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Report of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO)

20–24 March 2007

Tenerife, Spain

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Executive summary

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met 20-24 March 2007 in Santa Cruz de Tenerife, Spain. The meeting was chaired by S. MacLean (USA) and attended by 18 participants and guests representing 13 ICES Member Countries. Considerable intersessional work done by WGPDMO members and a large number of working documents provided in advance of the meeting allowed all 10 Terms of Reference to be addressed in an efficient manner.

The agenda items covered a wide range of topics related to diseases and pathology in wild and farmed finfish and shellfish, with additional attention to environmental concerns.

Highlights of the meeting were:

- a report on new disease trends in wild and farmed fish and shellfish in ICES Member Countries, which is the only annual expert report available on this topic (ToR a, report section 5)
- a review of the hyperpigmentation condition in dab (*Limanda limanda*) from the North Sea, a condition only rarely found in other flatfish species in the North Sea and not in Baltic Sea dab (ToR b, report section 6)
- an update of information on salmon louse vaccine development and lice management strategies (ToR c, report section 7)
- a review of progress made on international collaborative actions that include disease and pathology components, such as the ICES Regional Ecosystem Study Group of the North Sea (REGNS), the ICES/HELCOM Working Group on Integrated Assessment of the Baltic Sea (WGIAB), the Baltic Sea Regional Project (BSRP), and the Biological Effects Quality Assurance in Monitoring Programme (BEQUALM) (ToR d, report section 8)
- a review of newly developed molecular techniques for diagnosis of pathogens in bivalves, emphasising the need for formal validation and intercalibration of methods in order to move these from research tools to diagnostic tools (ToR e, report section 9)
- a review of progress on constructing a ‘fish disease index’, a tool to be used in ecosystem health assessments using empirical dab (*Limanda limanda*) disease data (ToR f, report section 10)
- a progress report on ICES publications on pathology and diseases of marine organisms, including ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, and publications in the ICES Techniques in Marine Environmental Science Series (TIMES) (ToR g, report section 11)
- the WG encouraged ICES Member Countries to submit their fish disease data to the ICES Environmental Databank by using the ICES Environmental Data Reporting Format 3.2 and recommends to ICES to provide as soon as possible a data screening programme facilitating a data check by submitting labs prior to data submission to ICES. (ToR h, report section 12)

The WGPDMO concluded that all Terms of Reference for the 2007 meeting were considered in a comprehensive and satisfactory manner and identified a number of issues for further joint work and publication.

Since several important issues in the field of pathology and diseases of marine organisms were identified for further consideration, it was agreed that a further WGPDMO meeting is required in 2008. The meeting will be held in St. John’s, Newfoundland, Canada, during 4-8 March 2008.

1 Opening of the meeting

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) was hosted by the Centro Oceanografico de Canarias del Instituto Espanol de Oceanografia (IEO) in Tenerife, Spain with S. MacLean (USA) presiding. IEO suffered recent severe damage to their building and an alternative site was arranged by the IEO. The WGPDMO was very appreciative of the change in venue, particularly as this was on very short notice and the facility and staff provided were excellent. The meeting was opened at 10:00 hrs on Tuesday, 20 March 2007, with the Chair welcoming the participants, particularly the new members and guests who have not previously attended WGPDMO meetings. Felix Acosta, Universidad de Las Palmas de Gran Canarias, was welcomed as a local participant for one day. In total, 18 participants attended the meeting, representing 13 ICES Member Countries. A list of participants is appended in Annex 1.

Apologies were received from B. Hjeltnes (Norway), A. Karasev (Russia), O. Haenen (The Netherlands), M. Engelsma (The Netherlands), M. Wolowicz (Poland), W. Piasecki (Poland), and T. Wiklund (Finland). Enrique Garcia, Raul Laiz and IEO Deputy Director Luis Lopez Abellan warmly welcomed the participants on behalf of the IEO. Sergio Mesa Gonzalez provided instructions on in-house facilities and meeting logistics.

The meeting was held as a series of plenary sessions with the option to establish ad-hoc specialist subgroups as appropriate in order to consider some agenda items in detail before reporting conclusions back to the plenum for further consideration and endorsement.

2 Terms of Reference, adoption of the agenda, selection of Rapporteurs

2.1 Terms of reference

The WGPDMO noted the Terms of Reference published as C. Res. 2006/2/MCC04 (Annex 2). The agenda involved extensive intersessional work by the members of the WGPDMO who were asked to produce written working documents for review at the meeting and inclusion in the WGPDMO report as Annexes, as appropriate. As agreed by the WGPDMO members, all working documents were to be prepared four weeks before the meeting and were distributed by the Chair via e-mail. As a result, the majority of the national reports and most of the remaining working documents were sent to the participants prior to the meeting. This ensured that the Terms of Reference could be treated efficiently. A list of working documents provided prior to the meeting is presented in Annex 3.

2.2 Adoption of the agenda and timetable

A draft agenda (Annex 4) and a draft timetable were circulated and adopted without changes.

2.3 Selection of rapporteurs

Rapporteurs were accepted as indicated in Annex 5.

3 ICES items of relevance to WGPDMO

The Chair highlighted items of relevance to WGPDMO.

3.1 ICES Annual Science Conferences 2006, 2007 and 2008

The 2006 ICES Annual Science Conference was held 19-23 September 2006 in Maastricht, The Netherlands. Information on the ASC can be found on the ICES website at http://www.ices.dk/iceswork/asc/2006/index.asp. T. Lang (Germany), previous WGPDMO chair, and D. Vethaak (The Netherlands) co-convened Theme Session G "Human health risks and marine environmental quality", that included human health risks of potentially contaminated and/or parasitised seafood. This Theme Session was a good opportunity to raise the profile of the work conducted by WGPDMO in the ICES community.

T. Lang informed the WGPDMO that the WGPDMO National Reports of new disease trends are now published as ICES advice in the form of an ICES Advisory Document. This stresses the need for continued high quality and accuracy in the summarisation of the WGPDMO report.

The 2007 ICES ASC will be held in 17-21 September in Helsinki, Finland. Eighteen Theme Sessions are planned for this meeting, some specific for Baltic Sea fishes. Of interest to the WGPDMO members is the Theme Session on "Biological Effects of Contaminants in Coastal and Estuarine Ecosystems" which includes a pathology component. For those interested in presenting information at this Theme Session, the abstract and title should be submitted to ICES by the end of April and the paper submitted by August.

Through the focused efforts of T. Lang, a disease-related Theme Session has been accepted for the 2008 ICES ASC to be held in Halifax, Canada, entitled "New disease trends in marine organisms: causes and effects". Co-conveners are projected to be S. MacLean, T. Lang, and S. McGladdery (Canada). Abstracts and titles should be submitted by end of April 2008. This Theme Session aims to gather scientists from ICES Member Countries contributing paper and poster presentations on the following and other relevant aspects:

- causes and effects of emerging diseases in wild fish, shellfish and other marine organisms,
- causes and effects of new diseases in farmed fish and shellfish,
- the use of diseases of wild marine organisms as indicators in integrated ecosystem health monitoring and assessment,
- new trends in the disease interactions between wild and farmed fish and shellfish,
- effects of introduced species on the health status of native fish and shellfish stocks,
- new methodologies related to disease diagnosis and control.

The Theme Session will provide a forum for discussion of recent findings among scientists of many ICES Member Countries and for proposal of new research, monitoring and assessment priorities. WGPDMO members were encouraged to participate in this Theme Session and to spread the word to their colleagues.

3.2 ICES Mariculture Committee (MCC)

T. Lang informed the WGPDMO that the WGPDMO 2006 report and ToRs from the meeting were accepted by the Mariculture Committee.

4 Other relevant activities for information

Information was given on scientific conferences/workshops and projects with relevance to the work of WGPDMO.

4.1 Conferences/Workshops (chronological order)

- Fish Immunology Workshop. 15-19 April 2007, Wageningen University, The Netherlands
- International Symposium of Viruses in Lower Vertebrates. 22-25 April 2007, Oslo, Norway

- American Fisheries Society/Fish Health Section and Western Fish Diseases Workshop. 4-6 June 2007, Jackson Hole, WY, USA
- Eastern Fish Health Workshop. 18-22 June 2007, Gettysburg, PA, USA
- 7th International Symposium on Fish Immunology. 18-22 June 2007, University Stirling, Scotland
- Scandinavian-Baltic Society for Parasitology. 30 August – 1 September 2007, Rovaniemi, Finland
- 12th European Congress of Ichthyology. 9-13 September 2007, Dubrovnik, Croatia (**http://www.biol.pmf.hr/~ecixii/index.html**)
- 10th International Helminthological Symposium. 10-14 September 2007, Stará Lesná, Slovakia
- Workshop for the Analysis of the Impact of Perkinsosis to the European Shellfish Industry (WOPER). 12-14 September 2007, Vigo, Spain
- ICES Annual Science Conference. 17-21 September 2007, Helsinki, Finland
- 13th International conference of the European Association of Fish Pathologists. 17-21 September 2007, Grado, Italy
- International Symposium on Fish Parasites. 24-28 September 2007, Viterbo, Italy

4.2 Relevant Projects and Activities

- **Permanent Advisory Network for Diseases in Aquaculture (PANDA)**: Discussions on WGPDMO's association with PANDA have been held during the 2004 and 2005 WGPDMO meetings. The final PANDA Workshop "Progress report and future perspectives" was being held at Cefas in Weymouth (UK) concurrent with the WGPDMO 2007 meeting and the PANDA Coordinator requested further definition of the association between the two organizations to be included in a presentation at the workshop. Recognizing that the interests and activities of WGPDMO and PANDA are complementary, the WGPDMO wholly supported technical linkages from the PANDA website to the WGPDMO reports and encouraged members to register with PANDA. Depending on the future of PANDA, the WGPDMO will consider how to improve the exchange of information.
- **Working Group on the Biology and Life History of Crabs (WGCRAB)**: WGCRAB is a new ICES Working Group having been formed recently from a Study Group of the same name. The WGCRAB will meet in May 2007 in Lowestoft, UK, and has a term of reference "assess and report on environmental effects including diseases on crab fisheries". The WGPDMO recognizes the importance of diseases in stock assessment but expressed concern that this ToR did not come to the WGPDMO, the ICES Expert Group on Pathology and Diseases of Marine Organisms. Direct and immediate communication between the WG chairs could be accomplished when mutually relevant information is presented during either WG meeting.
- **ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON)** The chair reported that the 2007 WKIMON report is currently available on the ICES website (http://ices.dk/reports/ACME2007/WKIMON.pdf) in which the Fish Disease Index produced by WGPDMO was given strong support and recognition for its outstanding work on this topic. The recommendation by WKIMON was for WGPDMO to continue this effort to its completion and forward the final product to OSPAR for consideration. WGPDMO noted that the monitoring of fish diseases is considered by WKIMON to be an important component for the integrated monitoring and assessment of contaminants and their biological effects.
- **Working Group on the Biological Effects of Contaminants (WGBEC)** The chair reported on a recent request by the chairs of the Advisory Committee on Ecosystems (ACE) and WGBEC for information on the changes in distribution, abundance and condition of marine species in the OSPAR area that are driven by

contaminants or by the interactions of effects of contaminants and changes in hydrodynamics and sea temperature. Since this request relates to a paper in preparation by WGPDMO members on effects of climate change on marine fish and shellfish diseases, the chair of WGPDMO will forward the draft manuscript to the chairs of WGBEC and ACE for their perusal and use as appropriate.

- **Workshop on the Integrated Assessment of Contaminants (ICON)** Prior to the WGPDMO meeting T. Lang had distributed a prospectus for the integrated assessment of contaminants in the North Sea. The objective of this workshop is to assess the health of the North Sea ecosystem through an international sea-going workshop. Components of the workshop are 1) discussion of approaches and methods; 2) data compilation for the locations and areas selected for study; and 3) field studies at the selected locations and areas in 2008. Outlines of proposals are to be submitted by 1 May 2007 to Ketil Hylland (ketilhy@bio.uio.no). This workshop is linked to the development of integrated monitoring guidelines within OSPAR (WKIMON) which is aimed at ensuring a core set of analyses are performed at each location.
- **Reform of the ICES Advisory Structure** The chair reported to the WGPDMO that the ICES Council recommendation for reforming the advisory process in ICES is a priority of the new ICES President. All members of the ICES community can be involved in the debate by joining the discussion forum available: http://portal.ices.dk/ICESAdvisorySystem/default.aspx or by emailing comments to: REFORM@ices.dk.
- **ICES SharePoint** The chair discussed the availability of the ICES SharePoint, an internet accessible location for storing documents for use before, during and shortly after the meeting. All members and guests attending this meeting were contacted by ICES and provided with log-in information. All reports received prior to the meeting were posted on SharePoint and were accessible during the meeting. SharePoint has many excellent features and should prove to be a very valuable tool for report handling.

5 Produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports

The update in the following sections is based on national reports for 2006 submitted by Canada, Denmark, England and Wales, Finland, France, Germany, Ireland, Latvia, Norway, Poland, Russia, Scotland, Spain, Sweden, The Netherlands, and USA. It documents significant observations and highlights the major trends in newly emerging diseases and in those identified as being important in previous years.

5.1 Common and Scientific Names of Host Fish and Shellfish Species Reported in Section 5

Asian oyster (*Crassostrea ariakensis*)

Atlantic salmon (*Salmo salar*)

Baltic herring (*Clupea harengus membras*)

Baltic macoma clam (*Macoma balthica*)

blueback herring (*Alosa aestivalis*)

bream (*Abramis brama*)

brown trout (*Salmo trutta*)

capelin (*Mallotus villosus*)

hermit crab (common) (*Pagurus bernhardus*)

Japanese carpet clam (*Ruditapes phillipinarum*)

largemouth bass (*Micropterus salmoides)*

mummichog (*Fundulus heteroclitus*)

Northern abalone (*Haliotis kamtschatkana*)

Norwegian lobster (*Nephrops norvegicus*)

Pacific oyster (*Crassostrea gigas*)

pink salmon (*Oncorhynchus gorbuscha*)

Chinese mitten crab (*Eriochier sinensis*)

chum salmon (*Oncorhynchus keta*)

cod (*Gadus morhua*)

crested oyster (*Ostreola equestris*)

dab (*Limanda limanda*)

Eastern oyster (*Crassostrea virginica*)

European abalone (*Haliotis tuberculata*)

European clam (*Ruditapes decussatus*)

European edible crab (*Cancer pagurus*)

European eel (*Anguilla anguilla*)

European flounder (*Platichthys flesus*)

European lobster (*Homarus gammarus*)

flat oyster (*Ostrea edulis*)

fourbeard rockling (*Enchelyopus cimbrius*)

Great scallop (*Pecten maximus*)

haddock (*Melanogrammus aeglefinus*)

halibut (*Hippoglossus hippoglossus*)

hard clam (*Mercenaria mercenaria*)

pollack (*Pollachius pollachius*)

red fish (*Sebastes mentella*)

red king crab (*Paralithodes camtschaticus*)

red turban snail (*Astraea gibberosa*)

sea bass (*Dicentrarchus labrax*)

seabream (*Pagellus bogaraveo*)

soft shell clam (*Mya arenaria*)

sole (*Solea senegalensis*)

spot (*Leiostomus xanthurus*)

striped bass (*Morone saxatilis*)

striped killifish (*Fundulus majalis*)

turbot (*Scophthalmus maximus*)

tusk (*Brosme brosme*)

weakfish (*Cynoscion regalis*)

white perch (*Morone americana*)

whiting (*Merlangius merlangus*)

winter flounder (*Pseudopleuronectes americanus*)

5.2 Wild Fish

5.2.1 Viruses

Viral Haemorrhagic Septicaemia Virus (VHSV) – The virus has spread rapidly further in the Great Lakes (USA and Canada) and was detected in Lake Erie, Lake Ontario, Lake St. Clair, the St. Lawrence River and most recently in Lake Huron. Mortalities have been reported in 15 fish species and the virus has been isolated from more than 40 species in the Great Lakes. Genotyping indicates this pathogen should be considered sublineage IVb, distinct from the other North American types (IVa).

Lymphocystis - The prevalence of lymphocystis in dab in the German Bight, North Sea, continued to be low. The prevalence of 2.7% recorded in Aug./Sept. 2006 was among the lowest ever recorded (since 1981). German Bight data suggested the prevalence of lymphocystis is higher in winter compared to summer. In European flounder from the Polish EEZ of the Baltic Sea the prevalence of lymphocystis increased only in subdivision 25 compared to previous years. In Baltic herring from Polish waters the prevalence decreased to a very low level (0.05%).

***Herpesvirus anguillae* (HVA)** - HVA was isolated from diseased European eel in Lake Mälaren. This represents the first isolation of HVA from Sweden. The virus was also found in wild eels one month after capture on the Mediterranean Spanish coast. This is the first isolation of HVA in Spain.

5.2.2 Bacteria

Acute/healing skin ulcerations –Russian data showed the prevalence of ulcers in European flounder collected in March was 0.3 % from the Russian EEZ (ICES Subdivision 26) and 1.7 % from the Polish EEZ (ICES Subdivision 26). Polish data collected between November and February confirmed the low prevalence of skin ulcers in flounder sampled in ICES Subdivision 25 and 26 (0.26%).

The prevalence in dab from the German Bight, the Firth of Forth (North Sea, Scotland) and Rye Bay has decreased compared to 2005. In dab at West Dogger Bank, an increase has been observed (6.2% to 13.0%). A prevalence of 4.7% in dab at Flamborough was the highest observed since 1993.

The prevalence in Baltic cod from the Russian EEZ was 2.2%, 2.3% and 1.4% in March, August and October, respectively. The prevalence in the Polish EEZ (ICES Subdivision 26) was 2.6% in March and 0.85% in October. Polish surveys revealed an increasing trend from 2004 to 2006. The highest prevalence of 5% was observed in Subdivision 26.

***Francisella* sp. -** A *Francisella* sp. has been confirmed in cod collected from the Skagerrak on the west coast of Sweden in 2004 and stored frozen. The genetic sequence of the Swedish isolate was almost identical to a Norwegian isolate. Clinical signs included external skin lesions and whitish granulomas in many internal organs. This represents the first isolate from Sweden. Koch's postulates were established using Swedish isolates in an experimental study with Atlantic cod in Scotland. Joint work between FRS, Aberdeen, and the National Veterinary Institute, Sweden, is being planned to develop diagnostic methods. Cases of a similar but undiagnosed visceral granulomatosis, were reported in 2006 in juvenile cod angled off the Norfolk coast (UK). The potential for infections in the mid-1980s is being assessed through analysis of archived tissues from wild cod that exhibited visceral granulomatosis.

***Mycobacterium* spp.** - Approximately 15 *Mycobacterium* spp. have been reported from diseased striped bass collected from the Chesapeake Bay, Maryland, USA. The predominant isolate belongs to the *M. chesapeakii-shotsii-pseudoshottsii* complex. The infection and disease in Chesapeake Bay striped bass are age dependent with prevalence up to 80% in age-6 fish. The most severe disease occurs in the late summer and fall. Vertical transmission is suggested by the isolation of mycobacteria from reproductive fluids. Mycobacteria have also been identified in blueback herring, winter flounder, striped killifish, mummichog, largemouth bass, weakfish, spot and white perch from Chesapeake Bay. The disease occurs as either visceral granulomatous lesions or skin lesions. Skin pathology may be a non-lethal indicator of overall disease and a predictor of visceral lesions. The impact of mycobacteriosis on populations of striped bass in Chesapeake Bay is unclear. Results of a recent workshop on Mycobacteriosis were compiled and published online (http://www.nccos.noaa.gov/documents/SIR2006-5214-complete.pdf).

Vibrio vulnificus – The bacterium was isolated from eels from coastal areas in southern Zealand, Denmark. High mortalities were observed and clinical signs included widespread external haemorrhages. The bacterium was also isolated from the water and these observations have been linked to unusually high water temperatures.

5.2.3 Parasites

Diplomonadida

Spironucleus torosa - Identified in three gadoid species: tusk, whiting, and fourbeard rockling. The whiting and fourbeard rockling were caught in the southern Baltic Sea. The tusk was caught in the Northern Oslo Fjord.

Myxozoa

Tetracapsuloides bryosalmonae – Proliferative kidney disease (PKD) was identified for the first time in yearling Atlantic salmon and brown trout experiencing significant mortalities in two Norwegian rivers, concurrent with increased water temperature.

Trematoda

Data from bream from the Russian EEZ of the Curonian Lagoon (Baltic Sea) revealed the prevalence of "black spot" (metacercaria of *Posthodiplostomum cuticola*) was 7.0% in May, 43.0% in July and 25.0% in October. The prevalences of parasitic cataracts caused by metaceracariae of *Diplostomum spathaceum* and *Tylodelphys clavata*, and of gross external parasites were low (< 2%).

Nematoda

***Anisakis simplex* (larvae)** – A long-term decreasing trend in prevalence of infection of *A. simplex* among Baltic herring in the Polish and Russian EEZs continued. As previously reported, the prevalence remained negatively correlated with the mean mass of individual herring. Since 2004 in the Barents Sea, prevalences greater than 85% have been observed in cod, redfish and haddock. However, in those species there was a trend of increasing parasite abundance. The abundance increased in cod from approximately 10 to 30; in redfish from 4 to 16.7 and in haddock from 10.3 to 24.4. In contrast, prevalence and abundance in capelin both increased during this period from 24% to 54% and from 0.4 to 0.9, respectively.

***Pseudoterranova decipiens* (larvae)** – A decreasing trend in prevalence (40% to 0%) was observed in cod from the Barents Sea between 2003 and 2006.

Philometra ovata – The prevalence in bream from the Curonian Lagoon (Baltic Sea) ranged from 14.5% in July to 1.2 % in October.

Crustacea

Lepeophtheirus salmonis – There was a declining trend in the prevalence and intensity of salmon lice on early marine phase pink and chum salmon in British Columbia. Since 2004 the prevalence has dropped from 63% to 14% and abundance has dropped from 2.6 lice per pink salmon and 7.1 lice per chum salmon to 0.2 lice per fish of both species.

Lepeophtheirus pectoralis - The prevalence in dab from hot spot areas of the North Sea (German Bight, Dogger Bank) has decreased since 2004 but still seems to be elevated compared to the long-term average.

5.2.4 Other diseases

Hyperpigmentation – The prevalence in North Sea dab remained high in the majority of examined areas (German Bight, Dogger Bank and Firth of Forth). A prevalence of 53% was observed in the German Bight, the highest ever recorded in this area. This is consistent with long-term data showing a dramatic increase over the past 20 years.

Liver nodules – The prevalence in dab increased, especially in the German Bight, North Sea, and in some areas of the Irish Sea. A similar trend was recorded in flounder from the German Bight.

Hepatic neoplasia - Recorded in mummichogs from creosote-contaminated sites in the USA. Laboratory exposures of mummichogs to creosote conducted under environmentally realistic conditions produced similar hepatic neoplasms.

Epidermal hyperplasia and skin papilloma – The prevalence of these conditions in bream from the Russian EEZ of the Curonian Lagoon (Baltic Sea) was highest in May (28% epidermal hyperplasia; 34% skin papilloma).

Toxic algae - *Aphanizomenon flosaquae* and *Microcystis aeruginosa* were found in the Curonian Lagoon (Russian EEZ of the Baltic). Widespread tissue damage in visceral organs of bream was associated with the algae.

A metal - containing exotoxin produced by *Pfiesteria piscicida* was recently identified during experimental studies conducted in the USA. The toxin required specific environmental conditions for production and rapidly degraded into lethal free radicals.

M74 Syndrome – An increasing trend in Atlantic salmon fry was recorded in Sweden between 2004 and 2006 (3% to 18%).

5.2.5 Conclusions

1) There is concern that the rapid spread of a new sub-group of VHS virus (IVb) among 40 species of fish that has occurred in the Great Lakes may have a significant impact to the ecology of fishes in the region.

2) *Herpesvirus anguillae*, identified for the first time in European eels in Sweden and Spain, raises concern that this is an emerging threat.

3) *Francisella* sp. associated with visceral granulomatosis was confirmed in Swedish cod for the first time and points to the potential for wild and farmed cod interactions.

4) There is a potential for significant impact on the striped bass population in the Chesapeake Bay (USA) due to the high prevalence of visceral mycobacteriosis.

5) Severe PKD (caused by *Tetracapsuloides bryosalmonae*) resulting in mass mortality of yearling salmon and brown trout in Norway was noted in association with increased temperature, raising concern for the sustainability of wild salmonid populations.

6) A declining trend of *Lepeophtheirus salmonis* observed in British Columbia on juvenile pink and chum salmon may be associated with chemotherapeutant treatment of adjacent farm populations.

7) The prevalence of *Anisakis simplex* larvae in Baltic herring continued to decline from 1997.

8) Hyperpigmentation in North Sea dab is one of the most prevalent anomalies observed and continued to increase in prevalence in some areas.

9) Laboratory exposures of mummichogs to creosote, conducted under environmentally realistic conditions, produced hepatic neoplasms similar to those found in wild mummichogs collected from creosote contaminated sites.

5.2.6 Recommendations

The WGPDMO recommends that:

i) ICES Member Countries continue to fund fish disease monitoring programmes to sustain fish health surveillance of wild stocks. Information obtained is of vital importance to integrated assessments of the health of marine ecosystems and will provide useful baseline data, e.g., to serve as a reference prior to establishing the culture of non-salmonid marine species. In addition, fish disease monitoring data will be useful in evaluating the effects of climate change on fish health and provide better understanding of pathogen interactions between wild and farmed fish.

ii) WGPDMO reviews the status of Proliferative Kidney Disease epidemics caused by *Tetracapsuloides bryosalmonae* in wild salmonid populations at its 2008 meeting.

5.3 Farmed Fish

5.3.1 Viruses

Heart and skeletal muscle inflammation (HSMI) – An increase in HSMI is reported from Norwegian marine farms rearing Atlantic salmon, from 54 cases in 2004 to 94 in 2006.

Infectious Pancreatic Necrosis Virus (IPNV) –IPNV in Ireland has caused low level losses in Atlantic salmon marine sites. However, more serious problems were encountered in the freshwater phase of production with losses up to 30%

Infectious Salmon Anaemia Virus (ISAV) – The number of Atlantic salmon farms in Norway with ISA has declined from 12 to 4 between 2005 and 2006. There are no new trends in Canada, although ISA is still present in the Bay of Fundy. The USA and Canada have implemented a single bay management area for ISA that covers all Maine and New Brunswick sites in Cobscook and Passamaquoddy Bays.

Salmonid Alphavirus (also known as Salmon Pancreas Disease Virus) – An increase in occurrence of pancreas disease (PD) in farmed Atlantic salmon is reported in Norway. PD also causes serious problems for Atlantic salmon in Ireland, although losses appear to have stabilised in 2006.

Viral Nervous Necrosis Virus (VNNV) – VNNV was observed for the first time in farmed cod in Norway. Sequenced PCR products show similarity to virus causing VNN in halibut. Nodavirus also was detected for the first time in turbot in Spain with prevalence less than 5% in asymptomatic carriers.

Viral Haemorrhagic Septicaemia Virus (VHSV) – VHSV was detected in sole in Spain for the first time, with no associated mortality.

5.3.2 Bacteria

Aeromonas salmonicida and ***Tenacibaculum maritimum*** – Despite the use of vaccines and the concomitant decrease in outbreaks, significant losses continue in turbot in Spain due to these two bacterial species.

***Edwardsiella tarda* –** The strains isolated from turbot in Spain belong to a new serotype and hence are different from those reported in other fish species. An experimental vaccine gave good protection (better than 80%) but the duration of immunity requires further work.

Francisella – *Francisella* is one of the main bacterial infections of cod in Norway. Six cod culture sites in Norway were diagnosed with francisellosis and there are strong indications that more cases occurred. Significant problems with francisellosis have been reported, with some affected stocks being destroyed. Information for early 2007 indicates that this trend is continuing.

Photobacterium damsela* subsp. *piscicida (Pasteurellosis) **–** Incidence was greater than 45% in seabream from the Canary Islands.

Listonella (Vibrio) anguillarum - During routine analysis the serotype O2 was isolated from asymptomatic pollack in Spain. *L. anguillarum* is a significant problem of cod in Norway.

