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

9–13 March 2004
Åbo, Finland

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark
Telephone + 45 33 38 67 00 · Telefax +45 33 93 42 15

www.ices.dk · info@ices.dk

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0 EXECUTIVE SUMMARY

The ICES Working Group on Pathology and Diseases (WGPDMO) met from 9–13 March 2004 in Åbo/Turku, Finland, with 19 participants from 11 ICES Member Countries, and was chaired by T. Lang (Germany). In order to consider all 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 that were subsequently reviewed at the meeting.

A number of new disease trends in wild and farmed fish and shellfish were reported by Member Countries for 2003: In Finland, the first isolation of the Viral Haemorrhagic Septicaemia Virus (VHSV) in wild fish was made from lamprey, the genotype of which was different from that found during outbreaks at distant rainbow trout farms. A high mortality rate (20–90%) of wild fall pre-spawning coho salmon females, suspected to be due to non-point pollution associated with run-off following autumn storm events, was observed in several streams in Washington state (USA). A number of new bacterial diseases emerging as problems for farmed fish was identified (e.g., *Pasteurella skyensis* in Atlantic salmon, *Streptococcus parauberis* in sea bass, *Candidatus* sp. in seabream and *Pseudomonas anguilliseptica* in blackspot seabream and cod). The mass mortality recorded in farmed salmon in Ireland was believed to be initiated by an initial insult caused by swarms of jellyfish/siphonophores and high water temperatures. Salmon pancreas disease has increased in severity in Norway and the condition has been diagnosed for the first time in northern Norway. A new syndrome with yet unresolved aetiology named ‘rash syndrome’ causing significant losses was described in farmed seabream from Spain. The number of cases of heart and skeletal muscle inflammation has increased significantly in farmed Atlantic salmon in Norway. A new species of *Bonamia*, considered to be a previously unrecognised enzootic parasite, was associated with mortalities in the introduced oyster, C*rassostrea ariakensis*, undergoing testing in North Carolina, USA. A new species, *Perkinsus mediterraneus* sp. nov., has been described in flat oysters in the Balearic Island in Spain. A neoplastic condition of gill epithelial cells possibly associated to sediment toxicity was recorded at high prevalences (mean value 32% over the period 1996–2002) in wild clams, *Macoma balthica*, in the Gulf of Gdansk, Baltic Sea. The causes of the Summer Mortality Syndrome affecting *Crassostrea gigas* still remained unresolved. Shell disease continued to affect American lobsters along the US Atlantic coast at high prevalences (30–35%) (Section 5).

A review of national environmental monitoring programmes in the ICES area revealed that only a few countries carry out regular surveys on pathology and diseases of marine organisms, most of which are focused on offshore rather than on coastal and estuarine areas of the North Sea (including adjacent areas such as the English Channel and the Irish Sea) and the Baltic Sea. The major target species are flatfishes, however, there is a growing interest in other fish species as well as in shellfish species. It is expected that these studies will increase in importance and will be expanded with the further development and implementation of the OSPAR CEMP/JAMP (Section 6).

The WGPDMO made recommendations on techniques to be used to differentiate between species of *Perkinsus* spp., a parasite with a world-wide distribution and important economic implications for commercial mollusc species (Section 7).

A review of viral diseases of crustaceans revealed that there is a significant amount of data available on viruses in farmed crustaceans but relatively little on the prevalence of these in wild populations. As population and environmental studies on wild crustaceans increase (see above), the expectation is that further viral pathogens will be disclosed. There is evidence that stress due to adverse environmental conditions may exacerbate viral diseases of crustaceans and that some of the viruses identified so far (e.g., in brown shrimp) may have significance at the population level of commercial species (Section 8).

The WGPDMO recommended on the use of epidemiological methods for the assessment of diseases and the risk of population effects in wild fish. As a next step, the availability of appropriate data for a pilot study will be assessed (Section 9).

Information on disease/parasite interactions between wild and farmed fish and on related management control methods was updated. It was concluded that, in general, there is little information regarding disease/parasite interactions between wild and farmed fish and that, therefore, emphasis should be given to research in this area. As one of the problems encountered, the use of wild broodstock for the enhancement of aquaculture activities, with the risk to introduce pathogens, was identified. Since it is expected that mariculture will continue to expand into new geographical areas, it will be of importance to further develop and implement measures (e.g., predictive risk assessment models) to assess and minimise the risk of disease transfer from wild to farmed fish and vice versa (Section 10).

From an analysis of national reports, there is evidence that *Ichthyophonus* sp. (now classified as Mesomycetozoa) continued to be endemic in herring stocks of the northern North Sea at a low prevalence. Compared to 2002, a decreasing mortality of salmon fry in the Baltic Sea due to M74 was observed. Although the ultimate causes of the M74 syndrome remain unknown, there is increasing evidence that the syndrome is linked to the thiaminase activity of prey species (herring rather than sprat, as previously thought) in the Baltic Sea which seems to be influenced by general stress factors associated with herring population density (Section 11).

The new ICES Environmental Data Reporting Format 3.2 to be used, e.g., for the submission of fish disease data to the ICES Environmental Databank was reviewed and considered as an improvement in the integrated management of the data maintained by ICES. Recommendations regarding disease data quality assurance procedures were made (Section 12).

Regarding WGPDMO publications on pathology and diseases of marine organisms, improvements in the ICES web-based report on trends in diseases of North Sea dab were suggested that will be implemented once the disease data basis is updated and a new trend analysis is made. Progress in the manuscript on statistical methods for the analysis of disease data to be published in the ICES TIMES series was presented. The WGPDMO nominated a new editor (S.W. Feist, UK) for the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, replacing the previous editor whose term had ended, and suggested ways how to improve the publication of old and new leaflets on the ICES web site. The report on trends in important diseases affecting the culture of fish and molluscs in the ICES area 1998–2002 was published shortly before the 2004 WGPDMO meeting (Section 13).

Based on an OSPAR request [OSPAR 2004/2], WGPDMO provided background material to be used by the 2005 ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Area (WKIMON). This consisted of answers to questions raised by the WKIMON Co-Chairs and background documents on integrated health assessment and on epidemiological methods (Section 14).

The WGPDMO discussed and agreed upon WGPDMO contributions to the initiated ICES integrated regional assessment of the North Sea Ecosystem coordinated by the ICES Regional Ecosystem Study Group for the North Sea (REGNS). Contribution will focus on an assessment of data on the health status of North Sea biota for the period 2002–2004, and any trends in the prevalence of diseases over the recent decades as well as on the results of an analysis on cause-effect relationships between diseases and environmental factors. A strategy how to accomplish this according to the time frame suggested by REGNS was developed and endorsed and WGPDMO members were nominated for the various tasks involved (Section 15).

WGPDMO concluded that all Terms of Reference for the 2004 meeting were considered in a comprehensive manner. Since several important issues in the field of pathology and diseases of marine organisms requiring further consideration were identified, it was agreed that a further WGPDMO meeting is required in 2005. An invitation was received and acknowledged to organise the 2005 meeting in France (either La Tremblade or La Rochelle). The proposed dates are 8–12 March 2005.

1 OPENING AND STRUCTURE OF THE MEETING

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at the Old Mill Conference Centre, Åbo/Turku, Finland, with T. Lang as Chair. The meeting was opened at 10:00 hrs on Tuesday, 9 March 2004, with the Chair and the local organiser, T. Wiklund, welcoming the participants, particularly the new members who had not previously attended WGPDMO meetings.

A list of participants is appended in Annex 1.

Apologies were received from S. Bower, C. Couillard, S. McGladdery and S. Jones (Canada), I. Dalsgaard (Denmark), A. Köhler (Germany), J. Palsson (Iceland), F. Geoghegan (Ireland), R. Medne and I. Savecka (Latvia), S. Mortensen (Norway), J. Rokicki, W. Piasecki and M. Podolska (Poland), T. Bezgachina, (Russia), G. Díez (Spain) and O. Haenen (The Netherlands).

The Chair thanked the local organiser for inviting WGPDMO to Finland and for providing excellent meeting facilities.

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 AGENDA, SELECTION OF RAPPORTEURS

2.1 Terms of Reference

The WGPDMO took note of the Terms of Reference published as C. Res. 2003/2F01 (Annex 2). The agenda once again demanded extensive intersessional work by the members of the WGPDMO who were requested to produce written working documents (Annex 3) 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 two 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 ensured that the Terms of Reference could be treated efficiently.

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 ANNUAL SCIENCE CONFERENCE 2003, ITEMS OF RELEVANCE TO WGPDMO

The Chair highlighted items of relevance to WGPDMO.

3.1 ICES Annual Science Conference 2003

The 2003 ICES Annual Science Conference (ASC) took place in Tallinn, Estonia, 24–27 September 2003. The ASC was organised in the form of Theme Sessions on various marine research topics that were held concurrently. The ASC was preceded by two days for business sessions of the three ICES Advisory Committees and the eight ICES Science Committees, including the ICES Mariculture Committee (the parent Committee for WGPDMO) and the newly established ICES Diadromous Fish Committee.

At last year’s ASC, no mariculture-related Theme Sessions were held, resulting in a low attendance of relevant experts and official members of the Mariculture Committee. Pathology/disease aspects were only considered in Theme Session M entitled ‘Biological Effects Monitoring in the Baltic Sea’ that was mostly dedicated to results from the EU-funded project ‘Biological Effects of Pollution in Coastal Marine Ecosystems, BEEP’ (see under Section 4.2).

3.2 ICES Mariculture Committee (MARC)

The Chair presented the 2003 WGPDMO Report to the MARC at its business session during the ICES Statutory Meeting. The report and its recommendations for Terms of Reference were accepted. Two new Terms of Reference (j, k) were added: ToR j based on a request from OSPAR regarding the planning of the 'ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas' to be held in January 2005 in Copenhagen and ToR k referring to the ICES initiative for an integrated assessment of the status of the North Sea ecosystem (see Annex 2).

The Mariculture Committee (MARC) regretted the unsatisfactory attendance of MARC members and attributed this to the fact that no mariculture-related Theme Session were held at the ASC 2003. However, it was noted that the situation will improve in 2004 because there will be a number of Theme Sessions on mariculture at the ASC in Vigo, Spain, 22–25 September 2004:

- **Towards Sustainable Aquaculture**. Co-Conveners: Hans Ackefors (Sweden), Pauline Kamermans (Netherlands), and Jacqueline Doyle (Ireland) (Session V);
- **Shellfish Culture: Perspectives and Limitations. Co-Conveners**: Alain Bodoy (France), and Aad Smaal (Netherlands), (Session W);
- **Water Treatment in Intensive Fish Cultures**. Co-Conveners: Anders Mangor-Jensen (Norway), Terje van der Meeren (Norway), Uwe Waller (Germany), and Ed Trippel (Canada) (Session X);
- **Mariculture in Integrated Coastal Zone Management Systems**. Co-Conveners: Edward Black (Canada) and Josianne Støttrup (Denmark) (Session BB).

Furthermore, there will be an environmentally-related Theme Session where pathology/disease aspects will be considered:

- **How Useful are Biological Effects Measurements in Marine Ecosystem Management?** Co-Conveners: Ketil Hylland (Norway), Thomas Lang (Germany), and Kris Cooreman (Belgium) (Session Z)

A number of Symposia co-sponsored by ICES and other organisations are in preparation:

- **Gadoid Mariculture - Development and Future Challenges** (13–16 June 2004, in Bergen, Norway);
- **Interactions of Wild and Cultured Atlantic Salmon** (2005, dates and venue yet to be decided);
- **Marine Bioinvasions** (2006 or 2007, dates and venue yet to be decided, possibly Boston, USA).

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 crucial for both the Theme Sessions and the Symposia. Furthermore, the participation in the ASC 2004 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 2004 ASC to the ICES Secretariat is 3 May 2004 and that more information on the ASC can be found on the ICES website (http://www.ices.dk/iceswork/asc/2004/index.asp).

3.3 ICES Advisory Committee on the Marine Environment (ACME)

The Chair informed the WGPDMO that a great number of topics considered by WGPDMO at its 2003 meeting were subsequently reviewed by the ACME at its annual meeting in June 2003 and incorporated in the 2003 ACME Report (ICES Coop. Res. Rep. 263):

a) Trends in diseases of wild and farmed fish and shellfish;
b) Status of the M74 Syndrome in Baltic salmon and status of Ichthyophonus in herring;
c) Strategies to assess the prevalence of shellfish diseases in parallel to fish diseases and chemical contaminant levels in environmental Monitoring programmes;
d) Impact of diseases of farmed fish on wild fish stocks;
e) Effectiveness of salmon farming management control methods for sea lice in ICES Member Countries;
f) Quality assurance procedures for biological effects techniques, including fish diseases;
g) Web-based report on trends in fish disease prevalence and mariculture-relevant diseases.

4 OTHER RELEVANT REPORTS/ACTIVITIES FOR INFORMATION

Information was provided on scientific conferences/workshops in 2004 and 2005 and research projects with relevance to the work of WGPDMO:

4.1 Conferences/Workshops

- SETAC Europe 14th annual meeting, 18 - 22 April 2004, Prague, Czech Republic;
- Fish Vaccination Workshop, 19–23 April 2004, Wageningen, The Netherlands;
- Environmental Monitoring and Assessment Program (EMAP) Symposium, 3–7 May 2004, Newport Rhode Island, USA;
- 6th Symposium on Fish Immunology, 26–29 May 2004, Åbo/Turku, Finland;
- Society of Protozoologists, 2–6 June 2004, Smithfield, Rhode Island, USA;
- 5th International Conference on Molluscan Shellfish Safety, 14–18 June 2004, Galway, Ireland;
- 2nd International Congress on Aquaculture, Fisheries Technology and Environmental Management, 19–20 June 2004, Athens, Greece;
- Society of Protozoologists, 2–6 July 2004, Smithfield, Rhode Island, USA;
- European Federation of Parasitologists - EMOP IX, 19–23 July 2004, Valencia, Spain;
- American Society of Parasitologists, 24–28 July 2004, Philadelphia, Pennsylvania, USA;
- American Fisheries Society/Fish Health Section, 25–28 July 2004, Kearneysville, West Virginia, USA;
- VI International Congress on the Biology of Fish, 1–5 August 2004, Manaus, Brazil;
- Society of Invertebrate Pathology, 1–6 August 2004, Helsinki, Finland;
- Internal Exposure - Linking Bioavailability to Effects Workshop. Monte Verità, Switzerland, 22–27 August 2004;
- XIX International Congress of Zoology, 23–27 August 2004, Beijing, China (symposia on ‘Environmental Impact’ and ‘Disease’)
- The 9th DCE/FECS Conference on Chemistry and the Environment (DCE9) “Behaviour of Chemicals in the Environment”, Bordeaux, France, 29 August - 1 September 2004;
- The 20th Congress of the Polish Parasitological Society, 2–4 September 2004, Warsaw, Poland;
- XI European Congress of Ichthyology, 6–10 September 2004, Tallinn, Estonia;
- Advanced Course on Diagnostic and Control of Diseases for the Mediterranean Aquaculture, 13–24 September 2004, Santiago de Compostela, Spain;
- 6th International Symposium for viruses of Lower Vertebrates 20–22 September 2004, Hakodate, Japan;
- International Giardia and Cryptosporidium Conference, 20–24 September, Amsterdam, Netherlands;
- ICES Annual Science Conference, 22–25 September 2004, Vigo, Spain;
- Aquaculture Europe 04: Biotechnologies for Quality. European Aquaculture Society, 20–23 October 2004, Barcelona, Spain;
- 4th SETAC World Congress, 14–18 November 2004, Portland, Oregon USA;
- National Shellfisheries Association, 10–14 April 2005, Philadelphia, Pennsylvania, USA;
- 1st Scandinavian and Baltic Society for Parasitology (SBSP) Symposium, 26–30 May 2005, Vilnius, Lithuania;
- American Society of Parasitologists, 8–12 July 2005, Mobile, Alabama, USA;
- EAFP, 12–17 September 2005, Copenhagen, Denmark;
- ICOPA-XI, 6–11 August 2006, Glasgow, UK;

4.2 Projects

- **Baltic Sea Regional Project (BSRP):** Sponsored by the World Bank, partly organised and managed by ICES through a project coordinator (J. Thulin) and various Study Groups under the Baltic Committee, e.g., the ICES/BSRP Study Group on Baltic Ecosystem Health Issues (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).
- **Biologial Effects of Pollution on Coastal Marine Ecosystems (BEEP):** Large EU-funded project with more than 30 partners studying biological effects of contaminants in the Baltic Sea, North-East Atlantic and the Mediterranean

Sea (including pathology/disease aspects). The project came to an end in January 2004, the final report will be submitted to the EU end of March 2004 (http://beep.lptc.u-bordeaux.fr).

- **Biological Effects of Contaminants in Pelagic Ecosystems (BECPELAG):** ICES-Workshop with participants form 12 countries, sponsored by participating countries and the Norwegian oil industry. Practical work (field sampling, cage experiments, bioassays) was finalized, results will be published in 2004 in a SETAC book (http://www.niva.no/pelagic/web).
- **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 (a website is in the process of being established).
- **Summer mortality in *C. gigas* oysters (Mortalités Estivales, MOREST):** National French project coordinated by IFREMER. This programme began in 2001. 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 mortalities observed (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)

5 ANALYSE NATIONAL REPORTS ON NEW DISEASE TRENDS IN WILD AND CULTURED FISH, MOLLUSCS AND CRUSTACEANS

5.1 Wild Fish

VIRUSES

Lymphocystis - The prevalence of lymphocystis in the Polish EEZ of the Baltic Sea was low in herring (*Clupea harengus*) (0.24% of 23,300 fish) and in flounder (*Platichthys flesus*) (0.39% of 7,916 fish). In contrast, prevalence of lymphocystis in Baltic flounder in adjacent areas ranged from 15.5% to 41.7%. Lymphocystis prevalence continued to decrease in dab (*Limanda limanda*) in the German Bight.

Infectious Hematopoietic Necrosis Virus (IHNV) - no new information.

Infectious Pancreatic Necrosis Virus (IPNV) - IPNV was isolated from 24 tissue pools (5 fish/pool) of 11,515 fishes representing 26 marine species from Scotland. Isolations were made from common dab, grey gurnard (*Eutrigla gurnardus*), lemon sole (*Microstomus kitt*), plaice (*Pleuronectes platessa*), saithe (*Pollachius virens*) and whiting (*Merlangius merlangus*) with no signs of disease (see Annex 10).

Infectious Salmon Anaemia Virus (ISAV) - ISAV was detected by RT-PCR in one of 120 alewife (*Alosa pseudoharengus*) collected from the Narraguagus River in Maine (USA). Cell cultures were negative. Genetic sequencing of the RT-PCR product showed 99–100% homology with the New Brunswick strain of ISAV. Fifty-five adult West Greenland salmon tested negative for ISAV by RT-PCR on kidney tissue. ISAV was not detected in more than 200 broodstock Atlantic salmon (*Salmo salar*) collected from the Penobscot River, Maine.

Nodavirus - no new information.

Viral Haemorrhagic Septicemia Virus (VHSV) - The first isolation of VHSV in wild fish in northern Finland was made from lamprey (*Lampetra fluviatilis*) caught in rivers of the Bothnian Bay. These isolates were from fish caught quite distant from VHS outbreaks in rainbow trout (*Oncorhynchus* mykiss) farms in Finnish coastal waters. The genotype of the isolates from lamprey were different from those isolated from the disease outbreaks in rainbow trout farms but similar to those previously reported from the southern Baltic Sea. In contrast to previous years, no mortalities of sardines (*Sardinops sagax*) due to VHSV occurred in British Columbia, Canada. VHSV was isolated from one apparently healthy herring (*Clupea harengus harengus*) captured along the Maine coast (USA). The closest genotype of this isolate is the North American strain.

BACTERIA

Edwardsiella tarda - *Edwardsiella tarda* was isolated from diseased silver eels (*Anguilla anguilla*) in Spain. The eels presented ulcers near the operculae and haemorrhaging in fins. Approximately 10% of the sampled populations was affected.

Juvenile coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon collected from rivers along the USA northern Pacific coast were infected with ***Renibacterium salmoninarum***, ***Listonella anguillarum***, ***Yersinia ruckeri*** and ***Aeromonas salmonicida***. During 1999–2001, which were La Niña years, the prevalence of *L. anguillarum* was significantly higher in coho and chinook than during 1996–1998, which were El Niño years.

Aeromonads and **pseudomonads** were the predominant bacterial species isolated from wild adult pink (*O. gorbuscha*) and chum (*O. keta*) salmon from the Sakhalin Island coast of Russia. Aeromonads represented 41.9% and 45.5% of all bacterial isolates from pink and chum salmon, respectively. Pseudomonads represented 32.3% and 31.3% of all isolates from pink and chum salmon, respectively.

Acute/healing skin ulcerations - Skin ulcers showed strong spatial distribution in Baltic cod (*Gadus morhua*) with the highest prevalences recorded in the Bornholm Sea (20.4%) and the Gdansk Deep (15.8%). The decreasing trend in prevalence in the Arkona Sea continued, whereas an increasing trend was noted for the Bornholm Sea and the Gdansk Deep. Observations in the Polish and Russian EEZs of the Baltic Sea also indicate an increasing trend. Central North Sea sites showed an increase in skin ulcer prevalence in dab. These included West Dogger (13.3% from 6.2% in 2002), North Dogger (28.4% from 12.2% in 2002) and the Hospital Ground (17.9% from 12.4% in 2002).

FUNGI

Ichthyophonus (now classified as belonging to the class Mesomycetozoea) - information is available in report Section 11.

Saprolegnia - caused mortalities in Atlantic salmon broodstock in Norwegian rivers.

PARASITES

X-cell lesions - Prevalence of X-cell gill lesions in dab was generally low in the North Sea but was unusually high (8.8%) in the Firth of Forth.

Myxosporidiosis - A previously undescribed myxosporean was found in spawning coho salmon collected from Vancouver Island, Canada, and some rivers in Washington State, USA. rDNA sequencing indicates this is related to *Sphaerospora oncorhynchi*. Effects on the spawning population are unknown.

***Stephanostomum baccatum* -** This parasite showed a pronounced spatial prevalence pattern in North Sea dab ranging from 9.1% to 63.7%. In Dec 2003, the prevalence recorded in the Firth of Forth dropped to 52.0% compared to 72.1% in December 2002. The highest prevalences (> 90%) of this parasite have consistently been recorded in two areas with offshore oil and gas installations. Prevalence of *S. baccatum* ranged from 0.2% at St. Bees (southern England coast) to 78.4% off St. Bees in the Irish Sea and 66.7% in dab caught at West Dogger in the North Sea.

***Gyrodactylus salaris* -** This monogenetic trematode remains a major threat to Atlantic salmon in Norway. However, no new rivers were found to be infected in 2003.

***Anguillicola crassus* -** This swimbladder parasite was detected for the first time in eels (*Anguilla anguilla*) in Finland.

Crustacean parasites- ***Lepeophtheirus salmonis*** has been implicated in unusually low returns of pink salmon in the Broughton archipelago in British Columbia, Canada. Consequently, a major sea lice monitoring programme for pink and chum salmon fry was implemented in the archipelago. Preliminary data are available on the website (http:www-sci.pac.dfo-mpo.gc.ca/mehsd/sea%20lice/pink_salmon_e.htm). Although still a problem for Atlantic salmon and sea trout (*Salmo trutta*) in Norway, *L. salmonis* infestations appear less severe than in 2002.

No major change in the prevalence of the copopod parasite ***Acanthochondria cornuta*** was observed in North Sea dab. However, there was a significant increase of ***Lepeophtheirus pectoralis*** at the Dogger Bank from 5.6% in August/

September 2002 to 19.2% in August/September 2003. Prevalence of *L. pectoralis* ranged from 1.1% at the Indefatigable Bank off the coast of England to 45.8% in dab from Burbo Bight (inner Liverpool Bay).

The prevalence of ***Sphyrion lumpi*** on redfish (*Sebastes mentella*) from the Barents Sea showed an increase from 25% in 2002 to 30% in 2003.

OTHER DISEASES

Epidermal hyperplasia/papilloma - Prevalence of epidermal hyperplasia/papilloma in dab from the North Sea and Irish Sea showed some changes compared to 2002. Red Wharf Bay, Liverpool Bay, Flamborough and Burbo Bight showed decreases in the prevalence from 2.3% to 0.4%, 5.5% to 1.4%, 3.2% to 1.6%, and 3% to 1.1%, respectively. Morecambe Bay and the Hospital Ground showed increases in the prevalence from 0.9% to 2.4% and 0.7% to 3.2% respectively. There is indication of a decrease in the German Bight.

Hyperpigmentation - A significant increase in prevalence in dab was noted from the Dogger Bank where the prevalence was 38.4% (from 18.2% in 2002) at the northern Dogger and 46.3% (from 30.1% in 2002) at West Dogger. Furthermore, the Hospital Ground site had a prevalence of 28.7% (up from 15% in 2002). The prevalence also continued to increase in the German Bight. As in previous years, the condition was present at low levels in the western Baltic Sea and Irish Sea, with prevalence rates ranging from 0% to 2.7% in the Irish Sea. A decrease in hyperpigmentation was noted in Inner Cardigan Bay from 20.7% to 3.5%.

Liver nodules/tumours/histopathology - In 2003, prevalences in dab from former hot-spot areas (German Bight, Dogger Bank) remained at recently observed low levels (< 2.0%). Red Wharf Bay, West Dogger, St. Bees and Burbo Bight all showed a decrease in prevalence to 0% from 4.8% in 2002, 7.6% from 10.8% in 2002, 2.2% from 7.2% in 2002, and 2.5% from 9.8% in 2002 respectively. Highest prevalence was recorded from fish from Liverpool Bay at 9.2% (n = 217), an increase from 5.1% compared to 2002.

A significant decrease in contaminant exposure parameters was noted for English sole (*Pleuronectes vetulus*) after a one meter-thick cap of clean sediment was placed over 0.2 km^2 of the most severely PAH-contaminated area of Eagle Harbor, USA. Over the entire monitoring period since cap initiation (up to 104 months, through 5/02), but particularly after ~3 years, there has been a significant decreasing trend in hepatic lesion risk in English sole, as well as for biliary FACs and hepatic DNA adducts in English sole and rock sole (*Pleuronectes bilineatus*), and starry flounder (*Platichthys stellatus*). CYP1A levels showed no trend relative to time of cap placement.

Skeletal deformities - The prevalence of skeletal deformities in southern Baltic cod varied between 0.0% and 8.3% and continues to be lower than at the end of the 1990s.

Pre-spawning mortality - A high mortality rate (20–90%) of fall pre-spawning coho salmon females has been observed in several streams in Washington State (USA). The affected fish are lethargic, disoriented, gaping, have lost equilibrium and splay fins prior to death. The precise cause remains unknown but the weight of evidence suggests that the widespread mortalities are due to non-point pollution associated with run-off following autumn storm events.

Conclusions

1) Different prevalences of skin ulcerations in Baltic cod and of lymphocystis and skin ulcerations in flounder were reported from adjacent areas in the Baltic Sea from ICES member countries. This may be due to methodological differences in the detection and recording of these lesions.
2) ISAV was detected by RT-PCR from one alewife in the USA.
3) The first isolations of VHSV in wild fish in Finland was made from lamprey, the genotype of which is different from those found in outbreaks at distant rainbow trout farms. VHSV of similar genotype to the North American strain was isolated from apparently healthy herring in the USA.
4) Prevalence of X-cell lesions in dab was generally low in the North Sea but was unusually high (8.8%) in the Firth of Forth.
5) A previously undescribed myxosporean related to *Sphaerospora oncorhynchi* was found in spawning coho salmon collected from Vancouver Island, Canada, and some rivers in Washington state, USA.
6) *Gyrodactylus salaris* remains a major threat to Atlantic salmon in Norway. However, no new rivers were found to be infected in 2003.
7) Hyperpigmentation in dab continued to show an increasing trend in most North Sea areas.

8) Prevalences of liver nodules in dab from North Sea areas remained static or decreased. The prevalence at Liverpool Bay increased to 9.2%, the highest level observed in 10 years.
9) A study examining liver nodules in English sole from Eagle Harbor, USA, showed a significant decreasing trend in hepatic lesion risk, as well as for biliary FACs and hepatic DNA adducts three years after applying a 1 m cap of clean sediment to a severely PAH-contaminated site.
10) A high mortality rate (20–90%) of fall pre-spawning coho salmon females has been observed in several streams in Washington state (USA) and is suspected to be due to non-point pollution associated with run-off following autumn storm events.

Recommendations

The WGPDMO recommends that:

i) ICES Member Countries are encouraged to continue to fund fish disease monitoring programmes to sustain health surveillance of wild fish stocks. This information is of vital importance to integrated assessments of the status of marine ecosystems, such as the initiated ICES Regional North Sea Assessment, and in light of the implementation of the OSPAR JAMP/CEMP;

ii) Baltic Sea countries are encouraged to continue their efforts to intercalibrate methodologies applied in fish disease surveys. This should be part of the Baltic Sea Regional Project (BSRP) and the Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM).

5.2 Farmed fish

VIRUSES

Infectious Salmon Anaemia Virus - During 2003 there has been a decrease in reported cases of ISAV in Atlantic salmon in Norway. No new clinical cases occurred in the previously most affected region, but the disease recurred in other areas. Screening for ISAV in Western Norway revealed 15 positive samples by PCR. No signs of disease were recorded.

In New Brunswick, Canada, 19 sites were positive for ISAV. In Nova Scotia, one clinical outbreak occurred (the second reported for this province) and at the same site as that reported in 2000. Affected cages were harvested and product marketed.

