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Report of the

Working Group on Pathology and Diseases of Marine Organisms

Copenhagen, Denmark

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International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 OPENING AND STRUCTURE OF THE MEETING

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the Danish Institute for Fisheries Research (DIFRES), Charlottenlund Castle, Denmark with S. Mellergaard as Chair. The meeting was opened at 10.00 hrs on Tuesday 12 March 2002 with the Chair welcoming the participants, particularly the new members who have not previously attended WGPDMO meetings.

A list of participants is appended in Annex 1.

Apologies were received from O. Haenen (Netherlands), V. Kadakas (Estonia), G. Díez (Spain), A. Figueras (Spain), S. McGladdery (Canada), M. Podolska (Poland), and T. Bezgachina (Russia).

The meeting took the form of a series of plenary sessions with specialist subgroups organised as necessary to consider some agenda items in detail before reporting conclusions back to the full WG for consideration and endorsement.

2 ICES, MARICULTURE COMMITTEE: ITEMS OF RELEVANCE TO WGPDMO

Items of relevance to WGPDMO were highlighted by the Chair.

a) The report of the Mariculture Committee:
   - i) accepted the report of the 2001 meeting of WGPDMO and its recommendations without change.
   - ii) the Mariculture Committee conducted its intersessional review of the 2001 WGPDMO report in order to get an early release. The WG Report was circulated at the beginning of May and the members of the Mariculture Committee were given three weeks to report back with comments. There were very few comments and the Report was released at the end of May 2001.
   - iii) In response to the ICES Strategic Plan, the Mariculture Committee has drawn up an Action Plan and the Chair pointed out the subjects involving WGPDMO.

b) ICES:
   - i) the ICES General Secretary circulated the 2001 WGPDMO report to the EU-Commission, OIE, FAO and PICES, all organisations dealing with regulatory aspects of fish health to raise the awareness that ICES provides information and advice on a broad range of fish and shellfish health aspects.
     WGPDMO proposed to ask the organisations for their opinion on the information presented in the WGPDMO report and the interest in receiving the report in the future.

c) As agreed at the 2001 meeting, a poster describing the WGPDMO activities was prepared and presented at the EAFP Conference in September 2001, Dublin, Ireland.

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

3.1 Terms of Reference

WGPDMO took note of the Terms of Reference published as C. Res 2001/2F02 (Annex 2). The agenda once again demanded extensive intersessional work by the members of the WGPDMO selected by the Chair. These persons were requested to produce written working/discussion documents to be included in the WGPDMO report as Annexes. As agreed, all working documents were to be prepared two weeks before the meeting and distributed by e-mail. As a result, all national reports and a considerable part of the remaining working documents were distributed to the participants before the meeting. The Chair thanked the members for preparing these reports in advance, a task which ensures the Terms of Reference can be treated efficiently.

3.2 Adoption of the Agenda

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

3.3 Selection of Rapporteurs

Rapporteurs were accepted as indicated in Annex 4.

4 OTHER RELEVANT INFORMATION

Information was given on a series of scientific conferences to be held in 2002:

- SETAC Europe 12th Annual Meeting, May 2002, Vienna, Austria;
- Fourth International Symposium on Aquatic Animal Health, September 2002, New Orleans, LA, USA;
- ICES Annual Science Conference, September 2002, Copenhagen, Denmark;
  Theme Sessions:
    - Immuno-modulators and Probiotics: Alternatives to Chemotherapeutics?
    - Salmon Aquaculture, Enhancement and Ranching: Are they a Threat to Wild Salmonid Stocks?
    - Interactions of Humans with Marine Ecosystems: Unaccounted Mortality in Fisheries;
    - Biological Effects of Contaminants in Marine Pelagic Ecosystems.
- SETAC North America 23rd Annual Meeting, November 2002, Salt Lake City, Utah, USA;
- 4th International Conference on Molluscan Shellfish Safety, June 2002, Santiago de Compostela, Spain;
- World Aquaculture Society, April 2002, Bejing, China;
- European Aquaculture Association, October 2002, Trieste, Italy;
- 5th International Symposium on Viruses of Lower Vertebrates: Comparative Virology of Amphibians, Reptiles and Fish, August 2002, Seattle, Washington, USA;
- ICPOA International Congress of Parasitology, August 2002, Vancouver, Canada;
- National Shellfisheries Association, April 2002, Mystic, Connecticut, USA;
- 8th International Conference on Copepoda, July 2002, Keelung, Taiwan;
- Impact of myxozoan parasites in wild and farmed finfish, July 2002, Nanaimo, Canada;
- 5th Triennial Symposium on Diseases in Asian Aquaculture (DAA5), Fish Health Section of the Asian Fisheries Society from 25–28, November 2002, Brisbane, Australia. Two satellite workshops will follow the Symposium: Epidemiology and Risk Assessment, 29–30 November 2002, and the Asia-Pacific Regional Molluscan Health Management Training Program Phase II, 2–6 December 2002.

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

5.1 Wild Fish Stocks

Viruses

There is an increasing effort to screen wild marine fish for viruses because of possible interactions between farmed and wild stocks.

Infectious pancreatic necrosis virus (IPNV) was isolated from winter flounder (*Pseudopleuronectes americanus*) from New Brunswick and Nova Scotia as well as brook trout (*Salvelinus fontinalis*) from New Brunswick. All positive fish were sub-clinical.

Viral haemorrhagic septicaemia virus (VHSV) was isolated for the first time from sand goby (*Pomatoschistus minutus*) in the western Baltic Sea. Studies in wild Baltic herring (*Clupea harengus*) from the Finnish south coast failed to isolate the virus from 1500 fish. Negative results were also obtained in Irish studies from 120 wild marine fish, mostly Atlantic cod (*Gadus morhua*) and a small number of sole (*Solea solea*), plaice (*Pleuronectes platessa*), turbot (*Scophthalmus maximus*) and flounder (*Platichthys flesus*). North American strains of VHSV were isolated from asymptomatic fish of several species (Euchalon smelt (*Thaleichthys pacificus*) and surf smelt (*Hypomesus pretiosus pretiosus*) from Oregon, Pacific sardine (*Sardinops sagax*) and Pacific mackerel (*Scomber japonicus*) from southern California).

Infectious salmon anemia virus (ISAV) was not found in 1600 wild fish belonging to several non-salmonid species (see Annex 5) collected from areas along the Atlantic coast of the USA near or far from Atlantic salmon (*Salmo salar*) aquaculture sites in Maine. Over 1850 wild fish (collected from the wild, including salmonids deemed as aquaculture escapees) from Atlantic Canada were also negative for ISAV.

Lymphocystis in dab (*Limanda limanda*) showed a decreasing trend in the North Sea. The highest prevalence in European flounder (*Platichthys flesus*) was found in the southwestern Baltic Sea (41.9 %). Considerably lower prevalences (between 0.0 % and 1.6 %) were recorded in ICES Subdivisions 24, 25, and 26 (Polish Exclusive Economic Zone).

Bacteria

Aeromonas salmonicida was isolated from wild Atlantic salmon associated with mortality in the Miramichi River drainage Basin, New Brunswick, Canada.

Multiple species of *Mycobacterium*, including several new species, have been isolated from dermal and/or visceral lesions of striped bass (*Morone saxatilis*) in the Chesapeake Bay, Maryland and Virginia, USA.

Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon juveniles are showing increasing levels of infection with a variety of pathogens, including *Renibacterium salmoninarum*, *Vibrio* sp., *Aeromonas* sp. and *Yersinia* sp., in Washington and Oregon, USA. The prevalence and composition of the pathogens varied between estuaries. Laboratory studies are under way to investigate the relationship between water quality and pathogen prevalence.

Fungi

Aphanomyces invadens continued to be isolated from dermal ulcers in Atlantic menhaden (*Brevoortia tyrannus*) in the Chesapeake Bay, Maryland and Virginia, USA. Although these lesions have been attributed to *Pfiesteria piscicida*, laboratory experiments by injection and bath immersion with zoospores of the fungus have reproduced the lesions in the absence of *Pf. piscicida*.

Toxic Algae

From a broad-scale survey on the presence of *Pfiesteria piscicida* in water samples taken from the tributaries of the Chesapeake Bay, Maryland, USA, there is growing evidence that fish kills and ulcerated menhaden frequently occur in the absence of the alga (see above).

Parasites

Parvicapsula minibicornis (Myxosporea) was detected in new host species in the Fraser River watershed, British Columbia, Canada (chinook salmon, pink salmon (*O. gorbusha),* rainbow trout (*O. mykiss*)) and in a new location (Okanagan River, British Columbia, Canada) in sockeye salmon (*O. nerka*).

Kudoa sp. (Myxosporea) infestation is decreasing in prevalence in wild marine sea trout (*Salmo trutta*) in France.

Gyrodactylus salaris was not found in wild salmonids from Irish river catchments. In Norway, the parasite remained a major threat for Atlantic salmon. Infested rivers are treated with rotenone, but re-infestations occur.

Pseudoterranova decipiens in American plaice (*Hippoglossoides platessoides*) continued to increase both in prevalence and in regional distribution in central and eastern Scotian shelves off Nova Scotia, Canada, and is associated with low abundance and recruitment of the fish host.

Infection of beaked redfish (*Sebastes mentella*) from the Barents Sea with *Anisakis simplex* continued to increase in prevalence.

Prevalences of *Contracaecum* sp., *Anisakis simplex* and *Hysterothylacium aduncum* recorded in anchovies (*Engraulis encrassicolus*) from French and Spanish parts of the Gulf of Biscay, Spain, were 12.5 %, 7.4 %, and 3.8 %, respectively.

The swimbladder nematode *Cystidicola farionis* was detected in 95 % of smelt (*Osmerus eperlanus*) in the Odra River estuary, Poland.

Echinorhynchus gadi was recorded in 97 % of Baltic cod collected close to the Hel Peninsula, Poland.

Lepeophtheirus salmonis is still considered a problem in wild Atlantic salmon and sea trout (*Salmo trutta*) in Norway. However, the infestation seemed to be less severe in 2001 compared to previous years.

The prevalence of the parasitic copepod *Sphyrion lumpi* has increased in beaked redfish in the Barents Sea.

In studies carried out in UK estuaries, a variety of parasites recorded in flounder showed differences in prevalence or intensity between PAH-contaminated sites and a reference site, suggesting that different taxa may provide useful information on environmental conditions. At polluted sites, higher intensities of *Trichodina* sp., *Lernaeocera branchialis*, *Gyrodactylus* sp., *Lepeophtheirus pectoralis* and *Acanthochondria cornuta* were observed compared with non-polluted sites. In contrast, lower prevalences of *Myxidium incurvatum*, *Anisakis simplex*, *Contracaecum osculatum* and gut Digenea were observed at PAH-contaminated sites.

Other diseases

Prevalences of neoplastic and pre-neoplastic liver lesions in North Sea dab (*Limanda limanda*) and Baltic Sea flounder (*Platichthys flesus*) remained at a low level. The prevalence of toxicopathic liver lesions in flatfish species in Puget Sound, USA, continued to decrease 92 months after the placement of a sediment cap covering a PAH-contaminated site. English sole (*Parophrys vetulus*) collected from the Seattle waterfront, USA, showed a sharp decline in the prevalence of liver lesions despite the fact that PAH levels remained at a high level.

In a pilot study of the pathology and parasitology of fish species in UK estuaries, it was shown that flounder from contaminated sites exhibited an increased prevalence of liver pathology (fibrillar inclusions, nuclear pleomorphism, adenoma). Viviparous blenny (*Zoarces viviparous*) from the Tyne estuary were affected by a high prevalence of ovotestis in males, significant kidney pathology (tubule pathology) and liver lesions (cholangioma, cellular and nuclear pleomorphism).

Prevalences of hyperpigmentation in North Sea dab remained high (up to 40 %) and showed an increase in some areas (German Bight, Dogger Bank, Firth of Forth). The aetiology has still not been resolved.

The prevalence of acute/healing skin ulcers in dab remained at a low level in the North Sea (<3 %). However, an increase was recorded in the inner Liverpool Bay, Irish Sea. The prevalence in cod from the southwestern Baltic Sea decreased compared to the period after 1998 (up to 30 %), but remained at an elevated level (up to 16 %) compared to the long-term average.

5.1.1 Conclusions

1) The host range of marine VHSV in wild marine fish species has continued to increase.
2) New species of *Mycobacterium* associated with pathology have been isolated from striped bass (*Morone saxatilis*) in the Chesapeake Bay, USA.
3) The hypothesis of a direct involvement of *Pfiesteria piscicida* in the occurrence of skin ulcers and mortalities in menhaden from the Chesapeake Bay, USA, is further weakened since there is increasing evidence that both events occur in the absence of *Pfiesteria*. In addition, it has been shown experimentally that *Aphanomyces invadens* causes skin ulcerations in menhaden in the absence of *Pfiesteria*.
4) The prevalences of putative toxicopathic liver lesions continued to decrease in flatfish from the Puget Sound, USA, as a result of sediment capping at a PAH-contaminated site.
5) Hyperpigmentation continued to show an increasing trend in dab from areas in the North Sea. The aetiology still needs to be resolved.
6) Results from studies carried out in estuarine fish species (flounder, viviparous blenny) from the North Sea and Irish Sea indicate the occurrence of biological effects of contaminants (e.g., liver and kidney histopathology, ovotestis). Results from parasitological studies in flounder revealed promising results as to the usefulness of parasites as indicators of ecological quality.

5.1.2 Recommendation

WPGDMO recommends that:

ICES Member Countries ensure that adequate funding is made available to continue health surveillance of wild fish stocks. Continued disease monitoring is necessary:

1) To be used as an indicator of environmental conditions, including anthropogenic effects;
2) To assess the impact of disease on wild fish stock performance;
3) To assess the potential for disease interactions between wild and farmed fish;
4) To recognise emerging diseases caused by infectious agents and/or contaminants.

5.2 Farmed fish

Atlantic salmon, *Salmo salar* - Viruses

Infectious salmon anaemia virus (ISAV). Clinical ISA was diagnosed at 21 sites in Norway, all in western areas. During the past five years, there has been an increasing trend in reported cases. A decreased focus on enforcement of management procedures, including fallowing, removal of dead fish, increased size of the industry and increased transportation, may be contributing factors. ISA remains a serious problem in infected farms, although the overall impact for the industry is low. Several vaccine companies are involved in the development of a vaccine and progress is being made. However, vaccination is not an official strategy to control ISA in the EU or in Norway. ISA in the Faroe Islands, Denmark, is of increasing concern.

In Scotland, no cases of ISA were reported for 2001. A report on the ISA epidemiology in Scotland is available "Epizootiological investigations into an outbreak of infectious salmon anaemia (ISA) in Scotland". FRS Marine Laboratory, Aberdeen. Report April 2001, 13/1, 60pp. It is expected that Scotland will be declared free of ISA in 2002.

During 2001, there were no new cases of ISA in Nova Scotia, Canada, but a slight increase in infected farms in New Brunswick, Canada, in 2001. The control and management programmes are considered practical and effective. Two experimental vaccines are available. Data exist to substantiate protection in laboratory trials but field trial data are lacking. Outbreaks of ISA have occurred in vaccinated fish, however, removal of infected cages is the prime disease management tool used, which leaves little material that can be used to verify the vaccine efficacy.

The appearance of ISA in several farms in Maine, USA, may have contributed to the increase in the number of ISA cases in 2001 in the Bay of Fundy. Mandatory surveillance is carried out across the Canada-US border. The current situation in the USA is presented in a separate report (Annex 5).

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

Infectious Haematopoietic Necrosis virus (IHNV). On rare occasions, losses in hatchery chum salmon (*Oncorhynchus keta*) in Canada have occurred. This is attributed to the presence of infected wild stocks in the vicinity.

Salmon Pancreas Disease Virus (SPDV). No new trends were reported, but data suggest the number of confirmed cases in single numbers in Scotland and Ireland. In Norway 13 cases were noted.

Epitheliocystis. This remains a problem in some parts of Norway in post-smolts 3–6 months after transfer. Recently, a paramyxo-like virus has been isolated from gills. The importance of this finding is unclear.

Atlantic salmon - Bacteria

Renibacterium salmoninarum. Outbreaks in Scotland and Norway remain low.

Aeromonas salmonicida. Outbreaks in Norway and Scotland are generally controlled by vaccination. In Norway, some clinical cases have been registered and the reasons are unclear. Several cases of *A. salmonicida* were identified in Newfoundland, Canada.

Vibrio* (*Listonella*) *anguillarum, V. salmonicida, Moritella viscosa. Reports of *V. salmonicida* and *V. anguillarum* are low in Norway. In Scotland, there were no isolations of *V. salmonicida* and cases of *M. viscosa* seem to be increasing. In Norway, there is an indication that a vaccine for *M. viscosa* has a positive effect, but disease outbreaks in vaccinated fish have also been recorded.

Piscirickettsia salmonis. In Norway, one case was recorded in 2001. None were recorded in Scotland.

Atlantic salmon -Parasites

Paramoeba. No new information was reported.

Lepeophtheirus salmonis. Sea lice numbers are declining in Scotland and Norway due to the use of in-feed treatments and management practices.

Non-infectious conditions. In Norway, there is a growing concern regarding so-called production problems including cataracts, heart and skeletal deformities that will result in downgrading at market. Gill pathology in Ireland occurred during the summer months due to phytoplankton blooms. Losses appear to have been low, in contrast to Scottish waters where phytoplankton blooms caused total finfish biomass losses of 1500–1700 tonnes.

Rainbow trout, *Oncorhynchus mykiss* - Viruses

Viral Haemorrhagic Septicaemia Virus (VHSV). VHSV spread within Finland in 2001, and the restriction zone now includes the whole Åland archipelago. In addition, a new single outbreak in the Gulf of Finland, with losses up to 50 % was recorded. The current situation in Finland is presented in a separate report (Annex 6). The taxonomic status of these VHSV strains is unclear (see Annex 11).

Rainbow trout – Bacteria

Renibacterium salmoninarum. No new cases were reported in Scotland. Two new cases have been reported in Norway and ten new cases in Finland.

Aeromonas salmonicida. Infection is generally controlled through vaccination, and no new trends were reported in Scotland and Norway. In Denmark, approximately 70 % of the diseases requiring treatment were attributed to furunculosis.

Yersinia ruckeri. Infection is generally controlled through vaccination and no new trends were reported.

Flavobacterium psychrophilum. Outbreaks occurred in Finland in fry but there is no significant trend.

Pseudomonas anguilliseptica. This bacterium is causing problems in Finnish stocks but there are no significant trends.

Whitefish (*Coregonus lavaretus*) – Bacteria

Renibacterium salmoninarum was recorded for the first time in Finland in whitefish. This industry is growing, but at present there is insufficient experience to evaluate the significance of BKD. Information on clinical signs is required.

Whitefish – Parasites

Henneguya zschokkei and *Triaenophorus crassus* are becoming significant problems for whitefish farming in Finland, particularly from the food hygienic (aesthetic) point of view.

Turbot (*Scophthalmus maximus*) - Viruses

Herpesvirus scophthalmi was reported for the second year in Norway during routine histological testing of imported fish. No clinical signs of disease were reported.

Turbot - Bacteria

Flexibacter maritimus and *Aeromonas salmonicida* cause important losses in Spain and France, but with no noticeable trends.

Pseudomonas anguilliseptica was isolated for the first time in turbot in Spain. Currently there is a limited distribution.

Turbot – Parasites

In Spain, the histiophagous ciliate *Uronema* sp. (probably, *Philasterides*) is becoming the most significant disease for cultured turbot. At present, moderate losses occur when the water temperature increases above 16 ^{o}C.

Sole (*Solea senegalensis*) -Viruses

Lymphocystis virus was detected in juvenile fish (size 5 g).

Sole - Bacteria

Co-infections of flexibacteriosis and pasteurellosis were reported for the first time in Spain.

Halibut (*Hippoglossus hippoglossus*) - Viruses

Nodavirus was not recorded in Norway.

Wolffish (*Anarhichas lupus, A. minor*) - Viruses

Histopathological changes similar to cardiac myopathy syndrome (CMS) have been reported in adult spotted wolffish.

Wolffish – Bacteria

Atypical *Aeromonas salmonicida* has been isolated occasionally but with low mortalities.

Wolffish-Parasites

***Pleistophora*.** This parasite has been detected in fillets of spotted wolffish.

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

Viral encephalopathy and retinopathy (VER) is still the major viral problem reported in sea bass farms in France.

Sea bass, seabream – Bacteria

Pasteurellosis in sea bass and seabream shows a stable trend in France and Spain. Vibriosis caused by *Vibrio harveyi* and *V. alginolyticus* is increasing in the seabream culture in Spain.

