Mariculture Committee **ICES CM 2001/F:02**
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REPORT OF THE

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Santiago de Compostela, Spain
13–17 March 2001

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 OPENING AND STRUCTURE OF THE MEETING

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the University of Santiago de Compostela, Spain with S. Mellergaard as Chair. The meeting was opened at 10.00 on Tuesday 13 March 2001 with the Chair welcoming the participants, particularly the new members who have not previously attended WGPDMO. The Spanish host, J. Barja, welcomed the participants to the University of Santiago de Compostela.

A list of participants is appended in Annex 1.

Apologies were received from O Haenen (Netherlands), V Kadakas (Estonia), A Hellström (Sweden), S McGladdery (Canada), H Grizel (France), Fiona Geoghegan (Ireland), S Helgason (Iceland), W Grygiel (Poland), R Dobberstein (Germany), W Wosniok (Germany), T. Bezgachina (Russia), S des Clers (UK) and S MacLean (USA).

This year only a few shellfish specialists have attended the meeting but contact will be needed during the meeting in order to deal with any recommendations that may be required for consideration at next year's meeting.

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

2 ICES, MARICULTURE COMMITTEE: ITEMS OF RELEVANCE TO WGPDMO

Items of relevance to WGPDMO were highlighted by the Chair.

a) Report of the Mariculture Committee:

   i) accepted the report of the 2000 meeting of WGPDMO and its recommendations without change.

   ii) the Mariculture Committee conducted its first intersessional review of the WGPDMO report in order to get an early release. The WG Report was circulated in the beginning of May and the members of the Mariculture Committee had three weeks to come up with comments. There were a few constructive comments and the Report was released at the end of May.

b) ICES:

   i) the ICES General Secretary circulated the report to the EU Commission, OIE, FAO and PICES, all organisations dealing with regulatory aspects of fish health to rise the awareness that ICES provides advice on a broad range of fish and shellfish health aspects.

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

Terms of Reference

The WGPDMO took note of the Terms of Reference published as C. Res 2000/2F02 (Annex 2). The agenda once again demanded extensive intersessional work by the members of the WGPDMO selected by the Chair. These persons were requested to produce written working/discussion documents to be included in the 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 task which ensures the Terms of Reference can be treated efficiently.

3.1 Adoption of the Agenda

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

3.2 Selection of Rapporteurs

Rapporteurs were accepted as indicated in Annex 4.

4 OTHER RELEVANT INFORMATION

Information was given on a series of scientific conferences to be held in 2001 and early 2002.

- SETAC Europe 11th Annual Meeting, May 2001, Madrid, Spain
- Nordic Society of Fish Immunology, June 2001, Trondheim, Norway
- American Fisheries Society, Fish Health Section, June 2001, Victoria, BC, Canada
- 11th PRIMO, July 2001, Plymouth, UK
- LARVI, August 2001, Gent, Belgium
- EAFP, September 2001, Dublin, Ireland
- ICES Annual Science Conference, September 2001, Oslo, Norway: Theme Sessions:

– Sustainable Development and Conservation of Natural Resources of the Coastal Zone

– Land-based Systems for Commercial Production on Saltwater Aquaculture

- Scandinavian Society for Parasitology, October 2001, Stockholm, Sweden
- SETAC North America 22nd Annual Meeting, November 2001, Baltimore, MD, USA
- National Shellfisheries Association, April 2002, Mystic, Connecticut, USA
- 4th International Conference on Molluscan Shellfish Safety, June 2002, Santiago de Compostela, Spain

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

5.1 Wild Fish Stocks

Viruses

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

Viral haemorrhagic septicaemia virus (VHSV) was isolated for the first time from dead and moribund mummichog (*Fundulus heteroclitus*) and three-spined stickleback (*Gasterosteus aculeatus*) in New Brunswick, Canada. In addition, VHSV was detected in 7 of 150 herring (*Clupea harengus*) sampled in the western Baltic Sea. VHSV was not detected in 400 Pacific herring (*Clupea pallasi*) collected from Prince William Sound, Alaska. VHSV, infectious pancreatic necrosis virus (IPNV), infectious haematopoietic necrosis virus (IHNV) and infectious salmon anaemia virus (ISAV) were not detected in Atlantic mackerel (*Scomber scombus*), Atlantic herring, winter flounder (*Pseudopleuronectes americanus*) and alewife (*Alosa pseudoharengus*) tested as part of a wild fish testing programme.

ISAV was detected by reverse transcriptase polymerase chain reaction (RT-PCR) in wild Atlantic salmon (*Salmo salar*) broodstock collected in Nova Scotia, New Brunswick and Prince Edward Island. Salmon head kidney (SHK) cultures were negative. Neither ISAV nor clinical ISA were detected in freshwater populations of brown trout (*Salmo trutta*) and Atlantic salmon in Scotland.

Salmon Swimbladder Sarcoma Virus (SSSV). PCR testing of 55 Atlantic salmon broodfish from each of the six downeast rivers in Maine showed one or two fish positive from each river, except the Dennys. No clinical signs were detected. It is the first report of asymptomatic carriers.

Lymphocystis in dab (*Limanda limanda*) continued to decrease in prevalence both in the German Bight and the Belgian continental shelf. The highest prevalence in European flounder (*Platichthys flesus*) was found in the southwestern Baltic Sea (35 %).

Bacteria

Little effort was put into screening wild marine fish for bacterial diseases. ***Aeromonas salmonicida*** was observed in 1 % and ***Aeromonas hydrophila*** in 5.3 % of prespawning hunchback salmon (*Oncorhynchus gorbuscha*) suffering sporadic furunculosis at Sakhalin, Russia (Pacific Region). This may be linked to prespawning mortality recently observed in North American *Oncorhynchus* spp.

Toxic Algae

Pfiesteria piscicida was detected using a gene probe in only 12 of 486 water samples from rivers draining into Chesapeake Bay, an area in which fish dermal ulcers had previously been attributed to *P. piscicida*. A highly significant relationship between the presence of dermal ulcers and reduced immunoglobulin levels was observed in a study of 315 menhaden (*Brevoortia tyrannus*). This supported other work that showed that the relationship between *P. piscicida* and dermal ulcers was weaker than previously believed.

Parasites

The prevalence of ***Glugea stephani*** in dab on the Belgian continental shelf (15.6 %) was the highest since 1985. This is a continuously increasing trend.

Parvicapsula minibicornis was detected in 95.6 % of mature sockeye salmon (*Oncorhynchus nerka*) from 18 stocks collected at or near spawning grounds in the Fraser River and was associated with prespawning mortality. Thus, the parasite is widespread among all spawning stocks of Fraser River sockeye.

Cryptobia salmositica was associated with significant prespawning mortality in pink salmon (*Oncorhynchus gorbuscha*) from two rivers on Vancouver Island.

Infection of herring in Polish waters with ***Anisakis simplex*** decreased slightly compared with previous years levels of approximately 60 %, but is still high. In the Barents Sea, infection of cod (*Gadus morhua*) with *A. simplex* and *Pseudoterranova decipiens* has decreased.

Gyrodactylus salaris was found in 23.4 % of a sample of approximately 800 Atlantic salmon parr (Baltic stock) occurring again in high density in the River Tornionjoki, a border river between Finland and Sweden. The highest prevalences (60 %) and intensities of the parasite were found in the uppermost parts of the water system near the boundary between Finland and Norway. The proximity of infected parr within a few hundred metres of a Norwegian river was considered to present a risk of infection to susceptible stocks of Norwegian salmon.

Skeletal Deformities. Vertebral malformations, mostly compressions, were detected radiographically in almost 20 % of 129 Atlantic tomcod (*Microgadus tomcod*) from the St. Lawrence River estuary. Vertebral density was reduced in fish with malformations. Levels of contamination with toxaphene, chlorinated pesticides and PCBs did not differ significantly between malformed and control fish.

Liver Nodules. Prevalences in dab, plaice (*Pleuronectes platessa*) and European flounder continued to decrease on the Belgian continental shelf. A similar trend was recorded in dab from the central and southern North Sea. In the northern and northeastern North Sea prevalences were stable. In the Gulf of Finland, prevalence of liver nodules in European flounder has decreased in the last 10 years. Neoplastic and pre-neoplastic lesions and hepatocellular fibrillar inclusions occurred in European flounder from the Mersey and Tyne estuaries. Hepatocellular tumours and a tumour of the exocrine pancreas were found in European flounder in the Estonian area of the Baltic Sea. Proliferative lesions of the exocrine pancreas were observed in flounder in the Gulf of Finland. The risk of hepatocyte hydropic vacuolation in white croaker (*Genyonemus lineatus*) was significantly greater near a sewage outfall in Orange County, California compared to reference sites. Up to 20 % pre-neoplastic and neoplastic lesions were observed in English sole (*Parophrys vetulus*) near an aluminium smelter and a pulp and paper mill in Kitimat Arm, British Columbia. Significant decline in the prevalence of microscopic hepatic lesions in English sole indicates the efficacy of a clean sediment cap placed over contaminated sediments in Eagle Harbor, Washington State six years earlier.

Hyperpigmentation. Prevalence continued to occur in high levels in dab (up to 35 %) from the North Sea. Increasing trends were observed in Cardigan Bay, the German Bight and the western Dogger Bank.

Intersex Condition was observed for the first time in viviparous blennies (*Zoarces viviparus*). The fish were collected from the Tyne estuary.

Acute/Healing Skin Ulcers. Prevalence in North Sea dab was exceptionally low. Prevalence in cod from the Baltic Sea increased up to 18 % and was approaching maximum levels recorded in 1998. Prevalences in cod and European flounder from the Polish Baltic Sea (ICES Subdivision 24) tended to decrease.

5.1.1 Conclusions

1) VHSV was isolated for the first time from mummichog and three-spined stickleback in New Brunswick, Atlantic Canada.
2) ISAV positive RT-PCR results were obtained from non-clinical wild Atlantic salmon collected in Prince Edward Island, Canada. No other detection methods used were ISAV positive, thus, the significance of these findings are being investigated further.
3) A relationship between *P. piscicida* and skin ulcers was further weakened by low occurrence of the algae in previously affected rivers. Undefined factors contributed to reduced immunoglobulin levels in fish with dermal ulcers.
4) *Gyrodactylus salaris*, found in Atlantic salmon parr in the River Tornionjoki, presents a risk to nearby susceptible Norwegian salmon stocks.
5) The prevalences of liver nodules decreased in flatfish from several North Sea areas, the Gulf of Finland and in Eagle Harbor, Washington State, USA.
6) Hyperpigmentation showed an increasing trend in dab from Cardigan Bay and the German Bight.
7) Intersex condition was observed for the first time in viviparous blennies from the Tyne estuary.
8) Prevalences of skin ulcers in cod from the southern Baltic Sea showed an increasing trend.

Recommendation

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

- to be used as an indicator of environmental conditions,
- to assess the impact of disease in wild fish stocks,
- to assess the potential for disease interactions between wild and farmed fish,
- to recognise emerging diseases caused by infectious agents and/or contaminants.

5.2 Farmed Fish

Atlantic salmon, *Salmo salar* - Viruses

Infectious salmon anaemia virus (ISAV). Clinical ISA was diagnosed at 18 sites in Norway in 2000 including one new area in the County of Rogaland. These data suggest a slight increase in the number of recorded outbreaks. However increasing transportation and greater awareness of the disease may be influencing these trends. In Scotland no new cases of ISA or ISAV were recorded in farmed fish during the past 14 months. Increasing mortalities associated with ISA virus were reported in two cages in Nova Scotia, Canada. At the Bay of Fundy aggressive management techniques have improved the overall situation and only a few cases were attributed to ISA. In New Brunswick, Canada field trials of ISA vaccination have not been evaluated. One outbreak has been reported from the Faroe Islands, Denmark. The stock was destroyed with no additional outbreaks.

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

Salmon Pancreas Disease Virus (SPDV). No new trends were reported, but data suggest the number of confirmed cases is declining in Scotland and Norway.

Togavirus continues to be found with no associated disease in USA.

Salmon Swimbladder Sarcoma Virus (SSSV). No further outbreaks have occurred in farmed salmonids in the USA.

Atlantic salmon - Bacteria

***Renibacterium salmoninarum*.** Outbreaks in Scotland and Norway remain low.

Aeromonas salmonicida outbreaks in Norway are generally controlled by vaccination but clinical outbreaks at some sites may be occurring through changes in vaccine components, production problems or improper use. Clinical outbreaks are low in Scottish stocks.

Vibrio anguillarum (*Listonella*), *V. salmonicida, Moritella viscosa* (*Vibrio viscosus*). Reports of *V. salmonicida* and *V. anguillarum* are low in Scotland and Norway. In Norway there is an indication that a vaccine for *M. viscosa* has a positive effect.

Piscirickettsia salmonis continues to be associated with low mortality in net pen reared Atlantic and chinook salmon on the west coast of Vancouver Island, Canada. No new cases were recorded in Norway.

Atlantic salmon - Parasites

Kudoa thyrsites continues to be associated with soft flesh syndrome in net pen reared Atlantic salmon in British Columbia. An increasing trend is suspected.

Paramoeba. No new information was reported.

Lepeophtheirus salmonis. Infestation with sea lice remains a major disease problem in Norway and Scotland. Recently area management schemes including synchronised treatment have been implemented (see separate report).

Non-infectious conditions. In Norway there is a growing concern regarding so-called production problems including cataracts and skeletal deformities in salmon and cod that impact on downgrading at market.

Rainbow trout, *Oncorhynchus mykiss* - Viruses

Viral Haemorrhagic Septicaemia Virus (VHSV). In 2000 clinical VHS was diagnosed in sea-reared rainbow trout from four farms in two areas from Finland. Exclusion orders and destruction of the stock were implemented at 3 farms (see Annex 5). Wild fish are strongly suspected as the source of infection. The testing of Baltic herring from the Finnish coast has been negative.

In Sweden VHS occurred in rainbow trout at a farm previously diagnosed with this disease in 1998. A possible reservoir in the environment was suggested as a possible cause.

Infectious Pancreatic Necrosis Virus (IPNV) was isolated from a trout farm in Mecklenburg-Vorpommern, Germany. A couple of isolations have been reported in Finland, but with no clinical disease.

Rainbow trout – Bacteria

Renibacterium salmoninarum. A few cases have been reported in Finland and Scotland but there is no new trend.

In Denmark approximately 70 % of the cases requiring treatment were attributed to ***Aeromonas salmonicida***. Minor outbreaks not requiring treatment were attributed to ***Yersinia ruckeri*** and ***Vibrio (Listonella) anguillarum***. Vaccination against *Vibrio* (*Listonella*) and *Aeromonas* outbreaks has been successful in Finland and Denmark and consequently the numbers of outbreaks are declining.

In Russia outbreaks involving *Aeromonas* and *Pseudomonas* are reported.

Flavobacterium psychrophilum outbreaks are occurring in sea farms in Finland; prophylaxis as well as therapy are problematic.

Whitefish (*Coregonus lavaretus*) – Bacteria

The main infectious agents affecting this stock in Finland are *Aeromonas salmonicida* and *Vibrio* (*Listonella*) *anguillarum.* The vaccines presently in use for other salmonids are also effective in whitefish.

Whitefish – Parasites

Henneguya zschokkei and ***Triaenophorus crassus*** are emerging problems for whitefish farming in Finland particularly from the food hygienic (aesthetic) point of view, although these parasites are not pathogenic to humans. Infected fish are removed from human consumption.

Turbot (*Scophthalmus maximus*) - Viruses

Herpesvirus scophthalmi has been detected in imported turbot in Norway. In Spain infection of the gills and skin were attributed to a herpesvirus based on histopathological findings. There does not appear to be significant mortality associated with this virus.

Turbot - Bacteria

Flexibacter maritimus outbreaks show an increasing trend in Spain.

Aeromonas salmonicida outbreaks have shown an increasing trend in Spain with conspicuous dermal ulcers.

***Vibrio* spp.** Outbreaks of vibriosis have been observed in Denmark despite vaccination. However, the serotypes of the *Vibrio* isolated were not included in the vaccine.

Halibut (*Hippoglossus hippoglossus*) - Viruses

Infectious Pancreatic Necrosis Virus (IPNV). A high mortality has occurred in fry in Scotland and in one case in Norway.

Nodavirus associated with vacuolating encephalomyopathy and retinopathy (VER) remains a problem in Norwegian Atlantic halibut fry. In Scotland mortality attributed to nodavirus has been recorded.

Sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) - Bacteria

***Pasteurella piscicida* (*Photobacterium damsela*)** is the main disease problem for sea bass and sea bream in Spain.

Sea bass - Parasites

In Spain chronic infestations of the gill by ***Microcotyle***, ***Furnestinia*** and ***Diplectanum*** were reported. ***Myxidium leei*** in adult fish is associated with low mortality. Microsporidia have been reported for the first time in sea bream but in this case there was no mortality.

Cod (*Gadus morhua*) - Viruses

Infectious Pancreatic Necrosis Virus (IPNV). An outbreak has been recorded in Scotland.

Nodavirus outbreak was reported in Scottish farmed stock.

Cod - Bacteria

Vibrio* (*Listonella*) *anguillarum is the main bacterial disease in farmed cod. Good protection is achieved by vaccination, although in semi-intensive production timing of an effective vaccination procedure can be difficult.

Cod - Aetiology unknown

Cod ulcer syndrome was diagnosed in Norwegian broodstock, however the significance is unknown.

5.2.1 Conclusions

1) There are no data available from the ISA vaccine field trials carried out in Canada.

2) ISA has not been recorded in Scotland for 14 months. In Norway outbreaks are reported at similar or slightly increasing levels. In Canada elevated mortalities occur in Nova Scotia with improvements in New Brunswick.
3) The first outbreaks of Viral Haemorrhagic Septicaemia (VHS) occurred in sea-reared rainbow trout in Finland. There is a possible link with wild fish such as herring.
4) Emerging problems relating to new species such as halibut and cod include IPNV and nodavirus.
5) Infections due to *Vibrio* spp. and *Aeromonas* spp. are generally under control through vaccination.

Recommendations

WGPDMO recommends that:

i. monitoring programmes and prevention measures for ISA should continue and data from field vaccination trials should be made available;
ii. continued efforts should be directed towards characterisation of marine and freshwater strains of VHSV strains and their link to pathogenicity.

5.2.1 Analysis by disease or parasite

Bacteria

Nocardia crassostreae (Nocardiosis in *Crassostrea gigas* and *Ostrea edulis*) – no new trends. Infections persist in British Columbia, Canada, but without observed mortality. A PCR detection assay is being developed and validated.

