Mariculture Committee

ICES CM 1998/F:4
Ref.: ACME + E

REPORT OF THE

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Gdynia, Poland
3–7 March 1998

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

1 OPENING AND STRUCTURE OF THE MEETING

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the Sea Fisheries Institute, Gdynia, Poland with Dr S. Mellergaard as Chairman. The meeting was opened at 10.00 on Tuesday 3 March 1998 with the Chairman welcoming the participants, particularly those who have not previously attended WGPDMO. Deputy Director Dr Daniel Dutkiewicz welcomed the participants and gave a short overview of the function and the work conducted by the Sea Fisheries Institute.

A list of participants is appended in Annex 1.

Apologies were received from P. van Banning (Netherlands), B. Hjeltnes (Norway), S. Mortensen (Norway), E. Lindesjöö (Sweden), S.W. Feist (UK), V. Kadakas (Estonia), S. Bower (Canada), S. Helgasson (Iceland) and T. Renault (France).

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

2 ICES ANNUAL SCIENCE CONFERENCE 1997: ITEMS OF RELEVANCE TO WGPDMO

Items of relevance to WGPDMO from the 1997 ICES Annual Science Conference (85th ICES Statutory Meeting) held in Baltimore, Maryland, USA were highlighted by the Chairman.

A new structure of the different ICES committees with a reduction of the number of committees was established. The background was to implement a more holistic view in the work of the committees. WGPDMO still works under the auspices of the Mariculture Committee.

a) The Report of the Delegates Meeting:

   i) stated that the recommendations originating from the Mariculture Committee were adopted by the Council with the addition of an extra item 'to define the scientific objectives of, and propose a strategic plan for, further statistical analysis of fish and shellfish data integrating other data types, e.g., contaminants, hydrographic and fisheries data, and report to ACME'.

   ii) ICES C.Res.1997/4:2 The ICES Secretariat will adapt the Fish Disease Data Entry Program and Environmental Data Reporting format to incorporate liver histopathology data.

b) The Report of the Publication Committee:

   i) noted that the editor of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, G. Olivier (Canada) had announced his withdrawal from this post at the end of 1997. S.E. McGladdery (Canada) had been recommended as his successor and Committee members were pleased to endorse her candidacy. It was hoped that ICES could find resources so that five Leaflets could be published in early 1998.

c) The report of the Mariculture Committee:

   i) accepted the Report of the Study Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks and its recommendations. The SG was then disbanded.

   ii) accepted the Report of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants and its recommendations.

   iii) accepted the report of the 1997 meeting of WGPDMO and its recommendations with the addition of one recommendation forwarded by ACME.

   iv) requested the WG to pay attention to the transfer of pathological agents through larvae, which are transported world-wide from hatcheries.

v) proposed to organise two Theme Sessions at the 1998 ICES Annual Science Conference in Lisbon—The Use of Genetics in Aquaculture, and - Farming Marine Fish beyond the Year 2000: Technological Solutions for Biological Challenges. The latter includes a topic on 'Hygienic and Sanitary Measures in Mariculture'.

vi) proposed to organise a Theme Session at the 1999 ASC in Stockholm, - Nutrient Enrichment and Mariculture.

vii) a Symposium on 'The Environmental Effects of Mariculture' is to be held 13–16 September 1999 in St. Andrews, Canada.

d) The Working Group on Biological Effects of Contaminants has a TOR -'review progress in the use of liver histopathology for monitoring the biological effects of contaminants'. This WG expects to use some of the information from the WGPDMO to fulfil this TOR.

e) The full Terms of Reference of the WGPDMO were agreed as ICES C.Res.1997/2:24.

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

3.1 Terms of Reference

WGPDMO took note of the terms of reference published as ICES C.Res.1997/2:24 (Annex 2). The heavy agenda still demanded extensive intersessional work by the members of the WGPDMO selected by the Chairman. These persons had been requested to produce written working/discussion documents which will be included in the Report as Annexes. The Chairman thanked the members for preparing these reports in advance, a work which ensures the Terms of Reference will 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 REPORTS FOR INFORMATION

Information was given on two scientific conferences to be held in 1998.

- Third International Symposium on Aquatic Animal Health, including Plenary Sessions on updates on the fish and shellfish pathogens listed in the revised OIE Aquatic Animal Health Code, as well as on emerging diseases of fish, crustaceans and bivalves. Aug. 30 - Sept. 3 1998, Baltimore, Maryland, USA
- The eighth International Symposium on Microbial Ecology, August 9–14, Halifax, N. S., Canada, including special workshop on microbial effects on culture of fish and shellfish.

5 NEW DISEASE TRENDS IN WILD FISH

5.1 Finfish

Lymphocystis: The prevalence tends to be stable in the North Sea. A considerable decrease in the prevalence was observed in flounder (*Platichthys flesus*) from the southwestern Baltic Sea, while the opposite was observed in southeastern areas. In the latter, an upward trend was also observed in herring (*Clupea harengus*).

Epidermal hyperplasia: In the North Sea, the prevalence was generally stable. However, an increase has been observed in dab (*Limanda limanda*) from the German Bight.

Skin ulcers: The prevalence of cutaneous ulcers continued to decrease in dab from Scottish waters. An increased prevalence was observed in dab sampled in the Dogger Bank in May with a subsequent decrease in December. In the Baltic Sea, a slight increase was observed in cod (*Gadus morhua*) in Polish waters and in flounder in Estonian waters.

Hyperpigmentation: The prevalence is still high in dab from the southwestern North Sea with a decreasing trend in the Flamborough off-Ground and an increasing trend in May in the Humber Estuary and in December in the Dogger Bank. A long term tendency for a wider distribution was observed in the North Sea. The aetiology remains unknown.

Liver nodules: In dab, a continuing decreasing trend was observed in the German Bight and the Dogger Bank. In flounder, a decreased prevalence was observed in coastal areas of the northern part of the Swedish coast of the Baltic Sea.

Liver lesions: In Puget Sound (Pacific USA) for the period 1989–1996, an increased prevalence of liver lesions occurred in English sole (*Parophrys vetulus*) in one reference site in the Straight of Georgia (from 0 to 8%) and two urban sites, Elliott Bay (from 13 to 33%) and Commencement Bay (from 20 to 35%) (Pacific USA).

Viral Haemorrhagic Septicaemia (VHS) like virus: In the northeastern North Sea and the Skagerrak, VHSV was isolated from pools of herring (*Clupea harengus*), blue whiting (*Micromesistius potassou*), whiting (*Merlangius merlangus*), cod (*Gadus morhua*) and lesser argentine (*Argentina silas*). The virus was detected in coastal waters of British Columbia (Canada) in Pacific herring (*Clupea pallasi*), shiner perch (*Cymogaster aggregata*) and stickleback (*Gasterosteus aculeatus*). In the United States, there was an increased infection rate in spawning Pacific herring in the spring in Prince William Sound followed by a drop below detection levels in the autumn.

Infectious Haematopoietic Necrosis (IHN) virus: In British Columbia (Canada), the infection was widespread in wild sockeye salmon and many were IHNV-positive at the end of their life cycle in freshwater and as newly hatched fry. The virus was isolated in one Pacific herring (distant from any fish farm) and in tubesnout (*Aulothynchus falvidus*) and shiner perch (collected near a salmon farm experiencing an IHN outbreak). These observations suggest a possible marine reservoir.

Other viruses: Cod from Caernarfon Bay (UK) were found to be positive for birnavirus serotypes A2 and B1, this being the first report of this virus in cod for UK waters. Herring from Liverpool Bay were found to be positive for birnavirus serotype B1, herring being a new host for the virus. In the St. Lawrence Estuary (Canada), oral epidermal papilloma, likely caused by herpes virus, were observed in 1 % of rainbow smelt (*Osmerus mordax*). This viral disease has been reported previously in the same species in the Gulf of St. Lawrence.

Various bacterial diseases: In British Columbia (Canada), *Renibacterium salmoninarum* was observed in moribund Pacific hake (*Merluccius productus*) collected from within a net-pen and was detected in several ocean-caught salmon.

On the Belgian continental shelf, an acid-fast bacteria, likely *Mycobacterium marinum*, was observed in the spleen of 0–2 year old cod (6%) with lesions. From The Netherlands, there were also some observations of mycobacteriosis in cod from southern areas of the North Sea. A total of 15 % of impounded striped bass (*Morone saxatilis*) in Chesapeake Bay (US) had mycobacteriosis.

In summer 1997, typical *Aeromonas salmonicida* was responsible for mass mortality in wild Atlantic salmon (*Salmo salar*) in the Miramichi River (Canada) drainage basin.

In Denmark, *Vibrio vulnificus* was isolated from flounder presenting severe necrosis of the tail muscle. This outbreak was considered significant and was associated with high water temperature (> 20°C).

Fungi: One case of fungal granulomatous hepatitis was observed in a large female Atlantic tomcod (*Microgadus tomcod*) in the St. Lawrence Estuary (Canada). The fish had multiple protruding black lesions (1 cm diameter) in liver and the fungus was identified as *Exophiala* upon histological examination.

Parasites: A high prevalence of *Hemogregarina* sp. was observed in sole (*Solea vulgaris*) from the southwestern North Sea. In the Baltic Sea, the distribution of *Anisakis simplex* in herring is extending eastward and the parasite occurred occasionally in other fish species such as flounder and pike-perch (*Lucioperca lucioperca*). In the Barents Sea, massive infections of *Gadus morhua* with *Anisakis simplex* were observed in the last 5 years, presumably due to a major shift in diet from capelin (*Mallotus villosus*) to other food items.

Miscellaneous: In North Carolina (USA), the dinoflagellate *Pfiesteria piscicida* was associated with 'punch-hole' ulcers most frequently around the vent of menhaden (*Brevoortia tyrranus*) and with mass mortalities in the same species. Several organisms (*Pfiesteria* complex) appear to be involved. An epidemiological study has recently demonstrated that there was a severe human health risk (neurological problems) resulting from contact with ulcerated fish and from laboratory exposure to the toxin.

Algal blooms were reported to be occurring more frequently in the Baltic Sea.

Jaw ulcers of unknown aetiology were observed in Atlantic tomcod (*Microgadus tomcod*) from the St. Lawrence Estuary (Canada). Mixed bacterial agents have been isolated from the lesions. Lesions were more frequently observed in females and the prevalence was up to 50 % in older fish, 4+.

Marked mortalities of adult grouper (*Epinephelus* sp.) have been observed for the third consecutive year in the Canary Islands. The aetiology is unknown.

5.2 Conclusions

1) No new trends in disease prevalence were noted in the North Sea. The first report received from Poland indicates an increasing trend in lymphocystis and skin ulcers in the southern Baltic Sea.
2) Toxic dinoflagellate blooms have been associated with skin ulcers and mass mortalities in menhaden in the USA. There are indications of increasing blooms of dinoflagellates and other algae in several ICES areas.
3) VHS-like and IHN viruses have been detected in various species of wild fish that may constitute reservoirs for these viruses.

5.3 Recommendation

WGPDMO should compile and review available information on the impact of marine biotoxins produced by dinoflagellates and algae on fish health and fish populations in order to provide a basis for evaluation of the significance and dynamics to provide recommendations for future research.

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

6.1 Finfish

6.1.1 Analysis by fish species

a) Atlantic salmon (*Salmo salar*)

No report was received from Ireland, one of the main salmon farming areas.

Infectious Salmon Anaemia (ISA)

Six new outbreaks of ISA were diagnosed in Norway in 1997, with a total of 24 farms under restriction because of the disease. In addition to diagnosis by culture on a Salmon Head Kidney (SHK) cell line and IFAT with monoclonal antibodies, a PCR diagnostic method is being developed. The Haemorrhagic Kidney Syndrome (HKS) reported from eastern Canada in the 1997 WGPDMO Report has been identified as ISA, with 21 sites affected in a restricted area of New Brunswick. The clinical signs of the disease were not always classical and may be influenced by local conditions. All affected fish will be slaughtered.

Togavirus

A Togavirus-like agent was reported from adult salmon, including broodfish, in New Brunswick and Maine without associated pathology.

Pancreas Disease (PD)

A virus identical to or very similar to Salmon Pancreas Disease Virus (SPDV) has been isolated from Atlantic salmon in Norway, supporting previous observations of PD pathology in salmon and rainbow trout.

Bacteria

The trend of decreasing dependence in salmon farming on antibiotics to control bacterial diseases has continued in all of the major salmon-producing areas in 1997. Some outbreaks of clinical furunculosis were reported in Norway for the first time for several years, possibly associated with the summer high seawater temperatures.

An unidentified species of *Rickettsia*-like organism was isolated from a salmon farm in Nova Scotia, Canada associated with high stress conditions.

Sea lice (*Lepeophtheirus salmonis*)

Although still a disease of major importance in salmon growing areas, infection levels in parts of Norway were low in late summer 1997, possibly because of exceptionally high water temperatures, and in eastern Canada because of the greater attention to control of the infection, compared to previous years.

Miscellaneous diseases

An outbreak of *Gyrodactylus salaris* in the River Kerit in Russia draining into the White Sea has led to a 100 % infection of salmon parr and a subsequent depletion of the salmon stocks in the river.

An increase in the occurrence of jellyfish causing significant mortalities has been reported from Scotland and Norway with the physonect siphonophore *Apolemia uvaria* being implicated in the latter.

There is increasing concern with skeletal deformities, ulceration conditions and eye cataracts in Norway, possibly associated with production factors. The latter condition has also been noted in other European production areas and may have a nutritional link.

b) Other salmonids

In Finland and Sweden, there has been a significant spread of BKD in rainbow trout culture in 1997, with strict controls being introduced in Finland to prevent further problems. Flavobacteriosis continues to be a significant problem in Finland requiring intensive research. Eye fluke (*Diplostomum* sp.), normally a problem in fresh water, caused problems in White Sea culture of rainbow trout, *Onchorhynchus mykiss*, associated with the low salinities of the area.

c) Turbot (*Scophthalmus maximus*)

An increasing trend in the occurrence of problems due to *Flexibacter martimus* and *Platyamoeba* was reported from localised areas of France and Spain. The systemic infection by the *Uronema*-like ciliate remains one of the main problems in turbot culture.

d) Eel (*Anguilla anguilla*)

Vibrio vulnificus has been isolated from eel for the first time, from two farms in Denmark.

e) Sea bass (*Dicentrarchus labrax*)

A new occurrence of *Vibrio carchariae* (two outbreaks) was recorded in Mediterranean culture. Although there is no trend concerning Nodavirus, it remains the main preoccupation in sea bass farming.

f) Sea bream (*Sparus aurata*)

A case of an asymptomatic nodavirus-like infection has been recorded in France from virological analysis. *Pseudomonas anguilliseptica* has, for the first time, been repeatedly isolated associated with mortalities during acute cases of Winter Disease.

g) Cod (*Gadus morhua*)

Heavy infections of *Gyrodactylus* spp were recorded in experimental cod farming near Murmansk, Russia.

6.1.2 Conclusions

1) For catadromous species, many of the important disease problems occurring in mariculture have been carried over from the freshwater environment. Consequently, WGPDMO believed that it was important that its consideration of diseases should not be rigidly restricted to marine problems, but should also take into account relevant conditions in fresh water.
2) The identification of ISA virus from farmed Atlantic salmon in New Brunswick, Canada, with associated pathology, represents a major extension to the known range of occurrence of this disease and its causative agent.
3) The occurrence of a *Rickettsia*-like agent in Nova Scotia, Canada is a new geographic record for this type of infectious agent, but more information is required on its prevalence, pathogenicity and relationship to *P. salmonis*.
4) Bacterial kidney disease is showing an increased prevalence in mariculture in the Baltic Sea.
5) Although there has been a previously published description of nodavirus-like disease in sea bream, the susceptibility of Sparidae for this disease requires further study. Until now, the disease has not been recorded from this family of fish despite their extensive use in mariculture, mainly in Japan. The possibility of this species acting as a reservoir should be considered.

6.1.3 Recommendations

WGPDMO recommends that:

1) because of the increase in the known range of ISA with its identification in salmon culture in eastern Canadian waters and its importance in salmon farming, it is recommended that the WGPDMO review available information on this disease;
2) considering the continuing importance of Nodavirus, the fact that it is known that more species can be infected, or carry the virus, and research is actively in progress on the identification of strains, pathogenicity and epidemiology, it is recommended that the WGPDMO again review current information on this disease.

6.2 Molluscs (Wild and Cultured)

6.2.1 Analysis by disease or parasite

Bonamia ostreae of European oysters (*Ostrea edulis*) continues to exist at low prevalences on sites in the Mediterranean Sea and the French Atlantic coast. Surveys of oysters in Lake Grevelingen, The Netherlands, showed a slight increase in prevalence (3 % to 5%) despite a sharp decline in oyster density to 1993 levels (less than half 1996 density). No changes in levels of *Bonamia ostreae* were reported in European oysters from the Pacific or Atlantic USA and, as with The Netherlands, *B. ostreae* continues to persist in Maine European oyster populations, despite low population densities (*see also Section 13*). Despite an exceptionally hot summer (> 20 °C for over a month) in the Limfjord, Denmark, in 1997, previously infected stocks of *O. edulis* continued to show no sign of infection using histopathological examination of samples collected in July and October.

Marteiliosis of European oysters was found in oysters from the French Atlantic coast (Vendee), but absent from Normandy and Mediterranean sites. Detailed investigation of the ultrastructural characteristics of *Marteilia refringens* and *Marteilia maurini* from mussels (*Mytilus edulis*) has led to the conclusion that the two species cannot be distinguished using electron microscopy. However, high variability in enzymatic activity, electrophoretic protein profiles, plus ultrastructure means that the possibility of there being two species still cannot be discounted. *Marteilia* *refringens* continues to be reported in *Crassostrea gigas* from localised areas on the Spanish northeast Mediterranean coast without clarification of specific identification.

Haplosporidiosis

i) A ***Haplosporidium*-like** parasite was reported for the first time in healthy European oysters (*Ostrea edulis*). The parasite was detected in a sample from Galicia (Spain) and its identity is currently under investigation.

ii) ***Haplosporidium nelsoni* (MSX)** showed high prevalences (75–90%) in American oysters (*Crassostrea virginica*) from Long Island Sound, USA, which were suffering mortalities approaching 60%. The cause of this sharp increase in infection prevalences and mortalities is unclear. Despite massive reductions in oyster populations in Maine and New Hampshire, prevalences up to 60 % persist, although intensity of infection is generally light. Low prevalences of light MSX infections also persist in South Carolina oyster populations.

Perkinsiosis

i) ***Perkinsus marinus* (Dermo)** infection levels in American oysters (*Crassostrea virginica*) appear to have been suppressed in 1997 by heavy rainfall and reduced salinities in the Gulf of Mexico, and Chesapeake and Delaware Bays, USA. Between Virginia and the Gulf of Mexico, infection levels appear stable with high prevalences but low intensities. The northernmost range of the disease (New Jersey to Massachusetts) continues to show higher levels of infection and associated mortalities in comparison to the southern range of Dermo. There is no evidence for further range extension north.

ii) **Scallop Protozoan X (SPX)** of Japanese scallops (*Patinopecten yessoensis*) has been conclusively identified as a new species of *Perkinsus* (in press) and continues to affect scallops at a site on Vancouver Island, British Columbia, Canada, which is used to monitor the disease. However, prevalences were lower (2%, compared with 10–60 % in previous years) and occurred earlier in the year (June).

iii) ***Perkinsus atlanticus*** in the carpet clam (*Tapes decussatus*) from Arcachon Bay, France, was associated with significant mortalities on several occasions.

Summer Pacific Oyster Mortality has shown a decreasing trend since the high levels reported in 1994–95 from the Atlantic coast of France. Studies are ongoing to assess the factors responsible for triggering these mortalities. Mortalities in *C. gigas* from California, USA, appear to affect all size-classes, but particularly juvenile and newly planted oysters. It does not appear to have an infectious aetiology and may be associated with a dinoflagellate bloom.

***Herpes* sp(p).** continue to affect larval and juvenile *C. gigas* from France. Diagnosis was confirmed using PCR. The same technique, along with electron microscopy, was used to identify *Herpes*-like viral infection of both *C. gigas* and Manila clam (*Tapes philippinarum*) larvae from a French hatchery. This is the first record of a *Herpes*-like infection of Manila clams.

Quahaug Parasite X (QPX) continues to spread among cultured hard-shell clams (*Mercenaria mercenaria* and *M. m.* var. *notata*) along the mid- to northeastern Atlantic coast of the USA. Lower prevalences reported from Massachusetts may be attributable to reduced planting densities. The most severely infected stocks are from cultured sources. QPX was reported in New Jersey for the first time in cultured clams. Surrounding wild clams were infected but showed no signs of mortality. Early development of this disease in this area may have been missed as grossly visible lesions require two years of exposure to develop. Younger infections can only be detected using histology or microbial culture.

Juvenile Oyster Disease continues to affect American oyster seed in Maine and Long Island Sound, New York, USA, although effects appear to be reduced in the progeny of oysters that survived previous infections. University of Maine research continues to provide chemotherapeutant evidence for a bacterial aetiology, along with the possible association with a marine alpha-proteobacterium.

Gonadal Neoplasia of soft-shell clams (*Mya arenaria*) was reported from the Bay of Fundy, Atlantic Canada, and the Gulf of Maine, USA, again this year. No changes in prevalence were reported.

Prosorhynchus squamatus (Digenea: Fellodistomatidae) of blue mussels (*Mytilus edulis*) was found in high prevalences (13.3%) for the first time at a major production site on the east coast of Nova Scotia, Canada, last summer. Infections were associated with high mortalities (10%), morbidity and shortened shelf-life.

Mortalities of the edible cockle (*Cerastoderma edule*) were reported from several locations in France during the summer of 1997. The cause of these mortalities is still under investigation, however, digeneans of the genera *Labratrema* and *Meiogymnophallus* were detected in affected cockles.

Polydora infestation of the shells of juvenile scallops (*Pecten maximus*) (7–15 mm in height) held in a flow through nursery system in Norway led to mass mortalities (100,000's) and destruction of the entire production stock.

Steinhausia mytilovum was reported from blue mussels (*Mytilus edulis*) from the Spanish northeast coast of the Mediterranean, for the first time. The prevalence of infection of the mussel gonads was high (45%).

Miscellaneous

i) **Imposex** continues to be monitored in coastal populations of dogwhelks (*Nucella lapillus*) from the Atlantic coast of Canada and the River and Gulf of St. Lawrence. Certain areas show prevalences of up to 95%, associated with active shipyards and the presence of TBT. German studies of the same phenomenon in deep-water whelk species (*Buccinum undatum* and *Neptunea antiqua*) found evidence of similar effects in offshore stations in the North Sea and Irish Sea. However, a correlation between imposex and body burden of TBT could only be observed in *B. undatum*.

ii) A tunicate (***Ciona intestinalis***) 'bloom' devastated blue mussel (*Mytilus edulis*) growing sites on the east coast of Nova Scotia, Canada, last summer and autumn. The rapid proliferation started on the mussel lines and spread over the mussels, smothering and stripping them off the lines.

iii) **An unexplained mortality of American oysters** (***C. virginica***) (30–50%) occurred in south and central South Carolina, USA, in summer and autumn, 1997.

6.2.2 Conclusions

1) Speciation still appears to be problematic for *Marteilia* infections of mussels, European and Pacific oysters.
2) The *Haplosporidian* in European oysters constitutes a new host species.
3) The Japanese scallop parasite SPX has now been identified as a new species of *Perkinsus*.
4) *Herpes*-like species continue to be detected in new bivalve species.

6.2.3 Recommendations

i) ICES Member Countries are recommended to clarify the host-specificity and pathogenicity of *Herpes*-like viral infections in hatchery-produced mollusc larvae. This is required to accurately assess the transmission potential and significance of cross-host transmission.

ii) The *Marteilia* reported from *Crassostrea gigas* from the Spanish northeast coast of the Mediterranean for the last three years must have its identification verified, as recommended in ICES CM 1997/F:6 Section 6.2.3 (2), especially since legislation may be based on the belief that *C. gigas* is not susceptible to infection by *Marteilia* spp.

iii) If sufficient specimens of the *Haplosporidium*-like parasite are found in *Ostrea edulis* from Galicia, these should be submitted to the Virginia Institute of Marine Science (E. Burreson) for PCR examination to determine whether or not the haplosporidian is *H. nelsoni* (MSX).

6.3 Crustaceans

6.3.1 Miscellaneous crustaceans

A **Baculovirus-like infection** was reported in the epithelia of the hepatopancreas of diseased spot prawns (*Pandalus platyceros*) which had been held in captivity on the west coast of Canada for almost a year. This is a new observation from a non-penaeid shrimp.

Dinoflagellate blood disease (***Hematodinium*** **sp.**) continues to be linked to high summer mortalities of blue crab (*Callinectes sapidus*) in commercially exploited populations off Maryland, USA (1996–97). This infection of the haemolymph has been detected from Texas, north to Delaware, with highest prevalences in enclosed, high salinity, bays. Low salinities (< 10) and temperatures (< 9 °C) appear to suppress proliferation of this parasite.

Taura virus continues to impact Texas, USA, penaeid shrimp culture, with tightened restrictions on the reporting of any abnormal mortalities.

Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) (putative Parvoviridae) was accidentally introduced from Ecuador to two penaeid shrimp farms in Hawaii, USA, despite two months quarantine and repeated dot-blot gene probe screening. The shrimp at both farms were destroyed and the facilities disinfected.

6.3.2 Conclusion

The number of viruses being detected in crustaceans continues to increase.

6.3.3 Recommendation

ICES Member Countries with wild and cultured crustaceans are recommended to gather more information on the relationships between known pathogens of penaeids in Asia and those found in wild penaeids (and other crustaceans) in North America. This is in order to accurately assess the disease risks for both the cultured and wild populations.

7 REVIEW AND ANALYSE THE DATA EXTRACTED FROM THE ICES FISH DISEASE DATABANK INTERSESSIONALLY AND AT THE WG MEETING

W. Wosniok presented an overview of the statistical analysis conducted intersessionally on the fish disease data in the ICES Environmental Data Centre which was updated and made available by the ICES Environmental Data Scientist, J.R. Larsen. A description of the methods used and a summary of the results are detailed in Annex 5.

WGPDMO noted, with appreciation, that a considerable amount of new data on diseases of dab (*Limanda limanda*) and flounder (*Platichthys flesus*) from the North Sea and adjacent areas (including the Baltic Sea) has been submitted to ICES by Member Countries since the 1997 meeting of WGPDMO. W. Wosniok informed the WGPDMO that the completed data bank allowed a more comprehensive analysis as compared to 1997 by applying a slightly modified statistical strategy.

