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

8–12 March 2005

La Tremblade, France

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

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer
H.C. Andersens Boulevard 44–46
DK–1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

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Executive summary

The **ICES Working Group on Pathology and Diseases (WGPDMO)** met from 8–12 March 2005 in La Tremblade, France, with 23 participants from 12 ICES Member Countries and Lithuania, chaired by T. Lang (Germany). In order to consider all of the 11 Terms of Reference in an appropriate way, intersessional work had been carried out by WGPDMO members and a large number of working documents had been provided in advance to the meeting.

A number of **new disease trends in wild and farmed fish and shellfish** (*ToR a, Report Section 5*) was reported by Member Countries for 2004: the Viral Haemorrhagic Septicaemia Virus (VHSV) was isolated for the first time in wild herring (*Clupea harengus*) from Finish coastal areas of the Baltic Sea. An intracellular bacterial pathogen of the gill epithelium was associated with mass mortalities of Atlantic croaker (*Micropogonias undulatus*) along the eastern US coast. Heart and skeletal muscle inflammation (HSMI), proliferative gill inflammation and infections with *Parvicapsula pseudobranchicola* are problems for farmed Atlantic salmon (*Salmo salar*) in Norway. A newly reported bacilliform virus was found in 100% of brown shrimp (*Crangon crangon*) in the Wash fishery, UK. The finding of a α-proteobacterium, *Roseovarius crassostrea*, in eastern oyster (*Crassostrea virginica)* from Maine and Massachusetts, USA, supports the contention that the bacterium is the proximate cause of Juvenile Oyster Disease (JOD). *Bonamia ostreae* was reported, at high prevalence (60%), for the first time in flat oysters (*Ostrea edulis*) in British Columbia, Canada. The newly discovered native *Bonamia* sp. found last year in an introduced oyster (*C. ariakensis*) in North Carolina, USA, was also found in a native oyster (*Ostrea equestris*) along with a second previously unknown *Bonamia* sp.

According to a review on **the role of plankton in gill-related mortality in farmed fish** (*ToR b, Report Section 6*), contact with planktonic organisms (e.g. jellyfish) may result in mass mortality in farmed Atlantic salmon (*Salmo salar*). In order to avoid mortalities, an early warning system should be developed, including better communication between individual farms. To identify causes if mortalities occur, water samples should be taken immediately.

Heart and skeletal muscle inflammation (HSMI) (*ToR c, Report Section 7*) is a major and increasing disease problem for Norwegian Atlantic salmon aquaculture. An outbreak of disease resembling HSMI has been reported in Scottish farmed Atlantic salmon. Evidence of a viral aetiology has been postulated for HSMI in Norway.

The **Summer Mortality syndrome** (*ToR d, Report Section 8, Annex 6*) affects Pacific oyster (*Crassostrea gigas*) and several other bivalve species in ICES Member Countries. The collective evidence (e.g. from the French MOREST project) suggests that the syndrome involves a suite of extrinsic and intrinsic causative factors. Specifically, the coincidence of elevated water temperature, gametogenesis and spawning place the animal in a relatively unstable physiological condition. Other external factors that exacerbate this instability (e.g. high food availability, physical stressors or pathogens) may have an additional impact.

A review of **effects of contaminants on the immune system of fish and shellfish** (*ToR f, Report Section 9, Annex 7*) revealed considerable evidence for such effects. However, a relationship between immunomodulation induced by contaminants and a higher susceptibility to infectious diseases has only rarely been demonstrated. Therefore, more research, applying a combined immunological approach including a suite of techniques, is needed before conclusions can be made as to the usefulness of immunological parameters for regular monitoring purposes and for predicting susceptibility to disease.

Due to multiple factors influencing wild fish populations, no firm conclusions can yet be drawn regarding the extent of **sea lice interactions between farmed and wild fish** and the effect on wild salmon (*ToR h, Report Section 10*). However, there is enough evidence of such

effects to further improve and implement measures to reduce the risks to wild fish, including a reduction in the number of escapees from farms that tend to stay in fjords and can, thus, provide a supply of sea lice to out migrating wild smolts.

WGPDMO reviewed and evaluated **'health indices' for the interpretation of data obtained from biological effects monitoring activities**, aiming at summarising information on the health status of marine organisms (*ToR e, Report Section 11, Annex 8*). However, the review revealed that all have their shortcomings and that none of them describe disease-related aspects of interest for WGPDMO appropriately. Therefore, WGPDMO recommended that a pilot study should be carried out as ToR for the 2006 WGPDMO meeting in order to construct a 'disease index', based on German data which incorporates disease intensity and parasite prevalence information from studies in common dab (*Limanda limanda*) in the North Sea.

WGPDMO evaluated **the availability of data for a risk assessment pilot study on population effects due to diseases in wild fish** (*ToR g, Report Section 12*). It was concluded that sufficient data is available in the ICES Databases (e.g. long-term diseases and CPUE data) enabling such a study, at least for the common dab in certain regions of the North Sea. Therefore, WGPDMO recommended that a pilot study should be carried out as ToR for the 2006 WGPDMO meeting.

Based on an OSPAR request [OSPAR 2005/6], the WGPDMO assessed the results of the **2005 ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON)** (*ToR i, Report Section 13, Annex 9*) and reviewed the OSPAR JAMP Guidelines/Technical Annexes for General and PAH-specific Biological Effects Monitoring. It was concluded that the Guidelines need to be amended with regard to the monitoring of externally visible fish diseases, liver nodules, liver pathology and liver neoplasia/hyperplasia, and relevant recommendations were made that should taken into account by OSPAR.

The WGPDMO recommended and specified **data on fish diseases for the ICES Integrated Assessment of the North Sea Ecosystem** coordinated by the ICES Regional Ecosystem Study Group for the North Sea (REGNS) (*ToR j, Report Section 14, Annex 10*). These comprise data on externally visible diseases in common dab (*L. limanda*) from the entire North Sea, covering a period from 1984 to 2004, submitted by ICES Member Countries Sea. The data can be extracted from the ICES Environmental Database for the REGNS Workshop, 9–11 May 2005.

In the discussion of progress made with regard to the fish disease component of the **Baltic Sea Regional Project (BSRP)**, WGPDMO recommended organisation of an ICES/BSRP Seagoing Workshop on Fish Disease Monitoring in the Baltic Sea, with the major aim to provide training and intercalibration of relevant methodologies. The workshop is supposed to be held in December 2005 (*Report Section 16.1*).

WGPDMO concluded that all Terms of Reference for the 2005 meeting were considered in a comprehensive and satisfying manner. 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 2006. An invitation was received and acknowledged to organise the 2006 meeting at ICES Headquarters, Copenhagen, Denmark. The proposed dates are 7–11 March 2006.

1 Opening and structure of the meeting

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the IFREMER Laboratoire de Génétique et Pathologie, La Tremblade, France, with T. Lang (Germany) as Chair. The meeting was opened at 10:00 hrs on Tuesday, 8 March 2005, with the Chair and the local organiser, T. Renault, welcoming the participants, particularly the new members and guests who have not previously attended WGPDMO meetings. A list of participants is appended in Annex 1.

Apologies were received from C. Couillard (Canada), L. Madsen and I. Dalsgaard (Denmark), M. Vigneulle (France), J. Palsson (Iceland), F. Geoghegan (Ireland), S. Mortensen (Norway), and O. Haenen (The Netherlands).

The Chair thanked the local organiser for inviting WGPDMO to France and for providing excellent meeting facilities. He informed WGPDMO that his term of office as Chair is due to expire this year (after three meetings) and the WG should consider a successor.

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. 2004/2F02 (Annex 2). The agenda once again demanded extensive intersessional work by the members of the WGPDMO who were 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 distributed by 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 ensures that the Terms of Reference could be treated efficiently. A list of working documents considered is in Annex 3.

2.2 Adoption of the agenda

A draft agenda was circulated and adopted without changes (Annex 4).

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 Conference 2004

The 2004 ICES Annual Science Conference (ASC) took place in Vigo, Spain, 24–27 September 2004. The WGPDMO Chair was unable to attend and 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).

Originally, there were plans to hold four mariculture-related Theme Sessions at the 2004 ASC:

- Towards Sustainable Aquaculture,
- Shellfish Culture: Perspectives and Limitations,
- Water Treatment in Intensive Fish Cultures, and
- Mariculture in Integrated Coastal Zone Management Systems.

However, due to a lack of paper submissions, only one Theme Session was organised. (Mariculture in the Coastal Zone: Sustainability, Perspective and Limitations), resulting in a low attendance of relevant experts and official members of the Mariculture Committee.

An Invited Lecture was given by A. Lane (Executive Director, European Aquaculture Society, EAS) on Sustainable Aquaculture Development in Europe, highlighting the EU strategy for the sustainable development of European Aquaculture and the plans to create a Platform for Sustainable European Aquaculture, steered by the principal European stakeholders, including the EAS, and assisting in the implementation of the EU strategy.

The 2005 ICES ASC will be held 20–24 September 2005 in Aberdeen, Scotland. Information on the ASC can be found on the ICES website at http://www.ices.dk/iceswork/asc/2005/index.asp .

3.2 ICES Mariculture Committee (MCC)

The MCC met for two business sessions during the 2004 ICES ASC/Statutory Meeting, addressing the progress achieved in its Working Groups and future activities of MCC.

The present ICES Working Groups under the MCC are:

- ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO),
- ICES Working Group on Environmental Interactions of Mariculture (WGEIM),
- ICES Working Group on Marine Shellfish Culture (WGMASC),
- ICES Working Group on Marine Fish Culture (WGMAFC),
- ICES Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM).

All WGs met in 2004 and their reports were presented to the MCC at its business session during the 2004 ICES Statutory Meeting. Since the WGPDMO Chair was unable to attend the ASC/Statutory Meeting, a summary of the 2004 WGPDMO Report was presented to the MCC by the MCC Chair T. Sephton (Canada). The report and its recommendations for Terms of Reference for the 2005 WGPDMO meeting were accepted by MCC and later by the ICES Council with minor amendments of the Terms of Reference f), i) and j) (see Annex 2).

The WGPDMO Chair informed WGPDMO that there are overlapping activities in the MCC WGs, e.g. between WGPDMO and WGEIM since both WGs are addressing disease-related issues. Furthermore, WGMASC was given two ToRs relevant to 2005 WGPDMO ToRs e) on health indices and ToR f) on contaminant effects on the immune system in shellfish (see Annex 2):

- **WGMASC ToR b)** prepare a state of knowledge report comparing and contrasting the standard methods used to measure stress indicators in shellfish and provide a discussion of how they would be used to diagnose incidents of cultured shellfish mortality,

- **WGMASC ToR c)** assess and provide a critique of the standard methodologies used to estimate shellfish performance indices as related to examining the carrying capacity of the growing area. This will include a review of the effects of HABS, disease and pollution in relation to the performance and carrying capacity of shellfish culture.

Since the deliberations of WGPDMO for its ToRs e) and f) will be of interest in this context, they will be forwarded to the WGMASC Chair in advance to the WGMASC meeting (13–15 May).

The MCC regretted the unsatisfactory attendance of MCC members and attributed this to the fact that only one mariculture-related Theme Session was held at the ASC 2004. The attendance might be even lower at the ICES ASC 2005 because, according to the present planning; there will be no mariculture-related Theme Session. Two Theme Sessions will be held that might be relevant for the environmentally-oriented side of WGPDMO:

- **Theme Session S:** Oil Spills in Marine Ecosystems: Impacts and Remediation (Session S) (Conveners: Joan Albaigés (Spain) and Kenneth Lee (Canada)),
- **Theme Session Z:** How to Improve Environmental Monitoring and Biological Studies – Integrating Ecology and Statistics (Conveners: Rob Fryer (UK) and Johan Craeymeersch (The Netherlands)).

Two Symposia co-sponsored by ICES and of relevance for MCC (and WGPDMO) are in preparation for 2005 and 2006 (see section 4.1):

- ICES-NASCO Symposium on the **Interactions between Cultivated and Wild Diadromous Fish Species** (18–21 October 2005, Bergen, Norway) (Co-Conveners: Lars Petter Hansen (Norway) and Malcolm Windsor, NASCO (UK))
- ICES-PICES Symposium on **"Marine Bioinvasions"** (Boston, USA for 3 days in early 2006) (Co-Conveners: James Carlton (USA), Erkki Leppäkoski (Finland) and Yasuwo Fukuyo, PICES (Japan))

The WGPDMO Chair encouraged the WGPDMO members to try to attend these Theme Sessions and the Symposia 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 both the Theme Sessions and the Symposia. Furthermore, the participation in the ASC 2005 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 2005 ASC to the ICES Secretariat is 25 April 2005.

3.3 ICES Advisory Committee on the Marine Environment (ACME)

The Chair informed the WGPDMO that several topics considered by WGPDMO at its 2004 meeting were subsequently reviewed by the ACME at its annual meeting in June 2004 and incorporated in the 2004 ACME Report:

- New disease trends in wild and cultured fish, molluscs and crustaceans,
- Causes of the M74 syndrome in Baltic salmon and progress in understanding relevant environmental factors,
- Status of *Ichthyophonus* in herring and salmon,
- Disease/parasite interactions between wild and farmed fish,
- Status of disease monitoring programmes and associated QA activities,
- Differentiation of *Perkinsus* species,
- Viral diseases in crustaceans,
- The use of epidemiological methods for the assessment of diseases and the risk of population effects.

The ACME Report is no longer being published in the ICES Cooperative Research Report Series but in the new ICES publication series ICES Advice (2004 ACME Report in ICES Advice Volume 1, Number 1, 283 pp.).

3.4 Changes related to ICES Expert Groups

The Chair informed the WGPDMO of changes concerning the membership of ICES Expert Groups (mainly Working Groups and Study Groups), quality assurance procedures implemented (Action Plan Progress Review) and the style and format of Expert Group Reports.

The **Membership of ICES Expert Groups** (EGs) is now more open since EGs from now on will not only constitute of member nominated by the national ICES Delegates (the old system) but may also involve members appointed by the respective EG Chair who can contribute to the work of the EG and who can obtain funds to do so. The latter members will be appointed for one year and the ICES Secretariat and the national ICES Delegates have to be informed of their appointment.

Since 2004, an **Action Plan Progress Review** is carried out by all ICES Committees. The purpose of this process is to ensure that progress is made in accordance with the ICES Action Plan. In this connection, each ICES Expert Group is requested to review the progress made with regard to its Terms of Reference at the end of the meeting, by using a Microsoft Excel Template provided by the ICES Secretariat. The following components need to be addressed:

- Progress satisfactory (S), no progress (0), progress unsatisfactory (U);
- Link to Report Section;
- Comments (explaining why no progress was made or to flag that a major milestone has been reached).

The ICES Secretariat developed and distributed a **New Template for Expert Group Reports** (for Microsoft Word**)** together with guidelines on how to use it. It is envisaged that the use of this template will simplify the drafting process within the Expert Groups as well as the work in the ICES Secretariat for finalising the reports and will, therefore, speed up the release of the final reports. The Chair distributed the new template and the instructions and asked the WGPDMO Rapporteurs to use the new template for preparing the draft section during the meeting.

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

- Fish Immunology Workshop, 10–14 April 2005, Wageningen, The Netherlands (http://www.zod.wau.nl/ceni/fish%20vaccination/);
- National Shellfisheries Association, 10–14 April 2005, Philadelphia, Pennsylvania, USA (http://www.shellfish.org/meetings/philadelphia.htm) ;
- 1st Scandinavian and Baltic Society for Parasitology (SBSP) Symposium, 26–29 May 2005, Vilnius, Lithuania (http://www.hi.is/pub/sbsp/);
- International Conference on Environmental Bioindicators, 6–10 June 2005, Praha, Czech Republic (http://www.uek.cas.cz);
- 130th Eastern Fish Health Workshop, 13–17 June 2005, Shepardstown, West Virginia, USA;
- 13th International, Symposium Pollutant Responses in Marine Organisms (PRIMO), 19–22 June 2005, Alessandria, Italy (http://www.disav.unipmn.it/Initiative/Primo13/index.htm);

- 5th Baltic Sea Science Congress "The Baltic Sea-a changing ecosystem", 20–24 June 2005, Sopot, Poland (http://www.iopan.gda.pl/bssc2005);
- 9th Canadian Workshop on Harmful Algae, 6–8 July 2005, St. John's, Newfoundland and Labrador, Canada (http://www.nfl.dfo-mpo.gc.ca);
- American Society of Parasitologists, 8–12 July 2005, Mobile, Alabama, USA (http://asp.unl.edu/);
- 6th International Crustacean Congress, 18–22 July 2005, Glasgow, Scotland, UK (http://www.gla.ac.uk/ibls/icc6);
- American Fisheries Society-Fish Health Section, 27–29 July 2005, Minneapolis, Minnesota. USA;
- 6th International Congress of Parasitology (ICOPA-XI), 6–11 August 2006, Glasgow, UK (http://www.icopa-xi.org/);
- 5th International Symposium on Monogenea, 8–12 August 2005, Guangzhon, China (http://www.diplectanum.dsl.pipex.com/ism5/);
- Aquaculture Europe 2005, 9–12 August 2005, Trondheim, Norway (http://easonline.org/home/en/default.asp);
- Sustainable Control of Fish Diseases in Aquaculture (SCOFDA) Workshop on environmental aspects of disease control in aquaculture, September 2005, Denmark (http://www.fishnet.dk/networks/scofda/seminars/w2005_environmental_aspects_of_disease_control_in_aquaculture.htm);
- European Association of Fish Pathologists (EAFP), 12–16 September 2005, Copenhagen, Denmark (http://www.eafp.org/EAFP2005.html);
- EAFP Histopathology Workshop on Early Life Stages, 17 September 2005, Copenhagen, Denmark (http://www.eafp.org/EAFP2005.html);
- ICES Annual Science Conference, 20–24 September 2005, Aberdeen, UK, (http://www.ices.dk/iceswork/asc/2005/index.asp)
- 8th International Conference on Shellfish Restoration, 2–5 October 2005, Brest, France (http://www.ifremer.fr/icsr05);
- 20th Meeting of the World Association for the Advancement of Veterinary Parasitology (WAAVP), 16–20 October 2005, Christchurch, New Zealand (http://www.waavp2005.org.nz);
- ICES/NASCO (NASCO: North Atlantic Salmon Conservation Organisation) Symposium on the Interactions between Cultivated and Wild Diadromous Fish Species, 18–21 October 2005, Bergen Norway (http://www.nasco.int/)
- ICES-PICES (PICES: North Pacific Marine Science Organisation) Symposium on "Marine Bioinvasions" (Boston, USA for 3 days in early 2006) (http://www.ices.dk/iceswork/symposia/Symposium-2006a.htm)

4.2 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 pro-

gramme, 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 through a project coordinator and various 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. Input from WGPDMO will therefore be required in the future (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/) (for further information, see report section 16);
- **Summer mortality in *C. gigas* oysters (Mortalités Estivales, MOREST, 2001–2005):** National French project coordinated by IFREMER. Teams from fifteen laboratories in eight different organisations, along with county or regional development bodies and professional groups are associated with the project. Its objective is to gather complementary expertise required to study a multifactor phenomenon causing the observed mortalities (http://www.ifremer.fr/anglais/rapp2001/defi6.htm) **;**
- **Anti-viral innate immunity in cultured aquatic species (AVINSI, QLRT-2001-01691, 2002–2005):** Non-specific anti-viral defence mechanisms (innate immunity) are important because they constitute the first line of defence in vertebrates, and the only one in invertebrates. Therefore, innate immunity will be investigated in fish, molluscs and crustaceans. Through this EU-funded project, conserved mechanisms and pathways of innate immunity may be identified. The project will be part of the research and technological development activities of Key Action 2 (Control of Infectious Diseases), Topic 2. 2. (Strategies to identify and control diseases) and Subtopic 2. 2. 1. (Treatment of and protection against human and animal infectious diseases). (http://www.ifremer.fr/latremblade/en/europeanprojects/Avinsi/avinsi.htm) ;
- **Disease interactions and pathogen exchange between farmed and wild aquatic animal populations – a 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) (for further information, see report section 16).
- **Sustainable management of interactions between aquaculture and wild salmonid fish (SUMBAWS):** This multidisciplinary EU-funded project addresses questions relating to: a) the decline in wild salmonid populations in NW Europe; b) aspects of the migratory behaviour and physiological responses of juvenile salmonids, as they adapt to seawater and as they respond to the additional environmental challenge and stress of ectoparasitic sea lice infestation; and c) up to date appraisals and modelling of the socio-economic importance and interaction of the aquaculture and angling industries in peripheral rural regions of northwestern Europe (http://www.st-andrews.ac.uk/~sumbaws/index.htm);
- 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 2004 submitted by Canada, Denmark, Estonia, Finland, France (only for shellfish), Germany, Latvia, Lithuania, Norway, Poland, Russia, Spain, The Netherlands, UK and USA. It attempts to document significant observations and 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

Infectious pancreatic necrosis virus – A total of 11,110 fish comprising 18 marine species were sampled for IPNV in Scotland. IPNV was isolated from dab (*Limanda limanda*) (16 isolations), grey gurnard (*Eutrigla gurnardus*), plaice (*Pleuronectes platessa*), long rough dab (*Hippoglossoides platessoides*) and flounder (*Platichthys flesus*) (one isolation each). In total, 20 IPNV isolations were made. The majority (19) were made from wild marine fish caught around the Shetland Islands; the remaining isolation was from dab caught east of the Fair Isle.

Lymphocystis – In UK waters slight changes in the prevalence of lymphocystis in dab (*Limanda limanda*) occurred in local areas in 2004. Liverpool Bay and Off Humber showed decreases and Cardigan Bay and in Scotland had slight increases. A marked decrease in prevalence (7.1% to 1.5%) was observed at West Dogger. German studies showed seasonal fluctuations in the prevalence of lymphocystis in North Sea dab; however, the overall trend appeared to be decreasing. In 2004 the lowest prevalence ever observed in the German Bight (1.8%) was recorded. Prevalences in flounder (*Platichthys flesus*) from the western Baltic Sea ranged from 14.3% to 38.5%. Summary data from Poland for 1998 to 2004 show herring (*Clupea harengus*) and flounder had mean prevalences of 0.13% and 0.41%, respectively. Decreasing trends in 2004 were observed in flounder and herring from this area. In the northeastern Baltic, lymphocystis in flounder ranged from 0.22% to 4.5%.

Rhabdovirus - A rhabdovirus was isolated from one asymptomatic starry flounder (*Platichthys stellatus*) collected during a survey of marine fishes from the Puget Sound, Washington, USA. PAGE of the structural proteins and PCR assays using primers specific for other known fish rhabdoviruses, including IHNV, VHSV, SVC and Hirame rhabdovirus, indicated this is a previously undescribed virus, tentatively termed starry flounder rhabdovirus (SFRV). Sequence analysis of 2,678 nucleotides of the amino portion of the viral polymerase gene indicated that SFRV is genetically distinct from other members of the family *Rhabdoviridae* for which sequence data are available.

Viral hemorrhagic septicaemia virus – The North American strain of VHSV was isolated from one brown trout (*Salmo trutta*) in Nova Scotia and one striped bass (*Morone saxatilis*) in New Brunswick, Canada, and one herring in Maine, USA. VHSV, preliminarily designated as genotype 2, was isolated from 25% of herring (*Clupea harengus*) samples taken from the Archipelago Sea, Finland.

5.1.2 Bacteria

Acute/healing skin ulcerations – Continued geographic variations were reported in dab (*Limanda limanda*) in the North and Baltic Seas. In the Irish Sea, prevalence for 2004 off Morecambe Bay was 8.7% compared to 10.1% and 11.9% for 2003 and 2002 respectively. Several sites showed clear increases in prevalence: Burbo Bight, Carmarthen Bay, Inner Cardigan Bay, Liverpool Bay and St Bees of 13.5%, 4.6%, 4.8%, 5% and 4.1%, respectively, since

2003. England and Wales reported a clear decrease in prevalence at all Dogger Bank sites since 2003. In contrast, the German report described slight elevations from the German Bight, Dogger Bank and Firth of Forth compared to 2003.

Prevalence in flounder (*Platichthys flesus*) from the Baltic Sea ranged from undetectable to 11.9%, with the highest level off the Lithuanian coast. Similarly, the prevalence in Baltic Sea cod (*Gadus morhua*) ranged from 0.6% to 9.4% at individual sites and was low compared to previous years. The exception was in ICES Subdivision 24, where prevalence increased from 4% (2003) to 8% (2004). Spatial variation was not as pronounced as in previous years. The mean prevalence for all species was lower in ICES Subdivisions 25 and 26. The number of flounder with skin ulceration varied irregularly over five years in the Barents Sea

Mycobacterium – Isolates from striped bass (*Morone saxatilis*) collected in the Chesapeake Bay, USA, over several years have been characterised. More than 76% of the 196 fish sampled were infected, and in 38% of the samples, mycobacterial densities were greater than 10^4 cfu per gram tissue. *Mycobacterium shottsii* was present in 57% of samples. Co-infections of *M. shottsii* and other mycobacteria were found in 25% of samples. *M. shottsii* clearly dominated among co-isolates of *M. interjectum*, *M. marinum*, *M. scrofulaceum*, *M. szulgai* and *M. triplex*.

Unknown bacteria – Mass mortalities were reported in adult Atlantic croaker (*Micropogonias undulatus*) between July and September extending from New Jersey to Florida, USA. The only sign of disease was haemorrhage from the gills. Histopathology confirmed the haemorrhage and degeneration of respiratory tissues associated with an uncharacterised bacterial infection. A universal molecular probe identified the organism as an intracellular bacterial pathogen infecting the respiratory epithelial cells. Water samples screened for toxic algae were negative.

5.1.3 Parasites

Mesomycetozoa

Ichthyophonus hoferi –The parasite is still endemic at low prevalence in herring (*Clupea harengus*) populations in the North Sea and the Baltic Sea.

Myxosporea

Parvicapsula* sp. –** Spores resembling a *Parvicapsula* sp. were identified in Gram-stained kidney imprints from four of 60 juvenile pink (*Oncorhynchus gorbuscha*) and one of 60 juvenile chum (*O. keta*) salmon. The spores were similar to those observed in 3 of 15 adult pink salmon collected from the Quinsam River, British Columbia, Canada, in 2003. DNA from pink salmon and chum salmon parasites was not amplified by PCR using primers for *P. minibicornis.**

Monogenea

Gyrodactylus salaris – Remains a major threat against Atlantic salmon (*Salmo salar*) in Norway. In 2004 *G. salaris* was detected on Arctic charr (*Salvelinus alpinus*) in the tributaries of the river Numedalslågen. This parasite belongs to a genotype previously only found on farmed Arctic charr.

Digenea

Stephanostomum baccatum – Prevalence of metacercariae in North Sea dab (*Limanda limanda*) ranged from 1.3% at the Indefatigable Bank to 61.3% at West Dogger. This parasite shows a pronounced spatial pattern, with high prevalences in the northern North Sea (e.g. the

Firth of Forth area). In the Firth of Forth area, the prevalence increased again after the drop observed in 2003.

Cryptocotyle lingua – Prevalence of metacercariae in Baltic cod (*Gadus morhua*) was similar to that of previous years. This parasite has a distinct spatial distribution pattern, with highest prevalences in the western Baltic Sea.

Prosorynchoides gracilescens – A total of 950 fish (cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*)) were sampled during a parasite survey from the west and east coast of Scotland as well as the Shetland Islands during 2003–2004. Metacercariae occurred at prevalences of 60% (west coast), 42% (east coast) and 67% (Shetland Islands).

Nematoda

***Anisakis simplex* (larvae)** – In Scotland the prevalence ranged from 3.6% to 27.3% in cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus)*. In Barents Sea cod, prevalence over the past decade appears steady but the abundance index (intensity) has decreased. Decreasing trends were observed in the prevalence and intensity of infection in Baltic herring (*Clupea harengus*) from Subdivisions 24–26 (Polish EEZ). A similar decreasing trend during 1999–2004 was observed in herring in the Russian EEZ (2004 prevalence was 1.3% in Subdivision 26). In Barents Sea redfish (*Sebastes mentella*) the prevalence remained the same but the abundance index was lower (2.6 vs. 5.7 in 2003). Pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta)* in the Far Eastern Region of Russia continued to have a high prevalence of infection. 75% to 96% of post-spawned pink salmon were infected in the muscle with an abundance index of 3–8. These values for chum salmon were 94% to 100% and 32–45, respectively.

Pseudoterranova decipiens (larvae) – An increase in liver infections in Barents Sea cod (*Gadus morhua*) has recently been recorded. Investigations conducted in 2004 show that the prevalence was 20% and the abundance index was 0.4.

Acanthocephala

***Corynosoma strumosum* (larvae)** – Prevalence was 4.5% in Baltic cod (*Gadus morhua*) and 6.1% in Baltic flounder (*Platichthys flesus*) (Russian EEZ, ICES Subdivision 26).

Crustacea

Lepeophtheirus salmonis – Fisheries and Oceans Canada continued monitoring juvenile pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta)* fry in the Broughton Archipelago and surrounding waters in British Columbia. The overall prevalence was approximately 62% on pink salmon and 64% on chum salmon. Mean intensities were approximately 4.1 and 11.1 lice per fish, respectively. Infestations are a problem in Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) in Norway, although infestations in salmon seem to be less severe than in previous years.

Lepeophtheirus pectoralis – Prevalence in dab (*Limanda limanda*) ranged from 1.2% at the Amble off the coast of northeast England to 62.7% from Liverpool Bay, Irish Sea. The prevalence at the Dogger Bank continued to increase to 30.2%.