Parasites

Bonamia ostreae in *O. edulis* – no new trends reported except in Spain, where prevalence shows a decreasing trend. The parasite remains present in France, England, The Netherlands, Spain, and Maine, USA, whereas no infections have been found in Scottish, Norwegian, Danish or Canadian European oysters. DNA sequence analysis supports the inclusion of *B. ostrea* as a member of the Haplosporidia, where it had originally been placed and later removed because it does not form spores.

Marteilia refringens in *O. edulis*, *Mytilus edulis*, and *M. galloprovincialis* in Europe – prevalence continues a downward trend that began 10 years ago.

Mickrocytosis mackini (Denman Island Disease of *C. gigas*) is now considered a minor and manageable problem for the British Columbia, Canada, oyster culture industry, but is still a potential threat to expansion of the industry.

Perkinsus spp**.**

P. marinus (Dermo) in *C. virginica*—no new trends. Prevalences remain near 100 % throughout most of its range, from New England through the Gulf of Mexico, USA.

P. atlanticus in *Ruditapes decussates*—no new trends. Prevalences ranged between 30–40 % in Spain, with some associated mortalities, which may also be caused, or aggravated, by other environmental stressors. Another parasite, newly named *Pseudoperkinsus tapetis*, was isolated from several clam species in Galicia, Spain, and described and propagated *in vitro*. This organism shares some morphological characteristics with the genus *Perkinsus* and produces hypnospores when incubated in fluid thioglycollate medium (the standard diagnostic method), raising the possibility that some positive identifications of *P. atlanticus* may be *P. tapetis.*

P. chesapeaki sp. nov. has been recently described in soft shell clams, *Mya arenaria*, in Chesapeake Bay, USA. Prevalence as high as 60 %, with heavy infections, was found in cultured clams in Virginia. Reported mortality of 50 % in older year classes may have been due to *P. chesapeaki*, although the link was not confirmed.

Haplosporidium spp**.**

H. nelsoni (MSX) in *C. virginica*—no new trends. Prevalences remain high (>50 %) in the high salinity regions of Chesapeake Bay, but are low (<30%) to the north and south along the Atlantic coast of the USA. It remains undetected in the Gulf of Mexico.

H. costale (SSO) in *C. virginica*—no new trends. The parasite continues to cause low mortalities in high salinity areas of the northeastern and the mid-Atlantic USA. *Haplosporidium costale* was originally reported as sporulating only in the spring, but has now been confirmed via DNA probes to sporulate in the fall also. Many cases of *H. costale* sporulation also had light to heavy *H. nelsoni* plasmodia present. The *H. costale* was not detected in the original diagnoses. It is not yet clear whether the fall sporulation represents a true change in seasonality or whether this parasite was simply missed in the mixed infections.

Quahog Parasite X (QPX disease in *Mercenaria mercenaria*) – no new trends. The parasite is found in cultured clams, >1 year old, in a few locations from Virginia to Massachusetts, USA, and associated with variable mortality.

Cockle Parasite X (CPX in *Cerastoderma edule*) - no new trends. The parasite remains associated with mortality in Galicia, NW Spain, but deaths may also be due to other causes. This parasite is usually present in cockles with disseminated neoplasia.

Digenean sporocysts resembling ***Prosorhynchus squamatus*** have been found in *M. edulis* in new geographically separated sites in Atlantic Canada, but with no apparent pathogenic consequences. Transfer risk assessments are under way.

Algae

Hematodinium sp. in tanner crabs (*Chionoecetes bairdi*) in southeast Alaska showed no new trends.

Hematodinium sp. in blue crabs (*Callinectes sapidus*) along the Atlantic and Gulf coasts of the USA: A summary of 8 years of data indicates that highest prevalence is in the autumn and in crabs <30 mm carapace width, but not related to sex or molt stage. Hematodinium-like infections were found in gammarid amphipods, xanthid crabs and the green crab, *Carcinus maenus*.

Diseases of Unconfirmed Aetiology

Haemic neoplasia in *Mya arenaria*, first reported from Prince Edward Island, Canada, where it was associated with a complete loss in one bed, appears to be spreading and increasing in prevalence. Results from transmission experiments suggest an infectious agent; however, environmental contaminant aetiology must also be considered in light of significant agricultural run-off in affected areas.

Haemic neoplasia in *Cerastoderma edule* - no new trends. The condition persists at prevalences up to 40 % in Galicia, Spain.

Withering Syndrome of abalone (mostly *Haliotis cracherodii*, occasionally *H. rufescens* and *H. corrugata*) – no new trends. The disease remains enzootic from San Francisco, California, USA south into Mexico. The disease is associated with a Rickettsia-like organism, which is now thought to be the causative agent.

Juvenile Oyster Disease – no new trends. Outbreaks were reported only in Maine, USA, including in two previously undocumented locations. The suspected bacterial agent, CvSP, was present in affected oysters. The disease is no longer considered a major problem to oyster growers in the area previously affected (northeastern USA).

Lobster (*Homarus americanus*) mortality, reported in 1999 in Long Island Sound, USA, did not continue in 2000. The ***Paramoeba*** sp. originally reported as the probable cause of the mortality is still found in lobsters, including those with no apparent signs of distress, and is now considered to be an opportunistic pathogen rather than the primary disease agent. Other factors, including unusually high summer temperatures and high population density probably contributed to the mortality. Additional information is provided in Section8, below.

Lobster shell disease in New England, USA. Surveys of over 80,000 lobsters in inshore waters off Rhode Island between 1996 and 1999 showed a significant annual increase in prevalence, from 1 % in 1996 to 20 % in 1999, with 50 % of ovigerous females being diseased. Shell disease was first noted in offshore lobsters in 1998, where overall prevalences remain less than 1 % (more than 87,000 lobsters examined) but with 5.2 % of ovigerous females affected. Monitoring in Massachusetts has revealed a shift in the complexion of the disease, especially in 1996–97 when the lesions, which had primarily been focal erosions, began to appear as more extensive lesions covering up to 100 % of the shell. In addition, the range of shell disease has expanded from southern into northern Massachusetts waters and now affects smaller lobsters. Despite the expansion and intensification of shell disease, it has not been linked to mortality as the lobsters shed the affected shell during molting.

Giant sea scallop (*Placopecten magellanicus*) mortalities are spreading along the lower north shore of the Gulf of St. Lawrence. A team including linkages to IFREMER La Tremblade, continues to investigate this problem.

Summer Mortality in *C. gigas* in France – no information reported.

Gill disease in *C. angulata* in Portugal – no information reported.

5.2.2 Conclusions

1) No new trends were reported for major molluscan disease agents (*B. ostreae, P. marinus, P. atlanticus, H. nelsoni, H. costale*, Quahog Parasite X, and Cockle Parasite X).
2) The severe and widespread lobster mortalities, reported in 1999 in Long Island Sound, USA, have not continued and the *Paramoeba* sp. originally proposed as the causative agent is now considered an opportunistic pathogen. Unusually high summer temperatures and high population density are likely to have contributed to the die off.
3) Shell lesions in lobsters off the New England, USA coast have been increasing in prevalence and intensity since 1996, and have been expanding northward.
4) Giant sea scallop mortalities of unknown origin are spreading along the lower north shore of the Gulf of St. Lawrence, Canada.

Recommendations

WGPDMO recommends that:

i. Information be obtained on progress in the ongoing investigations of the effect of temperature on *Bonamia ostreae* infection dynamics.
ii. Contact be established with groups working on the relationship between environmental contaminants and shellfish pathology in order to get such groups involved in the WGPDMO work as this field formerly has been poorly covered.
iii. Information be obtained on the EU project "*Marteilia refringens* studies: Molecular systematics and search for the intermediate host of the bivalve mollusc's parasite."

6 REPORT ON PROGRESS IN THE ONGOING INVESTIGATIONS OF THE EFFECT OF TEMPERATURE ON BONAMIA INFECTION DYNAMICS

No information available.

7 EVALUATE AND REPORT ON THE CONFIRMATION OF THE AGENT OF *CRASSOSTREA ANGULATA* GILL DISEASE AND ITS INFECTIVITY TO *CRASSOSTREA GIGAS* AND OTHER OYSTER SPECIES

No information available.

8 REPORT ON THE PROGRESS OF FURTHER INVESTIGATIONS ON THE ROLE OF *PARAMOEBAE* AND OTHER FACTORS IN THE MASS MORTALITY OF LOBSTERS IN LONG ISLAND, USA

High mortalities were reported in lobster, *Homarus americanus*, in the late summer and fall of 1999 in Long Island Sound, USA. Although localised mortalities had occurred in 1990, 1991, 1993, 1997, and 1998, the 1999 mortalities were much more severe and widespread. They were associated with the following conditions and events:

- A dramatic increase in population abundance in the mid-to-late 1990s, which reached a peak in 1998 that was 3-to-4 fold higher than the mean for the previous decade.
- Heavy harvest pressure, as indicated by the deployment of approximately half a million traps, each baited weekly with ~1.5 kg of menhaden.
- Unusually high summer temperatures. The year 1999 was the warmest of the decade and the decade of the 1990s was the warmest on record. *Homarus americanus* is at the southern end of its temperature range in Long Island Sound. This range is set by the highest temperatures, which occur in summer.

- Application of pesticides, specifically methoprene, around the western end of the Sound, where most of the mortalities occurred. This was an effort to kill mosquito vectors of West Nile Virus, which was found for the first time in and around New York City in the summer of 1999 and caused the deaths of several people.
- The passing of Hurricane Floyd in mid-September, which caused heavy rain and runoff. Most of the mortality is thought to have occurred after Floyd.

The following findings were reported from samples of dead and dying lobsters, and of water, from the affected area:

- No unusual concentrations of contaminants (volatile and semi-volatile organics, organo- cholorine pesticides, chlorinated herbicides, and heavy metals) were found in either water or tissue.
- No toxic algae were found.
- Tests for Gaffkemia were negative.
- A *Paramoeba* sp. was found in the tissues (mostly nervous tissues) of dead and dying lobsters; however, no prevalence data are available.

A trawl survey by the State of Connecticut in April–May, 2000 found the following:

- Lobster density had decreased 40 % from the 1998 peak (a decline that began in 1999), but was still the third highest since the survey began in 1986.
- Lobster density was highest in the western end of the sound where mortalities had been greatest the previous summer and where densities and fishing effort are typically highest.

The status as of early 2001 was as follows:

- The *Paramoeba* sp. is still found in lobsters, including those with no signs of distress, but no statistics are available and unusual mortalities have not been reported.
- Summer temperatures in 2000 were at or below average.
- A number of research projects have just been funded to examine hypotheses about causes of the mortality, including pesticides, the *Paramoeba*, high temperature, and low dissolved oxygen (perhaps exacerbated by the large quantities of bait present in traps), and to develop stress-related indices for lobster.

8.1 Conclusion

It is unlikely that the cause of the mortality will ever be known with certainty because deaths have not continued and none of the preliminary data point to a specific aetiology.

9 REVIEW AND ASSESS A PROGRESS REPORT ON THE DEVELOPMENTS AND INTERSESSIONAL ANALYSIS OF ICES FISH DISEASE AND RELATED DATA BANKS AND A DRAFT MANUSCRIPT FOR SUBMISSION TO ICES TIMES SERIES ON THE STATISTICAL METHODS DEVELOPED FOR THE ANALYSIS OF THE DATA IN THE ICES DATA BANKS IN RELATION TO FISH DISEASES

T. Lang presented a report highlighting progress made since the 2000 meeting of WGPDMO with regard to the statistical analysis of ICES fish disease data in relation to other data extracted from the ICES Environmental Data Centre, the ICES Oceanography Data Centre and the ICES Fishery Databank.

A paper (ICES CM 2000/S:12) produced by W. Wosniok (Germany) and co-authors (for title, co-authors and abstract: see Annex 6) was presented at the 2000 ICES Annual Science Conference providing information on the relationship between the prevalence of externally visible diseases of dab (*Limanda limanda*) from the North Sea and a wide range of potentially explanatory factors, including contaminants in water, sediments and biota, nutrients in water, general hydrographic parameters and catch per unit effort as a measure of population density. For this purpose, a previous analysis focusing only on one area in the southeastern North Sea (including the German Bight) was extended to cover an area in the central North Sea (including the Dogger Bank) and another one in the northwestern North Sea (including the Firth of Forth). The time span considered covered up to two decades, depending on the availability of relevant data.

The statistical analysis was carried out by means of logistic modelling, involving a newly applied interpolation technique for missing data, based on a Gaussian kernel smoother and generalised cross-validation and a bootstrap procedure to account for the effects of using interpolated values.

The multivariate analysis revealed a number of significant relationships between the prevalence of the diseases included in the analysis and potentially explanatory factors. However, some shortcomings were identified leading to problems in the analysis and subsequent interpretation of results:

- There is still a striking lack of environmental data, particularly for contaminants, in the ICES Environmental Data Centre. Even if data interpolation is considered feasible, it cannot replace real observed values and, therefore, may introduce a considerable bias and lead to misleading results and interpretation.
- The ICES fish diseases database is not complete because some of the historic data held by ICES Member Countries have not yet been submitted to ICES.
- Many of the potentially explanatory factors included in the analysis were highly correlated, possibly leading to ambiguous or erroneous conclusions. Cause-effect relationships between single factors and disease prevalence might be obscured by strong spurious statistical relationships with other factors that co-vary with the causal factor.

T. Lang provided an overview of the status of the manuscript on statistical methods for the analysis of fish disease prevalence data to be submitted to the ICES TIMES series. The objective of the paper is to give recommendations and guidelines on the use of statistical methodologies which have been used successfully for the analysis of the ICES fish disease data in relation to environmental factors potentially involved in the disease aetiology. It is intended to be written in a way that it can be used both by statisticians and other trained scientists.

A draft manuscript has been produced intersessionally by W. Wosniok and co-authors providing information on:

- Type of data typically generated within fish disease surveys;
- Estimation and statistical comparison of disease prevalences;
- Sample sizes required;
- Logistic models as a general parametric framework for investigation of relationships between the disease prevalence and potentially explanatory factors;
- Length and gender effects;
- Spatial and temporal analyses;
- Definition of standard populations as a means for spatial comparisons;
- General additive models as a nonparametric approach;
- Interpolation techniques.

In the discussion of the report, WGPDMO appreciated the progress made and emphasised that the results of the statistical analyses carried out using the ICES data have suggested that several relationships between disease prevalence and potentially explanatory factors exist and that these are worthy of future study. However, corresponding to concern already expressed, the WGPDMO pointed out that ICES Member Countries should again be encouraged to submit their data to the ICES Environmental Data Centre in order to facilitate a more comprehensive statistical analysis.

In this context, WGPDMO was informed of changes in the structure of the ICES Databanks and of the plans for establishing new data entry programs. These changes aim at a more user-friendly way to handle the data for submission, storage and subsequent analysis.

WGPDMO endorsed the structure and contents of the draft manuscript on statistical methods for the analysis of fish disease data to be submitted to the ICES *Techniques in Marine Environmental Sciences* (TIMES) series. WGPDMO stressed that the article constitutes a valuable documentation of the work undertaken by WGPDMO during the past years and will be useful as a guide for the analysis of results derived from present and future wild fish disease monitoring programmes, but also for the treatment of data obtained in other monitoring programmes.

9.1 Conclusions

WGPDMO appreciated the results of the statistical analysis of the ICES fish disease data carried out intersessionally. It emphasised the importance to continue this work, particularly in the light of the application of a more ecosystem-based approach aiming at an understanding of the relationship between environmental factors and the variation in disease prevalence. A lack of data in the ICES Environmental Data Centre was noted and it was pointed out that ICES should find ways to convince Member Countries to submit their national data.

The WGPDMO endorsed the progress made in the preparation of a draft manuscript on statistical methods for the analysis of fish disease data to be submitted to the ICES TIMES series and agreed to review further progress at the 2002 meeting of WGPDMO.

Recommendations

WGPDMO recommends that:

i. ICES Member Countries should strongly be encouraged to submit their national environmental data to the ICES Environmental Data Centre in order to facilitate a more comprehensive and holistic analysis of ICES fish disease data. An expanded database will also be crucial for more general assessments on the occurrence of biological effects of contaminants in the ICES area.

ii. Progress made in the preparation of the manuscript on statistical methods for the analysis of fish disease data to be submitted to the ICES TIMES series should be reviewed by WGPDMO at its 2002 meeting.

10 REVIEW AND ASSESS AN INTERSESSIONALLY PREPARED REPORT ON THE COMPILATION OF EXISTING DATA ON SPATIAL AND TEMPORAL TRENDS IN THE OCCURRENCE OF SELECTED PARASITES OF WILD FISH AND ON POTENTIAL ENVIRONMENTAL FACTORS OF RELEVANCE FOR THE EXPLANATION OF OBSERVED VARIANCE

At the meetings of WGPDMO in 1999 and 2000, the usefulness of fish parasites as indicators of environmental change was discussed. It was recommended that, before a final assessment can be made, existing national long-term data should be compiled in order to obtain an overview of data series that may be used for such a purpose.

Based on this recommendation, a report was prepared intersessionally and presented by T. Lang on spatial and temporal trends in the occurrence of selected grossly visible parasites of North Sea dab (*Limanda limanda*) and whiting (*Merlangius merlangus*) (Annex 7).

The report contains information on temporal variation in prevalence of the dab parasites *Acanthochondria cornuta* (Crustacea, Copepoda) and *Stephanostomum baccatum* (Digenea) and the whiting parasites *Lernaeocera branchialis*, *Clavella adunca* (both Crustacea, Copepoda), *Diclidophora merlangi* (Monogenea) and *Cryptocotyle lingua* (Digenea) in three areas (German Bight, Dogger Bank, Firth of Forth) for the period 1985–2000. The data were obtained in the course of the German long-term fish disease monitoring programme carried out on an annual or biannual basis. The data presented in the report were intended to be used as examples, from which a thorough assessment of the feasibility of studies on fish parasites as indicators of environmental change could be made.

The analysis of the data set revealed the following results:

- There is evidence that none of the parasites of dab and whiting considered in the study is restricted to certain areas of the North Sea. However, significant differences in prevalence were detected among the areas, indicating the existence of differences in the environmental conditions and/or the structure of the communities of intermediate or definite hosts, affecting the abundance of the parasites. Pronounced examples for this are the high prevalences of *Lernaeocera branchialis* and, to a lesser degree, of *Cryptocotyle lingua* in whiting from the German Bight and the high prevalence of *Stephanostomum baccatum* in dab from the Firth of Forth area.
- Patterns of temporal changes in prevalence did not reveal any clear long-term trends but were significantly different between parasites and areas, indicating that there were no major underlying environmental factors affecting the parasite prevalence in the same way in all areas.
- The mean length of the fish in the samples examined had a major impact on the prevalences of the parasites recorded. For an assessment of spatial and temporal variation, this length-dependence has to be taken into account and further statistical and demographic methods have to be developed in order to achieve this.

