# ICES WGPDMO Report 2006

**ICES Mariculture Committee**
**ICES CM 2006/MCC:01**
**Ref. ACME, MHC**

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

## 7–11 March 2006

## ICES Headquarters, Copenhagen

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

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# Contents

## Executive summary

The ICES Working Group on Pathology and Diseases (WGPDMO) met 7–11 March 2006 at ICES Headquarters, Copenhagen, Denmark. The meeting was well attended with 21 participants representing 12 ICES Member Countries and Lithuania and was chaired by T. Lang (Germany). In order to consider all 12 Terms of Reference in an appropriate way, considerable intersessional work had been carried out by WGPDMO members and a large number of working documents had been provided in advance of the meeting.

The agenda items covered a wide range of topics related to diseases and pathology in wild and farmed finfish and shellfish, with a focus on environmental and mariculture aspects.

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)*;
- an update of information on the causes and effects of Heart and Skeletal Muscle Inflammation (HSMI), a 'new' disease of concern (likely caused by virus) affecting farmed Atlantic salmon (*Salmo salar*) in ICES Member Countries (*ToR b, Report Section 6*);
- a report on the development of vaccines against sea lice and related management measures (*ToR c,Report Section 7*);
- a review paper on effects of climate change on the health status of marine fish and shellfish that will be further elaborated intersessionally in order to be published (*ToR d, Report Section 8*);
- a review of progress made with regard to major international collaborative actions including disease and pathology aspects (Baltic Sea Regional Project, BSRP; ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea, WKFDM; REGNS Integrated North Sea Assessment; Biological Effects Quality Assurance in Monitoring Programmes, BEQUALM) (*ToR h, Report Section 9, Annex 6*);
- two sections specifically dealing with farmed bivalves (diagnostic techniques for the identification and characterisation of microcell parasites in oysters; status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries) (*ToRs e and f, Report Sections 10 and 11, Annexes 7 and 8*);
- results of a pilot study assessing the feasibility of constructing a 'fish disease index' to be used as a tool in ecosystem health assessments using disease data from North Sea dab (*Limanda limanda*) (*ToR g, Report Section 12, Annex 9*);
- intersessional input to the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON II) (*ToR i, Report Section 13*);
- a progress report on ICES publications on pathology and diseases of marine organisms (disease report on the ICES website, ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, publications in the ICES Techniques in Marine Environmental Sciences Series, TIMES) (*ToR l, Report Section 14, Annex 10*);
- Guidelines for submission of disease data to the ICES Data Centre using the Environmental Data Reporting Format 3.2 (*ToR j, Report Section 15*);
- Suggestions for contributions on the health status of North Sea and of Baltic Sea fish to the ICES ecosystem overview advisory report (*ToR k, Report Section 16*).

WGPDMO concluded that all Terms of Reference for the 2006 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 2007. The meeting will be held either in Tenerife, Spain, or St. John's, Canada. The dates still have to be fixed. The WGPDMO nominated Sharon MacLean (USA) as new Chair.

# 1 Opening and structure of the meeting

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the ICES Headquarters, Copenhagen, Denmark, with T. Lang (Germany) as Chair. The meeting was opened at 10:00 hrs on Tuesday, 7 March 2006, with the Chair welcoming the participants, particularly the new members and guests who have not previously attended WGPDMO meetings. In total, 21 participants attended the meeting, representing 12 ICES Member Countries plus Lithuania. A list of participants is appended in Annex 1.

Apologies were received from C. Couillard and A.-M. MacKinnon (Canada), K. Lotman (Estonia), M. Vigneulle (France), K. Broeg and A. Köhler (Germany), S. Helgason and J. Palsson (Iceland), F. Geoghegan (Ireland), O. Haenen (The Netherlands), M. Wolowicz (Poland), G. Vialova (Russia), A. Figueras (Spain), A. Hellström (Sweden) and P. Fair (USA).

V. Piil welcomed the participants on behalf of ICES and provided instructions on in-house facilities and meeting arrangements. Later during the day, the WGPDMO was welcomed by the new ICES General Secretary, G. Hubold, and by the Head of the ICES Science Programme, A. Kellermann. The Chair thanked the General Secretary for inviting WGPDMO to Copenhagen and for providing excellent facilities.

The Chair informed WGPDMO that his term of office as Chair is due to expire this year (after four meetings) and the WG should nominate a successor to be approved by WGPDMO's parent committee, the ICES Mariculture Committee.

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 took note of the Terms of Reference published as C. Res. 2005/2/MCC01 (Annex 2). The Chair informed the WGPDMO that three new ToRs had been added to the list by ICES (ToRs j, k, l) and that, for compensation, two of the original ToRs recommended by WGPDMO at its 2005 meeting were deleted from the list (see Section 3.2).

The agenda once again had demanded extensive intersessional work by the members of the WGPDMO who had been requested to produce written working documents to be reviewed at the meeting and to be included in the WGPDMO report as Annexes, as appropriate. As agreed in WGPDMO, 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. The Chair thanked the members for preparing these reports in advance, a task that ensured that the Terms of Reference could be treated efficiently. A list of working documents considered is provided 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 2005 and 2006

The 2005 ICES Annual Science Conference (ASC) and Statutory Meeting took place in Aberdeen, UK, 18–27 September 2005. Except for the WGPDMO Chair, none of the WGPDMO members took part. The ASC was organised in the form of Theme Sessions on various marine research topics that were held concurrently and was, as part of the ICES Statutory Meeting, preceded by two days of business sessions, e.g. of the three ICES Advisory Committees and the ICES Science Committees, including the ICES Mariculture Committee (the parent Committee for WGPDMO).

In total, 18 Theme Sessions were held at the 2005 ASC. However, none of them was dedicated to disease/pathology or mariculture issues. The only Theme Session with some relevance to the work of WGPDMO was:

- **Theme Session S:** Oil Spills in Marine Ecosystems: Impacts and Remediation (Conveners: Joan Albaigés (Spain) and Kenneth Lee (Canada)).

The 2006 ICES ASC will be 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. According to the present planning, there will again be 18 Theme Sessions, three of which are of particular relevance to WGPDMO's activities:

- **Theme Session C:** Climatic variability in the ICES area – 2000–2005 in relation to previous decades: physical and biological consequences (Conveners: A. Lavin (Spain) and C. Reid (UK))
- **Theme Session G:** Human health risks and marine environmental quality (Conveners: A. D. Vethaak (The Netherlands) and T. Lang (Germany))
- **Theme Session P:** Integrated assessments in support of regional seas ecosystem advice – beyond quality status reporting (Conveners: A. Kenny (UK), B. Turrell, (UK) and K. Brander (GLOBEC, ICES))

The WGPDMO Chair encouraged the WGPDMO members to try to attend these Theme Sessions and to think of contributions from their field of expertise (papers, posters, oral presentations). He emphasised that the consideration of pathology/disease aspects is of relevance for the Theme Sessions. Furthermore, the participation in the ASC 2006 offers a good opportunity to raise the profile of the work carried out in WGPDMO in the ICES community. He informed WGPDMO that the deadline for submitting titles and abstracts for the 2006 ASC to the ICES Secretariat is 24 April 2006.

The Chair further informed WGPDMO that he, at the 2005 ASC, proposed to hold a disease-related Theme Session at the 2008 ICES ASC (probably to be held in Canada) entitled 'New disease trends in marine organisms: causes and effects'. Preliminary suggestions for conveners were T. Lang (Germany) and G. Olivier (Canada). The Chair, however, emphasised that the new WGPDMO Chair should act as co-convener, either in addition or as replacement.

## 3.2 ICES Mariculture Committee (MCC)

The MCC met for two business sessions during the 2005 ICES ASC/Statutory Meeting, addressing the progress achieved in its Expert Groups (EGs) and future activities of MCC.

The present ICES EGs under the MCC are:

- Working Group on Pathology and Diseases of Marine Organisms (WGPDMO),

- Working Group on Environmental Interactions of Mariculture (WGEIM),
- Working Group on Marine Shellfish Culture (WGMASC),
- Working Group on Marine Fish Welfare (WGMAFW) (formerly ICES Working Group on Marine Fish Culture (WGMAFW)),
- Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM).

All EGs (except WGMAFC) met in 2005 and their reports were presented to the MCC at its business session during the 2005 ICES Statutory Meeting. The WGPDMO report was presented by the Chair, was accepted by MCC and its recommendations for Terms of Reference (ToR) for the 2006 WGPDMO meeting were reviewed. On request from ICES bodies, WGPDMO was given three new Terms of Reference in addition:

| | |
|---|---|
| ToR j): | provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis |
| ToR k): | discuss and report on potential contributions for the ecosystem overview of the advisory reports describing the quantity and quality of marine habitat and/or the health of the marine ecosystem, and to consider and report on potential indicators of significant change in these ecosystem attributes |
| ToR l): | review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species and review selection of species, gender and size ranges (WKIMON) |

Because of the increase in the number of ToRs, the WGPDMO Chair suggested removing/postponing two ToRs originally recommended by WGPDMO at its 2005 meeting (see below) because he felt that otherwise the workload would be too large. This suggestion was adopted by the MCC.

| | |
|---|---|
| ToR e): | assess spatial and temporal variations in the pathogenesis of diseases of fish and shellfish and their effects |
| ToR h): | review the results of an intersessional risk assessment pilot study on population effects due to diseases in wild fish, using epidemiological methods and population dynamics modelling |

The WGPDMO Chair informed WGPDMO that there are partly overlapping activities in the MCC EGs addressing disease-related issues. For instance, WGMASC was given ToR a) on methods to measure stress indicators in shellfish, WGEIM ToR a) on the impact of escaped non-salmonid farmed fish, and WGMAFW ToR b) to develop guidelines for 'Fish Welfare in Mariculture'. He emphasised the need for a closer collaboration of these EGs.

Since the term of the MCC Chair T. Sephton (Canada) expired in 2005, I. Bricknell from the FRS Aberdeen, UK, was elected new MCC Chair.

## 3.3 ICES Symposia

A number of Symposia co-sponsored by ICES and of relevance for WGPDMO and MCC either were held or are in preparation for 2007 and 2008:

- **ICES-NASCO Symposium on "The Interactions between Cultivated and Wild Diadromous Fish Species",** 18–21 October 2005, Bergen, Norway (Co-Conveners: L.- P. Hansen (Norway) and M. Windsor, NASCO))

- **ICES-PICES Symposium on "Marine Bioinvasions",** 16–20 May 2007, Washington DC, USA (Co-Conveners: J. Pederson (USA), J. Carlton (USA), E. Leppäkoski (Finland) and Y. Fukuyo, PICES (Japan))
- **ICES/PICES/IOC Symposium on "Effects of Climate Change on the World Oceans",** Spring 2008, Gijón, Spain (Convener: L. Valdés (Spain))

The Chair again encouraged the WGPDMO members to attend the symposia and to think of contributions related to diseases and pathology in marine organisms.

### 3.4 ICES Advisory Committee on the Marine Environment (ACME)

The Chair informed the WGPDMO that several topics considered by WGPDMO at its 2005 meeting were subsequently reviewed by the ACME at its annual meeting in June 2005 and transformed into ICES Advice (see http://www.ices.dk/advice/icesadvice.asp ):

- New disease trends in wild and cultured fish, molluscs and crustaceans (WGPDMO Report 2005, Section 5 ),
- Review of the OSPAR JAMP Guidelines and Technical Annexes on fish disease monitoring (WGPDMO Report 2005, Section 13 and Annex 9),
- Effects of contaminants on the immune system of fish and shellfish (WGPDMO Report 2005, Section 9 and Annex 7),
- Health indices in relation to fish disease monitoring (WGPDMO Report 2005, Section 11 and Annex 8),
- Summer mortality syndrome in Pacific oyster and other bivalve species (WGPDMO Report 2005, Section 8 and Annex 6).

Since 2004, the ACME Report is no longer being published in the ICES Cooperative Research Report Series but in the new ICES publication series ICES Advice.

## 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

- CEFAS Histopathology Training Workshop, 20–24 March 2006, Weymouth, UK,
- Annual Meeting of the Shellfish Association USA, 26–30 March 2006, Monterey, California, USA,
- DIPNET Workshop Disease Interaction and Pathogen Exchange Between Farmed and Wild Aquatic Animal Populations, 3–5 April 2006, Prague, Czech Republic (http://www.dipnet.info),
- Fish Immunology Workshop, 18–22 April 2006, Wageningen, The Netherlands (http://www.zod.wau.nl/ceni/fish%20vaccination/),
- AQUA 2006, 9–13 May 2006, Firenze, Italy,
- Annual Meeting of the International Environmetrics Society, 18–22 June 2006, Kalmar, Sweden (www.mai.liu.se/ties2006/index.htm),
- 10th International Conference of the International Society For Developmental and Comparative Immunology, 1–6 July 2006, Charleston, SC, USA,
- ICOPA XI, 6–11 August 2006, Glasgow, UK,
- 5th International Symposium on Aquatic Animal Health, 2–6 September 2006, San Francisco, USA,
- ICES Annual Science Conference, 20–23 September 2006, Maastricht, The Netherlands, (http://www.ices.dk/iceswork/asc/2006/index.asp),

- Global Conference on Aquatic Animal Health, 9–12 October 2006, Bergen, Norway,
- Offshore Mariculture 2006, 11–13 October 2006, Malta,
- ICES-PICES Symposium on "Marine Bioinvasions", 16–20 May 2007, Washington DC, USA (http://www.ices.dk/iceswork/symposia/Symposium-2006a.htm).

## 4.2 Relevant Projects

- **Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)**: The project was initiated in 1998 as an EU-funded research programme. This project aimed to develop appropriate quality standards for a wide range of biological effects techniques and devise a method for monitoring compliance of laboratories generating data from these techniques for national and international monitoring programmes. The ultimate goal of this programme was to develop a Quality Assurance (QA) system for biological effects techniques that would be self-financing on the basis of fees recovered from participants. In essence, this would have similarities to the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) programme, which deals with quality issues in marine chemistry. The research programme was completed in April 2002. A self-funded scheme was launched in late 2004. This scheme comprises three components, biomarkers, whole organism (including QA for fish disease monitoring) and community analysis, each of which is organised by a lead laboratory/organisation which is responsible for establishing a QA programme, to include training workshops, intercalibration exercises and reporting the results. For fish diseases/liver histopathology, the lead laboratory is the CEFAS Weymouth Laboratory, UK (http://www.bequalm.org/about.htm).
- **Baltic Sea Regional Project (BSRP)**: Sponsored by the World Bank, organised and managed by ICES and HELCOM through a project coordinator and various ICES Study Groups under the Baltic Committee, e.g. the Study Group on Baltic Ecosystem Health Issues in support of BSRP (SGEH), that for instance is developing plans for coordinated monitoring programmes on the health status and on biological effects of contaminants in Baltic fish species. Continuous input from WGPDMO is therefore required (http://www.ices.dk/projects/balticsea.asp).
- **Permanent Advisory Network for Diseases in Aquaculture (PANDA)**: Network of Excellence under the 6th EU Framework Programme, with the aim to reinforce and expand the existing networks of the European Community and National Reference Laboratories for aquatic animal diseases (http://www.europanda.net/).
- **Disease interactions and pathogen exchange between farmed and wild aquatic animal populations – an European network (DIPNET**): A coordination action under the Scientific Support to Policy initiative under the 6th EU Framework The principal objectives of the DIPNET are: a) to strengthen the current scientific knowledge on transfer of pathogens and diseases between wild and cultured aquatic animal populations, b) to give scientific advice to support the development of European policies, and c) network building and dissemination of current knowledge to stakeholders and the wider European public (http://www.dipnet.info).
- **Summer mortality in *C. gigas* oysters (Mortalités Estivales, MOREST, 2001–2005)** (final meeting 14–15 March 2006, La Rochelle, France).
- **Sustainable Control of Fish Diseases in Aquaculture (SCOFDA**): Danish network of relevant researchers (http://www.fishnet.dk/networks/scofda/scofda.htm).

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

The update presented in the following sections is based on national reports for 2005 submitted by Canada, Denmark, Finland, France (only for shellfish), Germany, Iceland, Ireland, Latvia, Lithuania, Norway, Poland, Russia, Sweden, The Netherlands, UK and USA. It attempts to document significant observations and to highlight the major trends in newly emerging diseases and in those identified as being important in previous years.

## 5.1 Wild fish

### 5.1.1 Viruses

**Birnavirus** – Isolations of Birnavirus II (Tellina virus) were made from three out of 120 sea trout (*Salmo trutta*) broodfish screened in Denmark.

**Infectious Haematopoietic Necrosis Virus** – A database of genetic sequences of more than 600 isolates of IHNV is being established. Different patterns of diversity and evolution of IHNV throughout its range in western North America are apparent. When complete, the database will be available for researchers to determine epidemiological features of their isolates based on comparisons with sequences in the database.

**Infectious Pancreatic Necrosis Virus –** No isolates of IPNV were made from 600 brood salmonids (480 Atlantic salmon (*Salmo salar*), and 120 sea trout) in Denmark. In Finland, IPNV was isolated from whitefish (*Coregonus* sp.) broodfish and sea trout with no signs of disease.

**Lymphocystis –** In North Sea dab (*Limanda limanda*), the second lowest prevalence from the German Bight was recorded in 2005 (2.3%). The prevalence remained relatively unchanged in dab in the northern North Sea and the Irish Sea, although specific sites showed slight increases or decreases from 2004. In dab from the Dutch Wadden Sea there was a slightly decreasing trend in the period 2001–2005. No new trend was recorded in flounder (*Platichthys flesus*) from the Baltic Sea. Depending on the area, the prevalence ranged from 1.0% to 25.0%.

**Salmon Swimbladder Sarcoma Virus** – Variant strain 2 was identified by RT-PCR in Atlantic salmon smolts that were held for one week in rivers in Maine (USA). This is the same strain found earlier in landlocked salmon in New York state, which, when held for years in the laboratory, showed high viraemia but no signs of disease. There were no signs of disease in the Maine fish.

**Viral Haemorrhagic Septicaemia Virus –** The North American strain of VHSV (Genotype IV) was isolated from stocks of freshwater drum (*Aplodinotus grunniens*) and muskellunge (*Esox masquinongy*) experiencing mortalities in Canada and USA. These are the first mortalities in freshwater species in North America due to the marine genotype, suggesting a broader host range than expected for this virus. The outbreak in Canada occurred in eastern Lake Ontario with a connection to the St. Lawrence River, and the drum may have had a link with a diadromous species. VHSV (Genotype II) is still present in wild Baltic herring (*Clupea harengus membras*) in Finland. Genotyping of the G-gene segment showed that the isolate from Atlantic herring (*Clupea harengus harengus*) in Maine reported in 2004 is 99% homologous with the Pacific marine VHSV Genotype IV and 85% homologous with the European Genotype III.

### 5.1.2 Bacteria

**Acute/healing skin ulcerations** – The prevalence of skin ulcers in Baltic cod (*Gadus morhua*) increased in 2005 in comparison to 2004, but were generally lower than in 2003. The prevalence of ulcers in cod and plaice (*Platessa platessa*) in the Barents Sea decreased in

comparison to previous years. The prevalence of skin ulcers in 2005 in Baltic flounder from the Polish coast was higher than in 2004. The highest level of ulceration was noted in flounder from the Polish and Lithuanian EEZs. There was an increasing trend in ulcerations in North Sea dab from the German Bight and the Firth of Forth. In the Irish Sea, there was an increasing trend in Red Wharf Bay whereas a decreasing trend in Morecambe Bay was observed in the last two years.

***Yersinia ruckeri*/*Aeromonas salmonicida*** – The bacteria were isolated from captive sea run Atlantic salmon from the Penobscot River, Maine, USA. *Y. ruckeri* was isolated from 51% and *A. salmonicida* from 26% of the fish screened between 1976 and 1997, but were not isolated between 1998 and 2003.

***Renibacterium salmoninarum*** – No isolates of the pathogen (causative agent of Bacterial Kidney Disease, BKD) were made from 600 brood salmonids (480 Atlantic salmon and 120 sea trout) screened in Denmark.

**Putative Bacteria** - Of cod (100–400 g) caught by fyke-netfishing from a local area within the Skagerak Sea, Sweden, 15% to 20% had external lesions. The majority of those with lesions had whitish granulomas in liver, spleen, heart and kidney.

### 5.1.3 Parasites

#### Mesomycetozoa

***Ichthyophonus hoferi*** – The parasite is still endemic at low prevalence in herring populations in the North Sea, the Baltic Sea and the west coast of Sweden. Prevalence in Pacific herring (*Clupea pallasi*) in Puget Sound, Washington, USA, increased from 41% in 2000 to 59% in 2005. Experimental exposure of naive Pacific herring resulted in 80% mortality in 36 days. The parasite occurred in 12.5% of 304 Puget Sound rockfish (*Sebastes emphaeus*).

#### Myxosporea

***Myxobolus dermatobia* –** Observed in skin of 25% to 44% of chum salmon (*Oncorhynchus keta*) from far eastern Russia with intensities ranging from 2 to 203 cysts per fish.

#### Microsporidia

A decreasing trend of *Glugea stephani* was observed in dab from the Dutch North Sea coast.

#### Monogenea

***Gyrodactylus salaris*** – Remains a major threat to Atlantic salmon in Norway. Salmon in the Norwegian rivers Steinkjerelva and Figgja became re-infected following an earlier disinfection.

#### Digenea

***Stephanostomum baccatum*** – Prevalence of metacercariae in flatfishes (*Limanda aspera, Hippoglossoides elassodon, Cleisthenes herzensteini* and *Limanda proboscidea*) from the Sakhalin coast ranged from 12% to 96%.

#### 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 was observed. The prevalence was negatively correlated with the mean mass of individual herring from Polish waters. The mean number of parasites per all fish examined (abundance) in cod from the Barents Sea increased to 17.7 from 2004.

***Pseudoterranova decipiens* (larvae) –** A decreasing trend in prevalence in cod from the Barents Sea of 40% to 8% between 2003 and 2005 coincided with an increase in abundance from 0.2 to 1.6.

#### Acanthocephala

***Corynosoma strumosum* (larvae) –** Prevalence increased to 9.8% in cod and to 15.7% in flounder in the Baltic Sea (Russian EEZ, ICES Subdivision 26).

#### Crustacea

***Lepeophtheirus salmonis*** – The prevalence in 1,298 threespined sticklebacks (*Gasterosteus aculeatus*) collected in British Columbia, Canada, was 47.2%, with an intensity (mean number of parasites per infected host in a sample) of 4.4. In 2004, the prevalence and intensity on sticklebacks was 83.6% and 18.3, respectively. Adult stages were extremely rare.

***Lepeophtheirus pectoralis*** – Prevalence in dab ranged from 0.8% at Tees Bay (North Sea) to 45.9% at Morecambe Bay (Irish Sea).

***Sphyrion lumpi*** – In 2005, the prevalence in redfish (*Sebastes mentella*) from the Barents Sea declined, halting the increasing trend observed over the last four years.

***Clavella adunca*** – The prevalence in North Sea whiting (*Merlangius merlangus*) from Scottish waters ranged from 28.7% to 34%.

### 5.1.4 Other diseases

**Epidermal hyperplasia/papilloma** – Prevalence in dab at most sites sampled in North Sea areas during 2005 was reduced from 2004 with the exception of Central Dogger (2.3% from 1.7% in 2004). In the Irish Sea (Liverpool Bay), the prevalence increased to 2.4% from 0% in 2004.

**Liver nodules/tumours** – Prevalences of liver nodules in North Sea dab from former hot-spot areas (German Bight, Dogger Bank) remained at a low level in 2005. The occurrence of liver nodules greater than 2 mm in diameter in dab 20 cm and above showed some differences from 2004 with a general decrease in prevalence detected since 2002 at West Dogger (10.8% to 3.9%) and North Dogger (6.4% to 4.0%). The prevalences at Central Dogger/Hospital Ground, Red Wharf Bay, and Morecambe Bay remain steady at approximately 4.2%, 0.8%, and 1.3%, respectively. An increase in prevalence of 2.4% to 5.4% was seen at the Indefatigable Bank, southern North Sea. This is the highest prevalence recorded from this site and also the highest recorded in 2005. Liver nodules were observed for the second time at southeast Isle of Man (3.9%), however this was a decrease of 1.9% from levels recorded during 2004.

**Hyperpigmentation** – Dab in most North Sea areas showed a significant increase in prevalence of hyperpigmentation (maximum 2005 values: Dogger Bank, 42.8%; German Bight, 49.4%). Dab from the Irish Sea continued to show low levels of hyperpigmentation. Prevalence in Cardigan Bay (Irish Sea) in 2005 dropped from 15% to 5.8% after several years of an increasing trend. The condition was absent in dab from the western Baltic in 2005.

**Toxic algae** – Gill damage associated with *Karenia mikimotoi* blooms was observed along the north, west and south coasts of Ireland throughout the summer 2005. Associated losses of wild fish including flatfish, conger eel (*Conger conger*) and mullet (*Mugil* sp.) were noted. Blooms of *Noctiluca scintillians* affected large parts of the south and west coasts of Ireland in the summer of 2005.

**Intersex condition** – Previously reported from dab from the North Sea in 2004 was not detected in 2005.

**M74** – In Sweden, 3% of the batches of 2005 fry hatched from 765 female Atlantic salmon were affected, in comparison to 9% in 2003 and 36% in 2002. M74-associated mortality in Atlantic salmon fry in Finland between 1992 and 2002 has declined from a yearly average greater than 50% to less than 10% in the last three years. There is a clear decreasing trend in M74 syndrome in Sweden and Finland.

#### 5.1.5 Conclusions

1 ) New outbreaks of VHSV Genotype IV in freshwater fishes in North America suggest a broader host range for this marine virus than previously thought. The outbreak in drum (*Aplodinotus grunniens*) in Canada was the first due to Genotype IV in freshwater fish.

2 ) *Ichthyophonus* is an increasing disease problem in several species of fishes in Pacific coastal North America.

3 ) Early stages of *Lepeophtheirus salmonis* are commonly parasitic on threespined sticklebacks (*Gasterosteus aculeatus*) in British Columbia. The role of this host in the ecology of the parasite is not known.

4 ) A 27 year data set showed bacterial pathogens *Yersinia ruckeri* and *Aeromonas salmonicida* decreased significantly in the captive broodstock of sea run Atlantic salmon (*Salmo salar*) in Penobscot River, Maine, USA, since 1997.

5 ) There was a significant year effect on the prevalence of *Anisakis simplex* larvae infection in Baltic herring (*Clupea harengus*). Prevalence steadily declined since 1997 and was negatively correlated with average mass of herring.

6 ) The prevalence of hyperpigmentation in North Sea dab (*Limanda limanda*) continued to show a dramatic increase.

7 ) The impact of M74 has declined in wild Atlantic salmon fry in Sweden and Finland.

#### 5.1.6 Recommendations

The WGPDMO recommends that:

i ) ICES Member Countries are encouraged to 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.

ii ) WGPDMO reviews 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 at its 2007 meeting;

### 5.2 Farmed Fish

#### 5.2.1 Viruses

**Heart and Skeletal Muscle Inflammation (HSMI)** – This condition was first described in 1999 in Norway in Atlantic salmon (*Salmo salar*). A significant increase in prevalence was reported for 2005 compared to 2004. An outbreak of disease resembling heart and skeletal muscle inflammation in farmed Scottish salmon has been reported. Additional detail is provided under report Section 6.

**Infectious Pancreatic Necrosis Virus (IPNV)** – Clinical IPN was reported at one site with losses of 10% Atlantic salmon in Ireland. Atlantic salmon at a farm situated on the coastal zone of Sweden supplied with pumped freshwater were found to be infected with IPNV. The

farm has now been disinfected and will be fallowed for two years. IPN remains significant in sea water post smolts from Scotland but is now deregulated and consequently no longer notifiable. IPN remains one of the major diseases in Norway.

**Infectious Salmon Anaemia Virus (ISAV)** – ISAV continues to be detected in Atlantic salmon farms in Cobscook Bay, Maine, USA. Southwest of Cobscook Bay, ISAV has been detected by RT-PCR in *Caligus elongatus* taken from fish on a farm that was affected by an apparently non-pathogenic type of ISAV. This suggests that *C. elongatus* may serve as a vector of ISAV.

Sixteen separate incidents occurred in Maine in 2005, resulting in eradication of more than 125,000 fish and early harvest of substantial numbers of salmon. Genotyping of archived samples from Maine has revealed three genotypes as compared to 15 genotypes identified in New Brunswick, Canada. A model of hydrographic and epidemiological data collected on 32 sites with 2002 year class salmon of the Cobscook Bay (Maine) and Passamaquoddy Bay (New Brunswick) region suggests the movement of ISAV by water and tidal cycles played a relatively minor role in new outbreaks that occurred during a 28 month period. The model does not account for biosecurity, husbandry, fish strain or hatchery and may not apply to other areas.

**Salmon Pancreas Disease Virus (SPDV)** – This is an increasing problem in farmed Atlantic salmon in Scotland, Norway and Ireland.

**Viral Haemorrhagic Septicaemia Virus (VHSV)** – An increase in VHS outbreaks was reported in Finland. The virus was isolated from nine rainbow trout (*Oncorhynchus mykiss*) farms in the Åland Islands, SW Finland, compared to two isolations in 2004. VHS virus or clinical disease was not found within the two other VHS-restriction areas, or in other parts of the Finnish Baltic coastal areas. The genotype is Id. The North American (NA) strain of VHSV is reported to cause low level losses in 80–90 g Atlantic salmon introduced to seawater net pens in western Canada 1.5 months previously. VHSV was detected in all three Atlantic salmon examined. VHSV isolates from Pacific herring (*Clupea pallasi*) in the same netpen (reported above) were genetically identical to the salmon isolates. VHSV was identified in seventeen farmed chinook salmon (*Oncorhynchus tshawytscha*) in western Canada by RT-PCR and cell culture.

### 5.2.2 Bacteria

***Aeromonas salmonicida* subsp. *salmonicida*** – One new case of mortality was reported from a rainbow trout farm (coastal area of northern Baltic, Sweden). The trout farm had a vaccination programme, but during a warm summer period mortality coincided with a bloom of cyanobacteria.

***Francisella* sp**. – This is new and increasing problem in farmed Atlantic cod (*Gadus morhua*) in Norway. There is a potential link with reported disease in wild cod in Sweden where there is similar histopathology.