Sphyrion lumpi – In 2004 the prevalence showed a continued increasing trend in deep-water redfish (*Sebastes mentella*) from the Barents Sea to 38%.

Clavella adunca – The prevalence in North Sea cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*) from Scottish waters ranged from 9.2% to 12.6%.

5.1.4 Other diseases

Epidermal hyperplasia/papilloma – Variable prevalence between the areas visited previously with few sites showing general trends. In dab (*Limanda limanda*) from Morecambe Bay (Irish Sea) an increased prevalence was apparent between 2002 (0.9%) and 2004 (3.0%). Prevalence at the Indefatigable Bank (North Sea) shows a slight increase between 2002 (1.3%) and 2004 (2.9%). Off Flamborough and Off Tees (North Sea) both show a downward trend in prevalence since 2002 of 3.2% to 0.8% and 2.3% and 0.4%, respectively. The prevalence in summer samples remained steady at West Dogger (North Sea) at around 2.5% since 2002. Prevalence increased to 6.2% in Nov/Dec.

Liver nodules/tumours – In dab (*Limanda limanda*), differential prevalence of liver nodules > 2 mm, being low in the northern North Sea and high in the central and southern North Sea, consistently observed until the mid 1990s, has almost disappeared. The prevalence may have approached constant background levels in some areas. Similarly, the prevalence in former hotspot areas (German Bight, Dogger Bank) remained at a low level. In contrast, an increase in prevalence of 7.4% to 9.6% was seen at St Bees (Irish Sea). Furthermore, liver nodules were observed for the first time at South East Isle of Man (Irish Sea) (5.8%), since sampling began there in 2001. None were observed at Burbo Bight (inner Liverpool Bay, Irish Sea) during 2004. The highest prevalence was recorded in fish captured from Inner Cardigan Bay at 16.7%.

Tumours were detected in 0.02% of fish in the Barents Sea. Among tumours detected during 1999–2004 epithelial cancer (cod (*Gadus morhua*), long rough dab (*Hippoglossoides platessoides*)), reticulum cell sarcoma, fibrosarcoma, chondrosarcoma (haddock (*Melanogrammus aeglefinus*), wolffish (*Anarhichas* spp.)), papilloma (wolffish), fibroma (long rough dab), rhabdomyoma (wolffish) and poorly-differentiated liver cancer (haddock) were diagnosed.

Hyperpigmentation – Prevalences in North Sea dab (*Limanda limanda*) continued to be high in most North Sea areas, e.g. in the German Bight and at the Dogger Bank (27.4% and 53.1%, respectively). The England/Wales report showed somewhat lower values but concluded that the disease still appears to be most prevalent in the North Sea. An increase in prevalence of 5.8% in 2003 was observed at the Indefatigable Bank. Although the prevalence of hyperpigmentation still remains relatively low at sites in the Irish Sea, 2004 saw an increase of 11.1% to 15.0% at Inner Cardigan Bay, similar to those levels observed in 2002. In dab from the western Baltic Sea, the prevalence was below 0.1%.

Intersex – The condition has been detected for the first time in an offshore flatfish species. Two dab (*Limanda limanda*) from a total of 14 male fish from a station at the North Dogger Bank (North Sea) were found to exhibit the condition in 2004. Both cases exhibited only previtellogenic oocytes amongst the testicular tissue. In one fish where both lobes of the gonad were sectioned, only one was affected. In the second fish only one lobe was sampled. The significance of this condition in dab is currently unknown.

Skeletal deformities – The prevalence of skeletal deformities in Baltic cod (*Gadus morhua*) in 2004 varied between 0.3% and 3.8% and was lower than in previous years. Skeletal deformations were recorded in sprat (*Sprattus sprattus*) caught in ICES Subdivision 25 and 26 (0.1% and 0.02%, respectively). Percentage of sprat with deformations in Subdivision 25 was higher than in 2003. The deformations were found in 0.1% of flounder (*Platichthys flesus*) from the Baltic Sea (ICES Subdivisions 24 and 26).

Conclusions

1) Infectious pancreatic necrosis virus was isolated 20 times from wild-caught dab (Limanda limanda) (16 isolations), grey gurnard (Eutrigla gurnardus), plaice (Pleuronectes platessa), long rough dab (Hippoglossoides platessoides) and flounder (Platichthys flesus) around the Shetland Islands, UK.

2) A new rhabdovirus was isolated from starry flounder (Platichthys stellatus) in Puget Sound, Washington, USA.

3) Viral hemorrhagic septicaemia virus, preliminarily identified as genotype two, was isolated from herring (Clupea harengus) in Finland.

4) Isolates of Mycobacterium from striped bass (Morone saxatilis) in Chesapeake Bay, USA, are primarily M. shottsii and co-occur with five other Mycobacterium spp.

5) An intracellular bacterial pathogen of the gill epithelium was associated with mass mortalities of Atlantic croaker (Micropogonias undulatus) along the eastern US coast.

6) Gyrodactylus salaris was detected on Arctic charr (Salvelinus alpinus) in tributaries of the River Numedalslågen, Norway. The parasite belongs to a genotype previously found on farmed Arctic charr.

7) Lepeophtheirus salmonis was found on approximately 63% of juvenile pink (Oncorhynchus gorbuscha) and chum salmon (O. keta) in British Columbia, Canada. Prevalence and intensity of infections on these hosts were much higher than in 2003.

8) Prevalences of hyperpigmentation in dab continued to be high in most North Sea areas.

9) The intersex condition has been detected for the first time in dab from the North Sea.

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. This information is of vital importance to integrated assessments of the health of marine ecosystems, such as the ICES Integrated Assessment of the North Sea Ecosystem, the Baltic Sea Regional Project (BSRP), the OSPAR CEMP, and the revised HELCOM Monitoring Programme.

5.2 Farmed fish

5.2.1 Viruses

Viral Haemorrhagic Septicaemia Virus (VHSV) – A North American strain of VHSV was isolated from farmed Atlantic salmon (*Salmo salar*) showing visceral haemorrhage in British Columbia, Canada, during March 2004. This was associated with a low-level mortality from 150 g smolts that had entered seawater four months previously. Laboratory studies with previous isolates have shown this strain to be pathogenic.

Salmon pancreas disease virus (SPDV) – SPDV is widespread in Ireland in farmed Atlantic salmon (*Salmo salar*) and now affects the majority of marine farms. Mortality ranged from 5% to 30%. In Norway, an increase in losses associated with cases of clinical SPD (Salmon Pancreas Disease) has been noted together with the first diagnosis of this disease in Nordland and Rogaland.

Infectious pancreatic necrosis virus (IPNV) – IPNV was isolated in Ireland from two different Atlantic salmon (*Salmo salar*) farms in 2004. One isolate from one of these farms was the Ab serotype. In Scotland 44% of sites were positive for IPNV. Overall there is no major trend but the data indicates the widespread nature of the virus. IPNV has recently been deregulated and designated area orders revoked.

Infectious Salmon Anaemia Virus (ISAV) – In Norway there has been an increase in reported cases of ISAV in Atlantic salmon (*Salmo salar*). An investigation into high mortality

among farmed salmon post smolts in Scotland resulted in declaration of suspicion of ISAV at one farm following positive results from IFAT and RT-PCR. Significant gill pathology and ***Ichthyobodo* spp**. were recorded on these salmon in low to high numbers. The site was fallowed and the fish ensiled.

ISAV was confirmed at six Atlantic salmon farm sites in Cobscook Bay, Maine, USA. This virus was detected in June and continued through the year. Nearly all infected cages were voluntarily harvested before cell culture confirmation. During routine surveillance of all salmon culture sites in Maine, a second strain of ISAV was detected at a site south west of Cobscook Bay. This was the first detection of ISAV in Maine other than Cobscook Bay. ISAV was detected by RT-PCR in several pens, and appeared to spread to all cages at the net-pen site before dissipating over a period of 6 months. The new wild type strain (strain-2) did not cause disease in the cultured salmon and did not grow on normal cell lines. There was no spread to four other farms in the area. Segment 6 gene sequencing of PCR products indicates this strain is more closely related to the possible non-pathogenic or wild types from Scotland, Nova Scotia and Norway than to the New Brunswick strain that has caused mortality in Cobscook Bay. Preliminary results of sequencing of archived samples from the Cobscook Bay outbreaks shows that the strain-2 occurred with the pathogenic strain of ISAV in five out of the seven sites tested. In at least two cages, strain-2 infection was followed by a strain-1 disease outbreak; suggesting strain-2 may not be fully protective. Observational data demonstrated there were more ISAV-PCR positive sea lice (*Caligus elongatus*) than positive fish among the moribund fish examined.

Heart and skeletal muscle inflammation (HSMI) – In farmed Atlantic salmon (*Salmo salar*) in Norway, HSMI was first described in 1999 and a significant increase was reported for 2004. The outbreaks are generally severe with losses up to 25%. HSMI is an infectious disease of possible viral origin. A condition resembling HSMI has been reported in Scottish salmon with a cumulative mortality of 9%. Moribund fish showed swollen abdomen, dermal oedema, ascites and pericardial fluid with a thin gelatinous membrane over the liver. The heart appears soft and flabby. Histopathological changes in myocardial spongy layers were characterised by widespread vacuolation, degeneration and subsequent cavitation of cardiac myocytes, with loss of striation. Additional details are provided in report section 7.

5.2.2 Bacteria

Aeromonas salmonicida – An outbreak of furunculosis occurred at a federal US Atlantic salmon (*Salmo salar*) hatchery with significant mortality. Historically, incoming broodstock at this hatchery experienced 50% pre-spawn mortality due to terramycin-resistant *A. salmonicida*. The problem had been managed by inoculating incoming broodstock with oxolinic acid and a water-based vaccine. This recent outbreak occurred in post-spawned broodstock approximately two weeks post treatment.

Atypical *A. salmonicida* has been a problem in halibut (*Hippoglossus hippoglossus*) in Norway. In Ireland, furunculosis (typical and atypical strains) re-emerged on some freshwater rainbow trout (*Oncorhynchus mykiss*) sites to cause significant mortality.

***Aeromonas hydrophila, A. salmonicida*, myxobacteria and *Pseudomonas* spp. –** were detected in Atlantic salmon (*Salmo salar*) in Latvia, as well as several outbreaks of myxobacteriosis in some salmon hatcheries. *Pseudomonas spp.* constituted 23–35% of the isolates from hatchery juvenile Atlantic salmon in the Far East of Russia.

Edwardsiella tarda – The number of isolations of *Edwardsiella tarda* has increased from farmed turbot *(Scophthalmus maximus*) in Spain.

***Tenacibaculum (Flexibacter)* sp.** is frequently isolated from halibut (*Hippoglossus hippoglossus*) fry in Norway.

Moritella viscosa – Outbreaks of *Moritella viscosa* are causing significant losses, mainly due to decreased quality, both in salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). The losses are especially high in northern Norway, probably due to low temperature and a slow healing process. In Ireland, outbreaks are associated with mortality in S0 Atlantic salmon smolts.

Renibacterium salmoninarum – *R. salmoninarum* in Denmark was found in two new marine rainbow trout (*Oncorhynchus mykiss*) farms. The number of outbreaks of BKD in rainbow trout in Finland showed a slight decrease in 2004.

Piscirickettsia salmonis – *P. salmonis* outbreaks with associated high mortality occurred at three marine sites rearing Atlantic salmon (*Salmo salar*) in Ireland during the late summer. In Scotland there is a slight upward trend in reported cases.

Vibriosis – ***Vibrio*** (***Listonella anguillarum***) is the main disease problem in cod fry (*Gadus morhua*) in Norway.

5.2.3 Parasites

Amoebae

Paramoeba sp. – Amoebic gill disease affected Atlantic salmon (*Salmo salar*) at four sites in Ireland. Two sites had losses of 10–20% in some pens.

Ciliata

Philasterides dicentrarchi – An increasing trend in turbot (*Scophthalmus maximus*) culture in Spain is reported. A vaccine against *Philasterides* should be shortly available for field trials.

Mesomycetozoa

Ichthyophonus hoferi – A farmed Atlantic salmon (*Salmo salar*) in Scotland was reported with a proliferative granulomatous response with numerous encysted spores of *I. hoferi* at different developmental stages.

Myxosporea

Tetracapsuloides bryosalmonae – Proliferative kidney disease was observed on some Atlantic salmon (*Salmo salar*) farms in Ireland.

Parvicapsula pseudobranchicola – In 2002 the first clinical disease outbreaks caused by *Parvicapsula* were diagnosed in five Atlantic salmon (*Salmo salar*) farms in northern Norway. In 2004 the parasite was detected in several farms in northern Norway as well as in Trøndelag and Møre and Romsdal. The significance of the disease is uncertain.

Enteromyxum scophthalmi shows an increasing trend in turbot culture in Spain.

Cestoda

Eubothrium crassum – A possible increasing problem with *Eubothrium crassum* is reported from some regions in Norway rearing Atlantic salmon (*Salmo salar*). The effect of treatment (praziquantel) has in some cases been unsatisfactory and improper treatment procedures or emerging resistance has been proposed as possible causes.

5.2.4 Other diseases

Proliferative gill inflammation – Proliferative gill inflammation has been detected in Norwegian Atlantic salmon (*Salmo salar*) for at least 15 years, and generally occurs during the

first months following seawater transfer. Losses vary between 15–60% and growth is often seriously retarded. This condition has been associated with **epitheliocystis** and a newly described, **Atlantic salmon paramyxovirus (ASPV)**. In 2004 this has been a serious disease problem especially in the southern part of Norway (Rogaland).

Congenital deformities – A high prevalence of congenital deformities were present in some batches of imported Atlantic salmon (*Salmo salar*) fry in Ireland.

Conclusions

1) Heart and skeletal muscle inflammation (HSMI) is an emerging problem for Norwegian Atlantic salmon (*Salmo salar*) aquaculture and was recently recorded in farmed Atlantic salmon in Scotland.

2) Proliferative gill inflammation remains a problem for farmed Norwegian Atlantic salmon.

3) There is an increase in the number of cases of *Parvicapsula pseudobranchicola* in farmed Atlantic salmon in Norway.

4) A new wild type strain of Infectious Salmon Anaemia Virus (ISAV) was identified in farmed Atlantic salmon in Maine, USA.

5) A field trial vaccine for *Philasterides dicentrarchi* in turbot (*Scophthalmus maximus*) will be conducted in Spain.

Recommendations

The WGPDMO recommends that:

i) WGPDMO should update information on causes and effects of heart and skeletal muscle inflammation (HSMI) affecting Atlantic salmon culture in ICES Member Countries at its 2006 meeting;

ii) ICES Member Countries should investigate the role of epitheliocystis and Atlantic salmon paramyxovirus (ASPV) in proliferative gill inflammation of farmed Atlantic salmon.

5.3 Wild and farmed molluscs and crustaceans

5.3.1 Viruses

Herpesvirus in bivalves – No change reported in France and no new information from the US (but see report section 8 on Summer Mortality of Pacific oysters)

Viral Gametocytic Hypertrophy in Pacific oysters (*Crassostrea gigas*) – Continued rare presence in France.

Bacilliform virus in brown shrimp– Infections were detected for the first time in brown shrimp (*Crangon crangon)* from the North Sea collected from the offshore Wash fishery (UK). The virus, apparently the same as the *C. crangon* bacilliform virus (CcBV) previously described from *C. crangon* in estuarine environments, was present in 100% of shrimp sampled. Light to heavy infections affected the hepatopancreas.

White Spot Syndrome Virus in shrimp – WSSV was found in 20% of a sample of 100 shrimp (*Litopenaeus setiferus*) in Mississippi Sound, Gulf of Mexico, USA. The same virus was detected a few years ago in wild penaeid shrimp in the Gulf, but at a prevalence of <1%. Preliminary analysis indicates that this may represent a localised outbreak of an introduction or the eruption of an indigenous form of WSSV or related virus. The first outbreak of disease due to WSSV in the Pacific region of the USA occurred in a commercial shrimp farm on the island of Kauai, Hawaii. Quarantine restrictions were placed on the affected facility, prohibiting the movement of shrimp. Prior to this outbreak, WSSV in the USA was reported only in

commercial facilities in Texas and South Carolina in 1995 and from wild shrimp and crabs off shore in the Gulf of Mexico and near shore in Texas, Mississippi, Georgia and South Carolina.

Taura Syndrome Virus in shrimp – An outbreak of TSV occurred at four pond-culture facilities in Texas, USA, that were growing Pacific white shrimp (*Litopenaeus vannamei*). Sixteen of 38 ponds tested positive for the virus and experienced 80–90% mortality. Based on sequencing of the VP1 region, the isolated virus is 97% similar to isolates from the Americas, 96% similar to a Belize isolate and 98% similar to an Asian isolate in the OIE Reference Laboratory collection. Quarantines were placed on the facilities to prohibit water discharge and to restrict shrimp movement to processing facilities only.

5.3.2 Bacteria

***Rickettsia* in shore crabs** – A putative rickettsia-like organism (RLO) was found for the first time in shore crabs (*Carcinus maenas*) from Southampton Water, English Channel, UK. Two individuals, collected in July and October 2004 exhibited white opaque haemolymph upon dissection. Histopathology and transmission electron microscopy revealed a heavy RLO infection associated with connective tissues. Spongy connective cells, reserve inclusion cells, fixed phagocytes and haemocytes appeared the target for infection.

Nocardiosis of Pacific oyster – No new trends reported for *Crassostrea gigas* in Canada and USA.

Juvenile Oyster Disease of eastern oysters – No change in distribution reported. Sampling of eastern oyster (*Crassostrea virginica)* cohorts deployed experimentally in Maine and Massachusetts, USA, and followed during the summer of 2004 documented, for the first time, the presence and predominance of the α-proteobacterium, *Roseovarius crassostrea*, before and during the development of JOD in oysters. Previously, it had been found only in sick oysters. The disease has not been experimentally reproduced, but the new finding, along with earlier documentation that *R. crassostrea* is found in all JOD outbreaks over a wide geographical range, supports the contention that this newly described bacterium is the proximate cause of JOD, although other factors may be necessary to trigger disease outbreaks.

5.3.3 Fungi

Yeast in edible crabs – Continued low prevalence of yeast was found in edible crabs (*Cancer pagurus*) in UK waters. The yeast is possibly a co-infecting organism in immunosuppressed crabs infected with *Hematodinium sp*.

5.3.4 Parasites

Chlorophyceae

Picoeucaryot alga in blue mussels – Infections by a previously undescribed green alga were found in 3-year-old or older blue mussels (*Mytilus edulis*) in southern Norway. Infections were primarily on the edge and in the connective tissues of the mantle, and resulted in severe shell deformation.

Dinoflagellata

***Hematodinium* (Pink Crab Disease) in edible crabs (*Cancer pagurus*)** – continues to be present in UK waters at prevalences of 3% to 33%, depending on month of collection.

***Hematodinium* in blue crabs** – The end of a 4-year drought in the southeastern USA significantly reduced the prevalence (to 5%) o*f Hematodinium perezi* in blue crabs (*Callinectes sapidus*). This parasite was associated with marked declines in fishery landings during the drought. To the north, it remains more abundant in the Atlantic coastal bays of Maryland,

USA, where prevalences vary by month: 52% in September and October; 14% in November and 30% in December.

***Perkinsus marinus* in eastern oysters –** Another relatively cold wet winter/spring in 2003/04 led to the second year of marked decrease in the prevalence and intensity of *P. marinus* in Chesapeake and Delaware Bays, USA, oysters (*Crassostrea virginica*). Although the majority of sampled locations continue to be infected, prevalences fell to levels not seen since the early 1990s in Chesapeake Bay. In 2002 in the lower bay 26 of 29 stations had at least 80% prevalence in the fall survey; in 2004 this ratio was only 6 of 29. In the upper bay the prevalence of infected oysters fell from 94% to 52% between 2002 and 2004. In Delaware Bay the decrease was from 81% to 43% during the same period. No change was reported in New England, South Carolina, or around the Gulf of Mexico.

***P. andrewsi/chesapeaki* in clams** – During 2004 infections remained ubiquitous and prevalent among commercial soft shell clams (*Mya arenaria*) (45%) and stout razor clams (*Tagelus plebeius*) (72%) throughout the upper Chesapeake Bay, USA. A single sample of 18 *T. plebeius* examined for the first time in lower Delaware Bay, USA, had a prevalence of 84%, all very light infections. This pathogen, along with haemic neoplasia, is thought to be responsible for a serious decline in soft clam harvests in Chesapeake Bay.

***Perkinsus olseni* in Manila clams** – Infections continue to be prevalent in Manila clams (*Ruditapes philippinarum*) along the French coast *(*547 positive clams among 759 analysed).

Labyrinthomorpha

Quahog Parasite X (QPX) in hard clams – An outbreak of QPX in farms on the north side of Cape Cod, Massachusetts, USA, in 2004 resulted in the loss of several million clams (*Mercenaria mercenaria***)**, which were destroyed in an attempt to halt the spread of the parasite. The outbreak occurred in seed clams that had been transplanted earlier from an enzootic site. The prevalence at the time of transplantation was 1%, but it quickly increased, with concomitant mortalities, over the next several months. To date, there is no evidence that the parasite was transmitted to resident clams in the area. By using molecular methodologies, it has been possible to detect QPX-like particles in water samples and to further associate the parasite with marine aggregates, especially the seaweed portion of those aggregates. No change was reported in the remainder of the known range in Canada and the USA.

Haplosporidia

***Bonamia ostreae* in flat oysters** – Infections were reported for the first time in British Columbia, Canada in November 2004. A prevalence of about 60% was detected in 3-year old flat oysters (*Ostrea edulis*) sampled from one location. Re-examination of archived samples indicated that *B. ostreae* was present in flat oysters from the same location in 2003. Mortalities of 3–4 year old oysters over the past two years at the affected site were associated with severe algal blooms; thus, the exact correlation between these mortalities and *B. ostreae* infection is not clear. However, flat oysters from the infected stock held in the laboratory at ambient temperatures (9–10 °C) experienced continuous mortality associated with very heavy *B. ostreae* infections that reached about 40% over two months. Flat oysters account for less than 1% of the total British Columbia oyster production.

No change was found in France, Ireland, Spain and the UK. Samples of adult oysters in Scotland and the Limfjorden area of Denmark tested negative for *B. ostreae* in 2004 and the latter received approved status as being free from Bonamiosis by the EU in December.

***Bonamia* sp. in Asian oysters**– In 2003, a new species of *Bonamia* was discovered killing an exotic oyster species (*Crassostrea ariakensis*) experimentally deployed in South Carolina, USA. This oyster is being considered for introduction into Chesapeake Bay to replace the na-

tive eastern oyster (*C. virginica*), which has been devastated by diseases. The pathogen 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 *Bonamia exitiosa* and *B. roughleyi*. The parasite persisted throughout 2004, and was detected also in the native crested (horse) oyster *Ostrea 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 second *Bonamia* sp. (sequenced from *O. equestris*) 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.

***Mikrocytos mackini* in Pacific oysters** – In mid-February 2004, a mikrocytosis outbreak in Pacific oysters (*Crassostrea gigas*) within the known distribution of *M. mackini* in British Columbia, Canada, suspended harvesting of infected *C. gigas* because of the high prevalence of visible lesions. Unlike previous cases involving oysters harvested from the beach, this disease outbreak occurred among oysters in a suspension culture system. However, the affected stock had been maintained in culture for one year longer than the usual harvest regimen because of harvest closures due to toxic phytoplankton concerns. In April 2004, an estimated mortality of 7% was directly attributable to mikrocytosis. Three percent of juvenile oysters hung adjacent to the infected stocks on 8 April 2004 had light infections when sampled in June 2004.

***Haplosporidium nelsoni* (MSX) in eastern oysters –** To date, confirmed infections remain restricted to the Bras d'Or Lakes area of Cape Breton where the pathogen was first found in Canada in 2002. Surveillance of the buffer region around Cape Breton and oysters in the southern Gulf of St. Lawrence is ongoing. Continued above-average rainfall and consequently reduced salinities in 2003/04 depressed *H. nelsoni* prevalences and intensities for the second year in a row in Chesapeake Bay, USA. In the upper bay, mean prevalence fell from 28% to 0.3% between 2002 and 2004. In the lower estuary *H. nelsoni* was found at 28 of 29 sampling stations in 2002; in 2004 it was detected at just 5 of 29 stations. No change was recorded in Delaware Bay, where infection prevalence continues to be very low, apparently due to resistance in the native population, or in the southeastern USA, where prevalence has always been relatively low. No data were reported from New England.

***Haplosporidium costale* (SSO) in eastern oysters** – Infections, detected by PCR alone, with no confirmatory detection by histology and no associated mortality, were found in Atlantic Canadian oysters (*Crassostrea virginica*). No infections were detected in USA oysters examined by histology.

***Haplosporidium* sp. in blue mussels (*Mytilus edulis*) –** No new trends in Canada.

Paramyxea

***Marteilia refringens* in Pacific oysters** – No change in France**.** Samples of adult Pacific oysters (*C. gigas*) in Scotland and the Limfjorden area, Denmark, tested negative for *M. refringens* in 2004 and the latter received approved status as being free from Marteiliosis by the EU in December.

***Marteilia maurini* in blue mussels** – Infections were found in three of 50 blue mussels (*Mytilus edulis*) examined from Southampton Water, UK, in June 2003. A further sample of 150 mussels was examined in June 2004. Eight of these were infected. It was confirmed by 18s DNA sequence analysis using the OIE approved method that the species was *Marteilia maurini.* Nearby native flat oysters (*Ostrea edulis*) were negative for *Marteilia*. Further sampling of mussels from this site revealed one infected individual during October 2004 (n = 50) and another in November 2004 (n = 50).

Paramarteilia-like organism in edible crabs – One individual per sample of 30 edible crabs (*Cancer pagurus*) from UK waters was infected each month in January, February and April, 2004. The organism was found throughout the hepatopancreas and was also shown to be infecting the ovary and oocytes.

Microsporea

Microsporeans in crabs – An intranuclear microsporean infection was present in one individual per sample of 30 edible crabs (*Cancer pagurus*) collected each month from March through May, 2004, in the UK. The microsporean was distributed throughout the hepatopancreas tubules, infecting the nuclei of the endothelial cells. This is the first finding of an intranuclear microsporean infection of an invertebrate. Current work is attempting to classify this organism using ultrastructural and molecular tools. Another microsporean infection, with prevalence of over 60% in some months, was discovered in Chinese mitten crabs (*Eriocheir sinensis*) from the River Thames in London.

Digenea

***Prosorhynchus squamatus* in blue mussels –** Moderate infections were detected in 3% of wild mussels (*Mytilus edulis*) in Nova Scotia, Canada, collected from two sites: Aspy Bay, a new site for detection in October 2003 and Lennox Passage in June 2004. A three-year directed sampling program for this parasite has not detected it in the other Maritime Provinces nor has it revealed a significant impact to mussel resources either wild or cultured.

5.3.5 Other diseases

Neoplasia – No new trends were reported in Canada or in cockles (*Cardium edule*) in Spain. Disseminated neoplasia (DN) remains present (13% in a sample of 30) in soft shell clams (*Mya arenaria*) from upper Chesapeake Bay, USA. A similar, but also distinctive, haemolymph proliferative disorder was detected in stout razor clam (*Tagelus plebeius*) collected during both 2002 and 2004. This appears to be a previously undescribed disseminated haemocytological neoplasia of unknown aetiology, epidemiology, and pathology. To date, two 2002 samples and one 2004 *T. plebeius* sample have shown >90% prevalences of this neoplasm.

In 2004 neoplasia was for the first time observed in clams (*Macoma balthica*) from four new sampling stations in the Gulf of Gdansk, Baltic Sea, where the disease had not been noted before. Three of those at depth 40 m and one at depth 60 m had an average prevalence of 11%. Prevalence among all stations varied from 2.5% to 25%. When compared to a previous study it appears that the incidence of neoplasia has become more widespread and is now found throughout the whole area of the Gulf of Gdansk.

By using the nucleus-to-cytoplasm ratio of haemocytes it was confirmed that histopathological lesions detected in soft shell clams (*Mya arenaria*) from the German Wadden Sea area, and originally considered to be haemic neoplasia, were of an inflammatory nature.

Gill disease in blue mussels – A newly described gill anomaly was identified in blue mussels (*Mytilus edulis trossulus*) from the Gulf of Gdansk, Baltic Sea, sampled from the 45 m depth station in 2003 and 2004, with prevalence of 11% and 23%, respectively. Gills of affected mussels, viewed macroscopically, appeared highly eroded. Gill filaments were totally destroyed in advanced cases.

Mortalities of adult flat oysters (*Ostrea edulis*) – No new trends were reported in Canada.

Cockle mortality – Unusually heavy mortality, of up to 70%, occurred in some cockle (*Cerastoderma edule*) beds in November in Milford Haven, South Wales, UK. Most individuals were parasitised by a combination of digeneans in the muscle or gut lumen or by gregarines. These were in low numbers in all cases and were thus not considered significant. In addition,

there was some necrosis and infiltration present in the digestive gland but this was again felt to be within normal limits. Overall, there were no consistent features that would indicate a cause for the observed mortality and an environmental cause is suspected.

Mussel mortality – High mortality of blue mussels (*Mytilus edulis*) was reported in the UK in June, and along the northern Mediterranean coast of Spain between June and November. The mortality in Spain was attributed to unusually high water temperatures. These mussels had up to 80% prevalence of *Mytilicola* sp. and *Marteilia* sp. with associated alteration of internal organs.

