Mariculture Committee

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REPORT OF THE

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Lisbon, Portugal
2–6 March 1999

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

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1 OPENING AND STRUCTURE OF THE MEETING

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the Instituto de Investigação das Pescades e do Mar (IPIMAR), Lisbon, Portugal with S. Mellergaard as Chair. The meeting was opened at 10.00 hrs on Tuesday, 2 March 1999, with the Chair welcoming the participants, particularly those who have not previously attended WGPDMO. The Vice President, Dr. Costa Monteiro, welcomed the participants to the IPIMAR.

A list of participants is appended in Annex 1.

Apologies were received from P. van Banning (Netherlands), C. Couillard (Canada), S. Mortensen (Norway), E. Lindesjöö (Sweden), S. Bower (Canada) and W. Grygiel (Poland).

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

2 ICES ANNUAL SCIENCE CONFERENCE 1998: ITEMS OF RELEVANCE TO WGPDMO

Items of relevance to WGPDMO from the 1998 ICES Annual Science Conference (86th ICES Statutory Meeting) held in Cascais, Portugal were highlighted by the Chair.

a) The Report of the Delegates Meeting:
   - i) stated that the recommendations originating from the Mariculture Committee were adopted by the Council with the addition of an extra item 'to update information prepared for the HELCOM Third Periodic Assessment concerning diseases and parasites of 'Baltic fish stocks, diseases and ecosystem effects' for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994-1998 [HELCOM 1999/3]'.

b) The report of the Mariculture Committee:
   - i) accepted the report of the 1998 meeting of WGPDMO and its recommendations along with the addition of one recommendation forwarded by ACME.
   - ii) proposed to organise a Theme Session at the 1999 ASC in Stockholm, on M74 and similar reproductive disturbances in aquatic organisms

c) The Working Group on Biological Effects of Contaminants
   - i) has a TOR –'review and report on the impact of specific contaminants of concern, e.g., veterinary medicines used in fish farming and algal toxins', an item which may be of interest for the WGPDMO.

d) Through the auspices of the General Secretary, representatives from the EIFAC should be invited to attend the WGPDMO meeting. An invitation was forwarded from the WGPDMO Chair to Professor H. Ackerfors to represent EIFAC.

e) The final Terms of Reference of the WGPDMO were agreed as C.Res. 1998/2:44.

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

3.1 Terms of Reference

The WGPDMO took note of the terms of reference published as C. Res 2:44 (Annex 2). The heavy 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 Report as Annexes. As agreed at the 1998 WGPDMO meeting, all working documents were to be prepared 2 weeks before the meeting and distributed by E-mail. As a result, all national reports and a considerable part of the remaining working documents were distributed to the participants before the meeting. The Chair thanked the members for preparing these reports in advance, a work which ensures that the Terms of Reference will be treated efficiently.

3.2 Adoptation of the Agenda

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

3.3 Selection of Rapporteurs

Rapporteurs were accepted as indicated in Annex 4.

4 OTHER RELEVANT REPORTS FOR INFORMATION

Information was given on a series of scientific conferences to be held in 1999.

- ICES symposium: Environmental Effects of Mariculture, 13-16 September 1999, St Andrews, New Brusnwick, Canada
- ICES Annual Science Conference, Theme Session: M74 and similar reproductive disturbances in aquatic organisms. September 1999, Stockholm, Sweden.
- The IX International Conference on Diseases and Pathology in Fish and Shellfish, European Association of Fish Pathologists, 19-24 September 1999, Rhodes, Greece.
- World Aquaculture Conference, end April-May 1999, Sydney, Australia
- World Veterinary Conference, September 1999, Lyon, France (one day dealing with fish disease problems)
- The 10th PRIMO-meeting, 24-29 September 1999, Williamsburg, Virginia, USA
- American Fisheries Society, Fish Health Section Meeting, 29 August-2 Sept 1999, Charlotte, North Carolina, USA
- Fourth Symposium on Diseases in Asian Aquaculture, 22-26 November 1999, Cebu City, Philippines
- Fourth International Conference on Lice Control on Fish Farms, 28-30 June 1999, Trinity College, Dublin, Ireland
- European Aquaculture Society, Aquaculture Europe, 7-10 August, 1999, Trondheim, Norway.
- OIE International Conference on risk Analysis in Aquatic Animal Health, 8-10 February 2000, Paris France.

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

5.1 Wild Fish Stocks

VHS-like virus was detected in various wild marine fishes. From the west and north of Scotland VHS-like virus was found principally in Norway pout (*Trisopterus esmarkii*), while in the Kattegat/Skagerrak herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) were the predominant hosts. In the western Atlantic, co-infections of VHS-like virus and IPN virus was found frequently in Greenland halibut (*Rheinhardtius hippoglossoides*) and cod (*Gadus morhua*) from the Flemish Cap.

On the Pacific coast of North America, VHS-like virus was associated with mortalities of pilchard (*Sardinops sagax*) in Canada, and with Pacific herring (*Clupea pallasii*), Alaska pollock (*Theragra chalcogramma*) and Pacific hake (*Merluccius productus*) in the USA. VHS-like virus also was isolated from captive moribund Pacific cod (*Gadus macrocephalus*), Pacific hake and Alaska pollock in Oregon.

IHN-like virus was detected in Greenland halibut from the Flemish Cap.

Infectious Salmon Anemia Virus. No ISAV was detected in surveys of wild salmonids and a wide range of marine fish species in Scottish and eastern Canadian waters.

Infectious Pancreatic Necrosis Virus. A new IPNV serotype that cross-reacted with ab and sp serotypes was detected in feral brood salmon (*Salmo salar*) in Sweden. Normally, only the serotype ab is found in salmonids along the Swedish coast.

Lymphocystis. An increasing trend since 1992 in prevalence of lymphocystis was reported for dab (*Limanda limanda*) from the German Bight and Dogger Bank areas.

Bacteria. An epizootic of a new species of *Mycobacterium* was reported from striped bass in the Chesapeake Bay, USA. Ulcerative skin lesions and granulomatous lesions in the kidney, liver, heart and muscle marked the disease. In the southern part of the North Sea, 4 % of cod (*Gadus morhua*) had mycobacterial granulomata in liver, spleen, and kidney tissues.

Group B *Streptococcus* type 1b and *Vibrio cholerae* non-01 showed a decrease in prevalence over the past year but continue to be problems in Chesapeake Bay, USA, striped bass and white perch, respectively. Both diseases produced ulcerative skin lesions.

95 % of bacterial isolates from diseased pink salmon (*Oncorhynchus gorbuscha*) from Sakhalin were *Aeromonas hydrophila* and 5 % were *A. salmonicida*. Furunculosis also was observed in pink salmon and coho salmon (*Oncorhynchus kisutch*) from Kamchatka.

Parasites. A high prevalence (30 %) of *Kudoa* was reported in muscle of sardines (*Sardina pilchardus*) from Portugal and 25 % prevalence in mackerel (*Scomber japonicus*) from the Atlantic coast of North Africa. Also infected were whiting (*Merlangius merlangius*) and hake (*Merluccius merluccius*) from Portugal. Prevalences of *Kudoa* infections in seatrout (*Salmo trutta*) in France fluctuated widely from 50 % in 1990/1991 to 0 % in 1994, and rebounded to 40 % in 1996/97.

Anguillicola crassus. Eels (*Anguilla anguilla*) in many areas of France have become infected with *Anguillicola crassa*, with prevalences in the newly affected areas ranging from 80 %–100 %. In contrast, eels from rivers in Galicia, Spain, remain free of this parasite.

Erythrocytic infections of common sole (*Solea solea*) by *Haemogregarina* sp., first reported last year in the southwestern North Sea, remained at high levels of prevalence in fishes larger than 13 cm (35 %–98 %). No infections were found in sole measuring less than 13 cm.

Barents Sea cod (*Gadus morhua*) showed an increase in average intensity of infection by *Anisakis simplex* and *Pseudoterranova decipiens*. An increase in prevalence and average intensity of anisakid larvae in muscle of red fish (*Sebastes mentella*) and long rough dab (*Hippoglossoides platessoides limandoides*) was detected also.

Skin ulcers. A major new trend in cod is the markedly increased prevalence of acute/healing skin ulcers in cod from the southwestern Baltic, particularly in smaller fish. The usually low prevalence of ulcers in winter, approached 30 % in 1998 in certain areas.

Hyperpigmentation of dab has shown an increasing trend in the Dogger Bank of the North Sea reaching a peak prevalence of 35 % in 1998, while the condition remains undetected in the Irish Sea. A decreasing trend (22 % in 1996 to 13 % in 1998) was noted for dab from the Flamborough Ground.

Liver nodules. A decrease in prevalence in grossly observed hepatic nodules greater than 2 mm was reported for dab throughout the North Sea. The prevalence of nodules in 1998 for the Dogger Bank was the lowest recorded for that area.

Liver lesions. 1997 data from histologic examination of livers from English sole (*Parophrys vetulus*) in Puget Sound showed high prevalences (26 %–53 %) of toxicopathic lesions from the most 'impacted' sites based on sediment chemistry. No comparable lesions were found in liver of fish from 4 of the reference sites.

***Pfiesteria piscicida*.** Bacterial and fungal etiologies have been identified for skin ulcers on fish from the Chesapeake Bay that closely resemble what has been considered the typical '*Pfiesteria*' induced lesion without evidence of *Pfiesteria* toxin exposure.

New information. Eye pathology, including corneal opacity and rupture, leading to blindness, was reported from 10–20 % of pikeperch (*Stizostedion lucioperca*) in the Gulf of Riga (Baltic Sea). The aetiology is unknown.

All organs of Pacific salmonids from the Bering Sea were infected by the microsporidian *Pleistophora* sp.

Larvae of *Contracaecum* sp., and cercaria and metacercaria of trematodes (Diplostomidae, *Cryptocotyle* sp.) seem to be an important factor of mortality in 0+ smelt (*Osmerus eperlanus*) in the Gulf of Riga.

5.1.1 Conclusions

1) VHS-like virus appears to be widely distributed in wild marine fishes.
2) There is a marked increase in prevalence of skin ulcers in Baltic cod that deserves further attention.

5.1.2 Recommendations

The WGPDMO recommends that:

i) because of the widespread occurrence of VHS-like virus in the Baltic, North Sea and eastern North Atlantic areas, and the reported association of these viruses with high mortalities on the west coast of North America, it is recommended that information on the possible effects of this disease on wild fish populations be collated and reviewed by the WGPDMO.
ii) due to the marked increase in prevalence of skin ulcers in Baltic cod, it is recommended that further studies be conducted on the aetiology of this disease. Because of the possible implications of this disease for survival of young cod, it is recommended that this information be brought to the attention of the relevant fish stock assessment working group.

5.2 Farmed fish

Atlantic salmon (*Salmo salar*)

Infectious Salmon Anaemia (ISA)

With the reported occurrence of this disease in Norway over the last 15 years, in eastern Canada in 1997 and for the first time in Scotland in 1998, this disease is causing major financial losses and is one of the most serious threats to salmon farming. Despite the introduction of control measures in New Brunswick, the disease has continued to spread locally probably, at least in part, due to the late introduction of controls. The isolation of ISA virus (ISAV) in a broodstock population in Nova Scotia without obvious connections to New Brunswick, has been of particular concern. In Norway, the number of new cases of ISA has increased from 6 in 1997 to 13 in 1998, but it is not known if this represents a trend. ISA has now been confirmed on 10 farms in Scotland with a further 15 under official suspicion of the presence of the virus. No ISA has been detected in grower and broodstock Atlantic salmon in Maine.

In Scotland, all outbreaks can be linked to a single primary source. In all affected areas, the spread of ISA has been closely associated with farming practices, particularly with the transfer of live fish between farms, movement of contaminated equipment and untreated waste from salmon processing plants. Measures to contain the infection have been focused mainly on these risk factors. A strategy designed to eliminate the disease is being followed in Norway and Scotland while a containment policy is being pursued in Canada.

The origins of the disease have not been determined in any area. A large number of wild salmonid and other species of marine fish have been tested without evidence of ISAV. Unpublished results from Norway gave an indication that ISAV may be able to replicate within herring, *Clupea harengus*, possibly in several organs, but the infection could not be passed from these fish to salmon.

Togavirus

A togavirus-like agent was reported from an additional farm in Maine, USA but again without associated signs of disease.

Infectious Pancreatic Necrosis (IPN)

IPN continues to cause significant losses in early post-smolt salmon in Norway and Shetland. It was indicated that the use of treated sea water in hatcheries in Norway led to an increase in the IPN risk to these hatcheries/smolt farms.

Salmon Swimbladder Sarcoma Virus (SSSV)

SSSV was reported for the first time from wild salmon being held for stripping in Maine, USA. The only previous record of this condition was from Scotland in 1978 and it is not yet known if these represent the same infection.

Bacteria

Furunculosis remains under control in all salmon farming areas, largely through use of effective vaccines.

Miscellaneous diseases

The first case of Piscirickettsiosis in the United States was reported from a net pen farm in Washington State. *Piscirickettsia* caused the most significant disease problem in Ireland in 1998 and although oxytetracycline treatment was effective, recurrence of the infection was found. Similarly, Amoebic Gill Disease (AGD) has caused less serious losses in Ireland in 1998 compared with the last few years. The infection can be controlled using fresh water treatments, but this causes serious technical problems. Sea lice, *Lepeophtheirus salmonis*, although still remaining a problem in several areas largely because of the restricted availability of licenced chemotherapeutants, are being increasingly brought under control in most salmon farming areas.

Other salmonids

Viral Haemorrhagic Septicaemia (VHS)

An occurrence of VHS, with no associated pathology, was reported in 1998 from a hatchery in western Norway. This is the first record of this disease in rainbow trout, *Onchorhynchus mykiss*, in Norway since 1974. A containment and eradication policy was followed. The source of the infection was not found. The EU Fish Disease Reference Laboratory confirmed the strain of VHSV to be similar to the Danish VHSV from rainbow trout. VHS was recorded in a small rainbow trout fattening farm on the Swedish west coast. This was the first record of this disease since 1972 when the occurrence was associated with the import of fish. The origin of the 1998 outbreak is unknown. In Denmark, a programme attempting to eradicate VHS from the last approximately 20 remaining infected farms is in progress.

Miscellaneous diseases

The proper use of vaccines, particularly against Vibriosis and Yersiniosis, has led to good control of these diseases in the rainbow trout culture industries, despite a resurgence of outbreaks where complacency has occurred when the use of vaccines has decreased. Furunculosis was detected for the first time in a rainbow trout farm in Estonia. Only one new outbreak of Bacterial Kidney Disease (BKD) was reported in Finland, reversing a trend seen in previous years. A new report was given by Finland of a very aggressive strain of *Saprolegnia* causing high mortality levels (up to 40 %) in caged rainbow trout in the low salinities of the northern Baltic.

Halibut (*Hippoglossus hippoglossus*).

Nodavirus infection continues to cause disease problems (vacuolating encephalomyelopathy and retinopathy (VER)) in juvenile halibut production in Norway. Although wolf fish, *Anarhichas lupus*, experimentally injected with nodavirus developed the disease and died within 5 days, these were not able to transmit the infection through the water to other wolf fish. Canada reported the presence of a new filterable replicating agent in halibut culture, but the identification and significance to fish health is not yet clear.

Turbot (*Scophthalmus maximus*)

A serious outbreak of Yersiniosis which killed most fish on a farm in France and did not respond to antibiotic treatment was reported. This situation could be related to poor environmental conditions.

Sea bass (*Dicentrarchus labrax*)

Photobacterium damseli subsp. *piscicida* (Pasteurellosis) is an increasing problem in the culture of sea bass and sea bream in Spain, but decreasing as a problem in France. A high carrier status prevalence of this bacterium was demonstrated in broodfish (particularly in females). It has been demonstrated that vertical transmission occurs. There is an increasing occurrence of nodavirus in ongrowing fish on the Mediterranean coast of France. A possible new occurrence of nodavirus has been noted in Spanish cage culture of juvenile sea bass in the Mediterranean, but this identification has still to be confirmed. The protozoan *Ichthyobodo* and other flagellate ectoparasites represent the main problem to sea bass culture in Portugal. Rickettsiosis was diagnosed in two farms in France with mortalities in early juvenile fish occurring at approximately 10 %.

Sea bream *(Sparus aurata)*

A major new problem with gill amoeba infection was reported from Spain. The nematode parasite *Anisakis* sp. was reported in sea bream farm stocks in Portugal, associated with the feeding of raw marine fish.

5.2.1 Conclusions

1) The report of ISA from farmed Atlantic salmon in Scotland represents a major new extension of the known distribution of this disease and its viral causative agent.
2) The occurrence of VHS in a rainbow trout hatchery in western Norway and in a sea cage rainbow trout farm on the west coast of Sweden, when this disease has not been recorded in either area for many years, can be considered as a worrying new development in the distribution of this disease.
3) The trend towards an increasing spread of nodavirus in sea bass reported previously by WGPDMO has continued.

5.2.2 Recommendations

WGPDMO recommends that:

i) because of the further spread of ISA virus in Atlantic salmon farming, information on possible origins of the outbreaks, the mechanisms of spread of infection and the risks to wild fish populations should be collated and reviewed to provide a basis for disease risk advice to the Mariculture Committee and other relevant ICES bodies.
ii) because of the continued and increasing importance of nodavirus-associated disease in mariculture, the latest results of research programmes on this disease should be reviewed to provide a basis for disease risk advice to the Mariculture Committee and other relevant ICES bodies.

5.3 Wild and Farmed Shellfish

5.3.1 Analysis by disease or parasite

Bonamia ostreae. An *Ostrea edulis* restocking programme using stock imported to Spuikom (near Oostende), Belgium, from European origins, failed due to high mortalities associated with infection with *B. ostreae* (46 %). Only large oysters (from Canada) demonstrated apparent resistance. In Portugal, an area which was previously negative for *B. ostreae* suffered mass mortalities of imported European oysters due to the infection. Wild populations of *O. edulis* from the same area continue to show no signs of infection. Elsewhere in Europe, *Bonamia ostreae* infection levels have not changed significantly and the disease is still absent from Denmark, Norway, some areas of Galicia, and Scotland. In the USA, *B. ostreae* is still present in Atlantic and Pacific *O. edulis,* but absent from Canada.

Marteilia refringens **and** ***Marteilia*** **sp.** Increased screening, due to EU Directives 91/67 and 95/70, has enhanced surveillance. No sign of *Marteilia refringens* was detected in Belgian, Danish, Norwegian, Irish, Scottish, Swedish, English, Welsh, or North American samples of *O. edulis*. Mussel infections appear to have declined in Galicia. Continued reports from Spain of *Marteilia* (*"refringens"*) in *Crassostrea gigas* from Galicia and the Mediterranean still require confirmation, especially the new report of replicating stages from Galicia. Mussel mortalities associated with high prevalences of infection by the parasite in Portugal (Albufeira Lagoon) were linked to sub-optimal growing conditions. In France, *M. refringens* was detected in 1998 in most French coastal areas. In addition, screening for *M. refringens* using newly developed molecular probes (IFREMER, La Tremblade) revealed positive reactions in *Mytilus edulis, M. galloprovincialis* and *O. edulis.*

Herpes-like virus infections continue to affect larval and juvenile *C. gigas* in France, where PCR techniques are now applied to detect viral DNA. The same techniques were used to identify herpes-like virus infections in both *C. gigas* and grooved carpet clams (*Ruditapes decussatus*) larvae from a private French hatchery. This is the first report of a herpes-like infection in this species of clam. Reports of mortalities in *C. gigas* seed imported to Portugal from France, which showed signs of a possible viral infection, require further investigation.

SPOM (Summer Pacific Oyster Mortality). Reports of Summer Pacific Oyster Mortality (SPOM) in adult *C. gigas* in France do not appear related to herpes-like viral infections. Summer mortalities of adult *C. gigas* in Washington and California, USA, were found in association with blooms of *Gymnodinium splendens.*

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

***Vibrio tapetis* (Brown Ring Disease (BRD)).** Ireland's emergent clam industry has been adversely affected by BRD since the initial outbreak in 1997 and several growers have switched to alternative culture species. Increased prevalence with high mortalities was reported in wild clams in the Gulf du Morbihan, France, an area with no previous BRD problems.

***Perkinsus* spp.**

Perkinsus atlanticus has persisted in the grooved carpet clam, *R. decussatus*, since the first detection of the parasite in Portugal in 1985/86. Highest mortalities appear to be related to post-spawning, summer temperature and salinity increases as well as handling stresses.

Perkinsus marinus persists at or near 100 % prevalence in *C. virginica* from the Gulf of Mexico to Massachusetts. High recent prevalences in the northeastern USA appear related to the warm winter of 1997–1998 and the dry summer/fall of 1998. Work at Rutgers University and the Virginia Institute of Marine Sciences (VIMS) on production of dual pathogen (*P. marinus* and *H. nelsoni*) resistant oysters show signs of success over three-year trials in the enzootic waters of Chesapeake and Delaware Bays.

***Haplosporidium*-like parasites.** The observation of a *Haplosporidium*-like parasite in *O. edulis* from Spain is under taxonomic investigation. It was not associated with overt mortalities and may be related to *Minchinia* reports in the 1970s. Another *Haplosporidium* sp., with characteristics of both *H. nelsoni* and *H. costale*, was also reported at low prevalences in *C. gigas* from Oregon (Pacific, USA). In France, ***Haplosporidium*-like** infections were noted in *Crassostrea gigas*, *Ostrea edulis* and *Cardium edule*.

***Haplosporidium* spp.**

No new trends were reported from the northeastern United States for ***H. nelsoni*** (MSX) in *Crassostrea virginica* except for mass mortalities from 1996–1998 in Long Island Sound, NY, USA. The trigger for these outbreaks is as yet unknown, since low-prevalence, subclinical infections have been known from this area for several years.

H. costale (SSO) was reported at low prevalence in *C. virginica* in several locations from New Jersey to Massachusetts, USA, reflecting either an increase in occurrence or increased sample examination. Recent mortalities of *C. virginica* in Massachusetts, however, may be associated with SSO. An organism closely resembling SSO was found in Massachusetts and Connecticut, but its identification is under investigation (using a DNA probe developed for *H. costale*) since sporulation, in these cases, was in the autumn instead of spring, as is normal for SSO.

Quahaug Parasite X (QPX) continues to be found in cultured hard-shell (quahaug) clams, *Mercenaria mercenaria* in Cape Cod, MA, and the seasides of New Jersey and Virginia, USA. Pathology appears to decrease towards the southern part of the range. In eastern Canada, QPX continues to cause mortalities in broodstock clams, and has been found in local juvenile clams (16.0–29.0 mm) rising from 2 % to 42 % prevalence between August and October 1998, in association with approximately 13 % mortality.

Juvenile Oyster Disease (JOD) declined in 1998 with a maximum of 10 % in hatchery-produced juvenile *C. virginica* and < 1 % in wild oysters off Long Island Sound, NY, USA. A marine alphaproteobacter is consistently found in association with clinical signs of JOD, but culture challenges have not duplicated the disease. Experimental challenges with *O. edulis* show no evidence of cross-species transmissibility. Resistance to JOD is now apparent in lines selected for fast growth in the JOD-enzootic Damariscotta River, ME, USA.

Other infectious agents. A microsporidian-like agent was found in the digestive glands of wild Queen scallop (*Aequipecten opercularis*) from the Irish Sea at prevalences of up to 9 %. A possible haemic neoplasia was reported from *O. edulis* from Norway, along with rickettsial-like organisms.

5.3.2 Conclusions

1) Dual disease (MSX and Dermo) resistant *Crassostrea virginica* has been developed and shows promise for growth in enzootic waters.
2) *Bonamia ostreae* still appears to be absent from northern (cold water) European oyster culture countries (Scotland, Denmark, Canada).
3) The increasing number of hosts being found with herpes-like viruses now include non-oyster species.
4) The development and use of nucleic acid probes for *Haplosporidium* spp. has significantly enhanced detection capability for these species. This should enhance identification of vectors and reservoir hosts of described species, as well as unidentified species being found in new bivalve hosts.

5.3.3 Recommendations

The WGPDMO recommends to:

i) investigate the causative agent of gill disease in *Crassostrea angulata* in Portugal.

ii) as recommended in response to previous years' reports, clarify the report of *M. refringens* in *C. gigas* from Spain. PCR probes developed by IFREMER (La Tremblade) for *Marteilia* spp. should be used to expedite this recommendation, due to its European legislative implications.

iii) investigate the role of herpes-like virus in the summer mortalities of *C. gigas* spat reported in Portugal, as well as elsewhere.

iv) initiate experimental work to determine if the lack of infections detected in field observations from cold-water climates reflects parasite acquisition with subsequent loss over prolonged low water temperatures, or suppression of infectivity of the parasite.

5.4 Crustaceans

Miscellaneous

In 1998, American lobsters (*Homarus americanus*) were reported as dying in lobster traps and during transportation and impoundment. Haemolymph samples showed Gram negative motile rods (mostly identified as *Vibrio* spp.) and further testing for other possible causes is under way. No results are yet available from samples sent for viral examination.

5.4.1 Conclusion

1) Reports of diseases of wild and cultured crustaceans continue to be sporadic and show no evident trends. The emergence of a health problem in American lobsters, which does not appear linked to known handling-related infections, underlines a lack of baseline information for commercially important crustaceans.

6 ASSESS THE PROGRESS IN DATA SUBMISSION TO THE ICES FISH DISEASE DATA BANK

The list of submissions to the ICES Fish Disease Data Bank was presented. According to the list, the Netherlands was the only country having submitted data from 1998. However, Germany had also submitted data from 1998 but have not yet been listed.

Submitted files designated dis21 were old data files submitted before the Fish Disease Data Entry (FDE) program was made. As these files have been duplicated in the FDE or dis22 format, the ICES Secretariat asked for permission to delete the old dis21 files. The WGPDMO agreed that this could be done.

The ICES Secretariat will move to a new ICES Environmental Reporting Format version 2.3. This will be composed of a new ACCESS-based data entry program to include fish diseases since the old FDE has too many problems. In addition to this change, the WGPDMO had the opportunity to come up with proposals for changes or improvements to be included in the new program. The WGPDMO did not wish any changes at present but will wait for the BEQUALM to come up with recommendations for reporting liver histopathology data.

6.1 Conclusions

1) The ICES Secretariat is given permission to delete all old dis21 data.
2) The WGPDMO did not want any changes at present in the Fish Disease Data Entry Program.

7 DATA AVAILABLE IN ICES DATA BANKS TO BE USED FOR A HOLISTIC ANALYSIS IN RELATION TO DISEASE DATA

T. Lang and W. Wosniok presented a report providing an overview of data available in the ICES Environmental Data Centre, the ICES Oceanography Data Centre and the ICES Fishery Data Banks which may be used for a holistic analysis of the ICES fish disease data (Annex 5). The report also contains the results of a case study analysing a subset of data extracted from the ICES data banks for the presence of significant relationships between the prevalences of diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations) of North Sea dab (*Limanda limanda*) and a variety of potential explanatory factors. These include oceanographic parameters (water temperature, salinity, oxygen content, nutrients), organic and inorganic contaminants (in water, sediments, biota) and fisheries data (dab catch per unit of effort, CPUE).