In the USA, after more than a year of implementation of the ISAV management programme and monthly surveillance, ISAV was detected in net pens in Cobscook Bay, Maine. Two farms were affected, each with detection of ISAV in single cages in June 2003, followed by additional individual cage detection through January 2004. Affected cages were either removed or voluntarily harvested. In total, one farm had three cages affected and the second farm had four cages affected.

Nodavirus - Cases are reported in several species of larval fish (seabream, *Sparus aurata*; sea bass, *Dicentrarchus labrax* and sole, *Solea* spp..) in Spain with associated mortality.

Measures taken after the 2002 loss of 168,000 cod larvae due to nodavirus in the USA included increasing the UV treatment for incoming and recirculating water to 200,000 milliwatts and screening for virus at all the life stages from eggs in the hatchery to sub-adults in cages and broodstock reproductive fluids.

Infectious pancreatic necrosis virus- During 2003 in Scotland, 282 out of 654 Atlantic salmon sites were positive for IPNV (43%), compared with 2002, when 275 sites out of 703 were positive (39%). Overall there is no major trend, but data indicate the widespread nature of the virus.

Viral haemorrhagic septicaemia virus - The number of VHSV isolations in rainbow trout decreased from 14 in 2002 to 3 in 2003 in Finland. Clinical disease occurred at two farms in Åland Islands (SW Finland), compared to 14 in 2002. VHS was also found at one rainbow trout farm outside Åland Islands, and a new restriction area was set on the western coast of Finland. In contrast, VHS outbreaks have not occurred in farms in the Gulf of Finland since 2001. VHS occurred at three marine rainbow trout farms in Denmark. The cause was a highly pathogenic rainbow trout farm isolate (freshwater isolate) at all three farms.

Salmon pancreas disease -There has been an increase in reported cases of clinical SPD in Atlantic salmon in 2003 and the losses have been more severe than during past years in Norway. For the first time, SPD was diagnosed in Northern Norway. Until recently, all reported cases have been in Western Norway, indicating a spreading infection. In 2003, mortalities from SPD in Ireland ranged from 5–35%.

Epitheliocystis - Proliferative gill inflammation previously attributed to epitheliocystis has been detected for at least 15 years in Atlantic salmon in Norway, and occurs during the first months following seawater transfer. Losses vary between 15–30% and growth is often seriously retarded. In 2003 this was a serious disease problem especially in the Southern part of Norway (Rogaland). The newly described virus Atlantic salmon paramyxovirus (ASPV) has been isolated from certain cases. The significance of this virus has to be determined.

BACTERIA

***Renibacterium salmoninarum* -** In contrast to previous years, bacterial kidney disease (BKD) was not detected at Atlantic salmon farms in Cobscook Bay, Maine, USA, coincident with the measures implemented for ISA management (i.e., no carryover, 100 day fallow, extensive cleaning and disinfection).

R. salmoninarum was found in one marine rainbow trout farm in Denmark (a different site to the farm noted in 2002), but only a few fish died due to the disease. A decreasing prevalence has been observed for outbreaks of BKD in brackish water rainbow trout farms in Finland from 12 in 2001 and 15 in 2002 to three outbreaks in 2003.

Streptococcus parauberis re-emerged after six years of absence in the Iberian Peninsula. The diseased turbot (*Scophthalmus maximus*) were not vaccinated. The first isolation of *S. parauberis* has occurred in sea bass in 2003 (> 200 g weight) with high mortality, in one French farm.

***Listonella* (*Vibrio*) *anguillarum* -** In Denmark an outbreak was seen in non-vaccinated rainbow trout.

L. anguillarum remains a major disease in sea bass in France. Two unusual outbreaks occurred in the winter (in contrast to more typical summer outbreaks) with high mortality and loss of appetite, and, consequently, were difficulty to treat. Losses of 10% from *L. anguillarum* were experienced in cod cultured in New Hampshire, USA, due to serotype O2 alpha at one farm.

***Moritella viscosa* -** Infections causing ulceration resulted in significant losses and remain a significant problem in both Atlantic salmon and rainbow trout in Norway. The losses were especially high in Northern Norway, probably due to low temperatures and a slow healing process.

Pasteurella skyensis - Significant losses among Atlantic salmon farms in Scotland have been reported and a bacterium tentatively named *Pasteurella salmonicida* and more recently, as *P. skyensis* sp. nov., has been isolated. Pathological changes associated with these outbreaks were primarily septicaemic with various signs of systemic inflammation.

***Aeromonas salmonicida* -** A multi-resistant strain has been isolated from Atlantic salmon sites in New Brunswick and Nova Scotia, Canada. The isolate was resistant to standard commercially available antibiotics and was spread through movements of fish.

Aeromonas salmonicida and ***Tenacibaculum (Flexibacter) maritimum*** continued to be the main problem for turbot culture in Spain.

Photobacterium damselae subsp**.** ***piscicida*** and ***T. maritimum-*** These bacteria were reported with high frequency in unvaccinated sole in Spain.

Pseudomonas anguilliseptica - This has been isolated for the first time in juvenile and broodstock blackspot seabream (*Pagellus bogaraveo*) on different occasions during the winter in Spain. The broodstock appear to be asymptomatic carriers, although the incidence is unknown at present.

A 10% loss of cod larvae due to *P. anguilliseptica* was recorded in the Passamaquoddy Bay, Canada.

Filamentous bacteria - Bacteria have been isolated for the first time in seabream (similar bacteria have been observed in rainbow trout for several years in fresh water, proposed as *Candidatus* sp.). The role of these bacteria in the mortality and decrease in growth (due to a decrease of intestinal absorption) is unknown.

PARASITES

***Parvicapsula* spp.** - A presumptive *P. minibicornis* was associated with high mortality in pre-spawning sockeye salmon (*Oncorhynchus nerka*) in the Fraser River in British Columbia, Canada. Tagging studies conducted in 2003 suggested that significant prespawning mortality was associated with sockeye salmon that prematurely re-entered the Fraser River. Severe parvicapsulosis developed in 91% of adult sockeye collected at sea then exposed to Fraser River water. Light infections were observed in 11% of adult control sockeye salmon held in non-Fraser River freshwater. During 2002, the first clinical disease outbreaks caused by *P. pseudobranchiola* was diagnosed in five Atlantic salmon farms in Northern Norway. In 2003, the parasite was detected in several farms in Northern Norway as well as in Trøndelag and Møre and Romsdal.

***Myxobolus cerebralis* -** A new occurrence of a brain parasite caused 10% mortality on two Atlantic salmon farms in Ireland and was associated with lost of growth in survivors. Studies suggest this may be a pre- or extrasporogonic stage of *M. cerebralis.*

Cryptosporidium has occurred in juvenile seabream and turbot in Spain, as well as ***Cryptocaryon*** and ***Amylodinium***, which also occurred for the first time in 2003 in seabream.

DISEASES OF UNCONFIRMED AETIOLOGY

Heart and skeletal muscle inflammation - 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. The outbreaks are generally more severe and losses up to 25% have been reported.

Cardiomyopathy syndrome - An increase of CMS is reported in some regions of Norway in Atlantic salmon with associated mortality.

Winter disease syndrome - A decreasing prevalence in the winter disease syndrome of seabream was observed in Spain.

'Rash syndrome' - A new syndrome named **'rash syndrome'** was described during winter months in Spain from seabream causing significant losses. Sea bass cultured in the vicinity were not affected. The aetiology is unknown, although bacteria might be implicated since the disease seems to be alleviated with the use of chemotherapy.

Deformities - deformities in cod fry are reported in Norway, with a prevalence of 30–40%.

Mass mortality - In July 2003, a major mortality was reported in farmed salmon at Inver Bay and McSwynes Bay, Ireland. According to a recent report (Cronin *et al*. 2004: Salmon mortalities at Inver Bay and McSwynes's Bay finfish farms, County Donegal, Ireland during 2003. Marine Environment and Health Series, No. 15, 2004), several events have been ruled out as the cause of this mortality. These include infections with a primary fish pathogen, poor farm management, harmful algal bloom, pollution incidents, sediment disturbance or damages caused by dumping of dredged spoil material. In this report, it is suggested that the initial insult may have been caused by a biological event, such as a siphonophora bloom. This initial insult, coupled with high water temperatures, low oxygen and secondary bacterial infection of the gills, could have been enough to give rise to the loses observed.

Conclusions

- There are several new bacterial diseases emerging as problems for farmed fish, including *Pasteurella skyensis* in Atlantic salmon, *Streptococcus parauberis* in sea bass, *Candidatus* sp. in seabream and *Pseudomonas anguilliseptica* in blackspot seabream and cod.
- The mass mortality recorded in farmed salmon in Ireland was believed to be initiated by an insult caused by jellyfish/siphonophores and simultaneous high water temperatures. Due to the severe impact, further attention should be paid by WGPDMO to disease problems and mortalities associated with planktonic organisms.
- A new syndrome named 'rash syndrome' was described in seabream from Spain causing significant losses. Additional work is required to determine the aetiology.
- Salmon pancreas disease has increased in severity in Norway and the condition has been diagnosed for the first time in Northern Norway.
- A new restriction area for VHSV was established on the western coast of Finland.

- A multi-resistant *A. salmonicida* was isolated in Atlantic salmon in New Brunswick and Nova Scotia, Canada.
- The number of cases of heart and skeletal muscle inflammation has increased significantly in Atlantic salmon in Norway, warranting further studies on its aetiology and impact and further attention by WGPDMO.

Recommendations

The WGPDMO recommends that:

i) information available in ICES Member Countries on the role of plankton organisms in gill-related mortality in farmed fish be reviewed at the 2005 WGPDMO meeting;

ii) studies are being undertaken to determine the aetiology of the new 'rash syndrome' in Spain;

iii) information available regarding the continued increase of heart and skeletal muscle inflammation in farmed Atlantic salmon be reviewed at the 2005 WGPDMO meeting.

5.3 Wild and Farmed Shellfish

VIRUSES

Herpesvirus in bivalves - As a follow up to the finding of a herpesvirus, similar to that reported in France, associated with Pacific oyster (*Crassostrea gigas*) seed losses in Tomales Bay, California, USA, PCR testing was performed on seed oysters from other hatcheries in the USA (Oregon, Washington, Virginia, Maine, and Louisiana). All batches tested were negative for herpesvirus. No new trends were found in France.

Viral gametic hypertrophy in *C. gigas* - Histological examination of *C. gigas* oysters in France revealed several cases of abnormally large basophilic cells in gonadal tissues that resembled cells characteristic of ovacystis disease (Viral Gametic Hypertrophy) previously reported in *C. virginica* oysters from Maine and Long Island in the USA. Transmission electron microscopical examination of Feulgen-positive inclusions, observed in both males and females, revealed the presence of non-enveloped free viral particles ranging around 50 nm in diameter. Although similar abnormal cells have been observed occasionally before in *C. gigas* in France, viral particles were described for the first time in 2003. Further, the finding of this condition in seven batches of *C. gigas* from different geographical origins is considered unusual.

Picorna-like virus in the cockle *Cerastoderma edule* - Picorna-like viral particles were described for the first time on the Atlantic coast of Spain in cockles suffering from a condition first noted in 1997 as large foci of heavy hemocytic infiltration.

Herpes-like virus in spiny lobster (HLV-PA) - Follow-up, histologically based, surveys of Caribbean spiny lobsters (*Panulirus argus*) collected at 150 dive sites in the Florida Keys in the Gulf of Mexico, USA, indicate that the overall prevalence of HLV-PA (first reported in 2002) can be as high as 30% (mean 7%) of the juvenile lobster population, and it is most frequent among the smallest juveniles (mean 16%). In contrast, less than 1% of 1,548 adults and sub-adults presented visual signs of infection. All symptomatic lobsters were sub-adults. Experimental injections of the virus into spider crabs (*Libinia dubia*) and stone crabs (*Menippe mercenaria)* did not result in infection. Inoculation of adult spiny lobsters as yet has not resulted in disease. Two viral proteins (70kD and 200kD) were detected using a recently developed HLV-PA polyclonal antibody and Western Blot assay. There was no information on the results of molecular tests to determine the identity of the virus.

BACTERIA

Nocardiosis - No new trends in Canada and no new information from the USA.

Withering syndrome of abalone - The endangered white abalone, *Haliotis sorenseni*, on the west coast of the USA, has been determined to be susceptible to Withering syndrome.

Vibrio tapetis - No new trends in France and Spain.

Necrotizing hepatopancreatitis (NHP) in shrimp *(Litopanaeus vannamei)* in USA - No new information.

Vibriosis of the American lobster *Homarus americanus* - Several previously unidentified bacteria are believed to have caused losses of millions of dollars to the lobster industry in the late 1990s in the northeastern USA. Nineteen strains of *V. fluvialis*-like bacteria were isolated from diseased lobsters and Koch's postulates were met using several of the strains. Plasmids were found in seventeen of the strains, and studies with plasmid-positive and plasmid-negative strains suggest enhanced pathogenicity by plasmids. Additional laboratory assays (Chinese Hampster Ovary cell elongation; suckling mouse) suggest that toxins are involved in the infectious process.

FUNGI

Yeast in bivalves - No further information is available on the yeast-like organism found in the cockle, *Cerastoderma edule*, in France after the ERIKA oil spill.

Yeast in crustaceans – There was no new information on yeast in the brown shrimp, *Crangon crangon* (first reported in 2002), but a yeast infection was reported from a single individual edible crab, *Cancer pagurus*, from the English Channel in 2003.

ALGAE

Necrosis and haemocyte infiltration - were reported in several bivalve species (*C. gigas*, *Lutraria lutraria*, *Pecten maximus* and *Callista chione*) in association with a toxic algal bloom of *Cerataulina pelagica* in southern Brittany, France, in May 2003.

PARASITES

Bonamia ostreae* in the European flat oyster *Ostrea edulis – In England, *B. ostreae* was associated with high mortality (up to 50%) of native flat oyster seed at a farm site in the Fleet, Dorset. Prevalence was 30% and infection intensity was moderate to heavy. The oysters were hatchery produced and had been held off bottom in bags for only 3–4 months at a site where no native oysters had been cultivated for several years, although a low-density wild population of flat oysters is present. Advice was given to grow the surviving seed at much reduced densities. This was done and there have been no further mortalities to date. There were no new trends for wild or cultured oysters in other areas.

Following the outbreak of Bonamiosis in natural beds in Achill Sound, Co. Mayo (West coast of Ireland) late in 2002, surveillance for the disease was expanded according to European Directive 95/70/EC. In 2003, prevalence of *B. ostrea* ranged from 0 to 38% in the four beds examined in Achill Sound. The expanded survey included natural beds in the adjacent Black Sod Bay. There, *B. ostrea* was detected in one of 447 oysters (0.2%) in spring 2003. Consequently, Black Sod, as well as Achill, Bay lost the approved zone status for Bonamiosis (2003/378/EC and 2003/729/EC). The specific origin of infection has not been determined, but a number of sources are possible. One possibility is that the parasite was introduced in molluscs transferred from *Bonamia*-positive areas. The evolution and geographical spread of the disease was monitored again during autumn 2003. A sample of 100 oysters proved negative by cellular imprints and histology. Abnormal mortalities were not observed and spat settlement was noticed. No new trends were recorded in the rest of Ireland, with the overall level of infection in native oysters from wild fisheries historically affected by the disease remaining around 15%.

No new trends were reported in France, Norway or Scotland. There was no new information on Spain.

Bonamia* sp. in *C. ariakensis - A new species of *Bonamia* (based on rDNA sequence analysis) was found in the Suminoe oyster (*C. ariakensis*) in Bogue Sound, a high salinity site in North Carolina, USA, where the oysters were being evaluated for possible introduction. The oysters, hatchery-produced triploids reared following the ICES protocols, had experienced heavy mortality during the summer of 2003. *Bonamia* sp. was subsequently found in up to 60% of live *C. ariakensis* at this site. It has not been found in members of the same cohorts being tested at lower salinity sites in North Carolina or in the Chesapeake Bay, nor was it found in histological sampling of an earlier cohort tested in Chesapeake Bay and high salinity areas of coastal Virginia. The parasite is considered to be enzootic, rather than introduced, and a search for the local reservoir host is under way.

Marteilia refringens* in *O. edulis* and mussels *M. edulis – No new trends were reported in France, Norway or Scotland. One case, associated with mortality, was reported in *M. edulis* in Spain. Native flat oysters in UK and Ireland are considered free of Marteiliosis, as determined by histology.

Mikrocytos mackini* in *C. gigas - No new information was reported from Canada or the USA.

Perkinsus marinus* (Dermo) in *C. virginia - The parasite remains present on nearly all beds in the Chesapeake and Delaware Bay, but at significantly reduced prevalence and intensity compared to the four previous years. The reduction is associated with a very wet and relatively cold winter/spring. No change was observed in other areas where the parasite is enzootic.

P. atlanticus/olseni* in *Ruditapes philippinarum - No new trends were reported in France or Spain.

P. andrewsi/chesapeaki* in *Mya arenaria* and *Tagelus plebeius - Prevalence in a sample of 542 clams (both species combined) fell to 53% from 86% in 2002 (n=557) in the upper portion of Chesapeake Bay, USA. No change was reported for the lower Chesapeake Bay.

P. mediterraneus - A new species, *P. mediterraneus* sp. nov., has been described, using both morphological and genetic data, in flat oysters in the Balearic Islands in Spain.

Haplosporidium nelsoni* (MSX) in *C. virginica - Infections, reported for the first time in Canada in 2002, remained restricted to the Bras d'Or Lakes area of Cape Breton Island, Nova Scotia. Re-examination failed to detect the parasite in archived tissue sections, although only a relatively small number of sections have so far been examined. In the USA, freshwater inflow resulted in the restriction of *H. nelsoni* in the Chesapeake Bay to high-salinity, lower-bay, regions. In the upper portion of the estuary, infection prevalence among 1,290 oysters sampled in the regular autumn survey was 1% in 2003 compared to 28% in 2002. No change was reported for South Carolina and no new information was reported from other areas.

H. nelsoni* in *C. gigas - An *in situ* hybridisation test detected a probable infection of *H. nelsoni* in one of 119 *C. gigas* spat, originating from the Seudre River (Charente Maritime) in France and presenting abnormal mortality.

H. costale* (SSO) in *C. virginia - No new trends in Canada or USA. Limited re-examination of archived tissue sections failed to find this parasite before its initial detection in Canada in 2002.

Haplosporidium sp. was found in one single flat oyster from Clew Bay, Co. Mayo (west coast of Ireland).

Haplosporidium* sp. in *M. edulis - No subsequent finding of this parasite (reported for the first time in a single mussel in Canada in 2002) was reported, despite intensive surveillance of mussels for other infections.

Haplosporidium* sp. in the shore crab *Carcinus maenas - There was no new information on the status of this parasite (first reported in 2002), but a manuscript describing the finding has been accepted for publication and material has been sent to the Virginia Institute for Marine Science (reference laboratory for *Haplosporidium* spp.), USA, for molecular characterization.

Quahog Parasite X (QPX) in the hard clam *Mercenaria mercenaria* – An outbreak occurred in two-year old clams on a farm in Rhode Island, USA. Mortality was 50% and QPX prevalence was 80–90%. The clams were of southern origin (previously determined to be highly susceptible to QPX) and adjacent stocks of different geographic origin had low prevalence (17%) and no obvious mortality. Surveys of wild clams in Raritan Bay, New York, where a mortality associated with QPX was detected in 2002, confirmed the presence of QPX at generally low prevalence (<7%) in most locations, but reaching 30% at one site where the clam density was unusually high. QPX was also found at the eastern end of Long Island, New York, an area to which clams from Raritan Bay had been transplanted for field depuration. The highest prevalence (20%) was at a site distant from any transplant plot, suggesting that QPX was enzootic and not introduced with the Raritan Bay clams. No change in QPX was detected in Virginia, and no QPX was detected in 75 clams examined from two sites in South Carolina.

***Hematodinium* sp.** - No new information was reported for any species.

DISEASES OF UNCONFIRMED AETIOLOGY

Shell disease of *Homarus americanus* - Shell disease lesions associated with various bacterial and fungal species continue to be found at high prevalence (30–35%) on lobsters in the area from eastern Long Island Sound to southern Massachusetts, USA, and new cases have been found to the north in the Gulf of Maine and to the south in Maryland. Recent research resulted in isolation of *Pseudoalteromonas gracilis* and *Cytophaga* sp. from each affected lobster sampled to date, suggesting that these bacteria may play a significant role in the onset of shell disease. Laboratory studies have not been able to reproduce the disease in healthy lobsters either with surficial inoculation of bacteria or by cohabitation. Further study is focusing on the role of stress and a weak immune system in development of shell disease.

Juvenile Oyster Disease (JOD) of *C. virginica* - JOD epizootics occurred in New York, and were unusually widespread on Cape Cod and Marthas Vineyard, Massachusetts, ÚSA, where mortalities exceeded 50% during the summer. All were associated with a bacterium in the group *Roseobacter* (tentatively named *Roseimarina crassostreae*) previously isolated in JOD-affected oysters in Maine, but the strains were different both among sites and from the Maine strains. Although *R. crassostreae* is consistently found in oysters with JOD symptoms, it has not been detected in pre-symptomatic oysters and experimental challenge has so far not reproduced JOD symptoms. Consequently, the potential cause-effect relationship between the bacterium and the disease has yet to be demonstrated.

Neoplasia in bivalves - A neoplastic condition of gill epithelial cells, which can become invasive in other tissues, was described from clams, *Macoma balthica* in the Gulf of Gdansk, Baltic Sea (Annex 6). Chromosome numbers in the neoplastic cells ranged from 54 to 108, in contrast to the normal diploid number of 38. A multi-year study (1996–2002) found a mean prevalence of 32%. The mean was 30% from 1996 through 1999; rose to 43% in 2000 and 2001; and fell to 16% in 2002. Prevalences tended to be higher in spring and summer than in winter, and were highest in deep water, where the highest individual sample prevalence was 94%. A significant relationship ($p<0.005$) was found between sediment toxicity and the prevalence of the neoplasms in *M. balthica*. The condition was also found in *Mya arenaria*, which, like *M. balthica*, is a facultative filter/deposit feeder. In contrast, neoplasms were not found in two filter-feeding bivalves, the mussel *Mytilus edulis trossulus* and the cockle *Cerastoderma glaucum*. Consequently, the hypothesis was offered that the neoplasms are related to ingestion of contaminated sediment by deposit feeding, as well as by direct contact with the sediment. No new trends were reported for the cockle (*Cerastoderma edule*) in Spain and no new information was reported from other countries.

Unexplained mortalities of giant sea scallop (*Placopecten magellanicus*) in Canada - No new information.

Unexplained mortalities of mussels (*M. edulis*) in England - No new information.

Unexplained mortalities of adult Pacific oysters (*C. gigas*) - Summer Mortality Syndrome caused high mortalities in the Bannow Bay, Co. Wexford (south coast of Ireland) during the summer 2003. No disease agents were found in samples totalling 150 oysters, as determined by histology. No new trends were found in France and no new information came from the USA. An ongoing (2001–2005), large, multidisciplinary project (MOREST) in France is focused on understanding the causes of the Summer Mortality Syndrome and providing practical information for diminishing the problem.

Necrosis and haemocyte infiltration associated with mortalities in *C. edule* and *M. edulis* in France were reported during a period of exceptionally high temperatures in the summer of 2003.

Conclusions

1) Viral gametic hypertrophy (ovacystis) was documented in seven lots of *C. gigas* in France. Viral particles were associated with this condition for the first time.
2) Picorna-like viral particles were described for the first time in the Atlantic coast of Spain in cockles suffering from a condition first noted in 1997 as large foci of heavy hemocytic infiltration**.**
3) Follow-up studies of the herpes-like virus (HLV-PA) in the Caribbean spiny lobsters have confirmed high prevalence (up to 30%) in wild stocks, with the highest prevalence being in the smallest juveniles. The virus has not caused disease when injected into adult spiny lobsters or two sympatric crab species.
4) Following the outbreak of Bonamiosis in natural beds of flat oysters in Achill Sound, Ireland, in 2002, obligatory surveillance was expanded according to European Directive 95/70/EC. In the adjacent Black Sod Bay, *B. ostrea* was detected in one of 447 oysters (0.2%) in Spring 2003. Consequently, Black Sod, as well as Achill, Bays lost the approved zone status for Bonamiosis (2003/378/EC and 2003/729/EC). The specific origin of infection has not been determined. It is possible that the parasite was introduced in a species not known to be susceptible to *Bonamia*.
5) A new species of *Bonamia*, considered to be a previously unrecognised enzootic parasite, was associated with mortalities in the introduced oyster, *Crassostrea ariakensis*, undergoing testing in North Carolina, USA.
6) A cold winter and a very wet winter/spring greatly reduced the prevalence and intensity of both *Perkinsus marinus* and *Haplosporidium nelsoni* in *C. virginica* in the mid-Atlantic estuaries, USA.

7) A new species, *Perkinsus mediterraneus* sp. nov., has been described in flat oysters in the Balearic Islands in Spain.

8) Re-examination of archived tissue sections of *C. virginica* in Nova Scotia, Canada, has so far failed to detect the presence of either *H. nelsoni* or *H. costale* before the 2002 outbreak, but relatively few historical samples have so far been examined.

9) A neoplastic condition of gill epithelial cells was described in the clam, *Macoma balthica* in the Gulf of Gdansk, Baltic Sea. Prevalences were unusually high, with individual samples as high as 94% and a mean of 32% over a seven year period from 1996 to 2002. A significant relationship was found between sediment toxicity and the neoplasms, and a hypothesis was offered that the neoplasms are related to contact with, and ingestion of, contaminated sediment by deposit feeders such as *M. balthica*.

10) An ongoing (2001–2005), large multidisciplinary project (MOREST) in France is focused on understanding the causes of the Summer Mortality Syndrome and providing practical information for diminishing the problem.

11) The WGPDMO recognizes that non-commercial crustacean species harbour pathogens that may have significance for commercial fish and shellfish stocks.

Recommendations

The WGPDMO recommends that:

i) WGPDMO be kept informed of progress in studies of the herpes-like virus infecting the Caribbean spiny lobster in the USA, with particular attention to its impacts on the lobster population, and its identification and possible affiliation with the oyster herpes virus;

ii) studies be expanded on Viral Gametic Hypertrophy in French Pacific oysters, to specifically identify the viral agent and its effect, and to obtain information on viral prevalence;

iii) the ICES Working Group on Introductions and Transfers of Marine Organisms (WGITMO) be informed of the outbreak of bonamiosis in the introduced oyster, *C. ariakensis*, undergoing testing in North Carolina, USA, with special reference to the fact that the *Bonamia* sp. is not considered to have been introduced with the host oyster, but to be an enzootic parasite to which the introduced host is highly susceptible;

iv) tests of molluscan species including abalone and scallops be done to ascertain whether they might be carriers of *B. ostreae*;

v) WGPDMO be kept informed of progress on studies of neoplasms in Baltic Sea clams;

vi) WGPDMO compile and review information on the distribution, causes and significance of the Summer Mortality in the Pacific oyster (*C. gigas*) and in other bivalve species at its 2005 meeting.

6 REVIEW AND REPORT ON ENVIRONMENTAL MONITORING PROGRAMMES AND ASSOCIATED QUALITY ASSURANCE ACTIVITIES INCORPORATING STUDIES ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

6.1 National monitoring programmes

The following environmental monitoring programmes incorporating pathology and diseases of marine organisms are routinely performed in the ICES area:

- In the USA, the NOAA National Status and Trends (NS&T) Program was designed to assess historical trends of coastal contaminants and their biological effects. There are three major components: Benthic Surveillance Project (BSP), Mussel Watch Project (MWP) and Bioeffects Assessment (BA) Project.
- The US EPA Environmental Monitoring and Assessment Program (EMAP), is a research-program to develop the tools necessary to monitor and assess the status and trends of national ecological resources, including those in coastal environments. (Both the NOAA NS&T Program and the EPA EMAP are summarized in Annex 7).
- Regular wild fish disease monitoring programmes are conducted by the following ICES Member Countries:
  - *Germany*: Biannual surveys are carried out in offshore areas of the North Sea and the southwestern Baltic Sea. The major target fish species in the North Sea is dab (*Limanda limanda*), and in the Baltic Sea flounder (*Platichthys flesus*) and cod (*Gadus morhua*). Externally visible diseases/parasites and liver anomalies (gross and histopathological) are recorded according to ICES guidelines. The data are submitted to the ICES Environmental Data Centre.

- *The Netherlands*: Disease surveys are conducted annually in three North Sea offshore areas, sites in the western Wadden Sea and in coastal zone of the Eastern Scheldt with dab and flounder as target species. Externally visible diseases/parasites and liver anomalies (gross and histopathological) are recorded according to ICES guidelines. The data are submitted to the ICES Environmental Data Centre.
- *UK*: The UK National Marine Monitoring Programme (NMMP) was established to detect long-term trends in physical, biological and chemical variables at selected estuarine and coastal sites in the North Sea, Irish Sea and the English Channel. Ten to fifteen offshore areas are included. The biological effect component of this programme includes assessment of the disease status of target flatfish species (dab and flounder). In addition, data on diseases and parasites in commercial species are also collected. Estuarine monitoring activities have been undertaken more recently using flounder and viviparous blenny (*Zoarces viviparus*) as the target species. In Scotland, externally visible diseases/parasites and liver anomalies of dab, cod and haddock (*Melanogrammus aeglefinus*) are monitored at sampling sites in the Firth of Forth, east of Orkney and in the Moray Firth. Diseases are recorded according to ICES guidelines and the data are submitted to the ICES Environmental Data Centre.
- *Poland*: Externally visible diseases and some parasites (e.g., *Anisakis simplex* in herring) of Baltic cod, flounder, herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) are monitored in the Polish EEZ of the Baltic Sea.
- *Russia*: Disease prevalences are monitored in cod, herring, flounder and sprat in the Russian EEZ of the Baltic Sea. Since some years, diseases are also monitored in various fish species in the Barents Sea.