In the discussion of the working document, the WGPDMO noted that for most of the parasites considered in the working document, sufficient data series exist for a more thorough analysis of possible effects of environmental change on the occurrence of the parasites. It will be necessary to consider a wide range of host- and site-specific factors, including biotic and abiotic variables. For such an approach, data maintained in the ICES Databanks may be used (e.g., oceanographic, contaminant and stock assessment data). T. Lang informed WGPDMO that the data will be assessed in more detail within a national project and it was agreed that progress made should be reported to WGPDMO at its 2002 meeting as part of the German national report on new disease trends. WGPDMO took note of additional studies carried out in UK estuaries, which incorporate the assessment of spatial and seasonal differences in parasitic fauna in flounder,

viviparous blenny, and sand goby and their effects on the hosts as indicators of environmental change. Results from this study will also be reported to WGPDMO at its 2002 meeting.

WGPDMO expressed its concern that, due to the complexity of the interactions between parasites, their hosts and the environment, it will be difficult to establish clear cause-effect relationships between the occurrence of parasites and environmental factors. It was felt that this particularly will be the case for parasites with complex life cycles, involving different host species, and if large geographical areas are being monitored. The effort and resources needed to obtain a better understanding of the relationships will be enormous and it is doubtful whether such studies can be incorporated in routine monitoring programmes. If parasites are to be included in monitoring programmes, the selection of parasites and host species depends on the objectives of the programme. For regular long-term monitoring covering large areas, conspicuous parasites such as those considered in the working document are suitable since they can be recorded within existing fish disease monitoring programmes without any major additional efforts. For temporally and spatially restricted studies, an assessment of changes of the whole parasitic fauna may be more appropriate.

WGPDMO emphasised that the results of a number of studies clearly have indicated that environmental change may cause changes in the abundance and diversity of parasites of wild fish. However, before regular studies on parasites of wild fish can generally be recommended for monitoring purposes, further targeted research is required.

10.1 Conclusions

The WGPDMO agreed that studies on parasites of marine organisms may be a useful tool for environmental monitoring. However, it was emphasised that the identification of cause-effect relationships in most cases is complicated and requires significant resources. To improve the predictive capacity of parasites as bioindicators, further targeted research is required. This may involve laboratory studies on the susceptibility of individual parasites and their life cycle stages to environmental variables as well as studies on environmental factors that influence the susceptibility of fish to parasites. Furthermore, studies on the distribution of intermediate hosts and their response to environmental change are needed.

Recommendation

WGPDMO recommends that:

i. Multivariate statistical analyses should be carried out combining existing parasite and environmental data series to generate cause-effect hypotheses. These hypotheses should be the subject of targeted research. This could involve laboratory studies on the susceptibility of parasite hosts as well as individual parasites and their life cycle stages to environmental variables and studies on the distribution of intermediate hosts and their response to changes in environmental variables.
ii. Based on existing and developing knowledge, criteria should be carefully defined to select the parasites that can be used as bioindicators of environmental change and the marine fish species suitable for this type of monitoring.

11 REVIEW PROGRESS REPORTS FROM THE BEQUALM WORK PACKAGE "EXTERNAL FISH DISEASES AND LIVER HISTOPATHOLOGY" AND FROM THE EU PROJECT ON NODAVIRUSES AND OTHER RELEVANT INFORMATION TO PROVIDE ADVICE ON EFFECTIVE CONTROL MEASURES

11.1 BEQUALM

S. W. Feist presented a working document (Annex 8) describing the progress made regarding Work Package 6 (WP6) "External Diseases and Liver Histopathology" of the EU-funded BEQUALM project which has entered its final year. The programme aims to establish a European quality assurance framework for biological effects techniques used in environmental monitoring programmes. For the final year the milestones for WP6 consist of:

- Implementation of a full intercalibration programme based on sets of material collected during national monitoring programmes.
- Selection of materials for production of an atlas of common hepatic histopathological lesions.
- Second workshop to be held in early November 2001 at CEFAS Weymouth Laboratory.

S. W. Feist informed WGPDMO that good progress had been made in the collation of reference material, both for the intercalibration programme (ring test) and to fulfil the obligation of the reference laboratory to supply laboratory

reference materials (LRMs) to participants of the BEQUALM programme. WGPDMO noted that there had been a delay in the implementation of the ring test and that this exercise will be given priority in order to complete the analysis of the results prior to the planned workshop to enable a proper assessment of the performance of the participating laboratories.

A preliminary version of a CD-ROM designed for training purposes to encapsulate the procedures and protocols required for sampling and analysis, and reporting of externally visible fish diseases and in particular the assessment of liver histopathology was demonstrated to WGPDMO. This CD-ROM was conceived as a development of the original idea for the atlas of hepatic pathology of flatfish and it was decided during the first BEQUALM Workshop to broaden its scope to include all aspects of fish disease monitoring methodology. The CD-ROM incorporates images selected to complement brief descriptions of the procedures and of the different categories of the routinely monitored diseases, namely lymphocystis, acute and healing ulcerations, epidermal hyperplasia/papilloma and hyperpigmentation. A large section will be devoted to providing representative images of liver histopathology, including the normal structure of the target species according to the criteria established previously through ICES activities and the first BEQUALM Workshop.

S. W. Feist provided a brief outline of the proposed workshop to be held at CEFAS Weymouth in early November 2001. The main activities consist of:

- Evaluation of the ring test results, with review as necessary of the specimens circulated, with all participants.
- Review progress on the development of the CD-ROM and implement changes as required.
- Assessment of material collected during national monitoring programmes.
- Recommendations on the implementation of the quality assurance procedures established under BEQUALM.

11.2 Conclusion

WGPDMO acknowledged the progress made within the BEQUALM Work Package "External Fish Diseases and Liver Histopathology" and endorsed the plans for the workshop. WGPDMO agreed that emphasis should be given to the completion of the training CD-ROM and the evaluation of the intercalibration exercise.

Recommendation

WGPDMO recommends that:

i. the contents of the CD-ROM be made widely available via the BEQUALM website to interested parties in order to facilitate the collection and assessment of external fish disease and liver pathology data in environmental monitoring programmes applying clear quality assurance criteria.
ii. WGPDMO should review the final report of the BEQUALM Work Package 6 "External diseases and liver histopathology".

11.3 Nodavirus

A summary of the progress of the report for the FAIR project "Nodavirus disease of cultured marine fish in Europe" was presented by M. Vigneulle. A list of publications on the project is provided in Annex 9.

Virus characterisation

A total of 40 nodavirus isolates are now available. Strains were isolated from the following cultured fish species: sea bass (*Dicentrarchus labrax*), umbrina (*Umbrina cirrosa*), turbot (*Scophthalmus maximus*), halibut (*Hippoglosus hippoglosus),* Atlantic cod (*Gadus morhua*), Dover sole *(Solea solea*) and sea bream (*Sparus aurata*). Strains from halibut, turbot, Atlantic cod and Dover sole have been recovered for the first time in the UK. The isolation of a nodavirus isolate from asymptomatic carrier sea bream is of interest since this species is cultivated with sea bass, which are highly susceptible to the disease. Sequence data showed that there are at least three different genotypes of nodavirus in Europe: the halibut genotype and two genotypes recovered in sea bass.

Inactivation of nodavirus with chlorine, iodine, and ammonium was found effective. On the other hand, hydrogen peroxide (H_2O_2) treatment was totally ineffective. Nodavirus was more stable in fresh water than in sea water, which suggests that if nodavirus are restricted to marine fish species, release of nodavirus into the freshwater aquatic environment should be avoided.

Diagnosis

The RT-PCR technique was refined to provide a rapid RFLP genotyping assay for nodavirus detection.

Transmission

Comparison of the virulence of two nodavirus strains (Sb1 and Sb2) in sea bass larvae showed that Sb1 was pathogenic whereas Sb2 was not pathogenic. The difference between strains appeared to be correlated to their genetic classification.

So far, no evidence for vertical transmission of nodavirus in sea bass was found by monitoring the virus by ELISA in sexual products or organs from sea bass juveniles.

Sea bass juveniles were more sensitive to nodavirosis at 25 °C than at 20 °C. Turbot was found sensitive to the Sb2 sea bass strain at 20 °C and 25 °C.

The possibility of horizontal transmission from asymptomatic carrier sea bream to healthy sea bass was demonstrated.

Immune response and vaccination

Nodavirus antibodies were detected by ELISA in sea bass at day 6 post infection. In adult sea bass it was found that fish maintained at 13 °C did not developed serum antibodies for 125 days in contrast to fish that were maintained at warm water temperature over 18 °C.

Relevant Norwegian data concerning fish vaccination were presented to the group by B. Hjeltnes. These data are summarised in a recent publication entitled "Immune response to a recombinant capsid protein of SJNNV in turbot and Atlantic halibut and evaluation of a vaccine against SJNNV." by Husgard *et al*., accepted for publication in DAO.

Workshop

A workshop on nodavirus is planned for the 10th International EAFP Conference (Dublin, September 2001).

11.4 Conclusions

1) Infection caused by nodavirus continue to be a disease problem of significance in farmed halibut and sea bass.
2) Due to its wide host range and increasing reported geographical distribution, nodavirus is recognised as a potential problem in the culture of new marine species.
3) Since the ongoing EU project on nodavirus does not focus on control measures, it was difficult to give yet advice on possible control measures.

11.5 Recommendation

WGPDMO recommends that it reviews information from the EAFP workshop in Dublin on nodavirus, the final report from the ongoing EU project on nodavirus and other relevant information to provide advice on control measures.

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

12.1 *Ichthyophonus*

The present information on *Ichthyophonus hoferi* infection in herring indicates a decrease in the prevalence in the Norwegian spring-spawning herring stock. High prevalence (>5 %) was only observed on a few occasions north of 70°N. The general level is in the range of 1–5 %. The infection level in Icelandic herring stocks is negligible. Although limited information was presented there are indications that the infection rate in the North Sea herring is low. In the Skagerrak infection levels up to 6 % have been observed.

The indication of an increasing trend in the *Ichthyophonus* infection in Norwegian spring-spawning herring does not seem to have taken place.

In the Pacific herring in Puget Sound the prevalence was 60-70 %. At 3 months of age 5–6 % are infected.

In Chinook salmon from Yukon River 30 % of the fish sampled were infected by *Ichthyophonus.* The prevalence dropped to 10 % at the terminal spawning time suggesting significant mortality.

12.2 Conclusion

Ichthyophonus infection seems to be causing problems in both Pacific herring and Chinook salmon on the Pacific coast of North America while being of minor importance in the North Sea and North Atlantic region.

12.3 M74

A review report on the distribution and possible cause(s) of the M74 syndrome was presented by G Bylund (Annex 10).

A decreasing trend in M74 prevalence in salmon in most river systems was recorded in 2000 compared to 1999. However, the disease prevalence in most rivers is significantly higher than in 1998 when the prevalence reached a tolerable level. The prevalence of female salmon with 100 % fry mortality in salmon rivers monitored in Finland and Sweden during 2000 varied between 10–45 %. However, these figures are an underestimation as they only include female salmon with 100 % fry mortality. Partial mortality due to M74 occurs on all levels and the real disease prevalence is considerably higher. Moreover, in Sweden decreased thiamine levels were recorded in offspring from apparently normal salmon females in the absence of M74 syndrome.

The prognosis for the 2001 hatch is still preliminary. A Finnish prognosis for the River Tornionjoki indicates an increase in disease prevalence; a Swedish prognosis for River Dalälven indicates a slight decrease. At present there are no verified reports of M74 in salmonids in European areas outside the Baltic Sea. In Iceland however the mortality in turbot fry showing M74 symptoms was significantly reduced using thiamine baths indicating that thiamine deficiency may be a problem in non-salmonids.

Recent research projects in Sweden exposing salmon to thiamine antagonists such as pyrithiamine, oxythiamine and amprolium induced histopathological alterations typical for M74 fry and high fry mortalities. Utilising these models we will be able to study the pathogenesis of M74.

Ongoing research will focus on the role of environmental pollutants and oxidative stress in modulating the kinetics of thiamine in salmon. Exposure of rainbow trout to environmental pollutants in the laboratory activated the antioxidant enzyme systems but had no effect on the thiamine levels of the fish.

Finnish work has focused on the level of thiaminase activity in the prey fish species (sprat and herring) of Baltic salmon as well as on thiaminase activity in the intestinal tract of salmon. It was demonstrated that the concentration of thiaminase in Baltic herring varies on a wide scale. About 30 % of the herring had a thiaminase concentration at the same high level as in the salmon gastrointestinal contents.

Screening for thiaminase-producing or thiaminolytic bacteria in the intestinal tract of salmon and Baltic herring has so far given negative results.

Work is currently being carried out in Finland in order to evaluate the role algae and cyanobacteria blooms may play in the thiamine/thiaminase balance of organisms in the food chain of Baltic salmon.

12.4 Conclusions

1) Although there are fluctuations from year to year, M74 syndrome remains a significant threat to the Baltic salmon population.
2) It is important that scientists are aware that non-salmonids may also show thiamine deficiency, similar to M74 syndrome.

Recommendations

WGPDMO recommends:

to continue to update and maintain an overview on the M74 syndrome and any progress made with identification of possible causes.

WGPDMO regrets that the resources for M74 research have been reduced in Baltic countries and urges responsible authorities to recognise the importance of continued research in this field.

13 REPORT AND ASSESS THE EFFECTIVENESS OF SALMON FARMING MANAGEMENT CONTROL METHODS FOR THE CONTROL OF SEA LICE IN THE DIFFERENT ICES MEMBER COUNTRIES

A working document (Annex 11) on the control of sea lice infestations in Norway was presented to WGPDMO by B. Hjeltnes. Chemical treatment is used in major salmon producing ICES countries like Canada, Scotland and Norway. In order to reduce the infestation load during the period prior to the migration of wild fish to the sea different treatment regimes have been applied such as de-lousing at low infestation levels and organised de-lousing at cold temperatures. Synchronised treatment is to some extent in use in both Scotland and Norway. Wrasse is mostly used in Norway.

13.1 Conclusions

1) Sea lice infestations in farmed salmonids are effectively controlled by chemical treatment.
2) Control methods like de-lousing at low infestation levels, organised de-lousing at cold temperatures and attempts to minimise the number of escapees, are all believed to be important, but their effectiveness has so far been difficult to document in a scientific way.
3) Synchronised de-lousing would be particularly effective in areas with high densities of fish farms.

Recommendation

WGPDMO should review progress on the effectiveness of sea lice treatments in ICES Member Countries.

14 REVIEW AN INTERSESSIONALLY PREPARED DRAFT MANUSCRIPT FOR PUBLICATION IN THE *ICES COOPERATIVE RESEARCH REPORT* SERIES ON IMPORTANT TRENDS IN DISEASE PROBLEMS IN FINFISH AND SHELLFISH CULTURE IN THE ICES AREA DURING THE LAST FIVE YEARS

S. Mellergaard presented a preliminary draft proposal on the structure of the report. In order to standardise the description of the different diseases, WGPDMO agreed on the following template:

- Description of agent;
- Geographical range of distribution;
- Short description of clinical signs;
- Indications on impact/severity on stock level;
- Control/preventive measures;
- Temporal trends;
- Anticipated future trends.

Where appropriate, maps should be used to illustrate the geographical distribution. The table of contents should mainly reflect the disease problems where information on five-year trends exists, but diseases having shown significant impacts on farmed fish stocks – although five years of trend data do not exist – should be listed. In order to bring the content of the report out to a broader audience it was proposed that the report should be published on the ICES website, possibly as a part of the ICES Environmental Status Report. S. Mellergaard will contact the ICES Secretariat on this matter.

S. Mellergaard will coordinate the finalisation of the draft manuscript which will demand engagement of some of the WGPDMO members intersessionally.

Recommendation

WGPDMO recommended that the report on Important Trends in Diseases Occurring in Finfish and Shellfish Culture in the ICES area 1996–2000 should be prepared intersessionally and presented at the forthcoming WGPDMO meeting.

15 EVALUATE THE PROGRESS IN THE INTERSESSIONAL DEVELOPMENT OF MAPS OF MARINE FISH AND SHELLFISH DISEASES AS A CONTRIBUTION TO THE ICES ENVIRONMENTAL STATUS REPORT

At the 2000 meeting of WGPDMO, a contribution to the ICES Environmental Status Report consisting of maps and written information on the distribution and temporal trends of selected significant diseases of marine fish and shellfish species in the ICES area was reviewed and adopted. This report was later reviewed by the ICES ACME and published in the 2000 ACME Report (ICES Coop. Res. Rep., 241: 221–237).

Prior to the 2001 meeting of WGPDMO, the disease report was placed on the ICES website (www.ices.dk/status/fish_and_shellfish_diseases) after some modification of the format by the ICES Communications Officer.

T. Lang presented the web-based disease report and its components to WGPDMO:

- the presentation of temporal trends of regularly monitored externally visible diseases of North Sea dab (*Limanda limanda*) for the period 1993–1997;
- the distribution of VHS-like virus in wild marine fish from the North Sea and Baltic Sea;
- the distribution of the flat oyster (*Ostrea edulis*) parasites *Bonamia ostreae* and *Marteilia refringens* in Europe and North America;
- the distribution of *Perkinsus marinus*, a parasite of the eastern oyster (*Crassostrea virginica*).

He pointed out that WGPDMO was requested by ICES to discuss possibilities to improve the presentation. The following suggestions were made:

- The website should include images showing the diseases/parasites and information on the aetiology of the diseases/parasites and the distribution and significance of their host species.
- The maps should be coloured to be more illustrative.
- A regular update should be made of the maps providing information on the temporal trends of the dab diseases. However, it was agreed that this should be done once the changes in the ICES Environmental Data Centre have been finalised. This would also facilitate the incorporation of historic disease data that have so far not been incorporated in the data analysis.
- A brief description of the statistical methodologies used for the analysis of the disease data should be provided.
- The ICES disease website should be linked to other websites either planned or already under development, such as for BEQUALM, the ICES Fish Disease Leaflets and the WGPDMO report on disease trends in mariculture for the period 1996–2000 (see Section 14, above).

It was agreed that these suggestions will be forwarded to the ICES Communications Officer. WGPDMO nominated S. Mellergaard (mariculture diseases/parasites) and T. Lang (dab diseases) as contact persons for ICES who will collaborate with ICES in order to implement the changes of the website suggested.

Progress made in the next year will be reviewed by the WGPDMO at its 2002 meeting.

15.1 Conclusion

WGPDMO appreciated the progress made in the presentation of disease information as part of the web-based ICES Environmental Status Report and suggested a number of changes to improve the presentation. These will be forwarded to ICES and further developments will be done in collaboration with the ICES Communications Officer and two representatives of WGPDMO, S. Mellergaard and T. Lang.

