hepatis (known from North American flatfish species);
- clear cell foci;
- vacuolated foci;
- eosinophilic foci;
- basophilic foci;
- (mixed foci will be allocated to one of the above categories according to the predominating cell type);
- hepatocellular adenoma;
- cholangioma;
- pancreatic acinar cell adenoma;
- hemangioma;
- other benign tumours;
- hepatocellular carcinoma;
- cholangiocarcinoma;
- mixed hepatobiliary carcinoma;
- pancreatic acinar carcinoma;
- hemangiosarcoma;
- hemangiopericytic sarcoma;
- other malignant tumours.

As to the non-specific liver lesions, a unanimous consensus on the types of lesions to be included in the data bank could not be reached. It was therefore agreed to postpone a decision and to utilise the experience regarding diagnostic criteria and accuracy made in the forthcoming BEQUALM ringtests.

It was recommended that the ICES Fish Disease Data Reporting Format should be modified according to the above categories of liver lesions and that the lesions should be added to the already existing list of externally visible diseases. The participants of the BEQUALM Weymouth Workshop, however, endorsed the view expressed before by WGPDMO that the revision of the ICES Reporting Formats should ideally be postponed until the BEQUALM project is finalised since data will only be submitted to ICES after the quality assurance procedures for the diagnosis of histopathological liver lesions have been established successfully. It cannot be excluded that the experience made in the course of the project might possibly lead to a need for a further revision of the list of histopathological liver lesions to be reported to ICES.

Publications

The participants discussed the possibility to include the workshop report on the BEQUALM website and it was decided to discuss this at the next BEQUALM Steering Group meeting. In addition, the group discussed in detail sections of a draft publication for the *ICES Techniques in Marine Environmental Sciences* (TIMES) series. In particular, sections on histopathological diagnosis of liver lesions and technical aspects of histological protocols were addressed. Amendments will be included and it is anticipated that colour plates of the various histological lesions will be incorporated since the ability to visualise tinctoral differences between certain lesion types is essential for accurate diagnosis. It was suggested that a CD-ROM of the document might also be produced.

List of Workshop Participants

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Apologies for absence received from: Eric Lindesjöö (Sweden), Mark Myers (USA), and Sergei Bogovski (Estonia).

ANNEX 15: DEVELOP MAPS DEPICTING THE DISTRIBUTION OF FISH AND SHELLFISH DISEASES OF CONCERN FOR MARICULTURE AND TEMPORAL TRENDS OF WILD FISH DISEASES OF CONCERN FOR MARINE ENVIRONMENTAL MONITORING PROGRAMMES FOR INCLUSION IN THE ICES ENVIRONMENTAL STATUS REPORTS

Working document prepared by T. Lang, W. Wosniok, and S. Mellergaard

1 Introduction

In 1996, WGPDMO and a number of other ICES WGs were requested by ICES to provide suggestions for possible contributions to a proposed ICES Environmental Status Report. At that time, WGPDMO recommended to include:

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

Since that time some WGs took initiative and developed ICES Environmental Status Reports on oceanographic conditions, zooplankton monitoring results, and harmful algal bloom events in ICES Member Countries which were published for the first time in the 1998 ACME Report (ICES, 1999) and partly on the ICES website. It is intended to publish the complete ICES Report on the ICES website, when it is fully implemented.

In 1999, WGPDMO revisited this issue and agreed to develop proposals for the incorporation of disease data into the ICES Environmental Status Report. There was consensus that this should cover both wild fish disease data and data from farmed finfish and shellfish.

The ICES fish disease database as part of the ICES Environmental Data Centre contains a large data set on wild fish diseases covering large areas of the North Sea and Baltic Sea. These data have been analysed during the past two years with regard to spatial and temporal trends in disease prevalence and to relationships between the variation in prevalence and potentially explanatory parameters, utilising contaminant, oceanographic and fisheries-related data extracted from the ICES Data Banks (Wosniok *et al*., 1999; Lang and Wosniok, 2000 (in press)). Since the results of these analyses were considered promising and since diseases of wild fish are of interest as indicators of general biological effects in many national and international marine environmental monitoring programmes, WGPDMO reiterated possibilities to include parts of the results of the analysis as contribution to the web-based ICES Environmental Status Report. It was further considered to add information on new disease trends in farmed finfish and shellfish relevant for other international organisations (e.g., EU, OIE) and interest groups. (the latter will be dealt with separately in the 2000 WGPDMO report). WGPDMO agreed to develop plans for both aspects intersessionally and review and further elaborate proposals at the 2000 meeting.

2 Proposal for the presentation of wild fish diseases

As a basic requirement, it is felt that any information on fish and shellfish diseases placed on the ICES website should be as illustrative as possible, using coloured maps, graphs and photos accompanied by short and concise written background information. The information presented should be compiled in a way that it is sufficiently informative not only to scientists but also to other interested persons, e.g., managers, laypersons, politicians, etc.

As a first step, only information on dab diseases from the North Sea and adjacent areas, including the western Baltic Sea should be presented. Flounder and possibly other species (e.g., cod) can be added at a later stage.

An introductory page should provide information and links on fish species covered, source of data, national monitoring programmes, sampling strategies, QA, ICES WGPDMO, ICES Environmental Data Centre, methodologies used to generate data presented, ways to present data, etc.

All maps should be based on ICES statistical rectangles, possibly with OSPAR JAMP areas noted. The maps should be made in a way that they can easily be updated each year by ICES.

Map 1

- Should give number of time points with measurements for each rectangle to give an overview on data availability (see Figure A15.1. ICES Data Inventory.).

Maps 2–4

- Show temporal trends over the past 5 years, for lymphocystis, epidermal hyperplasia/papilloma, and acute/healing skin ulcerations (see Figures A15.2–A15.4. Temporal trends.).
- Calculation of trends is based on the method used for earlier statistical analysis (Wosniok *et al*., 1999).
- Presentation of trends by upward or downward arrows. Rectangles or other symbols may be used if no trends occurred.
- In each figure, the field with the name of the disease provides a link to a page with brief information on the disease (external characteristics (photo), aetiology, pathogenicity, diagnostic criteria, relevance for monitoring purposes etc.).

Figures

- In maps 2–4, each rectangle could contain a link to a full size plot giving the temporal development of the estimated disease prevalence together with a 95 % confidence interval per rectangle, either for a certain group of fish (e.g., females, 20–24 cm) (see Figures A15.1–A15.4) or for a standard fish population using a direct standardisation technique commonly used in human epidemiology.
- Absolute instead of relative prevalences could be used in order to enable a comparison of disease levels between rectangles. In this case, a disclaimer should be included, explaining that the values provided constitute estimates not observations and are not necessarily representative of the present conditions.
- Another possibility would be to calculate a mean prevalence for the standard population from all data available for a long fixed time interval (e.g., 1985–1995) (for method see Lang *et al.,* 1999) and to present only the deviations from the mean values by rectangle, subtracting the overall mean value from each individual value. That would prevent misuse and misinterpretation of data, but might, however, be confusing if not properly explained.

Proposals for presentations of diseases in farmed fish and shellfish

The following pages (see Figures A15.5–A15.8) contain a number of maps showing the distribution of the shellfish diseases *Bonamia oestrea*, *Marteilia refringens* and *Perkinsus marinum* which are of concern for mariculture and the fish disease, marine Viral Haemorrhagic Septicaemia which may be of concern for exploited fish stocks as well as mariculture.