Many of these programmes have increasingly evolved into integrated monitoring programmes, including studies on chemical contamination and on biological effects of contaminants.

- There is growing interest in effects of environmental factors (including contaminants) on the health status of marine mammals. Therefore, some countries such as Canada and Germany have started to monitor the health status of mammals (cetaceans and seals).

Methodologies and guidelines applied to monitor wild fish diseases, parasites, macroscopic liver nodules as well as liver histopathology were developed by ICES and relevant quality assurance procedures for biological effects techniques have been developed and implemented under the EU-funded project Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) (in depth reviewed by WGPDMO at its previous meetings).

Quality Assurance programmes in the USA for the NS&T Programme and the EMAP project have documented sampling protocols, analytical procedures and laboratory performance of intercalibration exercises that were conducted annually. Undocumented intercalibration exercises were conducted between the two laboratories performing the histological examinations of fish tissues. With the focus on hepatic lesions, the descriptive terminology used was well documented and accepted in published manuscripts.

In studies on the health status of marine mammals, quality assurance procedures comparable to those implemented in studies in wild fish have not been developed yet. However, more detailed information on the programmes was not available at the time of the 2004 WGPDMO meeting.

6.2 Research-oriented projects related to monitoring activities

The WGPDMO is aware that there are numerous research-oriented studies and projects under way aiming to identify biological effects techniques appropriate for monitoring purposes, including the use of diseases/pathology and other health parameters of marine organisms as indicators of environmental effects.

As an example, K. Broeg presented a brief summary of the use of histochemical techniques in integrated studies on pathological effects of contaminants. Parameters applied in these projects were lysosomal membrane stability, accumulation of neutral lipids and the activity of macrophage aggregates in the livers of fish. In addition, parasitological investigations as well as contaminant analyses were performed in identical animals in long-term studies in the German Bight (North Sea) from 1995 to 2000 (BMBF-funded projects MARS 1 and 2). Observations on a set of pathological endpoints in individual fish and progress on health deterioration (four stages) were characterised by certain pathological key-events in flounder from the German Bight. The Bioeffect Assessment Index (BAI), integrating these endpoints, was sensitive to disturbances and recoveries of the health conditions after a specific pollution event and intensive dredging activities in the river Elbe. The BAI was also applied on flounder and viviparous blenny (*Zoarces viviparus*) of the Baltic Sea (EU-funded project BEEP, 2001–2003) to quantify the effects of general toxicity on fish health and test its potential use as an indicator for environmental quality.

As another example, M. Wolowicz presented results of a study on the occurrence of neoplasms in bivalves in the Gulf of Gdansk (Baltic Sea) (*Macoma balthica*, *Mya arenaria*), possibly associated with sediment toxicity (see Section 5.3, Annex 6).

In the UK, research in support of the NMMP (see above) includes biomarker development and investigation of the health status of estuarine fish and shellfish, including flounder, viviparous blenny, shore crab (*Carcinus meanas*) and brown shrimp (*Crangon crangon*).

In Canada, a project on the health of Atlantic tomcod (*Microgadus tomcod*) in relation to environmental contaminants ended last year. A new project focuses on the health (mostly gonad histopathology) of stickleback (*Gasterosteus aculaetus*) in relation to pesticide concentrations in water in the marshes of the St. Lawrence Estuary.

Research-oriented programmes on changes in the health status of bivalves and gastropods with relevance for monitoring were reviewed by WGPDMO at previous meetings (e.g., ICES 2002, 2003).

Conclusions

1) With the further development and implementation of the OSPAR JAMP/CEMP, studies on general and contaminant-specific biological effects, including studies on diseases/pathology and other health parameters, will be of increasing importance for the assessment of the status of the marine environment.
2) Regular monitoring programmes incorporating studies on pathology and diseases of marine organisms are carried out only by a limited number of ICES Member Countries. While most emphasis is still placed on wild fish in offshore areas (mostly flatfish species), studies in coastal and estuarine areas with 'new' target fish as well as shellfish species (crustaceans and molluscs) are increasingly incorporated in regular monitoring programmes.
3) National monitoring programmes on the health status of marine fish and shellfish have increasingly been combined with the measurement of chemical contaminants and other types of biological effects, e.g., biomarkers of exposure and early effects, and to a lesser degree, bioassay-oriented work, in more integrated programmes.
4) There is growing interest in studies on environmental effects on health parameters in marine shellfish (crustaceans, bivalves and gastropods) and marine mammals and there is a need to develop appropriate methodologies and study designs that can be applied for monitoring.
5) New research/monitoring directions to be reviewed by WGPDMO address the establishment of 'health indices' based on a suite of health parameters. In this context, the WGPDMO considered changes in the immune functions in fish and shellfish as promising. However, tools applicable for monitoring still have to be developed.

Recommendations

The WGPDMO recommends that:

i) further studies are carried out in order to develop 'health indices' for the quantification of contaminant effects on the health status of fish and shellfish and that progress regarding their applicability for monitoring be reviewed by WGPDMO at its 2005 meeting;
ii) available information on the effects of contaminants on the immune system in fish and shellfish be reviewed at the 2005 WGPDMO meeting.

References

ICES 2002. Report of the Working Group on Pathology and Diseases of Marine Organisms, 2002. ICES CM 2002/F:02.

ICES 2003. Report of the Advisory Committee on the Marine Environment, 2003. ICES Cooperative Research Report 263, 66–70.

7 PROVIDE A RECOMMENDED TECHNIQUE TO DIFFERENTIATE AMONG *PERKINSUS* SPP., INCORPORATING INPUT RECEIVED FROM WEB-BASED INTERNATIONAL SOLICITATION OF COMMENTS

In 2003, the WGPDMO reviewed a report entitled "Review of molecular techniques used to differentiate the various species/isolates of *Perkinsus*" prepared by S. Bower (DFO, Canada), in collaboration with E. Burreson and K. Reece (VIMS, USA). The document outlined the criteria used for species identification in the genus *Perkinsus* and was annexed in the 2003 WGPDMO report (ICES 2003). The WGPDMO recommended that the document be circulated widely so as to solicit international comments and contribution. It was also recommended that an updated report on techniques used to differentiate among *Perkinsus* spp., incorporating input received as a result of the above solicitation, should be prepared for and reviewed at the 2004 WGPDMO meeting in Åbo.

T. Renault gave the PowerPoint presentation based on this document that was presented at a *Perkinsus* Workshop held in conjunction with the annual meeting of the European Association of Fish Pathologists (EAFP) in Malta on 23 September 2003. The aim of this workshop was to review current issues posed by perkinsosis with particular emphasis on taxonomy, epidemiology and impact on mollusc stocks, and identify where new developments in research are needed to improve knowledge and understanding. The workshop considered several scientific communications, including the contribution from the WGPDMO. Participants at the workshop considered the following recommendations and follow-up actions appropriate:

- Non-specific assays (i.e., Fluid Thioglycollate Medium and histopathology) are only suitable for the initial detection of perkinsosis and should not be used for the specific identification of any isolate regardless of host identity.
- Morphological differences between species need to be described by the accurate comparison of the morphology of various developmental stages *in vitro* of several isolates of named species under similar incubation conditions (e.g., media, parasite density, time in culture, incubation temperature, etc.). Studies conducted in parallel with the same isolates in several laboratories would be ideal.
- Verify if multiple polymorphic sequences of the ITS region of the rRNA gene complex occur in all *Perkinsus* spp.
- Assess if the NTS region of the genome is a good discriminator of species and strains of *Perkinsus.* Apparently, this region of the genome has low similarity between species and high similarity within species, but has not been adequately assessed for intra-specific variation.
- Identify other regions of the genome that may prove useful for species differentiation.
- Possibly all molecular assays should be tested in parallel and validated, and further sensitive diagnostic assays that will clearly discriminate between all “valid” species should be developed.
- Carry out surveys and transmission studies to accurately assess host and geographic ranges and distributions.
- Determine if strains have genetic and/or virulence differences.
- Description of new species must require the deposition of *in vitro* isolates in public repositories.

At the EAFP meeting, Dr A. Villalba (Centro de Experimental de Vilaxoan, Spain) offered to lead a group to try to obtain funding for a cooperative *Perkinsus* project at the EU level. In addition, there is a growing interest in Asia on this subject and the Network of Aquaculture Centres Asia-Pacific (NACA) supports the idea of launching a regional initiative with funds available in Brussels. It was also suggested that it would be good to have a common house for the different initiatives on the subject.

Conclusions

1) The WGPDMO endorses the recommendations of the EAFP workshop.
2) There is a real need for validation of existing techniques and development of new ones.
3) Criteria used to differentiate among species should include basic biological characteristics of the parasite, as well as its genetic sequence.
4) The WGPDMO appreciates the offer of Dr A. Villalba to organize a *Perkinsus* project and to seek funding for it at the EU level.
5) There is an increasing effort of the scientific community to exchange information on the *Perkinsus* topic as exemplified by the recent EAFP workshop and the “PerkID” discussion list.

Recommendations

The WGPDMO recommends that:

i) ICES Member Countries be encouraged to support the funding of research into improved methods of detecting and identifying *Perkinsus* spp. because of the worldwide distribution of *Perkinsus* spp. and the important economic damage that they inflict on commercial molluscan species;

ii) laboratories involved in *Perkinsus* research initiate collaborative testing, intercalibration, and validation of current and newly developed techniques for the purpose of recommending a technique or techniques to differentiate among *Perkinsus* spp.;

iii) the WGPDMO be kept informed on progress made in developing *Perkinsus* research programmes.

Reference

ICES 2003**.** Report of the Working Group on Pathology and Diseases of Marine Organisms, 2003. ICES CM 2003/F:03.

8 REVIEW THE EXISTING INFORMATION ON VIRAL DISEASES OF CRUSTACEANS WITH EMPHASIS ON COMMERCIALLY IMPORTANT SPECIES

A working document was presented by S.W. Feist and S.A. MacLean, which had been prepared intersessionally (Annex 8). The document reviewed the existing information on viral diseases of crustaceans, with the aim of 1) assessing the available information in this area; 2) highlighting evidence of significant infections, in particular pathological effects and any data on possible effects on wild populations and 3) providing a list of relevant references.

Knowledge on viral diseases of crustaceans is best developed for species of importance for aquaculture, in particular the penaeid shrimps, whereas relatively few studies deal with prevalence and pathogenicity of viral diseases in wild marine crustaceans. Since the first report of viral disease in crabs in 1966, there have been over thirty descriptions concerning such infections. Characteristic symptoms of some viral diseases in crab are "trembling movement" and paralysis, but in most infections no behavioural abnormalities are seen. Often viral disease outbreaks are seen in crabs after they have been taken into laboratories in connection with experiments of, for example, physiological parameters. Accounts of clinical signs of viral infections of crustaceans are limited. Although viruses appear to be widely distributed among wild crustaceans, there is little information on the prevalence and impact of virus-caused diseases on wild crustacean populations. It appears that several viruses can infect the same host but despite this, histopathologic changes in infected organs are usually mild. Intense infections can result in organ dysfunction but inflammatory responses are rarely reported. Several studies have suggested that stress is a significant factor in producing a disease state. As such, the detection of increased prevalence of diseased crustaceans could be used to indicate stress effects at the population level. It is suggested that investigations on disease prevalence in coastal regions used for biological effects monitoring be undertaken, as well as laboratory studies to understand the pathogenesis of the infections.

Many viral diseases are seen in cultured penaeid shrimps and nearly all have resulted from the use in culture of infected wild larvae, post-larvae or broodstock, and although reported from wild shrimp and other crustaceans, few data exist on their prevalence and impact on wild populations. The White Spot Syndrome Virus (WSSV), which infects a broad range of crustaceans including cold-water species, has been detected in wild shrimps, *Litopenaeus setiferus* and *Farfantepenaeus aztecus*, and blue crab (*Callinectes sapidus*) in the USA. Other recently described viral infections include intranuclear bacilliform virus (IBV) in brown shrimp (*Crangon crangon*) with prevalence up to 100% in the Clyde estuary, western Scotland, and a herpes-like virus infecting the haemocytes of the Caribbean spiny lobster (*Panulirus argus*), with prevalences around 17% in juveniles, the principal hosts of the infection.

Other pathogens often occur as co-infections with viral pathogens. These may adversely affect the host's ability to mount an effective immune response to viral infections with consequent increased severity of infection and prevalence. Usually, diagnosis of viral diseases is based on histopathology and ultrastructure of the viruses, although a number of viruses of cultured shrimp are diagnosed using molecular techniques. If the presence of specific viral pathogens is shown to be a good indicator of individual and population stress, the use of molecular and immunologically-based detection methods using haemolymph or tissue samples will be a practical approach for use in environmental monitoring programmes. However, the development of such methods for specific pathogens is needed.

Conclusions

1) There is a significant amount of data available on viruses in captive crustaceans but relatively few data on the prevalence of these in wild populations.
2) As the number of population and environmental studies on wild crustaceans increase, the expectation is that further viral pathogens will be disclosed. Some of these viruses may have significance at the population level of commercial species.
3) There is some evidence that stress, e.g., caused by adverse environmental factors, may exacerbate the intensity of viral diseases in crustaceans. Therefore, the presence of these infections may be of use in environmental effects monitoring studies.

Recommendations

The WGPDMO recommends that:

i) further studies be undertaken to assess the distribution and prevalence of viral pathogens in wild crustacean species that may be considered for inclusion in biological effects of contamination monitoring programmes;

ii) relevant ICES Working/Study Groups focusing on commercially exploited wild crustaceans (WGPAND, SGCRAB, WGCRAN, WGNEPH) should take note of the information on the occurrence of viral diseases of crustaceans with potential population impact, in particular the high prevalence of viruses observed in brown shrimp.

9 THE USE OF EPIDEMIOLOGICAL METHODS FOR THE ASSESSMENT OF DISEASES AND THE RISK OF POPULATION EFFECTS

The possibility to use epidemiological methods and the potential gain by their use for the analysis of fish disease data were reviewed in a paper prepared by W. Wosniok, S.W. Feist and T. Lang and presented by W. Wosniok. The paper (Annex 9) outlines basic terms, tools and approaches of epidemiological methods and identifies three fields in which such methods seem able to improve present analyses or to enlarge the amount of information that can be extracted from fish disease and related data.

From descriptive epidemiology, the concept of direct standardisation is useful to compare prevalences calculated from samples with different structures of, e.g., age or other factors that might influence the prevalence. The approach of Attributable Risk, which quantifies the prevalence proportion due to a specified exposure, is useful to assess the impact of the corresponding factor on the prevalence and also to assess scenarios of various exposures for e.g., regulatory purposes.

From analytical epidemiology, the concept of case-control studies can be adopted to improve the power of disease prevalence analysis by either obtaining higher probabilities to recognize effects (when maintaining present sample sizes) or achieving the same detection probabilities with less effort (using fewer cases). The basic idea of a case-control design is to analyse not a random sub-sample from the catch, but to select a specific sub-sample for analysis, which contains as many diseased as non-diseased specimens, a procedure which can be applied on board if externally visible diseases are studied.

Fish diseases may have effects on a population structure, if the disease affects growth, mortality or reproductive ability, for example. The study of population dynamics under the presence of diseases is an epidemiological approach to allow the assessment of disease effects. The result of such studies is a quantification of these effects in terms of changes in biomass, population size or structure. This means that the scope of risk assessment for fish diseases could be expanded beyond the immediate effects for the fish to aspects of general ecological and economic importance. Only a few studies of this kind are known so far (e.g., *Ichthyophonus* in herring stocks, reproductive effects in Puget Sound).

The prerequisite to study population effects by using a population dynamics approach is the availability of time series data of:

- stock sizes, including preferably their age structure (may possibly be replaced by length distributions plus age-length keys),
- fishing effort,
- fish disease prevalence,
- mortality due to natural causes, to the diseases under study, and due to fishing,
- reproductive ability.

For each location under study, data from all these categories are needed from identical or at least similar observation dates. Data exist for most of the mentioned kinds, however, it is open for some of them. It is also open whether the amount of data is sufficient and particularly whether the components can properly be linked to each other with respect to locations and observation times.

Conclusions

1) Standardisation methods from epidemiology may facilitate the comparison of disease prevalences from data with different demographic structures.

2) There are methods available that may improve the power of standard fish disease analysis for the effect of influencing factors.

3) There are epidemiological methods which allow assessment of the risk to populations due to fish diseases.

4) The availability of appropriate data for a risk assessment of population effects is not yet clear.

Recommendations

The WGPDMO recommends that:

i) WGPDMO should evaluate intersessionally whether sufficient data for a risk assessment pilot study on population effects due to diseases in wild fish, using epidemiological and population dynamics modelling, are available from ICES or related sources and review progress made at its 2005 meeting;

ii) the conveners of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Area (WKIMON) in Copenhagen, January 2005, should take notice of the methods outlined in Annex 9 of the present report as methods of relevance for the planning, conduct and analysis of integrated monitoring.

10 EVALUATE CURRENT INFORMATION ON DISEASE/PARASITE INTERACTIONS BETWEEN WILD AND FARMED FISH AND ADVICE ON RELATED MANAGEMENT CONTROL METHODS

A working document was presented by D.W. Bruno and B. Hjeltnes regarding current information on disease/parasite interactions between wild and farmed fish with advice on related management control methods (Annex 10).

There is continuing concern regarding the impact of diseases that occur in fish farms and their impact on wild stocks. However, there are very few documented examples of outbreaks that have had a significantly negative effect on populations of wild fish. Therefore, it is not easy to be confident about the frequency or significance of transfer of pathogens from farmed to wild stocks. Disease in wild fish often occurs at a low level, although epizootics are documented, and most diseases reported in farmed fish are identified in wild fish. In a similar manner, it is not easy to be confident about the frequency or significance of transfer of pathogens from wild to farmed stocks.

The infection with sea lice has been suggested as one cause for the decline in wild salmon and sea trout stocks, however this is unresolved and remains controversial. Advances in treatment and management tools, including modelling, are being developed in ICES Member Countries.

Conclusions

1) In general, there is little information regarding disease/parasite interactions between wild and farmed fish.

2) The greatest concern for wild fish in terms of disease impact is the exposure to a larger than normal concentration of indigenous pathogens or exotic pathogens from aquaculture sites. However, there are only few fully documented cases so far.

3) Where wild broodstock have been used to enhance aquaculture activities, diseases have been introduced with significant impact within the farm stock. Recently, cases of infections with nodavirus and *Pseudomonas anguilliseptica* have occurred with wild caught broodstock from sole (*Solea* spp.) and blackspotted seabream, (*Pagellus bogaraveo*) in Spain.

4) Cultured fish, particularly those in ocean net pens, could be exposed to pathogens from wild fish. The agents that affect farmed salmon have in general been reported in wild fish.

5) Aquaculture will continue to expand, and it will become increasingly important to locate farms in areas where both environmental and biological data can be combined and utilized to minimise risk of disease transmission. Where available, models such as on sea lice production and predicted impact on wild salmonids might be included.

6) Controlling emerging diseases on fish farms will benefit from continued development of mathematical models and risk analysis to understand the dynamics of fish epidemics and the use of these models to determine how the disease might persist and spread in a wild population. This approach could provide a rational way to identify potential risks and gaps in current knowledge.

7) Sea lice infestations and their impact on farmed fish have been reduced through the use of new treatments and fallowing options, but there is still controversy regarding the role and impact on wild fish in some areas. Work in

this area is under way and some countries are developing guidelines for minimising the risk of interaction with wild salmon, including compulsory reporting of lice numbers and co-ordinated treatment programmes.

8) Considerable emphasis has to be placed on studies of wild fish populations and the development of methodologies that will identify disease at an early stage and could include non-destructive test methods.

Recommendations

The WGPDMO recommends that:

i) despite the many difficulties in obtaining good data from disease interactions between wild and farmed fish, emphasis should be given to research in this area;

ii) consideration should be given to improving the management and disease screening of wild broodstock used to enhance farm stock;

iii) the development of models and risk analysis on disease persistence and their spread in wild populations should continue;

iv) the implementation of coordinated sea lice control management programmes should be encouraged;

v) WGPDMO continues to evaluate current information on sea lice interactions between wild and farmed fish and examines progress made in related management control methods at its 2005 meeting.

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

11.1 Ichthyophonus

Information was extracted from the national reports on diseases of wild fish.

Following an epidemic of *Ichthyophonus* in herring in the North Sea and adjacent areas in the early 1990s, the prevalences have decreased in previous hot-spot areas. However, data from a recent bottom trawl survey (February 2004) confirmed that *Ichthyophonus* is still endemic in the northern North Sea. The prevalence of grossly observed heart lesions in single hauls ranged from 0% to 17%, corresponding to data from previous years.

In the USA, approximately 35% of returning Yukon river chinook salmon (*Oncorhynchus tshawytscha*) were infected with *Ichthyophonus*, similar to 2002 levels, but much higher than in previous years.

According to the current taxonomy, the genus *Ichthyophonus* is no longer considered as belonging to the fungi but as a member of the Class Mesomycetozoea (a group of microorganisms at the animal-fungal boundary), Order Ichthyophonida (Mendoza *et al*. 2002).

11.2 M74 syndrome

S. Wistbacka (Laboratory of Aquatic Pathobiology, Åbo Akademi University) presented an overview of the present state and possible causes of M74 (Annex 11). The overview included information on 1) the history and present state of the syndrome, 2) current research activity and 3) future research needs.

The ultimate causes of the M74 syndrome still remain unknown, but there is increasing evidence that the syndrome is linked to the thiaminase activity of prey species (herring) in the Baltic Sea. It was also recognised that undefined stress factor(s) may cause variations in thiaminase activity of the prey species and are possibly linked to the density of pelagic fish populations in the Baltic Sea.

Accumulated information indicates that the wild salmon stock in the Baltic Sea is presently recovering from the critical decline faced in the 1990s. Several factors may have contributed to the improved situation:

- salmon quotas have been established that significantly have reduced the catches;
- thiamine treatment of M74-affected salmon eggs and fry has proven to be effective; and
- a decreasing trend in the prevalence of M74, compared to the situation in the middle of the 1990s.

However, based on the history of the M74 syndrome, it is known that its status can change again very rapidly. As long as the ultimate causes behind the M74 have not been identified, no reliable prognoses can be made on its future development.

Conclusions

1) Collected data indicate that *Ichthyophonus* is still present in the northern North Sea, but no new trends were observed. In the USA, chinook salmon were infected with *Ichthyophonus* at levels similar to those in 2002, but much higher than in previous years.
2) Compared to 2002, a decreasing mortality of salmon fry due to M74 was observed in the Baltic Sea.
3) The ultimate causes of the M74 syndrome remain unknown, but there is increasing evidence that the syndrome is linked to the thiaminase activity of prey species (herring) in the Baltic Sea which seems to be influenced by general stress factors associated with herring population density.
4) The WGPDMO agreed that there is no need to include *Ichthyophonus* and M74 as a separate WGPDMO Term of Reference for future meetings. It suggests that new information on *Ichthyophonus* and M74 is considered in the future as part of the review of national reports on new disease trends.

Recommendations

The WGPDMO recommends that:

i) ICES Member Countries be encouraged to continue monitoring the prevalence of *Ichthyophonus*, if appropriate in connection with herring stock assessments, so as to be aware of changes in status that may forecast an epizootic disease outbreak;

ii) relevant ICES Member Countries provide sufficient resources for continued studies into the aetiology of M74, because it is known that the prevalence of M74 can change again very rapidly. More information on the role of food organisms on the development of M74 is needed and especially on the role of stress factors influencing the thiaminase activity of the food organisms;

iii) new information on *Ichthyophonus* in herring stocks and on the M74 syndrome be considered by WGPDMO in the future as part of the review of national reports on new diseases trends and not as a separate Term of Reference.

Reference

Mendoza, L., Taylor, J.W., Ajello, L. 2002. The Class Mesomycetozoa: a heterogeneous group of microorganisms at the animal-fungal boundary. Annu. Rev. Microbiol. 56:315–344.

12 ADVISE ON THE MODIFICATIONS MADE TO RELEVANT ICES DATABASES AND THE REVISED ICES ENVIRONMENTAL DATA REPORTING FORMAT (VERSION 3.2)

The ICES Secretariat has recently released the new ICES Environmental Data Reporting Format Version 3.2. The background for the new reporting format is the restructuring of the ICES databases, which have been changed fundamentally with respect to the technical and logical organisation. The new reporting format is the result of a thorough discussion within ICES and with users, including WGPDMO members. As outlined by W. Wosniok, the former style of separate, hierarchically organized databases for different types of data (various types of environmental, oceanographic, fisheries data) has been changed to a single relational database with a unique technical format for all kinds of information (DOME: Databases on Oceanography and Marine Ecosystems). The relational format allows linking arbitrary types of information and is open to the inclusion of additional types of information without large effort. Both properties will facilitate the use of the database.

The new reporting format requires submitting data by arranging the information in a series of records which describe the conditions of the measurement/observation besides containing the measurements themselves. The record types are identified by a two-digit number (e.g., “00” for the header record, “10” for the parameter record). Each record contains fields characterized by a name (e.g., “SEXCO” for the sex code), each field has either a defined set of allowed values (“m”, “f”, “u” for the sex code field, representing male/female/undetermined), or a range of allowed values (e.g., “> 0” for the field describing an individual’s length). The actual structure of records, field names and allowed contents of fields can best be taken from the ICES website (www.ices.dk/datacentre/reco/reco.asp?), as some components are still under construction and may be subject to short-term change. However, field names and their contents have been taken from the earlier formats, where possible.

The major records for the reporting of fish disease data are:

- record type 00 (header record, identifies data supplier);
- record type 90 (platform records, identifies the cruise);
- record type 91 (station record, describes the sampling event);
- record type 03 (biological effects sample record, describes, e.g., the species sampled);
- record type 04 (biota specimen record, contains e.g., sex code for the (group of identical) fish);
- record type 10 (parameter record, contains the type and value of the observed quantity).

These records have an internally hierarchy in the sense that, e.g., the information from the station record (91) is valid for all subsequent records of the types 03, 04, 10 until another station or platform record appears. Typically, several parameter records will be necessary for one haul.

Quality assurance information is provided via record 93, where record 93 itself is prepared mainly for the type of QA information requested by OSPAR (see the column “Mandatory/optional”). QA information for fish disease data will be described by a link entered in the field QALNK. The type of QA information that may be entered for fish disease data is as yet not restricted; so every information about the QA protocol(s) used may (and should) be entered. As this part of the database is presently under development, ICES should be contacted for technical details concerning the reporting of QA information for fish disease data.

Conclusions

1) The new ICES Environmental Data Reporting Format Version 3.2 is considered an improvement in the integrated management of the data maintained by ICES.

2) The complex structure of the database and the reporting format might have caused not yet uncovered inconsistencies in the reporting format. Using the reporting format in practice is the most effective way to detect such problems.

Recommendation

The WGPDMO recommends that:

i) ICES Member Countries should be encouraged to submit new (and, where existing, not yet submitted historical) fish disease data using the new ICES Environmental Data Reporting Format Version 3.2.

13 PRODUCE UPDATED ICES PUBLICATIONS ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

13.1 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 presented an updated example version of a web-based report on the trends of fish disease prevalence in the North Sea. This report contains for each of the main diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulcerations):

- an overview map displaying symbols for the recent (1999–2004) prevalence trends per ICES statistical rectangles for those rectangles for which a sufficient amount of data is available;
- from those rectangles, a link from the map to the underlying trend curves (which include confidence bands);

- links from the map page to pages with information on the fish species examined, the features of the diseases, the data source and the method of trend calculation.

Examples for the overview map and a trend display are given in Figures 1 and 2. The calculation of long-term trends has been changed from the separate consideration of summer and winter data to a joint consideration of all data. This allows the use of more data than before for a trend assessment. Also, using only one symbol per year is more appropriate to the concept of a long-term trend. The new trend calculation became possible by adopting a more advanced statistical method that is able to deal with data from irregularly distributed observation times within the years. Consequently, the present version of a web-based report now contains only one trend symbol per year.

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

However, the web-based report would gain considerably if the trend assessments had a larger spatial coverage for recent time periods. It was agreed that it should be checked intersessionally whether an update of the present web page for disease trends would be worthwhile with respect to the amount of new and recent information that could be given to the reader on the basis of the currently available data.

Besides the maps on fish disease data trends, the ICES website contains also maps on distribution of VHS in wild fish and diseases of bivalves. These maps have not been updated for several years. It was decided that L. Madsen and T. Wiklund should evaluate the need for an update of the present situation concerning VHS, and S. Ford evaluate the need for an update concerning diseases in bivalves. The results should be reported to the WGPDMO Chair who will contact ICES for any further actions.

The ICES representative for web presentations, N. Fletcher, should be contacted to explore the technical conditions for a web publication of a (then updated and completed) web-based report.

Conclusions

1) The web-based report on the disease trends in dab as part of the ICES Environmental Status Report should be improved and updated according to the suggestions made by WGPDMO.
2) The publication of the report should occur only if justified by the amount of recent information that can be presented.
3) The web-based report should be published via ICES facilities, with appropriate information input by WGPDMO members
4) The need to update the maps on the distribution of VHS in wild fish and on diseases of bivalves should be assessed intersessionally by WGPDMO.

Recommendations

The WGPDMO recommends that:

i) WGPDMO should check on the amount of new fish disease data that are available from ICES.
ii) If sufficient new material is available, recent disease trends should be determined and the results incorporated in a web-based report as described above. This report should replace the report presently available at the ICES website after consultation of the ICES Secretariat.