Shell disease of crustaceans – American lobster (*Homarus americanus*) mortality/shell disease, which is associated with various bacterial and fungal species, continues to be a problem primarily from eastern Long Island Sound, New York to Buzzards Bay, Massachusetts, south of Cape Cod, USA. The appearance of shell disease off Salem and Cape Ann, Massachusetts, north of Cape Cod, is the first since extensive sampling began in 2000. In Maine and New Hampshire the prevalences are generally less than 0.1%, although in some areas of Maine the prevalence is as high as 5%.

Shell disease associated with carapace infestations of *Pseudomonas* spp. is common in crustaceans in Sakhalin Island, Russia. Prevalence ranges from 1% to nearly 60%, depending on host species. Chitinolytic fungus disease, caused by *Trichomaris invadens***,** is observed in 1% to 8.5% of snow crabs (*Chionoecetes opilio*). Hyphae penetrate the exoskeleton and invade the epidermis and other tissues and organs. Moderate infection prevents moulting, and invasion of the eyes probably causes blindness and a potential lethal outcome.

Summer Mortality of Pacific oysters – Oyster herpes virus was detected in juvenile oysters (C*rassostrea gigas*) in Tomales Bay, California, USA, an area that has experienced repeated episodes of Summer Mortality for the past decade. A causal relationship, however, has not been demonstrated and previous studies have associated a variety of physiological and environmental factors in the mortalities. Summer mortality studies continued in France in the MOREST Programme (see Report Section 8).

Mortality of Surf Clams – A widespread mortality of surf clams (*Spisula solidissima*), as documented by a marked (~50%) drop in abundance of live clams, occurred between 1999 and 2002 off the mid-Atlantic coast of the USA. A histological survey in 2003 found no evidence of a pathogen, but did document a loss of condition, gonadal "abnormality", and digestive gland atrophy that increased in a southerly direction and has been called a "malnourishment syndrome". It is hypothesized that regional increases in temperature due to global warming have resulted in a mismatching of food supply and feeding rate, leading to the slow starvation of the clams at the southern edge of their range. At the same time, there is evidence that clam densities are increasing at the offshore and the northern edge of the range, suggesting that the mortality is part of a range shift to regions of cooler water temperature in this species.

Black Spot Disease in brown shrimp (*Crangon crangon*) – No new trends have been reported in the German Wadden Sea.

5.3.6 Miscellaneous

Due to the heavy mortalities of eastern oysters (*Crassostrea virginica*) experienced in Chesapeake Bay, USA, from 1998 to 2002 caused by *Perkinsus marinus* and *Haplosporidium nelsoni*, the harvest of native oysters is now less than 2% of what it was in the 1970s. The state of Maryland, which borders the upper estuary, has proposed releasing diploid Asian oysters (*C. ariakensis*) in 2005 with the hope that this species, which is resistant to mortalities caused by these two pathogens, will restore oyster populations in the bay. The proposal has met with resistance from some adjacent states (Delaware and New Jersey), and with most of the scientific and environmental community. It is presently unclear whether Maryland will carry

through with the project, at least this summer. In Virginia, which borders the lower bay, growers have been experimenting with cage-culture of triploid Asian oysters and also triploid, selectively bred native eastern oysters, both of which have performed well in commercial-scale tests.

The project on effects of antifouling compounds (Irgarol, Diuron, and Copper) on wild mussels (*Mytilus edulis)*, Pacific oysters (*Crassostrea gigas*) and periwinkles (*Littorina littorea*) from a harbour area (Norderney) and a reference area at the German North Sea coast was finalised. Whilst the specimens from the reference area were affected only by lesions induced by parasites (trematode larvae and *Mytilicola intestinalis*), those from the harbour area displayed necrotic and inflammatory changes of the gonads and the hepatopancreas (*C. gigas*, *L. littorea*) as well as of the gills (*L. littorea*). A link with the antifouling compounds is not clear, however. In the harbour area, lesions known to be caused by tributyltin were also recorded (shell deformities in *C. gigas*, intersex and atrophy of gonads in *L. littorea*).

Conclusions

1) A newly reported bacilliform virus was found in 100% of brown shrimp (*Crangon crangon*) in the Wash fishery, North Sea, UK.

2) The α-proteobacterium, *Roseovarius crassostrea*, was found for the first time before and during the development of Juvenile Oyster Disease (JOD) in eastern oyster (*Crassostrea virginica)* in Maine and Massachusetts, USA. Previously, it had been found only in sick oysters. This new finding supports the contention that the bacterium is the proximate cause of JOD.

3) *Bonamia ostreae* was reported, at high prevalence (60%), for the first time in flat oysters (*Ostrea edulis*) in British Columbia, Canada. Associated mortality was documented in the laboratory, but was confounded with algal bloom-caused losses in the field.

4) The SSU rDNA of a newly discovered *Bonamia* sp., enzootic to North Carolina, USA, and infecting an introduced oyster (*Crassostrea ariakensis*), is similar in sequence to the southern hemisphere *Bonamia exitiosa* and *B. roughleyi*. The parasite was detected also in a native oyster *Ostrea equestris*. Also in *O. equestris* (but not *C. ariakensis)*, a second new *Bonamia* sp. was detected that is more similar in SSU rDNA sequence to the northern *B. ostreae* than to the southern hemisphere forms.

5) A previously undescribed intranuclear microsporean, the first for an invertebrate, was found in edible crabs (*Cancer pagurus*) in UK. Another microsporean infection was discovered in Chinese mitten crabs (*Eriocheir sinensis*) in UK.

6) A newly described disseminated neoplasia affecting >90% of stout razor clam (*Tagelus plebeius*) in the Chesapeake Bay, USA, was found in 2002 and 2004. The incidence of neoplasia in clams (*Macoma balthica*) has become more widespread and is now found throughout the whole area of the Gulf of Gdansk, Baltic Sea.

7) A previously undescribed gill anomaly was found in up to 23% of blue mussels (*Mytilus edulis*) in the Gulf of Gdansk, Baltic Sea.

8) Shell disease of American lobsters (*Homarus americanus*) was found for the first time north of Cape Cod, Massachusetts, USA.

9) The effect of local climate on shellfish diseases was illustrated by a significant decline in *Haplosporidium nelsoni* and *Perkinsus marinus* infections in eastern oysters (*Crassostrea virginica*), and in *Hematodinium perezi* infections in blue crabs (*Callinectes sapidus*) in the USA, all associated with the end of an unusually warm, dry period. High mortalities of surf clams (*Spisula solidissima*) off the US mid-Atlantic coast, of blue mussels (*Mytilus edulis*) in Spain, and summer mortality of oysters (*C. gigas*) in France were associated with high temperatures.

Recommendations

WGPDMO recommends that:

i) studies be expanded on the significance of the newly described bacilliform virus in brown shrimp (*Crangon crangon*) from the North Sea as a population modulator in this important European fishery;

ii) WGPDMO be kept informed of progress in investigation into the identification of *Bonamia* species infecting Asian oysters (*Crassostrea ariakensis*) and crested (horse) oysters (*Ostrea equestris*) in the USA;

iii) investigation be continued on mussel (*Mytilus edulis*) gill anomalies in the Gulf of Gdansk, Baltic Sea, including histopathological studies;

iv) WGPDMO be kept informed of progress in investigations to determine the causes of the Summer Mortality Syndrome in Pacific oysters (*Crassostrea gigas*).

6 Assess information available in ICES Member Countries on the role of plankton organisms in gill-related mortality in farmed fish

Information was presented by B. Hjeltnes and D. Bruno.

In 2002, 25 Atlantic salmon (*Salmo salar*) farms in Norway in three different areas experienced mortalities associated with planktonic blooms. In summer 2003, a rise in mortality was reported in three Atlantic salmon farms in Ireland. Approximately 1 million salmon died during the outbreak.

The affected salmon showed gill and mouth haemorrhage, and hyperplasia of the secondary gill lamellae. Histopathological studies also indicated the presence of invading bacteria in the gills. These bacteria were, however, considered secondary invaders.

The mortality in the Atlantic salmon in Norway was considered to be closely associated with the mass occurrence of a minute jellyfish *Muggiaea atlantica* (Siphonophora). Similar pathology was observed in the affected salmon in Ireland; however, the cause could not clearly be associated to jellyfish or planktonic blooms. In 2004, mortality and comparable pathology has also been observed in Atlantic salmon in Scotland due to jellyfish and phytoplankton blooms (Table 1).

Table 1: Scottish Atlantic salmon farms affected by planktonic organisms in 2004.

Location	Region	Date	Numbers dead fish	Av. Weight	Biomass (T)	Suspected cause	Notes of event	Temp (°C)
Shetland	Shetland	August	100	800g	0.08	*Phialidium* sp. (jellyfish)	Increased mortality amongst larger fish	13
Skye	Highland	June–August	670	4kg	2.69	*Ceratia furca* (phytoplankton)	Dead fish decomposed assumed plankton kill	Na

Na – not available

Conclusions

1) Contact with planktonic organisms may result in mass mortality in farmed Atlantic salmon (*Salmo salar*).

2) Causes of high mortality are difficult to identify where no water samples have been collected.

Recommendations

WGPDMO recommends that:

i) water samples should be collected immediately in cases of mass mortalities in farmed fish in order to identify possible harmful organisms;

ii) an early warning system should be available to mitigate the impact of planktonic organisms describing the water quality throughout farming regions, allowing personnel to make decisions regarding fish husbandry;

iii) communication between individual fish farms allowing the development of integrated bloom control strategies is required.

References

Hellberg, H., Olsen, A. B., Jensen, F. 2003. Clinical signs of histopathology in farmed Atlantic salmon and rainbow trout associated with large numbers of the jellyfish *Muggiaea atlantica* (Siphonophora). Poster presented at the 11th International Conference of the EAFP, Malta , 21–26 September 2003.

Cronin, M., Cusack, C., Geoghegan, F., Jackson, D., McGovern, E., McMahon, T., O´Beirn, F., O´Cinneide, M., and Silke, J. 2004. Salmon mortalities at Inver Bay and McSwyne´s Bay finfish farms, County Donegal, Ireland during 2003. Marine Environment and Health Series, 15.

7 Review current information on the continued increase of heart and skeletal muscle inflammation affecting farmed salmon

Information was presented by B. Hjeltnes and D. Bruno.

Heart and skeletal muscle inflammation (HSMI) was first diagnosed in Norwegian Atlantic salmon (*Salmo salar*) in 1999 and is becoming an increasing problem in post smolts. Affected fish are anorexic with pale heart, yellow liver, ascites and perivisceral petechiae. HSMI is characterised by extensive panmyocarditis and myositis particularly involving red skeletal muscle. Morbidity may be very high, while mortality is variable and may reach 20%.

Recently, a condition resembling HSMI has been reported in Scottish Atlantic salmon (Ferguson *et al.*, 2005). Currently there are no reports of HSMI in other salmon producing countries.

Based on infectivity studies, HSMI apparently is an infectious disease. A viral aetiology is suspected (Kongtorp *et al.* 2004 a, b). This disease has similarities with salmon pancreas disease (SPD) and cardiomyopathy syndrome (CMS) which affect heart and skeletal muscle (Table 1). Some researchers are of the view that this is a variant of salmon pancreas disease as clinical signs can be variable when different strains, time of outbreak and environmental conditions are taken into account. The presence of HSMI in regions with no SPD and evidence from epidemiological studies in Norway contradicts this hypothesis.

Table 2: Heart and skeletal muscle lesions with heart and skeletal muscle inflammation (HSMI), pancreas disease (SPD) and cardiomyopathy syndrome (CMS).

LESIONS	HSMI	SPD	CMS
Epicarditis	+	+	+
Myocarditis and degeneration of compact myocardium	+	+	-
Myocarditis and degeneration of spongy myocardium	+	+	+
Skeletal muscle inflammation and degeneration	+	+	-
Multifocal necrosis of hepatocytes	+	-	+/-
Necrosis of exocrine pancreas	-	+	-

Conclusions

1) Heart and skeletal muscle inflammation (HSMI) is a major and increasing disease problem for Norwegian Atlantic salmon (*Salmo salar*) aquaculture.

2) In Norway HSMI occurs in regions where Salmon Pancreas Disease (SPD) is considered absent.

3) Evidence of a viral aetiology has been postulated for HSMI in Norway.

4) An outbreak of disease resembling HSMI has been reported in Scottish farmed Atlantic salmon.

Recommendation

WGPDMO recommends that:

i) available new information on the causes and effects of heart and skeletal muscle inflammation (HSMI) affecting farmed Atlantic salmon (*Salmo salar*) in ICES Member Countries are reviewed by WGPDMO for the 2006 meeting.

References

Ferguson, H.W. Kongtorp, R.T. Taksdal, T. Graham, D. and Falk, K. 2005. An outbreak of disease resembling heart and skeletal muscle inflammation in Scottish farmed salmon, *Salmo salar* L., with observations on myocardial regeneration. Journal of Fish Diseases 28: 119–123.

Kongtorp, R.T., Kjerstad, A., Taksdal, T., Guttvik, A. and Falk, K. 2004a. Heart and skeletal muscle inflammation in Atlantic salmon, *Salmo salar* L; a new infectious disease. Journal of Fish Diseases 27: 351–358.

Kongtorp, R.T., Taksdal, T., Lyngøy, A. 2004b. Pathology of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms, 59: 217–224.

8 Compile information on the distribution, causes and significance of the summer mortality in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species

A working document was summarized by T. Renault (Annex 6).

The first part of the document, prepared by S. Ford, provided general information on Summer Mortality syndrome, including information on the bivalve species affected and the geographical distribution. The first description of the Summer Mortality syndrome concerned the Pacific oyster (*C. gigas)* in Japan in the 1940s. The syndrome was, and continues to be, associated with high mortality of Pacific oysters and other bivalves around the world (Japan, USA, Canada, China and France). The causes remain unclear, but a multifactorial aetiology is suspected.

The second part of the document, prepared by T. Renault and J. F. Samain, summarised results of the multidisciplinary MOREST Programme on Pacific oyster Summer Mortality in France. The project focuses on the interactions among environment, oysters and pathogens, and consists of a research network involving genetics, physiology, immunology, pathology, ecotoxicology and ecology. Researchers in each discipline have analysed the same biological material, and have coupled lab and field studies. This approach identified and assigned relative importance to the factors involved.

J. F. Samain (MOREST project director, IFREMER Brest, France) then provided an overview of MOREST programme results:

- A temperature of 19 °C or more is the primary necessary condition, but alone is not sufficient to produce mortalities.
- A genetic component evidenced by divergent selection in two generations was confirmed. Resistant oysters produce fewer gametes and spawn more completely than susceptible ones regardless of food supply.
- High nutrient levels favour reproduction over other metabolic needs and may lead to energetic imbalance.
- Triploids, which have greatly reduced gametogenesis, suffered the lowest mortalities and also demonstrated higher potential defence capacities than diploids.
- A stressor, such as a simple transfer of oysters, was necessary to induce mortality even when temperature and reproduction were favourable. Proximity to the sediment consistently exacerbated the mortalities.
- Herpesvirus (OsHV-1) was mostly detected in juvenile mortality events and when the temperature was high. Different species and strains of Vibrios (including *V. splendidus* and *V. estuarianus*) were also isolated from moribund oysters.

Conclusions

1) Several bivalve species are affected by the Summer Mortality syndrome in different countries. However, most research programmes are focused on the Pacific oyster (*Crassostrea gigas*) because of its worldwide commercial importance.

2) The collective evidence suggests that Summer Mortality involves a suite of intrinsic and extrinsic factors. The most important extrinsic factor seems to be elevated temperature coming at a time when the intrinsic factors gametogenesis and spawning place the animal in a relatively unstable physiological condition. Any other external factor that exacerbates this instability, including high food availability, physical stressors or pathogens, may push the animals over a threshold from which they cannot recover.

Recommendations

WGPDMO recommends that:

i) investigations continue to study the intrinsic and extrinsic factors associated with Summer Mortality in bivalves, and their interactions, in order to better define their roles in the phenomenon;

ii) coordinated studies be done by ICES Member Countries affected by Summer Mortalities in Pacific oyster (*Crassostrea gigas*) and be extended to other species.

9 Update and assess the current information on the effects of contaminants on the immune system in fish and shellfish

K. Broeg presented a review of the recent literature in the field of immunotoxicological studies (laboratory exposure experiments and field studies) in fish (Annex 7).

The main focus was placed on the innate immune system due to its important role as the first line of defence against pathogens and its evolutionary conservation. In summary, the following aspects were extracted from the reviewed studies:

- Nearly all known ecotoxicants have either stimulating or suppressing effects on innate immunity of fish. All factors of innate immunity might be affected: external factors, humoral internal factors, and cellular internal factors.
- Results obtained were dependent on toxicant doses, exposure time, toxicity of mixtures, methods used, cell type used, origin of the cells, species, gender, temperature, and salinity changes.
- Acute responses to sublethal contaminant concentrations often initiate general stress effects reflected by triggered immune activity, whereas chronic responses might be coupled with cytotoxic effects reflected by immunosuppression.
- Examples were given for effects of immunomodulation on protective actions. Several studies also indicated a potential interaction between reproduction, biotransformation, and immune response in fish.

A review prepared by T. Renault, M. Auffret and B. Gagnaire on recent studies on shellfish is included in Annex 7.

M. Auffret presented the results of some studies conducted on immunosuppressive effects of contaminants in shellfish. He reviewed studies on the potential risk for increased susceptibility to pathogens due to contaminant effects. Alterations of haemocyte numbers and viability and functional modulations (phagocytic capacity/ respiratory burst/ membrane potential) were applied in concert and have been shown to be reliable indicators for toxicity-induced immunomodulation in shellfish in both exposure experiments and field studies. The combined measurement is useful in order to account for the fact that immune parameters may compensate the decrease of others. Thus, the overall resistance may not be compromised by the impairment of a single immune parameter. For future perspectives, studies on host resistance to pathogens, and the derivation of shellfish disease models were suggested.

In the discussion it was emphasised that techniques measuring effects of contaminants on the immune systems of fish and shellfish are a promising tool in ecotoxicology. However, more research and validation is needed before they can be recommended for regular monitoring activities.

Conclusions

1) Studies demonstrate an effect of various pollutants on the innate immune system in fish and shellfish.

2) The relationship between immunomodulation induced by pollutants and higher susceptibility to infectious diseases is rarely demonstrated.

3) Although techniques measuring effects of contaminants on the immune systems of fish and shellfish are considered promising tools in ecotoxicology, more research and validation is needed before they can be recommended for regular monitoring activities.

Recommendations

WGPDMO recommends that:

i) methods in use to explore the innate immune system in fish and shellfish should be harmonised where appropriate;

ii) a combined approach including methods (functional and structural) on different factors of innate and acquired immunity should be used to obtain broader information about immunological disorders;

iii) information about the effects of immunomodulation caused by contaminants on disease susceptibility in fish and shellfish should be compiled.

10 Produce an update of current information on sea lice interactions between wild and farmed fish and examine progress made in related management control methods in ICES Member Countries

A summary of recent research and analyses of sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) data from Norway, Scotland and field data on Pacific salmonids from western Canada were presented by B. Hjeltnes, D. Bruno and S. Jones. It is generally accepted that assessing the impact of sea lice on wild salmon populations is a difficult task due to the many factors influencing wild fish stocks. Despite this, there are several points that can be made based on the Norwegian data regarding the interaction of sea lice between farmed and wild stocks.

There are sufficient data indicating that the infective burden of sea lice in fjords will impact Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*), but it is unclear to what extent. It is known that *Lepeophtheirus salmonis* is pathogenic to both farmed and wild Atlantic salmon and sea trout and it is generally believed that sea lice are hazardous to wild salmon populations. Wild Atlantic salmon stocks have fluctuated widely in Norway due to several contributing factors, including acid rain, hydroelectric dams, introduction of *Gyrodactylus salaris*, possible variations in oceanic feeding areas, sharply contrasting river and oceanic temperatures, and fishing pressure. Therefore, evaluating the contribution of sea lice to these population fluctuations is difficult.

However, studies aimed at determining the influence of sea lice on salmon populations have been made. Recent studies in Norway using Carlin-tagged salmon and sea trout fed SLICE-medicated feed showed higher returns of medicated fish than un-medicated fish. Further, Norwegian studies indicate that infection levels appear increased in fjords with intensive salmon farming activities. However, there also are conflicting data showing that no lice were found on smolts migrating through the intensively farmed Altefjord in November. These contrasting results may be related to temperature and the timing of smolt migrations in various parts of Norway.

National programmes have been developed in Ireland, Scotland and Norway to reduce sea lice numbers overall and to reduce the infectivity burden on both wild and farmed salmon. In Scotland, a treatment strategy for sea lice was adopted as a Code of Practice (COP) in 1998, promoting the concept of strategically timed co-ordinated lice treatment in late winter. This has been shown to have a subsequent beneficial effect in management areas. This is being revised and is to be an integral part of the new industry COP.

In Norway measures implemented appear to be effective, as evidenced by a decrease in the average number of lice per wild salmon. However, this decrease was not observed on sea trout. Although the mean level of infection on farms has decreased, the total number of infective sea lice may not have been reduced due to increased production overall. Increased catches of wild salmon are promising and add further support to the view that the treatment pro-

grammes may be having a positive effect. Reducing the number of escaped salmon may also aid in reducing the number of lice presented to outmigrating smolts since escapees tend to remain in fjords and, therefore, are a localised source of sea lice in the river system.

Research efforts to develop sea lice vaccines are underway in Norway and Canada.

Studies on sea lice in the Broughton Archipelago in western Canada were conducted after high sea lice numbers on juvenile pink salmon (*Oncorhynchus gorbuscha*) were associated with a sharp decline in returning pink salmon in 2002. Both pink and chum (*O. keta*) salmon were examined in 2003 and 2004. The results showed that *Caligus clemensi* dominated over *L. salmonis* in 2003 but the opposite was true in 2004, and in 2004 the sea lice infestations were 10-fold higher than the previous year. Copepodid and chalimus stages accounted for 82% of all life stages found on both fish species, although different patterns of *L. salmonis* stages were noted over time from different sampling areas. Ongoing and future studies will examine the significance of wild chinook salmon populations that inhabit in this region in winter as sources of sea lice.

Conclusions

1) Due to multiple factors influencing wild fish populations, no firm conclusions can be drawn regarding sea lice interactions between farmed and wild fish and the effect on wild salmon.

2) Enough evidence of effects of sea lice on wild populations is available to warrant further development and implementation of measures to reduce the risks to wild fish.

3) Equally important is reducing the risk of escapes from farms as escapees tend to stay in fjords and can provide a supply of sea lice to outmigrating smolts.

Recommendation

The WGPDMO recommends that:

i) relevant ICES Member Countries provide an update of developments on the sea lice vaccine and management measures for sea lice control at the next WGPDMO meeting.

11 Provide guidance on the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints

W. Wosniok presented a summary of a working document, providing a review of indices in use in the field of biological effects studies (Annex 8).

The purpose of a "health index" in the present context is to summarise information on the health status of marine organisms. While the original information on health status is expressed by several (many) quantities, an index is expected to represent the most relevant information by one (or at least few) number(s) or category(ies). Such an index should facilitate the interpretation of measurements as well as communication about health status based on a broad range of information. Monitoring results could be presented in a concise way via such an index. An index could also be the target quantity on the basis of which spatial comparisons and trend assessments could be performed.

Index components must exhibit a monotone (only up or only down, not variable) relationship between exposure and response and this relationship must be biologically plausible (to prevent

coincidental correlations from contributing to the index). The set of components contributing to the index should provide a proper summary of the measurements, which the index is to represent. This issue must clearly be defined to allow a sensible index construction. Finally, the index definition should be so detailed that subjective decisions about its calculation are avoided.

There is a wealth of possibilities to calculate a summarising index. The simplest and most frequently used way is to calculate a weighted sum of the original components, where necessary, after standardising components to non-negative values with a common dispersion and orientation for all components. However, arguments to define the weights are subject for discussion. In the six examples discussed in some detail in the working document, weights are set on the basis of various arguments, ranging from "set all weights to 1, because other choices are difficult to justify" to explicitly given variable weights which were felt optimal by the authors. In other cases the weights are obtained by rescaling the data in terms of a "normal range" or a background value.

The Integrated Biomarker Response (IBR) uses a different approach by arranging standardised index components in a star plot and using the star plot area as the IBR index value. This index is not a weighted sum of component values with fixed weights; instead the index is a sum of products of the component pairs in the star. This makes the IBR value depend on the order of the radii in the star, which is rarely naturally given, but instead depends on the more or less random preference of the user and thus introduces a high degree of subjectivity and randomness in this index. Another undesirable feature is that a component contributes only in cooperation with its neighbours in the star, which means that if these neighbours are equal or close to zero, then the middle component will contribute little or nothing to the IBR, however large its values. Summarising, the IBR might only be useful under few circumstances.

Besides defining an index as a sum of weighted component values by fixing weights on the basis of external consideration, another way is to derive these weights formally such that the resulting index carries as much as possible of the information in the set of the original components. Principal component analysis (PCA) is a standard technique for this purpose and other multivariate techniques may similarly be applied if the purpose of the index is, for instance to highlight the differences between groups in the data. The use of these methods obviously provides an elegant solution to the question of how to derive weights for a given data situation. However, the optimality holds only for the data set under study, which means that new data would require new optimal weights. On the other hand, if a summarising quantity for only a certain data set is required, PCA and related methods are reasonable instruments to generate an index.

In summary, none of the investigated indices described the disease-related parameters of interest for the WGPDMO appropriately. As in other cases, if an index for fish disease and/or parasite prevalence is to be constructed, then it must be specific for this particular problem. The construction, however, faces the same problem as in the situations discussed in the working document, namely that no *a priori* choice for a weighting or a construction principle exists. Therefore, to derive an index that quantifies the proportion of diseased fish, a stepwise procedure seems appropriate, starting with a simple index defined as the mean of the relevant disease prevalences, checking the amount of information that is lost in this way and then to revise the initial definition, as necessary.

In the discussion of this simple approach, several issues were raised:

- The prevalences that enter the index should be standardised for confounding factors such as age/size and possibly others.
- The decision on which factors should be considered for standardisation should be checked beforehand by appropriate statistical tests.

- The index might be improved by including intensity data in addition to prevalence. However, the ICES database presently does not provide intensity data, but could be made to do so in future. Those data are presently available only in national databases.
- The resulting index should better be termed a 'disease index', as effectively the disease (not the health) status is summarised.

Conclusions

1) None of the 'health indices' reviewed describe the disease-related parameters of interest for the WGPDMO appropriately, warranting the development of a disease-specific index.

2) A pilot study on the construction of a disease index for the prevalence of the main fish diseases could be initiated using German data which incorporates disease intensity and parasite prevalence information from studies in common dab (*Limanda limanda*) in the North Sea. Such a study can be used to identify potential problems that may be important for future larger scale studies.

Recommendations

The WGPDMO recommends that:

i) a pilot study be carried out intersessionally by WGPDMO members to determine the feasibility of constructing a 'disease index', using dab (Limanda limanda) disease data obtained from the North Sea and that the WGPDMO should review the results of the study during its 2006 meeting.

12 Evaluate the availability of data for a risk assessment pilot study on population effects due to diseases in wild fish, using epidemiological methods and population dynamics modelling

W. Wosniok presented a summary of progress on the assessment of available data within the ICES Database for undertaking a risk assessment pilot study on population effects of diseases in wild fish. Principal factors enabling such a pilot study to be undertaken relate to which population data exists within ICES, and which supporting information is available (e.g. fishing activity, nutrient concentrations, and contaminant levels). Availability of these quantities determines whether the intended study is possible.

The International Bottom Trawl Survey (IBTS) and Beam Trawl Survey (BTS) data which include size of catch, age-length information and catch per unit effort (CPUE) are available in principle. In addition, nutrients and contaminants and hydrographic CTD data are freely available. Other data may be available from EU sources. However, data on fish reproduction are not available. Extraction of data from the ICES Database and an overview from the ICES website were done by selecting parameters, time period and area of interest and then using the returned number of existing records for the decision to actually send the request. Data requests can be made on-line for biota, but paper requests are needed for fishery data as the additional agreement of the national representative will be required, obviously an inconsistency in the ICES data policy. The time perspective of the data delivery by ICES depends on the type and amount of data (experience: 1 week for biota and related data, > 4 weeks for fishery data, which has so far not arrived.)

Information from the data inventory on the ICES website leads to the conclusion that a risk assessment pilot study should currently be possible for a restricted geographical area, particularly for the expanded German Bight, using data on disease status, nutrients, contaminants and environmental factors.

The dependent quantity in such a study would be stock size of dab (*Limanda limanda*) expressed by IBTS catches, possibly involving only a subgroup of all dab (e.g. females, 20–24 cm length). All other quantities including fish diseases would serve as potentially explaining/intervening factors. The final decision on the execution of the study can be made only on arrival of the IBTS data, as a sufficient density of dab data can be suspected from the inventory. This must, however, be confirmed by the real data.

The possibility to use commercial catch data was discussed. It was agreed that such data could not be used in first instance since lack of size distribution information would be a problem. However, this could be utilised if preliminary analysis indicates strong correlations.

The use of data on fish reproduction data (data on fish eggs/larvae) was discussed. It was felt that the available data might be of limited use, as this data was collected for other purposes, and, additionally, it is not present in the ICES database.