The results of the analysis were presented as figures showing temporal trends in the estimated disease prevalence of lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations and liver nodules > 2 mm of dab (female, 20–24 cm), and lymphocystis, acute/healing skin ulcerations and liver nodules > 2 mm of flounder (female, 25–29 cm) for each ICES rectangle for which sufficient data were available. Furthermore, maps based on ICES rectangles were presented providing information on statistically significant temporal trends during the period 1992–1997 (either upward, downward or stable trends). WGPDMO endorsed this method of data presentation and considered it to be a useful tool for reporting fish disease data, e.g., in the framework of the OSPAR Joint Assessment and Monitoring Programme and the OSPAR Quality Status Report 2000.

WGPDMO noted that, from the results of the analysis, there is clear evidence for the presence of significant and consistent temporal trends in disease prevalence. However, the direction of these trends was not uniform for all areas. Some areas showed markedly distinct patterns which deserve particular attention in the coming years.

In the discussion of the results, the WGPDMO concluded that an identification of possible causes for the observed spatial and temporal trends in disease prevalence is still not possible, partly due to the unavailability of physical and biological data presently stored in the ICES Databanks. Therefore, the WGPDMO re-emphasised the need for a holistic data analysis involving available environmental, oceanography and fisheries data in the ICES Databanks. A strategy for how such an analysis can be accomplished is detailed in Section 16 and Annex 16 of the present report.

In order to disseminate the results of the data analysis and to increase awareness amongst relevant ICES Working Groups and other interest groups, the WGPDMO suggested that two publications should be produced:

- A description of the general strategy applied and the statistical methodologies used for analysis of the disease data in the *ICES Techniques in Marine Environmental Sciences* (TIMES) Series with W. Wosniok as first author and S. des Clers, J.R. Larsen, A.D. Vethaak, T. Lang, S. Mellergaard, A. McVicar and S.W. Feist as co-authors.
- An overview on the health status of North Sea dab and flounder including spatial and temporal disease trends in the ICES Journal of Marine Science with A.D. Vethaak as first author and T. Lang, V. Dethlefsen, S. Mellergaard, S.W. Feist, A. McVicar, P. v Banning, D. Bucke, W. Wosniok, S. des Clers and J.R. Larsen as co-authors.

7.1 Conclusions

1) WGPDMO noted with satisfaction that the Term of Reference was fulfilled successfully and expressed its gratitude to all individuals involved. In particular, the effort extended by J.R. Larsen and W. Wosniok was highly appreciated.
2) WGPDMO emphasised that the results of the statistical analysis provided important new information on differences between areas with respect to the temporal trends in the occurrence of fish diseases in the North Sea and Baltic Sea which deserve further attention. These results reinforce the need for and value of long-term fish disease data. Therefore, ICES Member Countries are encouraged to continue their fish disease monitoring programmes and report data to the ICES Environmental Data Centre for further analyses.
3) WGPDMO emphasised that the results of the analysis constitute an important contribution to the assessment of biological responses to environmental change and are of relevance for international monitoring programmes, such as the OSPAR Joint Assessment and Monitoring Programme (JAMP) and the related Quality Status Report 2000.
4) WGPDMO should maintain a regular overview of the status of the ICES Fish Disease Databank and decide when there is a need for further analyses.

7.2 Recommendations

WGPDMO recommended that:

i) ICES draw the attention of the OSPAR Commission to the results of the statistical analysis of fish disease data held in the ICES Environmental Data Centre in order to consider them for inclusion in the holistic Quality Status Report 2000;.
ii) the technical aspects of the statistical analysis of fish disease data be published in the ICES TIMES Series;
iii) the results of the statistical analysis of fish disease data be published as an overview paper on the health status of dab and flounder in the North Sea in the ICES Journal of Marine Science;
iv) ICES Member Countries be encouraged to continue their efforts in the monitoring of fish diseases of wild stocks and submit their data to the ICES Environmental Data Centre on an annual basis according to the established ICES standard procedures;
v) WGPDMO maintains an overview of the status of the ICES Fish Disease Databank on a regular basis in terms of new data submissions and the need for further statistical analyses. This task should be carried out intersessionally by W Wosniok, S desClers, T Lang and AD Vethaak in collaboration with the ICES Environmental Data Scientist.

8 REVIEW PROPOSALS FOR THE DEVELOPMENT OF A TRAINING AND INTERCALIBRATION PROGRAMME FOR THE DIAGNOSIS OF HISTOLOGICAL LIVER LESIONS AS PART OF A QUALITY ASSURANCE SCHEME

Background information on the Training and Intercalibration Programme (TIP) was provided by S W Feist and presented by T Lang (Annex 6). In 1996, the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants resulted in recommended guidelines for a fish liver histopathology sampling strategy and the identification of lesions used in monitoring. At that time it was recognised that the diagnosis of liver lesions must be intercalibrated, and that a quality assurance programme is required. A TIP for the diagnosis of histological liver lesions as part of a quality assurance scheme was first proposed at the 1997 WGPDMO meeting in Rhode Island, USA. Since then the TIP has been incorporated into an application for funds within the EU Standards, Measurement and Testing (SMT) Programme under the title of Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM). The rationale for BEQUALM is to standardise and intercalibrate the methods used in biological effects monitoring and to validate the data collected in field studies.

The proposal for the liver histopathology intercalibration programme would include expert laboratories from the participating countries that would be involved in standardising procedures, establishing agreement on neoplastic and non-neoplastic liver lesions and producing laboratory reference materials. Two workshops are proposed to establish protocols and provide exercises on diagnosis, and to assess results of earlier intercalibration exercises and data collected during national monitoring programmes. The production of a colour atlas of flatfish liver histopathology is proposed.

The CEFAS Weymouth Laboratory, UK (S.W. Feist) is proposed as the lead laboratory for the intercalibration exercises, with the Bundesforschungsanstalt für Fischerei, Institut für Fischereiökologie, Cuxhaven, Germany (T. Lang) as the secondary lead laboratory. The proposal as submitted through BEQUALM would provide funding to the expert laboratories from each participating country to attend workshops and participate in intercalibration and quality assurance exercises.

8.1 Conclusions

WGPDMO endorsed the BEQUALM proposal and emphasised that it constitutes an important step in the implementation of quality assurance procedures in international monitoring programmes such as the OSPAR JAMP.

8.2 Recommendations

WGPDMO recommends that, if the BEQUALM funding bid to the EU is not accepted, WGPDMO should consider further developments in the area of training and intercalibration of liver histopathology diagnosis.

9 COMPILE AVAILABLE EVIDENCE ON THE CAUSES OF THE M-74 SYNDROME IN BALTIC SALMON AND PROVIDE A SUMMARY OF THE PROGRESS IN UNDERSTANDING THE RELEVANT ENVIRONMENTAL FACTORS INFLUENCING THE OCCURRENCE OF M-74 ALONG WITH THE GEOGRAPHICAL EXTENT OF ITS DISTRIBUTION

9.1 Summary of Progress

Finnish and Swedish data on the prevalences of M-74 in 1997 are conflicting (Annex 7). Information from Swedish hatcheries indicates a steep decrease in prevalence from 1996 to 1997 with 68 % and 28 %, respectively, of the females producing M-74 offspring. In contrast, the Finnish data indicate that the prevalence of females producing M-74 offspring as well as the yolk sac fry mortality have remained at the same high level for the last five years. Apparently this disagreement is due to different methods of calculating these prevalences. In Swedish hatcheries, females with visible signs of M-74 (behavioural disturbances and pale pigmentation of eggs) were not used as spawners and were excluded from the calculations in 1997 whereas the Finnish data included all females.

So far, there are no reports on the occurrence of M-74 (or equivalent reproductive disorders) in areas other than the Baltic Sea and the Great Lakes region in North America. There has been no confirmation of the occurrence of M-74 in Galicia, Spain.

Recent Finnish investigations showed that the muscle tissue is the main site of thiamine reserves of the female salmon. Bath treatment of eggs with thiamine concentrations ranging from 0.1 to 0.3 % gave a dose-dependent response. Intraperitoneal injections of astaxanthin given to females significantly increased the astaxanthin concentrations of the eggs but had no influence on the survival of the yolk sac fry.

All present data indicate that no infectious agents are involved in the aetiology of this disease syndrome. Recent Finnish investigations showed a significant positive correlation between M-74 and the levels of certain coplanar PCBs. However, a clear cause/effect relationship between these contaminants and the development of the disease syndrome has not been demonstrated.

A web-site on the M-74 is available at the address: http://w1.185.telia.com/~18500254/m74eng.htm

9.2 Conclusion

Although intensive research is going on in order to explain the aetiology behind the M-74 syndrome (and the equivalent Early Mortality Syndrome in North America), no significant breakthrough in the research occurred during 1997. Preventive thiamine treatment helps in maintaining breeding and stocking programmes for the Baltic salmon but so far we do not understand the relevant environmental factors influencing the occurrence of the disease and the M-74 syndrome continues to require investigation.

9.3 Recommendations

WGPDMO recommends that:

i) countries in areas where the M-74 syndrome occurs standardise the methods for monitoring the disease levels in order to ensure compatibility of data on trends;

ii) all ICES countries continuously monitor salmonid populations for the occurrence of reproductive disorders similar to the M-74 syndrome. To facilitate the diagnosis, a description of the clinical signs of the disease is included in the present report (Annex 8).

10 OVERVIEW OF NEW INFORMATION ON *ICHTHYOPHONUS*

10.1 Current Information

Working papers dealing with the results of sampling herring, *Clupea harengus,* for *Ichthyophonus* were submitted by Iceland, Poland, Russia and Scotland. Additional data were obtained from the national reports on trends in marine fish diseases submitted by several countries.

A total of 650 summer spawning herring from Icelandic waters caught by research vessel midwater trawl showed no infection and 689 from fishing vessel seine net catches revealed one fish with *Ichthyophonus* infection (0.15 %). From 1,300 Atlanto-Scandian herring caught by Icelandic research vessel midwater trawl in May 1997, a total of 31 (2.38 %) were infected with *Ichthyophonus.* This is similar to the level reported for 1996 in 25+ cm fish (1.93 %) (Annex 9).

Data from Russia (PINRO, Murmansk) (Annex 9) indicated the continued occurrence of *Ichthyophonus* in herring from the Norwegian and Barents Sea. Differences were recorded in the prevalence of infection using different detection methods. In the Baltic Sea, monitoring for *Ichthyophonus* since 1995 in ICES Subdivision 26 indicated a prevalence of 0.2–0.5 % in different samples, taken in the autumn 1995 from commercial catches in the open sea.

No *Ichthyophonus* was detected in 5,773 herring examined from commercial catches in Scotland in 1997 from areas IVa, IVb, VIa and the Clyde (Annex 9).

On the Pacific coast, the USA noted that *Ichthyophonus* in Pacific herring of Prince William Sound and Sitka Sound decreased in prevalence from 25 % in 1996 to 18 % in 1997. There were no associated mortalities.

In Denmark, the fishing industry has reported that *Ichthyophonus* infected fish were still observed in fish landed for processing with a whole landing being rejected recently because of a high infection level. At least some of the herring were from North Sea stocks. Norway indicated that the prevalence of infection with *Ichthyophonus* remained unchanged in Norwegian spring spawning stocks in the Norwegian Sea.

Sweden noted that no new trends in the prevalence of *Ichthyophonus* have been reported in their sampling area.

The absence of *Ichthyophonus* in samples was noted by Estonia (from Baltic herring), by Germany (from 1053 North Sea herring 20.5–25.5 cm in length caught in May 1997 in the Orkney area), by Poland (during regular monitoring for the parasite in herring from the Gdansk Basin) and by England (in herring caught in the southern North Sea and the Irish Sea). Data from Poland (IMTM, SFI and the University of Gdansk) totalling 6876 Baltic herring collected over years 1992–1994 and in April 1996 and February 1998 caught in ICES Subdivision 26 (the Gdansk Basin) indicated that the prevalence of infection of *Ichthyophonus* was zero (Annex 9).

The Canadian wild fish disease report indicated that a screening of the hearts of herring collected during assessment and acoustic surveys off eastern Canada will commence in 1998.

10.2 Conclusions

There is still evidence that *Ichthyophonus* infection is persisting in some stocks of Atlantic and Pacific herring, but generally at a low or decreasing prevalence level.

11 REVIEW AVAILABLE INFORMATION ON PATHOLOGICAL ASPECTS OF ENDOCRINE DISRUPTING CHEMICALS (EDC) IN ESTUARINE AND MARINE ORGANISMS

A.D. Vethaak gave an overview on the problems related to endocrine disrupting chemicals (EDC) and their pathological effects (observed and suspected) in estuarine and marine organisms. He presented arguments for the need to augment existing marine monitoring programmes with methods for the detection of EDC effects. It was mentioned that a more general review on the effects of EDC will be considered by the ICES WG Biological Effect of Contaminants (WGBEC) during its March/April 1998 meeting (ICES C.Res./1997/2:12:6 e).

It was recognised that the definition of endocrine disrupting chemicals, as given in Annex 10, is very broad and needs further specification. It was then stated that most information exists on risks that can be expected from the direct receptor-mediated effects since different contaminants using the same mode of action may result in cumulative effects. However, other pathways of hormonal disruption (e.g., inhibition of P450-aromatase by organotin compounds in snails) may be of similar importance.

Furthermore, the following issues were raised:

- It can be expected that in areas such as the North Sea and the Baltic Sea, the impact by high fishing activity may mask, to a considerable extent, effects at the population level caused by endocrine disrupting chemicals, making detection of changes due to EDC difficult.
- The importance of detecting long-term ecological trends was emphasised, since effects might become apparent many years (e.g., several generations) after exposure to EDC.
- In addition to field surveys, controlled laboratory experiments and mesocosm experiments should be carried out to demonstrate causal relationships between effects observed in the field and exposure to EDC.
- Different species might respond differently to EDC, thus, further investigation must be carried out to identify the most sensitive marine species for experimental work and monitoring.
- New techniques for the detection of EDC have to be developed, e.g., immunocompetence function tests and a sperm quality bioassay for marine fish and its subsequent implementation in biological effects monitoring, should be encouraged.
- Data on long-term ecological changes in marine mammals, fish and shellfish should be included in the ICES Regional Data Centre.

WGPDMO stated that our knowledge to date on the risks of EDC for marine populations is very limited, in particular for invertebrates. It was mentioned that a special SETAC-Europe/US Workshop on Endocrine Disrupters and Aquatic Invertebrates will be held in Europe in September 1998, aiming to identify more specific needs in this field.

11.1 Conclusions

1) The number of observed/suspected cases of endocrine disrupting effects in estuarine and marine organism reported in the literature has recently increased.
2) Current biological effects monitoring programmes should be continued and expanded in the near future by including new endpoints/parameters for detection of general health effects and specific EDC effects. These parameters should include: gross reproductive/developmental disorders, Gonado-Somatic Index (GSI) and a general histological screening of the gonads.
3) For future monitoring activities, the inclusion of new indicator species should be considered. The new target species should be sensitive to EDC effects and include marine mammals, fish, molluscs and crustacean species, exhibiting different life styles/life cycles and representing different niches of the marine ecosystem.
4) Since reproductive disorders suspected to be linked to EDC have been observed particularly in marine mammals, the possibility of monitoring the health status of these animals using non-invasive techniques should be considered for further routine monitoring.

11.2 Recommendations

WGPDMO recommends that:

i) an intersessional evaluation of new techniques in pathology and other methods for the detection of effects of endocrine disrupting chemicals in estuarine and marine organisms be made and reported to the 1999 WGPDMO meeting by AD Vethaak, C Couillard and S McGladdery.
ii) a review of potential sentinel organisms, which could demonstrate pathological effects that may be linked to endocrine disruption, be prepared by A.D. Vethaak, C. Couillard and S. McGladdery. These organisms should represent the main ecological levels of the marine environment.
iii) all of the above recommendations should be carried out in collaboration with the ICES Working Group on Biological Effects of Contaminants.

12 REVIEW INTERSESSIONAL PROGRESS IN THE DEVELOPMENT AND IMPLEMENTATION OF A QUALITY ASSURANCE PLAN FOR THE INTEGRATION OF FLATFISH LIVER HISTOPATHOLOGY MEASUREMENTS IN THE OSPAR JOINT ASSESSMENT AND MONITORING PROGRAMME AND THE ADAPTATION OF THE ICES FISH DISEASE ENTRY PROGRAM AND FISH DISEASE REPORTING FORMAT FOR THE INCORPORATION OF LIVER HISTOPATHOLOGY DATA INTO THE ICES ENVIRONMENTAL DATABANK

T. Lang presented a progress report as detailed in Annex 11.

A quality assurance plan for the integration of liver histopathology into the OSPAR Joint Assessment and Monitoring Programme (JAMP) has not yet been developed. However, this issue is part of the activities connected with the EU funding bid 'BEQUALM' (details on this project can be found in Section 8 and Annex 6).

With regard to the integration of liver histopathology data into the fish diseases section of the ICES Environmental Data Centre, the WGPDMO noted with appreciation that the ICES Council decided (C. Res. 1997/4:2) that the ICES Secretariat will adapt the Fish Disease Data Entry Program (FDE) and Environmental Data Reporting Format to incorporate liver histopathology data.

WGPDMO recommended that the five categories of relevant liver lesions, as identified in the report of the 1996 ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants (ICES CM 1997/F:6) and listed in Annex 11, should be added to the existing list of diseases of dab (*Limanda limanda*) and flounder (*Platichthys flesus*) in the ICES Fish Disease Data Entry Program/Fish Disease Data Reporting Format. Confirmed cases of grossly visible neoplastic liver nodules > 2 mm in diameter should be maintained as a separate category. Whilst the first five categories will be used for the submission of data obtained during routine histopathological studies (second strategy according to Annex 11), the latter can be used for the submission of data obtained in studies applying the 'traditional' strategy (also see Annex 11). However, the WGPDMO emphasised that, before liver histopathology data can be submitted to the ICES Environmental Data Centre, a quality assurance programme for the diagnosis of liver lesions has to be developed and implemented. WGPDMO, therefore, expressed its hope that the BEQUALM proposal will be accepted and funded by the EU.

In the discussion, it was emphasised that, for future monitoring programmes focusing on fish liver lesions, it is advisable to follow the first strategy and to apply a routine histopathological study according to the methodological guidelines developed by the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants. This will increase the level of detection of neoplastic liver lesions and help identify a larger variety of other lesions as compared to the 'traditional' strategy.

12.1 Recommendations

WGPDMO recommended that the co-convenors of the 1996 ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants (S.W. Feist and T. Lang) collaborate with the ICES Secretariat after quality assurance procedures have been completed in order to initiate all necessary steps for the incorporation of liver histopathological data in the ICES Environmental Data Centre.

13 ASSESS THE PROGRESS IN THE POSSIBLE CAUSES OF *BONAMIA OSTREAE* PERSISTENCE IN AREAS WITH LOW DENSITIES OF OYSTERS AND THE POSSIBLE RELATIONSHIP TO DISEASE RESISTANCE AND WATER TEMPERATURE INHIBITION OF INFECTION

In order to accurately assess introduction and transfer risks associated with *Bonamia ostreae* infected European oysters (*Ostrea edulis*), it is necessary to understand the persistence of *B. ostreae* in low-density populations of oysters and the development (and manifestation) of *B. ostreae* resistance. Information contributing to the review of this question was provided by S. McGladdery (Canada), P. van Banning (The Netherlands), T. Renault (France), B. Barber and S. Ford (USA).

The best long-term survey of *B. ostreae* in an infected European oyster population is the study of The Netherlands at Lake Grevelingen, over the last ten years. Stocks decreased from 300–500 oysters m^{-2} in 1987 to an undetermined level between 1989 and 1992. In 1993, the levels were 15 m^{-2} with an average prevalence of 16 % associated with 64 % mortality. Stocks increased between 1994 and 1996 from 23 to 38 m^{-2}, while prevalences decreased from 16 % to 3 %, with a concomitant decrease in mortality from 38 % to 21 %, respectively. In 1997, the oyster population decreased again from 38 to 16 m^{-2}, but levels of *Bonamia* showed only a slight increase (3 % in 1996 and 5 % in 1997).

These low prevalences and mortality rates in low *Ostrea edulis* population densities appear to reflect the situation in other oyster populations, such as in Maine (USA). The exact relationship between population density and development of resistance/tolerance to infection is still not understood. A suggestion from the USA (B. Barber, University of Maine) is that there may be natural selection of particularly 'efficient' and/or virulent parasites during periods of low host densities. If there is a fitness cost to these qualities, such strains may be lost when the host population recovers. This may explain why differences in virulence of *Bonamia* are commonly encountered in experimental infection studies, and appears to be another interesting avenue for research into host-parasite dynamics and development of resistance. It is also important to note that there is strong evidence that many bivalve diseases can persist in numbers which are too low to detect using routine diagnostic methods (e.g., *Haplosporidium nelsoni* (MSX), *Perkinsus marinus* (Dermo) and Malpeque disease) which can re-emerge, when conditions (host density, environmental factors, etc.) become more favourable to the parasite. Such 'reservoir' infections may use residual hosts or exist in dormant external forms.

The question of the continuing absence of *Bonamia ostreae* from northern European oyster populations remains consistent, with negative findings from Canada, Denmark and Scotland. However, known introductions of oysters from enzootic areas have occurred in Denmark, as well as regularly in western Canada, without subsequent manifestation of disease. The reason for this is still under investigation. Studies are ongoing with Atlantic Canadian oysters, which have been isolated from known infected populations since the 1970s, to determine if they are resistant, 'sub-detectable' carriers, or are still susceptible to outbreaks if exposed to *Bonamia* or conditions changed to favour the parasite's proliferation. Stress testing (high water temperatures) has shown no expression of bonamiasis in Canada and abnormally high water temperatures in the Limfjord, Denmark, last summer showed no evidence of infection.

The Atlantic Canadian oysters are now being assessed using highly specific molecular tools at La Tremblade, France, as well as by challenge experiments.

13.1 Conclusion

No results are available yet.

13.2 Recommendations

WGPDMO recommends that:

i) ICES Member Countries enzootic for *Bonamia ostreae* should continue to assist ICES Member Countries with *Bonamia*-negative European oysters in order to assess possible temperature suppression of *Bonamia ostreae* and evaluate the accuracy of negative results obtained from histological analyses of northern grown European oysters.

ii) ICES Member Countries with European oysters which have been infected, or exposed previously, to *Bonamia ostreae* infection are recommended to submit samples for immunoassay diagnostic screening and *B. ostreae* challenges at La Tremblade (France), to determine whether or not negative results reflect absence.

14 PREPARE AN INFORMATION PACKAGE ON THE ICES ENVIRONMENTAL DATABANK AND DISEASE REPORTING FORMAT FOR SUBMISSION TO LABORATORIES IN MEMBER COUNTRIES IDENTIFIED AS CONDUCTING SHELLFISH DISEASE SURVEYS AND/OR MONITORING

T. Lang presented a working paper and information package on the ICES Environmental Data Centre, including a list of Shellfish Disease Laboratories or ICES Member contact addresses (Annex 13), prepared in collaboration with S. McGladdery. The working paper provided a detailed overview of the ICES Environmental Data Centre and the ICES Fish Disease Data Reporting Format/Fish Disease Data Entry Program, as well as a strategy which could be followed for future shellfish (molluscan and crustacean) data submission. The working paper and information package (Annex 12) will be edited prior to being mailed intersessionally to the laboratories listed.

In the discussion, the WGPDMO emphasised that the inclusion of shellfish disease data in the ICES Environmental Data Centre is worthwhile, since it will increase the diversity of data available for the assessment of spatial and temporal environmental changes.

Although many laboratories (Annex 12) perform shellfish disease diagnostics, few conduct regular disease surveys. Most current activities are centred around mariculture. Only some laboratories study shellfish diseases as indicators of anthropogenic environmental change. Others conduct intensive contaminant surveys involving shellfish, such as mussels (*Mytilus edulis*), but these involve chemical analyses in tissue extracts rather than histopathology or histochemistry. The lack of disease monitoring data for shellfish, in comparison to that available for finfish, was noted as a significant impediment to immediate shellfish data inclusion. However, it was also noted that there is growing interest in environmental effects monitoring which integrates shellfish disease data. As an example, an upcoming international workshop to be held in Europe in September 1998 will aim at developing guidelines for monitoring of endocrine disrupting chemicals in shellfish and other aquatic invertebrates. This meeting will be attended by A.D. Vethaak (The Netherlands) who will report what kind of shellfish data/survey expertise may be available for future ICES collaborative data collection.

WGPDMO discussed the need to determine which shellfish species and diseases would be most useful for the intended purpose of assessing environmental changes. At present, the studies on the effects of TBT contamination on marine gastropods appear particularly promising and data from these could be included in the ICES Environmental Data Centre, as a first step. Other potentially relevant studies include the monitoring of contaminant levels and the copepod parasite, *Mytlicola intestinalis*, in blue mussel (*M. edulis*). Data obtained in studies on diseases of crustaceans, such as black spot disease of brown shrimp (*Crangon crangon*), could also be considered.

WGPDMO recognised that there still is no complete overview of the types of data available in shellfish disease laboratories in ICES Member Countries. This information must be collected before any further action can be taken.

14.1 Conclusions

1) There is a need to assess the species of shellfish and diseases which could be sensitive and appropriate for environmental data collection.
2) There is a need to review the type of shellfish disease data available in ICES Member Countries.

14.2 Recommendation

WGPDMO recommended that:

i) information on appropriate shellfish species and diseases and on available data in ICES Member Countries be compiled intersessionally for presentation at the 1999 WGPDMO meeting. This task has been assigned to S. McGladdery, T. Renault, D. Vethaak and T. Lang.
ii) ICES review and approve the information package on the ICES Environmental Data Centre and the ICES Fish Disease Data Reporting Format/Fish Disease Entry Program, and distribute it to the shellfish disease diagnostic and research laboratories listed in Annex 13.

Justification of ii): WGPDMO has according to its Terms of Reference 1998, item j) prepared an information package and compiled an address list of laboratories that may be interested in receiving the information package.

15 REVIEW NEW PROGRESS IN DIAGNOSTIC, EPIDEMIOLOGY AND IMMUNOLOGY OF NODAVIRUS

F. Baudin Laurencin (France) presented a review paper (Annex 14) and J. Barja (Spain) provided two abstracts from the Japanese Society of Fish Pathology 1997 meeting in Hiroshima (Annex 15).