Vibrio vulnificus biotype 2, serotype A - Reported from eel farmed on the Spanish Mediterranean coast. This is a new serotype for this area and rarely reported from farmed fish.

***Vibrio* sp**. - Feed strategies in sea bass (*Dicentrarchus labrax*) culture have resulted in a fatty liver syndrome and secondary *Vibrio* infections in The Netherlands.

5.3.3 Parasites

Protista

Amoebic gill disease (AGD) - The first occurrence of AGD (*Neoparamoeba*) in Scotland was reported from farmed Atlantic salmon in October. Stress during well boat transfer probably resulted in increased infestation and consequent gill pathology.

Cestoda

***Eubothrium* sp.** – The prevalence of this cestode is declining in Norway, but the parasite caused significant losses in marine Atlantic salmon and rainbow trout farms due to reduced growth and is becoming resistant to therapeutic treatment (Praziquantel).

Myxozoa

Enteromyxum scophthalmi - Infection resulted in reduced growth in juvenile turbot in Spain.

Monogenea

Gyrodactylus marinus – Gill infestations have resulted in mortality in cod in Norway.

Ciliophora

***Miamiensis avidus* (*Philasterides dicentrarchi*)** – Infection by this ciliate has continued to result in significant mortality of turbot in Spain, increasing by 10-20% over a 2 year period.

Crustacea

Sea lice – Infestation by *Caligus elongatus* and *C. curtus* are increasing in farmed cod in Norway, whereas Atlantic salmon sea lice numbers are kept low through the use of wrasse and emamectin benzoate. *Lepeophtheirus salmonis* in Ireland is proving increasingly difficult to control as lice appear to be tolerant to authorised chemotherapeutants.

5.3.4 Other diseases

Cardiomyopathy syndrome (CMS) – Cardiac lesions attributed to CMS result in significant losses in some Atlantic salmon in Norway, particularly large salmon that are ready for market.

Gill disorders – These represent a growing problem and the most significant losses (1% to 80%) of Atlantic salmon in Ireland. Further studies are planned in relation to epidemiology, challenge experiments and dietary supplements. *Neoparamoeba* sp. is considered to be a secondary invader.

Algal blooms - Losses of farmed Atlantic salmon attributed to *Karenia mikimotoi* coupled with other factors including high stocking densities and losses due to jellyfish, including Lions mane (*Cyanea capillata*), occurred in Scotland. Biomass loss amounted to 109.7 tons between August and October.

5.3.5 Conclusions

1) Heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS) are of concern for the fish farming industries of Norway and Scotland.

2) New diseases or new biotypes are emerging, including *Edwardsiella* in turbot, *Francisella* in cod, amoebic gill disease in salmon and *Vibrio vulnificus* in eel.

3) There is circumstantial evidence of increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants.

5.3.6 Recommendations

The WGPDMO recommends that:

i) ICES Member Countries conduct comparative studies on *Francisella* sp. and on the visceral granulomatous condition in farmed cod (*Gadus morhua*) and review the potential for disease interaction between wild and farmed cod.

5.4 Wild and Farmed Molluscs and Crustaceans

5.4.1 Viruses

Herpesviruses in bivalves - No change reported in France and no new information from the USA.

Viral gametocytic hypertrophy - Continued to be rare in Pacific oysters in France. Histological examination of Pacific oysters in Germany (German Wadden Sea) revealed cases of abnormally large basophilic cells in gonadal tissues that resemble cells characteristic of ovocystis disease (viral gametocytic hypertrophy). However, the viral aetiology has not yet been demonstrated.

Picorna-like virus - Following mortalities of stock exported to France, samples of juvenile European clams from the source hatchery in southern England were analysed. Using transmission electron microscopy, these were found to contain picorna-like virus particles in their connective tissue cells. Mortalities were not experienced in the hatchery. Moreover, amplicons were obtained using primers targeting picornavirus for PCR analysis of animals collected during the mortality outbreak occurring in France. The finding needs to be verified using complementary molecular analyses.

Gill Epithelial Cell Nuclear Virus – Non-enveloped viral particles were reported among soft clam samples collected in 2006 from Chesapeake Bay, USA, suffering from a condition first noted in 2004 as gill lesions including intranuclear inclusions. The prevalence of this viral infection is high (85%). Similar viral lesions were previously described in the literature in soft clams collected during 1972 from Massachusetts.

White Spot Syndrome Virus - No new information from the USA.

5.4.2 Bacteria

Nocardiosis - No new trend in Canada and no new information from the USA. *Nocardia crassostreae* was isolated for the first time from Pacific oysters during mortalities in August 2006 in Lake Grevelingen, the Netherlands. *Nocardia*-like infections have been observed in routine monitoring for shellfish diseases since 2003 in Pacific oysters as well as flat oysters in the Dutch estuaries. Primary cause of the mortalities in 2006 was thought to be prolonged high water temperatures and low oxygen.

Withering syndrome – No new information from the USA. *Candidatus xenohaliotis californiensis,* the causative agent of Withering Syndrome was detected in moribund European abalone in Spain among animals exported from Ireland. A follow-up investigation indicated that the pathogen is present in four Irish hatcheries but in each case, the infection was sub-clinical.

Vibrio tapetis - Positive PCR results were obtained for *Vibrio tapetis* from two sites growing Japanese crested clams in Ireland while the brown ring disease was not observed.

***Vibrio* spp.** - In France some isolates of *V. splendidus* were reported in association with mortality of spat of great scallops. Norwegian lobsters landed in Skagen (Denmark) in February 2006 had lesions penetrating through the shell that appeared similar to “shell disease”. The bacteria isolated from the lesions of five lobsters were identified as *Vibrio* spp.

Juvenile oyster disease (JOD) – A JOD outbreak, diagnosed by PCR detection of *Roseovarius crassostreae*, occurred in an Eastern oyster hatchery in Connecticut, USA. The occurrence was the first for this hatchery. JOD continues to be a problem on Martha's Vineyard, off of Massachusetts, USA. Outbreaks are variable from year to year in Massachusetts but tend to recur in the same locations.

Rickettsia - Rickettsia-like organisms were associated with abnormal mortality in spat of great scallops in France.

Gaffkaemia - Gaffkaemia (*Aerococcus viridans*) was confirmed in European lobsters held at a storage facility in South Wales (UK) following reports of mortalities at this facility. Haemolymph smears and culture were also undertaken on lobsters from a storage facility in North Yorkshire (UK), following reports of Gaffkaemia at the receiving storage site in France. All these tests were negative. Further investigations into the outbreak in Wales are planned. Gaffkaemia was also recorded in September 2006 in Scotland from a batch of lobsters destined for export. The consignment was destroyed. This is the first occurrence of gaffkaemia in Scotland since 1991.

5.4.3 Fungal Infections

Cladosporium herbarum (Hyphomycetes) - The fungus was isolated from ulcers and hemolymph of diseased and moribund red king crab in the Barents Sea (Russian EEZ).

5.4.4 Parasites

Protista

Bonamia ostreae - *Bonamia ostreae* was detected in flat oysters for the first time in Lough Swilly (NW Ireland) in 2006 associated with significant mortalities. This is the nearest bay to L. Foyle which was found to be infected in 2005. The parasite has spread despite movement restrictions and an epidemiological investigation is ongoing.

B. ostreae was confirmed in native flat oysters in Loch Sunart in south west Lochaber (Scotland) in April 2006. This represents the first occurrence of this parasite in Scotland and was found during routine statutory monitoring. It is not known how or when the disease was introduced to Loch Sunart. The parasite was also detected for the first time in Wales (UK) during 2006. The original positive sample was taken from a natural bed of native flat oysters in the River Cleddau. Results from further sampling have shown that the disease organism is present at a low level of prevalence throughout the fishery areas in Milford Haven (south Wales).

Overall the prevalence of infection of *B. ostreae* in native flat oysters at farm sites and in fisheries in England and Wales increased during 2006 compared with 2005. The mean prevalence of infection in fisheries was the highest recorded in 14 years of sampling. The average for all fishery sites was 7.3%, compared with 4.5% the previous year and a ten-year average of 4.5%. Some east-coast fishery sites showed high levels of infection, boosting the average figure. This area has seen good natural recruitment in recent years and it may be that these increased levels of infection were a response to increasing densities of oysters on the bottom.

No change was found in France and Spain.

Bonamia perspora **-** A description of this unique spore-forming *Bonamia* species, known only from the crested oyster in South Carolina, USA, was published in 2006. There were no changes in its activity or distribution.

***Bonamia* sp.** - The *Bonamia* sp. in the experimentally introduced Asian oyster was also found in the native crested oyster from southern South Carolina, USA. PCR prevalence was 2/55 (3.6%), and *Bonamia* sp. cells were confirmed histologically. This finding expands the known range of this parasite by 500 km south of the site where it was originally found and confirms its status as an enzootic parasite.

Haplosporidium nelsoni – *Haplosporidium nelsoni* (MSX) in eastern oysters in the Bras d'Or Lakes, Nova Scotia, Canada has persisted in that location and expanded its range since 2002 to three new sites within the Lakes. In 2005, MSX was confirmed outside of the Bras d'Or Lakes and in 2006 a second site was confirmed on the north shore of Cape Breton. Both locations had historic oyster transfers from the Bras d'Or Lakes prior to 2002. Active surveillance of a buffer area around Cape Breton and oysters in the southern Gulf of St. Lawrence is ongoing. No evidence has been found to date of the presence of *H. nelsoni* (or *H. costale*) in historic samples from within the Bras d'Or Lakes.

Severe infections of *H. nelsoni* in eastern oysters, which resulted in heavy mortality, were recorded in 80 to 100% of 2-year old oysters collected from 5 different leases on the north side of Cape Cod, Massachusetts, USA in late autumn 2006. *H. nelsoni* infection levels were low and caused no apparent problems elsewhere in its range along the east coast of the USA.

***Haplosporidium* sp.** – A *Haplosporidium* sp. was detected during monitoring of local populations of European abalone in Spain but was not associated with disease. This is the first discovery of *Haplosporidium* sp. since monitoring began more than a year ago, after *Haplosporidium* was discovered in abalone transported to Spain from Ireland.

Microsporidia - A new genus and species has been described from the European edible crab (*Cancer pagurus*) and the common hermit crab captured in the UK. The new genus, *Enterospora,* is an intranuclear parasite and the first of its type described in an invertebrate host. No data is available on the impact of this pathogen on host populations. Another microsporidian parasite has been described infecting Chinese mitten crabs captured from the River Thames, UK, at up to 60% prevalence. The parasite is likely to be the same as that recently described during 2006 from the Chinese mitten crab from their native habitat in Asia, suggesting that the invasion of UK rivers by this crab species has also introduced an exotic microsporidian pathogen. No studies have been carried out on the transmission of this pathogen to other hosts or on the effect of the pathogen on populations of mitten crabs.

Kidney coccidian - A kidney coccidian, morphologically similar to *Margolisiella (=Pseudoklossia) haliotis,* was found in cultured northern abalone in British Columbia, Canada. Affected animals were considered as 'runts' (3 year old) and had a prevalence of 91%. Morphologically similar coccidia were detected at low levels in the kidney of red turban snails adjacent to the abalone hatchery effluent outlet. Subsequently, kidney coccidia were found in red turban snails over a wide distribution in BC. Research to identify the genetic relationship between the coccidia in the abalone and red turban snails is in progress.

Hematodinium - *Hematodinium* sp. was detected in 8.9% of male and 4.1% in female Norwegian lobsters from the Swedish west coast during July and August 2006, respectively, this being the highest prevalence recorded for five years. The infection was found in trawl-caught animals but not in those collected by trapping.

Crustacea

Parasite-associated mortality - Mortalities were investigated in lobsters in two on-growing facilities in Ireland. Gill damage associated with the copepod *Nicothoe astaci* was identified in each case.

5.4.5 Other diseases

Summer Mortality - As in the summer of 2005, considerable mortalities of Pacific oysters were noted at stations along the German coast of Lower Saxony (Wadden Sea) in July and in November 2006 (an exceptionally warm autumn). Juveniles were more frequently affected than adults and mortality was more severe in inner harbour areas as compared to outer areas. Histopathological changes in gonads, digestive tract and gill tissues have been reported for the 2005 samples; 2006 samples are still under investigation and a viral aetiology is suspected.

Neoplasms - Disseminated neoplasia occurring in the Baltic macoma clam continues to be a serious problem in the low biodiversity ecosystem of the Gulf of Gdańsk, Poland, with an average prevalence of 18%. Prevalences of disseminated neoplasia were low in samples of soft clams collected from the Chesapeake Bay, USA, during 2006. A single hard clam collected from waters near New York City was diagnosed with haematopoietic neoplasia. This condition has not previously been described in hard clams.

5.4.6 Conclusions

1) An enzootic *Bonamia* sp. parasitising the non-commercial native crested oyster and first identified in South Carolina, USA, in 2004 during testing of the non-native Asian oyster, was detected in crested oysters at a new site, 500 km south of the original site, indicating its endemic status over a wider range than previously known.

2) The only known spore-forming species of *Bonamia* (*B. perspora*) has been described from the crested oyster from South Carolina, USA.

3) *Bonamia ostreae* was diagnosed for the first time in flat oyster from Scotland and Wales.

4) Up to 85% prevalence of virus infections in the nucleus of gill epithelial cells of the soft clam have been detected for the past three years (2004-2006) in Chesapeake Bay (USA). An identical condition was first described from soft clams in Massachusetts, USA, in 1972 but has not been reported since.

5) Abnormally large basophilic cells in gonadal tissues that resemble cells characteristic of ovocystis disease (viral gametocytic hypertrophy) were reported for the first time in Pacific oysters in Germany (German Wadden Sea).

6) Picorna-like virus particles were described for the first time in European clams originating from a hatchery located in southern England with the absence of clinical signs and mortality.

7) *Candidatus xenohaliotis californiensis*, the causative agent of Withering Syndrome was detected in European abalone for the first time in four Irish hatcheries with the absence of clinical signs.

8) The first case of haematopoietic neoplasia was reported in a hard clam from waters near New York City, USA.

5.4.7 Recommendations

The WGPDMO recommends that:

i) studies be initiated or expanded on the significance of the newly described picorna-like virus in European clam *Ruditapes decussatus*.

ii) investigations continue on *Candidatus xenohaliotis californiensis* in European abalone, *Haliotis tuberculata*, in Europe.

iii) studies continue on gonadal tissue lesions in Pacific oysters, *Crassostrea gigas*, in Germany including transmission electron microscopy examination.

iv) studies be expanded on the virus of the gill epithelial cell nucleus in soft clams *Mya arenaria.*

6 Review the condition of hyperpigmentation in common dab (*Limanda limanda*) with special reference to histopathological and ultrastructure findings, analysis of prevalence and temporal changes, possible causes and similarities with other species.

T. Lang summarised a report by T. Lang, S. Feist, W. Wosniok and F. Baumgart (Annex 6) reviewing the results of a 18-year data set (1989-2006) of hyperpigmentation in flatfish, primarily dab (*Limanda limanda*), in the North Sea. Results of this long-term study indicated the following:

- Hyperpigmentation is more common in dab than in other flatfish species from the same habitats; lemon sole (*Microstomus kitt*), long rough dab (*Hippoglossoides platessoides*) and solenette (*Buglossidium luteum*) from the North Sea and, possibly, in Baltic flounder (*Platichthys flesus*) from a region on the Swedish east coast.
- Baltic Sea dab are not affected by hyperpigmentation.
- Macroscopic and histopathological findings indicate a condition with distinct features characterised by hyperplasia of melanocytes (mainly in the skin of the upper body side) and iridophores (mainly in the skin of the lower body side).
- Preliminary evidence suggests that the condition can be associated with an inflammatory response and degenerative processes.
- The prevalence of hyperpigmentation in the North Sea differs markedly between regions. For instance, in 2006, prevalence in female dab, size group 20-24 cm total length, ranged from <10% in areas in the northern central and in the southernmost North Sea to >50% in the German Bight, at the Dogger Bank and in the Firth of Forth area.
- A significant and dramatic increase in prevalence has occurred in many North Sea areas from <5% in most areas in 1989 to >50% in many areas in 2006.
- Hyperpigmentation is more prevalent in larger fish and in males as compared to females. This is presumably an age-related effect. The higher prevalence in males is thought to be due to lower growth rates and greater age at a given size than females.
- Advanced hyperpigmentation is correlated with low condition factor of the affected fish.
- No infectious agent has been identified to date in affected fish.

A number of hypotheses are proposed that could explain the condition:

- Hyperpigmentation is an infectious or non-infectious (i.e., neoplastic) disease;
- Contaminants in the sediments at sites inhabited by dab contribute to hyperpigmentation;
- UV-B radiation affecting early pelagic life stages (embryos, larvae) of dab at the water surface may activate pigment cells. However, this hypothesis does not directly explain the increase in pigmentation of older fish (vs. younger fish);
- A genetic change leading to greater pigmentation in the population. However, there is no obvious selective advantage to hyperpigmentation, especially considering its negative association with condition;
- Nutrition, algal blooms, and other factors possibly associated with climate change contribute to the condition.

A number of issues were discussed following the presentation:

- The necessity for further histopathological analysis of affected fish to verify preliminary evidence that hyperpigmentation is associated with inflammation and cellular degeneration;
- The requirement to search for a possible infectious agent;

- The need for age-specific and population density information;
- Explanations for the absence of this condition in other flatfish species inhabiting the same sites as the affected dab;
- The desirability of experimental work, including caging studies, and the capture and holding of fish to follow the development of hyperpigmentation in individual fish under varying experimental conditions;
- The benefits of adding chemical analyses of sediments and fish tissues in the affected areas.

6.1 Conclusions

1) The prevalence of hyperpigmentation in North Sea dab has increased significantly since the end of the 1980s.

2) The condition is characterized by clear histological signs, including evidence of cellular damage, and an association with low condition factor in the most advanced stages.

3) Hyperpigmentation has the appearance of a chronic condition in that it increases in prevalence and severity with age.

4) There is no known cause of this condition to date.

6.2 Recommendations

The WGPDMO recommends that:

i) WGPDMO be updated on the results of ongoing histopathological studies, and of virological and bacteriological analyses of affected fish.

ii) existing literature be reviewed on malpigmentation in farmed flatfish and fish pigment-cell tumours.

iii) funding be sought for additional research into the causes and effects of hyperpigmentation.

7 Update information on progress made in the development of salmon louse vaccines and sea lice management strategies in ICES Member Countries

A working document was presented by T.A. Mo, S. Jones, S. MacLean, D. Bruno and N. Ruane. The document (Annex 7) updated the information on progress made in the development of salmon louse vaccines and sea lice management strategies, with headings on chemical delousing, prophylactic treatment, development of salmon louse vaccine, sea lice management and sea lice counts, citing available information from Norway, Canada, USA, Scotland and Ireland.

7.1 Chemical Treatment

The document stated that there are indications of pyrethroid (deltamethrin and cypermethrin) resistance development in salmon lice in Norway. Prophylactic treatment of hatchery-reared wild salmon smolts with SLICE prior to seawater transfer resulted in higher return rates compared with untreated controls in Norway. Preliminary data from the US supports this.

7.2 Progress in Vaccine Development

Development of salmon louse vaccines is still progressing in Norway and Canada. Norwegian vaccination trials have shown a significant effect of experimental vaccines based on proteins isolated from salmon louse eggs.

7.3 Management Strategies

The Norwegian strategy against sea lice on salmonids based on the National Action Plan of 1987 has worked very well, as salmon lice are no longer a health problem for farmed salmon and wild Atlantic salmon mortality due to sea lice during their migration through the fjords in the spring has been significantly reduced. Scotland is proposing to introduce new legislation on sea lice management (see Annex 8). The integrated pest management strategy for sea lice in the Atlantic salmon farming industry in Maine (USA) is multifaceted, including fallowing sites after harvest and treatment with SLICE. A single-bay management area for Cobscook and Passamaquoddy Bays in USA and Canada, respectively, has been established. In British Columbia, Canada, monthly monitoring is conducted, increasing to biweekly monitoring when wild salmon are migrating between March and July (in this period juvenile wild Pacific salmon are also monitored). Ireland has had a programme on sea lice since 1991, including 14 inspections on each farm per year.

Withdrawal times vary depending on the drugs used as well as the country they are used in, e.g., withdrawal time for SLICE is 68 days in Canada, 60 days in USA, but 0 days in Ireland. Wrasse is still used with success as biological control of sea lice in Norway.

7.4 Conclusions

1) Development of salmon louse vaccines is progressing, but slowly.

2) The different national strategies against sea lice seem to work as intended.

3) Resistance in sea lice against pyrethroids has occurred in Norway. Development of resistance to other currently available chemotherapeutants may warrant additional vaccine development programs in the future.

7.5 Recommendations

The WGPDMO recommends that:

i) the progress of salmon louse vaccine development and sea lice management strategies made in ICES Member Countries should be reviewed in national reports at the WGPDMO 2008 meeting.

ii) ICES Member Countries review evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants.

8 Review progress made with regard to international collaborative actions including disease and pathology aspects

8.1 ICES Regional Ecosystem Study Group of the North Sea (REGNS)

W. Wosniok presented information on progress made by the ICES Regional Ecosystem Study Group of the North Sea (REGNS), the objective of which was to carry out an integrated assessment of the status of the North Sea ecosystem. W. Wosniok acted as contact point between WGPDMO and REGNS and took part in REGNS activities, e.g., by attending a workshop and supplying REGNS with fish disease data extracted from the ICES Environmental Databank.

The term of REGNS came to an end and the final REGNS meeting took place 15-19 May 2006. A report of that meeting is available (ICES 2006a). A Theme Session entitled ‘Integrated assessments in support of regional seas ecosystem advice – beyond quality status reporting’ (Theme Session P) was held at the 2006 ICES Annual Science Conference, presenting parts of the results of the REGNS activities.

The WGPDMO noted with disappointment that fish disease data submitted to REGNS were not included in the assessment because REGNS considered the data to be important but too regionally restricted for a North-Sea-wide overview assessment. WGPDMO regretted this and emphasised that the fish disease data held in the ICES Environmental Databank constitute one of the few long-term data series (some data go back to 1981) on biological responses of marine fish to environmental change covering a number of geographical areas in the North Sea and adjacent waters. WGPDMO considers these data to be of importance for any integrated assessment of the North Sea ecosystem health.

8.2 ICES/HELCOM Working Group on Integrated Assessment of the Baltic Sea (WGIAB)

The WGPDMO was informed by T. Lang of the establishment of a new joint ICES/HELCOM Working Group on Integrated Assessment of the Baltic Sea (WGIAB) which evolved from a workshop held in 2006 and which held its first meeting in March 2007. WGIAB was given Terms of Reference similar to the REGNS ones.

Contacts to WGIAB have been made by WGPDMO members and WGIAB expressed its interest in data on fish diseases in the Baltic Sea to be used for the assessment. However, only few Baltic Sea disease data have so far been submitted to the ICES Environmental Databank, so that, for the time being, only data from national sources (e.g., from fish disease monitoring programmes carried out by Germany, Poland and Russia) could be utilised. It must be confirmed, however, that these data are available in a format that can directly be used by WGIAB.

WGPDMO pointed out that for future WGIAB assessments or the HELCOM monitoring and assessment programme, it will be crucial that fish disease data available in national databanks in Baltic Sea countries are submitted to the ICES Environmental Databank which serves as the database for HELCOM. These data have to be generated according to established ICES and BEQUALM quality assurance requirements and laboratories conducting disease surveys and submitting disease data to ICES are, therefore, strongly encouraged to take part in the BEQUALM programme (see below in section 8.4).

8.3 Baltic Sea Regional Project (BSRP)

T. Lang provided an update on BSRP activities related to the monitoring and assessment of fish diseases in the Baltic Sea. He briefly described the objectives of the project (the first phase of which will end in June 2007) and the function of the BSRP Lead Laboratories implemented in eastern Baltic Sea countries, e.g. the Lead Laboratory for fish diseases, parasites and histopathology at AtlantNIRO, Kaliningrad, Russia, headed by the WGPDMO member G. Rodjuk.

As a follow-up to the 2005 ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM) (ICES 2006b), another practical workshop was proposed and adopted by the ICES Baltic Committee and subsequently by the ICES Study Group on Baltic Ecosystem Health Issues (SGEH) in support of the BSRP at its November 2006 meeting (ICES 2007, in preparation). The proposal (Annex 9) was developed by G. Rodjuk (AtlantNIRO, Kaliningrad, Russia, BSRP Lead Laboratory for fish diseases) and T. Lang (Fed. Res. Centre for Fisheries, Cuxhaven, Germany) and is based on a WKFDM recommendation and a request from HELCOM MONAS directed to SGEH.

Reasons for holding the workshop are:

- The 2005 WKFDM focused on fishes from offshore areas. However, the HELCOM fish monitoring activities are largely related to coastal fish species.
- There are plans to widen the scope of the HELCOM coastal fish monitoring and to integrate it with other coastal monitoring programmes in order to provide a basis for estimating the ecological status of the coastal fish compartment

(HELCOM 2006) as part of a more holistic ecosystem approach and related management objectives.

- The ICES SGEH, together with the group of HELCOM coastal fish monitoring experts, were requested by HELCOM to develop an indicator for fish disease with target levels in order to meet the demands of the Baltic Sea Action Plan.

According to the present planning, the workshop will be held for 4-5 days in late 2007 (or in 2008) at the field station of the AtlantNIRO near Kaliningrad and will be co-chaired by T. Lang, G. Rodjuk and an expert representing the group of HELCOM/BSRP Coastal Fish Monitoring Experts (possibly M. Appelberg, Sweden) as co-chairs. It is expected that scientists from all Baltic Sea countries as well as invited specialists from countries outside the Baltic Sea area will attend. The objectives of the workshop are as follows:

- provide baseline data on diseases and parasites in key fish species from coastal areas in the Baltic Sea to be used for future fish health assessments as part of the coastal fish monitoring;
- provide training and intercalibration of methodologies related to the diagnosis of diseases;
- produce draft guidelines for fish disease monitoring in coastal fish species in the Baltic Sea to be applied in the coastal fish monitoring programme, and
- propose indicators and target levels for diseases of coastal fish species to be used in Baltic Sea ecosystem health assessments.

It was recommended that ICES, HELCOM and BSRP act as co-sponsors of the workshop because there is interest in these organisations/projects in the issue of diseases in Baltic coastal fish species and in order to achieve a wide recognition of the workshop as well as a commitment from the Baltic Sea countries to contribute to the workshop.

It was pointed out to WGPDMO that, at present, it is not clear if a second phase of the BSRP will be launched. This will depend on the results of the evaluation process of the work done and the progress achieved within the BSRP and the various ICES Study Groups supporting the BSRP. T. Lang emphasised that the proposed workshop largely depends on the availability of funding in order to support participants and invited experts. If there will be no second BSRP phase, it is not clear if the workshop can be held at all because obtaining funds from other sources might be difficult.

The WGPDMO supported the progress achieved so far through the BSRP in relation to the intercalibration and standardisation methodologies for fish disease monitoring in the Baltic Sea and highlighted the improvements made with regard to the establishment of a Baltic Sea network of fish disease researchers. However, it was emphasised that the activities are to be seen as a dynamic process and have to be continued, e.g., by holding the workshop on diseases and parasites of coastal Baltic Sea fish species as proposed by the SGEH.

8.4 Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)

S.W. Feist gave an overview of new developments in the fish disease component of the BEQUALM programme (http://www.bequalm.org). Six laboratories have signed to the project and take part in regular intercalibration exercises and ring tests that so far have been focused on externally visible diseases and liver histopathology in European flatfish species (dab, *Limanda limanda*, and flounder, *Platichthys flesus*). The most recently completed ring test for liver histopathology was based on the virtual slide technology using electronic images that were distributed to the participants.

Further activities planned are:

- a practical workshop to be held in 2008
- further ring tests and intercalibration exercises

- consideration of more marine fish species (e.g., dragonet species, *Callionymus* sp.) and possibly also freshwater species (e.g., brown trout, *Salmo trutta*).