An overview was provided for three North Sea areas (German Bight, Dogger Bank, Firth of Forth) which are characterised by the availability of long-term disease data sets, some dating back to 1981. In contrast to the disease data, there is a striking lack of other long-term data, in particular with regard to contaminants in sediments and biota. However, other types of data, e.g., oceanographic and CPUE data, are available for almost the whole period, facilitating a statistical analysis.

For the case study, only the German Bight area was selected because of its comparatively good data coverage. However, since the intention was to relate the environmental and fisheries data to the disease data, the environmental and fisheries data had to be interpolated to obtain values for those time points for which prevalence data were available. As a first approach to analyse the data, univariate logistic models were fitted. The results revealed a significant relationship between the disease prevalence and the parameters considered in nearly half of the cases tested, possibly an indication of the multifactorial aetiology of the diseases considered, but also a consequence of correlations among the parameters. In a second approach, multivariate models were fitted for three scenarios (long-term, medium-term and short-term models), according to the time ranges of available data. Again, a number of parameters tested were significantly related to the disease prevalence. However, due to different time ranges analysed and the above-mentioned correlations, they were not in all cases identical to the parameters identified in the univariate approach.

Literature cited

ICES. 1999. Statistical analysis of fish disease prevalence data extracted from the ICES Environmental Data Centre. *In* Report of the ICES Advisory Committee on the Marine Environment, 1998. ICES Cooperative Research Report, No. 233.

7.1 Conclusions

1) The WGPDMO appreciated the outcome of the report as a valuable contribution to WGPDMO's efforts over the past years regarding the establishment of standardised procedures for disease data collection, submission, archiving and the development of appropriate statistical methods for a comprehensive data analysis. Whilst the report produced in preparation of the 1998 WGPDMO meeting (ICES, 1999) focused on the identification of spatial and temporal trends in the ICES fish disease data, the present study addressed for the first time the role of potentially explanatory environmental factors.
2) Since the results of the case study revealed some significant relationships between fish diseases and environmental factors, it was considered promising to continue the study and to extend it to larger geographical areas and time windows. However, this would require an extended database and the development of additional statistical methodologies adapted to the specific data requirements.
3) In order to overcome the apparent lack of data identified in the overview, the WGPDMO suggested that ICES Member Countries should submit relevant data known to exist in national data banks to the ICES Environmental Data Centre. It might be helpful for this purpose if WGPDMO members contact their national data coordinators in order to inform them of the objectives of the analysis and the need for additional data.
4) Additional statistical methodologies should focus on improved interpolation techniques, the use of disease data for all length classes and both sexes, the consideration of time lags and kinetics of biological responses, and the

detection of interaction terms. Furthermore, the precision of results should be assessed by appropriate techniques, e.g., a Markov Chain Monte Carlo simulation.

5) It was emphasised that other ICES Working Groups (e.g., WGSAEM, WGBEC, WGMS, MCWG) addressing corresponding topics should be made aware of the present report and should be encouraged to contribute to further activities in this field.

7.2 Recommendations

The WGPDMO recommends to:

i) encourage ICES Member Countries to submit historic and current data to the ICES Environmental Data Centre, ICES Oceanographic Data Centre and the ICES Fisheries Data Bank by using established ICES protocols, in order to facilitate a more comprehensive holistic analysis of the interactions between natural and anthropogenic environmental factors and the status and health status of marine organisms.

ii) review progress in data submissions and continue the study intersessionally in order to extend the analysis to enlarged areas and time windows and to develop and optimise suitable models and statistical method for a holistic analysis of the ICES fish disease data.

iii) make relevant ICES Working Groups (WGSAEM, WGBEC, WGMS, MCWG) aware of the data overview and the fish disease case study and encourage them to contribute to further activities related to the holistic analysis of fish disease data.

iv) ICES ACME to review the overview and the results of the case study and to consider recommendations for further actions.

8 COMPILE AND REVIEW AVAILABLE INFORMATION ON SUITABLE SHELLFISH SPECIES AND DISEASES FOR WHICH IT MAY BE APPROPRIATE TO SUBMIT DATA TO ICES, AND ON AVAILABLE DATA IN ICES MEMBER COUNTRIES

S. McGladdery provided a report on a survey conducted intersessionally in order to compile available information.

Potentially useful data are being collected in Norway, Germany, France, England and Wales, as well as in the USA and Canada. However, the diverse rationales for this data collection, along with its rare long-term nature, suggest that direct incorporation into the ICES Environmental Data Centre is not advisable at present.

In the discussion, the value of certain shellfish diseases, as a source of additional data for the assessment of environmental trends, was emphasised. In an ecosystem-oriented approach, shellfish data could fill the gap between information on physical sediment characteristics and on the health status of organisms at a higher trophic level, thus complementing findings from present monitoring programmes.

Examples of shellfish species and diseases/parasites were discussed and blue mussels (*Mytilus edulis*) were considered to be a possible suitable species, since they are widely distributed in the ICES area and are used already for contaminant monitoring programmes. In addition, there is information available on mussel diseases and parasites. It already was felt that crustaceans such as brown shrimp (*Crangon crangon*) have potential as indicator organisms of environmental changes, based on current collections of shell disease data, as well as gastropods which are being monitored for endocrine-disrupting chemical effects. It was stressed, however, that such data would only be useful if collected consistently, with a focused purpose and with stringent quality control.

8.1 Conclusions

1) There is potential for incorporation of shellfish disease data into the ICES Environmental Data Centre. However, the WGPDMO felt that Member Country collections are too diverse at present to permit immediate inclusion.

9 OVERVIEW OF NEW INFORMATION ON *ICHTHYOPHONUS*

9.1 Current Information

Information on data obtained during 1998 was requested intersessionally from WGPDMO participants.

In **Canadian waters,** herring hearts are now screened for *Ichthyophonus* but no indication of infection was obtained from the Gulf of St Lawrence (Baie des Chaleurs and Northumberland Strait). An infection of *Ichthyophonus* was recorded from the liver of yellowtail flounder *Limanda ferruginea* caught on Sable Island Bank, indicating the continued presence of the parasite in this area.

In **Icelandic waters,** 700 herring were examined for *Ichthyophonus* in 1998 (500 from commercial catches, 200 from research vessels) and no infection was found. However, in **Atlanto-Scandian** herring caught by an Icelandic research vessel, 169 of 1200 fish (14.1 % prevalence) had *Ichthyophonus* with the range in individual samples of 100 from 1 % to 38 %. This represents a considerably higher prevalence than that found in a comparable study in 1996 and 1997, when the average prevalence was 2 %.

In herring from the **Norwegian Sea**, Russian data indicated a prevalence of 25 % and 51.5 % in February/March and in June using Russian detection methods and 1.1 % and 6.1 % using ICES-recommended methods. In the **Barents Sea,** respective figures were 30.7 % and 3.0 %. Norwegian information indicated an unchanged prevalence of *Ichthyophonus* in Norwegian spring-spawning herring stocks.

From the **northern North Sea** east of Shetland, Scottish data showed *Ichthyophonus* infection in 2 of 2688 herring (0.1 % prevalence) from commercial catches and infection in 12 of 9899 herring (0.1 % prevalence) from research vessel catches. Information was obtained from the fish processing industry in Denmark that landings have to be occasionally rejected due to high levels of *Ichthyophonus*. It was indicated that these fish were mainly from North Sea herring stocks with two recent landings showing an infection level of 80–90 %.

Russian data from the southeastern **Baltic Sea** indicated a prevalence of *Ichthyophonus* of approximately 0.1 % in herring and sprat. Results from Danish research did not show *Ichthyophonus* in 1641 herring examined from the western Baltic but infection in 4 of 983 herring (0.5 % prevalence) examined from the **Kattegat**. Data from stock assessment samples from Danish waters indicate an infection level of less than 0.5 % in commercial catches.

No new information on the prevalence of *Ichthyophonus* off the Pacific coast of North America was obtained.

9.2 Conclusions

1) *Ichthyophonus* infection persists at low prevalence levels in herring stocks in the Kattegat, northern North Sea, Norwegian Sea and the Barents Sea. However, there are reports of increasing levels of infection in Atlanto-Scandian herring stocks and of high levels in some catches of North Sea herring stocks. Although, there are no epizootics in these areas, the situation should continue to be monitored as there is evidence of local high prevalence levels in the North Sea.
2) There was no evidence of significant *Ichthyophonus* infections in the Baltic Sea, southern North Sea, Icelandic waters or in the Northwest Atlantic.

9.3 Recommendation

The WGPDMO recommends that:

i) surveys for *Ichthyophonus* should be continued and incorporated into national routine herring stock assessment surveys. To assist in the standard diagnosis of infected fish, photographs of lightly and heavily infected hearts will be received before 1 May by the Chair and distributed to all members of the WGPDMO with a request to be circulated to the stock assessment staff in their laboratory.

10 COMPILE AVAILABLE EVIDENCE OF THE CAUSES OF THE M74 SYNDROME

An overview was presented to the WGPDMO by G. Bylund on recent observations on the M74-syndrome and on ongoing research (Annex 6).

Swedish and Finnish data on the prevalences of M74 continue to differ due to different methods for recording the disease prevalences. However, Swedish as well as Finnish data indicate that the decline in the M74 prevalences, as reported last year, is continuing. In the Finnish rivers the mean prevalence of affected salmon females was 34.1 %, the mean fry mortality in offspring from these females was 26.9 %. In the Swedish rivers the M74 prevalence was 7 %. In Estonia M74 was not recorded in 1998. There are strong indications, however, that the disease prevalence might increase again in offspring from the 1998 spawners since Swedish as well as Finnish observations indicate increased occurrence of broodfish with M74 symptoms (wiggling behaviour, pale eggs and flesh, etc.) among the 1998 spawners.

Ongoing Swedish research projects focus on different functional disturbances in salmon due to low thiamine status in combination with oxidative stress. Two new research projects were recently initiated, one in Finland and one in Sweden, in order to evaluate the possible role of algal blooms in the aetiology of the M74 syndrome.

In order to analyse and standardize the methods for monitoring disease levels, intercalibration studies were performed between Finnish and Swedish laboratories. One study focused on incubation of salmon eggs and evaluation of fry mortalities and the other on HPLC-methods for analysis of thiamine levels in fish tissues. The outcome of these studies will be published soon (see Annex 6).

The results from large-scale research projects on M74 performed during 1994–1998 will be published in a special issue of the journal Ambio during early 1999.

10.1 Conclusion

1) No significant breakthrough in the M74 research occurred during 1998. Research projects with new approaches, however, were initiated.

10.2 Recommendation

The WGPDMO recommends that:

i) the WG should continue to maintain an overview of the geographical distribution of M74 and progress made with regard to the identification of possible causes.

11 REVIEW NEW INFORMATION ON THE SPREAD, DIAGNOSIS AND CONTROL OF NODAVIRUS FOR FURTHER ADVICE ON POSSIBLE CONTROL MEASURES

F. Baudin Laurencin (France) presented a review paper (Annex 7) and B. Hjeltnes added pertinent information from Norway.

Only scattered data were obtained, mainly from France and Spain, concerning the spread of the disease. There is indication of an apparently higher incidence of nodavirus infections in juvenile sea bass (*Dicentrachus labrax*) in the Mediterranean. In Norway, nodavirus is still a major cause of mortalities in halibut (*Hippoglossus hippoglossus*) hatcheries.

Recently published data have added new knowledge of the disease and the virus:

- There is genomic variation among the European nodavirus isolates. Distinct genomes were described from two different isolates of sea bass. The Norwegian halibut isolate differs from the Mediterranean isolates, and tends to cluster closely with one of the Japanese isolates from barfin flounder (*Verasper moseri*).
- Diagnostic methods have been improved. An ELISA method is being used for the detection of seropositive and seronegative sea bass spawners. A nested-RT-PCR technique has been developed and demonstrated to be very sensitive, allowing monitoring for the presence of the viral genome in asymptomatic carriers. A modified RT-PCR method based on specific primers has also been developed for the detection of the virus in Norwegian halibut.
- Transmission trials in sevenband grouper (*Epinephelus septemfasciatus)* and in juvenile sea bass have demonstrated that higher mortality and earlier appearance of the disease signs occur at higher temperatures. Juvenile turbot (*Scophthalmus maximus*) were also experimentally infected, resulting in mortalities and characteristic lesions. On the other hand, experimental trials failed to infect juvenile seabream (*Sparus aurata*) and invertebrates (*Artemia salina* and *Brachionus plicatilis*) commonly used to feed fish larvae in hatcheries. To date, screening of halibut eggs in Norway has not demonstrated vertical transmission of nodavirus in this species.
- Immunisation of sea bass female spawners has been achieved. The antibody levels increased 4 weeks after injection of inactivated virus, and were still detectable after 41 weeks.

It was reported that an EC FAIR programme of research on nodavirus began in France and Scotland in November 1998. The main tasks of the study are: virus characterisation, assessment of diagnostic methods, epidemic parameters and immunological studies. The EC has requested that progress made by the programme be reported to the WGPDMO.

11.1 Conclusions

1) Progress has been made concerning the genomic viral diversity, the validation of diagnostic techniques, the environmental conditions for the experimental transmission of the disease and the immune response. The task areas to be studied in the EC FAIR programme should improve the knowledge of nodavirus infections.

11.2 Recommendation

i) In addition to the targets of the FAIR programme of research on nodavirus, which is to gain basic knowledge of the disease, it is also recommended to increase efforts to obtain a better knowledge of the distribution of the virus in farmed and wild fish species and carry out research in vaccine development and thus achieve a better control of the disease in farms and hatcheries. Greater collaboration between researchers is also recommended.

12 COMPILE AND REVIEW AVAILABLE INFORMATION ON THE IMPACT OF MARINE BIOTOXINS PRODUCED BY DINOFLAGELLATES AND ALGAE ON FISH POPULATIONS TO PROVIDE A BASIS FOR EVALUATION OF THE SIGNIFICANCE AND DYNAMICS AND FUTURE RESEARCH

Overviews of available information on microalgal blooms and effects on fish of toxins produced by microalgae were presented by G. Bylund and S. MacLean (Annexes 8 and 9).

Blooms of microalgae are more or less normal spring and autums events that have occurred for millenia. What stimulates nuisance algae to bloom and become toxic depends on a complex set of climatological, physical and chemical factors. Nutrient loading, particularly of phosphorus and nitrogen, and the ratio of these with other elements, such as silicate appears to be important. The global spread of toxic algal blooms has been recognized in the past 2 decades, however, there is little information on mechanisms contributing to this global trend.

Numerous biotoxins produced by the microalgae have been identified and chemically characterized, and are dermatotoxic, haemolytic, hepatotoxic, neurotoxic or mucin inducers. Some, such as microcystin and okadaic acid, are well-known tumour promotors.

There are numerous reports of massive fish kills associated with microalgal blooms. In some cases the role of the algal toxin in these fish kills is apparent, for example, in connection with kills at fish farms. In most cases, however, it has been difficult to positively establish the role of algal toxins in the mortalities due to the limited availability of toxin detection methods. Losses of larval fish are even less apparent in wild fish populations than those of juvenile and adult fish, but may be quite significant in evaluating population effects of toxic algal blooms. Although deleterious effects have been observed in several species of fish when exposed to sublethal concentrations of microalgl toxins under experimental conditions, there is little information on sublethal and subclinical effects of algal toxins in wild fish.

12.1 Conclusions

The WGPDMO recognized that much more basic research is needed before we can fully understand the effects of the microalgal blooms and algal toxins on wild fish populations. Attention has to be focused especially on the following areas of research:

1) the dynamics of the microalgal blooms and the factors which stimulate nontoxic algae to become toxic;
2) the spectrum of bioactive compounds produced by microalgae. Recent research has shown that microalgae produce, in addition to the "classical" toxins, unidentified compounds pharmacologically active on fish and fish eggs;
3) the role of microalgal toxins on fish eggs and fish fry. Laboratory tests as well as field observations have shown that biotoxins produced by microalgae can exert pronounced deleterious effects on fish eggs;
4) the subacute and subclinical effects of microalgal toxins on fish. We lack basic knowledge on the potential of effects from sublethal exposure, for example, of microalgae toxins on the immune parameters of fish;
5) improved diagnostics. Recently developed methods (DNA-probes, immunohistochemical assays) provide possibilities to identify algal toxins in fish tissues and to establish the role of these toxins in fish kills.

12.2 Recommendation

The WGPDMO recommends that it revisit this subject at a later date.

13 CLARIFY THE HOST SPECIFICITY AND PATHOGENICITY OF HERPES-LIKE VIRAL INFECTIONS IN MOLLUSC HATCHERIES, *MARTEILIA* SP. FROM *CRASSOSTREA GIGAS* AND THE HAPLOSPORIDIUM-LIKE PARASITE IN *OSTREA EDULIS*

T. Renault presented a progress report as detailed in Annex 10.

Herpes-like viruses have been now described in 7 different bivalve species (*Crassostrea virginica, C. gigas, Ostrea edulis, O. angasi, Triostrea chilensis, Ruditapes philippinarum* and *R. decussatus*) in different parts of the world. Concomitant high mortalities with herpes-like virus detection were reported in France in 1994, 1997 and 1998 among several bivalve species. These observations indicate a possible intergeneric transmission of herpes-like virus infections. Bioassays and specific molecular probes developed by IFREMER (La Tremblade, France) have been used to investigate this hypothesis; however strain diversity and pathogenicity of herpes-like viruses need to be studied further.

Specific probes were designed and successfully used to detect *Marteilia refringens* using PCR and *in situ* hybridization techniques (IFREMER, La Tremblade, France). The sequence used for probes and PCR primers is generated from the most divergent region of the 18s RNA gene of *Marteilia refringens* in reference to eukaryotic organisms. Positive reactions were obtained for *Marteilia refringens* in *Ostrea edulis* and *Mytilus edulis,* and for *Marteilia maurini* in *Mytilus galloprovincialis.* The results do not yet answer the specificity question, however the molecular tools now available can be used in studying *Marteilia* spp. infecting different bivalve species, e.g. the clarification of *M. refringens* in *C. gigas* reported from Spain.

Haplosporidan parasites are known to cause infections in many marine bivalve species. Two species, *Haplosporidium nelsoni* and *H. costalis,* cause extensive mortalities of *Crassostrea virginica* in USA. Haplosporidian parasites have also been found in *Ostrea edulis, O. lurida, Mytilus edulis* and *C. gigas.* For these affected bivalve species, there is no evidence that the haplosporidian parasites are responsible for significant mortalities similar to those reported in *C. virginica*; however, heavy haplosporidan infections in connective tissues of moribund animals have been reported. Until more is known about the identity and biology of these other *Haplosporidium* spp., their presence in any bivalve should be regarded as potentially serious. In order to clarify the taxonomy of Haplosporida found in European bivalve stocks, specific molecular probes (*in situ* hybridisation and PCR) will be tested (IFREMER, La Tremblade, France) on paraffin embedded material showing evidence of infection by *Haplosporidium* sp. in *C. gigas, O. edulis, Ensis ensis* and *Cardium edule* collected during epidemiological surveys.

13.1 Conclusions

1) Many of the shellfish pathogen identifications and the host specificity have not yet been resolved and cannot be resolved with routine technology. Molecular probe technology is particularly applicable for bivalve pathogens due to the lack of cell lines and alternative techniques available to detect sub-clinical carriers.

13.2 Recommendations

WGPDMO recommends that ICES Member Countries evaluate and define needs for new diagnostic techniques for the most important shellfish pathogens.

14 ASSESS THE DISEASE RISKS FOR WILD AND CULTURED CRUSTACEANS FROM KNOWN PATHOGENS OF PENAEIDS

S. MacLean presented information on the subject based on recent experiences in the USA shrimp industry (Annex 11).

Although various pathogens affect cultured and wild penaeids, the greatest threat to the continued growth or continuation of this industry has been introduction of exotic viruses into USA culture facilities. Four viruses presently most at issue are the Taura Syndrome Virus (TSV), White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV).

The impact of pathogens in culture facilities is well known, however, there is little information on the potential impact of pathogens, exotic or enzootic, on wild shrimp populations. Representatives of nearly all the major taxa containing infectious agents infect penaeid shrimps. Wild shrimp species are host to a wide array of infectious agents although reports of major losses in wild shrimp stocks due to pathogens are rare. One case is notable. Blue shrimp, *Penaeus stylirostris*, from the Gulf of California, México, experienced a major mortality due to infection with IHHN virus, perhaps as a result of escape of infected cultured shrimp.

The viruses of concern show broad host ranges of wild and cultured penaeids, with virulence varying with host species and host life history stage. WSSV especially is of concern because it has a wide host range in wild stocks of both penaeid and non-penaeid crustaceans in Asia.

Wild stocks of shrimp may be exposed to pathogens occurring in aquaculture through various pathways, including pond effluents, escapes of infected shrimp, losses during transport to shrimp processing facilities, disposal of pond sediment or solid waste, or through movements of infected bait shrimp. Shrimp processing plants pose a potentially serious problem as shrimp infected with virus have been identified in retail stores in the USA. Over 50 % of the shrimp processed in the USA is imported from Asia where viral diseases are a major problem, suggesting that processing may be a significant means of introduction of exotic viruses to coastal waters. WSSV infects various wild crustaceans in Asia, and WSSV-like virus has been found in several crustaceans from the east coast of the USA, however, there is no information on their impact on these wild species. Very little information is available on other viruses endemic in wild shrimp and other crustaceans in USA coastal waters.

The principal risk of disease to cultured shrimp has been the introduction of exotic viruses via infected broodstock or seed. Other sources include processing into shrimp feed the solid wastes from plants that process infected shrimp, transfer of viable pathogens by birds from waste in shrimp processing dumps, and transmission of virus by water insects.

14.1 Conclusions

1) More baseline information is needed on viruses existing in wild stocks of penaeid and non-penaeid species;
2) further evaluation is needed on the transfer of infective material from processing plant activities;
3) further work is needed on the role of birds and insects as vectors of viruses;
4) development of more rapid and accurate viral detection methods is needed;
5) the significance of disease outbreaks in aquaculture and the potential for predicting the potential of those diseases in wild populations needs to be examined;
6) shrimp population models are inadequate to explain the observed variability of wild shrimp populations and should be re-worked to include a disease factor.

15 REVIEW AVAILABLE INFORMATION ON THE USE OF PARASITES OF MARINE FISH SPECIES AS INDICATOR ORGANISMS FOR ENVIRONMENTAL CHANGES

H. Palm gave an overview on the use of marine parasites as indicator organisms for environmental changes (Annex 12).

It was recognised that parasites are currently in use as biological indicators of host biology, migration and stock separation as well as for other ecological parameters. Changes in parasite indices are often associated with biotic or abiotic environmental changes. While long-living species (digenean trematodes, cestodes, nematodes) mainly give information on the host migration habits, short-lived species with direct life cycles and high reproduction rates (protozoan ectoparasites, monogenean trematodes) are used to detect changing environmental conditions. It was emphasised that a change in parasite indices can be multifactorial, which causes problems in the interpretation of actual environmental effects.

Protozoan and metazoan parasites have been used as biological indicators for water quality. Peritrichous ciliates of the genus *Trichodina* indicate the general water quality, and respond to the presence of petroleum hydrocarbons, oil-contaminated sediments, pulp and paper mill effluents, as well as to a rise in temperature and bacterial concentrations. The presence and prevalence/intensity data on metazoans have been shown to be useful for detection of the presence and impact of a wide variety of contaminants including crude oil, petroleum aromatic hydrocarbons, industrial and sewage waste, lead, cadmium, zinc and benzene as well as general pollution and eutrophication. It was stressed that it is necessary to have a clear objective in using parasites as indicators for environmental changes. Some parasites may be highly pollutant specific and others may reflect exposure to a range of pollutants/environmental changes. These two objectives are different and will require a different type of parasite or set of parasite species. It was noted that some selection criteria for the use of parasites as biological indicators have already been established by MacKenzie (1983), and may be applicable for environmental correlation.

It was emphasised that changes and trends in the occurrence of marine parasites can be important as "alarm bell" indicators of possible environmental problems. Such information may not determine cause/effect, but serve to focus the resources of other specialist groups (e.g. toxicologists). However, such changes are only likely to be relevant if there are no concurrent changes in reference areas with the same parasite/host system.

The WGPDMO appreciated the report submitted and considered that the use of parasites as bioindicators of pollution/environmental change has a potential for application in monitoring programmes. It was also recognised that the use of parasites can be targeted according to the needs and requirements of the monitoring, including an adequate sampling strategy. In particular, this approach is able to detect local or regional fluctuations as well as short-term changes and long-term trends, by careful selection of hosts and their parasite species. Effects of seasonal and other variables can be reduced to acceptable levels through proper focus of the study and sampling strategy. It was stressed that the acquisition of long-term data sets is important for detecting trends in the occurrence of suitable parasite species. It was also recognised that fish species already being monitored could be those examined initially for the presence of externally visible parasites, using the current ICES guidelines (dab, flounder, cod).

Literature cited

MacKenzie, K. 1983. Parasites as biological tags in fish population studies. Adv. appl. Biol., 7: 251–331.

15.1 Conclusions

1) WGPDMO agreed that parasites have the potential to be used as indicators of environmental changes. However, for a more conclusive assessment, more research is required in order to obtain a better understanding of the complex interactions between parasites, hosts and the environment.
2) It was emphasised that not all parasites are equally useful, in particular with regard to regular monitoring programmes.
3) Depending on the environmental parameters to be assessed, the most useful host-parasite systems need to be selected.
4) It was suggested that the parasite sampling could be incorporated into existing disease monitoring programmes.

15.2 Recommendation

The WGPDMO recommends that it develop a proposal on possibilities to incorporate parasitological studies into existing disease monitoring programmes. For this purpose, it was considered useful to compile and evaluate long-term data sets already existing in ICES Member Countries.

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

The late withdrawal of WG members with the available expertise within this area precluded meaningful discussion of this agenda item. As a consequence it was agreed that this topic should be carried over to next year's meeting.

16.1 Recommendation

The WGPDMO recommends to:

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

17 REVIEW PROGRESS IN THE DEVELOPMENT AND IMPLEMENTATION OF A QUALITY ASSURANCE PROGRAMME FOR FISH LIVER HISTOPATHOLOGICAL DIAGNOSIS

An overview of the new European Commission-funded programme 'Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)' was provided by S. Feist (Annex 13). The overall aim of the programme is to establish a European quality assurance infrastructure for biological effects techniques used in biological effects monitoring programmes. Within this programme there are nine 'work packages', of which external fish disease and liver pathology constitute one.

Proposals for activities during the first year, including a workshop, were presented and members of the WGPDMO were asked to inform S. Feist of laboratories which should be included as participants in the programme and workshop.

17.1 Recommendation

The WGPDMO recommends:

i) that it reviews progress made within the BEQUALM work package 'Fish disease and liver pathology';

ii) that WGPDMO members contact relevant national institutes involved in biological effects monitoring and request that those interested in participating in the liver pathology component of BEQUALM contact S. Feist.