Population models for dab are not currently available and will need to be developed on the basis of available data. Before actually launching the study a power analysis should be undertaken in order to assess if any population effects of realistic size could be detected, given the available data.

Conclusions

1) It was agreed that a pilot study on population effects due to diseases in wild fish can be undertaken since there is sufficient data available for specific geographical regions such as the German Bight.. It was noted that prior to conducting the study a power analysis needs to be undertaken.

Recommendations

The WGPDMO recommends that:

i) a pilot study be undertaken intersessionally on population effects due to diseases in wild fish by WGPDMO members and that the results are presented at the 2006 meeting of the WGPDMO.

13 Assess the results of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) to resolve any outstanding issues and, together with WGMS, MCWG, and WGSAEM, finalise a draft set of guidelines for integrated monitoring for OSPAR [OSPAR 2005/6]

T. Lang presented a summary of the outcome of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON), held at ICES HQs in Copenhagen, Denmark, 10–13 January 2005. The workshop was co-chaired by K. Hylland (Norway) (ICES WGBEC Chair) and R. Law (UK) (ICES MCWG Chair). 22 participants attended the workshop; the WGPDMO was represented by four members (S.W. Feist, T. Lang, A.D. Vethaak, and W. Wosniok).

The WKIMON Terms of Reference given by ICES and OSPAR were as follows:

develop guidelines for integrated biological effects and chemical monitoring, including:

ii) specific guidelines for the integration of chemical and biological effects techniques with special emphasis on those parameters that have become mandatory in the OSPAR Coordinated Environmental Monitoring Programme;

iii) guidelines towards integrated chemical and biological effects monitoring for the entire range of issues in the OSPAR Joint Assessment and Monitoring Programme

The underlying objective of the workshop was to develop a methodology to evaluate the health of marine ecosystems in an integrated fashion, by defining guidelines for measurements of a suite of chemical and biological analyses. Monitoring in the past has been based mostly on chemical measurements, but biological effects techniques have become increasingly important over recent years. As such, they have become part of the OSPAR Joint Assessment and Monitoring Programme (JAMP) and, more recently, also of the OSPAR Coordinated Environmental Monitoring Programme (CEMP).

The OSPAR CEMP can be described as that part of monitoring within the OSPAR JAMP where the national contributions of countries that signed the OSPAR Convention overlap and are coordinated.

The major CEMP components are a) temporal trend monitoring and b) spatial monitoring, which are complementary. Three elements are essential for the realization of the CEMP. These are the existence of:

- Guidelines
- Quality assurance tools
- Assessment tools

The present status of the CEMP is based on the availability of these elements, and is provided in Annex 9.

The first part of the WKIMON workshop was dedicated to a more general discussion on the concept of integrated monitoring; the review of existing OSPAR monitoring guidelines and to presentations of participants on the extent to which integrated monitoring has already been implemented in national monitoring programmes. The second part was to develop guidelines for integrated monitoring and there was a general consensus that the guidelines should as far as possible be built upon the JAMP Guidelines already in place. The guidelines finally developed by WKIMON for integrated monitoring were annexed to the draft WKIMON Report (http://www.ices.dk/reports/ACME/2005/WKIMON05.pdf).

There was an agreement that fish disease monitoring should be part of the biological component of the integrated monitoring programme and that the existing JAMP Guidelines/Technical Annexes should form the methodological basis. However, by reviewing these it became apparent that some of them need to be revised because of conflicting contents and because partly new information has become available since the Guidelines/Technical Annexes were published.

Therefore, the WGPDMO was requested by WKIMON to deliver suggestions for amendments regarding the following issues:

- The Technical Annexes in the JAMP Guidelines for contaminant-specific and general biological effects monitoring should be reviewed and, if required, revised. This could be done by ICES WGBEC and, for disease aspects, by ICES WGPDMO.
- A review of the JAMP Guidelines for Contaminant-specific Biological Effects Monitoring showed that the Reporting Requirements in the Technical Annexes need to be harmonised with the ICES Database. This task should be carried out by relevant ICES Working Groups with the Data Centre taking the lead.
- Review available data for each biological effects method to clarify whether data can be compared across the range of recommended fish species: WGBEC, WGPDMO (the background for this request is that most species recommended

for biological effects monitoring are not available throughout the entire OSPAR area. A comparison between areas with different species is problematic because the species may show different diseases or, even if the diseases are identical, they may differ in disease susceptibility)

- Selection of species, gender and size ranges: WGBEC, WGPDMO.

Some of these issues will be addressed in the following sections.

Review of the OSPAR Guidelines for fish disease monitoring

The monitoring of fish diseases is included in two of the CEMP components (Ref. No. 2004_16), the PAH-specific biological effects monitoring (liver pathology) and the general biological effects monitoring (externally visible fish diseases, liver nodules, liver neoplasia/hyperplasia). In Table 2 of Annex 9, more details are provided.

The guidelines in place for fish disease monitoring (see Table 1) are included in the:

- JAMP Guidelines for Contaminant-specific Biological Effects Monitoring (Ref. No: 2003–10)
- JAMP Guidelines for General Biological Effects Monitoring (Ref. No. 1997–7)

and are based on information published in ICES publication series, including meeting reports (see Annex 9). In the following, some comments are made regarding the requirement to amend the CEMP Report and the JAMP guidelines.

Specific comments to the JAMP Guidelines for PAH-specific Biological Effects Monitoring (Ref. No.: 2003–10)

General recommendation: Monitoring of PAH-specific biological effects as part of the CEMP should include the monitoring of liver nodules (which are now only included in the CEMP General Biological Effects Monitoring component) because this technique detects the prevalence of macroscopically visible neoplastic liver lesions (liver tumours). Neoplastic liver lesions are one of the best documented biological effects of exposure to PAHs. In the monitoring, the quantification of liver nodules can easily be combined with the studies on liver histopathology (which are already part of the PAH-specific Biological Effects Monitoring component of the CEMP). More comments on liver nodules and liver histopathology follow below.

Page11, para 2: The guidelines state that liver histopathology is only recommended if the suite of biomarkers (CYP 1A, DNA adducts, PAH metabolites) demonstrate a PAH-related response. The WGPDMO does not agree to this. Liver histopathology should be incorporated in the suite of measures that are routinely applied because the simultaneous measurement of indicators of exposure and effect provide more comprehensive information.

Page 12, last para: The heading ‘Liver pathology’ should be changed to ‘Liver histopathology’ because histological techniques have to be applied. Furthermore, there is a need to update the categories of histopathological liver lesions to include the four categories as published in the ICES guidelines (Feist *et al.* 2004, see below) plus a ‘new’ category, the non-specific lesions (see Table 13.1), reflecting the BEQUALM categories.

Table 13.1: BEQUALM categories of histopathological liver lesions in fish that should be used for the CEMP PAH-specific Biological Effects Monitoring.

Histopathology Categories	Histopathological Lesions
Non-specific lesions	Coagulative necrosis Apoptosis Lipoidosis Haemosiderosis Variable glycogen content Increased numbers and size of macrophage aggregates Lymphocytic/monocytic infiltration Granuloma Fibrosis Regeneration
Early toxicopathic non-neoplastic lesions	Phospholipidosis Fibrillar inclusion Hepatocellular and nuclear polymorphism Hydropic degeneration Spongiosis hepatis
Foci of cellular alteration	Clear cell foci Vacuolated foci Eosinophilic foci Basophilic foci Mixed cell foci
Benign neoplasms	Hepatocellular adenoma Cholangioma Haemangioma Pancreatic acinar cell adenoma
Malignant neoplasms	Hepatocellular carcinoma Cholangiocarcinoma Pancreatic acinar cell carcinoma Mixed hepatobiliary carcinoma Haemangiosarcoma Haemangiopericytic sarcoma

Page 13, para 3 under '5. Field sampling and sampling equipment': For consistency reasons, the size ranges for dab should be 20–24 cm (instead of 20–25 cm) and for flounder 25–29 cm (instead of 15–35 cm) because this is in accordance with the monitoring of externally visible diseases. It is recommended to collect a minimum of 50 female fish per sample. The size measurement should be total length instead of 'total fork length' and should be in cm below instead of mm.

Page 16, bottom: The list with histopathological liver lesions needs to be updated according to Table 13.1 above.

Page 18 under 'Quality Assurance': This information is outdated since QA is achieved through BEQUALM.

Page 19 under '8. Reporting requirements': Information on liver histopathology is missing. Reporting requirements for liver histopathology are specified in the ICES Environmental Data Reporting Format for fish disease monitoring. Requirements are:

- Species
- Gender
- Size (cm below)
- Age (optional)
- Weight (optional)
- Presence of externally visible diseases (as specified in the OSPAR JAMP Guidelines for General Biological Effects Monitoring)

- Presence of macroscopically visible liver nodules > 2 mm in diameter (only confirmed cases of neoplastic lesions) (benign or malignant tumours, see Table 13.1) (see comments on the terminology made below)
- Data on other PAH-specific biomarkers

Page 21 under '9. References': Information needs to be updated, e.g. with 'Feist, S.W.; Lang, T.; Stentiford, G.D., Köhler, A. 2004. Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES TIMES No. 38, 42 pp.'

Specific comments to the JAMP Guidelines for General Biological Effects Monitoring (Ref. No. 1997-7)

The term 'liver pathology' should be changed to 'liver histopathology' and the term 'liver nodules' to 'macroscopic liver neoplasms' throughout the text.

Page 10, Technical Annex 7 on liver histopathology: The term 'liver neoplasia/hyperplasia' is incorrect and should be changed to 'Liver histopathology'. The list of effects (= histopathological lesions) given in the Technical Annex should be made identical with the list for PAH-specific biological effects monitoring in Table 13.1 (see above). QA/QC will be accomplished through BEQUALM. The reference is outdated and should be replaced by 'Feist, S.W.; Lang, T.; Stentiford, G.D., Köhler, A. 2004. Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES TIMES No. 38, 42 pp.'

Page 11, Technical Annex 8 on liver nodules: Since only histologically confirmed cases of liver neoplasms are to be recorded, the term 'liver nodules' could be changed to 'macroscopic liver neoplasms'. Another important point WGPDMO emphasised is that a sample of 50 dab of the size group ≥ 25 cm is hard to achieve owing to the fact that it is almost impossible at present to catch enough specimens of this size at most sampling sites in the North Sea. WGPDMO therefore strongly recommends that 50 specimens of the size group 20–24 cm should be examined in addition to the large specimens.

Page 11, Technical Annex 9 on externally visible fish diseases: The term 'Skin ulcerations' should be changed to 'Acute/healing skin ulcerations'.

WGPDMO is of the opinion that the list of 'effects measured' (= diseases) in dab and flounder should be extended, however, on a voluntary basis, e.g. by including hyperpigmentation, fin rot/erosion, *Stephanostomum baccatum*, *Acanthochondria cornuta*, *Lepeophtheirus pectoralis*.

WGPDMO further recommends adding whiting (*Merlangius merlangus*) to the list of species to be examined for externally visible diseases. This is mainly because of the disastrous situation of the cod stock in certain regions of the OSPAR area, e.g. the North Sea. Common externally visible diseases/parasites of whiting for monitoring purposes could be epidermal hyperplasia, *Lernaeocera branchialis, Diclidophora merlangi* and *Clavella adunca.* However, WGPDMO recommend conducting the monitoring of whiting on a voluntary basis until a conclusion can be drawn on its suitability.

Harmonisation of reporting requirements specified in the ospar guidelines with the requirements of the ICES Database/Data Reporting Formats

WGPDMO expressed its view that this task should be tackled once the OSPAR Guidelines for Integrated Monitoring are in a more advanced stage.

Comparison of disease data across the range of recommended fish species

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), and
- 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.

The only possibility to overcome this problem would be to use relative health/disease indices/indicators, e.g. deviation from reference prevalences or from long-term trends etc. However, such tools have so far not been developed. This issue is discussed in more detail in section 11 of the present report and the development of health/disease indices will be a task for the 2006 WGPDMO meeting.

Selection of species, gender and size ranges

This issue is partly already covered above and the suggestions made are summarised in Table 13.2 below:

Table 13.2: WGPDMO's suggestions for selection of species, gender, size ranges and sample sizes to be used for fish disease monitoring in the OSPAR area

Disease	Species	Gender	Size range (cm total length)	Sample size
Externally visible diseases	Dab (*L. limanda*)	Females + Males	15–19 20–24 ≥ 25	100 100 50
	Flounder (*P. flesus*)	Females + Males	20–24 25–29 ≥ 30	100 100 50
	Cod (*G. morhua*) *	Females + Males	< 29 30–44 ≥ 45	100 100 50
	Whiting (*M. merlangus*)	Females + Males	15–19 20–29 ≥ 30	100 100 50
Macroscopic liver neoplasms > 2 mm	Dab (*L. limanda*)	Females+ Males	20–24 cm ≥ 25	50 50
	Flounder (*P. flesus*)	Females+ Males	25–29 ≥ 30	50 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

*** Because of the disastrous situation of the cod stock in some OSPAR regions (e.g. the North Sea), cod may be replaced by whiting.**

**** Since dab and flounder are not available in all OSPAR regions, the usefulness of dragonet should be tested through a baseline study on liver histopathology of this species.**

Conclusions

1) The OSPAR JAMP Guidelines/Technical Annexes for General and PAH-specific Biological Effects Monitoring have to be amended with regard to the monitoring of externally visible fish diseases, liver nodules, liver pathology and liver neoplasia/hyperplasia.

Recommendations

The WGPDMO recommends that:

i) OSPAR takes note of the changes recommended by WGPDMO to the OSPAR JAMP Guidelines/Technical Annexes for General and PAH-specific Biological Effects Monitoring with regard to the monitoring of externally visible fish diseases, liver nodules, liver pathology and liver neoplasia/hyperplasia.

14 To prepare data on the prevalence and type of fish diseases by ICES rectangle for the period 1984 to 2004 where available. The data should be submitted to the secure REGNS website in preparation for the REGNS Integrated Assessment Workshop from 9–11 May 2005

T. Lang gave a short introduction to the plans to conduct an ICES Integrated Assessment of the North Sea Ecosystem coordinated by the ICES Regional Ecosystem Study Group for the North Sea (REGNS) and informed WGPDMO about the REGNS Workshop to be held 9–11 May 2005 at ICES Headquarters, Copenhagen, Denmark. He reminded the WGPDMO that this issue had already been dealt with at the 2004 WGPDMO meeting, where a suggestion had been made to include data on diseases of North Sea dab (*Limanda limanda*) held at the ICES Environmental Database in the assessment (ICES CM 2004/F:01).

For the 2005 WGPDMO meeting, REGNS requested (as from a number of other ICES WGs) the following actions:

- to identify the data sets the WGs feel will be of most value to an integrated assessment of the North Sea;
- to provide a data summary as maps showing location of sampling (spatial extent);
- to provide tables and relevant reports which provide additional detail on the data, or summaries of the data would also be useful for the workshop.

WGPDMO discussed the issue and, once more, emphasised the usefulness of the ICES disease data for the integrated assessment. It was highlighted that WGPDMO has previously carried out a number of such integrated assessments, combining ICES fish disease data with other ICES data (contaminants, nutrients, oceanography, fishery) in order to elucidate possible cause-effect relationships with regard to spatial and temporal patterns in disease prevalence (see Annex 10).

In order to use the most complete and consistent fish disease data set for the REGNS Integrated Assessment, the WGPDMO recommends to only use the data specified in Table 14.3, which will be readily available from the ICES Environmental Database for the REGNS Integrated Assessment Workshop, 9–11 May 2005.

Table 14.1: Fish disease data to be used in the ICES Integrated Assessment of the North Sea Ecosystem.

Region:	North Sea
Time period:	1984–2004
Species:	common dab (*Limanda limanda*)
Diseases:	Lymphocystis Epidermal Hyperplasia/Papilloma Acute/healing skin ulcerations X-cell gill disease
Data sets:	Denmark, Germany, The Netherlands, UK combined

More details on fish disease data sets considered being of particular suitability for the Integrated Assessment, a map and a table providing details on the spatial and temporal data coverage in the period 1981 to 2004 and some additional background information is provided in Annex 10.

The WGPDMO nominated Werner Wosniok (Germany) as key representative for the REGNS Workshop, since he has got the most experience in the statistical analysis and assessment of the ICES fish disease data. It is expected that he will attend the workshop. Other WGPDMO members involved (likely without attending) are Thomas Lang (Germany) (present WGPDMO Chair) and Steven Feist (UK).

Conclusions

1) Data on externally visible diseases of the common dab (*Limanda limanda*) held in the ICES Environmental Database should be included in the ICES Integrated Assessment of the North Sea Ecosystem to be coordinated by ICES REGNS.

2) Since the fish disease data are available in the ICES Database, WGPDMO sees no need to submit the data to the secure REGNS website as requested in the WGPDMO Term of Reference.

3) W. Wosniok (Germany) will represent WGPDMO at the ICES REGNS Workshop to be held 9–11 May 2005 at ICES HQ, Copenhagen, Denmark.

Recommendations

The WGPDMO recommends that:

i) data on the prevalence of externally visible diseases of the common dab (*Limanda limanda*) available from the ICES Environmental Database be included in the ICES Integrated Assessment of the North Sea Ecosystem coordinated by ICES REGNS;

ii) the WGPDMO reviews progress made in relation to the ICES Integrated Assessment of the North Sea Ecosystem at its 2006 meeting.

15 Produce updated ICES publications on pathology and diseases of marine organisms

15.1 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. Limited progress was reported as much of the data has yet to be received. (Details concerning data submission are described in report section 14, Annex 10.) Submitted data are used for mapping species and disease 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 detecting requires a minimum of 5-years of data. Information on fish species, diseases and on the statistical modelling employed will be added to the website (http://www.ices.dk/marineworld/fishdiseases/fishandshellfish.asp). Maps showing the distributions of diseases of finfish and shellfish are presently being checked for accuracy by identified WGPDMO members.

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

S. W. Feist, the editor, updated members on the leaflets. Since receiving files from the previous editor in August and a set of existing leaflets from ICES, the majority of the existing leaflets have been reviewed. A few need significant revision, but many need updating of diagnostic methods, addresses and in some cases authorship. Two (on nematodes) are currently being revised by R. Wootten and two new manuscripts have been received – Brown ring disease (C. Paillard) and Nodavirus (D. Bruno and M. Vigneulle). The editor will coordinate with the ICES publication representative, B. Anthony and the ICES Secretariat regarding progress made in the scanning of existing fiches and will notify authors of revisions. The WGPDMO expressed support of the need for electronic availability of fiches and the preference for colour images. Images are generally not available electronically and will require new images to be obtained. Scanning of text may be employed although image quality and ownership of existing images will have to be established. Another possibility is that files/images are saved in PDF-format and made available through the web-site. S. Ford will replace M. Lyons Alcantara on the publications subgroup.

15.3 WGPDMO website

T. Lang reviewed the status of the WGPDMO website (http://www.bfa-fish.de/WGPDMO/wgpdmo.html) His institution, The Federal Research Centre for Fisheries, Germany, has agreed to host the website which is presently under construction. It will include pull-down menus for general information, documents, members and useful links. Links will be available to WGPDMO information on the ICES website, including disease distribution maps and annual reports. WGPDMO member information, such as that in Annex 1 of the annual reports is proposed, with possible addition of areas of expertise, photos, and links to members' individual web-pages at their home institutions. T. Lang is in contact with members of a subgroup (S. Feist, W. Wosniok, L. Madsen and T. Wiklund) established last year to advise on site construction.

16 Any other business

16.1 Baltic Sea Regional Project (BSRP)

G. Rodjuk gave a report on the Baltic Sea Regional Project (http://www.ices.dk/projects/balticsea.asp). The project started in February 2004 and expected to continue for five years. The project is funded through the World Bank as part of the Global Environment Facility Programme (GEF). The first phase of the program will focus on Coordination. The main aim of the project is to strengthen institutional and technical capacity among countries bordering the Baltic Sea by improving and standardising national and regional cooperation and coordination for both coastal and open sea activities.

Aims of the project are:

- to develop a practical integration of institutes,
- to harmonise sampling techniques,
- to rationalize assessment and reporting
- to achieve a general upgrading and use of equipment and laboratories in a cost effective and quality-assured manner

The following BSRP Coordination Centres are be involved:

- CC Fish and Fisheries in Riga with 4 Leading Laboratories (LL);

- CC Ecosystem Health in Gdynia (Sea Fisheries Institute) with 2 LL and a 3rd recently established for Biodiversity studies. This CC will coordinate the work of the Lead Laboratory for histopathology, parasitology and fish diseases in Kaliningrad, Russia;
- CC Productivity in Riga with 2 LL and a newly established Co-Local Project Manager (LPM) position for management of Ships of Opportunity (SOOP) activities within BSRP;
- CC GIS/Data in Vilnius with a newly proposed co-LPM position for management of Multiple Marine Ecological Disturbances (MMED) component within BSRP;
- CC Socio-economy in Tallinn with newly proposed Co-LPM position for management of Integrated Coastal Zone Management (ICZM) activities within C1 of BSRP.

Two initial meetings were held in Riga and Vilnius to establish a list of experts, a working plan and a list of equipment.

BSRP Fish Disease Monitoring

A component of the BSRP focuses on diseases, parasites and liver histopathology. The identified experts for fish disease monitoring are from the eastern countries are M. Podolska (Poland), G. Rodjuk (Lead laboratory, Russia), E. Bacevicius (Lithuania), M. Kirjusina (Latvia) and K. Lotman (Estonia).

According to a tentative workplan, the following species were identified for inclusion in the off-shore monitoring program: cod (*Gadus morhua*), flounder (*Platichthys flesus*), herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). Species to be monitored during coastal surveillance include bream (*Abramis brama*), roach (*Rutilus rutilus*), eel (*Anguilla anguilla*) and flounder.

The idea is that the Baltic Sea fish disease monitoring should be part of an integrated monitoring programme, encompassing e.g. studies on coastal and offshore fish stocks and biodiversity, biological effects of contaminants (biomarker approach), on biodiversity and physical and chemical measurements. The fish disease monitoring programme should be coordinated by the WGPDMO and should ultimately be incorporated in the revised HELCOM monitoring programme. It was proposed that the ICES Marine Data Centre should act as central database for the Baltic Sea Project.

Plans for an ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea

Since there is an apparent need to further intercalibrate methodologies to be used for fish disease monitoring in the Baltic Sea, it was suggested to hold a sea-going practical workshop under the auspices of ICES/BSRP with specialists in this field and with trainees from the eastern countries. On invitation by T. Lang (Germany), the workshop is scheduled for 10–14 days in December 2005 on board the German RV 'Walther Herwig III'. It will be organised and co-convened by T. Lang (Fed. Res. Centre for Fisheries, Inst. of Fishery Ecology, Cuxhaven, Germany) and G. Rodjuk (BSRP LL for fish diseases, parasites, histopathology, AtlantNIRO, Kaliningrad, Russia). The ICES Study Group on Baltic Ecosystem Health Issues in support of BSRP (SGEH) and the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) will be involved in the programme planning. Links to the HELCOM expert network for coastal fish monitoring will be sought.

The major objectives of the workshop are to:

- provide training and intercalibration related to methodologies applied in fish disease monitoring in the Baltic Sea,

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

According to the present planning, the workshop will start in Kiel, Germany, and will end in a port in the eastern Baltic Sea yet to be decided.

12 scientists will participate, including training experts (on methodologies for fish disease surveys in the Baltic Sea, externally visible diseases/parasites, liver histopathology, data assessments, quality assurance) and trainees from Baltic Sea countries, with priority given to eastern BSRP countries.

The major target fish species will be flounder (*Platichthys flesus*), herring (*Clupea harengus*), sprat (*Sprattus sprattus*) and cod (*Gadus morhua*). These species will be sampled on a transect with selected sites representing different environmental conditions. If appropriate, samples can be taken for subsequent lab-based measurements, e.g. on biomarker responses (e.g. as part of the planned BSRP BIODEMO Project on Biological Effects of Contaminants).

The plans for the workshop will be presented and reviewed at the next ICES SGEH meeting in November 2005 in Kaliningrad, Russia, at the Meeting of the ICES Advisory Committee on the Marine Environment (ACME) in Copenhagen, June 2005, as well as at the ICES ASC/Statutory Meeting in September 2005, Aberdeen, UK.

Cost implications for BSRP: funding (travel and per diem) will be required for scientists from the eastern recipient countries. Funding will also be needed for a representative from the BEQUALM lead laboratory on fish diseases and liver histopathology at CEFAS, Weymouth, UK, whose participation is essential in order to guarantee compliance with the BEQUALM quality assurance activities. Ship time, accommodation and food on board, the use of equipment as well as time allocation by western experts constitute a significant in-kind contribution by western countries.

Conclusions

1) The WGPDMO appreciated the progress made in the development of the fish disease component of the Baltic Sea Regional Project (BSRP).

2) In particular, it endorsed the plan to hold a sea-going workshop on fish disease monitoring in the Baltic Sea in December 2005.

3) BSRP activities related to fish disease monitoring should be carried out in close collaboration with the WGPDMO.

Recommendations

The WGPDMO recommends that:

i) an ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea co-convened by T. Lang (Germany) and G. Rodjuk (Russia) shall be held in December 2005 on board the German RV 'Walther Herwig III'. Its objectives are to

- provide training and intercalibration related to methodologies applied in fish disease monitoring in the Baltic Sea,
- further develop and assess health indicators and indices appropriate for monitoring and assessment purposes,
- establish a closer collaboration between institutes involved in fish disease monitoring in the Baltic Sea,

- build the basis for incorporation of fish disease surveys into the revised HELCOM monitoring programme.

ii) future progress made in relation to the Baltic Sea Regional project (BSRP) be reviewed by WGPDMO at its 2006 meeting.

16.2 Permanent Advisory Network for Diseases in Aquaculture (PANDA)

I. Arzul and S. W. Feist provided a description of the EU-funded project 'Permanent Advisory Network for Diseases in Aquaculture' (PANDA) to the WGPDMO. It was explained that the rationale of the network is to strengthen expertise on infectious diseases of aquaculture species and to provide scientific advice to EU policy.

It was agreed that the activities of the WGPDMO are complementary to the aims of PANDA and members were encouraged to register with the network (www.europanda.net) as an appropriate forum for discussions on a variety of fish and shellfish health issues.

It was suggested that there could be a link from the PANDA website to the ICES website to facilitate access to the reports of the ICES WGPDMO and publications on disease issues. For example ICES Cooperative Research Report No. 265, 'Trends in important diseases affecting the culture of fish and molluscs in the ICES area 1998–2002' and the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish which are scheduled to be available on the ICES website.

The WGPDMO appreciated the offer from the PANDA Steering Group for a representative to participate in the forthcoming PANDA consortium meeting to be held at Hydra, Greece, in May 2005, to present the activities of the ICES WGPDMO and to discuss ways to further improve interaction between the two groups. The WGPDMO nominated S. W. Feist to represent the WGPDMO at that meeting.

16.3 Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)

S. W. Feist described the Biological Effects Quality Assurance in Monitoring Program (www.bequalm.org) project which was initiated in 1998 as an EU-funded programme. The aim was to develop appropriate quality standards for a wide range of biological effects techniques and to devise a method for monitoring compliance of laboratories generating data from these techniques for national and international monitoring programmes. Ultimately the goal was for BEQUALM to be self-financing based on fee recovery. The self-funded scheme comprises three components (biomarkers, whole organism and community analysis) and seeks to establish a QA programme including training workshops and intercalibration exercises.

Intercalibration exercises and training were organised around water and sediment bioassays, metallothionein measurements, ALA-D activity, DNA adduct measurements, P4501A activity, imposex/intersex measurement, lysosomal stability, liver histopathology, external disease measurements, chlorophyll a and phytoplankton assemblage analysis and benthic community analysis.

The WGPDMO noted the progress made regarding fish disease and liver histopathology components of BEQUALM The WGPDMO emphasised the anticipated future requirement of participation in BEQUALM, e.g. in order to ensure intercalibration of methodologies and diagnostic criteria used for generating data submitted for assessments. It is expected that this will become particularly important to meet the requirements within the OSPAR and HELCOM monitoring programmes.

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 Program (BEQUALM) project in order to achieve quality assurance regarding methodologies applied and data generated.

16.4 Disease Interaction and Pathogen Exchange Network (DIPNET)

A presentation by L. Miossec from IFREMER, La Tremblade, France was given to inform the group about the programme (www.dipnet.info/). She explained that DIPNET is a European Coordination Action (CA). It started in October 2004 and was funded for 24 months. Its objectives are:

- To integrate and strengthen current scientific knowledge on the potential transfer of pathogens and diseases between wild and cultured aquatic animal populations.
- To give support to the development of European policies protecting the health of aquatic animal populations while allowing responsible use of the aquatic environment for aquaculture purposes.
- To disseminate the current knowledge towards stakeholders and the wider European public.

The following institutes are represented in the project consortium.

- IFREMER (French Research Institute for Exploitation of the Sea, France) coordinator of the project: L. Miossec
- VESO (Centre for Veterinary Contract and Commercial Services - Norway): P. Midtlyng and A. H. Garseth
- FRS (Fisheries Research Services - UK): R. Raynard
- CEFAS (Centre for Environment, Fisheries and Aquaculture Science - UK): E. Peeler
- UZ (University of Zaragoza - Spain): I. de Blas

Five work packages have been established:

- WP1: Literature review of disease interactions and pathogen exchange, R. Raynard
- WP2: Risk assessment and modelling of pathogen exchange, E. Peeler
- WP3: Epidemiology of infectious diseases in wild fish and shellfish, I. de Blas
- WP4: Network building and knowledge dissemination, P. Midtlyng and A. H. Garseth
- WP5: Project management P. Midtlyng and L. Miossec

In the discussion it was emphasised that, as with PANDA (see section 16.3), the DIPNET website should provide links to the WGPDMO activities.