***Moritella viscosa*** – Ulcerations causing significant economic losses are mainly associated with decreased flesh quality and remain a significant problem in both salmon and rainbow trout farming in Norway. The impact is especially high in northern Norway, probably due to low temperatures and a slow healing process. Rough treatment of fish when they are sorted or handled seems to predispose fish to infection.

***Vibrio anguillarum*** – Serotype O2ß was responsible for losses in cod in Ireland in December 2005.

***Vibrio fluvialis*** – This pathogen was isolated from seahorse (*Hippocampus* sp.) in an aquarium in the west of Ireland following mortalities in late 2005.

***Vibrio ordalii*** – This pathogen was isolated from Atlantic cod for the first time in Norway with associated mortality.

### 5.2.3 Parasites

#### Myxozoa

***Kudoa* sp**. – The presence of *Kudoa* was confirmed at one Irish marine site rearing Atlantic salmon.

***Parvicapsula pseudobranchiola*** – Infections are common but mortality rates are low in Atlantic salmon Norway (2–3% accumulated).

#### Sarcomastigophora

***Spironucleus*** sp. – A systemic infection is regularly seen in marine-farmed Arctic charr (*Salvelinus alpinus*) in Norway. The mortality is low compared to infections in Atlantic salmon. A new case of systemic spironucleosis in Atlantic salmon was recently reported in northern Norway (2006).

#### Monogenea

***Gyrodactylus*** spp. and **Trichodinids** – Mixed infections are commonly reported from Atlantic cod farms in Norway. Both parasite types are often seen in fish reported to have "gill problems". Formalin treatment has been used successfully.

#### Cestoda

***Eubothrium crassum*** – Some problems were reported with resistance to Praziquantal treatment in farmed Atlantic salmon in Norway.

#### Crustacea

**Sea lice –** The potential of interactions between farmed and wild cod is a concern in Norway. Treatment of sea lice in Ireland has presented difficulties at some sites. There is concern that *Caligus elongatus* and *C. curtus* infestation in cod at certain farms are problems in Norway.

### 5.2.4 Other diseases

**Algal blooms** – High losses due to *Noctiluca scintillians* were encountered in some marine sites in the south west of Ireland. Some losses were also reported as a result of *Karenia mikimotoi* blooms in Ireland. In 2005, four incidents were recorded in Scotland in Atlantic salmon and attributed to a combination of *Thalassiosira* and *Crypophytos* spp., *Aurelia aurita*, *Pseudonitzschia* spp. and an unknown plankton/jellyfish.

**Epitheliocystis** – One case reported in Iceland in Atlantic halibut (*Hippoglossus hippoglossus*). This species appears to be particularly susceptible.

### 5.2.5 Conclusions

1 ) VHS appears to be the main threat for the Finnish rainbow trout (*Oncorhynchus mykiss*) farming in the Baltic Sea. The spread of the disease is controlled by the formation of restriction areas and by fallowing farming sites.

2 ) An increase in the number of isolations of IPNV in farmed Atlantic salmon (*Salmo salar*) is of concern in Ireland.

3 ) Salmon pancreas disease (PD), heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS) are virus-related diseases of growing concern for the Atlantic salmon farming industries of Ireland, Norway and Scotland.

4 ) There is concern that *Caligus elongatus* may act as a vector of Infectious Salmon Anaemia Virus (ISAV) in the USA.

5 ) A new and increasing problem in farmed Atlantic cod (*Gadus morhua*) in Norway is associated with *Francisella* sp. infections.

6 ) Pathogenic North American strains of VHSV appear to be transmitted to farmed Atlantic salmon from Pacific herring (*Clupea pallasi*).

#### 5.2.6 Recommendations

The WGPDMO recommends that:

i ) ICES Members Countries carry out comparative studies on *Francisella* sp. in farmed and wild cod (*Gadus morhua*) and a visceral granulomatous condition in wild cod.

### 5.3 Wild and farmed molluscs and crustaceans

#### 5.3.1 Viruses

**Herpesvirus** – No change in bivalves in France and no new information from the USA.

**Viral Gametotrophic Hypertrophy**– Continued rare presence in Pacific oysters (*Crassostrea gigas)* reported in France. The condition is also found regularly, but rarely, in eastern oysters (*C. virginica*) in the USA.

**White Spot Syndrome Virus –** WSSV was detected for the first time in the blue crab (*Callinectes sapidus).* It was diagnosed using real time PCR and an antibody test in a single blue crab in a sample of 300 collected in South Carolina and Georgia, USA. It was transmissible in an injection bioassay.

**Bacilliform virus** – A potentially novel bacilliform virus infection was found in up to 8% of pink shrimp *(Pandalus montagui*) collected from an offshore site in The Wash, UK. Light to heavy infections affected the hepatopancreas. Further work is being carried out to classify the virus and to compare it to the previously described brown shrimp (*Crangon crangon*) Bacilliform Virus (CcBV) found at the same site.

#### 5.3.2 Bacteria

**Vibriosis** – In France, *Vibrio harveyi* was associated with two mortality episodes affecting natural stocks of the abalone (*Haliotis tuberculata). Vibrio splendidus* was associated with mortality affecting great scallop (*Pecten maximus*) spat in the field. This is the first time that *V. splendidus* has been associated with scallop mortalities in France. As in 2004, *Vibrio* spp. were isolated from wild shrimp (*Palaemon adspersus*) with brown spot disease in Denmark.

**Nocardiosis** – In Pacific oysters, no new trends were reported in Canada and no new information was available from the USA.

**Withering syndrome** – No new information on occurrence in abalone.

**Juvenile Oyster Disease** – No outbreaks reported in eastern oysters.

#### 5.3.3 Fungal infections

**Fungal infection** – A fungal egg pathogen in externalised egg clutches was reported in the Chinese Mitten crab (*Eriocheir sinensis)* in the UK. The fungal infection appears to replace normal yolk storage and dividing embryonic cells and culminates in complete destruction of the egg mass (with fungal hyphae spreading between adjacent eggs via sporulating bodies). The necrotic egg mass is then infested with filamentous bacteria and other non-specific pathogens. Up to 75% of gravid females were affected at peak infection. The effect of fungal

infection on fecundity in this species has not been investigated but warrants further study (e.g. to control reproduction of this invasive species).

### 5.3.4 Parasites

#### Dinoflagellata

***Perkinsus marinus* –** After two years of marked decline in 2003 and 2004, the prevalence in eastern oysters increased in Chesapeake and Delaware Bays, USA, in 2005, but its impact was still low relative to historical levels. In these estuaries, maximum prevalence increased from 40–50% in 2004 to 50–60% in 2005. There was little change to the north in Long Island Sound and Narragansett Bay or to the south in South Carolina and Georgia, where prevalence remained high (≥ 80%), although with relatively light infections and little mortality.

***P. olseni*** – No change was reported in France where a survey of 836 carpet-shell clams (*Ruditapes decussates)* and manila clams (*R. philippinarum)* from the French coast found a prevalence of 43%. Prevalence was approximately equal in the two species.

***P. chesapeaki* –** The parasite remained prevalent in both soft-shell clams (*Mya arenaria*) and stout razor clams (*Tagelus plebeius*) in the upper portion of Chesapeake Bay, USA, where mortality of approximately 70% was associated with a prevalence of 80% in the razor clam. A *T. plebeius* sample of 20 clams collected in November 2005 from lower Delaware Bay, USA showed continued high prevalence (100%) of probable *P. chesapeaki*, first identified in this estuary in 2004. DNA samples of the Delaware Bay clams are preserved for species identification.

#### Labyrinthomorpha

**Quahog Parasite X (QPX)** – Histopathological surveys of the hard clam (*Mercenaria mercenaria)* found no change in prevalence in Massachusetts, New Jersey or Virginia, USA, but a decrease in New York. No new trends reported in Canada.

#### Haplosporidia

***Bonamia ostreae* –** No change was found in flat oysters (*Ostrea edulis*) in France where the annual survey found *B. ostreae* in 90 of 1,275 oysters (7%). In the spring of 2005, *B. ostreae* was found for the first time in a sample (13 of 30) of flat oysters from Lough Foyle, Ireland, during the routine screening programme. An investigation of the epizootic is ongoing. In England, the prevalence in native oysters at farm sites increased slightly and in wild populations decreased slightly, compared with 2004. The parasite was not detected in any of the samples taken from the Fal wild fishery, England, for the first time ever and regular surveillance shows that it has not spread to new areas, including Scotland and the Limfjorden area of Denmark. The pathogen was reported for the first time in Canada (British Columbia) in 2004 and examination of *O. edulis* from three grow-out facilities in the summer of 2005 revealed prevalences from 0.5% to 11.1%. Re-evaluation of historic *O. edulis* examination results between 1986 and 2000 from five locations in British Columbia and re-examination of archived samples (n = 343) collected from the reference site between 1999 and 2004 in conjunction with seed introduction records suggests that *B. ostreae* may have been inadvertently introduced into British Columbia around 2003 with *O. edulis* seed imports from enzootic areas in the Washington State, USA.

***Bonamia* sp. –** The *Bonamia* sp. pathogenic to the non-native Asian oyster (*C. ariakensis)* caused epizootic disease and mortality approaching 100% in every deployment of seed *C. ariakensis* (< 45 mm) to Bogue Sound, North Carolina, USA, through the warmer months of 2005. There is every indication the parasite is enzootic in Bogue Sound where the native, crested oyster (*Ostreola equestris*) in which *Bonamia* sp. was also detected again in 2005, may

serve as a reservoir. Maximum prevalence determined by PCR of this parasite in *C. ariakensis* in Bogue Sound was 91.7%; maximum prevalence in *O. equestris* was 1.3%. Infections were confirmed by histopathology. The *C. ariakensis*-pathogenic *Bonamia* sp. was also detected in *O. equestris* at Wilmington, North Carolina, over 100 km southwest of Bogue Sound and distant from any experimental deployments of *C. ariakensis*. PCR-detected prevalence was 1.5%. Sentinel *C. ariakensis* deployed to Wilmington in late September had 82.6% prevalence by November, and mortality was again high. The Wilmington results are significant in that they indicate an expansion of the known range of this parasite.

***Bonamia* sp. –** A second *Bonamia* sp., found in the crested oyster *O. equestris* in 2004 and never observed in *C. ariakensis*, persisted in Bogue Sound in 2005, and was also observed for the first time in Wilmington, North Carolina. Maximum prevalence determined by PCR was 1.3% in Bogue Sound and 3.5% at Wilmington.

***Mikrocytos mackini*** – No new trends were reported in the Pacific oyster in Canada.

***Haplosporidium nelsoni*** – The two-year decline in prevalence recorded in eastern oysters in 2003 and 2004 in the mid-Atlantic, USA, did not continue in 2005, although prevalences remained very low (< 5%) by historical standards. Elsewhere in the range examined in the USA (Rhode Island to South Carolina), prevalences were < 10%. In Nova Scotia, *H. nelsoni* persisted on farm sites in the Bras d'Or Lakes, where it was first discovered in Canada in 2002. In 2005, it was also confirmed in oysters from an isolated population on the north shore of Cape Breton, Nova Scotia. Although this is a new location, it does not affect the populations that remain under official protective measures elsewhere in Atlantic Canada. In France, *H. nelsoni* was detected in 6 of 450 (1.3%) Pacific oysters in 2005. Whereas this prevalence is not unusual, the spore stage of *H. nelsoni* was found for the first time in France in one of the infected oysters.

***Haplosporidium costale*** – No cases were detected in eastern oysters examined in New Jersey, Maryland, Virginia, or South Carolina, USA. No new trends in Canada

***Haplosporidium* sp. –** No new trends were reported in edible mussels (*Mytilus edulis)* in Canada.

### Paramyxea

***Marteilia refringens* in flat oysters** – No change was reported in France where the annual survey of flat oysters recorded a prevalence of 2.5% (25 of 1008). Approved-zone status has been maintained for UK and Danish waters monitored for *M. refringens.*

### Euglenosoa

***Isonema*-like parasite** – About 7% of the edible mussel larvae from a hatchery in southern British Columbia submitted in November 2005 for examination had systemic infections with a few to overwhelming numbers of a protozoan resembling the poorly understood *Isonema*-like parasite originally reported from geoduck clam (*Panopea abrupta*) larvae in an experimental hatchery in Washington State, USA. This is the first detection of this parasite since the initial report in 1987. However, the mussel hatchery manager claims that unreported outbreaks of this parasite with associated high mortalities have occurred in British Columbia over the past three years.

### Microsporea

**Microsporidian** – Work is continuing on identification of an intranuclear microsporidian infection in edible crabs (*Cancer pagurus*) from the English Channel, UK, reported for the first time in 2004. This is the first report of an intranuclear microsporidian in an invertebrate. A very similar pathogen has also been found in hermit crabs (*Eupagurus bernhardus*) from the Irish Sea, UK.

#### Digenea

**Trematodes –** Cercariae and metacercariae of trematodes were described for the first time in 2005 infecting gonads of the Baltic clam (*Macoma balthica*) (>13 mm shell length) from the Gulf of Gdansk (southern Baltic Sea), Poland. The metacercariae had characteristics typical of family Gymnophallidae, genus *Gymnophallus*. At 5 m depth, average prevalence was 6% and both cercariae and metacercaria were observed. At 45 m, prevalence was 12% and only metacercariae were found. Infected gonads appeared highly degenerated with a reduced number of eggs when viewed microscopically.

### 5.3.5 Other Diseases

**Toxic algae** – The *Karenia mikimotoi* blooms along the north, south, and west coast of Ireland killed large numbers of wild cockles, mussels and clams during the summer of 2005.

**Neoplasms** – No new trends in disseminated neoplasia were reported in Baltic clam populations inhabiting the Gulf of Gdansk, Poland. The disease remains present with prevalence between 10% (shallow sites) and 26% (deep sites) in the cold months. In one sample of 50 clams, 11 were diagnosed with neoplasia. Based on electron microscopy of these individuals, two types of neoplastic cells were described in 46% of clams, which also had advanced neoplasia: round cells and spindle-shaped cells, both with increased frequencies of cell division. This represents the first description of phenotypically different neoplastic cells in the same individuals, suggesting a co-occurrence of disseminated neoplasia with what might be a fibrosarcoma. No new trends were reported in the USA or in Canada.

**Shell disease –** A 10-year database on shell disease in American lobsters (*Homarus americanus*) from Rhode Island, USA, showed an increase from 0% (reported) in 1996 to 25–30% in 2002–2005. Prevalences in gravid females ranged from 45% to greater than 60%, with no apparent trend between 1998 and 2005, whereas prevalences in non-gravid females and males steadily increased from less than 5% in 1997 to approximately 20% in 2005. Tag and recapture studies showed that the mean growth increment per molt of all lobsters with signs of shell disease was significantly less than that for lobsters without disease signs (7.08 mm vs. 7.79 mm, respectively). Preliminary tag and recapture data suggest a greater proportion of shell-diseased females molt prematurely than non-diseased females (4.8% vs. 0.3%, respectively). Although these data indicate an effect on the diseased lobsters, the impact on lobster populations due to shell disease is uncertain due to the many other factors affecting population fluctuations. The cause of shell disease remains under investigation.

**Summer Mortality –** A farm site in the River Camel, Cornwall (SW England) continues to experience occasional late summer mortality in adult (2–3 year old) Pacific oysters without the presence of histologically detectable pathogens. It is thought that these mortalities may be due to excessive stocking densities. In France, summer mortalities continued with no change recorded. In the USA, summer mortalities continue in seed oysters in Tomales Bay, California, where oyster herpesvirus is likely involved. Mortalities were also reported in Washington State.

**Parasite-associated mortality** – Unusually heavy mortality, of up to 70% in some beds, was reported in cockles (*Cardium edule*) in Milford Haven, South Wales, UK, in 2004. Most individuals were infested by low numbers of digeneans or gregarines, which were not considered significant at the time. Monthly samples have been collected from August 2004 to the present to document seasonal dynamics of the parasites found and to discern any patterns that may indicate an effect of parasitism on host survivability. Two sites with similar habitats were selected: one experiencing ongoing population declines and another less affected by mortalities. To date, a total of 10 parasites have been identified in the two sites, including *Trichodina* sp. and other ciliates, Rickettsia-like organisms, gregarines and six digeneans. Two digeneans caused marked pathology in the host. In samples examined immediately after a

second mortality event in 2005, the prevalence of these two digeneans decreased compared with other parasites. Additionally, whilst they were found at both sites, they were more prevalent in the affected site. It is suggested that these digeneans may play a role in the observed mortalities. Studies are on-going to determine the link between cockle survival, environmental factors and parasitism.

**Gill disease signs** – In September 2005, high mortalities of wild introduced Pacific oysters were noted along the coast of Lower Saxony, Germany. Macroscopically, affected oysters were characterised by shell gaping, green discolorations on the gills and the mantle as well as a thickening and brown stripes on the gills. Histopathological signs were necrosis of the gills, infiltration of haemocytes (also in the gonads), the presence of large eosinophilic inclusion bodies in the oocytes, changes in the morphology of the oocyte nuclei (e.g. condensed ring-shaped basophilic material, possibly either condensed chromatin or 'foreign' DNA). A viral etiology is suspected.

**Shell anomalies** – North Sea brown shrimp collected at the inlet and outlet of a power plant located in the Weser estuary, Germany, were affected by white, crystal-like spots in the carapace, the composition and causes of which have so far not been identified. There seems to be a seasonal effect with elevated prevalence in late summer.

**Black Spot Disease** – No new trends have been reported in brown shrimp from monitoring in the German Wadden Sea.

**Disease signs in Icelandic scallops** – Signs of a previously unreported disease were observed in Icelandic scallops (*Chlamys islandica*) from the Svyatoy Nos area in the Barents Sea, Russia. It affects mature scallops with shell height exceeding 70 mm. Signs include soft body atrophy, a dull grey adductor muscle colour, a thin transparent mantle, change in colour of the gonad from orange to grey, light necrotic "spots" in internal organs, shell edge deformation and growth interruption marks on the inner surface of the shell. The affected scallops develop focal necrosis and dystrophy of conjunctive and muscular tissue. A histopathological investigation showed that 40% of examined scallops had severe necrotic changes in mantle, adductor muscle and gonads. An association of the disease with mortality has not yet been determined.

### 5.3.6 Miscellaneous

The states of Maryland and Virginia, bordering Chesapeake Bay in the USA, have again delayed a decision on the introduction of the Asian oyster into the Bay because numerous studies of its potential effect, including its extreme susceptibility to *Bonamia* sp., have not been completed.

In a 2003 study, *M. edulis* from offshore areas in Germany (moored suspended buoys and collectors) were free of parasitic trematodes and shell-boring polychaetes. Parasitic copepoda (*Mytilicola intestinalis*) were recorded at only one out of seven offshore sampling sites. In contrast, mussels from coastal inshore areas (moored on suspended buoys and collectors, and benthic subtidal and intertidal mussel beds) were infested by three parasite groups: (copepoda: *M. intestinalis*; trematodes: *Renicola roscovita*, *Himasthla continua*, *H. elongata*, *Psilostomum brevicole*; polychaetes: *Polydora ciliata*).

### 5.3.7 Conclusions

1 ) In 2005, the enzootic *Bonamia* spp. first identified in North Carolina, USA, in 2003 during testing of the non native Asian oyster (*Crassostrea ariakensis*) were detected 100 km south of the original site, thus extending the known range of these parasites, the natural host of which appears to be the native crested oyster (*Ostreola equestris*).

2 ) White Spot Syndrome Virus (WSSV) was detected for the first time in the blue crab (*Callinectes sapidus*) in the southeastern USA.

3 ) *Vibrio splendidus* was associated for the first time with mortality of the great scallop (*Pecten maximus*) in France.

4 ) The marked two-year decline in prevalences of *Perkinsus marinus* and *Haplosporidium nelsoni* in eastern oysters *(Crassostrea virginica*) in the mid-Atlantic estuaries of the USA ended in 2005.

5 ) The spore stage of *H. nelsoni* was found for the first time in a Pacific oyster (*Crassostrea gigas*) in France.

6 ) Shell disease in American lobsters (*Homarus americanus*) from Rhode Island, USA, has shown an increasing trend over the past 10 years. Population effects due to the disease are uncertain although significantly negative effects on gravid and non-gravid females have been documented.

7 ) Phenotypically different neoplastic cells co-occurring in individual Baltic clams (*Macoma balthica*) from the Gulf of Gdansk were described for the first time.

### 5.3.8 Recommendations

The WGPDMO recommends that:

i ) WGPDMO be kept informed of progress in investigations into the identification of *Bonamia* spp. infecting Asian oysters (*Crassostrea ariakensis*) and crested oysters (*Ostreola equestris*) in the USA;

ii ) studies be pursued on identifying the causes of gill disease in Pacific oysters (*Crassostrea gigas*) in Germany and on disease signs in Icelandic scallops (*Chlamys islandica*) from the Barents Sea.

# 6 Update information on the causes and effects of Heart and Skeletal Muscle Inflammation (HSMI) affecting farmed salmon in ICES Member Countries

B. Hjeltnes presented a short report on HSMI which is summarized below.

## 6.1 Pathology and etiology

The first description of Heart and Skeletal Muscle Inflammation (HSMI) was reported by Kongtorp *et al.* (2004a) in farmed Atlantic salmon (*Salmo salar*) in Norway. Fish are affected 5–9 months after transfer to sea water, although sometimes as early as 14 days post transfer. Although HSMI has been reported throughout the year, it is most common in spring and early summer. Affected fish appear anorexic and show abnormal swimming behaviour. Mortality varies from insignificant up to 20%. In Norway, the occurrence of HMSI is increasing and 83 cases were confirmed in 2005, but the condition was probably under-reported.

Extensive perimyocarditis and severe epicarditis characterised by massive infiltration of mononuclear cells occur (Kongtorp *et al.*, 2004b). The most common lesions are ventricular, in which the spongy and compact layers are infiltrated by mononuclear cells, comprised of macrophages, lymphocytes and plasma-like cells. The inflammatory cells are localised within and around myocytes in a diffuse or focal pattern, which is most evident in the compact layer.

Affected myocytes exhibit signs of degeneration, with condensation, loss of muscle striation, vacuolation and centrally located nuclei. Individual nuclei may undergo pyknosis or karyolysis. Necrotic cells appear in association with inflammatory infiltrates. Hypertrophic nuclei can be observed in a few myocytes within the spongiform muscle layer. Red skeletal muscle inflammation is also a feature of HSMI.

An outbreak of disease resembling HSMI has been reported in farmed Scottish salmon (Ferguson *et al.*, 2005).

Currently, the diagnosis is based on histopathological lesions as described. To avoid confusion and to differentiate HSMI from Salmon Pancreas Disease, diagnosis includes the absence of pancreatic lesions, Salmon Pancreas Disease (PD) virus or antibodies against PD-virus to avoid confusion and to differentiate HSMI from salmon pancreas disease.

Data available suggest that HSMI has a viral aetiology (Eliassen *et al.*, 2005) and can be artificially reproduced by cohabitation. Tissue homogenates have been injected into Atlantic salmon. Eight weeks later histological changes in the heart were recorded and a cytopathic effect was observed. Electron microscopy revealed particles of ~80 nm which were susceptible to chloroform, suggesting the presence of an enveloped virus. The virus was re-isolated, suggesting that River's postulates have been fulfilled. Antibodies have been prepared by PHARMAQ and a crude IFAT was developed. The potential for passive immunization is currently being investigated and protection using immune serum obtained from an earlier experiment has been demonstrated.

Notes regarding differential diagnosis for CMS, SPD and HSMI are listed in Table 6.1.1.

**Table 6.1.1: Diagnostic characteristics of Cardic Myopathy Syndrome (CMS), Salmon Pancreas Disease (SPD) and Heart and Skeletal Muscle Inflammation in Atlantic salmon (*Salmo salar*).**

| CARDIAC MYOPATHY SYNDROME (CMS) | SALMON PANCREAS DISEASE (SPD) | HEART AND SKELETAL MUSCLE INFLAMMATION (HSMI) |
|---|---|---|
| Characteristic lesions are found in the spongiform myocardium of the atrium and ventricular muscle. These lesions are comprised of muscular degeneration, proliferation of the endocardial cells with macrophage infiltration and lymphocytes subendocardially and in the degenerated muscle. Blood clots are frequently found in the atrium. Focal necrosis in the hepatic paren-chyma may also occur.<br>Within select sections of liver, a mild fibrosis of the central vein can be observed. | Pancreas disease is characterised by pancreatic atrophy followed by fibrosis. Acute cardiomyopathy characterised by a coagulative necrosis, myodegeneration and phagocytosis of fibre remnants affecting both spongy and compact layers with an increased eosinophilia and loss of striation. | Extensive panmyocarditis. Severe epicarditis characterised by a massive infiltration of mononuclear cells.<br>Deposits of fibrinous material in the epicardium.<br>The most common lesions occur in the ventricle, in which the spongy and compact layers are infiltrated by mononuclear cells, comprising macro-phages, lymphocyte and plasma-like cells. The inflammatory cells are localised within and around myocytes in a diffuse or focal pattern, and most evident in the compact layer. |

## 6.2 Conclusions

1 ) Heart and Skeletal Muscle Inflammation (HSMI) is an increasing problem for farmed Atlantic salmon (*Salmo salar*) in Norway.

2 ) A viral aetiology of HSMI is strongly suspected.

## 6.3 Recommendations

The WGPDMO recommends that:

i ) an update on HSMI should be included in the national reports on new disease trends to be reviewed by WGPDMO at its 2007 meeting.

## 6.4 References

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# 7 Produce an update of current information from ICES Member countries on the development of sea lice vaccines and management measures for sea lice control

A summary of recent research results on vaccine development against salmon louse (*Lepeophtheirus salmonis*) and a summary of current management measures for sea lice (*Lepeophtheirus salmonis, Caligus* spp.) were presented by E. Sterud, S. Jones and D. Bruno.

## 7.1 Effects of sea lice

Sea lice (*Lepeophtheirus salmonis, Caligus* spp.) constitute an economical problem for salmon farmers due to costs linked to control and treatment. Sea lice are also an ecological problem due to their negative effects on wild salmonids, i.e. Atlantic salmon (*Salmo salar*), Sea trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*). Sea lice can no longer be regarded as a health problem for farmed salmonids, due to low legal lice levels before treatment should be carried out.

## 7.2 Control measures

Biological control of sea lice is used (cleaner fish, species of wrasses). Pesticides are also necessary for lice control. Currently in use are pyrethroids (deltamethrin and cypermethrin) and emamectin benzoate. In Norway, chemical treatment is regionally coordinated through the "National Action Plan against Salmon Lice on Salmonids" implemented in 1997. In Canada (British Columbia), the management of salmon lice is part of the fish farmers "Fish Health Management Plan (FHMP)" which is a condition of license. The FHMP requires regular sea lice monitoring and counts submitted to the provincial regulatory agency. The use of pesticides for controlling sea lice is economically demanding, and ecologically negative. Resistance development against the chemicals used is also a potential problem. These are reasons why vaccines against sea lice are being developed.

## 7.3 Vaccines

### 7.3.1 Challenges when developing lice vaccines

The salmon louse (as an ectoparasite) is not very accessible to the immune system of its host. The interior of the parasite can, however, be reached by immune components in the host's blood and other tissues, which are ingested by the parasite. A model based on a cattle vaccine against ticks is used for development of a salmon louse vaccine. The cattle vaccine is based on a protein (Bm86) which is normally present in the digestive system of the parasite. The cattle develop antibodies against this protein, affecting the reproduction and digestive system of the ticks.

Vaccine development depends on a detailed knowledge of the salmon louse biology, so that the most appropriate biological processes in the parasite can be affected (reproduction,

digestion). Finding target antigens is a main challenge since few proteins in the louse (and the genes coding for them) have been characterised.

### 7.3.2 Working protocol

Salmon louse genes are localised by sequencing several thousand Expressed Sequence Tags (ESTs). Using microarray technology on these ESTs, the activity of thousand of genes can be studied simultaneously. The goal is to identify genes involved in the biological processes that the vaccine is intended to affect. The number of potential antigens mapped by these methods will be too high to study in clinical vaccine trials. The effect of potential target antigens is therefore tested using RNA interference where the expression of the antigens (proteins) is controlled by breaking the RNA translation necessary for protein production in the lice. These experiments are conducted on live salmon lice. The RNA interference studies will reduce the number of potential antigens significantly. Only those antigens whose blocking is observed to have serious impact on the lice will be used for clinical trials.

### 7.3.3 Current status

In Norway, trials of test vaccines based on proteins isolated from salmon louse eggs have shown significant positive results. In Canada, the National Research Council in collaboration with a Canadian vaccine manufacturer has identified and patented several potential antigens to be included in a recombinant sea lice vaccine and the efficacy of some of the antigens have been tested in lab trials.

### 7.3.4 Future work

In Norway, the Institute of Marine Research has initiated a new project (2006–2008) to increase the number of potential antigens to be included in a vaccine and to test these antigens by RNA interference.

## 7.4 Management measures

In Norway, Canada and Ireland there are official limits on the number of allowed sea lice on farmed fish before chemical treatments should be carried out (e.g. 0.5 adult female louse per fish in Norway, 3 mobile stages per fish in British Columbia, Canada). In the latter, the average time to first treatment of smolts is 266 days and on average, salmon are treated 1.5 to 1.75 times per production cycle. In Norway, biological control by using cleaner fish is commonly used the first year after sea transfer. Thereafter, pesticides are necessary.

In Norway there is a regional coordination of chemical treatment through the “National Action Plan against Salmon Lice on Salmonids”. This plan was implemented in 1997 and a review of its effect has recently been published (Heuch *et al.*, 2005). The authors conclude that there are now fewer salmon lice attacking wild salmon smolts during their migration to the open ocean in spring than a few years ago. Smolts of sea trout and sea-run arctic charr which spend all their marine phase in the fjords, still suffer from serious lice attacks. Premature return to fresh water is a consequence of excessive lice levels. The upper allowed lice limits should be much lower than today’s 0.5 adult female lice per fish. Even with a limit at 1/10 of today’s limits, the volume of the Norwegian salmon farming (450,000 tonnes a year) is probably high enough that physiological or ecological effects are likely to occur (Heuch *et al.*, 2005).

## 7.5 Conclusions

1 ) The presence of sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) in Atlantic salmon (*Salmo salar*) farms constitutes an economic problem for the farmers and an ecological problem due to a possible negative impact on wild salmonids.

2 ) Promising trials on Atlantic salmon using recently developed sea louse vaccines are currently being carried on both in Canada and Norway.

## 7.6 Recommendation

The WGPDMO recommends that:

i ) Progress of salmon sea louse vaccine development and sea louse management strategies made in ICES Member Countries should be reviewed by WGPDMO at its 2007 meeting.

