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

4–8 March 2008

Galway, Ireland

ICES International Council for the Exploration of the Sea

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Contents

Executive summary

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met 4–8 March 2008 at Oranmore, Galway, Ireland. The meeting, chaired by S. MacLean (USA), was well attended with 22 participants and guests representing 13 ICES Member Countries. In order to consider all 11 Terms of Reference in an efficient manner, considerable intersessional work was done by WGPDMO members and a significant number of working documents were provided in advance of the meeting.

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

Highlights of the meeting were:

- an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 in response to OSPAR request 13 (ToR a), report Section 5)
- a report on new disease trends in wild and farmed fish and shellfish in ICES Member Countries, which is the only annual expert report available on this topic (ToR b), report Section 6)
- a review of the status of proliferative kidney disease (PKD) epidemics caused by *Tetracapsuloides bryosalmonae* in wild salmonids (ToR c), report Section 7)
- a review of information on *Francisella* sp. and visceral granulomatosis in farmed cod (*Gadus morhua*) and the potential for disease interaction between wild and farmed cod (ToR d), report Section 8)
- a progress report on laboratory studies on hyperpigmentation in dab (*Limanda limanda*) from the North Sea (ToR e), report Section 9)
- a review of the increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants (ToR f), report Section 10)
- an update of international collaborative actions involving fish and shellfish disease and pathology activities (ToR g), report Section 11)
- an update on the validation and integration of molecular diagnostic and confirmatory techniques of pathogens of bivalves (ToR h), report Section 12)
- an update on use of the Fish Disease Index for Baltic cod and flounder (*Platichthys flesus*) and for other sets of available disease data (e.g. liver histopathology) (ToR i), report Section 13)
- a progress report on ICES publications on pathology and disease of marine organisms, including ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, and publications in the ICES Techniques in Marine Environmental Science Series (TIMES) (ToR j), report Section 14)
- in providing continuous advice to the ICES Data Centre and in preparation for the FDI assessments, the WG submits a request to the ICES Data Centre to contact the contributors of fish disease data and confirm the number of fish disease data submissions made (ToR k), report Section 15)

The WGPDMO concluded that all Terms of Reference for the 2008 meeting were considered in a comprehensive and satisfactory manner and identified a number of issues for further joint work and publication.

Since several important issues in the field of pathology and diseases of marine organisms were identified for further consideration, it was agreed that a further WGPDMO meeting is required in 2009. The meeting will be held in Riga, Latvia, from 3–7 March 2009.

1 Opening of the meeting

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) was hosted by the Marine Institute in Oranmore, Galway, Ireland, with S. MacLean (USA) presiding. The WGPDMO was very appreciative of the offer by N. Ruane (Ireland) and the Marine Institute to host the 2008 meeting, particularly as the original venue was cancelled on rather short notice before the meeting dates. The meeting was opened at 10:00 hrs on Tuesday, 4 March 2008, with the Chair welcoming the participants, particularly the new members (D. Cheslett (Ireland), N. House (Canada), K. Kramer (Germany) and V. Öresland (Sweden) and guests (E. Baceviscius (Lithuania) and K. Henshelwood (Ireland). In total, 22 participants attended the meeting, representing 13 ICES Member Countries. A list of participants is appended in Annex 1.

Apologies were received from T. Karaseva (Russia, new member), G. Rodjuk (Russia), A. Karasev (Russia), O. Haenen (The Netherlands), S. Helgason (Iceland), J. Palsson (Iceland), I. Dalsgaard (Denmark), S. Ford (USA), and T. Wiklund (Finland). N. Ruane provided instructions on in-house facilities and general meeting arrangements. D. Trill assisted in providing internet access.

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

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

2.1 Terms of reference

The WGPDMO noted the Terms of Reference published as C. Res. 2007/2/MCC01 (Annex 2). The agenda involved significant intersessional work by the members of the WGPDMO who had been requested to produce written working documents to be reviewed at the meeting and to be included in the WGPDMO report as Annexes, as appropriate. As agreed by the WGPDMO members, all working documents were to be prepared 4 weeks before the meeting and were posted to the ICES SharePoint by the Chair and members. As a result, most of the national reports were available to the participants prior to the meeting. This ensured that the Terms of Reference could be treated efficiently. A list of working documents provided prior to the meeting is presented in Annex 3.

2.2 Adoption of the agenda and timetable

A draft agenda (Annex 4) and a draft timetable were presented and adopted with minor changes.

2.3 Selection of rapporteurs

Rapporteurs were accepted as indicated in Annex 5.

3 ICES items of relevance to WGPDMO

The Chair and T. Lang (Germany) highlighted items of relevance to WGPDMO.

3.1 ICES Annual Science Conferences 2007 and 2008

S. MacLean reminded the WGPDMO that the WGPDMO National Reports of new disease trends are published as ICES advice in the form of an ICES Advisory Document. This emphasises the continued need for high quality and accuracy in the summarisation of the WGPDMO report.

T. Lang, having attended the ICES ASC 2007 meeting, provided the following information.

The 2007 ICES Annual Science Conference (ASC) was held 17–21 September 2007 in Helsinki, Finland, and was comprised of 18 Theme Sessions. Of interest to the WGPDMO members was the Theme Session on "Effects of Hazardous Substances on Ecosystem Health in Brackish Water Systems." The focus was on the biological effects of contaminants in the Baltic Sea and primarily involved biomarker studies. It was noted there was little attention given to diseases associated with contaminants.

The WGPDMO report and ToRs from the 2007 WGPDMO meeting were accepted by the Mariculture Committee (MCC). An additional ToR was included at the request of OSPAR in the final ToRs approved by the MCC, that being ToR a: provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010, to the extent possible by testing the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties (OSPAR request 13).

The ICES Advisory Committee on the Marine Environment (ACME) held its last meeting in 2007 as a result of ICES restructuring that merged 3 advisory committees into one Advisory Committee (ACOM). In light of the new structure and the possible greater emphasis on fisheries versus environmental issues, it may become more important for the WGPDMO to raise the significance of infectious and environmentally-related fish disease issues to fishery biologists/ecologists.

3.2 2008 Annual Science Conference and Theme Session D

The 2008 ICES ASC will be held 22–26 September in Halifax, Canada. Theme Session D, entitled "New disease trends in marine organisms: causes and effects" will be co-convened by S. MacLean, T. Lang (Germany), and S. McGladdery (Canada). The due date for on-line submission of abstracts and titles is 21 April 2008, with a manuscript submission due date of 11 August 2008. This Theme Session will provide a forum for discussion of recent findings among scientists of many ICES Member Countries and for proposal of new research, monitoring and assessment priorities. WGPDMO members were encouraged to participate in this Theme Session and to spread the word to their colleagues.

3.3 ICES Updated Strategic Plan

S. MacLean informed the WGPDMO that the ICES Strategic Plan was completed in 2007. Associated with this document is preparation of a 5-yr plan for ICES activities, processes, and the Science Programme. A draft Science Plan that proposes new research priorities was presented to the Delegates in February 2008, and now comments on the draft are being sought from the ICES community. The research priority on 'Understanding human impacts on Ecosystems' has relevance to the WGPDMO as

it includes issues concerning invasive species (which may serve to introduce new pathogens) and mariculture impacts on disease and biodiversity. WGPDMO members were encouraged to review the proposed Science Plan and provide comments to Vivian@ices.dk by 11 April 2008.

3.4 WGDEEP

The Working Group on the Biology and Assessment of Deep Sea Fisheries Resources (WGDEEP) had received a recommendation to consider the collection of information on contaminants, fish diseases, and parasites during deepwater surveys. WGDEEP planned to convene an ICES planning group in 2008 to develop the details of these surveys. The WGPDMO Chair designated T. Lang as the WG's representative for this planning group. At the time of the 2008 WGPDMO meeting, no information had been received concerning this planning group meeting.

4 Other relevant activities for information

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

T.A. Mo (Norway) participated in the North Atlantic Salmon Conservation Organisation (NASCO) *Gyrodactylus salaris* meeting held 10–12 Oct 2007 in Oslo, Norway. In addition to presenting information on *Gyrodactylus* in Norway, T.A. Mo discussed other currently significant diseases of Atlantic salmon, in particular Proliferative Kidney Disease (PKD) and red vent syndrome (due to *A. simplex*), both addressed in this WGPDMO report. NASCO generally was unaware of these diseases and the possible impacts on salmon populations.

4.1 Conferences/Workshops (chronological order)

- 7th International Sea Lice Conference. 31 March–1 April 2008, Puerto Varas, Chile
- SCOFDA Workshop on Molecular Techniques in Fish Health Research. 29–30 April 2008, Copenhagen, Denmark
- World Aquaculture Society. 19–23 May 2008, Busan, Korea
- International Symposium on the Effects of Climate Change of the World's Oceans. 19–23 May 2008, Gijon, Spain
- (Shell)Fish Immunology Workshop. 22–27 June 2008, Wageningen, The Netherlands
- American Fisheries Society/Fish Health Section. 9–13 July 2008, Charlottetown, PEI, Canada
- International Congress on the Biology of Fish. 9–13 July 2008, Portland, OR, USA (Symposium on Immunity and Disease Resistance in Fish)
- ICES Annual Science Conference. 22–26 September 2008, Halifax, NS, Canada

NB: With internet access available essentially worldwide, this section will not appear in future reports.