Sea bass – Parasites

Infestation of sea bass with an isopod (*Cerathotoa oestroides*) in the buccal cavity was reported to occur in France and Turkey resulting in decreased growth and some mortality (up to 10 %).

Increasing problems with infestation by *Myxidium leei* causing low mortality were observed in farmed seabream in France.

Sea bass - conditions of undetermined aetiology

Several cases of hypertrophic and granulomatous spleen were reported in on-growing seabream in Spain, although no mortalities were recorded.

Cod (Gadus morhua) - Viruses

Infectious Pancreatic Necrosis Virus (IPNV). No new trends were identified.

Cod - Bacteria

Vibriosis caused by *V. anguillarum* is the main disease in juvenile cod in Norway. Proper vaccination is difficult to achieve in semi-intensive production.

Cod – Parasites

Loma loma (Microsporidian) was reported as a problem in cod reared in research facilities in Newfoundland, Canada.

5.2.1 Conclusions

1) In Norway, outbreaks of ISAV are reported at similar/slightly increasing levels. In Canada, elevated mortalities occurred in Nova Scotia with decreases reported from the Bay of Fundy, New Brunswick.
2) The first outbreak of BKD in whitefish was recorded in Finland.
3) Infections due to *Vibrio* spp. and *Aeromonas* spp. are generally under control through vaccination.
4) *Uronema* sp. (probably, *Philasterides*) is becoming the most significant disease for cultured turbot in Spain.

5.2.2 Recommendations

WGPDMO recommends that:

i) Continued efforts should be directed towards characterisation of marine and freshwater strains of VHSV and their link to pathogenicity;
ii) The significance of *Uronema* sp. (probably, *Philasterides*) for cultured turbot should be determined.

5.3 Wild and Farmed Shellfish and Crustaceans

Viruses

Herpes-like virus in bivalves – PCR using the primer oyster herpes virus type 1 (OsHV-1) confirmed infection in larvae and spat (23 of 266 batches) of *Crassostrea gigas* from natural beds and hatcheries in France, and the virus was associated with abnormal mortalities among spat in June 2001. Herpes-like virus infections were also observed in larvae and spat of *Pecten maximus* (first report), *Crassostrea angulata*, *Ostrea edulis*, *Ruditapes philippinarum*, and *Ruditapes decussatus* in France.

Shrimp viruses - The first acute case of White Spot Syndrome Virus (WSSV) was detected in a wild shrimp, *Litopenaeus setiferus*, in South Carolina, USA, and the virus was confirmed from a wild shrimp in the Gulf of Mexico, USA.

Bacteria

Nocardiosis – no new trends (Canada). PCR assay was developed but was not as sensitive in detecting infection in comparison to classical techniques (histology and tissue imprints).

Withering syndrome of abalone (mostly *Haliotis cracherodii*, occasionally *H. rufescens* and *H. corrugata*) – no new trends. The suspected agent was detected in two new locations north of San Francisco, including near the Oregon-California border, but without evidence of the characteristic withering syndrome.

***Vibrio tapetis* (Brown Ring Disease)** – in France, not as many cases in 2001, but surveillance effort was reduced.

Parasites

Bonamia ostreae in *Ostrea edulis* – no new trends. Remains confined to four known areas in Ireland at prevalences <20 %. This parasite persists at low prevalences in France (5.8 % on the French Atlantic coast and 7 % on the Mediterranean coast) and the Atlantic coast of Spain (<20 %). Oysters (*O. edulis*) from Norway (Trondheim) became infected several months after transfer to the Mediterranean coast.

Marteilia refringens in *O. edulis, Mytilus edulis* and *Mytilus galloprovincialis* – no new trends. Low prevalence (<10 %) was recorded in Portugal and Spain (Ria de Vigo). High prevalences (i.e., 60 % in Port Leucate) were reported on the Mediterranean coast of France. Oysters (*O. edulis*) from Norway (Trondheim) became infected several months after transfer to the Mediterranean coast, indicating that they are susceptible to *M. refringens*.

Mikrocytos mackini **(Denman Island Disease)** – no new trends in Canada. Development of PCR assay and *in situ* hybridization techniques indicates that the parasite occurs in oysters throughout the year, with disease and detection via classical methods (histology) possible only in the spring months.

Perkinsus **spp.**

P. marinus **(Dermo)** in *Crassostrea virginica* – no new trends. Prevalence remains at or near 100 % along most of the parasite's range in the USA from the Gulf of Mexico to Massachusetts. Mortalities are reported from the northeastern and mid-Atlantic states, but the parasite does not appear to be causing reportable mortalities in the southeast. New molecular markers have been developed that distinguish genetic strains of *P. marinus*. Strong cross-reactivity of antisera developed against *P. marinus* occurs with other *Perkinsus* species and with parasitic dinoflagellates of crustaceans (*Hematodinium* spp.).

P. atlanticus in *Ruditapes decussatus* – reduction from last year (to about 15 %) with no associated losses in Spain.

P. chesapeaki* / *P. andrewsi in *Mya arenaria* and *Macoma balthica* – no new information. *Perkinsus chesapeaki* and *P. andrewsi* may be the same species.

Haplosporidium **spp.**

H. nelsoni **(MSX)** in *Crassostrea virginica* – no new trends. High prevalence continues in the high salinity regions of Chesapeake Bay, but was relatively low in other regions to the north and south.

H. costale **(SSO)** in *Crassostrea virginica* – no new trends. Low prevalence with low mortality continues in high salinity areas of the northeastern and mid-Atlantic states, USA. Maximum reported mortality, occurring in the spring, was 5 % to 12 % in Massachusetts.

Haplosporidium **sp.** – possibly *H. nelsoni* was detected in 0.07 % of *Crassostrea gigas* (n=1304) from the Atlantic coast of France.

Quahog Parasite X (QPX) disease in *Mercenaria mercenaria* – No new trends reported from Canada. Has now been identified in two new areas on Cape Cod, MA, USA with associated mortality estimates ranging from 20 % to 95 %. The first significant QPX-associated mortality (57 %) in Virginia was reported in the spring of 2001. The mortality occurred at a single site and was limited to clams from a Florida hatchery.

Protozoan parasite of spot prawns – phylogenetic affiliation still unknown (originally appeared to be a parasitic dinoflagellate but shows DNA affinities to Haplosporidia without morphological features of this phylum) and prevalence in the field remains low, at less than 1 % (was 19.6 % in 1992). A new host, *Pandalus dispar*, was detected in British Columbia, Canada.

Unidentified yeast in crabs – new report of a secondary infection in *Hematodinium*-infected *Cancer pagurus* and *Necora puber* in southern England.

Prosorhynchus squamatus **(Trematode)** in *Mytilus edulis* – no new trends.

Algae

Hematodinium **sp. (bitter crab syndrome)** in tanner crabs (*Chionoecetes* spp.) – new host species (*Chionoecetes tanneri*) and extension of range to British Columbia, Canada. Prevalence in *Chionoecetes opilio* from Newfoundland remains low (<4 %). The effect of this parasite on tanner crab population dynamics in Canada is not known.

Hematodinium perezi in blue crabs (*Callinectes sapidus*) – no new trends.

***Hematodinium* sp.** in Crustacea from UK - detected in one of two Norway lobsters (*Nephrops norvegicus*) from Dundrum Bay (northern Ireland) in the summer (usually only observed during the winter and spring periods). A similar species was observed in crabs (*Cancer pagurus*) from Guernsey (Channel Islands) and off Dorset and Cornwall and from *Necora puber* on the Dorset coast (England). The effects on natural mortality in the field are unknown.

Diseases of unconfirmed aetiology

Haemic neoplasia in *Mya arenaria* – no new trends despite increased surveillance in Atlantic Canada. Preliminary transmission experiments in 2001 suggest an infectious agent, however, repeat experiments and field-based proximity challenges were inconclusive. Observed for the first time in German coastal waters but with no clear link to anthropogenic contamination.

Haemic neoplasia in *Cerastoderma edule* – about 30 % prevalence in Galicia, Spain.

Haemic neoplasia in *Crassostrea virginica* - a notably high prevalence (50 %) was found in moribund yearling *C. virginica* near Cape May, NJ, USA. This is the first such report since an epizootic in 1991, also in juvenile oysters at the same location.

Shell disease of *Homarus americanus* - continues to affect large numbers of lobsters in nearshore areas from eastern Long Island Sound to southern Cape Cod Bay, USA. By autumn 2001, prevalence reached 60 % to 75 % in this area, the same as in 2000. Low prevalence in central and western Long Island Sound, two areas known to be more polluted with domestic sewage and industrial contaminants than areas to the east, argues against water quality degradation as a cause. Shell disease may be enhanced by increased transmission due to crowding as a result of the sharply higher abundance of lobsters in recent years, or to other factors such as warmer seawater temperature. While it may not result in significant mortalities, it affects the appearance, and thus the commercial value, of whole live lobsters.

Clam protozoan X (CPX) in *Cerastoderma edule* – no new trends. Associated with granulocytoma and high prevalence (about 50 %) in Spain.

Summer mortality of *Crassostrea gigas* – although less significant than in 1994 and 1995 (20–30 % in 2001 versus 80–100 % in 1994/1995), in some areas along the French coasts, a large research programme (MOREST) is in progress at IFREMER. Significant mortalities reported at one location on the south coast of Ireland and it still occurs in Washington and California, USA. In both Ireland and the USA, it affects multiple size classes, appears to be non-infectious and may be due to multiple environmental stressors.

Juvenile oyster disease (JOD) of *Crassostrea virginica* – the only serious outbreaks occurred in Maine with 40–50 % mortalities. In all cases, animals were heavily colonized by the same alpha-proteobacter species of *Roseobacter*. In all other areas previously affected by JOD, the disease no longer causes mortalities of economic significance.

Mortalities in *Placopecten magellanicus* – no new trends along Lower North Shore of Quebec, Canada, but similar new mortalities reported from some Bay of Fundy stocks. Cause is unknown and a multi-disciplinary team continues to investigate the problem.

Mortalities in *Ostrea edulis* – in southwest Nova Scotia, Canada, in late summer 2001 and persisted until October. Cause is unknown and both infectious aetiology and possible genetic in-breeding are being investigated.

Black spot disease in *Crangon crangon* – no new trend and prevalence remains higher in contaminated estuarine areas in the German Bight.

5.3.1 Conclusions

1) No new trends were reported for major molluscan disease agents (*B. ostreae*, *M. refringens*, *P. marinus, P. atlanticus, M. mackini, H. nelsoni, H. costale,* QPX, CPX, withering syndrome).
2) A new host species (*Chionoecetes tanneri*) and extension of range to British Columbia, Canada, were reported for *Hematodinium* sp. (bitter crab syndrome). The effect of this parasite on tanner crab population dynamics in Canada is not known.
3) *Placopecten magellanicus* mortalities reported last year from the Gulf of St. Lawrence were detected for the first time in the Bay of Fundy, Canada.
4) Mortalities were reported in *Ostrea edulis* in Nova Scotia, Canada but cause is unknown.

5) Shell disease of *Homarus americanus* continues to be a major problem for the lobster fishery in the northeastern USA.

5.3.2 Recommendations

WGPDMO recommends that:

i) Investigations continue to determine the cause of *O. edulis* mortalities in Atlantic Canada;

ii) A report on investigations into the molecular comparisons among the various species/isolates of *Perkinsus* in collaboration with the OIE reference laboratory for *Perkinsus* at the Virginia Institute of Marine Science (E. Burreson) be prepared intersessionally and reviewed at the 2003 WGPDMO meeting.

6 REPORT ON PROGRESS IN THE ONGOING INVESTIGATIONS OF THE EFFECT OF TEMPERATURE ON *BONAMIA OSTREAE* INFECTION DYNAMICS AND REPORT ON THE CONFIRMATION OF THE AGENT OF *CRASSOSTREA ANGULATA* GILL DISEASE AND ITS INFECTIVITY TO *CRASSOSTREA GIGAS* AND OTHER OYSTER SPECIES.

An investigation into the effect of temperature on *Bonamia ostreae* infection in *Ostrea edulis* is under way at IFREMER, La Tremblade, France, supported by a European Community programme, DISENV (Disease and Environmental Factors). This programme will finish early in 2002, after which results will be available. One of the objectives of this programme is to study, under experimental conditions, the effect of temperature on defence mechanisms in relation to the development of the disease.

Sixty *C. angulata* were obtained from the Ria Sado, Portugal and examined histologically at IFREMER, La Tremblade, for evidence of gill disease that caused large-scale mortalities in France in the late 1960s and early 1970s. No lesions or pathogens were detected. Transmission electron microscopy of selected samples also failed to detect any abnormalities. This study will be continued to determine whether the iridovirus, presumed causative agent of gill disease, is still present in *C. angulata*. A particularly interesting facet of the study is the differential susceptibility of *C. angulata* and *C. gigas* to gill disease, which could be used as a model to study mechanisms of defence against viruses.

6.1 Recommendations

WGPDMO recommends that:

i) results of the studies on effect of temperature on *Bonamia* infections in *Ostrea edulis* and on the aetiology of gill disease in *C. angulata* should be reported at the 2003 WGDPMO meeting.

7 REVIEW THE CURRENT STATUS OF STUDIES CARRIED OUT IN ICES MEMBER COUNTRIES ON THE RELATIONSHIP BETWEEN ENVIRONMENTAL CONTAMINANTS AND SHELLFISH PATHOLOGY.

C. Couillard presented a review on the most recent literature within this area (Annex 7).

Several shellfish diseases, with the potential to cause deleterious impacts on shellfish populations, have been associated with exposure to environmental contaminants. Most of these conditions have a multifactorial aetiology and may be triggered by a variety of natural and anthropogenic factors. New molecular biological tools are currently used to investigate the effects of environmental contaminants on genes of the p53 family that may be involved in the pathogenesis of haemic and gonadal neoplasia in bivalve species. Stereological and histochemical techniques are used to explore the link between exposure to environmental contaminants and digestive atrophy in marine bivalves. Imposex in gastropods is used successfully as a biomarker of exposure to tributyltin in the marine environment in several ICES countries. In some areas, parasitic infections or genetic adaptation may alter the prevalence of imposex. The potential of gastropods to be used to monitor the levels of endocrine disrupting substances other than TBT is under investigation. Multivariate analyses have revealed that the prevalence and intensity of *Perkinsus marinus* infections in oysters in the Gulf of Mexico, USA, is strongly influenced by large-scale climatic changes and have failed to reveal a clear effect of environmental contaminants. Few examples of field studies exist to show which exposures to contaminants can be linked to increased incidence of diseases in invertebrate species. Field and laboratory studies on the effects of environmental contaminants on the immune system of invertebrates are under way.

7.1 Conclusions

1) Diseases of shellfish that are possibly associated with exposure to environmental contaminants may have a significant impact on shellfish populations affecting growth, reproduction and survival.
2) Imposex in gastropods is used successfully to monitor exposure to tributyltin in the marine environment, and there are promising current studies on the use of gastropods to monitor exposure to other endocrine disrupting substances.
3) The chain of evidence linking contaminant exposure and neoplasia in shellfish is presently being investigated with molecular tools that may eventually be used to draw cause-effect relationships in field studies.
4) The number of ongoing studies on the association between shellfish diseases and contaminants is relatively limited.

7.2 Recommendations

WGPDMO recommends that:

i) ICES Member Countries conduct additional studies on the association between shellfish disease and environmental contaminants;
ii) the existing strategies to assess the prevalence of shellfish diseases in parallel to fish diseases and chemical contaminant levels in environmental monitoring programmes are reviewed in order to eventually implement this type of approach in the ICES Environmental Monitoring Programme;
iii) the results and progress of studies conducted on the association between shellfish diseases and environmental contaminants should be reported to WGPDMO as part of the national reports submitted on an annual basis.

8 OBTAIN INFORMATION ON THE EU PROJECT "*MARTEILIA REFRINGENS* STUDIES: MOLECULAR SYSTEMATICS AND SEARCH FOR THE INTERMEDIATE HOST OF THE BIVALVE MOLLUSCS PARASITE"

Under the EEC Council Directive 91/67, *Marteilia refringens* is included in list II of Annex A. This list includes serious pathogens causing important losses for the European shellfish aquaculture industry and these must be declared. However, the taxonomy of the genus *Marteilia* is still unclear. It is unknown if there is one or several species in Europe. Also, the life cycle of *Marteilia* is complex, with the possible existence of other host(s). The EU project (FAIR CT: PL97 – 3640) coordinated by Dr Antonio Figueras (Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello, 6, Vigo, 36208, Spain, Phone: 34-986-214462; 34-986-231930, Fax: 34-986-292762, E-mail: patol@iim.csic.es) focused on two main objectives, namely to:

1) Develop molecular tools for the detection of *Marteilia* infections at low prevalence and clarify the systematics of *Marteilia* in Europe;
2) Search for potential intermediate hosts of *Marteilia* using specific molecular tools for the parasite.

The gene coding for the small subunit of the ribosomal RNA (18S) of *Marteilia* sp. purified from *Ostrea edulis*, *Mytilus edulis* and *Mytilus galloprovincialis* from France, Spain and Croatia were sequenced. The sequences for all isolates were identical. Phylogenetic analysis of the relationships between the SSU rRNA sequence of *Marteilia refringens* and 26 homologous eukaryotic sequences indicates that *M. refringens* is not closely related to any eukaryotic organism whose SSU rDNA sequence is currently known. Notably, the SSU rRNA sequence of *M. refringens* is neither related to those of Myxosporea nor to haplosporidians.

Although all 18S sequences of all *Marteilia* from a variety of hosts and geographical locations were the same, polymorphism was found in the ITS region in different isolates of *Marteilia* in Europe. The analysis of the sequences of this ITS region indicated the existence of two genetic groups within *M. refringens*. The distinction between these two groups is supported by a bootstrap value of 98 %. The *Marteilia* sequences derived from mussels were named the "M" type and the *Marteilia* sequences derived from oysters were named the "O" type. The analysis of the PCR products by RFLP (restriction fragment length polymorphism) of the ITS confirmed the sequence polymorphism because two different profiles, corresponding to *Marteilia* from mussel ("M" type) or *Marteilia* from oyster ("O" type), were observed in agarose gels. Rarely, co-infections of "O" and "M" types were detected in both oysters and mussels indicating a lack of strict host specificity. Therefore, further studies must focus on the possible role of mussels as a vector for "O" type transmission and oysters as a vector for "M" type transmission.

Immunological and molecular tools were developed in order to carry out studies to find intermediate hosts in the life cycle of the parasite. A specific PCR assay for the parasite was instituted to analyse high numbers of samples in order to detect other forms of the parasite. This possibility of an intermediate host in the *Marteilia* life cycle was studied using two models: the claire pond/oyster model present in France and the raft/mussel model present in Spain. Because the claire ponds are enclosed and have fewer species of organisms, initial efforts were focused on the claire ponds with results to be applied to the raft/mussel model.

In claire ponds, the infection period for *M. refringens* is limited to the summer months (May to August) and the prepatent period is less than one month. Screening by PCR for *M. refringens* DNA was performed on all species sampled from the claire ponds in 1998 and 1999. Based on PCR results, two species were considered as potential hosts. One, the Cnidarian *Cereus pedunculatus*, was excluded during the second year of the study because the location of the parasite was always in the gut contents. The second species, the calanoid copepod *Paracartia grani*, gave positive results and was further investigated.

In situ hybridization on individuals of *Paracartia grani* indicated that the copepod was parasitised by *M. refringens*. Only the ovocytes of females proved to be infected. Infected *P. grani* were found in surveys of wild copepod populations. Also, the geographical range of *P. grani* matches *M. refringens* distribution and the copepod population increases in the summer corresponding to increased disease prevalence in oysters.

Experimental transmission of *Marteilia refringens* between the flat oyster *Ostrea edulis* and the copepod *Paracartia grani* were performed. Transmission occurred from the oysters to the copepods but not from the copepods to the oysters. Therefore, the life cycle was not completed. It is possible that the life cycle of *M. refringens* involves more than these two hosts or the experimental transmission parameters were not optimal.

In the second model (raft/mussel), it was not possible to find *Paracartia grani*. However, *M. refringens* occurred at very low prevalence at the study site (the Ría de Vigo) during the sampling period.

Some details of this study are available in the PhD thesis by C. Audemard, 2001, “Strategy of involvement of several species by the parasite *Marteilia refringens* to ensure its life cycle” (in French, copies can be obtained from F. Rivet, IFREMER, 17390 La Tremblade, France).

8.1 Conclusions

1) This report represents excellent work that provides significant information on the biology of *Marteilia refringens,* a severe pathogen of oysters (*Ostrea edulis)* and mussels (*Mytilus edulis* and *M. galloprovincialis)* in Europe.
2) Two genetic groups: “O” type from oysters and “M” type from mussels, with rare co-infection in both oysters and mussels, were identified.
3) Molecular tools were used to identify a potential intermediate host, the copepod *Paracartia grani*.
4) Once the life cycle has been completed, the new information may lead to changes in the current EU regulations pertaining to this disease.