## 7.7 References

Heuch, P.A., Bjørn, P.A., Finstad, B., Holst, J.C., Asplin, L., Nilsen, F. 2005. A review of the Norwegian "National Action Plan against Salmon Lice on Salmonids": The effect on wild salmonids. Aquaculture, 246: 79–92.

# 8 The effects of climate change on diseases of marine fish and shellfish

A multi-authored report on the effects of long-term climate change on the spatial distribution and prevalence of disease in fish and shellfish was presented by S. W. Feist.

Recent evidence has predicted that climate change is inevitable and that alterations in terrestrial and aquatic environments are likely to occur (Harvell *et al.*, 1999; 2002; 2004; Lafferty *et al.*, 2004; McCallum *et al.*, 2004). At the local level, these changes have the potential to be dramatic. In the marine environment, it is anticipated that climate change will precipitate alterations in salinity, temperature, current flow rate and direction, and lead to more extreme weather conditions.

Temperature is likely to be the most important factor in determining the impact of climate change on hosts and disease agents. Directly, temperature influences host physiology, virulence of pathogens, abundance and distribution of parasites, and therefore the general dynamic of the host-parasite relationship. Indirectly, change in temperature would be expected to lead to changes in species interactions, including predator-prey and host-parasite interactions, which will affect transmission dynamics. Depending on specific interactions, the effect of climate change could be to increase or decrease disease prevalence or intensity.

There are many different permutations of the effects of climate change on the prevalence and distribution of disease in fish and shellfish. When evaluating effects on fish diseases, the types of anticipated environmental changes noted above necessitate consideration of effects on fish distributions, prey type, habitat conditions, as well as on pathogens.

Potentially important to climate change effects on disease prevalence in fish and shellfish are regime shifts in marine ecosystems. Defined as a rapid shift or reorganisation from one relatively stable ecosystem to another type, regime shifts have been associated with climate changes and other factors. These shifts may be significant in altering, for example, the nutritional and physiological conditions of hosts, making them more or less susceptible to disease; the distribution of host species, extending or restricting their ranges and exposing them to novel disease agents or increased concentration of pathogens, and the composition of prey species that may serve as vectors or intermediate hosts of pathogens and parasites.

Current evaluations of effects of climate change on fish disease trends are mostly hypothetical due to the lack of decadal scale fish disease data and corresponding environmental data. However, data held in the ICES Environmental Database for the northeast Atlantic offer the potential to detect long-term trends in environmental conditions and the association with fish diseases.

There is a significant amount of long-term data on host-parasite relationships in molluscs, and the effects of temperature and salinity on most molluscan host-parasite relationships are well documented. Both laboratory and field in vivo experiments confirm strong dependence of

infection levels on salinity and temperature. An example is the expansion of the known range of *Perkinsus marinus*, a warm water parasite, in eastern oyster (*Crassostrea virginica*) from the southern US to the northeastern US during periods of pronounced winter warming. Therefore, climate change that results in shifts in temperature and salinity is likely to affect the distribution of many molluscan diseases.

An assessment of the likely impact of climate change on the occurrence of disease in the marine environment is extremely complex. It requires an understanding of the behaviour of marine fish and shellfish under changing environmental conditions and the same for pathogenic organisms and their intermediate hosts. Knowledge on the interactions between species is also required. Consequently, evaluation of disease effects in a changing environment needs to be considered as an ecosystem component, using modelling approaches similar to those currently applied to marine fisheries.

Predicting the nature of the future health of marine organisms offers the potential to manage changes, e.g. by altering harvest practices in wild stocks. Furthermore, areas requiring research will be identified, such as:

- diseases likely to increase or decrease in importance,
- potential threats to new mariculture species,
- potential threats to resident native fish and shellfish stocks.

## 8.1 Conclusions

1 ) From information currently available there is indication that climate change has an effect on the health status of marine organisms, such as host physiology, virulence of pathogens and abundance and distribution of pathogens. The effect may be to increase or decrease disease prevalence or intensity and distribution of diseases.

## 8.2 Recommendations

The WGPDMO recommends that:

i ) The document on effects of climate change on diseases of marine fish and shellfish reviewed by WGPDMO at its 2006 meeting be expanded by the authors during the intersession and submitted for publication. Suggestions for aspects to be added to the manuscript are:

- identification of data types necessary to confidently state the effect of climate change on fish diseases,
- identification of criteria necessary for linking climate change with changes in disease status, (suggestions were: accessibility of long-term data; emphasis on non-commercial finfish species; correlated climatic and population trends; identification and exclusion of other factors affecting population size or disease status; knowledge on the effect of temperature on hosts and pathogens),
- information on vibriosis in cod (*Gadus morhua*), M74 in salmonids (Baltic salmon, *Salmo salar*, and sea trout, *Salmo trutta*) and lobster (*Homarus americanus*) shell disease as possible examples,
- analysis of North Sea dab (*Limanda limanda*) and molluscan diseases.

## 8.3 References

Harvell, C. D., Kim, K., Burkholder, J. M., Colwell, R. R., Epstein, P. R., Grimes, D. J., Hofmann, E. E., Lipp, E. K., Osterhaus, A. D. M. E., Overstreet, R. M., Porter, J. W., Smith, G. W., Vasta, G. R. 1999. Emerging marine diseases – climate links and anthropogenic factors. Science, 285:1505–1510.

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S.; Samuel, M. D. 2002. Climate warming and disease risks for terrestrial and marine biota. Science, 296: 2158–2162.

Harvell, C. D., Aronson, R., Baron, N., Connell, J., Dobson, A., Ellner, S., Gerber, L., Kim, K., Kuris, A., McCallum, H., Lafferty, K., McKay, B., Porter, J, Pascual, M., Smith, G., Sutherland, K., Ward, J. 2004. The rising tide of ocean diseases: unsolved problems and research priorities. Frontiers in Ecology and the Environment, 2: 375–382.

Lafferty, K. D., Porter, J. W., Ford, S.E. 2004. Are diseases increasing in the ocean? Annual Review of Ecology and Systematics, 35: 31–54.

McCallum, H. I., Kuris, A., Harvell, C. D., Lafferty, K. D., Smith, G. W., Porter, J. 2004. Does terrestrial epidemiology apply to marine ecosystems? Trends in Ecology and Evolution, 19: 585–591.

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

## 9.1 The REGNS Integrated Assessment of the North Sea Ecosystem (REGNS)

W. Wosniok gave a report on the REGNS workshop (May 9–11, 2005) and the subsequent activities of the WGPDMO related to the REGNS project. The REGNS project aims to produce an integrated assessment of the North Sea status. ICES Expert Groups had been requested to contribute to this activity by submitting data or (initially) descriptions of data that were considered useful for the REGNS purpose. The information provided in response to this request was reviewed during the workshop and groups that had offered data were subsequently asked to submit their data, as this had not already happened. On behalf of the WGPDMO, seasonally adjusted data on the prevalence of the major fish diseases of dab (*Limanda limanda*) in selected ICES statistical rectangles was submitted to REGNS by W. Wosniok. Details on further processing including preliminary results can be found in the REGNS Workshop and the REGNS Study Group meeting report for 2005 (ICES 2005). A central part of these results is a “traffic light” display of the temporal change exhibited by the quantities that were included in the analysis. For unknown reasons, the fish disease data do not appear there.

In the discussion it was pointed out that the “traffic light” display is a reasonable approach to summarise changes. However, this type of display is still of descriptive nature, and not yet a qualitative assessment, even though the green/yellow/red colour coding might suggest that. Similarly, the temporal variation of the first principal component, which is interpreted in the REGNS report as signal for a “regime shift”, is a summary description (see Figure 4 of the REGNS Report 2005; ICES 2005). A determination of whether the observed changes constitute a matter of concern has still to be done.

The WGPDMO discussed how the future REGNS activities could be supported. It was decided to maintain the spectrum of parameters to supply, but to update the corresponding time series according to the presently available fish disease data. Further, the Chair of REGNS should be contacted by W. Wosniok to identify obstacles that had prevented the inclusion of fish disease data into the analysis in the past, and to modify the future data submission, where necessary.

### 9.1.1 Recommendations

The WGPDMO recommends that:

i ) the Chair of REGNS should be contacted by W. Wosniok to identify obstacles that had prevented the inclusion of fish disease data into the analysis carried out by REGNS in the past and to define the required data structure for the submission of updated data;

ii ) the WGPDMO submits updated data on fish disease prevalence trends to REGNS (diseases: lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations, X-cell gill disease; species: *Limanda limanda*; gender; female; size: 20–24 cm; spatial resolution: ICES statistical rectangles; temporal resolution: months; adjusted for seasonal variation).

### 9.1.2 References

ICES. 2005. Report of the Regional Ecosystem Study Group for the North Sea. ICES CM 2005/D:08, 45 pp.

## 9.2 The Baltic Sea Regional Project (BSRP)

T. Lang provided a report on progress made with regard to the Baltic Sea Regional Project (BSRP), in particular in relation to its Ecosystem Health component and to the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM).

The workshop was held 5–12 December 2005 on board the German RV 'Walther Herwig III' (see Annex 6) and started and ended in Gdynia, Poland. The workshop was attended by scientists from Estonia, Finland, Germany, Latvia, Lithuania, Poland, Russia, Sweden and the UK.

According to ICES Council Resolution 2005/2/BCC02, the objectives of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring on the Baltic Sea (WKFDM) were:

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

The workshop consisted of practical work and training in five areas in the southern Baltic Sea, encompassing sampling sites in German, Polish and Lithuanian waters, with flounder (*Platichthys flesus*), cod (*Gadus morhua*) and herring (*Clupea harengus*) as target fish species. The workshop also included theoretical work, addressing various aspects relevant to fish disease monitoring, data assessment and quality assurance (e.g. BEQUALM activities).

In the discussion it was emphasised that the workshop constituted an important step forward in standardising methodologies applied in fish disease monitoring in the Baltic Sea, a prerequisite for comparability of data obtained in national monitoring programmes and for the incorporation of fish disease data into internationally coordinated monitoring and assessments programmes, e.g. as part of the HELCOM monitoring.

WGPDMO endorsed the recommendations made by WKFDM that the WKFDM report and its recommendations are communicated to relevant national ministries/agencies responsible for monitoring the environmental status of the Baltic Sea and to international organisations (HELCOM, OSPAR, EU, EEA). It was furthermore emphasised that fish disease studies in the

Baltic Sea should be part of an integrated ecosystem health monitoring and assessment programme, encompassing studies on biological effects of contaminants (biomarker approach), eutrophication, biodiversity, physical and chemical measurements, and methods applied in fish stock assessment.

WGPDMO supported the idea to organise

- an international ICES/BSRP/HELCOM sea-going demonstration project on the ecosystem health of the Gulf of Finland (scheduled for 2007 or 2008) and
- a land-based ICES/BSRP/HELCOM workshop on monitoring of diseases and parasites in coastal fish species (scheduled for 2006 or 2007, venue suggestions: AtlantNIRO, Kaliningrad, or the Estonian Marine Institute, Tallinn) in the context of the HELCOM coastal fish monitoring.

### 9.2.1 Conclusions

1 ) The ICES/BSRP Sea-going Workshop on Fish Diseases Monitoring in the Baltic Sea (WKFDM) is considered an important step in the attempts to standardise methodologies for fish disease monitoring in the Baltic Sea.

2 ) Further activities, e.g. the planned ICES/BSRP/HELCOM workshops on the health of the Gulf of Finland ecosystem and on diseases and parasites of coastal fish species, will strengthen the collaboration between Baltic Sea Countries in the context of ecosystem health monitoring and assessment and will help to establish relevant guidelines and quality assurance protocols.

### 9.2.2 Recommendations

The WGPDMO recommends that:

i ) an international ICES/BSRP/HELCOM sea-going demonstration project on the ecosystem health of the Gulf of Finland (scheduled for 2007 or 2008) and a land-based ICES/BSRP/HELCOM workshop on monitoring of diseases and parasites in coastal fish species (scheduled for 2006 or 2007; venue suggestions: AtlantNIRO, Kaliningrad, or the Estonian Marine Institute, Tallinn) in the context of the HELCOM coastal fish monitoring be organised. The planning and organisation of both activities should involve the ICES Study Group on Ecosystem Health Issue in support of the BSRP (SGEH), with contributions from other relevant ICES Expert Groups, e.g. WGPDMO, WGBEC, MCWG and WGMS.

## 9.3 BEQUALM

S.W. Feist presented a progress report on the programme Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM), a self-funding activity aiming at implementing a QC/QA programme for techniques for measuring biological effects of contaminants (http://www.bequalm.org/about.htm).

The primary objective of the BEQUALM Fish Disease Measurement component is to establish a quality assurance programme in the accurate diagnosis and reporting of externally visible diseases and liver pathology in fish used in monitoring programmes.

The work programme for 2004–2005 involved the distribution of training/reference materials, including a CD-ROM containing comprehensive guidance with images of fish diseases of target species used in monitoring. Intercalibration exercises and a full histopathology ring-test were also undertaken during 2005. Intercalibration exercises consisted of a series of electronic images for which participants were asked to identify the lesion types present. Feedback was provided as part of the on-going training. The ring-test consisted of 25 histopathology slides that were sent to all participants. Participants were required to diagnose the principal pathologies present according to the guidelines provided in the ICES TIMES paper. The ring-

test has now been scored and feedback on lesion diagnosis will be provided at the 1st Cefas Histopathology Training Workshop, 20–24 March, 2006 (Cefas Laboratory, Weymouth, UK). Ring-test results will be provided individually to participating laboratories shortly thereafter.

Two full days of this workshop will be dedicated to BEQUALM giving participants the chance to provide feedback on aspects of the BEQUALM Fish Disease Measurement programme with a view to improving the quality and scope of this BEQUALM component.

The planned work programme for 2006 is as follows:

| | |
|---|---|
| April 2006: | Distribution of reference materials |
| May 2006: | Intercalibration exercise 1 |
| June 2006: | Full slide ring test initiated |
| August 2006: | Intercalibration exercise 2 |
| November 2006: | Intercalibration exercise 3 |
| December 2006: | Deadline for ring test |
| February 2007: | Summary report of the year's results. |

### 9.3.1 Recommendations

The WGPDMO recommends that:

i ) laboratories in ICES Member Countries studying diseases and liver histopathology in wild fish as part of national and international environmental monitoring and assessment programmes take part in the relevant component of the Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) project in order to achieve quality assurance regarding methodologies applied and data generated.

## 10 Propose a set of diagnostic techniques for the identification and characterisation of microcell parasites in oysters

A working document was presented by T. Renault. The document (Annex 7) reviewed the taxonomy, distribution and impact of microcell parasites on mollusc stocks, as well as criteria and diagnostic techniques for the identification and characterisation of these parasites. Research into developments needed to improve knowledge of microcell parasites, with special emphasis on diagnostic methods, was identified.

Diagnosis of a particular microcell parasite needs to include information on known susceptible host species, epizootiology and disease progression patterns, as well as morphological identification criteria and molecular characteristics. The *Manual of Diagnostic Tests for Aquatic Animals* (OIE 2003) recommends histology as the principal screening method for examining tissues of molluscs in abnormal mortality episodes, as well as when performing regular surveillance. Histology can be supplemented by tissue imprints or PCR, although the latter is not specifically mentioned in the OIE diagnostic methods for microcells. Nevertheless, routine application of molecular techniques for diagnosing microcell parasites might be a possibility in the future. At the present time, however, problems remain with the use of molecular diagnosis. For instance, closely related microcell species, including *Bonamia ostreae* and *B. exitiosa*, present high sequence similarities and not all regions of parasite DNA are useful as targets for molecular detection. Testing has not yet been undertaken to determine the most appropriate tissues for analysis, especially for early infections. Moreover, molecular tools detect DNA and not necessarily a true infection.

The working document contains a table of recommended diagnostic techniques for the three tiers of examination procedures currently proposed by OIE: routine surveillance and presumptive and confirmatory diagnoses in cases of abnormal mortality. The current OIE procedure is to use histology or tissue imprints for surveillance and electron microscopy for confirmatory diagnosis of molluscan parasites, including microcells. In known susceptible species within the known geographical range of *B. ostreae*, the only recommended method is tissue imprints for a suspect case of *B. ostreae* infection..In other host species or outside the geographical range of *B. ostreae*, the recommended method is histopathology. Transmission electron microscopy and PCR-RFLP are further recommended for a confirmatory diagnosis and discrimination among species.

## 10.1 Conclusions

1 ) Criteria used to identify microcell species should include basic biological and ecological characteristics of the parasite, as well as information on its genetic sequence.

2 ) There is a need for refinement and validation of existing molecular techniques and development of new ones.

## 10.2 Recommendations

The WGPDMO recommends that:

i ) ICES Member Countries be encouraged to use international standards proposed by the OIE with the inclusion of molecular methods for microcell parasite identification;

ii ) ICES Member Countries be encouraged to support the funding of research into improved methods of detecting and identifying microcell parasites due to their worldwide distribution and importance;

iii ) laboratories involved in microcell research initiate collaborative testing, intercalibration and validation of current and newly developed techniques for the purpose of recommending improved techniques to differentiate among microcell parasites;

iv ) WGPDMO be kept informed on progress made in developing microcell research programmes

v ) WGPDMO produce a review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves for its 2007 meeting;

## 10.3 References

OIE. 2003. Manual of Diagnostic Tests for Aquatic Animals. OIE, Paris, 4th edition. (http://www.oie.int/eng/normes/fmanual/A_00036.htm )

# 11 Produce a review on the current status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries

A working document was presented by T. Renault. The document (Annex 8) emphasized the recent increase in the use of hatchery-produced bivalve seed and reviewed existing information on viral and bacterial diseases in larval and juvenile bivalves reared in hatcheries and nurseries. The aim was to

- assess the available information in this area;
- highlight the need to assess the risk of pathogen transfer among hatcheries and nurseries, and between hatcheries/nurseries and field grow out sites;
- provide a list of relevant references.

Strains selectively bred for rapid growth and disease resistance are becoming increasingly useful in molluscan aquaculture. Hatcheries are the only source of such animals, leading to the expectation of increased intra- and international trade in bivalve gametes, larvae and seed, and the possibility that disease agents might be transferred at the same time. Most infectious agents identified in hatchery and nursery disease outbreaks are viral or bacterial. Such agents are of particular concern since no specific chemotherapies or vaccines are available. Some infectious disease episodes in hatcheries and nurseries are traced to opportunistic pathogens, which are also present in the wild, and that proliferate under the high-temperature, high-stocking densities typical of hatcheries and nurseries. Consequently, disease can be controlled by improved animal husbandry. The possibility exists that virulent pathogens could be transferred among hatcheries and then to wild stocks when hatchery-produced molluscs are transferred to growout sites. The risk of this to happen should be assessed in a scientific fashion. Such an assessment should include any historical evidence for such transfers in molluscan aquaculture, similar evidence from other aquacultured species, and new studies of potential pathogens in molluscan hatcheries and nurseries.

## 11.1 Conclusions

1 ) There is a significant amount of data available on viral and bacterial pathogens infecting bivalves in hatcheries and nurseries.

2 ) There is need for a risk assessment of the potential threat of transferring pathogens among hatcheries/nurseries and from there to field growout sites.

## 11.2 Recommendations

The WGPDMO recommends that:

i ) further studies be undertaken in ICES Member Countries to identify bivalve pathogens present both in hatcheries/ nurseries and in the field;

ii ) ICES Member Countries be encouraged to support funding of research for assessing the risk of disease for bivalves in growout sites associated with the use of hatchery products.

# 12 Conduct a pilot study assessing the feasibility of constructing a 'disease index' using disease data from North Sea dab (*Limanda limanda*)

W. Wosniok and T. Lang reported on progress in the construction of a Fish Disease Index (FDI) that could be used to demonstrate spatial and temporal patterns in disease prevalence (Annex 9).

Indices help simplify complex and diverse data. Measures of changes in environmental quality are becoming more important, and consequently, fish health/disease indices are becoming more desirable as indicators of ecosystem health.

Most fish disease data collected include data on intensity or severity in addition to prevalence data. It is recognized that changes in severity may be as significant, or perhaps more so, than changes in prevalence. Similarly, many diseases may affect fish; however, some diseases may impact individual fish more than other diseases. It is known that prevalence of disease varies with length, age and gender.

Taking all these factors into consideration and using data from the North Sea dab, an FDI was constructed that included what could be reasonable for scoring disease intensity, weighting the significance of those diseases, and adjustments for length and gender. FDIs generated for four hypothetical specimens of dab were presented to the group.

The next steps in the development of the FDI will be to address which diseases to include in the model, how to assign weights for various diseases and how to deal with missing values.

The WGPDMO is very supportive of this project and is interested in seeing it developed further.

## 12.1 Conclusions

1 ) A Fish Disease Index can be a useful means of simplifying complex fish disease data for use in fish health and ecosystem health assessments.

2 ) Due to the importance of information on disease intensity/severity, such data should be submitted to the ICES fish disease databank. Guidelines for data submitters are in preparation and will need to be incorporated into BEQUALM guidelines (see report Section 15).

## 12.2 Recommendations

The WGPDMO recommends that:

i ) the pilot study on constructing a 'Fish Disease Index' by using empirical dab (*Limanda limanda*) disease data should be continued by selected WGPDMO members and progress made be reviewed at the 2007 WGPDMO meeting.

ii ) for establishing a 'Fish Disease Index' for dab (*Limanda Limanda*), a consensus should be achieved among dab pathologists of a scale for scoring disease intensity (grades) and for weighting significance of various diseases;

# 13 Review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species and review selection of species, gender and size ranges (WKIMON)

This agenda item was dealt with by WGPDMO intersessionally as input to the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON) that took place during 17–19 January 2006 at ICES Headquarters, Copenhagen Denmark, and was attended by the WGPDMO members T. Lang, W. Wosniok and A.D. Vethaak. A summary is given below.

Externally visible disease studies are being conducted by ICES Member Countries in a variety of fish species, including dab (*Limanda limanda*), flounder (*Platichthys flesus*) and cod (*Gadus morhua*) and WGPDMO emphasised that methodologies are easily adaptable for other species such as whiting (*Merlangius merlangus*) and haddock (*Melanogrammus aeglefinus*). Methodologies and diagnostic criteria involved in the monitoring of contaminant-specific liver nodules and liver histopathology have largely been developed based on studies with flatfish species, in Europe mainly dab and flounder, but can also be adapted to other flatfish species (e.g. plaice (*Pleuronectes platessa*) or long rough dab (*Hippoglossoides platessoides*)) and possibly also to bottom-dwelling roundfish species, such as dragonet species (*Callionymus spp*.) or viviparous blenny (*Zoarces viviparus*).

However, there is no way to directly compare disease prevalence data derived from studies using different fish species because:

- species recommended in the OSPAR JAMP Guidelines for Contaminant-Specific and for General Biological Effects Monitoring have different diseases (except a few cases),
- even if the same diseases (would) occur, a comparison between species is not possible because species are characterised by marked variation in disease prevalence, possibly reflecting differences in disease susceptibility.

One way to overcome this problem is to develop synthetic disease indices (e.g. deviation from a natural background/reference prevalence or from long-term trends) as assessment tools. The use of indices would also offer the advantage that information on a large set of diseases in an individual can be summarised in one (or a few) quantitative figure(s) to display the general health status.

It was concluded that techniques for monitoring externally visible diseases and liver pathology are applicable across the OSPAR maritime area. However, since fish species monitored for diseases are partly affected by a great number of externally visible diseases/parasites and histopathological liver changes, and since species and diseases may differ between geographical regions of the OSPAR area, assessment tools (health/disease indicators and indices) have to be developed facilitating regional comparisons (see Section 12 of the present reports on fish diseases indices).

Table 13.1 provides information on fish species suitable for fish disease monitoring and on selection of gender, size ranges and sample sizes.

**Table 13.1: Fish species suitable for fish disease monitoring and selection of gender, size ranges and sample sizes.**

| DISEASE | SPECIES | GENDER | SIZE RANGE (CM TOTAL LENGTH) | SAMPLE SIZE |
|---|---|---|---|---|
| Externally visible diseases | Dab (*L. limanda*) | Females + Males | 15-19<br>20–24<br>≥ 25 | 100<br>100<br>50 |
| | Flounder (*P. flesus*) | Females + Males | 20–24<br>25–29<br>≥ 30 | 100<br>100<br>50 |
| | Cod (*G. morhua*) | Females + Males | < 29<br>30–44<br>≥ 45 | 100<br>100<br>50 |
| | Whiting (*M. merlangus*) | Females + Males | 15–19<br>20–29<br>≥ 30 | 100<br>100<br>50 |
| Liver Nodules > 2 mm | Dab (*L. limanda*) | Females+ Males | 20–24 cm<br>≥ 25 | 50<br>50 |
| | Flounder (*P. flesus*) | Females+ Males | 25–29<br>≥ 30 | 50<br>50 |
| Liver Histopathology | Dab (*L. limanda*) | Females | 20–24 | 30–50 |
| | Flounder (*P. flesus*) | Females | 25–29 | 30–50 |
| | Dragonet (*C. lyra*) | Females | 10–15 | 30–50 |

## 13.1 Conclusions

1 ) The WGPDMO strongly supports the development of strategies and guidelines for integrated chemical and biological effects monitoring for the OSPAR CEMP/JAMP and emphasised the need to incorporate studies on diseases of wild fish stocks as indicators of ecosystem health. The development of fish disease indices, summarising information on the occurrence and severity of a set of diseases into one robust figure, will help to establish assessment tools that can be used both on a regional and convention-wide basis.

# 14 Produce updated ICES publications on pathology and diseases of marine organisms

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

W. Wosniok reviewed the status of the web-based information system on diseases and parasites of fish and shellfish on the ICES website (http://www.ices.dk/marineworld/fishdiseases/fishandshellfish.asp). Data on diseases of dab from the North Sea between 1981 and 2005 have been compiled from the ICES Data Centre. Submitted data are used for mapping species and disease trend information in ICES statistical rectangles. Clicking on symbols identified within rectangles will in the future provide access to available trends that are represented by predicted values along with associated confidence intervals. Trend analysis requires a minimum of 5 to 10 years of data that further satisfies size and gender conditions. Information on fish species, diseases and on the statistical modelling employed will be added to the ICES website. The website will also link to further pages depicting images of the disease conditions under analysis. Fish species and data processing will also be described. A more detailed presentation of maps and the associated trend analyses are provided in Annex 10.

The website provides for each disease and ICES statistical rectangle trend information, as far as the available data allows to do so. It contains for each disease a map with symbols for

recent trends (up/down/constant), and, as links originating from the map, displays of season-adjusted trend curves as well as descriptions of the species involved (so far only dab, *Limanda limanda*), the diseases, the database, and the method used for season adjustment.

The need for updates to the maps depicting the geographical distribution of selected fish and shellfish pathogens (VHS in wild fish; *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*; *Perkinsus marinus* in *Crassostrea virginica*) was discussed. Revisions of maps are required for marine VHS virus as well as for shellfish diseases. T. Wiklund (Finland) volunteered to take responsibility for coordinating this process. The possibility that new maps are posted was also discussed, and that this process may be linked to the production of revised disease leaflets. Researchers working on a disease or pathogen should preferably be involved in the preparation of the maps.

### 14.1.1 Recommendations

The WGPDMO recommends that:

i ) the report on disease trends in dab (*Limanda limanda*) from the North Sea and adjacent areas and the distribution maps for VHS virus and shellfish diseases published on the ICES website is updated according to the suggestions made;

ii ) the calculation of trends and subsequent update of the maps on the ICES website should be taken over by the ICES Data Centre (see Section 15.3 of the 2006 WGPDMO report). The WGPDMO will provide a template for the website structure and support in statistical issues, if needed.

## 14.2 ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish

S. W. Feist, the editor, updated members on the leaflets. Many leaflets need updating or complete revision. Scanning original leaflets into an electronic format will permit their archiving on the ICES website. These will be deleted from the archive following the availability of a revised leaflet. Revised leaflets will retain their original ICES number with a new date. The decision to revise a leaflet will be based on its importance as determined by the editorial sub-group of the WGPDMO. Wherever possible, original authors will be retained for the revisions. If this is not possible, revisions will be completed by a competent authority designated by the WGPDMO sub-group.

The editor will inform WGPDMO on progress made at its 2007 meeting.

## 14.3 WGPDMO website

T. Lang reviewed the status of the WGPDMO website. No work has been conducted on this issue during the last year and his institution, the Federal Research Centre for Fisheries, Germany, may not now be in a position to host this web site. Since a substantial amount of information generated by WGPDMO is available on the ICES website, there was consensus that at present there is no need to maintain a WGPDMO website.

## 14.4 Publication in the TIMES series

W. Wosniok discussed the status of the manuscript on statistical methods for the analysis of fish disease data to appear in the *ICES TIMES* series. Work is progressing towards a more practical-orientated approach rather than a theoretical one. The manuscript will be submitted to ICES as soon as possible.

## 14.5 Other Publications

Several potential publications were recognised as deriving from WGPDMO intersessional activities or Terms of Reference. A preference would be given to publish in ICES publication

series (e.g. the ICES Journal of Marine Science). However the journal would depend on the target audience. Some examples of publications considered included:

- The Effects of Climate Change on Marine Fish and Shellfish – ICES J Mar Sci suggested;
- Fish Disease Index publication – ICES Times Series;
- Documents prepared to address WGPDMO Terms of Reference. Suitability for adaptation to submit for publication will be decided by WGPDMO members.

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

A subgroup of the WGPDMO (D. Bruno, T. Lang, S. Feist, W. Wosniok) together with M. Sörensen (ICES Data Centre) discussed problems that had emerged in the context of fish disease data submission and its processing by ICES.

## 15.1 Type of information submitted using the ICES Environmental Data Reporting Format 3.2

The following decisions with regard to the type of information that is to be supplied as part of fish disease data in Reporting Format 3.2 were made:

- Recommended fields were reduced to SEXCO, FINFL, STATN, NODIS, REFSK
- Recommended parameters were reduced to LNMEA since length should always be reported in centimetres, not as a length class (the latter would require LNMIN and LNMAX).

Submitted data generated for the purpose of the OSPAR CEMP/JAMP and according to standardised ICES procedures should be marked as data for OSPAR-CEMP by setting "MPROG" to "CEMP". This generates a need to officially define station names for OSPAR in a station dictionary. This is under development with the OSPAR-ICG-DOME sub-group. The actual state of development can be found under www.ices.dk/env/commissions/ospar/Station_Name_Project/.