4 Important points to be clarified

- Is this a useful way of illustrating the geographical distribution of diseases?
- Is there any risk of the public (e.g., journalists) making misinterpretations of the data presented?
- Which diseases should be illustrated in this way?
- What is the most convenient format for the specific diseases?
- Who will do what kind of work?
- What is ICES' contribution?
- Who will be responsible for maintenance and update of the report?
- What about copyrights for the material presented?
- Will there be a need to produce written reports for inclusion in the ACME Report?

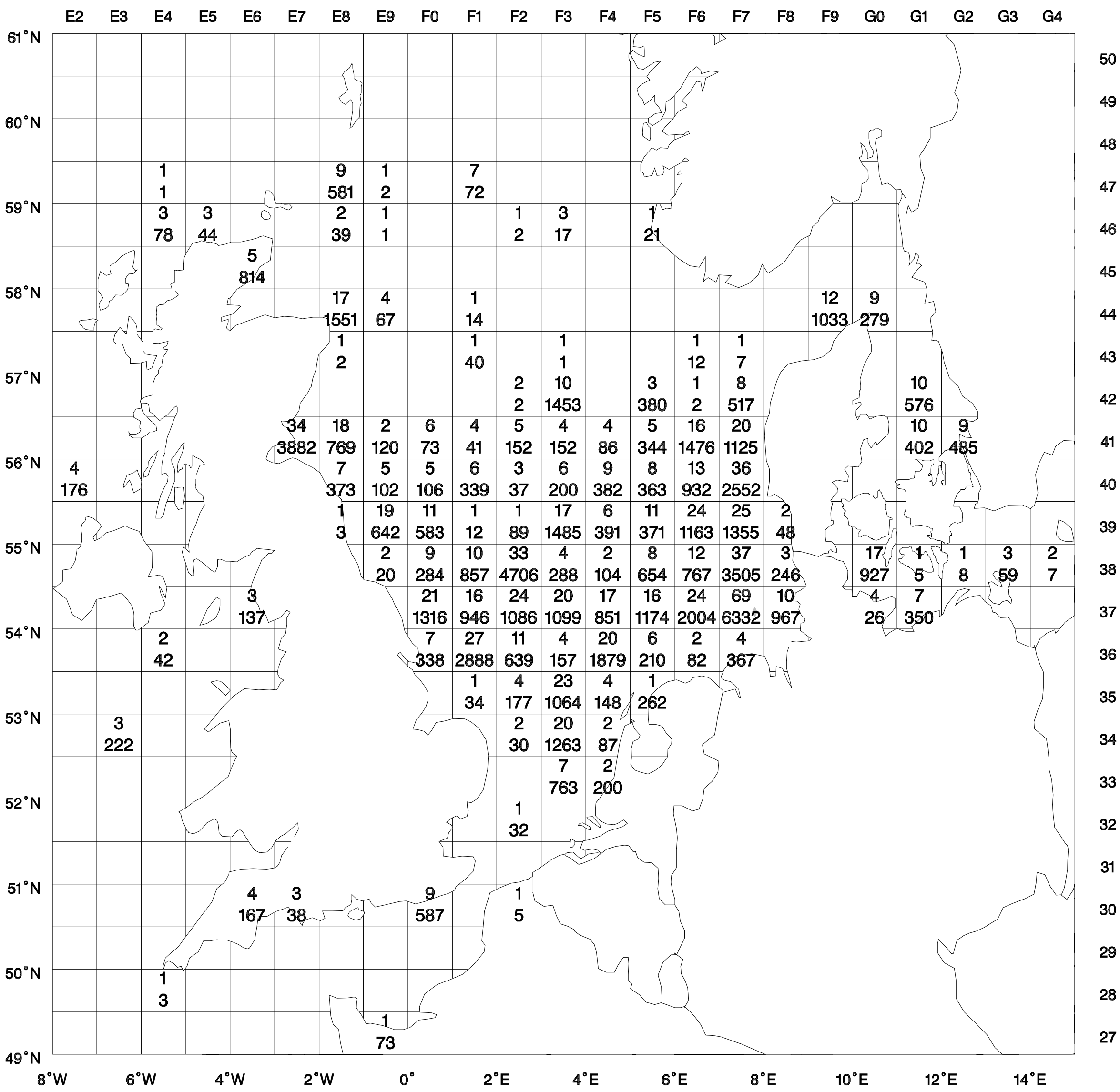
5 Literature cited

Lang, T., Mellergaard, S., Wosniok, W., Kadakas, V., and Neumann, K. 1999. Spatial distribution of grossly visible diseases and parasites in flounder (*Platichthys flesus*) from the Baltic Sea: a synoptic study. ICES Journal of Marine Science, 56: 138–147.

Lang, T., and Wosniok, W. 2000. ICES data for an holistic analysis of fish disease prevalence. *In* Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Cooperative Research Report, 239: 193–209.

Wosniok, W., Lang, T., Vethaak, S., des Clers, S., and Mellergaard, S. 1999. Statistical analysis of fish disease prevalence data from the ICES Environmental Data Centre. *In* Report of the ICES Advisory Committee on the Marine Environment, 1998. ICES Cooperative Research Report, 233: 297–327.

Diseases of Dab (*Limanda limanda*): ICES Data Inventory



ICES copyright statement

FIGURE A15.1. ICES Data Inventory.

Background. Data used for the statistical analysis of trends in the prevalence of externally visible diseases of the common dab (*Limanda limanda*) have been extracted from the fish disease database of the ICES Environmental Data Centre. The fish disease database consists of data on the prevalence of wild fish diseases submitted by ICES Member Countries running fish disease monitoring programmes.

All steps involved in the practical work during fish disease surveys (sampling strategies, inspection of fish for target diseases, disease diagnosis) as well as reporting and validation of data submitted to the ICES Environmental Data Centre are done according to ICES standard quality assurance procedures. Coordination of these activities is within the long-term remits of the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO).

Content of Figure A15.1. For each ICES statistical rectangle (0.5 ° latitude, 1.0 ° longitude), the map provides information on the number of observations (sampling dates) in the period 1981–1997 (upper number) and on the number of fish examined and incorporated in the statistical analysis (lower number).

In order to enable a regional comparison of trends, information is only presented for female dab of the size group 20–24 cm total length. These criteria have been selected because:

- *fish of this size are generally abundant in the study area;*
- *variation in age in this size group is smaller than in larger fish;*
- *female fish are more abundant than male fish;*
- *sex-specific variation in disease prevalence occur and data for females and males should, therefore, not be combined;*
- *selection criteria are corresponding to those applied in other monitoring programmes (e.g., chemical monitoring within the OSPAR Joint Assessment and Monitoring Programme, JAMP).*

Conclusions from Figure A15.1. Data on the occurrence of externally visible diseases in dab (females, size group 20–24 cm) are available for many ICES statistical rectangles. However, the number of observations (sampling dates) and the number of fish examined differ considerably between rectangles. That means that not for all of these rectangles temporal trends can be calculated.