8.2 Recommendations

WGPDMO recommends that:

i) work continue to verify that the copepod *Paracartia grani* is a true intermediate host for *Marteilia refringens*, complete the description of the life cycle of *Marteilia* sp. and resolve the current systematic questions pertaining to *Marteilia* sp. in Europe.

9 REVIEW AND REPORT ON THE PROGRESS MADE IN THE BEQUALM WORK PACKAGE 6 “EXTERNAL DISEASES AND LIVER HISTOPATHOLOGY”

S. W. Feist presented a working document (Annex 8) describing the progress made regarding Work Package 6 “External Diseases and Liver Histopathology” of the EU-funded BEQUALM project which aims to establish a quality assurance framework for biological effects techniques used in environmental monitoring programmes.

Progress during 2001 involved convening a workshop for participating laboratories and, as part of the intercalibration programme, undertaking a ring test of histological sections of liver lesions. The ring test was designed to assess the diagnostic accuracy of different laboratories for the main toxicopathic lesions encountered in flatfish livers. It was noted that there were some problems in encouraging laboratories to participate, and only five laboratories took part. The results of the ring test revealed considerable variation in diagnostic accuracy among laboratories, ranging from approximately 90 % to 30 %. Two key areas of discrepancy were identified relating to the "cut off" criteria between foci of cellular alteration/adenoma and adenoma/hepatocellular carcinoma. A set of measures was agreed to improve these results and to encourage greater participation. Further ring tests are planned.

A workshop was held at CEFAS Weymouth Laboratory in November 2001. The main objectives were to:

1) assess the current status of national activities regarding the use of fish diseases and liver histopathology in biological effects monitoring;
2) review the draft BEQUALM CD-ROM and finalise the content;
3) review the ring test results and decide on the scope of future intercalibration exercises;
4) provide training in histopathological assessment of flatfish livers collected from monitoring programmes; and
5) review the plans for the future of BEQUALM and provide feedback to the BEQUALM steering committee.

The workshop successfully addressed these objectives and further developed detailed diagnostic criteria to discriminate between the lesion types, which caused problems in the ring test. In addition, the content of the CD-ROM was finalised and material provided by several participants has been included. It was agreed that the CD-ROM provides the necessary information to undertake field studies using externally visible diseases and laboratory-based assessment of liver histopathology.

The EU BEQUALM programme comes to an end on 31 March 2002 and is intended to become self-funding thereafter. Future activities of this work package were discussed and a number of activities were agreed for the coming year. These related to the organization of an additional ring test, the continuing provision of reference materials in a variety of ways, including images of histological sections as well as stained sections where possible, and the distribution of the finalized CD-ROM. In order to continue to attract new participants to BEQUALM, it was agreed to investigate ways to expand the scope of this work package. Since liver tumours and pre-neoplastic lesions occur in many fish species, possibilities include expanding the number of fish species covered. These could also include freshwater species where assessment of toxicopathic lesions in a variety of organs and tissues could be used in ecosystem health assessments.

9.1 Conclusion

1) WGPDMO acknowledged the progress made within the BEQUALM Work Package "External Fish Diseases and Liver Histopathology" and emphasised that the project has been successful in establishing the basic QA procedures required for monitoring programmes.
2) Based on the results achieved, WGPDMO emphasised the clear need to continue the activities in order to maintain and improve the QA standards already achieved. This should include the organisation of further ring tests with greater participation, provision of histological reference material, and the distribution of the finalised CD-ROM.

9.2 Recommendation

WGPDMO recommends that:

i) the BEQUALM programme be continued in order to maintain and improve the QA standards already achieved in relation to biological effects techniques. ICES Member Countries conducting biological effects monitoring programmes should be strongly encouraged to participate;
ii) WGPDMO should review progress made in the "fish diseases and liver histopathology" component of the BEQUALM self-funding scheme.

10 REVIEW AND REPORT ON INFORMATION FROM THE WORKSHOP AT THE EAFP CONFERENCE IN DUBLIN, THE FINAL REPORT FROM THE ONGOING EU PROJECT ON NODAVIRUS, AND OTHER RELEVANT INFORMATION TO PROVIDE ADVICE ON CONTROL MEASURES

The workshop on nodavirus planned for the 10th EAFP conference in Dublin did not take place. However, a meeting took place in Padova (Italy) during 11–16 June 2001, organized by the EU Community Reference Laboratory, Denmark, for fish disease. The aim of the workshop was to provide information on aquaculture fish diseases of importance for the Mediterranean area, including Viral Encephalopathy Retinopathy, to the national reference laboratories of Europe.

10.1 Virus isolates

Ten new virus isolates were obtained from non-European, imported ornamental fish. These included the first record of nodavirus infection from wild ornamental fish (*Acanthurus triostegus, Apogon exostigma,* etc.). It is of particular significance since disease interactions between ornamental and aquaculture species is gaining more attention as a method of the spread of pathogens. These isolates belong to one of the three genotypes described previously. It seems that this genotype has a very widespread geographic distribution (Indo-Pacific area, Mediterranean area and Scotland) and can infect many species.

A new isolate was obtained from Dover sole (*Solea solea*), which further confirmed the existence of nodavirus infection in this species.

Recent transmission trials from Norway have shown that juvenile cod (*Gadus morhua*) are susceptible to nodavirus infection.

10.2 Diagnostic methods

The use of a RT-nested PCR assay appears to increase the sensitivity of the assay but it remains difficult to use routinely due to the possibility of cross contamination between samples. A novel nucleic acid amplification procedure NASBA (Nucleic Acid Sequence Based Amplification) for the detection of nodavirus is being developed.

10.3 Transmission

Vertical transmission of nodavirus in sea bass (*Dicentrarchus labrax*) from parent to eggs has been demonstrated experimentally. The virus was detected in unfertilized and fertilized eggs from spawners and in newly hatched larvae which developed clinical signs of the disease.

It has been shown that nodavirus strains pathogenic to sea bass at 25 ^{o}C are not pathogenic to turbot (*Scophthalmus maximus*) at 17 ^{o}C. Conversely, some strains having low pathogenicity to sea bass at 25 ^{o}C were highly pathogenic to turbot at 17 ^{o}C. The existence of "cold" and "warm" water nodavirus strains is suspected. The new isolates from ornamental fish were pathogenic to sea bass at 25 ^{o}C.

10.4 Immune response and vaccination

The nodavirus affects the total blood cell composition. The depletion of the B–lymphocyte population and the increase of the phagocytic cell population from day 7 post-infection are typical findings. These observations suggest that B-lymphocytes are possible targets for nodavirus. However, as specific anti-nodavirus antibodies were detected in the fish before changes in the blood composition, it is suggested that the humoral immune response takes place before the drop in the B-lymphocyte population.

In sea bass, inactivated vaccines failed to provide protection against nodavirus. Alternative strategies including the use of genetically engineered vaccines are in progress. In turbot, a recombinant vaccine has been shown to give significant protection.

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

11.1 *Ichthyophonus hoferi*

In 2001, no new trends were observed in *Ichthyophonus hoferi* infection in Atlantic herring in the North Atlantic, Norwegian Sea, Barents Sea, and Baltic herring in the Baltic Sea.

Russian investigations have shown that most herring in the feeding areas are present in the upper water layers and fish with *I. hoferi* are easily caught by both trawl, which samples the bottom, and purse seine, which samples the upper layers.

In the spawning areas herring aggregate near the bottom layer. Therefore, when analysing samples from a purse seine, fish prevalence may be underestimated (Annex 9).

11.2 M74

The prevalence of M74 (defined as the percentage of females producing fry mortality) in Finnish and Swedish rivers in 2001 was higher than that in 2000 (Annex 10). The prediction, based on thiamine analysis of eggs stripped in autumn 2001, is for a further increase in M74 disease prevalence in the 2002 hatch. Recent studies in Sweden indicate that the first-feeding stage fry, as well as yolk-sac fry, suffer from thiamine deficiency, for example, by not initiating feeding. Early bath treatment with thiamine serves as a prophylactic against abnormalities in both fry stages. However, delayed treatment can result in deformities in fish at later stages in development.

Research in Finland has demonstrated that Baltic herring have very high levels of thiaminase compared to sprat, which were previously thought to be involved in the development of M74. It is now thought that herring are more likely to be responsible for M74. Laboratory experiments have shown the necessity for a co-substrate to potentiate the thiaminase activity levels in herring. Possible co-substrates under consideration include environmental contaminants and algal bloom products. Similar approaches are under investigation in North America as factors in the aetiology of the Early Mortality Syndrome (EMS).

11.3 Conclusions

1) The accurate determination of prevalence of *Ichthyophonus hoferi* requires appropriate sampling gear.
2) Untreated salmon larvae that survive the yolk-sac fry stage can still suffer effects of thiamine deficiency (clinical signs of M74) as later stage fry.

11.4 Recommendation

WGPDMO recommends that:

i) relevant ICES Member Countries be urged to provide sufficient resources for continued studies into the aetiology of M74.

12 ASSESS THE EFFECTIVENESS OF SALMON MANAGEMENT METHODS FOR THE CONTROL OF SEA LICE

12.1 Chemotherapeutics

Infestations of salmon lice are mainly controlled by chemotherapeutics.

The main therapeutic products for control of sea lice currently licensed for use in the UK are as follows:

Product name	Type	Active Constituent	Manufacturer
Excis	Bath	Cypermethrin	Novartis
Salmosan	Bath	Azamethipos	Novartis
Calicide	In-feed	Teflubenzuron	Nutreco/Trouw
Slice	In-feed	Emamectin benzoate	Schering-Plough

In Norway, the following therapeutics are in use:

Product name	Type	Active Constituent
Exis, Betamax	Bath	Cypermetrin
Alpha Max	Bath	Deltamethrin
Lepsidon	In-feed	Diflubenzuron
Slice	In-feed	Emamectin benzoate
Ektobann	In-feed	Teflubenzuron

In Ireland, Slice is being widely used as an in-feed treatment, particularly for the first year in the sea and is proving very effective.

Bath treatments are usually administered by placing a tarpaulin bag around the cages. With the use of bath treatments, re-infestation can occur within a very short period, particularly if not all the lice are killed. Such treatments are effective only against the mobile stages of lice, and their administration stresses the fish. A critical period exists during which the use of these products is most effective. Hydrogen peroxide, which was widely used in the industry until the late 1990s, is a less popular choice, due to growing sea-lice resistance to this product.

In-feed treatments are administered over a 7-day period, to cover the varying appetites and feeding patterns in individual fish. The advantages to farmers of in-feed treatments are that they are easy to administer, non-weather-dependent and (in the case of Slice) 100 % effective against all developmental stages of the louse. Calicide exerts its effects at the moulting stage, and so must be used before adult lice appear.

12.2 Wrasse

In Norway, wrasse are frequently used to control sea lice numbers. However, this has declined recently as it is difficult to combine with an almost zero tolerance limit of lice (de-lousing at low infestation levels). In addition, wrasse are less active during the winter months.

12.3 Other management control methods

Other control methods include organised de-lousing at low temperatures and synchronised de-lousing in specific areas. In Norway it is believed that synchronised de-lousing during the early winter and spring is a useful procedure to reduce the infectivity pressure on smolts recently transferred to sea, as well as on migrating wild fish.

12.4 Temporal trends

During recent years, sea lice infestations in farmed fish in Scotland and Norway have been controlled by chemotherapeutics.

In Ireland, there is a decreasing trend in the numbers of sea lice. The national mean for ovigerous sea lice in 2001 was less than 0.5 per fish. The spring levels, measured in May, were lower for both ovigerous and total mobile lice than in the previous year and have continued the downward trend observed since 1998. Management protocols are in place nationally, which trigger treatments once ovigerous lice numbers exceed 0.3–0.5 per fish during the spring period and 2.0 per fish over the rest of the year.

The economic costs, because of the level of control achieved, are largely in terms of treatment costs rather than negative impacts on fish health/growth.

12.5 Anticipated future trend

The trend in lice numbers is apparently declining but it is considered too early to judge if current practices are beneficial over the long term.

12.6 Conclusions

1) Sea lice infestations are at present effectively controlled by chemotherapeutics.
2) The use of wrasse as a control method is declining.
3) There are indications that management control measures such as delousing at low lice levels, synchronised treatment, and delousing at low temperatures are beneficial.

12.7 Recommendations

WPGDMO recommends that:

i) reports from the Norwegian National Committee on Sea Lice and other relevant information on the effectiveness of salmon management control methods be reviewed by B. Hjeltness and presented at the 2003 WGPDMO meeting.

13 CURRENT STATUS REGARDING THE CHARACTERIZATION AND PATHOGENICITY OF MARINE VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS IN FARMED AND WILD FISH

A working document was prepared by H. Frank Skall, EU-Reference Laboratory for Fish Viruses, Århus, Denmark (Annex 11).

Preliminary work done at the Danish Veterinary Laboratory indicates that a panel of newly developed monoclonal antibodies may be able to differentiate between a) freshwater and non-Baltic marine isolates; and b) Baltic marine isolates. In addition, genetic characterisation of the VHSV isolates has identified four major genotypes:

Genotype I European freshwater isolates;
Genotype II a group of Baltic Sea marine isolates;
Genotype III North Sea, Skagerrak and Kattegat isolates;
Genotype IV North American isolates.

Although not as discriminatory as genetic sequencing, the RNAse protection assay is a rapid method for initial characterization. Restriction fragment length polymorphism (RFLP) is a new, promising method for genetic differentiation of isolates based on enzymatic digestion of RT-PCR amplified G-gene products.

VHSV is endemic in the Baltic Sea, Kattegat, Skagerrak, the North Sea and the coastal areas of the UK. The apparent prevalence of VHSV is highest in the Baltic Sea and lowest in the Norwegian Sea, North Coastal area and the Barents Sea, usually in schooling fish such as sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) in the Baltic Sea and Norway pout (*Trisopterus esmarkii*) from the Atlantic coast of Britain. VHSV also has been isolated from wild marine fish in North America, including Pacific herring (*Clupea pallasi*), Pacific mackerel (*Scomber japonicus*), euchalon smelt (*Thaleichthys pacificus*), surf smelt (*Hypomesus pretiosus pretiosus*) and sardine (*Sardinops sagax*), and from wild Japanese flounder (*Paralichthys olivaceus*) in Japan. The first outbreak of VHS in Japan was detected in farmed Japanese flounder in 1996. In 2001, 27 flounder farms were infected, resulting in approximately 50 isolations of VHSV.

13.1 Conclusion

The host spectrum, as well as the geographic distribution of the VHSV complex, is still expanding.

14 COMPILE AND REVIEW INFORMATION ON IPNV IN SALMONID FISH FARMING

Historically, IPN has been associated with high mortality in first feeding salmonid fry and subsequent low mortality in parr up to the smolt stage. However, during the last few years, IPN infection has become a serious cause of acute mortality and increasing economic impact in Atlantic salmon shortly after transfer to sea water in Scotland and Norway. In addition, turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossoides hippoglossodes*) and Atlantic cod (*Gadus morhua*) are all susceptible species that have considerable potential for intensive farming. Some of the information for this report is taken from the Interim Report of the Aquaculture Health Joint Working Group, subgroup on IPN, in Scotland, and the National Veterinary Institute in Norway.

14.1 Regional and temporal patterns of IPNV in Scottish and Norwegian salmon farms

Prevalence values were obtained from the fraction of salmon samples tested for IPN virus that are positive. For salmon in fresh water, the IPNV prevalence was lower than at sea water sites, with an overall prevalence of 8 % and 42 %, respectively. These data are broken down into regions: mainland Scotland, Shetland, Orkney, and the Outer Hebrides. The Shetland Isles have the highest prevalence of IPNV for both fresh water and sea water. In addition, the prevalence of IPN in Shetland has increased with an almost significant regression up to 1999–2000. In addition, there is clear evidence of increasing prevalence of IPNV in Orkney and the Outer Hebrides. Between 1998–1999, 30–40 % of Norwegian hatcheries and 40–70 % of seawater farms had clinical IPN. However, the presence of other disease infections may have contributed to these figures.

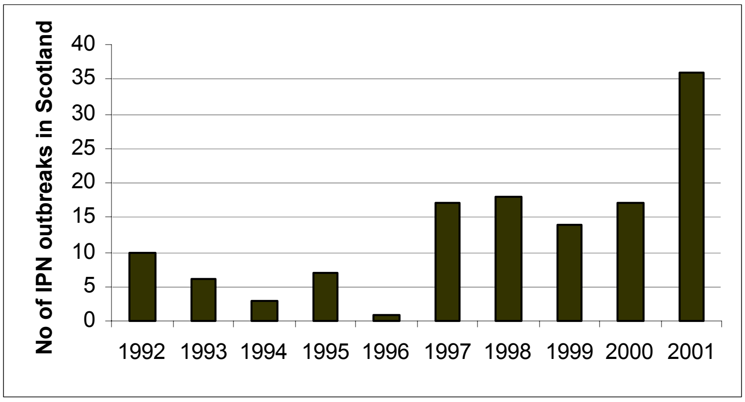
14.2 Clinical IPN

Clinical IPN in farmed Atlantic salmon S1 and S½ (salmon being at sea for 1 and ½ year, respectively) stock is reported from Scottish marine waters. In H&E slides, IPN infection comprised a focal or coagulative necrosis of the pancreatic tissue with mild to acute enteritis, focal necrosis and sloughing of the intestinal epithelium. Between 1992 and 1996, the number of farms with IPN ranged between 3–10 outbreaks. In 1997, 17 cases of clinical IPN were recorded throughout Scotland, and it was present in 36 farms by the end of 2001.

These data expressed as a proportion of the active producing marine sites in each year show an upward change in IPN from 1996. Based on the number of active sites in Scotland, clinical IPN was affecting 15.4 % of the active sea producing sites in 2001, compared with 3.7 % in 1990. The majority of IPN outbreaks have occurred in stock in waters off the Shetland Isles, however, the trend is also upward in the rest of Scotland. Most outbreaks of IPN were recorded in S1 fish and a low number in S½ fish.

In Scotland, clinical IPN was recorded in post-smolts in the months following their transfer to sea in April–June and for the years 1992–2001 (Figure 14.2.1). Most outbreaks of IPN disease occur in July and clinical IPN became uncommon after October of the same year. Clinical IPN has also been recorded in fish transferred to sea as S½ smolts since 1996, and further outbreaks in this group have remained low to date. Smolt first transferred to sea as S½ smolts exhibit clinical IPN from November, reaching a maximum in April of the following year. During July–August outbreaks of clinical IPN also occur in these fish.

Figure 14.2.1. Outbreaks of IPN in post-smolts in Scotland.



14.3 IPN mortality

In Scotland, mortality attributed to IPN occurs among S1 post-smolts during the spring months shortly after transfer to sea. Losses recorded from all causes were variable between farms. Data indicated that mortality from sites with confirmed IPN but covering all causes varied between 0.03–0.1 % per day in May, from 0.04–0.2 % in June, and up to 0.5 % per day in July. Later in the year, losses per day declined to early spring levels.

In Norway, losses at confirmed IPN sites averaged 11.2 % during the post-smolt season, although losses from all causes are included.

14.4 Vertical transmission

There is evidence of true vertical transmission, both intra-ovum and sperm-mediated in brook trout and rainbow trout. The current literature suggests that the probability of vertical transmission to the gametes is proportional to the virus levels in the gonads. Although vertical transmission has not been proven in salmon, some of the experimental evidence supports the notion of IPN entry and survival in eggs. Risk reduction measures include Statutory broodstock testing (30 fish per site), 100 % broodstock testing, separate water supplies for all egg batches and egg disinfection. Work is also required to establish if true vertical transmission occurs in salmon and to gather information on virus persistence within pre-brood cod and halibut.

14.5 Horizontal transmission

Horizontal transmission is the lateral spread of IPN virus and may occur between freshwater and marine environments by a variety of routes, and involving various reservoirs and vectors. The proportion of fish infected varies considerably at different times of the year. An examination of nine sites in Scotland at spawning time showed detectable levels from 0–65 %. Horizontal transmission is regarded as the major route of infection in Atlantic salmon in Norway and Scotland. In general, the risk associated with farmed salmon in freshwater is low compared with that of farmed salmon in seawater. Fish transferred to marine waters appear to show an increasing prevalence and susceptibility to clinical IPN.

14.6 Vaccination

The lack of evidence of clinical IPN in salmon during their second seawater season agrees with evidence of carrier status in this group. Antibody-positive juveniles with no history of IPN-outbreaks in freshwater appear to be protected against later outbreaks of IPN in seawater. The efficacy of existing vaccines remains to be proven, and the effect of vaccines on carrier-state needs to be assessed as well as identifying the risk posed by carriers in relation to vertical and horizontal transmission of the virus.