18 UPDATE OF INFORMATION PREPARED FOR THE HELCOM THIRD PERIODIC ASSESSMENT ON THE STATE OF THE MARINE ENVIRONMENT OF THE BALTIC SEA

The WGPDMO was informed of the plans to produce the HELCOM Fourth Periodic Assessment, scheduled for publication in the year 2001, which will include a section on diseases and parasites of Baltic fish. It was suggested that this section should be based on an updated version of an article prepared by T. Lang that was published in the HELCOM Third Periodic Assessment (HELCOM 1996).

T. Lang volunteered to produce the update intersessionally and suggested that it should be reviewed and adopted at the 2000 WGPDMO meeting. In order to incorporate new information, WGPDMO members from Baltic Sea countries will be contacted in the near future and requested to provide appropriate information.

With regard to new information available, the WGPDMO noted with appreciation that the results of the 1994 BMB/ICES Sea-going Workshop 'Fish diseases and parasites in the Baltic Sea' will be published in the *ICES Journal of Marine Science* (either in the April or June issue 1999) as a series of 8 scientific papers/short communications preceded by a section providing an introduction and major conclusions.

Literature cited

HELCOM. 1996. Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1989–1993; Background document. Baltic Sea Environ. Proc. No. 64B.

18.1 Recommendation

The WGPDMO recommends:

i) that an updated version of an article on diseases and parasites of Baltic fish, to be included in the HELCOM Fourth Periodic Assessment, be produced intersessionally for review at the 2000 meeting of WGPDMO and subsequent consideration by the ICES ACME.

19 ICES DISEASE PUBLICATIONS, DIAGNOSTIC FICHES UPDATE

A report on the current status of the *ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish* and suggestions for updating the series were presented by the editor S. McGladdery.

The ICES Publications Committee approved revision of the Fiches series at the ICES Statutory Meeting in Lisbon, September 1998, as recommended by the WGPDMO 1997 and S. McGladdery circulated a copy of the English version of the new Guidelines to Authors to the WGPDMO members at the 1998 meeting in Lisbon, in March 1999, for final approval before translation into French. Similar disease information, which is now available (especially on the Internet) was discussed in relation to the objective and application of the Fiches. It was felt that the Fiches, in their present format, still represent the most eclectic identification leaflet series, applying to wild as well as cultured fish and shellfish, especially where directed specifically to the objective of pathogen/parasite identification. It was felt that this information might be more effectively distributed, using the operational ICES Website. It was also noted that the present site for the Fiches solely contains a list of titles. Additional information on Fiche content, as well as electronically accessible copies of the French and English Guides to Authors, are required.

Titles which require revision or which may also be dropped from the this revised series of Fiches were circulated for WGPDMO their input. New titles and proposed authors are listed in Annex 14.

19.1 Recommendations

The WGPDMO recommends that:

i) the editor contact the ICES Publication Committee to request the installation of new and revised Fiches onto the ICES Publication website to enhance their dissemination and reduce publication challenges;

ii) the fiche format should be as agreed upon in 1998 and include peer review to ensure citable authorship;

iii) WGPDMO review the current list of titles and inform the editor of those which should be updated as well as appropriate authors before 1 May 1999.

20 ANY OTHER BUSINESS

20.1 Contribution to the ICES Environmental Report

Reference was made to the web-based ICES Environmental Report and possibilities for WGPDMO to contribute were discussed. An idea that was considered was the production of maps showing a) the distribution of fish and shellfish diseases of concern for mariculture and b) temporal trends of wild fish diseases of concern for marine environmental monitoring programmes. It was concluded that this should be considered intersessionally and proposals for this material be presented to the WGPDMO at the next meeting.

20.2 Collaboration with Other Organisations

In consideration of the ICES Strategic Plan, the remit and future activities of the WGPDMO were discussed. In particular, its relationship with other international fora such as OIE and EU Groups concerned with diseases of marine organisms was addressed. It was recognised that there is a certain lack of information exchange between these groups. Therefore, the WGPDMO discussed ways in which communication with these groups could be improved. There was consensus that the WGPDMO could provide useful information on new disease trends and emerging problems that may not be available elsewhere. In addition, it was felt that the WGPDMO could play an important role in considering in more depth topics of particular interest, both for specific diseases and more general issues.

It was recognised that the delay in official approval by ICES of the WGPDMO report is a constraint for the timely dissemination of relevant information, especially concerning disease outbreaks. WGPDMO emphasised that ICES should explore ways in which this problem may be overcome.

Following discussion, it was suggested that the WGPDMO could be officially represented at meetings of other relevant groups and *vice versa* in order to enhance collaborative links. ICES and the relevant organisations should consider possibilities on how this could be accomplished.

20.3 Recommendations

The WGPDMO recommends:

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

ii) that ICES contact other organisations concerned with studies and regulations with regard to diseases of marine organisms in order to consider ways to improve collaboration.

21 ANALYSIS OF PROGRESS WITH TASKS

An analysis of progress of tasks in the terms of reference was conducted and presented in Annex 15. All items had been dealt with in a comprehensive manner. Several intersessional tasks were identified during the meeting.

22 FUTURE ACTIVITY OF WGPDMO

There being several issues of importance in the field of pathology and diseases of marine organisms requiring further consideration, it was agreed that a further meeting of WGPDMO was required in 2000 to consider the results of intersessional work and to discuss outstanding items. It was agreed that the invitation to host the meeting from Dr W. Wosniok, University of Bremen, Germany would be accepted. The proposed dates are 29 February – 4 March 2000, the full recommendation is contained in Annex 16.

23 APPROVAL OF RECOMMENDATIONS

The recommendations contained in this report to ICES Council were discussed by WGPDMO and approved. The recommendations and justifications for recommendations to Council are added in Annex 16.

24 APPROVAL OF DRAFT WGPDMO REPORT

A copy of the draft report of the 1999 meeting was submitted to all WG members present before the end of the meeting and approved. The conclusions on the Terms of Reference and associated Annexes where advice was specifically sought by other ICES bodies would be extracted and sent separately to ICES.

25 CLOSING OF THE MEETING

On behalf of the participants of the 1999 meeting of the WGPDMO, the Chair expressed appreciation to Dr Francisco Ruano for his hospitality and the excellent facilities provided for the WGPDMO in the IPIMAR, for his considerable organisation and support before and during the meeting and for much assistance from other members of staff of the IPIMAR, which in no small way contributed to the success of the meeting.

The meeting was closed at 14.00 hrs on 6 March 1999.

ANNEX 1

LIST OF PARTICIPANTS

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ANNEX 2

TERMS OF REFERENCE

ICES C.Res. 1998/2:44

The Working Group on Pathology and Diseases of Marine Organisms [WGPDMO] (Chair Dr. S. Mellergaard) will meet in Lisbon, Portugal from 2–6 March 1999 to:

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

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

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

d) compile and review available information on suitable shellfish species and diseases for which it may be appropriate to submit data to ICES, and on available data in ICES Member Countries;

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

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

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

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

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

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

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

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

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

n) update information prepared for the HELCOM Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea concerning diseases and parasites of "Baltic fish stocks, diseases and ecosystem effects" for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994-1998 [HELCOM 1999/3].

ANNEX 3

AGENDA

1) Opening of the meeting 10.00.
2) ICES ASC 1998; items of relevance to WGPDMO.
3) Terms of Reference, adoptation of the agenda, selection of Rapporteurs.
4) Other relevant reports for information.
5) Analyse national reports on new disease trends in wild and cultured fish, crustaceans and molluscs (TOR a).
6) Assess the progress in data submissions to the ICES Fish Disease Databank (TOR b).
7) Provide an overview report of data available in ICES Data Banks, which may be used for a holistic analysis in relation to disease data (TOR c).
8) Compile and review available information on suitable shellfish species and diseases for which it may be appropriate to submit data to ICES, and on available data in ICES Member Countries (TOR d).
9) Overview of the *Ichthyophonus* (TOR e).
10) Compile available evidence on the causes of the M-74 syndrome in Baltic salmon (TOR f).
11) Review new information on the spread, diagnosis and control of nodavirus to further advise on possible control measures (TOR g).
12) Compile and review available information on the impact of marine biotoxins produced by dinoflagellates and algae on fish populations to provide a basis for evaluation of the significance and dynamics and future research (TOR h).
13) Clarify the host specificity and pathogenicity of herpes-like viral infections in mollusc hatcheries, *Marteilia* sp. from *Crassostrea gigas* and the *Haplosporidium*-like parasite in *Ostrea edulis* (TOR i).
14) Assess the disease risks for wild and cultured crustaceans from known pathogens of penaeids (TOR j).
15) Review available information on the use of parasites of marine fish species as indicator organisms for environmental changes (TOR k).
16) Provide a report with advice on new techniques in pathology and other methods for the detection of endocrine disrupting chemicals in marine and estuarine organisms and appropriate new target species representing the main ecological levels of the marine ecosystem (TOR l).
17) Review progress in the development and implementation of a quality assurance programme for fish liver histopathological diagnosis (TOR m).
18) Update information prepared for the HELCOM Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea concerning diseases and parasites of "Baltic fish stocks, diseases and ecosystem effects" for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994–1998 [HELCOM 1999/3] (TOR n).
19) ICES Disease publications. Diagnostic Fiches update.
20) Any other business.
21) Analysis of progress with tasks.
22) Future activity of WGPDMO.
23) Approval of recommendations.
24) Approval of draft WGPDMO Report.
25) Closing of the meeting.

ANNEX 4

RAPPORTEURS

Session(s)	Rapporteurs
1–4. Introductory sessions	S. Mellergaard,
5. Wild fish Wild shellfish Farmed fish Farmed Shellfish	S. MacLean, A. Karasev, D.Declerck S. McGladdery, T. Renault J. McArdle, J. Barja, A. McVicar S. McGladdery, T. Renault, F. Ruano
6. Progress in submissions to ICES Fish Disease Databank	S. Mellergaard, T.Lang
7. Data available in ICES Data Banks to be used for a hollistic analysis	W. Wosniok, T. Lang, A. McVicar
8. Information on suitable shellfish species and diseases appropriate to submit data to ICES	S. McGladdery, T. Renault, S. Ford
9. *Ichthyophonus*	A. McVicar, B. Hjeltnes
10. M-74	G. Bylund, A. Hellström, V. Kadakas
11. Nodavirus	F. Baudin-Laurencin, B. Hjeltnes
12. Marine biotoxins produced by dinoflagellates and algae	G. Bylund, S. Ford, F. Baudin-Laurencin
13. Host specificity and pathogenicity of various mollusc pathogens	S. McGladdery, F. Ruano, T. Renault,
14. Disease risks for wild and cultured crustaceans from known penaeid pathogens	S. MacLean, J. McArdle
15. Parasites as indicator organisms for environmental changes	H. Palm, S. Feist
16. New techniques in pathology for the detection of endocrine disrupting chemicals	G. Bylund, S. Ford
17. Progress in the development and implementation of quality assurance programme for fish histopathology	S. Feist, S. MacLean
18. Update information prepared for HELCOM Fourth Periodic Assessment Of the State of the Baltic Marine Environment	T. Lang, J. McArdle
19. ICES Disease Publications	H. Palm, W.Wosniok
20–23. AOB, Progress, Recommendations	S. Feist
24–25. Approval of report, closing	S. Mellergaard

ANNEX 5

OVERVIEW REPORT OF DATA AVAILABLE IN ICES DATA BANKS WHICH MAY BE USED FOR A HOLISTIC ANALYSIS IN RELATION TO DISEASE DATA

T. Lang and W. Wosniok

1 Introduction

At its 1998 meeting, the ICES WGPDMO reviewed the results of the statistical analysis of the ICES fish disease data and a written report was presented providing information on location-specific temporal trends in the prevalence of lymphocystis, epidermal hyperplasia/papilloma and skin ulcers (dab, *Limanda limanda*) and lymphocystis and skin ulcers (flounder, *Platichthys flesus*), as well as a comparison of trends found at different geographical locations (ICES, 1999).

The results of the analysis revealed marked spatial differences with respect to both the absolute levels and the temporal changes of the disease prevalences. Furthermore, areas characterised by either decreasing, increasing or stable time trends over the past years were identified. In some areas, temporal changes in the prevalence of two or more diseases were similar, thus, indicating the presence of common underlying ecological factors affecting the disease prevalence. However, the results of the analysis did not provide any information on possible causes of the observed trends, since potential explanatory factors known or suspected to be involved in disease aetiology and pathogenesis were not included in the analysis at that time.

The WGPDMO emphasised that the integration of the ICES fish disease data and other types of data (e.g., contaminant, oceanographic and fisheries-related data) would constitute a subsequent next step in the attempt to analyse the ICES fish disease data in a more holistic way, with the aim to obtain better insight into cause-effect relationships between diseases and environmental factors. Since ICES has established different data banks with relevant data, it was recommended that available data should be assessed with respect to their usefulness for such a holistic approach.

When reviewing the 1998 WGPDMO Report, the ICES ACME endorsed the view of WGPDMO that a more holistic statistical analysis is desirable and that the various ICES data banks could serve as a suitable data pool from which the information required could be extracted. ACME emphasised that the first step taken, before any further actions are decided upon, should be to obtain a detailed overview of the data availability and compatibility. This should be done in close collaboration between selected WGPDMO members and the ICES Secretariat. A second step, a pilot study, could be started subsequently, using a selected subset of suitable data extracted from the ICES databases, in order to assess the practicality and perspectives of a future holistic data analysis.

In the following, the results of intersessional activities carried out according to the above recommendations prior to the 1999 WGPDMO meeting are presented. The first part of the report describes the outcome of the assessment of the ICES data banks with respect to the types and amount of data available, with particular emphasis on spatial and temporal data coverage and overlap. In the second part, preliminary results of a multivariate statistical analysis of a selected sub-set of data is presented. The third part gives conclusions and perspectives, e.g., by focusing on data limitations identified and on ways to accomplish a more comprehensive analysis.

2 Overview of Data Available in ICES Data Banks

In the following, information is presented on the ICES Data Banks from which data can be extracted for a holistic analysis and on strategies applied to obtain an overview of available data. Furthermore, some of the data are presented in order to demonstrate temporal trends.

2.1 ICES Data Banks

ICES data considered relevant for a holistic analysis are available from the following data banks:

1) ICES Environmental Data Centre
2) ICES Oceanographic Data Centre
3) ICES Fishery Data Banks

ICES provides a detailed overview (partly interactive) on the data included on its website http://www.ices.dk. Most of the following information on the data banks is extracted from this website.

A ICES Environmental Data Centre

contains data on

- contaminants in marine invertebrates, fish, birds, and mammals (ca. 275,000 records)
- contaminants in sea water (ca. 280,000 records)
- contaminants in sediments (ca. 80,000 records)
- biological effects of contaminants: EROD, oyster embryo bioassay (ca. 4,000 records)
- fish disease prevalences (ca. 110,000 records)
- additional data: nutrients, oxygen, temperature, salinity
- quality assurance information.

B ICES Oceanography Data Centre

maintains two data banks in the ICES Secretariat:

- the ROSCOP databank (information on cruise activities)
- the hydro-chemistry databank (temperature, salinity, nutrients, oxygen etc.)

In addition, there is access to a number of Project Data Sets (including oceanographic data from the International Young Fish/Bottom Trawl Survey).

C ICES Fishery Data Banks

Includes 5 fisheries-related data banks:

- STATLANT 27A (Official Statistics on nominal catches of fish and shellfish)
- ICES Fisheries Assessment Package (for use by approx. 20 WGs for ICES stock assessments. Includes catches in tonnes, fishing effort, catch in number at age and relevant biological data.)
- International Bottom Trawl Survey (IBTS)(results from an international survey conducted each year in the North Sea since 1970 which provides an annual index of abundance by ICES Statistical Rectangle. Additional data: temperature, salinity, nutrients)
- North Sea data bank (contains details of catches and fishing effort originally set up by the EC)
- North Sea Multispecies data bank (contains stomach content data for each of the main predatory fish species in the North Sea. For use in multispecies models).

2.2 Strategies applied to obtain an overview

Since the ICES data available and potentially relevant for a holistic analysis are overwhelming in terms of parameters measured and results of measurements, it was considered impossible to present a full overview on their spatial and temporal distribution patterns. It was, therefore, decided to extract some of the data by using *a priori* selection criteria which were mainly based on the availability of ICES disease prevalence data for common dab (*Limanda limanda*).

Sites: Three North Sea areas (German Bight, Dogger Bank, Firth of Forth) were selected for which a considerable amount of disease data is available and which differ both in the absolute prevalence levels and the temporal trends recorded over the past years. As shown in Figure A5.1, the areas were relatively large and consisted of 4–9 ICES Statistical Rectangles in order to get sufficient data for the subsequent statistical analysis (case study, requested by ICES ACME).

Time span: Since the fish disease data date back to 1981, this year was used as the starting point for the temporal overview.

Parameters: From the ICES Fishery Data Banks, data on catch per unit effort (CPUE) for dab (all specimens, independently of size) derived from the International Young Fish/Bottom Trawl Survey (IYFS/IBTS) were selected. Originally, it was also planned to incorporate fishing effort data (STECF data, EU Scientific Technical and Economical Committee for Fisheries). However, since these are not structured in an easily-accessible way (e.g., effort data are only available for a short period of time and separately for 60 different fishing fleets) they were excluded. From the ICES Oceanographic Data Centre, information on water temperature, salinity, dissolved oxygen, total phosphorus, phosphate, ammonium, nitrite, nitrate, silicate and chlorophyll were considered, partly derived from the International Young Fish/Bottom Trawl Survey. From the ICES Environmental Data Centre, data on contaminants (Pb, Hg, Cd, γ-HCH, HCB, CB118, CB153, *o,p*-DDT) in water, sediments, dab muscle and liver, and blue mussel (*Mytilus edulis*) were selected.

After selections were made, the ICES Secretariat was contacted and requested to provide the data in electronic form. According to the ICES data policy, no raw data were available since this would have required permission from the data originators. Instead, aggregated data, consisting of, e.g., calculated mean values, were received.

2.3 Brief overview

Figures A5.2–A5.4 give an overview of data available according to the above selection criteria. In addition to the parameters mentioned above, ICES disease prevalence data for female dab, size group 20–24 cm, are included. The figures clearly show that most data are available for the German Bight, followed by the Dogger Bank and, finally, the Firth of Forth. Apart from the disease data, there is relatively good temporal coverage of data on CPUE, water temperature, salinity and nutrients for all three areas over almost the whole period, but only few data are available on contaminants in water (exception: German Bight since 1985), sediments (exception: 1990–1992) and dab (exception: German Bight, Dogger Bank 1990–1996.

In order to provide an overview of temporal trends, Figures A5.5a–f show the data for selected parameters measured in the German Bight. Both the observed values and the values derived from interpolation in preparation for the subsequent analysis (see below) are shown, and, in addition, the confidence intervals. For contaminants in dab, only data for female dab, size group = 20 cm are included since they correspond to the standard disease prevalence data used in previous statistical analyses (females, 20–24 cm; see ICES, 1999). In the ICES Environmental Data Centre, also contaminant and disease data for other size groups and for males are available.

3 Case study (Statistical analysis)

As requested by ACME, a case study was performed for which only the data set from Area 1 (German Bight, extended) was used since it constituted the largest set with the best temporal coverage of parameters.

3.1 Material and methods

Figures A5.2–A5.4 reveal that not all data to be compared were recorded at the same time and, therefore, some values had to be interpolated before analysis. Since the intention was to relate the data to the disease prevalence data, interpolations were calculated for those time points (days) for which disease data were available. A kernel smoother with Gaussian kernel was used to interpolate and to remove random fluctuation. The smoother bandwidth was selected by generalised cross-validation. No extrapolation outside the time range covered by real observations was done. Pointwise confidence intervals (dashed lines in Figures A5.5a–f) for interpolated values were derived as two standard deviations around smoothed values.

As a first approach to identify relationships between the disease prevalence and the parameters as listed in Section 2.2, univariate logistic models involving observed and interpolated values were fitted (see Table A5.1).

As a second approach, a multivariate logistic model was fitted for each of three scenarios comprising different sets of parameters (see Table A5.2). In the long-term model, parameters with observations for nearly the whole range 1981–1997 were included. The medium- and short-term models contained additional parameters which covered approximately one-half and one-third, respectively, of this range. A stepwise selection was used to identify parameters with the highest explanatory values. A multivariate analysis comprising all parameters simultaneously was considered inappropriate at this stage as it would have required either massive extrapolations or would have been restricted to a very narrow time span.

3.2 Results and Discussion

The results of the univariate analysis are given in Table A5.1. A comparison of the ratio of the number of observations (n_o) and the number of interpolated data points (n_i) used in the analysis for each parameter provides not only an overview of the data availability but also a rough indicator of the reliability of the results of the analysis: if the balance is towards the number of observations, interpolated data can be considered more valid than if the balance is towards the interpolated values.

For each parameter and disease tested, the table includes information on the direction of the relationship and the significance levels. In total, a significant relationship was identified in 31 out of 66 cases which could be taken as an indicator of the multifactorial aetiology of the diseases considered. Water temperature was the parameter with the strongest impact and the prevalences of all three diseases were positively and highly significantly related to temperature. Also CPUE was significantly related to the prevalence of the diseases. However, a positive relationship was only found for lymphocystis and epidermal hyperplasia/papilloma, whilst it was negative for acute/healing skin ulcerations. Information on the significance of other parameters can be taken directly from the table.

Table A5.2 provides information on the parameters found significantly related to the disease prevalence by using the stepwise multivariate analysis. Assuming an 'ideal', e.g., consistent, relationship between the disease prevalence and certain parameters, one would have expected that terms with a highly significant impact in the long-term model would also appear in the medium-term and short-term model for the respective disease. However, this was only the case for water nitrate and epidermal hyperplasia/papilloma. Other significant terms occurring at least twice were salinity and cadmium in *M. edulis* (lymphocystis), CB153 in *M. edulis* and cadmium in unfiltered sea water (epidermal hyperplasia/papilloma) and cadmium in unfiltered sea water and CPUE (acute/healing skin ulcerations).

The inconsistency between parts of the univariate and the multivariate analyses with regard to the parameters identified as significant (e.g., for water temperature) is at least partially due to the fact that these analyses referred to different time ranges. Furthermore, some of the parameters were found to be highly correlated (e.g., water temperature with CPUE, nitrate and phosphate), which means that, in the multivariate models, certain parameters might replace others in order to explain variation in prevalence.

For interpretation of the results of the analyses, it has to be taken into account that a considerable part of the data used was derived from data interpolation, which is always problematic since one does not know how the parameters have behaved in between two sampling dates. With increasing distance between sampling dates this problem becomes more serious and it is self-evident that the reliability of results from any statistical analysis suffers from this. Furthermore, automatic interpolation without adaptation to the type of data used might lead to biased data, e.g., if data series characterised by pronounced seasonal variation are not consistent in terms of temporal coverage of data points. An example from the existing ICES data set is the measurement of water temperature (Figure A5.5a). For the period 1981–1991, only winter data are available and interpolated values used for the analysis were generally low. Since 1991, however, also spring/summer values are available and, therefore, interpolated values were considerably higher although the winter values remained at about the same level as in previous years. This example clearly demonstrates that, for further analyses, it will be more appropriate to employ specific parameter-oriented interpolation techniques.

4 Conclusions and Perspectives

First of all, a little bit of 'back-clapping' with regard to the activities of the WGPDMO during previous years seems to be justified: the data overview revealed that the fish disease data constitute one of the most comprehensive and consistent data sets of the ICES Environmental Data Centre, both in terms of spatial and temporal coverage.

In contrast, there is a striking lack of other data, in particular for contaminants in biota and sediments, creating major problems in the initiated holistic data analysis. However, there are undoubtedly more data around held by national data banks. Therefore, ICES Member Countries should be encouraged to submit these data to the ICES Environmental Data Centre by using standard procedures already developed by ICES and, therefore, applicable without major effort.

The availability of additional data would improve the spatial and temporal data coverage and would, therefore, possibly facilitate an analysis based on smaller geographical areas than those used in the present case study. Large areas create problems since conditions normally are not the same all over the area. For instance, Area 1 (German Bight, extended) includes coastal, estuarine and offshore areas and a comparison of, e.g., contaminant levels in mussels from estuarine areas with disease prevalences of dab collected offshore can only be regarded as a rough approach to identify possible relationships. Furthermore, additional data could minimise the need for temporal interpolation and related problems (see above).

Despite the shortcomings identified, the results of the analysis seem to be promising since for a number of parameters included, a close relationship with the variation in disease prevalence could be identified. It is, therefore, concluded that further activities with respect to enhance the data basis and to improve the models applied and the methods for statistical analysis are desirable and should be initiated.

5 Suggestions for Recommendations

ICES Member Countries should be encouraged to submit relevant data to the ICES Environmental Data Centre, ICES Oceanographic Data Centre and the ICES Fisheries Data Bank by using established ICES protocols, in order to facilitate a more comprehensive holistic analysis of the interactions between natural and anthropogenic environmental factors and the status and well-being of populations of marine organisms.

The ICES WGPDMO should review progress in data submissions and continue the study intersessionally in order to develop and optimise suitable models and statistical methods for a holistic analysis of the ICES fish disease data.

Relevant ICES Working Groups (e.g., WGSAEM, WGBEC, MCWG, WGMS) should be made aware of the outcome of the present exercise and should be encouraged to provide additional information and their ideas with regard to further proceedings and possible collaboration.

ICES ACME should review the overview and the results of the case study and should consider recommendations for further actions.

Literature cited

ICES. 1999. Statistical analysis of fish disease prevalence data extracted from the ICES Environmental Data Centre. *In* Report of the ICES Advisory Committee on the Marine Environment, 1998. ICES Cooperative Research Report No. 233.

Acknowledgement

Thanks are due to the ICES Secretariat for advice and rapid supply of data requested.