Diseases of Dab (*Limanda limanda*): Trends 1993 – 1997 for Lymphocystis

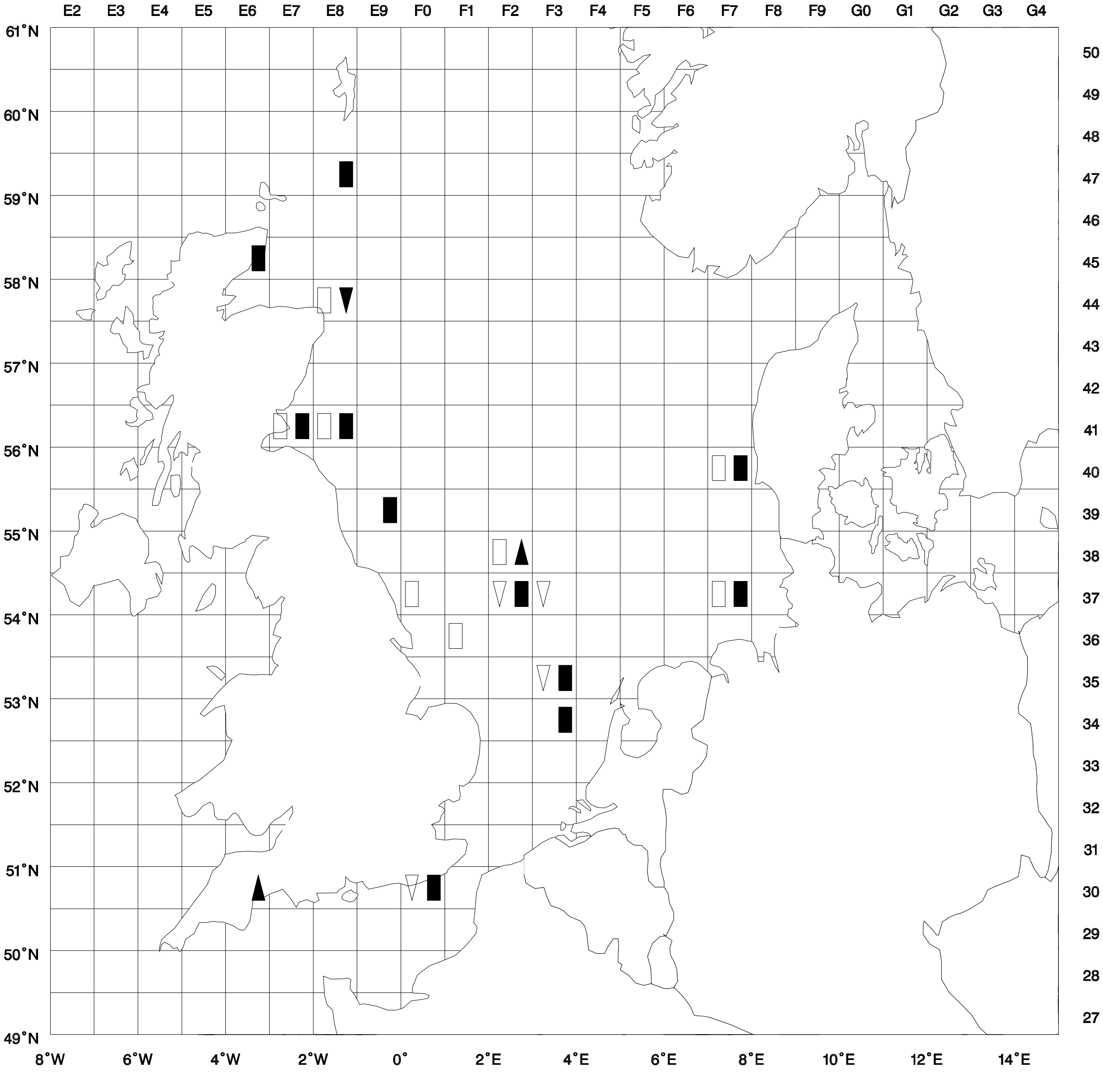


FIGURE A15.2. Temporal trends in the prevalence of lymphocystis in common dab (*Limanda limanda*) in the period 1993–1997.

Background. Since the intention of the ICES Environmental Status Report is to provide current information on the quality of the marine environment, Figure A15.2 shows trends in the prevalence of lymphocystis in dab for the period 1993–1997, calculated from the most recent data available in the ICES Environmental Data Centre.

Trends have been identified using statistical procedures based on logistic regression analysis. More details on the method are provided elsewhere.

Content of Figure A15.2. Current temporal trends for the estimated prevalence of lymphocystis in female dab, size group 20–24 cm (see Figure A15.1), are presented as upward or downward arrows or as rectangles, representing significantly increasing, decreasing or "stable" prevalences in the period 1993–1997.

Since seasonal effects on the prevalence have been observed and bias due to combining data from different seasons should be avoided, data are shown for two seasons (*Season 1*: April–September, filled symbols; *Season 2:* October–March, empty symbols).

Trends are only shown for those rectangles for which the data available meet the following criteria:

- *prevalence data must be available for a minimum of four out of the five years considered;*
- *these data must originate either from Season 1 or Season 2 or from both.*

Conclusions from Figure A15.2. Only for nine rectangles are sufficient data available for trend calculation for both seasons. With the exception of only two rectangles (30E6, 38F2), prevalences of lymphocystis were either decreasing or did not show a trend in the period 1993–1997.