4.2 Relevant Projects

- **Workshop on the Integrated Assessment of Contaminants (ICON)** T. Lang provided background information on the origins of ICON. The purposes of the ICON Workshop are: 1) to test the strategy for chemical and

biological effects monitoring proposed by OSPAR, and 2) to provide a status report on the impacts of North Sea contamination. Dab (*Limanda limanda*) has been the primary species of study in the North Sea on the effects of environmental contaminants, and long rough dab (*Hippoglossoides platessoides*) and haddock (*Melanogrammus aeglefinus*) are being considered as well.

- **Reform of the ICES Advisory Structure** The Chair reported to the WGPDMO that the ICES Council recommendation for reforming the advisory process in ICES was accepted. One aspect of the new advisory process is that advice from the Expert Groups will be subject to review by external reviewers. As such, the request by OSPAR for an assessment of data on fish diseases in the OPSPAR maritime area will be reviewed at a special meeting of the Review Group, 19–20 May 2008, in Copenhagen, Denmark.

5 Provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible by testing the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR (OSPAR Request 13/2008)

T. Lang and W. Wosniok presented a report (Annex 6) describing progress on the development of a Fish Disease Index (FDI). Data on diseases of the North Sea dab (*Limanda limanda*) were used to illustrate the index.

5.1 Purpose

The purpose of the FDI is to provide a summary of the prevalence, severity of and trends in diseases in a particular population, which can be used as an assessment tool. The presented version of the FDI, as used for dab, takes into consideration externally visible lesions and parasites, macroscopic liver neoplasms, and histologically detectable liver lesions.

5.2 Implementation

To implement the FDI, each observable disease condition or parasitic infection is assigned a numerical weighting factor depending on its impact on the animal, as determined by advice from a panel of experts. To overcome the problem of ranking many items (diseases) according to severity, the Bradley-Terry approach is used to derive a severity weighting scheme from pairwise comparisons originally given by the experts. Then the grade of severity of each disease is determined according to existing ICES/BEQUALM guidelines and coded by 1–3. If no grading exists, the presence of a disease is coded by 1/0. The FDI is then calculated by multiplying the weighting factor by the level of severity and summing these products over all diseases. Due to sampling variations between populations (different sex and length distributions) and different sampling seasons, adjustments for those variations are made before applying the FDI to any assessment.

Using the dab in the North Sea as a model, the externally visible lesions of the following diseases were used to illustrate the FDI: lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulceration, X-cell gill disease, hyperpigmentation, acute/healing fin rot/erosion, and the parasites *Stephanostomum baccatum, Acanthochondria cornuta* and *Lepeophtheirus pectoralis* (macroscopic liver neoplasm and histopathological liver lesions were not considered). FDI assessment ranges were

developed after adjusting the data for sex, length and season of collection, by dividing the range of FDI values into three equal groups representing the lower, middle, and upper portions of the range. All available data in an area was used for this purpose, while the assessment of level and trend refers only to observations since January 2001.

The level component of the assessment procedure used the counts of FDI values lying in the lower, middle und upper ranges, respectively. These were weighted by −1, 0, +1, and summed up to give the level component. For the trend component, the Mann-Kendall trend test statistic was calculated. The sum of counts and the trend statistic were added to give the FDI assessment statistic. Small values indicate low level and decreasing trend, high values indicate high level and increasing trend, values around zero indicate intermediate level and no change, i.e. purely random fluctuation. The range of random fluctuation was determined by Monte Carlo simulation. According to the resulting p values, different "smiley faces" were assigned to individual ICES regions. A $p < 0.025$ resulted in a "green smiley face"; $0.025 < p < 0.975$ in a "yellow indifferent face"; and $p > 0.975$, a "red frowny face". These faces, placed on a chart of the ICES statistical rectangles in the North Sea, provide a visual general assessment of levels and trends in overall disease status.

Performing an assessment of fish diseases in the OSPAR maritime area using data from the ICES Data Centre

In order to meet the OSPAR request, ICES member countries had been requested in 2007 to submit all existent data on fish diseases (externally visible diseases, macroscopic liver neoplasms and liver histopathology) in the actual Fish Disease Reporting Format 3.2 to the ICES Databank. This included a request for re-submission of data that had been reported earlier in other formats than 3.2. All data files that had been submitted to ICES and passed the ICES data screening procedure prior to 15 February 2008 were made available to the WGPDMO.

These data consist of 95 files with a total size 388 MB. The main fish species reported are dab (*Limanda limanda*), flounder (*Platichthys flesus*) and cod (*Gadus morhua*). The primary regions covered are the North Sea including the Channel, parts of the Irish Sea and the western Baltic Sea. Observation dates range from 1981 to 2007. Diseases reported are the principle diseases for the main species plus other diseases, the latter to a highly variable extent.

A first inspection of the data revealed that the regional as well as the temporal coverage is patchy and fragmented. Also, there are few areas for which time series of all diseases used by the original FDI definition are available. When the total number of fish available in this data set was compared to the numbers involved in earlier fish disease analyses (based on earlier data submissions to the ICES Data Centre), it turned out that the number of fish contained in the current data set is considerably smaller than the number available from the ICES Data Centre for earlier analyses. The reason for this discrepancy is as yet unknown and still needs clarification. Consequently, results from the present analysis must be considered as preliminary. A final assessment must be preceded by the completion of the data set, for which actions are proposed in Report Section 15 (ToR k).

In order to make use of the available data, ways to calculate a possibly modified version of the FDI were explored. To this end, the set of diseases involved in the FDI calculation was modified in various (and biologically acceptable) ways, with the aim to enable the calculation of an FDI for as many ICES statistical rectangles as possible. Three modifications of the original FDI with different characteristics were defined.

Their values were calculated and the FDI trend assessment was performed in the way outlined above. Computational aspects and details of the present analysis are given in Annex 6.

Main results of the analysis were:

- the FDI approach and assessment criteria could be applied to the data set available as requested by the Term of Reference
- it is advisable to include as many diseases as possible in the FDI
- the present (presumably incomplete) data set allows assessments only for certain ICES statistical rectangles

Once the data set is updated, preferably including the submission of data that is known to exist but has not yet been submitted to ICES, an updated FDI calculation and assessment can easily be accomplished by executing the established procedure with the updated data.

5.3 Discussion

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

- It is important to have a set of relevant diseases in characterising overall fish health to avoid having one disease dominate the index.
- FDI can be calculated for data collected from previously unsampled sites. However, a trend assessment is possible only if data from adjacent sites are used.

5.4 Conclusions

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

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

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

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

5) The OSPAR Request for a data assessment count not completely be finalised because a significant amount of data were missing in the ICES fish disease databank. Therefore, FDI values and assessment for the actual data should be re-calculated after completion of the data.

5.5 Recommendations

The WGPDMO recommends that:

i) the FDI be submitted to ICES for review by independent experts,

ii) following the review, ICES submits the FDI to OSPAR for consideration and publication,

iii) the WGPDMO complete the task given by OSPAR with the updated and completed disease data set and revisit the issue at the 2009 WGPDMO meeting,

iv) the technical aspects of the FDI calculations be published in the ICES TIMES series.

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

The update in the following sections is based on national reports for 2007 submitted by Canada, Denmark, England and Wales, Finland, France, Germany, Iceland, Ireland, Latvia, Norway, Poland, Russia, Scotland, Spain, Sweden, The Netherlands, and USA. It documents significant observations and highlights the major trends in newly emerging diseases and in those identified as being important in previous years.

6.1 Common and scientific names of host fish and shellfish species reported in Section 6

abalone	*Haliotis kamtschatkana*	oyster, Kumamoto	*Crassostrea sikamea*
barramundi	*Lates calcarifer*	oyster, Pacific	*Crassostrea gigas*
clam, geoduck	*Panope abrupta*	perch, European	*Perca fluviatilis*
clam, hard	*Mercenaria mercenaria*	periwinkle	*Littorina littorea*
clam, soft	*Mya arenaria*	plaice, American	*Hippoglossoides platessoides*
cockle	*Cardium edule*	plaice, European	*Pleuronectes platessa*
cod	*Gadus morhua*	salmon, Atlantic	*Salmo salar*
crab, European edible	*Cancer pagurus*	salmon, chum	*Oncorhynchus keta*
crab, snow	*Chionoecetes opilio*	salmon, pink	*Oncorhynchus gorbuscha*
crayfish	*Procambrus clarkia*	salmon, sockeye	*Oncorhynchus nerka*
dab, common	*Limanda limanda*	snail, red turban	*Lithopoma gibberosum*
herring, Atlantic	*Clupea harengus harengus*	snail, sentinel	*Assiminea grayana*
herring, Baltic	*Clupea harengus membras*	tilapia	*Oreochromis* spp.
lobster, European	*Homarus gammarus*	sole	*Solea solea*
mussels, blue	*Mytilus edulis*	trout, brown	*Salmo trutta*
oyster, Asian	*Crassostrea ariakensis*	trout, rainbow	*Oncorhynchus mykiss*
oyster, crested	*Ostrea equestris*	trout, sea	*Salmo trutta*
oyster, eastern	*Crassostrea virginica*	turbot	*Scophthalmus maximus*
oyster, European flat	*Ostrea edulis*	whitefish	*Coregonus* sp.

6.2 Wild Fish

6.2.1 Viruses

Infectious Pancreatic Necrosis Virus (IPNV) – The virus was identified from ovaian fluid from sea run Atlantic salmon during routine health testing of incoming brook-stock to a national fish hatchery in Connecticut, USA. Two of nine 5-fish pools were confirmed positive for the virus using cell culture and PCR assays. No clinical signs

of disease were noted. Genetic sequencing of the isolates indicated they were closely related to the Canada 3 strain of IPNV. The source of the virus is unknown, but the fish are presumed to have been infected prior to capture and holding at the facility.