Abbreviations used in the figures and tables, if not further specified

CPUE	catch per unit of effort (number of fish/1 hour of trawling)
W	water
DIS OXY	dissolved oxygen
P TOTAL	total phosphorus
W B	unfiltered sea water
S	sediment
U00	unfractionated
F63	fraction < 63 μm
F20	fraction < 20 μm
MYTI	*Mytilus edulis*
Sb	soft body tissue
LIMA	*Limanda limanda*
Mu	muscle tissue
Li	liver tissue
M4	males, size group ≥ 25 cm
M3	males, size group 20–24 cm
M2	males, size group 15–19 cm
M1	males, size group 10–14 cm
F4	females, size group ≥ 25 cm
F3	females, size group 20–24 cm
F2	females, size group 15–19 cm
F1	females, size group 10–14 cm

Table A5.1.:Area 1 (German Bight, extended): case study on the relationship between the prevalence of dab (*L. limanda*) diseases and parameters available from the ICES Data Banks, results of a univariate analysis. Shaded areas indicate significance at p <0.05.

n_o: number of observations, n_i: number of interpolations

dir: direction of relationship, p: significance level

Parameter	n_o	n_i	Lymphocystis		Ep. Hyp./Pap.		Ac./Heal. Ulc.	
			dir	p	dir	p	dir	p
W TEMPERATURE	261	98	+	< 0.0001	+	< 0.0001	+	< 0.0001
W SALINITY	239	98	+	< 0.0001	+	< 0.0001	-	0.8911
W PHOSPHATE	48	71	+	0.1688	-	0.0638	-	0.0418
W DIS OXY	20	29	+	0.0013	+	0.1358	-	0.0733
W NITRATE	48	71	+	0.0362	-	0.7437	-	0.0832
WB CB153	120	43	+	0.2149	-	0.7491	+	0.9132
WB HCB	114	43	-	0.6210	-	0.7535	-	< 0.0001
WB Cd	185	52	-	0.5397	+	0.2417	-	< 0.0001
WB Hg	186	52	-	0.0066	-	0.0051	-	0.0555
S F63 CB153	5	42	+	0.5098	-	0.0098	+	0.2030
S F63 HCB	3	27	-	0.0006	-	0.0534	+	0.0317
S F63 Cd	7	34	+	0.2290	+	0.7014	+	0.9162
S F63 Hg	8	42	+	0.0114	-	0.4048	+	0.8843
MYTI Sb CB153	36	96	+	< 0.0001	+	< 0.0001	+	0.4567
MYTI Sb HCB	33	39	-	0.0183	-	0 0544	-	0.0128
MYTI Sb Cd	50	96	+	0.1833	+	0.1119	-	0.0238
MYTI Sb Hg	51	96	+	< 0.0001	+	< 0.0001	-	0.1370
LIMA Li CB153	4	13	+	0.0192	-	0.4267	+	0.9582
LIMA Li HCB	3	5	+	0.1218	-	0.7319	+	0.6986
LIMA Li Cd	5	28	+	0.0235	+	0.8689	-	0.0115
LIMA Mu Hg	5	28	+	0.0019	-	0.8375	-	0.0064
CPUE	295	97	+	< 0.0001	+	< 0.0001	-	0.0060

Table A5.2. Area 1 (German Bight, extended): case study on the relationship between the prevalence of dab (*L. limanda*) diseases and parameters available from the ICES data banks, results of a multivariate analysis.

n_i: number of interpolations (data values) remaining for the analysis
dir: direction of relationship, p: significance level

Long-term model	Parameters included		
	W TEMPERATURE, W SALINITY, W PHOSPHATE, W NITRATE, WB CB153, WB HCB, WB Cd, WB Hg, MYTI Sb CB153, MYTI Sb Cd, MYTI Sb Hg, CPUE		
n_i	43		
Disease	*Significant terms*	*Dir*	*p*
Lymphocystis	W SALINITY	-	< 0.0001
	MYTI Sb Cd	-	< 0.0001
Ep. Hyp./Pap.	W NITRATE	+	0.0003
	WB Cd	+	< 0.0001
	MYTI Sb CB153	-	< 0.0001
	MYTI Sb Cd	-	< 0.0001
Ac./Heal. Ulc.	WB Cd	-	0.0008
	CPUE	-	0.0163
Medium-term model	Parameters included		
	W TEMPERATURE, W SALINITY, W PHOSPHATE, W NITRATE, WB CB153, WB HCB, WB Cd, WB Hg, S F63 CB153, S F63 Hg, MYTI Sb CB153, MYTI Sb Cd, MYTI Sb Hg, CPUE		
n_i	33		
Disease	*Significant terms*	*dir*	*p*
Lymphocystis	W SALINITY	-	< 0.0001
	MYTI Sb Cd	-	< 0.0001
	CPUE	-	0.0213
Ep. Hyp./Pap.	W NITRATE	+	< 0.0001
	WB CB153	-	0.0089
	S F63 CB153	-	< 0.0001
	MYTI Sb CB153	+	0.0004
Ac./Heal. Ulc.	CPUE	-	0.0016
Short-term model	Parameters included		
	W TEMPERATURE, W SALINITY, W PHOSPHATE, W NITRATE, WB CB153, WB HCB, WB Cd, WB Hg, S F63 CB153, S F63 Hg, MYTI Sb CB153, MYTI Sb HCB, MYTI Sb Cd, MYTI Sb Hg, LIMA Li Cd, LIMA Mu Hg, CPUE		
n_i	23		
Disease	*Significant terms*	*dir*	*p*
Lymphocystis	W NITRATE	+	0.0093
	MYTI Sb CB153	+	< 0.0001
	LIMA Li Cd	-	0.0070
Ep. Hyp./Pap.	W NITRATE	+	0.0005
	WB Cd	+	0.0288
Ac./Heal. Ulc.	WB Cd	-	0.0070

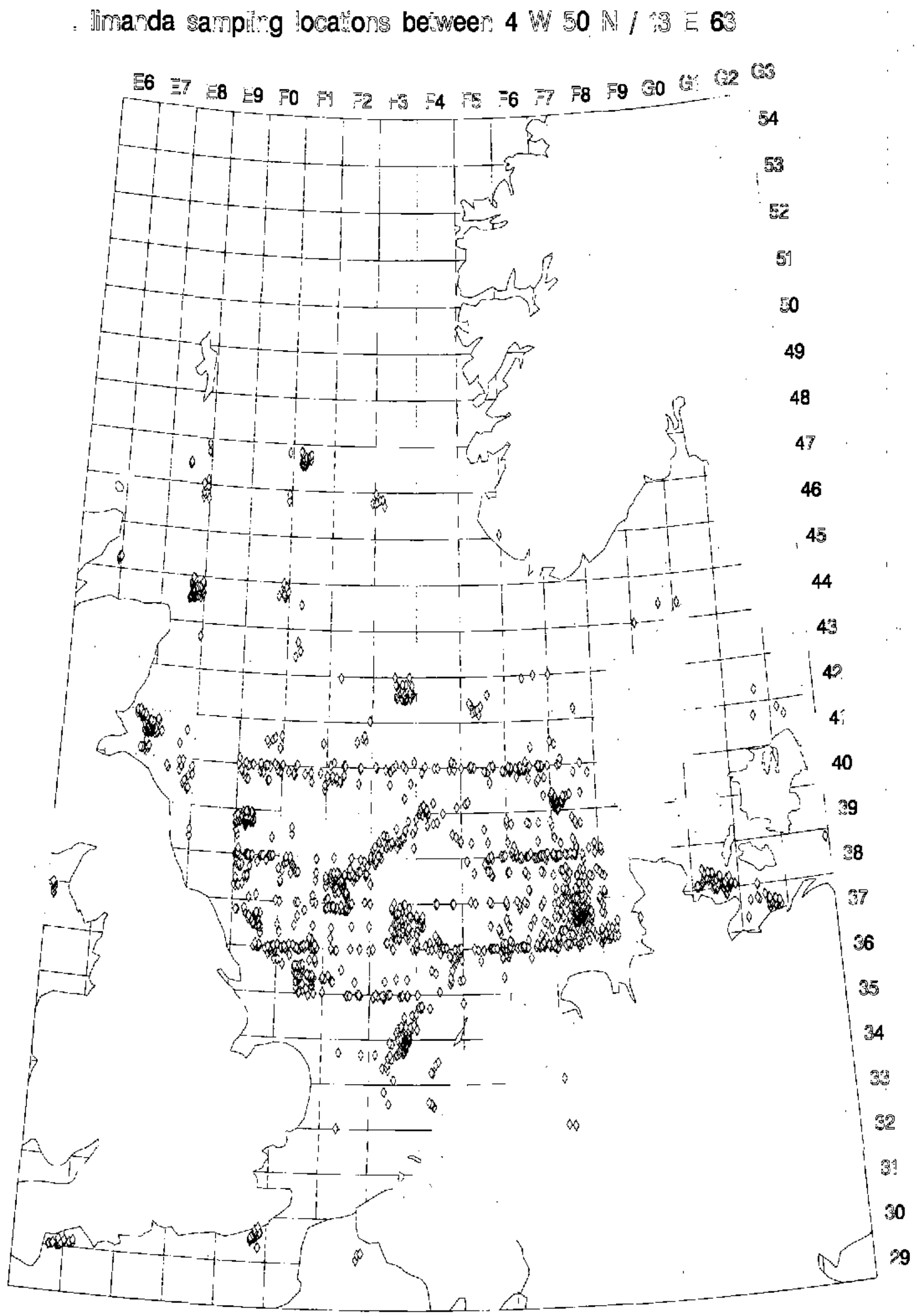


Figure A5.1. Location of the three North Sea areas for which an overview of available ICES data is presented (single marks indicate positions for which prevalence data of dab (*L. limanda*) diseases are available).

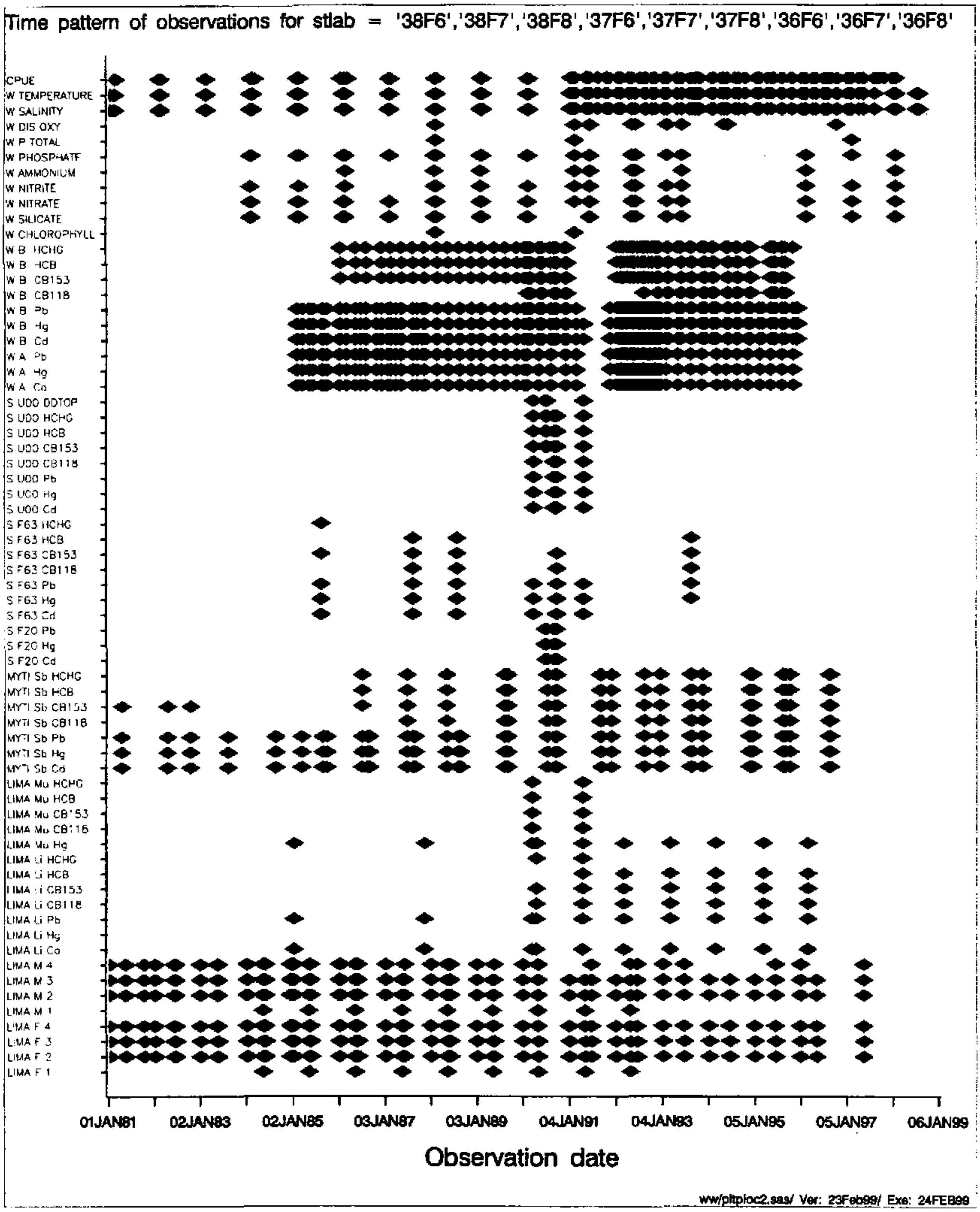


Figure A5.2. Area 1 (German Bight, extended): overview of ICES data available for a holistic analysis of fish disease data (extract).

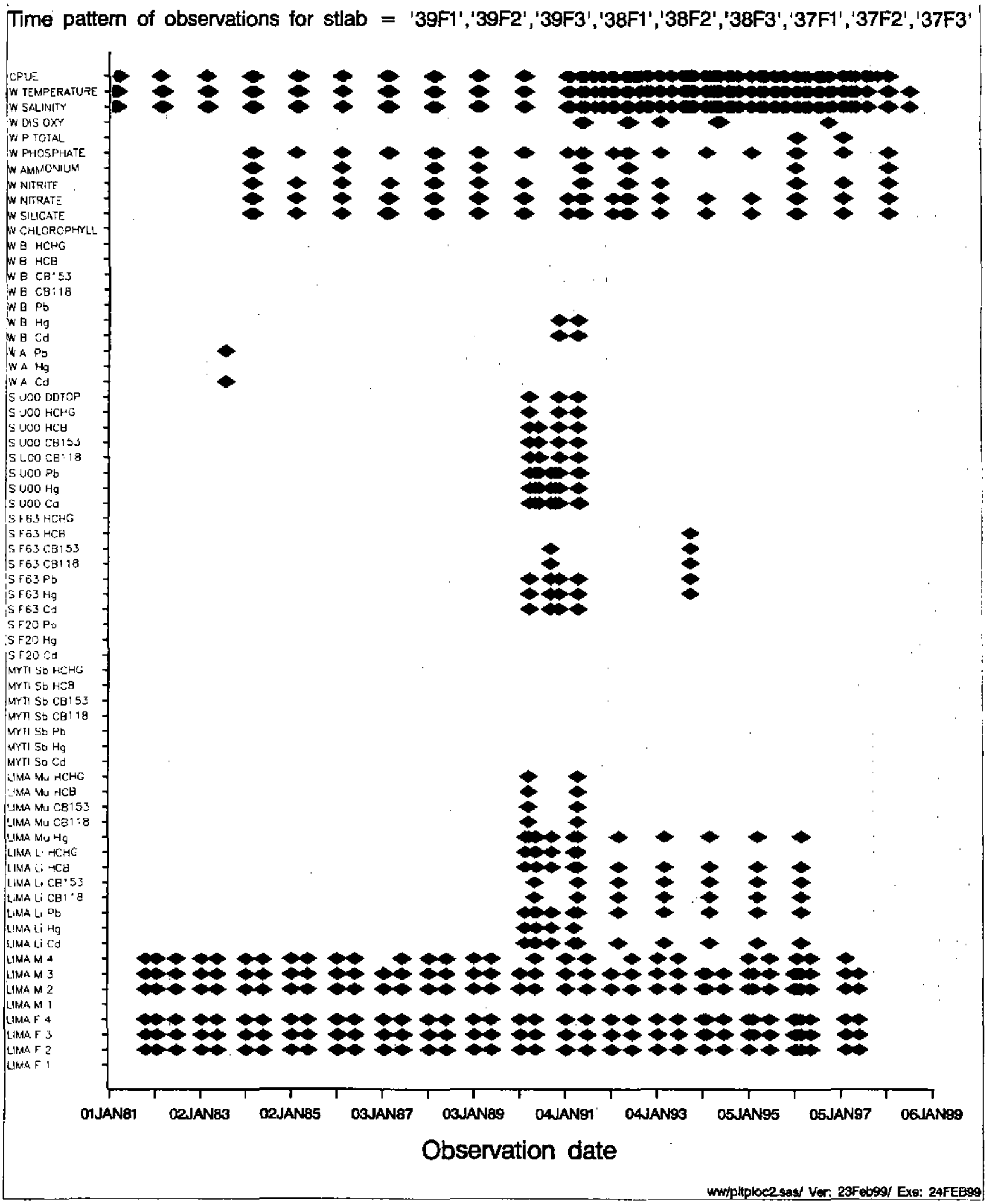


Figure A5.3. Area 2 (Dogger Bank, extended): overview of ICES data available for a holistic analysis fish disease data.

Figure A5.4. Area 3 (Firth of Forth, extended): overview of ICES data available for a holistic analysis of fish disease data (extract).

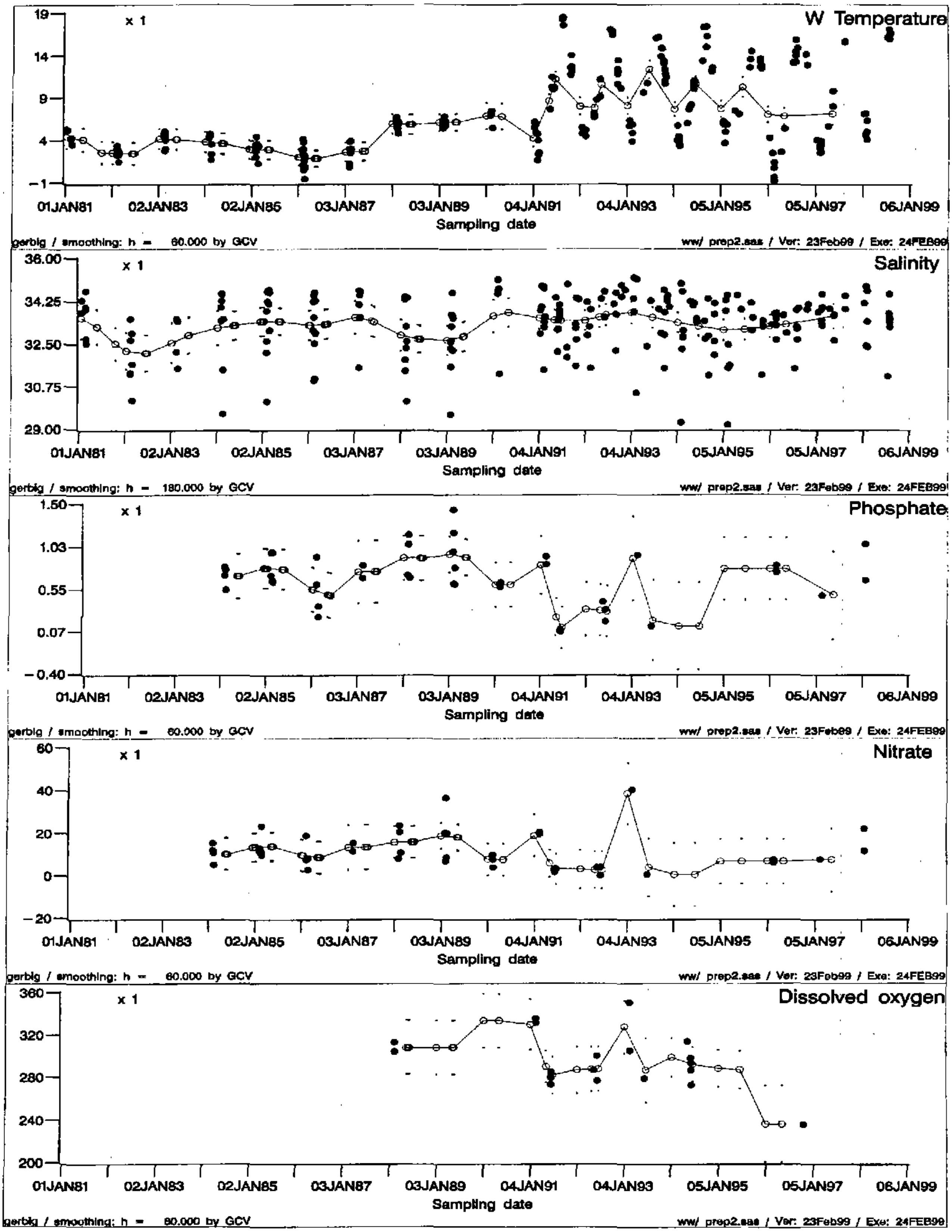


Figure A5.5a. Area 1 (German Bight, extended): water data from the ICES Oceanographic Data Centre (extract) (filled circles: empirical data, empty circles: interpolated values).

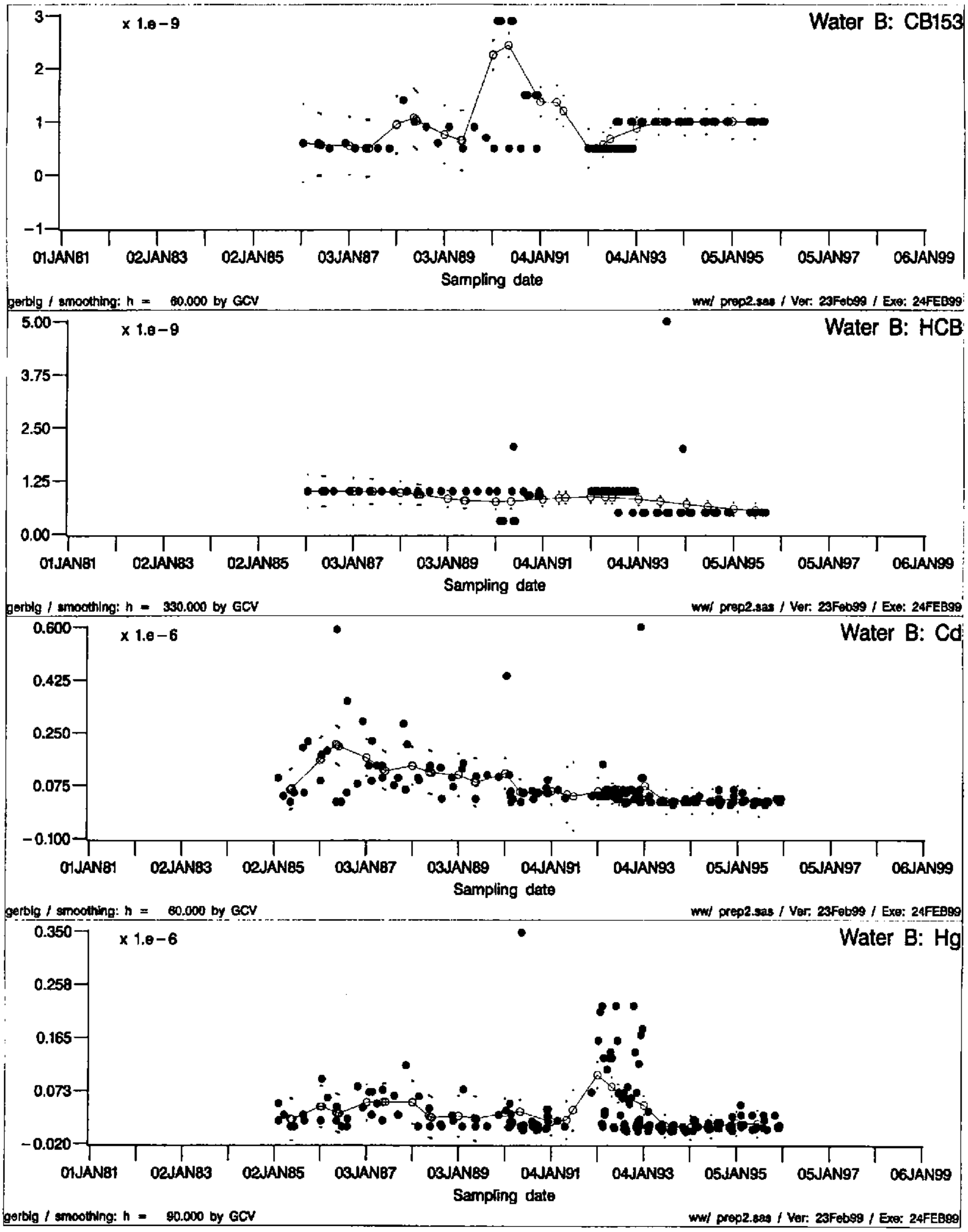


Figure A5.5b. Area 1 (German Bight, extended): data on contaminants in sea water (before filtration) from the ICES Environmental Data Centre (extract) (filled circles: empirical data, empty circles: interpolated values).

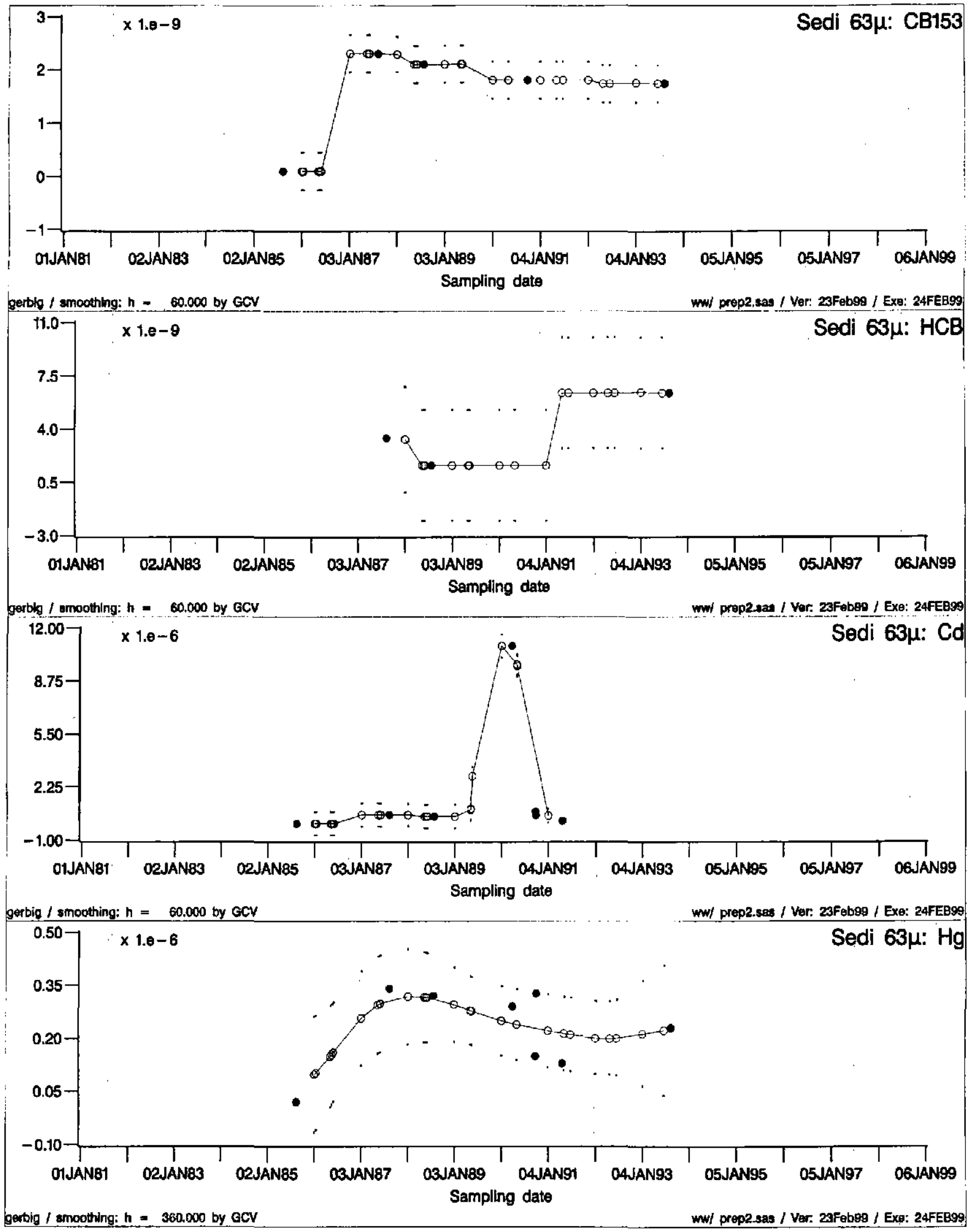


Figure A5.5c. Area 1 (German Bight, extended): data on contaminants in sediments (fraction < 63 µm) from the ICES Environmental Data Centre (extract) (filled circles: empirical data, empty circles: interpolated values).