As in 1996, nodavirus has caused significant mortalities world-wide in 1997 in several fish species, particularly in the Mediterranean Sea. The disease was usually associated with high water temperature (> 20 °C). However, it has also been observed at relatively low water temperature (15 °C) in Atlantic halibut in Norway. The existence of two strains of nodavirus in European sea bass was demonstrated. In France, the disease was transmitted experimentally in sea bass by several routes, including *per os*, bath immersion and intramuscular (IM) and intraperitoneal (IP) injections. Transmission by IM injection caused up to 100 % mortality. In Japan, the virus was demonstrated in striped jack (*Pseudocaranx dentex*) in various organs including gonads and resulted in infection of eggs. An ELISA method to detect serum antibodies, PCR methods, and cell-culture techniques have been developed and successfully used as diagnostic tools in France. The disease was effectively controlled in Japan by selecting virus-free broodstock and disinfecting fertilised eggs and influent seawater in hatcheries using ozone (0.5 ppm during 1 to 5 minutes).

15.1 Conclusion

Diagnostic methods and control measures by the selection of virus free broodstock are improving.

15.2 Recommendations

WGPDMO recommends that:

i) ICES Member Countries investigate the presence of the virus in wild fish species and evaluate the risk of transmission between wild and cultured fish.

ii) ICES Member Countries establish procedures to prevent the spread of nodavirus by transfer of eggs, larvae and juveniles.

16 DEFINE THE SCIENTIFIC OBJECTIVES OF, AND PROPOSE A STRATEGIC PLAN FOR, FURTHER STATISTICAL ANALYSIS OF FISH AND SHELLFISH DATA INTEGRATING OTHER DATA TYPES, E.G., CONTAMINANTS, HYDROGRAPHIC AND FISHERIES DATA, AND REPORT TO ACME

T. Lang provided background information on the rationale behind this Term of Reference, given to the WGPDMO by the ICES ACME, and presented an overview of the ICES activities in previous years on submission and statistical analysis of fish disease data held in the ICES Environmental Data Centre. Reference was also made to the results of the statistical analysis of data in the ICES Fish Disease Databank. which was presented separately to the WGPDMO (Section 7, above). T. Lang also outlined the scientific objectives which could be addressed in future analyses, incorporating disease, contaminant, oceanography and fisheries data available in the different ICES Databanks. A strategy to accomplish a more holistic analysis, in collaboration with other expert ICES Working Groups was also proposed (Annex 16).

WGPDMO endorsed the proposals made and emphasised that such an approach is highly desirable and appears promising, given the availability and quality of the data in ICES Databanks. These data may be used for an integrated statistical analysis to provide a more detailed picture of the relationship between fish/shellfish diseases and natural as well as anthropogenic environmental factors. It was emphasised that there is a need to collaborate with other ICES Working Groups in order to conduct such a project (e.g., the WGBEC, WGSAEM, MCWG, WGMS, WGMDM, relevant Fisheries Working Groups under the ICES Living Resources Committee and the ACFM).

WGPDMO emphasised that, as a first step, a detailed overview of the suitability and compatibility of the data available in the ICES Databanks is required in order to identify those which can be included in the holistic analysis. T. Lang, W. Wosniok, A.D. Vethaak and S.W. Feist were identified to take over this task intersessionally and report to the WGPDMO at its 1999 meeting. Contact with other relevant ICES Working Groups should also be established in order to discuss further these actions. Contacts with the WGBEC and the WGSAEM will be established by A.D. Vethaak and W. Wosniok, respectively, who will attend the forthcoming meetings of the Working Groups in Mont-Joli, Quebec, Canada, in March 1998, and will represent the views of WGPDMO.

16.1 Conclusion

The possibility of establishing an ICES Study Group consisting of representatives from the ICES Secretariat and relevant ICES Working Groups should be considered when the conclusions from the overview report are presented to the WGPDMO in 1999.

16.2 Recommendations

WGPDMO recommends that:

ii) an overview report on the data available in the ICES Databanks, which may be of use for a holistic analysis in relation to fish/shellfish disease data, be produced intersessionally by W Wosniok, T Lang, AD Vethaak and SW Feist and presented to the WGPDMO at its 1999 meeting;

iii) relevant ICES Working Groups (Biological Effects of Contaminants; Statistical Aspects of Environmental Monitoring; Marine Chemistry; Marine Sediments in Relation to Pollution; Marine Data Management) and relevant Fisheries Working Groups under the Living Resources Committee and the ACFM, should be made aware, through the ICES Secretariat, of the plans for holistic data analysis and should provide comments and ideas on this project.

17 UPDATE ON THE CURRENT STATUS OF THE ICES IDENTIFICATION LEAFLETS FOR DISEASES AND PARASITES OF FISH AND SHELLFISH

17.1 Introduction

A thorough review of published Identification Leaflets for Diseases and Parasites of Fish and Shellfish (Fiches), those in press and proposed titles, revealed a disordered assemblage of important disease problems and passing observations (Annex 17). The information contained is of high quality, however, some Fiches are seriously out-dated and require review. Others maintain applicability to the goal of providing a concise summary of the information required to identify a disease or parasite problem. The list of published and in press Fiches is given in Annex 17, along with proposed titles and authors for near-future manuscript submissions.

17.2 Fiche Updating

Dependent upon the Publications Committee approval, the revision of out-dated Fiches will be initiated. The original authors, where available, will be approached to provide the update or suggest an alternative author. If revision of the Fiches is not possible, publication of updated Fiches, in addition to outdated versions, will be requested. This is especially important for diseases currently falling under regulated controls (e.g., *Bonamia ostreae* = 'Haemocyte disease of flat oysters' - # 18), updating diagnostic techniques, e.g., for bacterial infections such as 'Vibriosis in cultured salmonids' (# 29), providing distinguishing characteristics for similar, non-disease agents, and providing clearer and consistent titles, with common and scientific names of the host and infectious agent, where applicable.

17.3 Format

The format used for the Fiches is thorough and meets most objectives of the leaflets (provision of diagnostic aid), however:

i) 'gross clinical signs' and 'histopathology' need to be changed to reflect non-histopathological diagnosis and effects on the host which are not reflected as classic gross clinical signs. For example, 'gross clinical signs' could be changed to 'effect of infection and clinical manifestations' and 'histopathology' to 'diagnostic methods and features of infection'. This would permit inclusion of tissue-culture, new immnoassays and nucleic acid assays, as well as diagnosis of non-clinical infections, e.g., parasite infections.

ii) The 'comments' section varies significantly between Fiches and should concentrate on noting similar organisms, or other factors, which can confuse diagnosis.

iii) Key references also vary in detail and should be restricted to those useful for diagnostics.

17.4 Organisation

Publication in a series of ten, with no particular theme, or timeframe for release, appears responsible for the present disjointed collection. The following changes to publication organisation are, therefore, suggested:

1) all published (and revised) Fiches should be assigned a subject label, as well as a release number. The subject groups could be similar to those used by the WGPDMO, as shown in Annex 18. This will assist selection by people with narrow subject interests and organisation by general interest users;
2) An ICES binder with subject area dividers should be made available to help file the Fiches;
3) Titles 'in press' should be sent out to previous purchasers, pertinent journals (Bulletin of the European Association of Fish Pathologists, Journal of Fish Diseases, etc.) and Web Sites, with a deadline for orders, every two years or so (irrespective of the number of titles 'in press'). This will assist in deciding how many copies to print and the Fiches can be ordered when they are still up-to-date.

17.5 Title Selection and Relevance to Fiche Series

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

17.6 Conclusion and Recommendation

WGPDMO welcome and endorse the suggestions proposed by the new editor of the ICES Identification Leaflet for Diseases and Parasites of Fish and Shellfish, and recommend that the ICES Publication Committee take them into consideration.

18 ANY OTHER BUSINESS

The Chairman proposed that, to give Working Group members time for a proper preparation of the meeting, the working documents should be submitted to the Chairman for further distribution to the members not later than 12 February 1999. The proposal was approved unanimously by WGPDMO participating members.

19 ANALYSIS OF PROGRESS WITH TASKS

An analysis of progress in tasks in the Terms of Reference was conducted, as presented in Annex 19. All items had been dealt with in a comprehensive manner. Several intersessional tasks were identified during the meeting.

20 FUTURE ACTIVITY OF WGPDMO

There being 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 was required in 1999 to consider the results of intersessional work and to discuss outstanding items. It was agreed that the invitation to host the meeting from Dr C. Azevedo, University of Oporto, Portugal be accepted. The proposed dates are 1–5 March 1999; the full recommendation is contained in Annex 20.

21 APPROVAL OF RECOMMENDATIONS

The recommendations contained in this Report to ICES Council were discussed by WGPDMO and approved. The recommendations and justifications for recommendations to Council are added in Annex 20.

22 APPROVAL OF DRAFT WGPDMO REPORT

A draft copy of the report of the 1998 meeting was submitted to all WGPDMO members present before the end of the meeting and approved. 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.

23 CLOSING OF THE MEETING

On behalf of the participants of the 1998 meeting of the WGPDMO, the Chairman expressed appreciation to Professor D. Dutkiewicz for his hospitality and the excellent facilities provided for the WGPDMO in the Sea Fisheries Institute, Gdynia, to Dr W. Grygiel for his considerable organisation and support before and during the meeting and for much assistance from other members of staff of the Sea Fisheries Institute, which in no small way contributed to the success of the meeting.

The meeting was closed at 18.00 hours on 07 March 1998.

ANNEX 1

LIST OF PARTICIPANTS

Name	Address	Telephone	Fax	E-mail
Stig Mellergaard (Chairman)	Danish Institute for Fisheries Research, Stigbøjlen 4 DK-1870 Frederiksberg C Denmark	+45 31 35 27 67 (from 23 June 1998: +45 35 35 27 67)	+45 35 28 27 11	sme@dfu.min.dk
Juan Barja	University of Santiago Departamento de Microbiologia Facultad Biologia 15706 Santiago de Compostela Spain	+34 9 81 56 31 00	+34 9 81 59 69 04	mpaetjlb@usc.es
Felix Baudin - Laurencin	CNEVA Brest Lab. de Pathologie des Animaux Aquatiques B.P. 70 29280 Plouzane France	+33 2 98 22 44 62	+33 2 98 05 51 56	fbaudinl@ifremer.fr
Göran Bylund	Institute of Parasitology Abo Akademi University Artillerigatan 6 20520 Abo Finland	+358 2 21 54 301	+358 2 21 54 748	gbylund@abo.fi
Catherine Couillard	Institut Maurice-Lamontagne 850 Route de la Mer C.P. 1000 Mont-Joli, Quebec G5H 3Z4 Canada	+1 418 775 06 81	+1 418 775 05 42	CouillardC@dfo-mpo.gc.ca
Daniel Declerck	Rijksstation voor Zeevisserij Ankerstraat 1 8400 Oostende Belgium	+32 59 32 08 05	+32 59 33 06 29	ddeclerc@mail.dma.be
Wlodzimierz Grygiel	Sea Fisheries Institut ul. Kollataja 1 81-332 Gdynia Poland	+48 58 6202825	+48 58 6202831	
Andrey Karasev	Knipovich Polar Research Institute of Marine Fisheries and Oceanography 6, Knipovich Street 183763 Murmansk Russia	+7 815 2 47 37 21	+47 789 10 518 (Norwegian line)	paralab@pinro.murmansk.ru
Thomas Lang	Bundesforschungsanstalt für Fischerei Institut für Fischereiökologie Deichstrasse 12 27472 Cuxhaven Germany	+49 4721 38 034	+49 4721 53 583	
Sharon A. MacLean	NMFS/NOAA Northeast Fisheries Center Narragansett Laboratory 28 Tarzwell Drive Narragansett, RI 02882 U.S.A.	+1 401 782 3258	+1 401 782 3201	sharon.maclean@noaa.gov

Name	Address	Telephone	Fax	E-mail
Alasdair McVicar	The Marine Laboratory Victoria Road Aberdeen AB11 9DB Scotland, UK	+44 1224 29 56 05	+44 1224 29 56 20	mcvicara@marlab.ac.uk
Sharon McGladdery	Department of Fisheries and Oceans Canada Gulf fisheries Centre P.O.Box 5030 Moncton, New Brunswick Canada E1C 9B6	+1 506 851 2018	+1 506 851 2079	mcgladderys@mar.dfo-mpo.gc.ca
Harry Palm	Institut für Meereskunde an der Universität Kiel Düsternbrooker Weg 20 D - 24105 Kiel Germany	+49 431 597 39 55	+49 431 597 39 94	hpalm@ifm.uni-kiel.de
Dick Vethaak	RIKZ Ecotoxicology Section P.O. Box 8039 4330 EA Middelburg The Netherlands	+31 118 67 23 11	+31 118 65 10 48	a.d.vethaak@rikz.rws.minvenw.nl
Werner Wosniok	University of Bremen P.O.Box 330 440 Germany	+49 42 12 18 34 71	+49 42 12 18 40 20	werner@math.uni-bremen.de
Observers:				
Jerzy Rokicki	University of Gdansk Department of Invertebrate Zoology Al. Pilsudskiego 46 81-378 Gdynia Poland	+48 58 21 70 28	+48 58 62 02 165	biojr@paula.univ.gda.pl
Galina Rodjuk	Atlantic Research Institute of Marine Fisheries and Oceanography 5 Dmitry Donskoy Street 236 000 Kaliningrad Russia	+7 0112 22 53 69	+7 011 2 21 99 97	scomber@soram.com
Ewa Sobecka	University of Agriculture Division of Fish Diseases ul. K. Krolewicza 4 71-550 Szczecin Poland	+48 91 423 10 61 ext 225	+48 91 423 13 61	
Przemyslaw Myjak	Institute of Maritime and Tropical Medicine Department of Tropical Parasitology 81-332 Gdynia Poland	+48 58 622 30 11	+48 58 622 33 54	pemyjak@immt.gdynia.pl
Magdalena Podolska	Sea Fisheries Institute ul. Kollataja 1 81-332 Gdynia Poland	+48 58 6201728	+48 58 6202831	bilbo@mir.gdynia.pl
Jolanta Morozinska-Gogol	Sea Fisheries Institute Al. Zjednoczenia 1 81-332 Gdynia Poland	+48 58 621 70 21		

Name	Address	Telephone	Fax	E-mail
Leszek Rolbiecki	University of Gdansk Department of Invertebrate Zoology Al. Pilsudskiego 46 81-378 Gdynia Poland	+48 58 21 70 28	+48 58 62 02 165	rolbiecki@ocean.com.pl

ANNEX 2

TERMS OF REFERENCE

ICES C.Res.1997/2:24

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chairman: Mr S. Mellergaard, Denmark) will meet in Gdynia, Poland from 3–7 March 1998 to:

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

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

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

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

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

f) maintain an overview of the *Ichthyophonus* issue as part of its regular agenda and report to ACFM and ACME if new information becomes available;

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

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

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

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

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

l) define the scientific objectives of, and propose a strategic plan for, further statistical analyses of fish and shellfish data integrating other data types, e.g., contaminants, hydrographic and fisheries data, and report to ACME.

WGPDMO will report to ACME before its June 1998 meeting and to the Mariculture and Marine Habitat Committees at the 1998 Annual Science Conference.

ANNEX 3

AGENDA

1) Opening of the meeting.
2) ICES ASC 1997; items of relevance to WGPDMO.
3) Terms of Reference, adoptation of the agenda, selection of Rapporteurs.
4) Other Relevant reports for information.
5) Analyse national reports on new disease trends in wild fish, crustaceans and molluscs (TOR a).
6) Analyse national reports on new disease trends in mariculture for fish and shellfish (TOR b).
7) Review and analyse the data extracted from ICES Fish Disease Databank intersessionally and at the WG meeting (TOR c).
8) Review proposals for the development of a training and intercalibration programme for the diagnosis of histological liver lesions as part of a quality assurance scheme (TOR d).
9) Compile available evidence on the causes of the M-74 syndrome in Baltic salmon and provide a summary of the progress in understanding the relevant environmental factors influencing the occurrence of M-74 along with the geographical extent of its distribution (TOR e).
10) Maintain an overview of the *Ichthyophonus* issue as part of its regular agenda and report to ACFM and ACME if new information becomes available (TOR f)
11) Review available information on pathological aspects of endocrine disrupting chemicals in estuarine and marine organisms (TOR g).
12) Review intersessional progress in the development and implementation of a quality assurance plan for the integration of flatfish liver histopathology measurements into the OSPAR Joint Assessment and Monitoring Programme and in the adaptation of the ICES Fish Disease Data Entry Program and Fish Disease Reporting Format for the incorporation of liver histopathology data into the ICES Environmental Databank (TOR h).
13) Assess the progress in studies on the possible causes of *Bonamia ostrea* persistence in areas with low densities of oysters and the possible relationship to disease resistance and water temperature inhibition of infection (TOR i)
14) Prepare an information package on the ICES Environmental Databank and Disease Data Reporting Format for submission to laboratories in Member Countries identified as conducting shellfish disease surveys and/or diagnostics TOR j).
15) Review new progress in diagnostics, epidemiology and immunology of nodavirus and new nodavirus-like associated diseases (TOR k)
16) Define the scientific objectives of, and propose a strategic plan for, further statistical analyses of fish and shellfish data integrating other data types e.g., contaminants, hydrographic and fisheries data, and report to ACME (TOR l)
17) ICES Disease publications. Diagnostic Fiches update.
18) Any other business.
19) Analysis of progress with tasks.
20) Future activity of WGPDMO.
21) Approval of recommendations.
22) Approval of draft WGPDMO Report.
23) Closing of the meeting.

ANNEX 4

RAPPORTEURS

Session(s)	Rapporteurs
1–4. Introductory sessions	S. Mellergaard,
5. Wild fish/Wild shellfish	C. Couillard, D. Declerck,S. McGladdery, J. Barja
6. Farmed fish/Farmed Shellfish	F. Baudin-Laurencin, A. Karasev, A. McVicar,S. McGladdery, J. Barja
7. Statistical analysis of wild fish data	W. Wosniok, T. Lang, D. Vethaak,A. McVicar
8. Training and intercalibration programme for diagnosis of histological liver lesions	T. Lang, S. MacLean
9. M-74	G. Bylund, J. Rokichi
10. *Ichthyophonus*	A. McVicar, W. Grygiel
11. Endochrine disrupting chemicals	D. Vethaak, H. Palm
12. Quality assurance plan for liver histopathology	T. Lang, S. MacLean
13. *Bonamia oestrea* persistence	S. McGladdery
14. Information package on reporting to ICES databank	T. Lang, S. McGladdery
15. Nodavirus	F. Baudin-Laurencin, C. Couillard
16. Strategic plan for future data analysis	D. Vethaak, S. MacLean
17. Publications	S. McGladdery, H. Palm
18–21. AOB, Progress, recommendations	A. McVicar, T. Lang, D. Vethaak
22–23. Approval of report, closing	S. Mellergaard

ANNEX 5

SUMMARY REPORT ON THE INTERSESSIONAL STATISTICAL ANALYSIS OF THE ICES FISH DISEASE DATABANK

Werner Wosniok
Institute of Statistics
University of Bremen
Germany

Status of the Databank

This report refers to the analysis of the data available at the end of January 1998. At that time, the database contained information about a total of 424,998 fish submitted by 6 laboratories. Species considered were dab (*Limanda limanda*, n = 399,262) and flounder (*Platichthys flesus*, n = 25,736). The fish were sampled between 1981 and 1997 at positions within 131 different ICES statistical rectangles. The most frequently visited locations are

- 37F7 with 40 samplings / 178 data records from the time interval 1981–1997,
- 38F2 (32 samplings / 132 records, 1981–1997)
- 40F7 (28 samplings / 138 records, 1984–1997)
- 38F7 (24 samplings / 133 records, 1981–1992).

Samplings did not generally occur in equally spaced time intervals within the total sampling period. Depending on the particular situation, the number of fish examined varied from sample to sample. The number of fish within a size class and per sex was variable, classes are empty in some cases. For each sample, the following information is recorded (among others):

- geographical position
- date
- species
- size class (≤ 14, 15–19, 20–25, ≥ 25 cm)
- gender
- number of fish examined
- number with disease (for all labs: lymphocystis, epidermal papilloma, skin ulcers, skeletal deformations, liver nodules).

Statistical analysis

In two previous meetings, the SubGroup on Statistical Analysis of Fish Disease Data in Marine Fish Stocks had developed a scheme for the analysis of data from the Fish Disease Databank. This scheme consists essentially of a sequence of logistic regressions, where the members of this sequence increase in complexity by containing more and more variables (sex, size, season, location). The inclusion of interaction terms was considered, but in the 1997 analysis finally rejected as a consequence of the manifold imbalances in the data with respect to the distribution of sex, size, and sampling times. In that analysis, a general non-linear trend over time was found appropriate for most of the diseases, possibly with a location-specific level.

A corresponding analysis procedure was applied to the present data body. However, as the amount of data has increased considerably, a check of the previous approach with respect to appropriateness of modelling and variable selection had become possible and seemed advisable. While the incorporation of sex and size group as factors in the model is not a critical issue, the former assumption of a common trend for all locations, which was made mainly because the data did not allow a more detailed treatment, seemed to be a candidate for revision. Therefore, as a more sensible alternative, a completely non-parametric estimate for the trend was calculated individually for each location. This estimate was compared with the station-specific linear trend and a general non-station-specific linear trend (which was the kind of trend suggested by the 1997 analysis). The non-parametric approach means to allow for each station its individual trend, where 'trend' in the non-parametric case can be any smooth curve, i.e., it is not restricted to a straight line or simple low-order polynomials. The requirement that the trend be smooth is necessary to remove sampling fluctuation. A test was performed for each station in order to check whether non-linear trend components existed. A minimum of 5 time points is required to calculate a meaningful smooth trend.

By using a non-parametric component in the description of prevalence time series, the class of linear logistic models has been left and instead a 'Generalised Additive Model' was employed. Its use involves iteratively a combination of non-linear estimation techniques and the calculation of smoothing splines, together known as backfitting (Hastie and Tibshirani, 1991). The result of this model fitting procedure is most easily summarised by reporting the estimated parametric model components and test statistics, if such if exist, and by additionally plotting the finally calculated smooth functions. The calculations here were made using the SAS software for the analysis following the 1997 scheme, and by using the SPLUS software for the general additive modelling.

A non-smooth trend would be the observed time series of prevalences itself, which does not give any insight into the amount of random (meaningless) influences and also does not allow the assessment of the significance of any changes.

Results of statistical analysis (summary)

The Model fitted A general additive model was fitted to each station (ICES rectangle), with a smooth function for the trend, and size class and sex entering as factors. By performing this separately for each rectangle, it was implicitly assumed that an interaction (in the standard terminology) between station and size and station and sex may exist.

Trends The inspection of the individual non-parametric trends and the comparison with the linear logistic parameters, which were computed in parallel, showed that in many (not all) cases a non-parametric trend exists, which is not properly captured by the linear logistic approach. The non-parametric trends show a variety of shapes, which can roughly be classified into the categories:

- a local maximum, mostly around 1989/1990, with a flat horizontal form before and after that date, approximately constant or still decreasing around 1996/97 (example: lymphocystis in 35F3);
- a local maximum around 1989/90, increasing again since 1995 (example: lymphocystis in 37F7 and in 40F7);
- slowly increasing over many years;
- more than one maximum (example: lymphocystis in 37F6).

The shapes vary with location and depend on the disease.

The use of a linear logistic model can be misleading in the assessment of current trends. An example is lymphocystis in 40F7, where the linear trend is a practically horizontal line, which, besides ignoring the maximum in 1989/1990, gives no indication that since 1994 the prevalence has increased every year until the last sample (middle of 1997).

Prevalence levels A comparison of two or more locations to answer the question whether they have the same level of disease prevalence is only reasonable if their trend shapes are identical or similar. A comparison for a fixed point in time is always possible by comparing the prevalence predicted by the model, using estimated confidence intervals.

Gender and size Both factors have, in general, an effect on the disease prevalence. The size of this effect varies over stations and depends on the disease. They should consequently be reported individually for each station.

Displaying results

A bubble plot for each year, each station, each disease and one gender, one size class still seems to be an acceptable characterisation of stations, if the purpose is to describe the spatial distribution of prevalences. In the 1997 report, these plots were generated for females of size class 2. However, the amount of plots of this kind has increased considerably. It should be discussed whether this approach is still technically feasible and desirable.

A characterisation of stations with respect to their temporal trends could theoretically simply have the form of plotting all estimated trends (for one sex/size class) in one plot. This has the disadvantage that a large number of irregular curves will be overlaid and the additional inclusion of confidence limits is likely to obscure the picture. Several options should be discussed:

- plotting subgroups, defined by area;

- not producing all paper plots, but instead preparing single plots as WWW pages which could be downloaded by interested researchers and overlaid in arbitrary combination either by image processing software (xv on UNIX machines), or by printing individual curves on transparencies
- try a formal characterisation of trend shapes, define symbols for these and produce maps of them.

ANNEX 6

PROPOSALS FOR THE DEVELOPMENT OF A TRAINING AND INTERCALIBRATION PROGRAMME FOR THE DIAGNOSIS OF HISTOLOGICAL LIVER LESIONS AS PART OF A QUALITY ASSURANCE SCHEME

by Steve Feist
UK

The original concept of a Training and Intercalibration Programme (TIP) for the diagnosis of histological liver lesions as part of a quality assurance scheme was first proposed at the 1997 meeting of the ICES WGPDMO in Rhode Island. The CEFAS Weymouth laboratory (UK) was identified as the lead laboratory for this programme. A draft proposal was prepared by the co-organisers of the TIP and circulated for comment and to M Myers (USA) for advice on the lesion types to include and for the provision of reference material. It was expected that participating laboratories were to bear the costs associated with this programme, including attendance at Workshops. Subsequently, the TIP was incorporated into a European Commission application for funding within the ***Standards, Measurement and Testing (SMT) Programme****, under the title* ***'Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)'****. This proposal was supported by the ICES Advisory Committee on the Marine Environment (ACME) as the ideal means to coordinate the development and implementation of a wide variety of biological effects techniques. The application is currently being considered for funding by the Commission.*

The following is the liver pathology component of the BEQUALM application.

LIVER HISTOPATHOLOGY, LIVER NODULES AND EXTERNAL FISH DISEASES.

Work content

Biological effects monitoring programmes incorporating externally visible fish diseases have largely been co-ordinated through ICES activities, including sea-going workshops, and utilise the detailed procedures laid down in ICES publications (Bucke *et al.*, 1996). Similarly, quality assurance procedures have been developed and assessed by ICES and are now fully established for most aspects of fish disease monitoring. These aspects include: fish species to be monitored, sampling strategies, externally visible diseases and parasites suitable for monitoring purposes, grading systems for these diseases, monitoring of liver nodules, the requirement for histological confirmation of liver nodules by a reference laboratory, data reporting and data analysis. The quality assurance plan for the incorporation of fish diseases and liver pathology in OSPAR JAMP can therefore be considered as essentially complete.