IPNV was isolated from wild Atlantic salmon fingerlings/parr in Poulmounty River, south east Ireland, but no clinical signs of disease were noted. It is believed that the virus originated in a nearby salmon hatchery which had clinical IPN on site in 2006.

Infectious Haematopoietic Necrosis Virus (IHNV) – The virus was detected in 79 of 379 (21%) spawning sockeye salmon at channels located on the Fraser and Skeena Rivers in British Columbia, Canada.

Viral Haemorrhagic Septicaemia Virus (VHSV) – The genotype IVa continues to be detected in marine fish off Vancouver Island, BC, Canada in 2007. Genotype IVb has been detected in two new locations within the Great Lakes region. The first isolations of the IVb genotype in inland waters in the US (no connections to the Great Lakes Basin) originated from lakes in New York State, Wisconsin and Michigan, with some associated mortality.

A marine VHSV genotype (III) and fresh water genotype (I) was found in Norway in apparently healthy Atlantic herring using molecular testing.

Lymphocystis – The low prevalence recorded in dab in recent years in the German Bight has continued with a lowest value of 0.4% recorded in August– September 2007, partly due to the small size of fish examined as compared to earlier years. Data from the German Bight obtained during 2003 through 2007 suggest the prevalence of lymphocystis is higher in winter compared to summer.

A high prevalence of lymphocystis was observed in American plaice in Massachusetts Bay, USA, particularly in the vicinity of Cape Ann in May 2007. In spring 2007, 8 stations had a prevalence >10% (6 of the 8 stations had a prevalence >25% and 4 of the 8 stations had a prevalence >50%) and contrasts sharply with prevalence recorded during 16 spring and autumn surveys conducted in 1999 through 2006. The prevalence of lymphocystis surpassed 10% at only 5 stations sampled between 1999 and 2006. It also appears that all but one positive lymphocystis observations between 1999 and 2006 were south of Thacher Island, while in spring 2007 three of the stations with the highest prevalences were north of Thacher Island.

6.2.2 Bacteria

Renibacterium salmoninarum – Each year salmonid juveniles and smolts of wild Atlantic salmon brood stock origin are used for restocking rivers in Iceland and are screened for *R. salmoninarum* by ELISA. The prevalence of infection has been 0.5–3% over the last 15 years. During the last two years (2006 and 2007) the prevalence has increased to 17%. Only eggs from non-infected females are used for hatching.

Acute/healing skin ulcerations– Compared to 2005 and 2006, the summer prevalence of acute/healing skin ulcerations in common dab in the North Sea has declined.

The mean prevalence of acute/healing skin ulcerations in Baltic cod from the southwestern Baltic Sea (Kiel Bight, Mecklenburg Bight, Arkona Sea) was 4.9% (n=1521) in August-September 2007 and 5.2% (n=1590) in December. In December 2007, the mean prevalence in cod from more eastern areas was 6.1% (n=639) and therefore low compared to the end of the 1990s and beginning of 2000. Polish and Russian surveys revealed lower prevalence of skin ulceration in cod from the southern Baltic (ICES Subdivisions 25 and 26) in comparison to 2005 and 2006 data.

6.2.3 Parasites

Crustacea

***Lepeophtheirus salmonis* and *Caligus clemensi*–** The prevalence of *L. salmonis* was 13% on juvenile pink (n=3,476) and 16% on juvenile chum salmon (n=4,643) in British Columbia, Canada in 2007. The abundance was 0.19 lice/fish and 0.25, respectively. The prevalence of *C. clemensi* on pink and chum salmon was 5% and 7%, respectively, and the abundance was 0.07 and 0.13, respectively. The prevalence of *L. salmonis* represents a declining trend.

***Lepeophtheirus pectoralis* –**The prevalence of *L. pectoralis* on common dab has continued to decrease since 2004 in the German Bight, North Sea.

Monogenean

***Gyrodactylus salaris* –** *G. salaris* reappeared on Atlantic salmon in the river Lærdalselva, Norway, after treatment with a combination of aluminium sulphate and rotenone in 2005 and 2006.

Myxozoa

***Tetracapsuloides bryosalmonae* –** Mortality attributed to proliferative kidney disease (*T. bryosalmonae* etiologic agent) caused high mortality and negative population effects on wild Atlantic salmon in two Norwegian rivers in 2007.

Alveolata

***Ichthyodinium* –** In June 2007 a batch of Baltic cod yolk sac larvae originating from Danish wild cod were found to be infected with a dinoflagellate-like parasite previously found in Baltic yolk-sac larvae in 1992. Molecular techniques indicated these dinoflagellates belong to the genus *Ichthyodinium.*

Mesomycetozoea

***Ichthyophonus* –** From 1995 the prevalence of *Ichthyophonus* infection in European plaice has been monitored in Faxafloi, west coast of Iceland. This has been done macroscopically since 1995 (500–600 fish inspected annually) and microscopically since 1996 (200 fish inspected annually) along a longitudinal transect through the commercial fishing area. The weighted total prevalence of macroscopically visible infection in the area increased sharply from 4.6% in 1995 to a peak of 47.9% in 1998. Microscope examination revealed 80.7% prevalence in 1998. In the following two years the level of macroscopically and microscopically visible infection dropped rapidly down to 11.7% and 20.2% respectively in the year 2000. During the years 2000 to 2004 the prevalence of *Ichthyophonus* infection was relatively stable between 10.9–17.9% macroscopically and 13.7–28.1% microscopically. Since the year 2004 the prevalence of infection has been steadily decreasing and reached 1.5% macroscopically and 1.3% microscopically in 2007. Higher estimates of macroscopically than microscopically visible infections in some of the later years can be explained by the additional 300 fish inspected in macroscopic estimates at different stations across the transect.

Nematoda

***Anisakis simplex* –** The prevalence of Baltic herring infection with *A. simplex* larvae successively declined in the Polish and Russian EEZs of the Baltic Sea. The negative correlation between the prevalence and mean mass of individual herring was still observed.

Red vent syndrome – Notification of rivers or catchment areas across the UK with returning wild Atlantic salmon showing a swollen, protruding, haemorrhagic vent lesion started in July 2007. No bacteria or viral agent was found but the lesion was associated with a high number of nematode larvae localised in the discrete area of the vent. Affected salmon were generally grilse weighing between 2–4kg and recently returned from the sea. Fish were in good external condition with moderate to high fat storage in the body cavity. The severe stage of the condition comprised a pronounced swelling with haemorrhaging and tissues protruding from the vent and a breakdown of the integument, scale loss and spontaneous bleeding under gentle pressure. The nematodes within the vent tissue were identified on the basis of morphology and molecular analyses as *Anisakis simplex* s. s. Although this condition was noted occasionally in past years, this year showed widespread occurrence and high numbers of affected fish.

6.2.4 Other Diseases

Epidermal hyperplasia/papilloma – The prevalence of epidermal hyperplasia/papilloma in dab from the German Bight was low compared to previous years. In other North Sea areas, the prevalence remained unchanged.

Hyperpigmentation – The prevalence of hyperpigmentation in North Sea common dab continued to be high in most North Sea areas, especially at the Dogger Bank and at the Firth of Forth area. In the German Bight, the August - September 2007 prevalence of 3.5% was exceptionally low (in the same season of 2006 it was 17.6%), probably due to the small mean size of dab examined. In December 2007, the prevalence increased to 31.5% which was low compared to December 2006 (53.2%). In dab from the western Baltic Sea, the condition was again absent in 2007. Studies from Scotland show no viral agent or consistent bacterial type was present from normal and pigmented fish.

Liver nodules – The prevalence of liver nodules > 2 mm in common dab had increased in August - September 2006 and again in December 2006, especially in the German Bight, however in 2007 the prevalence has since declined.

6.2.5 Conclusions

1) Viral Haemorrhagic Septicaemia Virus has been detected in two new locations within the Great Lakes region, Canada, and in inland waters (no connection to the Great Lakes Basin) from lakes in New York State, Wisconsin and Michigan, USA.

2) A fresh water VHSV genotype (I) was reported in herring in Norway using molecular testing.

3) Lymphocystis has shown a significant increase in prevalence and distribution in American plaice in Massachusetts, USA, during 2007 as compared to data collected during the previous eight years.

4) During 2006 and 2007 the prevalence of *Renibacterium salmoninarum* has increased to 17% in wild Atlantic salmon broodstock used for restocking rivers in Iceland.

5) *Gyrodactylus salaris* has reappeared in the river Lærdalselva, Norway, after treatment with a combination of aluminium sulphate and rotenone in 2005 and 2006.

6) Mortality attributed to proliferative kidney disease (*Tetracapsuloides bryosalmonae* agent) caused high losses of Atlantic salmon in two Norwegian rivers in 2007.

A 'red vent syndrome' in returning Atlantic salmon across the UK was recorded in high numbers from July 2007. Swollen, haemorrhagic vents were noted and attributed to the presence of nematodes which were identified as *Anisakis simplex* s.s.

No viral or consistent bacterial agent was isolated from normal and hyperpigmented common dab from the northwestern North Sea.

6.2.6 Recommendations

The WGPDMO recommends that:

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

ii) the WGPDMO reviews the status and impact of red vent syndrome in wild Atlantic salmon at its 2009 meeting.

iii) the WGPDMO reviews the information on the aetiology of hyperpigmentation in common dab.

iv) in light of the red vent syndrome in UK salmon, the WGPDMO reviews the information on *A. simplex* in marine mammals, fish and other intermediate hosts (zooplankton and other invertebrates).

v) the WGPDMO reviews the information on diagnostic methods for *Francisella* and the impacts of francisellosis on populations of wild cod and other fish species.