Diseases of Dab (*Limanda limanda*): Trends 1993–1997 for Epidermal Papilloma

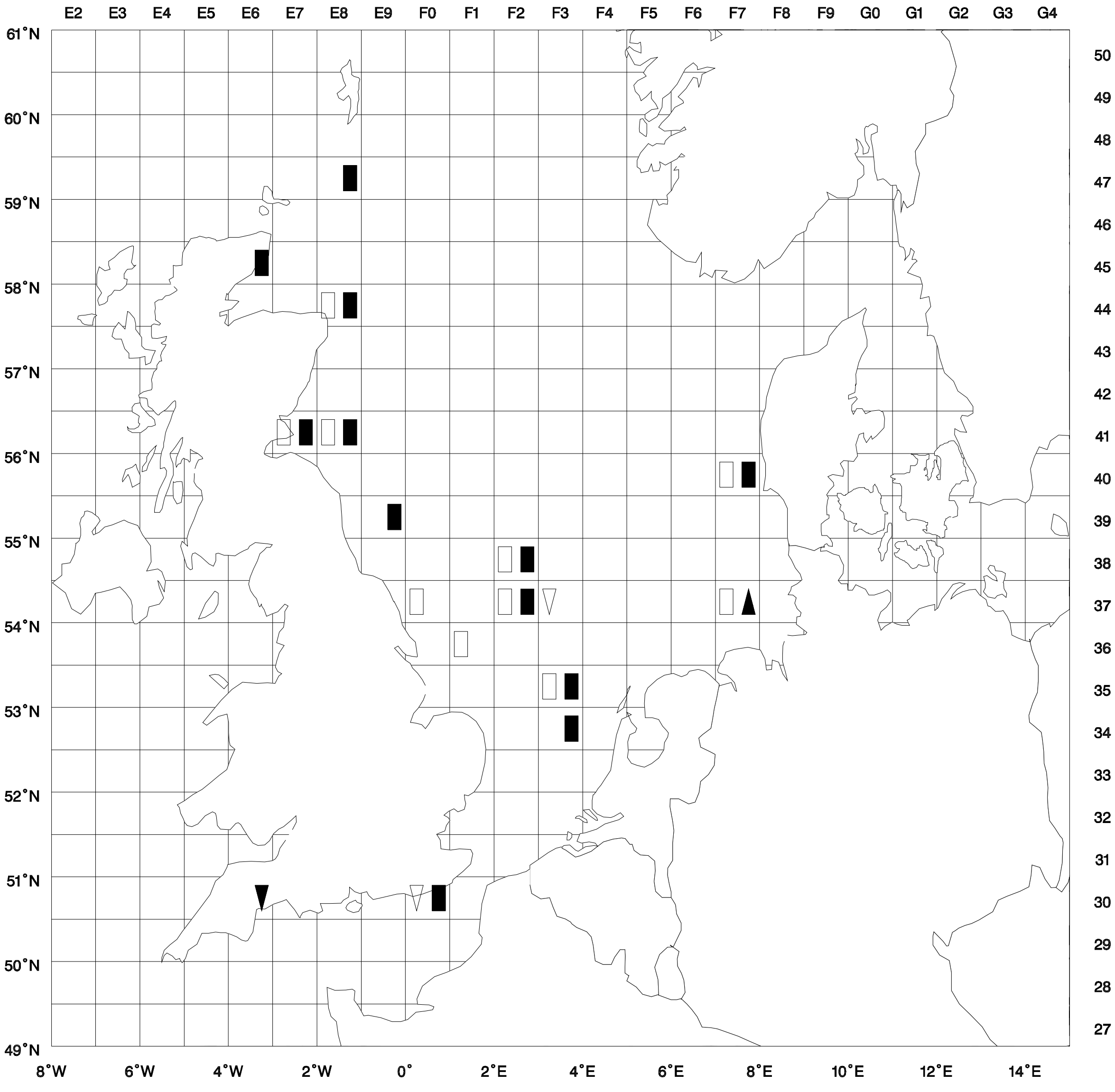


FIGURE A15.3. Temporal trends in the prevalence of epidermal hyperplasia/papilloma in common dab (*Limanda limanda*) in the period 1993–1997.

Background. Since the intention of the ICES Environmental Status Report is to provide current information on the quality of the marine environment, Figure A15.3 shows trends in the prevalence of epidermal hyperplasia/papilloma in dab for the period 1993–1997, calculated from the most recent data available in the ICES Environmental Data Centre.

Trends have been identified using statistical procedures based on logistic regression analysis. More details on the method are provided elsewhere.

Content of Figure A15.3. Current temporal trends for the estimated prevalence of epidermal hyperplasia/papilloma in female dab, size group 20–24 cm (see Figure A15.1), are presented as upward or downward arrows or as rectangles, representing significantly increasing, decreasing or "stable" prevalences in the period 1993–1997.

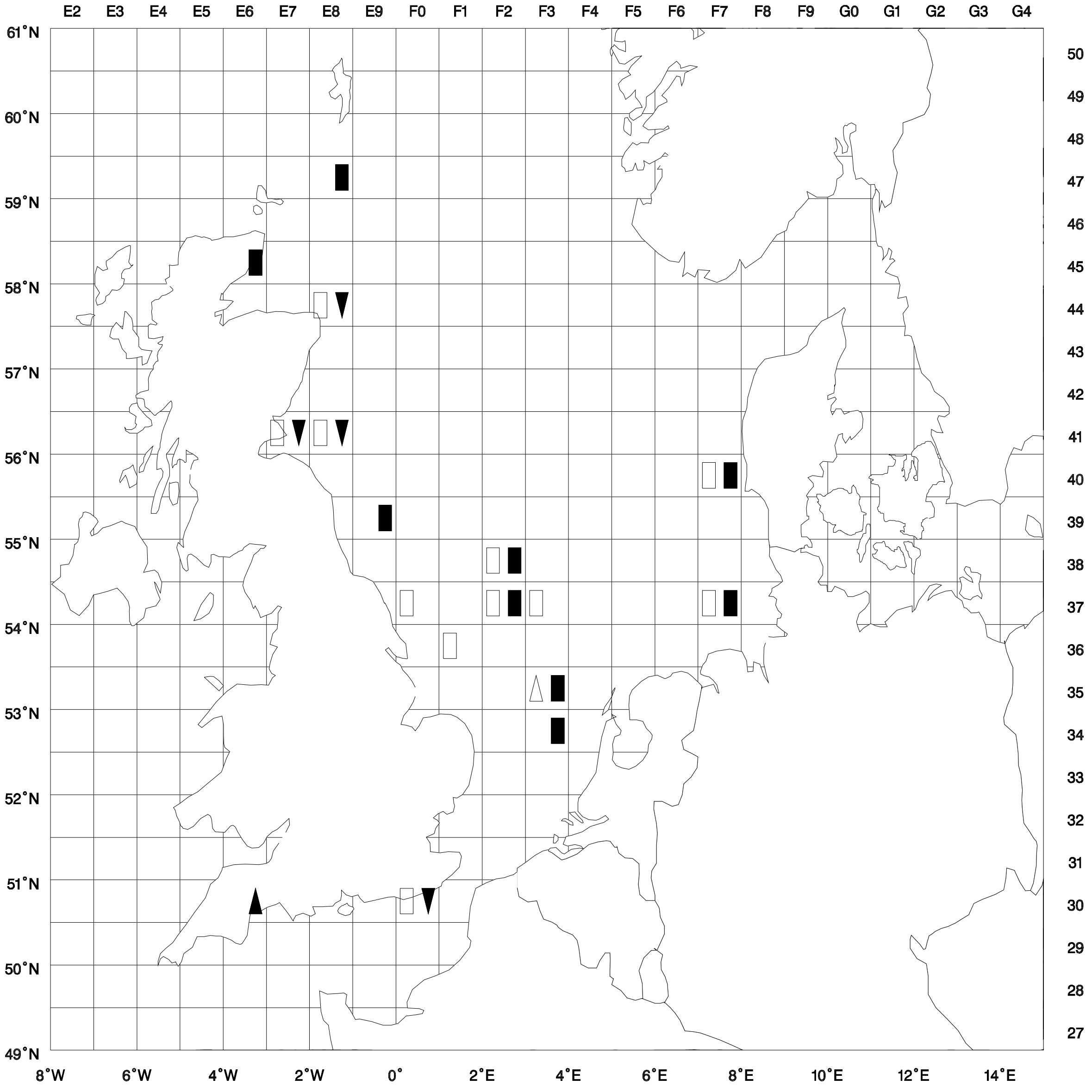
Since seasonal effects on the prevalence have been observed and bias due to combining data from different seasons should be avoided, data are shown for two seasons (*Season 1*: April–September, filled symbols; *Season 2*: October–March, empty symbols).

Trends are only shown for those rectangles for which the data available meet the following criteria:

- *prevalence data must be available for a minimum of four out of the five years considered;*
- *these data must originate either from Season 1 or Season 2 or from both.*

Conclusions from Figure A15.3. Only for nine rectangles are sufficient data available for trend calculation for both seasons. With the exception of only one rectangle (37F7), prevalences of epidermal hyperplasia/papilloma were either decreasing or did not show a trend in the period 1993–1997.