14.7 Conclusion

1) IPN prevalence is increasing in post-smolts in Norway and Scotland and clinical disease becomes apparent shortly after seawater transfer.
2) IPN has a significant impact on the salmon production industries of Scotland and Norway, but the reasons for IPN prevalence changes are unknown.
3) WGPDMO considers the observed trend in IPN prevalence to merit further investigation with respect to impact on salmonid fish farming in Scotland and Norway.

15 REVIEW PROGRESS MADE WITH REGARD TO THE UPDATE OF ICES PUBLICATIONS ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

15.1 Report on the document "Important Trends in Disease Problems in Finfish and Shellfish Culture in the ICES Area 1997–2001"

A draft of the document, which had been prepared intersessionally by various members, was distributed to WGPDMO. The group decided that following the standard ICES procedure for a printed report would unduly delay publication. The group reconfirmed the recommendation made at last year's meeting to place the document on the ICES website rather than having it printed. This would speed publication and allow regular updating. WGPDMO discussed methods for revising and editing the current draft document and obtaining information on web publication.

WGPDMO agreed that the section "Future Trends" was speculative, might be taken out of context, and should be eliminated. The group also agreed that references should appear at the end of the section in which they are cited, rather than being grouped together at the end of the document.

15.2 Web-based report on diseases and parasites of wild and farmed fish and shellfish as part of the ICES Environmental Status Report

At its 2001 meeting, WGPDMO agreed to update and modify the disease information presented on the ICES website (www.ices.dk/status) and to review progress made at the WGPDMO meeting in 2002.

W. Wosniok and T. Lang presented progress achieved in the data analysis and suggestions for changes in the presentation of data on diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations) of North Sea dab (*Limanda limanda*). These concerned:

- an update of the statistical analysis of time trends in prevalence, now covering the period 1995 to 2001;
- the colouration of the maps showing the time trends;
- the provision of information on the diseases considered (gross appearance, aetiology, significance, effects on the host).

In the discussion, it was suggested that the report should not only provide information on the presence of upward, downward or stable trends in prevalence, but in addition more detailed information on the general prevalence levels recorded in different regions (ICES statistical rectangles) in order to facilitate spatial comparisons. One way to achieve this would be to display the actual prevalence data. However, there was agreement that these data could easily be misused or misinterpreted and that there may be problems with copyrights. It was, therefore, decided to provide instead relative prevalence information about regional patterns by using a colour grading system, highlighting areas with generally high, medium or low disease prevalences. It was decided that a method to accomplish this will be developed intersessionally by W. Wosniok and T. Lang and will be made available to WGPDMO members on a website (www.math.uni-bremen.de/statistik/WGPDMO.html) for commenting and final adoption. The revised version of the maps and accompanying information will subsequently be submitted to ICES for incorporation in its website, replacing the version presently available.

There was agreement that the maps providing information on mariculture-relevant diseases and parasites will be updated as soon as new information becomes available. A request for information to WGPDMO members will be made by the WGPDMO Chair.

15.2.1 Conclusions

WGPDMO acknowledged the update made in the presentation of disease trends in North Sea dab as part of the ICES Environmental Status Report and decided that further modifications should be made in the design of the maps in order to facilitate regional comparisons of general prevalence levels.

15.2.2 Recommendations

WGPDMO recommends that:

i) the updated version of the maps and accompanying information providing an overview of temporal trends and the spatial distribution of diseases of North Sea dab be placed on the ICES website as soon as available.

15.3 Manuscript on methods for the statistical analysis of fish disease data for submission to the *ICES Techniques in Marine Environmental Sciences* (TIMES) series

W. Wosniok gave an overview of the present status of the manuscript. While the basic structure introduced during the 2001 WGPDMO meeting has been maintained, several parts of the text have been modified and expanded according to the WGPDMO recommendations and intersessional discussions among authors. Particular emphasis was given to the provision of statistical guidelines as close as possible to the needs of the presumed readership, e.g., by demonstrating the benefit of using trend assessments from long-term data compared to using data from a few isolated observations, precision considerations and issues of sampling design. Also, illustrative examples using real or realistic data will be added. The tentative table of contents is given in Annex 12.

Chapter 1 (Introduction) provides the background of the analysis: typical data structure, typical problems in the real data such as non-coherent data records (prevalence and corresponding explanatory quantities collected at different times or locations), correlated observations, and the nomenclature, which is used in the text. Chapter 2 (Comparison of fish disease prevalences) deals with methods for the comparison of prevalences without linking them to potentially explanatory factors. These comparisons aim to detect whether a change at all has occurred over time, or to detect a monotonic trend (only increase or only decrease) of the prevalence during the period under study. The precision of an estimated prevalence is discussed, and from this the aspects of sampling designs and efficiency of comparisons are derived. Approaches to dealing with missing values due to non-coherent data are presented. In Chapter 3 (Fish disease prevalence and potentially explanatory quantities), the considerations of Chapter 2 are expanded by including potential explanatory quantities into the statistical model and methods. The logistic model is introduced as the fundamental tool in dealing with prevalences as target quantities. Various ways to incorporate the variables of interest into the model are discussed, with special reference to interaction terms. The consequence of correlated observations as a typical feature of environmental data is demonstrated. Here also methods for the treatment of non-coherent data (interpolation methods, logistic bootstrap regression) are presented. Generalized Additive Models are introduced as useful generalizations of the logistic model for the prevalent situation of non-linear and non-monotonic trends. Additional chapters will be devoted to further exploratory approaches (classification and regression trees, neural networks) and presentation methods (standardisation), if space allows. Chapter 4 deals with technical aspects like available software and data access.

At present, a manuscript size of 40–50 pages is to be expected. The final version of the manuscript is expected prior to the 2003 meeting of WGPDMO.

15.3.1 Conclusion

WGPDMO acknowledged the progress made in preparing the manuscript on the statistical analysis of fish disease data to be submitted to the *ICES Techniques in Marine Environmental Sciences* (TIMES) series and agreed to review the final version of the manuscript at the 2003 meeting of the WGPDMO.

15.3.2 Recommendations

WGPDMO recommends that:

i) the final version of the manuscript on the statistical analysis of fish disease data should be reviewed and adopted by WGPDMO at its 2003 meeting;

ii) The adopted manuscript should be submitted for publication in the ICES TIMES series.

15.4 Current Status of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish

ICES has compiled and published a series of Identification Leaflets and WGPDMO recommended that the authors should be contacted to ensure they are completed or at least a status report is obtained. S. McGladdery (editor) has completed this task, and an update has been obtained, although not all authors have replied.

WGPDMO discussed the continued value of the ICES Leaflets in paper format and suggested that availability through the ICES website could be an option for some titles. This would allow updates to be carried out easily and colour pictures included. Ten ICES Leaflets require updating and two Leaflets have been revised. In addition, there are a further 29 titles proposed by WGPDMO for publication, three of which (SPX, M-74 and SPDV) are nearly ready for editorial review.

The OIE is preparing a series of web-based tutorials on diseases and pathogens notifiable to OIE; however, it was agreed that the Leaflet titles in general did not overlap with OIE. However, OIE will be contacted on this matter. Leaflets that overlap with those proposed by the OIE will be given a lower priority. WGPDMO decided that the web format was the most beneficial forum for some Leaflets, and S. Mellergaard was asked to confirm this as a possibility and to obtain guidelines on the format. A sub-group was established (D. Bruno - aquaculture, S. Feist - wild fish, and S. Bower - shellfish) that will review the list of proposed Leaflets suggested by S. McGladdery for revision and review the existing titles proposed by WDPDMO, and make recommendations for web-based Leaflets.

During the meeting, J. Barja proposed three new titles for consideration by the sub-group: Pasteurellosis, *Pseudomonas anguilliseptica* and *Flexibacter maritimus.* The Identification Leaflet on nodavirus required an additional author and will now be drafted by M. Vigneulle and D. Bruno.

15.4.1 Recommendations

WGPDMO recommends that:

i) S. Mellergaard evaluate the possibility of putting revised and new ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish onto the ICES website;

ii) B. Hjeltnes contact T. Håstein to obtain information on OIE web disease tutorials;

iii) The sub-group review the existing and proposed Leaflets with S. McGladdery, review the proposed titles by WDPDMO and make recommendations to WGPDMO.

16 ANY OTHER BUSINESS

16.1 Revision of the ICES Databank structure and the ICES Environmental Reporting Formats, implications for WGPDMO activities regarding the statistical analysis of ICES fish disease data in relation to other ICES environmental data sets

M. Sørensen from the ICES Secretariat informed WGPDMO about changes made in the structure of the ICES data banks maintained in the ICES Marine Data Centre and in the ICES Environmental Reporting Formats used for data submission.

The future structure of the environmental database will be a relational one, allowing variable linking of information records down to the level of individual fish, depending on the objectives of the data analysis.

The present reporting format versions 2.2 (used for fish disease, contaminant and biological effects data) and version 3.1 (biological community data) will be replaced by a new version 3.2, expected to be available in autumn 2002. This new format will combine all of the above data types into one format, in order to facilitate a better data integration and accessibility for more holistic assessments on the relationship between environmental factors and associated biological responses. The new format will, for example, enable the joint reporting of disease/biological effects data and contaminant data and will include multiple QA information.

Further modifications of the databases are under way in order to achieve an improved integration of the environmental database with the ICES fisheries and oceanography databases.

WGPDMO was requested to provide the ICES Secretariat with information on requirements for possible modifications in the structure of the reporting formats and on requirements regarding data products.

There was agreement that the new structure should meet the following requirements:

- The data format should allow an easy incorporation of new diseases. As a first step, the list of histopathological liver lesions identified in the BEQUALM Work Package 6 on external fish diseases and liver pathology should be incorporated (ICES 2000, Report of the ICES Advisory Committee on the Marine Environment, Section 20.5, p. 162).

- Since diseases are being partly recorded in different organs (e.g., skin surface, liver or other internal organs), it might be useful to place the "Fish Disease" information under the "Tissue" information on the same level as "Biological effects" and "Contaminants".

- In order to facilitate specific analyses of ICES data by ICES Working Groups, an extraction of data from the ICES databases by the ICES Secretariat based on requests from ICES Working Groups should be possible without major efforts. This should not only apply for the environmental database, but also for other ICES databases. For example, earlier WGPDMO activities required access to environmental, oceanographic and fisheries data.

- A product derived from the environmental ICES data is the web-based report on trends in wild fish diseases as part of the ICES Environmental Status Report (www.ices.dk/status). Ways should be explored as to how this product can be produced by the ICES Secretariat on request by WGPDMO in a timely manner. Protocols on the structure of this report and the procedures that have to be applied can be provided by WGPDMO.

- WGPDMO considers the environmental data inventory available on the ICES website (www.ices.dk/env/index.htm) as a useful instrument. However, the inventory should be completed by including an overview of the ICES fish disease data that comprise one of the largest data sets in the ICES Environmental Data Bank.

16.1.1 Conclusions

WGPDMO welcomed the intended changes in the ICES database structure and the Environmental Data Reporting Formats because they will facilitate a better integration of available data for ecosystem-based assessments of the state of the marine environment of the ICES area.

16.1.2 Recommendations

WGPDMO recommends that:

i) the requirements specified above be implemented by the ICES Secretariat in the process of modifying the structure of the ICES Databases and the ICES Environmental Data Reporting Format;

ii) the WGPDMO review progress made in the modifications of the ICES Databases and the ICES Environmental Data Reporting Format at its 2003 meeting.

16.2 Document on "Biodiversity of stickleback parasites"

WGPDMO discussed the summary document of the project "Biodiversity of stickleback parasites" provided by D. Marcogliese (david.marcogliese@ec.gc.ca) (Annex 13). It was noted that many countries are involved and that a key aim is to provide a database of information on parasite species affecting sticklebacks. These data will be used to address environmental and ecological issues and will provide a means for monitoring biodiversity and environmental stress.

16.2.1 Conclusion

WGPDMO supports this initiative and encourages scientists to contact D. Marcogliese with a view to contributing to the project.

16.3 Template for reporting

The analysis of national data on disease trends in wild and cultured fish and shellfish is part of the regular terms of reference for WGPDMO. A proposal to facilitate the collection and assessment of these data by using a standard template was discussed.

16.3.1 Conclusion

It was agreed that S. Mellergaard would develop such a template for use by members of the WGPDMO for submission of information for the 2003 WGPDMO meeting.

17 PROGRESS WITH TASKS

An analysis of progress of tasks in the Terms of Reference was conducted and presented in Annex 14. All items had been dealt with in a comprehensive manner. Several intersessional tasks were identified during the meeting.

18 FUTURE ACTIVITIES OF WGPDMO

Since there are several issues of importance in the field of pathology and diseases of marine organisms requiring further consideration, it was agreed that a further meeting of WGPDMO is required in 2003 to consider the results of intersessional work and to discuss outstanding items. It was agreed that the invitation to host the next meeting of the WGPDMO from Dr D. Bruno, Marine Laboratory, Aberdeen, Scotland should be accepted. The proposed dates are 11–15 March 2003.

19 APPROVAL OF RECOMMENDATIONS

The recommendations contained in this report to the ICES Council were discussed by the WGPDMO and approved. The recommendations and justifications for recommendations to the Council are appended as Annex 15.

20 APPROVAL OF THE DRAFT WGPDMO REPORT

The report of the 2002 meeting was approved before the end of the meeting and the draft report was circulated to the participants on 17 March. The conclusions on the Terms of Reference and associated Annexes where advice was specifically sought by other ICES bodies would be extracted and sent separately to ICES.

21 CLOSING OF THE MEETING

The meeting was closed at 14.00 hrs on 16 March 2002.

ANNEX 1: LIST OF PARTICIPANTS

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ANNEX 2: TERMS OF REFERENCE

ICES C.Res. 2001/2F02

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: S. Mellergaard, Denmark) will meet in Copenhagen, Denmark from 12–16 March 2002 to:

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

b) report on progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics and report on the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oysters species;

c) review the current status of studies carried out in ICES Member Countries on the relationship between environmental contaminants and shellfish pathology;

d) obtain information on the EU project "*Marteilia refringens* studies: Molecular systematics and search for the intermediate host of the bivalve mollusc's parasite" and review the results;

e) review and report on the progress made in the BEQUALM Work Package 6 'External diseases and liver histopathology';

f) review and report on information from the workshop on nodavirus at the EAFP Conference in Dublin, the final report from the ongoing EU project on nodavirus and other relevant information to provide advice on control measures;

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

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

i) review the current status regarding the characterisation and pathogenicity of VHSV strains in farmed and wild fish;

j) compile and review information on IPNV in salmonid fish farming;

k) review progress made with regard to the update of ICES publications on pathology and diseases of marine organisms:

   i) report on important trends in diseases occurring in finfish and shellfish culture in the ICES area in the period 1996–2000,

   ii) web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report,

   iii) manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series,

   iv) ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish.

WGPDMO will report by 10 April 2002 for the attention of the Mariculture Committee and ACME.

ANNEX 3: AGENDA

1. Opening of the meeting (10.00 hrs).
2. ICES ASC 2001; items of relevance to WGPDMO.
3. Terms of Reference, adoption of the agenda, selection of Rapporteurs.
4. Other relevant reports for information.
5. Analyse national reports on new disease trends in wild and cultured fish, molluscs and crustaceans;
6. Report on progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics and report on the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oysters species.
7. Review the current status of studies carried out in ICES Member Countries on the relationship between environmental contaminants and shellfish pathology.
8. Obtain information on the EU project "*Marteilia refringens* studies: Molecular systematics and search for the intermediate host of the bivalve mollusc's parasite" and review the results.
9. Review and report on the progress made in the BEQUALM Work Package 6 "External diseases and liver histopathology".
10. Review and report on information from the workshop on nodavirus at the EAFP Conference in Dublin, the final report from the ongoing EU project on nodavirus and other relevant information to provide advice on control measures.
11. Maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome.
12. Report and assess the effectiveness of salmon farming management control methods for the control of sea lice in the different ICES Member Countries.
13. Review the current status regarding the characterisation and pathogenicity of VHSV strains in farmed and wild fish.
14. Compile and review information on IPNV in salmonid fish farming.
15. Review progress made with regard to the update of ICES publications on pathology and diseases of marine organisms:
    a. Report on important trends in diseases occurring in finfish and shellfish culture in the ICES area in the period 1996–2000;
    b. web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report;
    c. Manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series;
    d. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish.
16. Any other business.
17. Analysis of progress with tasks.
18. Future activity of WGPDMO.
19. Approval of recommendations.
20. Approval of draft WGPDMO Report.
21. Closing of the meeting, Saturday 15.00 hrs.

ANNEX 4: RAPPORTEURS

Session (s)	Rapporteurs
1-4. Introductory sessions	Stig Mellergaard
5. Wild fish Farmed fish Wild and farmed shellfish and crustaceans	Thomas Lang/Catherine Couillard/Wojciech Poasecki David Bruno/Juan Barja Maria Lyons Alcantara/Susan Bower
6. Report on progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics and report on the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oysters species;	Susan Ford/Tristan Renault
7. Review the current status of studies carried out in ICES Member Countries on the relationship between environmental contaminants and shellfish pathology;	Catherine Couillard/Steve Feist
8. Obtain information on the EU project "*Marteilia refringens* studies: Molecular systematics and search for the intermediate host of the bivalve mollusc's parasite" and review the results;	Tristan Renault/Susan Bower
9. Review and report on the progress made in the BEQUALM Work Package 6 'External diseases and liver histopathology';	Thomas Lang/Steve Feist
10. Review and report on information from the workshop on nodavirus at the EAFP Conference in Dublin, the final report from the ongoing EU project on nodavirus and other relevant information to provide advice on control measures;	Brit Hjeltness/Martine Vigneuille
11. Maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome;	David Bruno/Andrey Karasev Göran Bylund/Sharon MacLean
12. Report and assess the effectiveness of salmon farming management control methods for the control of sea lice in the different ICES Member Countries;	Brit Hjeltness/Maria Lyon Alcantara
13 Review the current status regarding the characterisation and pathogenicity of VHSV strains in farmed and wild fish;	Göran Bylund/Sharon MacLean
14. Compile and review information on IPNV in salmonid fish farming;	David Bruno/Brit Hjeltness
15. Review progress made with regard to the update of ICES publications on pathology and diseases of marine organisms:	
• Report on important trends in diseases occurring in finfish and shellfish culture in the ICES area in the period 1996–2000	Göran Bylund/ Susan Ford
• web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report	Thomas Lang
• Manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series	Werner Wosniok/Thomas Lang
• ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish	David Bruno
16-19. AOB, Progress with tasks, Future activities, Recommendations	Steve Feist/Thomas Lang
20-22. Approval of report, closing	Stig Mellergaard

ANNEX 5: REPORT ON ISA IN THE US

By *Sharon A. MacLean, NOAA National Marine Fisheries Service.*

ISA outbreaks in the US occurred in Cobscook Bay, Maine, the primary focal point of the Atlantic salmon industry in the United States. No other areas in Maine or the US have been affected. The first reported case of ISA in the US was in February 2001 from an Atlantic salmon cage in Cobscook Bay, Maine. The second and third reported cases occurred within three and five weeks, respectively. Despite industry's efforts to control the spread of the disease through biosecurity measures and voluntary depopulation of infected cages, by early September, 11of 17 active Cobscook Bay culture sites reported at least one diseased cage.

On 10 September, the State of Maine Department of Marine Resources (DMR) put into effect an emergency rule which mandated the following:

- monthly testing for ISA using rtPCR and IFAT (cell culture also recommended) at sites within Cobscook Bay and quarterly testing for sites outside Cobscook Bay. Prior to this, no testing was required unless fish were to be moved;
- reporting of all test results to the DMR, confirmed positive sites being subject to immediate remedial action;
- restrictions on the movement of aquaculture vessels and equipment out of or into Cobscook Bay.

The industry depopulated infected cages without State enforcement, but new cases at previously diseased and uninfected sites continued to break out through November. By December approximately 925,000 fish, year classes 1999 through 2001, had been removed at an estimated production cost loss of $3.5 million.