6.3 Farmed Fish

6.3.1 Viruses

Infectious Salmon Anemia Virus – No new cases of the pathogenic strain were detected in New Brunswick, Canada or Maine, USA. This is the first time that the pathogenic strain of the virus has not been detected since the original identification. Management plans implemented in both countries likely explain the absence of new detections.

Viral Hemorrhagic Septicaemia Virus –VHSV was detected in rainbow trout at three marine locations in Norway for the first time since 1974. Mortality was limited. Rainbow trout from two brackish water farms in Finland tested positive, which represents a drop from 10 farms in 2006 and 9 in 2005. Genotype IVa was isolated from Atlantic salmon in western Canada and was associated with 1.2% mortality. VHSV was isolated from wild sole in Spain captured for broodstock.

Infectious Pancreatic Necrosis Virus – The number of new outbreaks of IPN in Norway dropped to 165 from 208 in 2006. The virus continued to be detected in rainbow trout in Finland and in Atlantic salmon in Ireland and Latvia. Clinical disease is rare in Ireland. Serotype Sp virus was isolated from captive sea trout

brood-stock in Sweden and in progeny from this stock following transfer to a new site.

Heart and skeletal muscle inflammation – HSMI continued to increase in Norway, to a high of 162 farms. The disease was found for the first time in a freshwater pre-smolt Atlantic salmon farm in Norway.

Pancreas Disease – A continued increasing trend was observed in Norway (to 98 farms from 58). In Ireland PD was found on >90% of marine salmon sites in 2007 with variable mortality.

Viral nervous necrosis virus –VNNV was observed in six cod farms in Norway which is twice the number as in 2006.

European perch rhabdovirus – The virus was isolated for the first time at one farm in Sweden associated with increased mortality during the spring. Diseased perch, raised in coastal waters, were pale and showed transparent yellowish ascites in the abdomen. The same virus, previously isolated from brown trout in Sweden, was referred to as laketrout rhabdovirus.

6.3.2 Bacteria

Aeromonas salmonicida – A decreased number of outbreaks was observed in rainbow trout in Finland.

Francisella philomiragia – The occurrence of francisellosis in farmed cod in Norway continues to increase. In Denmark francisellosis was found in live cod imported from Norway.

Listonella (Vibrio) anguillarum – Several isolates of *L. anguillarum* O2β from cod in Norway have shown reduced antibiotic sensitivity (oxolinic acid). Sporadic outbreaks were found in Atlantic cod in Iceland, while the number of outbreaks in rainbow trout increased in Finland.

***Moritella viscosa* –** Skin ulceration involving *Moritella viscosa* was observed to be a larger problem in farmed rainbow trout than in farmed Atlantic salmon in some areas of Norway.

***Vibrio fisheri* –** The bacterium was isolated from turbot in The Netherlands associated with clinical signs and mortalities.

Vibrio scophthalmi/ichthyoenteri – A 12.5% mortality in turbot with systemic infection occurred in The Netherlands.

Vibrio vulnificus – An outbreak in one barramundi farm using recirculated seawater in The Netherlands resulted in >50% mortality.

Yersinia ruckeri – Biotype 2 has been isolated from farmed rainbow trout in Finland since 2005 with an increasing trend, and in 2007 it was isolated for the first time from farmed whitefish. All isolates were from farmed fish in the Baltic. This biotype has previously been isolated in Denmark, UK, and Spain, but from fresh water species. It is thought this biotype was introduced to Finland in 2004 or 2005.

6.3.3 Parasites

Protista

Amoebic gill disease (AGD) – Over 40% loss of Atlantic salmon (first year at sea) at a farm in Scotland was attributed to a gill infection. The mortalities occurred between mid-September, when the fish were transferred to this site, and November. Mortality peaked at 4000 fish/day. All fish sampled were moribund and lethargic with pale gills. Internally fish exhibited focal haemorrhaging in the liver and pyloric caeca. Severe lamellar hyperplasia and fusion were recorded in all fish. Amoeba-like protists were identified in all fish. Some fish also had epitheliocystis. The liver exhibited mild to moderate focal necrosis throughout, with cell degeneration and pyknosis, which also suggests the fish had a bacterial infection. The presence of amoeboid protists and a bacterial infection contributed to the losses. This is believed to be an emerging condition for winter growers. The specific identity of the amoeba-like protist is not known.

Ciliophora

Philasterides dicentrarchi – Infection by this ciliate continued to increase in turbot in Spain. Mortality was observed in fish up to 500 grams.

Crustacea

Lepeophtheirus salmonis – Reduced effectiveness of emamectin benzoate (SLICE) treatments against salmon lice was observed in some areas in Norway. However, it is not clear whether this reduced effect was due to reduced sensitivity of salmon lice to the drug or due to management practices. An Integrated Pest Management Program in Maine, USA, has contributed in part to significantly reducing sea lice. Fallowing and early treatment aid in keeping lice counts low and drug use minimal.

6.3.4 Other Diseases (including problems caused by toxic algae)

Red Mark Syndrome – The 'red mark syndrome' represents a chronic dermatitis primarily involving the basement membrane and scale pockets, suggesting this is a hypersensitivity reaction and could be the result of a sub-lethal, chronic infection involving *Flavobacterium psychrophilum*. This is now observed in rainbow trout reared in salt water for part of their life cycle in Scotland.

Jellyfish – A swarm of *Pelagia noctiluca* killed 100,000 Atlantic salmon at one farm in Northern Ireland in November.

6.3.5 Conclusions

1) The absence of new detections of Infectious Salmon Anaemia Virus in eastern Canada and the USA likely is due to more stringent surveillance and management practises.

2) Viral Haemorrhagic Septicaemia Virus was detected in rainbow trout in Norway for the first time since 1974.

3) A declining trend of Infectious Pancreatic Necrosis Virus was observed in Norway.

4) Heart and Skeletal Muscle Inflammation and Pancreas Disease continue to show an increasing trend in Norway. HSMI was seen for the first time in freshwater fish. PD remains endemic in Irish salmon farms.

5) Viral Nervous Necrosis Virus shows an apparent increasing trend in cod in Norway.

6) *Yersinia ruckeri* biotype 2 was isolated from rainbow trout and for the first time from whitefish in Finland.

7) Amoebic gill disease was associated with high mortality in Atlantic salmon in Scotland.

8) A swarm of the jellyfish, *Pelagia noctiluca*, caused for the first time 100% mortality at an Atlantic salmon farm in Northern Ireland.

6.3.6 Recommendations

The WGPDMO recommends that:

i) ICES member countries conduct further research to refine diagnostic tools and to develop treatments or vaccines for francisellosis in farmed cod.

6.4 Wild and farmed molluscs and crustaceans

6.4.1 Viruses

Herpesviruses in bivalves – A batch of farmed Pacific oysters at the nursery stage, exhibited post-grading mortalities of 60–70% in Kent, UK. Virus-like particles were observed and they presented some features characteristic of iridoviruses. Molecular biology results using OsHV1-specific primers were negative.

A herpes-like virus was detected using TEM in association with gill hyperplasia in soft clams from Long Island Sound, USA.

White Spot Syndrome Virus in crayfish – An outbreak of WSSV in the crayfish occurred in St. Martin Parish, Louisiana, USA, beginning in February 2007. This is the first OIE reported occurrence in the US since an outbreak in Hawaii in 2004. All farms were ordered to be fallowed of crayfish stock. Surveillance of wild crayfish populations in the region, the source of the farmed crayfish brood stock, is ongoing. WSSV has previously been found in wild shrimp and crabs offshore in the Gulf of Mexico and near shore in Texas, Mississippi, Georgia and South Carolina.

Virus infecting edible crabs – A virus in juvenile European edible crab was reported following a survey of legal and sub-legal sized crabs from the English Channel fishery, UK. *Cancer pagurus* bacilliform virus (CpBV) was only found in juvenile crabs and is the first recorded virus in wild specimens of the *Cancer* genus. The discovery highlights the potential for juvenile crustaceans to harbour different diseases from their adult conspecifics, even at the same geographic locations. Effects on juvenile mortality have not been investigated.

6.4.2 Bacteria

***Vibrio* spp.** – During 2006 and particularly 2007, a re-emergence of vibriosis caused by *Vibrio tubiashii* was documented in shellfish hatcheries (including Pacific and Kumamoto oysters and geoduck clams) on the west coast of North America from Mexico to Alaska, and in hard clam hatcheries in Florida, USA. The occurrence of vibriosis was associated with intermittent upwelling and intrusion of unusually elevated sea surface temperature on the west coast.

In France some isolates of *V. splendidus* and *V. aestuarianus* were again reported in association with mortality outbreaks in Pacific oysters.

Juvenile oyster disease of eastern oysters – The disease has been renamed Roseovarius Oyster Disease (ROD) to avoid potential confusion between the disease caused by *Roseovarius crassostreae* and other diseases of juvenile oysters (Maloy *et al*., 2007. Aquaculture 269: 71–83). Two ROD outbreaks were reported in the USA: one at the beginning of September in suspended cases on the Connecticut shore of Long Island Sound, which was diagnosed on the basis of shell deposits, and a second, at the end of July, in upwellers in Point Judith Pond, Rhode Island. Mortalities in the latter ranged between 40 – 86% and the presence of *R. crassostreae* was confirmed by PCR and immunoprecipitation.