- The current proposal recognises the need to produce a formal quality control structure to validate the data obtained from field studies which will involve the establishment of protocols for assessments of data submitted from participating laboratories.

The above quality assurance measures concentrate on externally visible fish diseases and macroscopic liver nodules but not the use of histopathology in the confirmation of macroscopic lesions or as an indicator of biological effects of contaminants. This issue was addressed at the ICES Special Meeting on the 'Use of liver pathology of flatfish for monitoring biological effects of contaminants', Weymouth, October, 1996. The report of this meeting provided detailed guidelines on suitable techniques for monitoring purposes including sampling strategies, processing of fixed samples, diagnostic criteria for liver histopathology and quality assurance requirements which now need to be developed and implemented.

In fulfilment of the above an intercalibration programme will be designed incorporating:

- The establishment of appropriate standards and reagents whose suitability has been assessed by the lead laboratory.
- The establishment of agreed descriptions of neoplastic and non-neoplastic liver lesions.
- To establish reproducibility between participating laboratories by the development of standard operating procedures (SOPs) for the various methodologies utilised including preparation of liver tissue for each of the analyses required, fixation and preservation, processing, quantification of histopathological/histochemical changes, archiving and reporting of data.
- The production of laboratory reference materials (LRMs). These will consist of sets of approximately ten stained tissue sections representing the major categories of liver lesion encountered in flatfish of importance for biological

effects monitoring. The reference sets will be accompanied by supplementary notes on the specific interpretation of each lesion and may include colour print-outs of representative features of each specimen to assist in identification of specific diagnostic features.

- The design and implementation of an intercalibration programme for diagnosis of liver lesions, including performance limits where practical, since pathological diagnoses are often based on consensus opinion rather than absolute criteria. Material collected during national monitoring programmes will be tested, with material to be supplied by the lead laboratory and at least one other. This will incorporate liver material for the confirmation of gross pathology and randomly selected livers from specific size groups of fish. Twelve participating laboratories from across Europe will be involved in the evaluation of the test materials which will be sent by post.
- Two workshops, in years 1 and 3 are proposed. The first will involve the establishment of protocols and practical exercises on diagnostic criteria. These will determine the degree of agreement in the interpretation of pathology between participants and enable agreed limits of acceptable variation in diagnostic reporting to be set. The main aim of the year 3 will be to assess the results obtained from earlier intercalibration exercises and also data obtained from national monitoring programmes
- Establishment of procedures for appropriate action in the event of consistent disagreements in diagnoses of particular lesions between participating laboratories.
- The production of a colour atlas of common histopathological liver changes, possibly using CD ROM technology.

Project milestones

Year 1

1) Establishment and implementation of an intercalibration programme for liver pathology diagnosis using material supplied by the lead laboratory.
2) Preparation of LRMs, including processed tissues, micrographs of relevant lesions and associated documentation.
3) Workshop for establishment of protocols, practical exercises on diagnostic criteria in order to determine degree of agreement in the interpretation of pathology between participants and to set in place agreed limits of acceptable variation in diagnostic reporting.

Year 2

1) Implementation of a full intercalibration programme based on sets of material collected during national monitoring programmes from at least two participating countries.
2) Discussion, commence selection of materials for production of an atlas of common histopathological lesions.

Year 3

1) Workshop for assessment of data obtained during national monitoring programmes
2) Production of an atlas of common histopathological lesions.
3) Announce availability of atlas and distribute to participants

Partnership details

The lead laboratories (S.W. Feist, CEFAS Weymouth Laboratory, UK, and T. Lang, Bundesforschungsanstalt für Fischerei, Institut für Fischereiökologie, Cuxhaven, Germany) have led fish disease monitoring programmes for the UK and Germany for many years using standardised methods established through ICES activities, and have in addition, played a leading role in the utilisation of liver pathology of flatfish as a biological indicator of contaminant effects. Most recently, culminating in the organisation of an ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants, Weymouth, UK, 22–25 October, 1996. Both laboratories have been heavily involved in the development of quality assurance programmes for these activities and together form a strong partnership to implement the current proposal.

Exploitation plans

The establishment of a fully effective quality assurance programme for external fish diseases, liver pathology and histopathology will form an essential component of national fish disease monitoring programmes. The procedures adopted will ensure that the data obtained which will be submitted to the ICES Environmental Databank will be of consistently high quality and fully intercalibrated between participating countries. This consistency in reporting is essential for the accurate interpretation of biological effects data which forms an important component of regional Quality Status Reports (QSRs) which in turn will be amalgamated into QSR 2000 for the whole OSPAR.

Convention area to be produced by the end of 1999. A major output from this component of the project will be the publication of an atlas of hepatic pathology, which will provide a ready reference for workers in the field. As a CD ROM version the costs of production would be minimised. It is also anticipated that relevant information could be made available on the Internet, provided it is clarified that this method of dissemination is appropriate.

ANNEX 7

PROGRESS IN RESEARCH ON THE M-74 SYNDROME IN BALTIC SALMON

G. Bylund and E. Lindesjö

Finnish and Swedish data on the prevalences of M-74 in 1997 (i.e., females spawned in the autumn 1996) are conflicting. Information from Swedish hatcheries on the Baltic coast indicate a very steep decrease in the prevalence from 1996 to 1997 with 68 and 28 % of the females, respectively, producing M-74 offspring. In contrast, the Finnish data from the river Simojoki, indicate that the prevalence of females with M-74 offspring as well as the yolk sac fry mortality has remained on the same high level for the last five years (see enclosed Diagram). Apparently, however, the disagreement, is due to different methods for calculating these prevalences. In Swedish hatcheries females with visible signs of M-74 (behavioural disturbances and pale pigmentation in muscles and eggs) are excluded from the broodfish material when spawned, the eggs from these are not taken into the hatcheries and are excluded from the calculations wheraes the Finnish data included all females.

Finnish investigations showed that the muscle tissue is the main thiamin reserve for the females. It was shown that bath-treatment of salmon eggs with thiamin gave a dose-dependent response. The preventive thiamin level for bath treatment is in the region 0.1–0,3%. Astaxantine injections given to females, significantly increased the astaxantine concentrations in eggs but had no influence on the survival of the yolk sac fry.

In a recent publication by Vuorinen *et al.* (1997) dioxin-like contaminants, especially 1,2,3,7,8 pentachlorodibenzofuran and 3,3',4,4',5-pentachlorobiphenyl (coplanar) were found to have a significant connection to the occurrence of M-74 in Baltic Salmon ascending for spawning in the Simojoki River on the north-east coast of the Gulf of Bothnia. Nevertheless, it should be stressed that it is not established if these contaminants are the primary cause for the development of the M-74 syndrome. Vuorinen *et al.*(1997) also suggested that changes in thyreoid hormone status and retinoids may play a role in the M-74 syndrome but the results in this respects are still inconclusive.

An impression on the topics in focus for M-74 research in Sweden is given in the enclosed lists on recent and fortcoming publications. The project coordinator for the Swedish national research program on M-74 (B.-E. Bengtsson) has produced a Web-site on M-74 which will be regularely updated and which can be directly picked up from the Internet-address:

http://w1.185.telia.com/~u18500254/m74eng.htm

It must be concluded that although intensive research is going on in order to explain the etiology behind the M-74 syndrome (and the equivalent EMS-syndrome in North America), no significant 'break-through' has happened in this research during 1997.

Although preventive thiamin treatment helps us to maintain the breeding programs for Baltic salmon, we do not fully understand the relevant environmental factors influencing the occurrence of this disease syndrome.

A LIST OF SOME RECENT AND FORTHCOMING ARTICLES ON M-74

Amcoff, P., Börjeson, H.,Eriksson R., Norrgren L. (1998) Effects of thiamin treatments on survival of M74-affected feral Baltic salmon. American Fisheries Soc. (in press)

Amcoff, P., Börjeson, H., Norrgren, L. and Pesonen, M. (1998) Cytochrome P4501A-activity in rainbow trout hepatocytes after exposure to PCB-126 and astaxanthin: In vivo and in vitro studies. Marine Env. Res. 46, 5

Amcoff P, Börjeson H, Lindeberg J. and Norrgren L (1998) Thiamine (vitamin B1) in feral Baltic salmon suffering from the M-74 syndrome. American Fisheries Soc. (in press)

Börjeson H and Norrgren L (1997) The M-74 syndrome: A review of etiological factors. Soc. Environ. Tox. Chem. SETAC ISBN 1-880611-19-8.

Lundström, J., Andersson, T., Berqwist, P-A., Börjeson, H., Förlin, L,. Gessbo, Å. and Norrgren L. (1998) Microinjection of lipophilic extracts from feral Baltic salmon roe predicted to develop M-74 in yolk-sac fry of farmed salmon (Salmo salar) and brown trout (Salmo trutta). Marine Env. Res. 46, 5

Lundström, J. Börjeson, H and Norrgren, L. (1998) Clinical and pathological studies of Baltic salmon yolk-sac fry suffering from M74. American Fisheries Soc. (in press)

Norrgren L, Amcoff, P, Börjeson H and Larsson P-O (1998) Reproduction disturbances in Baltic fish-A rewiev. American Fisheries Soc. (in press).

Vuorinen, P.J., J. Paasivirta, M. Keinänen, J. Koistinen, T. Rantio, T. Hyötyläinen & L.Welling (1997) The M-74 Syndrome of Baltic Salmon (Salmo salar) and Organochlorine Concentrations in the Muscle of Female Salmon. Chemosphere 34 (5–7): 1151–1166.

Vuorinen, P.J., Keinänen, M., Brouver, A., Vartiainen, T., Spenkelink, A. and Schipholt, I. 1997. Retinoids and thyreoid hormones and toxicant loads with the occurrence of the M-74 syndrome in Baltic salmon. In 'Proceedings. Third Finnish Conference of Environmental Sciences, Jyväskylä 1997. *Ambiotica* 1997.

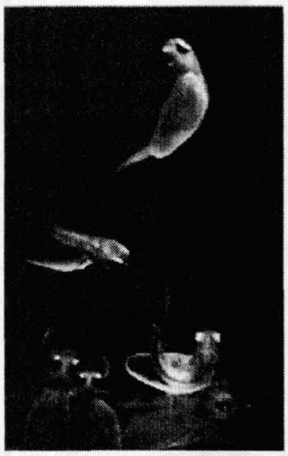
The Swedish 'FiRe' programme (Reproductive isturbances in Baltic fish) will publish a special issue with results from the M-74 research program in the journal AMBIO No 1 1999. The preliminary titels for this issue are:

1) Reproduction disturbances in Baltic fish: a synopsis of the FiRe project B.-E. Bengtsson, C. Hill, Å. Bergman, I. Brandt, N. Johansson, C. Magnhagen, A. Södergren and J. Thulin.
2) The Early Mortality Syndrome (EMS) in North American salmonids S.B. Brown, J. Fitzsimons, D.C. Honeyfield, and G. McDonald (and Sue Marcquenski?).
3) Histopathological studies of Baltic salmon yolk-sac fry (Salmo salar) that develop the M-74 syndrome. J. Lundström, H. Börjeson and L. Norrgren.
4) Physiological status of Baltic salmon yolk-sac fry (Salmo salar) suffering from the M-74 syndrome. J. Lundström, P. Amcoff, H. Börjeson, L. Förlin, A. Pettersson and L. Norrgren.
5) Reconditioning of female Baltic salmon (Salmo salar) that have produced offspring affected by the M-74 syndrome. H. Börjeson, P. Amcoff, B. Ragnarsson and L. Norrgren.
6) The diet of salmon (Salmo salar) in the Baltic Sea and connections with the M-74 syndrome. Lars Karlsson, E. Ikonen, A. Mitans and S. Hansson.
7) The connection between low astaxanthin levels and the M-74 syndrome in Baltic salmon (Salmo salar) A. Pettersson and Å. Lignell
8) The role of thiamine and thiaminase in the EMS-M-74 syndrome in salmonid fish. C. Haux.

ANNEX 8

RESEARCH ON THE M-74 SYNDROME IN THE BALTIC SALMON

Report from the Swedish FiRe-project, Dec. -97



by Bengt-Erik Bengtsson

http//w1.185.telia.com/~u18500254/m74eng.htm

Summary

The Swedish FiRe project was established in 1994 to study the M-74 syndrome in salmon (*Salmo salar*) and some other reproductive disruptions in Baltic fish. It is financed jointly by the Swedish Environmental Protection Agency, the National Board of Fisheries, the Swedish Council for Forestry and Agricultural Research, the World Wide Fund for Nature and the Power Board. The similarity between M-74 and EMS (Early Mortality Syndrome) in salmonids of the North American Great Lakes is striking and scientific cooperation has been established between researchers working with these two syndromes. Recent observations in the Great Lakes and the Baltic support a possible connection to dietary factors, such as high levels of thiaminase or low levels of thiamin in some food items. Treatment with thiamin (vitamin B1) has been proven to be efficient to alleviate the symptoms of both M-74 and EMS. Today all Swedish salmon hatcheries on the Baltic coast are treating egg and fry with thiamin, but the long term effect of this procedure is yet not known. The remaining wild spawning populations (representing 10 % of the smolt production) are not possible to treat with thiamin and are seriously threatened to be eliminated as a combination of M-74 and non-selective fishing.

THE M-74 SYNDROME

Increased fry mortality in Swedish salmon hatcheries

Feral Baltic salmon has suffered from increased fry mortality since the beginning of the 1970s (Figure 1). The symptoms were first discovered in the Bergeforsen salmon hatchery, river Indalsälven in 1974. The symptoms were named M-74 ('environmentally related disease 1974'). In order to better control egg and fry mortality the Swedish hatcheries are now separating the offspring of every single female. The symptoms occur during the phase of yolk-sac resorption of the fry development, i.e., before the stage when it starts to take external food items. The fry becomes apathetic and demonstrates only weak or no escape reactions (which are normal for the healthy fry). A darker pigmentation, hemorrhages close to the heart, swollen yolk sac (sometimes also with white precipitates) and exophtalmia ('pop-eye') are typical symptoms and death usually occurs already within a few days after the first external symptoms were seen.

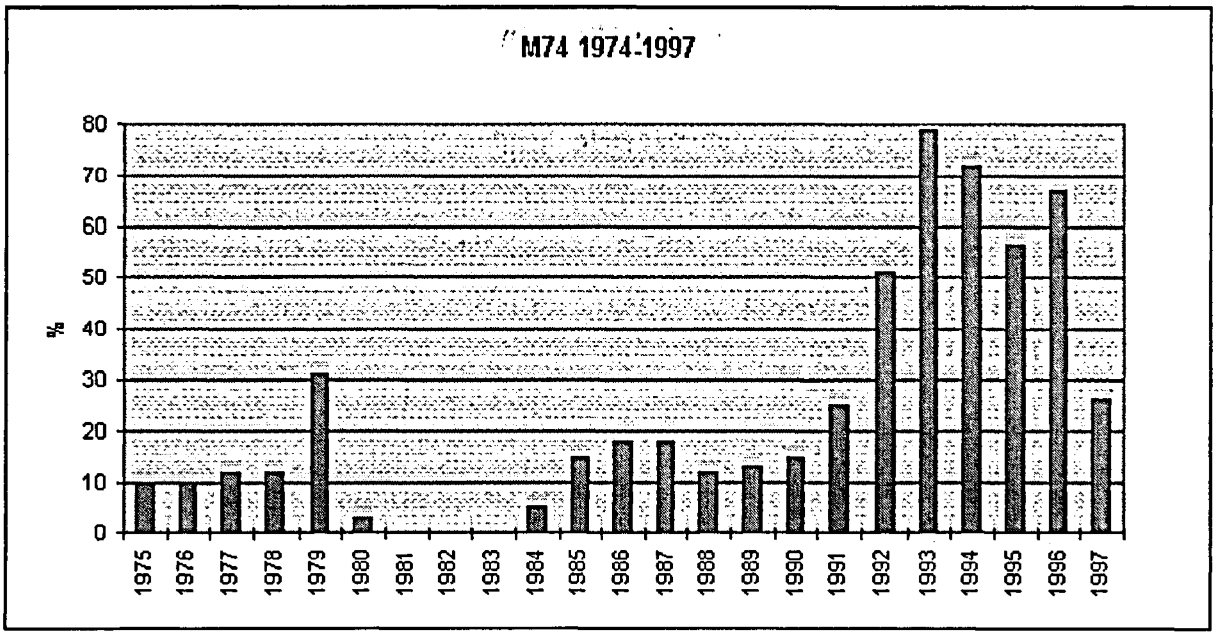


Figure 1. Number of Baltic salmon (*S. salar*) females producing offspring developing the fatal M-74 syndrome in Swedish hatcheries in 1974 to 1997 (Hans Börjesson, pers. comm).

The M-74 syndrome does not occur among salmon on the Swedish west coast and in the great lake Vänern. Baltic Sea salmon kept in cages (and fed commercial feed) for years for the purpose to serve as parental fish (e.g., as generally applied in Finland to support their hatcheries with parental fish) have also been free of symptoms. This points to the conclusion that there is a factor in the Baltic Sea that causes the problem. However, hatcheries in Latvia has not confirmed the existence of M-74 in their salmon populations and it remains to be investigated to what extent Latvian salmon populations have a different migratory and feeding pattern.

For a number of rivers there has existed a rather good correlation between the catch of potential spawners in the sea and the number of new fish (0+) in the rivers the following season. In the years 1992 and 1993, however, this correlation was broken for the wild spawners. No other factors, such as water flow, water temperature, presence of older stages of trout or salmon (as predators and competitors) could explain the losses of offspring, which were in the order of 60 to 90 % for this period. This further contributes to the suspicion that M-74 is a serious threat also to the wild salmon populations. The frequency of M-74 is still very high and the average for Swedish hatcheries by the Baltic Sea coast was 68 % in 1996 but decreased to 28 % in 1997 (Figure 1).

Initially the M-74 syndrome was observed only in Baltic salmon, while the sea trout (*Salmo trutta*) living in the Baltic and spawning in the same rivers as the affected salmon did not demonstrate any similar effects. In the last two/three years, however, there are indications from some Swedish and Finnish hatcheries that also the Baltic sea trout may be developing a syndrome very similar to M-74 (Nils Johansson, Antti Soivio, pers. comm.).

The present research situation

Besides the need to currently monitor the development of wild salmon populations and the records from the hatcheries of the Baltic salmon a Swedish research program was established in 1994: Reproduction Disturbances in Baltic Fish (the FiRe project). The FiRe project is financed jointly by the Swedish Environmental Protection Agency, the National Board of Fisheries, the Swedish Council for Forestry and Agricultural Research, the World Wide Fund for Nature and the Power Board. The project will run until mid-1998. The Nordic Council of Ministers (NMR) is also supporting Nordic scientific collaboration on fish reproduction disturbances including M-74 from 1995 to the end of 1997 (the REDFISH project).

Since M-74 was discovered there has been a suspicion of a possible connection with the presence of environmental pollutants and in particular chlorinated organic compounds, such as PCBs and chlorinated dioxins. The levels of such compounds tend to be elevated in salmon females who produce offspring that develop M-74, but there are so far no significant differences established in the Swedish analytical records, in spite of several attempts to find a correlation between these substances and the occurrence of M-74. It also deserves to be mentioned that the occurrence of these substances in the Baltic has been reduced during recent years (Mats Olsson, pers. comm.) and does not covary with the occurrence of M-74 (as seen in Figure 1). Results from Finnish studies (Vuorinen et al.,1997), however, point to a significant correlation between planar PCBs in salmon females and the occurrence of M-74 in the offspring. The activity of some 'detoxification systems' in the liver (the cytochrome P450 system or EROD-activity) that are frequently used as biomarkers for organic pollutants indicates that fry suffering from M-74 and their mothers have a higher toxicant load than normal fish. It deserves to be mentioned in this context, however, that a correlation is not necessarily equivalent to a proven cause.

Studies have so far not found any correlation between M-74 and heavy metals, parasites, bacteria or virus. The fact that it has not been possible to infect healthy fry with M-74-stricken fry points to that M-74 is a non-contagious phenomenon. The presence of e.g., retro-virus in M-74 material is, however, being investigated.

There is a strong correlation between salmon roe pigmentation and the occurrence of M-74. A comparison of the roe pigmentation in 1977 and 1992/1993 demonstrated a reduced pigmentation over that period (Hans Börjesson, pers. comm.). The pigmentation is caused by various carotenoids (e.g., astaxanthin) and roe with a high (normal) level of pigmentation is dark red-orange and roe with weak pigmentation is palish yellow. The M-74 mortality in pale roe has increased and today the roe pigmentation is a useful marker for the prognosis of M74. This has been known by hatchery people for many years and pale roe has been discarded as far as possible. Low pigmentation may be a sign of high load of (chlorinated?) organic pollutants since the pigment, i.e., the carotenoids, are metabolized by the same cell system that that is activated by some organic compounds (i.e., the cytochrome P450 system). Another and perhaps contributing explanation to the roe pigmentation is the selection of food items or what they in their turn are eating. Such relationships are presently being investigated in two studies financed by the FiRe project.There seems to exist a relationship between the occurrence of M-74 and the biomass of a high priority food item of the Baltic salmon, e.g., the sprat, *Sprattus sprattus* (Hans Börjesson and Gunnar Anéer, pers. comm.). Many professional fishermen are also of the opinion that the salmon flesh becomes 'pale' when sprat dominates in the food intake over Baltic herring, *Clupea harengus*. Among abiotic factors that seem to covary with M-74 is the winter temperature in the southern Baltic sea, i.e., high winter temperature seems to result in high levels of M-74 two years later.

Status of the Baltic Sea

The Baltic Sea environment is under heavy load of nutrient effluents together with known, less known and unknown toxic compounds. There are several indications of that the reproduction of other fish species in the Baltic sea is affected as well (Bengtsson et al. 1994; Johansson et al., 1995), e.g., cod (*Gadus morrhua)*, burbot (*Lota lota*) and perch (*Perca fluviatilis*), in spite the fact that the levels of DDT and PCB have decreased in Baltic Sea fish in recent years (Bignert et al., 1993). From earlier studies the negative effects of these substances have been well described for seals and birds, but for these organisms the situation has stabilized and possibly also improved (Blomqvist et al., 1992).

There are also suspicions that the whole ecosystem of the Baltic Sea is changing (Bengtsson et al., 1994) resulting in indirect effects (M74) on the salmon. There are accordingly suspicions that that the production of plankton carotenoids is decreased by increased eutrophication of the Baltic sea and that the food chain has been shifted for the fishes that serve as food for the Baltic salmon. There are also those who claim that a contributing factor to the occurrence of M-74 is the high ratio of hatchery-reared salmon over wild salmon, e.g., loss of genetic diversity.

EMS in the Great Lakes - a parallel phenomenon

In the early 1970s, American Great Lakes salmonids reproductive disturbances were correlated to chlorinated organic compounds, i.e., planar PCBs and chlorinated dioxins. Today's situation in Great Lakes salmonids is still bad (e.g., lake trout, *Salvelinus namaycush*, mortality is about 80 % in Lake Ontario) but there is no longer a clear correlation with pollutants (Phil Cook, in AFS, 1996). Instead the situation seems to be of a dietary origin and the symptoms (called EMS, the Early Mortality Syndrome) are today much more similar to the M-74 syndrome (more correctly, M-74 should be considered as just a variety of EMS). An intensive exchange of information has been established between the FiRe-project and scientists working on the EMS in Canada and USA.

EMS in Great Lakes and the Baltic Sea (M-74)

species	location	max. mortality
coho salmon	Lake Michigan	70 %
chinook salmon	Lake Michigan	60 %
steelhead	Lake Michigan	35 %
lake trout	Lake Ontario	80 %
Atlantic salmon	Finger Lakes	100 %
Baltic salmon	Baltic Sea	90 %
sea trout	Baltic Sea	confirmed

It has been known for some time that an important food organism for salmonids suffering from EMS, the alewife (*Alosa pseudoharengus*) contains high levels of thiaminase, an enzyme that inactivates thiamin (vitamin B1). These observations and laboratory experiments (first by Fitzsimons, 1995) have also resulted in a successful thiamin treatment (single or repeated bathing of egg or fry in thiamin) of M-74 and EMS stricken fry in US, Canadian, Swedish and Finnish hatcheries. The results are promising, but it is yet too early to judge whether the treated fry will result in viable and fertile parental fish later on. Another problem is the fact that only hatchery reared fish can be treated, but that the wild spawners might vanish successively as a result of untreated M-74 and indiscriminate fishing of hatchery reared and wild salmon in the open Baltic sea. In that way 'the cure' for the hatchery reared salmon may be 'the kill' for the wild salmon.

Causes?

As a result of the US/Canadian, Finnish and Swedish investigations three common hypotheses for the development of EMS/M-74 have been formulated:

- diet deficient in thiamin?
- diet rich in thiaminase?
- diet contains a contaminant interfering with thiamin dynamics?

All three hypotheses are today under investigation in the Swedish FiRe project to find an explanation to the M-74 syndrome in the Baltic salmon.

EARLY MORTALITY SYNDROME (EMS) and M74

Definition

'Excess mortality from eyed egg through to first feeding which cannot be explained by rearing environment, husbandry or infectious disease'

EMS/M-74

- develops when yolk sac absorption is well advanced
- symptoms develop very rapidly
- hyperexcitability
- lethargy, laying on tank bottom or surface
- spiral or side swimming
- dark coloration
- anemia
- ruptured blood vessels
- yolk sac and pericardial oedema
- yolk opacity
- feeding difficulties

Cause(s):

Three hypotheses:

- diet deficient in thiamin?
- diet rich in thiaminase?
- diet contains a contaminant interfering with thiamin dynamics?

Evidence

- correlation between egg thiamin content and EMS incidence
- adults, expressing EMS in eggs, are exhibiting:
- lower thiamin levels in liver (lake trout)
- symptoms of a neurological disorder consistent with a thiamin deficiency.

LITERATURE

AFS (1996) Early Mortality Syndrome: Reproductive Disruptions in Fish of the Great Lakes, New York Finger Lakes, and the Baltic Region. Symposium as part of 126th Annual Meeting of the American Fisheries Society, August 26–29, 1996, Dearborn, Michigan, USA. Abstract volume, 172 pp.