Experience with recent data submissions had shown that submitters followed different interpretations of the 3.2 Format when coding their data. The WGPDMO subgroup decided that data submission should follow the example given in the Section 15.2. Also, it was agreed that the ICES Data Centre should generate a summary of the disease prevalence reported by each data submission as a plausibility check (see report Section 15.3).

## 15.2 Example for the submission of fish disease data in the ICES Environmental Data Reporting Format 3.2

### 15.2.1 General rules

- Each fish (or pool of identical fish, see below for conditions that allow pooling) must have a unique identification. A fish is identified by the combination of CRUIS/STNNO/SMPNO/SUBNO, where typically "CRUIS" is the cruise identification, "STNNO" is the station number, "SMPNO" is the haul number, and "SUBNO" is the number of an individual fish. The station number must not be repeated when the same geographical position or area is visited a second time, instead a new number has to be used.
- Disease data should be reported either on the basis of individual fish or as pools of fish with all attributes (species, length in cm, sex, set of diseases examined, set of diseases present) identical. Pooling is not allowed if further measurements (e.g. chemistry, other biomarkers) are made on the members of a potential pool and these measurements are reported by a separate data submission.

- If pools are reported, the number of fish in the pool is given in the field "NOINP". For individual fish, the field "NOINP" contains a "1".
- Fish length has to be reported in full centimetres, not as a length interval, i.e. not as a size group.
- The presence/absence of a disease is coded in a type '10' record, in which the name of the disease is reported in the field "PARAM", and the presence/absence of the disease is coded by setting "VALUE" to 1 (presence) or 0 (absence). The field "MUNIT" must then contain "AFNR" to indicate that presence/ absence information is given. Disease grades cannot be reported if "MUNIT" = "AFNR". The next paragraph describes how to report disease grades.
- If disease grades are to be reported (new guidelines will soon be publicised), the grade of a disease is coded in a type '10' record, in which the name of the disease is reported in the field "PARAM", and the grade of a disease is coded by setting "VALUE" to the grade of the diseases. The field "MUNIT" must then contain "GRADE" to indicate that disease grades are reported.
- Type "10" records must be included only for those diseases that actually were examined.
- The number of diseases reported may be specific for each fish or pool of fish. This number must always be given in the field "NODIS".

### 15.2.2 Example: Reporting prevalence information

The following data example describes a part of a hypothetical fish disease data submission. The first number in each line denotes the record type, where the biota specimen record (type '04') is the start of the information for an individual fish (or a pool of fish with all attributes identical). Commas separate data fields. Note that the information for record '91' is split over two lines because of its length. The example below describes information for 7 fish, where the first two data blocks, each headed by a '04' record, describe individual fish which each were examined for 3 diseases, while the third block describes a pool of 4 fish which were examined for 2 diseases and the fourth block describes again one fish, but here disease grades are reported instead of the pure presence/ absence information which was given for the first 6 fish. All fish were *Limanda limanda*. The values of 'NOINP', 'SEXCO', 'NODIS', 'VALUE' are shown here in boldface for easier identification. The individual attributes reported by the data lines below are:

**fish 1:** male, length 17 cm, examined for lymphocystis (found present), skin ulcerations (absent) and epidermal hyperplasia / papilloma (absent),

**fish 2:** female, length 18 cm, examined for lymphocystis (found present), skin ulcerations (absent) and epidermal hyperplasia / papilloma (present),

**fish 3-6:** females, length 21 cm, examined for lymphocystis (found present), skin ulcerations (absent)

**fish 7:** female, length 23 cm, examined for lymphocystis (absent), skin ulcerations (grade 3 found) and epidermal hyperplasia / papilloma (grade 1 found).

```
00,DOUK,74,2004,3.2,,,,,,,,,,,,,,
20,DOUK,1,GRT,,,,4,,,,,,,,,,,
21,1,DOUK,,T19,NA,NON,NA,,,GRS,,,,,,,,,
90,74E9,CEND7/04,DEFRA,,,,,,,,,,,,,,,,
91,CEND7/04,77,+5519.234,-
                  00115.123,HLS,20040709,1358,1425,63,AMBLE,CEMP,MO,RH,,,,,,
03,CEND7/04,77,CF,1,1,,1,LIMANDA LIMANDA,ITLN,RS,,,,,,,,,
04,CEND7/04,77,1,1,1,OW,M,NS,,K,3,,,,,,,,,
10,CEND7/04,77,1,1,WO,,,LNMEA,CM,,,,,17,,,,,,
10,CEND7/04,77,1,1,EP,,,LYMP CYS,AFNR,,,,,1,,,,,,
10,CEND7/04,77,1,1,EP,,,SKIN ULC,AFNR,,,,,0,,,,,,
10,CEND7/04,77,1,1,EP,,,EPID PAP,AFNR,,,,,0,,,,,,
04,CEND7/04,77,1,2,1,OW,F,NS,,K,3,,,,,,,,,
10,CEND7/04,77,1,2,WO,,,LNMEA,CM,,,,,18,,,,,,
10,CEND7/04,77,1,2,EP,,,LYMP CYS,AFNR,,,,,1,,,,,,
10,CEND7/04,77,1,2,EP,,,SKIN ULC,AFNR,,,,,0,,,,,,
10,CEND7/04,77,1,2,EP,,,EPID PAP,AFNR,,,,,1,,,,,,
04,CEND7/04,77,1,3,4,OW,F,NS,,K,2,,,,,,,,,
10,CEND7/04,77,1,3,WO,,,LNMEA,CM,,,,,21,,,,,,
10,CEND7/04,77,1,2,EP,,,SKIN ULC,AFNR,,,,,1,,,,,,
10,CEND7/04,77,1,3,EP,,,LYMP CYS,AFNR,,,,,0,,,,,,
04,CEND7/04,77,1,3,1,OW,F,NS,,K,3,,,,,,,,,
10,CEND7/04,77,1,3,WO,,,LNMEA,CM,,,,,21,,,,,,
10,CEND7/04,77,1,2,EP,,,SKIN ULC,GRADE,,,,,3,,,,,,
10,CEND7/04,77,1,2,EP,,,EPID PAP,GRADE,,,,,1,,,,,,
10,CEND7/04,77,1,3,EP,,,LYMP CYS,GRADE,,,,,0,,,,,,
```

## 15.3 Data products to be prepared by ICES

The WGPDMO discussed what kind of data products can be delivered by the ICES Data Centre based on the fish disease data submitted.

It was agreed that the ICES Data Centre should generate a summary of the disease prevalence reported by each data submission. This summary should contain the raw prevalence per disease, separately for each combination of station and disease, where “station” should be the station name given the variable “STATN” in the submission. Such a rough overview will serve as a plausibility check for the detection of incorrect information that might be contained in a formally correct file. Errors of the latter type could not be detected by the ICES screening programme (see www.ices.dk/datacentre/datsu).

Furthermore, ICES is requested to update the fish disease maps on the ICES website (http://www.ices.dk/marineworld/fishdiseases/fishandshellfish.asp) providing information on spatial and temporal patterns in the prevalence of selected diseases of dab (*Limanda limanda*) from the North Sea and adjacent areas. The WGPDMO has provided new information on the contents and layout of the maps and their accompanying information in Section 14.1 and Annex 10 of the present report.

## 15.4 Conclusions

1 ) There is a need to modify the fish disease data submitted to ICES using the Environmental Data Reporting Format 3.2. This related to the fields and parameters of the format, the reporting of individual or pooled data and the submission of disease grades. Guidance is given accordingly.

2 ) The WGPDMO requests ICES to provide some specified fish disease data products on a regular basis (preferably annually).

## 15.5 Recommendations

The WGPDMO recommends that:

i ) ICES Member Countries submitting fish disease data to the ICES Data Centre follow the instructions given by WGPDMO in Section 15 of the 2006 WGPDMO report;

ii ) ICES generates the following fish disease related data products:

- a summary of the disease prevalence reported by each fish disease data submission to the ICES Data Centre as plausibility check. This summary should contain the raw prevalence per disease, separately for each combination of station and disease, where "station" should be the station name given the variable "STATN" in the submission. (see above);
- yearly prepared maps for the fish disease report on the ICES website, indicating trends and spatial distribution of diseases in dab (*Limanda limanda*) from the North Sea and adjacent areas (also see Section 14.1 of the 2006 WGPDMO report;
- yearly updated trend figures to be used in combination with the dab disease maps (also see Section 14.1 of the 2006 WGPDMO report).

# 16 Potential WGPDMO contributions to an ecosystem overview advisory report describing the quantity and quality of marine habitat and/or the health in the marine ecosystem, and to consider and report on potential indicators of significant change in these ecosystem attributes

The WGPDMO discussed the possibilities for contributing sections on the health status of fish in the North Sea and the Baltic Sea for the ecosystem overviews of the ICES Advisory Report.

There was consensus that a it will be useful to prepare chapters on the health of fish and shellfish. As a start, it was suggested to prepare a chapter for the North Sea overview summarising the available data on the health status of dab (*Limanda limanda*) which is the most abundant flatfish species in the North Sea and is, therefore, of high ecological relevance. Additional information on certain commercial species could also be incorporated in the chapter.

A similar chapter can be prepared for Baltic Sea fish. However, concerns were raised regarding the ability to prepare such a report since the available data are scattered spatially and temporally.

Current initiatives within the WGPDMO on the development of a 'Fish Disease Index' (FDI) and temporal trend analysis of fish disease prevalence were considered to offer useful tools to detect significant changes in the ecosystems under review.

## 16.1 Recommendations

The WGPDMO recommends that:

i ) written documents are produced on the health status of fish in the North Sea (responsible: S. Feist, with input from other WGPDMO members) and in the Baltic Sea (responsible: T. Lang, with input from other WGPDMO members) as contributions to the ecosystem overview advisory report. It was noted that the chapters should be prepared by May 2006 before the meetings of the ICES Advisory Committees.

# 17 Any other business

## 17.1 WGPDMO working procedures

The WGPDMO felt that its working procedures can be further improved by holding parts of the meeting in thematic sub-groups. It was agreed that, at the 2007 meeting, three sub-groups will be created, whose responsibility will be to review the national reports on new trends in diseases of wild fish (sub-group 1), farmed fish (sub-group 2) and wild and farmed shellfish (sub-group 3) and to report back to plenum.

### 17.2 Nomination of a new Chair

Since the term of the present Chair T. Lang will expire at the end of the year, the WGPDMO had to elect a successor and unanimously agreed to nominate S. MacLean (USA) as new Chair. The outgoing Chair congratulated the incoming Chair and promised to provide any support needed. The WGPDMO thanked the outgoing Chair for his service over the past four years.

## 18 Analysis of progress with tasks

An analysis of progress of tasks in the Terms of Reference was conducted and it was concluded that almost all items had been dealt with in a satisfactory manner. Table 18.3 provides more information on items completed and those that require further action. Several intersessional tasks to be fulfilled prior to the 2007 WGPDMO meeting were identified.

**Table 18.1: Analysis of progress with task of WGPDMO's Terms of Reference for 2006.**

| 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 2007 as part of ToR a |
| b | update information on the causes and effects of Heart and Skeletal Muscle Inflammation (HSMI) affecting farmed salmon in ICES Member Countries; | will be revisited in 2007 as part of ToR a |
| c | produce an update of current information from ICES Member Countries on the development of sea lice vaccines and management measures for sea lice control; | will be revisited in 2007 as part of ToR b |
| d | compile a report on effects of climate change on host-pathogen interactions; | completed; a manuscript for publication will be produced by WGPDMO members |
| e | propose a set of diagnostic techniques for the identification and characterisation of microcell-type parasites in oyster species; | completed |
| f | produce a review on the current status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries; | completed |
| g | conduct a pilot study assessing the feasibility of constructing a 'disease index', using disease data from North Sea dab (Limanda limanda); | will be revisited in 2007 as part of ToR c |
| h | review progress made with regard to international collaborative actions including disease and pathology aspects: | on-going task; will be revisited in 2007 as ToR f |
| i | produce ICES publications on pathology and diseases of marine organisms; | on-going task; will be revisited in 2007 as ToR h |
| j | provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis; | on-going task; will be revisited in 2007 as ToR g |
| k | discuss and report on potential contributions for the ecosystem overview of the advisory reports describing the quantity and quality of marine habitat and/or the health of the marine ecosystem, and to consider and report on potential indicators of significant change in these ecosystem attributes; | completed; WGPDMO members will prepare written documents to be considered by the ICES Advisory Committees |
| l | review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species and review selection of species, gender and size ranges (WKIMON). | completed |

## 19 Future activities of WGPDMO

Since there are several issues of importance in the field of pathology and diseases of marine organisms requiring further consideration, it was agreed that a further meeting of WGPDMO is required in 2007 to consider the results of intersessional work and to discuss new and outstanding items.

Venue (suggestions: Tenerife, Spain, or St. John's, Canada) and dates still have to be decided.

## 20 Approval of recommendations

The recommendations to the ICES Council contained in this report were discussed by the WGPDMO and approved (Annex 11). The proposals and justifications for new Terms of Reference for the 2007 WGPDMO meeting are appended in Annex 12.

## 21 Approval of the draft WGPDMO report

The draft report of the 2006 WGPDMO meeting was approved before the end of the meeting and outstanding issue were identified and delegated to WGPDMO members. The conclusions from the Terms of Reference and associated annexes where information was specifically sought by or provided to other ICES bodies will be extracted and sent separately to ICES or the Chairs of relevant Working Groups.

## 22 Closure of the meeting

The Chair thanked the local host, ICES, for providing excellent meeting facilities and arrangements and the participants for their hard work and input during and in preparation of the meeting. The 2006 WGPDMO meeting was closed at 13:00 h on 11 March 2006.

# Annex 1: List of participants

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## Annex 2: WGPDMO Terms of Reference for 2006

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: T. Lang, Germany) will meet at ICES Headquarters, Denmark, from 7–11 March 2006 to:

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

b ) update information on the causes and effects of Heart and Skeletal Muscle Inflammation (HSMI) affecting farmed salmon in ICES Member Countries;

c ) produce an update of current information from ICES Member Countries on the development of sea lice vaccines and management measures for sea lice control;

d ) compile a report on effects of climate change on host-pathogen interactions;

e ) propose a set of diagnostic techniques for the identification and characterisation of microcell-type parasites in oyster species;

f ) produce a review on the current status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries;

g ) conduct a pilot study assessing the feasibility of constructing a 'disease index', using disease data from North Sea dab (*Limanda limanda*);

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

- the REGNS Integrated Assessment of the North Sea Ecosystem. Review and update sub-regional data tables and where necessary include new data (parameters) and/or existing data (parameters) updated where relevant. The data tables will be subject to thematic assessment to be undertaken at a REGNS thematic assessment workshop, and
- the Baltic Sea Regional Project (BSRP);

i ) produce 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;

k ) discuss and report on potential contributions for the ecosystem overview of the advisory reports describing the quantity and quality of marine habitat and/or the health of the marine ecosystem, and to consider and report on potential indicators of significant change in these ecosystem attributes;

l ) review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species and review selection of species, gender and size ranges (WKIMON).

WGPDMO will report by 31 March 2006 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) 4.7, 3.14 c) 2.6, 3.14, 4.7 d) 1.3, 2.6, 4.7 e) 3.3, 3.14, 4.7 f)1.10, 2.10, 3.14 g) 2.6, 3.11 h) 2.2, 2.8, 3.2, 3.6 i) 2.2, 2.8, 3.2, 3.6 j) 1.10, 2.2, 2.8, 3.2, 3.3, 3.6, 4.6, 4.12, 5.4 k) 6.1, 6.3 l) 6.1 m) 1.10, 2.2, 2.8, 3.2, 3.3, 3.6, 4.6, 4.12, 5.4 n) 1.10, 2.2, 2.8, 3.2, 3.3, 3.6, 4.6, 4.12, 5.4<br>**Term of Reference a)**<br>New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained.<br>**Term of Reference b)**<br>Heart and skeletal muscle inflammation (HSMI) is an emerging important disease for farmed Atlantic salmon with reported high mortality. WGPDMO considered the issue at its 2005 meeting; however, it is envisaged that new information, e.g. on the causative agent of HSMI and a possible link to other diseases with similar symptoms, will be available to be considered at the 2006 WGPDMO meeting.<br>**Term of Reference c)**<br>Based on recent evidence, the issue of sea lice interactions between farmed and wild fish remains important. Information presented in the 2005 WGPDMO report points to continuing research in treatment, prevention and management of sea lice that WGPDMO should review at its 2006 meeting.<br>**Term of Reference d)**<br>A significant component of the work of the WGPDMO is to assess trends in disease occurrence, both in aquaculture and in wild populations. It is recognised that long-term climate change will have an effect on the spatial distribution and prevalence of disease in fish and shellfish and therefore the potential for such effects needs to be considered in assessments of disease trends. The WGPDMO considers it necessary to review the available information on this topic.<br>**Term of Reference e)**<br>Microcell-type parasites of oysters are associated with a complex of diseases in different oyster species around the world. Several methods have been used to identify and characterise these parasites. Newly developed genetic methods appear very useful to elucidate the taxonomy of the parasites. A scheme for differential diagnosis incorporating relevant morphological features, host, pathological features, and molecular markers needs to be developed by the widest possible group of molluscan disease investigators.<br>**Term of Reference f)**<br>Selective breeding of mollusc stocks appears suitable for aquaculture development. Only shellfish hatcheries are able to supply such animals. There may be substantial international trade in mollusc gametes and larvae, allowing for the distribution of seedstocks improved through selective breeding. Although hatchery technology is constantly being improved, significant production problems including infectious disease must be solved before hatcheries become a major supplier of juveniles for the industry. Thus, infectious diseases affecting mollusc hatcheries should be reviewed and the preparation of an ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish on diseases in bivalve hatcheries should be considered by WGPDMO.<br>**Term of Reference g)**<br>A disease index could serve as a useful instrument to illustrate temporal and spatial patterns in the prevalence of diseases used as indicators of ecosystem health. The construction of such an index must, however, secure that, by summarising the multidimensional basic information in a single number, no relevant information is lost. The pilot study proposed has the task to establish the properties of the index discussed and to assess its suitability for future use as an assessment tool.<br>**Term of Reference h)**<br>This is in direct response to a request by REGNS. The REGNS Integrated Assessment of the North Sea Ecosystem to be carried out at a workshop at ICES HQs, 9–11 May 2005, will include ICES data on the prevalence of fish diseases and since WGPDMO will be involved in the assessment, 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. Of particular interest will be the results of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea to be held in December 2005 on board the German RV 'Walther Herwig III', with T. Lang and G. Rodjuk as co-conveners.<br>**Term of Reference i)**<br>A number of ICES publications related to disease and pathology aspects, either web-based or in ICES publication series, are being prepared or updated, the progress of which |

| | |
|---|---|
| | has to be reviewed by WGPDMO at its next meeting. It will be necessary to consider ways by which these can be linked to each other.<br>**Term of Reference j)**<br>This is in compliance with a request from the ICES Data Centre.<br>**Term of Reference k)**<br>This is in response to a request by ACME. ICES is moving towards providing scientific advice for the integrated management of all human activities that affect marine waters. Information on the quantity and quality of habitat and the health of marine ecosystems will be essential to the achievement of this goal.<br>**Term of Reference i)**<br>This is in response to a request from WKIMON. The work has to be undertaken in advance of the OSPAR meeting schedule and therefore must be progressed intersessionally. |
| **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

| | 2006 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 | WGPDMO2006_ToR_a_Denmark.doc<br>WGPDMO2006_ToR_a_England&Wales.doc<br>WGPDMO2006_ToR_a_Finland.doc<br>WGPDMO2006_ToR_a_France_Shellfish.doc<br>WGPDMO2006_ToR_a_Germany.doc<br>WGPDMO2006_ToR_a_Iceland<br>WGPDMO2006_ToR_a_Ireland.doc<br>WGPDMO2006_ToR_a_Latvia.doc<br>WGPDMO2006_ToR_a_Poland.doc<br>WGPDMO2006_ToR_a_NL_wild_fish.doc<br>WGPDMO2006_ToR_a_Canada.doc<br>WGPDMO2006_ToR_a_Russia.doc<br>WGPDMO2006_ToR_a_Scotland.doc<br>WGPDMO2006_ToR_a_Sweden.doc<br>WGPDMO2006_ToR_a_USA.doc | 20.02.2006<br>20.02.2006<br>20.02.2006<br>20.02.2006<br>20.02.2006<br>05.03.2006<br>20.02.2006<br>20.02.2006<br>20.02.2006<br>20.02.2006<br>28.02.2006<br>28.02.2006<br>28.02.2006<br>28.02.2006<br>28.02.2006 |
| b) | update information on the causes and effects of Heart and Skeletal Muscle Inflammation (HSMI) affecting farmed salmon in ICES Member Countries | | |
| c) | produce an update of current information from ICES Member Countries on the development of sea lice vaccines and management measures for sea lice control; | | |
| d) | compile a report on effects of climate change on host-pathogen interactions; | WGPDMO2006_ToR_d_Climate | 03.03.2006 |
| e) | propose a set of diagnostic techniques for the identification and characterisation of microcell-type parasites in oyster species; | WGPDMO2006_ToR_e_Microcell | 03.03.2006 |
| f) | produce a review on the current status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries; | | |
| g) | conduct a pilot study assessing the feasibility of constructing a 'disease index', using disease data from North Sea dab (*Limanda limanda*); | WGPDMO2006_ToR_g_Fish_Disease_Index | 05.03.2006 |
| h) | review progress made with regard to international collaborative actions including disease and pathology aspects:<br>the REGNS Integrated Assessment of the North Sea Ecosystem. Review and update sub-regional data tables and where necessary include new data (parameters) and/or existing data (parameters) updated where relevant. The data tables will be subject to thematic assessment to be undertaken at a REGNS thematic assessment workshop, and the Baltic Sea Regional Project (BSRP); | WGPDMO2006_ToR_h_WKFDM | 28.02.2006 |
| i) | produce 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; | | |
| k) | discuss and report on potential contributions for the ecosystem overview of the advisory reports describing the quantity and quality of marine habitat and/or the health of the marine ecosystem, and to consider and report on potential indicators of significant change in these ecosystem attributes; | WGPDMO2006_ToR_k_ToR letter to EG Chairs<br>WGPDMO2006_ToR_k_ICES Advice 2005 | 05.03.2006<br>05.03.2006 |

| | 2006 WGPDMO TERMS OF REFERENCE | WORKING DOCUMENT (FILE) | SENT BY EMAIL |
|---|---|---|---|
| l) | review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species and review selection of species, gender and size ranges (WKIMON). | WGPDMO2006_ToR_l_OSPAR CEMP Review fish disease | 03.03.2006 |
| | Terms of Reference | WGPDMO2006_TOR | 18.11.2005/26. 01.2006 |
| | Draft Agenda | WGPDMO2006_Draft_Agenda | 28.02.2006 |
| | Provisional Timetable | WGPDMO2005_Draft_Timetable | 28.02.2006 |
| | Rapporteurs | WGPDMO2005_Rapporteurs.doc | 03.03.2006 |
| | List of Participants | WGPDMO2005_Participants<br>WGPDMO2006_Partcipants_V2 | 28.02.2006<br>03.03.2006 |
| | List of Working Documents | WGPDMO2006_Working_Documents | 03.03.2006 |
| | Template for National Reports | WGPDMO2006_Template_NatlReports | 26.01.2006 |
| | Instructions for meeting | Instructions_26012006 | 26.01.2006 |
| | ICES Template for Report Section | 1ICES TEMPLATE | 26.01.2006 |
| | ICES Template Instruction | ICES Template Instructions14-04-05 | 26.01.2006 |

## 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 2005 and 2006, items of relevance to WGPDMO

4 ) Other relevant reports/activities for information

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

6 ) Update information on the causes and effects of Heart and Skeletal Muscle Inflammation (HSMI) affecting farmed salmon in ICES Member Countries (ToR b)

7 ) Produce an update of current information from ICES Member Countries on the development of sea lice vaccines and management measures for sea lice control (ToR c)

8 ) Compile a report on effects of climate change on host-pathogen interactions (ToR d)

9 ) Review progress made with regard to international collaborative actions including disease and pathology aspects (ToR h):

the REGNS Integrated Assessment of the North Sea Ecosystem. Review and update sub-regional data tables and where necessary include new data (parameters) and/or existing data (parameters) updated where relevant. The data tables will be subject to thematic assessment to be undertaken at a REGNS thematic assessment workshop, and

the Baltic Sea Regional Project (BSRP)

10 ) Propose a set of diagnostic techniques for the identification and characterisation of microcell-type parasites in oyster species (ToR e)

11 ) Produce a review on the current status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries (ToR f)

12 ) Conduct a pilot study assessing the feasibility of constructing a ‘disease index’, using disease data from North Sea dab (Limanda limanda) (ToR g)

13 ) Review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species and review selection of species, gender and size ranges (WKIMON) (ToR l)

14 ) Produce ICES publications on pathology and diseases of marine organisms (ToR i)

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

16 ) Discuss and report on potential contributions for the ecosystem overview of the advisory reports describing the quantity and quality of marine habitat and/or the health of the marine ecosystem, and to consider and report on potential indicators of significant change in these ecosystem attributes (ToR k)

17 ) Any other business

18 ) Analysis of progress with tasks

19 ) Future activities of WGPDMO

20 ) Approval of Recommendations

21 ) Approval of draft WGPDMO Report

22 ) Closing of the meeting

## Annex 5: Rapporteurs

| AGENDA ITEM(S) | 2005 WGPDMO TERMS OF REFERENCE | RAPPORTEURS |
|---|---|---|
| 1-4 | Introductory session | T. Lang |
| 5 | Produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports (ToR a)<br>• wild fish<br>• farmed fish<br>• wild and farmed shellfish | <br><br>S. MacLean/S. Jones/A. Karasev/M. Podolska<br>D. Bruno/J. Barja/B. Hjeltnes/A. Alfjorden<br>S. Ford/T. Renault/L. Madsen |
| 6 | Update information on the causes and effects of Heart and Skeletal Muscle Inflammation (HSMI) affecting farmed salmon in ICES Member Countries (ToR b) | T. Wiklund/B. Hjeltnes/ D. Bruno/I. Briede |
| 7 | Produce an update of current information from ICES Member Countries on the development of sea lice vaccines and management measures for sea lice control (ToR c) | T. Wiklund/B. Hjeltnes/ D. Bruno/E. Sterud |
| 8 | Compile a report on effects of climate change on host-pathogen interactions (ToR d) | S. MacLean/M. Podolska/S. Feist/A. Mansour |
| 9 | Review progress made with regard to international collaborative actions including disease and pathology aspects (ToR h):<br>• the REGNS Integrated Assessment of the North Sea Ecosystem. Review and update sub-regional data tables and where necessary include new data (parameters) and/or existing data (parameters) updated where relevant. The data tables will be subject to thematic assessment to be undertaken at a REGNS thematic assessment workshop, and<br>• the Baltic Sea Regional Project (BSRP) | <br><br>A. Mansour/S. Feist/W. Wosniok<br><br><br>G. Rodjuk/M. Podolska/A. Alfjorden/<br>E. Bacevicius/M. Kirjusina |
| 10 | Propose a set of diagnostic techniques for the identification and characterisation of microcell-type parasites in oyster species (ToR e) | L. Madsen/ S. Ford/T. Renault/E. Sterud |
| 11 | Produce a review on the current status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries (ToR f) | S. Ford/L. Madsen/T. Renault/J. Barja |
| 12 | Conduct a pilot study assessing the feasibility of constructing a 'disease index', using disease data from North Sea dab (*Limanda limanda*) (ToR g) | S. MacLean/S.W. Feist /W. Wosniok |
| 13 | Review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species and review selection of species, gender and size ranges (WKIMON) (ToR l) | W. Wosniok/S.W. Feist/T. Lang |
| 14 | Produce ICES publications on pathology and diseases of marine organisms (ToR i) | S. Jones/S.W. Feist/W. Wosniok |
| 15 | Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis (ToR j) | S. Feist/W. Wosniok/T. Lang |
| 16 | Discuss and report on potential contributions for the ecosystem overview of the advisory reports describing the quantity and quality of marine habitat and/or the health of the marine ecosystem, and to consider and report on potential indicators of significant change in these ecosystem attributes (ToR k) | S. Feist/A. Mansour/T. Lang |
| 17-20 | Any other business, analysis of progress with tasks, future activities of WGPDMO, approval of recommendations | S. Jones/A. Mansour/ D. Bruno |
| 21-22 | Approval of draft report, Closing of the meeting | T. Lang |

# Annex 6: ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM): Summary

**T. Lang and G. Rodjuk**

## Introduction

The ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM) was held 5–12 December 2005 onboard the German RV 'Walther Herwig III'. It was Co-Chaired by T. Lang (Germany) and G. Rodjuk (Russia) and was attended by scientists from Estonia, Finland, Germany, Latvia, Lithuania, Poland, Russia and Sweden (see Table A6.1). The workshop started and ended in Gdynia, Poland. Local arrangements and support in Gdynia were kindly provided by the Polish Sea Fisheries Institute (MIR).

According to ICES Council Resolution 2005/2/BCC02, the objectives of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring on the Baltic Sea (WKFDM) were:

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

## Background

From a large variety of studies there is general consensus today that fish diseases are an appropriate indicator of ecosystem health and that the prevalence of diseases/parasites responds to natural and anthropogenic environmental change, including exposure to contaminants. Furthermore, many fish diseases/parasites are of ecological and economical relevance since they may affect growth, reproduction and survival in affected fish populations and may even cause human health problems. Therefore, a number of ICES Member Countries carry out fish disease surveys as part of their national marine monitoring and assessment programmes and results from these surveys are being used for internationally coordinated assessments, e.g. as part of the OSPAR JAMP/CEMP.

In the Baltic Sea, only Poland, Germany and Russia are presently conducting regular fish disease monitoring programmes. However, from data assessments carried out by the ICES Working Group on Pathology and Diseases (WGPDMO), there is indication of methodological problems, particularly regarding the comparability of disease prevalence data, and a clear need for more intercalibration has, thus, repeatedly been emphasised.

Besides these countries, there is also interest in other Baltic Sea countries to implement fish disease monitoring as part of the coastal or offshore monitoring, but there has been an apparent lack of either capacities or experience. Within the Baltic Sea Regional Project (BSRP), this has been realised and funding was provided for capacity building related to fish disease monitoring in the BSRP beneficiary countries. The AtlantNIRO, Kaliningrad, Russia, was appointed as BSRP Lead Laboratory for Fish Diseases, Parasites and Histopathology in order to coordinate relevant activities and the ICES Study Group on Baltic Ecosystem Health in support of the BSRP (SGEH) is reviewing progress made.