16.5 Improvement of working procedures and profile of the WGPDMO

The WGPDMO again emphasised the importance to start working on the Terms of Reference well in time prior to the annual meetings. Intersessionally produced working documents should be sent to the Chair one month before the meeting at the latest. The Chair will then distribute the documents to the WGPDMO members. Distribution will possibly be accom-

plished in the future through the installation of a members-only download section on the WGPDMO website.

For the written national reports on new disease trends in wild fish, farmed fish and wild and farmed shellfish to be submitted by WGPDMO members, clear guidelines on its expected contents and structure are needed, particularly for those WGPDMO members who do not attend the meetings and are therefore less familiar with the procedures. The Chair will provide these guidelines in advance to the 2006 meeting.

There was an agreement that a fixed structure for the WGPDMO report sections on new disease trends should be implemented, e.g. regarding the headings for the single sections, the use of common/scientific names, information on prevalences and sample size on which calculations are based (also relevant for the national reports, see above) etc. Reporting of new trends should be related to data from the previous year in order to provide a clearer picture of the magnitude of change observed.

A suggestion was made to change the review process for draft report sections carried out during the meetings. All sections available at the meeting used to be reviewed in plenum and the idea put forward was to split up into smaller specialist subgroups for doing the editorial changes needed and to subsequently review only the scientific contents of the sections in plenum. It was agreed to try and assess this approach at the 2006 WGPDMO meeting.

A number of suggestions were made to further raise the profile of WGPDMO:

- The WGPDMO report should be published as soon as possible after the meeting since it contains important information that should reach the interested groups in a timely fashion.
- It should be possible to quote the WGPDMO reports once published on the ICES website without consulting the ICES General Secretary because this procedure and the delay associated may prevent scientists or others to use the reports.
- Links on websites of institutions/projects to the published WGPDMO reports and to the ICES/WGPDMO website should be established.
- The work of WGPDMO should result in more publications, e.g. in scientific journals focusing on aspects of disease and pathology.
- Themes on disease and pathology aspects for the ICES Annual Science Conferences and for ICES Symposia should be identified and put forward.

16.6 Election of a new WGPDMO Chair

T. Lang was asked by WGPDMO to continue to serve as Chair and he agreed to do this for another year.

17 Analysis of progress with tasks

An analysis of progress of tasks in the Terms of Reference was conducted and the relevant information was entered into the 'Action Plan Progress Review' Excel template provided by the ICES Secretariat. Almost all items had been dealt with in a satisfactory manner. Several intersessional tasks to be fulfilled prior to the 2006 WGPDMO meeting were identified.

18 Future activities

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 2006 to consider the results of intersessional work and to discuss new and outstanding items.

It was agreed that the invitation by the ICES General Secretary to hold the 2006 WGPDMO meeting at ICES Headquarters in Copenhagen, Denmark, be accepted. The proposed dates are 7–11 March 2006. The Chair thanked the ICES General Secretary on behalf of WGPDMO for the kind invitation.

19 Approval of draft Terms of Reference 2005

The proposed draft Terms of Reference 2005 contained in this report were discussed by the WGPDMO and approved. The recommendations and justifications for recommendations to the Council are appended in Annex 11.

20 Approval of the draft WGPDMO Report

The draft report of the 2005 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.

21 Closing of the meeting

The Chair thanked the local host, T. Renault, for providing excellent meeting facilities and arrangements and the participants for their hard work and input during and in preparation of the meeting. The 2005 WGPDMO meeting was closed at 13:00 h on 12 March 2005.

Annex 1: List of participants

NAME	ADDRESS	PHONE/FAX	EMAIL
Michel Auffret (guest)	LEMAR UMR 6539/Biologie des interactions et de l'adaptation Institut Universitaire Europeen de la Mer Technopole Brest-Iroise 29280 Plouzane France	+33 2 98 49 86 49/	michel.auffret@univ-brest.fr
Egidijus Bacevicius (guest)	Lithuanian Centre for Pisciculture and Fishery Research Fishery Research Laboratory P/O Box108 LT 91001, Klaipeda Lithuania		ebacevicius@mail.lt
Juan L. Barja	University of Santiago Departamento de Microbiologia Instituto de Acuicultura Fac. Biologia 15782 Santiago de Compostela Spain	+34 981 56 31 00/ +34 981 56 69 04	mpaetjlb@usc.es
Katja Broeg	Alfred-Wegener-Institut for Polar and Marine Research Am Handelshafen 12 D-27570 Bremerhaven Germany	+49 471 4831 1618/ +49 471 4831 1425	kbroeg@awi-bremerhaven. de
David W. Bruno	FRS Marine Laboratory PO Box 101 375 Victoria Road Aberdeen AB11 9DB Scotland	+44 1224 295615/ +44 1224 295667	d.bruno@marlab.ac.uk
Stephen W. Feist	CEFAS Weymouth Laboratory The Nothe Weymouth Dorset DT4 8UB United Kingdom	+44 1305 206600/ +44 1305 206601	s.w.feist@cefas.co.uk
Susan E. Ford	Rutgers University Haskin Shellfish Research Laboratory 6959 Miller Avenue Port Norris, NJ 08349 USA	+1 856 785 0074 4305/ +1 856 785 1544	susan@hsrl.rutgers.edu
Brit Hjeltnes	National Veterinary Institute PO Box 1263 Sentrum 5811 Bergen Norway	+47 55363824 or +47 91889376/ +47 55321880	brit.hjeltnes@vetinst.no
Simon R.M. Jones	Department of Fisheries and Oceans Pacific Biological Station 3190 Hammond Bay Road Nanaimo, British Columbia V9T 6N7 Canada	+1 250 729 8351/ +1 250 756 7053	joness@pac.dfo-mpo.gc.ca

NAME	ADDRESS	PHONE/FAX	EMAIL
Andrey B. Karasev	Knipovich Polar Research Institute of Marine Fisheries and Oceanography 6, Knipovich Street 183763 Murmansk Russia	+7 815 2 47 37 21/ +7 815 2 47-33-31	paralab@pinro.ru
Muza Kirjušina (guest)	Division of Parasitology and Ichthyopathology State Veterinary Medicine Diagnostic Centre 3 Lejupes str. Riga, LV-1076 Latvia	+371 7 620 718/ + 371 7 620 434	muza.kirjusina@vvmdc.gov.lv
Thomas Lang (Chair)	Bundesforschungsanstalt für Fischerei Institut für Fischereiökologie Deichstrasse 12 27472 Cuxhaven Germany	+49 4721 38034/ +49 4721 53583	thomas.lang@ifo.bfa-fisch.de
Kaja Lotman (guest)	The Matsula National Park Administration, Penijoe 90305 Lihula Läänemaa Estonia	+37 25247899/	kaja@matsalu.ee
Sharon A. MacLean	NOAA/NMFS Narragansett Laboratory 28 Tarzwell Drive Narragansett, RI 02882-1199 USA	+1 401 782 3258/ +1 401 782 3201	sharon.maclean@noaa.gov
Atef A.H. Mansour	Science Branch, Aquaculture Research Dept. of Fisheries & Oceans Northwest Atlantic Fisheries Centre P.O. Box 5667 St. John's, NL A1C 5X1 Canada	+1 709 772 4133/ +1 709 772 5315	mansoura@dfo-mpo.gc.ca
Magdalena Podolska	Sea Fisheries Institute ul. Kollataja 1 81-332 Gdynia Poland	+48 58201728 (ext. 225)/ +48 58202831	bilbo@mir.gdynia.pl
Tristan Renault	IFREMER Laboratoire de Génétique, et Pathologie La Tremblade 17390 France	+33 5 46762649/ +33 5 46762611	trenault@ifremer.fr
Galina N. Rodjuk	Atlantic Scientific Research Institute of Marine Fisheries and Oceanography (AtlantNIRO) 5, Dm. Donskogo Street 236000 Kaliningrad Russia	+7 0112 225782 or +7 0112225369/ +7 0112 219997	rodjuk@atlant.baltnet.ru
Jean-François Samain (guest)	IFREMER Centre of Brest 29280 Plouzané France	+33 2 9822 4402/ +33 2 9822 4653	jfsamain@ifremer.fr
Galina P. Vialova	Sakhalin Research Institute of Fisheries and Oceanography 196 Komsomolskaya Str. Yuzhno-Sakhalinsk, 693023 Russia	+7 4242 45 67 79 or +7 4242 45 67 62/ +7 4242 45 67 89 or +7 4242 45 67 78	vyalova@sakhniro.ru vstex@sakhniro.ru

NAME	ADDRESS	PHONE/FAX	EMAIL
Tom Wiklund	Laboratory of Aquatic Pathobiology Department of Biology BioCity, Artillerig. 6 Åbo Akademi University 20520 Åbo Finland	+358 2 2154301 or +358 400 758957/ +358 2 2154748	twiklund@abo.fi
Maciej Wolowicz	Laboratory of Estuarine Ecology Institute of Oceanography University of Gdańsk Al. Marszałka J.Piłsudskiego 46 81-378 Gdynia Poland	+48 58 6601617/ +48 58 6202165	ocemw@univ.gda.pl
Werner Wosniok	University of Bremen Institute of Statistics P.O.Box 330 440 D-28334 Bremen Germany	+49 42 12 18 34 71/ +49 42 12 18 8944	wwosniok@math.uni-bremen.de

Annex 2: Terms of Reference for the 2005 WGPDMO meeting

2F02 The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: T. Lang, Germany) will meet in La Tremblade, France, from 8–12 March 2005 to:

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

b) assess information available in ICES Member Countries on the role of plankton organisms in gill-related mortality in farmed fish;

c) review current information on the continued increase of heart and skeletal muscle inflammation affecting farmed salmon;

d) compile information on the distribution, causes and significance of the Summer Mortality in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species;

e) provide guidance on the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints;

f) update and assess the current information on the effects of contaminants on the immune system in fish and shellfish;

g) evaluate the availability of data for a risk assessment pilot study on population effects due to diseases in wild fish, using epidemiological methods and population dynamics modelling;

h) produce an update of current information on sea lice interactions between wild and farmed fish and examine progress made in related management control methods in ICES Member Countries;

i) assess the results of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) to resolve any outstanding issues and, together with WGMS, MCWG, and WGSAEM, finalise a draft set of guidelines for integrated monitoring for OSPAR [OSPAR 2005/6];

j) to prepare data on the prevalence and type of fish diseases by ICES rectangle for the period 1984 to 2004 where available. The data should be submitted to the secure REGNS website in preparation for the REGNS Integrated Assessment Workshop from 9–11 May 2005;

k) produce updated ICES publications on pathology and diseases of marine organisms:

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

ii) ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish,

iii) WGPDMO website.

WGPDMO will report by 25 March 2005 for the attention of the Mariculture Committee and ACME.

Supporting Information

Priority:	WGPDMO is of fundamental importance to the ICES science and advisory process.
Scientific Justification:	Action Plan References: a) 2.4, 2.6, 2.10 b) 4.7, 3.14 c) 2.6, 3.14, 4.7 d) 2.6, 4.7 e) 3.3, 4.6 f) 2.12, 3.14 g) 3.11 h) 2.6, 2.7 i) 4.7, 4.9, 5.4 j) 1.3, 2.12, 3.3, 4.14 k) 6.1, 6.3 a) New disease conditions and trends in diseases of wild and cultured marine

	organisms continue to appear and an assessment of these should be maintained**.** b) Gill-related pathology in farmed salmon, likely to be caused by plankton organisms, resulted in the loss of almost 1 million fish in two adjoining bays in the northwest of Ireland in 2003. Gill pathology was also associated with moderate levels of mortality in other bays along the west coast of Ireland in 2003. Similar phenomena have been reported from Scotland and Norway over the past number of years. Because of the significance of these phenomena, the WGPDMO considered it necessary to assess available information more comprehensively. c) A condition associated with heart and skeletal muscle inflammation was first described in 1999 in Atlantic salmon from Norway. In 2003, there has been a significant increase in diagnosed cases of this condition. Current outbreaks are generally more severe and losses up to 25% have been reported. The WGPDMO will review the current findings on this syndrome and its impact from ICES salmon farming countries. d) The first description of Summer Mortality Syndrome in the Pacific oyster was in Japan in the 1940s. The syndrome was, and continues to be, associated with high mortality rates in Pacific oysters and other bivalves around the world. The causes remain unknown, but a multifactorial aetiology is suspected. Recently, a multidisciplinary approach including studies on pathology, genetics, physiology and immunology has been conducted in France (MOREST). The WGPDMO considered it important to have an overview of summer mortalities in Pacific oysters and in other molluscan species, with special emphasis on the results of the MOREST project. e) There are several examples of 'health indices' that have been published and others that are currently under development. There is a need to evaluate these in the context of their applicability for biological effects monitoring activities undertaken by ICES Member Countries. There is possible overlap with the WGMASC ToR b and this report should be made available to WGMASC. f) The impact of contaminants and other environmental factors on the immune system of fish and shellfish is an issue of ecological and economical concern, because it may result in clinical pathology and disease, by increasing the susceptibility of affected organisms to pathogens. Contaminants known to induce alterations of immune functions, e.g., including pesticides, heavy metals, organochlorines, PAHs, are present in almost all coastal areas, many of which are used for bivalve culture and fish farming. The WGPDMO considers it important to be updated on the most recent knowledge within this field. WGPDMO to provide examples of the environmental factors that it will be studying. g) The assessment of the potential risk to the fish population due to fish diseases is of considerable ecologic and economic concern. However, a prerequisite for such an assessment is the availability of appropriate data on stock sizes, their age structure, fishing effort, disease prevalence, mortality due to natural causes, fishing and diseases, and reproductive ability. Locations and times of observations of these data must coincide or differ only slightly. The availability of such data is as yet unclear and should be checked by WGPDMO in order to allow a further decision about the future conduct of a pilot study with the objective of a risk assessment for population effects due to fish diseases. h) Sea lice have been blamed for the decline in wild sea trout populations in several countries and perceived as a threat to salmon migrating in coastal areas. For the WGPDMO meeting in 2005, a progress report will be prepared to review current information on the interaction between lice on farmed and wild salmonids and the implementation and effectiveness of national management control programmes for sea lice. i) Health parameters form an important component within integrated monitoring activities. As such, the WGPDMO needs to be involved in the WKIMON and assess the outcomes of the Workshop at the next WGPDMO meeting. j) This is required as the WGPDMO input on the health status of North Sea biota for the period 2002–2004, and on trends in the prevalence of diseases over recent decades to the thematic writing panels working under the coordination of REGNS, to develop an integrated assessment of the North Sea. k) A number of ICES publications, either web-based or in ICES publication

	series, are being prepared or updated at present, the progress of which has to be reviewed by WGPDMO at its next meeting. It will be necessary to consider ways by which these can be linked to each other.
Resource Requirements:	None required other than those provided by the host institute.
Participants:	Representatives of all Member Countries with expertise relevant to pathology and disease of wild and cultured finfish and shellfish.
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	There is a close link to ACME activities.

Annex 3: Working documents

	2005 WGPDMO Terms of Reference	Working document (file)	Title/Content
a)	produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports	WGPDMO2005_Canada.doc	National Report on New Disease Trends in 2004
		WGPDMO2005_Denmark.doc	"
		WGPDMO2005_Finland.doc	"
		WGPDMO2005_Latvia.doc	"
		WGPDMO2005_Poland.doc	"
		WGPDMO2005_Russia.doc	"
		WGPDMO2005_Scotland.doc	"
		WGPDMO2005_TheNetherlands.doc	"
		WGPDMO2005_USA.doc	"
		WGPDMO2005_England_Wales.doc	"
		WGPDMO2005_France.doc	"
		WGPDMO2005_Germany.doc	"
		WGPDMO2005_Ireland.doc	"
		WGPDMO2005_Norway.doc	"
		WGPDMO2005_Scotland_V2.doc	"
		WGPDMO2005_Estonia.doc	"
b)	assess information available in ICES Member Countries on the role of plankton organisms in gill-related mortality in farmed fish;	WGPDMO2005_WD_B1.pdf	Rapid monitoring of toxic phytoplankton in Scottish coastal waters (Poster, ICES CM 2004/V:10)
		WGPDMO2005_WD_B2.pdf	Early warning systems to mitigate the impact of algal blooms and jellyfish on mariculture developments (Poster, ICES CM 2004/V:12)
c)	review current information on the continued increase of heart and skeletal muscle inflammation affecting farmed salmon;	WGPDMO2005_WD_C1.pdf	Kongtorp, R.T., Kjerstad, A., Taksdal, T., Guttvik, A. and Falk, K. (2004a). Heart and skeletal muscle inflammation in Atlantic salmon, *Salmo salar* L; a new infectious disease. Journal of Fish Diseases 27: 351–358.
		WGPDMO2005_WD_C2.pdf	Kongtorp, R.T., Taksdal, T., Lyngøy, A. (2004b). Pathology of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms, 59: 217–224.
		WGPDMO2005_WD_C3.pdf	Ferguson, H.W. Kongtorp, R.T. Taksdal, T. Graham, D. and Falk, K. (2005). An outbreak of disease resembling heart and skeletal muscle inflammation in Scottish farmed salmon, *Salmo salar* L., with observations on myocardial regeneration. Journal of Fish Diseases 28: 119–123.
		Hardcopy	Hellberg, H., Olsen, A.B., Jensen, F. (2003/2004). Clinical signs and histopathology in farmed Atlantic salmon and rainbow trout associated with large numbers of the jellyfish *Muggiaea atlantica* (Siphonophora) (Poster)
d)	compile information on the distribution, causes and nnn significance of the Summer Mortality in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species;	WGPDMO2005_WD_D1.doc	Distribution, causes and significance of the Summer Mortality in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species (T. Renault, S. Ford, J. F. Samain)

	2005 WGPDMO Terms of Reference	Working document (file)	Title/Content
e)	provide guidance on the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints;	WGPDMO2005_WD_E1.doc	On the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints (Werner Wosniok, Katja Broeg, Steve Feist)
f)	update and assess the current information on the effects of contaminants on the immune system in fish and shellfish;	WGPDMO2005_WD_F1_V2.doc	Effects of Contaminants on the Immune System of Fish and Shellfish (K. Broeg, T. Renault, M. Auffret and B. Gagnaire)
h)	produce an update of current information on sea lice interactions between wild and farmed fish and examine progress made in related management control methods in ICES Member Countries;	Hardcopy	McVicar, A.H. (2004). Management actions in relation to the controversy about salmon lice infections in fish farms as a hazard to wild salmonid populations. Aquaculture Research 35: 751–758.
		Hardcopy	Revie, C. and Gettinby, G. (2005). Do Scottish and Norwegian salmon farmers face different challenges in controlling sea lice ? Fish Farmer January/February 2005:10–11.
i)	assess the results of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) to resolve any outstanding issues and, together with WGMS, MCWG, and WGSAEM, finalise a draft set of guidelines for integrated monitoring for OSPAR [OSPAR 2005/6];	WGPDMO2005_WD_I1.doc	Report on the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) (T. Lang)
		WGPDMO2005_WD_I2.pdf	OSPAR Coordinated Environmental Monitoring Programme (Reference Number 2004–16)
		WGPDMO2005_WD_I3.pdf	JAMP Guidelines for Contaminant-specific Biological Effects Monitoring (Reference Number 2003–10)
		WGPDMO2005_WD_I4.pdf	JAMP Guidelines for General Biological Effects Monitoring (Reference Number 1997–7)
j)	to prepare data on the prevalence and type of fish diseases by ICES rectangle for the period 1984 to 2004 where available. The data should be submitted to the secure REGNS website in preparation for the REGNS Integrated Assessment Workshop from 9–11 May 2005;	WGPDMO2005_WD_J1.doc	Email from A. Kenny (REGNS Coordinator) with tasks for WGPDMO
		WGPDMO2005_WD_J2.doc	REGNS: Potential requirements for an Integrated Assessment
		WGPDMO2005_WD_J3.xls	Table: Integrated Assessment (IA) of the North Sea: datasets available for contributing to an IA
		WGPDMO2005_WD_J4.doc	Letter from the REGNS Coordinators to WG Chairs

Annex 4: Agenda

1) Opening of the meeting
2) Terms of Reference, adoption of Agenda, selection of Rapporteurs
3) ICES Annual Science Conference 2004, items of relevance to WGPDMO
4) Other relevant reports/activities for information
5) Produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports (ToR a)
6) Assess information available in ICES Member Countries on the role of plankton organisms in gill-related mortality in farmed fish (ToR b)
7) Review current information on the continued increase of heart and skeletal muscle inflammation affecting farmed salmon (ToR c)
8) Compile information on the distribution, causes and significance of the Summer Mortality in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species (ToR d)
9) Update and assess the current information on the effects of contaminants on the immune system in fish and shellfish (ToR f)
10) Produce an update of current information on sea lice interactions between wild and farmed fish and examine progress made in related management control methods in ICES Member Countries (ToR h)
11) Provide guidance on the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints (ToR e)
12) Evaluate the availability of data for a risk assessment pilot study on population effects due to diseases in wild fish, using epidemiological methods and population dynamics modelling (ToR g)
13) Assess the results of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) to resolve any outstanding issues and, together with WGMS, MCWG, and WGSAEM, finalise a draft set of guidelines for integrated monitoring for OSPAR [OSPAR 2005/6] (ToR i)
14) To prepare data on the prevalence and type of fish diseases by ICES rectangle for the period 1984 to 2004 where available. The data should be submitted to the secure REGNS website in preparation for the REGNS Integrated Assessment Workshop from 9–11 May 2005 (ToR j)
15) Produce updated ICES publications on pathology and diseases of marine organisms (ToR k):
    i) web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report
    ii) ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish
    iii) WGPDMO website
16) Any other business
17) Analysis of progress with tasks
18) Future activities of WGPDMO
19) Approval of recommendations
20) Approval of draft WGPDMO Report
21) Closing of 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)	
	• wild fish	S. MacLean/S. Jones/A. Karasev/M. Podolska
	• farmed fish	D. Bruno/J. Barja/B. Hjeltnes/A. Mansour
	• wild and farmed shellfish	S. Ford/T. Renault/M. Wolowicz
6	Assess information available in ICES Member Countries on the role of plankton organisms in gill-related mortality in farmed fish (ToR b)	T. Wiklund/B. Hjeltnes/ D. Bruno
7	Review current information on the continued increase of heart and skeletal muscle inflammation affecting farmed salmon (ToR c)	T. Wiklund/B. Hjeltnes/ D. Bruno
8	Compile information on the distribution, causes and significance of the Summer Mortality in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species (ToR d)	S. Ford/T. Renault/M. Wolowicz
9	Update and assess the current information on the effects of contaminants on the immune system in fish and shellfish (ToR f)	M. Wolowicz/K. Broeg/T. Renault/
10	Produce an update of current information on sea lice interactions between wild and farmed fish and examine progress made in related management control methods in ICES Member Countries (ToR h)	S. MacLean/B. Hjeltnes/ D. Bruno
11	Provide guidance on the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints (ToR e)	S.W. Feist/K. Broeg/ W. Wosniok
12	Evaluate the availability of data for a risk assessment pilot study on population effects due to diseases in wild fish, using epidemiological methods and population dynamics modelling (ToR g)	S.W. Feist /W. Wosniok/T. Lang
13	Assess the results of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) to resolve any outstanding issues and, together with WGMS, MCWG, and WGSAEM, finalise a draft set of guidelines for integrated monitoring for OSPAR [OSPAR 2005/6] (ToR i)	W. Wosniok /T. Lang/S.W. Feist
14	To prepare data on the prevalence and type of fish diseases by ICES rectangle for the period 1984 to 2004 where available. The data should be submitted to the secure REGNS website in preparation for the REGNS Integrated Assessment Workshop from 9–11 May 2005 (ToR j)	W. Wosniok/S.W. Feist/ T. Lang
15	Produce updated ICES publications on pathology and diseases of marine organisms (ToR k):	

Agenda Item(s)	2005 WGPDMO Terms of Reference	Rapporteurs
	• web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report;	S. Jones/W. Wosniok/T. Lang
	• ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish;	S. Jones/D. Bruno/M. Wolowicz
	• WGPDMO website	S. Ford/ S. Jones/M. Podolska
16–19	Any other business, Analysis of progress with tasks, Future activities of WGPDMO, Recommendations	S. Jones/A. Mansour/ D. Bruno
20–21	Approval of draft report, Closing of the meeting	T. Lang

Annex 6: Information on the distribution, causes and significance of the summer mortality syndrome in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species

by T. Renault, S. Ford, J. F. Samain

The first description of Summer Mortality Syndrome in the Pacific oyster was in Japan in the 1940s. The syndrome was, and continues to be, associated with high mortality rates in Pacific oysters and other bivalves around the world. The causes remain unknown, but a multifactorial aetiology is suspected. Recently, a multidisciplinary approach including studies on pathology, genetics, physiology and immunology has been conducted in France (MOREST). The WGPDMO considered it important to have an overview of summer mortalities in Pacific oysters and in other molluscan species, with special emphasis on the results of the MOREST project.

The first description of the phenomenon known as "summer mortality" concerned oysters of the species *Crassostrea gigas* being cultured in numerous embayments along the Japanese Pacific coast. According to Koganezawa (1974), the mortalities began occurring in 1945 with the large-scale use of hanging culture methods (raft, longline, and rack). These "mass mortalities" (50 to 60%, or more) all had certain common features:

- The animals affected were one year old or older, and were the largest and fastest growing.
- The mortalities occurred in eutrophic waters, mostly during the spawning season, but shortly thereafter in some regions.
- Deaths began with the rise of temperatures to 21–22°C or above and took place gradually over a spawning season.
- There was no (apparent) association with hydrographical conditions other than temperature.
- Bacteria were present in the affected oysters, but no other infective organism was associated with the mortalities.

The association with spawning and the highest temperatures of the season led Japanese investigators to examine the relationship between gonadal maturation, energy metabolism, and the mortalities (Mori, 1979). They reported that oxygen consumption, ciliary activity, and glycogen reserves all decreased during maturation and reached low points just before mortality began. The conclusion of these studies was that Summer Mortality in Japan was due to a "physiological disorder and metabolic disturbance derived by heavy gonad formation and massive spawning under high water temperature and eutrophication" (Koganezawa, 1974). In particular, it was thought that "overmaturation" of germ cells, i.e., a long residence time of ripe gametes within the gonad, induced pathological changes in the biochemical composition of oocytes that in turn led to death. The bacteria found in moribund oysters were considered secondary invaders of already weakened oysters.

Crassostrea gigas seed had been imported regularly for growout on the west coast of the United States and Canada since the early 1900s (Chew, 1990). In the late 1950s, mortalities of *C. gigas* were first noted on this coast (Glude 1975 in Cheney *et al*. (2000). They had many of the same features as those in Japan:

- The animals affected were 2 years old or older and had a high condition index.
- The mortalities occurred in high nutrient and high productivity areas, at water temperatures approaching 20°C, varied greatly from area to area, and did not occur in all years.

- Results of early bacteriological assays were inconsistent, with some showing presence of bacteria and other failing to do so, but no other infective agent was associated with the mortality.

A Summer Mortality episode in Alaska in 1987 also involved unusually high temperatures (~20°C) occurring during a period when the oysters contained mature or nearly mature gametes (Meyers *et al*., 1990). Experimental studies in the US further implicated gonadal maturation and loss of carbohydrate reserves in the mortality (Perdue *et al*., 1981) and showed that experimentally elevating temperature (to 21°C) or adding high doses of microalgae to the tank seawater significantly increased mortality (Lipovsky and Chew, 1972).

Studies during the 1980s described histopathological evidence of bacterial involvement in Summer Mortality in the USA. The agent, a Gram-positive bacterium was later named *Nocardia crassostrea* (Friedman *et al*., 1991; Friedman *et al*., 1998). Pacific oyster nocardiosis, the disease caused by *N. crassostrea*, and Summer Mortality overlap both spatially and temporally (Elston, 1987) although not all instances of Summer Mortality involve the bacterium. Friedman *et al*. (1991) considered that *N. crassostrea* might be opportunistic, rather than the primary cause of Summer Mortality, although Elston *et al*. (1987) pointed out that the bacterium could be lethal.

A selective breeding program at the University of Washington produced families that showed greatly improved survival in both the field and in elevated-temperature laboratory trials (Beattie *et al*., 1980). Although mortality consistently occurred shortly after spawning, Perdue (1981) found no correlation between high and low mortality groups and carbohydrate levels. Unfortunately, the selected stocks were thinner, smaller and had slower growth than unselected stocks (Cheney *et al*., 2000). Meanwhile, techniques to induce triploidy in oysters were developed (Allen and Downing, 1986). Triploid oysters were commercially valuable because their reproductive output was minimal and they could be marketed during the entire year (the mass of gametes produced by diploid *C. gigas*, negatively affected taste and texture and made them unmarketable during the reproductive season.) Triploid oysters had another advantage: their reduced gamete production was likely to make them less susceptible to Summer Mortality.