Diseases of Dab (*Limanda limanda*): Trends 1993–1997 for Acute/Healing Skin Ulcerations



ICES copyright statement

FIGURE A15.4. Temporal trends in the prevalence of acute/healing skin ulcerations in common dab (*Limanda limanda*) in the period 1993–1997.

Background. Since the intention of the ICES Environmental Status Report is to provide current information on the quality of the marine environment, Figure A15.4 shows trends in the prevalence of acute/healing skin ulcerations in dab for the period 1993–1997, calculated from the most recent data available in the ICES Environmental Data Centre.

Trends have been identified using statistical procedures based on logistic regression analysis. More details on the method are provided elsewhere.

Content of Figure A15.4. Current temporal trends for the estimated prevalence of acute/healing skin ulcerations in female dab, size group 20–24 cm (see Figure A15.1), are presented as upward or downward arrows or as rectangles, representing significantly increasing, decreasing or "stable" prevalences in the period 1993–1997.

Since seasonal effects on the prevalence have been observed and bias due to combining data from different seasons should be avoided, data are shown for two seasons (*Season 1*: April–September, filled symbols; *Season 2*: October–March, empty symbols).

Trends are only shown for those rectangles for which the data available meet the following criteria:

- *prevalence data must be available for a minimum of four out of the five years considered;*
- *these data must originate either from Season 1 or Season 2 or from both.*

Conclusions from Figure A15.4. Only for nine rectangles are sufficient data available for trend calculation for both seasons. With the exception of only two rectangles (30E6, 35F3), prevalences of acute/healing skin ulcerations were either decreasing or did not show a trend in the period 1993–1997.

Figure A15.5. The geographical distribution of the shellfish disease *Bonamia oestrea* in Europe and the USA. The red line indicates infected areas.

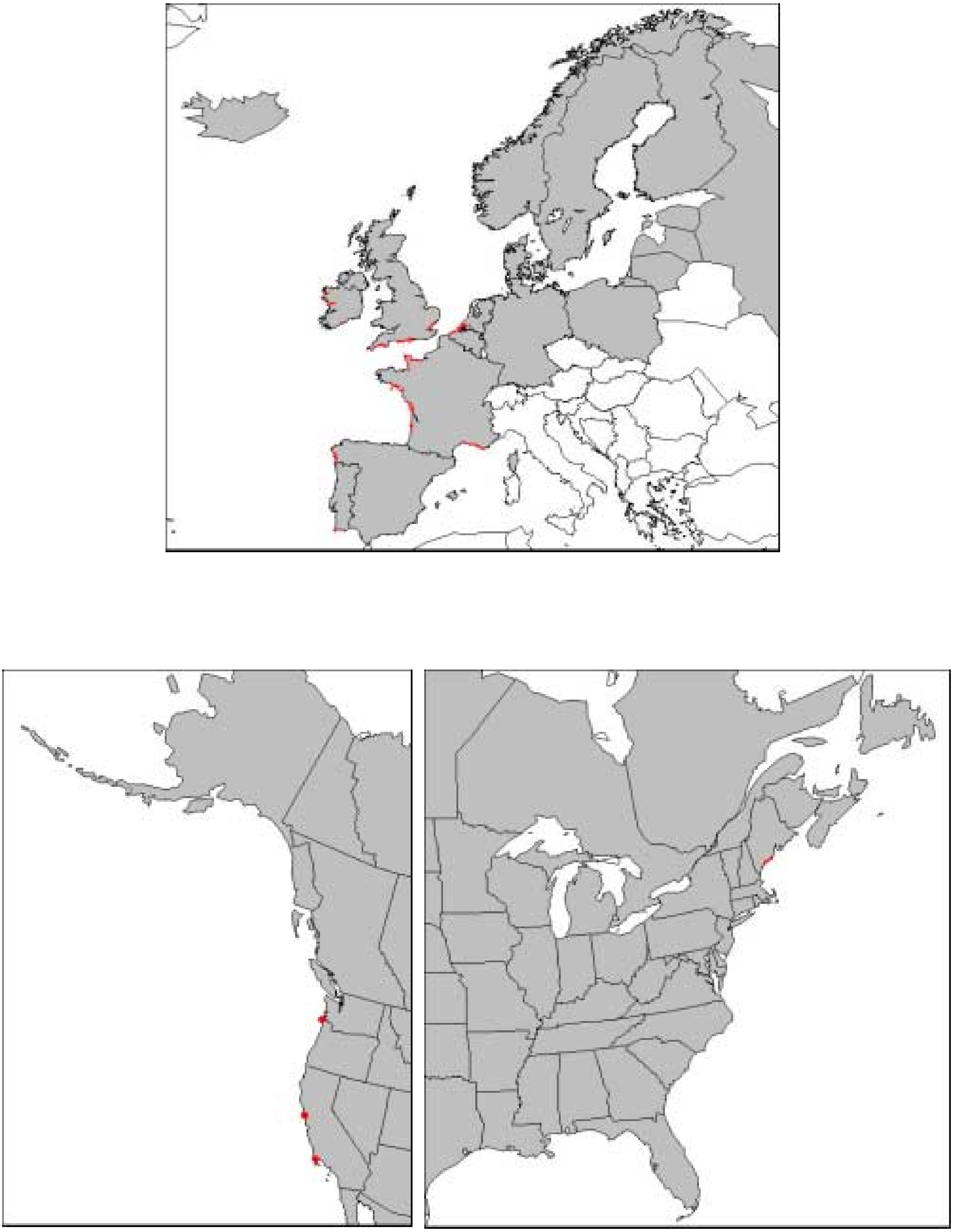


Figure A15.6. The geographical distribution of the shellfish disease *Marteilia refringens* in Europe. The red line indicates infected areas.