On 13 December, the US Department of Agriculture, Animal Plant Health Inspection Service (USDA-APHIS) was designated the lead federal agency in controlling ISA, with two years of funding for eradication, disinfection, surveillance and epidemiological programs. USDA-APHIS declared ISA an exotic pathogen and on 7 January 2002, DMR and USDA-APHIS jointly ordered the eradication of the remaining 1.5 million salmon in Cobscook Bay that were infected with or exposed to ISAv in order to begin a fallowing period for the entire bay. The fallowing requires the removal of all the fish as well as all the associated net pens, barges, and equipment at all the farms and disinfection of nets, barges/boats and equipment. The State's emergency rule became a permanent rule in January 2002 and increased DMR's authority to depopulate ISAv-exposed sites, as well as positive ones, to comport with the USDA objective of eradication of the pathogen. Bay-wide area management, indemnification and early reporting, single year-class stocking, final stocking density and coordination with the New Brunswick ISA management program are components of the ISA management plan for Maine. Details of implementation of the plan still need to be worked out with the industry, but to begin implementation of the joint DMR and USDA-APHIS ISA management plan, Cobscook Bay will be divided into two management areas; the southern portion will be stocked in spring 2002, after a 3-month fallowing period, and even-numbered years thereafter, and the northern portion will be stocked first in spring 2003.

In efforts to identify potential reservoirs of the ISA virus in wild fishes, a survey began late in 2000 to test non-salmonid fishes for the presence of salmonid pathogens. Viral isolations, DFAT for *R. salmoninarum*, rtPCR and/or IFAT for ISAv assays were run on nearly 1600 fishes including alewife (*Alosa pseudoharengus*), American eel (*Anguilla rostrata*), Atlantic herring (*Clupea harengus harengus*), Atlantic mackerel (*Scomber scombrus*), pollack (*Pollachius virens*), and winter flounder (*Pseudopleuronectes americanus*) collected from areas near or afar from salmon aquaculture sites, and pollack, cod (*Gadus morhua*), and lumpfish (*Cyclopterus lumpus*) collected from inside ISA-diseased cages. All fishes collected outside cages tested negative for viral pathogens and *R. salmoninarum*. Two of 16 pollack taken from inside ISA-diseased cages were weakly ISAv-rtPCR positive and cell culture negative, whereas ninety pollack taken directly outside a diseased cage tested negative for viruses and *R. salmoninarum*. PCR products from the two pollack did not result in good sequencing reactions; the bands may represent a degraded ISAv or low number of virus particles. One of 24 pools (5 fish/pool) of tissues from 120 cod collected from the wellboat of a harvested clinically diseased cage produced CPE characteristic of ISAv on SHK cells. This was confirmed by rtPCR testing of the cell culture supernatant. Repeated rtPCR assays of kidney tissue from the individual fish that comprised the positive pool of tissues did not detect ISAv. Since the viral isolations include gill tissues, it is possible that the positive cell culture resulted from an exogenous virus adherent to the gill lamellae, however, it would be expected that more than one pool would have tested positive in this case. Genetic sequencing of this isolate is under way. Viral pathogens and *R. salmoninarum* were not detected in 26 lumpfish collected from inside the diseased cages. These results suggest the potential for various non-salmonids to act as reservoirs of ISAv and may have implications toward biosecurity measures in harvest operations. The significance of these potential carriers to the epidemiology of the disease remains to be investigated. Monitoring for salmonid pathogens in wild non-salmonids will continue and testing of estuarine/coastal salmonids will begin in the coming months.

Atlantic salmon in the Greenland commercial fishery were sampled in attempts to estimate the level of marine mortality in US Atlantic salmon stocks attributed to fishing. This provided an opportunity for pathogen testing of these fish as well. Tissues of 19 Atlantic salmon caught commercially and landed in Nuuk were taken for viral culture, IFAT and rtPCR assays for ISAv, and DFAT assay for *R. salmoninarum*. No CPE was observed, and DFAT and IFAT assays were negative. The PCR test gave a weak positive band for ISAv; sequencing of the PCR product showed the closest similarity to the North American strain of the virus. Genetic testing of the fish is under way to determine the country of origin.

Preliminary research has been conducted on the comparative susceptibility of Pacific salmonids to ISAv. The European strain of the virus was used to challenge chinook salmon, coho salmon, chum salmon and steelheads (sea-run *Oncorhynchus mykiss*) and Atlantic salmon. Early results indicate that all the Pacific salmonid species have relatively higher resistance to ISA disease than Atlantic salmon. Several labs have begun to investigate other cell lines for ISAv susceptibility and isolation.

ANNEX 6: VHS IN FINLAND IN 2001

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The first outbreaks of VHS were diagnosed in Finland in 2000. By order from the Ministry of Agriculture and Forestry, the National Veterinary and Food Research Institute conducted an intensified VHS-survey program at seawater farms in the Åland Islands and on the south and southwestern coast of Finland in May and June 2001. Almost all farms in these areas were checked by local veterinarians, samples were taken and tested for virus infections before the seawater temperature reached 14 °C. In the Åland Islands, altogether four new VHS-positive farms were detected in May–June 2001, one farm inside the existing restriction zone and three farms outside this zone. The size of the infected fish varied in the range 50–2000 g. The mortality had been less that 10 %. In the Pyhtää restriction area in the eastern Gulf of Finland, VHS was detected in one new farm and also in the farm which was found infected in November 2000. The new farm is situated close to the "old" farm, where VHS caused about 50 % mortality in rainbow trout during spring 2001. Altogether, VHS was detected at six rainbow trout farms in 2001. Freshwater hatcheries which had delivered fish to the infected farms were also sampled and found negative.

Restriction measures are in force for the infected farms. However, mandatory eradication procedures are not carried out anymore. The restriction zone in Pyhtää was not altered, but in the Åland Islands the restriction zone was expanded to include the whole province. Launching of an eradication programme based on voluntary fallowing (needs to be accepted by the EU Commission) is under evaluation by the Finnish fish farmers association and other stakeholders.

The screening program for VHS virus in wild fish has been continued, so far with negative results. Totally about 1500 fish, mainly Baltic herring, were studied in 2001. An intensified survey is planned for autumn 2002. According to our experience in 2001, VHS has been spread between adjacent farms and between farms owned by same farmer.

Typing of the viruses from the first three outbreaks in Finland, in year 2000, has been performed in the Community Reference Laboratory in Denmark. According to the latest information from this laboratory, the Finnish isolates belong to the same serotype as the marine isolates as well as some of the first Danish isolates. Genetic analyses revealed that the two different Finnish isolates have a unique RFLP pattern, differing, e.g., from the isolates found in Sweden in 1998 and 2000. However, they seem to be closely related to the first Danish freshwater isolates. The isolates from Kumlinge (Åland) and Pyhtää were not identical according to the G-gene analysis (*Einer-Jensen, K, Ahrens, P. and Lorenzen, N. 2001. Genotyping of continental and marine VHS virus isolated by restriction fragment length polymorphism (RFLP). Poster and abstract at the 10th International Conference of the EAFP, Dublin, Ireland*). Typing of the isolates from the 2001 outbreaks is in progress.

ANNEX 7: REVIEW THE CURRENT STATUS OF STUDIES CARRIED OUT IN ICES MEMBER COUNTRIES ON THE RELATIONSHIP BETWEEN ENVIRONMENTAL CONTAMINANTS AND SHELLFISH PATHOLOGY

By C.M. Couillard, S.E. McGladdery, and S.W. Feist

Summary

Several shellfish diseases, with the potential to cause deleterious impacts on shellfish populations, have been associated with exposure to environmental contaminants. Most of these conditions have a multifactorial etiology and may be triggered by a variety of natural and anthropogenic factors. New molecular biological tools are currently used to investigate the effects of environmental contaminants on genes of the p53 family that may be involved in the pathogenesis of haemic and gonadal neoplasias in bivalve species. Stereological and histochemical techniques are used to explore the link between exposure to environmental contaminants and digestive atrophy in marine bivalves. Imposex in gastropods has been used successfully to monitor levels of tributyltin in the marine environment. In some areas, parasitic infections or genetic adaptation may alter the prevalence of imposex. The potential of gastropods to be used to monitor the levels of endocrine disrupting substances other than TBT is under investigation. Multivariate analyses of the relationship between the prevalence and intensity of parasite infections in oysters have revealed a strong influence of large-scale climatic changes on these infections but have failed to reveal a clear effect of environmental contaminants. Few examples of field studies exist to show which exposures to contaminants can be linked to increased incidence of diseases in invertebrate species. Field and laboratory studies on the effects of environmental contaminants on the immune system of invertebrates are under way.

1. Haemic neoplasia

The cause of the disease is unknown; possibly it is due to a virus. Haemic neoplasia was transmitted to healthy clams by injection with whole neoplastic haemocytes but not with cell-free filtrates. Reverse transcriptase activity was demonstrated in haemolymph with high numbers of neoplastic haemocytes (House *et al*., 1998). It may cause heavy mortalities and the severity appears to increase with age. The disease may also be temperature related, with the highest prevalences being recorded in the autumn and winter.

High frequencies of haemic neoplasia are often found in association with high levels of environmental contaminants such as pesticides or PCBs. However, high prevalences have also been reported from sites with no evident industrial or agricultural contamination (McGladdery *et al*., in preparation). A parallel distribution of disseminated neoplasia and the presence of certain dinoflagellate biotoxins has also been reported (Landsberg, 1996).

1.1 Current studies in *Mya arenaria* and other clams

In 1999, 95 % prevalence of advanced haemic neoplasia with mortalities was detected in soft-shell clam populations in Prince Edward Island, Canada (McGladdery *et al*., in preparation). Environmental factors at positive and negative sites are being studied at the Atlantic Veterinary College to see whether there are any common parameters. Preliminary transmission experiments in 2001 suggested an infectious agent; however, field-based proximity challenges and repeat experiments have been inconclusive. Significant levels of the disease were also found in clams from Sydney Mines, NS, and Kitimat Arm, BC, and at sites along the east coast of the U.S. with high levels of anthropogenic substances. Environmentally induced alterations in p53 may contribute to the pathogenesis of leukemia in *M. arenaria* in polluted environments. Analysis of the p53 gene obtained from PCB-exposed soft-shell clams revealed a mutation in exon 6 (Barker *et al*., 1997). Monoclonal antibodies belonging to the 1E10 series have been shown to react with neoplastic cells but not with healthy cells (Stephens *et al*., 2001). These proteins have possible linkages to the p53 gene family. The appearance of p73 and the disappearance of p97 coincided with leukemia-specific protein synthesis. In contrast, levels of p53 remain constant (Stephens *et al*., 2001; Kelley *et al*., 2001).

1.2 Current studies in *Mytilus edulis*

Haemic neoplasia is endemic to mussel populations in Puget Sound, Washington. No relationship between the body burden of environmental contaminants and the prevalence of haemic neoplasia in mussels has been identified. To evaluate the short-term ability of chemical contaminants to induce haemic neoplasia, mussels were fed microencapsulated PAHs or PCBs and the prevalence of neoplasia was assessed after 30 days or 180 days of exposure. No significant change in the prevalence of the disease was detected in treated mussels compared to controls. Thus, there is no experimental evidence that chemical contaminants induce or promote the development of haemic neoplasia in these mussels (Krishnakumar *et al*., 1999)

2. Gonadal neoplasia

The cause of this condition in mussels is unknown and it is relatively rare, compared with haemic neoplasia. The relationship between sex, size, season, reproductive cycle and with the occurrence of gonadal neoplasia has been described. Gonad neoplasia has also been studied in relation to environmental contaminants, such as oil spills and herbicides (Hillman *et al*., 1992; Hillman, 1993). Advanced stages of the disease in other bivalves (soft-shell clams, see below) limit gamete development, but this does not appear to be a significant factor for mussels due to the relative rarity of the condition. A parallel in the distribution and incidence of germinomas and blooms of toxin-producing dinoflagellates (*Alexandrium* sp.) has also been reported (Landsberg, 1996).

2.1 Current studies in *Mytilus galloprovincialis*

Gonadal neoplasia was found in several specimens of *Mytilus galloprovincialis* collected from the Ria de Vigo (northwest Spain) (Alonso *et al*., 2001). The highest prevalence was found in the spring, coincidental with mussel raft cleaning and maintenance. More studies are needed to evaluate the potential carcinogenic effects of the chemical substances used in raft maintenance.

2.2 Current studies in *Mya arenaria* and other clams

The relationship between gonadal neoplasia and exposure to environmental contaminants is under investigation at the University of Maine (Barber, 1996; Barber and Bacon, 1999), and the U.S. Environmental Protection Agency laboratory in Narragansett, RI. One epidemiological investigation identified the prevalence of gonadal cancers as high as 40 % in softshell clams (*Mya arenaria*) in Maine and 60 % in hardshell clams (*Mercenaria* spp.) from Florida. In the same geographical areas, human mortality rates with ovarian cancer were significantly higher than the national average. NIH3T/ transfection assays were used to examine DNA isolated from these molluscan tumours for the presence of activated oncogenes. DNAs isolated from advanced tumours in both species were able to transform NIH3T3 cells and induce tumours in athymic mice. Studies are under way to identify the gene identified in this assay (Van Beneden, 1994).

Clams were exposed to 2,4-D and to TCDD with or without DEN and were sacrificed six months after exposure. Although histological analysis did not indicate tumour formation, both 2,4-D and TCDD inhibited gametogenesis to an extent that gender was indeterminate (Butler *et al*., 2001). The expression of Ahr, E3, p53, and p73 was analysed in normal and neoplastic gonadal tissue from an affected population of Maine. Preliminary results indicate that tumourous tissue expressed higher levels of E3, an ubiquitin-protein ligase, that may potentially target p53 for degradation. There is an inverse relationship between E3 and p53 (Harring *et al*., 2001).

3. Imposex and intersex

Imposex and intersex in gastropods has been successfully used as a biological-effect monitoring system to determine the degree of environmental tributyltin (TBT) pollution (Matthiessen and Gibbs, 1998). Continuing use of TBT on large vessels is causing problems despite the widespread ban on the use of TBT on smaller boats (for example Bright *et al*., 2001, around Vancouver Island, Canada).

In some areas, parasitic infections or genetic disorders may interfere with the monitoring of TBT-related imposex in gastropods. In the northern Gulf of St. Lawrence, Canada, the castrating trematode, *Neophasis* sp., may cause atrophy of the penis in the male common whelk, *Buccinum undatum* (Tetreault *et al*., 2000). Elevated levels of imposex have also been found in dogwhelk (*Nucella lapillus*) collected in areas adjacent to a gull roost. Compounds in the birds' excreta and/or parasites may have caused the imposex (Evans *et al*., 2000). A genetic disorder, "Dumpton syndrome", was found in several populations of *N. lapillus* sampled at 56 stations along the coast of Galicia from 1996 to 1998 (Quintela *et al*., 2001; Barreiro *et al*., 1999). "Dumpton's syndrome" is the name given to a dogwhelk mutation found near to Dumpton, Ramsgate, UK, in the early 1990s (Gibbs, 1993). It causes a reduction in penis size in males. The population of dogwhelks almost disappeared from the area owing to TBT coming from nearby ships. This mutation appears to protect the females from imposex. This syndrome was also found in 1992 in *N. lapillus* populations in the vicinity of Brest, France (Huet *et al*., 1996).

The effects of various endocrine disrupting chemicals on freshwater and marine (*Nucella lapillus, Nassarius reticulatus*) pseudobranch species were analysed in laboratory experiments. Xeno-estrogens (e.g., bisphenol A, octylphenol) primarily cause induction of superfemales, resulting in an increased female mortality by the enhancement of spawning mass and egg production. Male sex organs may be reduced. Xeno-androgens (triphenyltin, tributyltin) cause virilization of females (imposex) and a marked decrease in fecundity. Anti-androgens (cyproterone acetate, vinclozolin) have less effect, causing reduced male sexual organs and suppression of imposex development (Tillmann *et* *al*., 2001). Thus, morphological changes in the genital tract of gastropods may be used to monitor exposure to endocrine-disrupting compounds other than tributytin.

4. Intensity of parasitic infection as an indicator of environmental health

As part of the NOAA Mussel Watch Program, oysters and mussels are sampled yearly from the East, West, and Gulf Coasts of the United States and the Great Lakes. Biological responses are evaluated in parallel to concentrations of metals, PAHs, and pesticides. Dr E. Powell (The State University of New Jersey) and his team have examined the influence of climate change and contaminant body burden on the prevalence and intensity of infection of oysters by various parasites. Their studies reveal that the intensity of parasitic infections was strongly influenced by large-scale climatic changes. In the Gulf of Mexico, the health of each oyster population sampled was evaluated by measuring size, condition index, reproductive stage, and the prevalence and intensity of infection by the parasite responsible for "Dermo" disease, *Perkinsus marinus*. Length, condition index, reproductive stage and *P. marinus* infection intensity were characterized by strong concordance in interannual variations between 1986 and 1990, when a strong El Niño/La Niña shift occurred, and a weak concordance in the period of 1990–1993, characterized by weak climatic shifts (Kim and Powell, 1998). The distribution of some contaminants, particularly metals, also appears to be markedly influenced by weather and less by watershed-dependent processes, such as land use and river flow. This may be correlated to food supply and feeding rates being influenced by climatic changes, thereby affecting the body burdens of contaminants (Kim *et al*., 2001).

Mussels are often exposed to high hydrocarbon concentrations in their natural habitat from petroleum seep and, thus, offer the opportunity to examine the relationship between parasitism, disease, and contaminant exposure. The parasitic fauna was highly variable between populations. Forty percent of the populations were severely reproductively compromised by a *Bucephalus* sp. digenean flatworm infection. Variation in two parasite infection levels: gill ciliates and *Bucephalus* sp., explained most of the variation in PAH body burden between mussel populations. PAHs are known to be sequestered preferentially in gametic tissue. *Bucephalus* sp. may reduce the PAH body burden by replacing gametic tissue (Powell *et al*., 1999).

5. Digestive gland atrophy

Digestive gland atrophy has been observed in bivalves exposed to a variety of contaminants. This condition has been correlated with contaminant burdens, disease, condition and nutritional states. Cell-type replacement in the digestive gland of mussels in response to pollution is under investigation (Ferreira and Bebianno, 2000; Soto *et al*., 2001; Syasina *et al*., 1997). Both the severity of the atrophic changes observed and the type of cells that are affected may be typical of a pollutant-induced response, compared with physiological changes associated with nutrition (Winstead, 1995). Histochemical and stereological techniques are used to characterize the changes associated with exposure to environmental contaminants. For example, auto-metallography, stereology of the lysosomes, and morphometry of the digestive epithelia were used to demonstrate the effect of metal contamination in mussels (*Mytilus galloprovincialis*), transplanted from a relatively pristine site to a polluted one in the Lagoon of Venice (Italy) (Da Ros *et al*., 2000). The seasonal and the site-specific variations in the structure of peroxisomes and in the activity of the peroxisomal marker enzyme catalase in digestive epithelial cells of mussels were studied in mussels sampled monthly for fourteen months in two Basque estuaries with different degrees of pollution. Stereological procedures were applied to detect changes in peroxisome structure in response to organic pollution. Further studies are needed before changes in peroxisomal structure can be used as a biomarker to assess environmental quality (Orbea *et al*., 1999).

6. Effect of contaminants on the function of the immune system

Few examples of field studies exist for which exposure to contaminants can be linked to increased incidence of diseases in invertebrate species. More research is needed on the effects of natural factors (gonad maturation, temperature, nutrition, and stress) on the immune function of invertebrates and on the relationship between observed changes in immunological responses and incidence of diseases in natural populations. The relative simplicity of invertebrate immune functions offers a good model to study the complex interactions between exposure to environmental contaminants and immune dysfunction (Galloway and Depledge, 2001). Investigations are under way. For example, in France, T. Renault (IFREMER) is studying the correlation between summer mortalities of *Crassostrea gigas* with environmental contaminants and immunotoxicity. Preliminary studies carried out after the "Erika" wreck indicate that ciliates are more abundant in mussels, and fungal infections increased in the cockle, *Cerastoderma edule,* after the oil spill. In Canada, S. St. Jean is studying the immune responses of bivalves exposed *in situ* to pulp mill and municipal effluents.

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ANNEX 8: REVIEW AND REPORT ON THE PROGRESS MADE IN THE BIOLOGICAL EFFECTS QUALITY ASSURANCE IN MONITORING PROGRAMME (BEQUALM) WORK PACKAGE 6 EXTERNAL DISEASES AND LIVER HISTOPATHOLOGY

By Steve Feist and Thomas Lang

The EU-funded BEQUALM programme will shortly come to an end (March 2002). Thereafter the programme will continue on a self-funding basis. The external fish disease and liver histopathology component (WP6) has made good progress in its tasks during the final year. These included organisation of a ring test and a final workshop during November 2001 held at CEFAS Weymouth Laboratory, UK. The following report provides a summary of the key activities of WP6 which are of particular relevance to the activities of the ICES WGPDMO.

For the third year of this BEQUALM work package, the primary objectives are:

1 Implementation of a full intercalibration programme based on sets of material collected during national monitoring programmes from at least two participating countries.

This task was completed. The following is an abbreviated version of the workshop report relating to the ring test.