A preliminary report of a comprehensive, recent investigation of the factors involved in Summer Mortality on the US west coast concluded that it is most likely caused by a suite of stressors: those already implicated, but also including possible links with low dissolved oxygen and phytoplankton blooms (Cheney *et al*., 2000). Interestingly, in this study, mortality of triploid oysters began earlier, rose more rapidly, and reached levels 8 to 28 percentage points greater than did mortality of diploids. The final report of this multi-year study has not yet been published, but members of the same investigative team have recently found oyster herpes virus in *C. gigas* from Tomales Bay, California, USA, where Summer Mortality has been reported for the past decade, although a causal relationship has not been established (Friedman *et al*., 2005).

A "summer mortality" of sorts has also been described in cultured eastern oysters, *C. virginica*, on the northeastern United States. It, too, affects fast growing oysters and begins when water temperatures approach or exceed 20–22°C, and can kill up to 90% of affected stocks within a few weeks (Bricelj *et al*., 1992; Ford and Borrero, 2001). There is a genetic component, which has permitted the development of resistant strains (Lewis *et al*., 1996), and it has even been associated with plankton blooms (Lee *et al*., 1996). However, it affects only juvenile (<1 year old) oysters, as its name Juvenile Oyster Disease (JOD) implies. It further differs from *C. gigas* Summer Mortality in that JOD produces a distinct symptom: an organic deposit on the inner shell more reminiscent of Brown Ring Disease in the Manila clam, *Ruditapes philippinarum* in western Europe (Paillard and Maes, 1994). A newly described proteobacterium, *Roseovarius crassostrea*, predominates on the inner shell surface, including the organic

deposit, of symptomatic oysters and increases in prevalence before symptoms or mortality begin; however, attempts to reproduce the characteristic organic deposit have been inconsistent Boettcher (2000).

Summer Mortality episodes, with losses of 80 to 90%, have been reported for other bivalves, including the blue mussel, *Mytilus edulis*, in eastern Canada and in Maine, USA, and the Zhikong scallop, *Chlamys farreri*, in China (Xiao *et al*., submitted); Newell and Lutz, 1991; Myrand and Gaudreault, 1995). Mortalities of these species have important elements in common with *C. gigas* Summer Mortality:

- they occur in off-bottom culture and in late summer when temperatures are maximal;
- they typically affect reproductively mature or post-spawning animals;
- they can kill up to 80–90% of affected groups;
- there is no histological evidence of a specific pathogen;
- in the case of mussels, at least, there is a genetic component to resistance and susceptibility (Myrand and Gaudreault, 1995).

Tremblay and colleagues (Tremblay *et al*., 1998a; Tremblay *et al*., 1998b; Tremblay *et al*., 1998c) have described a suite of physiological and biochemical differences between resistant and susceptible *M. edulis* stocks: compared to resistant stocks, susceptible mussels have lower heterozygosity, higher maintenance metabolism, lower scope for growth, lower O/N ratio, higher lysosomal membrane instability, and appeared less able to acclimate to the high temperatures of late summer when mortalities occur. Xiao *et al*. (submitted) reported somewhat reduced heterozygosity (9%) in cultured scallops, which experience mortality, compared to wild stocks. They also found an accumulation of organisms, including the large ciliate *Trichodina* sp., within the gill cavities of scallops experiencing summer mortalities. They postulated that heavy fouling and high stocking densities resulted in poor circulation, and perhaps oxygen depletion, in the growout cages and within the scallops' gill cavities, which would severely stress the scallops at high temperatures and during the period of reproduction. It might also foster the accumulation of potentially damaging gill symbionts. Some evidence also exists of viral involvement in the scallop deaths (Wang *et al*., 2002).

The collective evidence available at the start of the French MOREST program suggested that Summer Mortality affecting adult bivalves is not caused by a single etiological agent, but involves complex interactions between environment, oyster and pathogens. The most important external factor seemed to be elevated temperature coming at a time when the intrinsic factors gametogenesis and spawning place the animal in a relatively unstable physiological condition. Any other external factor that exacerbates this instability, including further stress and the presence of opportunistic invaders, may push the animals over a threshold from which they cannot recover.

Main results of the "MOREST" project on Summer Mortality in the Pacific oyster (*Crassostrea gigas*)

By J. F. Samain, P. Boudry, L. Degremont, P. Soletchnik, M. Ropert, E. Bédier, J. L. Martin, J. Moal, M. Mathieu, S. Pouvreau, C. Lambert, J. M.Escoubas, J. L. Nicolas, F. Le Roux, T. Renault, T. Burgeot, C. Bacher (MOREST network of French laboratories)

Pacific oyster production on the French coasts has experienced periodic mass mortalities for at least 20 years (Renault *et al*., 1994; Goulletquer *et al*,. 1998; Soletchnik *et al*., 1999). The syndrome, too, is known as Summer Mortality. Factors such as food limitation, oxygen depletion, salinity and temperature variations do not appear as single direct causative agents of the syndrome (Soletchnik *et al*., 1998). Some authors suggest that many of the mortalities occur-

ring in *C. gigas* are the result of multiple factors or stressors, including elevated temperatures, physiological stress associated with maturation, aquaculture practices, pathogens, or pollutants (Goulletquer *et al*., 1998). It could be assumed that a background rate of mortality due to environmental conditions, physiology and genetic makeup might be affected by infectious agents. As with many filter-feeding benthic invertebrates, oysters are permanently exposed to various microorganisms. Efficient humoral and cellular defence mechanisms normally help to limit the proliferation of microorganims in animals (Harris-Young *et al*., 1995; Cheng, 1996). Since 1996, the Laboratory of Shellfish Farming of Poitou-Charentes has studied the phenomenon of mortality in a culture area in the south of Marennes-Oleron Bay (Ronce-Perquis, Charente-Maritime, France) (Lodato, 1997). Despite inter-annual and inter-stock variability, mortality of oysters reared on sediment (or a few centimetres above) is significantly higher, by 20–30%, than mortality of animals on racks 50 cm or 70 cm above the sediment (Soletchnik *et al*., 1999). Previous studies also showed that energy allocation to reproduction, as well as shell and soma growth, were less important near the sediment than on racks (Goulletquer *et al*., 1998; Soletchnik *et al*., 1999; 2003).The MOREST project on *C. gigas* oyster Summer Mortality was focused on the complex interactions hypothesized by many authors between environment, oysters and pathogens. Such multifactorial associations led us to organize a research network (including genetics, physiology, immunology, pathology, ecotoxicology, and ecology) all using the same biological material. This strategy, and the coupling of field sampling and experimental studies, allowed us to progressively classify the importance of the different factors involved in Summer Mortality. Natural and hatchery-produced spat were compared at three oyster-production areas in France. Regardless of whether they were naturally set or of hatchery origin, oysters died during the reproduction period after temperatures reached 19 °C. Thus, in southern areas, temperatures accelerated gametogenesis in small spat (10 mm) as well as in adults, and mortality appeared in both development stages. Sexual maturation proceeded more slowly in northern France and consequently spat mortality was low compared to 18-month old oysters.

- A temperature over 19 °C appeared the primary necessary condition for Summer Mortality in France, but was not, by itself, sufficient to produce mortalities. This temperature is associated with the final stage of gametogenesis, when energy storage is minimal and when scope for growth becomes very low. It is also an optimal temperature for bacterial (Vibrio spp.) proliferation as well as for anaerobic production of H2S and NH4+ from accumulated organic matter in the sediment, which is considered one of the major stressing factors in the environment.
- Nutrient level is a second critical parameter as high levels favour reproductive effort and may lead to energy imbalance. Haemocyte numbers decreased during the gametogenesis period, regardless of the food supply. Phagocytosis capacity decreased inversely to food level and reproductive effort. Experimental challenges or natural infection under these conditions led to mortality rates related to this decrease. Other immune parameters or tools are under study to better document these relationships (Lambert *et al*. 2003; Gueguen *et al*., 2003; Montagnani *et al*., 2004; 2005).
- Gonadal maturation is known as an intrinsic factor of risk in Summer Mortality (Glude, 1975; Mori, 1979; Perdue *et al*., 1981). Thus, triploid oysters (with reduced maturation) were also tested. Results showed that triploids, which suffered the lowest mortalities, presented higher potential defence capacities than did diploids. Because Summer Mortality may result from a combination of environmental factors, physiological status, and presence of pathogens (Lacoste *et al*., 2001; Leroux *et al*., 2002), higher defence capacities may be an important factor to resist this phenomenon. In some rare observations, triploids demonstrating an unusually high reproductive state were as susceptible to summer mortality as diploids.
- Some stressor was necessary to induce mortality even when temperature and reproductive condition were favourable for Summer Mortality outbreaks. A simple transfer of oyster could induce mortality in these conditions. Proximity to sedi-

ment appeared to be a consistent detrimental factor (Soletchnik *et al*., 2005), probably because of its high organic content. High fresh water run-off from surrounding rivers was also correlated with elevated mortality.

- A genetic component evidenced by divergent selection in two generations was confirmed during the project (Dégremont *et al*., 2005). In the same area or in controlled experimental conditions, "Susceptible" (S) strains invested more energy in reproduction than did "Resistant" (R) oysters under similarly high food conditions. Molecular studies comparing these two phenotypes are under study (Huvet *et al*., 2004) and are now focused on pathways of glucose metabolism and on immune mechanisms (Bacca *et al*., 2005).
- Different pathogens were isolated from moribund oyster. Herpesvirus (OsHV-1) was mostly detected in juvenile mortality events and when temperature was high. Different strains and species of Vibrios (including V. splendidus and V. estuarianus) were isolated from moribund one- and two-year old oysters and identified by molecular techniques (Le Roux *et al*., 2002; 2004). Their virulence, as tested by injection in the adductor muscle, varied according to strain and species (Labreuche *et al*., 2005). The effect of environmental factors on transmission and expression of virulence is also under study. One of the Vibrio splendidus strains has been sequenced, and numerous genes coding for virulence factors were found. This model will offer possibilities to better understand the expression of virulence under differing environmental and host conditions. It emphasizes that necessity for interactions between oyster physiology and environmental parameters to occur in order to produce Summer Mortality.

None of these different factors can separately induce summer mortality, and all of these conditions seem necessary to reproduce the event. According to these interactions, strategies to forecast and prevent the risk will be devised.

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Annex 7: Effects of contaminants on the immune system of fish and shellfish

by K. Broeg, T. Renault, M. Auffret, B. Gagnaire

Introduction

The impact of contaminants and other environmental factors on the immune system of fish and shellfish is an issue of ecological and economical concern, because it may result in clinical pathology and disease, by increasing the susceptibility of affected organisms to pathogens. Contaminants known to induce alterations of immune functions, e.g., including pesticides, heavy metals, organochlorines, and PAHs, are present in almost all coastal areas, many of them are used for bivalve culture and fish farming.

Fish innate immunity and pollutants

The innate immune system is something common to all multicellular organisms. Innate or non-specific immunity comprehends defence mechanisms that protect an organism against infection without depending upon prior exposure to any particular microorganism. Many components of the innate immune system appear to be evolutionary conserved (Hoffmann *et al*., 1999;Ulevich, 2000). Thus, sensitivity of immune system mechanisms to a particular contaminant might be similar among different species. For fish populations, a link between environmental contamination and disease has long being discussed. Understanding the impact of contaminants on fish immunity is of economic relevance for fisheries as well as aquaculture. A comprehensive review on toxically-induced immunomodulation of fish innate immunity is given by Bols *et al.* (2001). Studies on more than 30 different fish species are mentioned in this article. Due to the complexity of innate immunity, many parameters (factors) have been measured as biomarkers.

External factors

First barriers are the mucous membranes. Mucus contains a variety of humoral factors with anti-microbial factors (lysozyme, complement, lectins, proteolytic enzymes, NOS (gill)). The gill is an important site for the entry of pathogens (thin mucus and only one layer of epithelial cells).

Mucus

Excessive mucus secretion of gills has been reported, caused by heavy metals (Lock and van Overbeeke, 1981), malathion (Richmonds and Dutta, 1989), 1-naphthyl-N-methylcarbamate (Pfeiffer *et al*., 1997), and outboard motor exhaust emissions (Brenniman, 1979). A decrease of number and density of goblet cells in fish skin and a very specific alteration of mucous cells in trout are caused by Nonylphenol (Burkhardt-Holm *et al*., 2000).

Interference with the protective actions?

Numbers of ectoparasites are increasing following to exposure of fish to some pollutants (Khan and Thulin, 1991), and under field conditions at polluted sites (Broeg *et al*., 1999, Schmidt *et al*., 2003). Some pathogenic *Vibrio* strains use mucus as carbon source (Bordas *et al*., 1996).

Gill lesions

Gill lesions can be provoked by heavy metals, pesticides, organotins, organic solvents, organic xenobiotics, and surfactants (in Bols *et al*., 2001).

Interference with the protective actions?

Gill lesions allow rapid entry of bacteria into the blood stream after gill damage (Bowers and Alexander, 1981).

Internal factors/humoral

Lysozyme

Lysozyme disrupts the cell walls of bacteria and has been most frequently measured in plasma and serum of fish. The nature of modulation by heavy metals and other environmental contaminants is complex. Different results (increase/decrease) depending on contaminant, species, and organ make their interpretation difficult.

Acute-phase proteins

Acute-phase proteins are plasma or serum proteins whose levels change in response to tissue damage, infection, or inflammation (Gaby and Kushner, 1999). In fish, the c-reactive protein (CRP) is measured in most of the cases. Ecotoxicants have been reported to increase serum CRP levels in fish, but the disadvantage is that the reaction is too transitory to be a useful bioindicator. Since hepatocytes are responsible for the production of acute-phase proteins, liver cell damage may lead to decreased acute-phase response.

Internal factors/cellular

Physiology and functions of leukocytes: macrophages, monocytes, granulocytes

Chemotaxis

Methods: Boyden chambers, microporous filters, random migration.

Metals appear to enhance chemotaxis in exposure experiments (in Bols, 2001). Reduced chemotaxis was reported at polluted stations in field studies (Weeks et al., 1986).

Ingestion

Methods: Yeast, bacteria, plastic beads, sheep red blood cells, often labelled with fluorescence. Ingestion can be monitored by flow cytometry, plate reader, or microscopically.

Cadmium enhances ingestion by rainbow trout macrophages at low doses and inhibits it at high doses (Zelikoff *et al*., 1995). Inhibition was also observed after exposure to: Chromium, copper, and zinc (spleen and kidney cells, different species), pulp mill effluents, pentachlorophenol, and PAHs. Field studies on oyster toadfish showed decreased ingestion along an increasing PAH-gradient in the sediment (Seeley *et al*., 1991). Creosote provoked an increase of ingestion with increasing doses (Karrow *et al*., 1999).

Respiratory burst (RB)

The respiratory burst of granulocytes and macrophages is the generation of superoxide anions, converted into different reactive oxygen species (ROS) which are acting as potent microbicidal agents. Different methods are available to measure RB which makes a comparison of results difficult (standardisation/ harmonisation is required). Variable and even contradictory results were obtained in different experimental settings.

Most studies were performed with metals and organometals. TBT stimulates RB, but longer periods of exposure decrease it (Rice and Weeks, 1986; 1991).

Field studies showed suppressed RB activity in areas contaminated with PCB and mercury (Rice *et al*., 1996). Different studies on Cu, Cd, and Hg gave contradictory results. Several

pesticides (trichlorfon, dichlorvos, chlorothalonil, in: Bols *et al*., 2001) have been shown to inhibit RB. Sewage sludge provoked suppression of ROS generation by kidney leukocytes at low exposure (Secombes *et al*., 1991). Exposure to sediments contaminated with PAH and other experiments with different kinds of PAHs showed variable results. PCB 126 decreased RB at the lowest dose that maximally induced cytochrome P450 (Rice and Schlenk, 1995).

Interference with the protective actions?

Decreased superoxide generation caused by exposure to sewage sludge lead to decreased bactericidal activity against *A. salmonicida* (Secombes *et al*., 1992).

Macrophage aggregates (MA)/melanomacrophage centres

Methods: Histology, histochemistry.

Toxically-induced alterations of size and numbers of MAs are extremely variable (in Bols *et al*., 2001).

Enlargement of MAs was observed caused by exposure to crude oil (Khan and Kikeniuk, 1984) and at sites contaminated with metals and organic contaminants (Hartley *et al*., 1996). An increase in number but a decrease in size was observed caused by chromium exposure, suggesting impairment of macrophages to aggregate. Macrophages are complex structures and toxic effects may alter different features from the chemotactic activity involved in MA-formation to the digestive capacity of the aggregates. The activity of relevant enzymes of MAs like acid phosphatase was shown to be suppressed in chronically exposed feral fish, but enhanced after acute pollution events (Broeg 2003, Sturve *et al*., in press).

Interference with the protective actions?

Incomplete digestion of ingested pathogens caused by suppressed acid phosphatase activity may lead to the ability of pathogens to reproduce in the host (for example Mycobacteria).

Physiology and functions of leukocytes: Nonspecific cytotoxic cells (NCC)

The activity of NCCs is assessed by testing their ability to spontaneously lyse several different targets (tumour cells, virus-infected fish cell lines and specific protozoan parasites). Significant suppressive effects were measured following zinc, copper and TBT exposure (Grinwis *et al*., 2000; Rougier *et al*., 1994). NCC activity showed no sensitivity to phenols, PAHs, and PCBs. An exception was DMBA, which nearly abolished NCC activity at all doses (Seeley and Weeks-Perkins, 1997). PAH exposure inhibited the recognition and binding of tumour target cells by NCCs (Faisal *et al*., 1991).

Discussion

The interpretation of toxically-induced alterations of innate immunity of fish is difficult due to different results depending on doses of toxicants, exposure time (acute and chronic response), mixture toxicity, species sensitivities, gender, temperature and salinity changes, just to name a few. Acute responses are often coupled with effects of general stress and reflected by increased immune activity, whereas chronic responses might be coupled with cytotoxic effects, reflected by immunosuppression. Standardisation and/or harmonisation of promising methods are urgently needed to overcome these difficulties at least partly. Several studies indicated a potential interaction between reproduction, biotransformation and immune response in female fish. Therefore, specific live stages seem to be more sensitive to immunomodulation exposure of contaminants than others.

Shellfish immunity and pollutants

In contrast to the vertebrate immune system which consists of innate and acquired mechanisms, invertebrates have only non-specific (innate) defence mechanisms. The fact that invertebrates represent more than 90% of the total number of species living on earth demonstrates the efficiency of their «primitive» host defence systems. In the last 10 years, it became more and more obvious that some of these innate mechanisms are conserved in invertebrates (*e. g. Drosophilae*) **and** vertebrates (Medzhitov *et al*., 1997; Means *et al*., 2000). Thus, the fundamental importance of the toxically-induced modulation of non-specific immune functions has increasingly been perceived in the last decade.

The invertebrate immune system comprises cellular (*i.e.* phagocytes) and humoral (*i.e.* complement) components (Marchalonis and Schluter, 1994; Kuby, 1997). Various strategies are employed by invertebrates to kill invasive or opportunistic micro-organisms. These include phagocytosis-mediated killing, agglutination, encapsulation, release of microbicidal molecules and apoptosis. Bivalve defence mechanisms are supported by haemocytes which are considered to be the counterpart of vertebrate inflammatory cells and participate directly in eliminating pathogens by phagocytosis. In addition, haemocytes produce compounds including lysosomal enzymes (esterases and aminopeptidases) and antimicrobial molecules which contribute to the destruction of pathogenic organisms.

Shellfish farming is an ancestral activity all around the world. It has been expanded and intensified in the last century and represents nowadays a major economic activity in several countries. In the majority of cases, bivalve species are reared in estuary zones, continually impacted by pollutants. Natural and man-made toxicants enter marine ecosystems by various routes, including direct discharge, land run-off, atmospheric deposition, *in situ* production, abiotic and biotic movements and food-chain transfer. Xenobiotics like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and heavy metals originate from anthropogenic activities including industry, fuel transport, and agriculture.

Bivalves in culture are continuously affected by environmental factors including the presence of pollutants, potentially increasing their susceptibility to a wide range of infectious diseases. The effects of environmental contaminants may result from direct toxic actions on tissues or cells or from alterations of the homeostatic mechanisms including the immune system (Coles and Pipe, 1994; Carajaville *et al*., 1996).

Haemocyte mortality

After 24 hours of exposition with 10^{-5} to 2.10^{-3} M of cadmium chloride ($CdCl_2$), haemocytes of *Mytilus galloprovincialis* showed a dose-dependent increase of mortality (Olabarrieta *et al*., 2001). Other studies reported mortalities of haemocytes of *Mya arenaria*, *M. edulis* and *Mactromeris polynyma* at methylmercury concentrations of 10^{-4} M (Brousseau *et al*., 2000; Sauvé *et al*., 2002). Mercury chloride caused dose-dependent haemocyte mortality in Pacific oyster, *Crassostrea gigas*, (2.10^{-6}, 2.10^{-5} and 2.10^{-4} M) after four hours of incubation (Gagnaire *et al*., 2004). This rapid effect was previously reported in haemocytes of Eastern oyster, *C. virginica* (Cheng and Sullivan, 1984). Haemocyte mortality increased in a dose-dependent way after two hours of contact with 2.10^{-6}, $.10^{-6}$ and 2.10^{-5} M of $HgCl_2$ (Cheng and Sullivan, 1984). *In vitro* studies also showed an effect of mercury at higher concentrations: increasing haemocyte mortality was detected in *Mya arenaria* L., *Mya truncata, M. edulis* and *Mactromeris polynyma* at a concentration of 10^{-3} M and higher (Brousseau *et al*., 2000; Sauvé *et al*., 2002). Triforine, a fungicide, induced decreased haemocyte viability in *C. virginica* (Alvarez and Friedl, 1992).

Enzymatic activities

After 24 hours of incubation, aminopetidase (AP) positive haemocytes from *Crassostrea gigas* showed increased enzyme activity with increasing mercury concentrations (Gagnaire *et al*., 2004). Aminopeptidase is involved in the generation of MHC class I-presented peptides by trimming N-extended precursors into peptides of the correct length to be presented on class I molecules (Rock *et al*., 2002; York *et al*., 2002). Further studies are needed to elucidate the specific role of AP in the invertebrate defence system. Aminopeptidases are hydrolytic enzymes first recorded in the haemolymph of *Biomphalaria glabrata* and *Crassostrea virginica* (Cheng *et al*., 1978). These enzymes belong to the humoral immunity and are known to degrade surface proteins of parasites (Cheng, 1983).

Organophosphorous compounds and carbamates including paraoxon and carbaryl are known to inhibit acetylcholinesterase (AChE) and carboxylesterase (CE) (Cooreman *et al*., 1993). Paraoxon inhibited the activity of AChE in the hepatopancreas of *M. edulis in vitro* at concentrations ranging from 1 µM to 1 mM (Ozretic and Krajnovic-Ozretic, 1992). Inhibition by carbaryl was less distinct. AChE from *M. edulis* haemocytes was inhibited *in vitro* by 0.1–3 mM paraoxon, eserine and DFP (Galloway *et al*., 2002). However, cholinesterases found in *C. gigas* appeared to be insensitive to organophosphorous insecticides (Bocquene *et al*., 1997).

Lysosomal stability

Effects of anthracen (PAH) on the integrity of lysosomal membranes of *M. edulis* digestive cells were reported following an injection of 1 to 5 mM (Moore *et al*., 1978). Lysosomes are organelles capable of ingesting and degrading macromolecules of intra- and extracellular origin, including chemical contaminants and therefore an important target of contaminants (Moore *et al*., 1978; Lowe *et al*., 2000; Moore, 2002). Another study reported an *in vivo* effect of 500 nM fluoranthen on neutral red retention time of lysosomes (Lowe *et al*., 1995). The effects of PCB 153 and 77 on haemocytes of *M. galloprovincialis* were tested *in vitro*. Only PCB 153 induced a release of lysosomal enzymes at a concentration of 30 µM (Canesi *et al*., 2003). Among all haemocyte parameters tested, lysosomal functions (also esterase activity) appeared to be most sensitive to pollutants. As demonstrated before, functions of lysosomes are reliable biomarkers for cellular integrity (Moore *et al*., 1978; Lowe *et al*., 2000; Moore, 2002).

Phagocytosis

At lower concentrations than 10^{-3} M, cadmium did not inhibit phagocytic activity of *Tapes philippinarum* haemocytes (Matozzo *et al*., 2001). Another study reported increased phagocytosis at a mercury concentration of 4.10^{-7} M and dose-dependent decrease at 2.10^{-6}, 4.10^{-6} and 2.10^{-5} M (Cheng and Sullivan, 1984). A mercury concentration of 10^{-6} M also caused a decrease of haemocyte phagocytosis in *Mya arenaria* L., *Mya truncata, M. edulis* and *Mactromeris polynyma* (Brousseau *et al*., 2000; Sauvé *et al*., 2002). Exposure studies of a mixture of anthracen, fluoranthen and phenanthren showed a decrease of phagocytosis and damages to lysosomes of *M. edulis* with concentrations ranging from 40 to 250 µM (Grundy *et al*., 1996a; 1996b). Chlordan, an insecticide, demonstrated effects on *C. virginica* haemocyte phagocytosis at 250 µM *in vitro* (Larson *et al*., 1989). Triforine, a fungicide, induced a decrease of phagocytic activity of *C. virginica* haemocytes (Alvarez and Friedl, 1992). A pesticide mixture (alachor, metolachlor, terbutylazine, glyphosate, diuron, atrazine, carbaryl and fosteyl aluminium) representative for surface waters of the Marennes-Oleron Basin (0.25 nM to 4 nM) induced an increase of phagocytic activity (Gagnaire, personal comm.).

Respiratory Burst (RB)

In *M. edulis* an *in vivo* exposure of 1 to 2 µM fluoranthen (PAH) for 7 days decreased the number of total circulating haemocytes, their ROS production and release of peroxidase and phenoloxidase (Coles *et al*., 1994). Other studies presumed peroxidase release and ROS production (both mechanisms are part of the oxidative burst) as possible targets for PAHs (Coles *et al*., 1994; Gomez-Mendikute *et al*., 2002). Haemocytes of *Mytilus galloprovincialis* exposed to BaP, (HAP, 2-150 µM) for 1 hour showed an increase of superoxide anion production, a decrease of neutral red retention time and a disruption of cytoskeletal actin filaments (Gomez-Mendikute *et al*., 2002). Naphthalene induced a decrease of ROS-induced chemoluminescence of haemocytes in *C. virginica* at concentrations from 80 to 800 µM (Larson *et al.,* 1989). Generation of superoxide anions was modulated by paraoxon (insecticide) (Gagnaire, personal comm.). PCB 77 increased the generation of ROS by *Paracentrotus lividus* coelomocytes, whereas PCB 153 had no effect (Coteur *et al*., 2001). Pentachlorophenol decreased the production of ROS by the inhibition of NADPH production in *C. virginica* (Baier-Anderson and Anderson, 1996). Dieldrin, tested *in vitro* on *C. virginica* haemocytes induced a decrease of chemoluminescence at concentrations ranging from 3 to 300 µM (Larson *et al*., 1989). Haemocytes of *C. virginica* exposed to chlorothalonil (fungicide) for 20 h at concentrations between 4 nM and 2 µM showed no modification of cell mortality and phagocytosis, but a decrease of ROS production (Baier-Anderson and Anderson, 2000).

A lot of studies indicated additive and antagonistic effects of mixture toxicity, for example the presence of zinc or cadmium can decrease the toxic effects of mercury (Gutierrez-Galindo *et al*., 1981; Breittmayer *et al*., 1984). Additional studies on effects of contaminant mixtures as they occur in most of the estuarine areas where bivalve cultures are located are of specific interest. Another point of interest is the question if pollutants increase the susceptibility to diseases. Chronic exposure of *Crassostrea virginica* to TBT has been shown to increase the progression of *Perkinsus marinus* infection (Anderson *et al*., 1996). Anderson *et al*. (1981) also demonstrated that *Mercenaria mercenaria* exposed to PCP were unable to kill injected bacteria.

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Annex 8: On the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints

by W. Wosniok, K. Broeg, S.W. Feist

Introduction

Why an index?

The purpose of a "health index" in the present context (WGPDMO 2004a, p.17–18) is to summarize information on the health status of marine organisms. While the original information on health status is expressed by several (many) quantities, an index is expected to represent the most relevant information by one (or at most few) number(s) or category(ies). Such an index should facilitate the interpretation of measurements as well as the communication about health status based on a broad range of information. It could also be a monitoring criterion itself, or monitoring results could be presented in a concise way via such an index. The index could also be a target quantity on the basis of which spatial comparisons and trend assessments could be performed.

A health index of this kind is closely related, if not identical to what is elsewhere called an "indicator" – see e.g. ICES (2004b), Annex 5, Nicholson and Fryer (2002). The general considerations about desirable properties as set forth there apply to the present problem as well, so we refer to these sources. However, the problem of formulating a suitable health index in concrete terms remains.

This contribution describes approaches of index constructions, some technical requirements on the quantities that are candidates to contribute to an index, and discusses some indices that have been proposed and applied. The selection of indices presented is neither exhaustive nor representative, but was made to illustrate the variety of approaches.

General requirements

Most of the proposed health indices require, sometimes implicitly, that

- index components (the quantities contributing to the index) must exhibit a monotone relation between exposure and response;
- this relation must be biologically plausible;
- index components are oriented or can be oriented by a transformation in the same way or (e.g. higher value means stronger response).

Deviations from the third requirement may be accounted for by the index construction. However, violations of the first requirement are in general disastrous to the usefulness of an indicator. They can be compensated by the index construction only under very special conditions.

An index should generally

- provide a proper summary of the situation (no loss of important information);
- should be objective (not depend on the researcher's personal taste how to deal with the measurement results obtained);
- be calculable from accessible data.