The WGPDMO appreciated the progress made with regard to the fish disease component of BEQUALM and it was noted that the fish disease component is a particularly active part of the BEQUALM programme. It was emphasised once more that laboratories involved in fish disease monitoring should take part in the BEQUALM programme in order to ensure implementation of quality assurance procedures. This will become ever more important as only fish disease data quality assured through participation in BEQUALM will be accepted for use in international assessments, e.g., as part of the OSPAR Coordinated Environmental Monitoring Programme (CEMP).

8.5 Conclusions

1) WGPDMO considers data on diseases and parasites of wild fish populations to be of importance and ecological relevance for the assessment of the health of marine ecosystems.

2) There is a need to incorporate fish disease data generated by Baltic Sea countries into the ICES fish disease database in order to make them available for future environmental assessments, e.g., carried out by the ICES Working Group on Integrated Assessment of the Baltic Sea (WGIAB) and as part of the periodic HELCOM assessments. It was pointed out that data submitted must meet QA requirements covered by the fish disease component of the BEQUALM programme.

3) The WGPDMO emphasised the need to continue the activities aiming at standardisation and intercalibration of methodologies for fish disease monitoring and assessment in the Baltic Sea as incorporated in the work of the first phase of the BSRP. In this context, WGPDMO strongly supports the proposal to organise a workshop on monitoring of diseases and parasites in coastal fish species in the Baltic Sea.

4) Participation of laboratories conducting fish disease monitoring programmes in the BEQUALM programme is considered essential in order to achieve implementation of quality assurance procedures needed for acceptance of data for international monitoring and assessment programmes.

8.6 Recommendations

The WGPDMO recommends that

i) Member Countries conducting fish disease monitoring programmes in the Baltic Sea make attempts to submit their disease data to the ICES Environmental Databank in order to make them available for integrated assessments, such as those carried out by the ICES Working Group on Integrated Assessment of the Baltic Sea (WGIAB) and as part of the periodic HELCOM assessments,

ii) a workshop on methodologies for monitoring fish diseases/parasites in coastal fish species from the Baltic Sea be organised in 2007 (or in 2008, depending on funding requirements) under the co-sponsorship of ICES, HELCOM and the BSRP, at the BSRP Lead Laboratory for fish disease issues, AtlantNIRO, Kaliningrad, Russia. G. Rodjuk, T. Lang and a representative of the group of HELCOM coastal fish monitoring experts should act as co-chairs. The main objectives of the workshop are:

a) to provide baseline data on diseases and parasites in key fish species from coastal areas in the Baltic Sea to be used for future fish health assessments as part of the coastal fish monitoring;

b) to provide training and intercalibration of methodologies related to the diagnosis of diseases;

c) to produce draft guidelines for fish disease monitoring in coastal fish species in the Baltic Sea to be applied in the coastal fish monitoring programme, and d) to propose indicators and target levels for diseases of coastal fish species to be used in Baltic Sea ecosystem health assessments.

iii) laboratories running fish disease monitoring programmes participate in the BEQUALM programme in order to achieve implementation of quality assurance procedures needed for acceptance of data for international monitoring and assessment programmes. Member Countries are urged to provide funding for BEQUALM membership fees for participating laboratories.

8.7 Literature cited

ICES (2006a). Report of the Regional Ecosystem Study Group of the North Sea. ICES CM 2006/RMC:06, 107 pp.

ICES (2006b). Report of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea. ICES CM 2005/BCC: 02, 85 pp.

ICES (2007). Report of the Study Group on Baltic Ecosystem Health Issues in support of the BSRP (in preparation).

9 Review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves

A working document (Annex 10) was prepared by T. Renault, S. Bower, L. Madsen, S. Feist and S. Ford that reviewed molecular techniques recently developed for diagnosing bivalve infectious agents.

9.1 Molecular Techniques

These methods are moving from a developmental phase for research purposes to routine application and are expected to be increasingly used in monitoring programmes for the detection of specific pathogens. Examples are provided of recently developed diagnostic assays for bonamiosis, microcytosis, haplospridiosis, marteiliosis, perkinsosis and infections caused by bacteria and viruses. International standards proposed by the OIE include molecular techniques for the detection and identification of certain bivalve pathogens (OIE, 2006). However, molecular tools require formal validation against traditional techniques and testing for specificity.

Concerns regarding the routine use of existing DNA-based diagnostic tools were identified. For example, not all regions of pathogen DNA are equally useful as targets for molecular detection, molecular tools detect DNA and not necessarily the viable pathogen, and molecular assays often have not been thoroughly tested for inclusivity or specificity. Finally despite its sensitivity, PCR is still subject to the limitations associated with sampling technique, particularly from low intensity infections.

9.2 Validation

The need for formal validation of new molecular methods was recognised. As recommended by OIE, validation activities should include comparisons with standard methods, comparisons with reference standards (if available), field and/or epidemiological studies, collaborative studies among laboratories using the same documented method and including the exchange of samples, reproducibility from accepted standards or from reputable publications, experimental infection studies, and analysis of internal quality control data. In addition, further research is necessary to identify additional regions of the pathogen genome that are appropriate for species differentiation. Validation needs to be a balance between costs, risks and technical possibilities.

9.3 Conclusions

1) Molecular detection assays for pathogens infecting molluscs are moving from development in specialised laboratories for research purposes to routine application and are expected to be increasingly used in pathogen monitoring programmes.

2) Some of the developed molecular tools have been submitted to validation tests against other methods, including histology.

3) However, major concerns are identified (no information on diagnostic sensitivity and specificity, no ring tests in progress between laboratories involved in mollusc pathogen diagnosis) and molecular tools need formal validation.

9.4 Recommendations

The WGPDMO recommends that:

i) studies be carried out to resolve which DNA sequence differences are consistently significant in the identification of pathogen species and how these differences can be used to describe and differentiate between closely related species;

ii) laboratories involved in research on pathogens of molluscs initiate collaborative testing, intercalibration, and validation of current and newly developed techniques for the purpose of recommending molecular diagnostic techniques;

iii) the WGPDMO be kept informed of progress made in validating molecular tools for the purpose of pathogen diagnosis in bivalves.

9.5 References

OIE (2006). International Aquatic Animal Health Code. OIE, Paris, 7th edition.

10 Review progress made inter-sessionally by selected members of WGPDMO on the pilot study on constructing a 'fish disease index' by using empirical dab (*Limanda limanda*) disease data

W. Wosniok presented a report by T. Lang and W. Wosniok (Annex 11) describing progress on the development of a Fish Disease Index (FDI). Data on diseases of the North Sea dab (*Limanda limanda*) were used to illustrate the index.

10.1 Purpose

The purpose of the FDI is to provide a summary of the prevalence, severity of and trends in diseases in a particular population, which can be used as an assessment tool. It does not summarise variation as do methods such as principal component analysis and multi-dimensional scaling. The presented version of the FDI, as used for dab, takes into consideration externally visible lesions and parasites, macroscopic liver neoplasms and histologically detectable liver lesions.

10.2 Implementation

To implement the FDI, each observable disease condition or parasitic infection is assigned a numerical weighting factor depending on its impact on the animal, as determined by advice from a panel of experts. Then the grade of severity of each disease is determined according to existing ICES/BEQUALM guidelines and coded by 1-3. If no grading exists, the presence of a disease is coded by 1. The FDI is then calculated by multiplying the weighting factor by the level of severity and summing these products over all diseases. To overcome the problem of ranking many items (diseases) according to severity, the Bradley-Terri approach can be used. Due to sampling variations between populations (e.g., different sex and length distributions) and different sampling seasons, adjustments for those variations are made before applying the FDI to any assessment.

Using the dab in the North Sea as a model, the externally visible lesions of the following diseases were used to illustrate the FDI: lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulceration, X-cell gill disease, hyperpigmentation, acute/healing fin rot/erosion, and the parasites *Stephanostomum baccatum*, *Acanthochondria cornuta* and *Lepeophtheirus pectoralis* (macroscopic liver neoplasms and histopathological liver lesions were not considered but will be added at a later stage). FDI assessment ranges were developed after adjusting the data for sex, length and season of collection, and plotted along a time line. The range of points was then divided into three equal groups representing the lower, middle, and upper portions of the range. The time series assessment strategy was continued by counting the number of observations since January 2000 in the three groups. After weighting these counts by −1, 0, +1, for the lower, middle and upper ranges, respectively, they were summed. In parallel, the Mann-Kendall trend test statistic was calculated. The sum of counts and the trend statistic were added to give the FDI assessment statistic. As presently constructed, the trend statistic is the more important factor. According to the resulting statistic, different "smiley faces" were assigned to individual ICES regions. A $p < 0.025$ resulted in a "green smiley face"; $0.025 < p < 0.975$ in a "yellow indifferent face"; and $p > 0.975$, a "red frowny face". These faces, placed on a chart of the ICES statistical rectangles in the North Sea, provide a visual general assessment of trends in overall disease status.

10.3 Discussion

The WGPDMO appreciated the progress achieved regarding the FDI and pointed out that the FDI is a useful tool to be applied in monitoring and assessment programs on diseases in wild fish populations. In the discussion a number of points were raised:

- It is important to have a set of relevant diseases in characterising overall fish health to avoid having one disease dominate the index.
- There may be a problem in using a linear disease-specific weighting system. For example, data from shellfish indicate that linear visual scoring systems actually represent exponential parasite counts. This can be overcome be replacing the present system of weighting by a system of nonlinear weights.
- FDI can be calculated for data collected from previously unsampled sites. However, a trend assessment is possible only if data from adjacent sites are used.

10.4 Conclusions

1) The Fish Disease Index (FDI) summarises and visually presents information on trends in the prevalence and severity of disease in wild fish populations.

2) Since assessment criteria for the FDI have been developed, changes in the FDI can serve as an alarm bell that signals undesired developments in fish health, relevant for monitoring and assessment purposes.

3) Its design principle allows the FDI to be applied to other species with other sets of diseases. Therefore, the FDI approach is applicable for wider geographical areas, e.g., as part of the convention-wide OSPAR monitoring and assessment programme.

10.5 Recommendations

The WGPDMO recommends that:

i) the FDI be applied to disease data sets for other geographical areas and species, e.g., flounder and cod in the Baltic Sea, and other diseases (e.g., liver histopathology) and the results will be reported to WGPDMO at its 2008 meeting.

ii) the results of the FDI development and analysis on dab should be published.

iii) OSPAR note the progress achieved in relation to the Fish Disease Index and consider acceptance of the FDI as a tool for the assessment of fish disease monitoring data.

11 Produce ICES publications on pathology and diseases of marine organisms

11.1 ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish

S. W. Feist, the editor, updated members on progress with the leaflets.

All original leaflets are now available on the ICES website. Several leaflets have been updated and will be placed on the website shortly. T. Renault presented leaflets on bonamiosis, marteiliosis and haemocytic and gonadal neoplasia. A template for the leaflets will be distributed by the editor. Updated leaflets will be sent to the editorial sub-group for reviewing before they are finally placed on the ICES website.

The editor will inform WGPDMO on progress made at its 2008 meeting.

11.2 Publications in the TIMES Series

Guidelines for fish disease monitoring in the Baltic Sea have been drafted (co-authors T. Lang and G. Rodjuk) as an outcome of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM), with emphasis on diseases and parasites of fish species characteristic for offshore areas (e.g., cod, flounder, herring). WGPDMO supports the submission for publication of the guidelines in the ICES TIMES Series once they have been reviewed by selected WGPDMO members.

A paper on histopathology of mussels is planned in collaboration with colleagues from WGBEC.

11.3 Other Publications

A manuscript entitled “The Effects of Climate Change on Marine Fish and Shellfish Disease” is in preparation.

12 Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis

In light of the anticipated change to the mandatory status for fish disease monitoring under the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP) and the developments within HELCOM regarding fish disease monitoring in the Baltic Sea, the importance for ICES Member Countries to submit fish disease data for future assessments of environmental status in the OSPAR or HELCOM maritime areas was stressed. It is currently time consuming to submit such data to the ICES Environmental Databank because of the complexity of the ICES Environmental Data Reporting Format 3.2 (EDRF 3.2), particularly where large data sets are concerned. It was discussed in WGPDMO if a new data entry programme would greatly facilitate the submission. It was noted that, e.g., the ICES WGBEC has requested that ICES accepts data submission in a free format designed by the institutes submitting data to ICES. However, the ICES Secretariat confirmed that all labs submitting fish disease data have already used EDRF 3.2 and, thus, WGPDMO did not see a need to design a new data entry programme. Therefore, the WGPDMO encouraged labs submitting fish disease data, or intending to do so, to convert their data into EDRF 3.2 and submit the data in this format. This offers the following advantages:

- No further delay in data submission (which would be the case if ICES either has to generate a new data entry programme or has to convert data submitted in a free format; both because of a lack of resources in the data section of the ICES Secretariat);

- No further sources of errors in the data (which might happen if new entry formats are created or if data are submitted in a free format);
- Fish disease data will be available for the assessments to be done in preparation for the 2010 OSPAR Quality Status Report;

The WGPDMO strongly urged ICES to, as soon as possible, establish the screening programme for fish disease data on its website that can be used by submitters to check their data before sending them to ICES.

12.1 Conclusions

1) It is increasingly important for ICES Member Countries to submit historic fish disease data not yet submitted to the ICES Environmental Databank and to promptly submit newly collected data.

2) These data should be submitted to ICES using the ICES Environmental Data Reporting Format (current version 3.2).

3) ICES should establish a screening programme at its website enabling submitting labs to check their data sets before submission to ICES.

12.2 Recommendations

The WGPDMO recommends that:

i) ICES Member Countries submit their fish disease data generated according to established ICES/BEQUALM guidelines as soon as possible to the ICES Environmental Databank by using the ICES Environmental Data Reporting Format (current version 3.2).

ii) ICES provides a screening programme on its website to be used by labs to check their fish disease data sets converted into the ICES Environmental Data Reporting Format (current version 3.2) before submission to ICES.

iii) The fish disease data submitted to ICES are used by OSPAR for assessments made in preparation of the 2010 OSPAR 2010 Quality Status Report (QSR 2010).

13 Other Business

13.1 Working Group procedures

As a result of the unique circumstances under which the WGPDMO met, in a venue only confirmed the day of opening the meeting, the WGPDMO procedures were modified to maximise time, space and equipment available to us. One result of these circumstances was a paperless meeting. It was agreed this was a positive outcome and should be continued in the future. In addition, assignments for rapporteurs and timing of breakout sessions were made in such a manner as to maximise the involvement of the participants in all discussions. This also will become standard procedure when possible.

14 Progress on tasks

Progress of tasks in the Terms of Reference was reviewed and it was concluded that all items had been dea1t with in a satisfactory manner. Table 14.1 provides more information on items completed and those which require further action. Several intersessional tasks to be fulfilled prior to the 2008 WGPDMO meeting were identified.

Table 14.1 Progress on tasks s of WGPDMO's Terms of Reference for 2007

	TERM OF REFERENCE	STATUS
a	produce a report on new disease trends in wild and cultured fish, molluscs, and crustaceans based on national reports	on-going task; will be revisited in 2008 as part of recommended ToR a
b	review the condition of hyperpigmentation in common dab (Limanda limanda) with special reference to histopathological and ultrastructure findings, analysis of prevalence and temporal changes, and possible causes and similarities with other species	will be revisited in 2008 as part of recommended ToR d
c	update information on progress made in the development of salmon sea louse vaccines and sea louse management strategies in ICES Member Countries	further updates of salmon louse vaccines will be made in Country Reports; issue of salmon louse tolerance of chemotherapeutants will be addressed in 2008 as part of recommended ToR e
d	Review progress made with regard to international collaborative actions including disease and pathology aspects:	on-going task; will be revisited in 2008 as part of recommended ToR f
e	produce a review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves	review completed; will be revisited as an update on the formal incorporation of molecular techniques as diagnostic methods as part of recommended ToR g
f	review progress made intersessionally by selected members of WGPDMO on the pilot study on constructing a 'fish disease index' by using empirical dab (Limanda limanda) disease data	will be revisited in 2008 using Baltic cod and flounder data as part of recommended ToR h
g	produce ICES publications on pathology and diseases of marine organisms	on-going task; will be revisited in 2007 as recommended ToR i
h	provide expert knowledge and advise on fish disease and related data to the ICES Data Centre on a continuous basis	On-going task; will be revisited in 2008 as part of recommended ToR j

15 Future activities of WGPDMO

There are several important issues in the field of pathology and diseases of marine organisms that require further consideration. It was agreed that a further meeting of WGPDMO is required in 2008 to consider the results of intersessional work, and to discuss new disease trends and new and outstanding items. The next meeting is planned for St. John's, Newfoundland, Canada, during 4-8 March 2008.

16 Approval of recommendations

The recommendations to the ICES Council contained in this report were discussed by the WGPDMO and approved. The recommendations and justifications for new Terms of Reference for the 2008 WGPDMO meeting are appended in Annex 13.

17 Approval of the draft WGPDMO report

A rough draft of the 2007 WGPDMO report was approved before the end of the meeting and outstanding issues were identified and delegated to WGPDMO members. Information specifically sought by or provided to other ICES bodies will be extracted from the Terms of

Reference conclusions and annexes and sent separately to ICES or the Chairs of the relevant Working Groups.

18 Closure of the meeting

The Chair thanked the local host of the facility for providing excellent meeting facilities and arrangements and thanked the WGPDMO participants for their hard work and input during and in preparation of the meeting. The 2007 WGPDMO meeting was closed at 11:30 am on 24 March 2007.

Annex 1: WGPDMO 2007 List of participants

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Annex 2: WGPDMO 2006 Terms of Reference

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: Sharon MacLean*, USA) will meet in Tenerife, Spain (due to recent severe damage to the Institute, the venue was changed to a facility in Santa Cruz de Tenerife) from 20–24 March to:

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

b) review the condition of hyperpigmentation in common dab (*Limanda limanda*) with special reference to histopathological and ultrastructure findings, analysis of prevalence and temporal changes, and possible causes and similarities with other species;

c) update information on progress made in the development of salmon sea louse vaccines and sea louse management strategies in ICES Member Countries;

d) review progress made with regard to international collaborative actions including disease and pathology aspects:

- i) REGNS Integrated Assessment of the North Sea Ecosystem,
- ii) Baltic Sea Regional Project (BSRP),
- iii) BEQUALM;

e) produce a review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves;

f) review progress made intersessionally by selected members of WGPDMO on the pilot study on constructing a 'fish disease index' by using empirical dab (*Limanda limanda*) disease data;

g) produce ICES publications on pathology and diseases of marine organisms;

h) provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis.

WGPDMO will report by 20 April 2007 for the attention of the Mariculture Committee, ACME, and Marine Habitat Committee.

Supporting information

PRIORITY:	WGPDMO is of fundamental importance to the ICES science and advisory process.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	**Action Plan References:** a) 2.2, 2.4, 2.5, 2.6, 2.8, 2.10, 6.1 b)1.2, 1.6, 2.2 c) 2.6, 2.7, 3.14, 4.7 d) 1.10, 1.12, 2.2, 3.3, 4.12, 5.4, 5.6 e) 2.6, 3.10, 3.14, 4.7 f)1.10, 2.2, 2.8, 4.6 g) 6.1, 6.3 h) 2.8, 6.1, 6. **Term of Reference 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. **Term of Reference b)** Hyperpigmentation has continued to increase dramatically in the common dab (*Limanda limanda*) populations in the North Sea. Evaluation of the condition and its possible causes are needed. **Term of Reference c)** Sea lice have been blamed for the decline in wild salmonid populations in several countries and perceived as a threat to salmonids migrating in coastal areas. Vaccines against sea lice are currently under development in some Member Countries. For the WGPDMO meeting in 2007, a progress report will be prepared to review most current information on vaccine development and sea lice management strategies. **Term of Reference d)** Since the REGNS Integrated Assessment of the North Sea Ecosystem includes ICES

	data on the prevalence of fish diseases compiled by WGPDMO, the outcome of the assessment has to be reviewed by WGPDMO. Another major international activity of concern, the progress of which has to be reviewed by WGPDMO, is the Baltic Sea Regional Project and its fish disease monitoring component. BEQUALM is the major quality assurance programme for biological effects monitoring in Europe and includes a fish diseases/histopathology component, the progress of which should be reviewed by WGPDMO on a regular basis. **Term of Reference e)** Molecular techniques for diagnosing bivalve infectious agents have been developed during the last decade and are now moving from development in specialized laboratories for research purposes, to routine application and are expected to be increasingly used in pathogen monitoring programs. International standards proposed by the OIE are including molecular techniques for the detection and identification of some bivalve pathogens. However, molecular tools may need formal validation against traditional techniques and testing for their specificity. A work package of the PANDA project also deals with this topic and results obtained may be included in the review. **Term of Reference f)** For its 2006 meeting, the WGPDMO produced a report on the construction of a Fish Disease Index (FDI) based on diseases in common dab (Limanda limanda) and carried out a pilot study on its applicability using empirical disease data. The FDI was considered as a promising tool in the context of ecosystem health monitoring and assessment and it was recommended to further develop the index by validating its component and by testing its applicability with a larger set of empirical data. **Term of Reference g)** *Justification:* 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. It will be necessary to consider ways by which these can be linked to each other. New publications have to be considered. **Term of Reference h)** This is in compliance with a request from the ICES Data Centre
RESOURCE REQUIREMENTS:	None required, other than those provided by the host institute.
PARTICIPANTS:	Representatives of all Member Countries and specialists invited by the Chair with expertise relevant to pathology and disease of wild and cultured finfish and shellfish. In total, normally 20 participants
SECRETARIAT FACILITIES:	Required to a limited extent, e.g. for data and publication issues
FINANCIAL:	None required
LINKAGES TO ADVISORY COMMITTEES:	There is a close link to ACME activities.
LINKAGES TO OTHER COMMITTEES OR GROUPS:	MCC, MHC, DFC, WGBEC
LINKAGES TO OTHER ORGANISATIONS:	BEQUALM, OIE, EU

Annex 3: Working documents distributed prior to the meeting

	2007 WGPDMO Terms of Reference	Working document (file)	Sent by Email
a)	produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports;	WGPDMO2007_Canada_NatlReport WGPDMO2007_Denmark_NatlReport WGPDMO2007_Finland_NatlReport WGPDMO2007_France_NatlReport WGPDMO2007_Latvia_NatlReport WGPDMO2007_Netherlands_NatlReport WGPDMO2007_Germany_NatlReport WGPDMO2007_Poland_NatlReport WGPDMO2007_Scotland_NatlReport WGPDMO2007_Sweden_NatlReport WGPDMO2007_UK_NatlReport WGPDMO2007_USA_NatlReport WGPDMO2007_Norway_NatlReport WGPDMO2007_Ireland_NatlReport WGPDMO2007_Russia_NatlReport	2/26/07 2/26/07 2/26/07 2/26/07 2/26/07 2/28/07 2/28/07 2/28/07 2/28/07 2/28/07 2/28/07 2/28/07 3/9/07 3/15/07 3/15/07
b)	review the condition of hyperpigmentation in common dab (*Limanda limanda*) with special reference to histopathological and ultrastructure findings, analysis of prevalence and temporal changes, possible causes and similarities with other species;	WGPDMO2007_ToR_b_Hyperpigmentation	3/16/07
c)	update information on progress made in the development of salmon sea louse vaccines and sea louse management strategies in ICES Member Countries;	WGPDMO2007_ToR_c_development of sea louse vaccines and sea louse management	3/9/07
d)	Review progress made with regard to international collaborative actions including disease and pathology aspects: i) REGNS Integrated Assessment of the North Sea Ecosytem; ii) Baltic Sea Regional Project (BSRP) iii) BEQUALM;	WGPDMO2007_ToR_d_BSRP	3/15/07
e)	produce a review of testing, intecalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves;	WGPDMO2007_ToR_e	3/9/07
f)	review progress made intersessionally by selected members of WGPDMO on the pilot study on constructing a ‘fish disease index’ by using empirical dab (*Limanda limanda*) disease data;		3/23/07
g)	produce ICES publications on pathology and diseases of marine organisms;		
h)	provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis.		
	Terms of Reference	WGPDMO2007_TOR	11/30/07
	Draft Agenda, Preliminary Attendee List, Draft Rapporteurs	WGPDMO2007Attendees-ToRs-Agenda-Rapporteurs	3/16/07
	Preliminary Meeting Information		11/30/07
	Additional Meeting Information-1		1/4/07

	Additional Meeting Information-2		2/12/07
	Additional Meeting Information -3		2/26/07
	Additional Meeting Information-4		2/28/07
	Additional Meeting Information-5		3/9/07

Annex 4: Agenda

1) Opening of the meeting

2) Terms of Reference, adoption of Agenda and Timetable, selection of Rapporteurs

3) ICES Annual Science Conferences 2006 and 2007 (input from members), and items of relevance to WGPDMO

3.1) 2008 ASC, Special Theme Session on pathology and diseases of marine organisms

3.2) PANDA

3.3) WGCRAB

3.4) WKIMON

3.5) WGBEC

3.6) ICES Advisory Structure reform

Discussion forum: http://portal.ices.dk.ICESAdvisorySystem/default.aspx

Comments: REFORM@ices.dk

3.7) ICES SharePoint http://portal.ices.dk/wgpdmo2007

4) Other relevant reports/activities for information (ICON)

5) Produce a report on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports (ToR a)

6) Review the condition of hyperpigmentation in common dab (*Limanda limanda*) with special reference to histopathological and ultrastructure findings, analysis of prevalence and temporal changes, and possible causes and similarities with other species (ToR b)

7) Update information on progress made in the development of salmon sea louse vaccines and sea louse management strategies in ICES Member Countries (ToR c)

8) Review progress made with regard to international collaborative actions including disease and pathology aspects (ToR d)

- the REGNS Integrated Assessment of the North Sea Ecosystem
- the Baltic Sea Regional Project (BSRP)
- BEQUALM

9) Produce a review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves (ToR e)

10) Review progress made intersessionally by selected members of WGPDMO on the pilot study on constructing a 'fish disease index' by using empirical dab (*Limanda limanda*) disease data (ToR f)

11) Produce ICES publications on pathology and diseases of marine organisms (ToR g)

12) Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis (ToR h)

13) Other business

14) Analysis of progress with tasks

15) Future activities of WGPDMO

16) Approval of Recommendations

17) Approval of draft WGPDMO Report

18) Closing of the meeting

Annex 5: Rapporteurs

AGENDA ITEM(S)	2007 WGPDMO TERMS OF REFERENCE	RAPPORTEURS
1-4	Introductory session	S. MacLean
5	Produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports (ToR a) • wild fish • farmed fish • wild and farmed shellfish	M. Podolska/ A. Alfjorden/S. Jones/G. Rodjuk J. Barja/D. Bruno/T.A. Mo S. Feist/A. Mansour/T. Renault/N. Ruane
6	review the condition of hyperpigmentation in common dab (*Limanda limanda*) with special reference to histopathological and ultrastructure findings, analysis of prevalence and temporal changes, possible causes and similarities with other species (ToR b)	S. Ford/ I. Briede/ T. Lang
7	update information on progress made in the development of salmon sea louse vaccines and sea louse management strategies in ICES Member Countries (ToR c)	L. Madsen//S. MacLean/T.A. Mo/N. Ruane
8	review progress made with regard to international collaborative actions including disease and pathology aspects i) REGNS Integrated Assessment of the North Sea Ecosystem ii) the Baltic Sea Regional Project (BSRP) iii) BEQUALM (ToR d)	T. Lang/G. Rodjuk
9	produce a review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves (ToR e)	S. Jones/T. Renault
10	review progress made intersessionally by selected members of WGPDMO on the pilot study on constructing a 'fish disease index' by using empirical dab (*Limanda limanda*) disease data (ToR f)	A. Mansour/ S. Ford
11	produce ICES publications on pathology and diseases of marine organisms (ToR g)	I. Briede/L. Madsen
12	provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis (ToR h)	A. Alfjorden/S. Feist
13-16	Any other business, analysis of progress with tasks, future activities of WGPDMO, approval of recommendations	D. Bruno/M. Podolska
17-18	Approval of draft report, Closing of the meeting	S. MacLean

Annex 6: Review of Hyperpigmentation in dab (*Limanda limanda*) with special reference to histopathological and ultrastructure findings, analysis of prevalence and temporal changes, possible causes and similarities with other species

T. Lang, S.W. Feist, W. Wosniok, F. Baumgart*

Introduction

Hyperpigmentation is a condition affecting primarily North Sea dab (*Limanda limanda*) and is known since the beginning of regular fish disease surveys at the end of the 1970s. The term

* Some of the results presented in this document are part of the Diploma Thesis of Felix Baumgart, University of Rostock, Germany.

hyperpigmentation describes the occurrence of patchy green to black spots in the skin on the upper pigmented body side and pearly-white or green to black spots on the non-pigmented lower body side at varying degree of discolouration. The condition is different from the olive to brown 'normal' pigment spots commonly seen in dab and in other flatfish species such as flounder (*Platichthys flesus*).