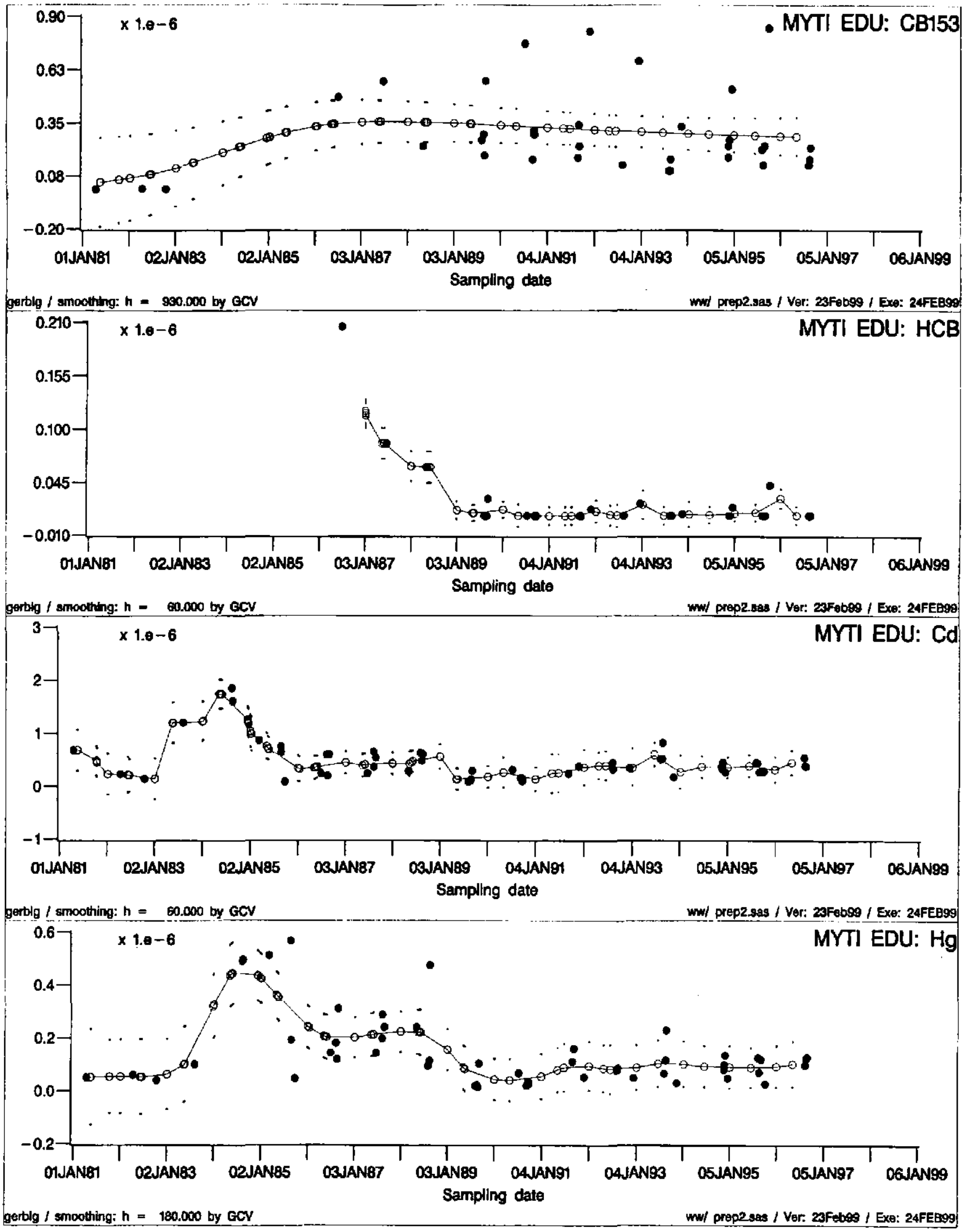


Figure A5.5d. Area 1 (German Bight, extended): data on contaminants in *Mytilus edulis* (soft body) from the ICES Environmental Data Centre (extract) (filled circles: empirical data, empty circles: interpolated values).

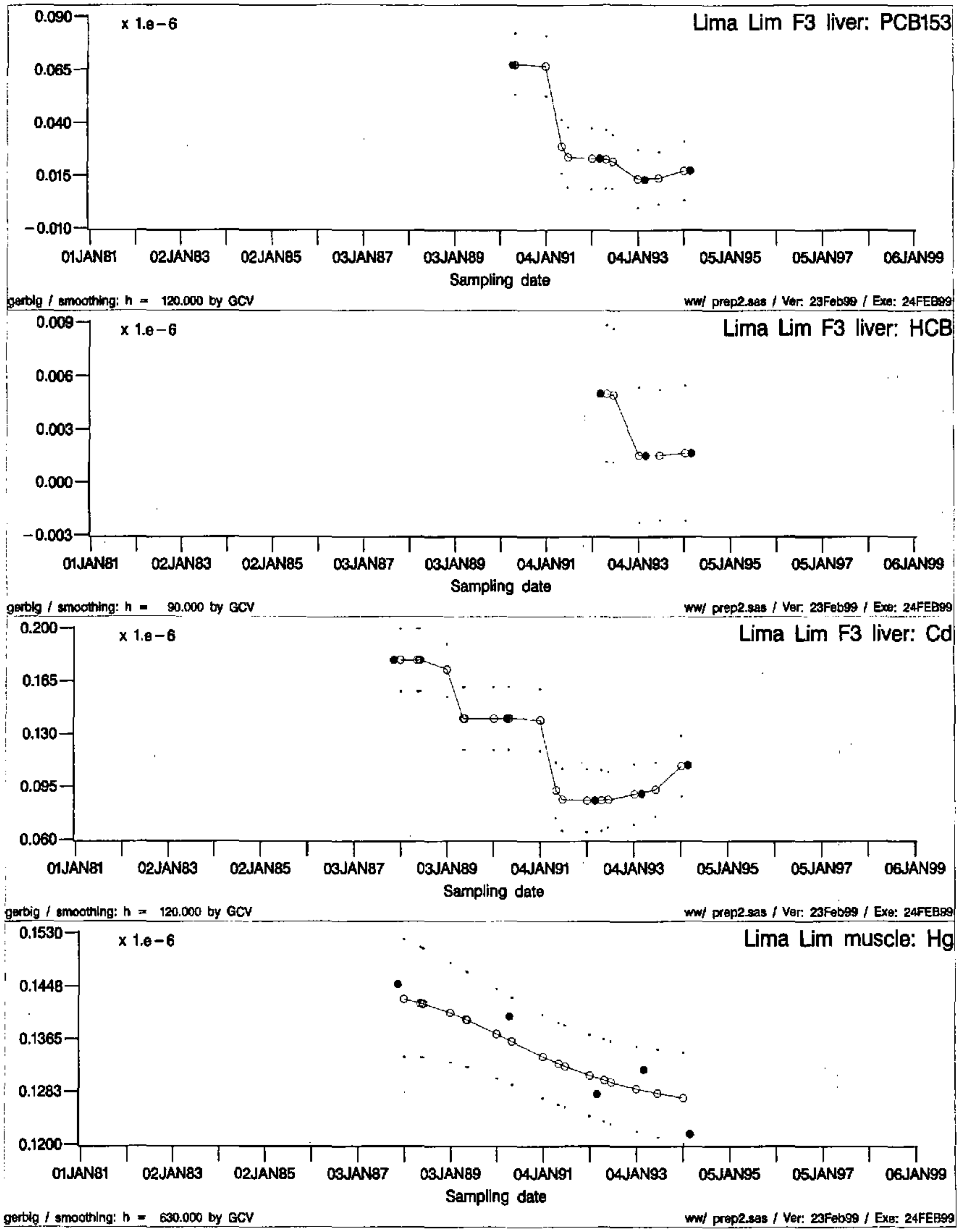
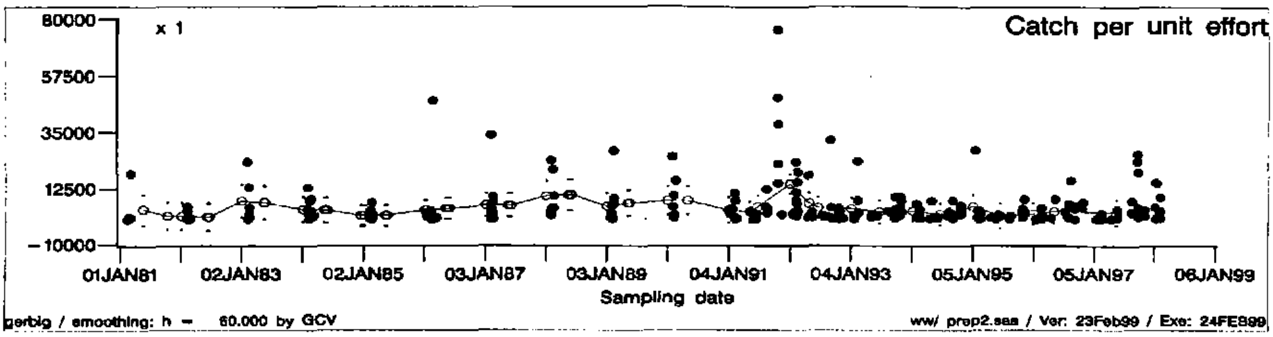


Figure A5.5e. Area 1 (German Bight, extended): data on contaminants in liver and muscle of *Limanda limanda* from the ICES Environmental Data Centre (extract).



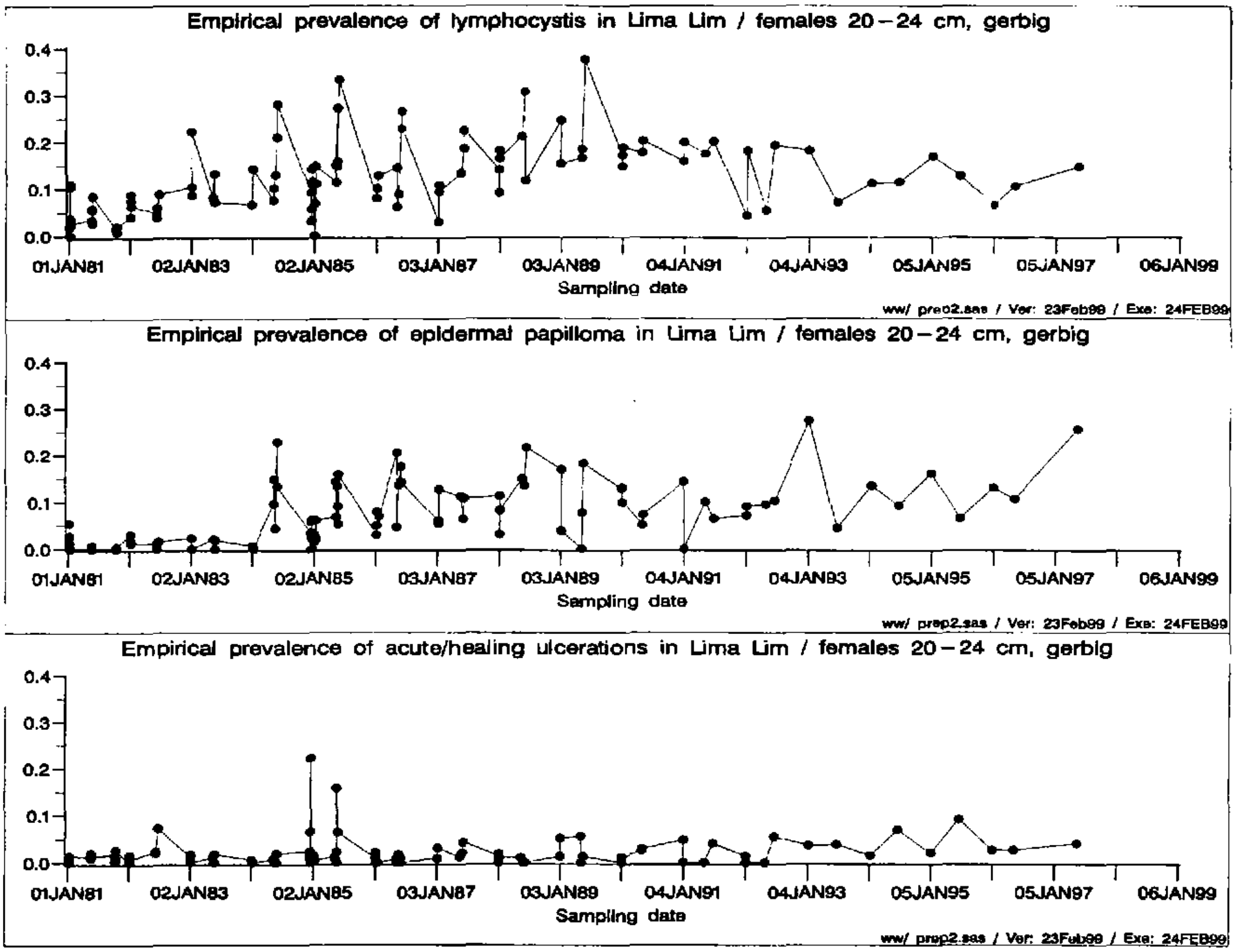


Figure A5.5. Area 1 (German Bight, extended): *Limanda limanda*, data on catch per unit of effort (CPUE) (all dab) from the ICES Fishery Data Bank and on diseases (females, 20–24 cm) from the ICES Environmental Data Centre (extract) (filled circles: empirical data, empty circles: interpolated values).

ANNEX 6

MAINTAIN AN OVERVIEW OF NEW INFORMATION ON M74

G. Bylund

Present state

Finnish as well as Swedish data indicate that there was a decline in the prevalence of M74 in 1998 (i.e., broodstock spawned in autumn 1997) compared to the previous years.

In the Finnish rivers monitored (River Tornionjoki, River Simojoki and River Kymijoki), the mean prevalence of affected salmon females was 34.1 %. The mean fry mortality in offspring from these females was 26.9 %. These figures are based on observations on offspring from 85 females.

In the Swedish rivers, the M74 prevalence was 7 % as a mean of 8 different salmon populations, estimated on 1071 family groups. All 8 salmon populations are of hatchery reared origin and the rivers enter the Gulf of Bothnia. The M74 prevalence ranged from 1 % to 17 %.

In the Swedish rivers with wild salmon, the production of 0+ parr, as measured by electro-fishing, was the highest registered since 1976, when the measurements started. Thus the situation regarding M74 seems to be rather good, but the situation can change in the coming years, i.a., for the following reasons:

a) in the wild salmon populations the ascending spawners during the following 4–5 years will originate from the year classes hatched 1992–1996 which suffered from high M74 mortality, thus giving weak spawning runs if effective fishing regulations are not implemented.

b) another apprehension is the observed increase in autumn 1998, in Finland as well as in Sweden, of broodstock with behavioural symptoms more or less pathognomic for M74. The results from this spawning season, however, are available only in spring 1999. At the broodfish fishery in River Dalälven, a rather high incidence was observed of wiggling males, in the weight class 1.8–5.5 kg indicating that A1+ and A2+ males in the spawning run may have been low in thiamine. Next year the females of these year classes (A2+ and A3+) will return to the spawning run and if the thiamine status of the Baltic salmon is linked to the year class of the smolt run, the situation can turn worse next year. As there is no effective way to treat salmon in the wild salmon rivers, the production of wild parr can fall again. In the hatcheries different techniques and strategies have been developed to manage such a situation.

Ongoing and initiated research

In Sweden experiments are performed where salmon are given feeds with different thiamine content and exposed to different oxidative agents in order to produce broodfish which are presumed to give M74 offspring. The experiments have been run for two years in a netpen in the coastal region of the Baltic Sea and started with post-smolt; they are planned to terminate after stripping in 1999. Measurements of xenobiotics, anti-oxidative capacity, pigmentation and thiamine status will be performed in adult fish (A3+) and in their eggs and alevins.

Similar experimental studies will be performed on alevins from sea-run Baltic salmon. Their eggs have been micro-injected with different oxidant agents at the eyed stage. This study will be started during April 1999 and will presumably give information on different functional disturbances of low thiamine status in combination with oxidative stress.

Two new research projects have recently been initiated in order to evaluate the possible role of eutrophication in the etiology of the M74 syndrome in the Baltic salmon. A project perfomed in Finland focuses on the algal blooms and possible production of thiamine-splitting agents by the blue-green algae (cyanobacteria). In Sweden another project focuses on the significance of microalgae in astaxanthin and thiamine dynamics in the lower trophic levels of the Baltic salmon food web. Results from these efforts will hopefully be available within the next 2–3 years.

Harmonization of research methods and reporting

In order to standardize the research methods and the methods for monitoring disease levels, the following steps have been taken:

- An intercalibration study was performed where 15 batches of eggs (i.e. eggs from 15 females) from salmon from River Dalälven were incubated in parallell in the Swedish Salmon Research Institute as well as in the laboratory of the Finnish Game and Fisheries Research Institute. The aim of this study was to compare observations of symptoms and final results from the incubations and, moreover, to use the results in harmonizing the reporting of the M74 situation in Sweden and Finland. A report of this study will be written and submitted for publication as soon as some results from the biochemical analysis are completed.
- An harmonizing study of thiamine analysis was performed in which three laboratories took part: one from Sweden and two from Finland. The Swedish laboratory analysed only total thiamine, but the Finnish laboratories analysed the different thiamine components according to the method of Brown *et al.* (Brown, S.B, Honeyfield, D.C. and Vandenbyllaard, L. 1998. Thiamine analysis in Fish Tissues. *In* Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and the Baltic Sea, Ed. by G. McDonald, J. Fitzsimons, and D.C. Honeyfield. American Fisheries Society, Symposium 21, Bethesda, Maryland. pp. 73–81.). This study will be reported in the near future (see below: Löflund *et al.*).
- It is stressed that during an international workshop in Uppsala in 1993 it was agreed that the name of the syndrome should be written: M74, and that the use of the name M74 should be restricted to the salmon syndrome.

Papers published in 1998 or coming out in the near future

A special issue of the journal Ambio, containing 15 articles and summarizing much of the research so far performed on the M74 syndrome will be published in early 1999. Moreover the following papers have been published or are in print:

Löflund, M., Amcoff, P., Eerola, S., Hartikainen, K., Vuorinen, P.J., Soivio, A., and Sistonen, S. 1998. Quality assurance of thiamine analyses in connection with investigations of reprodcutive disturbances in Baltic fish: a proficiency study. Nord (in print).

Soivio, A., Ikonen, E., Koski, P. and Vuorinen, P.J. 1998. M74 syndrome in Baltic salmon (*Salmo salar*): A short review of the situation in Finland. Boreal Env. Res. (in print).

Soivio, A., and Piironen, J. 1998. The aquacultural project to maintain natural stocks of Baltic salmon (*Salmo salar*) in Finland. Nord (in print).

Vuorinen P.J., Haahti, H., Leivuori, M., and Miettinen, V. 1998. Comparisons and temporal trends of organochlorines and heavy metals in fish from the Gulf of Bothnia. Mar. Poll. Bull. 36: 236–240.

Vuorinen, P.J., and Keinänen, M. 1998. Environmental toxicants and thiamine in reproduction disorder (M74) of Baltic salmon (*Salmo salar* L.). Nord (in print).

Vuorinen, P.J., Keinänen, M., Paasivirta, J., and Koistinen, J. 1998. Dioxin-like organochlorines in the M74 syndrome of Baltic salmon (*Salmo salar* L.). Mar. Environ. Res. 46: 177–178.

A web-site on the M74 syndrome can be accessed via the Internet at
http://w1.185.telia.com/~u18500254/m74eng.htm

ANNEX 7

NEW INFORMATION ON THE SPREAD, DIAGNOSIS AND CONTROL OF NODAVIRUS TO FURTHER ADVISE ON POSSIBLE CONTROL MEASURES

F. Baudin Laurencin

A review of information was reported in 1997 to the WGPDMO and completed in 1998. Hereafter are gathered together information on further progress in the knowledge of nodavirus infections. In the first part of this report are presented some papers, which have been recently published or accepted for publication. In the second part is presented a FAIR programme of research on nodavirus infection, as a progress report to this WG.

1 Newly published data

1.1 Genetic diversity of nodavirus strain (Thiery *et al.* in press, a)

The capsid protein coding sequences of two nodavirus isolates originating either from Atlantic sea bass or Mediterrannean sea bass were found to differ by numerous substitutions, in particular in the regions used for RT-PCR. Hence, a new primer set was tested that permits detecting the viral genome of the Atlantic isolate.

1.2 Diagnostic methods

Breuil and Romestand (1999) give the results of the use of an indirect ELISA for the detection of sea bass antibodies against nodavirus. The sera of 110 adult sea bass were analysed, and a large proportion of the fish was classified as seropositive or seronegative.

Thiery *et al.* (in press, b) have developed a nested RT-PCR detection method. Used comparatively with RT-PCR or cellule culture, the nested-RT-PCR was demonstrated as more sensitive, in particular for monitoring the presence of viral genome in asymptomatic carriers, before or after the clinical episode.

1.3 Disease transmission trials

Tanaka *et al.* (1998) transmitted the disease to young sevenband grouper (*Epinephelus septemfasciatus*) or *E. akaara*, by IM injection with a filtered homogenate of organs of diseased fish, fulfulling Koch's postulates. The pathogenicity of the virus was found to be associated with rearing water temperature: higher mortality and earlier appearance of the disease signs were observed at higher temperatures. The virus antigen was detected in survivors until 50 days post inoculation.

Peducasse *et al.* (in press) tested 5 infection ways on juvenile sea bass (3g): IM and IP injection, oral contamination, bath exposure and cohabitation. Depending on the way of infection, the time elapsed prior to the outbreak varied and the mortalities and lesions were more or less severe.

Also on juvenile sea bass, Latrous (1998) showed the effects of the water temperature. After IM injection at 20°C, mortality begins at day 7 post infection and reaches 50 %; at 25 °C, the mortality begins at day 4 and reaches 93 %. After bath exposure, the figures are respectively day 11 and 10 % at 20 °C and day 7 and 33 % at 25 °C. Juvenile turbots were also experimentaly infected, resulting in mortality and the usual lesions. On the contrary, juvenile seabream were apparently not susceptible: their experimental infections did not lead to mortality or lesions.

In order to investigate the possibility for invertebrates to act as a reservoir and source of nodavirus infection, Skliris *et al.* (1998) experimentaly tried to infect *Artemia salina*. Just a transitory infectivity appeared, but no evidence of degenerative changes, or of the persistance of viral particles, can be demonstrated.

1.4 Immune response

Breuil and Romestand (1999) immunized sea bass females of the broodstock with heat-killed nodavirus. The antibody level increased 4 weeks post-immunization and was still detectable after 41 weeks.

2 Programme FAIR PL98-4036

2.1 General objectives

The objectives of the project are to study nodavirus disease in cultured fish through characterization studies of nodavirus, comparative assessment of new and established diagnostic methods, experimental disease transmission trials and evaluation of the fish immune response to infection. The project should allow development of practical, effective and reliable methods for the diagnosis, prevention and control of nodavirus infections of cultured sea bass and other marine fish in Europe and other Mediterranean countries. Because the proposal deals with a disease which is of major importance to the aquaculture industry, especially in Italy and Greece, participation of an industry representative from these countries, proposed by the Federation of European Aquaculture Producers, is associated to the project as an expert consultant directly linked to the coordinator.

2.2 List of research tasks and subtasks

Task 1: Virus characterisation
- *Sub-task 1.1:* Virus collection and cell culture
- *Sub-task 1.2:* Effect of physical and chemical treatments *in vitro*
- *Sub-task 1.3:* Phylogenetic study of nodavirus isolates
- *Sub-task 1.4:* Antibodies production and serotyping

Task 2: Assessment of diagnostic methods
- *Sub-task 2.1:* Histopathology and immunohistochemistry
- *Sub-task 2.2:* Cell culture isolation
- *Sub-task 2.3:* Polymerase Chain Reaction
- *Sub-task 2.4:* *In situ* hybridization
- *Sub-task 2.5:* ELISA and seroneutralisation test

Task 3: Disease transmission trials and pathogeny
- *Sub-task 3.1:* Horizontal transmission to sea bass larvae
- *Sub-task 3.2:* Vertical transmission to sea bass larvae
- *Sub-task 3.3:* Horizontal transmission to sea bass juveniles
- *Sub-task 3.4:* Horizontal transmission to sea bass spawners
- *Sub-task 3.5:* Comparison of the pathogenicity of different strains in sea bass
- *Sub-task 3.6:* Infection trials in other fish species fish

Task 4: Immunological studies
- *Sub-task 4.1:* Immune response after prior infection
- *Sub-task 4.2:* Immune response delivered by inactivated virus

2.3 Partnership

CNEVA Brest (France)

Institute of Aquaculture, University of Stirling (Scotland, UK)

IFREMER-Palavas (France)

2.4 Deliverables

Among other deliverables it was planned to report each year to the WGPDMO on the occasion of its annual meeting, and also to present the results in EAFP International Conferences/Workshops.

2.5 Present state of the programme

Officially beginning: 1 November 1998

A recent meeting in Brest (3 February 1999) allowed a summary of what has already been performed:

-selection by ELISA of seropositive and seronegative individuals of the sea bass broodstock

-research on the ways of infection

-research on the effect rearing water temperature

-sequencing of nodavirus isolates (at Stirling and at Brest)

Results on the above points are published or soon to be.

The Brest meeting has also allowed a better clarification of the role and responsibilities of each partner in the beginning of the project.

Bibliography

Breuil, G., and Romestand, B. 1999. A rapid ELISA method for detecting specific antibody level against nodavirus in the serum of sea bass, *Dicentrarchus labrax* (L.): application to the screening of spawners in a sea bass hatchery. Journal of Fish Diseases, 22: 45–52.

Latrous, A. 1998. Transmission expérimentale de la nodavirose chez le bar *(Dicentrarchus labrax)*, la daurade *(Sparus aurata)* et chez le turbot *(Scophtalmus maximus)*. Rapport de DEA. 47p. (*Ce rapport, établi pour obtenir le diplome universitaire, n'a pas valeur de publication. Certains des travaux effectués ont été effectués en collaboration avec S. Péducasse*).

Peducasse, S., Castric, J., Thiery, R., Jeffroy, J., Le Ven, A., and Baudin Laurencin, F. In press. Comparative study of viral encephalopathy and retinopathy in juvenile sea bass (*Dicentrarchus labrax* L.) infected by different ways. Diseases of Aquatic Organisms. *Accepted for puublication.*

Skliris, G.P.,and Richards, R.H. 1998. Assessment of the susceptibility of the brine shrimp *Artemia salina* and rotifer *Brachionus plicatilis* to experimental nodavirus infections. Aquaculture, 169: 133–141.