General construction principles

The basis of most index constructions is a set of measured quantities like concentrations, enzyme activities, disease status, or categorical attributes. Other indices are derived from only one quantity, for which a time series of observations is available. A mixture of longitudinal (time series) and cross-sectional data could also form the basis of an index construction. In all situations it is the task of the index to highlight important aspects of the data.

Obviously, the decision about which quantities to use for index definition is crucial. It depends on the intended use of the index and requires support by biological reasoning as well as some mathematical-statistical considerations.

In simple (and most) cases an index is formally a weighted sum of measurement results. The weights may be negative to account for orientation of the quantity (e.g. to make larger values indicate stronger response). Usually, measurements are required to lie on the same scale (e.g. all measurements continuous, like concentrations). Mathematical transformations may be used to generate a common scale for all components. Also, standardization procedures are frequently used prior to index calculation in order to prevent the dominance of a component over others as a simple consequence of the units in which they are measured.

Proposed indices differ in the weights they assign to the original measurements and in the rationale that led to these weights. Usual principles to derive weights are

- Parameters expressing the presence of a worse health state are assigned a higher weight compared to those expressing a less severe state.
- Weights are chosen such that the constructed index preserves as much as possible of the variation in all measurements.
- Weights are chosen such that the constructed index has maximum ability to predict adverse effects (disease, loss of reproduction ability, death).

Obviously there is much room to cast any of these principles into a formal rule, with, unfortunately, relatively few guidance to do so. The first principle requires more biological knowledge than is often available. The second one can be followed relatively easy, but may fail to generate a generally relevant index (see 0). The third principle is likely to extract the most useful information out of the basic parameters, but the data base required for its derivation is frequently not available.

The following sections describe some published indices and their properties as well as the construction principles used to follow the principles 2 and 3.

A selection of published proposals for health indices

Health Assessment Index (Adams *et al.*, 1993)

Definition

The Health Assessment Index (HAI) is a quantitative index that allows statistical comparisons of data sets. Index variables are assigned numerical values based on the degree of severity or damage incurred by an organ or tissue from environmental stressors. To assign a numerical value of condition to each variable within the HAI, all variables are first given a field code designation according to the original necroscopy classification criteria of Goede and Barton (1990). All codes that represent a normal condition are replaced by a zero, and all codes representing an abnormal condition or anomaly are replaced by a value of 30. To account for differences in severity of damage or level of effect, some variables of the HAI are assigned values of 10, 20, or 30, depending on the extent of the observed damage. The HAI is calculated for each fish within a sample by summing the numerical values of all variables. The HAI for a

sample population is calculated by summing all individual fish HAI values and dividing them by the total number of fish.

Properties

The calculation of this index is based on the diagnostic study of nearly all organs of each individual fish which gives complete information about the health status of the respective population. The grading of severity of damage follows well known and defined pathological criteria. Even though the necroscopy classification criteria are explained in detail, some subjective aspects remain. Nevertheless the HAI has been proven to give consistent results in several studies, using different fish species at locations with different qualities of environmental contamination. It is used by the Tennessee Valley Authority (TVA) to evaluate the general health status of fish in a wide range of reservoir types in the entire Tennessee valley (North Carolina, Tennessee, Alabama, Kentucky).

Biomarker index (Narbonne *et al.*, 1999)

Definition

The biomarker index proposed by Narbonne *et al*. (1999) is defined as a sum of "index values" (scores) which are assigned to the original measurements via the following steps.

a) Calculate mean and confidence interval of the biomarker for the whole experiment (e.g. a cruise). The authors state here to calculate confidence intervals with a level of 0.05. Presumably they specify the proportion of probability mass outside the confidence interval, otherwise they would use extremely unusually small intervals.

b) Calculate the site-specific means.

c) Calculate the response range RR, defined as (maximum of site specific means) – (minimum of site-specific means). The authors refer to "higher and "lower" means without further specification – the interpretation as maximum and minimum is due to the present authors.

d) Calculate the discriminatory factor DF = (RR + CI) / CI. The authors define DF = RR + CI / CI, which is identical to DF = RR +1, which would make DF contain the same information as RR. Also, CI is presumably to be understood as the width of the confidence interval, not the interval itself (which would give no sense).

e) Use the DF as new measurement unit and express biomarker values in DF units, using the smallest site-specific mean as origin.

f) Assign a "response index" RI to the DF units, where the RI values for a DF value are given by a table, which is specific for the range of DF values.

To characterize a site at which several biomarkers have been measured, the RI values of the markers involved are added.

It should be noted that the paper by Narbonne *et al*. (1999) lacks some details and seems to include errors as indicated above. Therefore the present summary might contain misinterpretations.

Properties

The index attains values that are independent of the underlying original scales, which is desirable. It allows the comparison of stations for which several markers are available within an "experiment". However, the ranking and scoring system depends on the ranges found in the "experiment". Future results may not fit into this system and therefore require to set up a new scaling, including a re-calculation of previous results.

A severe drawback is that the final scoring (the assignment of RI values) seems arbitrary. No particular merits of the scoring system in their Table 1 are highlighted. Though the authors

state as the aim of the paper "to validate the use of a biomarker index for the quality assessment of the coastal environment" (p. 419), they do not present criteria for a validation. Their discussion, which ends with saying that "a global biomarker index seems to be able to translate scientific data into useful information to estimate the quality level …. of coastal environments", simply contains some examples in which the biomarker index behaved as the authors expected, but does not mention objective criteria.

Integrated Biomarker Response Index (IBR) (Beliaeff and Burgeot, 2002)

Definition

Observed parameters (biomarkers) are first oriented in the same direction, then standardized to standard deviation unity and mean zero, then the minimum of all transformed values is added. This standardization generates nonnegative values with initial scale differences removed. Then, the standardized values are arranged in a star plot with star radii length equal to the standardized values. Adjacent radii include an angle of 360°/n, where n is the number of measured parameters per station. The ordering of the radii within the star is arbitrary and left to the user. Outer endpoints of adjacent radii are connected by straight lines. The index, called IBR (Integrated Biomarker Response), is defined as the area circumscribed by these lines.

Properties

This index is not simply a weighted sum in which each marker contributes to the index value via a fixed weight; instead the contribution of each measurement to the IBR depends on the values of the two measurements which happen to be its neighbours in the star plot. This means that, in general, reordering the markers in the star changes the IBR value. Unfortunately, no argument is given why the impact of a measurement on the IBR should not so generally depend on the values of its neighbours in the plot, nor in what order the markers should be arranged. As there is no natural "order" among biomarkers, a considerable freedom remains to the IBR users. An unfortunate consequence of this freedom is not only that the same biomarker data can be summarized by different IBR values, but that even the rank order of the IBRs for different stations can be reversed by choosing different orderings in the star. The following Table 1 gives an example, with the corresponding star plots given in Figure 1.

Table 1: Fictitious data from 3 stations and resulting IBR values for 2 different orderings of the radii in the star plot: ordering 1 (left part of table): A – B – C – D, ordering 2 (right part of table): A – C – B – D.

	Star radius direction	North	East	South	West		North	East	South	West	
Stat. No.	Biomarker assigned	A	B	C	D	IBR	A	C	B	D	IBR
		Measurements					Measurements				
1		1	2	0	0	**1.0**	1	0	2	0	**0.0**
2		1	0	4	0	**0.5**	1	4	0	0	**2.0**
3		1	0	0	1	**1.0**	1	0	0	1	**0.5**

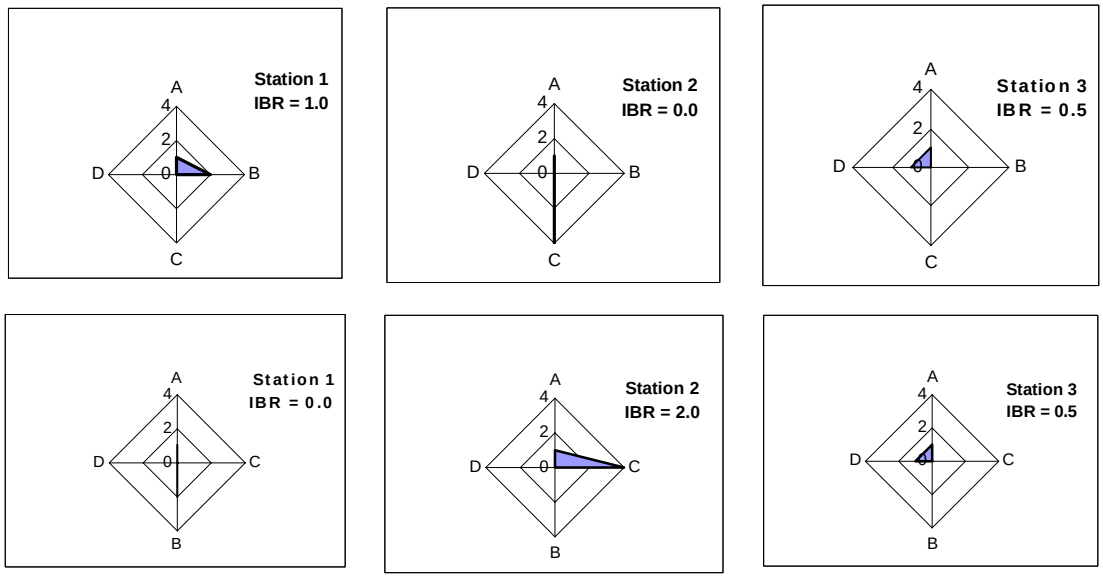

Figure 1: Star plots for the fictitious data from Table 1. Upper row: radii ordering 1, lower row: radii ordering 2.

The example in Table 1 and Figure 1 shows that a fixed set of measurement may lead to different IBR values as a consequence of a (slight) change in the ordering of the radii. Moreover, even the rank order of the stations has been reversed: under ordering 1, IBR values increase in the sequence station 2 / 3 / 1, while under ordering 2 the station sequence is 1 / 3 / 2. This means that IBR values depend to a high degree on the ordering of the radii, for which no common rule is available. Hence, the IBR should be used only, if there is a natural ordering of the radii, and if the fact that each marker affects the IBR only in cooperation with its neighbours in the star, is a desired property.

Similar restrictions apply to the use of a star plot as a graphical display technique for biomarkers, as the observer's eye is likely to be impressed mainly by the area include between the radii, which has the very restricted usability outlined above, while the only genuine information in the plot, namely the radii lengths, attracts much less attention.

Biomarker Index (Chèvre *et al.*,2003)

Definition

This index summarizes the values of 6 biomarkers (MT, DNA, LPO, Vn, PHAG, NspE) by adding their group ranks, where groups are formed by an entropy based algorithm (cut points are chosen such that the loss of information due to grouping is minimal) and the resulting groups are assigned ranks. In this way the problem of different measurement scales is overcome. The authors justify the assignment of equal weights to all components by saying that "it is difficult to justify the selection of weight values" (p. 289).

Properties

The first part of the construction principle (transforming measured / observed data to group ranks) is a useful general principle. The application of this principle to the 6 biomarkers of the study is specific for the purpose of that study. The justification for the choice of weights seems somewhat preliminary. By its construction, after having fixed the index components, the index depends only weakly on the researcher's subjective decisions via details of the grouping step.

The authors use their index to compare their sampling sites and discuss spatial and temporal differences. As significant differences are found, the authors consider their index a useful tool. However, arguments for its appropriateness on a broader scale outside the (freshwater) region of their study are not given.

It should be pointed out that the definition of the Biomarker Index is data-dependent in the sense that if the data set were increased by later measurements of the same type, then all Biomarker Index values including the old ones are likely to change. The reason is that the grouping of data via the entropy principle would hardly be the same for the augmented data set. Maintaining the grouping would avoid this problem, but this is possible only if new data fell into the initial data ranges.

The Biotic Coefficient/Biotic Index (BC, BI) (Borja *et al.*, 2000)

Definition

The biotic coefficient (BC) and biotic index (BI) are the continuous and discrete versions of a characterization of benthic communities. The basic data for their construction is the percentage of benthic species in the communities studied, where species are categorized into 5 groups according to their way of reaction to contamination. Groups are ordered in the sense that group I contains species which are present under "unpolluted conditions", group V contains species which accept "pronounced unbalanced" situations, and the remaining groups show intermediate forms of reaction between these two. The BC is then calculated as a weighted sum of the percentages, whereby, interestingly, group I is assigned a weight of zero, i.e. plays no role for the BC. The weights increase with group number (i.e. the amount of pollution tolerated by the species), but their particular values are given without further reasoning. The BI is derived as a system of 8 integer numbers, which represent a grouping of the BC. They do not contain any more information than the BC, in fact they contain less. However, the BI values are given short descriptions between "normal" and "azoic" as an interpretation aid.

Properties

Assuming that the authors used the correct assignment of species to the 5 groups in the sense that the species lives nearly exclusively under the degree of pollution assigned to its group, then every increasing set of weights for the groups produces an index the value of which increases with pollution. The weight values used in the BC definition of Borja *et al*. select a certain one among the infinity of possible rank orderings, but no particular merits of these weights or of the resulting index are presented. The authors calculate their index for measurements of various locations in Europe and demonstrate that these values lie in range they expected.

The Bioeffect Assessment Index (BAI) (Broeg *et al.*, 2003; Broeg *et al.*, 2005)

Definition

The "Bioeffect Assessment Index" (BAI) is based on the integration of several pathological endpoints measured in the liver of European flounder (*Platichthys flesus* (L.)). The BAI represents a modification of the "Health Assessment Index" (section 0) since it includes solely validated biomarkers reflecting toxically-induced alterations at different levels of biological organisation in order to quantify the effects of environmental pollution. The concept of the BAI is based on the observation of progressive deleterious effects from early responses to late effects. Specific "key events" were detected, representing progressive stages of functional deterioration. The biomarkers selected for the BAI calculation reflect deleterious effects of various classes of contaminants such as heavy metals, organochlorines, pesticides, PAHs, and therefore reflect general toxicity in an integrative manner. Selected biomarkers were: lysosomal perturbations (reduced membrane stability), storage disorders (lipid accumulation) as

early markers for toxic effects of liver cells, and the size of macrophage aggregates and their acid phosphatase activity. The latter two markers are indicative for the modulation of non-specific immune response which represents longer time scale responses after chronic exposure.

Properties

The use of the BAI allows:

- statistical comparison of large data sets obtained from sampling of different geographical areas;
- integration of different parameters by substituting each parameter value according to the progression of functional deterioration with a numerical value: 10 = stage 1, 20 = stage 2, 30 = stage3, 40 = stage 4;
- the calculation of individual BAI values and mean values for the single locations by summing up all individual fish BAI values and dividing them by the total number of fish in the sample, with higher BAI values indicating a poorer health condition.

The BAI has also successfully applied on other fish species (eelpout, *Zoarces viviparous*) and blue mussel (*Mytilus edulis*).

Index construction via multivariate approaches

Definition

Multivariate procedures like principal components analysis (PCA), factor analysis, multidimensional scaling (MDS), discriminant analysis, canonical correlation, Partial Least Squares (PLS) and the large class of Generalized Linear Models (GLM) are frequently used to derive one or more indices that summarise(s) in one (or few) number(s) the information originally provided by a set of many variables. All the methods mentioned, which are far away from constituting an exhaustive list, generate representations of originally high-dimensional data in few dimensions (the indices). They follow different principles to do so, depending on the type of data and the particular problem under study.

PCA, factor analysis and MDS operate on a single set of variables with no distinction between "dependent" and "explaining" variables, where MDS constructs a spatial representation of data that is given as a set of distances between objects. The principle to generate low-dimensional representations here is to calculate new (hopefully few) variables (principal components, factors, canonical variates, the name depending on the method) such that each of the new variables contains as much information as possible and that there is no redundancy within the new variables..

Canonical correlation, discriminant analysis, PLS and GLMs use a distinction between "dependent" and "explaining" variables and derive relationships between these two groups. Here the principle is to derive low-dimensional representations which capture as much explanatory value as possible, and, where more than one new variable is generated, the new ones contain no redundancy.

In all these cases, the new variables are calculated as weighted sums of the original variables, where the weights are optimal in the sense that the resulting new variables are the best ones possible among all weighted linear sums to summarize variation (PCA, factor analysis), to discriminate between groups (discriminant analysis), to establish a low-dimensional map (multidimensional scaling), or to "explain" (predict) the "dependent" variable(s), respectively.

Usually, a representation of data in fewer dimensions than originally measured goes along with a loss of information. The multivariate methods mentioned allow quantifying this loss by calculating the amount of total information that is represented by each of the derived new

variables. If one or two new variables are sufficient to jointly represent a major part of the original information, a convenient graphical display of the data is possible.

Properties

An index constructed by a multivariate approach has the advantage that its inherent weights are uniquely defined. There is no need to discuss the weights which define the new variables, as these are the optimised by the statistical method. This optimality holds, however, only for the data set from which the index was constructed. So, a multivariate approach might well provide a good representation of the data under study, but a transfer to other data requires additional justification.

A formal criterion like "explain as much variation as possible" does not mean the same as "represent as much of what is biologically relevant". For the methods not distinguishing between explaining and dependent variables, the aspect of biological relevance can be introduced into the construction of the index only via the selection of the variables that enter the procedure. For the other methods, biological relevance is usually introduced to the statistical method by the selection of the "dependent" set of variables, e.g. by deriving the index from a problem of the type "Which linear combination of explaining variables explains best the appearance of disease Y?"

Summary and outlook

The availability of a single health index (or few such indices) to summarize a set of many individual variables, which each describe different aspects of health, is highly desirable. Several indices have been proposed, also some approaches how an index could be developed.

Technically, nearly all of these indices are weighted sums of the original variables, mostly after some standardization. However, the weights involved are either tailored for specific questions, or are given without arguments that support a general appropriateness. This means that no index among those discussed qualifies immediately for universal use. Instead, it will be necessary to construct an index appropriate for its specific purpose, where different purposes will in general require different components that contribute and possibly even different technical ways of construction.

There seem to be two major ways to derive a useful health index in the framework of the WGPDMO activities: after identification of biological parameters that describe the relevant aspects of health, definition of an index by assigning weights to these parameters

- on the basis of biological reasoning, i.e. expert's assessment,
- or on the basis of a formal derivation e.g. by PCA,

and calculating the index as a weighted sum.

Considering the example task to develop a health index for dab (*Limanda limanda*) with the purpose to quantify the proportion of diseased fish, there are relatively few parameters for which long and regularly updated time series are available: the prevalence of 4 diseases and of 2 major parasite species. As long as no arguments favour a different way to proceed, an initial health index could be simply the mean of all prevalences. If there are indications that some of these 6 parameters have a larger impact than others on the health of dab, the weights could be modified accordingly, possibly involving one of the statistical methods from section 0.

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Annex 9: Status of chemical and biological effects monitoring in the OSPAR Coordinated Environmental Monitoring Programme (CEMP), with emphasis on fish disease aspects (OSPAR Ref. No. 2004–16)

Table 1: Status and components of the OSPAR Coordinated Environmental Monitoring Programme (CEMP) (grey shading: fish disease monitoring).

	Compounds/ Parameter	Matrix	Status	Guidelines	Quality Assurance	Assessment Tools
Chemical monitoring	Hg, Cd, Pb	Biota	Mandatory	JAMP Contaminants in Biota, Tech. Ann. 2	QUASIMEME	B/RC, EAC
		Sediments		JAMP Contaminants in Sediments, Tech. Ann. 5/6		
	PCB	Biota		JAMP Contaminants in Biota, Tech. Ann. 1		
		Sediments		JAMP Contaminants in Sediments, Tech. Ann. 2		
	PAH	Biota		JAMP Contaminants in Biota, Tech. Ann. 1		
		Sediments		JAMP Contaminants in Sediments, Tech. Ann. 3		
	TBT	Sediments		JAMP Contaminants in Sediments, Tech. Ann. 4		EAC
	Nutrients	Seawater		JAMP Eutrophication Monitoring		Developed
Monitoring of direct and indirect eutrophication effects	Chlorophyll, Phytoplankton species composition, Macrophytes, Oxygen, Benthic communities	Seawater, Sediments	Discretionary in non-problem areas, mandatory in potential problem areas	JAMP Eutrophication Monitoring	QUASIMEME, SGQAE, BEQUALM	Developed
Monitoring of PAH-specific biological effects	CYP1A	Fish liver	Awaiting outcome of development of QA	JAMP Contaminant-specific Biological Effects, Tech. Ann. 2	Under development in BEQUALM	-
	DNA adducts	Fish liver				
	PAH metabolites	Fish bile				
	Liver pathology	Fish liver	Awaiting outcome of development of QA	JAMP Contaminant-specific Biological Effects, Tech. Ann. 2	Under development in BEQUALM	-
Monitoring of metal-specific biological effects	Metallothionein	Fish liver	Awaiting outcome of development of QA	JAMP Contaminant-specific Biological Effects, Tech. Ann. 1	Under development in BEQUALM	-
	ALA-D	Fish red blood cells				

	Compounds/ Parameter	Matrix	Status	Guidelines	Quality Assurance	Assessment Tools
Monitoring of TBT-specific biological effects	Imposex, Intersex	Gastropods	Mandatory	JAMP Contaminant-specific Biological Effects, Tech. Ann. 3	BEQUALM	-
Monitoring of general biological effects	Whole sediment bioassays	Mortality in invertebrates	Awaiting outcome of development of QA	JAMP General Biological Effects, Tech. Ann. 1	[BEQUALM]	-
	Sediment pore-water and elutriate bioassays	Abnormal development, effects on mortality and reproduction in invertebrates		JAMP General Biological Effects, Tech. Ann. 2/3		
	Water bioassays	Abnormal development, effects on mortality and reproduction in invertebrates		JAMP General Biological Effects, Tech. Ann. 4		
	CYP1A	Fish liver		JAMP General Biological Effects, Tech. Ann. 5		
	Lysosomal stability	Fish liver		JAMP General Biological Effects, Tech. Ann. 6		
	Liver neoplasia/hyperplasia	Fish liver	Voluntary	JAMP General Biological Effects, Tech. Ann. 7	BEQUALM	-
	Liver nodules	Fish liver		JAMP General Biological Effects, Tech. Ann. 8		
	Externally visible fish diseases	Skin surface		JAMP General Biological Effects, Tech. Ann. 9		
	Reproductive success in fish	Fish	Awaiting outcome of development of QA	JAMP General Biological Effects, Tech. Ann. 10	[BEQUALM]	-

Table 2: Fish disease monitoring in the OSPAR Coordinated Environmental Monitoring Programme (CEMP).

Table 2a: PAH-specific biological effects monitoring				
	Species	**Diseases**	**Numbers**	**Guidelines**
Liver Pathology	Dab (1st priority) (*Limanda limanda*)	General non-specific lesions, necrotic/degenerative change Unique degenerative lesions Histopathological changes indicative of affected storage conditions Inflammatory changes Non-neoplastic proliferative lesions Vascular abnormalities Foci of cellular alteration Benign neoplasms Malignant neoplasms	Size group 20-24 cm : 50	*JAMP Guidelines based on:* ICES 1997. Report of the Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants. ICES CM 1997/F:2. *Relevant in addition:* Feist *et al*. 2004. Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES TIMES 38, 42 pp. BEQUALM
	Flounder (*Platichthys flesus*)		Size group 25-30 cm : 50	

Table 2b: General biological effects monitoring				
	Species	**Diseases**	**Numbers**	**Guidelines**
Externally visible fish diseases	Dab (1st priority) (*Limanda limanda*)	Lymphocystis Epidermal hyperplasia/papilloma Acute/healing skin ulcers X-cell gill disease Hyperpigmentation	Size group 15–19 cm: 100 Size group 20–24 cm: 100 Size group ≥ 25 cm : 50	*JAMP Guidelines based on:* Bucke *et al*. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES TIMES No. 19. *Relevant in addition:* ICES 1989. Methodology of fish disease surveys. ICES Coop. Res. Rep. 166. BEQUALM
	Flounder (*Platichthys flesus*)	Lymphocystis Acute/healing skin ulcers	Size group 20–24 cm: 100 Size group 25–29 cm: 100 Size group ≥ 30 cm: 50	
	Cod (*Gadus morhua*)	Acute/healing skin ulcers Skeletal deformities Pseudobranchial swelling *Cryptocotyle sp.*	Size group < 29 cm: 100 Size group 30–44 cm: 100 Size group ≥ 45 cm: 50	
Liver nodules	Dab (1st priority) (*Limanda limanda*)	Macroscopic liver nodules > 2 mm in diameter, subsequent quantification of histologically identified liver neoplasms	Size group ≥ 25 cm: 50 (if not available in sufficient numbers, include size group 20–24 cm)	*JAMP Guidelines based on:* Bucke *et al*. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES TIMES No. 19. *Relevant in addition:* ICES 1989. Methodology of fish disease surveys. ICES Coop. Res. Rep. 166. Feist *et al*. 2004. Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES TIMES 38, 42 pp. BEQUALM
	Flounder (*Platichthys flesus*)		Size group ≥ 30 cm: 50 (if not available in sufficient numbers, include size group 25–29 cm)	
Liver neoplasia/ hyperplasia	Dab (1st priority) (*Limanda limanda*)	Unique degenerative change *(hepatocellular and nuclear polymorphism, megalocytic hepatosis/hepatic megalocytosis, hydropic vacuolization of biliary epithelial cells and/or hepatocytes)* Foci of cellular alteration Benign tumours Malignant tumours	Size group 20–24 cm : > 30	*JAMP Guidelines based on:* ICES 1997. Report of the Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants. ICES CM 1997/F:2. *Relevant in addition:* Feist *et al*. 2004. Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES TIMES 38, 42 pp. BEQUALM
	Flounder (*Platichthys flesus*)		Size group 25–35 cm : > 30	

Annex 10: Data on the prevalence and type of fish diseases for the period 1984 to 2004 for the REGNS Integrated North Sea Assessment Workshop (9–11 May 2005)

Introduction

The regular and systematic monitoring of diseases and parasites of North Sea fish started in the late 1970s. Depending on the availability of funding and experts, most of the North Sea countries have been carrying out more or less regular fish disease surveys (Lang, 2002). At present, most activities are being done by Germany, The Netherlands and the UK.

Fish disease surveys in the North Sea are largely focused on the common dab (*Limanda limanda*), which is, based on the experience made in long-term monitoring, the most appropriate species for large-scale monitoring in the North Sea because of its abundance, wide geographical distribution and susceptibility to diseases.

In most national programmes, the regular surveys are targeted on the inspection of fish for grossly and externally visible infectious and non-infectious diseases and parasites. While some of the diseases/parasites were recommended by ICES for fish disease surveys (ICES, 1989; Bucke *et al*., 1996), others were added to the national programmes because of their prevalence and relevance. The selection of diseases is largely based either on the known or suspected responsiveness of the diseases/parasites to environmental factors (including contaminants) or on their potential impact on fish stocks.

In recent years, fish diseases studies were extended to studies on liver histopathology, encompassing a quantification of early and late neoplastic and non-neoplastic liver lesions (Feist *et al.*, 2004).

Standard methodologies involved in fish disease surveys in the North Sea have been developed by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) (ICES, 1989; Bucke *et al*., 1996, Feist *et al.*, 2004) and have been intercalibrated repeatedly between institutes involved, e.g. through two sea-going workshops organised in 1984 and 1988 (Dethlefsen *et al.*, 1986; ICES, 1989) and, more recently, within the formerly EU-funded and now self-financing Biological Effects Quality Assurance in Monitoring (BEQUALM) programme (www.bequalm.org) .

Disease prevalence data generated based on the standardised ICES methodology are submitted by ICES Member Countries to the Fish Disease Databank of the ICES Environmental Database and standard operating procedures for data submission, validation and statistical analysis have been developed by ICES WGPDMO in close collaboration with the ICES Secretariat (Wosniok *et al*., 1999; Lang and Wosniok, 2000).

A number of data assessment on spatial and temporal pattern in disease prevalence as well as on the relationship between disease prevalence and potentially explaining parameters have been carried out, using ICES fish disease data in combination with data extracted from the ICES fisheries, oceanography and environmental databases (Wosniok *et al*., 2000; Lang and Wosniok, 2000).

Fish disease data to be used for the REGNS Integrated North Sea Assessment

Figure 1 provides an overview of disease data for the common dab (*L. limanda*) from the North Sea and adjacent areas available in the ICES Environmental Database by ICES statistical rectangle. Table 2 gives details regarding the temporal coverage (5 years intervals) for

each of the rectangles. In total, data from almost 500,000 dab are included in the database at present. Some data were obtained in areas far outside the North Sea (e.g. at Iceland). Besides information on the presence of diseases, the ICES data include information on length and sex of the fish.

The disease data sets submitted to the ICES Database by country are listed in Table 3. Countries involved are Denmark, Germany, The Netherlands and the UK. WGPDMO's input to a table distributed by REGNS to relevant ICES Working Groups prior to their meetings is provided in Table 4.

In order to use the most complete and consistent fish disease data set for the REGNS Integrated Assessment, the WGPDMO recommends to only use the data specified in Table 1, which will be readily available from the ICES Environmental Databank for the REGNS Integrated Assessment Workshop, 9–11 May 2005

Table 1. Fish disease data to be used in the ICES Integrated Assessment of the North Sea Ecosystem

Region:	North Sea
Time period:	1984–2004
Species:	*Limanda limanda*
Diseases:	Lymphocystis Epidermal Hyperplasia/Papilloma Acute/healing skin ulcerations X-cell gill disease
Data sets:	Denmark, Germany, The Netherlands, UK combined

Note of caution:

Since the ICES fish disease data are derived from national monitoring programmes that use different sampling strategies, they are based on observations done in different areas, seasons (which are known to affect the prevalence) and not in all years of the period 1984–2004. Some of the national programmes started after 1984, some were modified or even terminated so that not all data sets cover the whole period 1984–2004 (for details, see Table 2).