Bengtsson, B.-E., Bergman, Å., Brandt, I., Hill, C., Johansson, N., Södergren, A. and Thulin, J. (1994). Reproductive Disturbances in Baltic Fish. Research Programme for the Period 1994/95–1997/98. Swedish Environmental Protection Agency Report 4319. 30 pp.

Bengtsson, B.-E., Hill, C. and Nellbring, S. (1996) Report from the Second Workshop on Reproduction Disturbances in Fish, 20–23 Nov. 1995, Lidingö, Stockholm, Sweden. Swedish Environmental Protection Agency Report No. 4534. 114 pp.

Bignert, A., Göthberg, A., Jensen, S., Litzén, K., Odsjö, T., Olsson, M. and Reutergårdh, L. (1993). The need for the adequate biological sampling in ecotoxicological investigations: a retrospective study of twenty years pollution monitoring. Sci. Total Environ. 128: 121–139.

Blomqvist, G., Roos, A., Jensen, S., Bignert, A. and Olsson, M. (1992). Concentrations of DDTs and PCB in seal from Swedish and Scottish waters. Ambio, 21(8): 539–545.

Fitzsimons, J. D. (1995). The effect of B-vitamins on a swim-up syndrome in Lake Ontario lake trout. Journal of Great Lakes Research 21 (Suppl. 1): 286–289.

ICES (1994) Report of the Study Group on Occurrence of M-74 in Fish Stocks, Copenhagen, Denmark, 1–3 March 1994. International Council for the Exploration of the Sea, C.M. 1994/ENV:9, 23 pp.

Johansson, N., Jonsson, P., Svanberg, O., Södergren, A. and Thulin, J. (1995). Reproduction Disorder in Baltic Fish: research enquiry. Swedish Environmental Protection Agency Report No. 4347. 33 pp.

Vuorinen, P.J., J. Paasivirta, M. Keinänen, J. Koistinen, T. Rantio, T. Hyötyläinen & L. Welling (1997) The M-74 Syndrome of Baltic Salmon (Salmo salar) and Organochlorine Concentrations in the Muscle of Female Salmon. Chemosphere 34(5–7): 1151–1166.

Updated 98.01.16

ANNEX 9

PREVALENCE OF *ICHTHYOPHONUS* IN HERRING (*CLUPEA HARENGUS*) IN SCOTTISH WATERS DURING 1997 DETERMINED FROM RESEARCH VESSEL AND COMMERCIAL CATCHES

A.H. McVicar

1 Introduction

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) has been requested, as part of its regular tasks, to monitor the levels of the parasitic infection *Ichthyophonus* in herring stocks in the ICES area, with the objective of being able to advise ICES of early changes in prevalence, possibly leading to a recurrence of the epizootic which occurred in the early 1990s. ICES C.Res.1997/2:24(f) refers to the current request by ICES.

2 Materials and Methods

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

3 Results

From a total of 5,773 herring examined from commercial catches, none showed evidence of infection.

Month	Area IVa	Area IVb	Area VIa	Clyde
1–3	-	-	0/88	0/94
4–6	0/444	0/265	0/60	0/105
7–9	0/2160	0/449	0/1787	-
10–12	-	-	0/137	0/184

4 Discussion and Conclusions

Ichthyophonus was not detected in North Sea herring stocks in Scottish waters, in 1997.

5 Acknowledgements

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

Ichthyophonus in herring (data collected by PINRO, Murmansk, Russia)

Andrey Karasev

	Year	Russian method	ICES method
Norwegian Sea	1996	82,9	7,5
	1997	17,1	12,7
Barents Sea	1996	26,7	3,3
	1997	16,9	3,1

Summerspawning herring from Icelandic waters examined for *Ichthyophonus* in 1997:

Jonbjorn Palsson
Marine Research Institute
Reykjavik, Iceland

1 From research vessels (midwater trawl):

Number examined: 650

Number affected: 0

2 From fishing vessels (purse seine):

Number examined: 689

Number affected: 1

Total number examined: 1339

Total number affected: 1

Prevalence (%): 0.07

These results indicate an unchanged situation from 1996. The prevalence in the Icelandic summerspawning herring (25 + cm) has ranged from 0.11 to 0.24 % since the monitoring started in 1992, and it still remains very low

Atlanto-Scandian herring caught by Icelandic research vessel in May 1997 (midwater trawl) and examined for *Ichthyophonus*:

Total number examined: 1300

Total number affected: 31

Prevalence (%): 2.38

This also indicates a similar situation as in 1996 when the prevalence in the Atlanto-Scandian herring (25 + cm) was found to be 1.93%.

Polish investigation of Baltic herring for the prevalence of *Ichthyophonus*

P. Myjak, W. Grygiel, J. Rokicki

In 1991, the epidemic spread of a disease among herring, caused by *Ichthyophonus*, was observed in Europe (Anon. 1991, Hjeltnes and Dankert 1992, Korsbrekke et al. 1992, Lang 1992, Thulin and Hoglund 1992). The herring mortality rates were as high as 54%. The largest quantities of infected fish were observed in the North Sea, along the coast of Norway, in Skagerrak and Kattegat, and in the west part of the Baltic Sea (Anon. 1992, 1994). It was revealed that the fungus-infected herring were also infected with *Anisakis simplex* larvae (Thulin 1992 and 1994, unpubl.). The analyses performed on central and southern Baltic herring have shown that the infection rates in 25–29 cm length-class fish were low and not larger than 1.4%. Yet, no traces of disease were found in fish caught in the northern Baltic, including the Gulf of Finland (Anon. 1992).

In the years 1992–1994, and in April 1996, and February 1998, in total 6876 Baltic herring from the ICES Subdivision 26 (the Gdansk Basin) were analyzed for the presence of *Ichthyophonus*. Investigations were performed by the Institute of Maritime and Tropical Medicin in Gdynia, the Sea Fisheries Institute in Gdynia and University of Gdansk. The samples of fish were taken from the commercial catches, landings and research surveys, performed within the Polish EEZ of the Baltic Sea. The fish were divided into length-classes, following the recommendations of the ICES WGPDMO (Anon. 1992). In none of the fish examined the macroscopic signs, indicative of the disease caused by the fungus in question, were found. Neither were the hyphae or spores identified in the microscopic specimens prepared from the heart tissue of herring infected with *Anisakis simplex* larvae (Table 1). The hearth and other organs of each fish were examined macroscopically for the presence of *Ichthyophonus*.

The scientific research, conducted by the Institute of Maritime and Tropical Medicin in Gdynia in the years 1992–1994, consisted mainly of standardized macroscopic examinations of the heart muscle and did not resulted in detection of the fungus in any of the herring examined. Although, the fungus was found in herring and sprat from coastal Estonian waters (Turovsky et al. 1992). Since the late 1970s, *Ichthyophonus* has been detected as single cases in eelpout in the Puck Bay (Gras-Wawrzyniak et al. 1979).

The lack of fungal infection in herring from the ICES Subdivision 26 can be explained as follows:

a) the epidemic has not reached the central and southern Baltic;

b) the presented scientific investigations have been initiated during the period of decreasing epidemic (ICES 1994);

c) because of high mortality rates among the infected fish, they might have not migrated as far east, despite the fact that the percentage of herring infested with *A. simplex* larvae, which is- 2 - indicative of fish coming to spawn from the North Sea and Skagerrak, had been systematically increasing f or the past few years.

Table 1. The results of herring analyses performed in 1992–1994 for the presence of *Ichthyophonus*.

Length-class [cm]	Male	Female	Not determined	Total	Mean length [cm] ### SD	Type of analysis macro- micro- scopic scopic		Prevalence [%]
< 15.0	2	1	0	3		3	0	0
15.0–19.5	157	153	0	310	18.8 ± 0.86	310	0	0
20.0–24.0	1247	1625	3	2875	22.3 ±1.26	2875	20	0
> 24.0	701	1004	8	1713	25.6 ±1.12	1713	123	0
total	2107	2783	11	4901		4901	143	0

References

Anon. 1991. Special meeting on the *Ichthyophonus* problem in the European herring held at the Institute of Marine Research, Lysekil, Sweden, November 7, 1991.

Anon. 1992. Evaluation of the impact of *Ichthyophonus* spp. on affected herring stocks, including extent of mortality. ICES WGPDMO Meeting in Copenhagen, March 1992.

Anon. 1994. Herring. Assessment Working Group for the Area South of 62°N. ICES C.M. 1994/Assess:13, Copenhagen 21–31 March, 1994.

Hjeltnes, B., D. Skagen. 1992. *Ichthyophonus hoferi* disease in the herring in Norwegian waters. ICES C.M. 1992/H:27, P.F.C.

Lang, T. 1992. Results of macroscopical examination of the occurrence of *Ichthyophonus* spp. in herring (*Clupea harengus*) of the Baltic and North Sea. ICES C.M.1992/F:7, Sess.O.

Korsbrekke K. et. al. 1992. Macroscopic organ manifestations and reduced condition factor in herring with *I. hoferi* disease: field studies and analysis of historical data. ICES C.M.1992/F:9, Sess.O.

Thulin, J., J. Hoglund. 1992. Status report from Sweden. Working document of the ICES WGPDMO Meeting, March 1992.

ANNEX 10

REVIEW OF AVAILABLE INFORMATION ON PATHOLOGICAL ASPECTS OF ENDOCRINE-DISRUPTING CHEMICALS IN ESTUARINE AND MARINE ORGANISMS, WITH REMARKS ON REQUIREMENTS FOR FUTURE MONITORING PROGRAMMES

A.D. Vethaak

Introduction

The endocrine system includes several feedback pathways between the central nervous system, the pituitary and the target organs. These pathways are concerned with the regulation of different metabolic functions and in the maintenance of homeostasis. They also have a role in growth regulation and reproduction. Reproduction in all vertebrates is regulated by a complex system of hormones, known as classical hormones. Hormones with similar functions, such as ecdysone, are found in several invertebrate groups. As well as sexual development, hormones also play a critical role in brain development and immune system function. Thus the effects of endocrine-disrupting chemicals (EDCs) on populations of estuarine and marine organisms are unlikely to be confined to reproduction. They may also include, for example, abnormal behaviour, altered immunomodulation and increased risk of developing cancer.

In this review, EDCs are broadly defined, following the 1996 European workshop on the impact of endocrine disrupters on human health and wildlife, held in Weybridge (UK). They are defined as exogenous substances that cause adverse health effects at the whole-organism level as a result of changes in endocrine function. Thus all potential effects arising from endocrine disruption are considered. These effects include, but may not be restricted to:

Effects via mediation of specific hormone receptors (oestrogen, androgen, progesterone and thyroid hormone receptors).

Effects through interference with hormone metabolism.

Effects through interference with feedback mechanisms involving the hypothalamus or pituitary gland.

Direct effects on endocrine organs.

Receptor-mediated effects, particularly those involving the oestrogen receptor, have received most attention in the literature. However, other proposed mechanisms such as altered steroid metabolism are also important. An example of the latter is the inhibition of P450 aromatase by tributyltin oxide (TBTO) as a cause of imposex in marine gastropods.

The study of EDCs poses several problems. Firstly, although the majority of known EDCs are persistent and bioaccumulative, they are often excluded from routine monitoring programmes because of a lack of adequate analytical methods. Secondly, most of the EDCs actually present in the environment seem to be unknown compounds, concentrated in particular in the hydrophilic fraction of samples. The use of in vitro oestrogen-receptor assays indicates that the greater part of the measured response cannot be explained in terms of conventionally identifiable chemicals samples (Legler et al. 1997). Thirdly, EDCs with similar modes of action may have synergistic, cumulative effects. Fourthly, the critical period for exposure is often in the very early stages of the organism's life. Lastly, the effects of EDCs may be manifested at very low concentrations (ppt level or lower).

Specific sources of EDCs in the marine environment include:

Estuarine discharges (e.g., oestrogenic discharges in UK and Dutch estuaries).

Offshore oil and gas installations (e.g., certain polyaromatic hydrocarbons (PAHs), nonylphenol (NP)).

Ships (e.g., TBT).

Aerial deposition (e.g., certain pesticides, toxaphenes).

Literature review

An overview of pathological and non-pathological changes associated with exposure to EDCs is given in Table 1. Published work suggests an increase in the number of observed or suspected instances of endocrine disruption in recent years. There are recent reports of effects on a wide range of freshwater, estuarine and marine animals, including mammals, fish and molluscs. Most instances have apparently been discovered by accident and appear to be local effects. An exception may be the worldwide occurrence of imposex in marine snails, caused by organotin compounds.

Observed effects associated with EDCs can be broadly categorised as follows:

Decreased fertility in marine mammals, fish and molluscs.

Demasculinisation and feminisation in fish.

Defeminisation and masculinisation in fish and gastropods.

Decreased hatching success in fish.

Abnormal thyroid function in marine fish and seals.

Altered immune function in marine mammals and fish.

Liver tumours and skin lesions in fish.

Most of the above involve associations or circumstantial evidence rather than proven causal relationships. A problem with the interpretation of field observations, in particular, is that causal relationships cannot be proven because other factors could be involved. These factors include other environmental contaminants, as well as environmental and host-related stress factors.

It should be noted that, strictly speaking, not all the conditions listed in Table 1 can be considered as pathological disorders. The population consequences of several observed effects are still unknown. For example, the significance of increased vitellogenin concentrations in the blood of male fish and of intersex in fish (see Table 1 for details) is not yet fully understood and requires further investigation. The background levels of intersex are not known, nor is the extent to which affected males remain functionally male. Recent studies, however, indicate that high vitellogenin levels may lead to kidney failure and death in rainbow trout(Nimrod and Benson, 1996). They may also lead to decreased testicular growth and inhibition of spermatogenesis (Jobling et al. 1996).

The nature of the observed effects, together with the fact that they seem to be occurring with increasing frequency, underlines the potential risks posed by EDCs for marine ecosystems, fisheries and human consumers.

Future monitoring

When considering monitoring requirements, it is useful to start with the recommendations of the workshop on Endocrine Modulators and Wildlife: Assessment and Testing (EMWAT), held in Veldhoven (Netherlands) in April 1997 (Tattersfield et al. 1997). The workshop proposed a general monitoring programme involving screening surveys to determine the general health status of wildlife populations (Vethaak et al. 1997).

In accordance with the workshop recommendations, the current ICES monitoring programme on fish diseases should be continued and extended to include the following:

Reproductive and developmental disorders.

Relevant reproductive end-points, such as the gonado-somatic index and general histological assessment of gonadal tissues. The latter would allow the occurrence and prevalence of phenomena such as intersex and hermaphrodism to be quantified in the target populations.

Population-related parameters such as age structure, abundance and sex ratios.

Suitable biomarkers of EDC effects, such as plasma vitellogenin concentration as a measure of the effects of oestrogenic compounds.

Immunological methods, in order to establish the immunological status of the target populations (e.g., Boonstra et al. 1996; Vos et al. 1996).

In addition, the use of computer-assisted sperm analysis (CASA), as recently developed for measuring pollution effects on sperm quality in freshwater fish (Kime, et al. 1996), should be investigated.

New target species should also be chosen. They should include both fish and invertebrate species, covering a variety of different lifestyles and levels in the food chain. Where possible, several different stages in the life cycle of each species should be investigated.

Table 1: Effects associated with exposure of estuarine and marine animals to endocrine-disrupting chemicals

Marine mammals

High incidence of premature births, associated with unusually high concentrations of DDT-like compounds (sea lion; California) (Delong et al. 1973; Addison, 1989).

Low reproductive success and reproductive disorders, associated with high lipid concentrations of polychlorinated biphenyls (PCBs) (seal; Baltic Sea) (helle et al. 1976a,b).

High degree of skull asymmetry indicative of disrupted development, attributed to pollution (grey seal; Baltic Sea) (Zakharov and Yablokov, 1990).

Poor reproductive performance, associated with immunosuppression and presence of PCBs and polyhalogenated aromatic hydrocarbons (PHAHs) in body fat and food (common and grey seal; Baltic, North and Wadden Seas) (Reijnders, 1996; Brouwer et al. 1989; Reijnders and Brasseur, 1992; De Swart et al. 199..1994; Ross et al. 1995).

Decreased testosterone levels, associated with high concentrations of PCBs and DDE in blubber (Dall's porpoise; northwest Pacific) (Subramanian et al. 1987).

Occurrence of thyroid and adrenal cortex lesions, hermaphroditism and reproductive disorders (beluga whale; Martineua et al. 1988).

Contamination-related immunosuppression (striped dolphin; Mediterranean Sea) (Scott et al. 1988).

High prevalence of neoplasms and frequent infection by mildly pathogenic bacteria, indicating contamination-related-immunosuppression (beluga whale; Lahvis et al. 1995; De Guise et al. 1995).

Fish

Occurrence of testicular abnormalities (flounder; UK estuaries).

Elevated vitellogenin levels and testicular abnormalities, associated with pollution (flounder; UK estuaries) (Allen et al. 1997).

Elevated blood vitellogenin in female fish, associated with pollution (flounder; Scottish estuaries) (Lye et al. 1997).

Elevated blood vitellogenin in female fish, associated with estuarine pollution (winter flounder; Boston harbour) (Pereira et al. 1992).

Premature vitellogenesis and reduced Vitamin A levels resulting from exposure to polluted dredged spoil in mesocosms (flounder; the Netherlands) (Janssen et al. 1997; Besselink et al. 1996, 1997).

Increased vitellogenin levels in the blood of caged male fish (rainbow trout; UK estuaries) (Harries et al. 1995, 1996).

Increased vitellogenin levels in the blood of male fish (flounder; UK and Dutch estuaries) (Allen et al. 1997; Allen, unpubl. results).

Occurrence of intersex at high prevalence in male fish (flounder; certain UK estuaries, but apparently absent from the Netherlands) (Feist et al. in press; Vethaak, unpl. results).

Various reproductive effects (precocious maturation, reduced reproductive output, reduced hatching success, and reduced larval growth and survival), associated with increased concentrations of lipophilic xenobiotics (cod; Baltic Sea) (Peterson et al. 1997).

Sterility, associated with high levels of plastic constituents and natural hormones in sewage outflows (trout; UK rivers) (Jobling and Sumpter, 1992; Jobling et al. 1996).

Reduced reproductive success and larval mortality (eelpout; Baltic Sea) (Draganik et al. 1995).

Occurrence of thyroid disorders, reduced fertility and reduced embryonic survival (salmon; Great Lakes) (Moccia et al. 1981; Leatherland,1992).

Masculinisation of appearance and behaviour of female fish, apparently caused by the concentrated mixture of phytosterols (from trees) and chlorine in pulp-mill effluent (mosquito fish; Howell et al. 1980, 1989; Davis and Bartone, 1992)).

Altered serum steroid levels, associated with exposure to bleached Kraft mill effluent (white sucker; Lake Superior) Munkittrick et al. 1991.

Disrupted ovarian development, associated with exposure to contaminants (English sole; Puget Sound, USA) (Johnson et al., 1988).

Delayed gonadal maturation, associated with exposure to bleached Kraft mill effluent and other anthropogenic influences (mummichog; Canada)(Leblanc et al. 1997).

Inhibition of spawning in female fish exposed to diluted sewage sludge under laboratory conditions (sand goby; Waring et al. 1996).

Increased occurrence of embryonic malformation in pelagic eggs (various fish species; North Sea coastal waters) (Cameron et al. 1992).

Changes in the sex ratio of North Sea dab (*Limanda limanda*) in the period 1981-1995 (Lang et al. 1995).

A decrease in the size and age at first maturation in North Sea plaice and sole between 1960 and 1995 (Rijnsdorp and Vethaak, 1997).

Molluscs

Occurrence of imposex, caused by TBT/TFT (gastropods; North Sea) (Hallers–Tjabbes et al. 1994; Mensink et al. 1997; Matthiessen, 1997).

Crustaceans

Disturbance of host defence capability, associated with exposure to polluted dredged spoil in mesocosms (common shrimp, the Netherlands) (Smith et al. 1995).

Occurrence of intersex, associated with exposure to sewage discharges (several species of harpacticoid copepod; UK) (Moore and Stevenson, 1991, 1994).

Literature cited

Addison, R.F. (1989). Organochlorines and marine reproduction. Can. J. Fish. Aquat. Sci. 46: 360-368.

Allen, Y.; Thain, J.; Matthiessen, P.; Scott, A.P.; Haworth, S.; Feist, S.W. (1997). A survey of oestrogenic activity in UK estuaries and its effects on gonadal development of the flounder *Platichthys flesus*. ICES CM 1997/U:01: 20 pp.

Besselink, H.T.; Brouwer, A.; Vethaak, A.D. (1996). Effects on retinoid homeostasis in the flounder (Platichthys flesus) following chronic exposure to contaminated harbour dredg spoil from Rotterdam harbour. Dioxine 1996.

Besselink, H.T.; Flipsen, E.M.T.E.; Eggens, M.L.; Vethaak, A.D.; Koeman, J.H.; Brouwer, A. (1997). Alterations in plasma and hepatic retinoid levels in flounder (*Platichthys flesus*) after chronic exposure to contaminated harbour sludge in a mesocosm study. Aquat. Toxicol. xx-xx.

Boon, J.P.; Ten Hallers, C.C.; Hattum, B.G.M.van; Vethaak, A.D.; Mensink, B.P. (1998). The development of imposex and intersex in marine snails after exposure to organotin compounds. An overview. J. Sea Res (in press).

Boonstra, A.; Grinwis, G.C.M.; Wester, P.; Loveren, H.van; Vaal, M.; Vethaak, A.D.; Vos, J.G. (1996). Development of ex vivo/in vitro immune fuction tests in flounder, *Platichthys flesus*. RIVM, Bilthoven, The Netherlands report 732501001, 37 pp.

Bortone, S.A.; Davis, W.P. (1994). Fish intersexuality as indicator of environmental stress. Bioscience 44: 165–172.

Brouwer, A.; Reijnders, P.J.H.; Koeman, J.H. (1989). Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*). Aquat. Toxicol. 15: 99-105.

Cameron, P.; Berg, J.; Dethlefsen, V.; Westernhagen, H.von. (1992). Developmental defects in pelagic embryos of several flatfish species in the southern North Sea. Neth. J. Sea Res. 29 (1–3): 239–256.

Davis, W.P.; Bortone, S.A. (1992). Effects of kraft mill effluent on the sexuality of fishes: an environmental early warning? IN: Colborn, T.; Clement, C. (ed.). Chemically–induced alterations in sexual and functional development: the wildlife/human connection. Advances in modern environmental toxicology. (Vol. 21). Princeton Scientific Publishing Co., Inc., Princeton, NJ. p. 113–127

De Guise, S.; Martineau, D.; Beland, P.; Fournier, M. (1995). Possible mechanisms of action of environmental contaminants on St Lawrence beluga whales (*Delphinapterus leucas*). Environ. Health Perspect. 103 (Supplement 4): 73-77.

De Swart, R.L.; Ross, P.S.; Vedder, L.J.; Timmerman, H.H.; Heisterkamp, S.H.; Loveren, H.van; Vos, J.G.; Reijders, P.J.H.; Osterhaus, A.D.M.E. (1994). Impairement of immune function in harbour seals (*Phoca vitulina*) feeding on fish from polluted waters. Ambio 23: 155-159.

De Swart, R.L.; Visser, L.K.G.; Ross, P.S.; Reijders, P.J.H.; Uytenhaag, F.G.C.M.; Vos, J.G.; Osterhaus, A.D.M.E. (1992). The influence of environmental contaminants on the functioning of the immune system of pinnipeds. Neth. J. Sea Res. 20: 295-297.

Delong, R.L.; Gilmartin, W.G.; Simpson, J.G. (1973). Premature briths in California sea lions: association with high organochlorine pollutant residue levels. Science 181: 1168-1170.

Draganik, B.; Bykowski, P.; Domagala, M.; Barska, I.; Polak-Juszezk, L.; Kuczynski, J. (1995). Heavy metals in adults and fry of the Baltic viviparous eelpout (Zoarces viviparus L.). ICES C.M.1995/E:16.

Hallers-Tjabbes, C.C.T.; Kemp, J.F.; Boon, J.P. (1994). Imposex in whelks (*Buccinum undatum*) from the open North Sea: relation to shipping traffic intensities. Mar. Pollut. Bull. 28: 311–313.

Harries, J.E.; Sheahan, D.A.; Jobling, S.; Matthiessen, P.; Neall, P.; Routledge, E.; Rycroft, R.; Sumpter, J.P.; Tylor, T. (1996). Survey of Estrogenic activity in UK inland waters. Environ. Toxicol. Chem. In press

Harries, J.E.; Sheahan, D.A.; Jobling, S.; Matthiessen, P.; Neall, P.; Sumpter, J.P.; Tylor, T.; Zaman, N. (1996). Further evidence of estrogenic activity in U.K. rivers - vitellogenesis and testicular retardation in caged male fish. in prep.

Helle, E.; Olsson, M.; Jensen, S. (1976). DDT and PCB levels and reproduction in Ringed seal from the Bothnian Bay. Ambio 5: 188-189.

Helle, E.; Olsson, M.; Jensen, S. (1976). PCB levels correlated with pathological changes in seal uteri. Ambio 5: 261-263.

Hose, J.E.; Cross, J.N.; Smith, S.G.; et al. (1989). Reproductive impairment in fishes inhabiting a contaminated coastal environment off Southern California. Environ. Pollut. 57: 139-148.

Howell, W.M.; Black, D.A.; Bortone, S.A. (1980). Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence for environmental-induced masculinization. Copia: 676–681.

Howell, W.M.; Denton, T.E. (1989). Gonopodial morphogenesis in female mosquitofish, *Gambusia affinis affinis*, masculinized by exposure to degraded products from plant sterols. Environ. Biol. Fish. 24: 43–51.

Janssen, P.A.H.; Lambert, J.G.D.; Vethaak, A.D.; Goos, H.J.T. (1997). Environmental pollution causes elevated concentrations of estrogens and vitellogen in the female flounder, *Platichthys flesus* (L.). Aquat. Toxicol. 39: 195-214.

Jobling, S.; Sheahan, D.A.; Osborne, J.A.; Matthiessen, P.; Sumpter, J.P. (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environ. Toxicol. Chem. 15: 194-202.

Jobling, S.; Sumpter, J.P. (1993). Detergent components in sewage effluent are weakly oestrogenic to fish: an in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes Aquat. Toxicol. 27: 361-372.

Johnson, L.L.; Casillas, E.; Collier, T.K.; McCain, B.B.; Varanasi, U. (1988). Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Can. J. Fish. Aquat. Sci. 45: 2133-2146.