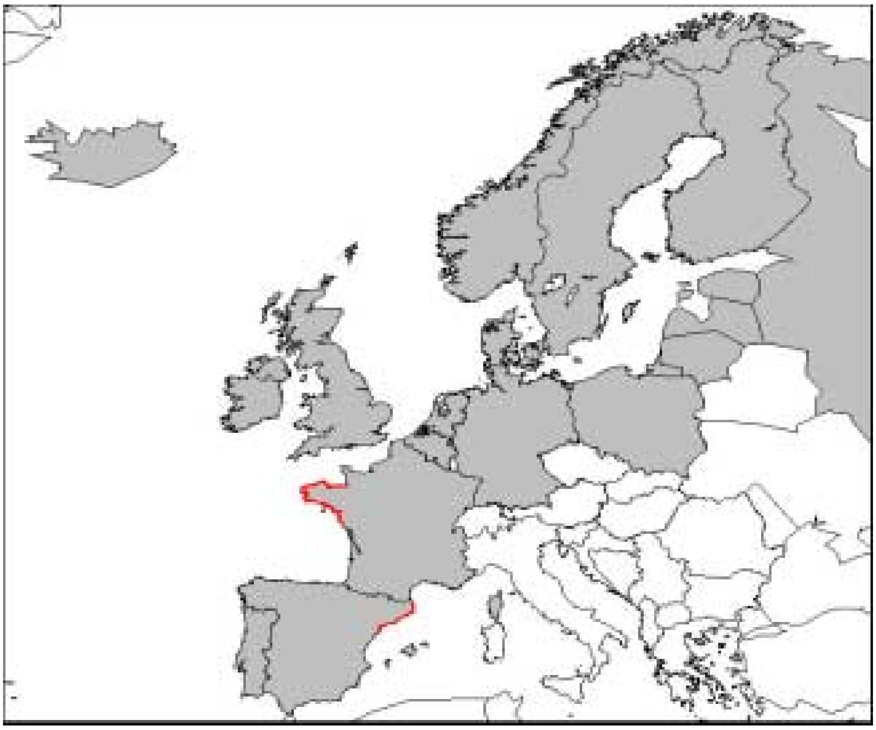
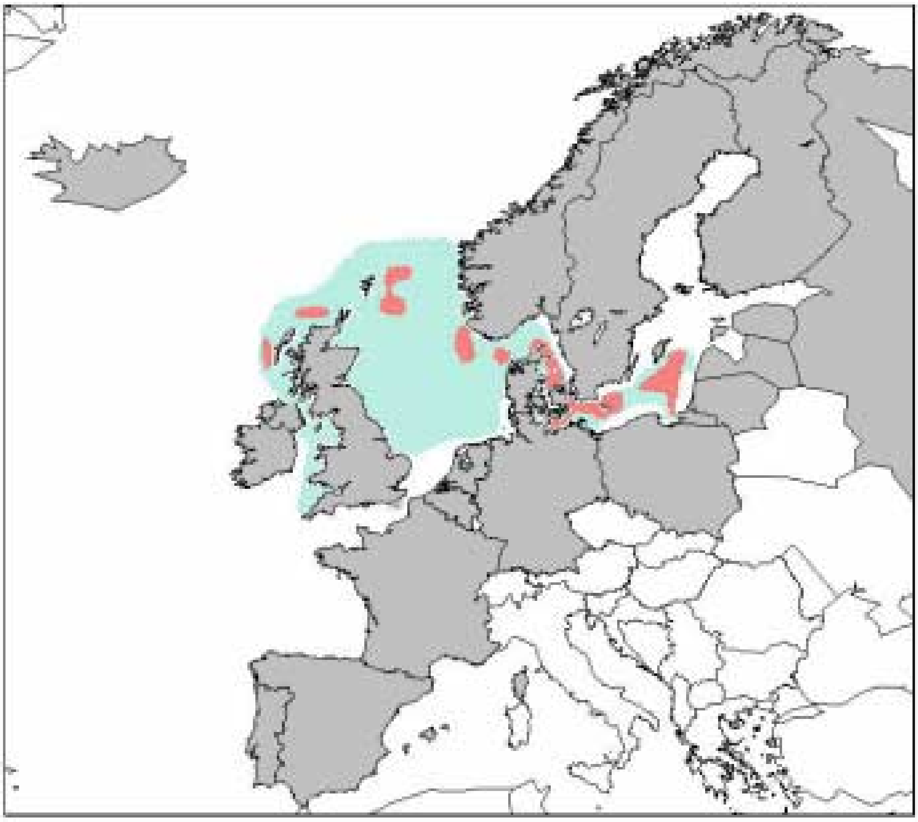


Figure A15.7. The geographical distribution of the shellfish disease *Perkinsus marinum* in the USA. The red line indicates infected areas.

Figure A15.8. The geographical distribution of the occurrence of marine Viral Haemorrhagic Septicaemia Virus (VHSV)-like virus in marine fish species in Europe and in the Pacific USA. The green shaded area indicates areas investigated and the red areas indicate where VHSV like virus has been isolated from marine fish species.



ANNEX 16: REPORT ON THE STATUS OF THE *ICES IDENTIFICATION LEAFLETS FOR DISEASES AND PARASITES OF FISH AND SHELLFISH* AND SUGGESTIONS FOR UPDATING THE SERIES

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New Fiches

Five additional titles (Nos. 51–55), listed in the 1998 report on the status of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish as "Leaflets edited, accepted and 'in press'are now being released via the ICES printing service in England. These are:

i) Stephanostomum tenue in marine aquaculture of rainbow trout (Oncorhynchus mykiss);
ii) Gaffkemia, a bacterial disease of lobsters: Genus Homarus;
iii) Diplostomum spathaceum larvae (Diplostomosis) (Digenea) in fish;
iv) Pasteurellosis;
v) Flexibacter maritimus, a causal agent of flexibacteriosis in marine fish;
vi) Streptococcosis of marine fish."

Fiche Updating

The authors of the following Fiches have been contacted for an update or suggestion of an alternative author for Fiches already published:

vii) Fiche # 4 – *Ichthyophonus*, a systemic fungal disease of fish (McVicar);
viii) Fiche # 11 – Haematopoietic neoplasm in the blue mussel (Alderman and Green);
ix) Fiche # 12 – Haematopoietic neoplasm in the flat oyster (Balouet, c/o Baudin-Laurencin);
x) Fiche # 16 – Shell disease of oysters (Alderman);
xi) Fiche #18 – Haemocytic Disease of the flat oyster (Comps);
xii) Fiche # 19 – Digestive gland disease of the flat oyster (Comps);
xiii) Fiche # 21 – Bacterial Kidney Disease (Vigneulle, c/o Baudin-Laurencin);
xiv) Fiche # 22 – Viral Erythrocytic Necrosis (Newman, c/o MacLean);
xv) Fiche # 29 – Vibriosis in cultured salmonids (Baudin-Laurencin);
xvi) Fiche # 30 – *Perkinsus marinus* parasitism, a sporozoan disease of oysters (Kern, c/o Ford);
xvii) Fiche # 37 – Furunculosis (Munro, c/o McVicar);
xviii) Fiche # 38 – *Haplosporidium nelsoni* disease of American oysters (Andrews, c/o Ford).

New Titles proposed by WGPDMO

The following authors have been contacted for the following titles:

i) QPX of hard-shell clams (*Mercenaria mercenaria*) – McGladdery and Smolowitz (in prep.);
ii) Denman Island Disease of Pacific oysters (*Crassostrea gigas*) – Bower (in prep.);
iii) SPX Disease of Japanese scallops (*Patinopecten yessoensis*) (in prep.);
iv) Brown Ring Disease of the clams *Ruditapes decussatus* and *R. philippinarum* – Paillard and Ford;
v) Herpes Virus – Renault and Hine;
vi) Infectious Salmon Anaemia (ISA) – Hjeltnes;
vii) Haemic neoplasia of soft-shell (*Mya arenaria*) and hard-shell (*Mercenaria mercenaria*) clams – Barber;
viii) M74 – Bylund;
ix) Pancreas Disease – McVicar;
x) Pseudophyllidean cestodes in marine fish – Palm;
xi) Trypanorhynch cestodes in marine fish – Palm;
xii) Nodavirus – Peducasse (in prep.);
xiii) Pfeisteria – MacLean;

xiv) *Gyrodactylus salaris* – McVicar;
xv) Flavobacterium – Dalsgaard;
xvi) Flounder Liver Tumours – Vethaak;
xvii) Gonadal neoplasia of hard-shell clams – Barber.

Format

The new Guide for Authors of the Fiches has been updated as per the draft submitted for Publications Committee review in 1998. The English version (attached) is currently being translated into French.

Organisation

The WGPDMO meeting in Lisbon in March 1999 discussed the possibility of using the ICES Publications webpage for Fiche presentation and automated downloading. This was seen as an *addition* to the printed word, which many felt was important for users who do not have web access. It was considered to be an effective alternative method to:

i) enhance dissemination of parasite and pathogen identification information;
ii) update the Fiches as new information emerges; and
iii) use colour illustrations.