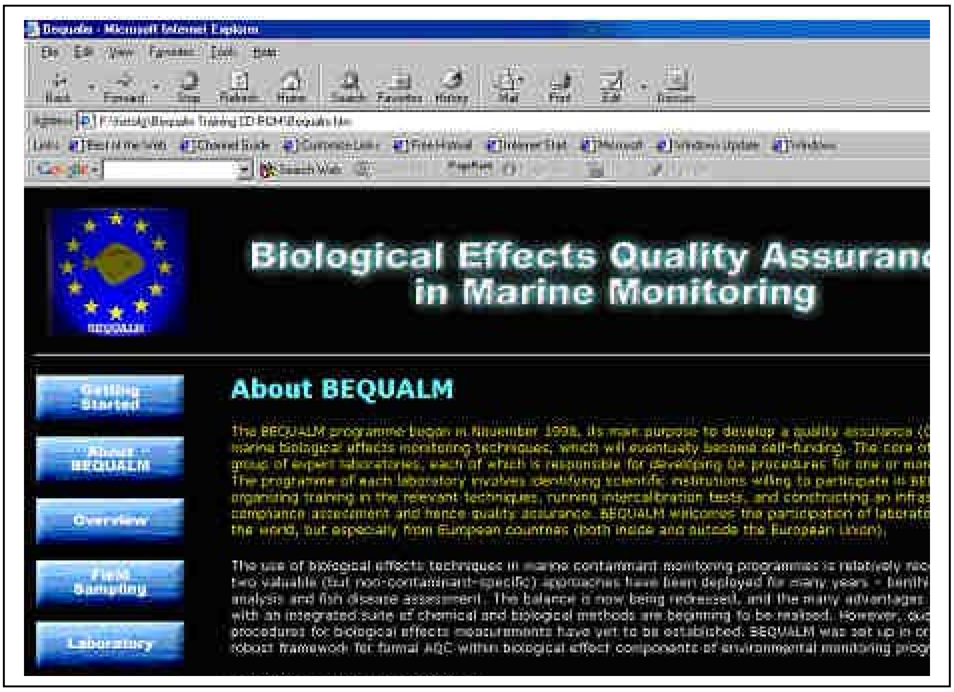
The main objective of the ring test was to assess the diagnostic accuracy of different laboratories for the main potentially toxicopathic lesion types encountered in the liver of fish. The results obtained allowed for an assessment of problem areas and possible weak points in diagnostic criteria. It was suggested that, for future trials of this type, a nucleus of twelve to fifteen laboratories should be involved. It was also stated that such ring tests are vital in ensuring that all participating laboratories are tied into a quality assurance programme that allows for accurate and transferable reporting of data to international databases.

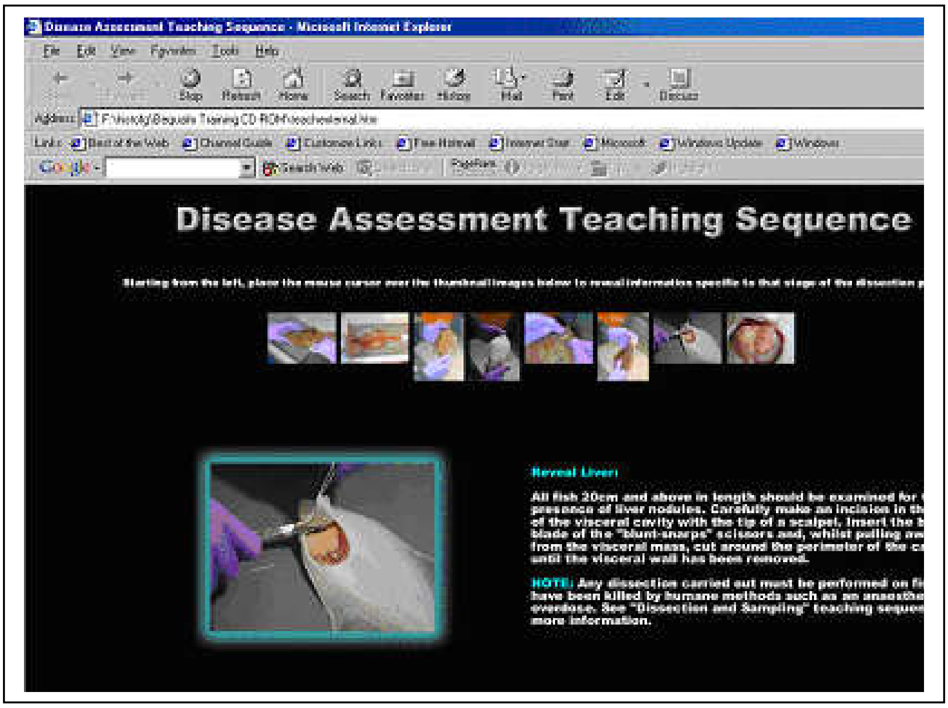
A number of important points were highlighted during the meeting:

- Considerable variation in diagnostic accuracy was observed between different participating laboratories. The highest level of accuracy for diagnostic scoring was ~ 70 %, while the lowest score was ~ 30 %, compared to the master score sheet;
- Participating laboratories generally over-scored for the presence of foci of cellular alteration (FCA);
- Participating laboratories generally under-scored on the presence of malignant neoplasms (such as hepatocellular carcinoma);
- There appeared to be two major “cut-off” areas that caused diagnostic problems for participating laboratories. The first one was in the differentiation between the pre-neoplastic lesions (FCAs) and the benign neoplasms (such as adenoma) and the second one was between the benign neoplasms and the malignant neoplasms (carcinoma). In light of this information, a flow diagram was designed to show which characteristics each group of lesions possess and how these criteria should be used to make a diagnosis. The flow chart had been designed by incorporating the results of the ring test and Dr Myers’ description of the lesion types from these slides. The chart was discussed by all participants and used for the diagnosis of some “test” lesions. Although all participants agreed that this is exactly the sort of approach that is needed for quality assurance in liver histopathology, it was suggested that more criteria are required for the accurate diagnosis of carcinoma. All of the details required for the successful diagnosis of pre-neoplastic lesions are in place. However, it became clear that for diagnosis of FCA type, a further flow diagram is required to describe characteristic cellular staining. An amended version of the flow diagram can be seen below.
- All workshop participants agreed that resending of the original ring test slides along with Dr Myers’ descriptions and electronic images of the principal lesion to all ring test participants would be beneficial for training purposes. Furthermore, it was suggested that a liver histopathology training workshop should be held, possibly in conjunction with the next EAFP meeting. It is possible that some funds may be available from the EAFP for such a workshop.

2 Selection of materials for production of an atlas of common hepatic histopathological lesions.

This aspect of the programme has now been completed. Several participants have contributed material for the CD. This has been incorporated and the final version of the CD has been completed and will be distributed to participants in the near future. The appearance of the front page and a teaching sequence section is shown below.





3 Second workshop held in November 2001 at CEFAS Weymouth Laboratory.

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

- It was noted with concern that there had been a reduction in the number of long-term fish disease monitoring programmes carried out in the North Sea, Baltic Sea and Irish Sea. However, it was also noted that several shorterterm national and EU-funded investigations on biological effects of contaminants including pathological studies are currently under way.

- The integration of existing routine disease studies with other biomarkers, environmental and contaminant measurements continues to improve.

- A number of successful research projects aiming to correlate biomarker responses during contaminant exposure have been carried out.

- The BEQUALM workshop was successful in establishing criteria for external disease assessment and liver pathology diagnosis and has identified areas that require further refinement.

- The ring test for the diagnosis of flatfish liver histopathology was completed successfully. The results showed that there was variation in the accuracy of diagnosis between laboratories and that this mainly arose from difficulties in discriminating between foci of cellular alteration and adenoma and between adenoma and carcinoma.

- It was recommended that additional reference material should be provided to participating laboratories and that further ring tests should be instigated on a regular basis.

- It was agreed that the CD-ROM (v.1) provides the necessary information to undertake field studies using externally visible diseases and laboratory-based assessment of liver histopathology.

- It was recommended that the incorporation of disease assessment quality assurance criteria (including multi-organ histopathology) for other fish species be investigated, thereby broadening the interest for this component of BEQUALM to other potential participants in Europe and further afield.

4 Future activities

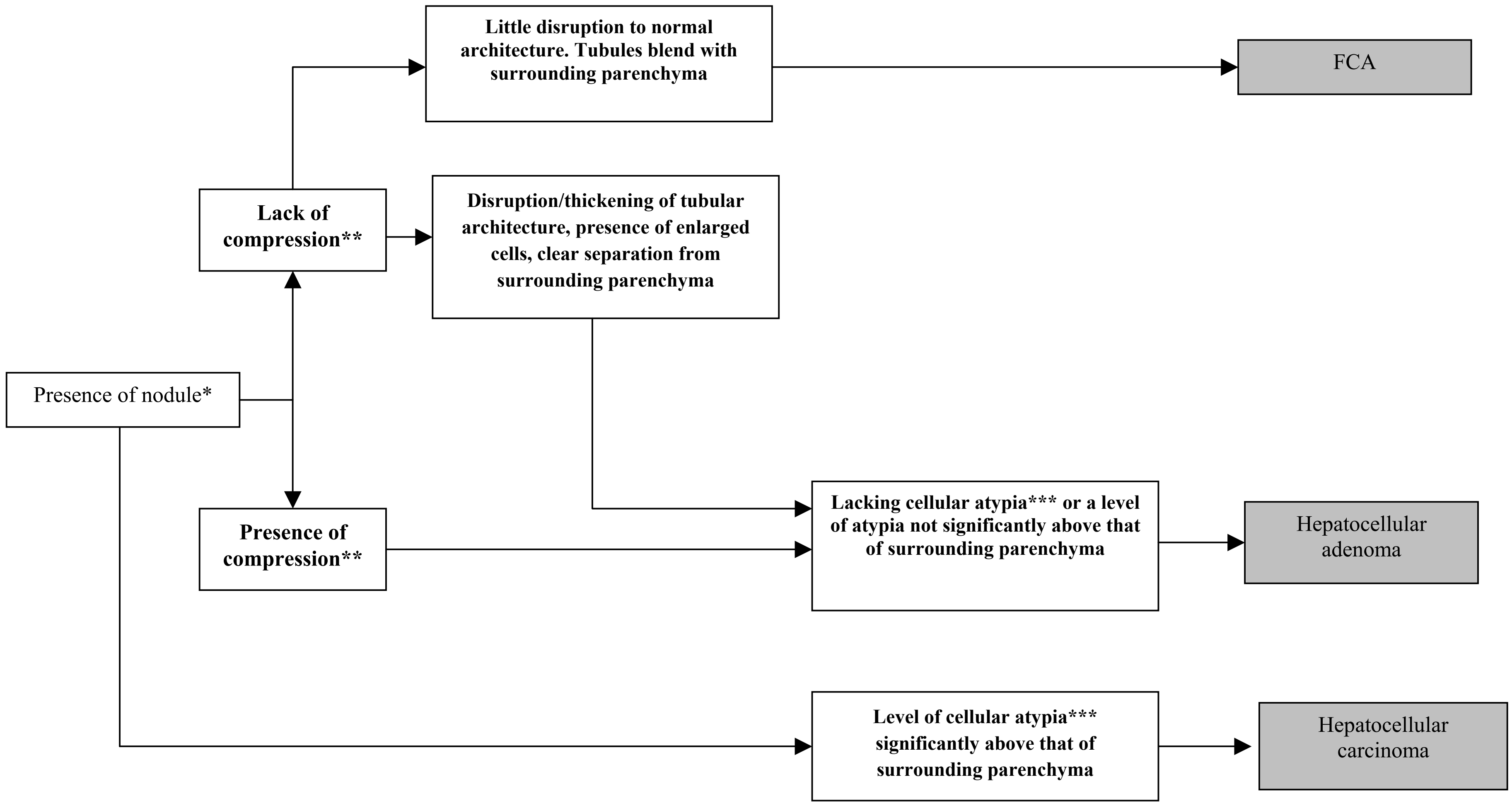
BEQUALM, currently funded by the EU, is due to officially end in March 2002. Following this, it may be possible for BEQUALM to become a sister-group to QUASIMEME. The steering group foresees that the whole group will be split into three major sub-headings, these being “Biomarker”, “Community” and “Organism”. As such, each of the sub-groups would report to a main project office, while each subsection (external diseases and liver histopathology being part of the “Organism” package) would have a lead laboratory and individual participating laboratories would be associated with this lead laboratory.

The future of BEQUALM and this work package in particular was discussed at the workshop. The following points were made:

- More reference material is needed for training purposes.
- Further ring tests are required, possibly containing a reduced number of slides, thus encouraging more laboratories to take part. It was also suggested that having more than one set of slides might help to speed up the process.
- It is critical for laboratories involved in this area of work to be part of this group in order for their data to be accepted internationally for inclusion into, e.g., the environmental section of the ICES Marine Data Centre. It was agreed that BEQUALM must continue to be an internationally accepted scheme (at present, biological effects monitoring does not have the same official regulatory structure as for chemical monitoring). The need to promote BEQUALM as an ongoing concern and the desire to bring biological effects monitoring to the same regulated standard as chemical monitoring was discussed.
- It was further suggested that it might be useful to broaden the outlook to quality assurance parameters for pathology of freshwater fish and for parasites. At present, freshwater work was currently not driven by quality assurance, though it seems likely that this will be the case in the future. In addition, it would be useful to discover the “network” of potential participants around Europe who may wish to be involved in the BEQUALM continuation programme. A questionnaire, distributed to likely participant laboratories, would assist with determining the extent of interest.
- From a non-European perspective, it was stated that U.S. and Canadian laboratories would find it particularly useful to be involved in an international quality assurance scheme. Additionally, since liver tumours apply to all species, the criteria developed for diagnosis can be used for fish species from seawater, freshwater and estuarine environments.

- It was decided by all participants that liver pathology would remain an important indicator of ecosystem health status, but in the future, other organs and tissues should also be included (such as the kidney and the gonad) to assess biological effects. Different species (apart from flatfish) should also be incorporated and all lesion types should be included from as many fish species as possible. This may help to draw in “isolated” laboratories that require an aspect of quality assurance in their monitoring schemes.
- It was decided that the CD-ROM of reference images will be sent to Dr Myers for his comment and a list of lesion descriptions will be included with these images. These will then be sent to all participants for training purposes. An action on all participants was to provide suitable images for inclusion onto this CD-ROM.
- The pathology flow chart and a lesion recording sheet (with the different key categories included) could be used by participants in the next ring test. They can then use this system to explain why they have arrived at a particular diagnosis for a particular lesion.
- A ring test “pack” will be compiled which contains the following: Slides, explanations of lesions (from Dr Myers), the results (from the ring test), images of the key lesion(s), the diagnostic key and a completed master score sheet for all ring test slides.

Diagnostic Key: Hepatocellular nodules (after Couillard, Stentiford, Feist, Myers, Fournie)



* **Nodule:** A discrete accumulation of hepatocytes (more than 10 cells). These lesions have usually a decreased concentration of pigmented macrophage aggregates relative to the surrounding parenchyma.

** **Compression**: Complete compression around the circumference of a lesion is rare. More commonly, compression is seen at certain sections of the lesion periphery. Adjacent parenchyma will appear relatively compressed. The lesion periphery may be marked by the presence of MMC's and flattened blood vessels.

*** **Atypia**: Most livers will contain a certain degree of nuclear and cellular atypia (though proportions of atypical nuclei and cells will be very small in most cases). In terms of lesion diagnostics, it is important to consider the degree of nuclear (size, shape, chromatin pattern) and cellular (size, shape, cytoplasmic content) atypia relative to the surrounding parenchyma. For the diagnosis of carcinoma *in situ*, the degree of cellular and nuclear atypia within the suspect carcinoma should be greater than that of the surrounding nodule parenchyma.

ANNEX 9: IMPACT OF SOME TRAWL FISHING PARAMETERS ON ESTIMATE OF PREVALENCE OF *ICHTHYOPHONUS HOFERI* DISEASE IN ATLANTO-SCANDIAN HERRING *(CLUPEA HARENGUS HARENGUS* L.)

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Abstract

Data on prevalence of *Ichthyophonus hoferi* disease in Atlanto-Scandian herring, obtained in the Norwegian Sea in the course of trawl and acoustic surveys carried out by research vessels of PINRO in 1992 and 1996, are analysed. The paper aims at studying the impact of some trawl fishing parameters upon the estimate of prevalence of *I. hoferi* disease in herring.

It is shown that analysis of samples from trawl catches allows to objectively estimate the prevalence of *I. hoferi* in herring during feeding and spawning.

Key Words: Atlanto-Scandian herring, relationship, the Norwegian Sea, parameters, samples, trawl fishing, *Ichthyophonus*, disease prevalence, epizooty.

Introduction

When studying epizooty of *Ichthyophonosis* in Atlanto-Scandian herring a question of reliability of estimation of the disease prevalence in herring by samples taken from trawl catches has arisen more than once.

According to the opinion of Norwegian scientists, sampling under purse seine fishing provided a more precise estimate for disease prevalence compared to that with a trawl in use. In accordance with this hypothesis, a herring infected by *I. hoferi* is not able to avoid a trawl, and therefore occurs in a trawl more frequently than a healthy fish (Hjeltnes and Skagen, 1992; ICES, 1993; Kvalsvik and Skagen, 1995).

However, by the data of J. Palsson (1994), in May–June 1994, the amount of Icelandic summer-spawning herring infected by *I. hoferi* was similar when fishing with a purse seine and a trawl, and in September–November 1994 the herring infected by *I. hoferi* were found only in samples from purse seine catches.

At the ICES WG on Pathology and Diseases of Marine Organisms, it was decided to further continue a comparative analysis of data using the samples taken from different fishing gears (ICES, 2000).

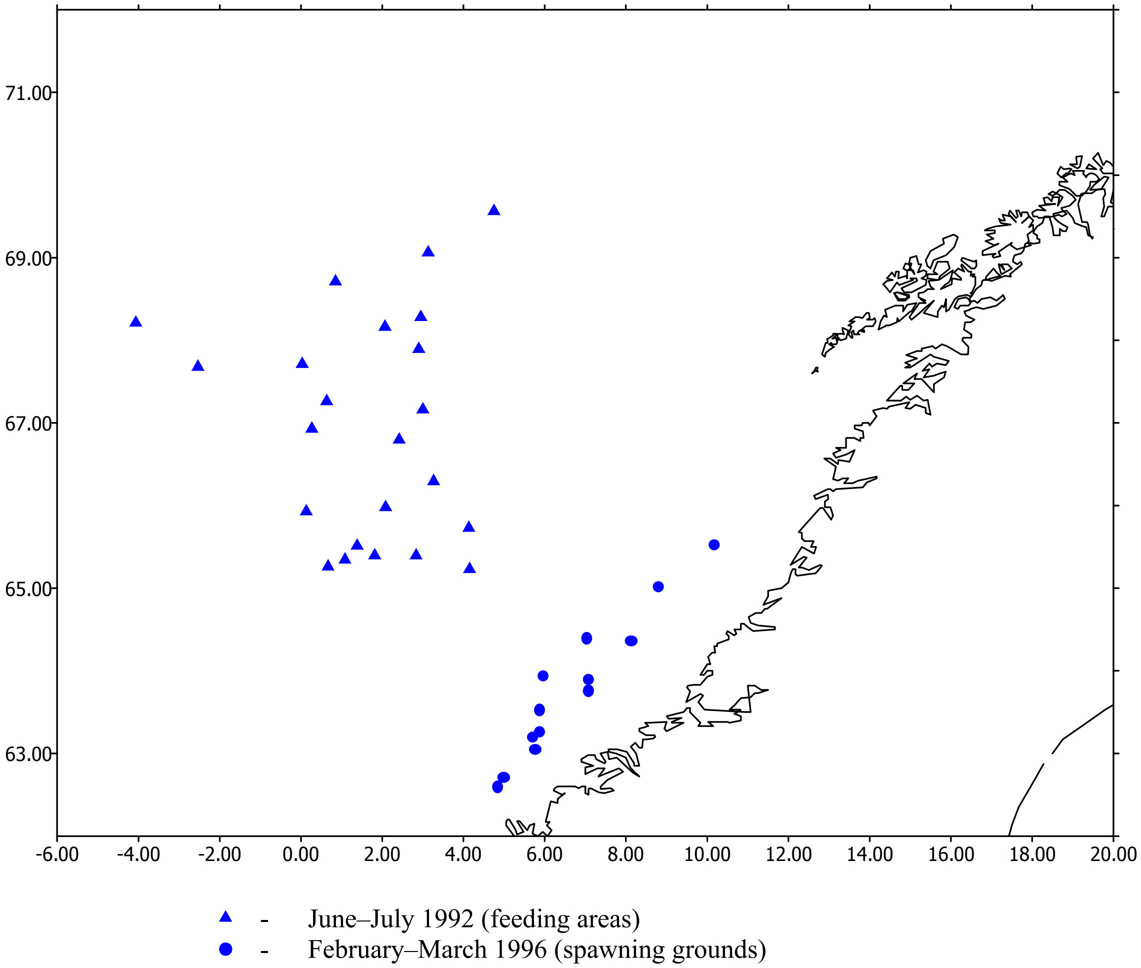
Russian scientists had no possibility to take samples from purse seine catches as the vessels participating in pelagic fishery use a mid-water trawl.