Gaffkaemia (*Aerococcus viridans*) – Following confirmation of Gaffkaemia in European lobsters held at a storage facility in South Wales, UK, in 2006, further samples were taken of wild caught lobsters in 2007. Gaffkaemia was not detected in a sample of 30 lobsters taken directly from catch pots in late March but two lobsters in a sample of 30 animals from holding pots in June tested positive for the disease organism.

6.4.3 Parasites

Protista

***Bonamia exitiosa* in European flat oysters** – *B. exitiosa* has been described to occur in 57% of flat oysters sampled in Galicia, Spain. This is the first report of this parasite in a European country.

***Bonamia ostreae* in flat oysters** – In Scotland, *Bonamia ostreae* was confirmed from flat oysters from the waters of West Loch Tarbert following histological examination, and confirmed by PCR product sequence that is identical to that expected for *B. ostreae*.

The prevalence of infection of *B. ostreae* in native oysters at farm sites and in fisheries in England, UK, decreased compared with 2006. The average for all fishery sites was 5.5%, compared with 6.8% the previous year and a ten-year average of 4.7%. High average infection rates were found in 2007 in the samples from the fished area in southwest Wales where the disease was first detected in 2006.

***Bonamia* sp. in the Asian oyster –** *Bonamia* sp. was detected at two new sites in the USA: one, at Ocracoke, North Carolina, USA, was generated from re-analysis of a previously collected (2004) sample of Asian oysters and is near the location where the parasite was first observed in 2003. The second site is Fort Pierce, Florida. The environmental source of these infections in experimentally maintained Asian oysters is in both cases unknown, though the crested oyster, susceptible to *Bonamia* sp. parasitism and occurring naturally in both locations, is the only plausible wild host. Sites in which *Bonamia* sp. has been observed now range over 1000 km of the southeastern coast of the USA. The parasite was not found in samples of Asian oysters deployed in upper Chesapeake Bay and assayed histologically (n=96) or analyzed by PCR (n=24).

There is uncertainty that the identity of the *Bonamia* sp. found in the southeastern USA is Office International des Epizooties-notifiable pathogen *Bonamia exitiosa* or *Bonamia roughleyi*, the Australian (*not* OIE-notifiable) parasite of *Saccostrea glomerata*, or if it represents a new species.

Haplosporidium nelsoni (MSX) – During routine disease surveillance the parasite was detected in 6% (2/35) of Pacific oysters tested from one location in British Columbia, Canada. This is the first time *H. nelsoni* has been detected on the West Coast of Canada. However, this occurrence was not associated with disease or mortality and further surveillance is ongoing in attempts to better define the prevalence and distribution of *H. nelsoni*. In 2007 in eastern Canada, a third site located within the

buffer zone around Cape Breton Island was confirmed positive for MSX. Although this is a new site, it does not affect populations under official protective measures elsewhere in Atlantic Canada.

In the upper Chesapeake Bay, USA, an expanded geographic range for detectable infections in eastern oysters was coincident with elevated 2007 salinities from severe and prolonged regional drought. The number of positive sites in the typically low-salinity, upper Chesapeake increased from 9 to 30%.

Kidney coccidian – The prevalence in hatchery reared abalone from B.C., Canada, declined from 91/100 (91%) in January 2006 to 10/74 (14%) in January 2007. No new information is available concerning the genetic relationship between the coccidia in the abalone and red turban snails.

***Hematodinium* sp. in snow crabs** – There is concern that an outbreak of bitter crab disease (BCD) in snow crabs in Canada was climate-related and that further trends in climatic warming will enhance transmission and spreading of the parasite into additional fishing areas.

Marteilia refringens – In 2006 and 2007, a survey focused on the detection of *Marteilia refringens* in blue mussels has been carried out along French coasts. In 2007, the parasite was detected by classical histology in 22 mussels of the 390 analysed individuals originating from 6 different areas. Positive samples were reported in Normandy (3/30), Brittany (2/90) and Mediterranean coast (10/125). The molecular characterization of the parasite in positive samples is in progress. All the parasites already characterised by PCR-RFLP are *M. refringens*, M type.

***Perkinsus marinus* in the eastern oyster** – Drought conditions in the eastern US that became progressively severe in a southerly direction, caused increased prevalence and intensity of infections. In Delaware Bay, infection and mortality levels continued an increasing trend begun in 2005 with a Bay-wide mean autumn prevalence of 88% compared to 78% in 2006 and a low of 43% in 2004. The long-term pattern shows a cycle of approximately 7 years, which may be tied to large-scale climatic patterns such as the North Atlantic Oscillation. In the upper Chesapeake Bay, autumn mean prevalence was 68% compared to 61% in 2006 and a low of 52% in 2004. In the lower Chesapeake Bay, autumn mean prevalence of 74% was the same as in 2006, but also considerably higher than the low of 54% in 2004. Markedly increased *P. marinus* infection levels in the eastern Gulf of Mexico and the upper Chesapeake Bay were associated with higher drought-related salinities.

Crustacea

Parasite-associated mortality – Heavy infestations of *Nicothoë astaci* (Copepoda) on the gills of wild European lobster were associated with mortalities in lobster storage facilities in Norway.

6.4.4 Other diseases

Summer Mortality – As in the summer of 2005 and 2006, mortalities of Pacific oysters associated with organ pathology were noted at stations along the German coast of Lower Saxony. However, mortality was much lower, possibly linked to low temperatures in summer 2007. CPE was observed in cell cultures (CHSE-214) but no agent was identifiable.

In Ireland losses were reported in cultured Pacific oysters in five bays. No pathogenic agent was isolated. The losses occurred during August in four of the bays and are consistent with summer mortality syndrome.

Mass mortalities in cockles – Since July 2003 mass mortalities of 1 year old cockles and older have been observed in the managed cockle fishery in the Burry Inlet in South Wales, UK. The same sequence of events is repeated annually: high spatfall in May and June culminates in densities approaching 9,000 individuals per square metre. From July onwards, mass mortalities of the previous year class occur, resulting in the obliteration of almost all 1-year-old cockles. High bacterial loads and a number of parasite species have been found, but the results so far do not give a clear indication of the possible cause of mortalities.

Intersex condition in periwinkle – Intersex conditions in the female periwinkle were investigated at 9 stations along the coast of Lower Saxony, northern Germany, and increased intersex conditions were recorded at 2 stations. In addition to these effects in female snails, some pathological changes were recorded in reproductive organs of male snails, indicating estrogenic endocrine contaminant effects.

Pathological changes in sentinel snail – Pathological changes (resorption of mature eggs and vacuolation of cells of protein gland) in gonad were recorded in the sentinel snail (a species inhabiting the supra-littoral) from sampling sites in the vicinity of a leisure boat marina in the Weser estuary, Germany. Snails sampled the same day from a reference site did not show these changes. Contaminants in the Weser estuary may play a role in the occurrence of these changes. Intersex or imposex conditions, observed in other species from the Weser estuary, were not detected.

6.4.5 Conclusions

1) A virus was recorded in juvenile European edible crab in the English Channel and this is the first recorded virus in wild specimens of the *Cancer* genus.

2) A parasite interpreted as *Bonamia exitiosa*, based on molecular analyses, was detected for the first time in Europe at a high prevalence in European flat oysters in Galicia, Spain.

3) *Bonamia* sp. was detected in the Asian oyster at two new sites in the USA.

4) *Haplosporidium nelsoni* was reported for the first time on the West Coast of Canada in Pacific oysters from one location in British Columbia.

6.4.6 Recommendations

The WGPDMO recommends that:

ii) studies are expanded in ICES Member Countries on gill epithelial cell nuclear virus in soft clams;

iii) investigations are initiated or be continued in ICES Member Countries on *Bonamia exitiosa* in European flat oyster in Europe;

iv) studies continue in Germany on summer mortality in Pacific oysters including transmission electron microscopy examination of gonadal tissue lesions.

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

A working document was prepared by T. A. Mo, S. W. Feist and S. Jones (Annex 7) reviewing the effect of proliferative kidney disease (PKD) on salmonid populations. A presentation was also given by T. A. Mo on the current research on outbreaks of the disease in juvenile Atlantic salmon (*Salmo salar*) in one (River Åelva) of the two Norwegian rivers where PKD-induced mass mortalities have been observed. In addition, the parasite (*Tetracapsuloides bryosalmonae*) causing PKD has been found in 16 Norwegian rivers, but so far the effect on the salmon populations has not been studied. In River Åelva salmon parr mass mortalities were observed in 2002, 2003, 2004, and at that time were linked to environmental factors. PKD initially was identified there in 2006 and most likely is also the explanation for mass mortalities in previous years. The mass mortality continued in 2007.

T. bryosalmonae requires a bryozoan host (definitive host) within which spores are produced that are infective to salmonids. Naturally occurring fish-to-fish transmission does not occur, but spores released from salmonids have recently been shown to be infective to bryozoans. This important finding means that infected fish can potentially transmit the parasite to naïve bryozoan populations following migration or anthropogenic translocation to new areas.

It has long been recognised that PKD is a major constraint to salmonid aquaculture in many countries in the northern hemisphere, with significant mortalities occurring when temperatures rise in the summer months and in particular when additional stress factors occur. The effect of the disease on wild anadromous populations is less well understood but also has recently been the focus of attention in Switzerland where declines in freshwater brown trout (*Salmo trutta*) populations have been associated with PKD. Research into the ecology of the disease, particularly for the distribution and density of the bryozoan definitive hosts in rivers and lakes, is required.