Tanaka, S., Aoki, H., and Nakai, T. 1998. Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. Fish Pathology, 33(1): 31–36.

Thiery, R., Arnauld, C., and Delsert, C. In press, a. Two different isolates of sea bass (*Dicentrarchus labrax*) nervous necrosis virus with distincts genomes. Journal of Fish Diseases. *Accepted for publication.*

Thiery, R.,Raymond, J-C., and Castric, J. In press, b. Natural outbreak of viral nervous necrosis in juvenile sea bass *Dicentrarchus labrax*: study by nested RT-PCR. Virus Research. *Accepted for publication.*

ANNEX 8

AVAILABLE INFORMATION ON THE IMPACT OF MARINE BIOTOXINS PRODUCED BY DINOFLAGELLATES AND ALGAE ON FISH POPULATIONS AS A BASIS FOR EVALUATION OF THE SIGNIFICANCE AND DYNAMICS AND FUTURE RESEARCH

Göran Bylund

Eutrophication induces the blooms

Algae blooms are not a new phenomenon. Harmful effects of microalgae blooms were originally recognized more than a century ago when toxic cyanobacteria were killing livestock around Australian lakes. Eutrophication of waterbodies and coastal areas due to increases in nutrient output, however, has resulted in an increase in the frequency and extensiveness of microalgae blooms during recent decades. The blooms occur as a response to a combination of climatic and hydrographic events and the availability of nutrients and trace elements (Bruno *et al.*, 1989). The term "blooms" of course refers to mass occurrences of the organisms, sometimes visible as thick scums, in the surface waters. When assessing the impact of the microalgae blooms, however, we have to recognize that the toxic algae can occur in a stratified manner at different depths, invisible in the surface waters.

Freshwater microalgae occur frequently in coastal and estuarine areas
In offshore marine areas the toxic algae blooms usually include species of dinoflagellates, chloromonads, and haptophyteans. In coastal and estuarine areas cyanobacteria (blue-green algae) of the genera *Microcystis, Oscillatoria, Anabaena, Nodularia* and *Apanizomenon* are most frequently implicated in toxicosis in vertebrates and also their toxic effects in zooplankton is well demonstrated today. It is important to recognize that many microalgae groups have a very wide spectrum concerning their salinity tolerance and can be represented in marine as well as in fresh waters (for example the *Prymnesium*-group). It should also be recognized that members of these groups are not always toxic. We do not know today why members of the same species sometimes produce bioactive toxins, sometimes not.

Cyanobacterial toxins and their pathophysiological mechanisms
Much of the knowledge we have today on the microalgae toxins and their effects concerns toxins produced by the cyanobacteria. Several different toxic substances have been isolated from cyanobacteria. The most toxic substances can be classified as neurotoxins and hepatotoxins. Among the neurotoxins the aphantoxin from *Aphanizomenon flos-aquae* and anatoxins from various species of *Anabaena* are best studied. The aphantoxins are identical with the saxitoxin and neosaxitoxin produced by marine flagellates and act as sodium channel blockers, the anatoxins act, i.a., as cholinesterase inhibitors (Eriksson, 1990). Our knowledge on the effects of algae neurotoxins on fish are still very poor.

Pathophysiological mechanisms of hepatotoxins

Most of the knowledge we have today on the pathophysiological effects of microalgae toxins are on the hepatotoxic microcystins and nodularins, produced by *Microcystis, Nodularia* and other species. In higher vertebrates these toxins act specifically in the liver, where they are thought to be taken up into the hepatocytes via multispecific bile acid transport systems (Eriksson *et al.*, 1990). At acutely toxic doses microcystins cause rounding of the hepatocytes and loss of normal hepatocyte structure. This disorganization of the tissue leads to massive intrahepatic hemorrhage, in severe cases followed by death of the animals from hypovolemic shock or hepatic insufficiency (Carmichael, 1992). The changes observed in the hepatocytes have been shown to result from a collapse of the intermediate filament and microfilament network of the cells (Eriksson *et al.*, 1989, 1990). This process appears to be triggered when the toxins bind to and thereby inhibit regulatory enzymes, e.g., the phosphatases 1 and 2A in the cytosol of the hepatocytes (Eriksson *et al.*, 1992).

Effects and symptoms in fish

Experimental work performed during recent years indicates that the effects of cyanobacterial hepatotoxins in fish are rather similar to those in higher vertebrates. Also in fish the toxins are highly organotrophic for the liver, where they induce more or less total inhibition of protein phosphatases (Tencalla *et al.*, 1997). Most of the fish studies have been done with carp-fish and salmonids and show that omnivorous (i.e., algae-feeders) as well as carnivorous fish are susceptible. Exposing the fish to living algae cultures seems to have minor or no effects, but exposure after lysis of the algae, or if the toxins are administered orally or by IP injection, can have dramatic effects.

Although the pathological and subcellular effects of these toxins in fish in many ways are comparable to those in higher vertebrates, there are also basic structural and physiological differences. The cause of death in exposed fish is not considered to be a hemodynamic shock but rather malfunction of the liver and kidney as a consequence of severe cell necrosis in these organs (Råbergh *et al.*, 1991). Thus the symptoms induced by the toxins appear to be less specific in fish than in higher vertebrates.

From experimental work with cyanobacterial hepatotoxins in fish, i.a., the following symptoms have been reported: *hepatocellular swelling, focal areas of congestion in liver, necrosis of hepatocytes, liquifactive necrosis of liver parenchyma, periportal necrosis and periportal infiltration in liver parenchyma; kidney lesions consisting of coagulative tubular necrosis and dilation of Bowmans space; ballooning of gill epithelium and necrosis of gill epithelial cells.*

Difficult to establish cause/effect relationships in fish kills

There are lots of reports on massive fish kills associated with algae blooms (Eriksson *et al.*, 1986; Toranzo *et al.*, 1990; Andersen *et al.*, 1993, etc.). In most of these cases, however, it has been difficult to establish the final cause of the fish kills, i.e. the role of the biotoxins. This is partly due to the fact that in association with the algae blooms the water chemistry usually changes in many ways (high pH, low oxygen content during night, H_2S-production etc.) and factors other than the toxins may have been fatal for the fish in some/many of these cases. Also, as mentioned above, the symptoms induced by the toxins may be non-specific and do not tell the final etiology of the mortality. And, until recently we had small possibilities to analyse the fish tissues for toxins.

Gaps in knowledge

From all the reports accumulating on fish kills associated with algae blooms it is apparent that these phenomena might have an enormous (and increasing) impact on natural fish populations. However, in order to correctly evaluate the role of algae blooms and biotoxins on fish populations we urgently need much more basic knowledge, i.a., on the following topics:

a) the dynamics of the microalgae blooms.

b) the spectrum of bioactive compounds produced by microalgae. So far most of our knowledge concerns only the lethal hepatotoxins and neurotoxins. However, we know that for example the cyanobacteria produce other pharmacologically active compounds too (dermatotoxins, endotoxins producing gastrointestinal symptoms or pyrogenic reactions, tumour-promoting as well as antitumor factors etc). In a recent work Oberemm *et al.* (1997) showed that crude extracts of toxic algae strains (*Aphanizomenon, Anabaena, Microcystis*) had much more severe effects on fish embryos/fry than the 'classical' toxins and they isolated a new toxin (muggelone) causing these effects.

c) the effects of microalgae toxins on fish eggs and fry. The knowledge we have today concerns almost only lethal effects on adult fish. Fry are generally more sensitive to harmful influences than adult fish. There are reports on massive mortalities of fish eggs associated with algae blooms. There are also indications that algae blooms may be associated with fry mortality syndromes. And, Oberemm *et al.* (1999) demonstrated the harmful effects of cyanobacteria on fish fry.

d) the subacute and subclinical effects of the biotoxins on fish. As stated above, most of the knowledge we have today are on lethal effects on adult fish. But the biotoxins might influence the health state of fish in many ways before leading to acute mortality, i.e., suppressing resistance against infective diseases. We do not know anything today, for example, on the effects of the microalgae biotoxins on fish immune parameters.

e) improved diagnostics. In many of the reports on fish kills associated with microalgae blooms the final cause of the mortality remains obscure because the symptoms registered were non-specific. Utilizing improved methods available today (histopathology on EM-level, analyses on liver phosphatases for hepatotoxins, identifying the toxins in fish tissues with immunohistochemical assays etc.), we have possibilities to evaluate the role of biotoxins in fish kills much more exactly than a few years ago.

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ANNEX 9

AVAILABLE INFORMATION ON THE IMPACT OF MARINE BIOTOXINS PRODUCED BY DINOFLAGELLATES AND ALGAE ON FISH POPULATIONS AS A BASIS FOR EVALUATION OF THE SIGNIFICANCE AND DYNAMICS AND FUTURE RESEARCH

Sharon MacLean

Introduction

Blooms of phytoplankton are important events in the aquatic environment and have received much attention because of their profound effect on the environment, fish and shellfish stocks and industries, and human health. Sediment core analysis indicates blooms of toxic dinoflagellates have occurred for millenia (Dale *et al.*, 1993), however, the incidence of harmful algal blooms appears to be increasing globally, due in part to greater awareness and research effort, increased coastal aquaculture that may serve as sentinels of bloom events, environmental changes that contribute to bloom events, and possible transfer of cyst-forming dinoflagellates in ballast waters (Smayda, 1990; Hallegraeff, 1993). A few of the many volumes addressing the occurrence, ecology, and monitoring methods of harmful algal blooms are listed in the bibliography (Okaichi *et al.*, 1989; Graneli *et al.*, 1990; Smayda and Shimizu, 1993; Yasumoto *et al.*, 1996; Kahru and Brown, 1997; Anderson *et al.*, 1998), and a recent publication by Brusle (1995) reviews much of the information of the topic at hand. Several websites listed in the bibliography were additional sources of information for this report. This segment of the report will address primarily toxic dinoflagellates.

Blooms of algae are normal spring and autumn events, but what stimulates nuisance algae to bloom and to become toxic depends on a complex set of physical and chemical factors, including nutrient loading of the coastal environment, particularly of phosphorus and nitrogen, wind turbulence, and unusual cliimatic conditions such as El Niño years. Altered ratios of silicate with phosphorus or nitrogen are suspected in favoring blooms of noxious species rather than that of the more beneficial siliceous diatoms. Exposure to atypical nutrient regimes also can render non-toxic species toxic, e.g. non-toxic *Chrysochromulina* becomes toxic when phosphate is limited (Edvardsen *et al.*, 1990).

The toxic dinoflagellate events that receive most recognition and are most well studied are those that are planktonic, visually dramatic in their bloom characteristics, acutely toxic resulting in mass mortalities of fish and shellfish, or that affect human health. In addition to direct toxic effects on fishes, are the effects due to anoxia or ammonia generation following bloom die-offs, the initiation of epidermal lesions and subsequent mortalities, and indirect effects on fish larvae through prey organisms.

Toxins and their Effects

Of the more than 5000 microalgal species known, approximately 1 % are toxic (Steidinger *et al.*, 1998). Phytotoxins which are directly toxic to fish are produced by several genera of microalgae. These toxins have various chemical structures, and physical and toxicologic properties. Any one dinoflagellate species may produce a suite of toxins, for example, *Gymnodinium breve* produces at least 8 toxins, one of which is a lethal neurotoxin. The algal toxins may be endotoxins released when the soft-bodied algae rupture, e.g., *Gymnodinium breve* and the widely distributed but fragile Prymnesiophytes, or exotoxins secreted by the intact organism, e.g., *Gyrodinium aureolum*. The mode of toxicologic action varies with the toxins produced. Well known are the saxitoxins and neosaxitoxins that cause paralytic shellfish poisoning, which are sodium channel blockers. On the other hand, the neurotoxic brevetoxin produced by *Gymnodinium breve* is a sodium channel stimulator. The toxic mode of action of *Heterosigma* species is an oxidative attack on the gills of fish by superoxide and hydroxyl radicals (Brusle, 1995).

The gill appears to be the primary site of uptake by fish of the toxins which are variously hepatotoxic, hemolytic, cytolytic, neurotoxic or mucin inducers, with the results being osmoregulatory, neurologic and respiratory distress. Varying degrees of injury to gills, liver and gut have been reported and likely are related to the type and potency of the toxin, mode of action, and duration of exposure. Mortalities occur when epithelial cell sloughing in primary and secondary gill lamellae is severe, gill chloride cells malfunction, and nerve tissue is severely damaged. Larval through adult stages of fish are affected by these toxins, although sensitivity to toxin varies with species and life stage of the fish, and with algal species producing toxin.

At present, direct detection of algal toxins is limited to an ELISA for brevetoxins and of several toxic algal species using monoclonal and polyclonal antibody probes, consequently, concentrations of toxic algal cells standardly have been used to designate toxin exposure. No clear relationship between algal concentrations and potential harmful effects exist. Fish kills have been observed when cell concentrations reach 1×10^7/L (*Gyrodinium aureolum*) (Dahl and

Tangen, 1993) and 2×10^5/L (*Gymnodinium breve*) (Steidinger *et al.*, 1998a). The type and concentration of toxin, its mode of action on the fish, the physiology of the impacted fish species, and hydrologic and environmental conditions (such as pH and oxygen levels) all have a role in the kill event. As several species of adult fishes are known to show avoidance behavior in the presence of toxic algae, entrainment of fish in open-water netpen systems exposes fish to high levels of toxin during bloom events that may be avoided under free-ranging conditions. Indeed, the expansion of coastal aquaculture may have helped raise awareness of toxic blooms due to the significant economic impact in some parts of the world.

Effects on Larvae

Kills of adult fishes are fairly well documented as they are quite visible events. Losses of larval fishes are less apparent in the wild than those of juvenile and adult fishes, but may be rather significant in evaluating population effects of toxic algal blooms. A long-term field study on fish populations determined that five species of fish larvae showed immediate declines in density during a bloom of *Gymnodinium breve* and two of those species appeared to suffer long-term effects (Warlen, *et al.*, 1999). Larval fish depend on dinoflagellate prey during the first week of life and apparently show little ability to distinguish toxic from non-toxic species. Experimental studies have shown that as few as 6–11 cells of *Alexandrium tamarense* were lethal to sea bream larvae (White *et al.*, 1989), and a single cell of a more highly toxic dinoflagellate could kill fish larvae (Gosselin *et a.l*, 1989). Larvae feeding on toxic dinoflagellates show neurotoxic effects and behavioral modifications including erratic swimming, loss of equilibrium, swimming on the side and in circles, and paralysis. After the first week post-hatch, fish larvae shift to larger prey and begin to feed on zooplankton. At this time, copepods and other zooplankton, which feed on toxic dinoflagellates and tolerate the toxins, can serve as vectors of toxin to the fish larvae and adult herbivorous fishes such as herring (White, 1980). Mortality experiments showed losses of 30 % per day of larval herring fed copepods that had ingested toxic dinoflagellates. Invertebrates, typically insenstive to the effects of certain marine biotoxins, can remain toxic for weeks after a bloom event (White, 1981). Indirectly, fish larvae may be impacted by negative effects on copepods and other zooplankton that are their primary prey items, as some copepods species have shown declines in egg production after feeding on toxic dinoflagellates.

Pfiesteria piscicida

Recent blooms of the dinoflagellate *Pfiesteria piscicida* in the mid-Atlantic region of the United States have been associated with the development of fish lesions, fish kills and health problems of fishermen and others in frequent contact with affected waters (Burkholder *et al.*, 1998). Detailed electron microscopical examination of cultures and field-collected samples revealed a complex of *Pfiesteria*-like organisms similar in size and morphology to *Pf. piscicida*, which also causes fish kills and is associated with lesions, emphasizing the importance of accurate identification of these organisms (Landsberg and Steidinger, 1998).

Pfiesteria piscicida, a new genus and species of dinoflagellate (Steidinger *et al.*, 1996), was first discovered in aquaria of *Tilapia* experiencing mortalities shortly after being transferred to water taken from the Pamlico Sound, North Carolina. Since then the organism has been implicated in fish kill events in Pamlico Sound tributaries, primarily of young of the year menhaden (*Brevoortia tyrranus)*, and has been found in sediments of Chesapeake Bay and Florida embayments (Lewitus *et al.*, 1995; Burkholder *et al.*, 1995). Laboratory and field studies indicate that numerous species of fish and shellfish may succumb to the toxin(s) produced by *Pfiesteria.*

Pfiesteria piscicida is reported to have a complex life cycle, consisting of 24 flagellated, amoeboid and encysted stages. The flagellated zoospore stages exist in the non-toxic form in the water column and feed on other algae, bacteria and protozoa. In the presence of high densities of fish, the non-toxic flagellated stage is stimulated to bloom and transform into the toxic zoospore stage (TZ) in response to an unknown substance excreted/secreted from fish. TZs tolerate wide temperature (12–33 °C) and salinity ranges (0–35 ppt) but peak in occurrence at > 25 °C, and in toxicity at 15 ppt. Exotoxin(s), as yet unidentified and uncharacterized, immobilize and eventually kill the fish. The zoospores transform to amoeboid forms which feed on the fish flesh and within hours may revert to the encysted stage if conditions are unfavorable (Burkholder *et al.*, 1995).

A high density of live fish is needed to sustain the toxic forms. As several species of *Pf.*-like organisms exist, the ecological conditions that stimulate toxin production may vary. In the case of *Pfiesteria piscicida*, it appears that inorganic phosphorous enrichment and availability of microbial prey have roles in transformation and blooming of toxic zoospores and low hardness (<10 mg/L $CaCO_3$) inhibits blooming and toxin production. Complicating the picture is the close association of bacteria with TZs. The role bacteria play in toxin production of dinoflagellates is rather complex. Depending on the species of dinoflagellate and of bacteria, there is evidence of bacterial production of recognized algal toxins, of bacterial enhancement of toxin produced by dinoflagellates, as well as no effect on toxigenesis by certain dinoflagellate-associated bacteria (Doucette *et al.*, 1998). Bacteria may or may not play a role in the toxigenesis of *Pfiesteria*.

Fish kills typically occur in shallow coastal estuaries that have poor flushing and high water temperature. Although dissolved oxygen levels have been reported at saturation during *Pfiesteria* associated kills, low DO is more typical of these estuarine environments in summer, when most toxic blooms and kills have been reported. Ninety percent of the fish in kills associated with *Pfiesteria piscicida* blooms have been young of the year menhaden, although other species of fishes may be abundant in the estuarine systems at the same time. Menhaden have a thin epidermis and are filter-feeders that ingest phytoplankton, which may render this species more susceptible to toxin exposure and ulcer formation in nature than other species.

Skin Ulcers associated with *Pfiesteria piscicida*

Laboratory exposure to *Pfiesteria piscicida* TZs has demonstrated initiation of epidermal lesions in striped bass (*Morone saxatilis*), hybrid striped bass (*M. saxatilis* female × *M. chrysops* male) and juvenile tilapia (*Oreochromis niloticus, Tilapia aurea, Oreochromis mossambicus*) (Noga *et al.*, 1995). Lesions that developed were marked by intra- and extra-cellular oedema and necrosis of the epithelium which progressed to epithelial erosion to the basement membrane. These lesions appeared in fish prior to signs of neurotoxicity (depression, loss of equilibrium, episodic hyperexcitability, and decreased respiration) noted in the initial discovery of *Pfiesteria* in the tilapia aquarium.

At least one decade prior to the discovery of *Pfiesteria piscicida*, an epizootic of ulcerative mycotic skin lesions was discovered in menhaden from Pamlico Sound, NC. Lesions in these fish often were quite advanced, penetrating through the musculature and exposing the visceral cavity. Commonly seen throughout the lesion and viscera was a severe mycotic infection by *Aphanomyces*. This same ulcerative mycosis is apparent today in fish in North Carolina and the Chesapeake Bay. These lesions now are considered by some to be the characteristic *'Pfiesteria'* lesions, although fish with these lesions occur at times without evidence of toxin exposure. Whether that has to do with delayed timing of the response to fish kills or other factors that may contribute to the inability to detect *Pfiesteria* is not clear. Numerous other pathogens can produce ulcerative lesions in fish that strikingly resemble and may be confused with the *'Pfiesteria'* lesions, e.g., *Aeromonas hydrophila, A. salmonicida, Mycobacterium* spp., *Flexibacter* spp., fungi, and parasites (Baya *et al.*, 1998; Blazer *et al.*, 1998; Kane *et al.*, 1998; Vogelbein *et al.*, 1998).

Sublethal Effects of Algal Toxins

In general, there is little information on the sublethal effects of algal toxins on fish. Deleterious effects have been observed in several species of fishes when exposed to sublethal concentrations of toxic dinoflagellates, including reduced appetite, mild sloughing of respiratory epithelium and altered behavior (swimming near surface). In addition, hepatotoxic effects and enteric lesions have been reported for many species of fishes exposed to dinoflagellate blooms. These reported effects are unlikely to have long-term effects on fish populations. Few studies exist like that of DeMendiola (1978) that demonstrated reduced growth rate, fat content and spawning output of adult *Engraulis ringens* fed on *Gymnodinium splendens*.

Exposure to algal toxins may have some role in the occurrence of certain diseases. Initiation of ulcerative lesions by *Pfiesteria* toxin was mentioned above, and other toxins have been associated with the occurrence of tumors in marine animals. Okadaic acid, better known for its role in diarrhetic shellfish poisoning in humans, has been shown to be a tumor promoter in mammals (Fujiki and Suganama, 1993). Okadaic acid produced by toxic benthic dinoflagellates recently has been linked with the promotion of viral-associated fibropapillomas in sea turtles (Landsberg and Shumway, 1998). Mapping of the global occurrence of neoplasms in molluscs against the recorded presence of dinoflagellate toxin in shellfish tissues offers circumstantial evidence of an association between toxins and tumor production in molluscs (Landsberg, 1996).

Recent attention is being given to the possible immunosuppressive action of brevetoxin in manatees and of ciguatera-associated toxins in reef fishes (Landsberg, 1995), and decrease in splenic lymphoid tissue suggests immunosuppression by *Pfiesteria* toxin in experimentally challenged fish (Noga *et al.*, 1996). Sublethal effects of toxic algae are well documented in shellfish (Shumway, 1990; Shumway and Cembella, 1993), and possible effects on shellfish defense systems are implicated by a dramatic increase in parasite intensity in clams after a devastating red tide event in China.

Future Needs

1) More attention to the effects of chronic, low-level exposure on fish. Due to low visibility, minor toxic algal blooms not associated with fish kills may occur more often than we are aware. There is surprisingly little work

assessing the effects on fish of chronic exposure to low levels of algal toxins. Attention in fish research needs to be given to effects of algal toxins on reproduction, egg and larval survival, and larval/juvenile growth, and disease promotion and immunosuppression.

2) New techniques or modification of old techniques to identify and monitor algal blooms and toxins. Short of awaiting reports of fish kills, paralytic shellfish poisonings or visibly discolored waters, there are few techniques available to monitor the environment for the presence of toxic algae. An ELISA exists for brevetoxin in tissues and is being adapted for testing natural waters, and a probe exists for *Alexandrium tamarense* and other toxic organisms. Satellite imagery of coastal waters provides a synoptic perspective but does not have the ability to discriminate between different phytoplankton taxa.

3) Determine the role of bacteria in *Pfiesteria* cultures in toxin production. Experimental work by (Kodama *et al.*, (1989) demonstrated that endosymbiotic bacteria derived from toxic dinoflagellates produced saxitoxin in culture medium, whereas no bacteria were isolated from non-toxic strains. Axenic cultures of algae from a toxic bloom were non-toxic, while the original bloom water samples were (Edvardsen, 1993; Meldahl *et al.*, 1994). Axenic cultures of *Pfiesteria* may be difficult to achieve due to its heterotrophism, however, attempts to establish bacteria-free cultures of *Pfiesteria* should be investigated and further studied as regards toxin production.

4) Determine the unique properties of menhaden that make them so susceptible to the effects of *Pfiesteria* toxins. Menhaden comprise 90 % of the fish in *Pfiesteria*-associated mortalities, although other species in the surroundings may be affected. Ulcerative lesions reported in association with *Pfiesteria* blooms predominate in menhaden and frequently occur in the ventral region near the anus.

5) Develop certified pure cultures of *Pfiesteria* to clearly define the toxic species from the non-toxic, yet similar appearing, species.

6) Develop molecular probes for detecting toxic algae and their toxins in tissues and in the environment. Probes should be capable of distinguishing between toxic and non-toxic stages of the dinoflagellates.

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Table A9.1. Microalgae Exclusive of Cyanobacteria Toxic to Fishes.

Dinoflagellates

Alexandrium monilatum (=Gonyaulax monilata)
Alexandrium tamarense (=Gonyaulax (Protogonyaulax) tamarensis)
Gymnodinium galatheanum
Gymnodinium breve (=Ptychodiscus breve)
Gymnodinium mikimotoi -species complex
  (=G. nagasakiense; Gyrodinium aureolum)
*Gymnodinium pulchellum**
Heterocapsa triquetra (=Peridinium triquetrum)
Pfiesteria piscicida -species complex
Cochlodinium sp.

Rhaphidophytes

Heterosigma akashiwo
Heterosigma (=Chattonella) antiqua
Heterosigma (=Chattonella) marina
Heterosigma (=Chattonella) subsalsa

Prymnesiophytes

Prymnesium calathiferum
Prymnesium parvum
Prymnesium patelliferum
Prymnesium saltans
Chrysochromulina polylepis
Chrysochromulina leadbeateri

Silicoflagellates

Dictyocha speculum (=Distephanus speculum)

Modified from Smayda (1991), Brusle (1995), Hallegraeff (1998), and Johnsen (1998).
* Steidinger *et al.*, 1998b.

ANNEX 10

HOST SPECIFICITY AND PATHOGENICITY OF HERPES-LIKE VIRAL INFECTIONS IN MOLLUSC HATCHERIES, *MARTEILIA* SP FROM *CRASSOSTREA GIGAS* AND THE *HAPLOSPORIDIUM*-LIKE PARASITE IN *OSTREA EDULIS*

T. Renault

1 Herpes-like virus infections in bivalves

The first description of a virus infection in bivalves was reported in adult Eastern oyters, *Crassostrea virginica,* with the detection of particles indicating membership of the *Herpesviridae* (Farley *et al.*, 1972;, Farley, 1978). More recently, in 1991, viruses interpreted as belonging to the *Herpesviridae* were associated with high mortality rates of hatchery-reared larval *Crassostrea gigas* in France (Nicolas *et al.*, 1992) and in New Zealand (Hine *et al.*, 1992). Since 1992 sporadic high mortalities of larval *C. gigas* have been regularly observed in some private French hatcheries, occurring each year during the summer period in association with a herpes-like virus (Renault *et al.*, 1994b). The pathogenicity of the virus has been demonstrated by experimental transmission of the infection to axenic *C. gigas* larvae (Le Deuff *et al.*, 1994). The influence of temperature and genitor origin was also investigated on the viral infection development among larval *C. gigas* (Le Deuff *et al.*, 1996).