In this connection, to settle the question of precision and reliability of the data on herring samples taken from trawl catches, the impact of such parameters as catch size, time of day and trawling depth per occurrence of fish infected by *I. hoferi* in samples was studied.

Materials and methods

Data were collected in the feeding areas in June–July 1992 and in February–March 1996 on the spawning grounds of spring-spawning herring in the Norwegian Sea in the course of trawl-acoustic surveys carried out by PINRO research vessels (Figure A9.1).

Figure A9.1. Areas of check hauls and collecting of ichthyopathologic samples of herring in the Norwegian Sea in 1992 and 1996.



Depth of sampling reached 3000 m in the open Norwegian Sea, and it did not exceed 300 m on the spawning grounds in the Norwegian Shallows.

In total, 1076 individuals of Atlantic herring and 395 mature specimens from 36 research trawl catches were diagnosed in 1992 and 1996, respectively.

Check hauls were done by acoustic indication using a mid-water trawl (50-metre vertical opening, 16 mm mesh-size insertion) at depths from 5 m to 300 m in different times of day and night. Duration of trawling was from 5 min to 1.5 hour at the speed of a vessel of 3.5–4.5 knots. Trawling depth was defined by a headline of a trawl.

Catch size of herring was recalculated per time unit of trawling (kg/trawling hr) for the hauls with a different duration.

The number of fish with connective tissue-nodes on heart being typical of the *Ichthyophonosis* chronic form was counted in herring samples from catches (ICES method) (Anon., 1991; Donetskov, 1995).

All the data on prevalence of infected fish in samples taken from trawl catches depending on parameters considered were grouped into classes (30–390 specimens) for further statistical analysis.

As for the data collected in 1992, the prevalence of infected herring was calculated for the catches below and above 60 kg/hr taken during a trawling from 0–5 m to 150 m depths and performed from 00:00 to 12:00 hrs and from 12:01 to 0:00 hrs (GMT ± 1 hr) —in total, 16 groups of data were obtained.

For the data collected in 1996, a similar calculation was done for catches up to 500 kg/hr (small catches) and to 501–120,000 kg/hr (large catches) taken in trawling depths 50–100 m, 101–200 m, 201–300 m from 0:00 to 12:00 hrs and from 12:01 to 0:00 hrs (GMT ± 1 hr), in total, 12 groups of data.

Reliability of impact of the day time, catch size and trawling depth upon the number of herring infected by *I. hoferi* in samples was determined by means of comparing the portions using Student's method, a two-factor dispersion analysis for a small group of data without repetition, as well as correlation and regression analyses using a method of data extrapolation.

The paper considers differences in values being reliable for a 95–99 % significance level.

Results and discussion

According to the results from the trawl-acoustic survey, no large aggregations of mature herring were formed at the depths below 60 m in the open Norwegian Sea in June–July 1992. In this connection, check hauls were mainly done in 5–15 m depths.

The analysis of our data on prevalence of herring infected by *I. hoferi* has shown that during that period the amount of fish with symptoms of the disease constituted on the average 15.9 % in the first half of day and 34.7 % in the second half (Table A9.1). These mean values were apparently formed due to significant differences in the number of infected fish caught in a 0–5 metre surface layer during the first and the second halves of a day.

No differences were revealed in the number of infected fish during different times of day in 10–50 m trawling depth.

In spite of that 67 % of catches taken in 50–150 m depth consisted of infected fish, the size of these catches and, respectively, the number of fish infected by *I. hoferi* were minor and could not have a strong impact on the average values of the disease incidence.

Table A9.1. Prevalence of herring infected by *I. hoferi* (%) in samples from catches taken in different trawling depths during different times of day in the Norwegian Sea, June–July 1992.

Trawling depth, m	Time of day, hour			
	00:01–12:00		12:01–00:00	
	Prevalence, %	n	Prevalence, %	n
0–5	13.8±3.3	109	35.5±2.1	513
10–50	16.5±1.9	394	24.5±6.1	49
50.1–150	---	-	66.7±27.2	3
Fish analysed, indiv.		503		565
Average prevalence, %	15.9±1.6		34.7±2.0	

A dispersion analysis did not reveal specific impact of trawling depth and time of day upon the number of herring caught with the symptoms of *Ichthyophonosis* under a joint action of these two factors. Probably, some other factors, unknown to us, also have an impact on distribution of infected herring during feeding.

Table A9.2. Prevalence of herring infected by *I. hoferi* (%) from different size catches taken in different trawling depths in the Norwegian Sea, June–July 1992.

Trawling depth, m	Catch, kg/hr			
	To 60 kg/hr		61–3000 kg/hr	
	Prevalence, %	n	Prevalence, %	n
0–5	53.0±3.3	236	18.0±1.9	390
10–15	23.0±3.5	140	11.2±1.9	270
45–150	50.0±7.9	40	-------	-------
Fish analysed, indiv.		416		660
Average prevalence, %	42.5±2.4		15.2±1.4	

As can be seen in Table A9.2, the infected fish (50.0–53.0 %) most frequently occurred in samples from small catches (to 60 kg/hr) taken in 0–5 m and 45–150 m trawling depths.

The amount of infected fish in samples from such small catches taken in 10–15 m depth was 2 times lower than in 0– 5 m and 45–150 m and constituted 23.0 %.

In large catches (61–3000 kg/hr), when trawling in 0–5 m and 10–15 m, the amount of infected fish was lower than in small catches and did not exceed 18%. Actually, the amount of infected fish from large catches taken in 0–15 m depth was similar to that from small catches taken in 10–15 m depth.

Thus, during feeding the infected fish aggregated in a 0–5 metre surface layer and in the depth exceeding 45 m.

Norwegian scientists have also come to a similar conclusion, that the per cent of infected fish being outside large aggregations of school was higher than that within such aggregations (Hjeltnes and Skagen, 1992; Holst, 1996; Hodneland *et al.,* 1997).

Redistribution of the major amount of herring school in mid-water could be caused by a temperature gradient or by a concentration of feeding plankton, since herring do not make direct vertical migrations during feeding (Zusser, 1971). In that case, transporting of fish weakened by illness to the surface layers was passive.

It is probable that the presence of infected fish (about 30 %) in the school resulted in a change of the whole herring school behavior that in its turn affected the distribution of infected fish and their amount in trawl catches.

It is impossible to exclude the fact that during an outbreak of *Ichthyophonosis* epizooty the dying herring descended below 150 m. However, hauls were not performed in such depth and we were not able to prove this hypothesis.

Sinderman (1962), when studying epizooty of *Ichthyophonosis* in the Gulf of St. Lawrence during 1954–1955, noted that herring infected by *I. hoferi* aggregate mainly in deeper layers. An occurrence of infected fish in samples caught in near-bottom layers of the coastal areas was higher than in the samples from deeper layers.

A similar relationship was revealed by us during the herring spawning on the banks of the Norwegian Shallows.

In February–March 1996, the amount of infected fish in samples from small (to 500 kg/hr) and large (501–120,000 kg/hr) catches was approximately similar and made up 9.4 % and 12.1 %, respectively (Table A9.3).

Table A9.3. Prevalence of herring infected by *I. hoferi* (%) in different size catches taken in different trawling depths in the Norwegian Sea, February–March 1996.

Trawling depth, m	catch, kg/hr			
	To 500 kg/hr		501-120000 kg/hr	
	Prevalence, %	n	Prevalence, %	n
50–100	20.0±7.3	30	1.7±1.7	60
101–200	7.5±2.4	120	14.4±5.9	35
201–300	6.7±4.5	30	16.7±3.4	120
Fish analysed, indiv.		180		215
Average prevalence, %	9.4±2.2		12.1±2.2	

Besides, no differences were found between the amount of infected fish in samples from small catches taken in different depths on spawning grounds.

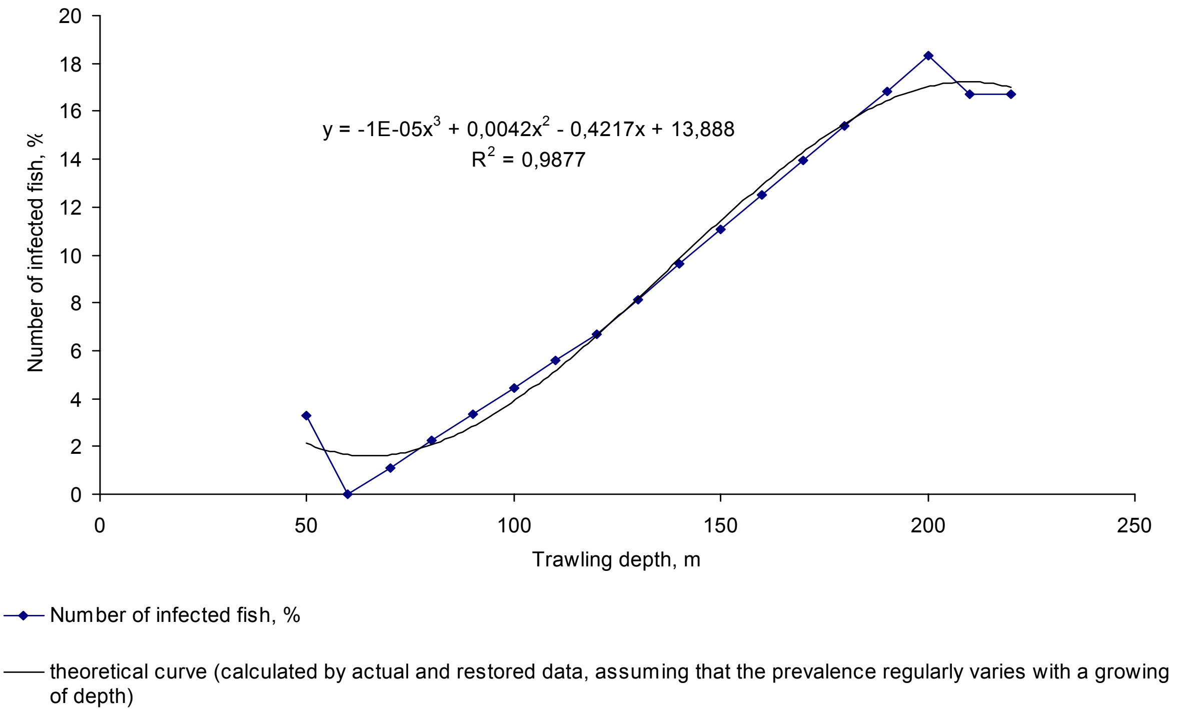
In large catches taken in 50–100 m trawling depths the amount of infected fish constituted only 1.7 %. With growing depth, the infected fish occurred more frequently in large catches. In the samples taken in 200–250 m (near-bottom layer), their number constituted 14–17 %.

As a result of the analysis of the data, a theoretical curve of relationship between the amount of infected herring and trawling depth was calculated for the samples taken from large catches. This relationship was described by the equation of polynomial function (Figure A9.2).

It is known that in healthy populations herring are slow-moving on spawning grounds and form large aggregations on banks. Fish perform vertical migrations during 24 hours. In the daytime, herring are distributed as a dense aggregation, at the bottom, and with an approaching of dusk fish ascend a 50–70 m depth and occupy the vast area (Okonski and Konkol, 1957; Zusser, 1971; Retingen, 1990).

Apparently, herring infected by *I. hoferi* did not perform daily migrations during the spawning period. Fish were permanently distributed in the depth below 100 m, creating major aggregations in a near-bottom layer. This was probably due to the fact that *Ichthyophonosis* causes pathologic variations in the infected fish organism, that result in a reduction of moving activity and disturbance of behavioral reactions.

Figure A9.2. Empirical and theoretical curves of relationship between the amount of fish with a heart affected by *I. hoferi* and trawling depth (catches below 500 kg/hr of trawling).



Conclusion

Thus, it is shown that during herring feeding, the largest proportion of fish infected by *I. hoferi* is distributed in the surface layer that is well fished off both by a trawl and a purse seine.

During spawning the infected herring create major aggregations in a near-bottom layer that is usually not fished off by a purse seine. Depth of purse seine fishing is restricted by the height of its wall, that as a rule does not exceed 150 m, and depth on spawning grounds attain 300 m and below. In this connection, when analysing samples from a purse seine catch, the per cent of infected fish is probably underestimated.

During trawl fishing of the feeding herring, the highest proportion of infected fish was registered in catches below 60 kg/trawling hour. No relationship between the catch size and amount of infected herring in samples was revealed during spawning.

Estimation of the prevalence of *I. hoferi* disease in herring is reliable and possible by samples selected from trawls both during a feeding and a spawning period of fish.

In our opinion, a comparison of results from the investigations on samples, taken from different fishing gears, is impossible to be considered as correct.

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ANNEX 10: OVERVIEW OF THE DISTRIBUTION AND POSSIBLE CAUSES OF THE M74 SYNDROME

By G. Bylund

Present state

Compared to one year earlier (2000) there was again an increasing trend with higher disease prevalences in most of the Baltic rivers The disease prevalences (%) for the most important salmon rivers monitored are presented in Table A10.1 below. The prognoses for the 2002 hatch indicate further increase in the disease prevalences, both in Finnish and in Swedish rivers.

Table A10.1 Disease prevalence (%) for salmon rivers.

River system	1998	1999	2000	2001
Simojoki	31	38	22	41
Tornionjoki	25	56	32	41
Lule älv	6	34	21	29
Skellefteälven	4	42	12	14
Ume/Vindelälven	9	53	45	39
Ångermanälven	3	28	21	25
Indalsälven	1	20	14	7
Ljungan	10	25	10	n.d.
Ljusnan	6	41	25	46
Dalälven	9	33	27	33
Kymijoki	42	0	10	n.d.
Mean total	13.3	33.6	21.7	30.6

Recent results and ongoing research

No very significant breakthrough has been reported in the research on the etiology of M74.

Recent observations in Sweden indicate that the consequences of the thiamine deficiency are not restricted to the yolk sack stage of salmon fry. Fry originating from females with insufficient thiamine levels may survive up to the first feeding stage but refuse feeding and gradually die off unless treated with thiamine. Bath treatment with thiamine at that stage leads to immediate recovery of the fry. Other observations have demonstrated the importance of early thiamine treatment of fry with insufficient thiamine levels. Populations of yolk sac fry which have already developed the typical M74 symptoms (uncoordinated swimming movements, grayish color, congestion and fragility of blood vessels, precipitates in the yolk sac, etc.) show rapid recovery after bath treatment with thiamine. At later stages, however, the prevalence of fish with different sorts of deformities is high in these populations. This is also reflected in an increased prevalence of individuals with deformities among returning spawners in recent years.

The research in Finland focuses on the thiaminase activity of organisms in the food chain of Baltic salmon. From this work, it appears clearly that Baltic herring rather than sprat (as previously suggested) is responsible for the low thiamine level in salmon. The thiaminase activity is many-fold higher in herring than in sprat. Also there is pronounced seasonal as well as geographical variation in the thiaminase activity of herring. Accumulating data support the hypothesis that the Baltic salmon might selectively feed on herring with high thiaminase activity.

Finnish work also shows that not only the presence of the enzyme thiaminase but also the presence of a suitable co-substrate is decisive for the thiaminase activity in fish. Herring may contain a high level of the enzyme thiaminase (i.e., have high potential thiaminase activity) but show low thiaminase activity (actual thiaminase activity) until a suitable co-substrate is added. It has been shown experimentally that a large number of chemicals can act as co-substrate, for the thiamine splitting reaction (e.g., pyridine, aniline, nicotinic acid, proline, hypotaurine, cysteine, etc.) thus influencing the thiamine level in organisms. It is suggested that changes in the occurrence of suitable co-substrates in the food chain of Baltic salmon might have influence on the thiaminase activity in the prey fish and thus finally on the thiamine level of salmon. As possible co-substrates we have to consider, i.a., environmental contaminants, agents connected to the eutrophication with accompanying algae blooms, etc. Work in order to evaluate this hypothesis is in progress.

Also work on the etiology of the Early Mortality Syndrome (EMS) in North America focuses presently on the thiaminase activity and thiaminase kinetics in salmonid fish.

ANNEX 11: CURRENT STATUS REGARDING THE CHARCTERISATION AND PATHOGENICITY OF MARINE VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS IN FARMED AND WILD FISH

By Helle Frank Skall, Danish Veterinary Institute (EU-Reference Lab).

Serologic identification

It is not possible to assign VHSV isolates to the freshwater or marine group using the monoclonal antibodies available today. Preliminary work done at the Danish Veterinary Laboratory, however, indicates that a panel of newly developed monoclonal antibodies may be able to differentiate between a) freshwater and non-Baltic marine isolates and b) Baltic marine isolates (N. Lorenzen, unpublished data). More isolates should be analysed before making final conclusions regarding the efficacy of this assay.

Genetic characterisation

Sequence comparisons of many VHSV isolates by several laboratories have shown that genetic differences appear to be stronger related to geographic location than to year of isolation or host species (Basurco *et al*., 1995; Benmansour *et al*., 1997; Stone *et al*., 1997; Snow *et al*., 1999).

Four major genotypes have been identified by Snow *et al*. (1999):

Genotype I:	European freshwater VHSV isolates and a group of marine isolates from the Baltic Sea;
Genotype II:	a group of marine isolates circulating in the Baltic Sea;
Genotype III:	isolates from the North Sea, Skagerrak and Kattegat;
Genotype IV:	North American isolates.

RNAse protection assay is a fast method to initially characterise many isolates in a short time. It is not, however, as discriminatory as sequencing (Snow *et al*., 1999).

Also, restriction fragment length polymorphism (RFLP) is a promising new method for genetic differentiation of VHSV isolates (K. Einer-Jensen, unpublished data). This molecular typing system is based on enzymatic digestion of RT-PCR amplified G-gene products and subsequent grouping of the obtained restriction patterns. The RFLP method has been developed on the basis of sequence data from more than 60 isolates, representing a broad geographical area of isolation. The developed method has so far allowed grouping of VHS virus isolates into the above-mentioned major geographical areas as well as detailed geno sub-grouping according to the performed phylogenetic analysis. The assay is at present undergoing further development and validation.

Distribution and host species of marine VHSV

VHSV is endemic in the Baltic Sea, Kattegat, Skagerrak, the North Sea and the coastal areas of the British Isles.

The apparent prevalence of VHSV is highest in the Baltic Sea, and lowest in the Norwegian Sea, North Coastal area and the Barents Sea (H.F. Skall, personal communication).

The species most frequently found infected are schooling fish such as sprat and herring in the Baltic Sea and Norway pout at the Atlantic coast of the British Isles (Mortensen *et al*., 1999; King *et al*., 2001). But bottom-dwelling fish are also found infected.

VHSV in the marine environment is not only a North European phenomenon. It has also been isolated among wild marine fish in North America and Japan. In Japan the first VHS outbreak was detected in a farm producing Japanese flounder (*Paralichthys olivaceus*) in 1996 (Isshiki *et al*., 2001). In 2001 the number of infected farms was 27, and to date approximately 50 isolations from Japanese flounder farms has been done (Dr Nakajima, personal communication). Two isolations of VHSV have also been done from farmed black rockfish (*Sebastes inermis*) (Dr Nakajima, personal communication). VHSV has also been isolated from wild Japanese flounder (Takano *et al*., 2000).

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ANNEX 12: TENTATIVE TABLE OF CONTENTS OF THE MANUSCRIPT ENTITLED "STATISTICAL METHODS FOR THE ANALYSIS OF FISH DISEASE DATA"

W. Wosniok, T. Lang, A.D. Vethaak, S. desClers, S. Mellergaard, S.W. Feist, A.H. McVicar, and V. Dethlefsen

ANNEX 13: BIODIVERSITY OF STICKLEBACK PARASITES

Since 1997, David J. Marcogliese has been coordinating a Canadian National Stickleback Parasite Survey (see: http://www.biology.ualberta.ca/parasites/indexen/modulei.htm). This project has been expanded to an international context and has been approved as a core project for the International Biodiversity Observation Year (IBOY: 2001–2002) (see: http://www.nrel.colostate.edu/IBOY/index2.html), an initiative of DIVERSITAS, the International Programme on Biodiversity Science.