Since ICES has a long-term experience in developing and intercalibrating methodologies for fish disease surveys and organised a number of practical practical methodological workshops

before (e.g. 1994 in the Baltic Sea, co-sponsored by the Baltic Marine Biologists, BMB), it was recommended in the ICES SGEH and the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) to organise an ICES/BSRP sea-going training workshop in December 2005, aiming at a standardisation and intercalibration of methodologies, addressing aspects from fish sampling, disease diagnosis, data reporting to statistical data assessment.

**Table A6.1: Participants of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring on the Baltic Sea (WKFDM).**

| NAME | AFFILIATION |
|---|---|
| Anders Alfjorden | National Veterinary Institute<br>Department of wildlife, fish and environment<br>Uppsala, Sweden |
| Egidijus Bacevicius | Lithuanian State Centre for Pisciculture and Fishery Research<br>Fishery Research Laboratory<br>Klaipeda, Lithuania |
| Natalia Chukalova | Atlantic Scientific Research Institute of Marine Fisheries and Oceanography (AtlantNIRO)<br>Kaliningrad, Russia |
| Nico Geveke<br>(Student) | University of Oldenburg<br>Olddenburg, Germany |
| Muza Kirjusina | Division of Parasitology and Ichthyopathology<br>State Veterinary Medicine Diagnostic Centre (SVMDC)<br>Riga, Latvia |
| Paul Kotterba<br>(Student) | University of Hamburg<br>Hamburg, Germany |
| Thomas Lang<br>(Co-Chair) | Federal Research Centre for Fisheries<br>Institute of Fishery Ecology<br>Cuxhaven, Germany |
| Kari Lehtonen | Finnish Institute of Marine Research<br>Helsinki, Finland |
| Kaja Lotman | The Matsalu National Park Administration,<br>Lihula Läänemaa, Estonia |
| Galina Rodjuk<br>(Co-Chair) | Atlantic Scientific Research Institute of Marine Fisheries and Oceanography (AtlantNIRO)<br>Kaliningrad, Russia |
| Grant D. Stentiford | CEFAS Weymouth Laboratory<br>Weymouth, UK |
| Kordian Trella | Sea Fisheries Institute<br>Gdynia, Poland |
| Thomas Tepperies<br>(Technician) | Federal Research Centre for Fisheries<br>Institute of Fishery Ecology<br>Cuxhaven, Germany |
| Werner Wosniok | University of Bremen<br>Institute of Statistics<br>Bremen, Germany |

## Workshop programme

The workshop was organised according to the timetable shown in Table A6.2 and consisted of practical work and training with flounder (*Platichthys flesus*), cod (*Gadus morhua*) and herring (*Clupea harengus*) as target fish species as well as of theoretical work addressing aspects such as:

- **Practical work:** Methods for fish sampling, disease diagnosis (externally visible diseases/ parasites and liver histopathology), intercalibration exercises, sampling for histo-pathology., fixation and preservation techniques, age determination, data entry software, hydrographic measurements, sampling for biomarker measurements.

- **Theory:** Overview of national and international programmes and databases (e.g. ICES Data Centre), sampling design, data recording, analysis and assessment, development of health indicators, confounding factors with impact on diseases (host-specific, site-specific), quality assurance (e.g. BEQUALM) etc.

**Table A6.2: Timetable for the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM).**

| | |
|---|---|
| 4 Dec. 2005 | RV Walther Herwig III arrives in port of Gdynia |
| 5 Dec. 2005 | Participants arrive in Gdynia, start of workshop on board RV Walther Herwig III |
| 6-11 Dec. 2006 | Field work and training at selected sampling sites (see Figure 1) |
| 11 Dec. 2005 | RV Walther Herwig III returns to Gdynia, reception on board with participants and invited guests |
| 12 Dec. 2005 | End of workshop, RV Walther Herwig III leaves Gdynia |

Practical work was carried out in five Baltic Sea areas encompassing sampling sites in German, Polish and Lithuanian waters (see Figure A6.1 and Table A6.3).

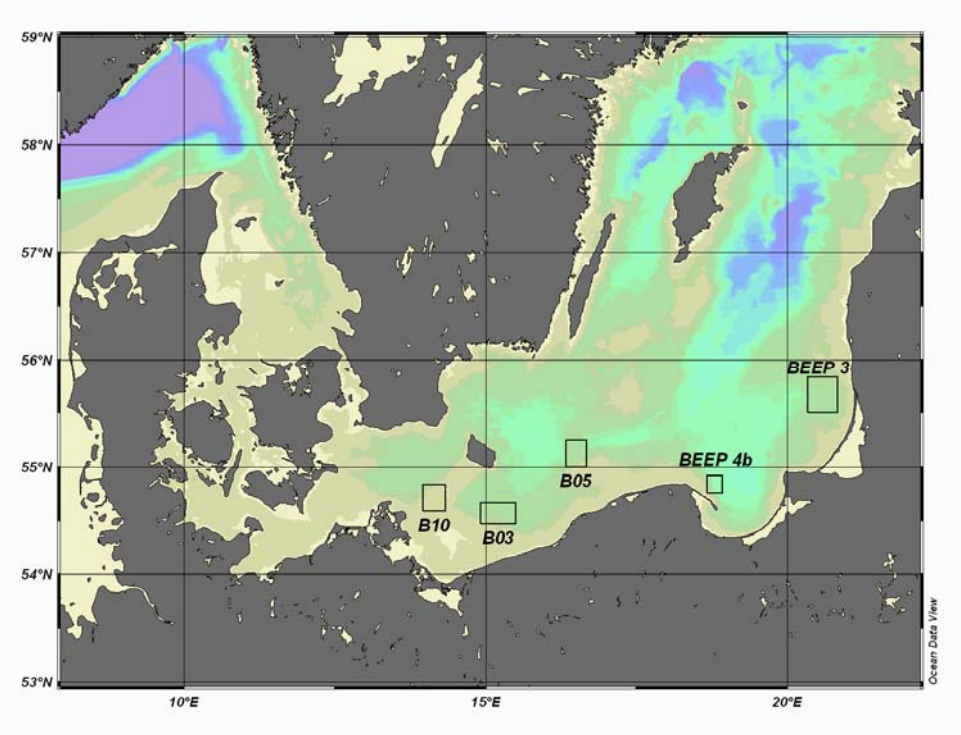


**Figure A6.1: Location of sampling areas of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM).**

**Table A6.3: Geographical position of sampling areas of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM).**

| SAMPLING AREA | LATITUDE | LONGITUDE |
|---|---|---|
| B10 | 54° 35'N – 54° 50'N | 13° 58'E – 14° 20' E |
| B03 | 54° 28'N – 54° 40'N | 14° 55'E – 15° 30'E |
| B05 | 55° 00'N – 55° 15'N | 16° 20'E – 16° 40'E |
| BEEP 3 | 55° 30'N – 55° 50'N | 20° 20'E – 20° 50'E |
| BEEP 4b | 54° 45'N – 54° 55'N | 18° 40'E – 18° 55'E |

As part of the training programme, externally visible diseases and parasites of cod (*Gadus morhua*) and flounder (*Platichthys flesus*) were recorded (Table A6.4). In addition, herring were inspected for the presence of nematode larvae (*Anisakis simplex*) in the body cavity as well as for the presence of granulomas in the heart caused by *Ichthyophonus hoferi*, and flounder livers were examined for the presence of liver nodules (macroscopic liver neoplasms) and parasites.

**Table A6.4: Grossly visible diseases recorded in Baltic cod (*Gadus morhua*) and flounder (*Platichthys flesus*) and their causes.**

| COD (*G. morhua*) | | FLOUNDER (*P. flesus*) | |
|---|---|---|---|
| DISEASE/PARASITE | AETIOLOGY | DISEASE/PARASITE | AETIOLOGY |
| Acute/healing skin ulcers | Bacterial | Lymphocystis | Viral |
| Skeletal deformities | Multifactorial | Acute/healing skin ulcers | Bacterial |
| Pseudobranchial swelling (X-cell disease) | Parasitic (Amoeba-like) | Acute/healing fin rot/erosion | Bacterial |
| *Cryptocotyle lingua* | Metacercariae of a parasitic digenean trematode | Skeletal deformities | Multifactorial |
| *Lernaeocera branchialis* | Parasitic copepode | *Cryptocotyle spp.* | Metacercariae of two parasitic digenean trematode species (*C. lingua, C. concavum*) |
| | | Liver nodules > 2 mm | Contaminants likely |
| | | Liver parasites | Nematodes, Acanthocephaleans |

## Outcome of the workshop/recommendations

Some results of the practical work are shown in Figures A6.2 and A6.3.

The participants considered the workshop to be successful since the major Terms of Reference were fulfilled. There was an agreement that the results of the workshop should be communicated to a wider forum, including responsible national authorities and international organisations coordinating marine environmental monitoring programmes. A number of follow-up activities were suggested, building on the experience made during the sea-going workshop.

The workshop participants agreed on the following (draft) recommendations:

- ICES to communicate the WKFDM report and recommendations to relevant national ministries/agencies responsible for monitoring the environmental status of the Baltic Sea and to international organisations (HELCOM, OSPAR, EU, EEA);

- Baltic Sea countries use fish disease as a ‘top-level indicator‘ of health in national integrated chemical and biological effects monitoring programmes;
- Baltic Sea countries conducting fish disease studies should apply the guidelines developed by ICES and through the BEQUALM programme summarised in the present report as Annex 6.
- Baltic Sea institutes involved in fish disease monitoring in the Baltic Sea participate in the Biological Effects Quality Assurance in Monitoring Programme (BEQUALM);
- ICES/BSRP organise a land-based workshop on methodologies for coastal fish disease monitoring. The workshop could be held in 2006 or 2007 at the AtlantNIRO, Kaliningrad, Russia, or at the Estonian Marine Institute, Tallinn;
- Baltic Sea countries harmonise the components of their national marine monitoring and assessment programmes in order to implement an integrated programme on contaminants (and other anthropogenic stressors) and their biological effects;
- Baltic Sea countries, ICES/BSRP and HELCOM investigate the potential for an internationally coordinated integrated monitoring programme in the Baltic Sea, encompassing joint sampling campaigns and the involvement of appointed expert laboratories in the Baltic countries responsible for the conduct of specific analytical measurements;
- ICES/BSRP and Baltic Sea countries consider to organise an international demonstration project in 2007 or 2008 on the ecosystem health of the Gulf of Finland, providing baseline data and assessing the feasibility of coordinated sample collection and analysis.

The WKFDM results will be published in 2006 as ICES/BSRP Report (ICES CM 2006 xx:xx) and will be presented to and reviewed by the ICES SGEH, the ICES WGPDMO, the ICES Baltic Committee (BCC) and the ICES Mariculture Committee (MCC). The report will provide:

- an overview of present activities in Baltic Sea countries related to fish diseases,
- the results of the practical work,
- methodological guidelines for fish disease monitoring in the Baltic Sea.

It is hoped that the workshop and related future activities will build the basis for the incorporation of coordinated and standardised fish disease studies into national marine monitoring and assessment programmes of the Baltic Sea countries and eventually into the HELCOM monitoring programme. There is consensus that fish disease studies should be part of an integrated monitoring approach, encompassing e.g. studies on biological effects of contaminants (biomarker approach), eutrophication, biodiversity, physical and chemical measurements, and methods applied in fish stock assessment.

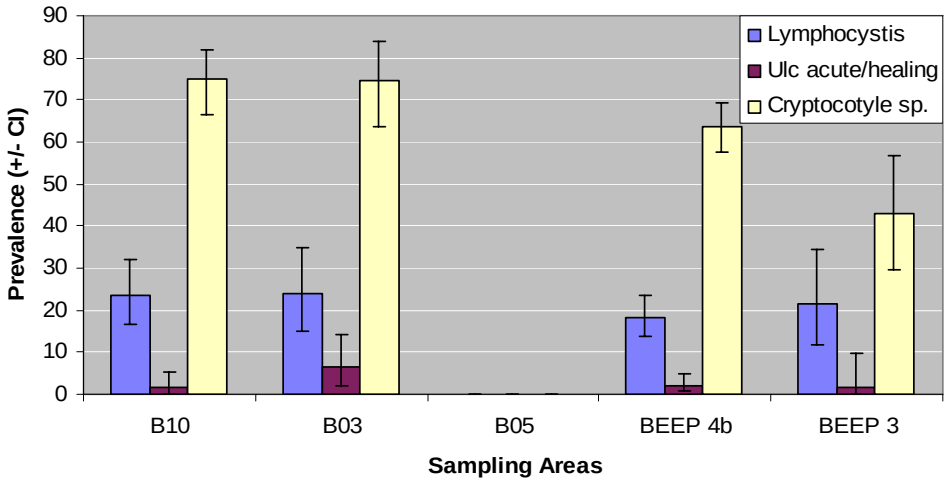


**Figure A6.2: Prevalences (% with 95% confidence intervals) of externally visible diseases in Baltic flounder (*Platichthys flesus*) recorded during the ICES Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM).**

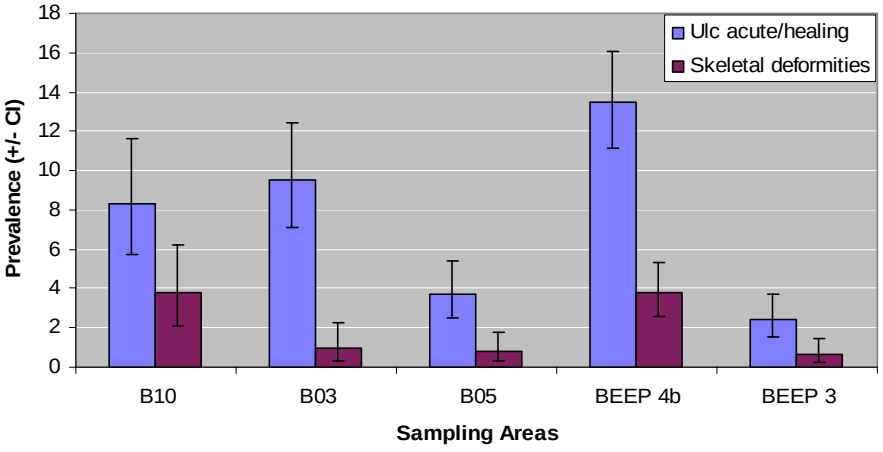


**Figure A6.3: Prevalences (% with 95% confidence intervals) of externally visible diseases in Balic cod (*Gadus morhua*) recorded during the ICES Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM).**

# Annex 7: Diagnostic techniques for the identification and characterisation of microcell-type parasites in oyster species

**T. Renault, I. Arzul, S.W. Feist and S. Ford**

Description of the first microcell-type parasites resulted from studies that were carried out to determine the cause of serious mortalities in flat oysters, *Ostrea edulis*, in California, USA (Katkansky *et al.,* 1969) and in Pacific oysters, *Crassostrea gigas*, at Denman Island, British Columbia, Canada (Bower 1988; Farley *et al.,* 1988). In total, four species of microcell-type parasites belonging to two distinct genera have been described and they are now recognized as major parasitic threats to oyster populations worldwide. The type species of the genus *Bonamia*, *Bonamia ostreae*, believed to have been first observed by Katkansky *et al.* (1969; Elston *et al.,* 1986), has caused recurring mass mortalities of the flat oyster, *O. edulis*, in Europe since its discovery in the late 1970s (Pichot *et al.,* 1980). *Bonamia ostreae* infecting flat oysters, *O. edulis*, and *B. exitiosa* (Hine *et al.,* 2001) which infects *O. chilensis* are major concerns for oyster aquaculture in Europe, California and Maine, USA (Barber and Davis 1994; Comps *et al.,* 1980; Friedman and Perkins 1994; Friedman *et al.,* 1989) and in New Zealand (Dinamani *et al.,* 1987), respectively. The genus *Mikrocytos* was first proposed to include the Denman Island disease agent *Mikrocytos mackini* and *Mikrocytos roughleyi*, the aetiological agent of winter mortality in Sydney rock oysters, *Saccostrea glomerata*, in Australia (Farley *et al.,* 1988). However, recent phylogenetic studies based on molecular analysis led to the conclusion that *B. ostreae*, *B. extiosa*, and *M. roughleyi* are closely related (Carnegie *et al.,* 2000; Cochennec-Laureau *et al.,* 2003; Hine *et al.,* 2001), but that *M. mackini* is not obviously related to members of any described taxon (Carnegie *et al.,* 2003). Moreover, an interesting new finding is molecular phylogenetic support for inclusion of the genus *Bonamia* in the phylum Haplosporidia (Carnegie *et al.,* 2000; Reece and Stokes 2003; Reece *et al.,* 2004). On the basis of 18S rRNA sequence analysis, species of *Bonamia* formed a monophyletic clade nested within the traditional haplosporidian taxa, as sister taxa to *Minchinia* spp. (Reece *et al.,* 2004).

In 2003, a new species of *Bonamia* was discovered killing a non-native oyster species (*Crassostrea ariakensis*) experimentally deployed in South Carolina, USA (Burreson *et al.*,, 2004) This oyster is being considered for introduction into Chesapeake Bay to replace the native eastern oyster (*C. virginica*), which has been devastated by diseases. The oysters, hatchery-produced triploids reared following the ICES protocols, experienced heavy mortality during the summer of 2003. The *Bonamia sp*. is considered to be a previously unrecognized enzootic parasite infecting a susceptible introduced host. Subsequent analyses demonstrated that the pathogen is similar in SSU rDNA sequence to the southern hemisphere *B. exitiosa* and *M. roughleyi*. The parasite persisted throughout 2004, and was detected also in the native crested (horse) oyster *Ostreola equestris*. Also *in O. equestris*, another new *Bonamia sp.* was detected that is more similar in SSU rDNA sequence to the northern *B. ostreae* than to the southern hemisphere forms. Neither North Carolina *Bonamia sp.* was observed in *O. equestris* at (PCR) prevalence greater than 6%. The *O. equestris Bonamia sp.* was never found in *C. ariakensis* by species-specific PCR. The geographical distribution of the North Carolina *Bonamia* spp. is unknown, although neither has been detected in *C. ariakensis* being tested in Chesapeake Bay. In addition, another potentially new Bonamia species has recently been described in oysters, *Ostrea peulchana*, farmed in Argentina (Kroeck and Montes, 2005). These 3 new recent findings illustrate the widespread distribution of Bonamia species and the high likelihood that additional species will be found.

For many pathogens of mollusc bivalves, available diagnostic techniques are rather limited, and investigation, restricted to histological and ultrastructural examination. Microcell-type parasites may be diagnosed by applying simple methodologies, such as stained tissue imprints

and tissue-section histology. However, the small size of these parasites makes them difficult to detect using standard histopathology and cytology. Moreover, these techniques do not permit identification at generic or species levels. The effective control of microcell diseases requires diagnostic tests that are specific, rapid, reliable and sensitive, and that can discriminate between genera and species. Molecular detection assays for microcell parasites are being developed at an increasingly rate. The routine use of DNA based diagnostic tools is however hampered by a number of major concerns. The assays often have not been thoroughly tested for inclusivity (detection of all strains of the pathogen) or specificity (cross reaction with other organisms). The main concern is that molecular tools too often are developed from a few sequences without sufficient understanding of the overall sequence variability within the species.

The first DNA-based diagnostic assay for a microcell was designed for *M. roughleyi* (Adlard and Lester 1995). Questions of specificity and sensitivity, however, limit the usefulnes of the assay. "*Bonamia ostreae*-specific" PCR assays were then developed by Carnegie *et al.* (2000) and Cochennec *et al.* (2000). While neither assay amplifies oyster DNA, these assays are now known to have broader specificity than originally supposed. The first assay (Carnegie *et al.*, 2000) should detect *B. ostreae* and *B. exitiosa*, but perhaps not *M. roughleyi*. The second assay (Cochennec *et al.*, 2000) should detect the SSU rRNA of all microcell haplosporidians. Both PCR assays have undergone validation against the light microscopic screening of fixed and stained haemolymph smears (Carnegie *et al.*, 2000; Diggles *et al.*, 2003). Results indicated that PCR assay detected more infections than the tissue-imprint samples. PCR-restriction fragment length polymorphism (PCR-RFLP) assays may provide the most useful molecular tool to distinguish *B. ostreae* from *B. exitiosa* and to distinguish *M. roughleyi* from the other *Bonamia* spp. (Hine *et al.*, 2001; Cochennec-Laureau *et al.*, 2003). PCR and fluorescent *in situ* hybridisation (HIS) assays for *M. mackini* were also recently developed (Carnegie *et al.*, 2003).

Molecular techniques for diagnosing microcell parasites are now moving from development in specialised laboratories for research purposes, to routine application and are expected to be increasingly used in microcell monitoring programs International standards proposed by the OIE now include molecular techniques for the detection and identification of molluscan parasites (OIE 2003). Not all regions of parasite DNA, however, are equally useful as targets for molecular detection. Closely related parasites, including *B. ostreae* and *B. exitiosa*, may present high sequence similarities. Moreover, molecular tools detect DNA and not necessarily a viable pathogen. To help 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.

The following tasks are recommended:

- Morphological differences between species need to be described by detailed morphological comparison of several isolates of named species. Studies conducted in parallel with the same isolates in several laboratories would be ideal.
- Identify regions of the genome (other than SSU rRNA) that may prove useful for species differentiation.
- Molecular assays should be tested in parallel and validated, and diagnostic assays that will clearly discriminate between all "valid" species should be developed.
- Conduct surveys to assess host and parasite geographic distributions.
- Determine if parasite strains have genetic and/or virulence differences.

Diagnosis of microcell-like parasites requires information on susceptible host species, disease patterns, histopathology, and parasite morphology and molecular characterisation. Thus prescribed methods for *targeted surveillance* to declare areas "free from infection" as outlined

in the *Aquatic Manual (OIE)* are histology, tissue imprints (heart or gills) or PCR. An examination procedure at three levels is currently recommended by the OIE: routine screening or surveillance, and presumptive and confirmation methods in the case of abnormal mortalities.

For example, in known susceptible species within the known geographical range of *Bonamia ostreae*, a suspect case of infection with *B. ostreae* is considered positive if the parasite is detected 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 can be considered positive if diagnosed by histopathology, tissue imprints, PCR or *in situ* hybridization. A confirmed case of *Bonamia ostreae* is a positive result by tissue imprints or histology combined with a positive result with PCR or *in situ* hybridization. Transmission electron microscopy and PCR-RFLP are recommended for a confirmatory diagnosis..

Factors to be considered in the diagnosis of Bonamia infections:

## Host factors

Susceptible host species or strains
Susceptible stages of the host
Target organs and infected tissue

## Disease pattern

Transmission patterns
Prevalence (stages of development, seasonal variation)
Geographical distribution
Mortality and morbidity
Economic and/or production impact of the disease

## Agent detection and identification methods

### Direct detection methods

#### *Microscopic methods*

**Tissue imprints:** After blotting tissue sections from oyster spat, or ventricles or gills from adult oysters on an absorbent paper, several imprints are made on a glass slide. Slides are air-dried, fixed in methanol or in absolute ethanol and stained using a commercially available blood-staining kit (Hemacolor, Merck). Slides are mounted with a cover-slip using an appropriate synthetic resin. Slides are firstly observed X 200 magnification and then under oil immersion X 1000 magnification. Positive controls need to be included and are available from the OIE Reference Laboratory. This technique demonstrates a low specificity. The sensitivity is better than tissue-section histological examination which is the “gold standard” (Bachère *et al.,* 1982; Culloty *et al.,* 2003) but the heart imprint technique is apparently not reliable for detecting latent infections (Da Silva and Villalba 2004).

**Tissue-section histology**: Sections of tissue from live or recently dead oysters that include gills, digestive gland, mantle, gonad should be fixed for 24 hours in Davidson’s fixative followed by standard processing for paraffin histology and staining for example with haematoxylin and eosin. Positive controls need to be included and are available from the OIE Reference Laboratory. Histology demonstrates a low specificity with a good sensitivity for moderate to high intensity infections, but low for low intensity infections. Histology is the gold standard and is the recommended surveillance method.

**Transmission electron microscopy**: Transmission electron microscopy may help to differentiate *B. ostreae* from other closely related microcells like *B. exitiosa*. Dense forms of

*B. ostreae* are more dense, slightly smaller in size (2.4 ± 0.5 µm mean diameter n = 64 in comparison to B. *exitiosa* with a mean diameter of 3 ± 0.3 µm, n = 61), have fewer haplosporosomes, mitochondrial profiles and lipoid bodies per ultrastructure section, and have larger tubulo-vesicular mitochondria than *B. exitiosa*. In addition, dense forms of *B. ostreae* lack nuclear membrane-bound Golgi/nuclear cup complexes and a vacuolated stage (Hine *et al.,* 2001).

***Agent isolation and identification***

To date, no cell culture/artificial media are available. An early immunofluorescent technique based on monoclonal antibodies was developed and presented sensitivity similar to tissue imprints (Boulo *et al.,* 1989). Although direct monoclonal antibody sandwich immunoassay for the detection of *B. ostrea*e in haemolymph samples of *O. edulis* was developed (Cochennec *et al.,* 1992) and marketed commercially for a few years in the mid 1990s, it is no longer available. The specificity and sensitivity of this last technique against histology were 76.7% and 106% respectively (Cochennec *et al.,* 1992).

**Agent purification**: *Bonamia ostreae* can be purified from highly infected oysters (Miahle *et al.,* 1988).

**Molecular techniques: polymerase chain reaction (PCR)**: Tissue samples from live or recently dead oysters are placed in 95% ethanol or frozen at -80°C until DNA is extracted. DNA extraction is accomplished by proteinase K digestion overnight and phenol-chloroform extraction and ethanol precipitation (Carnegie *et al.,* 2000; Cochennec *et al.,* 2000) or spin-column methodology using commercially available kits (e.g. QIAGEN) (Carnegie *et al.,* 2000). Two PCR protocols with two different primer pairs targeting the SSU rDNA have been developed for *B. ostreae.* 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, and amplifies a 300 bp product (Cochennec *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, and amplifies a 760 bp product (Carnegie *et al.,* 2000). Positive controls are DNA from purified parasites, or genomic DNA from heavily infected hosts. Negative controls are genomic DNA from uninfected hosts.

The first assay should amplify all microcell haplosporidians and the second one, at least *B. ostreae* and *B. exitiosa* (Carnegie *et al.,* 2004). Sensitivity of both assays is higher than histocytological methods, but either PCR assay has been formally validated against histology for the detection of *B. ostreae*. A positive result is an amplicon of the appropriate size, with all negative controls negative and all positive controls positive. Neither assay is species specific, however. The sequence of the SSU rDNA gene of *B. ostreae* can be distinguished from that of *B. exitiosa* or *B. roughleyi* by restriction fragment length polymorphism (RFLP) analysis, when the Bo-Boas PCR product is digested with Hae II and Bgl I. The obtained profiles vary according to the parasite species. *B. ostreae* and *B. exitiosa* present the same profile (2 products of 115 and 189 bp) when digested with Hae II while *B. roughleyi* is not digested. The *B. ostreae* profile consists of 2 bands of 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).

**Molecular techniques: *in situ* hybridization (ISH):** Two ISH protocols have been developed (Carnegie *et al.,* 2003; Cochennec *et al.,* 2000). Tissue samples are placed in Davidson's fixative for 24 hours and then embedded in paraffin. Sections are cut at 5 µm, placed on silane-coated slides and then baked overnight in an oven at 50 to 60°C. After dewaxing, slides are treated with proteinase K in TE buffer at 37°C for 30 minutes in the first protocol or in PBS buffer for 15 minutes at 37°C in the second protocol. In the first protocol, the probe is produced by PCR using the previously described primer pair Bo/Boas with digoxigenin incorporation. In the second protocol, probes consist of a cocktail of oligo-fluoroscein-labeled 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. Positive controls are histological sections from infected hosts. Negative controls are histological sections from uninfected hosts.

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). 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* (Carnegie *et al.*, 2004). ISH has not yet been validated against histology.

**Table A7.1: The methods currently available for surveillance, detection, and diagnosis of *Bonamia ostreae*. The designations used in the Table indicate: A = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; B = the method is a standard method with good diagnostic sensitivity and specificity; C = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and D = the method is presently not recommended for this purpose These designations are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility.**

| METHOD | SURVEILLANCE | | PRESUMPTIVE | | CONFIRMATORY |
|---|---|---|---|---|---|
| | In known host and geographical range | Outside known host and geographical range | In known host and geographical range | Outside known host and geographical range | |
| Gross signs | D | D | D | D | D |
| Tissue imprints | A | B | A | B | D |
| Histopathology | B | A | B | A | D |
| PCR | B | B | B | B | B |
| PCR-RFLP | D | D | D | D | A |
| Transmission EM | D | D | D | D | A |
| *In situ* hybridization | C | C | D | D | B |

**EM = electron microscopy; PCR = polymerase chain reaction, RFLP = Restriction Fragment Length polymorphism**

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# Annex 8: Review on the current status of studies carried out in ICES Member Countries on infectious diseases in shellfish hatcheries

**T. Renault, S.W. Feist and S. Ford**

The potential of aquaculture to meet the challenge of food security and to generate employment and foreign exchange is clearly demonstrated by the rapid expansion of this sector, which has grown at an average annual rate of almost 10% since 1984 compared with 3% for livestock meat and 1.6% for capture fisheries production. According to FAO data (1996), aquatic production (including plants) has steadily increased since 1984, and in 1996 total world production of finfish and shellfish from capture fisheries and aquaculture reached 120.3 million tons. A significant proportion of this increased production was of cultured origin. The annual contribution of cultured species to total finfish and shellfish production rose from 13% in 1990 to 22% in 1996. Global production of molluscs, the greatest proportion of which is bivalves, was estimated at 4,388,967 metric tons in 1994 (FAO, 1996). However, high density culture conditions mean that many aquacultured animals are under stress conditions and are generally more susceptible to infections. Major disease outbreaks result in loss of income and sometimes the complete shutdown of operations. The control of infectious diseases is a priority for sustainable aquaculture and attempts must be made to develop new approaches and technologies, suited to health improvement of farmed species, and which are not harmful for the environment.