The WGPDMO recognised the unusual severity of the PKD outbreaks affecting juvenile salmon in Norwegian rivers and it was pointed out that concerns on potential reductions in recruitment to adult populations have been raised in other countries such as Switzerland and the UK. Since affected fish suffer severe anaemia, they are particularly sensitive to decreased oxygen levels, which may occur during periods of increased temperatures. It was pointed out that in affected rivers, oxygen levels were not significantly reduced and was not thought to be a significant contributory factor in the mortalities. Specific questions regarding the proportion of fish surviving infection and whether these migrated to sea were discussed. It was noted that some large (older) fish were found with gross swelling of the kidney and it was considered that since previously infected fish gain some immunity to re-infection, it was likely that these fish were infected for the first time.

7.1 Conclusions

1) Mass mortalities in Norway of stocks of juvenile wild Atlantic salmon (*Salmo salar*) due to *T. bryosalmonae* are of concern because of the potential negative effect on recruitment.

2) The association of mortalities due to PKD with elevated temperatures suggests that the severity of PKD in wild salmonids may increase if the upward trend in mean temperature predicted in climate change models continues.

3) Key areas for further investigation include estimates of the impact of PKD on recruitment to adult populations and year class strength and determination of the extent of infected bryozoan populations.

7.2 Recommendations

The WGPDMO recommends that:

i) ICES Member Countries determine the distribution of *T. bryosalmonae* in bryozoan populations in watersheds with salmonids.

ii) field and laboratory studies on growth of bryozoans and on the life cycle of *T. bryosalmonae* under natural conditions in watersheds (with and without salmonids) be conducted. Because of recent changes in climatic conditions in the sub-arctic region, particular attention should be given by the ICES Member Countries of that region for these studies.

iii) investigations be conducted on *T. bryosalmonae*-infected Atlantic salmon smolts in the marine environment.

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

A working document (Annex 8) was prepared by T.A. Mo, A. Alfjorden, D. Bruno and L. Madsen reviewing the recent history of *Francisella* in wild and farmed cod and information on host susceptibility, associated pathology, and current diagnostic methods.

8.1 Background

Since 2004 a new bacterial disease, characterised by the presence of gross and microscopic granulomatous lesions in internal organs and the skeletal musculature, has been reported from farmed and wild cod (*Gadus morhua*) in Norway, from wild cod in Sweden and from farmed cod in Denmark that had been imported from Norway. Mortalities in farmed cod are variable. In Norway, cases have been confirmed with increasing frequency since 2005. The disease is seen most commonly during the summer but the infection can persist during winter months. Experiments have shown that the causal agent transfers readily among cohabiting cod.

Similar diseases have been reported from tilapia (*Oreochromis* spp.), three-line grunt (*Parapristipoma trilineatum*), hybrid striped bass (*Morone saxatilis x M. chrysops*) and Atlantic salmon (*Salmo salar*). Genetic data indicate the causal agents in these cases are related to, but distinct from, the agent in cod. Atlantic salmon are susceptible to the cod isolate but disease is absent.

8.2 Diagnosis and Pathology

Infected fish show internal lesions consisting of whitish nodules in most organs (liver, spleen, heart and kidney). External lesions involve the skin, eyes and pseudobranchs. Histopathologic changes include chronic inflammatory reactions characterised by numerous granulomas of various sizes and stages. The causal agent is also visualised in histologic sections by immunohistochemistry using specific diagnostic antisera.

8.3 Identification and culture of the causative agent

The intracellular bacterium *Francisella philomiragia* subsp. *noatunensis* has been cultured from infected fish and was identified as the causal agent through laboratory exposure studies. The organism may grow slowly and should be isolated from fresh material onto a specialised agar containing cysteine and blood. The species identification was based on amplification and sequencing of 16S ribosomal DNA.

8.4 Conclusions

1) An emerging chronic, granulomatous disease has been described from wild and farmed cod.

2) The disease causes mortality and reduced growth in farmed cod with associated economic losses.

3) The intracellular bacterium *Francisella philomiragia* subsp. *noatunensis* is identified as the causal agent.

4) Diagnosis is obtained by observing gross or microscopic granulomas and identification of the agent by PCR, immunohistochemistry or cultivation on a cysteine-blood agar.

8.5 Recommendations

The WGPDMO recommends that:

i) further surveillance and research be conducted by ICES Member Countries to document the distribution, severity and host specificity of francisellosis in wild and farmed fish.

8.6 Key References

Mikalsen, J., A. B. Olsen, T. Tengs, and D. J. Colquhoun. 2007. *Francisella philomiragia* subsp *noatunensis* subsp nov., isolated from farmed Atlantic cod (*Gadus morhua* L). *International Journal of Systematic and Evolutionary Microbiology* 57:1960–1965.

Nylund, A., K. F. Ottem, K. Watanabe, E. Karlsbakk, and B. Krossøy. 2006. *Francisella* sp. (Family Francisellaceae) causing mortality in Norwegian cod (*Gadus morhua*) farming. *Archives of Microbiology* 185:383–392.

Olsen, A.B., J. Mikalsen, M. Rode, A. Alfjorden, E. Hoel, K. Straum-Lie, R. Haldorsen, and D.J. Colquhoun. 2006. A novel systemic granulomatous inflammatory disease in farmed Atlantic cod, *Gadus morhua* L., associated with a bacterium belonging to the genus *Francisella*. *Journal of Fish Diseases* 29:307–311.

Ottem, K.F., Nylund A., Karlsbakk E., Friis-Moller A., and Krossoy B. 2007. Characterization of *Francisella* sp., GM2212, the first *Francisella* isolate from marine fish, Atlantic cod (*Gadus morhua*). *Archives of Microbiology* 187:343–350.

9 Progress report on studies carried out on hyperpigmentation in common dab (*Limanda limanda*)

T. Lang presented a working document by T. Lang, F. Baumgart, D. Bruno, S.W. Feist, and P. Noguera (Annex 9) on new studies carried out on hyperpigmentation in dab (*Limanda limanda*) from the North Sea together with a list of relevant publications on this subject. This was a summary of a Diploma thesis conducted at the University of Rostock (Baumgart, 2007). The major findings were:

- The increase in prevalence of hyperpigmentation recorded in dab from almost all North Sea areas since the mid 1990s is largely caused by an increase in prevalence of the lowest severity grade 1.
- The prevalence of hyperpigmentation depends on the age of fish, with grade 1 dominant in younger fish and grades 2 and 3 more prominent in older fish. The prevalence increased from age groups 2 to 4 and decreased in age group 5 to 8.
- Dab with hyperpigmentation grade 3 have a faster growth rate than normally pigmented fish, but the condition factor is lower.
- The results of a virologic and bacteriologic studies carried out at the Fisheries Research Services, Aberdeen, UK, did not reveal involvement of pathogens in the aetiology of the condition.
- A hypothesis was proposed suggesting increased UV-B exposure of early dab life stages, as a possible cause of hyperpigmentation.
- Pigment anomalies have been described in aquaculture flatfish, most often albinism and ambicoloration. Causes are unresolved.

9.1 Conclusions

1) The increasing trend in prevalence of hyperpigmentation recorded in North Sea dab areas since the mid 1990s is largely caused by an increase in prevalence of the lowest severity grade 1.

2) Age – related changes in prevalence of hyperpigmentation may indicate increased mortality, e.g. caused by higher predator pressure on hyperpigmented and, thus, more visible fish, or resolution of the condition by adults.

3) The lower condition factor in faster growing fish may indicate a physiological effect of the condition on the fish.

4) There is no known cause of this condition. However, a bacterial or virological agent is unlikely. Further work on the aetiology is ongoing.

9.2 Recommendations

The WGPDMO recommends that:

ii) WGPDMO be updated on the results of ongoing histopathologic studies on organs other than the integument (e.g. liver, kidney, spleen, gonad, eye, thyroid gland, pineal gland, pituitary) in affected fish,

iii) a thorough review of the existing literature on malpigmentation in farmed flatfish and fish pigment-cell tumours should be conducted and reported to the WGPDMO,

iv) potential causes of hyperpigmentation be investigated. These could include analysis of the food composition in affected areas, pigment cell development/regulation in affected fish, the potential role of contaminants in hyperpigmentation, and factors which make the skin hypersensitive to UV-B irradiation.

9.3 Reference

Baumgart, F. 2007. Hyperpigmentierung bei Klieschen (*Limanda limanda*) in Nord- und Ostsee: regionale und zeitliche Muster sowie mögliche Ursachen (title in English: Hyperpigmentation in dab (*Limanda limanda*) from the North Sea and Baltic Sea: regional and temporal patterns and possible causes). Diploma Thesis, University of Rostock, 2007, 139 pp.

10 Review the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants

A working document was prepared by S. Jones, T.A. Mo and N. Ruane. The document (Annex 10) reviewed the information on classes of pesticides and medicines, including organophosphates, pyrethrum and pyrethroids, disinfectants, insect growth regulators and avermectins that are used to treat sea lice. Evidence of increased tolerance to chemotherapeutants was reviewed. S. Jones presented the highlights of this document.

The application of pesticides or medicines for the treatment of ectoparasitic copepods has a long tradition in the cultivation of freshwater fish, and farmed salmon have been treated for infestations with sea lice since the beginning of intensive aquaculture. Increased tolerance (or resistance) to the therapeutants is not an unknown phenomenon in the aquatic environment. A number of different chemical treatments have been used to treat against sea lice. Some of the first chemotherapeutants used were the organophosphates, a group of synthetic acetylcholinesterase inhibitors that are administered as bath treatments. They act on the nervous system, and affect the motile stages of the sea lice. In recent years their use has been limited. Pyrethrum and the semi-synthetic pyrethroids are also administered as bath treatments. They also act on the nervous system through the disruption of sodium channels and affect all parasitic stages. Another chemical which has been used as a bath treatment is hydrogen peroxide, which may disrupt the cuticle (outer shell), and is most effective on motile stages of sea lice. Sea lice may recover from this treatment though.