The project has thirty participants from twelve countries, including Canada, Czech Republic, Denmark, England, Germany, Iceland, Japan, Norway, Russia, Scotland, Ukraine, and the United States. Glenn Bristow of the University of Bergen is the European coordinator. While there is no financial support as of yet for the project as a whole, individual researchers have generously committed some of their time and resources to the project. Within Canada, our project has received the support of the Biodiversity Science Board of Canada, the Biological Survey of Canada, and the Biodiversity Committee of the Canadian Society of Zoologists, in addition to a number of Canadian national parks.

Sticklebacks of all species will be collected from various sites from around the world in 2001 and 2002, and most likely thereafter. To date we have collections from Canada, Faroe Islands, Germany, Iceland, Norway, Russia, Scotland and the United States. After collections, the participants will assemble when possible to describe their results and collate the data together for analysis. The database will be used to address evolutionary, ecological and environmental hypotheses. A workshop is planned for ICOPA X in Vancouver, 4–10 August, 2002 for those participants in attendance. Ultimately, in addition to developing a monitoring indicator for biodiversity and environmental stress, we hope to produce products such as pamphlets, picture keys, and CD-ROM learning tools to allow non-parasitologists including biologists and students to be able to look at stickleback parasites and implement monitoring systems.

Those wishing to participate in this continuing international effort should contact David J. Marcogliese (St. Lawrence Centre, Environment Canada, 105 McGill, 7th Floor, Montreal, Quebec, Canada H2Y 2E7; Tel: 514-283-6499; Fax: 514-496-7398; email: david.marcogliese@ec.gc.ca) for further information.

INFORMATION FORM FOR DIVERSITAS - IBOY PROJECTS
(INTERNATIONAL BIODIVERSITY YEAR 2001–2002)

Project title: Biodiversity of stickleback parasites

Leader:

Dr David J. Marcogliese, St. Lawrence Centre, Environment Canada, Montreal, Quebec, Canada

Other participants:

Dr Per-Arne Amundsen, Norwegian College of Fishery Science, University of Tromso, Tromso, Norway
Dr Duane Barker, School of Fisheries, Marine Institute of Memorial University, St. John's, Newfoundland, Canada
Dr Ann Barse, Department of Biological Sciences, Salisbury University, Salisbury, Maryland, USA
Dr Glenn Bristow, Zoological Laboratory, University of Bergen, Bergen, Norway
Dr Dan Brooks, Department of Zoology, University of Toronto, Toronto, Ontario, Canada
Dr Mick Burt, Huntsman Marine Sciences Centre, St. Andrews, New Brunswick, Canada
Dr David Cone, Department of Biology, St. Mary's University, Halifax, Nova Scotia, Canada
Dr David Gibson, Department of Zoology, Natural History Museum, London, UK
Dr Cam Goater, Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada
Dr Tim Goater, Department of Biology, Malaspina University-College, Nanaimo, British Columbia, Canada
Dr David Heins, Department of Ecology & Evolutionary Biology, Tulane University, New Orleans, Louisiana, USA
Dr Danjal P. Hoejgaard, Gerdisvegur, Soeldarfjoerdur, Faroe Islands, Denmark
Dr Kym Jacobson, NOAA, National Marine Fisheries Service, Newport, Oregon, USA
Dr Andrey Karasev, Laboratory of Fish Parasitology and Physiology, PINRO, Murmansk, Russia
Dr Greg Klassen, Biology Department, University of New Brunswick, Saint John, New Brunswick, Canada
Dr Armand Kuris & Kevin Lafferty, Department of Biological Sciences, University of California, Santa Barbara, California, USA
Dr Evgeny Leshko, Institute of Biology, Karelian Science Centre, Russian Academy of Sciences, Petrozavodsk, Russia

Dr Ken MacKenzie, Department of Zoology, University of Aberdeen, Aberdeen, UK

Dr Deborah McLennan, Department of Zoology, University of Toronto, Toronto, Ontario, Canada

Dr Kazuya Nagasawa, National Research Institute of Far Seas Fisheries, Fisheries Agency of Japan, Orido, Shi'izu, Shizuoku, Japan

Dr Oleg Pugachev, Zoological Institute, Department of Parasitic Worms, St. Petersburg, Russia

Dr Sigurður Richter, Institute of Experimental Pathology, University of Iceland, Reykjavik, Iceland

Dr Olga Rusinek, Limnological Institute, Russian Academy of Sciences, Irkutsk, Russia

Dr Vladimir L. Sarabeev, Department of Biology, Zaporizhzhia State University, Zaporizhzhia, Ukraine

Dr Tom Scholz, Institute of Parasitology, Academy of Sciences of the Czech Republic, Ceske Budejovice, Czech Republic

Dr Al Shostak, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

Dr Karl Skirnisson, Institute of Experimental Pathology, University of Iceland, Reykjavik, Iceland

Dr Willie Yeomans, Environment Agency, Reading, Berkshire, United Kingdom

Dr C. Dieter Zander, Zoologisches Institut, Zooligisches Museum, Hamburg, Germany

Note: Participants include parasitologists familiar with stickleback parasites (many), as well as expertise in historical ecology and parasite phylogenetics (D. Brooks), stickleback phylogenetics and biology (P. A. Amundsen, D. Heins, D. McLennan), ichthyology and database management (G. Klassen).

Organism, habitat or system focus:

Parasites of all species of sticklebacks (Gasterosteidae) will be studied. The fish include the threespine (*Gasterosteus aculeatus*), fourspine (*Apeltes quadracus*), ninespine (*Pungitius pungitius*), fifteenspine (*Spinachia spinachia*), blackspotted (*Gasterosteus wheatlandi*), and brook (*Culaea inconstans*) sticklebacks. Sticklebacks occur in shallow inshore areas of marine and fresh waters of North America, northeastern and central Asia, Europe, and Algeria. They can be found associated with most oceans and seas and inland into fresh waters in the boreal-temperate regions of the northern hemisphere. Emphasis will be placed on the threespine and ninespine sticklebacks, as these species are circumboreal in distribution, and span freshwater-estuarine-marine habitats.

Countries or regions involved:

The study will cover selected fresh, estuarine and marine habitats in Canada, the Pacific United States, the United Kingdom, Norway, continental Europe, Russia, Iceland, and Japan.

Brief summary of Project, along with timetables

Sticklebacks are among the most widely distributed fishes in the northern hemisphere. They are found in both the east and west hemispheres, in the Arctic, and in boreal-temperate regions. Many are euryhaline, occurring in marine coastal areas, brackish waters and fresh waters. The threespine (*Gasterosteus aculeatus*) and the ninespine (*Pungitius pungitius*) sticklebacks are the most widespread, with circumboreal distributions.

Sticklebacks are an ideal model system with which to examine the distribution of parasites on a regional scale. They are easy to catch with very simple collecting gear, and it does not require much time to examine specimens in sufficient numbers. In addition, there already exists some data on parasites found in sticklebacks from Canada, the U.K., continental Europe, Russia, and Japan. They also are tolerant of adverse conditions, and can be found in both perturbed and undisturbed habitats. Moreover, by spanning the freshwater-saltwater continuum, they provide a unique model with which to evaluate ecological conditions and biodiversity in aquatic habitats.

Parasites, because of their very nature, compose ideal organisms to use as indicators of biodiversity and ecosystem structure. Parasites within a single assemblage represent different phylogenetic lineages, which means they possess different life cycles, respond differently to various environmental conditions, and are not biased by phylogenetic constraints. They possess rapid generation times compared to their hosts so environmental effects will be manifested more quickly. Because many parasites possess complex life cycles and rely on the presence of intermediate hosts and predator-prey relationships for transmission, they are well suited to evaluate food web structure and trophic interactions, including those bridging the terrestrial-aquatic interface. As a result of the varied nature of their transmission patterns, parasite assemblages make excellent early bioindicators of impending ecosystem stress and biodiversity (Cone *et al*., 1993; Marcogliese and Cone, 1996, 1997a, 1997b). Furthermore, their importance in ecosystem processes cannot be ignored. Parasites can regulate the populations of their hosts and affect energy flow within a food web (Marcogliese and Cone, 1997a).

It is intended to build an international database on the distribution and abundance of stickleback parasites. Such a database may be used to examine questions pertaining to the biogeography of sticklebacks and their parasites, the local and regional distribution of stickleback parasites, and the use of parasite assemblages as environmental and biodiversity indicators. The application of modern phylogenetic techniques and principles of historical biogeography will permit further resolution of the stickleback clade, and predictions of ecological patterns of food web structure based on the distribution of parasites among species and habitats. This then will improve our predictive capabilities regarding the impacts of environmental stressors on biodiversity.

Protozoan and metazoan parasites will be surveyed from all species collected, with emphasis on the threespine and ninespine sticklebacks from freshwater, brackish and marine habitats in North America, Europe, and Asia. In each region, relatively undisturbed and perturbed sites will be chosen from the different habitat types. Thirty fish will be collected from each site in June–July 2001, and examined for parasites using standardized techniques. This international endeavour will build on the recently initiated Canadian National Stickleback Parasite Survey. Further information on this project and details concerning methodology can be found on the World-Wide Web at: http://www.biology.ualberta.ca/parasites/indexen/modulei.htm

Participants will assemble in the autumn 2001, for an international workshop to present results and compile data into an electronic database. The database will be accessible on the World-Wide Web, and maintained so that researchers internationally can have access and continue to contribute. Data collected and presented in 2001 will be analysed using statistical and phylogenetic techniques, and the results published.

Cone, D.K., Marcogliese, D.J., and Watt, W.D. 1993. Metazoan parasite communities of yellow eels (*Anguilla rostrata*) in acidic and limed rivers of Nova Scotia. Can. J. Zool. 71: 177–184.

Marcogliese, D.J., and Cone, D.K. 1996. On the distribution and abundance of eel parasites in Nova Scotia: influence of pH. J. Parasitol. 82: 389–399.

Marcogliese, D.J., and Cone, D.K. 1997a. Food webs: a plea for parasites. Trends Ecol. Evol. 12: 320–325.

Marcogliese, D.J., and Cone, D.K.1997b. Parasite communities as indicators of ecosystem stress. Parasitologia 39: 227–232.

Under which DIVERSITAS Programme or Special Target Area does the project fall?

Core Program Elements

PE1: The effect of biodiversity on ecosystem functioning:

- understand effects of complexity on eco-functions;
- understand effects of global change at different spatial scales.

PE3: Systematics, inventorying and classification:

- inventory and phylogenetic research on little-studied taxa.

PE4: Monitoring of biodiversity:

- develop standardised methods to sample, assess, and monitor biodiversity;
- testing new biodiversity indicators.

PE5: Conservation, restoration and sustainable use of biodiversity:

- monitoring global climate change, through changes in parasite assemblages;
- monitoring wetland restoration (sticklebacks are important components of wetlands).

Special Target Areas of Research (STARs)

PE 7: Marine biodiversity, including coastal and estuarine habitats.

PE 9: inland water biodiversity.

In addition, this project addresses biodiversity of parasites, which, despite being neglected taxa, are important components of ecosystems that have received little if any attention by DIVERSTAS and other biodiversity initiatives.

How does this project fit into the biodiversity science goals of IBOY?

This project is global in nature, and links biodiversity at the species and ecosystem levels. It addresses a little-recognized but important component of biodiversity, that being the parasites. Parasitism may be the most common lifestyle of organisms in existence on earth. The use of parasites as biodiversity indicators may be applicable to all ecosystems, and at the same time excellent indicators of environmental quality and degredation. Importantly, parasites also represent a neglected ecological component of biodiversity which demands attention, especially given that they can regulate host populations and greatly affect energy flow through an ecosystem.

How will the project draw attention to biodiversity in 2001?

By and large, parasites are unrecognized in the public arena, except as agents of disease. This project will serve to focus the attention of both the public and scientific communities on parasites as indicator organisms and significant components of biodiversity. Furthermore, the project will permit the public and the scientific community to establish the connection between parasites, ecosystem function, and energetics.

All participants will assist in publicizing their contributions to this international effort. An international workshop will be convened with a resulting publication. Moreover, we plan to produce manuals or guides outlining methodology and identification of stickleback parasites, the use of parasites as indicators of environmental conditions and biodiversity, and the use of sticklebacks and their parasites as teaching aids.

What are the expected products of the project?

1) An electronic database of stickleback parasites.
2) A publication in the form of a proceedings on workshop presentations.
3) A manual outlining methodology of use of parasites as indicators of environmental conditions and biodiversity.
4) A practical guide to the stickleback parasites of the world.
5) A manual for the use of the stickleback survey as a teaching aid.

What future ongoing activities beyond 2001, if any, will result from this project?

Essentially the Canadian National Stickleback Parasite Survey will continue as an international undertaking. Participants will be encouraged to continue collections in the future. New participants will be encouraged to collect and submit data on an international level. The electronic database will be maintained and updated with the new submissions. Data will be made available to researchers for phylogenetic and ecological analyses.

Which organizations are involved in your project?

Canadian Society of Zoologists.
Member institutions of the various participants.

Funding requirement

Is the project funded? No, the Canadian National Stickleback Parasite Survey is a newly initiated volunteer survey. There is not any funding for current or proposed activities.

If not have you plans to seek funding? Yes.

Approximate cost

Collections, examinations of fish ($1000 per participant)	$15 000
Travel to workshop (average of $2500 per participant)	$35 000
Coordination, travel for project leader	$10 000
Publication costs	$ 5 000
Database management	$ 3 000
TOTAL	**$68 000**

Submitted by: David J. Marcogliese **Date: February 3, 1999**

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ANNEX 14: ANALYSIS OF PROGRESS WITH TASKS

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

b) Report on progress in the ongoing investigations of the effect of temperature on Bonamia infection dynamics and report on the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oysters species; *A status of the present knowledge was presented but investigations are still ongoing. A summary report of an EU project on the effect of temperature on Bonamia infection dynamics will be finalized during 2002 and the final results will be presented at the next WGPDMO meeting.*

c) Review the current status of studies carried out in ICES Member Countries on the relationship between environmental contaminants and shellfish pathology; *Available published information was assessed and a summary report was presented.*

d) Obtain information on the EU project "*Marteilia refringens* studies: Molecular systematics and search for the intermediate host of the bivalve mollusc's parasite" and review the results; *Available information was assessed and a summary report was presented.*

e) Review and report on the progress made in the BEQUALM Work Package 6 "External diseases and liver histopathology"; *A summary progress report was provided with an outline of the future prospects of the project.*

f) Review and report on information from the workshop on nodavirus at the EAFP Conference in Dublin, the final report from the ongoing EU project on nodavirus and other relevant information to provide advice on control measures; *Available information on the EU project on nodaviruses was reviewed.*

g) Maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome; *A verbal report summarising available information was presented.*

h) Report and assess the effectiveness of salmon farming management control methods for the control of sea lice in the different ICES Member Countries; *Available information was assessed and a summary report was presented.*

i) Review the current status regarding the characterisation and pathogenicity of VHSV strains in farmed and wild fish; *A summary of the most recent information on the characterisation and pathogenicity of marine VHSV was provided.*

j) Compile and review information on IPNV in salmonid fish farming; *Available information was assessed and a summary report was presented.*

k) Review progress made with regard to the update of ICES publications on pathology and diseases of marine organisms:

- Report on important trends in diseases occurring in finfish and shellfish culture in the ICES area in the period 1997–2001; *A draft manuscript was presented and a strategy for the publication was agreed.*

- Web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report; *A proposal for improvements of the WGPDMO contribution to the ICES Environmental Status Report was presented and will be submitted to ICES for inclusion on the ICES website.*

- Manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series; *The content of the different sections of the publication was presented and agreed on.*

- ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish; *A strategy for the production of the Leaflets was discussed and a plan for revision was made.*

ANNEX 15: RECOMMENDATIONS

1. The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (New Chair: T. Lang, Germany) will meet 11–15 March 2003 in Aberdeen, Scotland to:

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

b) report on progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics and report on the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oyster species; **(Tristan Renault, Susan Ford)**

c) review the existing strategies to assess the prevalence of shellfish diseases in parallel to fish diseases and chemical contaminant levels in environmental monitoring programmes in order to eventually implement this type of approach in the ICES Environmental Monitoring Programme; **(Catherine Couillard, Steve Feist, Tristan Renault)**

d) review and assess a report prepared intersessionally on investigations into the molecular comparisons among the various species/isolates of *Perkinsus* in collaboration with the OIE reference laboratory for *Perkinsus* at the Virginia Institute of Marine Science (E. Burreson); **(Susan Bower)**

e) obtain information on the EU project “Diagnosis of oyster herpes-like virus: development and validation of molecular, immunological and cellular tools ” (FAIR-PL98-4334) and review the results; **(Tristan Renault)**

f) review progress made in the “fish diseases and liver histopathology” component of the BEQUALM self-funding scheme; **(Steve Feist, Thomas Lang)**

g) review and assess the impact of diseases of farmed fish on wild fish stocks; (**David Bruno, Brit Hjeltness**)

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

i) report and assess the effectiveness of salmon farming management control methods for the control of sea lice in the different ICES Member Countries; **(Brit Hjeltnes, David Bruno)**

j) reviews progress made in the modifications of the ICES Databases and the ICES Environmental Data Reporting Format; (**Thomas Lang, Werner Wosniok**)

k) review progress made with regard to the update of ICES publications on pathology and diseases of marine organisms:

   i) web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report, **(Thomas Lang, Stig Mellergaard)**

   ii) manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series, **(Werner Wosniok, Thomas Lang)**

   iii) ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. **(Sharon McGladdery)**

WGPDMO will report by 31 March 2003 for the attention of the Mariculture Committee and ACME.

Supporting Information

Priority:	WGPDMO is of fundamental importance to the ICES advisory process.
Scientific Justification:	a) New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained. b) Experimental work is required to confirm field observations and the hypothesis of *Bonamia* suppression vs. destruction over long periods of low temperatures. This question is important for accurately assessing climate effects on Bonamiasis and European oyster culture. There are historic records of an iridoviral infection of *Crassostrea gigas* gills, associated with low/transient pathology. This suggests that the gill disease agent may have multi-host infection potential, which needs to be addressed for like-to-like *C. gigas* and *Ostrea edulis* transfers. c) In recent years, an increasing effort in ICES Member Countries has been given to studies on shellfish diseases and pathology in relation to environmental contaminants. The WGPDMO considered it timely to obtain an overview of existing strategies within this field.

	d) Confusion occurs in the taxonomy of *Perkinsus* spp., which cause significant disease and mortalities in molluscs in many locations around the world. New species are being named based on differences in molecular sequences. The current status of species identification should be reviewed to document acceptable criteria for species identification. e) Herpesvirus infections have been reported in different bivalve species around the world. Specific diagnostic tools are needed in order to assess the causative role of these viruses in shellfish mortalities. Within the ongoing EU-funded project VINO, specific tools for diagnosing these viral infections have been developed and validated. WGPDMO will assess the most recent knowledge within this field. f) Within the EU-funded project BEQUALM, a quality assurance programme for fish diseases and liver pathology has been developed. In future, it is intended to be a self-funding scheme, which will form an essential part of wild fish disease monitoring programmes and be important for implementing data on liver pathology in the ICES Fish Disease Database. g) The impact of the increasing development of aquaculture on diseases in the wild fish populations is an issue of concern. The WGPDMO considers it important to be updated on the most recent knowledge within this field. h) ICES C.Res. 1993/2:23(m) requested that WGPDMO maintain an overview of the M74 syndrome and the *Ichthyophonus* issue as part of its regular agenda. i) In the process of assessing the effectiveness of salmon farming management control methods for the control of sea lice the WGPDMO will assess the most recent knowledge within this field which will be published in a report submitted by the Norwegian National Committee for sea lice. j) The WGPDMO considers it necessary to follow the process of modification of the ICES Databank structure in order to assist if required and to obtain an overview of changes suggested or introduced by other ICES Working Groups. k) A number of ICES publications, either web-based or in ICES publication series, are being prepared or updated at present, the progress of which have to be reviewed by the WGPDMO at its next meeting. It will be necessary to consider ways how these can be linked to each other.
Relation to Strategic Plan:	Responds to Objectives
Resource Requirements:	None required, other than those provided by the host institute.
Participants:	WGPDMO members
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	ACME
Linkages to other Committees or Groups:	MARC
Linkages to other Organisations:	BEQUALM, OIE, EU
Cost share	ICES:100 %

2. The report **Important Trends in Disease Problems in Finfish and Shellfish Culture in the ICES Area, 1997–2001**, by members of the Working Group on Pathology and Diseases of Marine Organisms, as reviewed and approved by the Chair of the Mariculture Committee, will be published in the *ICES Cooperative Research Report* series, preferably as a web-based publication only. The estimated number of pages is 30.