15.2 Recommendations

WGPDMO recommends that:

i) The ICES Communications Officer take note of the changes to the disease website suggested by WGPDMO and contact be established between the ICES Communications Officer and the representatives of WGPDMO, S. Mellergaard and T. Lang, in order to discuss and implement changes required.

ii) WGPDMO review progress made in the development of the ICES diseases website at its 2002 meeting.

16 ICES DISEASE PUBLICATIONS: DIAGNOSTIC FICHES UPDATE

Only two of 12 fiches recommended for updating and two of 18 proposed new titles have been received, although several are nearing completion. Contact with authors, in September 1999, was not very successful in stimulating them to submit manuscripts, although most confirmed their willingness to contribute to the series. Several new authors were assigned during the WGPDMO meeting.

WGPDMO members felt strongly that the fiches should be posted on the ICES website, with colour photographs, rather than appearing in printed form. Web posting would allow easier and more widespread public access, speedier updating, and linkage to other sites, including the manuscript on 1996–2000 trends in diseases of finfish and shellfish cultured in ICES countries, which is also proposed for the web. All fiches will require updating if they are to be put on the web, if for no other reason than to include colour photographs.

There was agreement that the fiches remain valuable diagnostic aids, even though they may repeat information found in textbooks and other diagnostic manuals. The fiches are good, short summaries and if they appear on the web, will be more readily available to many users than textbooks or other printed material.

The Editor's report is attached as Annex 12.

Recommendations

WGPDMO recommends that:

i) All fiches should be updated if they are to be put on the web, if for no other reason than to include colour photographs.

ii) A complete listing, discussion, and reassignment of fiches should be included as a Term of Reference for the next WGPDMO meeting.

iii) S. Mellergaard should contact the editor concerning changes in titles and authorship of proposed fiches.

17 ANY OTHER BUSINESS

Spanish *Anisakis* investigations

G. Diez gave a presentation of his work dealing with *Anisakis simplex* infestations in the flesh of commercial fish species. The background data are appended as Annex 13.

BEQUALM workshop on "External fish diseases and liver histopathology"

The workshop will be held at CEFAS Weymouth Laboratory, UK. The proposed dates for the workshop are 8–10 November 2001.

Collaboration with international organisations

The distribution of the WGPDMO report to other international organisations working in areas of similar responsibilities, such as OIE, PICES and FAO will continue. The Chair of WGPDMO will endeavour to get the WG report released by ICES by the end of May 2001 and thereafter will notify WG members that the report could then be distributed to interested parties.

Database of fish histopathology

On the request of Dr A. Kohler, Germany, WGPDMO considered proposals for the establishment of a web-based database of histological images based on the collection of Professor H. Kryvi (University of Bergen, Norway). WGPDMO supported the idea and agreed that such a website would be a valuable resource for anyone interested in fish pathology. WGPDMO drew attention to the efforts conducted under the BEQUALM programme, where a collection of

images of flatfish liver histopathology and associated information comprising a "training guide" will be placed on the BEQUALM website (http//www.cefas.co.uk/bequalm). In addition, the "Registry of Aquatic Pathology" (RAP) collection and database held at CEFAS Weymouth Laboratory (UK) and a similar collection at FRS Aberdeen Laboratory are also available for examination by interested parties. The RAP database was demonstrated at the last EAFP Conference in order raise awareness of this collection and Dr S. W. Feist offered to send details as presented at that meeting to Professor Kryvi.

18 PROGRESS WITH TASKS

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

19 FUTURE ACTIVITIES OF WGPDMO

WGPDMO recommended that more ecosystem-based holistic studies on the effects of diseases on wild fish stocks be carried out, involving experts in the fields of epidemiology, fish stock assessment, and reproduction biology. It was felt that there is sufficient expertise available in the ICES Working Groups to carry out such studies and that this kind of work would be a good way to improve the interdisciplinary collaboration between these groups within ICES.

Members of WGPDMO agreed that improvements in the dissemination of information on the activities of the WG were desirable. It was suggested that the presentation of a poster, depicting the key objectives of the WGPDMO and examples of "achievements" at conferences would in part accomplish this. In addition, the production of another more general poster providing basic information on ICES would also be valuable for presentation at meetings.

WGPDMO also suggested that in addition to the full WG reports, a brief summary report of all active Working Groups should be made available on the ICES website.

Since there are several issues of importance in the field of pathology and diseases of marine organisms requiring further consideration, it was agreed that a further meeting of WGPDMO is required in 2002 to consider the results of intersessional work and to discuss outstanding items. It was agreed that the invitation to host the next meeting of the WGPDMO from Dr S. Mellergaard, Danish Institute for Fisheries Research, Denmark, be accepted. The proposed dates are 12–16 March 2002.

APPROVAL OF RECOMMENDATIONS

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

20 APPROVAL OF THE DRAFT WGPDMO REPORT

The report of the 2001 meeting was approved before the end of the meeting and the draft report was circulated to the participants on 17 March. The conclusions on the Terms of Reference and associated Annexes where advice was specifically sought by other ICES bodies would be extracted and sent separately to ICES.

21 CLOSING OF THE MEETING

On behalf of the participants of the 2001 meeting of WGPDMO, the Chair expressed appreciation to J Barja for his hospitality and the excellent facilities provided for WGPDMO at the University of Santiago de Compostela, and for his considerable organisation and support before and during the meeting.

The meeting was closed at 13.00 hrs on 17 March 2001.

ANNEX 1: LIST OF PARTICIPANTS

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*ANNEX 2: TERMS OF **REFERENCE***

ICES C.Res. 2000/2F02

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

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

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

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

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

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

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

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

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

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

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

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

WGPDMO will report by 6 April 2001 for the attention of the Mariculture Committee and ACME.

ANNEX 3: AGENDA

1. Opening of the meeting.
2. ICES ASC 2000; items of relevance to WGPDMO.
3. Terms of Reference, adoption of the agenda, selection of Rapporteurs.
4. Other relevant reports for information.
5. Analyse national reports on new disease trends in wild and cultured fish, molluscs and crustaceans.
6. Report on progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics.
7. Evaluate and report on the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oysters species.
8. Report on the progress of further investigations on the role of paramoebae and other factors in the mass mortality of lobsters in Long Island.
9. Review and assess a progress report on the developments and intersessional analysis of ICES fish disease and related data banks and a draft manuscript for submission to ICES TIMES series on the statistical methods developed for the analysis of the data in the ICES data banks in relation to fish diseases (authors: W. Wosniok et al.);
10. Review and assess an intersessionally prepared report on the compilation of existing data on spatial and temporal trends in the occurrence of selected parasites of wild fish and on potential environmental factors of relevance for the explanation of observed variance.
11. Review progress reports from the BEQUALM Work Package "External Fish Diseases and Liver Histopathology" and from the EU project on nodaviruses and other relevant information to provide advice on effective control measures.
12. Maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome.
13. Report and assess the effectiveness of salmon farming management control methods for the control of sea lice in the different ICES Member Countries.
14. Review an intersessionally prepared draft manuscript for publication in the *ICES Cooperative Research Report* series on important trends in diseases problems in finfish and shellfish culture in the ICES area during the last five years.
15. Evaluate the progress in the intersessional development of maps of marine fish and shellfish diseases as a contribution to the ICES Environmental Status Report.
16. ICES Disease publications. Diagnostic Fiches update.
17. Any other business.
18. Analysis of progress with tasks.
19. Future activity of WGPDMO.
20. Approval of recommendations.
21. Approval of draft WGPDMO Report.
22. Closing of the meeting.

ANNEX 4: RAPPORTEURS

Session(s)	**Rapporteurs**
1-4. Introductory sessions	S.Mellergaard
5. Wild fish Farmed fish Wild shellfish Farmed shellfish	D. Declerck, C. Couillard, S. Jones A. Karasev, D. Bruno, B. Hjeltnes, R. Rahkonen S. Ford, J. Barja, S. Feist
6. report on the progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics;	S. Ford, A. Figueras
7. report on and evaluate the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oysters species;	S. Ford, A. Figueras
8. report on the progress of further investigations on the role of paramoebae and other factors in the mass mortality of lobsters in Long Island;	S. Ford
9. review and assess a progress report on the developments and intersessional analysis of ICES fish disease and related databanks and a draft manuscript for submission to ICES TIMES on the statistical methods;	T. Lang, S. Jones
10. review and assess a report on the compilation of existing data on spatial and temporal trends in the occurrence of selected parasites of wild fish;	T. Lang, G. Bylund, A. Karasev
11. review progress reports from the BEQUALM and from the EU project on nodaviruses;	S. Feist, M. Vigneulle, T. Lang
12. maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome;	D, Bruno, G. Bylund
13. report and assess the effectiveness of salmon farming management control methods for the control of sea lice in the different ICES Member Countries;	B. Hjeltnes, S. Jones
14. evaluate the progress in the intersessional development of maps of marine fish and shellfish diseases as a contribution to the ICES Environmental Status Report.	D. Bruno, T. Lang
15. review draft manuscript on important trends in diseases problems in finfish and shellfish culture in the ICES area during the last five years	S. Mellergaard
16. ICES Disease publications. Diagnostic Fiches update	S. Ford
17-19. AOB, Progress with tasks, Future activities of WGPDMO	S. Feist
20-22. Approval of recommendations and report, closing	S. Mellergaard

ANNEX 5: REPORT ON THE FIRST CASE OF VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS) IN FINLAND

Eija Rimaila-Pärnänen, Sanna Sainmaa, Christine Ek-Kommonen and Hannele Tapiovaara; National Veterinary and Food Research Institute (EELA), Department of Pathology and Field Extension and Department of Virology and Epidemiology

The first case of Viral Haemorrhagic Septicaemia (VHS) was identified in Finland and the virus isolated from farmed rainbow trout in the Kumlinge Island situated in the archipelago of Åland.

At autopsy the macroscopical changes in the fish were those of an acute infection and samples for bacteriology and virology were taken. A severe CPE in the cell lines and a non-specific toxicity was suspected. In the second sample the macroscopical changes were more advanced. The histological findings were typical of VHS. ELISA and IF-staining were also positive for VHS. The isolated virus was sent to the EU reference laboratory in Århus and on 18 May confirmed as VHSV. The official announcement of the first case of VHS in Finland was made on 19 May 2000. The governmental and municipal veterinarian ordered all fish, a total of 30 tonnes to be killed, starting on 25 May. The dead fish were treated with formic acid and covered in earth beds according to municipal practice. The disinfection of the farm began thereafter.

A restriction zone with a radius of 20 km around the infected farm was established. All 15 farms located inside this zone were sampled and investigated by virological testing. These tests were negative. The second VHS outbreak occurred in Pyhtää on the southeastern costal area of Finland, 330 km from Kumlinge. On 24 May a fish farmer sent eight fish to EELA with an anamnesis of 10 % mortality during the winter. The pathological findings at autopsy were identical to the first case of VHS. Histology and VHS-ELISA confirmed the disease three days later. IF-staining was also positive. In this restriction area there were four fish farms owned by three farmers. After sampling all the units a second farm was shown to be positive. In this restriction area over 150 tonnes of fish were destroyed and 20 tonnes slaughtered for consumption. The processing waste and the juvenile fish were transported in tanks to an incineration plant. The farms were disinfected according to instructions of the governmental and municipal veterinarian. All affected farms are prohibited to stock with new fish until the summer 2001.

Following the diagnosis of VHS epidemiological investigations began in both areas. No direct contact had occurred between the two infected areas. Transport of live fish, eggs and gametes from Åland to continental Finland as well as from the coastal area inland has been prohibited since 1987. All the farms that had delivered fingerlings to the positive farms were sampled but were found negative for VHS. Since the source of the VHS infection could not be found it has been suspected that wild fish might have carried the virus. All infected farms had some contact with Baltic herring caught by trawling. In Kumlinge the harbour ships wash nets and the herring packed for consumption are close to the positive farm. Until recently, in the Pyhtää area all affected farms had used semi-moist food made mainly of Baltic herring. In addition, one of the farmers trawls for herring in the Baltic Sea area between Helsinki and Tallinn.

Attempts to isolate virus from wild fish, especially from herring, were initiated as these were suspected to be virus carriers, however no VHSV was isolated from these samples. However, the number of fish investigated is too small to give reliable and conclusive results. Investigations on larger numbers of wild fish are needed and these investigations will continue.

During the summer and early autumn virological investigations were made from every fish disease sample investigated at EELA, but VHSV was not identified. In late November 2000 during EU inspection of the farms still active in the Pyhtää restriction area one farm was found positive for VHSV. This virus was isolated from several pools of the150 fish sampled from the slaughter line and one single fish from a cage

The Finnish VHS isolates are under further characterisation at the EU reference laboratory in Århus, Denmark

ANNEX 6: REVIEW AND ASSESS A PROGRESS REPORT ON THE DEVELOPMENTS AND INTERSESSIONAL ANALYSIS OF ICES FISH DISEASE AND RELATED DATA BANKS AND A DRAFT MANUSCRIPT FOR SUBMISSION TO ICES TIMES SERIES ON THE STATISTICAL METHODS DEVELOPED FOR THE ANALYSIS OF THE DATA IN THE ICES DATA BANKS IN RELATION TO FISH DISEASES

International Council for
the Exploration of the Sea CM 2000/S:12

Theme Session S: Temporal and Spatial Trends in the Distribution of Contaminants and their Biologic Effects in the ICES Area

ANALYSIS OF ICES LONG-TERM DATA ON DISEASES OF NORTH SEA DAB (*LIMANDA LIMANDA*) IN RELATION TO CONTAMINANTS AND OTHER ENVIRONMENTAL FACTORS

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

Abstract

ICES data on the prevalence of grossly visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations) of dab (*Limanda limanda*) submitted by Member Countries were statistically analysed with respect to potential relations with contaminants in water, sediments and biota as well as nutrients, water temperature, salinity, oxygen content and catch per unit effort (CPUE). Data were extracted from the ICES Environmental Data Centre, the ICES Oceanographic Data Centre and the ICES Fishery Databanks. The analysis was carried out for three regions located in the southeastern, central and northwestern North Sea which were selected on the basis of the availability of disease data. The time span considered partly covered almost two decades. Non-parametric interpolation techniques were used to obtain the necessary uniform time pattern for all time series. Parameter estimates and significances within a logistic model for the disease prevalences were calculated by means of a bootstrap procedure which accounted for the need to interpolate within observed time series. A variety of factors, including contaminants, were identified as being significantly related to the disease prevalence. However, depending on area, time range and data availability, different sets of factors were identified. This reflects the multifactorial aetiology of the diseases covered, but can also be attributed to some high correlations among the explaining quantities.

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ANNEX 7: TRENDS IN THE OCCURRENCE OF SELECTED PARASITES OF NORTH SEA DAB (*LIMANDA LIMANDA*) AND WHITING (*MERLANGIUS MERLANGUS*)

by
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Abstract

As a basis for a further evaluation of the usefulness of fish parasites as biological indicators of environmental change, information is provided on trends in prevalence of grossly visible parasites of dab (*Limanda limanda*) and whiting (*Merlangius merlangus*) from three sites in the North Sea (German Bight, Dogger Bank, Firth of Forth) for the period 1985–1999. Dab parasites covered are *Acanthochondria cornuta* (Crustacea, Copepoda) and *Stephanostomum baccatum* (Digenea). Parasites of whiting are *Lernaeocera branchialis*, *Clavella adunca* (both Crustacea, Copepoda), *Diclidophora merlangi* (Monogenea) and *Cryptocotyle lingua* (Digenea).

Introduction

The usefulness of parasites of wild fish as indicators of environmental change has been discussed controversially for a long time. One of the major criticisms has been that the parasite-host relationships are normally complicated, particularly if complex parasite life cycles involving a sequence of intermediate host species from different taxa are encountered, thus making the identification of causes for observed changes in the abundance and diversity of parasites of wild fish difficult. However, there are a number of examples in the literature that indicate that environmental change including anthropogenic impacts (e.g., eutrophication, pollution) may alter the parasite fauna of wild fish.

At its meetings in 1998 and 1999, the ICES WGPDMO reviewed available information on this issue and it was noted that one of the main obstacles for a proper assessment of the usefulness of fish parasites as indicators is the lack of long-term data series. It was, therefore, recommended that, before a more comprehensive assessment can be made, existing long-term data on spatial and temporal trends in the occurrence of selected parasites of wild fish should be compiled.

The present contribution provides information on data gathered in the course of the German long-term monitoring programme on the occurrence of diseases and parasites of wild fish species in the North Sea and adjacent areas, including the Baltic Sea, that is carried out according to ICES standard procedures. This programme started in the late 1970s and largely focuses on grossly visible fish diseases and parasites. Therefore, it does not generate data covering the whole parasitic fauna of single fish species and does not allow more general conclusions on changes in the parasite diversity. Nonetheless, the data obtained constitute to our knowledge one of the few long-term data series on the prevalence and intensity of selected parasites, covering almost a period of two decades for certain geographical areas in the North Sea (e.g., the German Bight and the Dogger Bank).

The aim of the working document is to show temporal and spatial trends for macroscopically and externally visible parasites of the common dab (*Limanda limanda*) (*Acanthochondria cornuta*, Crustacea: Copepoda; *Stephanostomum baccatum*, Digenea) and the whiting (*Merlangius merlangus*) (*Lernaeocera branchialis*, *Clavella adunca*, Crustacea: Copepoda; *Diclidophora merlangi*, Monogenea; *Cryptocotyle lingua*, Digenea) both of which belong to the most abundant and widespread fish species in the North Sea. The data presented are intended to be used as examples, based on which a more thorough assessment of the feasibility of studies on fish parasites as indicators of environmental change can be made.

Material and methods

Fish were collected during the regular German fish disease monitoring programme carried out by the Federal Research Centre for Fishery by means of bottom trawling using standard equipment and procedures. Dab and whiting were inspected macroscopically on-board the research vessels directly after sorting of the catches for the presence of externally visible parasites in the mouth, the gill chambers, on the gills and on the whole external surface including the fins (see Table A7.1). Methodologies applied were according to ICES standard procedures for fish disease surveys.