Long-term data from North Sea surveys reveal that the prevalence of hyperpigmentation in dab differs considerably between regions in the North Sea and adjacent waters and has significantly increased since the middle of the 1980s, particularly during the last decade. This is of concern because the significant increase in prevalence:

- may reflect changes in environmental quality,
- may be linked to population effects, and
- may affect marketability because commercial catches of dab with severe hyperpigmentation do not meet the general requirement for landing fish without obvious disease, parasitism or deformities and may be rejected for sale on that basis.

So far, there is no information available on the causes of hyperpigmentation and on reasons for its dramatic increase in prevalence. Therefore, the ICES WG on Pathology and Diseases of Marine Organisms decided at its 2006 meeting to review available information and to provide a *status quo* report (ICES 2006).

In the following sections information is provided on

- fish species affected,
- macroscopic, histopathological and ultrastructural features,
- spatial and temporal patterns,
- host-specific effects on the prevalence of hyperpigmentation, and
- effects of hyperpigmentation on the host.

In the discussion, possible causes of hyperpigmentation are briefly described.

Species affected

Apart from dab, hyperpigmentation has been observed in lemon sole (*Microstomus kitt*), long rough dab (*Hippoglossoides platessoides*) and solenette (*Buglossidium luteum*) from the North Sea and, probably, in Baltic flounder from a region at the Swedish east coast (not confirmed). However, in contrast to North Sea dab, these species have only rarely been found to be affected.

Macroscopic and histopathological features

Macroscopy

Hyperpigmentation is characterised by the occurrence of patchy green to black spots in the skin on the upper pigmented body side (Fig. 1) and pearly-white or green to black spots on the non-pigmented lower body side (Fig. 2). The degree of discoloration of the body surface varies greatly and has been categorised through the BEQUALM project (http://www.bequalm.org) for monitoring and assessment purposes into three grades (Figs. 1 and 2).

The condition is different from 'normal' olive to brown pigment spots seen to varying degrees in dab from different areas of the North Sea (sometimes associated with healing processes related to skin ulcers or mechanical damage) and in other flatfish species, especially in flounder (*Platichthys flesus*).

Histopathology

The structure of normal dab skin (Plate 1, Fig. A) comprises of an epidermis of semi-stratified epithelium with the outermost cells becoming flattened and eventually sloughed from the surface of the skin. Occasionally, some of these cells appear rounded and clearly losing attachment from adjacent cells. Immediately below the epidermis is the eosinophilic basal lamina overlying the dermis, which consists of a thin layer containing a network of capillaries and connective tissue within which occasional pigment cells (both melanocytes and iridophores in different proportions, depending on the location on the fish), lymphocytes and phagocytes may be present, and below this is the stratum spongiosum. The scale pockets sit underneath this layer and above the stratum compactum which defines the lower limit of the dermis. Below this is the adipose tissue and somatic muscle.

Figure 1: Three grades of hyperpigmentation on the upper body side of North Sea dab (*Limanda limanda*), according to the BEQUALM definition (http://www.bequalm.org)

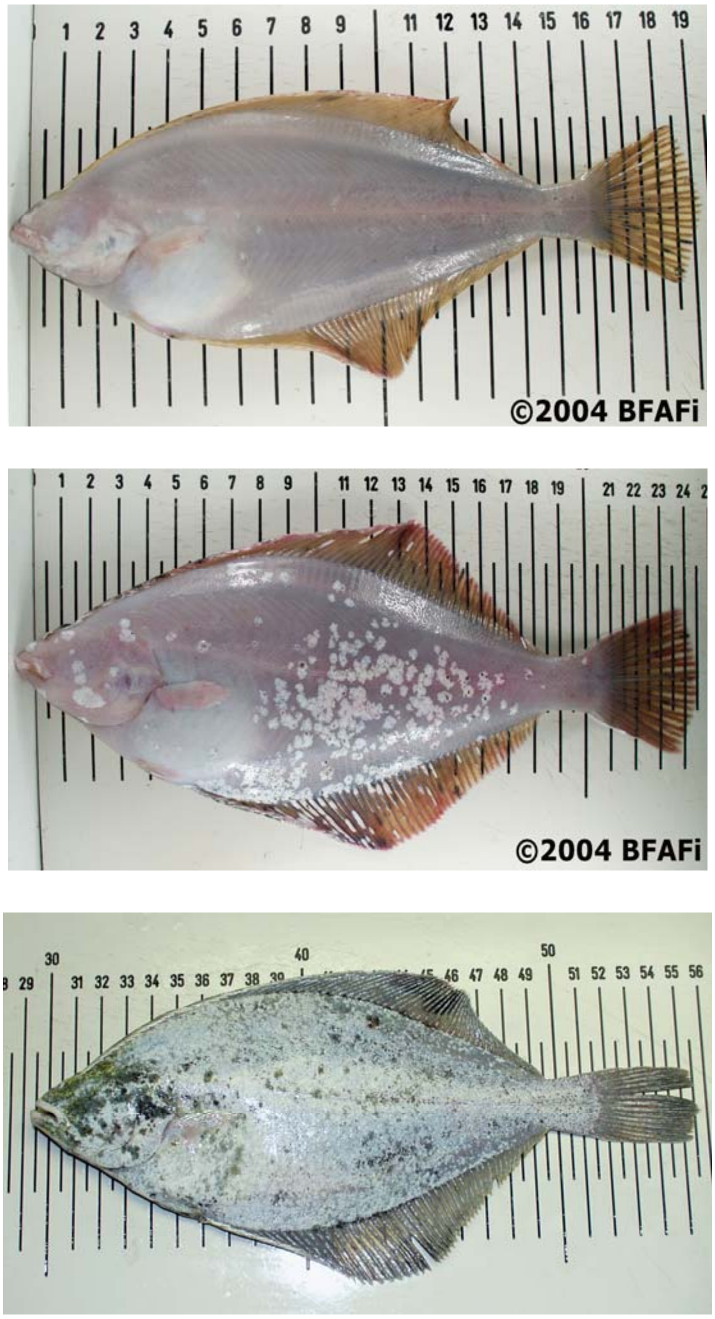

Figure 2: Three grades of hyperpigmentation on the lower body side of North Sea dab (*Limanda limanda*), according to the BEQUALM definition (http://www.bequalm.org)

In the upper surface of dab exhibiting moderate to marked hyperpigmentation (grade 2 and 3), there is a conspicuous hyperplasia of melanocytes arising within the uppermost layer of the dermis (Plate 1, Figs. B and C), which usually appear as discrete dendritic cells containing numerous melanin granules. In grade 3 fish, lymphocytic infiltration within this layer was sometimes observed (Plate 1, Fig. D), suggesting an active immunologic response. In some cases, melanocytes also occur within the epidermis and occasionally rupture, releasing the melanin granules (Plate 2, Figs. A and D). This was also noted in the layers immediately below the epidermis (Plate 2, Fig. B) and in these cases, degeneration and necrosis of adjacent cells, including iridophore cells, were observed (Plate 2, Figs. B and C).

Hyperpigmentation occurring on the underside of dab usually presents a different histopathological picture. In most cases, there is a hyperplasia of iridophore cells rather than melanocytes, although the latter are occasionally seen and in severe cases produce spots or patches of macroscopically visible melanisation amongst the pearly-white areas of iridophore cell hyperplasia. Iridophores characteristically contain numerous guanine platelets which appear as stacks of light olive brown cytoplasmic inclusions (Plate 1, Figs. E and F), which are birefringent in polarised light. By electron microscopy, the platelets appear as vacuoles since the contents have been dissolved during processing (Plate 2, Figs. E and F). Occasionally, iridophores can be seen within the stratum compactum (Plate 1, Fig. F) but have not been observed in the epidermis (Plate 2, Fig. D). In the specimens examined during the current investigation, lymphocytic infiltration amongst the 'layer' of hyperplastic iridophore cells was not observed.

No evidence of micro-organisms or parasites in tissues affected by hyperpigmentation was seen by light or electron microscopy.

Spatial and temporal patterns

Regional distribution

Hyperpigmentation has been observed in dab from areas in the entire North Sea, and less frequently in dab from the English Channel, the Irish Sea, and the Celtic Sea. In dab from the Baltic Sea, the condition has never been recorded.

Figure 3 shows a map of the North Sea with weighted mean prevalences of hyperpigmentation over the period 1989-2006, based on the trend curves and the width of confidence intervals (root of the width) shown in Fig. 4. Hotspot areas over the whole period have been area N22 in the south-western North Sea (mean prevalence 42.65 %), the Dogger Bank (N04, mean prevalence 32.52 %), the Firth of Forth (N06, mean prevalence 23.91 %) and the German Bight (N01, mean prevalence 22.65%). In some areas in the central northern (P02, mean prevalence 2.51 %; N10, mean prevalence 0.11 %) and the southernmost North Sea (N03, mean prevalence 3.83 %), the prevalence has been low throughout the entire period of investigation. The regional pattern observed indicate that hyperpigmentation is more prevalent in sampling areas that are located closer to the coast (except for area N04).

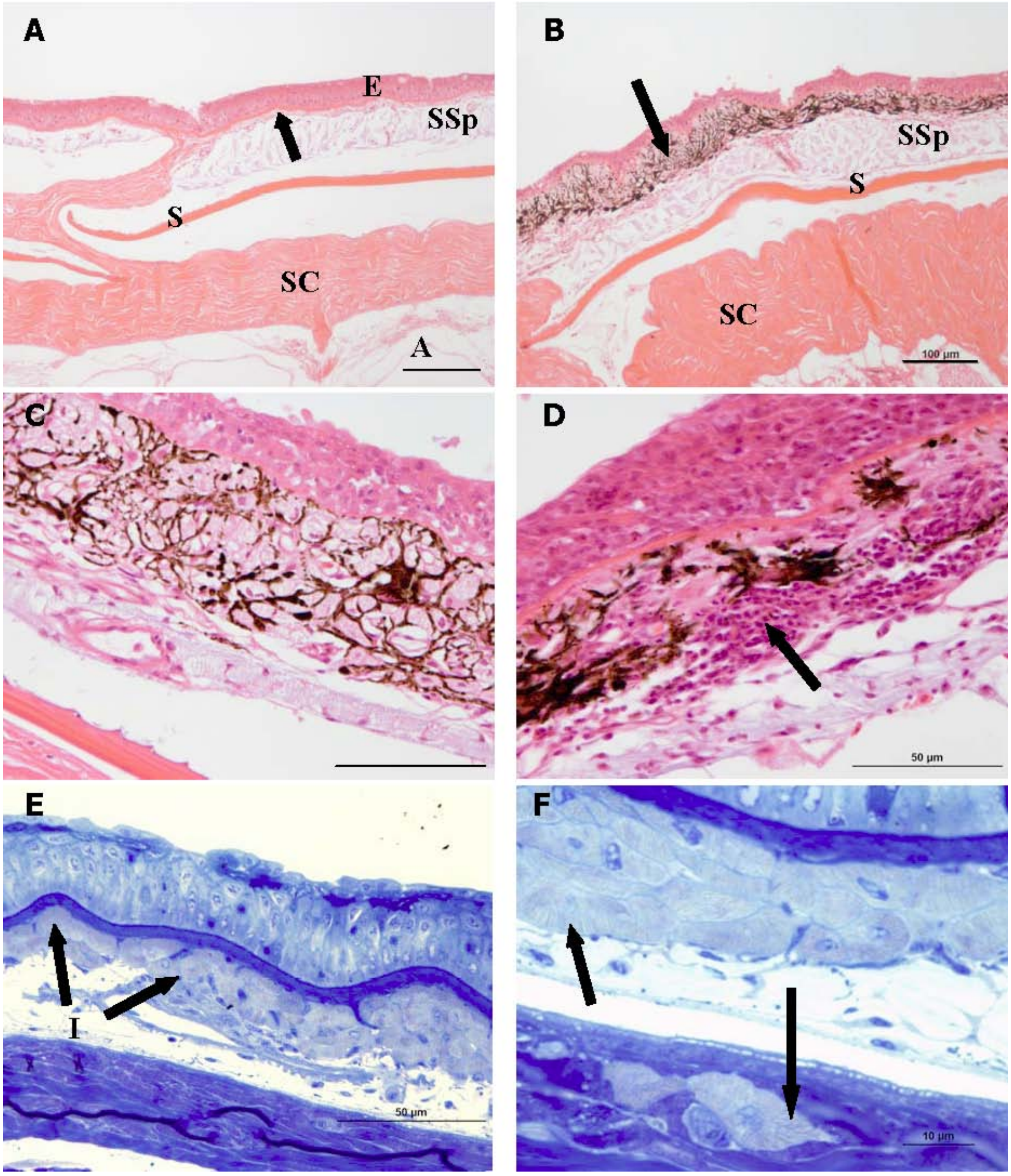

Plate 1. Histology of hyperpigmentation in dab. A. Section through normal skin of the under surface showing intact epithelium (E), stratum spongiosum (SSp), eosinophilic basal lamina (arrow), scale (S), stratum compactum (SC) and adipose tissue (A). There is no obvious pigmentation . Bar = 100µm. B. Section through the skin of a 'Grade 3' fish showing hyperplasia of melanocytes below the epidermis (arrow). Other labels as for A. Note the epithelium remains intact with little sloughing. Bar = 100µm. C. Extensive dendritic cytoplasmic extensions of melanocytes in a 'Grade 2' fish. Bar = 50µm. D. Section through the epidermis and partly the dermis of the underside of a 'Grade 3' fish. Note the presence of lymphocytic infiltration in the connective tissues of the dermis (arrow). Bar = 50µm E. Semithin resin section showing tightly packed iridophore cells (arrows) between the epidermis and stratum compactum. Bar = 50µm. F. High power view of iridophore cells containing characteristic platelets of guanine (arrows) and a central irregular shaped nucleus. Note the presence of iridophore cells in the stratum compactum (long arrow). Bar = 10µm.

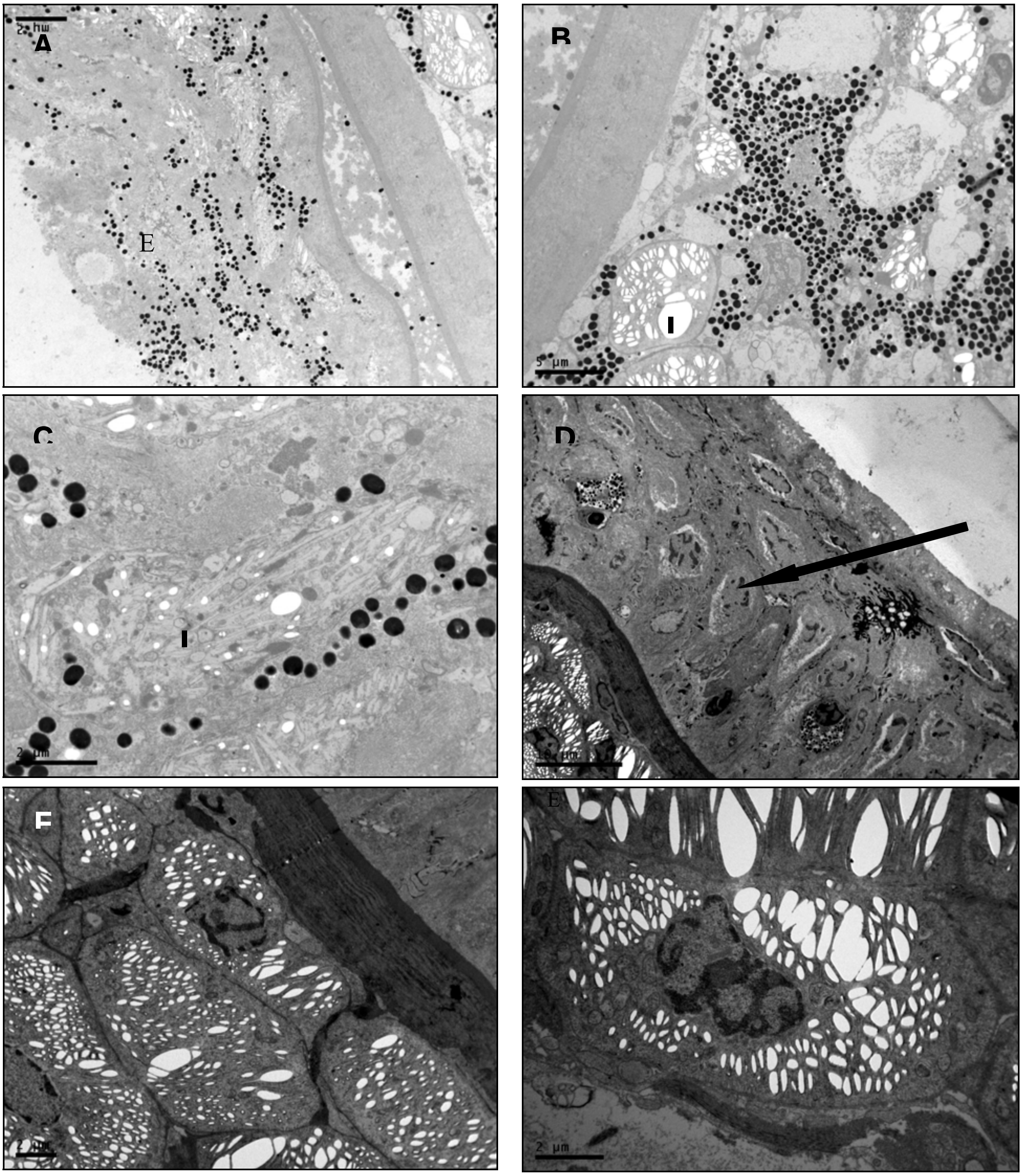

Plate 2. Ultrastructure of hyperpigmentation in dab. A. Section through the upper surface showing disruption of the epithelial layer (E) with numerous melanin granules dispersed between the epithelial cells. The latter appear degenerate. Bar = 5µm. B. Similar view to A, showing increased numbers of melanocytes in the sub-epithelial tissue. Note the presence of a few iridiphore cells (I). Bar = 5µm. C. Degenerate iridophore cell in the dermis (upper surface) of a 'Grade 3' fish. Bar = 2µm. D. Section through the epidermis and partly the dermis of the underside of a 'Grade 3' fish. The epidermis is intact although some epithelial cells show cytoplasmic degeneration (possibly fixation artefact) (arrow). Proliferating iridophore cells can be seen below the stratum compactum. Bar = 10µm. E. Higher power view showing tightly packed iridophore cells containing numerous spaces previously occupied by guanine crystals. Bar = 2µm. F. Iridophore cell with a characteristic irregular nucleus and coarse chromatin pattern. Distension of the spaces remaining after removal of guanine during processing is an artefact. Bar = 2µm.

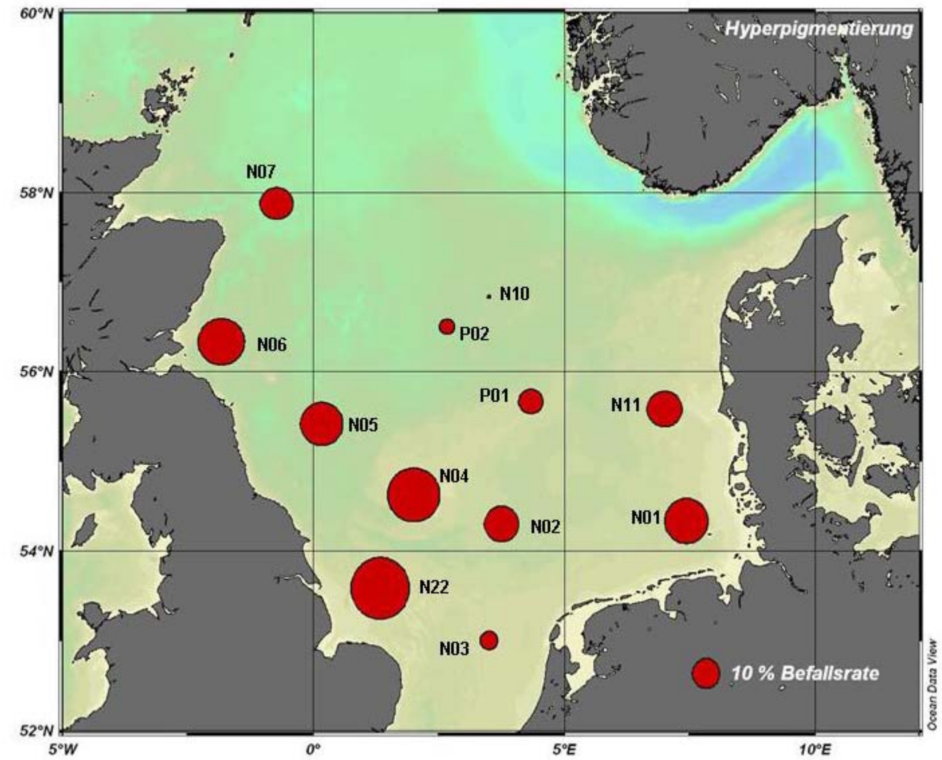

Figure 3: Mean prevalence of hyperpigmentation in female North Sea dab (*Limanda limanda*), size group 20-24 cm total length, in the period 1989-2006 (weighted estimated means based on a season adjustment trend model, see Figure 4)

Temporal trends

In Figure 4, the trends in the prevalence of hyperpigmentation in female dab of the size group 20-24 cm are given for the 12 North Sea sampling areas shown in Fig. 3. Data used are from the German fish disease monitoring programme (more data from other countries exist in the ICES fish disease databank but have not been used here). A season adjustment model was applied to generate trend figures (Wosniok et al., in preparation)

In 6 out of the 12 sampling areas (N01, N04, N05, N06, N11, N 22) a statistically significant increase (as can be seen from the confidence intervals) was recorded in the period 1989-2006. It is interesting to note that in most of these areas, except for areas N04 and N22, the starting levels from 1989 were below 5%. In contrast, highest prevalence recorded in December 2006 were in the range of 60-65%, reflecting the dramatic increase in prevalence of hyperpigmentation within the past 18 years. The fact that in the other 6 areas no such increase was recorded may partly be attributed to a lack of data. However, it is also clear from the trend curves that there are areas in the North Sea that have generally been characterised by low prevalences of hyperpigmentation (see also Fig. 3).

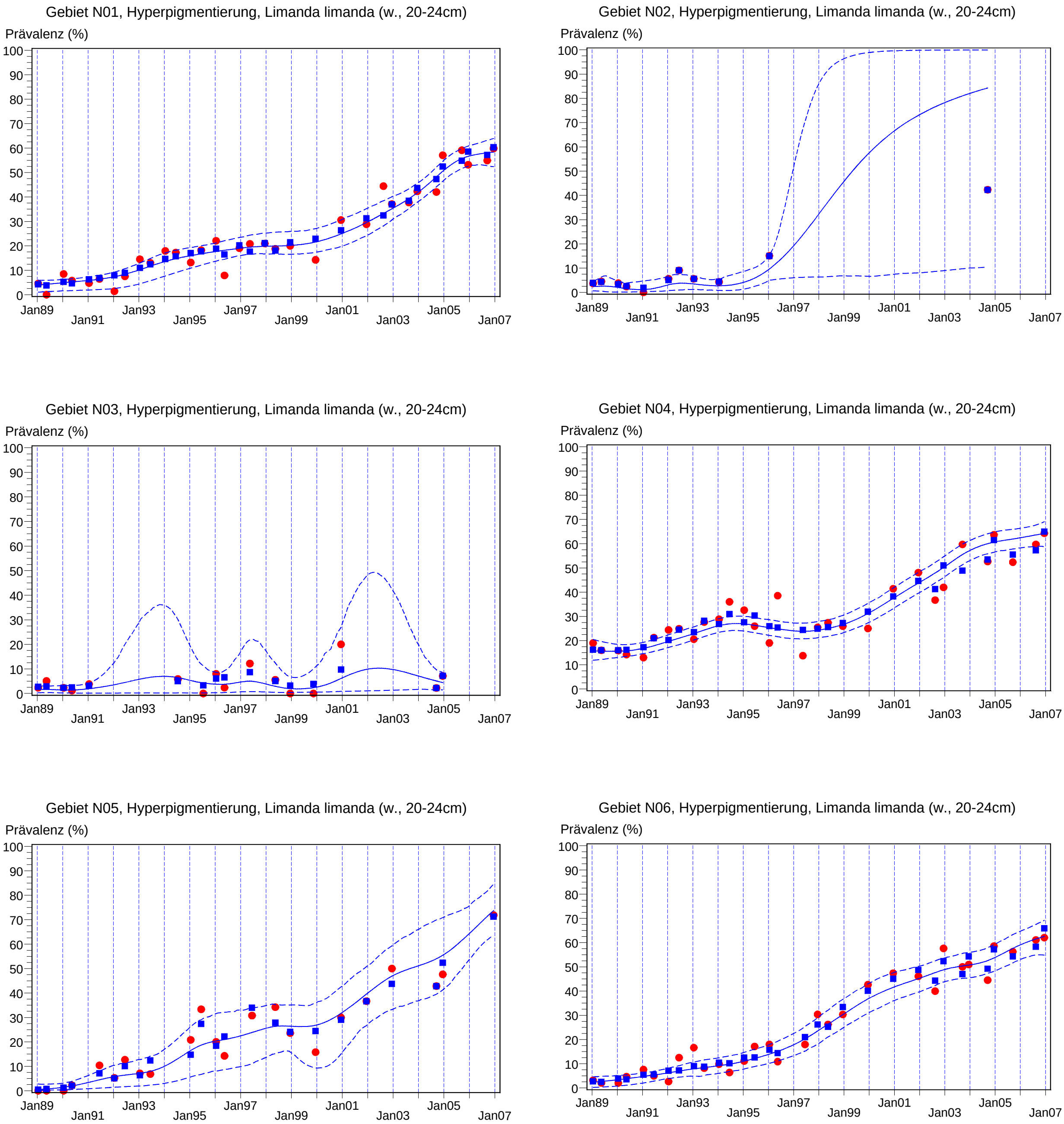

Figure 4: Changes in the prevalence of hyperpigmentation in female dab (*Limanda limanda*), size group 20-24 cm total length, from 12 North Sea areas in the period 1989-2006 (red dots: observed prevalence, blue squares: prevalence predicted based on a season adjustment trend model, solid line: trend, broken lines: confidence interval)

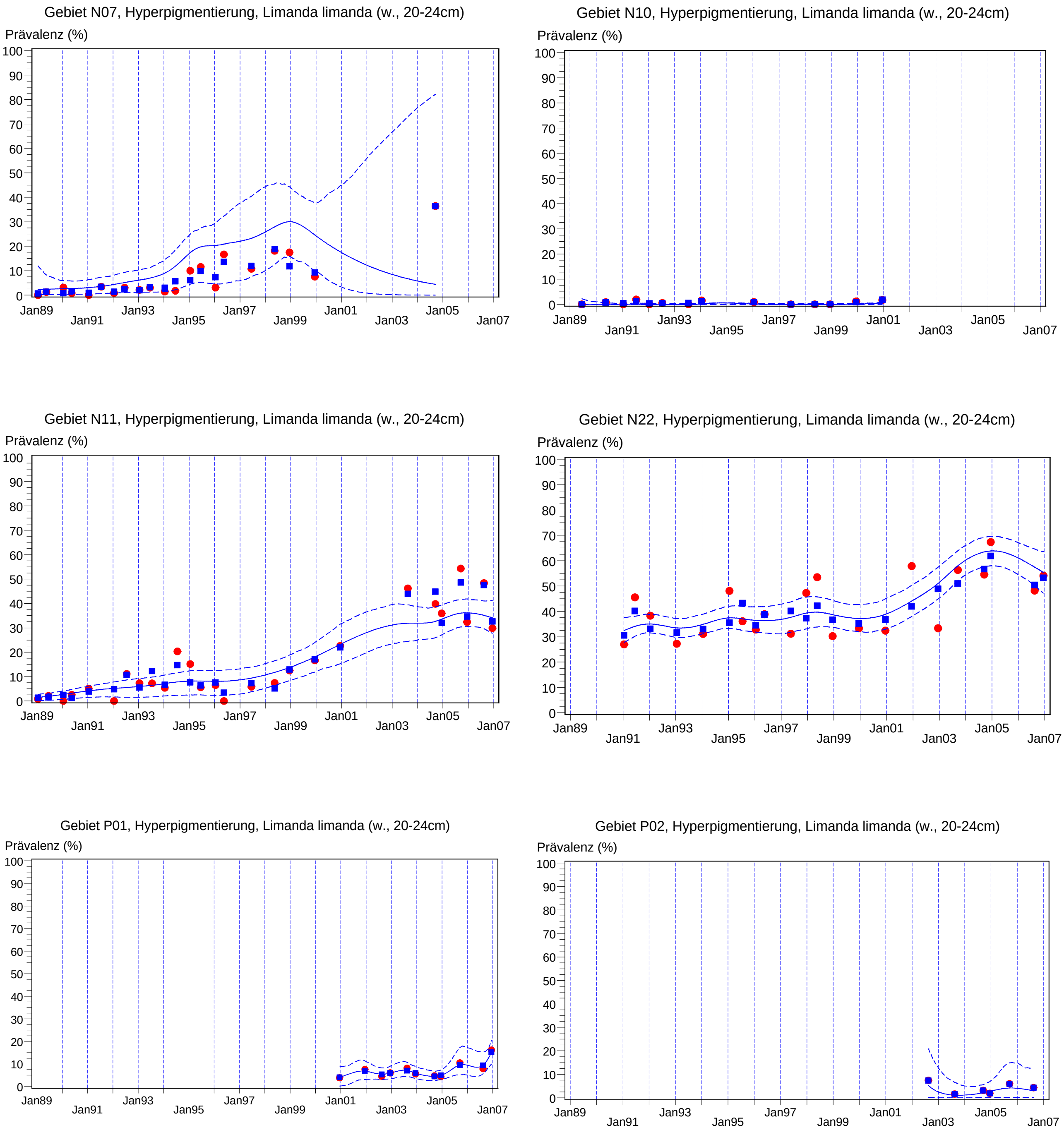

Figure 4: (cont.)