There are also a number of treatments which are administered in feed. These include insect growth regulators which interfere with the synthesis of chitin in the lice. They target the actively moulting early developmental stages. Avermectins are another group of chemicals which are administered in feed. They act on the nervous system of the parasites by increasing neural cell membrane permeability. Emamectin benzoate (SLICE) is an example of a semi-synthetic avermectin.

Over the last number of years, treatment of sea lice in farmed salmon in Norway has been characterised by a sequence of preferred chemotherapeutic compounds: use of organophosphates preceded pyrethroids, and most recently emamectin benzoate usage has predominated. The chitin inhibitors, ivermectin and hydrogen peroxide have been used less frequently. It may be inferred from the scientific literature that a similar trend occurred in Scotland, Ireland, Canada and the USA, although comprehensive data are lacking. These trends are related to the availability over time of products with improved margins of safety, efficacy and ease of delivery. Different treatment strategies as well as the availability of chemotherapeutants will also affect the usage trends regionally and nationally. A pattern of repeated single usage may, theoretically, lead to increased tolerance. There are a number of anecdotal reports in the literature of changes in efficacy over time which would suggest an increased tolerance to organophosphates, hydrogen peroxide and pyrethroids in Norway, Scotland and Canada. The anecdotes appear to be based on treatment failures and it is not clear whether alternative explanations such as inappropriate treatment concentration or exposure time were ruled out in these cases.

To accurately measure whether sea lice are becoming increasingly tolerant, the use of controlled tests such as bioassays or similar sensitivity tests is required. Techniques used for field bioassays for organophosphates, pyrethroids and emamectin benzoate have been described but the results of these trials have not been made public. It is also difficult to verify the accuracy of statements when comparing results from dif-

ferent authors. There are several published accounts of bioassay-confirmed, increased tolerance to organophosphates as well as a controlled study documenting resistance to hydrogen peroxide. Studies on increased tolerance to emamectin benzoate have given conflicting results. Two studies showed an increased tolerance over time, while a third study showed a seasonal and temperature variation in efficacy of treatments. Details of these publications and reports are included in Annex 10. More research is necessary to identify mechanisms of increased tolerance, and molecular techniques such as gene expression studies may provide more sensitive tools for screening sea lice populations.

10.1 Conclusions

1) Treatment of sea lice can be effective but this is costly and access to efficacious medicines and pesticides is limited to a small number of available compounds and by regional or national regulatory processes.

2) The limited available information provides evidence of increased parasite tolerance to four classes of compounds: organophosphates, pyrethroids, hydrogen peroxide and avermectins.

10.2 Recommendation

The WGPDMO recommends that:

i) ICES Member Countries encourage research to identify and license new classes of sea lice medications;

ii) ICES Member Countries encourage salmon aquaculture companies to practise integrated pest management, including synchronised treatments within management areas, use of alternating classes of sea lice medication, and routine sea lice monitoring;

iii) ICES Member Countries encourage coordinated use of bioassay techniques to screen for tolerance to medicines and pesticides;

iv) results of bioassay screens in all regions of ICES Member Countries should be made public to permit an adequate assessment of local parasite populations;

v) ICES Member Countries should encourage research in the development of alternative molecular tools for screening sea lice tolerance.

11 Provide an update of international collaborative actions involving fish and shellfish disease and pathology activities (ToR g)

11.1 Cooperation with the World Organization for Animal Health (OIE)

The WGPDMO noted with great appreciation that ICES has received a request for cooperation from the World Organization for Animal Health (OIE). ICES has signed and forwarded a Letter of Agreement (LOA) to cooperate with OIE on matters of mutual interest in preventing the international spread of unwanted organisms and diseases in the aquatic environment. The LOA will be considered for approval at the OIE Council meeting in May 2008. In the future it is likely that WGPDMO, WGITMO, and WGMAFW will be more involved with OIE activities and *vice versa*.

11.2 Baltic Sea Regional Project (BSRP)

T. Lang informed WGPDMO that the BSRP has come to an end in June 2007 and that there will be no continuation. This unfortunate and unexpected development affects, e.g. activities aiming at implementing an internationally standardised and harmonised fish disease monitoring programme in the Baltic Sea as part of an integrated Ecosystem Health Assessment of the Baltic Sea and the activities of the former BSRP

Lead Laboratory for fish diseases, parasites and histopathology at AtlantNIRO, Kaliningrad, Russia, headed by the WGPDMO member G. Rodjuk.

As an immediate effects of the termination of the BSRP, the workshop on methodologies for monitoring fish diseases/parasites in coastal fish species from the Baltic Sea originally planned for 2007 at AtlantNIRO, Kaliningrad, Russia with G. Rodjuk, T. Lang and a representative of the group of HELCOM coastal fish monitoring experts as Co-Chairs had to be postponed because of funding constraints. The workshop is supposed to be a follow-up activity to the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring (WKFDM) (ICES 2005) which was focused on diseases in offshore areas (ICES, 2006) (Report at: http://www.ices.dk/reports/BCC/2006/WKFDM06.pdf). The main objectives of the 'new' workshop are:

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

Meanwhile a decision was made to incorporate the workshop into the HELCOM FISH Project. At present, plans for the date and venue of the workshop are being made. (http://www.helcom.fi/press_office/news_helcom/en_GB/HELCOM_FISH_Project/)

11.3 4th ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their effects in Coastal and Open-sea Areas (WKIMON IV)

WKIMON IV took place 5–7 February 2007 at the ICES Headquarters, Copenhagen, Denmark, with representatives from OSPARCOM and from various ICES expert Groups, including the WGPDMO. The major objective of the WKIMON Workshops is to develop guidelines for integrated monitoring of contaminants and their biological effects as part of the OSPAR Coordinated Environmental Monitoring Programme (CEMP), including the development of assessment criteria.

Fish disease monitoring and assessment is included in the process, since the monitoring of externally visible diseases, macroscopic liver neoplasms and liver histopathology is part of the general and the contaminant-specific monitoring CEMP programme. The Fish Disease Index (FDI) and the related assessment criteria developed by WGPDMO have been accepted in the WKIMON process as component of such an integrated programme.

11.4 Workshop on Integrated Assessment of Contaminant Impact in the North Sea (ICON)

T. Lang informed WGPDMO about the ICON Workshop the practical component of which is going to take place in the second half of 2008 and which will include studies on externally visible diseases, macroscopic liver neoplasms and liver histopathology.

The objective of the practical ICON Workshop is to assess the health of North Sea ecosystems through an international sea-going workshop and subsequent integrated assessment of the impact of contaminants on the North Sea.

ICON aimed at: (1) to try out the core components of the WKIMON integrated chemical biological effect methods and guidelines (demonstration component) (2) investigate a range of additional biological effects techniques (research component).

The ICON initiative was started in 2006, led by Ketil Hylland (Norway) and with potential funding from the Norwegian oil industry and Research Council. A last steering group meeting was held in Copenhagen at the ICES Headquarters in May 2007 to discuss the program. Commitments were made by various Member countries at that time to have ship-time and scientific resource available to do the work. In December 2007, it became clear that no further Norwegian funding would be available to support the activities in ICON.

At the 4th ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their effects in Coastal and Open-sea Areas (WKIMON IV) it was discussed how best to take ICON forward under the new conditions, as OSPAR had expressed its support for such an opportunity to field test integrated monitoring schemes. It was decided that a realistic option will be to run a nation-based, but coordinated, programme. The UK, Germany, France, NL and Spain informed the WG that they would be willing to participate in such a reduced ICON project.

The following components/sites/species should be included:

Final plans for the programme will be made at an ICON steering group meeting to be held in Hamburg, Germany, 10 March 2008. Progress made will be reported to WGPDMO at its 2009 meeting.

11.5 Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM)

S.W. Feist gave an overview of new developments in the fish disease component of the BEQUALM programme (http://www.bequalm.org/fishdisease.htm). It is anticipated that 6 laboratories will sign to the 2008/2009 activities and take part in regular intercalibration exercises and ring tests that so far have been focused on externally visible diseases and liver histopathology in European flatfish species (dab, *Limanda limanda*, and flounder, *Platichthys flesus*). The most recently completed ring test for liver histopathology was based on the virtual slide technology using electronic images that were distributed to the participants.

Further activities planned are:

- a practical histopathology workshop to be held in May 2008
- further ring tests and intercalibration exercises
- consideration of more marine fish species (e.g. dragonet species, *Callionymus* sp.) and possibly also freshwater species (e.g. brown trout, *Salmo trutta*).

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

are not taking part due to the registration fee which, however, will be reduced the more participants will sign in.

11.6 International cooperation to further knowledge and understanding of salmonid alphavirus

Two years ago an international group of key salmon industry people and scientists from Ireland, France, Norway and Scotland was set up to speedily advance and address the gaps in our knowledge of pancreas disease (PD) and related pathologies. PD has re-emerged in recent years as a serious economic cost, both in terms of mortality and growth loss, within the European salmon industry. Sleeping disease (SD), a closely related alphavirus, is now present in France, Italy, Spain and the UK and is causing significant losses in the rainbow trout industry. In the two years the group has met six times, the most recent meeting being hosted by the Fisheries Research Laboratory in Aberdeen in April 2007. Over fifty people from various countries participated in this latest round of information exchange and project development discussions.