Table A7.1. Parasites of dab and whiting considered

Fish species	Parasite species	Taxonomy	Appearance
Dab (*Limanda limanda*)	*Acanthochondria cornuta*	Crustacea, Copepoda	White/yellow females in the gill cavity, < 10 mm
	Stephanostomum baccatum	Digenea	White encysted metacercariae in the skin, < 2 mm
Whiting (*Merlangius merlangus*)	*Lernaeocera branchialis*	Crustacea, Copepoda	Red/brown females in the gill cavity, < 20 mm
	Clavella adunca	Crustacea, Copepoda	White/yellow females in the gill cavity, mouth, fins, anus, < 5 mm
	Diclidophora merlangi	Monogenea	Brown parasites between the gill filaments, < 5 mm
	Cryptocotyle lingua	Digenea	Black spots on the body surface, < 1 mm

The data used for the present compilation are from the period 1985–1999 and are restricted to three representative sampling areas: German Bight (N01), Dogger Bank (N04) and Firth of Forth (N06). For most years, sampling was carried out in winter (December/January) and spring/summer (May–July). However, during the most recent years the sampling was restricted to winter cruises. In addition to the data on the prevalence of the parasites, information is provided on the mean size (cm below) of the fish samples examined. The statistical analysis of the data was done by means of logistic regression analysis.

Results and Discussion

Figure A7.1 gives the location of the threes sampling sites in the North Sea (N01: German Bight, N04: Dogger Bank, and N06: Firth of Forth) considered in this paper.

Parasites of dab (*Limanda limanda*)

In Figures A7.2a and A7.2b, changes in the prevalence of parasites of dab are shown, together with changes in the mean size of the fish examined.

The prevalence of *Acanthochondria cornuta* (Figure A7.2a) did not differ considerably between areas, but the differences were still highly significant ($p < 0.001$). Although the data indicate a generally increasing trend for all areas, the patterns of the temporal changes recorded were significantly different ($p < 0.001$) between areas, indicating that there was no general North-Sea-wide underlying factor triggering the prevalence. In all three areas, the mean size of the dab examined had a significant positive effect on the prevalence ($p < 0.001$). However, there was also a significant effect of the sampling time, indicating that the temporal changes in prevalence observed in the areas were not only due to changes in the mean size of the fish inspected.

For *Stephanostomum baccatum* (Figure A7.2b), which is by far the most frequent externally visible parasite of dab in the North Sea, the graphs indicate consistently highest prevalences in the Firth of Forth, followed by the Dogger Bank and the German Bight. These differences were statistically significant ($p < 0.001$). When comparing the graphs, some common temporal features seem to exist. In the beginning of the study, the prevalences increased in all areas until 1991/1992 and decreased thereafter, however on different levels. The most pronounced temporal effect was seen in the German Bight, where the prevalence dropped significantly from 35 % to 10 % within a few years and remained on a constantly low level thereafter The statistical analysis nevertheless revelaed that the patterns of the temporal changes in prevalence recorded differed significantly between areas ($p < 0.001$). The mean size of the fish had a significant effect on the prevalence only at the Dogger Bank (positive effect, $p < 0.001$) and in the Firth of Forth (negative effect, $p < 0.001$), but not in the German Bight. As for *Acanthochondria cornuta*, there was a significant site-specific effect of the time of sampling in all three areas, meaning that only parts of the temporal changes in prevalence could be explained by changes in mean size of the fish inspected.

A comparison between the prevalence of both parasites revealed for all areas that, despite the significant effects of size, both the prevalences and their temporal changes were significantly different, indicating that the temporal changes recorded occurred independently from each other.

Parasites of whiting (*Merlangius merlangus*)

Figures A7.3a to A7.3d show prevalence data for parasites recorded in whiting together with data on changes in the mean length of the specimens examined.

The highest prevalences of *Lernaeocera branchialis* were consistently found in the German Bight (Figure A7.3a). In certain years, maximum values of more than 40 % were recorded. Differences between all three areas were significant ($p < 0.001$). Apart from an increase in prevalence in the German Bight in the beginning of the 1990s that was followed by a decrease, no clear temporal change in prevalence was recorded. However, the patterns of the temporal changes were also significantly different between the areas. Mean size of the fish examined had a significant positive effect on the prevalence in all areas ($p < 0.001$), but again there were significant effects of the time of sampling ($p < 0.05$) indicating that changes in prevalence were not only driven by changes in mean size of the fish in the samples.

Prevalences of the second parasitic copepod in whiting, *Clavella adunca* (see Figure A7.3b), were generally higher than those of *Lernaeocera branchialis*, but did not differ between areas as much as in *Lernaeocera branchialis*. However, again the prevalences as well as the patterns of the temporal changes were significantly different ($p < 0.001$). In none of the areas could a clear trend be observed; the data from the Dogger Bank and the Firth of Forth indicate a slight increase in prevalence, however. The prevalences in all areas were significantly related to mean length (German Bight and Dogger Bank $p < 0.001$, Firth of Forth $p < 0.05$). This relationship was negative in all areas, meaning that prevalences dropped with increasing size of the fish. As for the other parasites, the observed temporal patterns in prevalence could not solely be explained by the mean length of the fish examined, because there were also significant effects of the sampling time.

The monogenean parasite *Diclidophora merlangi* occurred most frequently at the Dogger Bank and in the Firth of Forth area (see Figure A7.3c), without revealing any temporal trends. Both the prevalences and the patterns in the temporal changes differed significantly ($p < 0.001$) between areas. The mean size of the fish in the samples examined had a significant positive effect on the prevalence ($p < 0.001$), but there also was a significant effect of the time of sampling ($p < 0.001$). From the graphs, there is some indication for the occurrence of a seasonal effect on the prevalence of the parasite. Particularly the data from the Firth of Forth area revealed lower prevalences in winter than in spring/summer. However, a test for statistical significance of this finding was not performed.

Highest prevalences of the digenean parasite *Cryptocotyle lingua* were recorded in the German Bight (see Figure A7. 3d) followed by the Firth of Forth and the Dogger Bank. In the German Bight, the prevalence increased from the end of the 1980s until the middle of the 1990s, but decreased thereafter. An increase was also observed in 1997–1998 in the Firth of Forth area. Prevalences and patterns of the observed temporal changes differed significantly between the areas ($p < 0.001$) and the prevalences in all areas were again significantly and positively related to the mean length of the whiting examined. Again, the mean length alone did not explain the observed changes in prevalence, since the time of sampling had also a significant effect ($p < 0.001$). However, apart from the German Bight ($p < 0.05$), there was no effect of the time of sampling, again indicating that changes in prevalence recorded at the Dogger Bank and the Firth of Forth area could best be explained by changes in the mean length of the fish inspected.

A comparison of the temporal changes in prevalence of the four parasites for the presence of corresponding patterns revealed significant differences in all areas in most cases. Only the temporal changes in prevalences observed for *Lernaeocera branchialis* and *Clavella adunca* in the Firth of Forth area did not differ significantly.

Conclusions

From the data presented, there is evidence that none of the gross externally visible parasites of dab and whiting is restricted to certain areas of the North Sea, implying that these parasites cannot be used to geographically separate stocks. However, there are significant differences in prevalence between areas, indicating the existence of differences in the environmental conditions and/or the structure of the host communities, affecting the abundance of the parasites. Pronounced examples for this are the high prevalences of *Lernaeocera branchialis* and, to a lesser degree, of *Cryptocotyle lingua* in whiting from the German Bight and the high prevalence of *Stephanostomum baccatum* in dab from the Firth of Forth area.

In all cases (parasites and areas), the mean length of the fish in the samples examined had a major impact on the prevalences of the parasites recorded. Mostly, this effect was positive, indicating that the prevalence generally increases with length (and probably age) of the fish. This can for example be explained by a higher likelihood of larger fish to become infected (due to behavioural differences between larger and smaller fish in terms of feeding or migration or due to a longer time of exposure to the parasites in older/larger fish) or by a higher susceptibility/mortality of smaller fish. However, there are exceptions such as for *Clavella adunca* that occurred more frequently in smaller than in large fish in all areas studied. For a proper comparison of differences in prevalence between areas and periods, this length dependence has to be taken into account and further statistical and demographic methods have to be developed and assessed in order to achieve this. This should also be done for possible effects of gender, a factor that has not been considered in the present compilation.

With regard to the perspectives of further analyses and assessments of the usefulness of parasites of wild marine fish as environmental indicators, it can be stated that for most of the parasites considered in this document, sufficiently long enough data series exist worthwhile to be analysed in more depth in order to obtain a better understanding of possible effects of environmental change on the prevalence and intensity (the latter is not covered in here) of the parasites. For such an attempt, it will be necessary to consider a wide range of host- and site-specific factors, including biotic and abiotic parameters. For such an approach, data maintained in the ICES Databanks can be used (e.g., oceanographic and stock assessment data). However, on the basis of the experience gained throughout the past almost two decades with the monitoring of externally visible fish diseases, it can be predicted that it will be difficult to establish clear cause-effect relationships. For certain parasites with complex life cycles involving different host species it may even be more difficult than for the "classical" externally visible diseases of common North Sea fish species which have been monitored extensively for many years.

Figure A7.1. North Sea areas (N01: German Bight, N04: Dogger Bank, N06: Firth of Forth) considered in the data compilation on the occurrence of parasites of dab and whiting

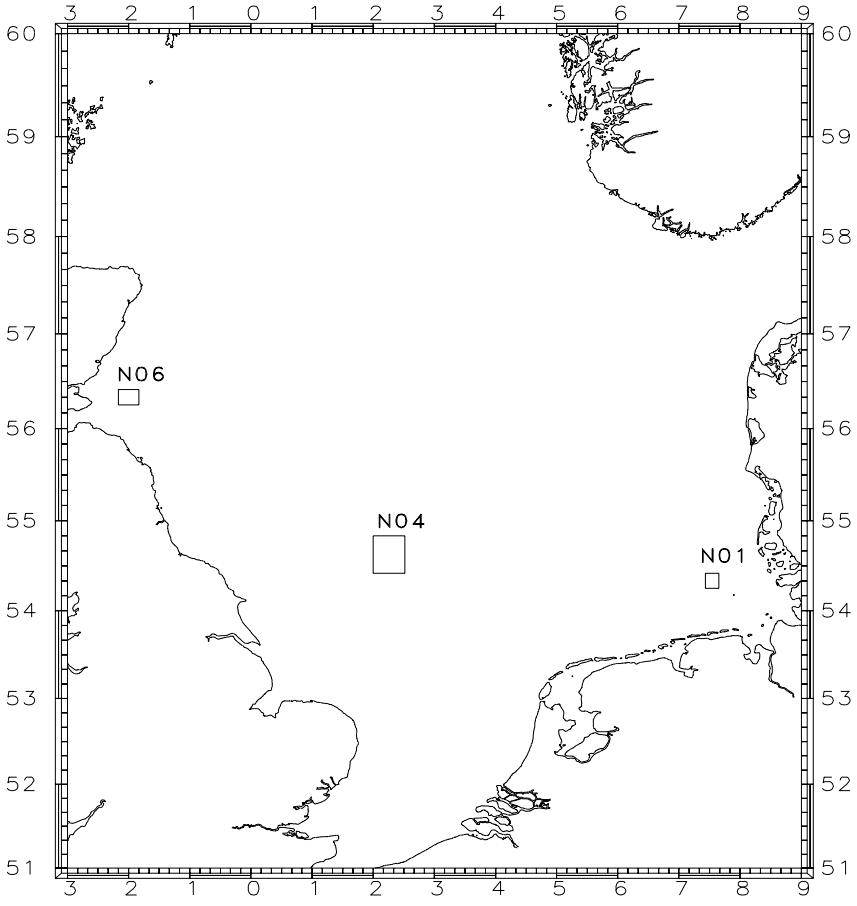


Figure A7.2a North Sea dab (*Limanda limanda*), prevalence of *Acanthocondria cornuta* and mean length of dab examined

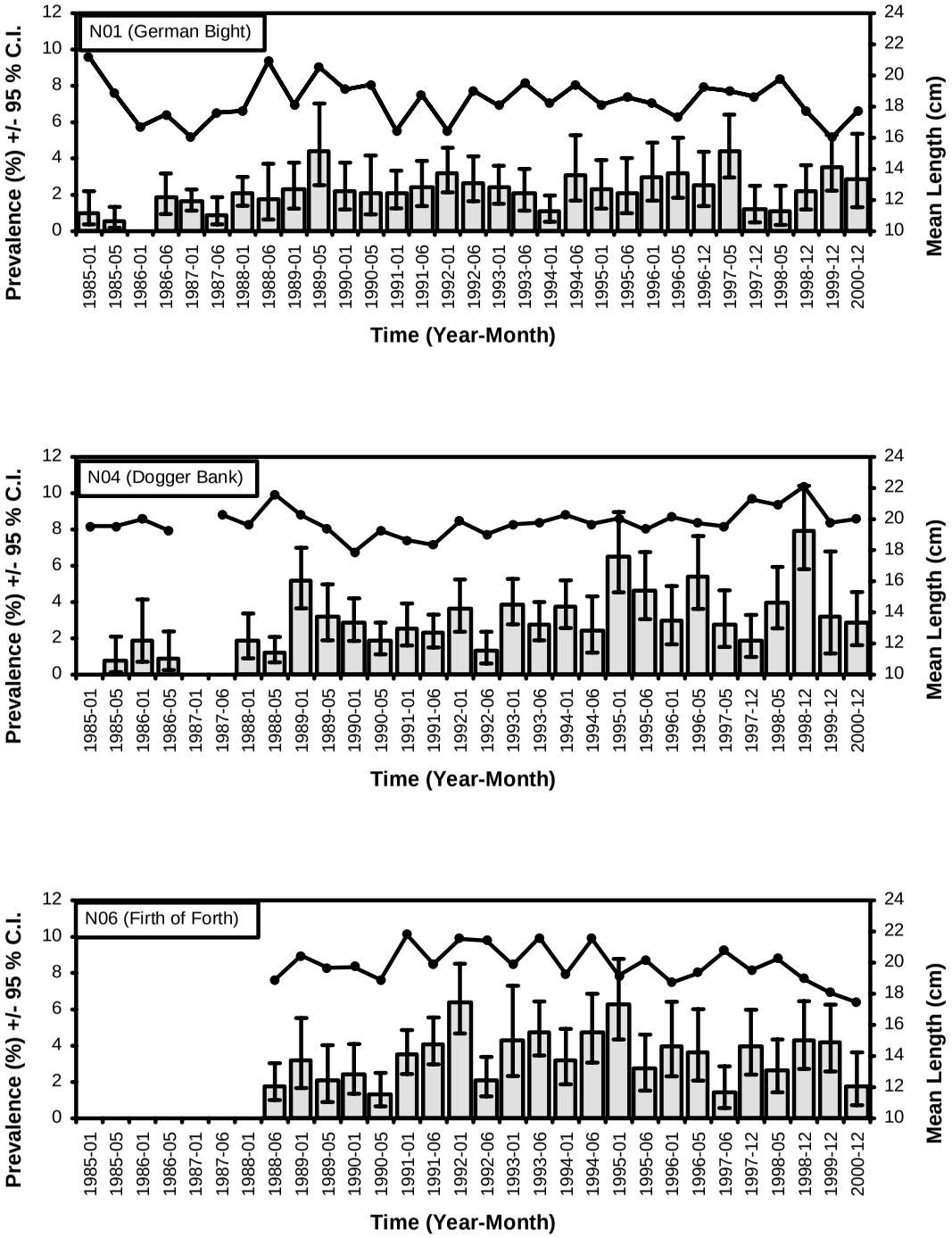
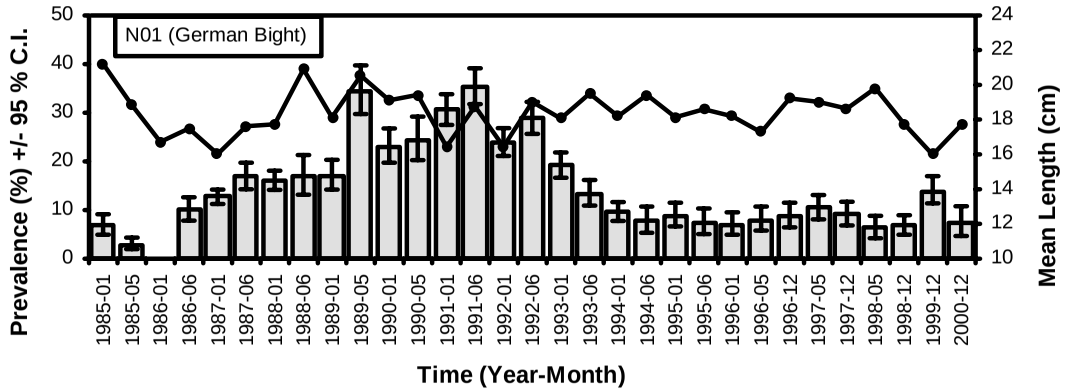
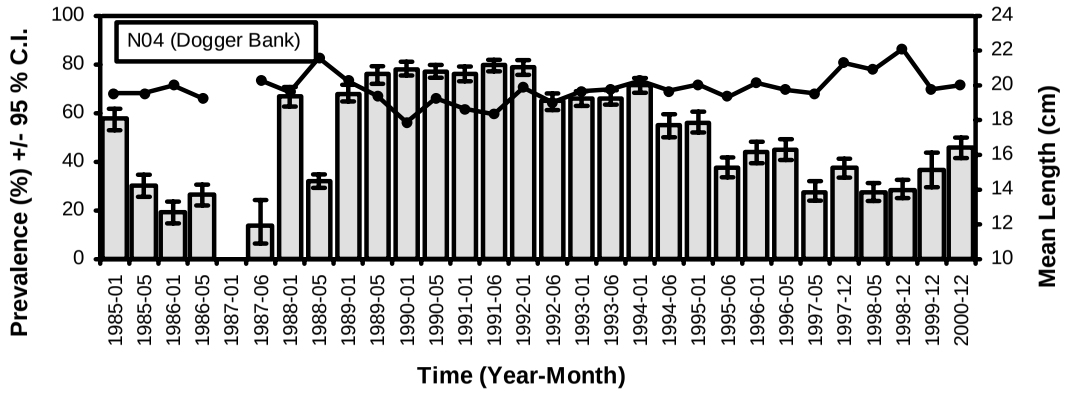


Figure A7.2b North Sea dab (*Limanda limanda*), prevalence of *Stephanostomum baccatum* and mean length of dab examined