Selective breeding of bivalve stocks appears suitable for aquaculture development. Examples have been reported for *Haplosporidium nelsoni* (Haskin and Ford 1979), and Juvenile Oyster Disease (Barber *et al.*, 2000) in *Crassostrea virginica* and for *Bonamia ostreae* in *Ostrea edulis* (Naciri-Graven *et al.*, 1998). Only shellfish hatcheries are able to supply such animals. There may be a substantial international trade in bivalve gametes and larvae, allowing for the distribution of seed stocks improved through selective breeding. Although hatchery technology is constantly being improved, significant production problems including infectious diseases must be solved before hatcheries become a major supplier of juveniles for the industry.

Current practise in the commercial shellfish hatcheries takes account of basic research findings about food provision and avoidance of bacterial infection but uncontrolled variables are still damaging the industry. Among these uncontrolled variables, infectious diseases seem to play a key role. The development of infectious diseases in aquaculture partly comes from high-density production systems including commercial hatcheries and nurseries. Bivalves in intensive culture are continuously affected by environmental fluctuations and management practices that can impose considerable stress, rendering them susceptible to a wide variety of infectious diseases. Viral and bacterial diseases in invertebrates are of serious concern in aquaculture since no specific chemotherapies and vaccines are available. Knowledge of viruses and bacteria in cultured bivalves is needed in order to develop new tools for disease control. This knowledge may give new insights into the management and control of infections in aquaculture.

## Viral infections in larval bivalves

### Oyster velar virus disease (OVVD)

Oyster velar virus disease (OVVD) was reported from hatchery-reared larval Pacific oysters, *Crassostrea gigas*, on the west coast of North America (Washington state) (Elston 1979; Elston and Wilkinson 1985). The virus was associated with mortality of oyster pediveliger larvae. Pacific oyster larvae exhibiting clinical lesions of OVVD were less active than normal

animals. The disease resulted in the sloughing of ciliated velar epithelial cells forming the characteristic "blisters" (Elston and Wilkinson 1985). Infected velar epithelial cells, which were in process of detaching from the velum, appeared as blebs along the periphery of the velum. Detailed hatchery records from 1976 through 1984 indicated that a disease consistent with the clinical lesions and behaviour described above occurred each year most often from mid-March through mid-June (Elston and Wilkinson 1985). Over the 8-year period and during the April to May period, losses of up to 50% of hatchery production were presumptively attributed to OVVD. However since 1985, no iridoviral infection has been reported in Pacific oyster larvae on the west coast of North America. Electron microscope examination of OVVD infected larvae demonstrated viroplasic inclusion bodies and viral particles. Complete particles were hexagonal in profile and average 228 nm in diameter. The properties of the virus, as well as its assemblage within the cytoplasm, characterize it as a member of the *Iridoviridae*. However, the exact affiliation of the virus cannot be made at this point. No molecular data is available**.** Iridoviral infections were also reported in France among adult Portuguese oysters *C. angulata* reared in the field and leading to the extinction of the species along the Atlantic French coast.

### Herpesvirus infections

In 1991, viruses interpreted as belonging to the *Herpesviridae* were associated with high mortality rates of hatchery-reared larval *Crassostrea gigas* in France (Nicolas *et al.,* 1992) and in New Zealand (Hine *et al.,* 1992). Since then, sporadic high mortalities of larval *C. gigas* are regularly observed in commercial European hatcheries, occurring each year during summer in association with herpes-like virus detection (Renault *et al.,* 1994; Renault and Arzul 2001). In addition, herpesvirus infections were reported in larvae of the European flat oyster, *Ostrea edulis* (Renault *et al.,* 2000), of clams *Ruditapes decussatus* and *R. philippinarum* and of the scallop, *Pecten maximus* (Arzul *et al.*; 2001b; Renault *et al.,* 2001a,b; Azul and Renault 2002). Herpesviruses were also detected in animals reared in the field.

Transmission experiments have demonstrated the virulence of the virus (Le Deuff *et al.,* 1994) and indicated that a single species is probably responsible for all the infections observed (Arzul *et al.,* 2001a and c). Infected larvae show a reduction in feeding and swimming activities (Hine *et al.,* 1992; Nicolas *et al.,* 1992). Moribund larvae swim weakly in circles with their velum noticeable more extended than healthy larvae. Shortly before death infected larvae settle at the bottom of the tanks. Viral particles were detected in basophilic fibroblastic-like cells in infected larvae. Capsids and nucleocapsids are scattered throughout the nucleus in infected cells. The virus isolated from infected *C. gigas* larvae has been classified as a member of the *Herpesviridae* under the name ostreid herpesvirus 1 (OsHV-1) (Minson *et al.,* 2000; Davison *et al.,* 2005) on the basis of ultrastuctural features, large double stranded DNA, capsid structure, genome structure and DNA content.

## Bacterial infections in larvae and spat

Bacterial diseases were commonly described in larval stages and were associated with high mortalities in hatcheries (Lauckner 1983; Sinderman 1990). Bacteria that provoke severe mortalities in larval culture generally belong to the genus *Vibrio* (for a review Paillard et al; 2004). Disease symptoms in moribund larvae were described first in *Crassostrea virginica* and *Mercenaria mercenaria* by Tubiash (1965) and Elston and Leibovitz (1980), and named Bacillary Necrosis. More recently, new species of vibrios have been isolated and associated with high mortalities of larvae in hatcheries. In 1986, severe mortalities occurred in clam *Ruditapes philippinarum* larvae and were associated to a *Vibrio* named VTB (*Vibrio Tapes philipinarum*) (Nicolas *et al.* 1992). Variants of vibrios designated as "*V. anguillarum*-like" (VAR) were isolated from an epizootic occurring in a commercial hatchery in Chile, producing the scallops, *Argopecten purpuratus* (Riquelme *et al.* 1995). A bacterial pathogen of scallop *Pecten maximus* larvae, *V. pectinicida* was also isolated and considered as the

aetiological agent of mortality outbreaks in a French hatchery (Lambert *et al.,* 1998). The experimental reproduction of mortality, pathogen identification and pathogenesis are documented only for *V. pectinicida* (Lambert *et al.* 1998; Lambert *et al.,* 2001). This bacterial pathogen does not induce mortalities in adult scallops.

Bacterial diseases have also been reported to affect juvenile bivalves. These include Juvenile Oyster Disease (JOD) in *C. virginica* (Bricelj *at al.,* 1992; Boettcher *et al.,* 1999, 2000), hinge ligament erosion disease in *C. gigas* (Dungan and Elston 1988; Dungan and Elston 1989), the chronic abscess syndrome in *C. gigas* (Elston *et al.,* 1999) and *C. gigas* oyster mortalities occurring in nurseries in France in association with the detection of Vibiros including *V.aestuarianus* (Labreuche *et al.,* 2006**).** In this last case, the same bacteria are also observed in the field associated with spat and adult oyster mortalities.

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# Annex 9: Construction of a Fish Disease Index (FDI) based on data on disease conditions in dab (*Limanda limanda*)

**T. Lang, W. Wosniok and S.W. Feist**

## Introduction

At the 2005 meeting of the ICES Working Group on Pathology (WGPDMO), a number of 'Health Indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies was reviewed (ICES 2005) and it was highlighted that the purpose of a health index in this context is to summarize information on the health status of individual organisms. While the original information on the health status is expressed by several (many) quantities (e.g. diseases, parasitoses, biomarkers, physiological parameters), an index is expected to represent the most relevant information by one (or at most few) number(s) or category(ies).

Health indices have been developed to simplify complex and diverse data sets to focus on key issues. They are becoming more and more important as easy-to-understand indicators of ecosystem health to be used in the assessment of the environmental status of ecosystems and, therefore, their development is on the agenda in many national and international bodies. In the maritime area, OSPAR (for the Northeast Atlantic area) and HELCOM (for the Baltic Sea) are amongst the major driving forces. Indices have already been established for a large variety of environmental parameters (e.g. benthic communities, contaminant levels in biota, water and sediment quality, coastal habitats, fisheries, fish diseases) and are being used as tools to define Ecological Quality Objectives (EcoQOs) and Background/Reference Values and as tools to detect negative deviations from defined environmental conditions (potentially requiring subsequent management actions) and to detect positive deviations, indicating an environmental improvement (e.g. following remediation actions).

It appears self-evident that a health index should provide information on the health status of defined target organisms in terms of their diseases and it is undisputable that this kind of information is valuable in the context of ecosystem health assessment. Furthermore, such an index could be used as an assessment tool in the context of the fish disease monitoring under the OSPAR Coordinated Environmental Monitoring Programme (CEMP). The development of such tools is an OSPAR requirement and the basis for changing the status of fish disease monitoring from 'voluntary' to 'mandatory' within the general and contaminant-specific biological effects monitoring programmes to be carried out by contracting parties (countries that signed the OSPAR Convention).

For these reasons, the WGPDMO recommended at its 2005 meeting to construct a) a 'Disease Index' which could serve as a useful instrument to illustrate temporal and spatial patterns in disease prevalence and b) to conduct a pilot study, using empirical fish disease data derived from marine fish disease monitoring programmes carried out by ICES Member Countries, aiming at a validation of the index in the light of its suitability for future use as an assessment tool. The term 'Disease Index' was chosen since it was felt that this term more clearly expresses what kind of information is provided by the index. In particular, it can be useful to define indices for several groups of diseases while it is not reasonable to define various health indices.

It was agreed to use data on diseases of the common dab (*Limanda limanda*) which is the most common flatfish species in the North Sea and adjacent areas and has been the major target species for monitoring fish diseases and biomarkers in these areas for more than two decades. Standardised methodologies for fish disease surveys have been established through ICES activities and as part of the BEQUALM programme and a number of ICES Member Countries

(Germany, The Netherlands, UK) are carrying out regular dab disease surveys in the North Sea and neighbouring areas (e.g. the Irish Sea, Celtic Sea, English Channel, western Baltic Sea). The data obtained in these programmes are submitted to the ICES Environmental Data Centre on an annual basis and have been used for various statistical data assessments in previous years.

The common dab is affected by a large variety of externally visible diseases and parasites as well as by a wide range of liver pathologies, including neoplastic changes, at varying degree of severity/intensity. However, no attempts have been made so far to summarise these data into one figure (index), reflecting the overall health/disease status of individual fish.

## How to construct a Fish Disease Index (FDI)

There are a number of questions that need to be answered before constructing a Fish Disease Index:

### What kind of diseases should be included in the FDI?

Different species often are afflicted with different diseases/parasites and, therefore, the components used for constructing a FDI very likely will have to be species-specific and will be selected based on the objectives of the assessment. If the FDI is to provide a quantitative figure of the general health status of an individual fish, ideally, all diseases, pathologies and parasites should be considered. However, this will not be possible due to practical reasons. A selection will have to be made, based on criteria to be defined. If the FDI, in contrast, is to provide a quantitative figure on the effects of a specific environmental stressor, specific diseases/pathologies/parasites have to be selected that are known (or at least suspected) to react to exposure to the stressor. Two criteria appear to be noteworthy in this context of monitoring and assessment in any case:

- For practical reasons, diseases/pathologies/parasites should be easily detectable.
- Diseases/pathologies/parasites should respond to environmental stressors.

In dab, there are, according to ICES and BEQUALM guidelines, and since recently also in the context of the OSPAR CEMP, three categories of diseases to be monitored:

- externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcers, x-cell gill disease, hyperpigmentation),
- macroscopically visible liver neoplasms > 2 mm in diameter (previously termed liver nodules > 2 mm), and
- histopathological liver lesions (early non-neoplastic toxicopathic lesions, pre-neoplastic lesions (foci of cellular alteration, FCA), benign tumours, malignant tumours).

However, since more diseases/parasites of dab are being recorded on a regular basis in the existing monitoring programmes, the authors suggest that these should be considered in addition. Table A9.1 shows the diseases which were decided to be included in the FDI constructed for dab.

**Table A9.1: Disease categories and diseases/parasites of dab (*Limanda limanda*) considered to be useful for constructing a Fish Disease Index (FDI) for dab from the North Sea and adjacent areas.**

| EXTERNALLY VISIBLE DISEASES (EVD) | MACROSCOPIC LIVER NEOPLASMS > 2 MM (MVLN) | LIVER HISTOPATHOLOGY (LH) |
|---|---|---|
| Lymphocystis<br>Epidermal hyperplasia/ papilloma<br>Acute/healing skin ulcers<br>X-cell gill disease<br>Hyperpigmentation<br>Acute/healing fin rot erosion<br>*Stephanostomum baccatum*<br>*Acanthochondria cornuta*<br>*Lepeophtheirus pectoralis* | Benign tumours<br>Malignant tumours | Non-specific lesions<br>Early non-neoplastic toxicopathic lesions<br>Pre-neoplastic lesions (foci of cellular alteration, FCA)<br>Benign tumours<br>Malignant tumours |

### Should all disease categories be combined for the calculation of a single FDI?

Referring to diseases monitored in dab (see Table A9.1), this can only be done if all disease categories (EVD, MVLN, LH) are included in the disease examination. However, since not all labs are in a position to do this due to a lack of resources and capacities and since small dab (size group 15–19 cm total length) are, according to the ICES/BEQUALM guidelines, only examined for externally visible diseases, the authors suggest to calculate three different FDIs for EDV, MVLN and LH, respectively, as a first step. If feasible and if data on the complete set of diseases is available, the FDIs may be combined (see below).

### Should the FDI only consist of data on presence/absence of a disease?

This obviously depends on the data availability. If only data on the presence or absence of a disease in an individual fish is available (this kind of data is used for the calculation of the disease prevalence in a given sample) (for example in the present ICES Fish Disease Database), the answer is simple (yes). However, if also data on intensity/severity of a disease affecting an individual fish is available, this information should also be included in the FDI since intensity/severity of a disease is also considered to be a useful indicator of ecosystem health. Most monitoring programmes are applying a grading system anyway, with three stages (guidelines for grading are, however, only available for parts if the diseases listed in Table 1, but can easily be developed) and, therefore, disease grades should be included when constructing a FDI. This approach was chosen accordingly for constructing the dab FDI.

### Should all diseases be given the same weight?

This is a crucial point for consideration, because the decision made has a large impact on the resulting FDI. If all diseases are given the same weight, the important fact that some diseases have a more severe impact on the general health of an individual than others would be neglected. Consequently, this would support the idea to define disease-specific weights and use them for calculating the FDI. Another reason for assigning weights to the diseases could be that the monitoring and assessment is targeted to identify and quantify effects of specific environmental stressors on the health of the fish (see comments made above). For instance, if effects of carcinogenic xenobiotics are to be assessed, it could be useful to assign higher weights to neoplastic and pre-neoplastic lesions when calculating a FDI.

However, there are two general problems encountered when assigning disease-specific weights. Firstly, the weighting would require a proper knowledge of the effects of the disease on the host (or of the responsiveness to a given stressor) which can be translated into quantitative figures (weighting factors). Secondly, if disease-specific weights are being assigned and if some diseases are not being looked for some reason, the resulting index would

be biased because of missing values and there would be no appropriate way how to adjust the FDI.

### What should be done if not all diseases to be incorporated in the FDI have been looked for?

This is another problem which is particularly evident when trends derived from long-term data series should be assessed or when data generated by different labs are to be combined or compared. If there are gaps in a data series (e.g. because of a temporary change in strategy or staff), there may be possibilities for interpolation when calculating a FDI, requiring careful expert judgment and the application of appropriate methods. If certain diseases have systematically been ignored or have not been looked for at the beginning or the end of a time series, there is no way to compensate for that because extrapolation does not seem to be justified. Figure A9.1 provides a simple example illustrating the problem.

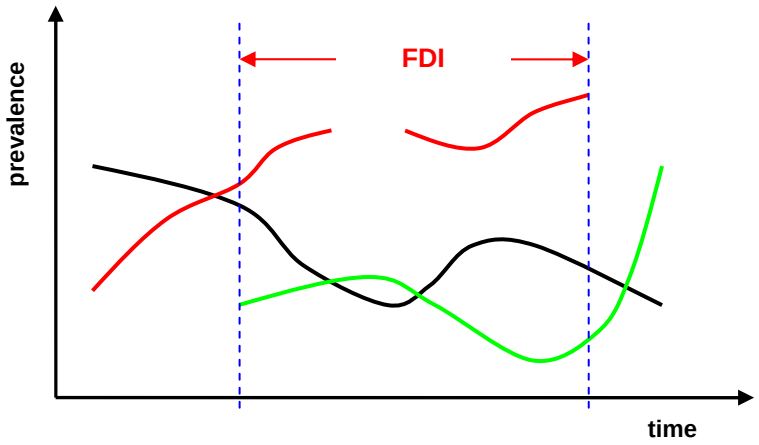



**Figure A9.1: Example of a period for which the calculation of a Fish Disease Index (FDI) based on three target diseases (red, black, green) is justified.**

### Are there other factors that should be taken into account when constructing a FDI?

From empirical data there is evidence that length, age and gender may have an effect on the prevalence of certain diseases, e.g. in dab. Therefore, it is problematic to compare prevalence data and resulting FDIs derived from the study of populations that differ in their length, age and gender structure. A solution to the problem is the use of adjustment factors calculated on the basis of the known relationship between prevalence and age, length and gender, respectively. The situation is complicated by the fact that these relationships vary according to the type of disease studied. For example, there is evidence that the prevalence of liver tumours in dab increases with age and that, in contrast, it decreases with age for the x-cell gill disease. Female fish seem to be more susceptible to tumours than male fish, while male fish seem to be more susceptible to lymphocystis and skin ulcers. Furthermore, the relationship between age/length and prevalence may not always be a linear one, e.g. because of acquired immunity in older fish (in case of infectious diseases) or increased mortality. Therefore, it should be tested if such effects exist and, if so, appropriate adjustment factors have to be developed which should be included in the calculation of a FDI. However, the question remains to be answered as to how such adjustment factors can be defined, e.g. on a regional or temporal basis.

## Construction of a Fish Disease Index (FDI) for dab

According to the above considerations, non-adjusted and adjusted (with correction factors for length and gender) FDIs were constructed for four (hypothetical) specimens of dab. For illustrative reasons, these specimens represent extremes in terms of their length and diseases.

### Non-adjusted FDI

The non-adjusted FDIs for these four fishes are provided in Table A9.2a, separately for the categories externally visible diseases/parasites (EVD), macroscopically visible liver neoplasms (MVLN) and liver histopathology (LH). In addition, total non-adjusted FDIs calculated from the three FDIs are given at the end of the table.

For each fish and disease, a 'Score' is calculated from the multiplication of the entries in the cells 'Disease code' (= Grade), 'Disease-specific weight' and 'Presence of disease' (values either 0 or 1). The disease-specific weights were allocated based on expert judgement. The FDIs for EVD, MVLN and LH were then calculated as proportion of the maximum possible score, with the low values reflecting a good and the high values reflecting a poor health status. The 'Total non-adjusted FDIs' (at the end of Table V9.2a) were calculated as the sum of the three single FDIs divided by the factor 3, assuming that each of the three disease categories has the same impact on the general disease status. For Fish 1 (male, 15 cm), no data on liver anomalies are given in Table A9.2a, because these small specimens are not examined for liver lesions according to ICES/BEQUALM guidelines. As a consequence, no total non-adjusted FDIs could be calculated.

### Adjusted FDI

The adjusted FDIs constructed (see Table A9.2b) take the effects of length and gender into account by including a (purely hypothetical) disease-specific length and gender adjustment factor (cell 'Adjust factor') into the calculation of the score for each of the diseases. The adjustment factors assigned are based on the function of the (hypothetical) relationship between length and likelihood to be affected as well as on the extent of the gender effect. The adjustment factors are calculated based on the definition of a standard fish (female, 22 cm) for which all adjustment factors are set to 1 (see Tables A9.4a–A9.4d). For Lymphocystis, a bell-shaped relationship between length and likelihood to be affected as well as a higher likelihood for males to be affected compared to females were assumed (Table A9.4a). For all other diseases, a positive linear relationship between length and disease likelihood was assumed, except for x-cell gill disease (Table A9.4c). For neoplastic and pre-neoplastic lesions, females were assigned a higher likelihood to be affected than males. For the rest of the diseases, a general adjustment factor as shown in Table 4d was assumed. The calculation of the scores and the adjusted FDIs was done by multiplying the cells 'Disease code', 'Disease-specific weight', 'Presence of disease' and 'Adjust factor'. The total adjusted FDIs (see end of Table A9.2b) were calculated in an analogous way to the total non-adjusted FDIs. Again, no total adjusted FDI was calculated for Fish 1 (see above)

Figure A9.2 summarises the results of the calculations. Differences between the non-adjusted and adjusted FDIs can mainly be seen in the smallest (Fish 1) and largest dab (Fish 4). For instance, the non-adjusted FDI for externally visible diseases (EVD) in Fish 1 is 29.6 while the adjusted one is 36.8, a difference of more than 20%. In the largest specimen (Fish 4), the non-adjusted FDI for liver histopathology (LH) is 60,0 and the adjusted FDI is 42.0, a difference of more than 40%. These differences reveal that an adjustment of the scores for length and gender effects may lead to significant changes in the scores and resulting FDIs compared to non-adjusted data and indicate that an adjustment should be carried out if significant effects of length or gender on the prevalence occur.

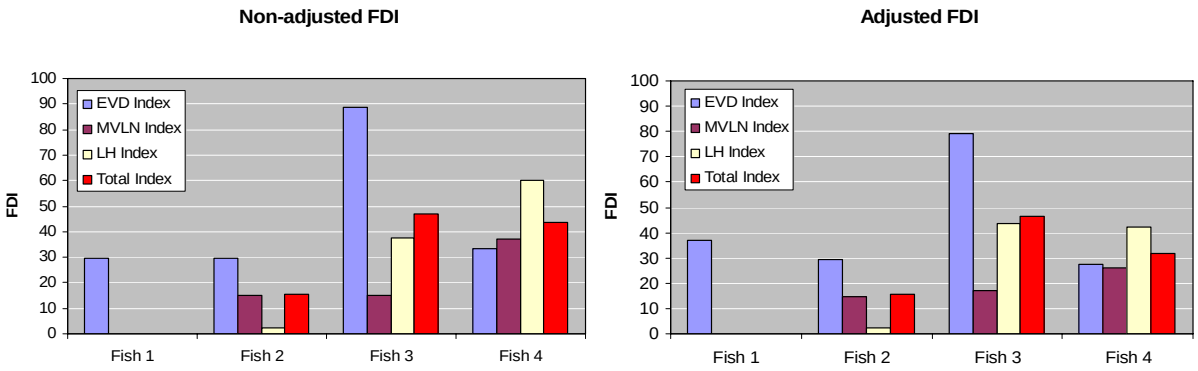



**Figure A9.2: Non-adjusted and adjusted Fish Disease Indices (FDIs) calculated for four specimens of dab (*Limanda limanda*) based on hypothetical assumptions ( = values) (see Tables A9.2a, 2b).**

## The pilot study: using empirical data to construct a Fish Disease Index (FDI) for dab

The calculation of Fish Disease Indices for historical data is limited by the availability of the parameters which constitute these indices. Historical data frequently contains no information about disease grades, information about macroscopically visible liver neoplasms is given only occasionally, and liver histology has been reported only sporadic. As a consequence, only German data (reporting lab: BFCG) on externally visible diseases observed between 1981 and 2002 is used for this pilot study, as this data set contains disease grades in a readily accessible form. Only the Fish Disease Index for externally visible diseases and parasites (EVD-FDI) is considered due to present data availability restrictions.

The purpose of this pilot study is to demonstrate the application of the approach to real empirical data and to provide a basis to discuss the appropriateness of the approach in general and the setting of weights in the definition of the indices. The proposed indices should represent the biological knowledge about the disease situation in the area and time period under study. They also should be able to indicate temporal changes and spatial differences with a reasonably high probability.

To illustrate the behaviour of the EVD-FDI, its value was calculated for dab (*Limanda limanda*, males and females, size group 15–33 cm) caught between 1990 and 2006 at three stations in the North Sea (ICES statistical rectangles 37F7: German Bight; 38F2: Doggerbank; 40F7: Danish coast) and one station in the Baltic Sea (ICES statistical rectangle 38G0: Kiel Bight) as a reference. Diseases taken into account were lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcers, acute/healing fin rot/erosion, hyperpigmentation, x-cell gill disease and three parasites (*Stephanostomum baccatum*, *Lepeophtheirus pectoralis*, *Acanthochondria cornuta*) (see Table A9.2). The grading of each disease (indicating its severity) and the assignment of disease-specific weighting factors were done according to the method described above and shown in Table A9.2.

Means and standard errors of the EVD-FDI were calculated per ICES statistical rectangle and sampling date, the latter given as the day of the catch. Figure A9.3 shows mean values with standard error added for the four rectangles, which were selected for display as for these further data exist (from ICES sources, not used in this study), which qualifies these areas for the assessment of recent prevalence trends (http://www.ices.dk/marineworld/fishdiseases/fishandshellfish.asp). The figure leads to the

conclusions that temporal and spatial structures are not only expressed qualitatively, but also that the variation of the index is small enough to generate significant differences between areas and sampling dates.

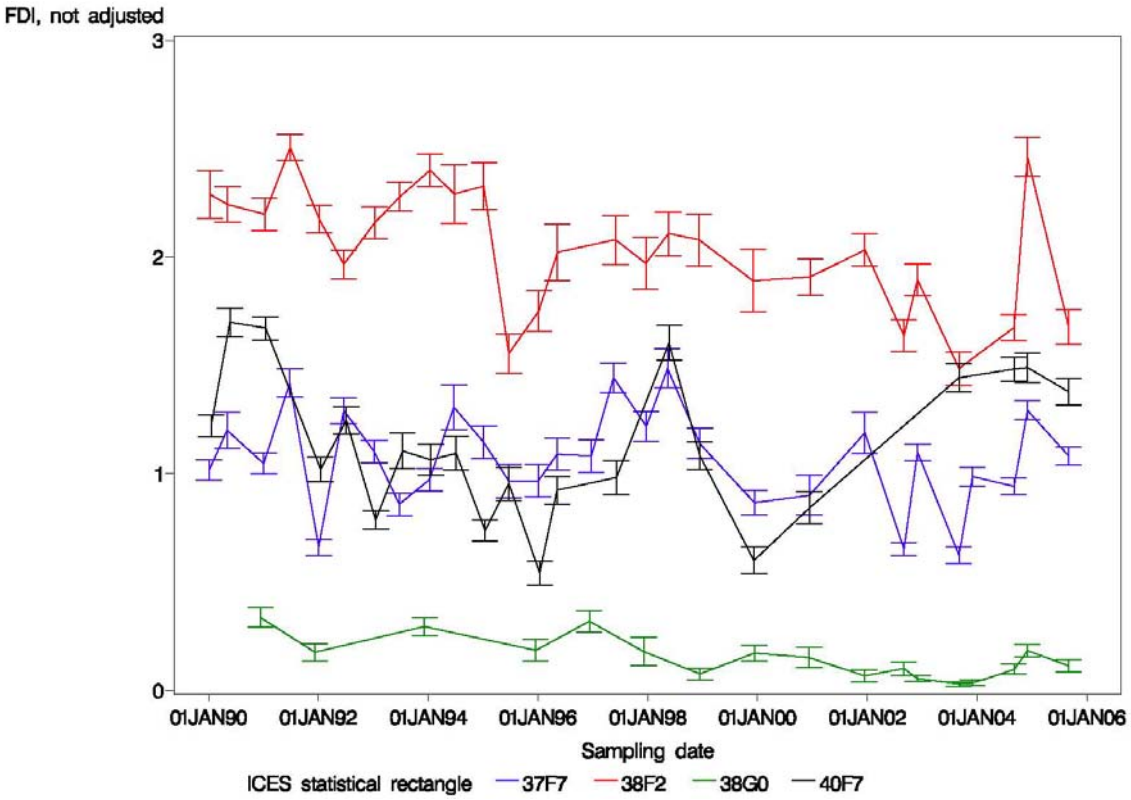



**Figure A9.3: Temporal change (1990–2006) in the non-weighted 'Fish Disease Index' (not adjusted for size effects) for externally visible diseases and parasites in dab (*Limanda limanda*) (males and females, size group 15–33 cm) from three North Sea areas (37F7: German Bight; 38F2: Dogger Bank; 40F7: Danish coast) and a Baltic Sea area (38G0: Kiel Bight).**

In order to account for the effect of size (total length) on the presence of a disease, an adjustment factor, which compensated the length effect (see above), was introduced. This factor was derived from the empirical relationship between length and each single disease prevalence, speparately for each disease grade. For most of the diseases this relationship had a bell-shaped form, but also J and U shapes were found. These shapes were approximated using a LOESS smoother, which provided an adjustment factor for each centimetre of length and specific for each gender. Weight factors are scaled such that the maximum raw value of the adjusted index is the same as the one for the not adjusted index. The resulting FDIs are shown in Figure A9.4.

The main consequence of the weighting procedure in this pilot example seems to be twofold: first, some level differences between stations are removed, and second, a common decreasing long-term trend, less visible in the „unadjusted" version, seems to become more clearly apparent. Using the length adjustment approach allows to use more than only one length class for the construction of an index, and also to combine data from both sexes.