With respect to cost recovery, downloading following subscription, or other such restricted access could make the Fiches available for viewing but not printing. Those who want printed versions could still purchase them using information from the editor or the ICES Secretariat.

Then other question raised was peer review. Some authors feel that, although intended as an information summary, the Fiches could be boosted by including as yet unpublished findings, if peer review could be incorporated. Bearing in mind the interest in website dissemination, this is considered a good idea to ensure a high degree of quality control/cross-checks. This peer review process could be managed through the Editor and confidential reviews filed at the ICES Secretariat, as well as with the Editor.

Title Selection and Relevance to Fiche Series

As done previously, proposed titles and authorship should be discussed and approved by WGPDMO prior to submission to the Publications Committee and solicitation of author participation.

Conclusions

WGPDMO supports continuation of the Fiche series, and welcomes the recent release of titles #51–55. It is recognised that similar disease information is becoming available, especially via the Internet, but the Fiche series continues to be the most eclectic, applying to wild as well as cultured fish and shellfish. In addition, it is the sole format available in both French and English. The hard-copy edition is a valuable baseline reference format and should be considered for adaptation to a parallel electronic format with peer review as well as editorial quality control.

ICES Guide for Authors of the Series

IDENTIFICATION SHEETS FOR DISEASES AND PARASITES OF FISH AND SHELLFISH ("FICHES")

Purpose

The Identification Sheets for Diseases and Parasites of Fish and Shellfish provide detailed information on diagnosis of parasites and diseases of fish and shellfish. Since many species are estuarine, or have freshwater phases in their life-cycle, the fiches are not limited to marine organisms. The information may be compiled from published research or on-going research, and authors are selected from experts on the specific disease or parasite problems. Subjects are proposed publication by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO). Authors may also contact the editor directly with suggestions, and these are submitted for WGPDMO approval. The authors are then provided with a schedule and guide for manuscript submission to the editor.

Format

The Fiches are bilingual (English and French). Bilingual authors are, therefore, encouraged to translate their own manuscripts, if possible, to reduce turn-around time and minimise errors from non-specialised translation. Manuscripts in French or English, however, are equally acceptable. Manuscripts submitted in French should indicate the method of accent insertion, and include accents over capital letters. Hard copies will assist where accent translation is scrambled electronically. American and English spelling is acceptable for English submissions.

Manuscripts should be double-spaced with a 3 cm left-hand margin. Hard copies should be mailed to the Editor, with one copy of original figures, for review. Electronic copies can also be submitted (MS Word 6.0 or compatible word-processor software), including scanned images, but hard copies of figures are also required. Duplicate copies of figures should be retained by the author in case of accidental loss. Font size and typeface are the authors' choice, since they will be changed at the galley-proof stage of preparation.

Titles

Titles should include the scientific names of the parasite or disease agent, common names, and the name of the disease, where applicable. Titles must also include the common and scientific names of the hosts affected. Scientific authorities are not necessary in the title but should be included in the text as indicated under headings, below.

Headings

In order to make the series as consistent as possible, the fiches use a standard set of headings to keep information concise and applicable to the purpose of the series as described above. The headings were reviewed in 1998 and slight changes made to take into account advances made in diagnostic technology over the last 20-30 years. The overall pattern of presentation, however, has been maintained and is designed to be used as reference material for scientists, diagnosticians, teachers and people involved directly in fishery and aquaculture industries. Concise, single paragraph, summaries are recommended for each section, since the entire Fiche is designed to be no more than 2-4 pages (including French and English parallel columns).

Host Species	Common and scientific names of host species. Include naturally and experimentally infected species, but clearly distinguish the two types of infection.
Disease Name	The common name(s) of the disease or parasite infection, e.g., bacterial kidney disease (BKD) for *Renibacterium salmoninarum* and anisakiasis for infections by larvae of the anisakiid nematode *Anisakis simplex*.
Aetiologic Agent	The common and scientific names of the infectious agent (where applicable). Include synonyms. Where the aetiologic agent is unknown, include known information on the aetiology of the disease which is used for diagnosis.
Associated Environmental Conditions	Include conditions such as temperature, salinity, pollutants, host population density, culture techniques, etc., which show a close correlation to the appearance of the disease or proliferation of the parasite.

Geographic Distribution	Distribution on record, as well as unpublished observations, which have been substantiated/reviewed.
Significance	Include losses due to mortality, spawning failure, abnormal or stunted growth, as well as impacts on fisheries or aquaculture due to human health issues, marketing/processing concerns and/or aesthetics.
Control	Only control methods with a proven record should be included, including chemotherapeutant use. If such direct controls are used, include appropriate protocols for safe application or direct the reader to contact their local veterinarian for advice on application. Control methods involving circumvention of acute losses should be included, where appropriate, such as modifying culture methods to minimise exposure to infective stages. Where there are no known methods of control, this should be clearly stated.
Impact on Host	Gross clinical signs and/or effects at the organism, organ, tissue or cellular level. Include, and differentiate between, non-specific and specific clinical effects.
Diagnostic Methods	Include techniques necessary to diagnose the disease (gross observations are seldom conclusively diagnostic, but can be included where appropriate by cross-referencing to "Impact on Host"). Histological diagnosis should include method of fixation, stains used and minimum magnification for microscopic detection. Immunoassay and nucleic acid probe diagnosis should clearly state whether or not the appropriate antibodies and PCR primers are commercially available or if they are available on request from specific laboratories. If the latter, please ensure that the laboratory approves the use of their name in the Diagnostic Fiches.
Comments	This section should include any information of relevance to the diagnosis of the disease or identification of the disease agent. Any similarities with other diseases or disease agents, or benign organisms, should be noted in this section, along with features used to distinguish them from the subject of the Fiche.
Key References	Include references which apply to accurate diagnosis of the parasite or disease, including sources of the information cited in the Fiche. Reference citations beside statements in the text portion of the Fiche are up to the discretion of the Author.

Cost of Publication

The cost of publication of the Fiche series is covered by ICES. Fiches are priced for sale by the ICES Secretariat in order to offset this cost. As with most ICES publications, there is no remuneration for authors asked to contribute to the series. However, it is hoped that the invitation to contribute will be accepted as respectful recognition of their expertise in the title area requested.