Kime, D.E.; Ebrahimi, M.; Nysten, K.; Roelands, I.; Rurangwa, E.; Moore, H.D.M.; Ollevier, F. (1996). Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish:application to effects of heavy metals. Aquat. Toxicol. 36: 223–237.

Lahvis, G.P.; Wells, R.S.; Kuehl, D.W.; Stewart, J.L.; Rhinehart, H.L.; Via, C.S. (1995). Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Turiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environ. Health Perspect. 103 (Supplement 4): 67-72.

Lang, T.; Damm, U.; Dethlefsen, V. (1995). Changes in the sex ratio of North Sea dab (*Limanda limanda*) in the period 1981-1995 ICES CM 1995/G:25 Ref E.

Leatherland, J.F. (1992). Endocrine and reproductive function in Great Lakes salmon IN: Colborn, T.; Clement, C. (ed.). Chemically-induced alterations in sexual and functional development: the wildlife/human connection. Advances in modern environmental toxicology. (Vol. 21). Princeton Scientific Publishing Co., Inc., Princeton, NJ. p. 129-145

Leblanc, J.; Couillard, C.M.; Brethes, J.-C.F. (1997). Modifications of the reproductive period in mummichoq (*Fundulus heteroclitus*) living downstream from a bleached kraft pulp mill in the Miramichi estuary, New Brunswick, Canada. Can. J. Fish. Aquat. Sci. 54: 2564-2573.

Legler, J.; Murk, A.J.; Vethaak, A.D.; Brouwer, A. (1997). Determination of estrogen-like activity in sediment extracts using chemical activtaed luciferase gene expression (CALUX) assays (abstract). Presented at Seventh Annual Meeting of SETAC-Europe, Amsterdam, The Netherlands, April 6-10, 1997.P12.11, p. 257.

Lye, C.M.; Frid, C.L.J.; Gill, M.E.; McCormick, D. (1997). Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works. Mar. Poll. Bull. 34: 34-41.

Martineua, D.; Lagace, A.; Beland, P. (1988). Pathology of stranded beluga whales (*Delphinapterus leucas*) from the St. Lawrence estuary, Quebec, Canada. J. Comp. Pathol. 98: 287-311.

Mensink, B.P.; Hattum, B.G.M.van; Hallers-Tjabbes, C.C.; Everaarts, J.M.; Kralt, H.; Vethaak, A.D.; Boon, J.P. (1996). Tributyltin causes imposex in the common whelk, Buccinum undatum. Mechanism and occurrence NIOZ report 1997-6, 56 pp.

Moccia, R.D.; Leatherland, J.F.; Sonstegard, R.A. (1981). Quantitative interlake comparison of thyroid pathology in Great Lakes coho (*Oncorhynchus kisuch*) and chinook (*Oncorhynchus tschawytscha*) salmon. Cancer Res. 41: 2200-2210.

Moore, C.G.; Stevenson, J.M. (1991). The occurrence of intersexuality in harpacticoid copepods and its relationship with polltuion. Mar. Poll. Bull. 22: 72-74.

Moore, C.G.; Stevenson, J.M. (1994). Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland. Journal Natural History 28: 1213-1230.

Munkittrick, K.R.; Port, C.B.; Van Der Kraak, G.J.; et al. (1991). Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a lake superior white sucker (*Catostomus commersoni*) population. Can. J. Fish. Aquat. Sci. 48: 1371-1380.

Nimrod, A.C.; Benson, W.H. (1996). Environmental estrogenic effects of alkylphenol ethoxylates. Crit. Rev. Toxicol. 26: 335–364.

Pereira, J.J.; Ziskowski, J.; Mercaldo-Allen, R.; et al. (1992). Vitellogenin in winter flounder (*Pleuronectes americanus*) from Long Island Sound and Boston harbour. Estuaries 15: 289-297.

Reijnders, P.J.H. (1986). Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324: 456-457.

Reijnders, P.J.H.; Brasseur, S.M.J.M. (1992). Xenbiotic induced hormonal and associated developmental disorders in marine organisms and related effects in humans; An overview IN: Colborn, T.; Clement, C. (ed.). Chemically-induced alterations in sexual and functional development: the wildlife/human connection. Advances in modern environmental toxicology. (Vol. 21). Princeton Scientific Publishing Co., Inc., Princeton, NJ. p. 159-174

Rijnsdorp, A.D.; Vethaak, A.D. (1997). Changes in reproductive parameters of North Sea plaice and sole between 1960 and 1995. ICES C.M. 1997/U:14.

Ross, P.S.; De Swart, P.J.H.; Reijnders, P.J.H.; Loveren, H.van; Vos, J.G.; Osterhaus, A.D.M.E. (1995). Contaminated-related suppression of delayed-type hypersensitivity and antibody responses in harbor seal fed Baltic Sea herring. Environ. Health Perspect. 103: 162-167.

Sheahan, D.A.; Bucke, D.; Matthiessen, P.; Sumpter, J.P.; Kirby, M.F.; Neall, P.; Waldock, M. (1994). The effects of low levels of 17?-ethynylestradiol upon plasma vitellogenin levels in malle and female rainbow trout, *Oncorhynchus mykiss* (Walbaum) held at two acclimation temperatures IN: Muller, R.; Lloyd, R. (ed.). Sublethal and Chronic Effects of Pollutants on Freshwater Fish. Fishing News Books, Oxford, UK. p. 99-112

Smith, V.J.; Swindlehurst, R.J.; Johnstone, P.A.; Vethaak, A.D. (1995). Disturbance of hostdefence capability in the common shrimp, *Crangon crangon*, by exposure to harbour dredge spoils. Aquat. Toxicol. 32: 43-58.

Subramanian, A.; Tanabe, S.; Tatsukawa, R.; Saito, S.; Miyazaki, N. (1987). Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of northwestern North Pacific. Mar. Poll. Bull. 18: 643-646.

Vethaak, A.D.; Jobling, S.; Waldock, M.; Bjerregaard, P.; Dickerson, R.; Giesy, J.; Grothe, D.; Karbe, L.; Munkittrick, K.R.; Schlumpf, M.; Sumpter, J.P. (1997). Approaches for the conduct of Field Surveys and Toxicity Identification and Evaluation in identifying the Hazards of Endocrine Modulating Chemicals to Wildlife IN: Tattersfield, L.; Matthiessen, P.; Campbell, P.; Grandy, N.; Lange, R. (ed.). In: SETAC-Europe/OECD/EC Expert Workshop on

Endocrine Modulators and Wildlife: Assessment and Testing. Veldhoven, The Netherlands, 10-13 April 1997 ed. SETAC-Europe, Brussels. p. 79-91

Vos, J.G.; Loveren, H.van; Osterhaus, A.D.M.E.; Vaal, M.; Vethaak, A.D.; Wester, P. (1996). Imunotoxicity of environmental pollutants: adverse effects on resistence to infectious diseases. Proceedings OECD Workshop 'Ecotoxicology: Responses,Biomarkers,and Risk Assessment',Maui,April 10-13 1996. SOS Publications,Fair Haven,NJ.

Zakharov, V.M.; Yablokov, A.V. (1990). Skull asymmetry in the Baltic grey seal effects of environmental pollution. Ambio 19: 66-269.

ANNEX 11

PROGRESS IN THE DEVELOPMENT AND IMPLEMENTATION OF A QUALITY ASSURANCE PLAN FOR THE INTEGRATION OF FLATFISH LIVER HISTOPATHOLOGY MEASUREMENTS INTO THE OSPAR JOINT ASSESSMENT AND MONITORING PROGRAMME AND IN THE ADAPTATION OF THE ICES FISH DISEASE DATA ENTRY PROGRAM AND FISH DISEASE REPORTING FORMAT FOR THE INCORPORATION OF LIVER HISTOPATHOLOGY DATA INTO THE ICES ENVIRONMENTAL DATABANK

by T. Lang

Introduction

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

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

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

The definition of the biological effects monitoring and assessment component of the JAMP, including the identification of suitable biological effects techniques, was addressed at the 1995 OSPAR/ICES Workshop on Biological Effects Techniques, and was further put forward by various ICES bodies (ACME, WGPDMO, WGBEC). Based on ICES advice, draft guidelines for biological effects monitoring under JAMP have been elaborated by the OSPAR Ad Hoc Working Group on Monitoring (MON). In these guidelines, studies on liver nodules and liver histopathology are amongst the suite of techniques recommended for use in the general biological effects monitoring and in the contaminant-specific biological effects monitoring. Information is provided on target organs/organisms for the above studies, effects measured, means of interpretation, methodologies, and the establishment of QA procedures.

However, the draft guidelines for biological effects techniques have not yet finally been adopted by OSPAR, mainly due to the fact that QA programmes are still missing for most of the techniques. According to the evaluation by OSPAR SIME (SIME(2) 97/12/1-E, Annex 11), QA programmes for biological effects techniques designated for incorporation in the OSPAR JAMP have only been implemented on a national level and not on a Convention-wide basis. Therefore, products (= data) will not be available in time for the QSR 2000 in terms of assessments based on a co-ordinated international programme. Nevertheless, information concerning biological effects monitoring will be included in the QSR 2000, on the basis of national assessments referred to in the regional QSRs.

However, in contrast to the assessment by OSPAR SIME on the status of QA programmes, it has to be emphasised that QA programmes for externally visible fish diseases and macroscopic liver nodules, which are on the list of techniques to be included in the JAMP, have already been developed and established in ICES Member Countries due to activities of the WGPDMO. These cover all aspects from fish sampling, disease diagnosis, annual data reporting to the ICES Environmental Data Centre, to statistical data analysis. The ICES Sub-group and Study Group on Statistical Analysis of Fish Disease Data in Marine Stocks in collaboration with the ICES Secretariat have developed standard procedures for the submission of fish disease data to the ICES Environmental Data Centre (Successor of the ICES Environmental Databank) by ICES Member Countries. They established the ICES Fish Disease Data Entry Program which can be used for data submission and which is compatible with the ICES Fish Disease Data Reporting Format. Furthermore, standard methods for statistical analysis of spatial and temporal trends in the fish disease data have been established (see also Section 7 of this report). Therefore, the QA plan for the incorporation of externally visible fish diseases and macroscopic liver nodules in the JAMP can be considered complete and operational and the disease data held in the ICES Data Centre are ready for integration in the OSPAR QSR 2000.

At its 1997 meeting, the WGPDMO recognised the need for a similar QA programme for the use of liver histopathology of flatfish. This issue had already been addressed at the 1996 ICES Special Meeting on the 'Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants'. In the report of the Special Meeting (ICES CM 1997: F:6), detailed guidelines are provided on

- suitable techniques for monitoring purposes;
- sampling strategies for monitoring of liver histopathology/histochemistry;
- processing of fixed samples;
- diagnosis of and classification criteria for liver histopathology;
- quality assurance/control requirements, e.g., intercalibration of diagnosis, identification of a lead laboratory, etc.

While quality assurance guidelines for fish sampling and processing of tissue samples have already been prepared at the ICES Special Meeting, training and intercalibration for the diagnosis of histopathological lesions were considered incomplete. Therefore, at the 1997 meeting of WGPDMO, it was recommended to organise a training and intercalibration programme (TIP) for the diagnosis of relevant histopathological liver lesions, as detailed in the report of the ICES Special Meeting. Preliminary information on the design of the TIP are provided in the report of the 1997 meeting of the WGPDMO (ICES CM 1997/F:6). WGPDMO recommended that S. MacLean, A.D. Vethaak, S.W. Feist and T. Lang would co-operate intersessionally to develop a more detailed proposal on the design of the TIP and on further actions required to be presented at the 1998 meeting of the WGPDMO (see Section 8 and Annex 8 of the present report).

In order to integrate liver histopathology studies into the ICES fish disease activities, the WGPDMO further recommended that the ICES Fish Disease Data Entry Program and the ICES Fish Disease Data Reporting Format be adapted to incorporate liver histopathology data and that the co-convenors of the ICES Special Meeting (S.W. Feist and T. Lang) should liaise with the ICES Secretariat in order to determine the best approach to accomplish this.

Progress in the development and implementation of a quality assurance plan for the integration of flatfish liver histopathology measurements into the OSPAR Joint Assessment and Monitoring Programme

As already mentioned above, QA programmes for biological effects techniques to be applied in the OSPAR JAMP have not yet been implemented. However, for some techniques programmes are in preparation, partly under the QUASIMEME programme (biological effects of TBT). Also, details of the TIP recommended by WGPDMO for liver histopathological studies have further been developed since the 1997 meeting of WGPDMO and are described in Section 8 and Annex 6 of this report.

At its 1997 meeting, the ICES Working Group on Biological Effects of Contaminants (WGBEC) agreed to apply for EU-funding for a project entitled 'Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)'. At the 1997 meeting of the ICES Advisory Committee on the Marine Environment (ACME) it was suggested to find ways to incorporate activities intended to be part of the TIP into the BEQUALM funding bid. In the meantime, the proposal has further been elaborated and submitted to the EC Standards, Measurement and Testing Programme by the project co-ordinator and new chairman of WGBEC, Dr P. Matthiessen, CEFAS Burnham-on-Crouch Laboratory, UK.

Similar to the QUASIMEME programme for chemical measurements, the purpose of BEQUALM is to establish a European quality assurance (QA) infrastructure for biological effects techniques used in marine environmental monitoring programmes such as the OSPAR JAMP. This will be achieved as follows:

- Development of appropriate reference materials or type collections;
- Development of an infrastructure for assessing the comparability of data from individual laboratories;
- Demonstration that biological effects analyses are under statistical control and are of known quality.

As project partners responsible for fish liver histopathology and, if considered necessary also for externally visible fish diseases, S.W. Feist (UK) and T. Lang (Germany) are included in the funding bid. Details of the histopathology part of the BEQUALM bid can be found under Section 8 and Annex 6 of this report.

If the funding bid will be successful, BEQUALM will certainly help considerably in implementing studies on liver histopathology of flatfish within the OSPAR JAMP.

Progress in the adaptation of the ICES Fish Disease Data Entry Program and the ICES Fish Disease Data Reporting Format

Discussions with the ICES Environmental Data Scientist (J.R. Larsen) have resulted in the following suggestions:

As a first step, types of histopathological liver lesions of dab and flounder to be incorporated in the ICES Environmental Data Centre have to be defined. The definition should be based on the outcome of the ICES Special Meeting. The following four disease categories could be considered according to the importance of the lesions to indicate contaminant exposure:

- Unique degenerative lesions
  - hepatocellular and nuclear polymorphism
  - megalocytic hepatosis
  - hydropic vacuolisation of biliary epithelial cells and/or hepatocytes

- Foci of cellular alteration
  - clear cell foci
  - vacuolated foci
  - eosinophilic foci
  - basophilic foci

- Benign neoplasms
  - hepatocellular adenoma
  - cholangioma
  - pancreatic acinar cell adenoma
  - hemangioma

- Malignant neoplasms
  - hepatocellular carcinoma
  - cholangiocarcinoma
  - mixed hepatobiliary carcinoma
  - pancreatic acinar carcinoma
  - hemangiosarcoma
  - hemangiopericytic sarcoma

A fifth category summarising other non-specific lesions identified during the ICES Special Meeting could be added:

- Non-specific lesions
  - necrotic/degenerative change
  - non-neoplastic proliferative lesions
  - storage conditions
  - inflammatory change

As a second step, these lesions could be added to the list of diseases of dab and flounder already existing in the ICES Fish Disease Data Entry Program and the ICES Fish Disease Data Reporting Format, respectively. This procedure would not cause any significant additional workload to the ICES Secretariat.

However, there is a problem with data comparability since at present two strategies for the examination of fish for liver lesions are applied:

a) The 'traditional' one consists of a quantification of grossly visible liver nodules > 2 mm in diameter in fish of a certain size group (dab: ≥ 25 cm, flounder: 25–29 cm) with a subsequent histological confirmation of the type of lesions (neoplastic or non-neoplastic) by a reference laboratory (Dr S.W. Feist, CEFAS Weymouth Laboratory, UK). Only the prevalence of those lesions confirmed as neoplastic (including putative neoplastic lesions, e.g., foci of cellular alteration, FCA) are reported to the ICES Fish Disease Databank, without distinguishing between different categories of neoplasms. The advantage of this method is that a relatively high number of fish can be checked for gross liver lesions and that liver nodules > 2 mm are easy to recognise. The major disadvantage of this method, however, is that the prevalence recorded always is an underestimation of the 'true' prevalence since only grossly visible nodules but no microscopic lesions are detected.

b) The second strategy, recommended in the report of the ICES Special Meeting as an additional measurement to the above method, consists of a random histological examination of livers from a), according to statistical requirements, of a given number of fish of a certain size group (e.g., dab: 20–24 cm), independently of whether the livers show any grossly visible lesions or not. The advantage of this method is that the level of detection of liver lesions associated with contaminant effects is increased since even microscopic early lesions can be identified. The disadvantage of this method is that it is much more time consuming and requires more specialised knowledge in fish liver histopathology than the above method, particularly for the identification and diagnosis of early liver lesions.

WGPDMO has to discuss whether data obtained by using both methodologies should be reported to the ICES Data Centre by ICES Member Countries or whether the data reporting should be restricted to one method. If data from both methods will be reported, there must be a clear distinction in the ICES Fish Disease Databank between prevalence data derived from the macroscopic inspection and those from the microscopic inspection since the two types of data are not comparable. This would require some further changes in the ICES Fish Disease Data Entry Program and the Fish Disease Data Reporting Format.

A suggestion could be that a final decision about what kind of data should be incorporated in the ICES Fish Disease Databank be postponed until there is evidence that the microscopic method constitutes a 'real' improvement to the macroscopic one and until a QA programme for liver histopathology has been established in ICES Member Countries. A compromise could be that, as a first step, only data from the macroscopic inspection are reported but that the lesions should be classified according to the five categories listed above. As long as the QA programme is not implemented, the classification of lesions should still be carried out by the reference laboratory (S.W. Feist CEFAS, Weymouth Laboratory, UK). Experts from other lead laboratories (S. MacLean, T.Lang, D. Vethaak) could be involved as part of the TIP/BEQUALM (see above).

ANNEX 12

INFORMATION PACKAGE ON THE ICES ENVIRONMENTAL DATABANK AND DISEASE DATA REPORTING FORMAT (FOR SUBMISSION TO LABORATORIES IN ICES MEMBER COUNTRIES IDENTIFIED AS CONDUCTING SHELLFISH DISEASE SURVEYS AND/OR DIAGNOSTICS)

by T. Lang and S. McGladdery

Introduction

Due to the increasing economical significance of shellfish mariculture in Member Countries of the International Council for the Exploration of the Sea (ICES), shellfish diseases became an issue of growing interest in the activities of ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO). Apart from addressing new trends in the spatial and temporal distribution, aetiology and therapy of shellfish diseases, it has been discussed repeatedly at the meetings of WGPDMO if it would be useful to compile shellfish disease data available in ICES Member Countries and to combine them in a database in order to utilise them together with other types of data available in ICES for a comprehensive analysis aiming at an assessment of the quality of the marine environment. A similar approach is being followed in ICES with data on diseases of finfish which are submitted to ICES by its Member Countries running regular fish disease surveys (see below).

At its 1997 meeting, the WGPDMO addressed the question whether shellfish disease data could be incorporated in the ICES Environmental Data Centre in more detail and it was recommended that, as first step, a list of shellfish disease laboratories in ICES Member Countries be compiled and an information package on the ICES Environmental Data Centre be prepared which could be distributed to those shellfish laboratories which might consider a submission of data to ICES.

In the following, a brief overview is given on the ICES Environmental Data Centre and its fish disease component (the ICES Fish Disease Data Reporting Format and the ICES Fish Disease Data Entry Program). Furthermore, problems encountered with the incorporation of shellfish disease data are addressed and possible strategies for an integration of shellfish disease data are suggested. A preliminary list with shellfish disease diagnosticians and laboratories inside and outside the ICES area is provided in addition.

The ICES Environmental Data Centre

One of the major strengths of ICES in marine research and monitoring is its function as data base. Data held in the ICES databanks can be categorised into three main types:

- fisheries data - ICES Fisheries Databank;
- oceanography data - ICES Oceanography Data Centre;
- environmental data - ICES Environmental Data Centre.

At present, these data are stored in different databanks the structures of which are not directly compatible. However, it is envisaged for the future that all databanks are integrated into the *ICES Regional Data Centre* in a way that they can easily be combined for a joint analysis. Information on the data stored in the different databanks are provided via the World Wide Web (http://www.ices.dk).

Data are submitted to ICES by ICES Member Countries running appropriate and internationally standardised research/monitoring programmes or are deriving from ICES activities such as workshops, baseline studies and special sampling programmes with representatives from Member Countries participating. Data held in the ICES databanks can be utilised by Member Countries, international environmental or fisheries regulatory organisations and research/monitoring programmes or, internally, by ICES Working/Study/Steering Groups and Workshops.

Environmental data are held in the *ICES Environmental Data Centre* (the former *ICES Environmental Databank*) which contains information on:

- contaminants in bio;a, sediments, sea water
- certain biological effects of contaminants (part of the contaminant database);

- fish disease prevalence data.

For submission of data to the ICES Environmental Data Centre and for internal use, the *ICES Environmental Data Reporting Formats* (Version 2.2) were created which have been released in 1994 and which are intended for use in reporting data for monitoring programmes coordinated by ICES such as the new Joint Assessment and Monitoring Programme (JAMP) of the OSPAR Commission, the Baltic Monitoring Programme (BMP) of the Helsinki Commission (HELCOM), the marine component of the Arctic Monitoring and Assessment Programme (AMAP) and the Cooperative ICES Monitoring Studies Programme (CMP).

A comprehensive information package providing detailed information on the structure of the Reporting Formats and the *ICES Environmental Data Screening Program* (Version 1.32) for validation of the data prior to submission to ICES are available from ICES on request as a loose-leaf binder together with floppy disks. Interactive information on the data stored in the ICES Environmental Data Centre are provided via the World Wide Web (http://www.ices.dk). The ICES Environmental Data Scientist can be contacted for further information.

The fish disease databank of the ICES Environmental Data Centre was established in collaboration with the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) and its Sub-group (in 1997 Study Group) on Statistical Analysis of Fish Disease Data in Marine Stocks (SGFDDS). Fish disease data are submitted to ICES using the *ICES Fish Disease Data Reporting Format* (part of the ICES Environmental Data Reporting Formats) (see below).

The ICES fish disease data so far comprise information from studies on externally visible diseases and macroscopic liver nodules/tumours in the common dab (*Limanda limanda*) and the European flounder (*Platichthys flesus*) from the North Sea and adjacent waters (including the Baltic Sea) and partly date back until 1981. However, the structure of the databank permits an extension regarding other species (including invertebrates) and other types of diseases.

Quality assurance programmes for externally visible fish diseases and macroscopic liver nodules, which are on the list of techniques to be included in the OSPAR JAMP, have already been developed and established in ICES Member Countries, also due to activities of the WGPDMO. These cover all aspects from fish sampling, disease diagnosis, annual data reporting to the ICES Environmental Data Centre, to statistical data analysis. Corresponding guidelines have been published by ICES (ICES, 1989, 1995; Bucke *et al.*, 1996). For histopathological liver lesions of flatfish, which are also on the list of techniques for the OSPAR JAMP, the planning of a quality assurance programme and the incorporation of data into the ICES Fish Disease Data Reporting Format are under way (see also Section 8 and Annex 6 of this report).

Standard procedures for the submission of fish disease data by ICES Member Countries to ICES have been developed and tested successfully. For this purpose, the *ICES Fish Disease Data Entry Program* (FDE, Version 2.0) (see below) was created in 1996 which is compatible with the ICES Fish Disease Data Reporting Format. The FDE computer software is also available from ICES on request.

Furthermore, standard techniques for the statistical analysis of spatial and temporal trends in the fish disease data have been developed by SGFDDS and applied successfully using the ICES fish disease data (see also Section 7 and Annex 5 of this report).

The ICES Fish Disease Data Reporting Format

The *ICES Fish Disease Data Reporting Format* (DF) is one of the four *ICES Environmental Data Reporting Formats* (RF) (Contaminants and selected biological effects in biota, contaminants and selected biological effects in sea water, contaminants and selected biological effects in sediments, fish diseases).

The ICES RF have been developed to provide a consistent mechanism for reporting marine environmental data at an international level. Provision is made, therefore, for the inclusion of items of information which are essential to the compilation and archiving of data in an international databank, and for ensuring, to the greatest possible degree, the integrity and validity of data for their eventual use in various scientific, monitoring and assessment programmes.

The DF (as are the other RF) is structured in a hierarchical (relational) way as shown in Table 1.

Table 1. Structure of the ICES Fish Disease Data Reporting Format.

01	Sample Master Record	Includes general information such as when and where the sample was obtained, together with administrative information in relation to the intended uses of the data for specimens collected as part of a single sample of fish.
02	Haul Information Record	The sample can be reported as one or more hauls, utilising the *Haul Information Record.* This record gives more detailed information concerning the gear, the total number and sex of individuals in the haul, and the scientists involved in examining the specimens.
06	Fish Disease Specimen/Sub-sample Record	A given haul of fish is sub-divided via Fish Disease Specimen/Sub-sample Records into different length classes/sex categories for individuals or bulks which represent a sub-sample. The total number of diseases looked for, the number of specimens examined in the specified category and general information concerning length and age of the specimens are given in this record type.
08	Fish Disease Data Record	Each *Fish Disease Data Record* records the number of individuals affected by a particular disease in a given sub-sample.

The ICES Fish Disease Data Entry Program

The *ICES Fish Disease Data Entry Program* (FDE) is a computer program that has been developed in order to support the handling of fish disease data within the ICES community and, particularly, to facilitate the re-entering of historical data stored by different laboratories in various formats.

The FDE runs on an IBM compatible PC with MS-DOS 3.3 or higher. Approximately 550 Kbyte of memory below the 640 Kbyte limit is required to run the programme. It constitutes a user-friendly way to submit the data in a format that can directly be converted into the ICES Fish Disease Data Reporting Format.

As with the ICES Fish Disease Data Reporting Format, the FDE is organised in a hierarchical way. It is organised as a number of *Menu Screens, Data Entry Screens* and *Check Screens.* After data entry, data can be validated using the *Submission Validation Menu* and the *Error List* Menu. By using the *Query Data Entry Screen* and the *Design Query Data Entry Screen,* a column/row structured ASCII-file containing all or a part of the data from a particular data submission can be created which is suited for further processing by a spreadsheet system.

Further information on the FDE are available from the ICES Environmental Data Scientist.