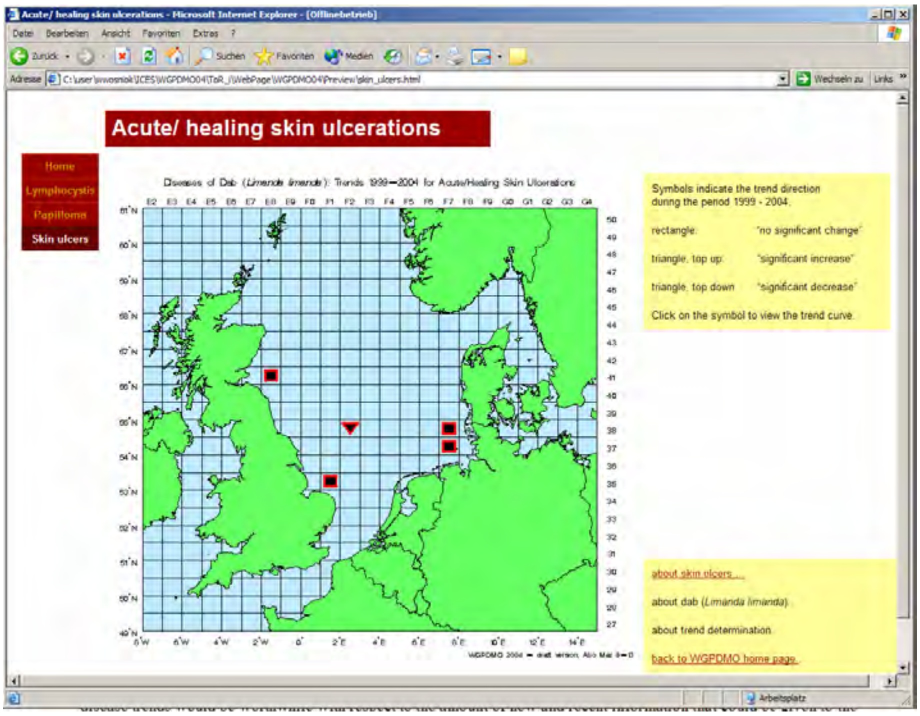

Figure 1. Example of an overview map. The symbols in the ICES rectangles denote recent trends. Clicking on the symbol leads to a display of the underlying long-term trend as shown in Figure 2. The displays are based on a preliminary data set.

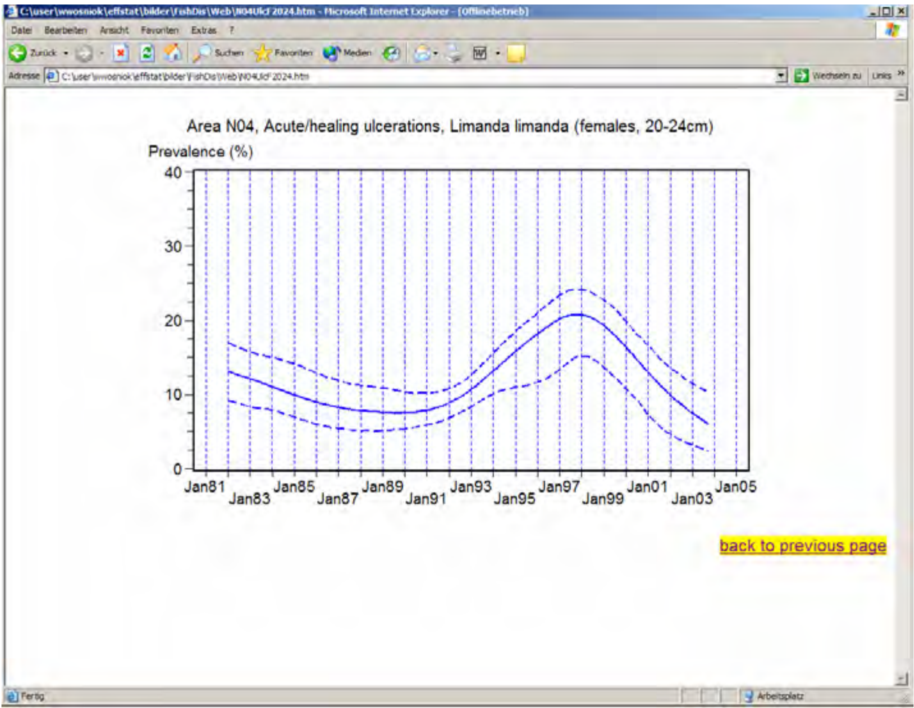

Figure 2. Example of a long-term trend with 95% confidence band. This display shows up after clicking at the corresponding ICES statistical rectangle (38F2). The displays bases on a preliminary data set, also area designations are preliminary. The latter will be changed to ICES statistical rectangle names.

13.2 Manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series

The TIMES manuscript on methods for the statistical analysis of fish disease data was presented by W. Wosniok. Compared with the previous version, some sections containing more general aspects had been shortened, while sections referring to more fish-disease specific topics have been enlarged:

- standardisation methods are described in more detail;
- the section on epidemiological methods contains additional material on optimised study design;
- the section on the analysis of data from prevalence time series with irregularly spaced observations includes methods for the calculation of long-term trends in the presence of seasonal fluctuations;
- more example calculations have been introduced.

Some minor editorial changes were found necessary. When these are done, the manuscript will be circulated for comments among the members of the Working Group on the Statistical Aspects of Environmental Monitoring (WGSAEM), as already arranged with the WGSAEM Chair.

Conclusion

1) After incorporation of changes initiated by WGSAEM, the manuscript on methods for the statistical analysis of fish disease data should be submitted to the TIMES series editor for publication.

Recommendations

The WGPDMO recommends that

i) the finalised manuscript on methods for the statistical analysis of fish disease data (authors W. Wosniok *et al.*) be reviewed by WGSAEM and submitted to the editor of the ICES TIMES Series thereafter.

13.3 Status of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish

ICES publishes a series of Identification Leaflets on the subject Diseases and Parasites of Fish and Shellfish for which S. McGladdery was the previous editor. The term of the editor has expired and S. McGladdery declined to be re-appointed. The sub-group on the leaflets established last year to assist the editor (wild fish: S. Feist and S. MacLean; aquaculture: D. Bruno and L. Madsen; shellfish: M. Lyons and S. Bower) met and discussed options for a new editor. S. Feist was nominated by the subgroup and accepted by the WGPDMO as the new editor for a term of three years. T. Lang will replace S.W. Feist in the sub-group.

Currently, the Disease Leaflets Series consists of 56 leaflets published between 1984 and 1999 (according to the ICES website publication list). They are now available in both English and French. However, after discussion, the subgroup suggested that they should be issued in English only so as to minimize publication delays. There has been a formal ICES Council Resolution (2003:1F03) that old leaflets are scanned by the ICES Secretariat (currently underway), issued on CD ROM, and placed on the ICES web site. However, old leaflets will have to be checked by the new editor and the subgroup and, if necessary, revised before they are scanned, published on CD ROM and made accessible on the ICES website.

WGPDMO agreed that the Chair contact ICES in order to inform about the nominee for the editorship. The new editor S. Feist, once approved, will have to contact S. McGladdery on the status of all leaflets (e.g., old, revised, new).

It will be necessary to negotiate with the ICES Secretariat on how to get new and revised leaflets on the ICES website, as well as to discuss the format of the leaflets on the website.

Conclusions

1) WGPDMO thanked S. McGladdery for her efforts as the previous editor of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish.

2) The WGPDMO nominated S.W. Feist as new editor of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish.

3) The new editor will have to contact S. McGladdery on the status of all leaflets.

4) The WGPDMO appreciated the efforts of the ICES Secretariat to digitise the old leaflets.

5) The old leaflets, that do not need to be revised, must be made available on the ICES website as soon as possible and the new editor will have to negotiate with the ICES Secretariat in what format the revised and future new leaflets will be placed on the website.

6) The new editor will have to contact authors of revised and new leaflets to advise them of the status of their leaflet and what needs to be done to complete them.

7) At the next meeting of the WGPDMO there will have to be decisions made on subjects for new leaflets.

8) The WGPDMO strongly suggested that the revised and new ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish be issued in English only so as to minimize publication delays.

Recommendations

The WGPDMO recommends that:

i) The new editor of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, S.W. Feist, contact ICES to make arrangements for the scanning and placement of leaflets on the ICES website and to obtain information on the most appropriate method for submitting revised and new electronic leaflets for direct placement on the website;

ii) a more structured procedure (including timetable) for publishing the leaflets be developed by the editor and the assisting sub-group;

iii) the sub-group assisting the editor review the existing and proposed leaflets with the editor and make recommendations to the WGPDMO regarding the need for new leaflets;

iv) the new editor, after consultation with the sub-group, contact all authors to advise them of any steps they need to take (e.g., submit colour photos, update) before the document can be published and that finalised versions be submitted to ICES as soon as possible,

v) the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish be issued in English only so as to minimize publication delays.

13.4 Review progress in the digitisation of the Disease Leaflets by the Secretariat

This issue is being dealt with in the previous Section 13.3.

13.5 Status of the report on Trends in Important Diseases Affecting the Culture of Fish and Molluscs in the ICES Area 1998–2002

The Chair informed WGPDMO that the report was published shortly before the 2004 WGPDMO meeting as *ICES Cooperative Research Report* No. 265 and thanked all members who had contributed to its preparation. The report can be found on the ICES website (http://www.ices.dk/pubs/crr/crr265/crr265.pdf).

14 DEVELOP PLANS FOR THE PREPARATION OF DETAILED BACKGROUND MATERIAL TO BE USED BY THE 2005 ICES/OSPAR WORKSHOP ON INTEGRATED MONITORING OF CONTAMINANTS AND THEIR EFFECTS IN COASTAL AND OPEN-SEA AREA [OSPAR 2004/2]

The ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) will be held in Copenhagen, Denmark, January 2005. Its basic objective is to develop guidelines for integrated biological effects and chemical monitoring, in particular for those parameters (contaminants and effects) that have become mandatory in the OSPAR Coordinated Environmental Monitoring Programme (CEMP), but also for the entire range of issues in the OSPAR Joint Assessment and Monitoring Programme (JAMP).

Because of its experience in the monitoring of externally visible fish diseases, macroscopic liver nodules and liver histopathology (all already included in JAMP) and the assessment of this kind of data in combination with other environmental data, WGPDMO was asked to contribute to the planning of the workshop.

In this context, the WGPDMO Chair received a letter (dated 21 January 2004) from the WKIMON Co-Chairs Robin Law (Chair MCWG) and Ketil Hylland (Chair WGBEC) with the following five questions, detailing what kind of input is expected to be delivered by WGPDMO (the same letter had been sent to the Chairs of WGMS, WGSAEM, BEWG, WGOH, SGQAE, SGQAB):

1) Which of the JAMP issues can be effectively addressed through integrated chemical and biological effects monitoring?
2) How should integrated monitoring be carried out to improve the quality of assessments?
3) Are there specific issues (with a focus on your field of expertise) that need to be addressed during the workshop?
4) Are there experts within your work area that you suggest be asked to participate in the workshop?
5) Are there other national or international activities that overlap with this workshop and that should be taken account of in its preparation?

In the following, answers to the questions raised by the WKIMON Co-Chairs are provided by WGPDMO according to their numbers:

1) In principle, all issues where effects of contaminants (potentially including effects of eutrophication and radioactivity) are to be assessed, can be addressed through integrated monitoring. More specifically, the questions in the 2003 Strategy for a Joint Assessment and Monitoring Programme (JAMP) (OSPAR reference number 2003/22) under 72c (Hazardous Substances), 79d (Offshore Activities) and, to a lesser degree, 85d (Radioactive Substances) seem to be most relevant.
2) A general answer is of course not possible here. The design of integrated monitoring programmes fully depends on their objectives, e.g., if it is for the assessment of inputs and effects from point or diffuse sources, or for coastal or offshore areas, for contaminant-specific or general biological effects, etc. However, in any case some have requirements to be met:
    - As already stated in the OSPAR outline of WKIMON, there are three principal approaches for integrated monitoring: a) field monitoring of organisms in their natural environment, b) bioassay-based monitoring and c) exposure experiments in cages. All three have their advantages and disadvantages. The major advantage of a) is that it reflects the natural conditions and, therefore, has a high ecological relevance. The advantage of b) is that this approach can be applied under controlled experimental conditions and, therefore, cause-effect relationships may more easily be established. The caging experiments (c) are somewhat in between the two others. However, caged organisms may not react in the same way to stressors as free-living organisms and this may lead to biased information. Again, the choice of one of these principal approaches depends on the objectives of the monitoring.
    - The sampling strategy for chemical and biological measurements (especially under *in situ* conditions) has to be done in a way that the data are comparable (same time, location and specimen) (relates to approaches a) and c)). The monitoring design has to be according to statistical requirements. WGDPMO may provide its long-term experience in this subject for the preparation of the workshop.
    - The monitoring design has to meet statistical requirements, e.g., for a case-control study on PAH-specific biological effects other types of samples are needed rather than the assessment of general toxicity. In the latter, biomarkers of acute and chronic effects reflecting the health status of target species are measured to obtain information about the toxicity of the complex contaminant mixture in coastal and estuarine environments (relates to approaches a), b), c)).
    - Pooling of samples (as often done for chemical monitoring) should be avoided since this leads to a major loss of information, e.g., on intra-specific variation (relates to approaches a), b), c)).
    - In addition, measurements of contaminants and biological effects, other host- and site-specific confounding factors have to be measured in parallel in order to assess their effects (relates to approaches a) and c)).
3) The following specific issues may be of relevance for the workshop:
    - For monitoring purposes, it is not sufficient to identify biological effects techniques that can be recommended for national/international monitoring or that are promising without providing information for what kind of monitoring these techniques are suitable (e.g., acute effects vs. chronic effects, 'warning bell' effects vs. ecologically relevant effects, offshore vs. coastal monitoring, point source vs. diffuse sources monitoring, etc.). The different purposes of monitoring are something that WKIMON should explicitly try to focus on when recommending guidelines.

- For the onset of 'classical' early-warning biomarkers (e.g., enzyme induction/suppression), the basic hypothesis is that the response reflects the current exposure situation. However, for the development of pathology/disease, exposure time in some cases has to be longer (e.g., in cases where exposure induces immunosuppression that subsequently leads to infection and ultimately to the endpoint clinical disease). Furthermore, there might be a considerable delay between the exposure and the occurrence of pathology/disease (e.g., for tumours). Thus, the question is to what extent the contaminant level measured at the same time when the biological sample is taken for pathology/disease inspection really can reflect a cause-effect relationship.
- For contaminants that are rapidly metabolised (e.g., PAHs), it is not clear if the concentrations measured reflect the exposure of the target species that might display biological effects. Intermediate metabolic products may be measured instead of the actual contaminant levels (e.g., PAH metabolites in the bile). However, more information is needed here, because there is not always an ideal correlation between the concentrations of the contaminant and of its metabolites.
- In the analysis of ICES fish disease data, the problem of correlation between potentially explanatory factors and its effect in the multivariate analysis ("true" causes may be mimicked by "false" causes that just correlate with the "true" ones) became evident. This has to be taken into account when defining parameters to be measured.

4) WGPDMO recommends that the organisers of the WKIMON invite WGPDMO members with long-term expertise in monitoring pathology/diseases in wild fish and in data treatment to attend the workshop. The WGPDMO nominated T. Lang, S. Feist, and W. Wosniok.

5) There are two major international projects on biological effects of contaminants in marine ecosystems: The ICES Workshop on Biological Effects of Contaminants in Pelagic Ecosystems (BECPELAG) and the EU-funded project Biological Effects of Pollution in Coastal Marine Ecosystems (BEEP)) that ended recently, the outcome of which should be considered at the workshop. Particularly BECPELAG focused on the usefulness of the three approaches (field sampling, cage experiments, and bioassays) for integrated monitoring purposes.

Conclusions

1) WGPDMO emphasises the need for integrated approaches (chemical and biological) for the assessment of environmental quality of marine offshore and coastal areas. The health status of marine animals reflected by their diseases and pathological endpoints is considered as an integrative indicator, useful for the assessment of environmental quality.

2) As part of the detailed background material for the planning of ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) requested by the ICES Council, Annex 12 of the present report on the contribution of WGPDMO to the initiated ICES integrated regional assessment of the North Sea ecosystem and Annex 9 on epidemiological methods for risk assessment are considered to be of great relevance. Basically, the first document provides a rationale for the incorporation of fish disease studies in environmental monitoring programmes as well as a description of previous and future WGPDMO activities with regard to an integrated data assessment, combining disease data and other types of data partly maintained in ICES databanks (e.g., contaminants, nutrients, particle drift, algae blooms, oceanography, population dynamics, migration behaviour of target species, and fishery). The second document addresses epidemiological (mostly statistical) methods recommended for the assessment of the relationship between wild fish diseases (and other indicators) and environmental factors, including contaminants.

Recommendations

The WGPDMO recommends that

- the organisers of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) take note of the answers of the WGPDMO to the questions raised by the WKIMON Co-Chairs, the conclusions made by WGPDMO on the workshop and Annexes 9 and 12 including background material considered useful for the planning, conduct and analysis of integrated monitoring;
- the ICES/OSPAR WKIMON organisers invite WGPDMO members with long-term expertise in monitoring pathology/diseases in wild fish and in data treatment to attend the workshop. The WGPDMO nominated T. Lang, S. Feist and W. Wosniok;
- the WGPDMO assess the results of ICES/OSPAR WKIMON at its 2005 meeting.

15 START PREPARATIONS TO SUMMARISE DATA ON THE HEALTH STATUS OF NORTH SEA BIOTA FOR THE PERIOD 2002–2004, AND ANY TRENDS IN THE PREVALENCE OF DISEASES OVER THE RECENT DECADES FOR INPUT TO REGNS IN 2006

A working document prepared by T. Lang, W. Wosniok and S. Feist was presented for discussion by T. Lang. The aim of the document is to provide a) information about the plans in ICES for an integrated regional assessment of the state of the North Sea ecosystem and b) suggestions for possible contributions of the ICES WGPDMO to the assessment (see Annex 12).

There is a growing demand for ICES to provide Member Countries and international commissions with integrated, holistic, ecosystem-centred advice, based on regional assessment of the state of marine ecosystems.

This issue has been discussed in various ICES bodies and it has been considered that the best way to learn how such an assessment can be achieved is by performing an integrated assessment for the North Sea as a pilot project within ICES. The Regional Ecosystem Study Group for the North Sea (REGNS) was established in 2003 as the coordinating body within ICES.

The actions and timetable suggested by REGNS to achieve the goals were reviewed by the WGPDMO. The following were acted upon:

- The WGPDMO contact person for REGNS is the Chair T. Lang.
- WGPDMO members responsible for preparing the ‘Draft Chapter’ are those that have long experience in monitoring fish diseases in the North Sea. They are: T. Lang, W. Wosniok, S. Feist, D. Bruno and a representative from The Netherlands to be identified.
- Representatives for the ‘Thematic Drafting Panels’ are T. Lang and S. Feist.

It is recognized that one of the most important first steps in this process is to update the ICES fish disease database, with priority given to data on the common dab (*Limanda limanda*). The completed and updated data will be reviewed and, subsequently, determinations will be made as to the appropriate analyses for these data.

Conclusions

The following strategy is to be applied by WGPDMO according to the schedule outlined in Annex 12:

1) Update the ICES fish disease database as soon as possible by incorporating historic data from the North Sea not yet submitted to ICES;
2) Obtain a spatial and temporal overview of the updated ICES disease data;
3) Define the fish species, diseases, geographical areas and time spans for which information on spatial patterns and temporal trends should be included in the regional North Sea assessment;
4) Carry out regional comparisons of prevalences (2002–2004) and trends (at least 2000–2004, preferably over a longer period), applying statistical methods as described in the draft manuscript to be submitted to the ICES TIMES series;
5) Carry out multifactorial analyses on cause-effect relationships with updated data extracted from the ICES fisheries, oceanography and environmental databases;
6) In collaboration with other ICES WGs compile additional information on the health status of North Sea invertebrates, fish, seabirds and mammals that is considered of use for the integrated assessment;
7) Prepare a draft paper with the results of the above analyses.

Recommendations

The ICES WGPDMO recommends that:

i) ICES Member Countries be encouraged to submit as soon as possible historic data on North Sea fish diseases to the ICES Environmental Databank in order to enable WGPDMO to carry out a comprehensive and up-to-date analysis of regional and temporal trends in disease prevalence as well as of the role of explanatory environmental factors, as input to the ICES integrated regional North Sea assessment;

ii) a progress report on the preparations to summarise data on the health status of North Sea biota for the period 2002–2004, and any trends in the prevalence of diseases over recent decades for input to REGNS in 2006, be reviewed by WGPDMO at its 2005 meeting.

16 ANY OTHER BUSINESS

16.1 "Prestige" oil spill

A summary of the events surrounding the sinking of the oil tanker "Prestige" off the coast of Spain in November 2002 and of the subsequent impact of the oil spill was presented to the WGPDMO by J. Barja. The main coastline affected has been the northern Galician coast and Atlantic Islands National Park of Spain. However, the oil has spread along northern Spane to the Atlantic coast of France up to Brittany. Estuarine areas and the Rias along the affected coastline remained relatively unaffected because the islands act as natural barriers. While the situation of the sandy beaches with regard to oil contamination has improved since the oil spill, large parts of the rocky shores are still covered with oil. The government issued a total ban on fisheries in the affected area for 10 months.

A large number of projects (partly in collaboration with adjacent countries) were started to assess the environmental effects and the development of strategies for remediation measures. However, it is unclear whether long-term monitoring will be carried out. In this context, the WGPDMO emphasised that long-term studies on pathobiological changes associated with the oil spill in both fish and shellfish species are required in order to assess the effects at the population and community level and to obtain information on recovery.

16.2 Permanent Advisory Network for Diseases in Aquaculture (PANDA)

S.W. Feist presented a short review of a new EU-funded project (PANDA) in Aquaculture. Work packages implementd cover risk analysis, epidemiology, diagnostic methods, environmentally safe disease control, training needs and opportunities. A website has been established to facilitate registration and as a convenient forum for discussions, details can be found at www.europanda.net. WGPDMO considered this is a valuable project with complementary links to ICES, OIE and other organisations. The WGPDMO emphasised that the expertise of the WGPDMO could be utilised within PANDA, particularly in the field of identifying new and emerging diseases and disease trends in the marine environment.

16.3 WGPDMO Website

The Chair explained that he had contacted the ICES Secretariat to explore possibilities of hosting a website. Due to staff limitations, ICES is not in a position to establish and maintain such a website and the Chair offered to host a site in Germany. WGPDMO agreed to establish its own website for exchange of information amongst WGPDMO members and as a means to facilitate the dissemination of information on the WGPDMO's activities to the wider scientific community and to other relevant organisations. The site will have to follow ICES guidelines, with links to the main ICES website. A sub-group consisting of T. Lang, S. Feist, W. Wosniok, T. Wiklund and L. Madsen will develop plans for developing the site and report back to the WGPDMO in 2005.

Recommendations

The WGPDMO recommends that:

i) a WGPDMO sub-group consisting of T. Lang, S.W. Feist, W. Wosniok, T. Wiklund, and L. Madsen will develop plans for developing a WGPDMO website and report back to the WGPDMO at its 2005 meeting.

16.4 Questionnaire from the ICES Study Group on Information Needs for Coastal Zone Management (SGINC)

The Chair presented a questionnaire on coastal zones related activities sent to the WGPDMO from the SGINC Chair J. Stottrup. The Chair agreed to draft responses to the questionnaire and distribute to WGPDMO members intersessionally before sending to the Chair of SGINC and before the next meeting of this group (April 2004).

16.5 Improvement of working procedures of the WGPDMO

The WGPDMO agreed on the relevance of sending intersessional working documents to the Chair one month prior to the meeting. The Chair would then distribute the documents to the rest of the working group members.

17 PROGRESS WITH TASKS

An analysis of progress of tasks in the Terms of Reference was conducted and presented in Annex 13. All items had been dealt with in a comprehensive manner. Several intersessional tasks to be fulfilled prior to the 2005 WGPDMO meeting were identified.

18 FUTURE ACTIVITIES OF WGPDMO

Since there are several issues of importance in the field of pathology and diseases of marine organisms requiring further consideration, it was agreed that a further meeting of WGPDMO is required in 2005 to consider the results of intersessional work and to discuss new and outstanding items.

It was agreed that the invitation by T. Renault (France) to host the 2005 WGPDMO meeting in France be accepted. The venue (either La Tremblade or La Rochelle) still has to be decided. The proposed dates are 8–12 March 2005. The Chair thanked T. Renault on behalf of WGPDMO for the kind invitation.

19 APPROVAL OF RECOMMENDATIONS

The recommendations to the ICES Council contained in this report were discussed by the WGPDMO and approved. The recommendations and justifications for recommendations to the Council are appended in Annex 14.

20 APPROVAL OF THE DRAFT WGPDMO REPORT

The report of the 2004 meeting was approved before the end of the meeting and the draft report was circulated to the participants on 23 April 2005. The conclusions from the Terms of Reference and associated Annexes where information was specifically sought by or provided to other ICES bodies would be extracted and sent separately to ICES.

21 CLOSING OF THE MEETING

The Chair thanked the local host, T. Wiklund, for providing excellent meeting facilities and arrangements and the participants for their hard work and input during and in preparation of the meeting. The 2004 WGPDMO meeting was closed at 14.00 hrs on 13 March 2004.

ANNEX 1 LIST OF PARTICIPANTS

Name	Address	Telephone	Fax	E-mail
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@meeresforschung.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 ext. 105	+1 856 785 1544	susan@hsrl.rutgers.edu
Brit Hjeltnes	National Veterinary Institute PO Box 1263 Sentrum 5811 Bergen Norway	+47 55363824 +47 91889376	+47 55321880	brit.hjeltnes@vetinst.no
Andrey B. Karasev	Knipovich Polar Research Institute of Marine Fisheries and Oceanography 6, Knipovich Street 183763 Murmansk Russia	+7 815 2 47 37 21	+47 789 10 518 (Norwegian line) +7 815 2 47–33–31 (Russian line)	paralab@pinro.murmansk.ru
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
Maria Lyons Alcantara	Fish Health Unit Marine Institute Snugboro Road Abbotstown Dublin 15 Ireland	+353 1 8 228 280	+353 8 228 303	maria.lyons@marine.ie
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

Name	Address	Telephone	Fax	E-mail
Lone Madsen	Danish Institute for Fisheries Research Fish Disease Laboratory Stigbøjlen 4 DK-1870 Frederiksberg Denmark	+45 35282763	+45 35282711	lm@dfu.min.dk
Tristan Renault	IFREMER Laboratoire de Génétique, Aquaculture et Pathologie La Tremblade 17390 France	+33 5 4636 9841	+33 5 4636 3751	trenault@ifremer.fr
Galina N. Rodjuk	Atlantic Scientific Research Institute of Marine Fisheries and Oceanography (AtlantNIRO) 5, Dm. Donskogo Street 236000 Kaliningrad Russia	+7 112 225369	+7 112 219997	rodjuk@atlant.baltnet.ru rodjuk_gn@rambler.ru
Galina P. Vialova *(Observer)*	Sakhalin Research Institute of Fisheries and Oceanography 196 Komsomolskaya Str. Yuzhno-Sakhalinsk, 693023 Russia	+7 4242 45 67 79 +7 4242 45 67 62	+7 4242 45 67 89 +7 4242 45 67 78	vyalova@sakhniro.ru vstex@sakhniro.ru
Martine Vigneulle	AFSSA Brest BP 70 29280 Plouzané France	+33 2 98 22 44 61	+33 2 98 05 51 65	m.vigneulle@brest.afssa.fr
Svante Wistbacka *(Observer)*	Laboratory of Aquatic Pathobiology Department of Biology BioCity, Artillerig. 6 Åbo Akademi University 20520 Åbo Finland	+358 2 215 46 04	+358 2 215 47 48	svante.wistbacka@abo.fi
Tom Wiklund	Laboratory of Aquatic Pathobiology Department of Biology BioCity, Artillerig. 6 Åbo Akademi University 20520 Åbo Finland	+358 2 2154301 +358 400 758957	+358 2 2154748	twiklund@abo.fi
Maciej Wolowicz	Laboratory of Estuarine Ecology Institute of Oceanography University of Gdańsk Ul. 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

2F01 The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: T. Lang, Germany) will meet in Åbo, Finland, from 9–13 March 2004 to:

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

b) review and report on environmental monitoring programmes and associated quality assurance activities incorporating studies on pathology and diseases of marine organisms;

c) provide a recommended technique to differentiate among *Perkinsus* spp., incorporating input received from web-based international solicitation of comments;

d) review the existing information on viral diseases of crustaceans with emphasis on commercially important species;

e) recommend on the use of epidemiological methods for the assessment of diseases and population effects risk;

f) evaluate current information on disease/parasite interactions between wild and farmed fish and advise on related management control methods;

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

h) advise on the modifications made to relevant ICES databases and the revised ICES Environmental Data Reporting Format (Version 3.2);

i) 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. manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series;

   iii. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish;

   iv. review progress in the digitisation of the Disease Leaflets by the Secretariat

j) develop plans for the preparation of detailed background material to be used by the 2005 ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas [OSPAR 2004/2];

k) start preparations to summarise data on the health status of North Sea biota for the period 2002–2004, and any trends in the prevalence of diseases over recent decades. Where possible, the causes of these trends should be outlined. For input to REGNS in 2006

WGPDMO will report by 14 April 2004 for the attention of the Mariculture Marine Habitat and Diadromous Fish Committees, and ACME.