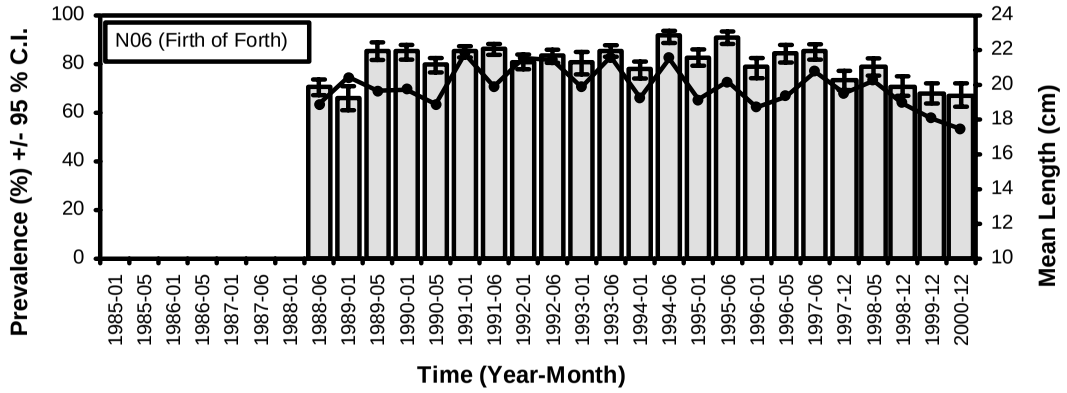


Figure A7.3a North Sea whiting (*Merlangius merlangus*), prevalence of *Lernaeocera branchialis* and mean length of whiting examined

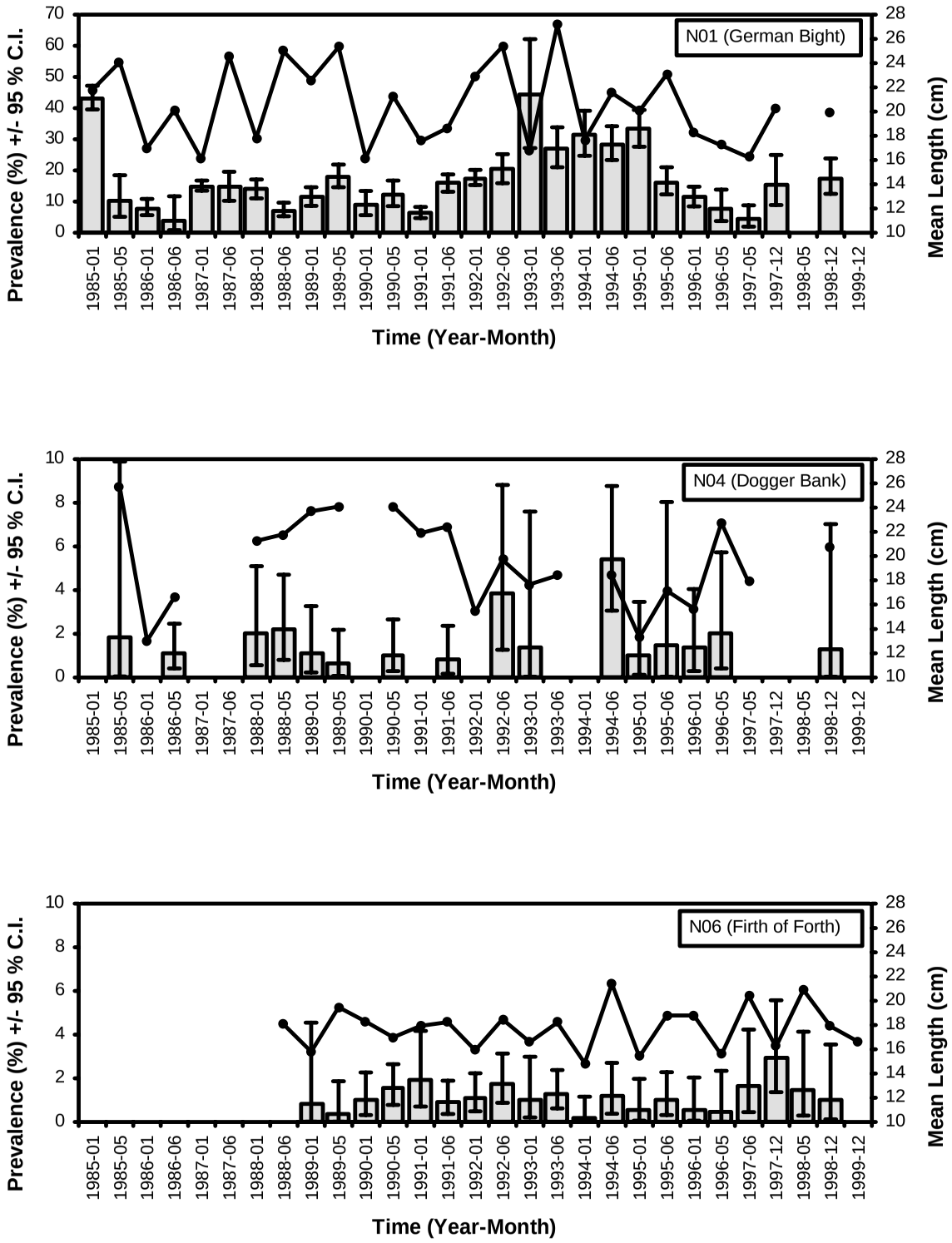


Figure A7.3b North Sea whiting (*Merlangius merlangus*), prevalence of *Clavella adunca* and mean length of whiting examined

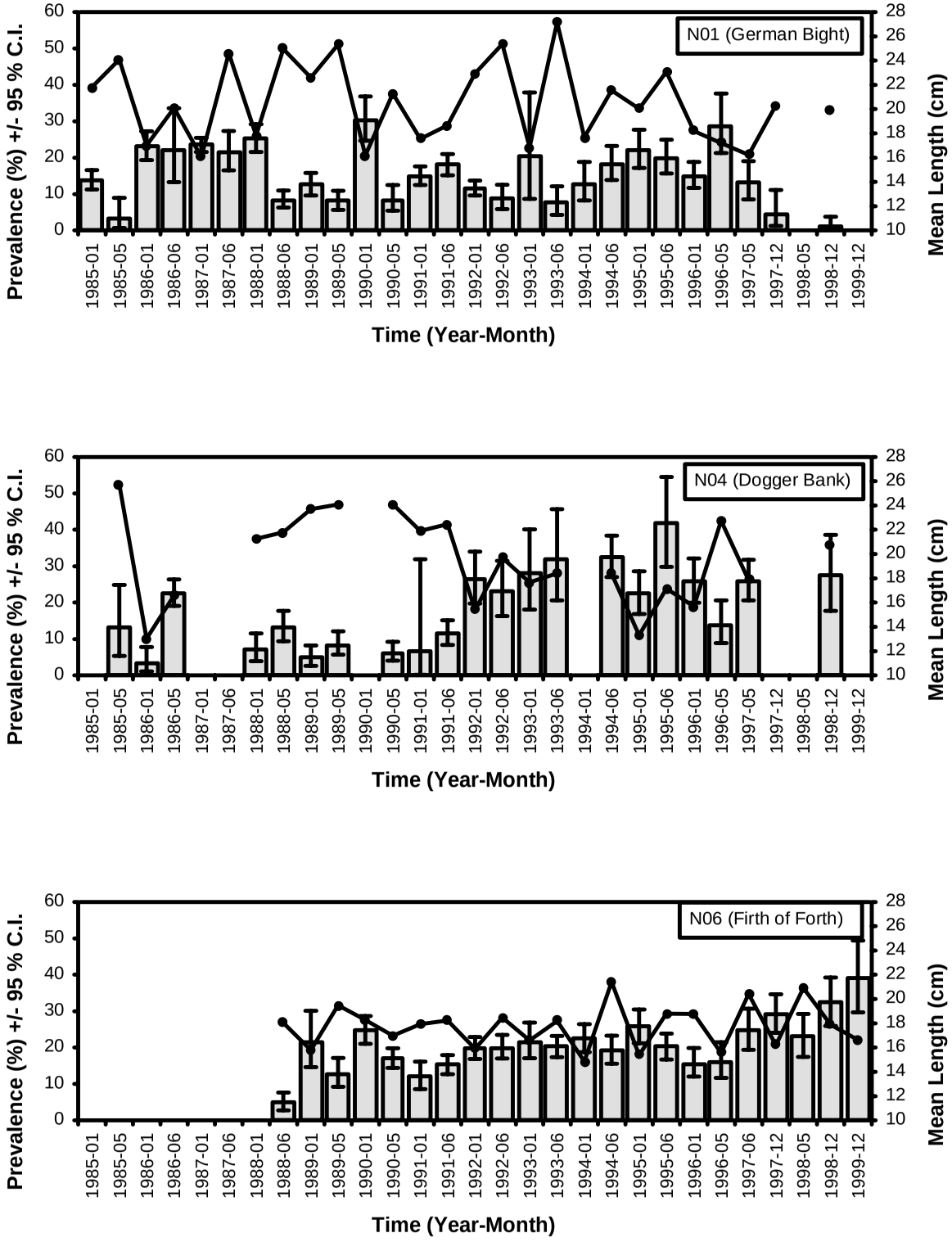
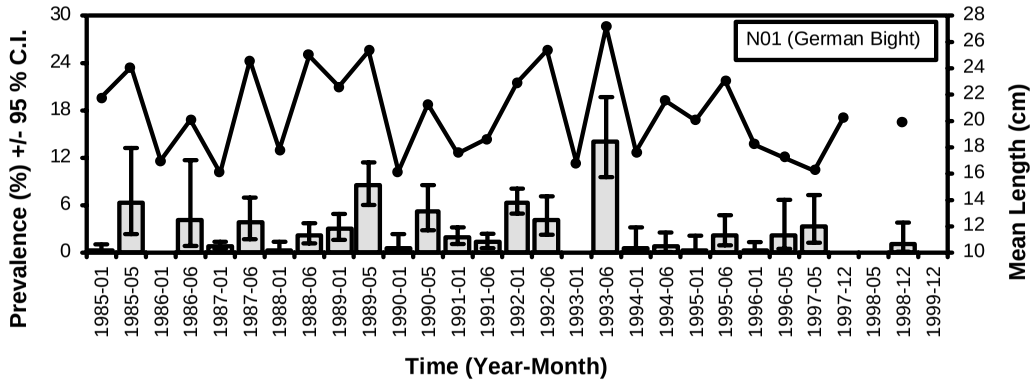
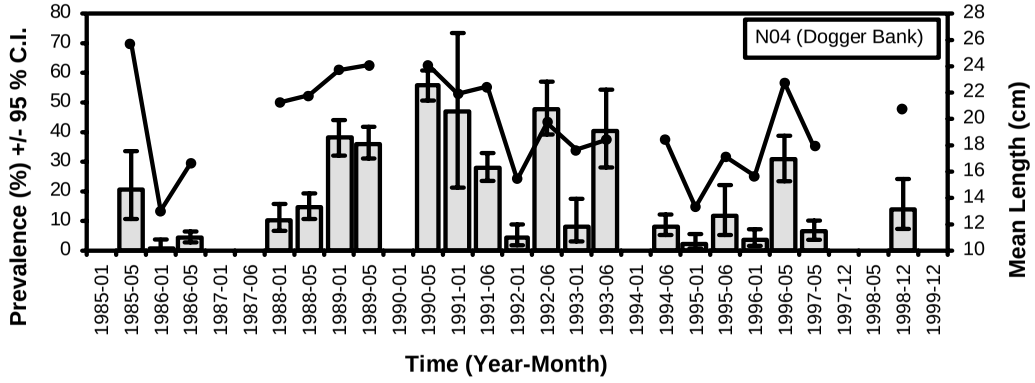
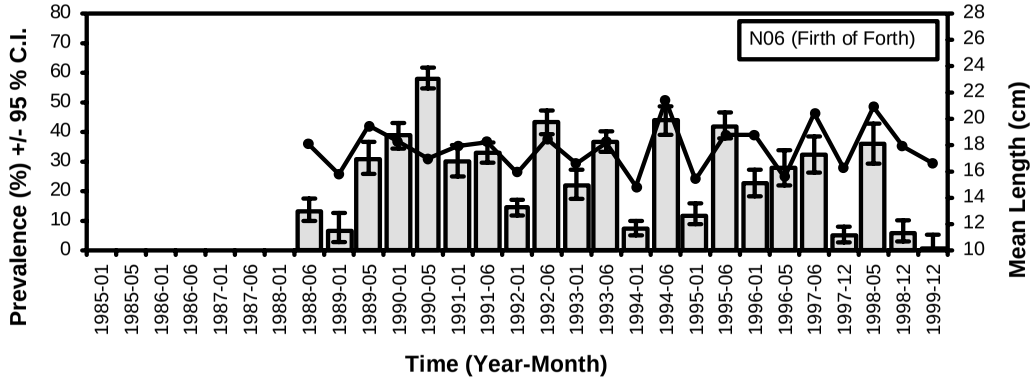


Figure A7.3c North Sea whiting (*Merlangius merlangus*), prevalence of *Diclidophora merlangi* and mean length of whiting examined







ANNEX 8: REVIEW PROGRESS MADE WITHIN THE BIOLOGICAL EFFECTS QUALITY ASSURANCE IN MONITORING (BEQUALM) PROJECT TITLED "FISH LIVER HISTOPATHOLOGY, LIVER NODULES AND EXTERNAL FISH DISEASE MEASUREMENT"

The EU-funded BEQUALM programme has now been in existence for two years and for most components significant progress has been made in the establishment of networks of participants for each work package (WP). The external fish disease and liver histopathology component (WP6) has made good progress in its tasks following the successful workshop held during October 1999 at CEFAS Weymouth Laboratory, UK. The following report provides a summary of the key activities of WP6 which are of particular relevance to the activities of the ICES WGPDMO.

Progress has concentrated on the collation of reference material, both for the intercalibration programme (ring test) currently under way on the diagnosis of flatfish liver histopathology and particularly for laboratory reference materials (LRMs). For this the extensive collections held at CEFAS Weymouth have been used together with sets of material loaned by Dr M Myers (USA) of representative material from North American flatfish species and additional material from European flounder donated by Dr A Köhler (Germany) and Dr Göran Bylund (Finland). The latter set is particularly useful since it contains examples of rare lesion types, which generally cause most confusion for pathologists. The material will be deposited in the Registry of Aquatic Pathology (RAP) housed at the CEFAS Weymouth Laboratory.

A comprehensive set of images of different hepatic lesions has been compiled each accompanied by descriptive text. This process has continued and the "database" of reference material will be expanded thereby increasing its usefulness throughout this contract and beyond. The main categories of lesions include the following:

- Early non-neoplastic toxicopathic lesions.
- Foci of cellular alteration.
- Benign neoplasms.
- Malignant neoplasms.
- Non-specific lesions.

The previous progress report provided the list of specific lesion categories, which fall into the above classes of hepatic pathology. The majority of the examples used within this BEQUALM work package are from the common dab (*Limanda limanda* L.) since this is at present the main species used in biological effects monitoring at offshore sites. Additional material is from the European flounder (*Platichthys flesus* L.), which is a major target fish species in inshore areas.

The examples of liver pathology selected meet the requirement of BEQUALM to provide adequate reference material for participants. In addition, it is planned to incorporate several of these in the *ICES Techniques in Marine Environmental Sciences* (TIMES) leaflet on "The use of liver pathology of flatfish for monitoring biological effects of contaminants" which is nearing completion (co-authors: S.W. Feist, T. Lang, A. Köhler). It should be noted that this publication concentrates on pathologies encountered in European fish species examined in monitoring programmes, namely, the dab and flounder. The manuscript is currently with the co-authors for their final amendments and for finalisation of the figures to be included. Janet Pawlak (ICES) has confirmed that colour plates are acceptable provided that their number is kept down! The current aim is to submit the manuscript by the beginning of January 2002.

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

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

A set of histological slides has been compiled and the diagnoses have been checked by Dr Mark Myers (US), an expert in the application of liver histopathology in biological effects monitoring. The slides have been sent to the first participants in the ring test and a total of twelve laboratories are involved. It has been arranged that each laboratory will have up to five working days to evaluate the material and submit the results to CEFAS Weymouth.

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

There has been significant progress in this aspect of the project. The original concept of the atlas of common histopathological lesions was quite specific and limited to the microscopic appearance of liver pathology. However, as indicated in the last progress report, it has been decided to broaden the scope of the atlas to include representative

images and data on all aspects of the sampling procedure from "ship to microscope". It is now intended to produce this as a CD-ROM training guide. The scope of the proposed CD-ROM can be seen below, together with the appearance of the "front page" as it appears in the current state of development.

As an additional part of the BEQUALM objectives, plans are being developed for an incorporation of relevant information on quality assurance for surveys on externally visible diseases. However, liver histopathology will be given priority, because guidelines and quality assurance procedures for externally visible diseases of dab and flounder have already been published elsewhere and have been implemented in the majority of national monitoring programmes.

3) Second workshop to be held in autumn 2001 at CEFAS Weymouth Laboratory.

The planned workshop will have several aims:

a) Following the completion of the ring test for flatfish liver histopathology, the results will be evaluated and assessed by the participants. Each slide (as required) will be examined and further training will be given as necessary.

b) The progress on the development of the CD-ROM training guide will be reviewed and changes implemented as decided by the participants.

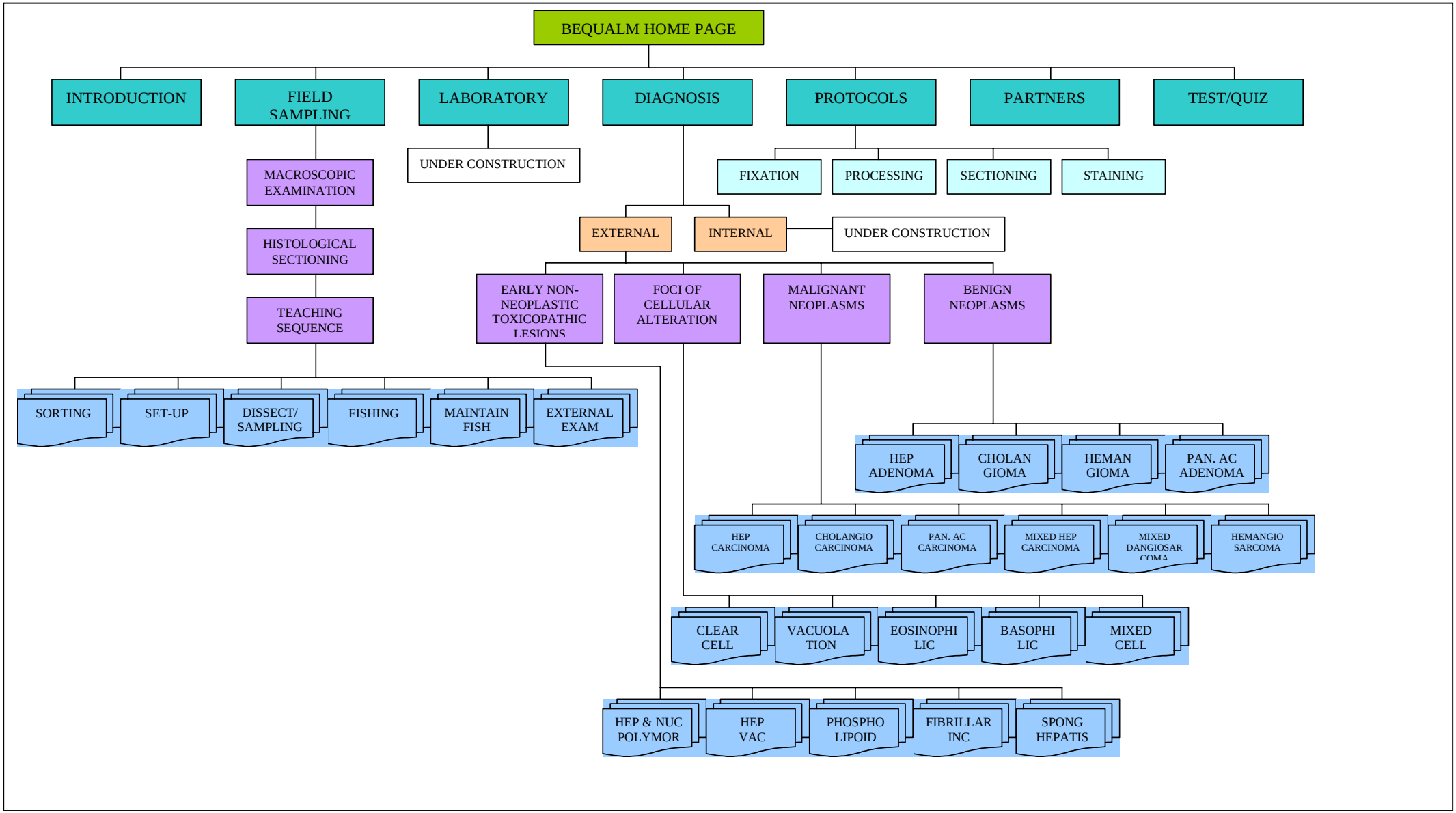
c) An assessment of national fish disease monitoring programmes will be made and histological material from them will be assessed as time permits during the workshop.

d) Discussion will be held on the future implementation of the QA procedures established under BEQUALM.