Strategy for an incorporation of shellfish disease data into the ICES Environmental Data Centre

Although, in principle, there has been a general consensus in the WGPDMO that an incorporation of shellfish disease data would be desirable, a number of problems were identified which have to be solved before such a project can start:

1) A compilation of what is being done in the various shellfish laboratories in ICES Member Countries is still missing.
2) There are only few shellfish disease surveys underway related to an assessment of environmental quality which meet the ICES requirements.
3) At present, data collected by different laboratories are incompatible since reasons for collecting the data and their uses are different.
4) The data are not directly applicable to the ICES Environmental Data Centre due to various formats in use.
5) Guidelines for suitable diseases and host species and methodologies for sampling, disease diagnosis and data reporting have not yet been developed or even discussed.

In order to implement a regular submission of shellfish disease data to the ICES Environmental Databank by ICES Member Countries, a number of requirements have to be met:

1) The objectives of an incorporation of shellfish disease data and a subsequent analysis have to be clearly defined (e.g., whether the data should be used for assessing anthropogenic environmental effects or whether they should serve more disease-related purposes such as disease control measures associated with mariculture operations etc.).
2) Shellfish disease laboratories in ICES Member Countries should be asked to provide an activity report in order to get an impression which laboratories could submit data meeting the above objectives and fulfilling the ICES requirements. Subsequently, laboratories performing relevant work have to be encouraged by ICES to submit data and to take part in associated activities (quality assurance etc., see below).
3) Based on the objectives and the above activity reports received, diseases and host species suitable for ICES purposes have to be defined and standard methodologies for sampling, diagnosis and data reporting have to be developed.
4) According to the requirements defined, the ICES Fish Disease Data Reporting Format and the ICES Fish Disease Data Entry Program have to be modified as appropriate.
5) Based on the experience made with finfish diseases, quality assurance procedures required for shellfish disease studies and data submission to ICES have to be developed and, subsequently, a quality assurance programme (e.g., intercalibration, training etc.) has to be implemented with laboratories participating which are willing to co-ordinate their studies and to submit data to ICES.

To meet these requirements it will certainly need a longer-term project. However, if the WGPDMO endorses this suggestion, the clarification of some of the items listed above could be dealt with in preparation of the 1999 WGPDMO meeting as an intersessional task allocated to WGPDMO members to be identified. These should involve shellfish disease specialists and experts familiar with the ICES Environmental Data Centre and with quality assurance requirements associated with disease studies within ICES.

For the future, it is envisaged that all data held in the ICES Environmental Data Centre are made compatible by modifying the Data Reporting Formats. Thus, it will be possible to combine disease data with biological effects (biomarker) data, contaminant data and hydrographic data for a statistical analysis. Furthermore, strategies for also integrating the fisheries and oceanography data held by ICES are being developed at present (see Section 16 and Annex 15 of this report). To complete the picture and to widen the scope of the ICES fish disease databank, the integration of shellfish disease data would certainly be advisable. The experience made during the process required could be useful for an evaluation of the possibility to further expand the databank in the future in order to include data on other types of diseases and host species, possibly from wild as well as cultured marine organisms.

Literature cited

ICES. 1989. Methodology of fish disease surveys. Report of an ICES Sea-going Workshop held on RV U/F 'Argos' 16–23 April 1988. ICES Cooperative Research Report, No. 166. 33 pp.

Bucke, D., Vethaak, A.D., Lang, T., and Mellergaard, S. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES Techniques in Marine Environmental Sciences, 19. 27 pp.

ICES. 1995. Report of the ICES Advisory Committee on the Marine Environment, 1995. ICES Cooperative Research Report, No. 212. 135 pp.

ANNEX 13

SHELLFISH HEALTH DIAGNOSTICIANS AND LABORATORIES

(the questionable applicability-to-ICES labs are put in italics)

Name	Phone	Fax	e-mail
Belgium Dr Daniel Declerck Department of Patholoy and Microbiology Fisheries Research Station Aukerstraat, 1, 8400 Ostend	32–5932–0388	32–5933–0629	ddeclerc@mail.dma.be
Canada - Atlantic Dr David Groman and Dr Jeff Davidson Department of Pathology & Microbiology Atlantic Veterinary College University of Prince Edward Island 550 University Avenue Charlottetown, PE C1A 4P3	1–902–566–0595	1–902–566–0745	
Dr Shona K. Whyte Atlantic Fish Health Institute Atlantic Veterinary College University of Prince Edward Island 550 University Avenue Charlottetown, PE C1A 4P3	1–902–566–0831	1–902–566–0745	
Mr Neil MacNair Prince Edward Island, Department of Fisheries and Environment Aquaculture Division P.O. Box 2000 Charlottetown, PE C1A 7N8	1–902–368–5615	1–902–368–5542	
Dr Sharon McGladdery Department of Fisheries & Oceans, Canada Gulf Fisheries Centre P.O. Box 5030 Moncton, NB E1C 9B6	1–506–851–2018	1–506–851–2079	mcgladderys@mar.dfo-mpo.gc.ca
Dr Roland Cusack Fish Health Veterinarian NS Department of Fisheries & Aquaculture P.O. Box 2223 Halifax, NS B3J 3C4	1–902–424–4560	1–902–420–5506	
Dr Cyr Couturier Marine Institute of Memorial University P.O. Box 4920 St. John's, NF A1C 5R3	1–709–778–0609	1–709–778–0535	
Dr Robert Claveau MAPAQ Laboratoire de Pathologie Animale de l'Agriculture et des Pêcheries 337 Moreault Rimouski QC J5L 1P4	1–418–722–3522		
Canada - Pacific Dr Susan Bower Department of Fisheries & Oceans, Canada Pacific Biological Station Nanaimo, B.C. V9R 5K4	1–250–756–7077	1–250–756–7053	bowers@pac.dfo-mpo.gc.ca

Name	Phone	Fax	e-mail
Dr Dominique Hervio Department of Fisheries & Oceans, Canada West Vancouver Laboratory 4160 Marine Drive West Vancouver, B.C. V7V 1N6	1–250–666–4805		
Denmark Dr Stig Mellergaard Danish Institute for Fisheries Research Fish Disease Laboratory Bülowsvej 13 DK-1870 Frederiksberg C Denmark	45–31–352–767	45–35–282–711	sme@dfu.min.dk
Estonia Dr Vello Kaldikas			
Finland Dr Goran Bylund BioCity, Artillerigatan 6 20520 Abo	358–22–154–301	358–22–154–748	
France Dr Tristan Renault IFREMER BP 133 17390 La Tremblade France	33–46–36–30–07	33–46–36–37–51	renault@ifremer.fr
Dr Philippe Maes & Dr Christine Paillard University of Bretagne Occidentale Institut Universitaire Européen de la Mer Unité de Recherche Associée CNRS D1513 B.P. 809 29285 Brest Cedex France		33–98–01–63–11	
Drs. Philippe Roch, Viviane Boulo, Evelyne Bachère IFREMER-DRIM (Défense et Résistance chez les Invertebrés Marins) 2 Place Eugène Batillon C.P. 71 - 34095 Montpellier Cedex 5 France	33–67–14–46–25	33–67–14–46–22	
Dr J.-R. Bonami Laboratoire de Pathologie Comparée Universiti des Sciences et Techniques du Languedoc Place Eugène Bataillon 34060 Montpellier Cedex 5 France	33–67–14–30–30	33–67–14–30–31	
Germany Dr Sven Bergmann Bundesforschungsanstalt für Viruskrankheiten der Tiere Institut für Angewandte Virologie 17498 Insel Riems Germany	49–38351–7-103	49–38351–7-219 49–38351–7-151	
Dr Burkard Watermann LIMNOMAR Bei der Neuen Mühle 11 22145 Hamburg Germany	49–40–6789911	49–40–6799204	

Name	Phone	Fax	e-mail
Iceland Dr Sigurdir Helgasson			
Ireland Drs. John McArdle & Dan Minchin Department of the Marine Fisheries Research Centre Abbotstown Dublin 15 Eire	44-353-1-821-0111	44-353-1-820-5078	
Latvia			
The Netherlands Dr Paul van Banning Netherlands Institute for Fisheries Investigations P.O. Box 68 1970 AB Ijmuiden The Netherlands	31-25-50-64-732	31-25-50-64-644	
Dr A. Dick Vethaak P.O. Box 8039 4330 EA Middelburg The Netherlands	31-118-67-23-11	31-118-65-10-48	vethaak@rikz.rws.minvenw.nl
Norway Dr Stein H. Mortensen Institute of Marine Research Department of Aquaculture P.O. Box 1870 5024 Bergen Norway	47-55-23-83-03	47-55-23-83-33	
Poland Wlodzimierz Grygiel ul. Kollataja 1 Gdynia 81-332 Poland	4858-620-17-28	4858-620-28-31	
Portugal Dr Carlos Azevedo Department of Cell Biology Institute of Biomedical Science University of Oporto Porto Portugal			
Russia Dr Andrey Karasev 6 Knipovich St. Murmansk 183763 Russia	7-815-2-47-25-32	47-789-10-423 (Norwegian line)	paralab@piro.murmansk.ru
Spain Dr Antonio Villaba Centro de Investigaciones Marinas Conselleria de Pesca Xunta de Galicia Aptdo 208 Vilagarcia de Arousa 36600 Pontevedra Spain			

Name	Phone	Fax	e-mail
Dr Juan Barja University of Santiago Department of Microbiology Fac. Biologia 15706 Santiago de Compestela Spain	34–81–56–31–00	34–81–59–69–04	
Dr Antonio Figueras Instituto de Investigaciones Marinas Muelle de Bouzas 36200 Vigo Spain	34–86–23–19–30	34–86–29–27–62	
Sweden Eric Lindesjoo			
UK Dr Alasdair McVicar SOAEFD Marine Laboratory P.O. Box 101 Victoria Road Aberdeen AB11 9DB Scotland, UK	44–1224–295–605	44–1224–295–620	
Dr James Turnbull & Mr David Currie Insitute of Aquaculture University of Stirling Stirling, FK9 4LA Scotland	44–1786–731–171	44–1786–721–133	
Dr Stephen Feist, Dr David Alderman and Mr E.B. Hudson CEFAS Weymouth Laboratory Barrack Road The Nothe Weymouth, Dorset, DT4 8UB UK	1305–206–662	1305–206–601	
Dr Susan D. Utting Directorate of Fisheries Research Fisheries Laboratory Benarth Road Conwy, Gwynedd Wales LL32 8UB	44–1492–593–883	44–1492–592–123	
USA - Atlantic Dr Bruce J. Barber University of Maine Fisheries and Aquaculture Research Group Dept. of Animal, Veterinary & Aquatic Sciences 5735 Hitchner Hall Orono, ME 04469–5735	1–207–581–2783	1–207–581–2729	
Dr Susan E. Ford Haskin Shellfish Research Laboratory Rutgers University Port Norris, NJ 08349			
Dr Sharon MacLean NMFS Narragansett Laboratory 28 Tarzwell Drive Narragansett, RI 02882	1–401–782–3258	1–401–782–3201	
Dr Roxanna Smolowitz Marine Biological Laboratory Woods Hole, MA 02543	1–508–289–7663	1–508–289–7392	

Name	Phone	Fax	e-mail
Dr Carol Reinisch Environmental Health Department School of Veterinary Medicine Tufts University Medford, MA 02155	1–508–839–7049		
Mr E. Jay Lewis and C. Austin Farley (Molluscs - Federal) United Stated Department of Commerce NOAA/NMFS Oxford Laboratory 904 South Morris Street Oxford, MD 21654	1–141–226–5193	1–141–226–5925	
Dr Christopher Dungan (Molluscs - State) United Stated Department of Commerce NOAA/NMFS Oxford Laboratory 904 South Morris Street Oxford, MD 21654	1–141–226–5193	1–141–226–5925	
Dr Gretchen Messick (Crustaceans) United Stated Department of Commerce NOAA/NMFS Oxford Laboratory 904 South Morris Street Oxford, MD 21654	1–141–226–5193	1–141–226–5925	
Dr Eugene Burreson Virginia Institute of Marine Science Gloucester Point, VA 23062	1–804–642–7000		
Dr Patrick Gaffney College of Marine Studies University of Delaware Lewes, DL 19958	1–302–645–4364	1–302–645–4028	
Dr Frank O. Perkins Department of Zoology Box 7617 NC State University Raleigh, NC 27695	1–919–515–8124	1–919–515–5327	
Dr Thomas C. Cheng Director, Shellfish Research Institute P.O. Box 12139 Charleston, SC 29422–2139	1–803–762–0022	1–803–795–6672	
Dr Norman J. Blake Department of Marine Science University of South Florida 147 7th Ave. Sth. St. Petersburg, FL 33701–5016	1–818–893–9521		
Dr William S. Fisher Environmental Protection Agency Gulf Breeze Laboratory Gulf Breeze, FL	1–904–934–9200		
Dr Robin Overstreet Gulf Coast Research Laboratory P.O. Box 7000 Ocean Springs, MI 39564–7000	*1–601–872–4243*	*1–601–872–4204*	
Dr Paul Frelier Texas A&M University Department of Veterinary Pathology College of Veterinary Medicine College Station, TX 77843	*1–409–845–5066*	*1–409–845–9972*	

Name	Phone	Fax	e-mail
Dr Sammy Ray Texas A&M University at Galveston P.O. Box 1675 Galveston, TX 77553	1–409–740–4525	1–409–740–5002	
USA - Pacific Dr J. Frank Morado NOAA - National Marine Fisheries Service Alaska Fisheries Science Center 7600 Sand Point Way NE Seattle, WA 98115	1–206–526–4172	1–206–526–6723	
Dr Ralph Elston AquaTechnics Inc. P.O. Box 687 Carlsborg, WA 98324	1–360–683–2376	1–360–683–2550	
Dr Carolyn Friedman Bodega Marine Laboratory University of California at Davis Bodega Bay, CA 94923	1–707–875–2211		
Dr Donald Lightner Department of Veterinary Science The Univesrity of Arizona Building 90, Room 202 Tucson, AZ 85721	1–520–621–8414	1–520–621–4899	
Dr James Brock Hawaii Department of Land and Natural Resources Anuenue Fisheries Research Center Division of Aquatic Resources Area 4, Sand Island Honolulu, Hawaii 96819	1–808–845–9561	1–808–845–4334	
Puerto Rico Dr Lucy Willliams University of Puerto Rico Department of Marine Sciences P.O. Box 908 Lajas, Puerto Rico 00667	1–809–899–2048	1–809–899–5500	
Guam Dr Ise Silva-Krott University of Guam College of Agriculture U.O.G Station Mangilo, Guam 96931	1–671–734–9431	1–671–734–6842	

ANNEX 14

NEW PROGRESS IN THE KNOWLEDGE OF NODAVIRUS AND NODAVIRUS-LIKE ASSOCIATED DISEASES

F. Baudin Laurencin

Since the 1997 WGPDMO meeting, where a review information on Nodavirus and Nodavirus-like agent was reported, several data were obtained, often published, which are reported thereafter.

1 Evolution of the distribution and importance of nodavirosis

No data were published on the importance of the disease in 1997. It seems that the situation was similar to 1996's. The effects of the Nodavirus on fish stocks has been very serious in certains farms where losses of up to 90 % have been reported, notably in Italy, Greece, and Malta (**Le Breton** *et al.*, 1997 b), where warmer summer temperatures have encouraged the proliferation of the disease symptoms and effects. In Malta, the effects of Nodavirus have contributed to a cessation of sea bass farming. Two in press scientific papers presented at the 1996 WGPDMO have since been published confirming Nodavirus as a cause of mass mortality in Atlantic halibut (**Grotmol** *et al.*, 1997 c) and the detection of a Nodavirus-like agent in the heart tissue from reared Atlantic *Salmo salar* suffering from cardiac myopathy syndrome (**Grotmol** *et al.*, 1997 a). The disease has been for the first time described in Taiwan (**Chi** *et al.*, 1997) and also for the first time in the grouper *Epinephelus fuscogutatus*. From **Comps and Raymond** (1996) and recent unpublished data, it seems that gilthead sea bream could be susceptible. From experimental results (**Skliris and Richards**, 1997 b) Tilapia *Oerochromis mossambicus* seems generally refractory to the infection but could be an asymptomatic carrier.

2. Virological studies

Sideris (1997) succeeded in the cloning, expression and purification of the coat protein of the virus infecting sea bass (DIEV). **Thiery** (unpublished results) has shown the existence at the least two genetically different strains in Mediterranean sea bass. Fish Nodavirus appears to be very stable at 15° C in cultured cell medium. A pH variation from 3 to 9 does not modify the titer after 6 weeks. The virus can resist 3 month either in sea water or in freshwater (**Frerichs & Tweedie**, 1997).

A molecular phylogenetic analysis of 25 isolates of fish nodaviruses, the causative agents of viral nervous necrosis of marine fish, was performed based on the nucleotide sequences (427 bases) of the coat protein gene. These fish nodaviruses were classified into four clusters: tiger puffer nervous necrosis virus, striped jack nervous necrosis virus, berfin flounder nervous necrosis virus, and red-spotted grouper nervous necrosis virus (**Nishizawa** *et al.*, 1997).

3. Pathogeny

Nguyen *et al.* (1997) have demonstrated (using FAT reaction and PCR) the presence of Nodavirus in the gonad, intestine, stomach, kidney and liver of infected striped jack *Pseudocaranx dentex* broodstokes. The data suggest that the virus originates in various organs and is shed from the intestine and gonads, resulting in contamination of eggs.

4. Diagnosis

The new 1997 edition of the OIE Diagnostic Manual for Aquatic Animal Diseases reports on the immunohistochemical detection in histological sections. In fact, as said in the 1997 WGPDMO report, that is the easiest method for diagnose the disease when clinical signs are obvious. On the other hand, the Manual does not take in account the ELISA method for highlighting serum antibodies: the method was successfully used at IFREMER for separate seropositive from seronegative brookfish (unpublished data). Also at CNEVA Brest, both the culture on cell line (SNN-1) and nested-PCR allow identification of asymptomatic carrier (unpublished data). Mammalian and fish cell cultures (COS 1 and SBL cells) have been demonstrated semipermissive for the sea bass Nodavirus DIEV (**Delsert** *et al.*, 1997).

5. Epidemiology

Thiery *et al.* (1997a, 1997b) and **Peducasse** (1997) succeeded in experimentally transmitting the disease, using several ways (annex 1) either with a brain homogenate from naturally contaminated fish or with a supernatant of contaminated

SSN-1 cell line. In the same way **Grotmol** *et al.* (1997 b) infected Atlantic halibut yolk-sac larvae by bath challenging causing a total mortality within few days. The infection was studied sequentially by immunohistochemistry and electromicroscopy

6. Control

Yoshimizu *et al.* (1997) succeeded in controlling the disease in berfin flounder offspring by selecting virus free brood stocks (using ELISA and PCR) and disinfecting fertilized eggs and sea water for culture

Bibliography

Chi S.C., Lo C.F., Kou G.H. & Chang P.S., 1997. Mass mortalities associated withviral nervous necrosis (VNN) disease in two species of hatchery reared grouper, *Epinephelus fuscogutatus* and *Epinephelus akaara* (Teeminck & Schlegel). *J. Fish Dis.*, 20, 185–193

Delsert C., Morin N. & Comps M., 1997. Fish nodavirus lytic cycle and semipermissive expression in mammalian and fish cell cultures. *J. Virol.*, **71**, 7, ?-?

Frerichs G.N. & Tweedie A., 1997. Survival and inactivation of piscine neuropathy nodavirus. *VIIIth International Conference 'Diseases of Fish and Shellfish'* (EAFP), Edinburgh Conference Centre Heriot-Watt University, Abstracts Book, p. 097

Grotmol S., Totland G.K. & Kryvi H., 1997 (a). Detection of a nodavirus like agent in heart tissue from rearedAtlantic salmon *Salmo salar* suffering from cardiac myopathy syndrome (CMS). *Dis. Aquat. Org.*, **29**, 79–84

Grotmol S., Totland G.K., Kryvi H., Bergh O. & Hjeltnes B.K., 1997 (b). Experimental infection of Atlantic halibut *(Hippoglossus hippoglossus)* yolk sac larvae with a nodavirus. *VIIIth International Conference 'Diseases of Fish and Shellfish'* (EAFP), Edinburgh Conference Centre Heriot-Watt University, Abstracts Book, p. 052

Grotmol S., Totland G.K., Thorud K. & Hjeltnes B.K., 1997 (c). Vacuolating encephalopathy and retinopathy associated with a nodavirus like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Dis. Aquat. Org.*, **29**, 85–97

Johnson K.L. & Ball L.A., 1997. Replication of flock house virus RNAs from primary transcripts made in cells by RNA polymerase II. *J. Virol.*, **71**, 4, 3323–3327

Le Breton A., Abela M. & Brinch-Iversen J., 1997 (a). Viral nervous necrosis of sea bass *Dicentrarchus labrax* L.: epidemiological data and prophylactic guidelines. *VIIIth International Conference 'Diseases of Fish and Shellfish'* (EAFP), Edinburgh Conference Centre Heriot-Watt University, Abstracts Book, p. 050

Le Breton A., Grisez L., Sweetman J. & Ollevier F., 1997 (b). Viral nervous necrosis (VNN) associated with mass mortalities in cage reared sea bass, *Dicentrarchus labrax* L. *J. Fish Dis.*, 20, 145–151

Nguyen H.D., Mushiake K. & Muroga K., 1997. Tissue distribution of striped jack nervous necrosis virus (SJNNV) in adult striped jack. *Dis. Aquat. Org.*, **28**, 87–91

Nishizawa T., Furuhashi M., Nagai T., Nakai T. & Muroga K., 1997. Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. *Appl. Environ. Microbiol.*, 64(4), 1633–1636.

OIE, 1997. Encephalopathy and retinopathy or viral nervous necrosis. In: *« OIE Diagnostic Manual for Aquatic Animal Diseases »*, Ed. 1997, pp. 99–107

Peducasse S., 1997. Mise au point d'un modèle expérimental de transmission horizontale de l'encéphalopathie et rétinopathie virale ou nodavirose sur des bars juvéniles *Dicentrarchus labrax*. *DEA Univ. Montpellier, CNEVA Brest*, soutenue le 9 juillet 1997

Sideris D.C., 1997. Cloning, expression and purification of the coat protein of encephalitis virus (DIEV) infecting *Dicentrarchus labrax*. *Biochem. Mol. Biol. Int.*, 42, 2, 409–417

Skliris G.P. & Richards R.H., 1997 (a). Nodavirus - historical analysis and future prospects. *VIIIth International Conference 'Diseases of Fish and Shellfish'* (EAFP), Edinburgh Conference Centre Heriot-Watt University, Abstracts Book, p. 095

Skliris G.P. & Richards R.H., 1997 (b). The pathogenesis of an experimental nodavirus infection induced in tilapia *(Oreochromis mossambicus). VIIIth International Conference 'Diseases of Fish and Shellfish'* (EAFP), Edinburgh Conference Centre Heriot-Watt University, Abstracts Book, p. 051

Thiery R., Peducasse S., Castric J., Le Ven A. & Jeffroy J., 1997 (a). Experimental transmission of viral encephalopathy and retinopathy to juvenile sea bass *Dicentrarchus labrax. Bull. Eur. Ass. Fish Pathol.*, **17**, 3/4, 118–122

Thiery R., Peducasse S., Castric J., Le Ven A., Jeffroy J. & Baudin Laurencin F., 1997 (b). Evidence for horizontal transmission of viral encephalopathy and retinopathy to juvenile sea bass *Dicentrarchus labrax. VIIIth International Conference 'Diseases of Fish and Shellfish'* (EAFP), Edinburgh Conference Centre, 14–19 sept. 1997, Heriot-Watt University. Abstracts book, p. 096

Yoshimizu M., Watanabe K., Nishizawa T. & Ezura Y., 1997. Control of viral nervous necrosis of barfin flounder by selection of virus free brood stocks, and disinfection of fertilized eggs and sea water for culture. *VIIIth International Conference 'Diseases of Fish and Shellfish'* (EAFP), Edinburgh Conference Centre Heriot-Watt University, Abstracts Book, p. 098

ANNEX 15

ABSTRACTS FROM THE JAPANESE SOCIETY OF FISH PATHOLOGY MEETING 1997

Current control methods of viral nervous necrosis in striped jack (*Pseudocaranx dentex*)

K. Mori, K. Mushiake and M. Arimoto
Japan Sea-Farming Association, Kamiura, Oita 879–26, Japan

Viral nervous necrosis (VNN) caused by nodaviruses, the family Nodaviridae, has been a serious problem in marine fish hatcheries in Japan. In the Japan Sea-Farming Associatiotl (JASFA), VNN disease was first recognized in reared laryae of striped jack *Pseudocaraijx dentex* with extremely high mortalities in 1989, and then intensive investigations on the disease and causative agent (SJNNV: striped jack nervous necrosis virus) were carried out in collaboration with Kyoto University atid Hiroshima University. The most important finding was that the causative virus was detected from gonads of striped jack brood stocks, this indicating vertical transmission of the virus from spawners to the offsprings. Because of the results obtained through studies, we developed some effective methods to prevent VNN of striped jack, and the, following prophylactic measures are currently adopted in the JASFA. 1) The broodstocks are examined by ELISA-based antibody detection from the plasma and PCR-based virus detection from the gonad fluids before spawning. One spawner is not induced to spawn more than 10 times in a season. 2) Fertilized eggs collected from virus-negative spawners are washed several times with seawater and then disinfected with ozone (0.5 ppm, 1 min). 3) Eggs and hatched larvae (and juveniles) are also kept in disinfected sea water.

Control of viral nervous necrosis of barfin flounder by selection of virus frfe brood stocks in the hatchery

K. Watanabe [1], S. Suzuki [1], K. Suzuki [2], T. Niishizawa [3], M. Yoshimizu [2] and Y. Ezura [2]
[1] Japan Sea-Farming Association, Akkeshi, Hokkaido, 088–11 Japan
[2] Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido, 041 Japan
[3] Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima, Hiroshima, 739 Japan

Mass mortality of Barfin flounder *Verasper moseri* occurred at Akkeshi station of Japan Sea-Farming Association in 1993. Diagnosis of viral nervous necrosis (VNN) was made on the basis of the light and electron microscopic observations and RT-PCR to detect striped jack *Pseudocaranx dentex* nervous necrosis virus (SJNNV) specific gene sequence. Since 1994, selection of virus free brood stocks was started to control the VNN of barfin flounder in the hatchery. ELISA for detention of antibody against barfin flounder nervous necrosis virus (BFNNV) using anti-barfin flounder IgM rabbit serum were applied to detect the BFNNV carrier fish, and their eggs and sperm were examined by PCR. In 1994, VNN outbreak was observed when 11 spawners were selected by PCR. PCR negative fish showed more than 1:40 ELISA antibody titer. In 1995, 3 months before the spawning, brood stocks were selected by ELISA and remaining fish of the low antibody titer were further measured during the spawning season. All the fish which indicated the ELISA antibody titer loss than 1:20 showed the negative results by PCR, and VNN outbreak did not occur except two seed production tanks. In 1996, barfin flounder spawners which showed 1:10 ELISA antibody titer were selected where VNN outbreak didn't occur in their offspring. To prevent the disease, fertilized eggs were disinfected in the morula stage using ozonated sea water (TROs; 0.5mg/l, 5min). All equipments were disinfected by ozonated sea water (TROS; 0.5mg/l, lh) and separated for each tank. Charccoal-treated ozonated sea water (TROS; 0.5 mg/l, 5min and then removed) was used for culture, and all tanks were separated for each other.