Supporting Information

Priority:	WGPDMO is of fundamental importance to the ICES science and advisory process.
Scientific Justification and Relation to Action Plan:	1.3, 2.3, 2.5, 2.6, 2.7, 2.10, 2.12, 3.3, 3.14, 4.6, 4.7, 4.9, 4.14, 5.4, 6.1. 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) Many ICES Member Countries are conducting marine environmental monitoring programmes incorporating studies on pathology and diseases of fish and shellfish species. The WGPDMO considers it important for its work to review the progress made, including the implementation of quality assurance programmes c) Since the first identification of a *Perkinsus* sp. in the late 1940s, several methods have been used to identify and characterise new species. The use of different criteria has caused confusion in the taxonomy of the genus, although newly developed genetic methods are helping to sort out the taxonomy. A set of identification criteria needs to be developed and agreed upon by the widest possible group of molluscan disease investigators.

	d) Several crustacean species are commercially highly valuable with many wild stocks being heavily exploited. Considerable information is available for cultured species. For instance, penaeid shrimp culture is a major aquaculture industry in several countries around the world and is seriously affected by the viral disease "White Spot Syndrome". In contrast to knowledge of cultured crustaceans, very little is known about viral diseases of wild-stock crustaceans. Recent studies indicate these infections to be more prevalent than previously thought and it is timely to review the available information on the types and pathogenicity of viruses in wild crustaceans. e) Risk assessment aims to quantify the risk of (usually adverse) effects and serves as a basis for many regulatory decisions. Epidemiological methods are tools to calculate risk quantifications and, given a particular data structure, are effective also for small samples. The applicability of epidemiological methods to existing or obtainable data and potential gain by future use should be reviewed. f) The impact of the increasing development of aquaculture on diseases in wild fish populations is an issue of concern. WGPDMO considers it important to be updated on the most recent knowledge within this field. g) ICES C.Res. 1993/2:23(m) requested that WGPDMO maintain an overview of the M74 syndrome and the *Ichthyophonus* issue as part of its regular agenda. h) WGPDMO considers it necessary to follow the process of the modification of the ICES Databank structure in order to assist, if required, and to obtain an overview of changes suggested or introduced by other ICES Working Groups. i) A number of ICES publications, either web-based or in ICES publication series, are being prepared or updated at present, the progress of which has to be reviewed by WGPDMO at its next meeting. It will be necessary to consider ways by which these can be linked to each other. j) This is response to a request from OSPAR. k) This is required as the working groups input to the thematic writing panels working under the coordination of REGNS to develop an integrated assessment of the North Sea. For the purposes of this study the North Sea comprises ICES Area IV and IIIa and does not include intertidal areas. As far as possible, significant seasonal variation should be described.
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:	ACME
Linkages to other Committees or Groups:	MARC, MHC
Linkages to other Organisations:	BEQUALM, OIE, EU
Secretariat Cost share	ICES:100 %

ANNEX 3 WORKING DOCUMENTS

Code	**Titel/Content**
WGPDMO2004_WD_A1_Canada.doc	Canada - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A2_Denmark.doc	Denmark - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A3_Finland.doc	Finland - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A4_France1.doc	France - National Report on New Disease Trends (Shellfish) in 2003
WGPDMO2004_WD_A5_Germany.doc	Germany - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A6_Latvia.doc	Latvia - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A7_Poland_Text.doc	Poland - National Report on New Disease Trends in 2003 (text)
WGPDMO2004_WD_A7_Poland_FigTab.xls	Poland - National Report on New Disease Trends in 2003 (figures and tables)
WGPDMO2004_WD_A8_E&W.doc	England and Wales - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A9_Scotland.doc	Scotland - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A10_USA.doc	USA - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A11_Norway.doc	Norway - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A12_Ireland.doc	Ireland - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A13_Spain.doc	Spain - National Report on New Disease Trends in 2003
WGPDMO2004_WD_A14_France2.doc	France - National Report on New Disease Trends (Finfish) in 2003
WGPDMO2004_WD_A15_Russia.doc	Russia - National Report on New Disease Trends in 2003
WGPDMO2004_WD_B1.doc	Neoplastic disease in Bivalvia from the Gulf of Gdańsk (Baltic Sea, Poland) (*M. Wolowicz et al.)*
WGPDMO2004_WD_C1.doc	Identification of Perkinsus species (*S. Bower*)
WGPDMO2004_Perkinsus_workshop_final.ppt	EAFP Powerpoint presentation: review of molecular techniques used to differentiate Perkinsus spp. (*S. Bower et al*.)
WGPDMO2004_WD_D1.doc	Review the existing information on viral diseases of crustaceans with emphasis on commercially important species (*S.W. Feist and S. McLean*)
WGPDMO2004_WD_E1.doc	Epidemiological Methods for the Assessment of Diseases and Population Risks (*W. Wosniok et al.*)
WGPDMO2004_WD_F1.doc	Evaluate current information on disease/parasite interactions between wild and farmed fish and advise on related management control methods (*D. Bruno and B. Hjeltnes*)
WGPDMO2004_WD_G1.doc	Overview of the distribution and possible causes of the M74 syndrome *(S. Wistbacka and G. Bylund)*

WGPDMO2004_WD_H1_overview.html	Overview of the ICES Environmental Data Centre (*ICES website*)
WGPDMO2004_WD_H2_submitting_data.html	Submission of Environment Data to ICES (*ICES website*)
WGPDMO2004_WD_H3_PartEnv32.doc	Structure of the ICES Integrated Environmental Reporting Format Version 3.2 (*ICES website*)
WGPDMO2004_WD_H4_ALUK1998CFBE.txt	Example of data in Reporting Format 3.2 (*ICES website*)
WGPDMO2004_WD_J1.pdf	Terms of Reference for the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (*OSPAR*)
WGPDMO2004_WD_J2.doc	ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) [OSPAR 2004/2], contributions from WGPDMO (*T. Lang*)
WGPDMO2004_WD_K1.doc	Data on the health status of North Sea biota as input to the ICES Regional Assessment of the North Sea Ecosystem (*T. Lang et al.*)

ANNEX 4 AGENDA

1. Opening of the meeting
2. Terms of reference, adoption of agenda, selection of rapporteurs
3. ICES Annual Science Conference 2003, 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
6. Review and report on environmental monitoring programmes and associated quality assurance activities incorporating studies on pathology and diseases of marine organisms
7. Provide a recommended technique to differentiate among *Perkinsus* spp., incorporating input received from web-based international solicitation of comments
8. Review the existing information on viral diseases of crustaceans with emphasis on commercially important species
9. Recommend on the use of epidemiological methods for the assessment of diseases and population effects risk
10. Evaluate current information on disease/parasite interactions between wild and farmed fish and advise on related management control methods
11. Maintain an overview of the spread of Ichthyophonus in herring stocks and the distribution and possible cause(s) of the M74 syndrome
12. Advise on the modifications made to relevant ICES databases and the revised ICES Environmental Data Reporting Format (Version 3.2)
13. Produce updated ICES publications on pathology and diseases of marine organisms:
    1. web-based report on diseases and parasites of wild and farmed marine fish and shellfish as part of the ICES Environmental Status Report
    2. manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series
    3. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish
    4. Review progress in the digitisation of the Disease Leaflets by the Secretariat
    5. Status of the report on Trends in Important Diseases Affecting the Culture of Fish and Molluscs in the ICES Area 1998–2002
14. Develop plans for the preparation of detailed background material to be used by the 2005 ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas [OSPAR 2004/2];
15. Start preparations to summarise data on the health status of North Sea biota for the period 2002–2004, and any trends in the prevalence of diseases over recent decades. Where possible, the causes of these trends should be outlined. For input to REGNS in 2006
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)	2004 WGPDMO Terms of Reference	Rapporteurs
1–4	Introductory session	T. Lang
5	a) produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports • wild fish • farmed fish • wild and farmed shellfish	 S. MacLean/T. Wiklund/A. Karasev D. Bruno/M. Vigneulle/J. Barja/B. Hjeltnes M. Lyons Alcantara/S. Ford/T. Renault
6	b) review and report on environmental monitoring programmes and associated quality assurance activities incorporating studies on pathology and diseases of marine organisms	K. Broeg/S. MacLean/S. Feist/ M. Wolowicz
7	c) provide a recommended technique to differentiate among *Perkinsus* spp., incorporating input received from web-based international solicitation of comments	M. Lyons Alcantara/ S. Ford/ T. Renault
8	d) review the existing information on viral diseases of crustaceans with emphasis on commercially important species	L. Madsen/S. MacLean/ S. Feist
9	e) recommend on the use of epidemiological methods for the assessment of diseases and population effects risk	B. Hjeltnes/ S. Feist/W. Wosniok
10	f) evaluate current information on disease/parasite interactions between wild and farmed fish and advise on related management control methods	J. Barja/ D. Bruno/B. Hjeltnes
11	g) maintain an overview of the spread of *Ichthyophonus* in herring stocks and the distribution and possible cause(s) of the M74 syndrome	B. Hjeltnes/T. Wiklund/D. Bruno
12	h) advise on the modifications made to relevant ICES databases and the revised ICES Environmental Data Reporting Format (Version 3.2)	K. Broeg/ S. MacLean/W. Wosniok
13	i) 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) manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series; iii) ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish; iv) review progress in the digitisation of the Disease Leaflets by the Secretariat.	 T. Wiklund/M. Lyons Alcantara/W. Wosniok D. Bruno/W. Wosniok/M. Wolowicz L. Madsen/S. Ford/M. Vigneulle L. Madsen/S. Ford/M. Vigneulle
14	j) develop plans for the preparation of detailed background material to be used by the 2005 ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas [OSPAR 2004/2]	K. Broeg/S. Feist/ W. Wosniok
15	k) start preparations to summarise data on the health status of North Sea biota for the period 2002–2004, and any trends in the prevalence of diseases over recent decades. Where possible, the causes of these trends should be outlined. For input to REGNS in 2006	S. MacLean/ S. Feist/W. Wosniok
16–19	Any other business, Analysis of progress with tasks, Future activities of WGPDMO, Recommendations	T. Wiklund, L. Madsen, D. Bruno
20–21	Approval of draft report, Closing of the meeting	T. Lang

ANNEX 6 NEOPLASTIC DISEASE IN BIVALVIA FROM THE GULF OF GDAŃSK (BALTIC SEA, POLAND)

Maciej Wołowicz and Katarzyna Smolar,
Laboratory of Estuarine Ecology, Institute of Oceanography, University of Gdańsk,

One of the most negative phenomena observed recently in bivalves is a tumour disorder. In disturbed coastal and estuarine systems, some bivalves develop fatal neoplasia in their hemolymph and tissues. Proliferative disorders in bivalve molluscs are characterised by enlarged undifferentiated cells, presenting a high nucleus to cytoplasm ratio and a high mitotic index and were for the first time reported by Farley (1969). Since then similar disorders have been described in 20 bivalve species all around the world (Elston *et al.* 1990, Peters *et al.* 1994, Alonso *et al.* 2001, Villaba *et al.* 2001) including the Baltic clam *Macoma balthica* (L.) (Elston *et al.* 1992, Pekkarinen 1993, Thiriot-Quiévreux and Wołowicz 1996, 2001). Most neoplasias reported in bivalves have been described as a disturbed growth pattern of hemolymph cells, characterized primarily by excessive cell proliferation. Three main cytological features are associated with neoplastic cells: nuclear polymorphism, nuclear hyperchromatism and cellular polymorphism. In molluscs, most identified neoplasms have been described as sarcomas of haematopoietic origin (Bower *et al.* 1994, Rodriguez *et al.* 1997) due to proliferation of enlarged cells with a large lobate nucleus. Gonad neoplasms have also been found (Bert *et al*. 1993, Peters *et al*. 1994, Alonso *et al*. 2001).

Due to potential deleterious effects that epizootium may have on monospecific cultivated communities, the etiology of neoplastic conditions have received considerable attention. At first, viruses, retroviruses and infectious agents have been commonly hypothesised as the main cause of neoplastic changes in marine bivalves (Farley *et al*. 1972, Oprandy *et al*. 1981, Oprandy and Chang 1983, Ford *et al.* 1997). Presently, environmental carcinogens such as hydrocarbons (Yevich and Barszcz 1977, Naes *et al.* 1995), herbicides (Van Beneden *et al.* 1993) and so-called biotoxins (Landsberg 1996, Roy *et al.* 1998) have been suggested as risk factors in the development of mutations that may induce the neoplastic disease. In many cases, although the increase in tumour incidence can be correlated with the increased aquatic toxicant levels, causality cannot be definitively proven.

Suspension/deposit feeders and detrivorous organisms play the most important role in the transfer of organic matter from water column to bottom sediments (through filtration and biodeposition), contributing to a high degree to energy fluxes between pelagic and benthic systems (Kautsky and Evans 1987). Direct consequences of accumulation of harmful and toxic substances in their tissues are an increased risk of cumulative pollutant transfer along the food chain to higher consumers including human beings, thus leading to a general threat to the ecological and economic environments. Recent studies on molluscan neoplasia have been induced by the question, whether bivalves can be used as biomonitors of environmental quality (Roy *et al.* 1998, Fournier *et al*. 2001, Sauve *et al*. 2002, Rainbow *et al*. 2004, van Beneden 1994).

In *M. balthica* from the Gulf of Gdańsk (Baltic Sea, Poland; Figure 1) neoplasia was identified by the occurrence of abnormal metaphases in the respiratory system and was first reported by Thiriot-Quiévreux and Wołowicz (1996). Chromosome analyses based on Giemsa-stained metaphases of the M. balthica gill tissue revealed two categories of animals, a normal one with a diploid chromosome number of 2n=38 (Figure 2a) and an abnormal one with hyperploidy and chromosomal abnormalities related to neoplasia.

Chromosome number scored in the abnormal mitosis using cytogenetics ranged from 59 to 109 with a mean of 84 (Figure 2b). Out of 47 specimens studied in 1996 six presented enlarged nuclei and abnormal mitosis while seven specimens showed intermediate features (Thiriot-Quiévreux and Wołowicz 1998). Thiriot-Quiévreux and Wołowicz (2001) reported that 33 % of the clams from the Gulf of Gdańsk were affected by the tumour. Such a high prevalence of the disease can be considered as “epizootic disseminated neoplasia” (Elston *et al.* 1992). Recent studies have confirmed an increasing prevalence of the tumor between 1996 and 2001 and revealed the neoplastic changes in another bivalve species, the soft-shell clam *Mya arenaria* (Wołowicz *et al*. 2000).

On histological sections neoplastic cells were detected in gills and other organs of the affected *M.*balthica. Four histological stages were classified according to Christensen *et al.* (1974) and Pekkarinen (1993). Early stage neoplasia were confined to gill epithelia and characterized by nuclear and nucleolar enlargement, multiple nuclei and a large number of abnormal mitoses. In later stages of the disease locally invasive epithelial lesions occurred and had invaded underlying tissues together with a small collection of neoplastic cells in other tissues. Heavily affected individuals showed wide spread proliferation of neoplastic lesions and numerous mitotic figures. A few cases of similar but morphologically distinctive disorder such as the germinoma (Elston *et al*. 1992) detected by histology examination have been distinguishable from typical circulating neoplasias.

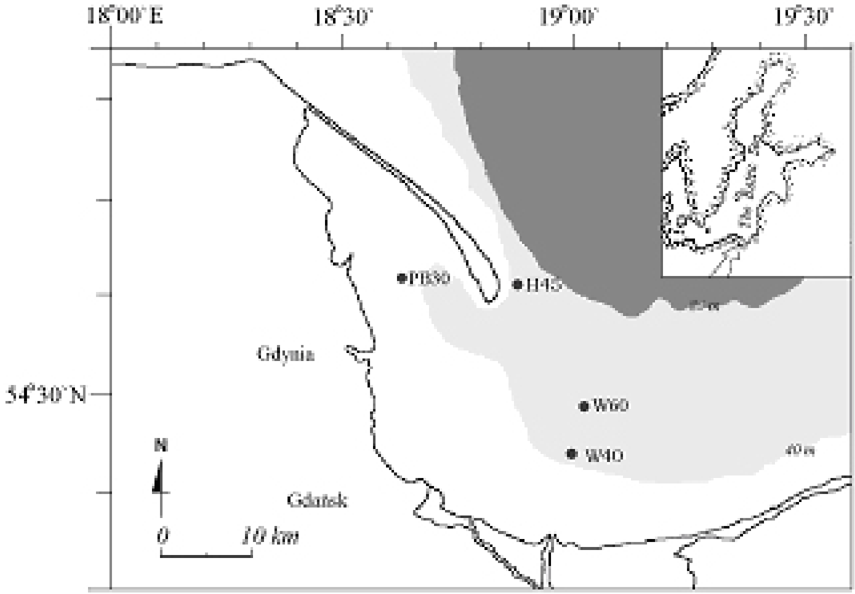

Figure 1. The sampling area in the Gulf of Gdansk (Baltic Sea, Poland).

(a) (b)

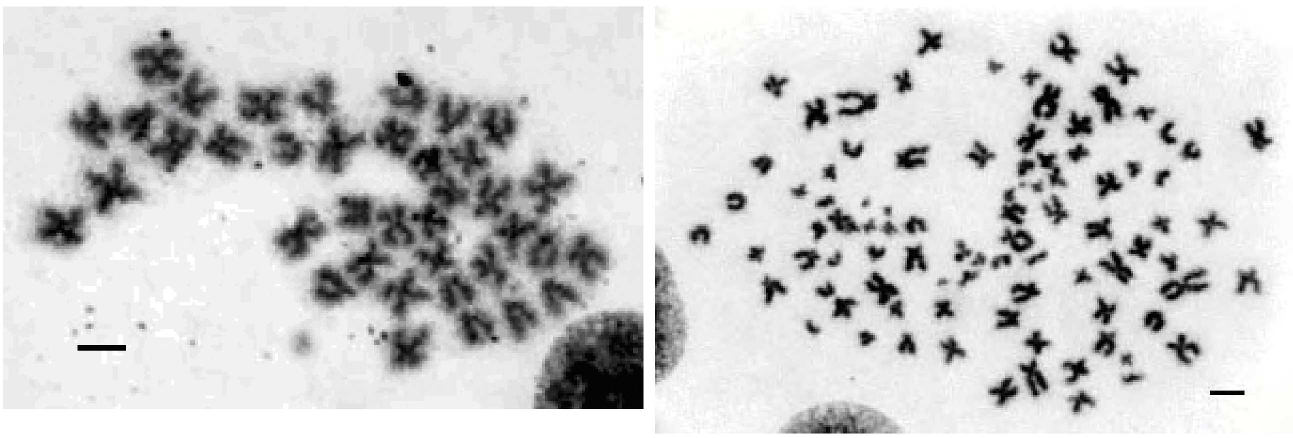

Figure 2. Giemsa-stained metaphases in gills of (a) non- affected *Macoma balthica* with 2n=38 and (b) affected *Macoma balthica* with 90 chromosomes, scale 5 µm.

Multi-year studies in the Gulf of Gdańsk revealed that the prevalence of disseminated neoplasia in the Baltic clam varied from year to year (Figure 3a) and changed seasonally with the increased proportion of the affected individuals in summer. At the site with the highest average prevalence of the tumour (H45) the affected clams were noted during all sampling seasons, while at the other sites (W40, W60 and PB30) the highest incidence of neoplasia occurred only in spring and summer (Figure 3b). Progression of the cancer during warm months can be attributed to a temperature- and spawning-induced increase in metabolic activity of the bivalves. This may further influence the animal's immunocompetence to environmental stress, including the disease. Moreover, elevated physiological activity (i.e., feeding, respiration, digestion) in summer, stimulated by good food availability, enhances apparently the uptake rate of toxicants from the ambient environment thus increasing a degree of exposure.

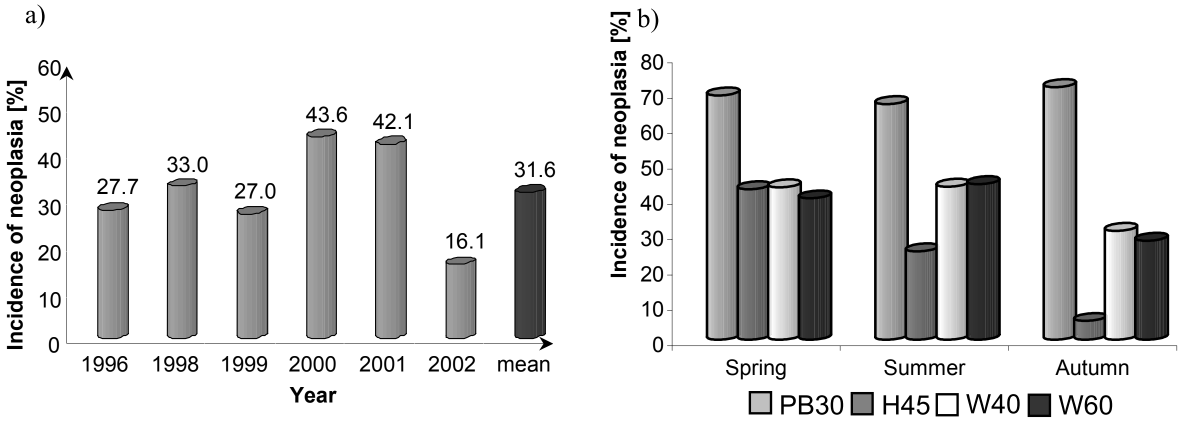

Figure 3. Average contribution of diseased Macoma balthica in the Gulf of Gdansk over a period of 1996–2002 (a), and seasonal changes of the incidence of the disease at three sites in 2000 (b).

A higher prevalence of neoplasia was generally recorded at deeper sites (Figure 3b). In deep regions the clams are subject to adverse conditions like hypoxia, anoxia or the presence of hydrogen sulphide that may induce an increase in sensitivity to stress (Janas and Szaniawska 1996). Differences in bivalve physiological performance with changes in oxygen conditions and hydrogen sulphide, phenomena often observed in eutrophied stagnant deep-water layers, have been well documented in various marine and estuarine regions (Jahn and Theede 1997, de Zwaan and Babarro 2001). Bacterial outbreaks are a part of every anoxic event and, therefore, strongly interfere with the toxic effects of sulphide and likely cause direct infection of the bivalve (de Zwann and Babarro, 2000). Moreover, Falandysz *et al*. (1996, 1997, 1998) reported that polychlorinated napthalenes (PCNs) were present in different fish species and other organisms collected from the south-western part of the Gulf of Gdańsk.

The sites located in the Vistula River plume receive a high load of different contaminants (particulate organic matter, nutrients, metals, organic compounds) that are drained to the Gulf of Gdańsk from nearly 50% of Poland (Andrulewicz 1996, Sokołowski *et al*. 1999, 2001). A direct relationship between metallic pollutants in sediments and physiological responses of *M. balthica* from the Vistula estuary has been observed (Sokołowski *et al.* 1999, Hummel *et al*. 2000). Adverse environmental conditions can appear in a bottom zone where oxygen deficiency, the presence of hydrogen sulphide and elevated trace metal concentrations (e.g., Cu, Pb, Zn, Cd, Fe, Mn) have been recorded. The highest concentration of Cu, Zn, Pb, Cd and Ag in sediments from the Gulf of Gdansk (Poland) occurred near mouth of the Vistula River, Puck Bay and Gdańsk Bay (Glasby and Szefer 1998).

Sediments from nearly all sites demonstrated the effect of acute toxicity to disappear only with a dilution below 10 % that allows classifying sediments as toxic according to the Recommendations of Helsinki Commission (HELCOM Recommendation 23/11 adopted March, 6 2002). A strong relationship of sediment toxicity to the prevalence of gill neoplasia in *Macoma balthica* was found ($p<0.005$), highlighting the importance of surficial sediments as a source of causal factors of the disease. An increased prevalence of neoplasia in the clams was recorded at deeper sites that cope with the results of the sediment toxicity test. Moreover, in the deep regions the clams are subject to adverse conditions that may induce an increase in sensitivity to stress and make the etiological agent more virulent.

The southern Baltic Sea with the Gulf of Gdańsk in its southern-most end is considered a seriously polluted water basin. Over the last tens of years a number of adverse alterations in the ecosystem of the Gulf have been noted (Glasby and Szefer 1998), most important was increasing contamination with nutrients (Nowacki *et al*. 1993, Andrulewicz 1996), heavy metals, radionuclides (Szefer *et al*. 1996, Szefer 2002), organic compounds (e.g., pesticides, cyclic hydrocarbons, organotins; Falandysz *et al*. 1997 1998, Konat and Kowalewska 2001, Albata *et al*. 2002), and military wastes that were deposited on the sea bottom after the Second World War (Korzeniewski 1999). Potential effects to the system may be also induced by invasive and non-indigenous estuarine and marine organisms (NEMO) bearing parasites and pathogenic microorganisms to which local species are not specifically resistant (Leppakoski and Olenin 2000).

Conclusions

Interspecific distribution of gill neoplasia in the Gulf of Gdańsk provides a new biological (behavioural) aspect into the disseminated neoplasia model. Neoplastic cells were recorded in two of four bivalve species commonly distributed in the Gulf, *M. balthica* and *Mya arenaria*. The two species are facultative (filter/deposit) feeders that are able to take up particles from the water column and surficial sediments but only the Baltic clam inhabits deeper regions. The cancer was not detected in the mussel *Mytilus edulis trossulus* and the common cockle *Cerastoderma glaucum* that are typical suspension feeders. Since a distribution range of the bivalve species often overlaps, specifically that of *Mya arenaria* and *Cerastoderma glaucum*, feeding behaviour might be indicative of the source of potential causal factors in the environment. Chemical compounds present in the sea bottom, available to deposit feeders but not to filter feeders, therefore may act as carcinogenic agents. A risk of induction of neoplasia in bivalves would then increase with depth, particularly in water basins with a trough-like geomorphological structure and enhanced accumulation/sedimentation of contaminants in deep areas. The highest prevalence and most advanced stage of neoplasia in the Baltic clam was observed at deeper sites. Deeper regions of the Gulf of Gdańsk represent the ultimate sink for effluents e.g., nutrients, metals and organic compounds associated with particles, discharged from the land via run-off and rivers (Glasby and Szefer 1998). This statement generates hypotheses for further studies, which can help to elucidate the environmental significance of bivalve disseminated neoplasia and to assess its biological risk at the population and ecosystem levels. Questions relating to causes and mechanisms of disease transmission in bivalves frame a priority challenge.

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ANNEX 7 US ENVIRONMENTAL MONITORING PROGRAMMES AND ASSOCIATED QUALITY ASSURANCE ACTIVITIES INCORPORATING STUDIES ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

S. A. MacLean, NOAA/NMFS, Narragansett, RI, USA

There are two national environmental monitoring programmes in the USA that include studies on pathology of bottom dwelling marine organisms. One is the NOAA National Status and Trends Program, and the other is the EPA Environmental Monitoring and Assessment Program.

The NOAA National Status and Trends (NS&T) Program was designed to assess historical trends of coastal contaminants. It has three major components: Benthic Surveillance Project (BSP), Mussel Watch Project (MWP) and Bioeffects Assessment (BA) Project. The NS&T web site (http://nsandt.noaa.gov/) provides the user with different web-based data analysis and display tools and protocols that provide access to the agency's data. This web site performs well using Internet Explorer 6.0 browser or later version. The tools provided in the NS&T web site on the three major NS&T projects (Benthic Surveillance, Mussel Watch, and Bioeffect Assessment) are:

- **Site profile (monitoring site data retrieval).** Query capability tool to locate and obtain information about a site.
- **Analysis tool.** It allows the user to analyze the data and compare contaminant concentrations and contaminant trends between two selections. It also provides a site contaminant query capability.
- **Mapping tool.** Mapping capabilities are offered making use of ArcExplorer enabling users to integrate local data sources with Internet data sources, query, and analysis in an easy-to-use web browser.
- **Data.** It allows users to download both raw data in ASCII tab delimited format and GIS ArcView format files.
- **Links.** It offers useful links to various local, state, and federal organizations web sites.
- **Publications.** It allows links to various NS&T publications, some of which are downloadable.

The **Benthic Surveillance Project (BSP)** was conducted from 1984 to 1993 at approximately 120 coastal sites around the USA. BSP has a completed long-term database of over 80 organic and inorganic contaminants in sediment, fish liver and fish bile, and of fish toxicopathic liver diseases. The primary objective was to determine concentrations of more than 80 organic and inorganic contaminants in the liver and bile of bottom dwelling fishes and associated surficial sediment from coastal and estuarine waters of the Atlantic, Gulf of Mexico, and Pacific coasts, including Alaska. In addition, incidences of visible lesions were noted and histopathological examinations of selected liver, kidneys, fins, gills, ovaries, and testes were conducted. In 1987, the BSP expanded to include measurements of biological effects due to contaminant exposure (toxicopathic liver diseases) and measurements of mixed function oxygenase (MFO) and xenobiotic-DNA adducts. Site selection was based on collecting samples from areas that would be representative of a particular body of water. Sites were not knowingly located near waste discharge points, local dump sites, or "hot spots". The primary target species were winter flounder (*Pseudopleuronectes americanus*) from the U.S. Northeast, white perch (*Morone americana*) from the mid-Atlantic, Atlantic croaker (*Micropogonias undulatus*) from the Southeast Atlantic and Gulf of Mexico, white croaker (*Genyonemus lineatus*) from the Southwest, English sole (*Pleuronectes vetulus*) from the Northwest, and flathead sole (*Hippoglosoides elassodon*) from Alaska. Field sampling procedures and analytical quantification methods for the period 1984–1992 are described in NOAA Technical Memorandum NOS ORCA 71.