Host-specific effects on hyperpigmentation

Effects of length

The smallest dab found to be affected by hyperpigmentation were 12 cm long (total length). As shown in Fig. 5, there is a clear positive relationship between size of the fish and the prevalence of hyperpigmentation, indicating an age effect on the prevalence. It can also be seen from the figure that hyperpigmentation grades 2 and 3 increase in prevalence with increasing size whereas the proportion of fish with hyperpigmentation grade 1 decreases in fish larger than 25 cm after an initial steep increase in the smaller size classes. The maximum prevalence of approximately 80% was reached in dab of the size 28 cm, thereafter the prevalence dropped. It has to be considered, however, that the number of fish in the largest size classes is comparatively small, possibly leading to somewhat biased data.

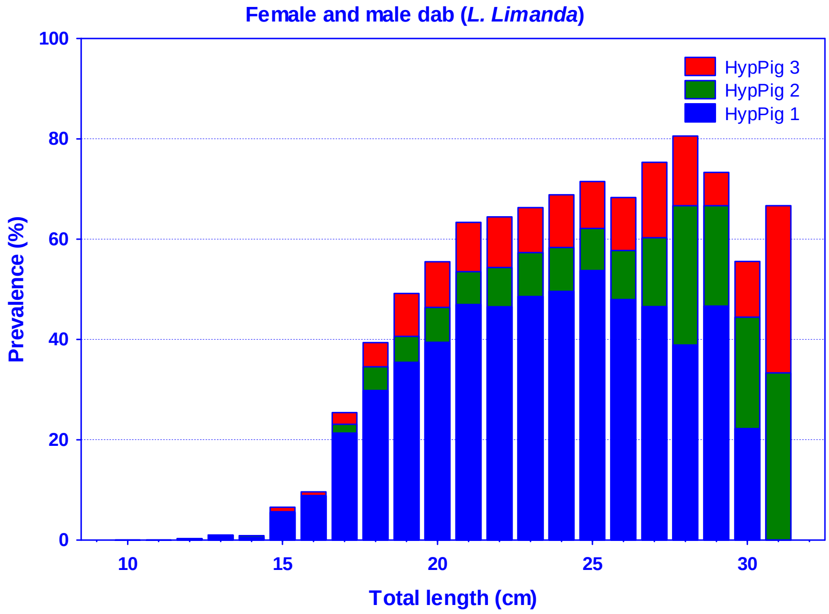

Fig. 5: Prevalence of hyperpigmentation (grades 1, 2 and 3) in North Sea dab (*Limanda limanda*) according to total length (cm below); data from males and females, from sampling areas N01, N04, N06 and N22 and from sampling campaigns 2004-2006 combined

Effects of sex

As shown in Figure 6, the prevalence of hyperpigmentation was higher in males than in females in the period 1988-2006 (exemplified by the trend curves for the size group 20-24 cm total length). In males, the maximum prevalence recorded in Dec. 2006 exceeded 80%, while it was approx. 60% in females. An explanation could be that males are older than females of the same size, again indicating that age is a risk factor.

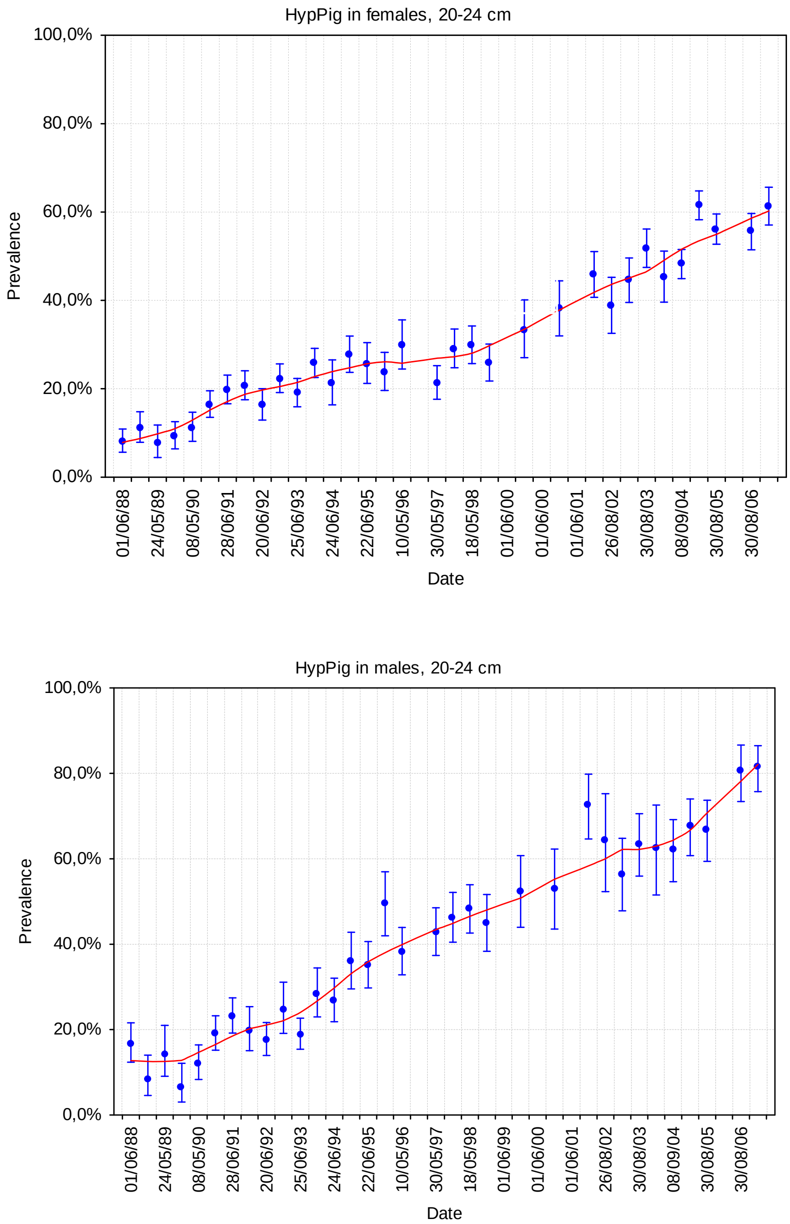

Fig. 6: Temporal change in the prevalence (with 95% confidence intervals) of hyperpigmentation in female (left figure) and male (right figure) North Sea dab (*Limanda limanda*), size group 20-24 cm total length, in the period 1988-2006; data from areas N01, N04, N06 and N22 combined

Effects on the host

Effects on 'robustness'

It has been observed that fish with severe symptoms of hyperpigmentation are more susceptible to stressors than healthy fish, e. g. when sorted from the catches and kept alive in containers with running seawater for subsequent tissue/fluid sampling. Particularly when the condition are suboptimal (e. g., in terms of ambient water temperature), specimens heavily affected by hyperpigmentation are mostly those that die first when maintained over longer periods of time.

Effects on condition

Fig. 7 shows the condition factors of affected and unaffected female and male dab of the size group 20-24 cm, calculated from total length and wet weight. Although the figure indicates that the differences between the groups of fish are only minor, there is a tendency for both females and males towards a decrease in condition factor with increasing severity of the condition. This could be a sign of effects of hyperpigmentation on the general physiological condition of fish affected.

Relationship to other diseases

The areas N04 and N22 that showed the highest start values of hyperpigmentation are those that always have had the highest prevalence of liver tumours. In area N05 that showed the steepest increase in the prevalence of hyperpigmentation, dab are characterised by a prevalent green discolouration of the livers, associated to an icteric condition due to a blocking of the bile ducts by parasitic myxozoan parasites. Apart from these coincidences there are no obvious links to other diseases and their temporal trends.

Discussion and outlook

The information presented in this document indicate the following features of hyperpigmentation in dab (*Limanda limanda*):

- Hyperpigmentation is more common in dab than in other fish species from the same habitats.
- Macroscopic and histopathological findings indicate a condition with distinct features consisting of a hyperplasia of melanocytes (mainly in the skin of upper body side) and iridophores (mainly in the skin of the lower body side), partly associated with inflammatory responses and degenerative processes.
- The prevalence of hyperpigmentation in the North Sea differs markedly between regions.
- There has been a significant and dramatic increase of the prevalence in many North Sea areas over the past 17 years.
- Hyperpigmentation is more prevalent in larger fish and in males compared to smaller fish and females, respectively.
- There is indication that there is a correlation between the presence of hyperpigmentation and the condition of the affected fish.

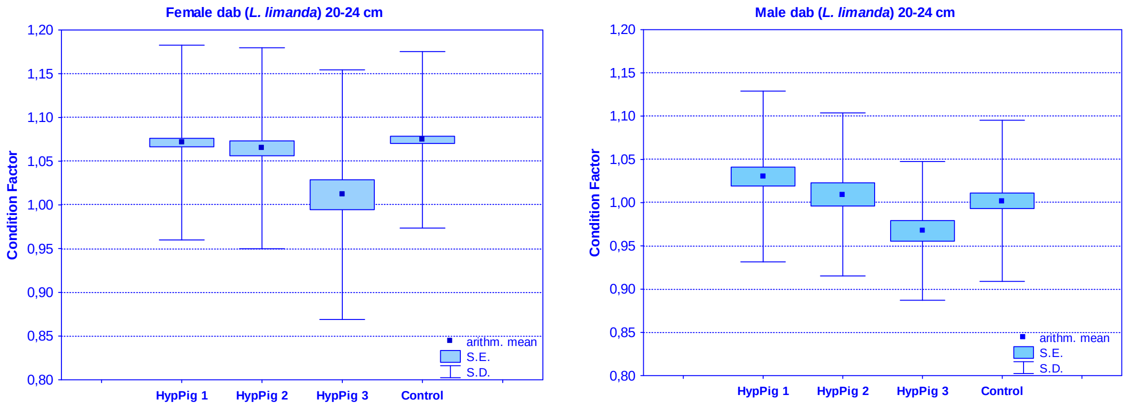

Fig. 7: Condition factors (CF = wet weight *100/length^3) of female and male North Sea dab (*Limanda limanda*) (size group 20-24 cm total length) with different grades of hyperpigmentation (control: unaffected); data from sampling areas N01, N04, N06 and N22 (see Fig. 3) and sampling campaigns 2002-2006 combined

So far, there is no information at all on possible causes of hyperpigmentation and the significant change in prevalence recorded in the North Sea. A number of hypotheses have been discussed that are briefly summarised below (it is emphasised, however, that other factors not mentioned here may also play a role):

Involvement of pathogens: Although there is no indication from (very preliminary) studies carried out so far for an involvement of pathogens as causes of hyperpigmentation, based on the spatial and temporal patterns observed and the relationship between prevalence and size and sex, it cannot be excluded that hyperpigmentation is an infectious disease. However, it may also well be a non-infectious disease, possibly related to neoplasia.

UV-B: It has been hypothesized that increased UV-B radiation affecting early pelagic life stages (embryos, larvae) of dab while drifting at the water surface during calm weather periods in spring/summer may be a cause of hyperpigmentation. It is known that organisms exposed to UV-B radiation react with increased deposition of pigment in their integument, preventing them from toxic UV-B effects and it cannot be excluded that early life stages of fish exposed react in the same way. However, in dab, this would imply that this effect is somehow conserved/fixed in the organism, leading to increased pigmentation in juvenile and adult fish that are very likely not anymore exposed to toxic levels of UV-B radiation due to increased attenuation of UV-B radiation with increasing water depth.

Contaminant exposure: Contaminants may affect all life stages of dab (e.g., accumulation of contaminants in the surface microlayer may have effects on the early life stages and accumulation in the sediments may affect the juveniles after metamorphosis or the adult stages). However, there is no information available so far on possible links between contaminants and hyperpigmentation.

Genetic change: It cannot be excluded that genetic changes have occurred leading to a wider regional distribution of hyperpigmentation and to higher prevalences. However, it has to be considered here that hyperpigmentation does apparently not offer any selective advantages as indicated by the negative effects on general condition.

Nutrition, algal blooms, climate change: These are factors that need to be considered, particularly in the context of climate change and its effects on marine ecosystems.

As a conclusion, it can be emphasised that more research (including experimental studies) and more analyses of existing data (e.g., in the ICES fish disease databank) are needed in order to identify the causes of hyperpigmentation and its effects at an individual and population level.

Literature cited

ICES 2006. Report of the Working Group on Pathology and Diseases of Marine Organisms. ICES CM 2006/MMC:01, 98 pp.

Wosniok, W., Lang, T., Vethaak, A.D., desClers, S, Mellergaard, Feist, S.W., McVicar, A.H. Statistical methods for the analysis of fish disease data (in preparation for publication in ICES Techniques in Marine Environmental Science)

Annex 7: Development of salmon louse vaccines and update on sea lice management schemes

T.A. Mo, S.R.M. Jones, S.A. MacLean, D.W. Bruno, N. Ruane

Chemical delousing

Norway

Currently in use for Atlantic salmon (*Salmo salar*) are pyrethroids (deltamethrin and cypermethrin) and emamectin benzoate (SLICE®). Treatment failure with pyrethroids has been observed in 4 farms in mid-Norway, one farm had to treat 5 times during a short period. There are indications of pyrethroid resistance developing in salmon lice and it is feared that this capability will spread to salmon lice in other farm areas.

Canada

SLICE® is used in virtually all treatments of farmed Atlantic salmon in New Brunswick and British Columbia. Clinical disease in farmed Atlantic salmon is extremely rare in British Columbia and delousing treatments are triggered by the legislated thresholds described in the previous report (a year round threshold of 3 mobile stages). In British Columbia, the efficacy of a SLICE® treatment is approximately 5 months and on average the drug is administered 1.75 times per production cycle. No evidence of resistance to emamectin benzoate has been reported.

Ireland

There are currently four licensed sea lice treatments in Ireland. CALICIDE® and SLICE® are in-feed treatments while EXCIS® and ALPHAMAX® are used in bath treatments. Withdrawal periods for these treatments are as follows: CALICIDE® - 7 days; SLICE® - no withdrawal period; EXCIS® - 1 day; ALPHAMAX® - 3 days.

Prophylactic treatment

USA

Preliminary field data suggest freshwater treatment of smolts with SLICE® prior to seawater transfer provides protection from lice predation to newly transferred smolts (Gustafson et al 2006; http://www.sciquest.org.nz/default.asp?pageid=2; Search under Non-peer reviewed publication, ISVEE, Author: Gustafson L). Smolts fed SLICE®-medicated feed in the hatchery for one week prior to transfer to sea had statistically significant fewer lice-attributed mortalities in the first 4 weeks in seawater than the untreated fish on the same site. Despite the small sample size, the trend suggests a substantial field benefit of SLICE® pre-treatment on sea lice prevention in the first several weeks of smolts in seawater. Comparisons of post-treatment protection of both transferred hatchery-treated smolts and sea cage-treated fish over the last 5 years have demonstrated comparable levels and spans of benefit under both treatment regimes.

Norway

In many Norwegian fjords, high numbers of salmon lice have been observed on wild smolt migrating from the rivers and out the fjords. High mortalities among the smolt have been estimated. In the most affected fjords, smolts produced for stock enhancement have been treated with SLICE one week prior to release. Several releases have been followed with untreated control groups. After one month in the sea, catches of treated smolts have been significantly higher than untreated smolts.

Development of salmon louse vaccine

Current status in Norway

Vaccination trials have shown significant positive effects of test vaccines that are based on proteins isolated from salmon louse eggs. The effect has not been linked to any specific antigens, but the effect of one of the proteins has been tested by RNA interference. Follow-up experiments using recombinant antigens will be carried out during 2007. This will provide data on whether *in vitro* produced protein from one of the promising candidates would be an effective vaccine antigen. It is crucial that candidate antigens can be produced *in vitro* and can induce protective immunity.

Future work in Norway

The Institute of Marine Research has initiated a new project (2006-2008) to increase the number of potential antigens and to test these antigens by RNA interference. This includes increasing the number of Expressed Sequences Tags for microarray probe production. Ideally, all salmon louse genes should be available (complete genome sequence) to increase the likelihood of identifying relevant vaccine candidates.

Canada

Researchers are continuing to test the efficacy of candidate recombinant *L. salmonis* antigens, in a variety of formulations, as described in the previous report.

Ireland

A strategic project entitled "*Novel vaccines for the control of sea lice on salmonids*" received national funding for the period 2005 – 2007. The lead partner is the Faculty of Veterinary Medicine, University College Dublin.

Sea lice management

Norway

The Norwegian strategy against sea lice on salmonids is based on the National Action Plan of 1987 which was drafted by relevant authorities, farmers and scientists. The strategy includes a) monitoring of lice abundance every 2 weeks by farmers, b) reporting of counts to the fisheries and food authorities at the same interval, and c) monitoring of rivers for prematurely returned sea trout and sea char in the spring. Delousing within 14 days is required by law if the reported farm lice abundance exceeds a set limit, currently 0.5 adult female lice/fish most of the year. If the farm is not deloused within this timeframe, a running fine based on the stock in the farm will be levied. There is no requirement for coordinated delousing in the strategy, but in some areas voluntary strategic treatments have been very successful.

Generally, this strategy has worked well. At present salmon lice no longer pose a health problem for farmed salmon. In general, wild Atlantic salmon smolts migrating through the fjords in the spring no longer suffer significant mortality due to sea lice, and premature returns of sea char and sea trout, triggered by heavy infestations of copepods, has decreased.

However, the significant increase in the farmed standing stock of Atlantic salmon in Norwegian coastal waters in recent years has made a revision of the current regulation imperative.

Key reference: *Heuch et al. 2005. A review of the Norwegian "National Action Plan against Salmon Lice on Salmonids": The effect on salmonids. Aquaculture 246: 79-92.*

Scotland

Scotland is proposing to introduce new legislation (Aquaculture and Fisheries (Scotland) Bill) that will be mandatory for farmers to keep records of data in relation to the prevention of sea lice, including the control and reduction of lice on their farms and make those records available to for up to three years (see Annex 8). The Bill is intended to issue an Enforcement Notice where control measures have not been carried out. Where appropriate a case for prosecution can be submitted. Sea louse management strategies seem to be working quite well in the form of Area Management Agreements (AMA) with synchronised treatments and all in/all out policies having largely been affective in areas where these agreements have been implemented. Farmers follow current guidelines, and lice counts may be weekly at specific times of year or when wild smolts are migrating to sea, for example.

Discharge and disposal of therapeutics at fish farm sites in Scotland are subject to control by licence and hence influences the ability of a fish farmer to conduct treatments and the frequency at which treatments can be made. It is envisaged there may be scope to permit emergency treatments in extreme cases where high sea lice levels are observed and the licence for a site does not permit the discharge of suitable medicines or where limits on the amount or rate of discharge in the existing licence may be exceeded.

USA

The integrated pest management strategy for sea lice in the Atlantic salmon farming industry in Maine is multifaceted and involves coordinated efforts with the salmon farmers and provincial authorities in neighbouring New Brunswick, Canada. The approach to control of sea lice over the last 5 years has included:

1) Fallowing sites/bays after harvest for 1 to 3.5 months.

2) Treatment with SLICE®-medicated (emamectin benzoate) feed when settlement of *Lepeophtheirus salmonis* is observed by the site veterinarian.

3) Encouraging higher level site or management personnel to perform counts.

4) Encouraging more stringent training to improve count accuracy by better identification of lice species, gender and life stages on site.

5) Encouraging post-treatment counts for at least 8 weeks to quickly identify treatment failures and the need for re-treatment, as well as the nature of the failure.

6) A change in bay management strategy to a 3 year production cycle, to include all sites on both sides of the US-Canada border within a zone delineated by hydrographic contact in a single complete tidal cycle (this was initiated in 2006, despite 2 carryover sites in Passamaquoddy Bay, and will require complete harvest by January '09, followed by a 3 month fallow).

7) Freshwater treatment of Maine smolts with SLICE® under an Investigational New Animal Drug (INAD) permit (Canadian-raised freshwater-treated smolts have been stocked in Maine for several years).

Canada

In British Columbia, monthly monitoring is conducted between August and February, whereas between March and July this increases to biweekly monitoring. Twenty fish are examined from each of one reference and two random pens per site. Data are collected on chalimus (all species), adult female *L. salmonis* (ovigerous and nonovigerous), all mobile *L. salmonis* and all mobile *Caligus*. Temperature, salinity and flow data are also collected from most sites. Aggregated data are posted on the provincial web-site. Sea monitoring complies with the Fish Health Management Plan which is a condition of licence. Industry surveillance is verified via a government audit of 25% of sites quarterly between July and March, and 50% of sites between April and June.

Sea lice counts on juvenile wild Pacific salmon are also made during the spring out-migration between March and July.

Ireland

In 1991, in response to concerns about the possible impacts of sea lice from salmon farms on wild populations of sea trout, a sea lice monitoring programme was initiated by the Department of the Marine. In 1992/93 the programme was expanded and culminated in the publishing in May 2000 of the 'Offshore Finfish Farms – Sea Lice Monitoring and Control Protocol'. The management strategy for sea lice has five principle components:

1) Separation of generations.
2) Annual fallowing of production sites.
3) Early harvest of two sea-winter fish.
4) Targeted treatment regimes, including synchronous treatments.
5) Agreed husbandry practices.

When lice levels exceed pre-set treatment figures, advice is given to treat the affected stock. These are designed to minimise any risk of transmission of sea lice from fish farms to wild sea trout stocks. The current treatment trigger level is 0.3 – 0.5 egg-bearing female lice per fish during spring. Outside the critical spring period, the treatment trigger level is set at 2.0 egg-bearing female lice per fish. Where numbers of mobile lice are high, treatments are triggered even in the absence of egg-bearing females.

Sea lice counts

USA

Variations in average weekly lice counts from year to year are attributed to wide year to year variations in sea water temperature, the greater effort to assure accuracy of lice counts in the last two years, the number of fish (i.e., potential hosts) stocked per year class, a gradual shift in lice species distribution over time and the variable rapidity with which ISA-infected cages of fish are removed from the sites. The presence or absence of financial compensation of depopulated cages and the varying and lengthy drug withdrawal interval for SLICE® in Maine (60 days) and New Brunswick (68 days) have had significant impacts on the rapidity of culling in both Passamaquoddy and Cobscook Bays.

Ireland

All marine fish sites undergo lice inspections 14 times per year (once per month except during spring when it is twice per month). Sea lice counts are published annually by the Marine Institute and can be found on the MI website www.marine.ie under publications (Irish Fisheries Bulletins).

Annex 8: Discussion Paper – Enforcement of sea lice controls proposed in the Aquaculture and Fisheries (Scotland) Bill

Sea Lice Records – Keeping and Inspection

The Aquaculture and Fisheries (Scotland) Bill (the Bill) proposes that Scottish Ministers have powers to make an Order requiring fish farmers to:

- keep records of data in relation to the prevention, control and reduction of sea lice on their farms;
- make those records available to the Scottish Ministers;
- retain those records for up to three years.

An offence is committed if a person fails to comply with the Order, provides or records false information or alters a record so that the information becomes false.

It is anticipated that such an Order will be made in the autumn. Following the making of such an Order, there are a number of options available with regard to the inspection and collection of the records required to be kept:

1) Inspectors may examine records during routine fish farm inspections and reserve the right to collect copies where the records are unsatisfactory.

2) Inspectors may examine records and collect copies during routine fish farm inspections (normally once per year).

Routine Inspections and Audits

It is envisaged that the vast majority of inspections will be conducted by FRS fish health inspectors in conjunction with their routine visits to fish farms to examine site records of live fish movements, mortalities of fish and veterinary medicines usage, inspect the fish on the farm and take whatever samples may be required. In addition, a percentage of farms will be subject to a more in-depth audit.

Audits may involve examination of staff training records, records of veterinary inspections, records of sea lice treatments, as well as records of sea lice counts. Audits may involve witnessing procedures, such as sea lice counting or administration of therapeutic treatments. Up to 60 audits (20% of farms) may be conducted per year. The proportion of sites audited will be influenced by the number of follow up visits required.

Advance notice of an audit may be given so that the inspection coincides with a sea lice count, a therapeutic treatment, or both. Inspectors will not normally carry out sea lice counts. They will audit site staff carrying out sea lice counts against standard procedures. However, in cases where there is an obvious failure by site staff to recognise or identify sea lice and/or a failure to maintain accurate records, then it may be necessary for FRS inspectors to conduct a sea lice count. The data generated may to be used as evidence in any future prosecution proceedings.

Inspectors will adopt a strategy to ensure a proportion of farms are audited each year.

The options for the strategy include:

1) A risk-based approach;

2) A random approach;

3) An intelligence-based approach; or

4) A combination of the above.

A risk-based approach may be favourable in terms of extracting the maximum benefit from the resources available. This is provided that the appropriate risks are identified. The disadvantage may be that farms that are considered low risk are never audited.

The following risk factors could be considered for selecting sites to be audited.

- Signed up to an Area Management Agreement (AMA) under the terms of the Tripartite Working group (TWG)
- Signed up to *A Code of Good Practice for Scottish Finfish Aquaculture* (CoGP)
- Appropriate licences under the Water Environment (Controlled Activities) (Scotland) Regulations 2005 (CAR)
- Proximity to sensitive or important wild fisheries
- Compliant with the CoGP with respect to sea lice provisions
- History of sea lice problems on site

Intelligence that may prompt an inspector to audit a site could include reports of high lice levels or malpractice received from fish health inspectors, other government agencies, neighbouring farmers, wild fishery interests and members of the public likely to have access to reliable information. At its discretion FRS will follow up and investigate claims from credible sources.

Enforcement Notices

The Bill proposes that powers are granted to the Scottish Ministers to issue Enforcement Notices (a Notice) to prevent, control and reduce sea lice. A Notice may be served upon any person carrying out the business of fish farming and may require the implementation of procedures to ensure the prevention, control or reduction of sea lice. An offence is committed if, without reasonable excuse, the person contravenes the conditions of a Notice.