ANNEX 16

SCIENTIFIC OBJECTIVES OF AND A STRATEGIC PLAN FOR FURTHER STATISTICAL ANALYSES OF FISH AND SHELLFISH DATA INTEGRATING OTHER DATA TYPES, E.G., CONTAMINANTS, HYDROGRAPHIC AND FISHERIES DATA

T. Lang and S. Mellergaard

Introduction

Data on the prevalence of fish diseases form a major part of the ICES Environmental Data Centre. The data are submitted to ICES by ICES Member Countries running fish disease monitoring programmes according to methodological guidelines established through the work of the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) (ICES, 1989; Bucke *et al.*, 1996).

The ICES fish disease data so far comprise information from studies on externally visible diseases and macroscopic liver nodules/tumours in the common dab (*Limanda limanda*) and the European flounder (*Platichthys flesus*) from the North Sea and adjacent waters (including the Baltic Sea) and partly date back until 1981. In 1997, a considerable amount of new data on dab and flounder diseases were added and the data can, therefore, be considered complete now and ready for a comprehensive statistical analysis. The importance of these data will increase in the future, since studies on externally visible diseases and liver nodules/tumours of dab are on the list of techniques recommended for the biological effects component of the new OSPAR Joint Assessment and Monitoring Programme (JAMP) and since ICES serves as data base for OSPAR and will provide environmental data (contaminant data and fish disease data) to be incorporated in the forthcoming OSPAR Quality Status Report (QSR) 2000.

In 1992, the ICES Sub-group on Statistical Analysis of Fish Disease Data in Marine Stocks (SGFDDS) was established according to a recommendation from the WGPDMO. The Sub-group existed until 1996 and was then replaced by an ICES Study Group with the same name, which only met once in 1997. The major tasks of the Sub-group/Study Group were:

- to establish standardised procedures for the submission of fish disease data to the ICES Environmental Data Centre (the former ICES Environmental Databank) by ICES Member Countries;

- to develop methods for and carry out a statistical analysis of fish disease prevalence data submitted to the ICES Environmental Data Centre.

At the 1997 meeting of WGPDMO, a progress report was presented on the activities of the SGFDDS and their tasks were considered fulfilled. Through the work of the SGFDDS carried out in collaboration with the ICES Secretariat, the submission of fish disease data to ICES has been standardised via the implementation of the ICES Fish Disease Data Reporting Formats (part of the ICES Environmental Data Reporting Formats), the ICES Fish Disease Data Entry Program and procedures for a validation of the data submitted (see also Section 12 and Annex 10 of this report). Statistical methods for analysing spatial and temporal trends in the disease prevalence have been elaborated and applied successfully (see also Section 7 and Annex 5 of this report).

When the ICES Advisory Committee on the Marine Environment (ACME) reviewed the reports of the WGPDMO and the SGFDDS at its 1997 meeting, it was discussed what else could be done with the fish disease data apart from analysing them for spatial and temporal characteristics and incorporating the results in the OSPAR QSR 2000. It was suggested to explore possibilities to combine the ICES fish disease data with other types of data held in the different ICES databanks (ICES Environmental Data Centre, ICES Oceanography Data Centre, ICES Fisheries Databanks) for a more holistic type of analysis and to evaluate the perspectives of such an approach. In order to start this process, the WGPDMO was given the above Term of Reference. However, it was emphasised that other appropriate ICES Working Groups should be involved as well.

In the following, some preliminary ideas are presented addressing the scientific objectives of a holistic data analysis and a possible strategy to accomplish such an analysis.

Scientific objectives of a further analysis

The following types of data available in the ICES databanks could be considered for a holistic analysis: contaminants (ICES Environmental Data Centre), oceanography data (ICES Oceanography Data Centre) and fisheries data (ICES Fisheries Databanks). Obvious objectives of an analysis can be summarised as to assess the impact of:

- environmental contaminants on spatial and temporal characteristics of fish disease prevalence data;
- hydrographic/oceanographic parameters on spatial and temporal disease trends;
- fisheries activity on spatial and temporal disease trends;
- diseases on fish stock performance.

Contaminants

Probably the most obvious objective is to assess the impact of contaminants on the spatial and temporal distribution of fish diseases, an issue which has been of great interest since the first fish disease surveys have started. The SGFDDS addressed this issue already some years ago with, however, only little success. Disease data and sediment contaminant data available at that time were considered not compatible due to different data formats and, more important, due to the fact that the spatial coverage of sampling sites were not identical.

However, since this first and not very comprehensive assessment, the ICES Environmental Data Centre has been extended considerably and now contains much more data enhancing both the range of contaminants and matrices (biota, sediment, sea water) and the spatial coverage of sampling sites. Therefore, it seem justified and promising to once more address the impact of contaminants.

A number of selections have to be made prior to an analysis:

- what kind of contaminants should be considered?
- from which matrix (sea water, sediment, biota)?
- if sediments, which fraction (whole sediment, only certain fractions, normalised data) should be used?

Of course, a selection has to be based on the data availability. Therefore, the first step will have to be to obtain an overview of the type of data available in the ICES Environmental Data Centre. An interactive inventory with maps showing sampling sites and lists with more detailed information are provided by ICES on the World Wide Web. There is, however, no direct access to the results of the various measurements. An access will be provided after consultation with ICES and the data originators.

Once there is a sufficient overview of the data, relevant contaminants and matrices have to be selected according to the scientific objectives of the data analysis. These may differ between diseases, e.g., for liver nodules/tumours, carcinogenic contaminants may be more important than for infectious diseases. According to statistical requirements and limitations, it will certainly be necessary to group the contaminant data rather than to consider single substances (e.g., for CBs and PAHs). It will probably further be necessary to restrict the analysis to certain regions for which sufficient data are present.

Oceanography

As for the contaminants, the objective of an analysis is to investigate the relationship between oceanographic factors and the spatial and temporal trends in the fish disease prevalence. Factors known or suspected to influence the prevalence of fish diseases are e.g., oxygen contents, salinity and water temperature. At least for the latter two parameters, there are numerous data available in the ICES Oceanography Data Centre. It is not clear yet what the situation is like for oxygen contents.

Again, the first step will have to be to get an overview of the data available. Based on the outcome, selection criteria will have to be defined according to the objectives of the analysis.

Fisheries

It appears as if this issue will be the most challenging project due to the complexity of data and problems encountered. Questions to be answered in a statistical analysis could be in what way the fisheries influence the spatial and temporal distribution of diseases. For example, it has been speculated repeatedly that the prevalence of the bacterial skin ulcer disease might be linked to the fishing intensity since an intensive fishery might increase the risk of fish to get into contact with fishing gears. These might cause injuries in fish either escaping through the meshes of the net or those being discarded and survive. The injuries might subsequently be invaded by pathogens, ultimately leading to the typical clinical symptoms of the skin ulcer disease.

Another interesting question which already has been addressed in association with the *Ichthyophonus* epidemics in North American and European herring stocks is to what extent fish diseases might influence fish stocks in terms of mortality, growth and reproduction and, thus, might lead to changes in the stock abundance. The main problem here will be to distinguish between stock fluctuations due to diseases and various other factors.

At present, ICES fisheries data are stored in five different data banks and a lack of compatibility of these data will certainly be one of the main problems to be solved.

Strategy for a further analysis

A strategy to be followed could be as listed below. If the WGPDMO endorses this type of project in principle, one of the major questions certainly will be: Who is going to do the work ? An option could be to recommend to create an ICES Study Group with a restricted life span working mostly by correspondence but having at least one or two meetings. The other option could be that WGPDMO alone tackles this project with the support from other WGs.

- Obtain a detailed overview of the relevant data available in the different ICES databanks
- Define the scientific objectives to be addressed
- Develop statistical methodologies considering all relevant data available
- Collaborate with the ICES Secretariat and other relevant ICES Working Groups which could contribute, such as the

  -WG on Biological Effects of Contaminants (WGBEC)

  -WG on Statistical Aspects of Environmental Monitoring (WGSAEM)

  -Joint Meeting of WGBEC and WGSEAM

  -Marine Chemistry Working Group

  -WG on Marine Sediments in Relation to Pollution

  -WG on Marine Data Management (WGMDM)

  -Fisheries WGs under the ICES Advisory Committee on Fisheries Management (ACFM)

  -and the new ICES Living Resources Committee.
- Establish a Study Group (?).

Literature cited

ICES. 1989. Methodology of fish disease surveys. Report of an ICES Sea-going Workshop held on RV U/F 'Argos' 16–23 April 1988. ICES Cooperative Research Report, No. 166. 33 pp.

Bucke, D., Vethaak, A.D., Lang, T., and Mellergaard, S. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES Techniques in Marine Environmental Sciences, No. 19. 27 pp.

ANNEX 17

LIST OF PUBLISHED FICHES
(THOSE NEEDING UP-DATING ARE MARKED IN THE RIGHT COLUMN)

Fiche #	Title		
1984-1987			
1	Papillomatosis of Eels.	FU	
2	Lymphocystis disease of fsh.	FV	
3	Ichthyophonus, a systematic fungal disease of fish.	FP	Fungus
4	Coccidiosis of the liver of blue whiting	FP	
5	Digestive tract microsporidiosis of fishes	FP	
6	Cranial myxosporidiosis of fishes	FM	
7	Pseudoterranova larvae ('codworm') (Nematoda) in fish.	FH	
8	Anisakis larvae ('herringworm') (Nematoda) in fish.	FH	
9	Phocascaris/Contracaecum larvae (Nematoda) in fish.	FH	
10	Larval paragnathiosis of mullets.	FC	
11	Haematopoietic neoplasm in the blue mussel.	MN	haematopoeitic terminology
12	Haematopoietic neoplasm in the flat oyster.	MN	haematopoeitic terminology
13	Portuguese oyster virosis.	MV	
14	Rickettisal infection of the flat oyster.	MB	
15	Rickettisal disease of Donax trunculus.	MB	
16	Shell disease of oysters.	MF	mercuric chloride mentioned as treatment. Culture to confirm putative diagnosis?
17	Minchinia armoricana disease of the flat oyster.	MP	
18	Haemocyte disease of the flat oyster.	MP	= Bonamia ostreae
19	Digestive gland disease of the flat oyster.	MP	= Marteilia refringens
20	Mytilicola orientalis Mori, parasitism.	MC	
21	Bacterial Kidney Disease.	FB	update diagnostic methods and review treatment suggestions
22	Viral Erythrocytic Necrosis.	FV	? = VHN
23	Gill Disease.	MV	
24	Mytilicola intestinalis, parasitism.	MC	
25	Viral Gametocytic Hypertrophy in oysters.	MV	
26	Salmon lice, Lepeophtheirus salmonis.	FC	
27	Vibriosis in Saithe.	FB	
28	Milk or cotton disease of shrimp.	CB	
29	Vibriosis in cultured salmonids.	FB	update serology and diagnostics - reword Control
30	Perkinsus marinus parasitism, a sporozoan disease of oysters.	MP	update diagnostics
31	Bacterial shell disease of crustaceans.	CB	
32	Fungal shell disease of crustaceans.	CF	

Fiche #	Title		
1984–1987			
33	Epidermal papilloma of dab.	FU	
34	Connective tissue inflammation in dab.	FU	
35	Ulcus syndrome in cod.	FU	
36	Spring ulcer disease in eels.	FB	
37	Furunculosis.	FB	update diagnostics, plus 'typical' vs. 'atypical' info
38	Haplosporidium nelsoni disease of American oysters.	MP	update diagnostics
39	Haplosporidium costale disease of American oysters.	MP	
40	Pasteurella disease of striped bass.	FB	
1991–1992			
41	Granulomatous hypertyrosinaemia	FU	
42	*Exophiala* spp., a systemiic fungal disease of fish.	FF	
43	Eye-maggot (*Lernaeenicus sprattae*) and body-maggot (*L. encrasicoli*) of the sprat.	FC	
44	*Hysterothylacium aduncum* (Nematoda) in fish.	FN	
45	Muscle myxosporidiosis of Pacific salmon.	FM	
46	Buccal granulomatosis of smelt.	FU	
47	Spawning papillomatosis of smelt.	FU	
48	Swimbladder nematode (*Anguillicola crassus*) in the European eel (*Anguilla anguilla*).	FH	
49	*Aporoctyle simplex*, a blood fluke in flatfish.	FH	
50	Vibriosis in sea bass.	FB	

Leaflets edited, accepted and 'in press' awaiting release.

Leaflets 'in press' - 1998	
a	*Stephanostomum tenue* in marine aquaculture of rainbow trout (*Oncorhynchus mykiss*).
b	Gaffkemia, a bacterial disease of lobsters: Genus *Homarus*.
c	*Diplostomum spathaceum* larvae (Diplostomosis) (Digenea) in fish.
d	Pasteurellosis.
e	*Flexibacter maritimus*, a causal agent of flexibacteriosis in marine fish.
f	Streptococcosis of marine fish.

Proposed Leaflets and those 'in preparation'

Proposed Leaflets - 1998 - *to be added to / discussed*		
	QPX of hard-shell clams (*Mercenaria mercenaria*).	McGladdery, Smolowitz
	Denman Island Disease of Pacific oysters (*Crassostrea gigas*).	Bower
	SPX disease of Japanese scallops (*Patinopecten yessoensis*).	Bower
	Brown Ring Disease of the clam, *Ruditapes decussatus*	Paillard, Maes

Proposed Leaflets - 1998 - *to be added to / discussed*		
	M-74	Goran
	Pancreas Disease	McVicar
	Pseudophyllidean cestodes in marine fish	Palm
	Trypanorhynch cestodes in marine fish	Palm
	Nodavirus	Baudin-Laurencin
	Pfeisteria	McLean
	Gyrodactylus salaris	McVicar
	Flavobacterium	Dalsgaard
	Flounder Liver Tumours	Vethaak
	Gonadal neoplasia of hard-shell clams	Barber, Bacon

Proposed Subject Areas

Finfish		Mollusc		Crustacean	
Viruses	FV	Viruses	MV	Viruses	CV
Bacteria	FB	Bacteria	MB	Bacteria	CB
Fungi	FF	Fungi	MF	Fungi	CF
Protists	FP	Protists	MP	Protists	CP
Myxosporeans	FM				
Helminths	FH	Helminths	MH	Helminths	CH
Crustaceans	FC	Crustaceans	MC	Crustaceans	CC
Neoplasias	FN	Neoplasias	MN	Neoplasias	CN
Undetermined Aetiology	FU	Undetermined Aetiology	MU	Undetermined Aetiology	CU

Application of Subject Areas to Published and 'in press' Leaflets (see Nos. Listed above)

	Finfish	Mollusc	Crustacean
Viruses	2, 22,	23, 25	
Bacteria	21, 27, 29, 36, 37, 40, 50, d, e, f	14, 15	28, 31, b
Fungi	3, 42	16	32
Protists	4, 5	17, 18, 19, 30, 38, 39	
Myxosporeans	6, 45		
Helminths	7, 8, 9, 44, 48, 49, a, c		
Crusteaceans	10, 26, 43	20, 24	
Neoplasias		11, 12, 13	
Undetermined Aetiology	1, 33, 34, 35, 41, 46, 47		

ANNEX 18

UPDATE ON THE CURRENT STATUS OF THE ICES IDENTIFICATION LEAFLETS FOR DISEASES AND PARASITES OF FISH AND SHELLFISH

Sharon McGladdery

Introduction

A thorough review of published Identfication Leaflets for Diseases and Parasites of Fish and Shellfish (a.k.a. Fiches), those in press and proposed titles, revealed a somewhat chaotic assembly of important disease problems and passing observations. The information contained is of high quality, however, some Fiches are seriously out-dated and require review. Others maintain applicability to the goal of providing a concise summary of the information required to identify a disease or parasite problem. The list of published and in press Fiches is given in Annex 17, along with proposed titles and authors for near-future manuscript submission.

Fiche Updating

Dependant upon Publications Committee approval revision of out-dated Fiches will be initiated. The original authors will be approached to provide the update or suggest an alternative author. If revision of the Fiches is not possible, publication of updated Fiches, in addition to outdated backnumbers will be requested. This is especially important for diseases currently falling under regulated controls (e.g., *Bonamia ostreae* = 'Haemocyte disease of flat oysters' - # 18), updating diagnostic techniqes, e.g., for bacterial infections such as 'Vibriosis in cultured salmonids' (# 29), providing distinguishing characteristics for similar, non-disease agents, and providing clearer, consistent, titles (with common and scientific names of the host and infectious agent (where applicable)).

Format

The format used for the Fiches is thorough and meets most objectives of the leaflets (provision of diagnostic aid), however:

i) 'gross clinical signs' and 'histopathology' need to be changed to reflect non-histopathologial diagnosis and effects on the host which are not reflected as classic gross clinical signs. For example,'gross clinical signs' could be changed to 'effect of infection and clinical manifestations' and 'histopathology' to 'diagnostic methods and features of infection'. This would permit inclusion of tissue-culture, new immno-assays and nucleic acid assays, as well as diagnosis of non-clinical infections, e.g., parasite infections;
ii) do we still want to include 'Key Laboratories' in addition to 'Key references'?;
iii) The 'comments' section varies significantly between Fiches and should probably concentrate on noting similar organisms, or other factors, which can confuse diagnosis;
iv) Key references also vary in detail and should be restricted to those useful for diagnostics.

Organisation

Publication in a series of ten, with no particular theme, or time-frame for release, appears responsible for the present disjointed collection. The following changes to publication organisation are, therefore, suggested:

i) all published (and revised) Fiches be assigned a subject label, as well as a release number. The subject groups could be similar to those used by the WGPDMO, as shown in Annex 17. This will assist selection by people with narrow subject interests and organisation by general interest users.
ii) An ICES binder with subject area dividers be made available to help file the Fiches.
iii) Titles 'in press' be sent out to previous purchasers, pertinent journals (Bulletin of the European Association of Fish Pathologists, Journal of Fish Diseases, etc.) and Web Sites, with a deadline for orders, every two (?) years or so (irrespective of the number of titles 'in press'). This will assist in deciding how many copies to print and the Fiches can be ordered when they are still up-to-date.

Title Selection and Relevance to Fiche Series

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

ANNEX 19

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

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

b) Analyse national reports on new disease trends in mariculture for fish and shellfish.
Reports on new diseases and trends on diseases were evaluated from national reports presented at the meeting and conclusions were drawn up.

c) Review and analyse the data extracted from ICES Fish Disease Databank intersessionally and at the WG meeting.
Analysis of the Fish Disease databank was undertaken intersessionally by ICES staff and during the meeting and a report produced on the interpretation of results.

d) Review proposals for the development of a training and intercalibration programme for the diagnosis of histological liver lesions as part of a quality assurance scheme.
A proposal for EU-funding establishing a training and intercalibration programme had been submittet and the WG endorsed the project. If funding is not obtained from that source, WGPDMO will reconsider the options open to further development in this area.

e) Compile available evidence on the causes of the M-74 syndrome in Baltic salmon and provide a summary of the progress in understanding the relevant environmental factors influencing the occurrence of M-74 along with the geographical extent of its distribution.
Available information was assessed and a summary report provided in the Annex of this report.

f) Maintain an overview of the *Ichthyophonus* issue as part of its regular agenda and report to ACFM and ACME if new information becomes available.
The current status of Ichthyophonus in the North Sea and the North Atlantic herring stocks was assessed and a report compiled for the presentation to the ACFM and ACME.

g) Review available information on pathological aspects of endocrine disrupting chemicals in estuarine and marine organisms.
A summary of the current stage of knowledge on the subject was compiled and recommendations made on research required.

h) Review intersessional progress in the development and implementation of a quality assurance plan for the integration of flatfish liver histopathology measurements into the OSPAR Joint Assessment and Monitoring Programme and in the adaptation of the ICES Fish Disease Data Entry Program and Fish Disease Reporting Format for the incorporation of liver histopathology data into the ICES Environmental Databank. the WG appreciated the ICES Council's decision to incorporate liver histopathology data into the ICES Environmental Databank.
A quality assurance plan has not yet been established but action in this drection has been taken by members of the WG (see item d).

i) Assess the progress in studies on the possible causes of *Bonamia ostrea* persistence in areas with low densities of oysters and the possible relationship to disease resistance and water temperature inhibition of infection.
The assessment was conducted but no results are yet available yet. A recommendation to encourage progress in this field was made.

j) Prepare an information package on the ICES Environmental Databank and Disease Data Reporting Format for submission to laboratories in Member Countries identified as conducting shellfish disease surveys and/or diagnostics.
An information package has been prepared and will be submitted to laboratories dealing with shellfish diseases.

k) Review new progress in diagnostics, epidemiology and immunology of nodavirus and new nodavirus-like associated diseases.
Current knowledge on nodavirus was assessed and a summary of the most recent progress was provided as an Annex to this report.

l) Define the scientific objectives of, and propose a strategic plan for, further statistical analyses of fish and shellfish data integrating other data types, e.g., contaminants, hydrographic and fisheries data, and report to ACME.
Scientific objectives of further analysis of the fish and shellfish data were established and are included as an Annex to this Report.

ANNEX 20

RECOMMENDATIONS TO COUNCIL

Recommendations

The Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) recommends that it meets at the University of Oporto, Portugal on 1–5 March 1999 under the Chairmanship of Dr S Mellergaard to:

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

b) assess the progress in data submissions to the ICES Fish Disease Databank;

c) provide an overview report of data available in ICES Databanks which may be used for a holistic analysis in relation to disease data;

d) compile and review available information on suitable shellfish species and diseases and on available data in ICES Member Countries;

e) maintain an overview of new information on *Ichthyophonus hoferi* and report to ACFM and ACME;

f) maintain an overview of new information on M-74 and report to ACME;

g) review new information on the spread, diagnosis and control of nodavirus to further advise on possible control measures;

h) compile and review available information on the impact of marine biotoxins produced by dinoflagellates and algae on fish populations to provide a basis for evaluation of the significance and dynamics and future research;

i) clarify the host specificity and pathogenicity of herpes-like viral infections in mollusc hatcheries, *Marteilia* sp. from *Crassostrea gigas* and the *Haplosporidium*-like parasite in *Ostrea edulis*;

j) assess the disease risks for wild and cultured crustaceans from known pathogens of penaeids;

k) review available information on the use of parasites of marine fish species as indicator organisms for environmental changes;

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) review progress in the development and implementation of a quality assurance programme for fish liver histopathological diagnosis.

Justification for Recommendations to Council

There are major developments in the field of pathology and diseases of marine organisms to warrant a further meeting of the WGPDMO

a) New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of them should be maintained.

b) The fish disease data being submitted on a regular annual basis to ICES should be monitored to ensure that continuation and quality is maintained and assess whether a new in-depth analysis is required.

c) WGPDMO emphasised that a holistic statistical analysis involving fish disease, contaminant, oceanographic and fisheries data held in the ICES Databanks is highly desirable in order to provide a more detailed picture of the relationship between fish diseases and natural as well as anthropogenic factors.

d) In preparation for the future expansion of the ICES Environmental Data Centre to include data on shellfish diseases it is necessary to get an overview of which shellfish species may be suitable for inclusion into the Databank and if such data already exist in files of some shellfish laboratories in ICES Member Countries.

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

g) Nodavirus remains a disease of major importance as a pathogen in mariculture. With the increasing range of host species and the rapid progress in research in diagnosis and epidemiology, it is necessary to maintain close awareness of new developments in order to provide sound advice to ICES.

h) Biotoxins produced by dinoflagellates and algae have caused mass mortalities and pathologies in recent years in wild and farmed fish populations in many ICES areas (Atlantic and Pacific coasts of North America, Norway, the northern Baltic Sea). It is evident that these organisms have the potential to have an influence on fish health, either directly or indirectly, especially in coastal and estuarine sea areas. While several aspects of the dinoflagellate/algal blooms are dealt with by other scientific bodies, the evaluation of pathological aspects and possible impacts on fish populations from a fish health point of view should be dealt with by the WGPDMO.

i) The appearance of herpes-like viruses in an increasing number of species of bivalves is cause for concern, since these viruses are known to be transmissible in open water nursery areas. More information on their host specificity is necessary to avoid accidental spread to other hatchery produced species or populations, via seed distribution. *Marteilia* species continue to require differentiation since morphological features are inadequate. This will clarify the host specificity question, which encompasses European oysters, blue mussels and possibly Pacific oysters. The latter host species observation is particularly serious since some legislative shellfish movement controls are based on the belief that Pacific oysters do not host *Marteilia* spp. Lastly, the haplosporidian found in European oysters opens the possibility of another host species for the MSX species *H. nelsoni* and this needs to be clarified.

j) The increase in number of viral inclusions being detected in wild and cultured shrimp (penaeids and other groups) has increased the difficulty in differentiating potentially pathogenic viruses from benign inclusion bodies. There is thus a growing need to assess the current knowledge on shrimp (and other crustacean) viruses, their specificity and diagnosis.

k) Parasites are an integral part of each ecosystem and can serve as potential indicator organisms for environmental changes. For example, it could be demonstrated that the infection of fish species with trichodinid ciliates reflects the water quality, and the infestation with helminth species can be associated with different eutrophication and pollution levels. To evaluate the potential use of parasites in current monitoring programmes, a review of the literature is needed for further selection of possible target parasite species occurring in the North Sea and adjacent areas (including the Baltic Sea).

l) As international marine monitoring organisations (e.g., OSPAR), the European Environment Agency, US EPA and national environment agencies have been requested to consider the relevant recommendations from the EMWAT Workshop held in Veldhoven, The Netherlands in April 1997, WGPDMO should similarly take appropriate action.

m) WGPDMO re-emphasised the need to develop and implement a QA programme for training and intercalibration with respect to the diagnosis of fish liver histopathology as a prerequisite for the intcorporation of studies on liver histopathology into the international monitoring programmes.