The **Mussel Watch Project (MWP)** is an ongoing project that began in 1986 sampling over 250 sites in coastal waters around the USA, including the Great Lakes. The long-term database includes data on sediment and bivalve tissue chemistry of over 80 organic and inorganic contaminants, bivalve histology, and of *Clostridum perfringens* as sewage marker. The sampling sites are selected to be representative of large coastal areas and avoid small scale patches of contamination. Mussel Watch primary target species are blue mussels (*Mytilus edulis*) from the USA North Atlantic, zebra mussels (*Dreissena polymorpha*) and quagga mussel (*D. bugensis*) from the Great Lakes, foolish mussel (*Mytilus trossulus*), the Mediterranean mussel (*M. galloprovincialis*), and the California mussel (*M. californianus*) along the Pacific Coast, eastern oyster (*Crassostrea virginica*) from the mid-Atlantic southward through the Gulf of Mexico, smooth-edged jewelbox (*Chama sinuosa*) from the Florida Keys, tropical oyster (*Ostrea sandvincensis*) and the Pacific oyster (*C. gigas*) from Hawaii, and Caribbean oyster (*C. rhizophorae*) from Puerto Rico.

The **Bioeffects Assessment Project** began in 1986 and is an ongoing project of intensive regional studies, of 2 to 4-year duration, to assess bioeffects associated with contaminant exposure at over a thousand sites in nearly 30 coastal locations (estuaries, bays, lagoons) in the United States. Bioeffects Assessment studies consist of sediment toxicity surveys, evaluation and application of biomarkers, development of effect-based numerical guidelines to infer toxicological relevance of sediment contamination, and formulation of indices to describe the condition of the coastal ecosystems. Sediment toxicity surveys are conducted in specific coastal regions where contaminant monitoring data from the National Status and Trends Program, and other auxiliary information from state and local sources, indicate that the potential for substantial environmental degradation and associated likelihood of biological effects exists.

A secondary criterion is collaboration with other Federal, state, and local agencies that would assure direct and immediate use of the study results. Sediment toxicity assessments are based on bioassays for acute toxicity to amphipods, impaired fertilization and abnormal larval development in sea urchins, and measures of physiological stress in bacterial cells or transformed cell lines. Biomarkers, suborganismic responses to contaminant exposure at the histological, physiological or biochemical levels, are being developed or tested to validate their field performance using endemic or transplanted bivalves or demersal fish. In recent years, the bioeffects assessments studies have been expanded to develop an integrated approach to assessing sediment contamination and its ecological implications by measuring the differences in benthic biological community structure between contaminated and reference sites. The intent is to derive a composite index or indices of coastal environmental degradation that are comparable across regions, and address the concept of sustainable use resources.

Relational databases of seven study areas (Biscayne Bay, Saint Lucie Estuary; Chesapeake Bay, Delaware Bay, San Francisco Bay, Puget Sound, and Galveston Bay) and non-relational data from twenty three other study areas are provided on the NS&T website. In addition, written reports and the following information are available:

- sediment, tissue, and water chemistry of over 80 organic and inorganic contaminants;
- toxicity assays [e.g., Sediment extract (MicroTox), Whole sediment (10-day survival Amphipod Assay), Sediment porewater (Sea urchin fertilization) and others];
- biomarker assays;
- biota histopathology;
- benthic community assessment.

Significant Findings of NS&T Program regarding Finfishes: Prespawning female winter flounder collected from two highly contaminated embayments and four nearby less urbanized embayments were evaluated for liver pathology, ovarian development and exposure to xenobiotic contaminants. Indicators of contaminant exposure (bile fluorescent aromatic compounds; hepatic aryl hydrocarbon hydroxylas acitivity; concentrations of polychlorinated biphenyls in liver ovary and brain; levels of xenobiotic –DNA adducts in liver) and toxicopathic lesions were highest in the fish from the contaminated sites. Liver PCB levels and FACs in bile positively correlated with hepatic hydropic vacuolation and biliary or hepatocellular proliferation. Fecundity and other measures of ovarian development were not negatively impacted by contaminant exposure. The latter finding may be due to the offshore habitat of the winter flounder during early vitellogenesis.

Analysis of data on histologic lesions, and sediment and fish tissue contaminant concentrations of several different species of fishes from embayments on the Atlantic and Pacific coasts of the USA, demonstrated that fish age was a significant risk factor in the occurrence of hepatic neoplastic and non-neoplastic lesions and of necrotic and sclerotic kidney lesions. The relationship between contaminant exposure and specific lesion risk were frequently species-specific, so that data from some species consistently demonstrated a positive association between organic contaminant exposure and increased risk of hepatic lesions. This suggests that not all hepatic lesions identified in these studies could be used as biomarkers of contaminant exposure in all fish species. Associations between kidney lesions and measures of contaminant exposure showed that renal lesions may have potential as biomarkers of contaminant exposure in various species of fishes. PCBs alone does not appear to be a significant risk factor for hepatic or renal lesions, however, fish from embayments contaminated by PCBs and high levels of aromatic hydrocarbons (a frequent co-occurrence) showed high risk of hepatic lesions.

Key references relating to bioeffects of environmental contaminant exposure resulting from the NS&T program include:

Bioindicators of contaminant exposure, liver pathology, and reproductive development in prespawning female winter flounder (*Pseudopleuronectes americanus*) from urban and nonurban estuaries on the Northeast Atlantic coast. 1992. NOAA Technical Memorandum NMFS-NWFSC-1.

National Benthic Surveillance Project: Northeast Coast. Fish histopathology and relationships between lesions and chemical contaminants (1987–1989). 1992. NOAA Technical Memorandum NMFS-NWFSC-4.

National Benthic Surveillance Project: Pacific Coast. Fish histopathology and relationships between toxicopathic lesions and exposure to chemical contaminants for cycles I to V (1984–1988). 1993. NOAA Technical Memorandum NMFS-NWFSC-6.

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EPA Environmental Monitoring and Assessment Program (EMAP) is a research program to develop the tools necessary to monitor and assess the status and trends of national ecological resources. EMAP's goal is to develop the scientific understanding for translating environmental monitoring data from multiple spatial and temporal scales into assessments of current ecological condition and forecasts of future risks to our natural resources. The Demonstration Project of EMAP's Virginian Province began in 1990 and involved 500 sampling visits to 217 sites in estuaries from Cape Cod, Massachusetts to the Chesapeake Bay. Additional provinces around the coastal USA were added to the program as EMAP evolved into a national monitoring network that includes various components, one of which is the National Coastal Assessment program. The National Coastal Assessment (also known as Coastal 2000) is a five-year effort to survey the condition of the Nation's coastal resources by creating an integrated, comprehensive coastal monitoring program among the coastal states. EMAP's National Coastal Assessment comprises all the estuarine and coastal sampling done by EMAP beginning in 1990. Data collected during these surveys include water quality measurements, water column nutrients, sediment chemistry and toxicity, benthic infaunal biomass abundance, organic and inorganic toxicant body burdens of fish, and visible external fish lesions. The list of target species of fish varies with each region. In the Virginian Province targeted species of interest to WGPDMO included: Atlantic croaker (*Micropogon undulatus*), hogchoker (*Trinectes maculatus*), summer flounder (*Paralichthys dentatus*), spot (*Leiostomus xanthurus*), weakfish (*Cynoscion regalis*), winter flounder (*Pseudopleuronectes americanus*), windowpane flounder (*Scophthalmus aquosus*), and white perch (*Morone Americana*). All fish captured in the trawl are examined for external lesions and representative tissues are excised and preserved for histologic examination.. Further information on EMAP and data from the program are available at the EMAP website (http://www.epa.gov/emap/index.html). The EPA publishes a National Coastal Condition Report that consists of information from four federal agencies and several state and regional/local organizations. The report addresses eutrophication, water clarity and quality, benthic community condition, and sediment and fish tissue contaminants. Copies of the report can be obtained from the USEPA, Office of Research and Development/ Office of Water, Washington, DC 20460.

Quality Assurance

The quality of the analytical data generated by the NS&T Program was overseen by its Quality Assurance Project component that documented sampling protocols, analytical procedures and laboratory performance, and the reduce intralaboratory and interlaboratory variation. Intercalibration exercises were conducted annually, results of which are published (e.g., NOAA Technical Memorandum NOS ORCA 66; NOAA Technical Memorandum NOS ORCA 69). Other references to intercalibration exercises can be found on the NS&T website. Two laboratories performed gross and histopathologic examinations of bottom-dwelling fishes collected under the BSP. Meetings were convened between them to jointly examine the most common lesions in liver, kidney, gill, skin and fin and to establish consistent coding and terminology for their description. The focus was on hepatic lesions and the descriptive terminology used is that presented in Hendricks, *et al.,* (1984: National Cancer Institute Monograph 65, pp.321–326) and Myers, *et al.,* (1987: Journal of the National Cancer Institute 78:333–363). By the third year of the NS&T program, only one of these two laboratories was performing the histological examinations and continued to do so until the end of the program.

Details of the QA Project Plan for the EMAP program has been published (National Coastal Assessment Quality Assurance Project Plan 2001–2004, EPA/620/R-01/002, May 2001). QA standards have not been set for histopathological evaluations. However, standard procedures for the routine laboratory examination of finfish pathologic conditions are described in Section 4 of the EMAP-Estuaries Laboratory Methods Manual Vol. 1 (US EPA, 1995). The QA/QC recommendations for these studies are that the samples be properly preserved and that qualified pathologists conduct the histologic examinations

ANNEX 8 OVERVIEW OF VIRAL DISEASES OF CRUSTACEANS WITH EMPHASIS ON COMMERCIALLY IMPORTANT SPECIES

S. W. Feist, CEFAS Weymouth Laboratory,Weymouth, Dorset, UK and
S. A. MacLean, NOAA/NMFS, Narragansett, RI, USA

Knowledge on viral diseases of crustaceans is best developed for those species of importance for aquaculture, in particular the penaeid shrimps. Although viruses in crustaceans are widely distributed in wild populations, there are relatively few studies that have investigated the prevalence and pathogenicity of viral diseases in wild marine crustaceans, either as objects of scientific interest or as potential hosts for inclusion in biological effects monitoring programmes in estuarine or offshore environments. The purpose of this working document is 1) to provide a listing of relevant references; 2) to assess the available information in this area and 3) to highlight evidence of significant infections, in particular pathological effects and any data on possible effects on wild populations.

Viruses of crabs

1) Paralysis virus of *Macropipus depurator*. First reported by Vago (1966) from the Mediterranean coast of France. Transmission of purified material from infected crabs induced clinical signs 6 days post inoculation, 'trembling movement' and paralysis ensues and mortalities reach 70–85%. Large amounts of virus occur in gill and intestinal tissue. Co-infections with the S-virus may also occur. No information on the impact of these infections in natural populations.
2) Reo-like virus of blue crab (*Callinectes sapidus*) from the east coast of the United States. Infected crabs appear lethargic and 'tremble', become paralysed with poor blood clotting. Necrosis of hematopoietic tissues, hemocytes and nervous tissue, mortality can occur in captive crabs. Prevalence in natural populations is unknown but the infection has been recorded in juvenile and adult animals in Chesapeake Bay and Chincoteague Bay.
3) Picorna-like virus of blue crab (Chesapeake Bay virus (CBV)). Causes abnormal behaviour and blindness in young crabs. Infects nervous cells, epidermis and epithelial tissues with significant pathology evident. Mortality occurs several weeks after initial onset of the disease. Prevalence in natural populations is unknown.
4) Crab Hemocytopenic virus (Bunyaviruidae) infects hemocytes of the European shore crab (*Carcinus maenas*). Some affected crabs lose clotting ability but others appear asymptomatic. Impact on wild animals appears slight.
5) Rhabdo-like viruses "A" and "B" of blue crab from other incidental findings, which came to light following other investigations. Infects hemocytes and basal lamina of the mandibular organ respectively. Do not elicit significant pathology. Thought to be associated with 'stress'.
6) Baculo-A of blue crab appears to be ubiquitous throughout the range of its host at prevalences up to 52%. Nuclei of hepatopancreas epithelial cells are infected but mortalities have not been reported. Several other baculo-like viruses have been reported but there is little data on the prevalence in wild populations.
7) Bifacies virus (BFV) (= Herpes-like virus) of blue crabs infects hemocytes, inhibits clotting and causes mortality. Prevalence up to 13% in juveniles has been reported. Mortality amongst naturally infected animals may take up to 60 days.
8) *Cancer pagurus* bunya-like virus (CpSBV) has recently been reported for European edible crab (*Cancer pagurus*). Mortalities from experimental infections occur 7–12 days post challenge. No data on prevalence in wild populations.
9) There are a few other incidental reports of viruses in crabs (e.g., Y-organ virus in *C. mediterraneus*) but there are no data on the significance to the host at the individual or population level. In several cases, mixed infections have been reported.

Overall, there is little information on the prevalence and impact of viral diseases affecting crabs along the coastlines of the North Atlantic, even though some consider that viruses are the most important pathogens of this group (Corbel *et al*., 2003). It appears that several viruses can infect a single host but pathogenicity may be limited. Several studies on pathogenesis indicate that mortalities occur, but most researchers conclude that "stress" is a significant factor in producing a disease state and that this is often a chronic process. For this reason it could be suggested that the detection of increased prevalence of diseased crustaceans could indicate stress effects at the population level. As such, investigations aimed at gaining background information of disease prevalence in coastal regions used for biological effects monitoring and laboratory studies to understand the pathogenesis of the infections under controlled conditions are recommended.

Viruses of shrimps and prawns

There are many viral diseases of cultured penaeid shrimps (Lightner, 1996), nearly all of which have resulted from the use in culture of infected wild larvae, post-larvae or broodstock. However, despite the huge economic impact these diseases have had in many Asian and Central American countries culturing penaeids, there is little data on their prevalence and impact on wild populations. Due to the extensive literature available on penaeid shrimp viruses, we will address here only some key pathogens currently of interest in wild populations.

1) Parvo-like virus was found in 2/30 *Penaeus merguiensis* sampled from North Queensland, Australia (Roubal *et al*, 1989). Infected animals showed intranuclear inclusions in the hepatopancreatic tubule epithelial cells. Overt signs of disease were not reported.
2) *Baculovirus penaei* (PvSNPV) was the first virus described in penaeid shrimp and was found to be enzootic in adults and juveniles of several species of wild penaeids in the Gulf of Mexico (USA) (Couch, 1974; Couch *et al*., 1975; Overstreet, 1994). Although widely distributed and known to cause mortalities of juveniles, the impact of infection on natural shrimp populations has not been demonstrated. Toxicants may enhance natural infections.
3) Baculovirus from adult shrimp (*Metapenaeus bennettae*) (Bennettae Baculovirus, BBV) has been reported from Moreton Bay, Australia. Affected animals showed no external lesions or other disease signs. DNA probes for Monodon Baculovirus (MBV) proved negative. Intranuclear inclusions in the hepatopancreatic epithelial cells.
4) White Spot Syndrome Virus (WSSV) has caused devastating losses in farmed shrimp production in Asia. WSSV impacted a culture facility in the USA as well, with evidence suggesting the infections came from the wild. Since WSSV infects a broad range of crustaceans, this is a virus of particular concern. WSSV has been detected in asymptomatic blue crabs from the Atlantic and Gulf coasts of the USA (Chang *et al*., 2001) and from unhealthy wild srhimps *Litopenaeus setiferus* and *Farfantepenaeus aztecus* from the vicinity of the culture outbreak in the USA. Exposure to tissues from these infected shrimp caused disease in healthy shrimp (Chapman *et al*., in press).
5) Diseased *Carcinus mediterraneus* from the Mediterranean were found infected with a Tau virus localized in the epithelia of hepatopancreas and midgut. Experimental transmissions resulted in mortalities of infected animals (Pappalardo, *et al*., 1986).
6) Intracellular bacilliform Virus (IBV) in brown shrimp (*Crangon crangon*) termed *C. crangon* bacilliform virus (CcBV) was described by Stentiford *et al*. (2004). Prevalence was up to 100% in the Clyde estuary, western Scotland (see appendix 1).
7) Shrimps (*Palaemon elegans*) from the Bay of Piran, Adriatic Sea were found to be infected with two viral pathogens. The Palaemon B-cell Reo-like virus (PBRV) and "Bay of Piran Shrimp Virus" (BPSV) (parvo- or picorna-like virus) were detected in one specimen each of 7 animals examined. Neither infection produced any disease symptoms (Vogt and Strus, 1998). Pathology appears to be limited to the individual cells infected. No evidence of an inflammatory response. The authors suggested that these pathogens and other co-infections (rickettsia and bacteria) may cause problems under less favourable nutritional and environmental conditions.
8) A virus has recently been described infecting the haemocytes of the Caribbean spiny lobster *Panulirus argus* from the Florida Keys (Shields and Behringer, in press). Juveniles, with a mean prevalence of 17% are the principal hosts of the virus. Inoculations of infected tissue filtrates into adult spiny lobsters, spider crab and stone crab, two cohabitants of the spiny lobster, did not produce infections. The virus is thought to be spread by commercial practices and may cause significant mortalities in wild stocks.

The disease status of crabs and other crustaceans should be taken into account when considering the impact of specific viral pathogens. The presence of facultative pathogens may enhance prevalence and severity of infection (Lightner, *et al*., 1983), perhaps by affecting the hosts ability to mount an effective response to the primary pathogen. These can generally be assessed using histological methods but more detailed investigations using electron microscopy and virological methods will be needed for accurate diagnosis of particular viral infections. This is both time consuming and expensive. However, if the presence of specific viral pathogens are shown to be good indicators of individual and population stress, molecular and immunologically based detection methods for analysis of haemolymph or tissue samples may be relatively easy to develop and will be a practical approach for use in environmental monitoring programmes.

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Appendix 1

Paper 'in press' DAO

Pathology and ultrastructure of a non-occluded, intranuclear bacilliform virus (IBV) infecting brown shrimp *Crangon crangon* (Decapoda: Crangonidae).

Stentiford, G.D.*, Bateman, K., Feist, S.W.

Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Barrack Road, Weymouth, Dorset DT4 8UB, UK.

ABSTRACT

The brown shrimp (*Crangon crangon*) supports a very important fishery in Europe (over 25,000 t, value 80 M ECU in 2000). Through the course of histopathological screening of crustaceans from the Clyde estuary, western Scotland, for the biological effect of contaminants, we have discovered a highly prevalent (up to 100 %) non-occluded intranuclear bacilliform virus (IBV) infection in the hepatopancreatic tubule epithelia and midgut epithelia of wild *C. crangon*. This is the first report of an IBV in this family. We have termed this virus Crangon crangon bacilliform virus (CcBV). Histological and ultrastructural observations suggest that this virus is similar to other IBVs (or non-occluded baculoviruses) previously described from crabs and penaeid shrimps. Virus-infected epithelial cells contained eosinophilic, hypertrophied nuclei with marginated chromatin. The basement membrane of infected cells was often separated from that of its neighboring cells and their nuclei appeared apoptotic. In heavily infected shrimp, apoptotic cells were expelled into the lumen of the hepatopancreatic tubule or the midgut. Following this stage, some hepatopancreatic tubules became degenerate, with remnants of the basement membrane and myoepithelial lining remaining. Transmission electron micoscopy of hypertrophic nuclei revealed the presence of rod-shaped and cylindrical, envelope-bound virions. These virions did not form arrays and were not encapsulated within occlusion bodies but did appear to be partially occluded in an amorphous matrix which corresponded to a granular viroplasm. The ultrastructure, morphology and size of the nucleocapsid and of the complete virion associated them most closely with the intranuclear bacilliform viruses (IBVs) previously reported from other decapod crustaceans. Due to the pathological manifestation of IBV infection in C. crangon, it seems likely that it can act as a population modulator, particularly at sites where infection prevalence is high, such as that observed in the Clyde estuary.

ANNEX 9 EPIDEMIOLOGICAL METHODS FOR THE ASSESSMENT OF DISEASES AND POPULATION EFFECTS RISK

W. Wosniok, Institute of Statistics, University of Bremen, Germany
S.W. Feist, CEFAS, Weymouth, UK
T. Lang, Federal Research Centre for Fisheries, Cuxhaven, Germany

1 Abstract

This paper describes central terms and methods of epidemiology as well as main approaches that might be useful to increase the power of certain fish disease analyses and to enlarge the scope of risk assessment for fish diseases regarding effects on the population.

2 Introduction

The term "epidemiological methods" summarizes a collection of statistical methods, which are used to describe and analyse the frequency, spread, causes and effects of diseases in a population. Historically these methods had been developed for diseases of humans, but a part of the method spectrum has been used quite some time also for diseases of marine organisms. Historically, methods used in studies on diseases of, for example, wild fish were focused on the assessment of their frequency and spread, and more recently on their causes. However, the application of more epidemiological methods seems useful, e.g., in order to

- optimise the analysis of disease data by accounting for confounding factors
- improve the sampling strategy applied in disease surveys
- analyse effects of diseases on the population structure

Epidemiological methods start from disease data of the type that is common in fish disease research: random or length-stratified samples from wild populations are examined for the presence of certain diseases, which is recorded as a yes/no information per individual fish, plus possibly additional information on the individual like sex, length, age, contaminant concentration in the individual etc. The methods may as well be applied to laboratory data with the same structure. Laboratory studies may even allow a sequence of observations on the same individual, which expands the range of questions that may be addressed to e.g., survival time studies.

This text summarizes some epidemiological methods, which have the potential to make presently performed fish disease analyses more effective or to expand the scope of these analyses towards the consideration of disease effects on the population.

3 Descriptive epidemiology

3.1 Quantification of disease frequency

Descriptive epidemiological methods provide ways to quantify the disease frequency. The central quantity for fish diseases is the prevalence, the proportion of diseased fish among the examined ones. In human epidemiology, the incidence, defined as the proportion of newly diseased cases per time period, is also used. However, the calculation of the incidence requires repeated and frequent observations of the same fish population, which seems feasible only for studies under controlled conditions (laboratory, aquaculture).

An alternative characterisation of disease frequency are the odds that correspond to a prevalence. The odds are defined as the ratio of the proportions with and without disease

$$odds = \frac{p}{1-p}.$$

The odds are equivalent to the prevalence in the sense that the prevalence can be calculated from the odds (p = 1/(1+odds) and vice versa, which means that the decision which of both to use is essentially a matter of tradition or taste.

3.2 Comparison of prevalences

Frequently there is the need to compare two prevalences p_0 and p_1, e.g., if these originate from a study (p_1) and a reference area (p_0). Of course, the difference p_1 - p_0 describes the difference between both, but sometimes it is more elucidating to express the study group value relative to the reference. This is done by the relative risk (RR), which expresses p_1 as a multiple of the reference p_0:

$$RR = \frac{p_1}{p_0}.$$

A value of RR = 1 means equality of both prevalences, a value below (above) 1 indicates that $p_1 < p_0$ ($p_1 > p_0$). The relative risk is useful for group comparisons but, in contrast to prevalence and odds, does not give an absolute quantification.

An alternative quantity for comparisons is the odds ratio (OR), defined by

$$OR = \frac{p_1}{1-p_1} \cdot \frac{1-p_0}{p_0}$$

An OR of 1 indicates equality of both odds, OR < 1 (> 1) means that the odds in group 1 are smaller (larger) than in group 0.

3.3 Comparison of prevalences in the presence of concomitant factors

A disease prevalence may depend on various factors, e.g., on the type of disease (for lethal diseases, other prevalences are to be expected than for non-lethal ones) as well as on the age and length distributions within the populations involved. For the comparison of the prevalences of a given disease from different geographical areas or over time, the length or age effect should be removed. This is usually done by using length-stratified samples, which contain a pre-specified number of specimens per length class. As an example, it is common to report disease rates for female dab for the size classes 15–19 cm, 20–24 cm, > 24 cm (e.g., Wosniok *et al.* 1999). Stratification by age may have a stronger biological justification, but is hardly possible on board. But also with length stratification, there usually still remains a variation of individual lengths within the length class between samples. Even more important is that the intended age stratification is not automatically achieved by using length stratification as a surrogate. The epidemiological approach of standardisation is useful to remove interfering effects of (in this example) age in a proper way.

Direct standardisation allows comparing prevalences from differently structured samples while removing the effect of those influencing parameters, which are of no interest for the research question. For age effects, the technique of direct standardisation means to re-calculate the observed prevalences, which are affected by the specific age distributions of their parent samples, as if they were observed in a common “standard population”, yielding standardized prevalences. If these differ, then the reason can no more be a difference in the age distributions of the original samples, but must be due to other reasons. The standard population (more precisely: their age distribution) is defined by the researcher, usually by using a well-known average population structure over a long time and a large area. In analogy to human epidemiology, which uses some (few) standard populations for worldwide comparisons, one could think of using e.g., the average age distribution of dab caught in a certain ICES statistical rectangle in the North Sea between 1990 and 2000 as the age distribution of a standard population. There is no problem to define a general standard population for several parameters (e.g., length, age, sex) simultaneously and to refer only to a subset of it, (e.g., females only), if the problem requires so. An example of the application of direct standardisation for the analysis of fish disease data is given in Lang *et al.* (1999).

The example given in Table 1 demonstrates the approach. The necessary calculations can be done in a simple way by standard spreadsheet software.

3.4 Attributable risks

Closely related to the comparison of observed prevalences is the question of how the population disease frequency would change if level of the factor changes from the present value to zero. The Attributable Risk (AR) quantifies the proportion of the disease prevalence that can be attributed to the present level of x and is calculated by

$$AR = \frac{\Pr(Disease \mid x\ as\ presently) - \Pr(Disease \mid x = 0)}{\Pr(Disease \mid x\ as\ presently)}$$

Here, "Pr(…)" stands for the disease probability, i.e., the prevalence. However, the hypothetical prevalence given that x = 0 is usually unknown and must be calculated from a mathematical model. This is demonstrated in the next section, using the logistic model.

Table 1. Raw and standardised prevalences and odds for (fictitious) age-dependent disease data.

age class (years)	number examined		proportion in age class			number diseased			
	in sample		observed in sample		standard population	observed in sample		standardised in sample	
	1	*2*	*1*	*2*		*1*	*2*	*2*	*2*
≤2	5	5	0.10	0.10	0.06	1	1	0.60	0,60
3	11	6	0.22	0.12	0.12	2	1	1.09	1.00
4	26	20	0.52	0.40	0.40	13	10	10.00	10.00
5	6	16	0.12	0.28	0.32	4	10	10.67	11.43
≥6	2	5	0.04	0.10	0.10	2	5	5.00	5.00
total	50	50	1.00	1.00	1.00	22	27	27.36	28.03
prevalence						0.44	0.54	0.55	0.56
odds						0.79	1.17	1.21	1.28

4 Analytical epidemiology

Though standardisation seems at first sight a purely descriptive approach, it is in fact on the borderline to analytical statistical approaches, because the choice of the parameter to standardise for implies the assumption that this parameter does actually influence the prevalence. Analytical approaches, however, typically do not remove the effect of influencing parameters from the data, but incorporate them in a mathematical-statistical model in order to quantify their effect and hence allow to assess the risk associated with them. The following section describes briefly the main mathematical tool involved and those main approaches, which are suitable for standard fish disease data.

4.1 Logistic regression as a basic tool

The most prominent representative of analytical epidemiological approaches is logistic regression, which is well known in general epidemiology and is also a standard tool for the analysis of fish diseases. The purpose of logistic regression is to link one or more explaining quantities $x_1, x_2, \ldots, x_n$ (influencing quantities or suspected causes) with the probability p of the presence of a disease (the prevalence). The result of logistic regression shows the contribution of each explaining quantity x_i to the prevalence p. The result also contains information about the random error associated with these contributions, which means that their significance can be tested against zero (being non-existent). From the regression approach, also the prevalence to be expected for arbitrary sets of ($x_1, x_2, \ldots, x_n$) values can be calculated. This means that scenarios can be investigated ("what is the change in disease prevalence p if the contaminant concentration x is reduced by 10%?"). Odds ratios (OR) and Attributable Risks (AR) as standard parameters to characterise the changes due to different scenarios can easily be calculated from the logistic regression.

Technically, logistic regression generalises the idea inherent in standard linear regression and analysis of variance, which postulate as the relation between a (continuous) dependent quantity y and explaining quantities $x_1, x_2, \ldots, x_k$ the linear form

$$y = a_0 + a_1 x_1 + a_2 x_2 \ldots + a_k x_k + e\,, \qquad (1)$$

where e is a normally distributed error term, which absorbs the random variation. The explaining quantities x_i are either continuous (e.g., concentration, length, age, …), or they are indicator variables with values 0 or 1 coding the absence or presence of a categorical attribute (e.g., 0 for "male", 1 for "female"). This purely linear approach is not appropriate for the analysis of disease data, because there the dependent quantity is a prevalence, i.e., a probability with values restricted to the interval [0,1], while y in (1) may attain any value in [-∞,+∞]. Also, the random variation of a prevalence has a binomial (not a normal) distribution, the variance of which is automatically linked to the predicted value of the prevalence, while the variance of a normally distributed error is in no way linked to the predicted value of y. To account for these differences, logistic regression postulates as a relationship between (expected) prevalence p and explaining quantities $x_1, x_2, \ldots x_k$ the nonlinear form

$$p = \frac{1}{1 + \exp[-(a_0 + a_1 x_1 + a_2 x_2 \ldots + a_k x_k)]} \qquad (2)$$

and the random variation of the number r of diseased fish is assumed to have a binomial distribution with coefficients n (the number examined) and p (from equation (2)). The coefficients a_0, a_1, …, a_k in (2) are calculated from prevalence data (n, r) and the observed $x_1, x_2, \ldots x_k$ by an iterative procedure and can be tested for being significantly different from zero. A coefficient of zero implies that the corresponding variable x does not influence the prevalence p. This identifies unimportant parameters.