Any expenses, which have been reasonably incurred by the Fish Health Inspectorate when taking remedial action to fulfil the requirements of a Notice that has been contravened, may be recovered from the person on whom the Notice was served. Scottish Ministers may publicise the serving of an enforcement notice to the extent and in such a manner as they consider fit.

Enforcement action will be in line with the FRS Enforcement Policy (Annex I).

Where and when will a Notice be served?

- If records of sea lice counts are not available on site at the time of the inspection, an inspector may issue a Notice requesting that the records are made available to FRS within a specified timeframe. If the records are not supplied within the timeframe specified in the Notice, and there is no reasonable excuse for failure to supply the records, further action may be considered.
- If the records are unsatisfactory, then copies of the records may be taken and may be used as evidence in subsequent proceedings. The site concerned may be subject to a comprehensive audit of procedures and record keeping. Further action may be considered.
- Failure to conduct sea lice counts at the required frequency may result in a Notice being issued.
- Failure to conduct sea lice counts according to standard procedures may result in a Notice being issued. For example, the Notice may require that staff undertake further training.
- Failure to seek veterinary advice if sea lice counts exceed recommended trigger values may result in a Notice being issued.
- Failure to control sea lice through the effective application of therapeutic treatments, or other authorised means, may result in a Notice being issued. For example, if veterinary advice has been ignored.

A Notice will stipulate why it was served and highlight any corrective action required to be taken within a specified timeframe. Follow-up inspections or audits may be conducted to establish whether corrective action has been taken. A second Notice may be served, if the requirements of the first Notice are not met in full. If no action is taken to comply with a Notice, or if the response is not adequate, then a case may be put to the Procurator Fiscal.

The following are examples of corrective actions that may be specified in a Notice:

- Instruction to carry out sea lice counts
- Instruction to keep records of sea lice counts
- Instruction to furnish FRS with records of sea lice counts
- Instruction to provide further staff training in relation to sea lice identification or application of therapeutic treatments
- Instruction to seek veterinary advice
- Instruction to follow veterinary advice with a view to control of sea lice.

Standard procedures for sea lice counts and measures to control sea lice

Section 3.4.3 of the CoGP is dedicated to control and management of sea lice on Scottish fish farms. It is envisaged that the standard for establishing best estimates of sea lice numbers that fish health inspectors will audit farmers against, will be based on the existing CoGP, which includes advice from statisticians as to current best practice.

Section 6 of the Bill stipulates that fish farmers are required to have "satisfactory measures" in place to control sea lice. This discussion paper does not attempt to define satisfactory measures. Section 7 of the Bill provides that Scottish Ministers may approve a code of practice, giving practical guidance to persons who carry on the business of fish farming, and promoting what appear to be desirable practices by such persons. If guidance on what constitutes satisfactory measures is to be issued, then that will be formally adopted as a code of practice.

At the present time it has been agreed not to use section 7. This provision will only be implemented if problems arise with the industry's CoGP and it becomes unworkable. Approving the Code now would not allow sufficient time for the CoGP to prove itself.

It has, however, been agreed that guidance shall be made public on how inspectors will act when undertaking the new duties provided for in the Bill. This 'guidance to inspectors' will state that inspectors will be bound to take the relevant parts of the industry's CoGP into account when coming to a view on the whether a site has satisfactory measures in place to control sea lice. In this way, the Code has meaning and influence without being formally put into law.

SEPA licences and CAR

A licence under the Water Environment (Controlled Activities) (Scotland) Regulations 2005 (CAR) is necessary for fish farm companies seeking to discharge and dispose of therapeutics at their fish farm site. As most sea lice treatments occur within the cages or at the facilities of a site the licence controls or influences the ability of a fish farmer to conduct treatments and the frequency at which treatments can be made. Within Scotland, SEPA are the authority responsible for issuing such licences.

At the discretion of Scottish Ministers, there may be scope to permit emergency treatments in extreme cases where high sea lice levels are observed and the licence for a site does not permit the discharge of suitable medicines or where limits on the amount or rate of discharge in the existing licence may be exceeded. It may be that the use of well boats and discharging chemicals in a controlled manner at a designated location could form part of this emergency procedure. Such operations are likely to require a licence under the provisions relating to Deposits in the Sea as set out in Part II of the Food and Environment Protection Act 1985 (FEPA). Where emergency treatments have been required at a given site, SEPA may review the licence for that site and may limit the maximum biomass permitted to that which can be treated by the amount of medicine licensed for that site, as appropriate.

Hypothetical case studies / scenarios

Wherever possible advice will be given to assist and encourage fish farmers to comply with the requirements of the legislation but an Enforcement Notice may be issued where necessary. The following examples are designed to illustrate situations where advice has been followed up by an Enforcement Notice.

PROBLEM	ENFORCEMENT NOTICE	FOLLOW UP ACTION/COMMENTS
1. Frequency of sea lice count insufficient, although standard procedures are followed.	Instruction to carry out regular sea lice counts	Inspection/audit to verify frequency of sea lice counts has been increased.
2. Standard sea lice counting procedures not followed by site staff.	Instruction to follow standard procedures or to obtain further staff training, as necessary	Satisfactory evidence of training obtained submitted to FRS.
3. Standard sea lice counting procedures followed but misidentification of sea lice by site staff.	Instruction to obtain further staff training	Satisfactory evidence of training obtained submitted to FRS.
4. Failure to take action despite recording lice numbers above threshold for treatment on consecutive counts.	Instruction to seek veterinary advice	Satisfactory evidence of veterinary input submitted to FRS or site inspection.
5. Sea lice counts above threshold for treatment and failure to act on veterinary advice.	Instruction to follow veterinary advice with a view to controlling sea lice	Inspection/audit to verify that appropriate action has been taken.
6. Sea lice counts above threshold for treatment despite therapeutic treatments having been conducted.	Instruction to seek and follow veterinary advice	Satisfactory evidence of veterinary input submitted to FRS or site inspection.
7. Sea lice counts above threshold for treatment, veterinary advice sought but treatment constrained by CAR licence.	Instruction to follow veterinary advice with a view to controlling sea lice	Consult with SEPA. Emergency measures may be necessary.
8. Organic site not willing to treat as there is potential to lose organic status.	Instruction to follow veterinary advice with a view to controlling sea lice	Consultation with organic accreditation body to determine the scope for treatments and the impact upon organic status.

Appendix I: FRS Enforcement Policy

As part of our duties we may be required to take enforcement action. Our enforcement activity will reflect the Government's principles on good regulation. Whenever possible, we will advise and assist on compliance with the regulations, explaining clearly what constitutes best practice and what is a legal requirement. In situations where immediate action is necessary, we will explain why it is necessary and confirm this in writing within five working days.

We will ensure that:

- Any enforcement action taken is proportional to the risks posed and the seriousness of the offence. As far as the law allows, we will take account of the individual circumstances of the case and the attitude of the operator when considering action.

- Before formal enforcement action is taken, inspectors will provide an opportunity to discuss the circumstances of the case and, if possible, resolve points of difference unless immediate action is required.
- Warning letters and cautions may be issued. Where there are rights of appeal against formal action, advice on the appeal mechanism will be clearly set out in writing at the time the action is taken.
- Where possible and the law permits, we will consider alternatives to prosecution. If a serious offence or persistent minor offences are considered to have taken place, we will investigate and report directly to SEERAD. If appropriate, a case for prosecution will be submitted to the Procurator Fiscal. Upon successful prosecution, the penalties may include a fine.

Annex 9: Proposal: HELCOM/ICES/BSRP Workshop on Monitoring of Diseases and Parasites in Coastal Fish Species of the Baltic Sea

(extracted from the 2006 Draft Report of the ICES Study Group on Baltic Ecosystem Health Issues in Support of BSRP, SGEH)

T. Lang and G. Rodjuk

Abstract

Based on a recommendation from the 2005 ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM) and on requests from HELCOM, a land-based workshop is proposed on methodologies for monitoring diseases and parasites in coastal fish species of the Baltic Sea. The workshop shall be held for 4-5 days in 2007 (or 2008) at the BSRP Lead Laboratory for Fish Diseases and Histopathology, AtlantNIRO, Kaliningrad, Russia (priority 1) or at the Estonian Marine Institute, Tallinn, Estonia (priority 2). Expected deliverable are (a) baseline data on diseases and parasites of coastal fish species in the Baltic Sea to be used for future assessments, (b) training and intercalibration of methodologies for disease studies, (c) guidelines for fish disease monitoring in coastal fish species in the Baltic Sea to be applied in coastal fish monitoring programmes, and (d) proposals for disease indicators and target levels for coastal fish species in the Baltic Sea.

Introduction and Rationale

In December 2005, the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM) was held on board RV 'Walther Herwig III'. Its main objectives were to

- provide training and intercalibration related to methodologies applied in fish disease monitoring in the Baltic Sea,
- further develop and assess health indicators and indices appropriate for monitoring and assessment purposes,
- establish a closer collaboration between institutes involved in fish disease monitoring in the Baltic Sea,
- build the basis for incorporation of fish disease surveys into the revised HELCOM monitoring programme.

As a major output of the workshop, draft guidelines for fish disease monitoring in the Baltic Sea were provided (ICES 2005) (http://www.ices.dk/reports/BCC/2006/WKFDM06.pdf). However, it was clearly recognised that these guidelines are mainly applicable to studies carried out in offshore areas of the Baltic Sea, with cod (*Gadus morhua*), flounder (*Platichthys flesus*) and herring (*Clupea harengus*) as major target species, and cannot directly be used for studies in coastal fish communities, largely consisting of different fish species that may be affected by different diseases (incl. parasites). In order to fill this gap and to establish a link to the existing coordinated HELCOM coastal fish monitoring programme in the Baltic Sea, the WKFDM recommended that:

- ICES/BSRP organise a land-based workshop on methodologies for coastal fish disease monitoring to be held in 2006 or 2007 at the AtlantNIRO, Kaliningrad, Russia, or at the Estonian Marine Institute, Tallinn.

The present monitoring and assessment of coastal fish in the Baltic Sea is part of the HELCOM and BSRP activities and is coordinated by the Co-ordination Organ for Baltic Reference Areas (COBRA). Three coastal fish monitoring workshops were held under the auspices of HELCOM and the BSRP in the years 2004-2006. The coastal fish monitoring is carried out annually (in August) in up to 15 coastal sampling locations, encompassing areas around the entire Baltic Sea. COBRA maintains a database with time series data (partly since 22 years) submitted by countries participating on the programme (HELCOM 2006). So far, the data provide numeric information on spatial and temporal patterns in fish population characteristics (e.g., species abundance and richness, biomass, catch per unit effort), enabling an assessment of population/community changes and, by utilising biotic and abiotic data, of the role of environmental factors.

However, new perspectives on marine ecosystem management and conservation, including an ecosystem approach to coastal zone management, as well as recent EU directives such as the Water Framework Directive and the Habitats Directive, call for revised objectives in monitoring practices (HELCOM 2006). From originally being focused on mainly detecting the effects of local pollution, including toxic substances and eutrophication, coastal fish monitoring should be developed to provide a basis for estimating the ecological status of the coastal fish compartment (HELCOM 2006) as part of an ecosystem approach and related management objectives. Amongst other management objectives, HELCOM (2006) has identified the following:

- to restore and maintain healthy fish on an individual level and to ensure healthy fish populations, without causing harm either to other marine biota or to human populations

This management objective is in line with the Baltic Sea Action Plan objective related to hazardous substances that no health problems among animals should occur.

It has further been emphasised (HELCOM 2006) that relevant indicators for coastal fish management objectives should be further developed and that coastal fish monitoring should be integrated with other coastal monitoring programmes. It seems evident that the coastal fish monitoring should, amongst others, be linked to the monitoring and assessment of hazardous substances and their biological effects.

In this context, as an outcome of the 2004 HELCOM Workshop on Coastal Fish Monitoring, a number of indicators to be used for ecological assessments was proposed, amongst others the prevalence of diseases and parasites as health/stress indicator (also included in the strategy proposed for hazardous substances). Furthermore, at the 2006 HELCOM/BSRP Workshop on Coastal Fish Monitoring, there was consensus that biomarkers (including health indicators and indicators for reproductive success) fit well to the coastal fish monitoring programme and that further research should be supported, e.g. as part of the BONUS-169 programme.

(http://sea.helcom.fi/dps/docs/documents/Monitoring%20and%20Assessment%20Group%20(MONAS)/BSRP-HELCOM%20Coastal%20Fish%20Monitoring%202,%202005/2-2.pdf).

(http://sea.helcom.fi/dps/docs/documents/Monitoring%20and%20Assessment%20Group%20(MONAS)/MONAS%20Coastal%20Fish%20Monitoring%203,%202006/FINAL%20MINUTES.pdf).

In the Minutes of the 9th Meeting of HELCOM MONAS (2-6 October 2006), the ICES/BSRP SGEH and the HELCOM/BSRP Coastal Fish Monitoring Experts were requested to develop an indicator for fish disease with target levels for the Baltic Sea Action Plan.

For the reasons highlighted above and as a consequent next step in the development of appropriate ecosystem health objectives and indicators for fish health, a workshop is proposed focusing explicitly on diseases and parasites in coastal fish species and on related methodological aspects relevant for monitoring and assessment. The workshop could be co-sponsored by ICES, BSRP and HELCOM.

Objectives of the workshop

The major objectives of the workshop are to

- provide baseline data on diseases and parasites in key fish species from coastal areas in the Baltic Sea to be used for future fish health assessments as part of the coastal fish monitoring;
- provide training and intercalibration of methodologies related to the diagnosis of diseases;
- draft guidelines for fish disease monitoring in coastal fish species in the Baltic Sea to be applied in the coastal fish monitoring programme, and
- propose indicators and target levels for diseases of coastal fish species to be used in Baltic Sea ecosystem health assessments.

Organisation of the workshop

It is proposed to organise the land-based workshop to be held either at the BSRP Lead Laboratory for Fish Diseases and Histopathology, AtlantNIRO, Kaliningrad, Russia (priority 1) or the Estonian Marine Institute, Tallinn, Estonia (priority 2). The workshop should be held for approx. 4-5 days in late 2007 (or early 2008) under the co-chairmanship of G. Rodjuk (AtlantNIRO, Kaliningrad, Russia) and T. Lang (Fed. Res. Centre for Fisheries, Cuxhaven, Germany). It is recommended to identify a third co-chair representing the group of HELCOM/BSRP Coastal Fish Monitoring Experts. ICES, BSRP and HELCOM may act as co-sponsors of the workshop.

Participants should preferably represent all of the Baltic Sea countries and should consist of trainees and a number of invited trainers (possibly including experts from countries outside the Baltic Sea). Representatives of the co-sponsoring organisation are welcome. It is anticipated that, for logistic reasons, the maximum number of participants should not exceed 20 persons.

The workshop should consist of two tiers: (a) lectures and seminars providing background information and for discussions and drafting purposes and (b) practical work in the lab with fresh material (key fish species) and preserved samples (e.g. parasite specimens).

Requirements for the workshop

Amongst others, the following requirements have to be met:

- The ICES/BSRP SGEH has to endorse the plan to organise the workshop.
- A steering group consisting of at least three dedicated experts (e.g., the co-chairs) should be identified at the 2006 SGEH meeting and should start working by correspondence to plan details of the workshop.
- Co-sponsors have to be identified who have to adopt the plans for the workshop and to provide support in the planning phase and during the workshop.
- A final decision has to be made on the venue and on the workshop dates.

- The hosting institute has to provide equipment needed (e.g., meeting rooms, lab, microscopes, fresh material for dissection, specimens collections) and logistic support.
- Potential experts acting as trainers have to be identified and contacted.
- Participants (trainees) have to be identified, preferably representing all Baltic Sea countries and institutes therein engaged in coastal fish monitoring.
- Funding sources have to be identified. It is hoped that a considerable contribution from the BSRP will be available, including support for western experts if required. Further contributions are expected from Baltic Sea countries (and ICES, HELCOM ?).

Expected deliverables of the workshop

It is expected that the following products will be generated:

- a full (ICES) report with all information generated by the workshop and with recommendations for further actions;
- a compilation of available background data on diseases and parasites of coastal fish species in the Baltic Sea to be used a reference for future assessments;
- training and intercalibration of methodologies;
- national reports from Baltic Sea countries on the status of relevant activities;
- draft guidelines for monitoring of diseases and parasites in coastal fish species in the Baltic Sea;
- proposals for indicators and target levels related to diseases in coastal fish species in the Baltic Sea to fulfil the demands of the HELCOM Baltic Sea Action Plan.

Literature cited

HELCOM 2006. Assessment of Coastal Fish Species in the Baltic Sea. Baltic Sea Environment Proceedings No. 103 A.

ICES 2005. Report of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea. ICES CM 2005/BCC: 02.

Annex 10: Review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves

T. Renault, S. Bower, S. Ford

Molecular techniques for diagnosing bivalve infectious agents have been developed during the last decade and are now moving from a developmental phase in specialized laboratories for research purposes, to routine application and are expected to be increasingly used in disease monitoring programs for the detection of specific pathogens. International standards proposed by the OIE are including molecular techniques for the detection and identification of certain bivalve pathogens. However, molecular tools will need formal validation against traditional techniques and testing for their specificity.

For many pathogens of molluscs, available diagnostic techniques are rather limited, and investigation restricted to histological and ultrastructural examination. Some infectious agents can be tentatively diagnosed by applying simple methodologies, such as stained tissue imprints usually in association with gross pathology. In addition, histopathological examination provides valuable information on the intensity and severity of infection at the individual level, co-infections with other notifiable pathogens as well as potential emerging pathogens and non-infectious conditions. These techniques including histology do not allow identification to the species level for many pathogens. There is a need for diagnostic tools to discriminate between genera and species. In this context, molecular detection assays for pathogens infecting molluscs appear clearly as suitable tools. They are being developed at an increasingly rate. Molecular techniques for diagnosing pathogens in molluscs are now moving from development in specialised laboratories for research purposes, to routine application and are expected to be increasingly used in pathogen monitoring programs. International standards proposed by the OIE are including molecular techniques for the detection and identification of listed pathogens (OIE 2006).

The routine use of DNA based diagnosis tools is however hampered by a number of major concerns.

i) Not all regions of pathogen DNA are equally useful as targets for molecular detection. Closely related pathogens may present high sequence similarities.

ii) Moreover, molecular tools detect DNA and not necessarily a viable pathogen.

iii) The assays often have not been thoroughly tested for inclusivity (detection of all strains of the pathogen) or specificity (cross reaction with any other organism). The main concern is that molecular tools too often are developed from a few sequences without a good understanding of the overall sequence variability within the species.

iv) PCR - despite its sensitivity - is still subject to the same problems that other diagnostic methods are: the tiny piece of tissue used for amplification may simply not contain the pathogen.

Moreover, molecular tools need formal validation against traditional techniques and testing for their specificity. Studies conducted in parallel with the same isolates in several laboratories would be ideal. It will also be necessary to identify regions of the pathogen genome that can be utilised for species differentiation. Moreover, all molecular assays specific for a pathogen should be tested in parallel and validated, and further sensitive diagnostic assays that will clearly discriminate between all "valid" species should be developed.

Bonamiosis

The first DNA-based diagnostic assay for a microcell was designed for *Bonamia* (=*Mikrocytos*) *roughleyi* (Adlard and Lester 1995). Question of specificity and sensitivity, however, limit the usefulness of the assay. "*Bonamia ostreae*-specific" PCR assays targeting the small subunit (SSU) rDNA were then developed by Carnegie et al. (2000) and Cochennec-Laureau et al. (2000). A first primer pair is 5' CAT TTA ATT GGT CGG GCC GC 3' and 5' GGG GGA TCG AAG ACG ATC AG 3', designated Bo and Boas, respectively, amplifies a 300 bp product (Cochennec-Laureau et al. 2000). A second primer pair is 5' CGG GGG CAT AAT TCA GGA AC 3' and 5' CCA TCT GCT GGA GAC ACA G 3', designated C_F and C_R, respectively, amplifies a 760 bp product (Carnegie et al. 2000). The first assay (Carnegie et al. 2000) should detect *B. ostreae* and *B. exitiosa*, but perhaps not *B. roughleyi*. The second assay (Cochennec-Laureau et al. 2000) should detect the SSU rRNA of all microcell haplosporidians. Both PCR assays have undergone some validation against the light microscopic screening of fixed and stained hemolymph smears (Carnegie et al. 2000, Diggles et al. 2003). Results indicated that both PCR assays detected more infection cases than histocytology. Also, the diagnostic sensitivity of a new real-time PCR that amplifies a 68-bp target DNA fragment using forward primer, 5′CCCGGCTTCTTAGAGGGACTA3′; reverse primer, 5′ACCTGTTATTGCCCCAATCTTC 3′; and probe, 5′FAM-CTGTGTCTCCAGCAGAT-MGBNFQ on frozen tissue samples or tissue in paraffin sections was consistently greater than histopathology (Marty et al. 2006). However, the species specificity of this assay has not been assessed. Recently, a real-time TaqMan PCR assay was developed for the detection of *Bonamia* spp. (but not *Haplosporidium nelsoni* nor *Haplosporidium costale*) that was comparable to conventional PCR in sensitivity but produced more rapid results with a low risk of sample cross-contamination and can be optimised to determine the intensity of infection (Corbeil et al. 2006). Primers applicable to PCR and fluorescent *in situ* hybridization (FISH) were described for the detection of a new species *Bonamia perspora* (Carnegie et al. 2006). Although these primers are theoretically specific to *B. perspora*, they have not been assessed in practice.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays may provide the most useful molecular tool to distinguish *B. ostreae* from *B. existiosa* and to distinguish *B. roughleyi* from these two *Bonamia* spp (Hine et al. 2001, Cochennec et al. 2003). The sequence of the SSU rDNA gene of *B. ostreae* shows polymorphism with that of *B. exitiosa* or *B. roughleyi* by restriction fragment length polymorphism (RFLP) analysis, by digesting the SSU PCR product (amplified using primers Bo and Boas) with *Hae* II and *Bgl* I. The digestion profiles vary according to the parasite species. *Bonamia ostreae* and *B. exitiosa* present the same profile (two products at 115 and 189 bp) when digested with *Hae* II while *B. roughleyi* is not digested. The *B. ostreae* profile consists of two bands at 120 and 180 bp when digested with *Bgl* I while *B. exitiosa* and *B. roughleyi* are not digested (Cochennec et al. 2003, Hine at al. 2001).

Two ISH protocols were developed for *B. ostreae* (Carnegie et al. 2003, Cochennec-Laureau et al. 2000). In the first protocol, probes consist of a cocktail of fluorescein-labeled oligonucleotide probes specific for *Bonamia ostreae:* UME-BO-1 (5' CGA GGC AGG GTT TGT 3'); UME-BO-2 (5' GGG TCA AAC TCG TTG AAC 3') and UME-BO- 3 (5' CGC TCT TAT CCA CCT AAT 3'). All these probes target the SSU rDNA gene. The specificity of the oligoprobe cocktail UME-BO-1, 2 and 3 has been tested and proved against *H. nelsoni* (Carnegie et al. 2003) but this ISH assay probably detects other microcells including at least *B. exitiosa*. In the second protocol, the probe is produced by PCR using the previously described primer pair Bo/Boas with digoxigenin incorporation. The probe Bo-Boas is able to detect *Haplosporidium nelsoni* in *Crassostrea virginica, Bonamia exitiosa* in *Ostrea chilensis* but not *Mikrocytos mackini* in *C. gigas* (Cochennec et al. 2000). ISH has not yet been validated against histology.

To confirm the presence of a viable pathogen, molecular approaches should be used in conjunction with other methods including histology in order to allow pathogen visualization. In known susceptible species within the known geographical range of *Bonamia ostreae*, a suspect case of infection with *B. ostreae* is a positive result by one of the following methods: histopathology, tissue imprints, or PCR. In other host species or outside the known range of *Bonamia ostreae*, a suspect case is detection by histopathology, tissue imprints, PCR or *in situ* hybridisation. A presumptive case of *Bonamia ostreae* is detection by tissue imprints or histology combined with a positive result with PCR or *in situ* hybridisation. PCR-RFLP and possibly, transmission electron microscopy are recommended for a confirmatory diagnosis.

The PCR assay developed by Cochennec-Laureau et al (2000) has been submitted to several validation tests against histological methods. However, this assay is not species specific and PCR-RFLP analysis must be applied to the amplified products to assess species identity by comparison with the profiles of known species. Validation is still required for in situ hybridization methods.

Mikrocytosis

PCR and fluorescent *in situ* hybridisation (FISH) assays for *Mikrocytos mackini* were also recently developed (Carnegie et al. 2003). A digoxigenin-labelled DNA probe (DIG-ISH), was used to reveal *M. mackini* in digestive gland tissues, an organ not previously known to be inhabited by this parasite (Meyer et al. 2005). Although these molecular assays do not cross-react with other molluscan pathogens of concern, the possibility of cross-reaction with other as-yet-undescribed species closely related to *M. mackini* has not been fully assessed.

Haplosporidosis

Molecular diagnostic tools were first developed to detect *Haplosporidium nelsoni*, the causative agent of MSX disease in eastern oysters. The small subunit (SSU) rRNA gene was targeted for the design of DNA probes and PCR primers (Fong et al 1993, Stokes and Burreson 1995, Day et al 2000, Penna et al 2001, Stokes and Burreson 2001, Russell et al 2004). These molecular detection assays were used by various teams involved in mollusc disease diagnosis to identify *H. nelsoni* in oysters in different geographical locations.

Although light microscopy is well suited to detect haplosporidian parasites on histological sections, this technique does not allow the differentiation of plasmodial stages from different species. Parasite location, host species and morphological features can be used to help in differential diagnosis. However, in absence of spore differential diagnosis between *H. nelsoni* and *H. costale* is problematic. In this context, Stokes and Burreson (2001) have developed PCR primers targeting the SSU rRNA gene of *Haplosporidium* species. These authors testing the primers showed that both species, *H. nelsoni* and *H. costale*, are differentially diagnosed. Moreover, both parasite species may be detected in the same animals indicating that mixed infections can occur (Stokes and Burreson 2001). PCR is currently being used on the east coast of Canada as a surveillance tool for both of these parasites.

Marteiliosis

A PCR protocol targeting the first internally transcribed spacer (ITS1) of the rRNA region has been developed for the detection of *Marteilia refringens* (Le Roux et al. 2001). No cross-reaction with parasites from other genera has occurred in tested samples. However, this assay can not differentiate *Marteilia refringens* and *M. maurini*. A protocol of RFLP applied on PCR products obtained using ITS-1 primers has been developed and facilitates the differentiation of *Marteilia refringens* and *M. maurini*. (Le Roux et al. 2001). This technique needs to be validated.

An *in situ* hybridization protocol has been developed and is based on the use of Smart2, a 266 bp digoxygenin-labelled PCR-generated probe targeting the SSU rDNA (Le Roux et al. 1999). Smart2 is able to detect *Marteilia* species including *Marteilia refringens*, *M. maurini* and *M. sydneyi* (Le Roux et al. 1999, Kleeman et al. 2002). Values of specificity and sensitivity for *in situ* hybridization were estimated at 0.9 and 0.99 respectively when co-validated with histology (Thébault et al. 2005). *In situ* hybridization can help to detect early infections which are more difficult to detect in traditional histological sections.

In situ hybridization developed by Le Roux et al (1999) has been co-validated with histology (Thébault et al. 2005). However, validation is still required for PCR.-based assays.

A nested-PCR using primers OPF-2-OPR-2 and OPF-3-OPR-3 and amplifying 672 and 447 bp of the SSU rDNA respectively was developed to detect the parasite *Marteilioides chungmuensis* in *Crassostrea gigas* (Itoh et al. 2003). The detection limit of this technique has not been determined yet. However, PCR detected the parasite in some oysters found negative by histology suggesting that the PCR assay is more sensitive than histology. These primers could not amplify other Paramyxean like *Marteilia refringens* and *M. sydneyi*. An *in situ* hybridization (ISH) protocol has also been developed using three DIG-labelled oligonucleotide probes MCSP-05, MCSP-06 and 6-R (Itoh et al. 2003). No non-specific binding was observed when tests were performed with other Paramyxean like *Marteilia refringens* and *M. sydneyi*. ISH can help to detect immature stages of the parasite which are more difficult to detect in traditional histological sections.