BEQUALM Liver Histopathology Training CD-ROM "home" page.

The original demonstration version of the "BEQUALM Liver Histopathology CD-ROM" was produced using basic html programming with Microsoft Front Page 98 and received a good reception at the previous BEQUALM Steering Group meeting. However for the final version it is intended to produce a CD-ROM to a much higher standard. The program of choice for the final phase of the CD-ROM production is Macromedia, Dreamweaver & Fireworks Studio. It is considered to be the industry standard and is more than capable of achieving the high standards required of this CD-ROM.

As can be seen from the flow diagram below, the content of the CD-ROM is comprehensive, with images to illustrate all aspects of the sampling and interpretation process. The diagram shows basic CD-ROM structure only; it is intended that the final version will be fully navigable with all sections accessible within three clicks of the mouse. Training will be achieved by a user-defined combination of classical atlas, slide show tutorials and interactive training tools. The final version is currently in production with initial reports being very promising and already there is a higher degree of interactivity than in the previous demonstration version.


BEQUALM HOME PAGE
INTRODUCTION
FIELD SAMPLING
LABORATORY
DIAGNOSIS
PROTOCOLS
PARTNERS
TEST/QUIZ
UNDER CONSTRUCTION
FIXATION
PROCESSING
SECTIONING
STAINING
MACROSCOPIC EXAMINATION
HISTOLOGICAL SECTIONING
TEACHING SEQUENCE
EXTERNAL
INTERNAL
UNDER CONSTRUCTION
EARLY NON-NEOPLASTIC TOXICOPATHIC LESIONS
FOCI OF CELLULAR ALTERATION
MALIGNANT NEOPLASMS
BENIGN NEOPLASMS
SORTING
SET-UP
DISSECT/ SAMPLING
FISHING
MAINTAIN FISH
EXTERNAL EXAM
HEP ADENOMA
CHOLAN GIOMA
HEMAN GIOMA
PAN. AC ADENOMA
HEP CARCINOMA
CHOLANGIO CARCINOMA
PAN. AC CARCINOMA
MIXED HEP CARCINOMA
MIXED DANGIOSAR COMA
HEMANGIO SARCOMA
CLEAR CELL
VACUOLA TION
EOSINOPHI LIC
BASOPHI LIC
MIXED CELL
HEP & NUC POLYMOR
HEP VAC
PHOSPHO LIPOID
FIBRILLAR INC
SPONG HEPATIS

ANNEX 9: REVIEW PROGRESS REPORTS FROM THE EU PROJECT ON NODAVIRUSES AND OTHER RELEVANT INFORMATION TO PROVIDE ADVICE ON EFFECTIVE CONTROL MEASURES

Articles:

Péducasse S., Castric J., Thiéry R., Jeffroy J., Le Ven A., Baudin Laurencin F. , 1999

Comparative study of viral encephalopathy and retinopathy in juvenile sea bass *Dicentrarchus labrax* infected in different ways. *Dis. Aquat. Org*., 36(1), 11-20.

Thiéry R., Arnauld C., Delsert C., 1999

Two isolates of sea bass, *Dicentrarchus labrax* L., nervous necrosis virus with distinct genomes. J. *Fish Dis*., 22, 201-207.

Thiéry R., Raymond J.C., Castric J., 1999

Natural outbreak of viral encephalopathy and retinopathy in juvenile sea bass, *Dicentrarchus labrax*: study by nested reverse transcriptase-polymerase chain reaction. *Virus Research,* 63, 11-17.

Breuil G. & Romestand B., 1999

A rapid ELISA method for detecting specific antibody level against nodavirus in the serum of the sea bass, *Dicentrarchus labrax* (L.): application to the screening of spawners in a sea bass hatchery. *J. Fish Diseases,* 22, 45-52.

Breuil G, Pépin JF, Castric J, Fauvel C & Thiéry R., 2000

Detection of serum antibodies against nodavirus in wild and farmed adult sea bass: application to the screening of the broodstock in sea bass hatcheries. *Bull. Europ. Assoc. Fish. Pathol., 20*, 95-100.

Frerichs GN, Tweedie A, Starkey WG, Richards RH. 2000

Temperature, pH and electrolyte sensitivity, heat, UV and disinfectant inactivation of sea bass (*Dicentrarchus labrax*) neuropathy nodavirus. *Aquaculture*, 185, 13-24.

Starkey WG, Ireland JH, Muir KF, Shinn AP, Richards RH & Fergusson HW. 2000

Isolation of nodavirus from Scottish farmed halibut, *Hippoglossus hippoglossus* (L). *J. Fish diseases*, 23, 419-422.

Breuil G, Mouchel O, Fauvel C & Pepin JF. 2001

Two isolates of sea bass, *Dicentrarchus labrax*, nervous necrosis virus with distinct pathogenicity to sea bass larvae. *Dis. Aquat. Org*. in press

Breuil G, Mouchel O & Pepin JF.

A sandwich ELISA to detect Nodavirus in the sea bass (*Dicentrarchus labrax*). Submitted to *Dis. Aquat. Org.*

Castric J, Thiéry R, Jeffroy J, de Kinkelin P& Raymond J-C.

Seabream (*Sparus aurata*), an asymptomatic contagious fish host for nodavirus. Submitted to *Dis. Aquat. Org.*

Posters:

Péducasse S., Castric J., Baudin Laurencin F. 1999

Physical and chemical inactivation of sea bass nodavirus on SSN-1 cell line. IXth International Conference "Diseases of Fish and Shellfish," Rhodes, Greece.

Thiéry, R. Arnauld, C. Boscher, S. Castric, J. 1999

Genotyping of nodavirus isolates from sea bass *Dicentrarchus labrax*: implication on PCR-based diagnosis. IXth International Conference "Diseases of Fish and Shellfish," Rhodes, Greece.

Oral communications:

Péducasse S., Boscher S., Le Ven A., Baudin Laurencin F. 1999

Study of portals of entry and progression for nodavirus in juvenile sea bass *Dicentrarchus labrax*. IXth International Conference "Diseases of Fish and Shellfish," Rhodes, Greece.

Skliris GP, Sideris DC, Krondiris GV, Shinn AP, Starkey WG, Richards RH 1999.

Nucleotide sequence and phylogenetic analysis of the coat protein gene of piscine nodaviruses. IXth International Conference "Diseases of Fish and Shellfish," Rhodes, Greece.

Péducasse S, Quentel C. 2000.

Etude des voies de pénétration et du tropisme du nodavirus chez le bar *Dicentrarchus labrax*. 2ème journées francophones de virologie, Paris, 6-7 avril 2000, Abstract published in Virologie, 2000, 4, pp 149.

WORKSHOP

A new workshop on nodavirus is planned in the frame of the 10th international conference on the EAFP (Dublin, September 2000).

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

by G. Bylund

Present state

Compared to one year earlier (1999) there was a positive trend with decreasing disease prevalences in most river systems. However, the disease prevalences in most rivers are still significantly higher than in 1998 when the prevalences were down on a fairly tolerable level. The prevalences of salmon females with 100 % fry mortality in the most important salmon rivers monitored in Finland and Sweden during 1997–2000 are given in the table below:

River system	1997	1998	1999	2000
Simojoki	86	31	38	22
Tornionjoki	-	25	56	32
Lule älv	38	6	34	21
Skellefteälven	16	4	42	12
Ume/Vindelälven	37	9	53	45
Ångermanälven	21	3	28	21
Indalsälven	22	1	20	14
Ljungan	29	10	25	10
Ljusnan	22	6	41	25
Dalälven	38	9	33	27
Kymijoki	79	42	0	10
Mean total	38.8	13.3	33.6	21.7

It has to be emphasised, however, that these figures are too optimistic as they include only salmon females with 100 % fry mortality. In fact partial mortality due to M74 occurs on all levels and the real disease prevalence is considerably higher. In the Finnish monitoring programme also females with fry mortalities less than 100 % are recorded and the total prevalences of M74-affected females are frequently twice as high as those indicated in the table above. As an example it can be mentioned that although no females with 100 % fry mortality were recorded in River Kymijoki in 1999 the prevalence of females affected with lower levels of fry mortality was 42 %. Moreover it has been demonstrated in Sweden that also offspring from “normal” females, i.e., salmon females without significant fry mortality, suffer from subnormal thiamine levels.

The prognosis for the 2001 hatch is still very preliminary. A Finnish prognosis for the River Tornionjoki indicates disease prevalence around 50 %, a Swedish prognosis for River Dalälven indicate a disease prevalence of 17 %.

So far there are no verified reports on M74 in European areas outside the Baltic Sea.

Recent results and ongoing research

Recent research projects in Sweden have, i.a., focused on experimental induction of thiamine deficiency in salmon fry and females. Microinjection of pyrithiamine, a thiamine kinase inhibitor, or oxythiamine another thiamine antagonist, into salmon fry, induced histopathological alterations typical for M74 fry and high mortalities. Including the thiamine antagonist amprolium into the feeds also induced a significant reduction in the thiamine contents of eggs and muscles of salmon females. Utilising these models we will be able to study the pathogenesis of M74 more in detail.

Ongoing research also focuses on the role that environmental pollutants and oxidative stress may play in the kinetics of thiamine in salmon. Although for example paraquat, when injected into rainbow trout, significantly interferred with the enzyme systems (hepatic glutathione reductase, hepatic glucose-6-phosphate dehydrogenase) there was no effect on the thiamine level of the fish.

Finnish work have focused, i.a., on the level of thiaminase activity in the prey fish species of Baltic salmon as well as on thiaminase activity in the intestinal tract of salmon. It was demonstrated that the concentration of thiaminase in Baltic herring measured as potential thiaminase activity varies on a very wide scale. About 30 % of the herring had a

thiaminase concentration on the same high level as in the salmon gastrointestinal contents which might even indicate that salmon selectively prey on “high activity” herring.

Screening for thiaminase-producing or thiaminolytic bacteria in the intestinal tract of salmon and Baltic herring has so far given negative results.

Work is currently being carried out in Finland in order to evaluate the role algae and cyanobacteria blooms may play in the thiamine/thiaminase balance of organisms in the food chain of Baltic salmon.

Information on M74 works performed by the Finnish Game and Fisheries Research Institute is available on: http://www.rktl.fi/english/fisheries/enviro/m74eng/m74eng.html.

Recent articles on M74

Keinänen M., Tolonen T., Ikonen E., Parmanne R., Tigerstedt C., Rytilahti J., Soivio A. & Vuorinen P.J. 2000. Reproduction disorder of Baltic salmon (the M74 syndrome): research and monitoring. Finnish Game and Fisheries Research Institute, Kalatutkimuksia - Fiskundersökningar No. 165, 38 p. (in Finnish, abstract)

Hansson S., Karlsson L., Ikonen E., Christensen O., Mitans A., Uzars D., Petersson E. & Ragnarsson B. 1999. Yolk-sac-fry mortality (M74) in Baltic Sea salmon (*Salmo salar* L.): Analyses of its relation to the diet. ICES, M74 Syndrome and similar Reproductive Disturbances in Marine Animals. ICES CM 1999/U:09, 17 p.

Ikonen E. & Soivio A. 1999. Does the feeding area choice of Atlantic salmon (Salmo salar L.) affect M74 mortality? ICES, M74 Syndrome and similar Reproductive Disturbances in Marine Animals. ICES CM 1999/U:01, 9 p.

Ikonen E., Karlsson L., Mitans A. & Hansson S. 1999. Yolk-sac-fry mortality (M74) in Baltic salmon (*Salmo salar* L.): Indications from where and when they feed. ICES, M74 Syndrome and similar Reproductive Disturbances in Marine Animals. ICES CM 1999/U:07, 10 p.

Karlsson L., Ikonen E., Hansson S. & Mitans A. 1999. Yolk-sac-fry mortality (M74) in Baltic Sea salmon (*Salmo salar* L.): Its relation to thiamine levels during migration and at spawning. ICES, M74 Syndrome and similar Reproductive Disturbances in Marine Animals. ICES CM 1999/U:08, 18 p.

Karlsson L., Ikonen E., Mitans A. & Hansson S. 1999. The diet of salmon (*Salmo salar*) in the Baltic Sea and connections with the M74 syndrome. Ambio 28: 37-42.

Karlsson L., Ikonen E., Mitans A., Hansson S. & Uzars D. 1999. Thiamine levels in migrating salmon spawners (Salmo salar) in the Gulf of Riga and in the Gulf of Bothnia. In: Bengtsson B.-E., Hill C. & Nellbring S. (eds), Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project. TemaNord 1999:530: 67-88.

Koski P. 1999. Thiamine concentrations in herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) in stomach contents of Baltic salmon (*Salmo salar*) - an experimental study. In: Bengtsson B.-E., Hill C. & Nellbring S. (eds), Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project. TemaNord 1999:530: 59-62.

Koski P., Pakarinen M., Nakari T., Soivio A. & Hartikainen K. 1999. Treatment with thiamine hydrochloride and astaxanthine for the prevention of yolk-sac mortality in Baltic salmon fry (M74 syndrome). Dis. Aquat. Org. 37: 209-220.

Löflund M., Amcoff P., Eerola S., Hartikainen K., Sistonen S., Vuorinen P.J. & Soivio A. 1999. Quality assurance of thiamine analyses in connection with investigations of reproductive disturbances in Baltic fish: a proficiency study. In: Bengtsson B.-E., Hill C. & Nellbring S. (eds), Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project. TemaNord 1999:530: 49-58.

Soivio A. 1999. Book review: Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. (Proceedings of the Symposium “Early Mortality Syndrome: Reproductive Disruptions in Fish of the Great Lakes, New York Finger Lakes, and the Baltic Region”, Dearborn, Michigan, USA, 28 August 1996. Edited by McDonald G., Fitzsimons J.D. & Honeyfield D.C. American Fisheries Society, Bethesda, MD, 1998. 177 p.) Rev. Fish Biol. Fish. 9, p. 204.

Soivio A. & Hartikainen K. 1999. Thiaminase activity in the forage fish of Baltic salmon (*Salmo salar*). In: Bengtsson B.-E., Hill C. & Nellbring S. (eds), Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project. TemaNord 1999:530: 63-66.

Soivio A., Piironen J. & Eskelinen P. 1999. The aquacultural project to maintain natural stocks of Baltic salmon (*Salmo salar*) in Finland. In: Bengtsson B.-E., Hill C. & Nellbring S. (eds), Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project. TemaNord 1999:530: 93-99.

Strandman T., Koistinen J., Kiviranta H., Vuorinen P.J., Tuomisto J., Tuomisto J. & Vartiainen T. 1999. Levels of some polybrominated diphenyl ethers (PBDEs) in fish and human adipose tissue in Finland. Dioxin99. 19th International Symposium on Halogenated Environmental Organic Pollutants and POPs. September 12 - 17, 1999, Venice, Italy. Organohalogen Compounds 40: 355-358.

Vuorinen P.J. 1999. Summary of M74 research in Finland. Early mortality syndrome workshop, Ann Arbor, 17–18 May 1999, p. 15. (Abstract).

Vuorinen P.J. & Keinänen M. 1999. Environmental toxicants and thiamine in connection with the M74 syndrome in Baltic salmon (*Salmo salar*). In: Bengtsson B.-E., Hill C. & Nellbring S. (eds), Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project. TemaNord 1999:530: 25-37.

Vuorinen P.J., Paasivirta J., Keinänen M., Vartiainen T. & Parmanne R. 1999. Organochlorines and thiamine in salmon (Salmo salar L.) and prey species, Baltic herring (*Clupea harengus* L.) and sprat (*Sprattus sprattus* (L.)), in connection with the M74 syndrome. ICES, M74 Syndrome and similar Reproductive Disturbances in Marine Animals. ICES CM 1999/U:12, 16 p.

ANNEX 11: METHODS FOR THE CONTROL OF SEA LICE INFESTATIONS IN NORWAY

Working document prepared by Brit Hjeltnes, Institute of Marine Research, Norway

In 1996 a National Working Group (WG) on salmon lice infections in salmonids was established in Norway. One of the main duties of this group was to establish a National Program for Action on Sea Lice Infections in Salmonids. Such a programme was approved in 1997 and has since been revised in 1998. Members of the WG include authorities, fish health veterinarians and the fish farmer's association. It is believed that this WG and the national programme for action have played a key role in the control of sea lice infections in Norway. Key elements of the plan include:

Documentation of the occurrence of lice in fish farms.

This involves counting, registration and reporting of all louse stages. Regional regulations are in force in all relevant fish farming counties. The reporting routine and quality of the reported data have improved considerably following application of the regulations.

Non-acceptable levels of lice infection.

Treatment is required when the level of infection exceeds a certain value. From December to July, fewer lice are tolerated before treatment is obligatory. The reduced toxicity of some of the new chemical compounds has motivated the fish farmers to de-louse at lower infection levels. Regulations are now in force.

Organised de-lousing during the cold periods.