If the prevalence is modelled by the logistic approach (2), then the odds are simply

$$odds = \exp(a_0 + a_1 x_1 + a_2 x_2 \ldots + a_k x_k)$$

The Attributable Risk (AR) for the case of only one explaining parameter being present (k=1), which is hypothetically from $x_1 > 0$ reduced to $x_1 = 0$, is calculated by

$$AR = \frac{\exp[-(a_0 + a_1 x_1)] - \exp[-a_0]}{1 + \exp[-(a_0 + a_1 x_1)]}.$$

For the comparison of two groups A and B with coefficient sets (a_0, a_1, …, a_k), (b_0, b_1, …, b_k) the odds ratio calculated as

$$OR = \exp[(a_0 + a_1 x_1 + a_2 x_2 \ldots + a_k x_k) - (b_0 + b_1 x_1 + b_2 x_2 \ldots + b_k x_k)].$$

The relative risk for the same comparison is

$$RR = \frac{1 + \exp[-(b_0 + b_1 x_1 + b_2 x_2 \ldots + b_k x_k)]}{1 + \exp[-(a_0 + a_1 x_1 + a_2 x_2 \ldots + a_k x_k)]}$$

4.2 Study design for the analysis of suspected causes

The analysis of fish disease prevalence usually employs cross-sectional data as it emerges from standard catches, possibly stratified by length class. A "cross-sectional" sample is one taken at one point in time from a population with time-varying structural attributes (size, age distribution, biomass, sex ratio, …). For fish disease studies, a cross-sectional sample is formed either by the group of fish that was present in the way of the net during the trawl, or, if there were more fish than could be analysed, by a sub-sample taken at random from the catch. Length stratification also results in using only a sub-sample from the total catch.

A cross-sectional study is appropriate for exploratory questions of the type "Which effects are caused by which parameters?" with a large set of candidate effects and parameters available. While exploration was the main focus of early fish disease studies, there is now, after many years of exploratory analysis, often a more specific hypothesis to check (e.g., "Parameter x influences the prevalence of lymphocystis"). To study such a dedicated hypothesis a case-control study design as described in section d) is more effective

Other designs to collect data from a developing population might seem preferable at first sight. A cohort study, in which the health status of the same individuals within a population is recorded at several consecutive points in time, might provide a better basis to study the appearance and development of diseases. However, such cohort studies seem feasible at most under laboratory conditions.

4.3 Study design for the analysis of regional spread

Though there is considerable difference between examining the effect of a suspected cause and looking for a geographical pattern for the occurrence of diseases, both aspects can to a large extent be treated by the same mathematical machinery. Regional attributes that are suspected to affect disease prevalence can be coded as explaining quantities (the x's) in e.g., the logistic model and their significance can be assessed in the same way as it is done for suspected causes. Slightly different is the identification of "hot spots" (zones of uncommonly high prevalences) by the methods of disease mapping (cf. Alexander & Boyle, 1996, Lawson *et al*., 1999), which starts from a geographically dense net of observation points. As these are rarely available for marine disease data, this direction of analysis will not be treated here.

4.4 Case-control studies

The effect of a disease-causing agent can be most clearly be seen when the agent concentration ranges around EC50, because there a change in concentration results for most concentration-response-relationships in the highest change of the disease response. This means that to show a concentration-disease relationship needs least effort (cases), if the sample prevalence is close to 0.5. However, this can be expected in a cross-sectional study only if the true population prevalence also happens to be close to 0.5. In all other cases there is the chance to increase the effectiveness of the study by not using the cross-sectional sample itself, but instead to create a case control sample from it. A case-control study can be expected to detect a relationship either with higher confidence, compared with a cross-sectional study of the same size, or with the same confidence, but less effort. The latter is a particular attractive aspect if the effort to determine the x value is high, which demands a small number of measurements.

A case-control sample is created by a selection of specimens from the usual catch, the cross-sectional sample. The diseased fish are the "cases", while the non-diseased are the "controls", and the aim is to obtain a sample with as many cases as controls. The parameter x is determined only for specimens in this case-control sample. Logistic regression is used to analyse the effect of x as before. As outlined above, the advantage of case-control studies is that the statistical power to detect an effect of x is increased compared with cross-sectional studies of the same size. It should be noted that a case-control study is not meant to derive statements about the absolute disease prevalence in the population, but exclusively to quantify the effect of the agent x. This information is necessary to calculate risk quantifications like odds ratios, relative risks, attributable risks, and to feed models of population dynamics (see section f), which provide a more holistic risk assessment.

Table 2. Results from fitting a logistic regression model to simulated population data, a cross-sectional and case-control sample, the latter two selected randomly / randomly within disease status group from the population data set.

Sample	Sample size	Estimated a_0	SD of a_0	$p(a_0)$	Estimated a_1	SD of a_1	$p(a_1)$
"population"	253	-5.36	0.77	< 0.0001	1.53	0.25	0.0001
cross-sectional sample	50	-23.60	15.35	0.1341	7.32	4.69	0.1152
case-control sample	50	-2.65	0.87	0.0024	1.12	0.29	0.0001

In Table 2, the results from using different types of samples for the calculation of the relationship between disease prevalence and a causing quantity x are compiled. The "population" data was generated by a simulation, and the cross-sectional and case-control sample were then randomly drawn from this "population". In real life, the population would correspond to the total number of fish caught by one haul, and the cross-sectional or case-control sample is the part of the catch that undergoes further inspection. The relationship between prevalence and x is modelled by the logistic approach (1) with k= 1.

Using the whole population data set to estimate the intercept a_0 and the slope a_1, where the latter quantifies the effect of x, leads to the clear conclusion that x has a significant influence on the prevalence, expressed by $p(a_1) = 0.0001 < 0.05$. From the cross-sectional sample, no effect of x would be concluded because of $p(a_1) = 0.1152 > 0.05$. However, from the case-control sample, which has the same size as the cross-sectional, the presence of the x effect is clearly identified by $p(a_1) = 0.0001$. Similarly, the 3 estimated values of a_1 and their standard deviations show a similar pattern with a_1 from the population and the case-control sample being relatively close and both different from the cross-sectional sample estimate. If one wanted to carry out a cross-sectional study with the same precision (standard deviation) for a_1 as obtained here by the case-control sample, the cross-sectional sample size would have to be more than the tenfold of the case-control sample. This is, of course, no general rule, but it holds generally that a case control sample needs a smaller or at most the same size as a cross-sectional sample in order to achieve the same precision.

4.5 Matched-pairs designs

Case-control studies can be made even more effective if there are additional parameters besides the one under study, which are suspected to affect the disease prevalence, but are of no interest by themselves. Such parameters are sometimes call “nuisance parameters”. The effect of these can be removed by creating and analysing a matched-pairs case-control sample, a technique related to the standardisation and case-control approaches mentioned earlier. A matched-pair design means to form pairs consisting of a case and a control for which the third (matching) parameter has the same value (e.g., the same length). Pairs with the same value for the matching parameter form a “stratum”. The effect of the stratum parameter can be accounted for in different ways:

- by introducing a model component for the stratum effect,
- by conditioning on the stratum effect,
- by modelling the stratum effect as a “random effect”.

All these options can be realised within the framework of logistic regression. Details of the computational techniques involved depend on the nature of the explaining quantities (binary, ordinal or continuous), and in the case of a matched-pairs analysis also on the number of strata (standard, conditional logistic regression, or a non-linear mixed model). Standard software packages (SAS, S-plus, R, Statistica, SPSS, …) are able to deal with all these situations. There are also mathematical tools for situations in which the linear component in (2), which describes the essential part of the parameter effects, must be replaced by a more complicated form, either by including interaction terms or non-linear components. In the latter case, the logistic regression might be replaced by a Generalised Additive Model, which provides high flexibility in model building and can as well be fitted by many software packages.

4.6 Disease effects: Risk assessment on the basis of population effects

The occurrence of a disease does not only influence an individual’s well-being. It may also influence the size and structure of the whole population, if the disease has effects on e.g., growth, mortality, or reproductive ability. The assessment of the risk associated with a disease or the agent that causes the disease, should consequently incorporate population effects by considering, e.g.,

- population size,
- length distribution,
- age distribution,
- biomass

as target quantities. To do this, data on these quantities is required as well as a mathematical model for the link between agent / disease prevalence and the above target quantities. The population dynamics model will further require information about the mortality of the fish under real conditions with and without disease, about the reproductive ability, and possibly about the duration of the disease, if it is reversible. Part of this information is already available, e.g., from stock assessment activities (population size, biomass, length distributions), others can be inferred by mathematical methods from time series of observations of a population (e.g., lifetime from age distributions, cf. Kalbfleisch *et al*. 1983, Astheimer and Wosniok, 1984, Wosniok 1987). Obviously, the exposure to a causing agent must be measured in the real environment, as well as possibly other quantities that might be recognized as relevant for the dynamics in the future (climate, food, …). There is a vast literature available about mathematical population dynamics models (cf. Murray 1979, Royama 1992, Temming 1989, Webb 1985); however, simple models with simple plausible assumptions should serve for a beginning.

The risk assessment with respect to population effects involves steps similar to the calculation of the Attributable Risk mentioned above. The steps are:

- formulate the basic model,
- estimate unknown coefficients from real data, where possible, including the coefficients which quantify the effect of disease and / or of the causing agent on population parameters,
- plug in plausible assumptions where nothing else is available,
- calculate scenarios for the population development over time, given either the present disease prevalences and exposure situation, or as an alternative a situation with reduced prevalence or exposure,

- compare the two population developments: what are the differences in stock size, biomass or other relevant quantities?

For a beginning, studies should concentrate on the path from disease to population effects rather than from suspected agents to population effects, because the disease aetiology seems to be multi-factorial in most cases, which makes the start of such an analysis unduly complicated.

The procedure proposed above leads to an assessment of the disease-associated risk based on criteria that are relevant from a biological as well as from an economic point of view. It seems to be a consequent next step in the analysis of fish diseases, its sources and effects.

5 Further perspectives

Though it is strongly recommended to start the use of epidemiological methods, particularly those focussing on population effects, with simple approaches and to increase the degree of complexity according to previous experience, one should have the future steps in mind. As a long-term perspective, risk assessment will be improved by including more details in any modelling. These details will comprise

1) Knowledge on the biology of the species under investigation, migration patterns, feeding behaviour, stock structure, background levels of disease, age structure etc. Ideally, there would be information on temporal changes to the above. Current monitoring programmes target only a few species (usually flatfish), which have been selected for their apparently high disease susceptibility and responsiveness to contaminant exposure as well as availability throughout the study area. These species represent only a component of the marine ecosystem. Possibly more species will have to be considered.
2) Knowledge on the biology of the pathogens under consideration: transmission requirements, pathogenicity under different conditions, effects to the host.
3) Knowledge on the relevant environmental conditions, like temperature, dissolved oxygen, water movements, presence of contaminants and pollutants.

Most of these quantities will be needed as actual data, but also as long-term data to allow the detection of effects that have been initiated before the actual observation of effects.

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ANNEX 10 INFORMATION ON DISEASE/PARASITE INTERACTIONS BETWEEN WILD AND FARMED FISH AND RELATED MANAGEMENT CONTROL METHODS

D.W. Bruno, FRS Marine Laboratory, Aberdeen, Scotland

Introduction

Aquaculture has become established during the last two decades as an industry of considerable importance to many countries, in many cases bringing much needed employment opportunities to rural areas. The industry has grown rapidly over a relatively short period and is currently dominated by ICES countries Scotland and Norway through the farming of Atlantic salmon with a production of around 150,000 tonnes and 509,000 during 2003 respectively. The production of new species such as Atlantic cod has risen slowly at approximately 100 tonnes and 1500 tonnes respectively. Increasing the diversity of fish species in aquaculture provides increased opportunity for disease interaction of micro (viruses and bacteria) and macro-parasites between the established species (predominantly salmonids in the north Atlantic) and gadoids and flatfish (which are perceived as having the greatest potential for diversification). The risk with respect to the interaction of pathogens between farmed and wild fish can be summed up by the generality that fish sharing water are likely to share diseases (McVicar 1997).

Impact

Historically, fish diseases identified in wild stock have consequently occurred in farmed fish and the documentation of bacterial kidney disease (BKD) and furunculosis are good examples. Although there is evidence of transfer of infection into farmed fish from wild populations, based on the assumption that fish farm disease has its origin in the endemic range of the surrounding area, it is difficult to locate salmonids with notifiable or other significant infections in the marine environment. Possibly such animals succumb to predation typically encountered in the wild, or act as carriers where under natural conditions infectious agents co-exist with their host without causing significant disease. Furthermore, susceptibility to individual diseases will vary between strains of salmon (wild and farmed alike), the disease agents and the specific health status of the stock. Therefore, it is not easy to be confident about the frequency or significance of transfer of pathogens to wild stocks and consequently there are few recent documented examples of disease outbreaks that have significantly had a negative effect on populations of wild fish.

Management

Management control methods for farmed fish are wide ranging and include maintaining a high health standards thorough vaccination and farm hygiene, monitoring and reporting of escapes, disinfection of equipment, disease reporting, surveillance, modelling and risk analysis. The question of the increasing number of vaccines for juvenile fish and the potential for the immune system becoming compromised has to be addressed. For wild fish the options for control are limited and difficulties are encountered when it comes to determine the consequences of an infection in wild fish. Whereas, in a fish farm, any damaging effects of disease usually becomes obvious through a decrease in growth performance, loss of body condition, increased morbidity or in extreme cases, an increase in the rate of mortality, such changes are difficult to detect in wild fish populations unless mass mortality is registered. Countries developing new species for aquaculture have removed wild broodstock as a source of eggs for farming and this had led to the introduction of some bacterial infections directly to the farm. Recently cases of nodavirus and *Pseudmonas anguilliseptca* have occurred with wild caught broodstock from sole, *Solea* spp. and blackspotted seabream, *Pagellus bogaraveo* in Spain. Consequently there are lessons for improved management and the risk of wild and farmed fish interaction.

Monitoring and surveillance

Infectious pancreatic necrosis (IPN) is a highly contagious viral disease of young fish in freshwater particularly of salmonid species held under intensive rearing conditions. Evidence suggests that the prevalence of IPN in marine reared fish is also increasing. To further our understanding of IPNV and its prevalence in wild fish a survey was completed during 2003 in Scottish waters to determine the prevalence of IPNV in wild marine and freshwater fish in local waters. A total of 11,515 fish (26 marine species) were sampled and checked for IPNV. As a result 24 IPNV isolations were made from common dab (*Limanda limanda*), grey gurnard (*Eutrigla gurnardus*), lemon sole (*Microstomus kitt*), plaice (*Pleuronectes platessa*), saithe (*Pollachius virens*) and whiting (*Melangius melangus*). Three virus isolations were made from the East of Fair Isle, the remaining from inshore waters around the Shetland Isles. No clinical signs of disease were recorded and the findings support earlier studies that this virus is widespread in wild fish and adult marine finfish appear resistant. An assessment of risk from infected fish such as saithe entering sea cages has not been widely researched.

In addition, a total of 2,191 wild fish (Atlantic salmon, brown trout, rainbow trout, sea trout, eel and flounder) caught in freshwater from a wide geographical area of mainland Scotland including the Isle of Skye, Uist and Orkney were sampled and checked for IPNV during 2003. In this case no virus was isolated. The survey excluded Lewis, Harris and Shetland Isles.

Modelling and risk

There is an increasing use being made of risk analysis in support of legislative controls of fish disease and key components of such an analysis include an assessment (when risks are identified, estimated and the consequences evaluated), management (when measures to reduce risks are implemented) and communication.

The sea louse, *Lepeophtheirus salmonis* is a specific parasite of salmonids that occurs in the Atlantic and Pacific Oceans on both wild and farmed fish. Lice feed by grazing on the mucous and skin of the fish and maybe present on fish with little to no impact on their health and survival. If sea lice accumulate and/or the health of the fish is compromised then lice can potentially affect the survival of fish. The factors that are responsible for causing epizootics, especially in wild salmonid populations, are still largely unknown and controversy remains regarding possible disease interactions between farmed and wild fish, especially where lice have been blamed for the collapse of sea trout, *Salmo trutta* populations in several countries. The extent of interaction of lice populations on farmed and wild fish, and hence the risk to wild fish, depends on the transmission of larval lice between these populations. This transmission in turn depends on the larval lice's movement through the water. Fisheries Research Services in Aberdeen are developing a model to simulate the movement of lice through the waters of Loch Torridon, Scotland. Extensive data are available on adult lice population and larval lice distribution in this area. The hydrodynamic model generates water current velocities in response to tides and under different assumptions of wind forcing can be used to identify sites at risk and the behaviours that are necessary to explain the observed distribution of pelagic stages. The areas in which fish are at risk of infection from lice produced at a given source can be found from combining runs from many lice particles.

In Norway a programme that aims to reduce the effects of lice on farmed and wild fish to a minimum has been developed (Huech *et al*, personal communication). This includes compulsory reporting of lice numbers and the monitoring of infection in wild salmonids. Heuch and Mo (2001) modelled the production of salmon lice along the Norwegian coast. The model predicted that if the allowed limit for number of lice is below 0.5 adult female lice fish^{-1}, more lice eggs will be produced on escaped fish than on farmed fish. To maintain a constant egg production this value should be lower each year as production and potential for escapes increases. However, the level of lice at which wild stocks are undamaged by sea lice is unknown (Heuch *et al.*, personal communication).

The monogenean parasite, *Gyrodactylus salaris* was transferred from resistant Baltic salmon populations for stock enhancement into Norway and subsequent movements of infected fish stocks during the 1970s that resulted in the infection and loss of wild populations. Restrictions on the movement of live material between countries are enforced but this parasite still presents a significant potential threat to wild stocks. The prevalence of infestation in salmonids varies widely, but is generally highest in parr and smolts. The inadvertent transfer by anglers is considered to represent a significant risk. Recently, a population of hybrid brown trout-salmon have been found in the Vefsna river, Norway and can survive *Gyrodactylus* infestation and hence are considered a risk as a vector of the parasite to native stocks (Anon, 2004). Data indicates that no new river systems were found to be infected in Norway with *G. salaris* during 2003.

During 1998–1999 infectious salmon anaemia (ISA) was recorded in farmed salmon in Scotland. This disease was spread through fish movements and the use of well boat and other site to site contacts. Diffusive spread may have also operated locally. The outbreaks of ISA demonstrated the importance of measures required to manage and control an infection on individual fish farms. Codes of practice were agreed regarding the enforcement of disinfection procedures, limiting the movement of personnel, equipment and boats, the use of procedures for removal and disposal of dead fish and adopting precautions during harvesting to prevent spread of disease. Despite rigorous checking the conditions prompting the emergence ISA were not fully established in Scotland, but operational factors at the primary site could have enabled a particularly virulent or pathogenic form of ISA virus to emerge from a rare wild reservoir. However, from the widespread surveys carried out no clinical signs of ISA were detected in the wild fish examined. Subsequent sequencing demonstrated positive reactions by RT-PCR for segment 8 of ISAV in a few wild fish but there was no evidence that infection in farmed fish was driving infection in wild fish.

Viral haemorrhagic septicaemia (VHS) was historically a disease of farmed rainbow trout in fresh water in continental Europe. However, outbreaks in the marine environment have been recorded in cultured turbot in Germany, Scotland and Rep. of Ireland, and in cultured rainbow trout in Sweden and wild isolates reported to infect wild cod, haddock and other marine fish species. Surveillance programmes have found isolates of VHSV from a wide range of wild marine fish species in the North Sea, Baltic Sea and eastern Atlantic. The concern is concentrated on the possibility of one of the emerging aquaculture species being more susceptible to VHSV than salmonids and developing the disease. Experimental immersion challenges show that marine isolates of VHSV are low risk for salmon. The North American strain of VHSV has been isolated from wild coho and chinook salmon and also from sea-farmed Atlantic salmon. Although turbot have been reported as developing clinical VHSV halibut seem resistant when farmed in conjunction with turbot which subsequently developed a clinical VHSV infection (Cape Clear, Ireland). Cod, haddock and other gadoids have been found infected with VHSV in the wild. Immersion challenge of wild cod with a cod VHSV isolate has not caused mortality or clinical disease. Hence, the risk of mortality in gadoids is low although they could develop as carriers.

Escapes

Fish escapes especially from cage farms result from accidents of weather or operation, and through poor maintenance of nets and other equipment, inappropriate specification of containment equipment for the exposure characteristics of the site or through damage from animals such as seals. Hatchery-produced fish may also differ from wild fish in their behaviour, appearance, and/or physiology. The effects of disease on hatchery fish and their interaction with wild fish are not well understood, but wild fish may indeed come into contact with escaped fish. Although legislation requiring the notification of farmed fish escapes has been in place in some countries since May 2002, dealing with this problem is one of the major challenges facing the aquaculture industry.

The high level of escapes from Atlantic salmon farms, up to two million fishes per year in the North Atlantic, has raised concern about the potential impact on wild populations with respect to inter-breeding and diseases that they may carry. Offspring of farm and 'hybrids' in Ireland has shown a reduced survival compared with wild salmon but these fish grew faster as juveniles and displaced wild parr, which as a group were significantly smaller. Where suitable habitat for these emigrant parr is absent, this competition would result in reduced wild smolt production. In the experimental conditions, where emigrants survived downstream, the relative estimated lifetime success ranged from 2% (farm) to 89% (wild) of that of wild salmon, indicating additive genetic variation for survival. Wild salmon primarily returned to fresh water after one sea winter (1SW) but farm and hybrids produced proportionately more two sea-winter salmon. However, lower overall survival means that this would result in reduced recruitment despite increased two sea-winter fecundity. It has thus demonstrated that interaction of farm with wild salmon results in lowered fitness, with repeated escapes causing cumulative fitness depression and potentially an extinction vortex in vulnerable populations (McGinnity *et al.* 2003).

Summary

Overall, the greatest concern for wild fish in terms of disease impact would be exposure of a larger than normal concentration of indigenous pathogens or exotic pathogens from aquaculture sites. For cultured fish, particularly those in ocean net pens, they could also be exposed to pathogens from wild fish particularly as the agents that can affect farmed salmon have in general been reported in wild fish. Also, the conditions operating in the aquaculture environment will predispose these fish to a higher risk of developing clinical disease than the wild fish.

Some diseases such as IPNV are detected widely in farmed fish but also occur in a range of natural hosts. Controlling emerging diseases on fish farms will benefit from the development of mathematical models to understand the dynamics of fish epidemics and use of the models to determine how the disease might persist and spread in a wild population. Thus approach would provide a rationale way to identify potential risks.

Sea lice infestations and their impact on farmed fish have been reduced through the use of new treatments and fallowing options, but the controversy regarding their role on the decline in wild fish in some areas remains. Modelling work in this area is underway.

Aquaculture will continue to expand and it will become increasingly important to locate farms in an area where both environmental and biological data can be combined into a model of louse production and their impact on wild salmonids predicted.

In some countries wild broodstock are used as a source of eggs for farming and this had led to the introduction of infection directly to the farm. Consequently improved management in this area is recommended.

It is evident that in any evaluation of the consequences to wild fish of disease and interaction with farmed fish, considerable emphasis has to be placed on studies of wild fish populations and the development of methodologies that will identify disease at an early stage. Despite the many difficulties in obtaining good data from this area, more emphasis should be given to research in this area.

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ANNEX 11 OVERVIEW OF THE DISTRIBUTION AND POSSIBLE CAUSES OF THE M74 SYNDROME

Svante Wistbacka and Göran Bylund
Laboratory of Aquatic Pathobiology, Åbo Akademi University, Åbo/Turku, Finland

Present state

Accumulating information indicates that the wild salmon stock in the Baltic Sea is presently recovering from the critical decline faced in the 1990s. The number of salmon parr in rivers with wild salmon is now more than doubled compared to the years in the mid 1990s. Also the number of returning spawners entering rivers with wild salmon has increased. Thus, the catches in rivers with wild salmon in the late 1990s were three times as high as in the late 1980s, this in spite of the heavy losses of alevins in the middle of the 1990s due to the M74 syndrome.

Several factors may have contributed to the improved situation: International actions, i.e., the salmon quota established by IBFSC, reduced the catches in the Baltic Sea by more than 50 % from the end of the 1980s to the end of the 1990s (from 1 million to 450 000 specimens). Thiamine treatment of M74 affected salmon eggs and fry improved the conditions for compensatory release of salmon smolts. And, although the prevalence of M 74 is shifting from year to year, the trend has definitely been a decrease in the occurrence of this disease syndrome in recent years when comparing with the situation during the worst years in the middle of the 1990s (Figure 1). In the 2003 hatch, which was exceptionally successful, the mean fry mortality due to M74 in Finnish and Swedish rivers was 9–10 % compared to 80–90% during the worst years in the 1990s.

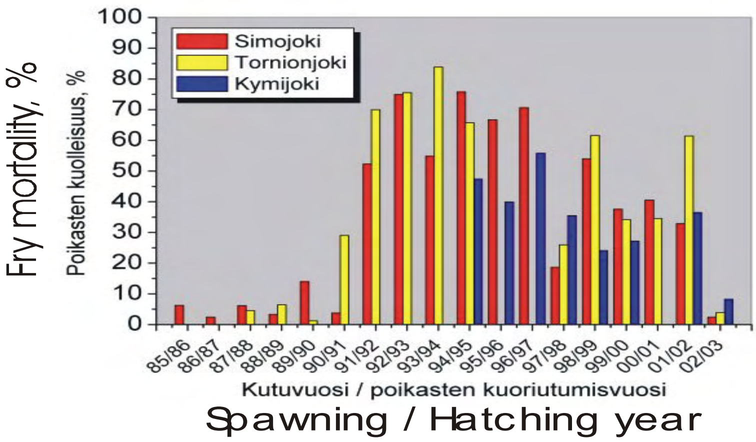

Figure 1. Fry mortality due to M74 each spawning/hatching year in three different rivers (Simojoki, Tornionjoki, and Kymijoki). (With permission, © 2004 Finnish Game and Fisheries Research Institute.)

A review of research and recent progress

The ultimate cause/causes of the M74 syndrome still remain unknown. However, there are correlations and experimental data accumulated during the history of M74 research that may facilitate the understanding of the aetiology of M74. Some of these data are reviewed below:

- There is a strong positive correlation between the M74 prevalence and the size of the sprat population in the Baltic Sea. However, the proportion of sprat in the salmon diet was less in 1994–1997, when M74 reached the highest prevalence, compared to 1959–1962 when M74 did not occur. Thus the correlation does not implicate that a diet shift towards more sprat is a factor in the aetiology of M74 as previously suggested. It is suggested, however, that the increasing sprat stock in the Baltic Sea may have induced qualitative changes in salmon prey species rather than shifts in the species composition in the diet.
- The mean weight-at-age as well as the condition factor of sprat and herring steeply decreased in all parts of the Baltic Proper from 1989 through the first half of the 1990s, when the M74 prevalence dramatically increased. From 1998 and onwards the condition factor of sprat as well as the mean weight-at-age of herring have been

slightly increasing in the Baltic Sea. Cardinale and Arrhenius (2000) and Cardinale *et al* (2002) stated that the condition factor of herring and sprat are dependent on the stock density and possibly related to the total abundance of pelagic fish and the individual food intake. It is suggested that some factor/factors related to the growth rate and condition of sprat and herring, or some other factor dependent on the stock density of sprat and herring in the Baltic Sea might release the expression of the M74 syndrome.

- There is increasing evidence linking the thiamine deficiency causing the Early Mortality Syndrome (EMS) affecting salmon in the Great Lakes to a thiamine-destroying factor, thiaminase, in salmon prey species. The most significant developments in recent EMS research in North America include: 1) the confirmation that adult salmon reared on diets with elevated thiaminase activity develop thiamine-deficient embryos that exhibit EMS; 2) the demonstration that thiamine in salmon prey species is adequate to meet the thiamine requirements of salmon but the thiaminase activity in prey fish can be highly variable within a species (temporal and geographic variability); 3) the suggestion that inadequate egg thiamine may be associated with sub-lethal and/or interactive effects that extend beyond the initial overt fry mortality; and 4) the indication that severely EMS-impacted adult fish may exhibit neurological effects associated with thiamine deficiency (Brown *et al*., 2002).
- The thiaminase activity of Baltic herring analysed during 2000–2002 varied on a large scale between specimens in each single trawl-net catch (ranging from 0 to about 34 nmol g^{-1} min^{-1} potential activity) and was on a substantially higher level than the thiaminase activity found in sprat (ranging from 0 to about 13 nmol g^{-1} min^{-1} potential activity). The distributions where skewed and more than 60 % of the specimens of herring and more than 80% of the sprats analysed had a rather low thiaminase activity (0–1 nmol g^{-1} min^{-1} potential activity). Comparison of the thiaminase activity of herring from trawl net catches with the thiaminase activity of recently ingested herring collected from oesophagus and stomach of salmon indicated that salmon is feeding selectively on individuals with high thiaminase activity (>1 nmol g^{-1} min^{-1}) (Wistbacka *et al.*, 2002; and Wistbacka, unpublished data).
- Analyses of Baltic herring collected seasonally in the years 2000 to 2001 from ICES Subdivisions 29–32 indicated only minor seasonal and/or regional variations in the thiaminase activity. Analysis on herring and sprat collected in spring 2002 from Subdivisions 24–28, however, indicated significantly higher thiaminase activities in herring from Subdivisions 24 and 25 (Wistbacka, unpublished data).
- We recently demonstrated that the thiamine levels in organs of salmon (liver, muscle, blood and female gonads) decreased significantly in a 144-day feeding trial, when salmon were fed on a diet of herring containing thiaminase activities between 0.9 and 3.7 nmol g^{-1} min^{-1} and with a thiamine level of 0.38 nmol g^{-1} (±0.05). Mature eggs (5–7 mm, diameter) in female gonads showed the steepest decrease in thiamine content, achieving thiamine levels under and just above the threshold value demonstrated empirically to induce development of M74 (Järvinen, 2003).
- An increase in the occurrence of organochlorines, especially dioxin-like compounds, in salmon female muscle samples was demonstrated to correlate with the outbreak of the M74 syndrome. The dioxin-like compounds in salmon are suggested to originate from the major prey species, sprat and herring (Vuorinen *et al*. 2002). The dioxin contents measured in Baltic herring suggest that a slow growth rate increases the dioxin accumulation while during a fast growth phase (young fish) or in fast-growing herring, the dioxin accumulates to a lesser extent (Anon., 2003). Asplund *et al*. (1999), however, did not observe any differences in organohalogen substance (OHS) concentrations between healthy Baltic salmon and salmon that produced offspring with M74. Neither did Honeyfield *et al*. (1998) find any clear evidence supporting a role of contaminants as a factor behind the EMS.