Additionally, some mortalities reported among *Ostrea edulis* and *C. gigas* spat in France since 1992 were also associated with detection of a herpes-like virus (Comps and Cochennec, 1993; Renault *et al.*, 1994a). More recently, replication of herpes-like viruses has been observed in *Ostrea angasi* adults in Australia (Hine and Thorne, 1997) and in larval *Tiostrea chilensis* in New Zealand (Hine, 1997; Hine *et al.*, 1998).

Concomitant sporadic high mortalities were reported in France, in May 1994 among batches of larval Pacific oyster, *Crassostrea gigas* and European flat oyster, *Ostrea edulis,* in two hatcheries, and in June and July 1994 among batches of cultured spat of the same species in another shellfish nursery. Electron transmission microscopy revealed the presence of herpes-like virus particles in infected larvae and spat of both oyster species. Viruses observed in diseased larvae and spat of both species are very closely related with respect to ultrastructure and morphogenesis. They have the same structural characteristics, the same cellular localizations with a high tropism for fibroblastic-like cells in all connective tissues and comparable sizes. They are detected both in *Crassostrea gigas* and *Ostrea edulis* larvae and are associated with high mortality rates in both oyster species. The observation of mortality in association with detection of virus particles in one oyster species a few days after appearance in the other underlines the possibility that the herpes-like viruses in *C. gigas* and *O. edulis* could be the same species. Moreover, this seems to suggest that interspecific transmission can occur very efficiently in hatchery and nursery rearing conditions. This possible interspecific transmission was previously investigated and transmission of herpes-like virus to axenic *C. gigas* larvae using material obtained from diseased European flat oyster larvae has been reported (Le Deuff *et al.*, 1994).

In addition, concomitant high mortalities were reported in 1997 and 1998 among *Crassostrea gigas*, *Ruditapes decussatus* and *Ruditapes philppinarum* larvae in private French hatcheries (Renault, 1998). These mortalities were associated with a herpes-like virus in both species using PCR. This result seems to indicate a possible intergeneric transmission of herpes-like virus among bivalve molluscs and underlines the need of futher study on virus diversity. In these last cases, the PCR-based technique used primers designed for specific amplification of herpes-like virus infecting *Crassostrea gigas* larvae. This technique also amplified herpes-like virus DNA from the other infected bivalve species. These results further indicate that the same herpes-like virus is capable of infecting several bivalve species and genera, or that bivalves are infected by very closely related herpes-like viruses.

Herpes-like virus infections in bivalves belonging to the genera *Ruditapes, Ostrea* and *Crassostrea* seem to be ubiquitous and are associated with substantial mortalities (Renault, 1998). These observations highlight the importance of studying virus diversity in order to assess their causative role in bivalve mortalities and to limit their possible economic impact. A breakthrough was achieved in the development of a protocol, based on sucrose gradient density centrifugation, for purifying oyster herpes-like virus particles from fresh infected larval *Crassostrea gigas* (Le Deuff, 1995; Le Deuff and Renault, in press). This advance has served as an appropriate platform for generating molecular biological reagents to diagnose virus infections (Renault *et al.*, 1997).

A study on the diversity of herpes-like viruses infecting bivalves has been started at the IFREMER laboratory in La Tremblade using molecular probes and bioassays of interspecific viral transmission. The first step involves analysis of larval bivalves belonging to different species and originating from different geographical locations in Europe (Project " Research objective mortality in European oysters ", FAIR CT97 9052) for herpes-like viruses and determination of their molecular diversity.

2 Marteiliosis in bivalves

In 1998, DNA-based detection assays for *Marteilia refringens* were developed at the IFREMER laboratory in La Tremblade (France). The 18s RNA gene of *Marteilia refringens* isolated from infected *Mytilus edulis* was sequenced and demonstrated both conserved and non-conserved regions which allowed development of both universal and specific PCR pimers. After alignment of the *Marteilia refringens* rDNA SSU sequence with several eukaryotic organisms, specific primers were designed and used to amplify DNA extracted from *Marteilia refringens*. In order to evaluate the primers' specificity, different DNA preparations were used: uninfected *Mytilus edulis*, uninfected *Ostrea edulis*, *Marteilia refringens*-infected *Mytilus edulis*, *Marteilia refringens* infected *Ostrea edulis* and purified *Marteilia refringens* from infected *Ostrea edulis* and *Mytilus edulis*. A specific primer was identified which only produced the amplicons of expected size from DNAs extracted from *Marteilia*-infected animals and from purified parasites. The specificity of amplified fragments was confirmed by southern blotting using a specific oligoprobe.

For *in situ* hybridisation, a specific probe was designed and succesfully used to detect *Marteilia refringens* on histological slides. Positive reactions were obtained for *Marteilia refringens*-infected *Ostrea edulis* and *Mytilus edulis* and for *Marteilia maurini*-infected *Mytilus galloprovincialis*. These results suggest that the probe sequence may be shared by different species belonging to the *Marteilia* genus or that there is only one *Marteilia* species in Europe which infects several bivalve species. The results do not yet answer the specificity question. However, the sequence used for the probe is generated from the most divergent region of the 18s aligment between *Marteilia* and eukaryotic organisms and the developed molecular tools can be used now in studying *Marteilia* spp. infecting different bivalve species.

3 Haplosporidiosis in bivalves

Haplosporidian parasites are known to cause infections in many species of marine bivalve molluscs. Two species, *Haplosporidium nelsoni* and *H. costalis*, cause extensive mortalities of *Crassostrea virginica* from the east coast of the United States (Andrews, 1966). Haplosporidian parasites have also been found in European flat oysters, *Ostrea edulis* (Van Banning, 1977; Pichot *et al.*, 1979, Vivares *et al.*, 1982; Bachere and Grizel, 1983), Olympia oysters, *O. lurida* (Mix and Sprague, 1974), mussels, *Mytilus edulis* (Taylor, 1966) and Pacific oysters, *C. gigas* (Rosenfield *et al.*, 1966; Katkansky and Warner, 1970; Kern, 1976, Friedman *et al.*, 1991; Comps and Pichot, 1991; Friedman, 1996). For these affected bivalve species, there is no evidence the haplosporidian parasites are responsible for significant mortality similar to those reported among American oysters, *C. virginica*; however, Katkansky and Warner (1991) descibed a heavy haplosopridian infection in the connective tissues of a moribund Pacific oyster.

Haplosporidian parasites have been detected in Pacific oysters from different parts of the world, including Korea (Kern, 1976), Taiwan (Rosenfield *et al.*, 1966), Japan (Friedman *et al.*, 1991) and France (Comps and Pichot, 1991) during routine histological examination for bivalve parasites.

Haplosporidium spp. infect oysters and other bivalves. Until more is known about the identity and biology of these other *Haplosporidium* spp., their presence in any bivalve should be regarded as potentially serious. In France, several reports have been made of *Haplosporidium* sp. in the European flat oyster, *Ostrea edulis* and the Pacific cup oyster, *Crassostrea gigas*, however, the taxonomic affiliation of these Haplosporida and there relationships with *H. nelsoni* remain unclear. In order to clarify the taxonomy of Haplosporida found in European bivalve stocks, molecular probes (*in situ* hybridisation and PCR) will be tested on paraffin-embedded material showing evidence of infection by *Haplosporidium* sp. collected during zoosanitary surveys.

A DNA probe and PCR primers specific for *H. nelsoni* will be used in order to identify *Haplosporidium* spp. infecting different bivalve species in Europe. The DNA probe recognizes an unique 21 base pair (bp) sequence of the *H. nelsoni* small subunit (SSU) rRNA gene and the PCR primers amplify a 565 bp fragment of the same gene (Stokes and Burreson, 1995; Stokes *et al.*, 1995; Burreson, 1996).

4 Development of molecular techniques for diagnosis of bivalve pathogens

The effective control of shellfish diseases needs rapid, reliable and highly sensitive diagnostic tests.

To diagnose bivalve infections, the basic method for examination is still light microscopy. This method is poorly adapted for viral diseases and for light or subclinical infection with parasite agents of significant diseases. In the case of viral infections, other techniques such as transmission electron microscopy are required to clarify the nature of the infectious agent. Both light and electron microscope techniques are time consuming and impractical for epidemiological surveys. In addition, research into virus cytopathogenic effects in cell cultures is impossible at present owing to the lack of bivalve cell lines. Serological methods are also not available owing to the absence of immunoglobulin production in molluscs.

Recent efforts to overcome these problems have led to the development of immunoassay techniques (FAT, ELISA) and nucleic acid-based diagnostic methods (Restriction Enzyme Analysis, *in situ* hybridization, dot blot hybridization, and Polymerase Chain Reaction). These techniques offer the advantages of high sensitivity and high specificity, and diagnostic kits permitting the rapid detection of pathogenic agents in bivalves.

Bearing such developments in mind, these new techniques are expected to find increasing use in routine disease monitoring and treatment programs in aquaculture, in field epidemiology and in efforts to prevent the spread of diseases. Therefore, it is important to develop and standardize rapid diagnostic techniques for major bivalve diseases. The routine use of DNA-based diagnostic techniques is hampered by a number of problems, such as contamination during processing which may result in false positives, or false negatives easily caused by the selection of inappropriate host tissue sources for pathogen detection, or incorrect choice of DNA extraction method, etc. It is important to:

- Review the existing techniques and tools for infection diagnosis among bivalves.
- Evaluate needs for rapid diagnostic techniques for the principal diseases of cultured shellfish.
- Promote research in fields where the need and the use of molecular techniques and tools is likely to have the most significant impact on improving disease diagnosis in molluscs.
- Identify problems related in establishing international standards for protocols and procedures for such tests and make recommendations towards their solution.

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ANNEX 11

DISEASE RISKS FOR WILD AND CULTURED CRUSTACEANS FROM KNOWN PATHOGENS OF PENAEIDS

Sharon MacLean

Shrimp farming in the USA has been growing steadily in states such as Texas, Hawaii and South Carolina showing combined production increases from less than 0.5 million pounds of tails in 1984 to over 4 million pounds in 1993. Although various pathogens affect cultured and wild penaeids, the greatest threat to the continued growth, or simply the continuation, of this industry has been introduction of exotic viruses into USA culture facilities. Four viruses presently most at issue are the Taura Syndrome virus (TSV), White Spot Syndrome virus (WSSV), Yellow Head virus (YHV) and Infectious Hypodermal and Hematopoietic Necrosis virus (IHHNV). Necrotizing hepatopancreatitis, a disease caused by a rickettsia and problematic in the Texas shrimp industry in the past, successfully is being controlled through close monitoring and treatment with oxytetracycline.

The impact of pathogens in culture facilities is well known, however, there is little information on the potential impact of pathogens, exotic or endemic, on wild shrimp populations. Penaeid shrimp are infected by representatives of nearly all the major taxa containing infectious agents (Table A11.1). Wild shrimp are host to a wide array of infectious agents although reports of major losses in wild shrimp stocks due to pathogens are rare. One case is notable. The beginning of severe declines in the wild populations of blue shrimp, *Penaeus stylirostris,* in Mexico, that could not support a commercial harvest for 7 years, was coincident with the onset of disease due to IHHNV infection. IHHNV is endemic in wild penaeids in the Indo-Pacific, Equador and western Panama and some of these species are used in culture in North America. Although the source of the virus in the Mexico epizootic has not been confirmed, the release of infected cultured shrimp is a possible source.

The wild penaeid shrimp species of commercial importance in the USA are the Atlantic white shrimp, *Penaeus setiferus*, the brown shrimp, *Penaeus aztecus*, and the pink shrimp, *Penaeus duorarum*. Although differences in the specific timing and location of life history events exist for these three species, the general life cycle is the same. Adult shrimp reside in the ocean where spawning and development of the young through the mysis stage take place. Mysid stage larvae enter the estuaries and develop through the post-larval stages to juvenile stage. Juveniles migrate out of the estuaries to the ocean for growth and maturation.

Spawning of shrimp in captivity has not been successful, therefore, the shrimp farming industry relies on a supply of post-larval shrimp as seed for culture. In the past, the source of seed was wild post-larvae. To decrease the chances of pathogen introductions into culture facilities, the USA Marine Shrimp Farming Program recently established a nucleus breeding center and quarantine centers to supply specific pathogen-free seed to the commercial industry. Nine viruses and many other pathogens are monitored in broodstock or in seed for commercial use.

The preferred species for culture is *Penaeus vannamei* (Pacific white shrimp) for its fast growth rate and market value. After 2 years of *P. vannamei* cultures being decimated by TSV, South Carolina farmers re-stocked with *P. setiferus.* Following restocking at these facilities, WSSV and YHV occurred for the first time in cultured shrimp in North America. The typical USA shrimp farm stocks 100,000–200,000 post-larvae per hectare and has an average pond covering less than 3 hectares. Ponds are open and usually are located near a source of brackish or saline water, and wastewater from the pond may be recycled back to the source water. Usually effluent is held in ponds to settle before discharge. At the onset of disease, farmers may be required to hold water on their farms until after harvest.

Viruses of concern

Taura Syndrome Virus (TSV) was first reported from Ecuador in 1992 and spread rapidly throughout most of the Americas. It is present in wild post-larvae and adult *P. vannamei* from Central and South American countries, the sources of much of the industry's nauplii, post-larvae and broodstock. Natural infections occur in *P. vannamei, P. stylirostris,* and *P. setiferus*; experimental infections have been produced in post-larvae of *P. setiferus* and juveniles of *P. chinensis*. Although *P. setiferus* juveniles can become infected with TSV, they appear refractory to disease. Post-larval and juvenile *P. duorarum* and *P. aztecus* may also be refractory.

White Spot Syndrome Virus (WSSV) is a complex of baculovirus reported from culture facilities throughout Asia and recently in the USA. The virus has a broad host range and high virulence; no resistance to disease has been reported for the 10 species of shrimp (native Asian and native USA species) tested. Shrimp packing plants located near the affected Texas shrimp farm process shrimp imported from affected Asian areas, and are implicated as the source of virus to the

USA. In South Carolina, WSSV-like virus may have existed in wild stocks and been the source of WSSV disease in captive shrimp. WSSV is unusual from the typical baculovirus in that it has a broad host range and has been found infecting a variety of crustaceans in Asia. Using PCR methods, WSSV-like genetic material has been found in white shrimp, water beetles, grass shrimp, fiddler crabs, blue crabs and stone crabs in South Carolina.

Yellow Head Virus (YHV) is widespread in cultured *P. monodon*, the primary species farmed in Asia. Brackish water shrimp resident in *P. monodon* culture ponds were found infected with YHV. Experimentally YHV caused serious disease in juveniles, but not in the post-larvae, of the American penaeids *P. vannamei, P. stylirostris, P. aztecus, P. duorarum* and *P. setiferus*. YHV generally co-occurs with WSSV in Asia.

Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) is widely present in aquaculture facilities in Asia and the Americas and is presumed to be enzootic in wild penaeids in the Indo-Pacific and Ecuador. It has been found also in wild shrimp in western Panama and western Mexico. Natural infections have been reported in *P. vannamei* and *P. stylirostris* and 6 Asian species of shrimp, and *P. setiferus, P. duorarum* and *P. aztecus* have been infected experimentally. *P. indicus* and *P. merguiensis* appear refractory to IHHN. Survivors of IHHN infections carry the virus for life and transmit it vertically and horizontally.

Risk of disease in wild stocks

The risk of disease depends on the pathogen and the penaeid species of concern. As noted above, the virulence of a pathogen can vary with host species and host life history stage. Although many pathogens have been reported from wild shrimp, no major losses in shrimp stocks have been attributed to them with the exception of the IHHNV infection of blue shrimp in Mexico.

Native species of shrimp may be exposed to pathogens occurring in aquaculture through various pathways, including pond effluents, escapes of infected shrimp, losses during transport to processing facilities, disposal of pond sediment or solid waste, or through infected bait shrimp. Temporal and spatial distribution of dense populations of post-larvae and juvenile shrimp during migrations in/out of the estuaries is important in considering the 'susceptibility' of a wild population to potential exposures. Dilution of pathogens from the source introduction to the wild, dispersion of the hosts, and general condition of hosts are factors that lessen the potential impact of introduced pathogens to wild populations of penaeids.

Processing plants pose a potentially serious problem regarding introduction of disease to wild shrimp. Over 50 % of the shrimp processed in the USA are imported from Asia, where shrimp diseases, particularly viral diseases, are a major problem. In some countries, the practice of harvesting shrimp at the early stages of a disease outbreak increases the chances that shrimp contaminated with viable pathogens will be brought into the USA. Shrimp infected with WSSV, YHV and TSV have been identified in retail stores in the USA, which suggests that processing infected shrimp may be a significant means of introduction of exotic virus to coastal waters. Discharge from processing plants often is directly into adjacent waters and without adequate wastewater treatment, wild shrimp and other crustaceans could be at risk of infection by exotic pathogens.

Other species of crustaceans may be affected by penaeid pathogens. The commercial species of concern include lobsters, blue crabs, non-penaeid shrimp, and bait shrimp. Largely ignored have been the ecologically important species of crustaceans, which also may serve as a source of pathogens to the wild penaeid populations. WSSV has been found broadly distributed among various crustacean species in Asia, and despite limited monitoring, WSS-like virus has been found in several commercially important crustaceans in the USA, the impact of which has not been studied. Local stocks of crustaceans, including penaeids, most likely are adapted to their parasite/viral fauna and the issues arise with introduction of exotic hosts or exotic pathogens.

Other less significant sources of pathogens to wild shrimp include: infected bait shrimp which are live-shipped between bays within a state or across state lines; ship ballast water that has been implicated in the introduction of more than 25 shrimp species into the USA; and research and display aquaria that unwittingly release organisms that may carry pathogens infectious to wild species.

Wild stocks, though, do not appear to be under immediate threat. Considering the recent history of devastating viral disease outbreaks in culture facilities in Texas and South Carolina, it is notable that no major declines in commercial shrimp harvests occurred. However, the role of disease in past years of unexplained fluctuations in shrimp populations is unknown.

Risk of disease in cultured shrimp

The principal risk of disease to cultured shrimp in the USA has been introduction of exotic pathogens, particularly viruses, via infected broodstock or seed. The currently available diagnostic methods and established quarantine periods recommended by the US Marine Shrimp Farming Program have not been totally adequate for detection of viruses, particularly in the important post-larval stages.

Once a pathogen enters the shrimp pond, likely its effect is more rapid and severe than in the natural environment due to crowding and stress of the cultured organisms. The cannibalistic nature of shrimp facilitates the rapid spread of disease as sick and dying shrimp are readily eaten by healthy shrimp. In the wild, sick shrimp are more likely to be eaten by other predators. Also, confinement to a pond system increases contact between pathogens and hosts over that which potentially occurs in the wild.

Solid wastes from processing plants that may handle infected shrimp, may be processed further into shrimp and fish feed at temperatures inadequate to kill pathogens, thereby providing an additional pathway for disease transmission. Discard of solid waste to landfills has also posed a potential problem, as transfer of viable virus by birds from processing dumps has been suspected in outbreaks of TSV in nearby shrimp ponds in Texas. Insects harboring shrimp viruses have been detected and it has been proposed that transfer of exotic shrimp viruses by native water insects is probable in spreading viral disease from pond to pond.

Movement of vehicles, boats, equipment and personnel from infected ponds/farms to uninfected areas can disseminate pathogens. Proper disinfection of equipment and personnel before movement between ponds/farms, and establishment of equipment dedicated for single pond use are management practices that can decrease the risk of disease transmission.

Figure A11.1, taken from the Joint Subcommittee on Aquaculture, summarizes the risks of viral disease in shrimp aquaculture and wild shrimp stocks. Although few other pathogens presently are of primary concern to the shrimp industry, the same risks apply for those pathogens having direct life cycles.

Further Needs

1) More baseline information is needed on the presence and distribution of pathogens in wild stocks of penaeids and non-penaeid species. At issue is whether or not pathogens observed in culture are truly exotic.
2) The significance of data from disease outbreaks in aquaculture needs to be identified in predicting the occurrence of disease in wild populations.
3) Shrimp population models are inadequate to explain the observed variability of wild shrimp populations. Disease, largely ignored in population models of marine resources, may play an unrecognized role in population fluctuations.
4) An evaluation of the significance of shrimp processing plants handling infected product is needed to realistically assess the potential for transfer of infective material into wild stocks and aquaculture facilities via waste effluent.
5) Further research is needed on the role of birds and insects in the transmission of viral diseases from pond to pond, farm to farm, and from processing disposal sites to shrimp farms.
6) Development of more accurate viral detection methods is necessary to diagnose farm outbreaks and for use in field monitoring studies. Recognizing that viral diseases are most threatening to the industry, focus on decreasing the time to develop detection methods when "new" viruses appear is critical. Concurrent with this is the need for certification of diagnostic laboratories.

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Table A11.1

Pathogens of Penaeid Shrimp found in:	Cultured Shrimp	Wild Shrimp
Virus		
Infectious hypodermal and hematopoietic necrosis	x	x
Hepatopancreatic parvovirus	x	x
Baculoviral midgut gland necrosis	x	x
Monodon baculovirus	x	x
Baculovirus penaei	x	x
REO-like III	x	
REO-like IV	x	x
Yellow-head	x	
Lymphoidal parvo-like virus	x	x
Lymphoid organ vacuolization disease	x	
White spot syndrome baculovirus complex	x	x
Taura syndrome	x	x
Rickettsiae	x	x
Bacteria		
Vibrio, Beneckea, Pseudomonas	x	
Mycobacterium	x	
Leucothrix	x	x
Fungi		
Lagenidium; Dermocystidium, Fusarium	x	
Sirolpidium (larvae)	x	
Protozoa		
Zoothamnium, Epistylis	x	x
Balanus		x
Microsporidans (Agmasoma; Ameson; Pleistophora)	x	x
Haplosporidans	x	x
Gregarines (Nematopsis, Cephalolobus)	x	x
Metazoa		
Trypanorhynch cestodes	x	x
Trematode metacercariae		x
Ascaroid nematodes		x

Based in part on: Lightner, D.V. (1996), Overstreet, R.M. and D. Cook (1972).

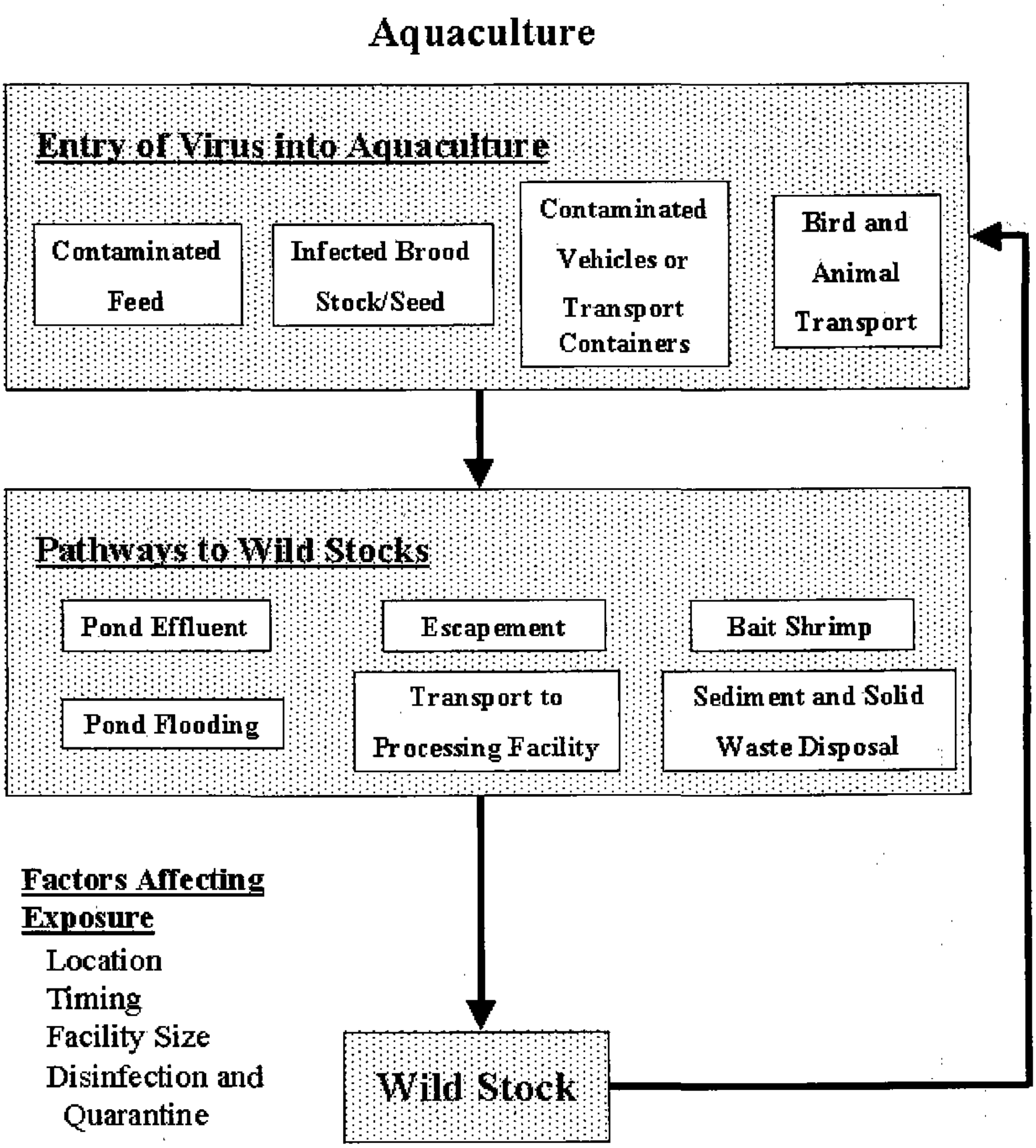

Figure A11.1. Conceptual model: virus sources and pathways for aquaculture (From: Joint Subcommittee on Aquaculture, 1997. An evaluation of potential shrimp virus impacts on cultured shrimp and wild shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters).

ANNEX 12

AVAILABLE INFORMATION ON THE USE OF PARASITES OF MARINE FISH SPECIES AS INDICATOR ORGANISMS FOR ENVIRONMENTAL CHANGES

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Parasites are an integral part of each aquatic community. Besides of their meaning as pathogenes which influence the host mortality (Heckmann *et al.*, 1995; Rohlwing *et al.*, 1998) and their negative impact on aquaculture and fisheries (Berland, 1997; Lester, 1978), parasites are useful tools to indicate, e.g. the host biology (Caira, 1990; Palm *et al.*, 1998) and its systematic position (Rokicki, 1983). The transfer of pathogenic parasite species into formerly uninfected host populations causes problems for the new hosts (Heckmann *et al.*, 1995), and might also have negative effects on fisheries (Chubb and Yeomans, 1995). Parasites were also used as biological indicators for host migration (Kabata *et al.*, 1987; MacKenzie, 1985) and stock separation (Khan and Tuck, 1995; MacKenzie, 1990; Moser and Hsieh, 1992; Pascual and Hochberg, 1996). MacKenzie and Abaunza (1998) summarised the procedures and methods for stock discimination of marine fish.

The presence of parasites within the environment often becomes evident after a mass development causing clinical signs or leading to mortality of infested hosts. Such a situation can be combined with biotic or abiotic changes in the environment (Möller, 1987). The knowledge on the biology of the parasite and their host(s), the host-parasite relationship and the environmental situation can help to detect such environmental changes. Long-living species (digenean trematodes, cestodes, nematodes) can give information on seasonal migrations of their hosts and migration habits of different age groups (feeding area/spawning area). Short-living species combined with a direct life cycle and high reproduction rates (protozoan ectoparasites, monogenean trematodes) can give information on the environmental conditions of the host (Lester, 1990).