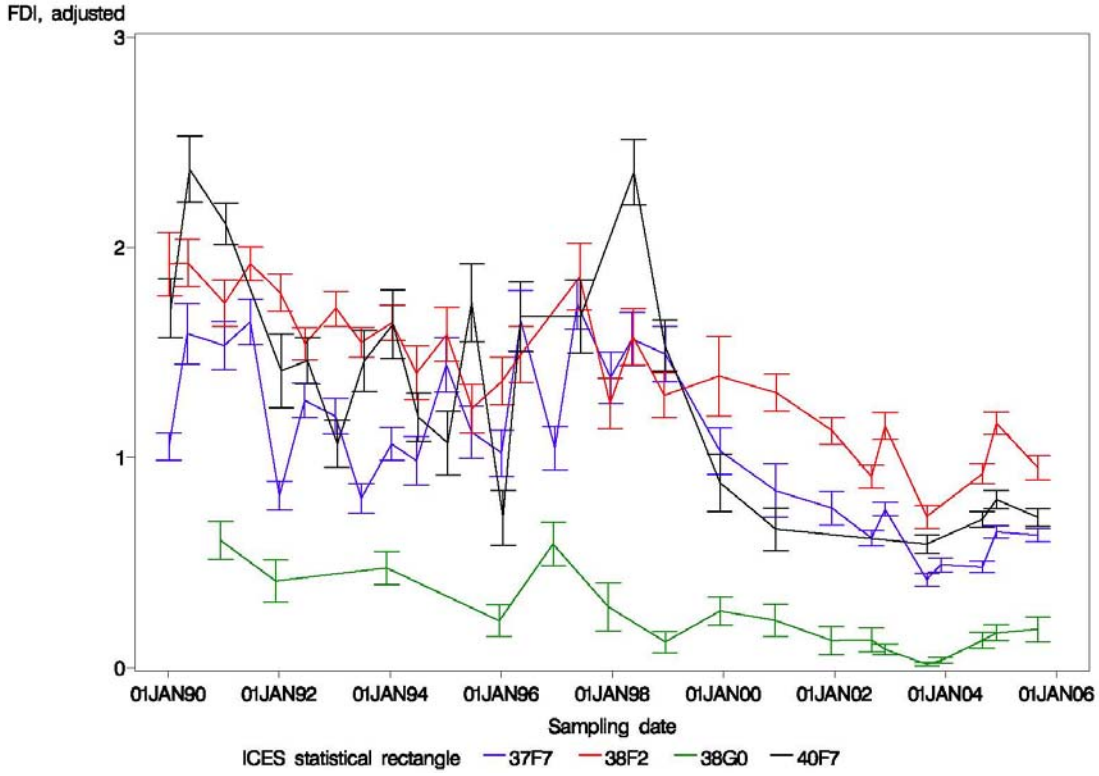



**Figure A9.4: Temporal change (1990-2006) in the weighted 'Fish Disease Index' (adjusted for size effects) for externally visible diseases and parasites in dab (*Limanda limanda*) (males and females, size group 15–33 cm) from three North Sea areas (37F7: German Bight; 38F2: Dogger Bank; 40F7: Danish coast) and a Baltic Sea area (38G0: Kiel Bight).**

## Discussion and outlook

The constructed Fish Disease Indices (FDIs) for dab and the results of the pilot study using empirical data should be seen as examples and there is a need for further discussion and certainly room for improvement, e.g. in terms of

- the diseases to be included,
- the assignment of disease-specific weighting factors,
- the calculation of adjustment factors, and
- how to deal with missing values.

Furthermore, a number of issues have to be dealt with before the FDI approach can be applied on a routine basis, e.g. convention-wide in the OSPAR area:

- the applicability of the FDI approach for other fish species,
- the development of statistical methods for spatial and temporal assessments,
- the definition of background/reference values,
- the definition of threshold values as a basis for management action, and
- the development of methods for a comparison of FDIs derived from different fish species.

However, despite these issues to be solved, the development of Fish Disease Indices is considered to be an appropriate approach to utilise complex fish disease data as tools for assessing ecosystem health and, therefore, further work into this direction is required.

**Table A9.2a: Construction of non-adjusted Fish Disease Indices (FDI) for externally visible diseases/parasites (EVD), macroscopically visible liver neoplasms > 2 mm (= liver nodules > 2 mm) (MVLN) and liver histopathology (LH) in dab (*Limanda limanda*).**

| | | | | Fish 1 | | Fish 2 | | Fish 3 | | Fish 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Size (cm total length) | 15 | | 22 | | 25 | | 32 | |
| | | | Gender | Male | | Female | | Male | | Female | |
| **A: Externally visible diseases (EVD)** | **Grade** | **Disease code** | **Disease-specific weight** | **Presence of disease** | **Score** | **Presence of disease** | **Score** | **Presence of disease** | **Score** | **Presence of disease** | **Score** |
| Lymphocystis | 1 | 1 | 2 | 1 | 2.00 | 1 | 2.00 | 0 | 0.00 | 1 | 2.00 |
| | 2 | 2 | 2 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 | 3 | 2 | 0 | 0.00 | 0 | 0.00 | 1 | 6.00 | 0 | 0.00 |
| Epidermal Hyperplasia/Papilloma | 1 | 1 | 2 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 | 2 | 2 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 | 3 | 2 | 0 | 0.00 | 0 | 0.00 | 1 | 6.00 | 0 | 0.00 |
| Acute/Healing Skin Ullcerations | 1 | 1 | 3 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 | 2 | 3 | 1 | 6.00 | 1 | 6.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 | 3 | 3 | 0 | 0.00 | 0 | 0.00 | 1 | 9.00 | 1 | 9.00 |
| X-cell gill disease | 1 | 1 | 9 | 0 | 0.00 | 0 | 0.00 | 1 | 9.00 | 0 | 0.00 |
| Hyperpigmentation | 1 | 1 | 3 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 3.00 |
| | 2 | 2 | 3 | 1 | 6.00 | 1 | 6.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 | 3 | 3 | 0 | 0.00 | 0 | 0.00 | 1 | 9.00 | 0 | 0.00 |
| Ac./Heal. Fin Rot/Erosion | 1 | 1 | 6 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| *Stephanostomum sp.* | 1 (1–10 cysts) | 1 | 1 | 1 | 1.00 | 1 | 1.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 (11–50 cysts) | 2 | 1 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 (> 50 cysts) | 3 | 1 | 0 | 0.00 | 0 | 0.00 | 1 | 3.00 | 1 | 3.00 |
| *Acanthochondria sp.* | 1 (1 specimen) | 1 | 1 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 1.00 |
| | 2 (2 specimens) | 2 | 1 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 (> 2 specimens) | 3 | 1 | 0 | 0.00 | 0 | 0.00 | 1 | 3.00 | 0 | 0.00 |
| *Lepeophtheirus sp.* | 1 (1 specimen) | 1 | 1 | 1 | 1.00 | 1 | 1.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 (2 specimens) | 2 | 1 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 (> 2 specimens) | 3 | 1 | 0 | 0.00 | 0 | 0.00 | 1 | 3.00 | 0 | 0.00 |
| | | | EVD raw score | | 16.00 | | 16.00 | | 48.00 | | 18.00 |
| | | | **EVD-FDI** | | **29.63** | | **29.63** | | **88.89** | | **33.33** |

**Table A9.2a: (continued)**

| | | | | Fish 1 | | Fish 2 | | Fish 3 | | Fish 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Size (cm total length) | 15 | | 22 | | 25 | | 32 | |
| | | | Gender | Male | | Female | | Male | | Female | |
| **B: Macroscopically visible liver neoplasms (MVLN)** | **Grade** | **Disease code** | **Disease-specific weight** | **Presence of disease** | **Score** | **Presence of disease** | **Score** | **Presence of disease** | **Score** | **Presence of disease** | **Score** |
| Benign tumours | 1 (2–5mm) | 1 | 4 | n.e. | 0.00 | 1 | 4.00 | 1 | 4.00 | 0 | 0.00 |
| | 2 (6–9mm) | 2 | 4 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 (>=10mm) | 3 | 4 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| Malignant tumour | 1 (2–5mm) | 1 | 5 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 (6–9mm) | 2 | 5 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 10.00 |
| | 3 (>=10mm) | 3 | 5 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | | | MVLN raw score | | - | | 4.00 | | 4.00 | | 10.00 |
| | | | **MVLN-FDI** | | **-** | | **14.81** | | **14.81** | | **37.04** |

**Table A9.2a: (continued)**

| | | | | Fish 1 | | Fish 2 | | Fish 3 | | Fish 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Size (cm total length) | 15 | | 22 | | 25 | | 32 | |
| | | | Gender | Male | | Female | | Male | | Female | |
| **C: Liver Histopathology (LH)** | **Grade** | **Disease code** | **Disease-specific weight** | **Presence of disease** | **Score** | **Presence of disease** | **Score** | **Presence of disease** | **Score** | **Presence of disease** | **Score** |
| Non-specific lesions | 1 | 1 | 1 | n.e. | 0.00 | 1 | 1.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 | 2 | 1 | n.e. | 0.00 | 0 | 0.00 | 1 | 2.00 | 1 | 2.00 |
| | 3 | 3 | 1 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| Early toxicopathic non-neoplastic lesions | 1 | 1 | 2 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 | 2 | 2 | n.e. | 0.00 | 0 | 0.00 | 1 | 4.00 | 0 | 0.00 |
| | 3 | 3 | 2 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 6.00 |
| Pre-neoplastic lesions (FCA) | 1 | 1 | 3 | n.e. | 0.00 | 0 | 0.00 | 1 | 3.00 | 0 | 0.00 |
| | 2 | 2 | 3 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 3 | 3 | 3 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 9.00 |
| Benign tumours | 1 | 1 | 4 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 | 2 | 4 | n.e. | 0.00 | 0 | 0.00 | 1 | 8.00 | 0 | 0.00 |
| | 3 | 3 | 4 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| Malignant tumours | 1 | 1 | 5 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | 2 | 2 | 5 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 10.00 |
| | 3 | 3 | 5 | n.e. | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| | | | LH raw score | | - | | 1.00 | | 17.00 | | 27.00 |
| | | | **LH-FDI** | | **-** | | **2.22** | | **37.78** | | **60.00** |
| | | | **Total non-adjusted FDI** | | **-** | | **15.56** | | **47.16** | | **43.46** |

**Table A9.2b: Construction of Fish Disease Indices (FDI) adjusted for length and gender effects for externally visible diseases/parasites (EVD), macroscopically visible liver neoplasms > 2 mm (=liver nodules > 2 mm) (MVLN) and liver histopathology (LH) in dab (*Limanda limanda*).**

| | | | | Fish 1 | | | Fish 2 | | | Fish 3 | | | Fish 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Size (cm total length) | 15 | | | 22 | | | 25 | | | 32 | | |
| | | | Gender | Male | | | Female | | | Male | | | Female | | |
| **A: Externally visible diseases (EVD)** | **Grade** | **Disease code** | **Disease-specific weight** | **Presence of disease** | **Adjust Factor** | **Score** | **Presence of disease** | **Adjust Factor** | **Score** | **Presence of disease** | **Adjust Factor** | **Score** | **Presence of disease** | **Adjust Factor** | **Score** |
| Lymphocystis | 1 | 1 | 2 | 1 | 1.456 | 2.91 | 1 | 0.998 | 2.00 | 0 | 0.500 | 0.00 | 1 | 1.806 | 3.61 |
| | 2 | 2 | 2 | 0 | 1.456 | 0.00 | 0 | 0.998 | 0.00 | 0 | 0.500 | 0.00 | 0 | 1.806 | 0.00 |
| | 3 | 3 | 2 | 0 | 1.456 | 0.00 | 0 | 0.998 | 0.00 | 1 | 0.500 | 3.00 | 0 | 1.806 | 0.00 |
| Epidermal Hyperplasia/Papilloma | 1 | 1 | 2 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 | 2 | 2 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 3 | 3 | 2 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 1 | 0.910 | 5.46 | 0 | 0.700 | 0.00 |
| Acute/Healing Skin Ullcerations | 1 | 1 | 3 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 | 2 | 3 | 1 | 1.210 | 7.26 | 1 | 1.000 | 6.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 3 | 3 | 3 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 1 | 0.910 | 8.19 | 1 | 0.700 | 6.30 |
| X-cell gill disease | 1 | 1 | 9 | 0 | 0.790 | 0.00 | 0 | 1.000 | 0.00 | 1 | 1.090 | 9.81 | 0 | 1.300 | 0.00 |
| Hyperpigmentation | 1 | 1 | 3 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 1 | 0.700 | 2.10 |
| | 2 | 2 | 3 | 1 | 1.210 | 7.26 | 1 | 1.000 | 6.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 3 | 3 | 3 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 1 | 0.910 | 8.19 | 0 | 0.700 | 0.00 |
| Ac./Heal.Fin Rot/Eros. | 1 | 1 | 6 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| *Stephanostomum sp.* | 1 (1–10 cysts) | 1 | 1 | 1 | 1.210 | 1.21 | 1 | 1.000 | 1.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 (11–50 cysts) | 2 | 1 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 3 (> 50 cysts) | 3 | 1 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 1 | 0.910 | 2.73 | 1 | 0.700 | 2.10 |
| *Acanthochondria sp.* | 1 (1 specimen) | 1 | 1 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 1 | 0.700 | 0.70 |
| | 2 (2 specimens) | 2 | 1 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 3 (> 2 specimens) | 3 | 1 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 1 | 0.910 | 2.73 | 0 | 0.700 | 0.00 |
| *Lepeophtheirus sp.* | 1 (1 specimen) | 1 | 1 | 1 | 1.210 | 1.21 | 1 | 1.000 | 1.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 (2 specimens) | 2 | 1 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 0 | 0.910 | 0.00 | 0 | 0.700 | 0.00 |
| | 3 (> 2 specimens) | 3 | 1 | 0 | 1.210 | 0.00 | 0 | 1.000 | 0.00 | 1 | 0.910 | 2.73 | 0 | 0.700 | 0.00 |
| | | | EVD raw score | 54 | | 19.85 | 54 | | 16.00 | 54 | | 42.84 | 54 | | 14.81 |
| | | | **EVD-FDI** | | | **36.76** | | | **29.62** | | | **79.33** | | | **27.43** |

**Table A9.2b: (continued)**

| | | | | Fish 1 | | | Fish 2 | | | Fish 3 | | | Fish 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Size (cm total length) | 15 | | | 22 | | | 25 | | | 32 | | |
| | | | Gender | Male | | | Female | | | Male | | | Female | | |
| **B: Macroscopically visible liver neo-plasms (MVLN)** | **Grade** | **Disease code** | **Disease-specific weight** | **presence of disease** | **Adjust Factor** | **score** | **presence of disease** | **Adjust Factor** | **score** | **presence of disease** | **Adjust Factor** | **score** | **presence of disease** | **Adjust Factor** | **score** |
| Benign tumours | 1 (2–5mm) | 1 | 4 | n.e. | 1.45 | 0.00 | 1 | 1 | 4.00 | 1 | 1.15 | 4.60 | 0 | 0.7 | 0.00 |
| | 2 (6–9mm) | 2 | 4 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.7 | 0.00 |
| | 3 (>=10mm) | 3 | 4 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.7 | 0.00 |
| Malignant tumour | 1 (2–5mm) | 1 | 5 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.7 | 0.00 |
| | 2 (6–9mm) | 2 | 5 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 1 | 0.7 | 7.00 |
| | 3 (>=10mm) | 3 | 5 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.7 | 0.00 |
| | | | MVLN raw score | 27 | | - | 27 | | 4.00 | 27 | | 4.60 | 27 | | 7.00 |
| | | | **MVLN-FDI** | | | **-** | | | **14.81** | | | **17.04** | | | **25.93** |

**Table A9.2b: (continued)**

| | | | | Fish 1 | | | Fish 2 | | | Fish 3 | | | Fish 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Size (cm total length) | 15 | | | 22 | | | 25 | | | 32 | | |
| | | | Gender | Male | | | Female | | | Male | | | Female | | |
| **C: Liver Histo-pathology (LH)** | **Grade** | **Disease code** | **Disease-specific weight** | **presence of disease** | **Adjust Factor** | **score** | **presence of disease** | **Adjust Factor** | **score** | **presence of disease** | **Adjust Factor** | **score** | **presence of disease** | **Adjust Factor** | **score** |
| Non-specific lesions | 1 | 1 | 1 | n.e. | 1.45 | 0.00 | 1 | 1 | 1.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 | 2 | 1 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 1 | 1.15 | 2.30 | 1 | 0.700 | 1.40 |
| | 3 | 3 | 1 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| Early toxicopathic non-neoplastic lesions | 1 | 1 | 2 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 | 2 | 2 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 1 | 1.15 | 4.60 | 0 | 0.700 | 0.00 |
| | 3 | 3 | 2 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 1 | 0.700 | 4.20 |
| Pre-neoplastic lesions (FCA) | 1 | 1 | 3 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 1 | 1.15 | 3.45 | 0 | 0.700 | 0.00 |
| | 2 | 2 | 3 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| | 3 | 3 | 3 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 1 | 0.700 | 6.30 |
| Benign tumours | 1 | 1 | 4 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 | 2 | 4 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 1 | 1.15 | 9.20 | 0 | 0.700 | 0.00 |
| | 3 | 3 | 4 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| Malignant tumours | 1 | 1 | 5 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| | 2 | 2 | 5 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 1 | 0.700 | 7.00 |
| | 3 | 3 | 5 | n.e. | 1.45 | 0.00 | 0 | 1 | 0.00 | 0 | 1.15 | 0.00 | 0 | 0.700 | 0.00 |
| | | | LH raw score | 45 | | - | 45 | | 1.00 | 45 | | 19.55 | 45 | | 18.90 |
| | | | **LH Index** | | | **-** | | | **2.22** | | | **43.44** | | | **42.00** |
| | | | **Total adjusted FDI** | | | **-** | | | **15.55** | | | **46.60** | | | **31.78** |

**Table A9.4a: Size adjustment factor for Lymphocystis (hypothetical model, only for illustration).**

| location | 25 | 25 |
|---|---|---|
| sd | 4 | 4 |
| max | 4 | 6 |
| vert shift | 1.5 | 2.13 |
| | | |
| **Size** | **males** | **females** |
| 15 | 1.456063066 | 2.0640946 |
| 16 | 1.420440491 | 2.010660737 |
| 17 | 1.364664717 | 1.926997075 |
| 18 | 1.283734833 | 1.80560225 |
| 19 | 1.175347533 | 1.643021299 |
| 20 | 1.042166638 | 1.443249957 |
| 21 | 0.89346934 | 1.22020401 |
| 22 | 0.745160398 | 0.997740597 |
| 23 | 0.617503097 | 0.806254646 |
| 24 | 0.530766766 | 0.676150148 |
| 25 | 0.5 | 0.63 |
| 26 | 0.530766766 | 0.676150148 |
| 27 | 0.617503097 | 0.806254646 |
| 28 | 0.745160398 | 0.997740597 |
| 29 | 0.89346934 | 1.22020401 |
| 30 | 1.042166638 | 1.443249957 |
| | | |
| max | 1.456063066 | 2.0640946 |

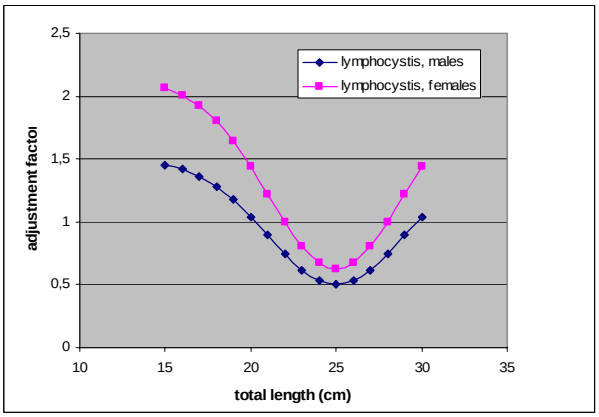



**Table A9.4b: Size adjustment factor for pre-neoplastic lesions (FCA) and tumours (hypothetical model, only for illustration).**

| intercept | 1.9 | 1.66 |
|---|---|---|
| slope | -0.03 | -0.03 |
| | | |
| **Size** | **males** | **females** |
| 15 | 1.45 | 1.21 |
| 16 | 1.42 | 1.18 |
| 17 | 1.39 | 1.15 |
| 18 | 1.36 | 1.12 |
| 19 | 1.33 | 1.09 |
| 20 | 1.3 | 1.06 |
| 21 | 1.27 | 1.03 |
| 22 | 1.24 | 1 |
| 23 | 1.21 | 0.97 |
| 24 | 1.18 | 0.94 |
| 25 | 1.15 | 0.91 |
| 26 | 1.12 | 0.88 |
| 27 | 1.09 | 0.85 |
| 28 | 1.06 | 0.82 |
| 29 | 1.03 | 0.79 |
| 30 | 1 | 0.76 |
| | | |
| max | 1.45 | 1.21 |

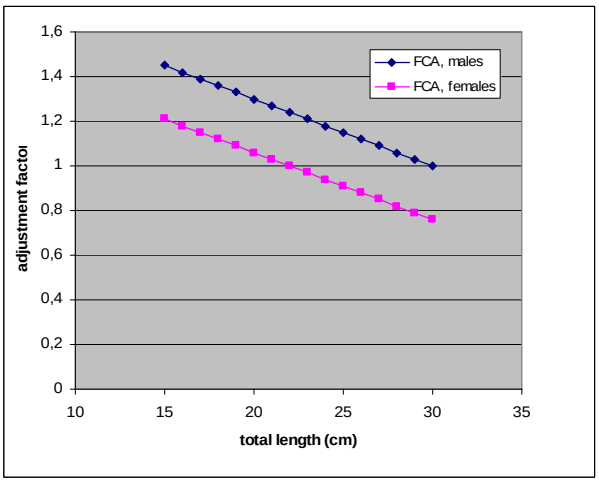


**Table A9.4c: Size adjustment factor for x-cell gill disease (hypothetical model, only for illustration).**

| | |
|---|---|
| intercept | 0.34 |
| slope | 0.03 |
| | |
| **Size** | **males+females** |
| 15 | 0.79 |
| 16 | 0.82 |
| 17 | 0.85 |
| 18 | 0.88 |
| 19 | 0.91 |
| 20 | 0.94 |
| 21 | 0.97 |
| 22 | 1 |
| 23 | 1.03 |
| 24 | 1.06 |
| 25 | 1.09 |
| 26 | 1.12 |
| 27 | 1.15 |
| 28 | 1.18 |
| 29 | 1.21 |
| 30 | 1.24 |
| | |
| max | 1.24 |

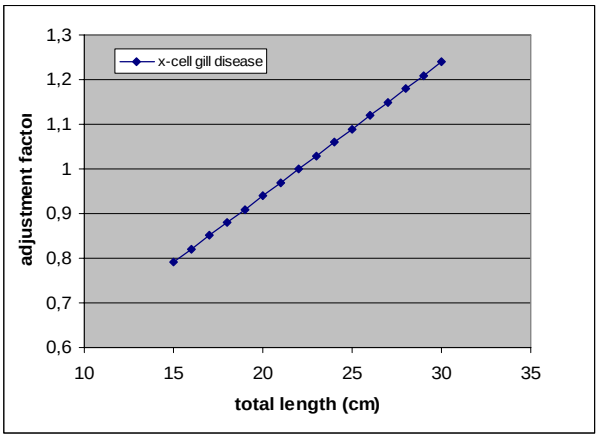
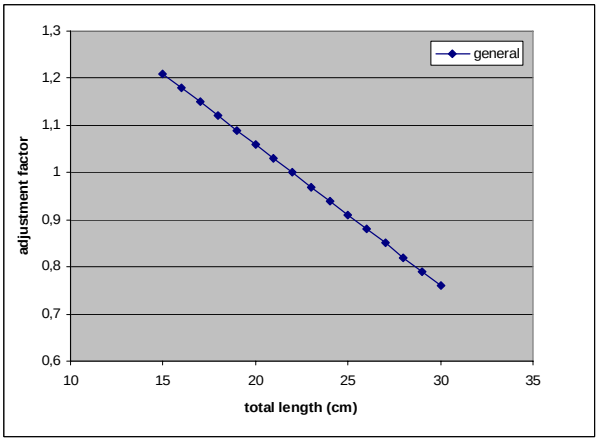



**Table A9.4d: General size adjustment factor (for all other diseases) (hypothetical model. only for illustration).**

| | |
|---|---|
| intercept | 1.66 |
| slope | -0.03 |
| | |
| **size** | **males+females** |
| 15 | 1.21 |
| 16 | 1.18 |
| 17 | 1.15 |
| 18 | 1.12 |
| 19 | 1.09 |
| 20 | 1.06 |
| 21 | 1.03 |
| 22 | 1 |
| 23 | 0.97 |
| 24 | 0.94 |
| 25 | 0.91 |
| 26 | 0.88 |
| 27 | 0.85 |
| 28 | 0.82 |
| 29 | 0.79 |
| 30 | 0.76 |
| | |
| max | 1.21 |



# Annex 10: Update of disease maps on the ICES website

**W. Wosniok and T. Lang**

The ICES website contains a section providing information on temporal trends in the prevalence of externally visible diseases (so far lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulcerations) of North Sea dab (*Limanda limanda*) that are monitored by North Sea countries on a regular basis (http://www.ices.dk/marineworld/fishdiseases/fishandshellfish.asp). The data generated in these national programmes are submitted to the ICES Data Centre and are assessed by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO). Based on these data, maps have been developed that are part of the website

In the following, new information is provided and suggestions are made for an update of the maps.

The first map (Figure A10.1) to be displayed on the website (replacing the old and out-dated black and white map) provides an overview (data inventory) of fish disease data available in the ICES Data Centre (number of observation days, number of fish examined in the period 1981–2005).

In Figure A10.2 (showing only the inner North Sea) (not to be displayed on the ICES website) it can be seen that there are marked differences between ICES statistical rectangles in the amount of data available which is important in the context of generating trend figures. Figure A10.3 (not to be displayed on the ICES website) provides more detailed information on data availability and specifies those rectangles that fulfil the criteria defined for conducting a trend assessment (at least 10 samples within the last 10 years). In total, only 7 of the North Sea rectangles (rectangle 38G8 is located in Kiel Bight, Baltic Sea) meet these criteria. There locations are shown in Figure A10.4 (not to be displayed on the ICES website).

Figure A10.5 (to be displayed on the ICES website), provides an overview map displaying symbols for the prevalence trends for skin ulcers (as an example) per ICES statistical rectangles for those rectangles for which sufficient amount of data are available. From those rectangles, a link from the map to the underlying trend curve (which includes confidence bands) should be established (see Figure A10.6) (to be displayed on the ICES website)[1].

The calculation of long-term trends has been changed from the separate consideration of summer and winter data (as shown in the present out-dated version on the ICES website) to a joint consideration of all data. This allows the use of more data than before for a trend assessment. Also, using only one symbol per year is more appropriate to the concept of a long-term trend. The new trend calculation became possible by adopting a more advanced statistical method that is able to deal with data from irregularly distributed observation times within the years. Consequently, the new version of a web-based report will contain only one trend symbol per year instead of two.

In addition to presenting the maps, the updated website will provide links to pages with information on the fish species examined, the features of the diseases, the data source and the method of trend calculation. Examples as to how information on the types of diseases can be displayed are given in Figures A10.7a and A10.7b.

1 **Note:** Both of these figures (A10.5, A10.6) need to be updated with new data before being placed on the ICES website since they only cover the period 1999-2004 .

### Conclusion and suggestion to the ICES Data Centre

The suggested display of trend curves is considered useful, as it provides more information to the reader than the earlier categorical trend assessment (up/down/constant) alone. It is felt that the new contents and style of the web-based report would be an improvement in communicating the results of the ICES WGPDMO's activities to a broader public.

However, the web-based report would gain considerably if the trend assessments had a larger spatial coverage for recent time periods. Therefore, it should be checked by the ICES Data Centre on an annual basis whether an update of the present web page for disease trends would be worthwhile with respect to the amount of new and recent disease data submissions that could be given to the reader on the basis of the currently available data. If new data is available, it is suggested that the ICES Data Centre itself conducts the update of the maps (as an ICES data product) and that the WGPDMO provides guidance as to how the trend calculations have to be made.