Ongoing research

The research efforts in our own laboratory (Laboratory of Aquatic Pathobiology, Åbo Akademi University, Finland) are presently focused especially on the kinetics of the thiaminase activity in fish and the role different stress conditions may play in the induction of thiaminase activity in fish. We are considering especially the effects pathological conditions may have on the level of thiaminase activity in fish. In collabaration with the Norwegian National Institute of Nutrition and Seafood Research (NIFES) we are performing preliminary experimental studies concerning stress response, immunology, expression of detoxification markers (CYP1A) and expression of thiaminase activity in herring.

Another research programme, focusing on the bioaccumulation of dioxin-like organochlorines in Baltic fish, might provide information elucidating the aetiology of the M74 syndrome. The aims of this project are:

- to study biomagnification of PCBs and dioxins from zooplankton via Baltic herring and sprat and to Atlantic salmon;
- to explain large variation between individuals and species in concentrations and congener profiles of PCBs, dioxins, and toxic 2,3,4,7,8-PeCDF in salmon and their prey;
- to construct a bioenergetics-based model.

Partners in this research programme are University of Jyväskylä, Finland; Finnish Environment Institute (SYKE); Finnish Game and Fisheries Research Institute; NIFES, Norway and ICES.

In Sweden P. Snoeijs and her co-workers at the Department of Plant Ecology, Evolutionary Biology Centre, Uppsala University, are working on several projects dealing with the M74 syndrome and the lower trophic levels in the food web of the Baltic Salmon. The Baltic salmon shows symptoms of oxidative stress, deficiency of antioxidants (astaxanthin), and deficiency of vitamin B1. In this context, they are studying how the production of vitamins, fatty acids and antioxidants is regulated in phytoplankton in the Baltic Sea and the Kattegat area and how these compounds are transferred to crustaceans and fish.

Another project going on in Sweden (Pickova *et al*, Dept of Food Science, Swedish University of Agricultural Sciences, Uppsala) is dealing with the levels of cholesterol, cholesterol oxidation products (COP) and carotenoids in salmon from different stocks and the correlation between these parameters and the M74 syndrome.

Conclusions

In summary, we can conclude that the M74 syndrome is currently not a threat to the Baltic salmon population of the same magnitude as we saw it during the 1990s. Especially through protective measures initiated by national and international authorities and through improved possibilities to treat M74-affected salmon (eggs, fry and broodstock), the population of wild Atlantic salmon in the Baltic Sea seems to be recovering. Also the prevalence of the M74 syndrome has shown a decreasing trend in recent years.

However, during the past thirty years since we detected this serious disease syndrome, we have seen that the trend can change again even very rapidly. As long as we do not know the ultimate causes behind or releasing the M74 syndrome, we cannot make reliable prognoses or draw any firm conclusions concerning the importance and magnitude of this disease problem in the future. Recognizing this, we have with great concern seen the reduction in research efforts and funds available for research on the M74 syndrome in recent years. This development is even more regrettable as there is information accumulating indicating that the factors underlying this syndrome, although most obviously reflected in the salmon population, might have influence on other organisms too and on a larger scale in the aquatic ecosystem.

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ANNEX 12 DATA ON THE HEALTH STATUS OF NORTH SEA BIOTA AS INPUT TO THE ICES REGIONAL INTEGRATED ASSESSMENT OF THE NORTH SEA ECOSYSTEM

T. Lang, Federal Research Centre for Fisheries, Cuxhaven, Germany
W. Wosniok, Institute of Statistics, University of Bremen, Germany
S.W. Feist, CEFAS, Weymouth, UK

Abstract

The aim of the present document is to provide a) information about the plans in ICES for an integrated regional assessment of the state of the North Sea ecosystem and b) suggestions for possible contributions of the ICES WGPDMO to the assessment.

Introduction

What is REGNS and what does it hope to achieve? *(extracted from information provided by REGNS and associated bodies)*

There is a growing demand for ICES to provide Member Countries and international commissions with integrated, holistic, ecosystem-centred advice, based on regional assessment of the state of marine ecosystems. The provision of integrated advice can be seen as a strategic shift in the ICES advisory process, from a thematic-based advice to a more regional-based advice, which will require the combined co-ordinated efforts of almost all disciplines and expertises represented in ICES.

The underlying desire for ecosystem-based ocean governance has been reiterated at meetings such as Rio de Janeiro (1992), Johannesburg (2002), and the Køge Stakeholder Meeting (2002). For the North Sea, these ideas have been clarified in the Bergen Declaration (2002), which all North Sea states signed. From a scientific point of view, many scientists have already taken up the banner of the ecosystem approach, and it is very much at the core of the new ICES Strategic Plan.

The issue of integrated assessment has been discussed in various ICES bodies and it has been considered that the best way to learn how such an assessment can be achieved is by performing an integrated assessment for the North Sea as a pilot area within ICES. As co-ordinating body within ICES, the Regional Ecosystem Study Group for the North Sea (REGNS) was established in 2003, with Andrew Kenny (CEFAS, Lowestoft, UK) as Chair. Other ICES bodies, such as the Advisory Committee on Ecosystems (ACE), through its former Chair, H.R. Skjoldal (IMR, Bergen, Norway) and the ICES-IOC Steering Group on GOOS (SGGOOS), through its Co-Chair B. Turrell (FRS Marine Laboratory, Aberdeen, UK) are also involved in the co-ordination.

Actions and timetable suggested by REGNS to achieve the goals

- The existing ICES structure will be used for the assessment, although perhaps in the future the need to provide integrated advice will itself lead to a reorganisation of ICES and how it works.
- The REGNS request (WGPDMO 2004 ToR k, see Annex 2) will be considered by relevant ICES Working Groups supposed to contribute to the regional assessment, and feedback will be given to REGNS on how sensible it is (*2004 meetings of 19 relevant ICES WGs*).
- A set of commissioned 'Chapters' on the trends and status of the key components of the North Sea ecosystem, including human pressures and impacts, will be prepared by the relevant ICES Working Groups, each focused on individual themes (*preparations within ICES WGs start at the 2004 meetings, the 'Draft Chapters' will be finalised at the 2005 WG meetings, the final 'Chapters' will be reviewed at the 2006 WG meetings*).
- The relevant ICES Working Groups nominate: 1) a WG contact person for REGNS, 2) members responsible for preparing the above 'Draft Chapters', and 3) representatives for the 'Thematic Drafting Panels' (see below). Individuals for 2) and 3) could be identical, individuals for 1), 2), and 3) should attend the ICES Annual Science Conferences 2004–2006 (*to be decided at the 2004 WG meetings*).
- The different 'Draft Chapters' will be pulled together through 'Thematic Drafting Panels' (consisting of REGNS co-ordinators and WG representatives, see above) (*nominees for the Panels meet for the first time at the 2004 ICES ASC and decide on further proceedings*).

- An 'Overarching Integrating Assessment Panel' (to be nominated at a later stage) will take the 'Chapters' from each 'Thematic Drafting Panels' and prepare the overall, integrated assessment (*starts in early 2006*).
- A Theme Session, convened by REGNS, will be held at the 2006 ICES Annual Science Conference, where each 'Chapter' will be presented as ICES Council Meeting paper, together with the overall assessment (*at the 2006 ICES ASC*).
- The combined ICES Council Meeting papers and integrating papers may form a Cooperative Research Report, which will be the first ICES integrated ecosystem assessment of the North Sea region (*after the 2006 ICES ASC*).

WGPDMO contributions to the regional integrated North Sea assessment

The most obvious contribution of WGPDMO to a holistic integrated assessment of the state of the North Sea ecosystem is information on trends in the health status of North Sea biota. While information available from national and international studies on the health status of marine invertebrates, sea birds and mammals from the North Sea is scarce in terms of spatial and temporal coverage, there is considerable data available from national long-term programmes on the prevalence and spatial distribution of wild fish diseases in wider areas of the North Sea. In the following, a short outline is provided on the role that ICES and its WGPDMO has played in this context

Studies on wild fish diseases under ICES – a short outline

Diseases of wild marine fish have been studied on a regular basis by many ICES Member Countries for more than two decades. Disease surveys are often integrated with other types of biological and chemical investigations as part of national monitoring programmes aiming at an assessment of the health of the marine environment, in particular in relation to the impact of human activities.

On an international level, fish disease data have been used for environmental assessments in the framework of the North Sea Task Force and its Quality Status Report (North Sea Task Force, 1993), the OSPAR Quality Status Report 2000 (OSPAR Commission, 2000) and in the 3rd and 4th HELCOM assessments (HELCOM, 1996, 2002). Studies on externally visible diseases and liver histopathology are on the list of techniques for general and contaminant-specific biological effects monitoring as part of the OSPAR JAMP/CEMP.

Since the early 1980s, ICES has played a leading role in the initiation and coordination of fish disease surveys and has contributed considerably to the development of standardised methodologies. Through the work of the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), its offspring, the Sub-Group/Study Group on Statistical Analysis of Fish Disease Data in Marine Stocks (SGFDDS) (1992–1994) and the ICES Secretariat, quality assurance procedures have been implemented at all stages, from sampling of fish to submission of data to ICES and to data assessment.

Three practical ICES sea-going workshops on board research vessels were organised by WGPDMO in 1984 (southern North Sea), 1988 (Kattegat) and 1994 (Baltic Sea, co-sponsored by the Baltic Marine Biologists, BMB) in order to intercalibrate and standardise methodologies for fish disease surveys (Dethlefsen *et al.*, 1986; ICES, 1989; Lang and Mellergaard, 1999) and to prepare guidelines. Whilst first guidelines were focused on externally visible diseases and parasites, WGPDMO developed guidelines for macroscopic and microscopic inspection of flatfish livers for the occurrence of neoplastic lesions at a later stage. Attempts to further intercalibrate and standardise methodologies used for studies on liver pathology of flatfish were a major issue of the 1996 ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants (ICES, 1997) and the EU-funded BEQUALM project..

A fish disease data bank has been established within the (former) ICES Marine Data Centre, consisting of disease prevalence data of key fish species and accompanying information, submitted by ICES Member Countries. To date, the data comprise information from studies on the occurrence of externally visible diseases and macroscopic liver lesions in the common dab (*Limanda limanda*) and the European flounder (*Platichthys flesus*) from the North Sea and adjacent areas, including the Baltic Sea, Irish Sea, and the English Channel. In addition, reference data are available from pristine areas, such as waters around Iceland. In total, data on length, sex, and health status of more than 500.000 individual specimens, some from as early as 1981, have been submitted to ICES, as well as information on sampling characteristics (Wosniok *et al.*, 1999). With the new ICES Environmental Reporting Format (version 3.2), it is envisaged that the fish disease database will be extended to include data from other species and areas as well as data on studies into other types of diseases, e.g., liver histopathology.

Current WGPDMO activities have focussed on the development and application of statistical techniques for an assessment of disease data with regard to the presence of spatial and temporal trends in the North Sea and western

Baltic Sea (Wosniok *et al.* 1999). An output of WGPDMO's activities is the ICES web-based report on wild fish diseases, consisting of trend maps and associated information.

In a subsequent, more holistic approach, pilot analyses have been carried out combining the disease data with oceanographic, nutrient, contaminant and fishery data extracted from the ICES data banks in order to improve the knowledge about the complex cause-effect relationships between environmental factors and fish diseases (Lang and Wosniok, 2000; Wosniok *et al.*, 2000;). These analyses constituted one of the first attempts to combine and analyses ICES data from various sources and can, therefore, be considered as a step towards a more comprehensive integrated assessment.

Causes of diseases - what kind of information can data on trends in disease prevalence provide?

Today, the multifactorial aetiology of disease is generally accepted. Most wild fish diseases monitored in past decades are caused by pathogens (viruses, bacteria). However, other endogenous or exogenous factors may be required before the disease develops. One of these factors can be environmental pollution which may either affect the immune system of the fish in a way that increases its susceptibility to disease, or may alter the number and virulence of pathogens. In addition, contaminants may also cause specific and/or non-specific changes at various levels of biological organisation (molecule, subcellular units, cells, tissues, organs) leading to disease without involving pathogens.

In the statistical analysis of ICES data on externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulceration) of dab from different North Sea regions (see 3.1), it could be demonstrated that there were significant spatial differences, both in terms of absolute levels and the temporal changes in disease prevalence in the North Sea. While data from the 1990s revealed stable or decreasing disease prevalences in the majority of sampling sites, some areas in the North Sea showed increasing trends for some of the diseases, indicating a change in environmental conditions adversely affecting the health status of dab (Wosniok *et al*. 1999).

The results from the subsequent multivariate analysis on the relationship between the prevalence of the diseases with potentially explanatory environmental and host-specific factors (also extracted form the ICES fishery, oceanography and environmental databases, see 3.1) clearly highlighted the multifactorial aetiology of the diseases under study. A number of natural and anthropogenic factors (stock composition, water temperature, salinity, nutrients, contaminants in water, sediments and biota) were found to be significantly related to the temporal changes in disease prevalence. However, depending on area, time range and data availability, different sets of factors were identified. This reflects the multifactorial aetiology of the diseases covered, but was also attributed to some high correlations among the explaining quantities (Lang and Wosniok, 2000; Wosniok *et al*., 2000).

Based on the experience in and the results of ICES coordinated programmes, some conclusions as to the strengths and limitations of wild fish disease surveys were drawn (see Lang, 2002). One of the major advantages fish disease surveys offer is that diseases are ecologically relevant and integrative endpoints of chronic exposure to stressors including contaminants. Disease tends to reduce the energy available for sustaining essential functions and structures, the resistance to concomitantly effective stressors (natural and man-made), the capabilities for defence and escape, and the potential for competition and counteracting additional disease-causing entities. Therefore, a high prevalence of diseases might have serious implications for fish populations in terms of growth, reproduction, and survival. However, the occurrence of significant changes in the prevalence of gross fish diseases can be considered a non-specific and more general indicator of chronic rather than acute (environmental) stress, and it has been speculated that they might, therefore, be an integrative indicator of the complex changes typically occurring under field conditions rather than a specific marker of effects of single factors. Because of the multifactorial causes of diseases, the identification of single factors responsible for observed changes in disease prevalence is difficult, and scientific proof of a link between contaminants and fish diseases is hard to achieve. Nevertheless, there is a consensus that fish disease surveys should continue to be part of national and international environmental monitoring programmes since they can provide valuable information on changes in environmental health and may act as an "alarm bell".

Wild fish diseases monitored - what kind of data are available for the regional integrated North Sea assessement?

At present, annual or biannual fish disease surveys in the North Sea are carried out by Germany (BFA Fishery, Inst. of Fishery Ecology), The Netherlands (RIKZ) and the UK (CEFAS, Weymouth; FRS Marine Laboratory, Aberdeen) (see Table 1). The type of diseases/parasites monitored regularly are listed in Table 2. However, more data is available from monitoring programmes that were terminated in the 1990s or early 2000s (e.g., carried out by Denmark and Belgium). Figure 1 provides an overview of the disease data available and of areas used for previous statistical analyses (see 3.1. and 3.2).

A large amount of data generated so far was submitted to the ICES fish disease database and can be used for an immediate assessment, once its goals and an appropriate strategy have been fixed. However, Member Countries should be encouraged to submit historic disease data that are still missing in order to be able to use a more complete dataset for the assessment. It has to be checked with the ICES Secretariat if data can still be submitted using the old version of the ICES Environmental Data Reporting Format (2.2).

Proposed strategy for contributions from WGPDMO to the regional integrated North Sea assessment

The following strategy to be applied by WGPDMO according to the schedule outlined in chapter 2.2 above is proposed:

1) Update ICES fish disease database as soon as possible by incorporating historic data from the North Sea not yet submitted to ICES (either by using the Environmental Data Reporting Format 2.2 or 3.2)
2) Obtain on overview on the updated ICES disease data (in terms of spatial and temporal coverage)
3) Define the fish species, diseases, geographical areas and time spans for which information on spatial patterns and temporal trends should be included in the regional North Sea assessment
4) Carry out regional comparisons of prevalences (2002–2004) and trends (at least 2000–2004, preferably over a longer period), applying statistical methods as described in the draft manuscript to be submitted to the ICES TIMES series (Wosniok *et al*., in preparation)
5) Carry out multifactorial analyses on cause-effect relationships (as done before, see above under 3.1., 3.2) with updated data extracted from the ICES fisheries, oceanography and environmental databases)
6) Compile (in collaboration with other ICES WGs) additional information on the health status of North Sea invertebrates, fish, sea birds and mammals that is considered of use for the integrated assessment
7) Prepare a draft paper with the results of the above analyses

WGPDMO members responsible to work intersessionally on the tasks will have to be identified at the 2004 WGPDMO meeting.

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Table 1. Present fish disease surveys in the North Sea.

Country	Institute	Surveys	Season	Area	Fish Species
Germany	BFAFi, Cuxhaven	Biannual	Aug./Sept. Nov./Dec.	10 offshore areas, covering large parts of the North Sea. In addition, sampling sites in the Baltic Sea	Dab (*L. limanda*), Cod (*G. morhua*) Other species also examined where available
The Netherlands	RIKZ	Biannual Annual Biannual	March September September	3 offshore areas western Waddensea coastal zone, eastern Scheldt	Dab (*L. limanda*) Flounder (*P. flesus*) Flounder (*P. flesus*)
United Kingdom	CEFAS, Weymouth	Annual	June/july for offshore. Spring and/or atumn for estuaries	10–15 offshore areas from the east coast of the UK to the Dogger Bank. English Channel and Irish Sea are also sampled. Up to 6 estuaries on the east coast of the UK	Dab (*L. limanda*), Cod (*G. morhua,* Flounder (*P. flesus*) (only estuarine), Blenny (*Z. viviparous*) (only estuarine). Other species also examined where available
	FRS Marine Laboratory, Aberdeen	Annual	May/June	Firth of Forth, east of Orkney, areas in the Moray Firth	Dab (*L. limanda*), Cod (*G. morhua*) Haddock (*M. aeglifinus*)

Table 2. Diseases (externally visible diseases/parasites; liver anomalies) of fish species in the North Sea that are currently monitored on a regular basis.

Disease/Parasite	Aetiology	Host Species	Data in the ICES database
(1) Externally visible diseases/parasites			
Lymphocystis	Viral	Dab (*L. limanda*) Flounder (*P. flesus*)	+ +
Epidermal Hyperplasia/Papilloma	Viral	Dab (*L. limanda*)	+
Acute/healing Skin Ulceration	Bacterial	Dab (*L..limanda*) Flounder (*P. flesus*) Cod (*G. morhua*)	+ + -
Acute/healing fin rot/erosion	Bacterial	Dab (*L. limanda*) Flounder (*P. flesus*)	- -
Hyperpigmentation	Unknown	Dab (*L. limanda*)	+
Skeletal deformities	Multifactorial	Dab (*L. limanda*) Flounder (*P. flesus*) Cod *(G..morhua)* Haddock (*M. aeglifinus*) Whiting (*M. merlangus*)	+ + - - -
X-cell-gill-disease	Parasitic (?)	Dab (*L. limanda*)	+
Pseudobranchial swelling	Parasitic (?)	Cod (*G. morhua*)	-
Acanthochondria cornuta	Parasitic	Dab (*L. limanda*)	+
Lepeophtheirus pectoralis	Parasitic	Dab (*L. limanda*)	+
Stephanostomum baccatum	Parasitic	Dab (*L. limanda*)	+
Lernaeocera branchialis	Parasitic	Cod *(G. morhua)*	-
Cryptocotyle lingua	Parasitic	Cod *(G. morhua)*	-
Glugea stephani	Parasitic	Dab (*L. limanda*)	+
(2) Liver anomalies			
Macroscopic liver nodules > 2 mm	Contaminants (?)	Dab (*L. limanda*) Flounder (*P. flesus*)	+ +
Liver histopathology - Early non-neoplastic toxicopathic lesions - Foci of cellular alteration - Benign neoplasms (tumours) - Malignant neoplasms (tumours)	Contaminants (?)	Dab (*L. limanda*) Flounder (*P. flesus*)	- -

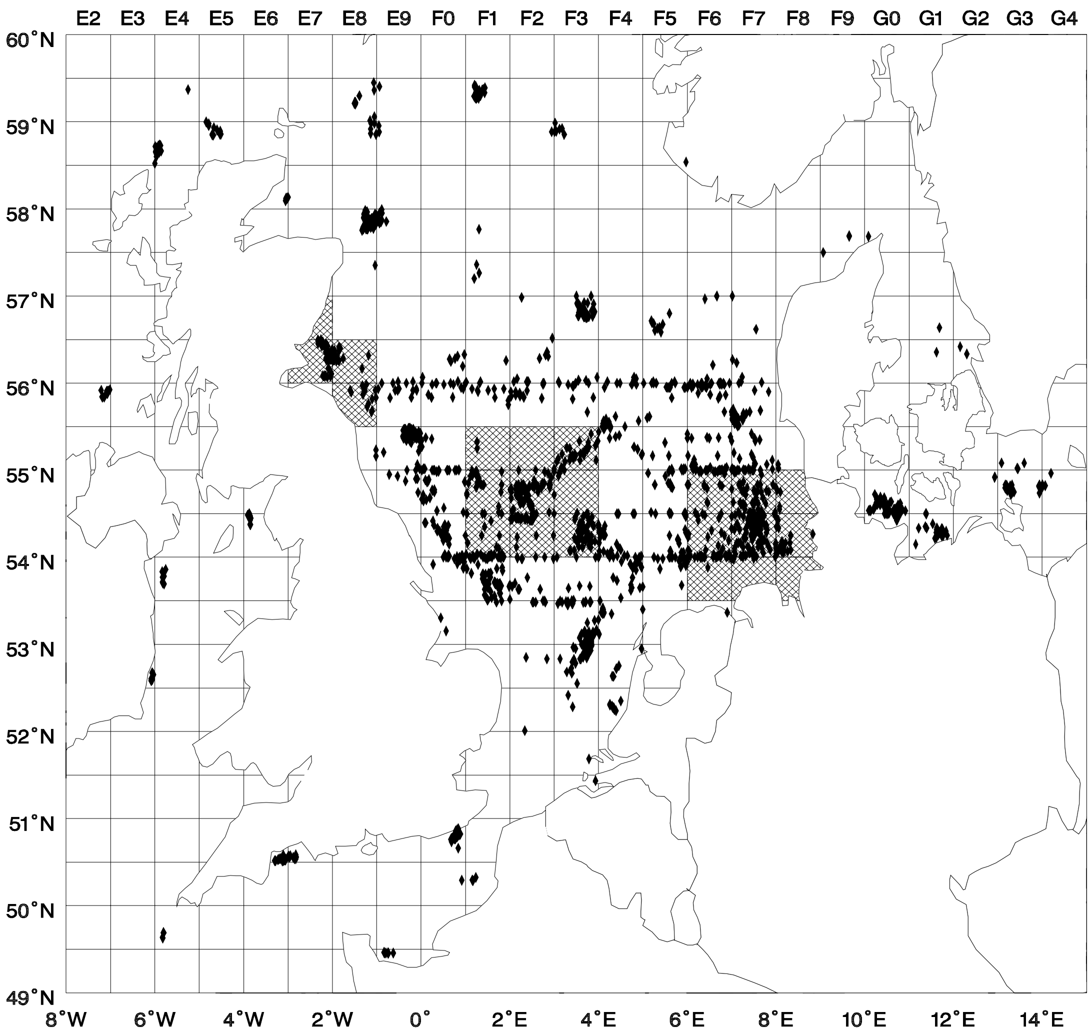

Figure 1. Location of the areas (grey shading) used for the statistical analysis of the ICES data on diseases of North Sea dab (*Limanda limanda)* in relation to contaminants and other environmental factors. Each diamond indicates one sample of dab examined for diseases in the period 1981–1999 (source: Wosniok *et al.,* 2000).

ANNEX 13 ANALYSIS OF PROGRESS WITH TASKS

a) produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports – as part of the continuing WGPDMO remits, reports on new diseases and trends of diseases were evaluated from national reports presented at the meeting and conclusions were drawn. Based on the output of the discussions, three recommendation for Terms of Reference for the 2005 WGPDMO meeting were drafted.

b) review and report on environmental monitoring programmes and associated quality assurance activities incorporating studies on pathology and diseases of marine organisms - available information was assessed and a summary report was presented. Based on the outcome of the deliberations, two recommendations for new Terms of Reference for the 2005 WGPDMO meeting were drafted.

c) provide a recommended technique to differentiate among Perkinsus spp., incorporating input received from web-based international solicitation of comments - available information was assessed and a summary report including recommendation for suitable techniques and further strategies was provided.

d) review the existing information on viral diseases of crustaceans with emphasis on commercially important species – available information was reviewed and a summary report was provided.

e) recommend on the use of epidemiological methods for the assessment of diseases and population effects risk – appropriate epidemiological methods were identified in a summary report, preparatory work for a future pilot study was suggested and a recommendation for a Term of Reference for the 2005 WGPDMO meeting was drafted.

f) evaluate current information on disease/parasite interactions between wild and farmed fish and advise on related management control methods - a status of the present knowledge was reviewed and, due to the importance of this topic, WGPDMO recommended to revisit this issue at its 2005 meeting.

g) maintain an overview of the spread of Ichthyophonus in herring stocks and the distribution and possible cause(s) of the M74 syndrome - reports summarising available information on Ichthyophonus (extracted from the national reports) and on M74 were presented. The WGPDMO recommended to revisit these issues at its 2005 meeting as part of the review of new disease trends and not under a separate Term of Reference as previously.

h) advise on the modifications made to relevant ICES databases and the revised ICES Environmental Data Reporting Format (Version 3.2) - the new Reporting Format was presented and discussed and WGPDMO provided information on its viewpoints to ICES.

i) 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 - *proposals for improvements of the WGPDMO contribution to the ICES Environmental Status Report were presented and will be submitted to ICES for consideration and later inclusion on the ICES web site.*

    ii. manuscript on methods for the statistical analysis of fish disease data for submission to the ICES TIMES series - *the content of the updated version of the manuscript was presented and agreed on.*

    iii. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish - *a progress report was presented, a new editor was nominated and strategies for the production of the Leaflets was discussed and endorsed. A recommendation to publish revised and new Leaflets only in English was given.*

    iv. review progress in the digitisation of the Disease Leaflets by the Secretariat - *the status was reviewed in the context of the discussions under item iii.*

j) develop plans for the preparation of detailed background material to be used by the 2005 ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas [OSPAR 2004/2] – answers were given to the questions raised by the ICES/OSPAR WKIMON Co-chairs and background material for the preparation of the workshop was provided. A recommendation for a Term of Reference for the 2005 WGPDMO meeting was drafted.

k) start preparations to summarise data on the health status of North Sea biota for the period 2002–2004, and any trends in the prevalence of diseases over recent decades. Where possible, the causes of these trends should be outlined. For input to REGNS in 2006 – WGPDMO contributions to REGNS and a strategy to achieve the goals were discussed and agreed upon, WGPDMO members who will serve as contact points for REGNS were identified and WGPDMO nominees for the drafting panels were appointed. A recommendation for a Term of Reference for the 2005 WGPDMO meeting was drafted.

ANNEX 14 RECOMMENDATIONS TO THE ICES COUNCIL

CATEGORY 2

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: T. Lang, Germany) will meet in (either La Tremblade or La Rochelle), 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) review available 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);
j) provide a progress report on the preparations to summarise data on the health status of North Sea biota for the period 2002–2004, and any trends in the prevalence of diseases over recent decades for input to REGNS in 2006;
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.

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) 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 (F. Geoghegan, B. Hjeltnes, D. Bruno). 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 (B. Hjeltnes, D. Bruno). 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 (T. Renault, S. Ford). 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. (K. Broeg, S. W. Feist, W Wosniok). 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 (K. Broeg, T. Renault). 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 should differ only a little. 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 (W. Wosniok, T. Lang, and S.W. Feist). 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 (B. Hjeltnes, S. MacLean, D. Bruno). 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 (T. Lang, S.W. Feist, and W. Wosniok). 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. (T. Lang, W. Wosniok, S.W. Feist). 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. (W. Wosniok, T. Lang, S. W. Feist).
Relation to Action Plan:	The Working Group directly addresses the remits of the Mariculture Committee. Its Terms of References are related to various Action Plan No., e.g., 1.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.10, 2.12, 3.3, 3.11, 3.14, 4.6, 4.7, 4.9, 4.12, 4.14, 5.4, 6.1
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	There is a close link to ACME activities.

Committees:	
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 %