Despite these shortcomings which have to be taken into account when selecting data for the Integrated Assessment, the data represent a unique source of information on biological responses to environmental change, considered to be of great value for the ICES Integrated Assessment of the North Sea Ecosystem.

References

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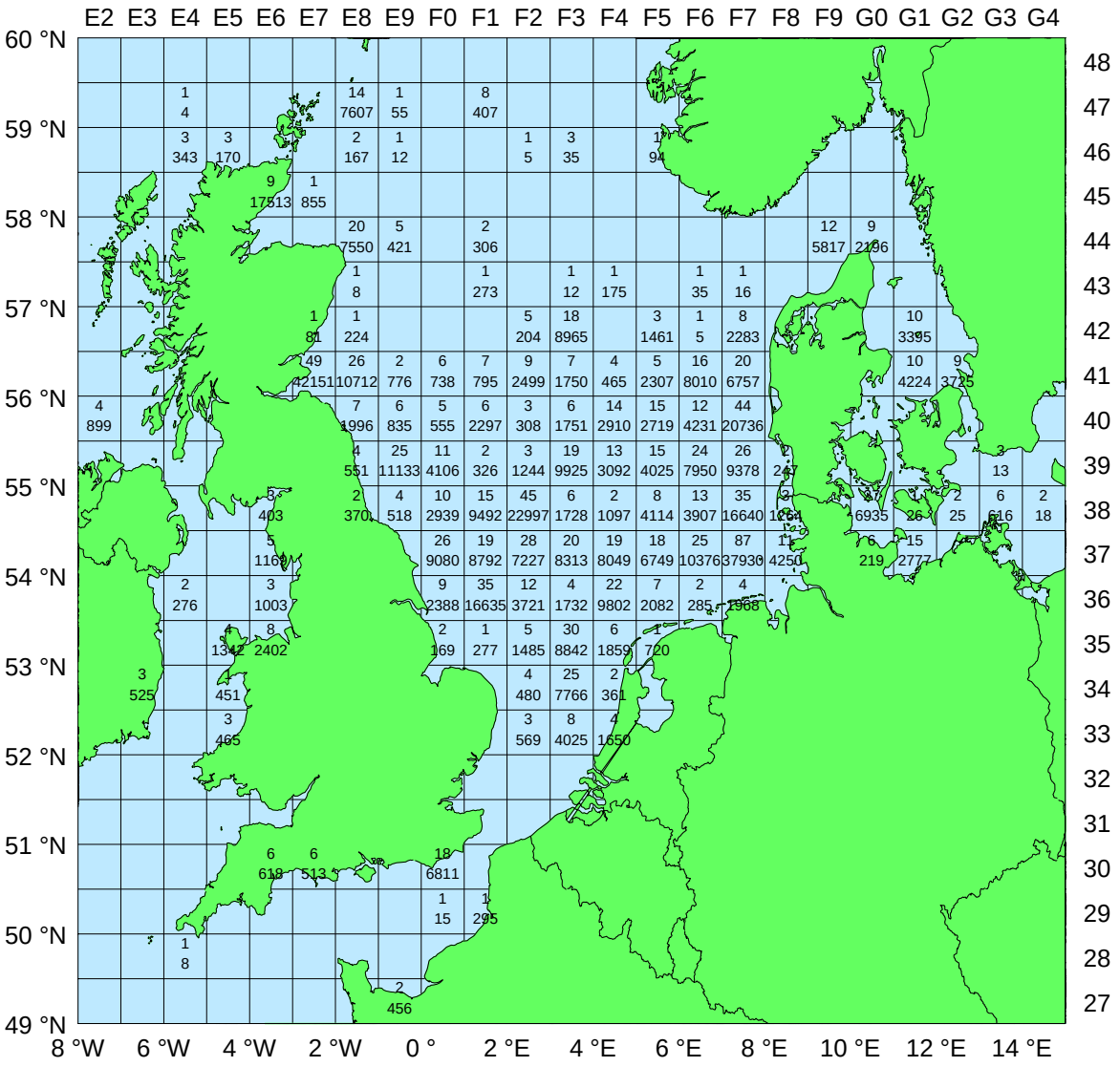

Figure 1: Disease data available for dab (*Limanda limanda*) in the ICES Environmental Databank per ICES statistical rectangle (upper number: number of days with observations in 1981–2004, lower number: number of dab examined).

Table 2: Disease data available for dab (*Limanda limanda*) in the ICES Environmental Databank per ICES statistical rectangle, period 1981 – 2005 (5 year intervals).

	sampling date					
	1981-85	1986-90	1991-95	1996-00	2000-05	All
	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum
ICES rectangle						
27E9	.	.	.	456	.	456
28E4	.	.	8	.	.	8
29F0	.	.	.	15	.	15
29F1	.	.	.	295	.	295
30E6	.	.	291	327	.	618
30E7	.	.	114	399	.	513
30F0	.	.	2114	4161	536	6811
33E5	.	.	.	232	233	465
33F2	.	.	177	174	218	569
33F3	.	2651	444	930	.	4025
33F4	.	.	.	1650	.	1650
34E3	.	.	495	30	.	525
34E5	.	.	.	.	451	451
34F2	14	.	171	.	295	480
34F3	788	1468	3845	1490	175	7766
34F4	.	.	159	202	.	361
35E5	.	.	.	612	730	1342
35E6	.	.	.	1377	1025	2402

(Continued)

Table 2: Lima lim until April 2005: number examined.

	sampling date					
	1981-85	1986-90	1991-95	1996-00	2000-05	All
	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum
ICES rectangle						
35F0	.	.	.	169	.	169
35F1	277	.	.	.	.	277
35F2	732	488	.	.	265	1485
35F3	631	2976	2086	2928	221	8842
35F4	.	669	191	999	.	1859
35F5	.	.	720	.	.	720
36E4	.	.	65	211	.	276
36E6	.	.	.	263	740	1003
36F0	935	196	250	1007	.	2388
36F1	1876	2843	5710	4819	1387	16635
36F2	2537	947	.	.	237	3721
36F3	878	547	307	.	.	1732
36F4	348	3537	2882	3035	.	9802
36F5	69	949	402	662	.	2082
36F6	200	.	85	.	.	285
36F7	1688	280	.	.	.	1968
37E6	.	.	727	164	278	1169
37F0	1794	1281	3249	2537	219	9080
37F1	6122	1883	.	261	526	8792
37F2	2158	1945	2034	852	238	7227

(Continued)

Table 2: Lima lim until April 2005: number examined.

	sampling date					
	1981-85	1986-90	1991-95	1996-00	2000-05	All
	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum
ICES rectangle						
37F3	639	4247	2510	525	392	8313
37F4	.	3555	2439	2055	.	8049
37F5	4336	190	915	1308	.	6749
37F6	6676	2653	275	772	.	10376
37F7	14485	9540	7656	2904	3345	37930
37F8	3469	231	.	550	.	4250
37G0	36	87	.	.	96	219
37G1	109	166	1171	651	680	2777
38E6	.	.	86	317	.	403
38E8	.	.	.	.	370	370
38E9	37	152	.	329	.	518
38F0	873	1860	206	.	.	2939
38F1	4685	279	1606	2660	262	9492
38F2	8357	4468	5297	2630	2245	22997
38F3	1375	200	153	.	.	1728
38F4	.	1097	.	.	.	1097
38F5	2509	1605	.	.	.	4114
38F6	2882	805	.	.	220	3907
38F7	11797	4133	710	.	.	16640
38F8	1264	.	.	.	.	1264

(Continued)

Table 2: Lima lim until April 2005: number examined.

	sampling date					
	1981-85	1986-90	1991-95	1996-00	2000-05	All
	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum
ICES rectangle						
38G0	1391	2366	1050	1352	776	6935
38G1	.	26	.	.	.	26
38G2	.	25	.	.	.	25
38G3	.	.	394	.	222	616
38G4	.	4	14	.	.	18
39E8	.	.	14	234	303	551
39E9	21	3219	4583	2924	386	11133
39F0	2837	409	187	103	570	4106
39F1	.	.	.	326	.	326
39F2	590	.	.	.	654	1244
39F3	3633	2589	1147	2556	.	9925
39F4	1163	544	462	296	627	3092
39F5	2629	616	.	159	621	4025
39F6	4757	2714	479	.	.	7950
39F7	4564	4226	588	.	.	9378
39F8	94	153	.	.	.	247
39G3	3	.	10	.	.	13
40E2	.	.	744	155	.	899
40E8	782	1214	.	.	.	1996
40E9	787	48	.	.	.	835

(Continued)

Table 2: Lima lim until April 2005: number examined.

	sampling date					
	1981-85	1986-90	1991-95	1996-00	2000-05	All
	number of spec. exa	number of spec. exa	number of spec. exa	number of spec. exa	number of spec. exa	number of spec. exa
	Sum	Sum	Sum	Sum	Sum	Sum
ICES rectangle						
40F0	555	.	.	.	.	555
40F1	2085	212	.	.	.	2297
40F2	251	57	.	.	.	308
40F3	782	969	.	.	.	1751
40F4	509	669	431	299	1002	2910
40F5	1313	823	.	98	485	2719
40F6	2494	1654	83	.	.	4231
40F7	2762	9192	5785	2066	931	20736
41E7	.	1617	18825	10218	11491	42151
41E8	.	1939	2154	801	5818	10712
41E9	.	776	.	.	.	776
41F0	148	590	.	.	.	738
41F1	.	408	.	.	387	795
41F2	1062	57	564	.	816	2499
41F3	1195	418	.	.	137	1750
41F4	68	397	.	.	.	465
41F5	2307	.	.	.	.	2307
41F6	3683	2495	1832	.	.	8010
41F7	1132	3194	2431	.	.	6757
41G1	760	1789	1675	.	.	4224

(Continued)

Table 2: Lima lim until April 2005: number examined.

	sampling date					
	1981-85	1986-90	1991-95	1996-00	2000-05	All
	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum	number of spec. exa Sum
ICES rectangle						
41G2	620	1618	1487	.	.	3725
42E7	.	81	.	.	.	81
42E8	.	.	.	224	.	224
42F2	.	2	67	.	135	204
42F3	.	1135	4980	2401	449	8965
42F5	5	1038	418	.	.	1461
42F6	5	.	.	.	.	5
42F7	154	1274	855	.	.	2283
42G1	483	1288	1624	.	.	3395
43E8	.	.	8	.	.	8
43F1	.	.	.	273	.	273
43F3	.	12	.	.	.	12
43F4	.	.	.	.	175	175
43F6	.	35	.	.	.	35
43F7	.	16	.	.	.	16
44E8	.	1577	4159	1814	.	7550
44E9	.	335	74	12	.	421
44F1	.	.	73	.	233	306
44F9	1178	2052	2587	.	.	5817
44G0	230	769	1197	.	.	2196

(Continued)

Table 2: Lima lim until April 2005: number examined.

	sampling date					
	1981-85	1986-90	1991-95	1996-00	2000-05	All
	number of spec. exa	number of spec. exa	number of spec. exa	number of spec. exa	number of spec. exa	number of spec. exa
	Sum	Sum	Sum	Sum	Sum	Sum
ICES rectangle						
45E6	.	.	2980	6835	7698	17513
45E7	.	.	855	.	.	855
46E4	.	.	198	145	.	343
46E5	.	.	59	111	.	170
46E8	.	167	.	.	.	167
46E9	.	12	.	.	.	12
46F2	.	5	.	.	.	5
46F3	.	30	5	.	.	35
46F5	.	.	94	.	.	94
47E4	.	4	.	.	.	4
47E8	.	156	1823	3817	1811	7607
47E9	.	55	.	.	.	55
47F1	.	232	167	8	.	407
55C8	.	.	102	.	.	102
56C8	.	.	149	.	.	149
56C9	.	.	78	.	.	78
56D3	.	.	895	.	.	895
57C7	.	.	615	.	.	615
All	**131608**	**115230**	**118551**	**82185**	**51111**	**498685**

Table 3: Status of fish disease data submissions to the ICES Environmental Database (as of 18 March 2005.

Denmark				
	Fish disease	***Accession Number***	***Status***	***Last Received Date***
	DFHU1984	19960001	Pending ICES	12/01/2005
	DFHU1985	19960002	Pending ICES	12/01/2005
	DFHU1986	19960003	Pending ICES	12/01/2005
	DFHU1987	19960004	Pending ICES	12/01/2005
	DFHU1988	19960005	Pending ICES	12/01/2005
	DFHU1989	19960006	Pending ICES	12/01/2005
	DFHU1990	19960007	Pending ICES	12/01/2005
	DFHU1991	19960008	Pending ICES	12/01/2005
	DFHU1992	19960009	Pending ICES	12/01/2005
	DFHU1993	19960010	Pending ICES	12/01/2005
Germany				
	Fish disease	***Accession Number***	***Status***	***Last Received Date***
	BFCG1981	19970001	Completed	28/01/1997
	BFCG1982	19970002	Completed	01/08/1997
	BFCG1983	19970003	Completed	01/08/1997
	BFCG1984	19970004	Completed	28/01/1997
	BFCG1985	19970005	Completed	01/08/1997
	BFCG1986	19970006	Completed	01/08/1997
	BFCG1987	19970007	Completed	01/08/1997
	BFCG1988	19970008	Completed	01/08/1997
	BFCG1989	19970009	Completed	28/01/1997
	BFCG1990	19970010	Completed	01/08/1997
	BFCG1991	19970011	Completed	01/08/1997
	BFCG1992	19970012	Completed	28/01/1997
	BFCG1993	19970013	Completed	01/08/1997
	BFCG1994	19970014	Completed	28/01/1997
	BFCG1995	19970015	Completed	01/08/1997
	BFCG1996	19970016	Completed	01/08/1997
	BFCG1997	19970017	Completed	01/08/1997
	BFCG1998	19990004	Completed	11/03/1999
	BFCG1999	20010016	Completed	20/11/2001
	BFCG2000	20010017	Completed	20/11/2001
	BFCG2001	20050048	Pending ICES	17/03/2005
	BFCG2002	20050049	Pending ICES	17/03/2005
	BFCG2003	20040159	Pending ICES	17/03/2005
	BFCG2004	20050050	Pending ICES	17/03/2005
The Netherlands				
	Fish disease	***Accession Number***	***Status***	***Last Received Date***
	DGWN1991	19960011	Completed	07/02/1996
	DGWN1992	19960012	Completed	07/02/1996
	DGWN1993	19960013	Completed	07/02/1996
	DGWN1994	19960014	Completed	07/02/1996
	DGWN1995	19960015	Completed	07/02/1996
	DGWN1996	19970018	Completed	01/09/1997
	RIVO1983	19970019	Completed	20/08/1997
	RIVO1984	19970020	Completed	20/08/1997
	RIVO1985	19970021	Completed	20/08/1997
	RIVO1986	19970022	Completed	20/08/1997
	RIVO1987	19970023	Completed	20/08/1997

	RIVO1988	19970024	Completed	20/08/1997
	RIVO1989	19970025	Completed	20/08/1997
	RIVO1990	19970026	Completed	20/08/1997
	RIVO1991	19970027	Completed	20/08/1997
	RIVO1992	19970028	Completed	20/08/1997
	RIVO1993	19970029	Completed	20/08/1997
	RIVO1994	19970030	Completed	20/08/1997
	RIVO1995	19970031	Completed	20/08/1997
	RIVO1996	19970032	Completed	20/08/1997
	RIVO1997	19990005	Completed	04/01/1999
	RIVO1998	19990006	Completed	04/01/1999
	RIVO1999	20000010	Completed	27/11/2000
UK				
	Fish disease	***Accession Number***	***Status***	***Last Received Date***
	ALUK1991	19960016	Completed	11/03/1996
	ALUK1992	19960017	Completed	14/01/1997
	ALUK1993	19960018	Completed	14/01/1997
	ALUK1994	19960019	Completed	14/01/1997
	ALUK1995	19960020	Completed	11/03/1996
	ALUK1996	19970033	Completed	16/01/1997
	ALUK1997	19970034	Completed	13/08/1997
	ALUK1999	19990007	Completed	30/09/1999
	ALUK2000	20000003	Completed	16/10/2003
	ALUK2001	20010010	Completed	16/10/2003
	ALUK2002	20020008	Completed	16/10/2003
	ALUK2003	20030203	Completed	16/10/2003
	DOUK1991	19960021	Completed	13/12/1996
	DOUK1992	19960022	Completed	13/12/1996
	DOUK1993	19960023	Completed	13/12/1996
	DOUK1994	19960024	Completed	10/12/1996
	DOUK1995	19960025	Completed	10/12/1996
	DOUK1996	19960026	Completed	10/12/1996
	DOUK1997	19970035	Completed	18/07/1997
	DOUK1998	19990008	Completed	13/10/1999
	DOUK1999	20030185	Completed	09/09/2003
	DOUK2000	20030189	Completed	11/09/2003
	DOUK2001	20030190	Completed	12/09/2003
	DOUK2002	20030191	Completed	17/09/2003

Table 4: REGNS table and WGPDMO input.

Integrated Assessment (IA) of the North Sea: datasets available for contributing to an IA											
data attribute	**Priority for IA 1 - essential 2 - useful 3 - desirable**	**specific programme or identifier**	**spatial extent**	**spatial resolution (eg m^{-2})**	**temporal range**	**temporal resolution (eg a-1)**	**Where is data Held (eg ICES)**	**format**	**restrictions on access?**	**guardian/ stewardship**	**comments: e.g. potential contribution to IA**
fish disease prevalence	1	national fish disease monitoring	North Sea and adjacent areas	very variable, ICES rectangle, partly smaller	1981–2004	very variable, partly twice a year	ICES	ICES Environmental Reporting Format 2.2 and 3.2	no	ICES	information on changes in fish health status as an important endpoint

Annex 11: Draft Terms of Reference 2005 (Cat. 2)

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: T. Lang, Germany) will meet at ICES HQs 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) assess spatial and temporal variations in the pathogenesis of diseases of fish and shellfish and their effects;

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

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

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;

i) review the results of an intersessional pilot study assessing the feasibility of constructing a 'disease index', using disease data from North Sea dab (*Limanda limanda*);

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

  i) the REGNS Integrated Assessment of the North Sea Ecosystem, and

  ii) the Baltic Sea Regional Project (BSRP);

k) produce ICES publications on pathology and diseases of marine organisms.

Supporting information

Priority:	WGPDMO is of fundamental importance to the ICES science and advisory process.
Scientific Justification:	a) New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained (**all members).** b) 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 **(B. Hjeltnes, D. Bruno)**. c) 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 **(B. Hjeltnes, D. Bruno, S. Jones)**. d) 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 (**S.W. Feist, D. Bruno, S. Jones, A. Mansour, S. Ford)**. e) Infectious diseases of aquatic organisms are recognised by characteristic pathogeneses. There are several examples from ICES Member Countries in which the pathogenesis of some important diseases has changed over time. WGPDMO will review the scientific literature and will report on factors influencing this phenomenon and on its consequences **(D. Bruno, S. Jones, A. Mansour)**. f) 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 ap-

	pear 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 **(T. Renault, S. Ford, S.W. Feist)**. g) 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 **(T. Renault, S. Ford, S.W. Feist)**. h) Because the potential risk to wild fish populations due to fish diseases is of considerable ecological and economical concern, WGPDMO wants to carry out a pilot study aiming at an assessment of potential population effects of diseases, based on data extracted from the ICES fisheries, oceanography and environmental databases **(W. Wosniok, T. Lang, S.W. Feist)**. i) 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 **(W. Wosniok, T. Lang, K. Broeg)**. j) Since 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 **(T. Lang, W. Wosniok, S.W. Feist, G. Rodjuk, M. Podolska)**. k) 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. (**W. Wosniok, T. Lang, S. W. Feist**).
Relation to Action Plan:	The Working Group directly addresses the remits of the Mariculture Committee and partly also that of other Science Committees. Its Terms of References are related to various Action Plan No., e.g., 1.3, 1.10, 2.2, 2.4, 2.5, 2.6, 2.7, 2.8, 2.10, 2.12, 3.2, 3.3, 3.6, 3.11, 3.14, 4.6, 4.7, 4.9, 4.12, 4.14, 5.4, 6.1, 6.3
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.
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:	The WGPDMO is parented by the Mariculture Committee and has strong links to the Marine Habitat Committee and Diadromous Fish Committee. On the Working Group levels, strong links exist to the Working Group on Biological Effects of Contaminants.
Linkages to other Organisations:	BEQUALM, OIE, EU
Secretariat Cost share	ICES:100%

Annex 12: Action Plan Audit

Year	Committee Acronym	Committee name	Expert Group	Reference to other committees	Expert Group report (ICES Code)	Resolution No.		
2004/2005	MARC	Mariculture	WGPDMO	ACME, MHC	2005/F:??	2F??		
Action	Action Required	ToR's	ToR	Satisfactory Progress	No Progress	Unsatisfactory Progress	Output (link to relevant report)	Comments (e.g., delays, problems, other types of progress, needs, etc.
No.	Text	Text	Ref. (a, b, c)	S	0	U	Report code and section	Text
2.4, 2.6, 2.10, 3.11, 3.14	Please see action item below	Produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports;	a)	yes			5	National reports submitted by 14 ICES Member Countries were review and the most important information was extracted.
4.7, 3.14	Please see action item below	Assess information available in ICES Member Countries on the role of plankton organisms in gill-related mortality in farmed fish;	b)	yes			6	information was only available from three countries
2.6,3.14,4.7	Please see action item below	Review current information on the continued increase of heart and skeletal muscle inflammation affecting farmed salmon;	c)	yes			7	Due to its importance in salmon farming countries, WGPDMO recommended to revisit this issue at the 2006 WGPDMO meeting
2.6, 4.7	Please see action item below	Compile information on the distribution, causes and significance of the Summer Mortality in the Pacific oyster (*Crassostrea gigas*) and in other bivalve species;	d)	yes			8	A background paper on this issue is in Annex 6 of the 2005 WGPDMO report
2.2, 3.3, 4.6	Please see action item below	Provide guidance on the applicability of the various available 'health indices' for the interpretation of data obtained from biological effects monitoring activities and associated research studies using pathology and disease endpoints;	e)	yes			11	A background paper on this issue is in Annex 8 of the 2005 WGPDMO report. As an outcome of this ToR, WGPDMO recommended to carry out an intersessional pilot study on the development of a 'disease index' for the common dab (*L. limanda*) in the North Sea and to review the results at the 2006 WGPDMO meeting
2.2, 2.8, 2.12, 3.14	Please see action item below	Update and assess the current information on the effects of contaminants on the immune system in fish and shellfish;	f)					
1.3, 2.2, 3.2, 3.6	Please see action item below	Evaluate the availability of data for a risk assessment pilot study on population effects due to diseases in wild fish, using epidemiological methods and population dynamics modelling;	g)	yes			12	there was a delay because of problems associated to data access. Therefore, WGPDMO recommended to revisit this issue at its 2006 meeting, by assessing the results of a pilot study to be carried out intersessionally, using data form the ICES Databases.
2.6, 2.7, 3.11, 3.14, 4.9	Please see action item below	Produce an update of current information on sea lice interactions between wild and farmed fish and examine progress made in related management control methods in ICES Member Countries;	h)	yes			10	due to its importance in salmon farming countries, WGPDMO recommended to revisit this issue at the 2006 WGPDMO meeting
1.10, 2.2, 2.8, 5.4,	Please see action item below	Assess the results of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) to resolve any outstanding issues and, together with WGMS, MCWG, and WGSAEM, finalise a draft set of guidelines for integrated monitoring for OSPAR [OSPAR 2005/6];	i)	yes			13	WGPDMO reviewed the OSPAR Guidelines/Tech, Ann. for biological effects monitoring and recommended changes for the parts on externally visible diseases, liver nodules and liver histopathology.
1.3, 2.2, 2.8, 3.3, 4.14	Please see action item below	Prepare data on the prevalence and type of fish diseases by ICES rectangle for the period 1984 to 2004 where available. The data should be submitted to the secure REGNS website in preparation for the REGNS Integrated Assessment Workshop from 9–11 May 2005;	j)	yes			14	The WGPDMO has a longterm experience in the assessement of ICES disease data in combination with other ICES environmental data and provided WGECO and REGNS with information requested. WGPDMO will be represented at the REGNS Workshop, 9-11 May 2005.
6.1, 6.3	Please see action item below	Produce updated ICES publications on pathology and diseases of marine organisms:	k)				15	
		i) web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report,	k)			yes		The web-based report needs to be updated according to suggestions made by WGPDMO. This will require some more intersessional work by WGPDMO and the ICES Secretariat.
		ii) ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish,	k)	yes				A major milestone is that the Leaflets have been scanned and, once reviewed by the Editor and his subgroup, can be placed on the ICES website.
		iii) WGPDMO website.	k)			yes		Progress made was not as good as expected.

Action Plan Nos.	
1.3	Increase knowledge of the effects of physical forcing, including climate variability, and biological interactions, on recruitment processes of important commercial species. [MHC/OCC/RMC/LRC/MARC/BCC/DFC]*
1.10	Develop better tools and training opportunities for monitoring and observation of physical, chemical and biological properties of marine ecosystems. [FTC]* [Other Science Committees]
2.2	Develop a process for conducting holistic assessments of the impact of human activities, and identify a suite of indicators or variables that will facilitate the monitoring of ecosystem status and evaluating whether ecosystem quality objectives (EcoQOs) are being met. This will be achieved by the following activities: 2.2.1 Contribute to the scientific advice for the development of EcoQOs that will ensure the environmental health of marine ecosystems. [MHC/LRC/OCC/BCC/DFC/ACFM/ACME/ACE] 2.2.2 Assist in the development of spatial and temporal assessments of the indicators for those EcoQOs. [MHC/LRC/OCC/BCC/RMC/DFC]* 2.2.3 Produce holistic assessments of spatial and temporal patterns of contaminants and their effects on marine ecosystems. [MHC/LRC/OCC/BCC/DFC]*
2.4	Update the ICES Code of Practice on Introductions and Transfers and Transfers of Non-indigenous Organisms, including genetically modified organisms. [MARC/ACME/DFC]
2.6	Evaluate and assess the intra- and interspecific interactions of wild and farm-reared stock as well as disease and genetic interactions. [MARC/LRC/DFC]
2.7	Assess the variety and amounts of chemicals used in mariculture and their potential environmental impacts. [MARC/MHC/ACME/DFC]
2.8	Continue and further improve assessments of the transport, fate, and biological effect of contaminants on the marine ecosystem through sampling, analyses, data collection, and evaluation of sampling, analytical, and data processing techniques. [MHC/OCC/LRC/BCC]
2.10	Evaluate and increase knowledge on the potential impacts of intentional and accidental introductions of non-native species and their vectors of introductions. [LRC/MHC/MARC/DFC/ACFM/ACME]*
2.12	Evaluate and increase knowledge of the effects of human activities on the productive capacity of estuarine and freshwater habitats of diadromous fish. [MHC/OCC/MARC/BCC/DFC]
3.2	Further develop, and evaluate performance of, indicators of the status of stocks and ecosystems, relative to effects of fishing and other human activities by new analyses and modelling. [ACFM/ACME/ACE/LRC/ RMC/MHC/OCC/BCC/DFC]
3.3	Develop a framework for an integrated evaluation of the impacts of human activities in the coastal zone, (e.g., mariculture, dredging/extraction, building structures), as an aid to coastal zone management. [MHC/MARC/RMC/OCC/DFC/ACE/ACME]*
3.6	Evaluate the sensitivity and robustness of analytical methods for assessing the impact of human activities, including fishing, on ecosystem properties and processes, through statistical analyses and modelling. [RMC/MHC/LRC/BCC/DFC]*
3.11	Evaluate information on technological change in mariculture, including the utilisation of new species, with particular emphasis on the consequences for production and the environment. [MARC/ACME]
3.14	Provide information to the mariculture industry regarding effects of organic loading, diseases, and chemical treatments. [ACME/MARC/DFC]
4.6	Develop document guidelines for the preparation of Environmental Impact Assessments, and appropriate monitoring programmes. [MARC/MHC/ACME/ACE]
4.7	Review issues of sustainability in mariculture, including interactions between mariculture and other users of resources in the coastal zone, and between cultured and wild stocks. [MARC/DFC/ACME/ACE]
4.9	Assess the effectiveness of salmon-farming management-control methods for the control of fish parasites. [MARC/DFC]
4.14	Provide scientific advice relevant to integrated coastal zone management, including guidelines for sand and gravel extraction and mariculture, and for monitoring programmes that would be included in integrated coastal zone management. [MHC/MARC/DFC/ACME/ACE]
5.4	Develop guidelines and standards for the participation of partners outside the traditional network of ICES-collaborating laboratories in monitoring programmes (such as RV surveys) that underpin on-going ICES science programmes. [All Committees]
6.1	Integrate and expand databases to support ICES programmes within a well-defined data management policy. [CONC/MCAP/all Science Committees]*
6.3	Encourage the production of high-quality scientific publications by ICES through a coordinated publications policy, involving continuous review of ICES scientific output and proactive support for its publications through diverse routes. [Publications Committee (PUB)/CONC/all Science Committees]