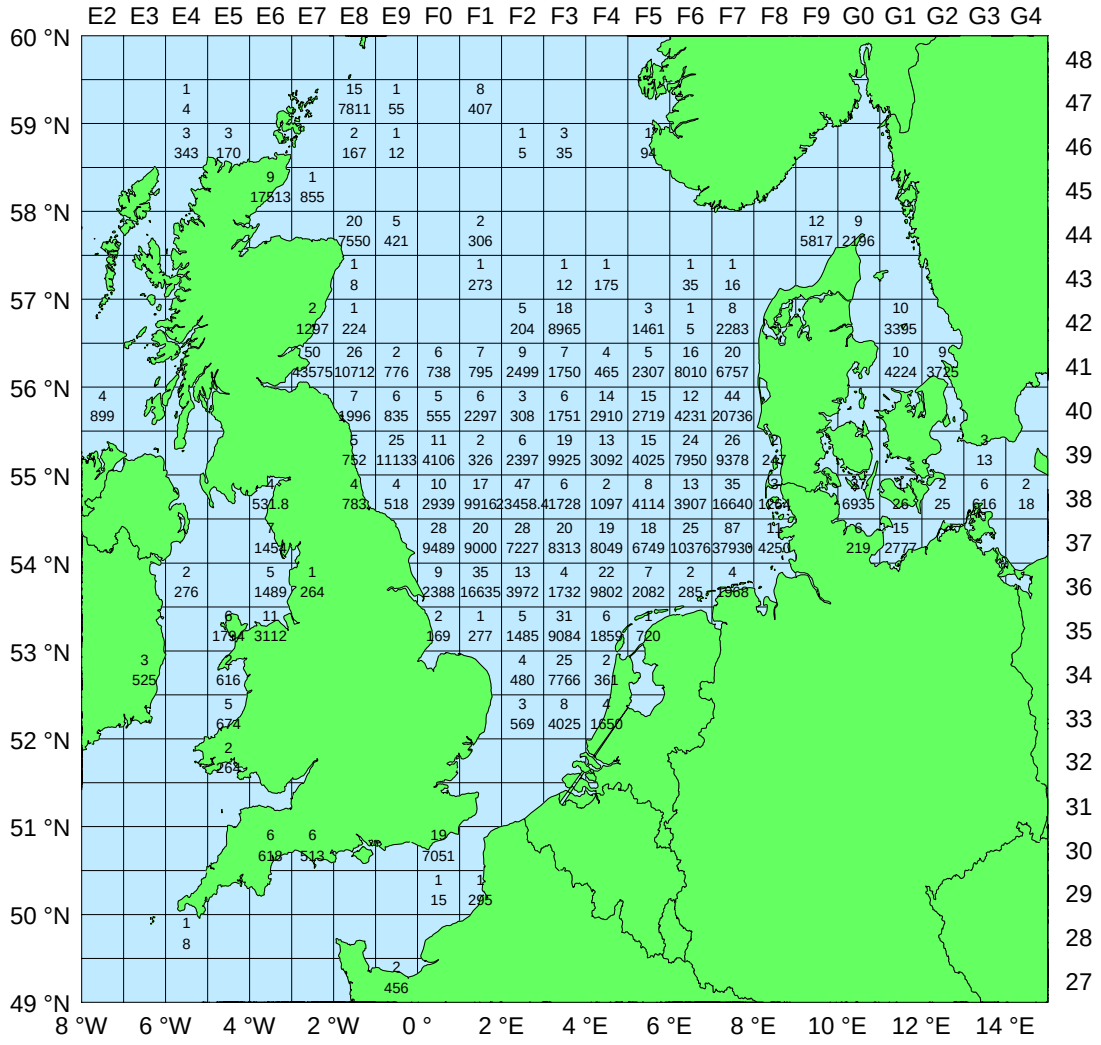



**Figure A10.1: ICES fish disease data inventory for the North Sea and adjacent areas (species: *Limanda limanda*; all size groups; females and males; squares: ICES statistical rectangles; numbers in squares: upper: # observation days, lower: # of fish; time period covered: 1981–2005) (last update: March 03, 2006; source: ICES Data Centre).**

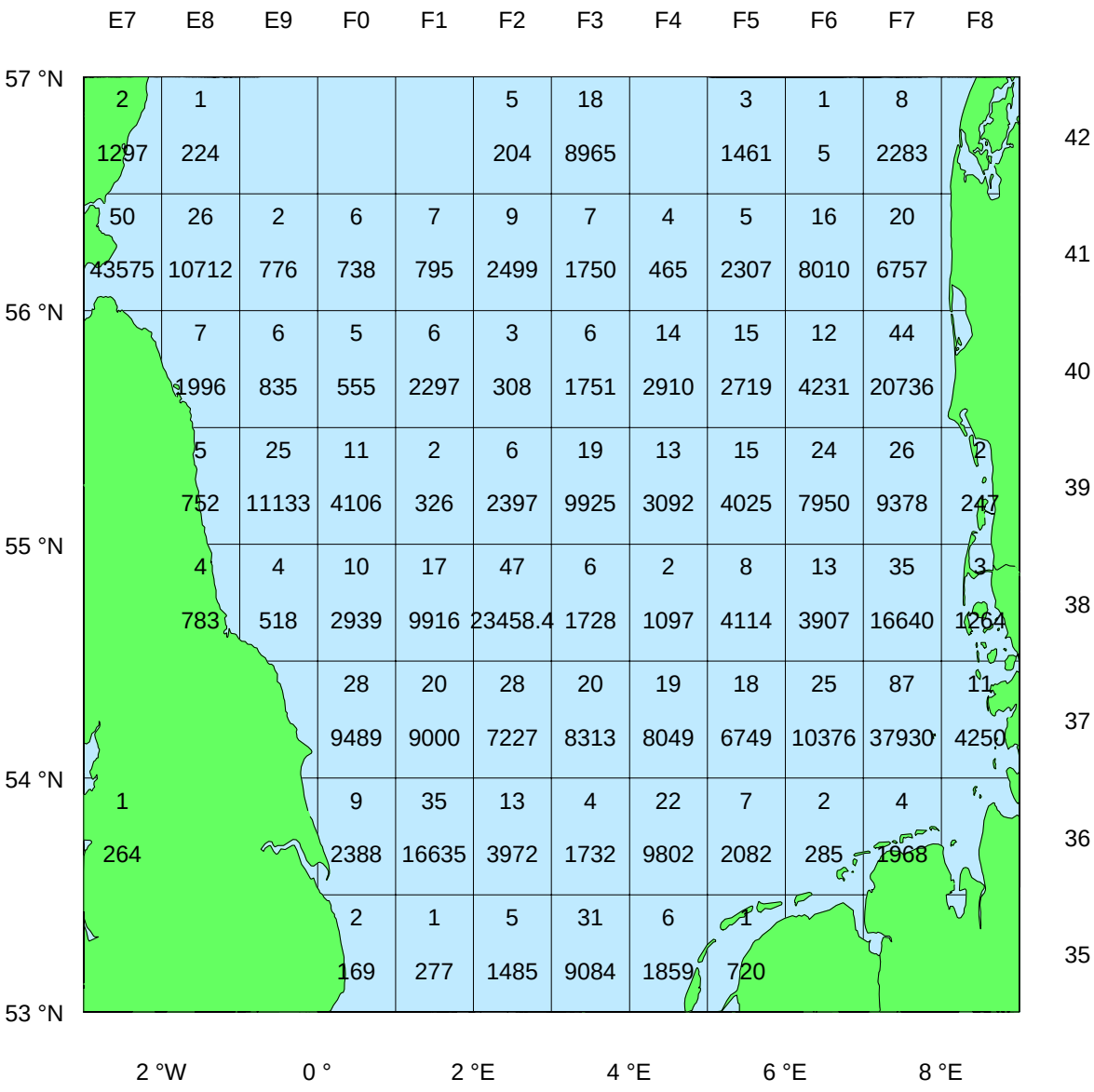



**Figure A10.2: ICES fish disease data inventory for the inner North Sea (species: *Limanda limanda*; all size groups; females and males; squares: ICES statistical rectangles; numbers in squares: upper: # observation days, lower: # of fish; time period covered: 1981 – 2005) (last update: March 03, 2006; source: ICES Data Centre).**

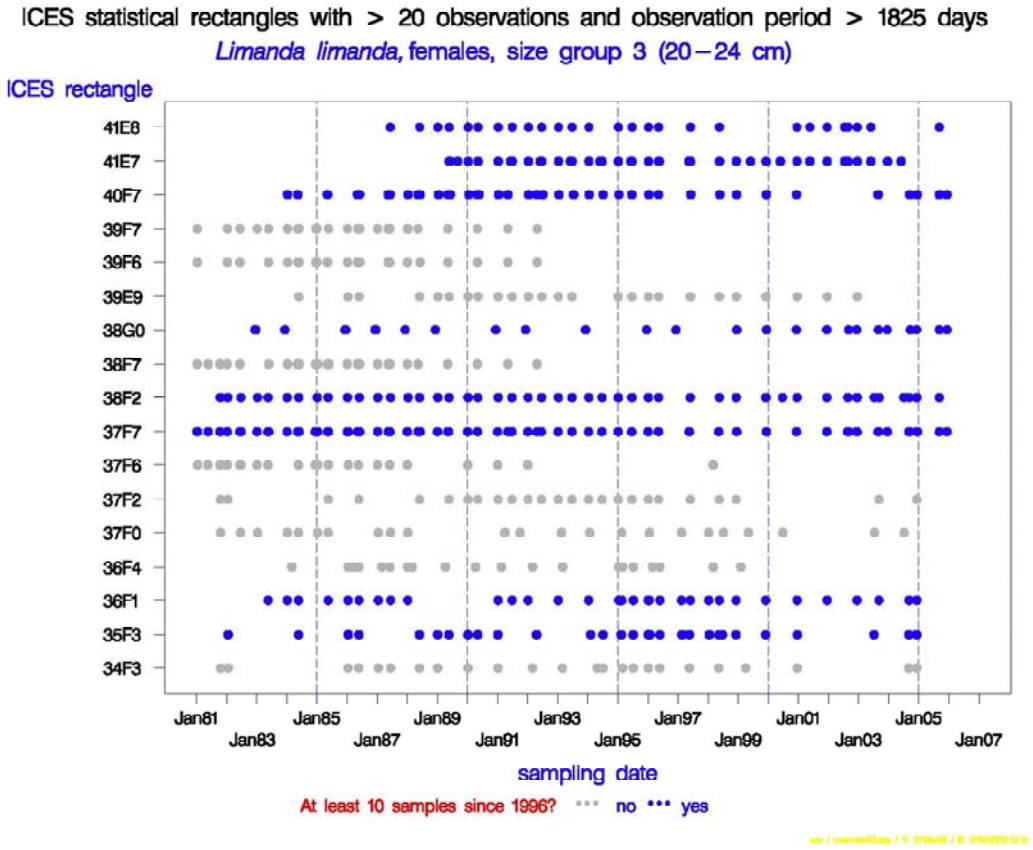



**Figure A10.3: Availability of ICES fish disease data meeting criteria defined for trend assessment (species: *Limanda limanda*; all size groups; females and males; squares: ICES statistical rectangles; numbers in squares: upper: # observation days, lower: # of fish; time period covered: 1981 – 2005) (last update: March 03, 2006; source: ICES Data Centre).**

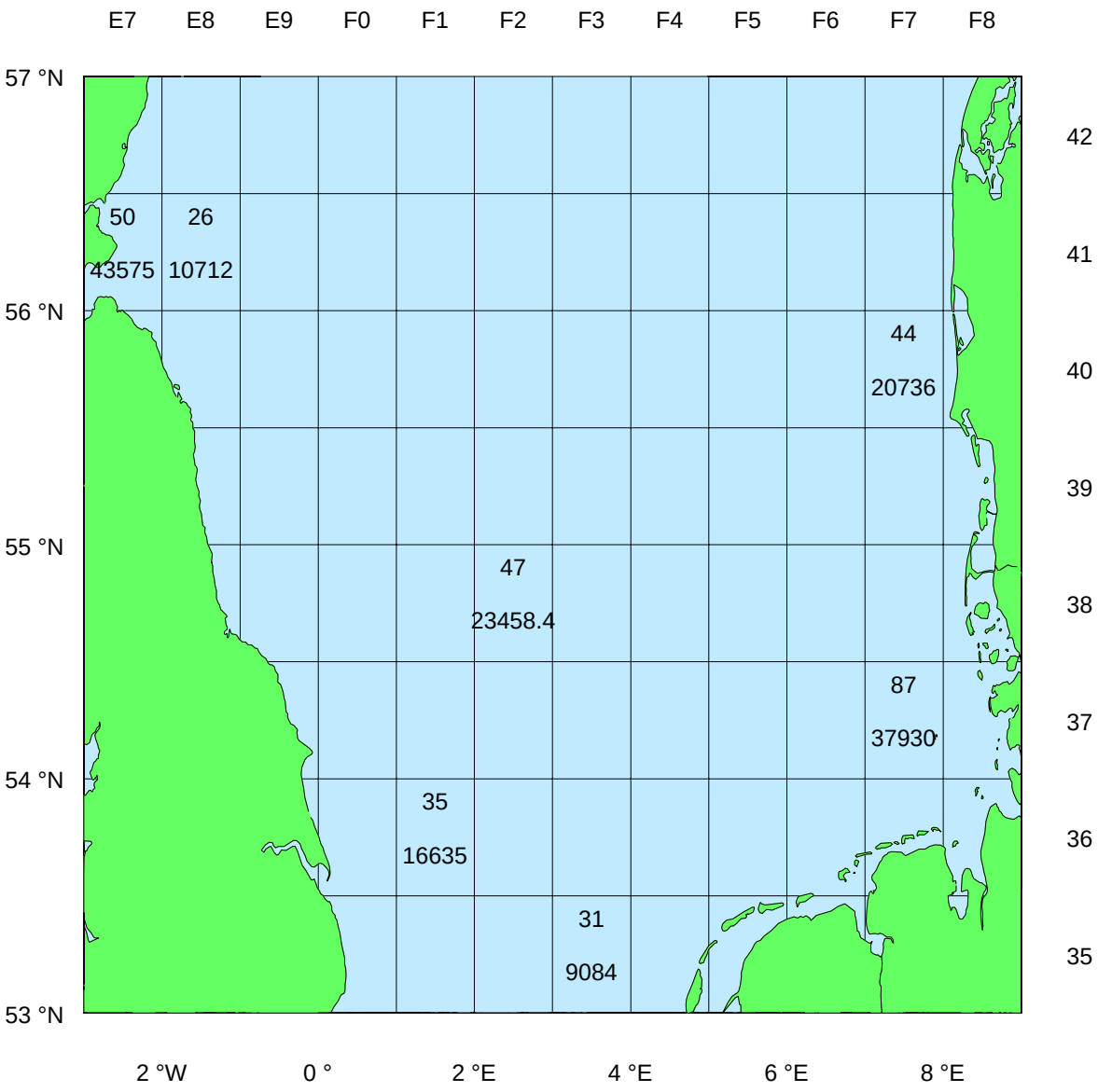



**Figure A10.4: ICES statistical rectangles with at least 10 samplings after 1996 (species: *Limanda limanda*; all size groups; females and males; squares: ICES statistical rectangles; numbers in squares: upper: # observation days, lower: # of fish; time period covered: 1981 – 2005) (last update: March 03, 2006; source: ICES Data Centre).**

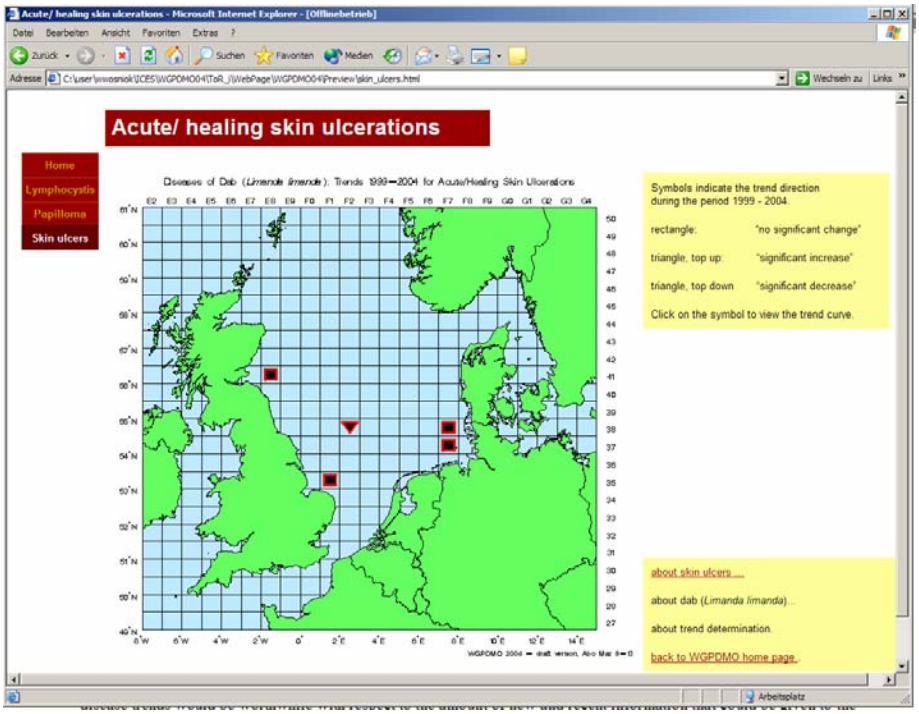



**Figure A10.5: Example of an overview map. The symbols in the ICES rectangles denote recent trends. Clicking on the symbol leads to a display of the underlying long-term trend as shown in Figure 2. The displays bases on a preliminary data set.**

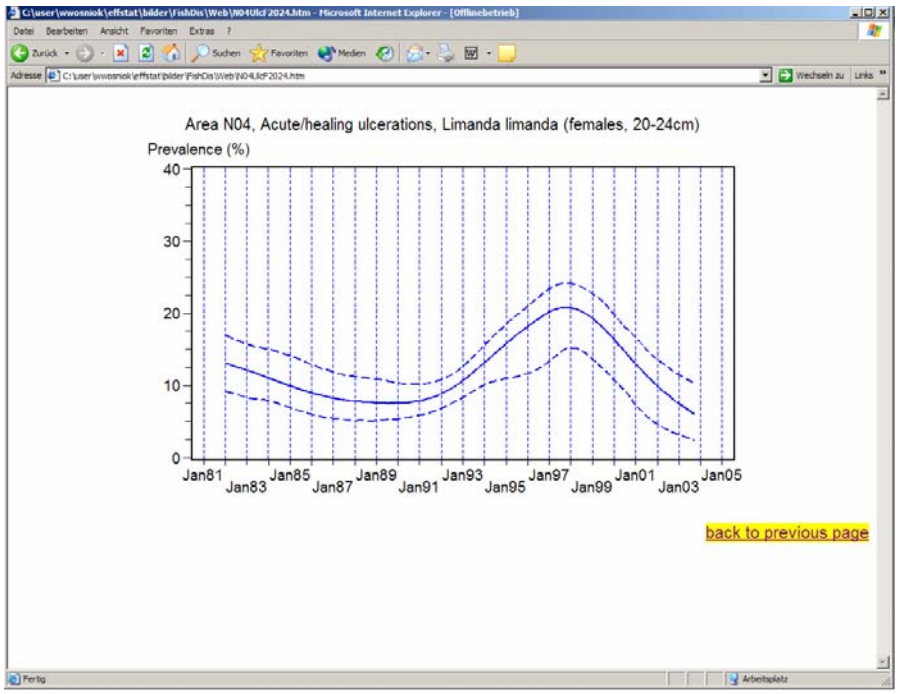
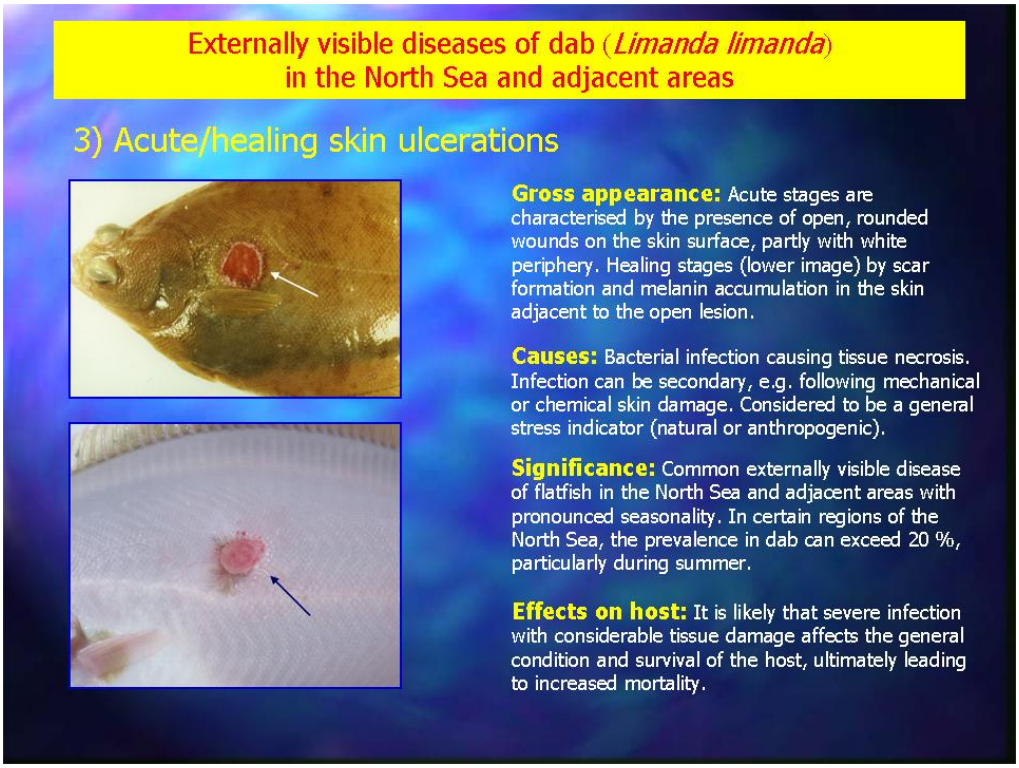



**Figure A10.6: Example of a long-term trend with 95% confidence band. This display shows up after clicking at the corresponding ICES statistical rectangle (38F2). The displays bases on a preliminary data set, also area designations are preliminary. The latter will be changed to ICES statistical rectangle names.**


**Figure A10.7a: Description of diseases (lymphocystis and epidermal hyperplasia/papilloma).**



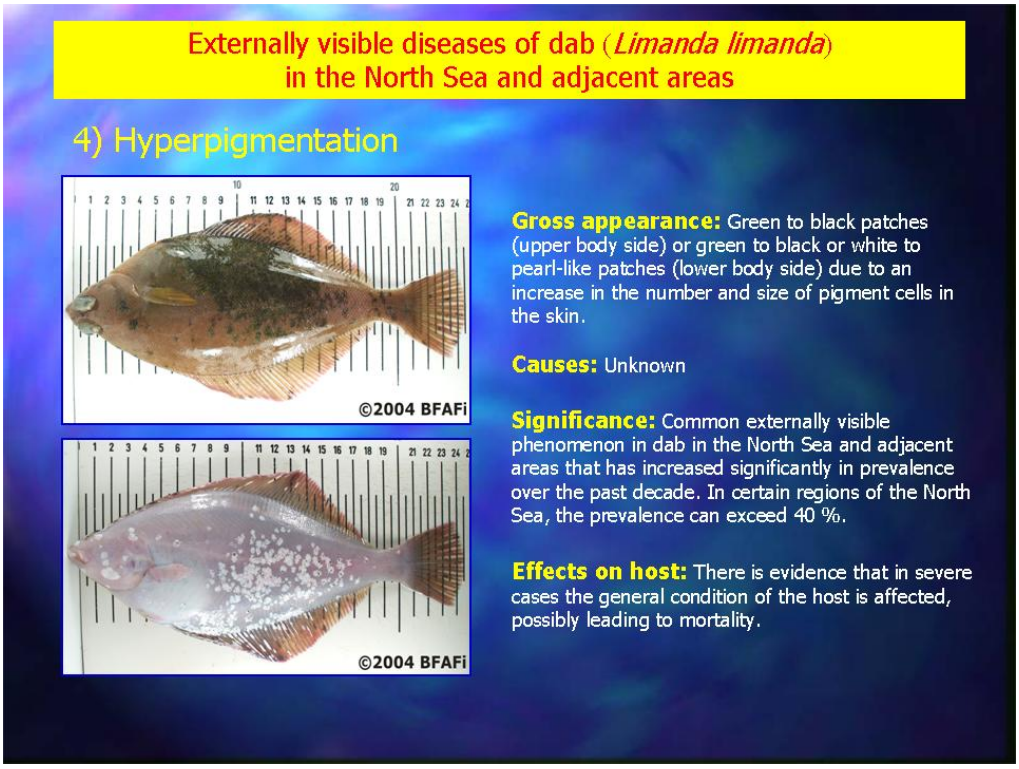




**Figure A10.7b: Description of diseases (skin ulcers and hyperpigmentation).**

# Annex 11: Recommendations

| Report Section | Recommendation | Action |
|---|---|---|
| 5.1 | 1. ICES Member Countries are encouraged to 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 Members Countries carry out comparative studies on *Francisella* sp. in farmed and wild cod (*Gadus morhua*) and a visceral granulomatous condition in wild cod. | ICES Member Countries |
| 5.3 | 3. WGPDMO be kept informed of progress in investigations into the identification of *Bonamia* spp. infecting Asian oysters (*Crassostrea ariakensis*) and crested oysters (*Ostreola equestris*) in the USA. | WGPDMO members |
| 5.3 | 4. Studies be pursued on the causes of gill disease in Pacific oysters (*Crassostrea gigas*) in Germany and on disease signs in Icelandic scallops (*Chlamys islandica*) from the Barents Sea. | ICES Member Countries |
| 6 | 5. An update on HSMI should be included in the national reports on new disease trends to be reviewed by WGPDMO at its 2007 meeting. | WGPDMO members |
| 8 | 7. The document on effects of climate change on diseases of marine fish and shellfish reviewed by WGPDMO at its 2006 meeting be expanded by the authors during the intersession and submitted for publication. Suggestions for aspects to be added to the manuscript are:<br>- identification of data types necessary to confidently state the effect of climate change on fish diseases,<br>- identification of criteria necessary for linking climate change with changes in disease status, (suggestions were: accessibility of long-term data; emphasis on non-commercial finfish species; correlated climatic and population trends; identification and exclusion of other factors affecting population size or disease status; knowledge on the effect of temperature on hosts and pathogens),<br>- information on vibriosis in cod (*Gadus morhua*), M74 in salmonids (Baltic salmon, *Salmo salar*, and sea trout, *Salmo trutta*) and lobster (*Homarus americanus*) shell disease as possible examples,<br>- analysis of North Sea dab (*Limanda limanda*) and molluscan diseases. | WGPDMO members |
| 9.1 | 8. the Chair of REGNS should be contacted by W. Wosniok to identify obstacles that had prevented the inclusion of fish disease data into the analysis carried out by REGNS in the past and to define the required data structure for the submission of updated data; | WGPDMO members |
| | 9. the WGPDMO submits updated data on fish disease prevalence trends to REGNS (diseases: lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations, X-cell gill disease; species: *Limanda limanda*; gender; female; size: 20–24cm; spatial resolution: ICES statistical rectangles; temporal resolution: months; adjusted for seasonal variation). | WGPDMO members |

| REPORT SECTION | RECOMMENDATION | ACTION |
|---|---|---|
| 9.2 | 10. An international ICES/BSRP/HELCOM sea-going demonstration project on the ecosystem health of the Gulf of Finland (scheduled for 2007 or 2008) and a land-based ICES/BSRP/HELCOM workshop on monitoring of diseases and parasites in coastal fish species (scheduled for 2006 or 2007; venue suggestions: AtlantNIRO, Kaliningrad, or the Estonian Marine Institute, Tallinn) in the context of the HELCOM coastal fish monitoring be organised. The planning and organisation of both activities should involve the ICES Study Group on Ecosystem Health Issue in support of the BSRP (SGEH), with contributions from other relevant ICES Expert Groups, e.g. WGPDMO, WGBEC, MCWG and WGMS. | ICES/BSRP/HELCOM |
| 9.3 | 11. Laboratories in ICES Member Countries studying diseases and liver histopathology in wild fish as part of national and international environmental monitoring and assessment programmes take part in the relevant component of the Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) project in order to achieve quality assurance regarding methodologies applied and data generated. | ICES Member Countries |
| 10 | 12. ICES Member Countries be encouraged to use international standards proposed by the OIE with the inclusion of molecular methods for microcell parasite identification; | ICES Member Countries |
| | 13. ICES Member Countries be encouraged to support the funding of research into improved methods of detecting and identifying microcell parasites due to their worldwide distribution and importance; | ICES Member Countries |
| | 14. Laboratories involved in microcell research initiate collaborative testing, intercalibration and validation of current and newly developed techniques for the purpose of recommending improved techniques to differentiate among microcell parasites; | ICES Member Countries |
| | the WGPDMO be kept informed on progress made in developing microcell research programmes; | WGPDMO members |
| 11 | 15. further studies be undertaken in ICES Member Countries to identify bivalve pathogens present both in hatcheries/ nurseries and in the field; | ICES Member Countries |
| | 16. ICES Member Countries be encouraged to support funding of research for assessing the risk of disease for bivalves in growout sites associated with the use of hatchery products. | ICES Member Countries |
| 12 | 17. For establishing a 'Fish Disease Index' for dab (*Limanda limanda*), a consensus should be achieved among dab pathologists of a scale for scoring disease intensity (grades) and for weighting significance of various diseases; | WGPDMO members |
| 14.1 | 18. The report on disease trends in dab (*Limanda limanda*) from the North Sea and adjacent areas and the distribution maps for VHS virus and shellfish diseases published on the ICES website is updated according to the suggestions made; | WGPDMO members |
| | 19. the calculation of trends and subsequent update of the maps on the ICES website should be taken over by the ICES Data Centre (see Section 15.3 of the 2006 WGPDMO report). The WGPDMO will provide a template for the website structure and support in statistical issues, if needed. | ICES Data Centre |
| 15 | 20. ICES Member Countries submitting fish disease data to the ICES Data Centre follow the instructions given by WGPDMO in Section 15 of the 2006 WGPDMO report; | ICES Member Countries |

| REPORT SECTION | RECOMMENDATION | ACTION |
|---|---|---|
| | 21. ICES generates the following fish disease related data products:<br>- a summary of the disease prevalence reported by each fish disease data submission to the ICES Data Centre as plausibility check. This summary should contain the raw prevalence per disease, separately for each combination of station and disease, where "station" should be the station name given the variable "STATN" in the submission. (see above);<br>- yearly prepared maps for the fish disease report on the ICES website, indicating trends and spatial distribution of diseases in dab (*Limanda limanda*) from the North Sea and adjacent areas (also see Section 14.1 of the 2006 WGPDMO report;<br>- yearly updated trend figures to be used in combination with the dab disease maps (also see Section 14.1 of the 2006 WGPDMO report). | ICES Data Centre |
| 16 | 20. written documents will be produced on the health status of fish in the North Sea (responsible: S. Feist, with input from other WGPDMO members) and in the Baltic Sea (responsible: T. Lang, with input from other WGPDMO members) as contributions to the ecosystem overview advisory report. It was noted that the chapters should be prepared by May 2006 before the meetings of the ICES Advisory Committees. | WGPDMO members |

# Annex 12: Proposed Terms of Reference for the 2007 WGPDMO meeting and related action list

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: Sharon MacLean*, Germany) will meet at [suggestions: Tenerife, Spain, or St. John's, Canada] from [dates to be decided] 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, 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; produce a report on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports;

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 [date to be decided] 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, 6.4<br>**Term of Reference a)**<br>New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained.<br>**Term of Reference b)**<br>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)**<br>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.<br>**Term of Reference d)**<br>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.<br>**Term of Reference e)**<br>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.<br>**Term of Reference f)**<br>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.<br>**Term of Reference g)**<br>*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.<br>**Term of Reference h)**<br>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 |

## ToR action list

| Report Section | ToR Recommendation | Action |
|---|---|---|
| 5 | a) produce a report on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports; | All WGPDMO members |
| 5.1 | b) WGPDMO reviews 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 at its 2007 meeting; | WGPDMO members:<br>T. Lang<br>S. Feist<br>D. Bruno<br>W. Wosniok<br>A. Mansour |
| 7 | c) update information on progress made in the development of salmon sea louse vaccines and sea louse management strategies in ICES Member Countries; | WGPDMO members:<br>B. Hjeltnes<br>(E. Sterud)<br>D. Bruno<br>S. Jones<br>S. MacLean |
| 9 | d) review progress made with regard to international collaborative actions including disease and pathology aspects:<br>REGNS Integrated Assessment of the North Sea Ecosystem,<br>• Baltic Sea Regional Project (BSRP),<br>• BEQUALM; | WGPDMO members:<br>W. Wosniok<br>T. Lang<br>G. Rodjuk<br>S.W. Feist |
| 10 | e) the WGPDMO produce a review of testing, intercalibration, and validation of current and newly developed molecular techniques for the purpose of pathogen diagnosis in bivalves for its 2007 meeting; | WGPDMO members:<br>T. Renault<br>S. Ford<br>L. Madsen<br>S.W. Feist |
| 12 | f) the pilot study on constructing a 'fish disease index' by using empirical dab (*Limanda limanda*) disease data should be continued by selected WGPDMO members and progress made be reviewed at the 2007 WGPDMO meeting. | WGPDMO members:<br>T. Lang<br>W. Wosniok<br>S.W. Feist<br>D. Bruno |
| 14 | g) produce ICES publications on pathology and diseases of marine organisms | WGPDMO members:<br>S.W. Feist<br>W. Wosniok<br>T. Lang<br>T. Wiklund<br>S. Ford |
| 15 | h) provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis; | WGPDMO members:<br>W. Wosniok<br>T. Lang<br>S.W. Feist<br>D. Bruno |