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ANNEX 17: ANALYSIS OF PROGRESS WITH TASKS

a) Analyse national reports on new disease trends in wild and cultured fish, molluscs and crustaceans; Reports on new diseases and trends on diseases were evaluated from national reports presented at the meeting and conclusions were drawn up.

b) Update information on the diseases and parasites of Baltic fish, to be included in the HELCOM Fourth Periodic Assessment (this is being produced intersessionally for review at the 2000 meeting of WGPDMO, and for subsequent consideration by the ACME) [HELCOM 2000/3]; A draft document was provided for consideration by WGPDMO. This was considered to be a useful document. Additional data from WGPDMO members would be provided.

c) Review progress in data submissions to the ICES Data Banks and continue the statistical analysis of ICES fish disease data in relation to environmental and fisheries data intersessionally, in order to extend the analysis to enlarged areas and time windows, and to develop and optimise suitable models and statistical methods; Information on significant progress on the development of methods for statistical analysis of fish disease data was presented and a summary report provided.

d) Maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome; Available information was assessed and a summary report provided.

e) Investigate gill disease in *Crassostrea angulata* adults, the cause of summer mortalities of *C. gigas* spat, and clarify the report of *M. refringens* in *C. gigas* from Spain; Available information was assessed and a summary report provided.

f) Collate and review available information on the distribution and effect of marine VHS-like virus on cultured and wild fish stocks; Current knowledge on marine VHS-like virus was reviewed and a summary report provided.

g) Collate and review available information on the distribution, origin, host range and impact on salmon culture of Infectious Salmon Anaemia (ISA); Current knowledge on ISA was reviewed and a summary report provided.

h) Review new information on the structure and diversity of nodavirus(es), the spread, diagnosis and epizootiology of the disease, and host immunity, to provide effective advice on possible control measures; Current knowledge on nodavirus was reviewed and a summary report was provided.

i) Initiate experimental work to determine whether the lack of *Bonamia ostreae* infections detected in field observations of *Ostrea edulis* from cold water climates reflects parasite acquisition with subsequent loss over prolonged low water temperatures, or suppression of infectivity of the parasite; Available information was assessed and a summary report provided.

j) Develop a proposal for incorporation of parasitological studies into existing disease monitoring programmes. For this purpose, it was considered useful to compile and evaluate long-term data sets already existing in ICES Member Countries; A verbal report on parasites of marine fish as biological indicators of environmental changes was presented, and conclusions and recommendations were made.

k) Review progress made within the Biological Effects Quality Assurance in Monitoring Programme (BEQUALM) work project titled “Fish disease and liver pathology”; available information was assessed and a summary report provided.

l) Provide a report with advice on new techniques in pathology and other methods for the detection of endocrine-disrupting chemicals in marine and estuarine organisms and appropriate new target species representing the main ecological levels of the marine ecosystem; Because of the last-minute inability of WGPDMO members with relevant expertise to attend the meeting it was not possible to make progress in this topic during the WG meeting. A written report is provided to be included in the report after approval of the WG members.

m) Develop proposals for the inclusion of maps of the distribution of fish and shellfish diseases of concern for mariculture and temporal trends of wild fish diseases of concern for marine environmental monitoring programmes. Different proposals were presented and future development discussed and a summary report was provided.

n) Considerations for the development of the ICES Data Centre. The forwarded request was discussed and a priority list provided.

ANNEX 18: RECOMMENDATIONS TO COUNCIL

The **Working Group on Pathology and Diseases of Marine Organisms (WGPDMO)** (Chair: Dr S. Mellergaard, Denmark) will meet in Santiago de Compostela, Spain, from 13–17 March 2001 to:

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

b) report on the progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics;

c) report on and evaluate the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oyster species;

d) report on the progress of further investigations on the role of paramoebae and other factors in the mass mortality of lobsters in Long Island;

e) review a progress report on developments and intersessional analysis of ICES fish disease and related data banks and a draft manuscript for submission to ICES TIMES on the statistical methods developed for the analysis of the data in the ICES data banks in relation to fish diseases (authors: W. Wosniok *et al.*);

f) review and assess an intersessionally prepared report on the compilation of existing data on spatial and temporal trends in the occurrence of selected parasites of wild fish and on potential environmental factors of relevance for the explanation of observed variance;

g) review progress reports from the BEQUALM Work Package "External Fish Diseases and Liver Histopathology" and from the EU project on nodaviruses and other relevant information to provide advice on effective control measures;

h) maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome;

i) report and assess the effectiveness of salmon farming management control methods for the control of sea lice in the different ICES Member Countries;

j) review an intersessionally prepared draft manuscript for publication in the *ICES Cooperative Research Report* series on important trends in diseases problems in finfish and shellfish culture in the ICES area during the past five years;

k) evaluate the progress in the intersessional development of maps of marine fish and shellfish diseases as a contribution to the ICES Environmental Status Report.

WGPDMO will report to ACME before its June 2001 meeting and to the Mariculture Committee at the 2001 Annual Science Conference.

Priority:	This group is of fundamental importance to the ICES advisory process.
Scientific Justification:	a) New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained. b) Experimental work is required to confirm field observations and the hypothesis of *Bonamia* suppression versus destruction over long periods of low temperatures. This question is important for accurately assessing climate effects on Bonamiasis and European oyster culture. c) There are historic records of an iridoviral infection of *Crassostrea gigas* gills, associated with low/transient pathology. This suggests that the gill disease agent may have multi-host infection potential, which needs to be addressed for like-to-like *C. gigas* and *Ostrea edulis* transfers. d) As the reported mortality in lobsters seems to have a serious effect on the lobster stock and the aetiological background is not fully clarified, the progress within this field should be assessed by WGPDMO. e) Based on the results of the statistical analysis carried out on the relationship between fish disease prevalences and environmental factors (data from ICES data banks), WGPDMO considered it promising to continue the statistical analysis of fish disease data, environmental and fishery data. It is expected that the results of an extended study will provide a better insight into possible cause-effect

	relationships. A publication for the ICES TIMES series should be prepared in order to document and disseminate the methodology applied in the statistical analysis of data originating from the different ICES data banks and fish disease data to be used in other group's analyses of environmental data. f) Before a detailed plan for the incorporation of parasites of wild marine fish as biological indicators of environmental change can be made, more data on temporal and spatial variation in the occurrence of fish parasites and on the role of environmental factors are needed as a basis for selecting appropriate parasite and host species. g) Where closely related work to the areas of concern to WGPDMO is being undertaken by other groups, it is necessary to maintain a good awareness of results from them. BEQUALM is currently developing a quality assurance programme for fish diseases and liver pathology which will form an essential part of wild fish disease monitoring programmes. In mariculture, the Nodavirus group of viruses contains pathogens of major importance and the development of efficient disease control should be encouraged. It is part of the contract of the EU-FAIR research programme that participants regularly report to WGPDMO. h) ICES C.Res 1993/2:23(m) requested that WGPDMO maintain an overview of the M74 syndrome and the *Ichthyophonus* issue as part of its regular agenda. i) Sea lice (*Lepeophtheirus salmonis*) continue to be a major disease problem in salmonid farming. New chemicals have currently been licensed and significant new national strategies are being implemented. This information should be compiled from relevant ICES Member Countries and incorporated into a working document to be assessed by WGPDMO. j) Annually, WGPDMO analyses national reports on new diseases and disease trends in farmed fish and shellfish within ICES member countries and offers advice in the form of recommendations on significant developments in its report. As these reports principally deal with changes which have occurred during the previous year, there is a need for an assessment of collated data from an extended period (5 years) to determine longer-term trends, which may be occurring. A publication as proposed will provide a wider dissemination of the work of WGPDMO to, e.g., OIE, EU and other international and national bodies. k) As ICES has planned to develop a more comprehensive web-based Environmental Status Report including information on diseases of wild and farmed fish and shellfish, further plans for development of an appropriate presentation should be elaborated.
Relation to Strategic Plan:	Responds to Objectives 1 (d), 2 (a, d) and 4 (a).
Resource Requirements:	None required, other than those provided by the host institution.
Participants:	WGPDMO members
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	ACME
Linkages to other Committees or Groups:	MARC, MHC
Linkages to other Organisations:	BEQUALM, OIE, EU-FAIR, HELCOM