Both techniques need to be validated and more specifically, specificity and sensitivity values are lacking.

Perkinsosis

Various PCR based diagnostic assays proposed to be genus-specific and/or species-specific have been developed. Hamaguchi et al (1998) designed a PCR method for the diagnosis of the *Perkinsus* sp. from *V. philippinarum* in Japan. Kotob et al (1999 a,b) used sequence analysis of the ITS regions of two isolates of *Perkinsus* sp. from *Mya arenaria* to suggest that the two isolates were different species of *Perkinsus*. Cross et al. (2001) developed a PCR-based diagnostic assay for *P. chesapeaki* (=*andrewsi* see Burreson et al. 2003) in the USA. Robledo et al. (2000) and De la Herrán et al. (2000) developed one for *P. atlanticus* from Spain. However, as cautioned by Burreson (2000), more research is necessary to compare PCR assays with standard diagnostic techniques before PCR can be recommended as the method of choice for perkinsosis diagnosis. Nevertheless, molecular sequence data is playing an increasingly important role in the identification of *Perkinsus* species and requires adequate DNA sequence data at the targeted loci from the same and related species over a wide geographic area in order to develop reliable, accurate and sensitive molecular diagnostic tools (Villalba et al 2004).

Further research is required to resolve which DNA sequence differences are consistently significant in the identification of species and how these differences relate to biological parameters that can be used to describe and differentiate between closely related species. This is especially important if some species and/or strains of *Perkinsus* prove to be non-pathogenic for some or all host species.

Perkinsus marinus

A PCR assay based on the amplification of a part of the rRNA non transcribed spacer (NTS) region has been developed (Marsh et al. 1995, Robledo et al. 1998). A set of primers (PmarITS-70F and PmarITS-600R) for two species specific standard or real-time PCR techniques has also been designed from the internally transcribed spacer sequence (ITS) (Audemard et al. 2004). These last two techniques can detect *P. marinus* DNA levels equivalent to less than 1 cell. DNA sequencing of the ITS region can be done to identify the species, by comparing the ITS region nucleotide sequences with reference sequences deposited in the GenBank database (http://www.ncbi.nih.gov/entrez/).

The NTS PCR assay has been validated against fluid thioglycollate culture (Robledo et al. 1998). The ITS PCR assay has not been validated against fluid thioglycollate culture. However the ITS primers are recommended over the NTS assay because they are more likely to amplify all *Perkinsus marinus* strains (Audemard et al. 2004). Also, intra-specific variations within the NTS region has not been broadly assessed creating a risk of false negatives due to polymorphism within a species if the PCR primers do not bind the target sequence of all strains of that species (Villalba et al. 2004).

Perkinsus olseni

A species-specific PCR assay based on the amplification of part of the rRNA non transcribed spacer (NTS) region has been developed (Robledo et al. 2000) (Forward sequence (PA690F): 5' ATG CTA TGG TTG GTT GCG GAC C 3' and reverse sequence (PA690R): 5' GTA GCA AGC CGT AGA ACA GC 3') producing an expected amplicon at 690-bp. PCR tested with *P. marinus* DNA extracted from *Crassostrea virginica* and *Perkinsus* sp. DNA extracted from *Macoma balthica* produced a negative result. The lowest limit of detection of *P. olseni* isolated DNA is 0.01 µmol of NTS DNA. Sequencing of the NTS can also be used to assess the species of *Perkinsus* observed in clams (Murrel et al. 2002).

A pair of primers has also been designed from the intergenic spacer (ITS) sequence between the 18S and 5.8S rRNA of *P. olseni* to produce a PCR-based diagnostic test (de la Herran et al. 2000) (forward sequence (PK1): 5' ACC AGT CAC CAC AGG GCG TAA T 3' and reverse sequence (PK2): 5’ GTA GCG TGC TCT GAT GAT CAC T 3’) producing an expected amplicon at 554 bp. Tested with *P. olseni* extracted from infected *Ruditapes decussatus* in Europe.

Bacterial diseases

Molecular detection methods have been developed for the identification of different bacteria infecting molluscs. The aetiological agent of brown ring diseases can be identified using dot blot hybridisation and a species-specific primer (SSP-PCR) (Paillard et al 2001). This tool has been used to detect *V. tapetis* in larvae and juveniles from hatcheries, and in adults from cultured stocks and natural beds. Maloy et al (2005) have also developed a PCR technique used for identification of the aetiological agent of Juvenile Oyster Disease (JOD).

A PCR assay incorporating a lysozyme treatment after proteinase K digestion of tissue has been recently developed (Bower et al. 2005). This PCR technique uses two primers specific of *Nocardia crassostreae*. In this case, the PCR assay had similar sensitivity for detecting nocardiosis in *C. gigas* as histological examination of tissue sections stained with Gram stain. An alternate technique is to get a specific pattern after PCR-randomly amplified polymorphic DNA (RAPD) fingerprinting from DNA extracted from bacterial culture (Isik and Goodfellow 2002).

A PCR reaction using species specific primers (RA 5.1 and RA 3.6) has been developed to detect genomic DNA of a rickettsiales-like prokaryote associated with withering syndrome in Califonia abalone (Andree et al. 2000). An *in situ* hybridisation test using four probes designed from the small-subunit of ribosomal DNA has also been developed (Antonio et al. 2000).

Although PCR techniques are useful for molecular identification of specific bacterial pathogens associated with disease, bacterial strains belonging to the same species may possess different levels of virulence (i.e. *V. splendidus*). In this context, genotyping appears a suitable tool in order to associate a specific strain to a disease. DNA typing may be carried out using different techniques targeting, intergenic rDNA spacer regions, individual genes, a gene cluster and the whole genome. However, this approach can be completed by the screening of genes encoding virulence factors and the virulence characterisation based on experimental trials.

Viral diseases

To diagnose viral infections, the basic method for examination of suspect samples is still light microscopy. This method appears poorly adapted to viral diseases and needs to be improved upon by other techniques including transmission electron microscopy. These procedures are time consuming and inappropriate for large epidemiological surveys; In addition, some viruses can be difficult to detect and identify. As molluscs lack antibodies, serological methods can not be used for viral diagnosis. In addition, the diagnosis of viral diseases based on the detection of cytopathogenic effects in cell cultures cannot be carried out due to the absence of mollusc cell lines. Within this context, purification of viruses infecting molluscs appears as a way out from methodological deadlocks. For an example, extraction and sequencing of OsHV-1 DNA from purified particles infecting *C. gigas* larvae (Le Deuff and Renault 1999) rendered the development of molecular diagnosis tools possible.

A PCR-based procedure for detecting the herpesvirus that infects the Pacific oyster, *Crassostrea gigas*, in France was first developed (Renault *et al.*, 2000). Two primer primer pairs (A3/A4 and A5/A6) were designed to provide specific amplification products ranging in size 917 and 1001 bp, respectively when performed on OsHV-1 DNA (Renault *et al.*, 2000). No amplification was observed on oyster genomic DNA nor on the DNA from vertebrate herpesviruses. A quick and convenient sample preparation using ground tissues allowed a sensitive detection of OsHV-1 infected oysters. Several PCR diagnostic protocols have then been developed. Different methods of DNA extraction (Renault *et al*. 2000a, Arzul *et al*. 2002, Batista *et al*., 2005, Friedman *et al.* 2005) as well as various primer pairs (Renault *et al*. 2000, Arzul *et al*. 2001 a, b, c, Renault and Arzul, 2001, Barbosa-Solomieu *et al*. 2004, 2005) have been designed and used to detect viral DNA using single-round or nested PCR. Thus, PCR has been successfully used to detect viral DNA in various bivalve species. In this context, PCR is considered a suitable tool for the diagnosis of OsHV-1 infections due to pathogen specificity, extreme sensitivity, ease of sample processing, availability of reagents, time and cost efficiency. A competitive PCR method was also developed that can be used to demonstrate the presence of PCR inhibitors or to quantify OsHV-1 DNA (Renault *et al*., 2004). In addition, the PCR conditions used for detection of OsHV-1 can also allow the amplification of DNA from other close related herpesvirus as it was the case of a variant of OsHV-1 (Arzul *et al.* 2001b, c). Despite the various methods that have been developed for the diagnosis of bivalve herpesvirus by PCR, it is noteworthy that the specificity has been assessed using DNA from vertebrate herpesvirus since OsHV-1 is the only known herpesvirus infecting invertebrates (Davison et al 2005). Therefore, existing PCR assays may have a limited value assuming that there are probably much more invertebrate herpesviruses in the marine environment. The analysis of the same samples with different primer pairs can be used to evaluate the diagnostic specificity of the primer pairs.

Another technique that has also been developed is *in situ* hybridisation that allows the detection of viral DNA on histological sections (Renault and Lipart 1998, Lipart and Renault 2002, Barbosa-Solomieu *et al*. 2004).

A limit of detection of OsHV-1 has been defined using the nested PCR protocol (0.1 µg of viral genomic DNA per 50µl). PCR, ISH and antibody based detection (immunochemistry) were compared. The comparison between the three techniques indicates that PCR is the more sensitive method (Arzul et al. 2002). Moreover, a validation approach has been carried out for PCR and ISH during the course of a project funded by EU (VINO, FAIR-CT98-4334). The same biological material (reference material) has been used in four European laboratories.

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Annex 11: The Fish Disease Index (FDI): a tool for ecosystem health assessment

by T. Lang and W. Wosniok

(not to be cited without prior reference to the authors)

Abstract

The present document described progress made in the construction of a Fish Disease Index (FDI) and its application using empirical data on diseases of North Sea dab (*Limanda limanda*). The FDI serves as ecological health indicator and summarises information on the presence/absence and the severity grades of 9 externally visible diseases, including 3 parasitoses, into one numeric value for individual fish reflecting its health status. Further components in the calculation of the FDI are the assignment of disease-specific weighting factors and the use of adjustment factors for effects of length, sex and sampling season. A strategy for the use of the FDI approach for the assessment of changes in the health status of dab populations is proposed. The strategy is based on a regional approach and is derived from the analysis and classification of longer-term changes in the mean FDIs within a region and the application of a trend statistic. Assessment criteria are defined and illustrated as colour bands (green, yellow and red bands represent a good, moderate and bad health status, respectively) or as smiley faces depicted on a geographical map showing the regions assessed. The latter approach can be used to compare recent developments in the health status of dab populations between regions. The FDI and the assessment procedures are constructed in a way that the approach can be adapted to other fish species with other diseases, provided that sufficient data are available and that a disease-specific weighting can be done. It is concluded that the approach is a useful tool to monitor and asses ecosystem health and that it can thus be used for national and international monitoring and assessment programmes, such as the OSPAR CEMP and the revised HELCOM monitoring.

Introduction

At the 2006 meeting of the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) meeting, the construction of a Fish Disease Index (FDI) for dab (*Limanda limanda*) was presented and discussed, the purpose of which was to summarise data from fish disease surveys on the presence of a range of diseases (externally visible diseases and parasites, macroscopic liver neoplasms, liver histopathology) and their severity grades into one figure representing the health status of individual fish (ICES 2006).

The FDI developed for the 2006 WGPDMO meeting was not only based on data reflecting the presence/absence and the severity of the diseases, but also included disease-specific weighting factors assigned to each of the diseases according to their known or suspected effects on the well-being of the host. Furthermore, adjustment factors for host-specific effects of length and sex on the likelihood for a disease to occur were introduced, enabling a joint assessment of fish of all lengths and both sexes with no restrictions to a standard size group or one sex group.

The FDI developed for the 2006 WGPDMO meeting was only applied using a restricted set of empirical data on externally visible diseases and parasites derived from studies in dab (*Limanda limanda*) from the North Sea and adjacent waters. The 2006 WGPDMO Report provides examples for changes in the FDI over time recorded in a number of ICES statistical rectangles. Major findings were:

- host-bound effects of length and sex on the FDI are present;
- seasonal patterns exist in the FDI time series;
- long-term trends exist in the FDI time series;
- geographical regions exhibit different trends.

At the 2006 WGPDMO meeting, some suggestions were made regarding improvements of the FDI and it was decided to further elaborate the FDI by taking into account the suggestions made and by using an extended set of empirical data. It was further agreed to develop a robust and illustrative scheme as to how the FDI can be used for assessing changes in the health status of a fish population in a given region and for a comparison between regions. In the following, a report on progress made is provided.

Modifications in the construction of the Fish Disease Index: methods

Only data on externally visible diseases and parasites of North Sea dab (*Limanda limanda*) were used, most of which have been recommended for monitoring purposes (Bucke et al. 1996). Table 1 provides details on the diseases considered, the grading and the assignment of disease-specific weighting factors (see below).

Table 1: Diseases of North Sea dab (*Limanda limanda*), their grading and weighting factors assigned used for construction of the Fish Disease Index (FDI) (the disease-specific weighting factors are derived from an expert judgement, see further below in the text)

Disease/parasite	**Severity grading**	**Disease-specific weighting factor**
Lymphocystis	3 grades	1.99
Epidermal hyperplasia/papilloma	3 grades	1.98
Acute/healing skin ulcers	3 grades	2.12
X-cell gill disease	no grading	9.00
Hyperpigmentation	3 grades	1.99
Acute/healing fin rot/erosion	no grading	6.01
Stephanostomum baccatum	3 grades	1.00
Acanthochondria cornuta	3 grades	1.90
Lepeophtheirus pectoralis	3 grades	1.89

The FDI is composed of the sum of the single disease scores calculated for individual fish of a sample based on the presence/absence, the severity grades and the disease-specific weighting factors. It is scaled in a way that FDI values can range from 0 to 100, with low values representing healthy and high values representing diseased fish. The maximum value of 100 can only be reached in the (purely theoretical and unrealistic) case that a fish is affected by all 9 diseases/parasitoses at their highest severity grades.

The disease scores are composed of the product of a value representing the presence or absence of each of the diseases (values are either '1' or '0') multiplied by the severity grade (values can be '1', '2' or '3') multiplied by the disease-specific weighting factor (see Table 1). Furthermore, adjustment terms may be added, compensating for effects of the length and sex composition in the population and compensating for seasonal effects on the likelihood that a disease occurs (see below).

Adjustment for confounding factors

For comparison purposes, Fish Diseases Indices were calculated with and without adjustment for effects of length, sex and season (see below) . In both cases, the FDIs are calculated in a way that the maximum value is 100, requiring a data transformation.

Length and sex

From a large number of epidemiological studies there is clear evidence that length, age and sex are risk factors for the occurrence of many diseases. For instance, large (= old) fish are more frequently affected by tumours than small (= young) fish and male fish are often more frequently affected by infectious diseases than female fish of the same length. Hence, in populations dominated by old fish one would *a priori* expect a higher prevalence of, e.g., tumours than in populations dominated by young fish and, in populations dominated by male fish, a higher prevalence of certain infectious diseases would be expected compared to populations dominated by female fish. Since the length/age composition and/or the sex ratios of populations may differ between regions, the prevalence of diseases recorded in these regions cannot directly be compared if data from large and small and from female and male fish, respectively, are combined. Hence, previous analyses have often focused only on certain length groups of fish and only on female fish (e.g., for dab: females; 20-24 cm total length, see Wosniok et al. 1999, 2000a,b). The main disadvantage of this approach is that only parts of the data sets available are used for the analysis. By introducing adjustment terms for length (or/and age, if data are available) and sex, this disadvantage can be overcome and data from all fish can be combined, enabling the utilisation of the full set of data available.

Adjustment terms for the FDI have been calculated based on empirical data on the prevalence of each of the diseases by length group (cm total length) for male and female fish separately, as shown in Fig. 1 for the diseases lymphocystis, hyperpigmentation and infestation with *Stephanostomum baccatum* as examples. From the figure it can be seen that the relationship between the length and the sex of the fish with the prevalence may differ considerably between the diseases and between sexes. The adjustment term depends on the empirical relationship between disease prevalence and length. It is assigned a value of '1' for the length associated with the lowest prevalence and a value of '-1' for the length associated with the highest prevalence. It is '0' for the mean empirical prevalence. These three conditions establish mathematically a power relationship between adjustment term and prevalence:

$$\text{adjustment} = 1-2*((\text{prevalence}-\text{prevalence minimum})/(\text{prevalence range}))^c$$

This equation uses a smoothed form of the prevalence-length relationship obtained by a LOESS smoother, and the constant 'c' is chosen such that the adjustment term becomes '0' if the mean prevalence is entered on the right hand side. The right horizontal scales in Fig. 1 give numerical examples for adjustment terms. The adjustment term is further designed so that, even after adjustment, fish with disease grade 2 (or 3) still get a higher resulting score than fish with disease grade 1 (or 2), independent of their length. That means that the effect of length adjustment cannot exceed the effect of the basic grades. The scheme in Fig. 2 illustrates this approach.

Season

An adjustment term for season effects was not included in the FDI version presented to WGPDMO at its 2006 meeting (ICES 2006). However, its inclusion makes sense because (a) a season adjustment is done anyway when calculating trends (e.g., for the ICES disease maps, see at http://www.ices.dk/marineworld/fishdiseases/fishandshellfish.asp) and (b) possible season effects introduced through changes in the time of sampling are smoothened and misinterpretations are avoided. Season adjustment terms were calculated separately for each ICES statistical rectangle by fitting a regression model to the sex/length adjusted FDI time series in each rectangle, where year and season (3 classes) served as explaining factors. Estimated season effects were then subtracted from the input time series, resulting in an FDI series adjusted for sex, length and season effects. Fig. 3 shows a series of non-adjusted FDI time series and their adjusted counterparts.

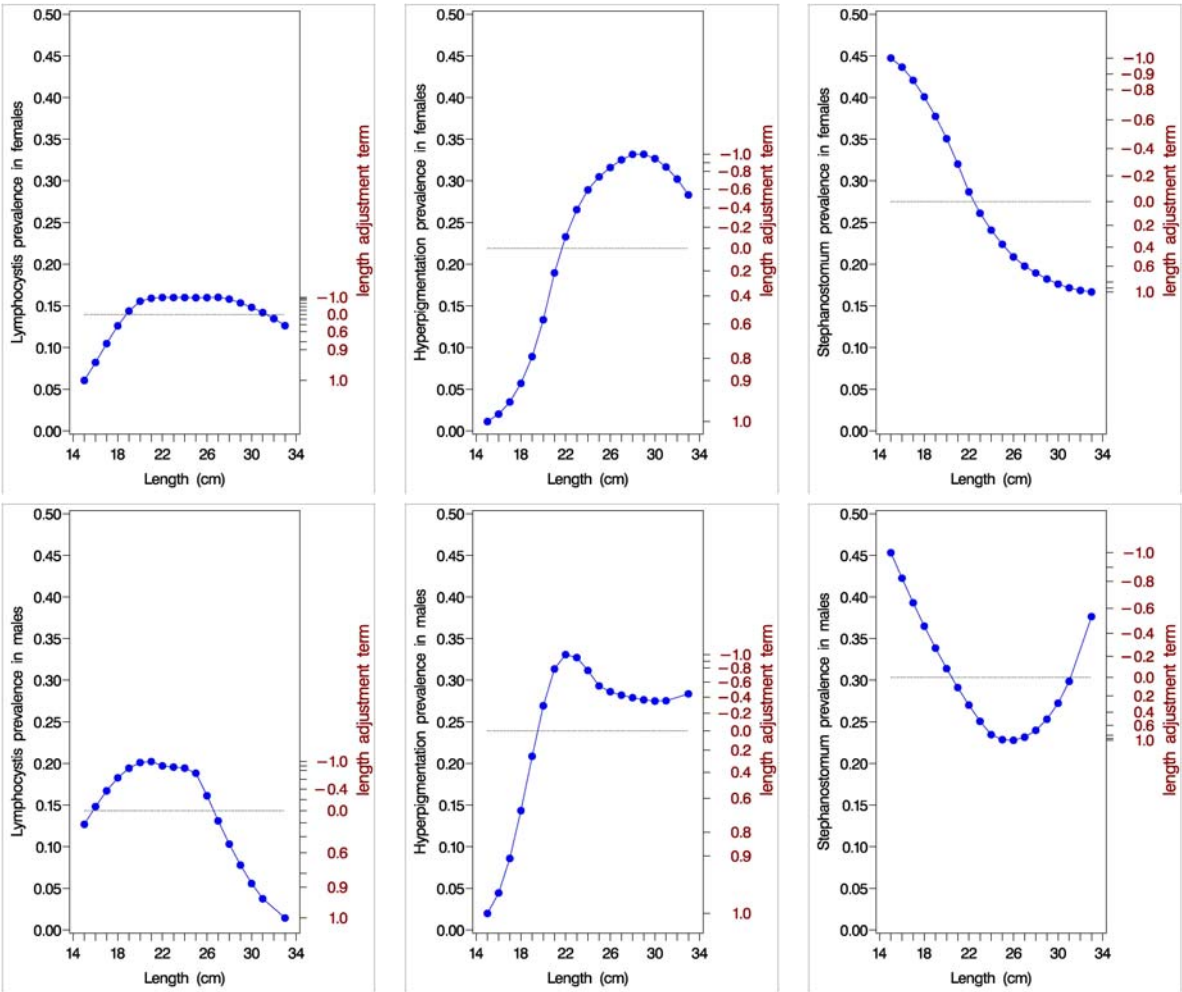


Fig. 1: Disease prevalence in *Limanda limanda,* by length and sex (left axis), and resulting length adjustment terms for the Fish Disease Index (right axis). The horizontal line shows the mean prevalence. Prevalences are smoothed values obtained from North Sea data collected in 1989–2005 as part of the German fish disease monitoring programme.

Introduction of disease-specific weighting factors

The assignment of disease-specific weighting factors is critical because the decision regarding the ranking of the diseases based on their importance for the well-being of the host and the resulting assignment of weighting factors have a major impact on the final FDIs. For instance, if a single disease known to increase (or decrease) in prevalence over time is assigned a particularly high weighting factor, the resulting FDI might be dominated by the disease and will very likely increase (or decrease), especially if only few diseases were taken into account or only few other diseases are present.

In order to reach a broad consensus on the weighting factors, the assignment of weighting factors was done on a basis of expert judgement, where the Bradley-Terry approach (Bradley and Terry 1952; Rao and Kupper 1967, Agresti 2002) was used to derive a joint assessment from individual statements. Independent expert judgements were provided by selected WGPDMO members and other specialists with a background in disease monitoring in dab or other flatfish with similar diseases (S.W. Feist, G.D. Stentiford, J.P. Bignell (all Cefas, UK), D. Bruno (FRS, UK), T. Lang (BFAFi, Germany), A.D. Vethaak (RIKZ, The Netherlands).

The experts had been asked to assess for each pair of diseases the relative severity of the pair's members. Possible statements were (i) "first disease more severe than the second", (ii) "second disease more severe than the first", (iii) "both diseases are similarly severe" and (iv) "cannot give an assessment". These statements served as input for the Bradley-Terry approach to compute the disease-specific given in Table 1. The rationale behind the Bradley-Terry approach is to compute weights w_i, ($i = 1, 2, \ldots, I$, index for disease) from the expert input such that the input assessments can be reproduced by comparing the weights: $w_i > w_j$ corresponds to the expert statement "disease i is more severe than disease j". If the input (the expert assessments) contains inconsistencies or contradictions, no set of weights can reproduce the input perfectly, however, the Bradley-Terry approach finds a set of w_i that generates the best possible approximation to the expert assessment. Table 1 contains the weights obtained by the Bradley-Terry approach, scaled to the range 1 – 9 to secure comparability with earlier reports.

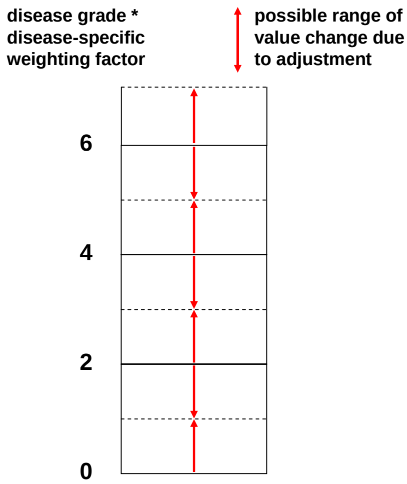


Fig. 2: Scheme illustrating the extent to which the adjustment influences the final disease scores.

Methods for assessment

The FDI is meant to be used for the assessment of disease patterns on a regional basis, where, e.g., ICES statistical units or a union of these can serve as geographical units. The outcome of the assessment is a classification of the disease situation in the region into one of three categories (low, medium, high). These categories can easily be communicated by using colours (green/yellow/red) and/or symbols (up/down arrows, smiling/frowning faces). For the FDI approach chosen, the boundaries which divide the range of FDI values into categories are defined on the basis of FDIs previously observed in the area. The boundaries divide the empirical range of FDIs into 3 subdivisions such that each subdivision contains on third of all observations. Values in the lower third are desirable (and achievable) values in that area, values in the upper third give reason for concern and further inspection, values in the middle third have no clear characterization (see Fig. 4). This approach can be used to assess changes in an area on a longer-term basis based on absolute FDI levels.

However, as FDI values may exhibit considerable random fluctuation even after the adjustments described above, the joint consideration of not only absolute FDI levels, but also of FDI trends is advisable in an assessment. This approach is considered particularly useful to assess recent changes in FDI patterns, For that purpose a joint test statistic is proposed, calculated in the following way, separately for each region:

- define a starting time for "recent" trends (e.g., 01 January, 2000)
- count the number of FDI means in each of the three ranges
- weight these counts such that higher ranges get a higher weight (e.g. '1' for the upper third, '0' for the middle third, '-1' for the lower third)
- for the same FDI means, calculate the Mann-Kendall trend test statistic (high values indicate an increasing trend, low (negative) values indicate a decreasing trend
- add up both previous terms to obtain the assessment statistic.

This test statistic has negative values for a decreasing trend and high positive values for an increasing trend. If the same increasing trend took place in the range of already high FDI values, the test statistic is larger than if the same trend occurred in the low FDI range. In this way it would be clearly signalled if two adverse conditions, high FD plus increasing trend, would occur jointly. To test if this assessment statistic has a value compatible with the null hypothesis of no trend, but only random fluctuation around the mean FDI, the statistical distribution of the statistic is determined by simulation. From this, the summarizing assessment is done as follows:

- If the observed test statistic is smaller than the 2.5% quantile of the simulated distribution, then this is considered to signal a desirable FDI level and trend ('green smiling face'),
- if the observed test statistic is larger than the 97.5% quantile of the simulated distribution, then this is considered to signal an undesirable FDI level and trend ('red frowning face'),
- in all other cases the test statistic is interpreted as giving an indifferent signal ('yellow neutral face').

This smiley approach generates information not only on the current disease level in a region compared to the long-term status, but also on recent changes (= trends). For instance, in two hypothetical areas were the majority of FDIs has been in the green range within the past 5 years, the area that does exhibit a statistically significant upward trend will be assigned a more 'negative' smiley than the area that does not exhibit a trend, because the upward trend (even if it is in the green range) is causing the alarm bell to ring.

Analysis of empirical data: results and assessment

Fish Disease Index values and assessment quantities were calculated according to the methods above for data on diseases of dab (*Limanda limanda*), obtained by surveys in the North Sea in the period 1990 to 2005. The data were provided by the German Federal Research Centre for Fisheries. Only data on externally visible diseases/parasitoses in dab were used, derived from the German fish disease monitoring programme. Data from the ICES fish disease database could not be used because to date they do not contain information on severity grades. The set of externally visible diseases and parasites considered was the one described in Table 1. Only data from the area spanned by the ICES rectangles 33E7 to 46F8 was involved, and only male and female dab with length between 15 and 33 cm (about 150,000 individuals) were included in the analysis. Adjustment terms for sex and length were calculated for each disease (irrespective of grade) from the pooled data of this area. Adjustment terms for seasonal effects were derived by a regression approach as described above.

Calculation of regional Fish Disease Indices

Fig. 3 shows the mean non-adjusted and adjusted FDIs calculated for dab from 11 ICES statistical rectangles fulfilling the data requirements defined (> 10 observations in the period

1990-2005). Although there are only slight differences between the figures, the adjustment clearly leads to a smoothening of the curves, making the general trends more visible. In most areas, the FDIs increased, with the exception of rectangle 35F3 in the southern North Sea.