Some free-living ciliated Protozoa (Boikova, 1990) as well as acanthocephalans, which specifically accumulate certain heavy metals in a higher amount than their host, were discussed as biological indicators of heavy metal pollution (Sures and Taraschewski, 1995). In marine coastal areas, human activities directly have influence on living communities, which can result in a heavier parasite-infestation compared to less-polluted sites (Skinner, 1982). However, ecological aspects of infestations by parasites and its relationship to environmental factors, such as fish-farming, industrial and communal wastewater and influx of fertilisers from agriculture, are presently at the beginning of being better understood.

Pollution indicators

Protozoa

1) Mobiline peritrichous ciliates (genus *Trichodina*) have been experimentally examined for their function as biological indicators of water quality (Voigt, 1993).
2) Mobiline peritrichs were reported as indicators for petroleum hydrocarbons (Khan and Thulin, 1991). Oil-contaminated sediment caused an increasing infestation with *Trichodina* sp. in *Myoxocephalus octodecemspinosus* after an exposure over a time of 3 and 6 months.
3) *Trichodina cottidarum* and *T. saintjohnsi* infested crude oil-exposed *Myoxocephalus octodecemspinosus* in laboratory experiments as well as at a oil receiving terminal in a significantly higher intensity as compared to uncontaminated control experiments/sites. Pulp and paper mill effluents showed similar effects on these trichodinid ciliates of *M. octodecemspinosus* (Khan, 1990; Khan *et al.*, 1993).

Metazoa

- The effect of crude oils on the gastrointestinal parasites of *Pseudopleuronectes americanus* (*Steringophporus furciger*; Trematoda) and *Gadus morhua* (*Echinorhynchus gadi*; Acanthocephala) resulted in a lower infection compared to control groups. Especially water-soluble fractions of the crude oil are discussed causing this effect (Khan and Kiceniuk, 1983; Khan, 1987)
- Petroleum aromatic hydrocarbons are known to cause lesions and hyperplaisia of secondary gill lamellae. Long-time exposure of cod to aromatic hydrocarbons (max. 30 weeks) caused increasing infestation with monogenetic

flatworms (prevalence as well as intensity), which was attributed to the already damaged gill tissue, and therefore to the better living conditions for monogenetic trematodes (Khan and Kiceniuk, 1988).

- The occurrence of heteroxenous parasites in an area affected by pollution can be related to the number of intermediate hosts at the studied sites (Liao Xinghua, 1987; Overstreet *et al.*, 1996). The intermediate hosts may react more sensitively to environmental changes than the parasite, which in case of endoparasites is buffered from the environment by the host's physiology (Paperna and Overstreet, 1981).

- The parasites and diseases of wild fish species from a lake in Greece were studied over two long periods (1984–1990 and 1994–1997) (Athanassopoulou and Ragias, 1998). The study concluded that the pollution in the lake increased together with the prevalence, intensity and pathology of most parasites. Tumors and bacterial infections were directly associated with increased pollution, especially industrial and sewage waste values.

- PAH caused increasing prevalences of *Ceratomyxa acadiensis* (Myxosporidia), parasitising in the gall bladder of winter flounder, *Pseudopleuronectes americanus* (Khan 1986 cited in Khan and Thulin, 1991).

- The parasite infrapopulations can be affected by changes of the host physiology and substances accumulated with the host's food. Studies of Read (1951) and Roberts (1961) have shown the decrease of weight, total tissue carbohydrate and reduced numbers of mature and gravid proglottids in adult *Hymenolepis diminuta*, after the hosts were exposed to a low carbohydrate diet (cited in Esch *et al.*, 1975).

- Adults of the acanthocephalans *Pomphorynchus laevis* and *Paratenuisentis ambiguus* accumulate lead and cadmium in a greater amount than the hosts (*Anguilla anguilla, Leuciscus cephalus, Perca fluviatilis*). Adults of *Acanthocephalus lucii* accumulated Cd in higher amounts than the larvae (Sures *et al.*, 1994)

- Lead and cadmium are concentrated in a significantly higher amount in the tissues of the cestode *Monobothrium wageneri* in comparison to their fish host tissues (*Tinca tinca* from Ruhr River). The marine cestode *Bothriocephalus scorpii* (Cestoda) from *Scophtalmus maximus* (Gdansk Bay) was found to accumulate these heavy metals especially in the posterior part of the proglottids. The anterior part revealed same amounts of the heavy metals than the fish host tissues (Sures *et al.*, 1997)

- Experimental exposure to sublethal concentrations of zinc and benzene in combination with larval *Anisakis* sp. infestations cause alteration of the liver-somatic index, decreased haematocrit values and higher mortalities in juvenile striped bass, whereas the pollutants alone affected the fish to a significantly lower extent (Sakanari *et al.*, 1984).

- Petroleum aromatic hydrocarbons (in combination with infestations of glochidia of *Anodonta oregonensis*) caused mortalities of *Oncorhynchus kisutch* fry. Susceptibility to the pollutants increased linearly with the parasitation. Fry parasitized with 20–35 glochidia were significantly more sensitive to these toxicants than uninfected fish (Moles, 1980; Rice *et al.*, 1977 in Khan and Thulin, 1991).

Eutrophication indicators

Protozoa

- A combination of eutrophication and rise of temperature caused epizootic outbreaks of the facultative episymbiontic *Epistylis* sp. and *Aeromonas hydrophila* (A-E complex) in centrarchid fish in some freshwater reservoirs in North Carolina, USA (Esch *et al.*, 1976).
- Sessiline peritrichous ciliates were discussed as biological indicators for water quality by Rustige and Mannesmann (1994).
- The prevalence of infestation with trichodinid ciliates of *G. morhua* from sampling sites in western Baltic Sea was significantly higher in *G. morhua* inhabiting the Kiel Fjord as compared to *G. morhua* from Kiel Bight. The eutrophic state and therefore the high total bacterial number within the Kiel Fjord is attributed to cause the effect, as peritrichous ciliates are primarily filter feeders on small algae and bacteria (Palm and Dobberstein, in press; abstract see below).

Metazoa

- *Trematomus bernacchi* (Nototheniidae) from the eutrophic east and oligitrophic west sides of McMurdo Sound (Antarctica) were differently infested with endoparasitic helminth species. Fish from eutrophic localities revealed high prevalences of *Echinorhynchus* sp., *Ascarophis nototheniae*, *Dinosoma* sp. and *Phyllobothrium* sp. Investigations on the abundance of arthropods at these two localities showed that these are more abundant at the eutrophic east side of McMurdo Sound. The intermediate hosts of these parasites trigger their prevalence in fish (Moser and Cowen, 1991).

- Parasites of piscean and other hosts were used as eutrophication indicators in the Baltic Sea (Reimer, 1995).
- The different infestation of gobiid fish from different sampling sites in the western Baltic Sea with helminth parasites was associated with the eutrophication level, influencing the abundance of herbivorous intermediate hosts (Zander and Kesting, 1996, 1998).

General articles

MacKenzie (1983), Lester (1990), Khan and Thulin (1991), MacKenzie *et al.* (1995), Lafferty (1997).

The published works suggest that in freshwater and marine environments, parasites can be used as indicators for different pollution or eutrophic situations. However, it has to be kept in mind that a proper selection of useful parasites is a prerequisite. Lafferty (1997) stated that the different environmental factors differed in their effects and parasites differed in their responses. For example, eutrophication increased parasitism while heavy metals and unspecified human disturbances reduced parasitism. Ciliates responded positively to impacts, while digenes responded negatively. Consequently, MacKenzie (1983) proposed 6 criteria to be fulfilled for useful indicator organisms: 1) being differently abundant in various localities; 2) attached to the host in a number that is unlikely to be removed within the short period until examination; 3) easily collected; 4) easily identifiable to the genus level; 5) ability to react directly on changing environmental conditions; and 6) not being pathogenic in free-living host populations.

Kennedy (1997) considered the usefulness of eel parasites in a freshwater environment to explain the observed environmental changes. However, he stated that in his case the helminth communities provide no clear indication of the nature of these environmental changes. This contrasts with his results in an earlier study (Kennedy *et al.*, 1994). Summarising the above, protozoan and metazoan parasites are useful indicators to detect environmental changes. The selection of useful hosts and parasites enables the detection on large- and small-scale levels. Though several open questions still remain, especially due to the absence of controls, parasites are useful indicators for environmental changes and should be included into regular monitoring programs to get access to long data sets. In combination with other indicator parameters, parasites will enable a better estimate of the degree of change and its possible reasons.

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Occurrence of trichodinid ciliates (Peritricha: Urceolariidae) in Kiel Fjord (Baltic Sea) and its possible use as a biological indicator

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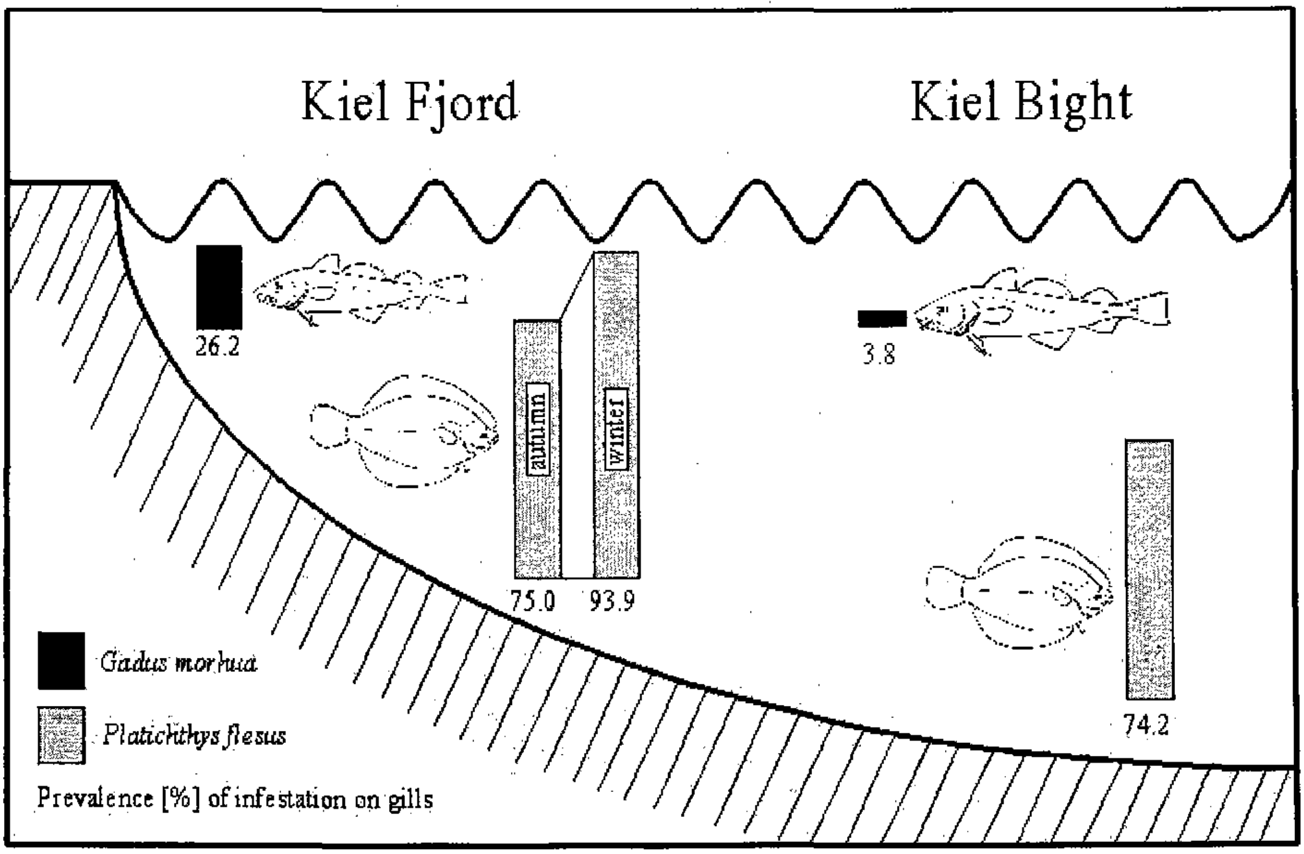
PARASITOLOGY RESEARCH: in press

Abstract

Investigations on the occurrence of trichodinid ciliates from fish caught in the Kiel Bight and Kiel Fjord (western Baltic Sea) were carried out between September 1996 and March 1997. Smears of the gills, fins and the skin of 120 *G. morhua* and 92 *P. flesus* caught by fish traps and trammel nets revealed the presence of trichodinid ciliates. According to fish species and locality different prevalences and intensities of trichodinid ciliates were found. Fish caught in the Kiel Bight revealed a lower prevalence of trichodinid ciliates on the gills (*Platichthys flesus*: 74.2%; *Gadus morhua*: 3.8%) in comparison to fish of the same species and size caught in Kiel Fjord (*P. flesus*: 75.0%; *G. morhua*: 26.2%). In both areas *P. flesus* was more heavily infested than *G. morhua*. Seasonal changes in the prevalence of infestation of *P. flesus* between autumn and winter in Kiel Fjord are proposed to be linked to an increasing bacterial biomass during winter. The fish ecology in combination with the total bacterial number in the fish environment is discussed as an important factor influencing the abundance of trichodinid ciliates. The present data suppose to use trichodinid ciliates as indicator for eutrophication situations in brackish-water environments (see below).



Scheme on the dependence of marine *Trichodina* spp. on host ecology and environment. A host of trichodinids, which, according to its ecology, lives in a bacteria-rich environment, is infested to a higher amount with trichodinids than a host living in the relatively bacteria-free water column. This can explain the nearly identical gill infestation of *P. flesus* from Kiel Bight and Kiel Fjord. In contrast *G. morhua* from Kiel Bight, which only temporarily feeds on benthic organisms but also feeds in the pelagic environment, consequently harbours a smaller trichodinid burden.

ANNEX 13

BIOLOGICAL EFFECTS QUALITY ASSURANCE IN MONITORING PROGRAMMES (BEQUALM)

Steve Feist

At the 1998 meeting of the WGPDMO background information on the proposal for the development of a training and intercalibration programme for the diagnosis of histological liver lesions as part of a quality assurance scheme was provided. This scheme became incorporated into a European Commission application for funding within the ***Standards, Measurement and Testing (SMT) Programme****, under the title '****Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)****'. This application for funding was accepted by the Commission and the BEQUALM project officially started on 1 November 1998.*

1) The first meeting of the BEQUALM Central Steering Group took place at the European Commission on 5 November, 1998 (list of participants attached for information). In addition to the externally visible fish disease and liver pathology component (WP6) the overall programme incorporates eight further work programmes as indicated below:

WP1. Water and sediment bioassays (CEFAS; John Thain)
WP2. Metallothionein and ALA-D (NIVA; Ketil Hylland)
WP3. DNA adducts (ITM; Lennart Balk)
WP4. P4501A and imposex/intersex (FRS; Ian Davies - presented by Dave Wells)
WP5. Lysosomal stability (PML; Dave Lowe)
WP6. External fish disease and liver pathology (CEFAS; Steve Feist)
WP7. Fish reproductive success (SNBF; Olof Sandström)
WP8. Chlorophyll-*a* and Phytoplankton assemblages (CAU; Franciscus Colijn)
WP9. Benthic communities (IfM; Heye Rumohr)

For each component the list of laboratories participating in the QA programmes are to be based on the following priorities:

1) Laboratories with a commitment to OSPARCOM, HELCOM, MEDPOL etc.
2) Laboratories that are linked to National Institutes
3) Others.

General principles on which the collaboration with participating laboratories are to be based are that budget for their travelling and subsistence should be kept as low as possible and that analytical costs will not be reimbursed. The next Steering Group meeting is scheduled for 7 May 1999, to be held at the CEFAS Weymouth Laboratory, UK.

2) The outlines of the work programme for WP6 were provided at the 1998 WGPDMO meeting in Gydnia, Poland. For the first year the milestones consisted of:

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

The major task for 1999 is the organisation and implementation of the first workshop (the second occurring in year 3 of the project). The list of participating laboratories and details of the workshop programme have yet to be finalised. However, the provisional list of participating laboratories is expected to be based on those represented at the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants which was held at CEFAS Weymouth in 1996 (see below) (ICES, 1997). It is anticipated that additional laboratories will wish to become involved as greater awareness of the BEQUALM project is achieved through the publication of information in the QUASIMEME Bulletin and at the Website (http://www.cefas.co.uk/bequalm).

The workshop is proposed to be held during October 1999 and to occupy three days in total. The basic aims of this workshop are as indicated in c) above. Initial progress has already been made in the preparation of materials and in the development of diagnostic criteria for histopathological lesions in dab and flounder livers. These have been based primarily on those agreed at the 1996 ICES Special Meeting at CEFAS Weymouth. Representative specimens have been identified for the majority of important lesion categories for biological effects monitoring and images from these specimens have been archived for possible eventual inclusion in the proposed Atlas of flatfish liver histopathology as one of the major outputs of WP6. In addition, a manuscript on techniques to be used in studies on fish liver histopathology is in preparation for publication in the ICES TIMES series, with S.W. Feist, T. Lang and A. Kohler as co-authors.

Day 1.

Effort will concentrate on the following aspects:

1) For all participants, establishment of current effort and level of familiarity with fish liver histopathology as applied to biological effects monitoring.
2) Preparation of protocols for the identification of externally visible diseases and macroscopic lesions, dissection and sampling of tissues, fixation, histological processing, sectioning and staining of histological sections.
3) Distribution of reference materials, initial training in diagnostic criteria.

Day 2.

1) Training on diagnostic criteria.
2) Define performance limits for diagnostic criteria. This will involve decisions on the detail required for reporting i.e., individual lesion types or categories of lesions.
3) Practical exercises on assessment of material collected from national monitoring programmes

Day 3.

1) Practical exercises as required to complete assessment.
2) Establishment of actions to be taken in cases of disagreement in diagnosis.
3) Agree scope and nature of a ring-test to be undertaken during year 2 of BEQUALM.

Literature cited

ICES. 1997. Report of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants. ICES CM 1997/F:2. 75 pp.

BEQUALM contact addresses and numbers

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Provisional List of Participating Laboratories for the First Bequalm Workshop, October 1999, Cefas Weymouth, UK.

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ANNEX 14

PROPOSED ICES IDENTIFICATION LEAFLETS FOR DISEASES AND PARASITES OF FISH AND SHELLFISH AND THOSE IN PREPARATION

Many authors have not yet been contacted, due to waiting for the Publications Committee approval of the WGPDMO proposed revisions (not received until October 1998) and the Editor's perceived need for further discussion on publication media/format before proceeding. The following will be contacted, along with other proposals, contingent upon clarification of the discussion items noted above.

	Proposed Leaflets - 1998	
	QPX of hard-shell clams (*Mercenaria mercenaria*).	McGladdery, Smolowitz
	Denman Island Disease of Pacific oysters (*Crassostrea gigas*).	Bower
	SPX disease of Japanese scallops (*Patinopecten yessoensis*)	Bower
	Brown Ring Disease of the clam, *Ruditapes decussatus*	Paillard, Ford
	ISA	Hjeltnes
	Haemic neoplasia of soft- and hard-shell clams	Farley, Sunila
	M74	Bylund
	Pancreas Disease	McVicar
	Pseudophyllidean cestodes in marine fish	Palm
	Trypanorhynch cestodes in marine fish	Palm
	Nodavirus	Baudin-Laurencin
	Pfeisteria	MacLean
	Gyrodactylus salaris	McVicar
	Flavobacterium	Dalsgaard
	Flounder Liver Tumours	Feist
	Gonadal neoplasia of hard-shell clams	Barber, Bacon

ANNEX 15

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

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

b) Assess the progress in data submissions to the ICES Fish Disease Data Bank. *An assessment was conducted and the Netherlands and Germany had submitted fish disease data for 1998.*

c) Provide an overview report of data available in ICES Data Banks which may be used for a holistic analysis in relation to disease data. *An overview was provided and an example of analysis of a subset of data extracted from the ICES Data Banks was presented.*

d) Compile and review available information on suitable shellfish species and diseases for which it may be appropriate to submit data to ICES, and on available data in ICES Member Countries. *An overview of available information on suitable shellfish species and diseases was presented.*

e) Maintain an overview of new information on *Ichthyophonus hofer*i and report to ACFM and ACME. *The current status of Ichthyophonus in the North Sea, the Kattegat, the Baltic Sea and the North Atlantic herring stocks was assessed and a report compiled for the presentation to the ACFM and ACME.*

f) Maintain an overview of new information on M-74 and report to ACME and ACFM. *Available information was assessed and a summary report provided.*

g) Review new information on the spread, diagnosis and control of nodavirus to further advise on possible control measures. *Current knowledge on nodavirus was reviewed and a summary of the most recent progress was provided as an Annex to this report.*

h) Compile and review available information on the impact of marine biotoxins produced by dinoflagellates and algae on fish populations to provide a basis for evaluation of the significance and dynamics and future research. *Current knowledge on the impact of marine biotoxins produced by dinoflagellates and algae on fish populations was reviewed and a summary of the most recent knowledge was provided as an Annex to this report.*

i) Clarify the host specificity and pathogenicity of herpes-like viral infections in mollusc hatcheries, *Marteilia* sp from *Crassostrea gigas* and the *Haplosporidium*-like parasite in *Ostrea edulis*. *The host specificity and pathogenicity of the different pathogens to different mollusc species were reviewed and it appeared that there are still gaps of knowledge.*

j) Assess the disease risks for wild and cultured crustaceans from known pathogens of penaeids. *The disease risk and potential sources of the diseases were assessed.*

k) Review available information on the use of parasites of marine fish species as indicator organisms for environmental changes. *A review was conducted and a summary of the most recent knowledge was provided as an Annex to this report. Recommendation made concerning its future inclusion in fish disease monitoring cruises.*

l) Provide a report with advice on new techniques in pathology and other methods for the detection of endocrine disrupting chemicals in marine and estuarine organisms and appropriate new target species representing the main ecological levels of the marine ecosystem. *This item was not thoroughly discussed as the lead person for this TOR was unable to participate in this meeting. The item will be taken up at the 2000 meeting.*

m) Review progress in the development and implementation of a quality assurance programme for fish liver histopathological diagnosis. *A review of the progress in the development and implementation of a quality assurance programme was conducted.*

n) Update information prepared for the HELCOM Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea concerning diseases and parasites of 'Baltic fish stocks, diseases and ecosystem effects' for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994–1998 [HELCOM 1999/3]. *Information on the task on updating the HELCOM report and on the need of feed-back from all members from Baltic countries was presented.*

ANNEX 16

RECOMMENDATIONS TO COUNCIL

Recommendations

The Working Group on Pathology and Diseases of Marine Organisms [WGPDMO] (Chair: Dr S. Mellergaard) recommends that it meet at the University of Bremen, Germany, from the 29 February to 4 March 2000 to:

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

b) update an article on the diseases and parasites of Baltic fish, to be included in the HELCOM Fourth Periodic Assessment. This is being produced intersessionally for review at the 2000 meeting of WGPDMO, and for subsequent consideration by the ACME;

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

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

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

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

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

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

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

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

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

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

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

Justifications for Recommendations to Council

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

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

b) The WGPDMO was requested by the ACME to produce a chapter on diseases and parasites of Baltic fish. This will be incorporated in the HELCOM Fourth Periodic Assessment. According to the time schedule given, the contribution will be prepared intersessionally by T. Lang with support from WGPDMO members from Baltic Sea countries. It should be reviewed at the WGPDMO meeting in the year 2000, and submitted to the ACME for consideration and adoption at its annual meeting.

c) Based on the overview of available ICES data, and the results of the case study presented on the relationship between fish disease prevalences and environmental factors, the WGPDMO considered it promising to continue the statistical analysis of fish disease data, environmental and fishery data. It is expected that the results of an extended study will provide a better insight into possible cause-effect relationships.

d) ICES C.Res 1993/2:23(m) requested that the WGPDMO maintain an overview of the M74 syndrome and the *Ichthyophonus* issue as part of its regular agenda.

e) The causative agent of gill disease in *Crassostrea angulata* in Portugal needs to be investigated in case it is due to an iridovirus, which has serious implications for hybrid *Crassostrea gigas* and *angulata* development and larval *C. gigas.* Summer mortalities of *C. gigas* spat also need to be investigated in case herpes-like virus is involved. Clarification of the *M. refringens* report from *C. gigas* in Spain is necessary due to the EC Directive and OIE listing of *C. gigas* as a non-susceptible species for this parasite. PCR probes developed by IFREMER (La Tremblade) for *Marteilia* spp. should be used to expedite this recommendation.

f) Because of the widespread occurrence of VHS-like virus in the Baltic, North Sea and eastern North Atlantic areas, and the reported association of these viruses with high mortalities on the west coast of North America, it is recommended that information on the possible effects of this disease on cultured and wild fish populations be collated and reviewed by the WGPDMO, in order to give effective advice to ICES and other relevant bodies dealing with fish diseases.

g) Infectious Salmon Anemia (ISA) caused by ISA-virus is a disease of major importance in the cultivation of Atlantic salmon. The increase in the geographic distribution of the ISAV indicates an urgent need to collect and review available information in order to give effective advice to ICES and other relevant bodies dealing with control of this disease.

h) Nodavirus remains a pathogen of major importance in mariculture. In addition to the activities, e.g., the EU-FAIR programme, collaboration of scientists working on the subject must be encouraged to enhance efficient disease control.

i) Initiate experimental work to determine whether the lack of *Bonamia ostreae* infections detected in field observations of *Ostrea edulis* from cold-water countries reflect: i) parasite acquisition with subsequent loss over prolonged low water temperatures, or ii) suppression of infectivity of the parasite. It is important to clarify this in order to accurately assess the disease risks associated with transfers between these countries and enzootic countries.

j) WGPDMO agreed that parasites have the potential to be used as indicators of environmental changes. To incorporate parasitological studies into existing disease monitoring programmes, a proposal has to be developed. For this purpose, long-term data sets already existing in ICES Member Countries need to be compiled and reviewed.

k) The development of a quality assurance programme for fish diseases and liver pathology is essential for monitoring programmes and the WGPDMO needs to maintain an overview of relevant activities in this area.

l) As international marine monitoring organisations (e.g., OSPAR), the European Environment Agency, US EPA and national environment agencies have been requested to consider the relevant recommendations from the EMWAT Workshop held in Veldhoven, The Netherlands in April 1997, WGPDMO should similarly take appropriate action. As the late withdrawal of WG members with the available expertise within this area precluded meaningful discussion of this agenda item it was decided to postpone this item, to the WGPDMO meeting in the year 2000.

m) Maps illustrating the geographical distribution of diseases in fish and shellfish will provide people (e.g., administrators) dealing with disease problems in marine organisms with an opportunity to obtain an easy overview of their distribution. It was therefore decided that disease maps could be the contribution of the WGPDMO to the web-based ICES Environmental Report.