The long-term goal for control is that no sexually mature lice are present in fish farms during the period prior to the migration of wild fish to the sea. Regional regulations are in force and they are partly implemented.

Synchronised de-lousing.

Synchronised de-lousing is implemented in some areas, but is not always feasible in areas where wrasse is used as cleaning fish.

Reduction of fish escapes.

A very large number of fish escape annually from Norwegian fish farms, and this biomass is considered to be a major source of lice infestation on wild fish. Escaped farmed fish mainly keep to fjord systems in the winter, and this may explain recent observations that there is a greater burden of louse infestation in the cold part of the year. There is thus a great need to reduce the number of fish that escape from Norwegian sea farms.

Control Methods.

Wrasse

The use of wrasse as cleaner fish is quite frequent in Norwegian fish farming. It is environmentally friendly and can be used over a long time period to maintain a low level of sea lice in the cages. By having a preference for adult lice, the wrasse will prevent the spread of infection. The cost of using wrasse is low (25 %) compared to chemical treatment. Used in the right way, the use of wrasse is effective, less stressful to the fish and does not result in reduced appetite and growth.

The use of wrasse is less effective at temperatures below 10°C as, with one exception, wrasse are passive at low temperatures. Normally it is problematic to over-winter wrasse in the cages. Excessive fouling on the nets will reduce the feeding activity of wrasse on the lice. It is important that the level of infection is not too high before wrasse are applied. However, absence of sea lice and lack of adequate food supply could induce a more aggressive behaviour (snapping). Only wrasse of good quality should be used and the number of fish has to be sufficient (1–3 % wrasse compared to the salmon population). Diseases can be a problem and outbreaks of atypical furunculosis and vibriosis have been reported. Although there is no indication that wrasse are susceptible to important diseases like ISA, the role of wrasse as disease reservoirs cannot be excluded.

Chemical treatment

The synthetic pyrethroids cypermethrin and deltamethrin (Excis/Alpha Max) are the most frequently used chemicals in treatment of sea lice infections in Norwegian fish farming (80 %). When used as a dip or bath these compounds are effective against all stages of sea lice and the toxicity is relatively low. In contrast, oral treatment has almost exclusively been used on smolts. The active compounds are teflubenzuron (Ektobann) and diflubenzuron (Lepsidon). They are not effective against adult sea lice, have a long withdrawal time (120 days) and are relatively expensive. Recently, a new compound, emamectin benzoate, belonging to the avermectin group (SLICE), has been launched. It is effective on all stages of sea lice (80–90 % at 5-15 $^{\circ}$C). Of the organo phosphorus compounds, only azamethiphos (Salmosan) is still in use, although its use is limited and declining. This compound will only be effective on adult/preadult stages of sea lice and the toxicity is relatively high. A growing concern with chemical treatment has been the risk of developing resistance and possible harmful effects on the environment. Variable treatment regimes have been discussed to reduce the risk of resistance development in sea lice.

Conclusion

For several years, sea lice infestations in salmonids have been effectively controlled by chemical treatment and the use of wrasse as cleaning fish. Management control methods like de-lousing at low infection levels, synchronised de-lousing, organised de-lousing during cold periods and attempts to minimise the number of escapes are all believed to be important, but the effectiveness has so far been difficult to document in a scientific way.

ANNEX 12: REPORT ON THE CURRENT STATUS OF THE ICES IDENTIFICATION LEAFLETS FOR DISEASES AND PARASITES OF FISH AND SHELLFISH

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Fiche Updating

WGPDMO has recommended re-contacting the authors, which was done in the first week of September 2000. This has not been too successful, with the exceptions of MSX and Dermo of American oysters (c/o Susan Ford). I suggest that, unless anyone has any burning concerns regarding maintaining the listing and availability of the Fiches listed below, we move forward with "new" fiches.

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

Titles proposed by WGPDMO

The following authors have been contacted for the following titles:

i. QPX of hard-shell clams (*Mercenaria mercenaria*) – McGladdery & Smolowitz (in preparation)
ii. Denman Island Disease (*Mikrocytos mackini*) of Pacific oysters (*Crassostrea gigas*) – Bower (in preparation)
iii. *Perkinsus qugwadi* (SPX Disease) of Japanese scallops (*Patinopecten yessoensis*) – Bower (in preparation)
iv. Brown Ring Disease, caused by *Vibrio tapetis* of the clams *Ruditapes decussatus* and *R. philippinarum* – Paillard and Ford **Received**
v. Juvenile Oyster Disease - Ford
vi. Herpes Virus – Renault and Hine
vii. Infectious Salmon Anaemia (ISA) – Hjeltnes
viii. Haemic neoplasia of soft-shell (*Mya arenaria*) and hard-shell (*Mercenaria mercenaria*) clams – McGladdery
ix. M 74 Disease – Mellergaard and Bylund **Submitted to WGPDMO**
x. Pancreas Disease – McVicar
xi. Pseudophyllidean cestodes in marine fish – Palm
xii. Trypanorhynch cestodes in marine fish – Palm
xiii. Nodavirus – Peducasse **(near completion in May 1999, but subsequent attempts to contact author failed - ? suggestions)**
xiv. Pfeisteria – MacLean
xv. *Gyrodactylus salaris* – McVicar
xvi. Flavobacterium – Dalsgaard
xvii. Flounder Liver Tumours – Vethaak
xviii. Gonadal neoplasia of hard-shell clams – Barber and McGladdery

All authors received the "Guide to Authors" (both languages) and I have received confirmation from most on willingness to contribute to this series. The WGPDMO suggested finding alternatives for some fiche requests which had not been responded to. Alternatives are currently being solicited.

Format

As with last year - discussions with ICES Headquarters on web formatting have been positive and they are now considering the free downloading option. Advantages will be (hopefully) real-time posting and inclusion of colour images to assist diagnosis. I am uncertain of what this may do to "hard copy" availability, due to cost of printing and mail-out vs. web access. Feedback on this direction would be appreciated….

Review

The question of peer review, which was raised at the WGPDMO meeting in Lisbon, 1999, was not re-addressed, since the opinion of the WGPDMO has not changed. Most members feel this would be beneficial. The review process can be managed through the Editor and reviews filed at ICES HQ and/or with the Editor. This should not slow down publication or posting significantly. For new fiches, please suggest appropriate reviewers, since the experts usually are the authors.

Other Comments

No feedback on Fiche direction/format has been received inter sessionally (cf. Fiche report submitted at Bremen). The wide range of similar "summary" diagnostic information continues to increase from other sources - notably for cultured species. We should seriously consider the value of continued effort on mariculture-based diseases, whereas wild fish diseases and parasites have a relative lack of diagnostic reference information. For example, should liver nodule diagnosis be formatted into a Fiche, or endocrine disruption-related pathology? Does lymphocystis need updating for different species affected. Another very important question with respect to viral isolates from wild fish (e.g., VHSV) is serological and DNA primer information for distinguishing virulent and avirulent strains - nothing like this is addressed to date in the fiches and will gain greater importance (not given under strictly aquaculture documentation, such as OIE) as more emphasis is placed on disease-screening of wild fish for health "zonation".

If the WGPDMO feels that the Fiches can no longer fill the niche information role for which they were originally intended, I would appreciate receiving that feedback. As noted above, this needs *ruthless* discussion at the WGPDMO level. The Fiches have to be something that the WG can be proud of. Right now, it continues to feel "piecemeal" and mea culpa also, with several outstanding Fiche titles bearing my name…

S.E. McGladdery

March 12, 2001

ANNEX 13: *ANISAKIS SIMPLEX* INFESTATION IN COMMERCIAL FISHES AND CEPHALOPODS OF BISCAY GULF

Document prepared by Guzmán Díez, AZTI Fundazioa, Fisheries Resources Department, Basque Country, Spain.

A study of *A. simplex* infestation in commercial fishes and cephalopods was carried out throughout the year 2000. The flesh, viscera and body cavity of several length classes of 10 different species caught by the Basque fleet in ICES Divisions VIIIc and VIIIab,d were examined. Larvae of *A. simplex* were found in flesh of the following species: hake, *Merluccius merluccius*; blue whiting, *Micromesistius putassou*; monk fish, *Lophius budegassa*; Atlantic mackerel, *Scomber scombrus*; horse mackerel, *Trachurus trachurus*; anchovy, *Engraulis encrasicolus*; and megrim, *Lepidorhombus whiffiagonis*. In the sardine, *Sardina pilchardus*, only larvae of *Histerothylacium aduncum* were observed in viscera and body cavity. No larvae of *A. simplex* were found in flesh of albacore (*Thunus alalunga*), or in flesh or viscera of squid *Loligo forbesi*. The biggest length classes of hake, horse mackerel, Atlantic mackerel, blue whiting and monk fish showed higher infestation in flesh than small ones. In all species the part of the flesh more heavily infested was the belly flaps. In some species the percentage of larvae in belly flaps reached the 94 % of the total larvae found in flesh. Only in hake were the values of abundance and intensity in flesh higher than those in viscera and body cavity. Results of prevalence, mean and standard deviation of abundance and intensity for each specie are given in the following tables:

Atlantic mackerel (data on flesh infestation)

size average (cm)	nºexamined	prevalence	abundance	intensity
22.8	51	0%	0	0
35.5	49	33%	0.90 (2.4)	2.8 (3.5)

Percentage of total larvae found in:	
body cavity	3.0%
viscera	86.8%
flesh	10.2%

Megrim (data on flesh infestation)

size average (cm)	nºexamined	prevalence	abundance	intensity
22.5	25	12%	0.2 (0.65)	1.7 (1.2)
29.5	25	0%	0	0
30.6	25	0%	0	0
42.5	25	4%	0.04 (0.65)	1

Percentage of total larvae found in:	
body cavity	13%
viscera	63%
flesh	24%

Hake (data onflesh infestation)

size average (cm)	nºexamined	prevalence	abundance	intensity
22.3	19	47%	0.79 (1.4)	1.7 (1.7)
31.4	27	63%	1 (1.1)	1.6 (0.9)
38.9	22	59%	10.9 (24)	18.5 (29.3)
41.9	22	100%	46.3 (72.9)	46.3 (72.9)
47.1	22	95%	72.4 (55.8)	75.9 (54.7)

Percentage of total larvae found in:	
body cavity	20.4%
viscera	19.2%

flesh	60.4%

Sardine (data on body cavity infestation)

Only larvae of *Histerothylacium aduncum* were observed in this species

size average (cm)	nºexamined	prevalence	abundance	intensity
18.9	72	6%	0.06 (0.23)	1 (0)
21.5	37	38%	0.68 (1)	1.63 (0.89)

Percentage of total larvae found in:	
body cavity	93.5%
viscera	6.5%
flesh	0%

Blue whiting (data on flesh infestation)

size average (cm)	nºexamined	prevalence	abundance	intensity
19.2	36	36.1%	0.64 (1.1)	1.77 (1.2)
21	33	45.5%	1.4 (2)	3 (2)
28.9	35	51.4%	2.6 (3.7)	5 (3.8)

Percentage of total larvae found in:	
body cavity	10.3%
viscera	77.1%
flesh	12.6%

Horse mackerel (data on flesh infestation)

size average (cm)	nºexamined	prevalence	abundance	intensity
15.3	35	0%	0	0
31.3	35	40%	0.94 (1.5)	2.4 (1.5)
34.3	33	70%	6.7 (16.9)	9.7 (19.7)

Percentage of total larvae found in:	
body cavity	4.4%
viscera	78.4%
flesh	17.1%

Monk fish (data on flesh infestation)

size average (cm)	nºexamined	prevalence	abundance	intensity
24.6	12	0%	0	0
38.1	54	14.8%	0.63 (2.9)	4.3 (6.9)
44.1	12	8%	1.3 (4.3)	15 (0.0)
59.9	22	40.9%	1.8 (3.9)	4.4 (5.2)

Percentage of total larvae found in:	
body cavity	44.2%
viscera	31.9%
flesh	23.9%

Anchovy (data on body cavity infestation)

Only a single larva of *A. simplex* was found infesting the body cavity of one specimen of anchovy.

size average (cm)	nºexamined	prevalence	abundance	intensity
13.7	35	0%	0	0
14.3	35	0%	0	0
16.2	36	0.94%	0.009	1

Albacore

In this species the presence of larvae of *A. simplex* was only observed in the viscera.

size average (cm)	nºexamined	prevalence	abundance	intensity
55.1	17	5.9%	0.94 (3,9)	16 (0)
71.2	17	65%	1.71 (2)	2.6 (2)
78.0	16	81%	4.25 (6,5)	5.2 (6,9)

Squid

No larvae of *Anisakis simplex* were found in flesh or viscera of *L. forbesi.*

ANNEX 14: ANALYSIS OF PROGRESS WITH TASKS

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

b) Report on progress in the ongoing investigations of the effect of temperature on *Bonamia* infection dynamics; Because of the absence of key WGPDMO members to present this report, it was not possible to sufficiently address this topic during the meeting.

c) Evaluate and report on the confirmation of the agent of *Crassostrea angulata* gill disease and its infectivity to *Crassostrea gigas* and other oyster species; Because of the absence of key WGPDMO members to present this report, it was not possible to sufficiently address this topic during the meeting.

d) Report on the progress of further investigations on the role of paramoebae and other factors in the mass mortality of lobsters in Long Island; A summary of the current knowledge of paramoeba infections and their possible role in lobster mortalities was provided.

e) Review and assess a progress report on the development and intersessional analysis of ICES fish disease and related data banks and a draft manuscript for submission to ICES TIMES series on the statistical methods developed for the analysis of the data in the ICES data banks in relation to fish diseases (authors: W. Wosniok *et al*.); A summary report and a paper (ICES CM 2000/S:12) were provided and a draft manuscript for submission to the ICES TIMES series was also presented and discussed.

f) Review and assess an intersessionally prepared report on the compilation of existing data on spatial and temporal trends in the occurrence of selected parasites of wild fish and on potential environmental factors of relevance for the explanation of observed variance; A report containing a preliminary assessment of the analysis of long-term data sets in dab and whiting was provided.

g) Review progress reports from the BEQUALM Work Package "External Fish Diseases and Liver Histopathology" and from the EU project on nodaviruses and other relevant information to provide advice on effective control measures; A summary progress report was provided and a preliminary version of a CD- ROM Training Guide was demonstrated. Available information on the EU project on nodaviruses was reviewed.

h) Maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome; A verbal report summarising available information on *Ichthyophonus* and a working document on the recent progress within M74 research were presented.

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

j) Review an intersessionally prepared draft manuscript for publication in the *ICES Cooperative Research Report* series on important trends in disease problems in finfish and shellfish culture in the ICES area during the last five years; A draft manuscript was presented for consideration by the WGPDMO. Amendments to the proposed format were discussed and included as appropriate.

k) Evaluate the progress in the intersessional development of maps of marine fish and shellfish diseases as a contribution to the ICES Environmental Status Report. Examples of maps of marine fish and shellfish diseases/parasites were presented and the WGPDMO also viewed these on the ICES website.

ANNEX 15: RECOMMENDATIONS TO COUNCIL

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

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

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

c) review the current status of studies carried out in ICES Member Countries on the relationship between environmental contaminants and shellfish pathology; **(Catherine Couillard, Steve Feist, Sharon McGladdery)**

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

e) review the progress made in the BEQUALM Work Package 6 "External diseases and liver histopathology"; **(Steve Feist, Thomas Lang)**

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

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

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

i) review the current status regarding the characterisation and pathogenicity of viral haemorrhagic septicaemia virus (VHSV) strains in farmed and wild fish; **(Stig Mellergaard, NN Denmark)**

j) compile and review information on infectious pancreatic necrosis virus (IPNV) in salmonid fish farming; **(Brit Hjeltnes, David Bruno)**

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

- report on important trends in diseases occurring in finfish and shellfish culture in the ICES area in the period 1996–2000, **(Stig Mellergaard, coordinator)**
- web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report, **(Thomas Lang, Stig Mellergaard)**
- manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series, **(Thomas Lang, Werner Wosniok)**

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

Priority:	WGPDMO is of fundamental importance to the ICES advisory process.
Scientific Justification:	a) New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained. b) Experimental work is required to confirm field observations and hypotheses of Bonamia suppression vs. destruction over long periods of low temperatures. This question is important for accurately assessing climate effects on Bonamiasis and European oyster culture. There are historic records of an iridoviral infection of *Crassostrea gigas* gills, associated with low/transient pathology. This suggests that the gill disease agent may have multi-host infection potential, which needs to be addressed for like-to-like *C. gigas* and *Ostrea edulis* transfers. c) In recent years, an increasing effort in ICES Member Countries has been given to studies on shellfish diseases and pathology in relation to environmental contaminants. WGPDMO considered it timely to obtain an overview of progress in this field. d) An EU project "*Marteilia refringens* studies: Molecular systematics and search for the intermediate host of the bivalve mollusc's parasite" will be finalised in 2001 and WGPDMO considered it important to review the results of the project, as *Marteilia refringens* is one of the most important notifiable disease agents affecting European flat oyster production. e) Within the EU-funded project BEQUALM, a quality assurance programme for fish diseases and liver pathology is currently developing which will form an essential part of wild fish disease monitoring programmes. f) In mariculture, the Nodavirus group of viruses contains pathogens of major importance and the development of efficient disease control should be encouraged. An EU-FAIR research programme on Nodavirus will be finalised during 2001. This will lead to the publication of a final report and the arrangement of a workshop on the subject where the most recent results are presented. WGPDMO should review this report and the outcome of the workshop. g) ICES C.Res. 1993/2:23(m) requested that WGPDMO maintain an overview of the M74 syndrome and the *Ichthyophonus* issue as part of its regular agenda. h) Sea lice (*Lepeophtheirus salmonis*) continue to be a major disease problem in salmonid farming. New chemicals have currently been licensed and significant new national strategies are being implemented. This information should be compiled from relevant ICES Member Countries and incorporated into a working document to be assessed by WGPDMO. i) There is a lack of knowledge regarding the significance of recently isolated strains of VHSV and their implications on cultured and wild fish stocks. j) IPN has been recognised as an important disease in salmonid fish farming, but factors contributing to clinical disease are poorly understood. Commercial vaccines have been launched, but their efficacy has been difficult to evaluate. k) A number of ICES publications, either web-based or in ICES publication series, are being prepared or updated at present, the progress of which has to be reviewed by WGPDMO at its next meeting. It will be necessary to consider ways how these can be linked to each other.
Relation to Strategic Plan:	Responds to Objectives

Resource Requirements:	None required, other than those provided by the host institute.
Participants:	WGPDMO members
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	ACME
Linkages to other Committees or Groups:	MARC
Linkages to other Organisations:	BEQUALM, OIE, EU-FAIR, OSPAR