Application of assessment criteria

Results are shown in Fig. 4 for the 11 ICES statistical rectangles already illustrated in Fig. 3. The data shown clearly indicate the general differences in the FDIs for the areas depicted. For instance, the FDIs have always been higher in rectangles 36F1 and 38F2 compared to, e.g., rectangles 35F3 and 40F7. Since the colour bands indicating low, intermediate and high FDIs were calculated for each rectangle separately, this leads to differences between areas in the threshold values calculated for the colour bands. From the figure there is evidence for an increase in FDIs in most areas, and recent FDIs are mostly located in the red areas, indicating high FDIs compared to the long-term values.

The application of the ‘smiley approach’ enables an assessment of the most recent trends over the past 5 years for each area and also a comparison of the recent trends between areas. As can be seen from Fig. 4, the FDI values as well as the trends mostly resulted in red (5 cases) or yellow (5 cases) smilies, except for a green smiley in area 42F3, indicating that the current health status of the dab in most areas has to be regarded as only moderate or even as poor. Figure 5 provides the results of the assessment in a map.

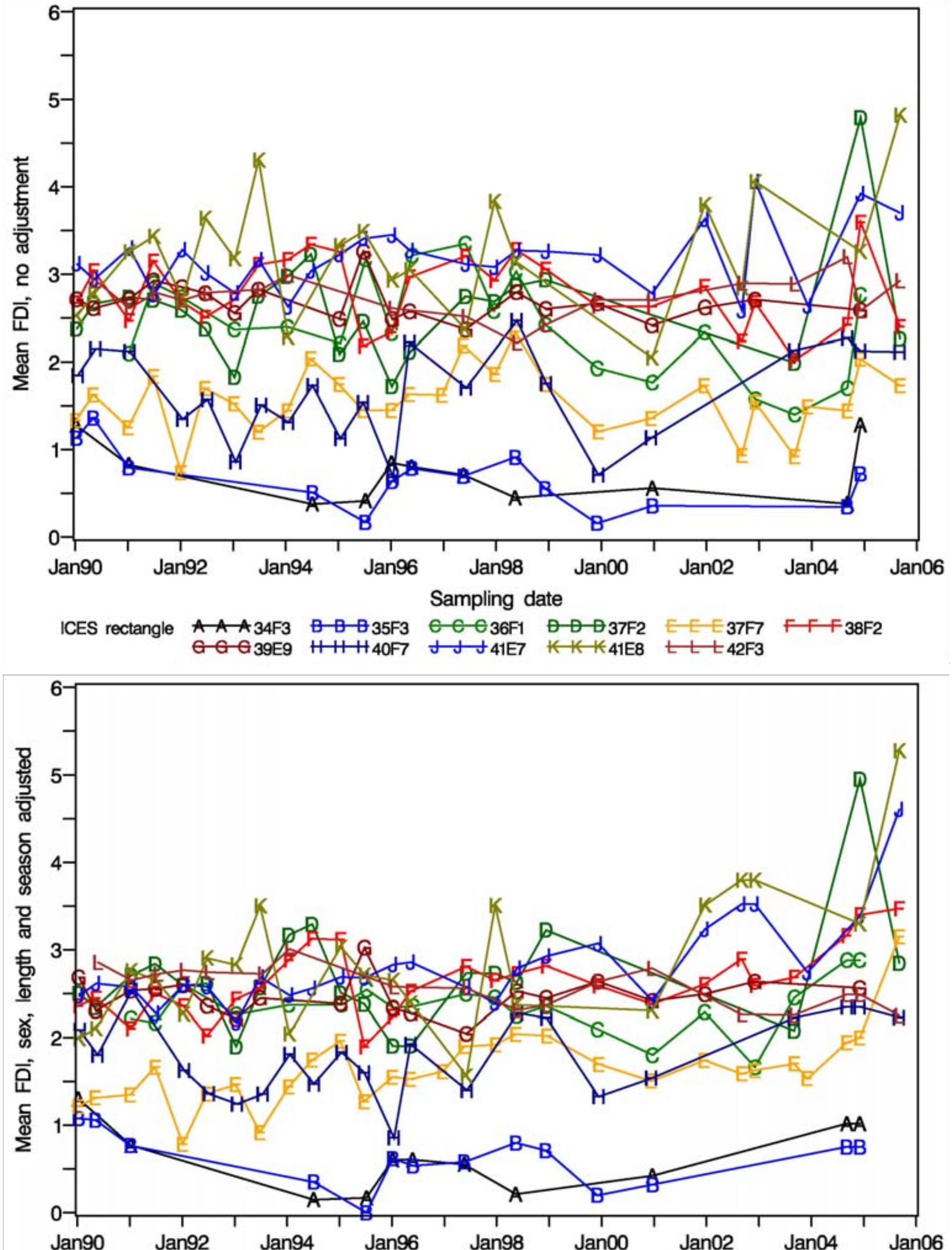
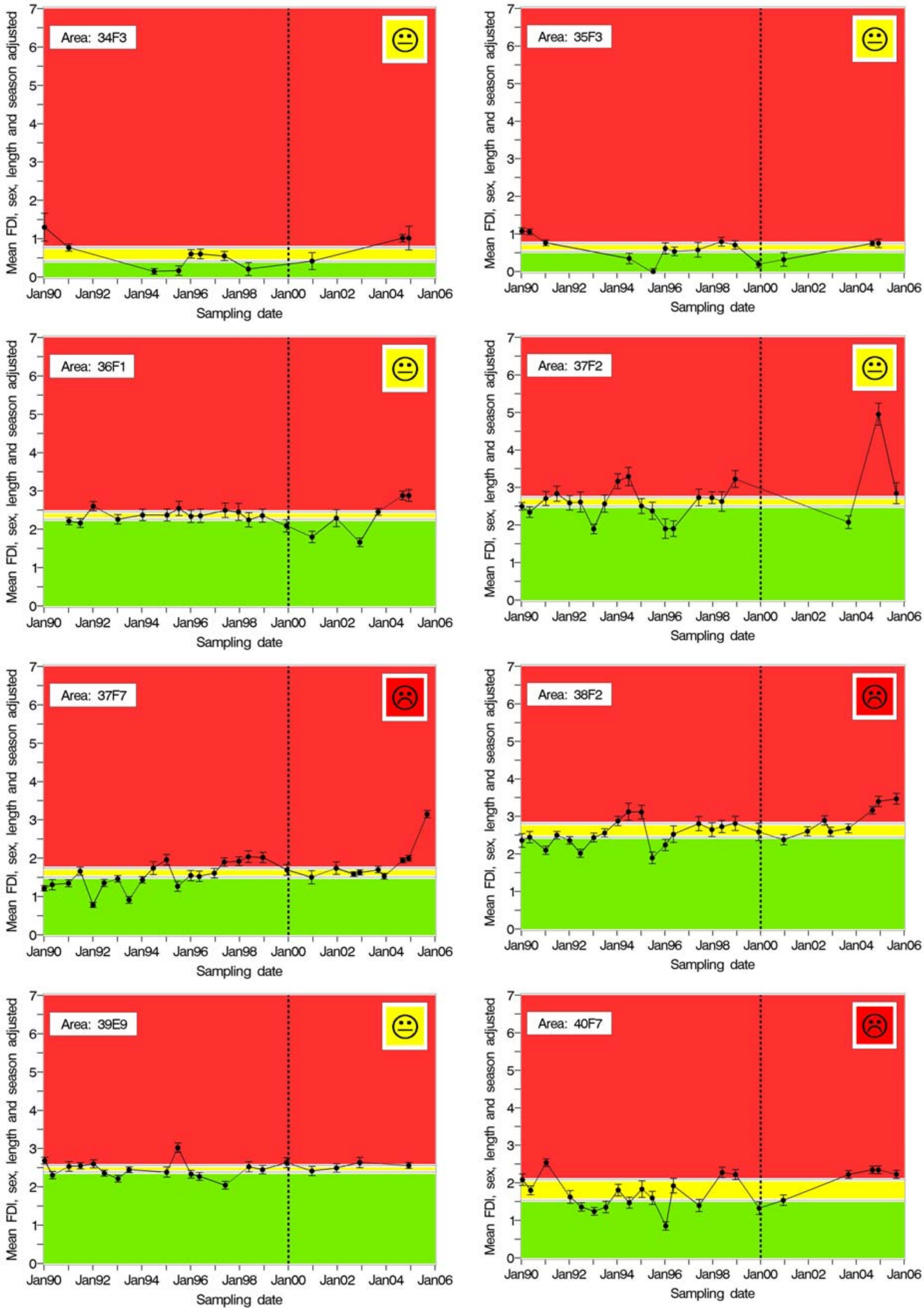


Fig. 3: Mean Fish Disease Index (FDI) in North Sea dab (*Limanda limanda*), size range 15-33 cm total length, females and males combined, from 11 ICES statistical rectangles (top figure: without adjustment for length, sex and season; bottom figure: with adjustment for length, sex and season


Area: 34F3
Area: 35F3
Area: 36F1
Area: 37F2
Area: 37F7
Area: 38F2
Area: 39E9
Area: 40F7
Mean FDI, sex, length and season adjusted
Sampling date
Jan90 Jan92 Jan94 Jan96 Jan98 Jan00 Jan02 Jan04 Jan06
0 1 2 3 4 5 6 7

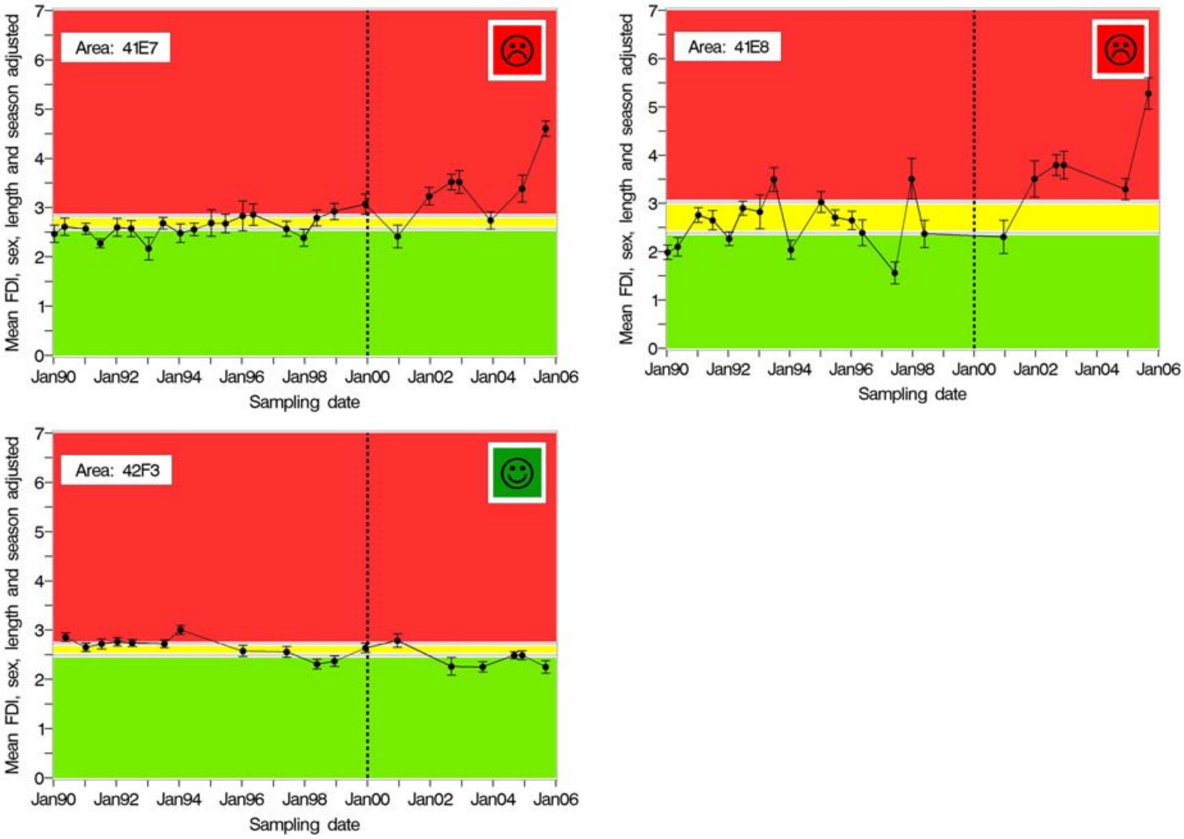


Fig. 4: Assessment of changes in the FDIs in dab (*Limanda limanda*) from different ICES statistical rectangles. The green, yellow and red colour bands represent levels of low, moderate and high FDIs, the vertical dashed line denotes the year 2000 from which onward recent FDI values and trends were assessed by using the 'smiley approach'

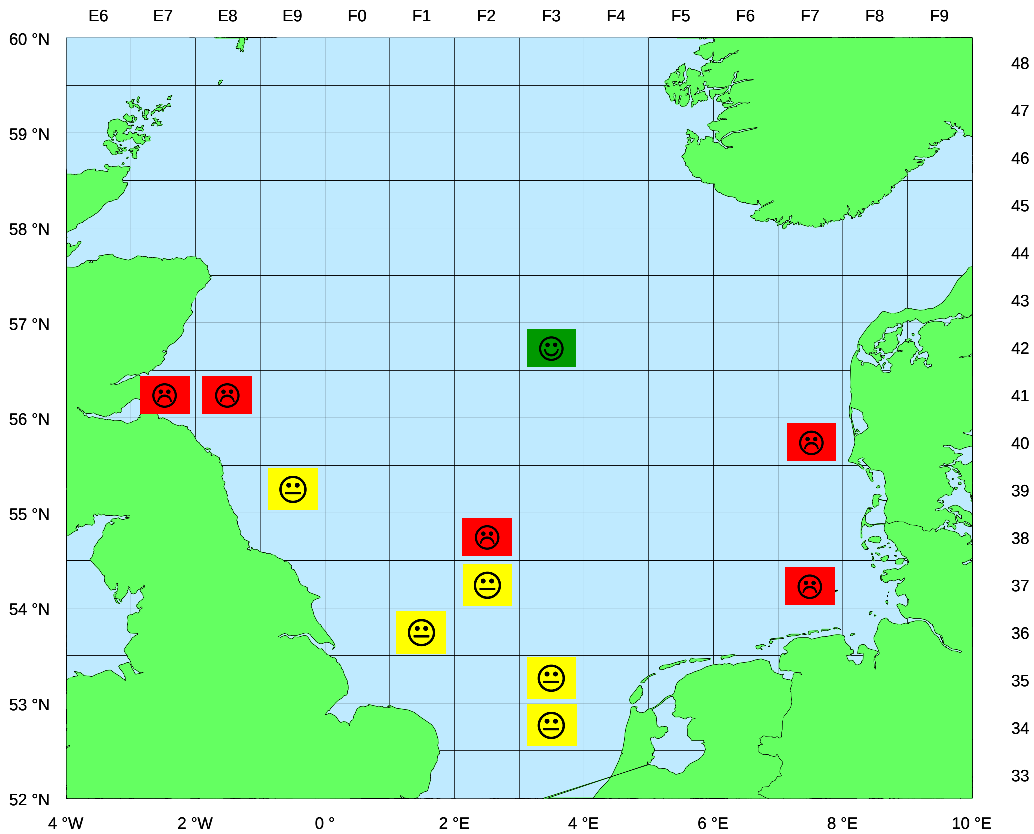


Fig. 5: Representation of recent Fish Disease Index trends in North Sea dab (*Limanda limanda*) by smiley symbols. Green, yellow and red symbols indicate a good, moderate or bad health status, resp., since January 1, 2000. The FDI trend is assessed with respect to trend direction and also to FDI level (see text).

Discussion and conclusions

The Fish Disease Index serves to summarize the presence/absence and the severity of the disease status of single fish specimen and of a whole fish population. By focussing on the severity of the diseases, the FDI differs from standard multivariate summaries like Principal Components Analysis and other ordination techniques, which aim at delivering the best possible summary of variation in the data. However, explaining variation of the disease situation and describing its severity are different targets that should not be confused.

By applying the FDI to the data set on diseases of North Sea dab, the following features were observed:

- long-term temporal trends in the FDI exist,
- seasonal patterns in the FDI exist,
- the total level of the FDI, the long-term trend and seasonal pattern depend on area,.
- after adjustment for sex, length and season, similar shapes in many of the FDI time series were apparent, however, with level shifts against another,
- the FDI assessment statistic shows significant developments (beyond random fluctuation) of the FDI in recent years. In 5 of 11 ICES statistical rectangles that qualified for assessment, there was a development in an undesirable direction reflecting a poor health status giving reason for concern..

The results show that summarizing the originally multivariate disease information by the FDI does not simply lead to a purely randomly varying quantity, but that instead changes in the disease status are in fact reflected by the FDI.

The FDI in its present form adjusts for the confounding effects of the sex proportion, the length distribution and of the season of catch. All these conditions might otherwise cause a variation of the FDI over time as a consequence of random sampling variation. If in the future further factors will be identified as confounders, these also could be adjusted for by the same procedures used so far for sex, length or season.

For the assessment, an important component of the FDI is the assignment of weights to the diseases. This is done by expert judgement, where for the example given here 6 experts was involved. An expert judgement can be done by applying the Bradley-Terry technique, which requires the experts to compare diseases pairwise, where not even all possible pairs need to be compared. From these pairwise statements, the Bradley-Terry approach constructs a scale of severity that in the best possible way reflects the expert's judgments, and which would serve as the then consolidated set of disease weights in the FDI calculation.

The construction principle of the FDI and the associated assessment procedure can easily be transferred to other assessment tasks (e.g., other fish species, other diseases), as long as sufficient data are available and an equivalent to the disease weight can be provided. Therefore, the FDI approach is applicable for wider geographical areas, e.g. as part of the convention-wide OSPAR monitoring and assessment, or in other seas such as the Baltic Sea.

A major advantage of the FDI is that it does not require a "background area" from which background values had to be derived. For many problems the search for a pristine area without human impact, which could provide background values, has turned out to be unsuccessful. Hence, in such cases, a background based assessment is impossible. The FDI approach is applicable, given that there is either long-term data from the study area to establish the assessment ranges, or if information from a similar area is available. For the case of the North Sea, assessment ranges on the basis of all North Sea data pooled could serve as the basis for the assessment of previously not examined areas (e.g., for exploratory monitoring and assessment).

It is concluded that the FDI approach is a useful tool for monitoring and assessment of the health status of fish populations. Since the FDI has been designed based on diseases of dab and since assessment criteria have been defined, it is considered to be directly applicable for the fish disease monitoring component of the OSPAR Co-ordinated Monitoring Programme (CEMP). Since the FDI is constructed in a way that it can be adjusted to other fish species and other diseases, it is anticipated that the FDI approach can eventually be used on a convention-wide basis, provided that sufficient disease data are available. The FDI furthermore offers potential as ecological indicator for the revised HELCOM monitoring programme in the Baltic Sea and the associated Baltic Sea Action Plan, with its ecological objective 'healthy wildlife'.

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Annex 12: Recommendations

General Recommendations

REPORT SECTION	RECOMMENDATION	ACTION
5.1	1. ICES Member Countries continue to fund fish disease monitoring programmes to sustain fish health surveillance of wild stocks. Information obtained is of vital importance to integrated assessments of the health of marine ecosystems and will provide baseline data, e.g., to serve as a reference prior to establishing the culture of non-salmonid marine species. In addition, fish disease monitoring data will be useful in evaluating the effects of climate change on fish health and provide better understanding pathogen interactions between wild and farmed fish.	ICES Member Countries
5.2	2. ICES Member Countries conduct studies on *Francisella* sp and visceral granulomatosis in farmed cod and review the potential disease interaction between farmed and wild cod.	ICES Member Countries
5.3	3. ICES Member Countries conduct further studies on the picorna-like virus in the clam *Ruditapes decussatus*, on *Candidatus xenohaliotis californiensis* in European abalone, *Haliotis tuberculata*, and on gill epithelia nuclear virus in soft-clam *Mya arenaria*.	ICES Member Countries
5.3	4. Electron microscopy studies should be conducted on gonadal tissue lesions in *Crassostrea gigas* from Germany.	ICES Member Countries
6	5. Bacteriological, virological and electon microscopy studies should be conducted on dab (*Limanda limanda*) affected by hyperpigmentation, and literature on malpigmentation in farmed fish be reviewed.	WGPDMO members
7	6. A review of the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants be conducted.	WGPDMO members
7	7. Updates on salmon louse vaccine development and sea lice management strategies made in ICES Member Countries be reviewed in national reports at the WGPDMO 2008 meeting.	WGPDMO members
8	8. Baltic Sea fish disease monitoring data be submitted to the ICES Environmental Databank in order to make them available for integrated assessments, such as those carried out by the ICES Working Group on Integrated Assessment of the Baltic Sea (WGIAB) and as part of the periodic HELCOM assessments.	ICES Member Countries

8	9. A workshop on methodologies for monitoring fish diseases/parasites in coastal fish species from the Baltic Sea be organised in 2007 (or in 2008, depending on funding requirements) under the co-sponsorship of ICES, HELCOM and the BSRP, at the BSRP Lead Laboratory for fish disease issues, AtlantNIRO, Kaliningrad, Russia.	ICES Member Countries
8	10. Laboratories conducting fish disease monitoring programmes participate in the BEQUALM programme in order to achieve implementation of quality assurance procedures needed for acceptance of data for international monitoring and assessment programmes.	ICES Member Countries
9	11. Laboratories involved in research on pathogens of molluscs initiate collaborative testing, intercalibration, and validation of current and newly developed techniques for the purpose of recommending molecular diagnostic techniques.	ICES Member Countries
10	12. The FDI be applied to disease data sets for other geographical areas and species, e.g., Baltic cod and flounder.	WGPDMO Members
10	13. Results of the FDI development and analysis on dab be published.	WGPDMO Members
12	14. ICES Member Countries submit their fish disease data generated according to established ICES/BEQUALM guidelines as soon as possible to the ICES Environmental Databank by using the ICES Environmental Data Reporting Format (current version 3.2).	ICES Member Countries
12	15. ICES provides a screening programme on its website to be used by labs to check their fish disease data sets converted into the ICES Environmental Data Reporting Format (current version 3.2) before submission to ICES.	ICES Secretariat
12	16. The fish disease data submitted to ICES be used by OSPAR for assessments made in preparation of the 2010 OSPAR 2010 Quality Status Report (QSR 2010).	OSPAR

Annex 13: WGPDMO terms of reference for the 2008 meeting

The **Working Group on Pathology and Diseases of Marine Organisms** (Chair: S. MacLean, USA) will meet in St. Johns, Canada, 4–8 March 2008 to:

a) Produce a report on new disease trends in wild and cultured fish, molluscs and crustaceans based on national reports.

b) Review the status of proliferative kidney disease (PKD) epidemics caused by *Tetracapsuloides bryosalmonae* in wild salmonid populations.

c) Review the information on *Francisella* sp. and visceral granulomatosis in farmed cod and the potential for disease interaction between wild and farmed cod.

d) Provide a progress report on studies carried out on hyperpigmentation in common dab (*Limanda limanda*) from the North Sea with special reference to pathological findings and possible causes.

e) Review the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants.

f) Provide an update of international collaborative actions involving fish and shellfish disease and pathology activities.

g) Provide an update on the validation and integration of molecular diagnostic and confirmatory techniques for pathogens of bivalves.

h) Provide update on the use of the fish disease index for other fish species (e.g., Baltic cod and flounder) and other sets of available disease data (e.g., liver histopathology data).

i) Provide an update on the status of ICES publications on pathology and diseases of marine organisms.

j) Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis.

WGPDMO will report by 20 April 2008 for the attention of the Mariculture Committee.

Supporting information

PRIORITY:	WGPDMO is of fundamental importance to the ICES science and advisory process.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	**Term of Reference 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. (all WGPDMO members) **Term of Reference b)** Epidemics of proliferative kidney disease with mass mortalities in wild salmonids are a major concern as a potential threat to the sustainability of anadromous populations. Information on the extent of fish mortalities, distribution of bryozoan hosts harbouring the infectious stage of the parasite, identification of salmonid populations at risk and improved understanding of the role of environmental factors, particularly temperature on the severity of epidemics, is urgently required. (T.A. Mo, S. Jones, and S. Feist) **Term of Reference c)** A systemic granulomatous disease in Atlantic cod (*Gadus morhua*) related to the presence of *Francisella* sp. has emerged and currently is one of the main bacterial diseases for farmed stock in Norway. This intracellular Gram negative bacterium has also been isolated from wild cod in Sweden. It is recommended that WPGDMO review the current status on control and diagnosis in farmed cod, the available literature regarding the potential for interaction between wild and farmed cod, and information on a visceral granulomatous condition in wild cod attributed to *Francisella* infection (T.A. Mo, A. Alfjorden, and D. Bruno) **Term of Reference d)** Hyperpigmentation has continued to increase in the common dab (*Limanda limanda*) populations in the North Sea. At the 2007 WGPDMO meeting a report was reviewed providing information on spatial and temporal trends in

	prevalence in North Sea areas, on histological and ultrastructural features of the condition, on host-specific effects on the prevalence and on effects of hyperpigmentation on the condition of the host. There still is a need for more information on pathology associated with hyperpigmentation, on possible causes of the condition (e.g., if pathogens are involved) and on similar conditions involving pigmentation anomalies in wild and farmed fish. Since more in depth studies on these issues are carried out at present, the results of which will be available for the next WGPDMO meeting, it is recommended to revisit the issue of hyperpigmentation at the 2008 WGPDMO meeting. (T. Lang, S.W. Feist, D. Bruno, W. Wosniok, and N. Ruane) **Term of Reference e)** The extensive use of chemotherapeutic agents against parasitic copepods in marine reared fish has led to a loss of susceptibility of sea lice to some of these agents. The limited availability of alternative medicines has raised a concern regarding the inevitability of increased tolerance. (S. Jones, T.A. Mo, and N. Ruane) **Term of Reference f)** WGPDMO is working collaboratively with other ICES and non-ICES groups in the field of diseases and pathology of marine organisms. It is always critical to keep WGPDMO members aware and able to review such international activities and report back to ICES. (T. Lang, S.W. Feist, N. Ruane, and A. Mansour) **Term of Reference g)** The effective control of pathogens infecting molluscs requires diagnostic tests that are specific, reliable and sensitive, and that can discriminate between genera and species. Several methods are used to identify and characterise molluscan pathogens, among them, newly developed molecular methods appear very useful. Due to their specificity and sensitivity they allow species and strain identification. International standards proposed by the OIE now include such molecular techniques. Criteria used to identify molluscan pathogens should, however, include basic biological and ecological characteristics of pathogens as well as information on their genetic sequence. Thus, schemes for differential diagnosis incorporating molecular techniques have been developed. (T. Renault and S. Ford). **Term of Reference h)** For the 2007 meeting, the WGPDMO produced a report on the application of the Fish Disease Index (FDI) on externally visible diseases in common dab (*Limanda limanda*) and on the assessment of geographical areas by FDI-based criteria. It was recommended to extend this assessment to other species (e.g. Baltic cod and flounder) and to other sets of diseases (e.g. liver histopathology), for which empirical data is available, in order to assess the applicability of the FDI under more general conditions. (W. Wosniok and T. Lang). **Term of Reference i)** 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. It will be necessary to consider ways by which these can be linked to each other. New publications have to be considered. (S. Feist, T. Lang, and W. Wosniok) **Term of Reference j)** This is in compliance with a request from the ICES Data Centre. (W. Wosniok, T. Lang, S.W. Feist, D. Bruno)

RESOURCE REQUIREMENTS:	None required, other than those provided by the host institute.
PARTICIPANTS:	Representatives of all Member Countries and specialists invited by the Chair with expertise relevant to pathology and disease of wild and cultured finfish and shellfish. In total, normally 20 participants
SECRETARIAT FACILITIES:	Required to a limited extent, e.g. for data and publication issues
FINANCIAL:	None required
LINKAGES TO ADVISORY COMMITTEES:	There is a close link to ACME activities.
LINKAGES TO OTHER COMMITTEES OR GROUPS:	MCC, MHC, DFC, WGBEC
LINKAGES TO OTHER ORGANISATIONS:	BEQUALM, OIE, EU