This initiative is quite unique in that it has succeeded in bringing together previously competing groups to focus on how they can learn from the past and forge new productive alliances to combat the losses caused by PD and related pathologies. Several new projects have been agreed and, while financed at an individual country level, there has been significant international cooperation and input. Some projects have already been completed and results published, some of which are cited in the scientific summaries section of this newsletter and many more interesting articles are in the pipeline. The group are going to make a concerted effort to acquire EU funding so that this important work can be progressed as quickly as possible. A special issue of the Journal of Fish Diseases to be published this autumn will contain a review of salmonid alphaviruses and a number of related papers. The coordinators of this project are Gordon Ritchie and Neil Ruane (source of information: http://aqua.intervet.com/news/2007-04-15.asp).

11.7 EU project 'Improved immunity of aquacultured animals' (IMAQUANIM)

An EU-funded project entitled 'Improved immunity of aquacultured animals' (IMAQUANIM) was launched to be run until 2010 (www.imaquanim.dfvf.dk/). This project involves 22 partners from all over Europe and is coordinated by the Technical University of Denmark, National Veterinary Institute. The major aim of the IMAQUANIM Project is to create a basis for the selective breeding of aquacultured animals that are immune to devastating infectious disease.

11.8 EU-funded project AQUAFIRST: Key genetic characteristics to improve selective fish breeding for disease resistance

AQUAFIRST (will run until 2008) (source of information: http://ec.europa.eu/research/fp6/ssp/aquafirst_en.htm) seeks to identify genes associated with stress and disease resistance in fish and molluscs to provide a physiological and genetic basis for marker-assisted selective breeding. It builds on earlier EU-funded projects: BRIDGEMAP, BASSMAP and STRESSGENES. To counter poor growth performance, impaired reproduction and increased susceptibility to disease, growers have increasingly used antibiotics and drugs. The development of selective breeding programmes using genetic markers offers a means to reduce, if not eliminate, the use of antibiotics.

- The project will help in attaining aquaculture objectives within the Common Fisheries Policy by establishing a healthier and more economically competitive aquaculture sector
- The development of 'targeted' selective breeding methods will reduce the need for antibiotics and help the EU meet targets for reducing residues in foodstuffs and the environment
- An aquaculture sector that relies on selective breeding rather than pharmaceutical intervention will reduce public reservations towards a key sector of the European seafood industry

11.9 EPIZONE

The mission of EPIZONE (http://www.epizone-eu.net/default.aspx) is to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks. The Network of Excellence EPIZONE, including 18 institutes from 12 countries, FAO and 1 SME, operates as a virtual institute. The activities are organized in 4 vertical scientific integration themes (Diagnostics, Intervention Strategies, Surveillance and Epidemiology, and Risk Assessment). In Theme 6 "Surveillance and Epidemiology", there is one Work Package Epizone WP 6.1 that works with: Surveillance & Epidemiology of emerging viral diseases in aquaculture. Participants in this project are Technical University of Denmark (DTU) – Denmark, Veterinary Research Institute of Venice (IZSVe)- Italy, Friedrich Löffler Institute (FLI) – Germany, National Veterinary Institute (NVI)(SVA) – Sweden, Central Institute for Animal Disease Control (CIDC)-The Netherlands, National Veterinary Research Institute (NVRI) – Poland, and National Veterinary and Food Research Agency (AFFSA) –France. The work of this group focuses on virus characterization, virus phylogeny, geographic spotting and tracing of diseases.

11.10 Conclusions

1) WGPDMO noted that there is an increasing number of international collaborative actions involving fish and shellfish disease and pathology, reflecting the importance of disease issues in relation to environmental monitoring and assessment as well as to mariculture. WGPDMO emphasised that the information provided in the section is far from being complete and only highlights particularly relevant activities.

2) The WGPDMO noted with appreciation that a formal cooperation with the OIE will be established which offers improved possibilities for further joint activities and representation of the WGPDMO at relevant OIE meetings and *vice versa*.

3) The WGPDMO once more endorsed the plans to organise a workshop on methodologies for monitoring fish diseases/parasites in coastal fish species from the Baltic Sea and welcomed that the workshop is now (after the end of the Baltic Sea Regional project, BSRP) included in the HELCOM FISH project. It is expected that this will lead to a greater commitment of the Baltic Sea countries to contribute to the workshop and to nominated participants. The Co-Chairs together with the project leaders should make detailed plans for the workshop as soon as possible, e.g. on dates and an appropriate venue.

11.11 Recommendations

The WGPDMO recommends that:

i) ICES Member Countries take note of the present international collaborative actions involving fish and shellfish disease and pathology activities and support the participation of the national institute in these activities;

ii) the workshop on methodologies for monitoring fish diseases/parasites in coastal fish species from the Baltic Sea be organised as part of the HELCOM FISH project with G. Rodjuk, T. Lang and a representative of the group of HELCOM coastal fish monitoring experts as Co-Chairs. A decision on the dates and venue should be made by the Co-Chairs and the HELCOM Fish project leaders as soon as possible. The main objectives of the workshop are:

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

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

iv) the WGPDMO be informed of progress of the various meetings at the 2009 meeting.

11.12 Reference

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

12 Provide an update on the validation and integration of molecular diagnostic and confirmative techniques for pathogens of bivalves

A working document prepared by T. Renault, S. Ford and L. Madsen was presented by T. Renault. The document (Annex 11) provides an update on the validation and integration of molecular diagnostic and confirmatory techniques for pathogens of molluscs based on the following aspects:

i) Comparison of diagnostic techniques for mollusc pathogen detection

ii) Interlaboratory evaluation

iii) Design of valuable molecular tools

iv) Diagnostic kits

For many pathogens of molluscs, diagnostic techniques have been based on histological and ultrastructural examinations. The accuracy and reliability of diagnosis by means of these methods is highly dependent on the experience of the investigator. In addition, histological diagnosis does not allow identification at the species level for most mollusc pathogens. In this context, in a large number of available reports and publications on pathogen speciation is based on host species and geographic range.

Recognising the need for diagnostic tools to discriminate between genera and species, efforts have been made to develop molecular detection assays for pathogens infecting molluscs. For the use of molecular based diagnostic tools in routine diagnosis, however, DNA-based assays need formal validation.

Therefore, the DNA-based assays must be compared to traditional methods. Lack of standardisation of tests and test protocols is a major impediment to the effective implementation of DNA-based methods. Standardisation requires international agreement and cooperation in test selection, practitioner training and laboratory accreditation. Improvements of reproducibility, validity and comparability of data will amend the suitability of DNA-based methods for the detection of listed pathogens. Interlaboratory assays have to be performed in order to confirm reproducibility not only of the assay but also with respect to sensitivity and the implementation of contamination control procedures. Differences may also occur in reagent quality and preparation, in controls, as well as in the interpretation of results. The standardisation of diagnostic tools is essential for their use in routine analysis and diagnosis.

In addition, it is necessary to identify regions of the pathogen genome that can be used for species differentiation. The taxonomic history of various pathogens including viruses, bacteria and protozoan parasites is the subject of ongoing discussions. In recent years molecular biology has lead to taxonomic clarification of some of the pathogens.

For diagnostic purposes, often only small sequences are used as probes without considering their true specificity. In addition, DNA probes are often designed from genes or clusters of genes of phylogenetic significance which often does not reflect the diversity in terms of virulence. There is a growing recognition of the need for strain differentiation in diagnostic procedures.

All molecular assays specific for a pathogen should be tested and validated in parallel, and further sensitive diagnostic assays that allow discrimination of all 'valid' species should be developed.

A further aspect of the working document is the development of commercial kits for the detection of mollusc pathogens and their potential use in hatcheries.

12.1 Conclusions

1) There are a number of mollusc pathogens for which DNA-based test methodologies are published. However, in general, further research is required before standardised and validated DNA-based test protocols can be implemented for disease diagnosis and pathogen detection in molluscs.

2) Pathogens that require long, complex culture or histology-based confirmatory diagnosis are prime candidates for rapid, pathogen-specific diagnostic methods. This applies predominantly to microbial pathogens, but may be equally appropriate for protozoan parasites which are difficult to distinguish morphologically at the light microscope level or which have a broad host-range.

3) Rapid, pathogen-specific diagnostics would be particularly appropriate for disease management and control when diseases emerge in new geographic locations or host species. However, in the case of molluscs, histology provides a large amount of information and should be applied together with any other type of examination.

4) There is a need for an international agreement on methodologies that have to be evaluated and accredited for specific applications in disease diagnosis and pathogen screening. There is also a need to ensure that tests are performed by trained staff with access to standardised reagents and suitably equipped laboratories. No one technique shows a significant advantage over another, and none appear sufficient to merit "stand-alone" application, with the possible exception of pathogen-specific research.

12.2 Recommendations

The WGPDMO recommends that:

i) communication networks of diagnostic practitioners and internationally recognised experts in aquatic animal health in ICES Member countries be established and maintained. Activities of the networks should include development of training programmes and cooperative programmes for test validation and laboratory accreditation;

ii) all molecular assays specific for a pathogen be tested in parallel and validated, and further sensitive diagnostic assays that will clearly discriminate between all “valid” species be developed;

iii) research into regions of the genome must be conducted to identify which regions are useful for species differentiation.

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

W. Wosniok gave a brief overview about the aspects of using the FDI for fish species other than dab, *Limanda limanda*. Although the FDI thus far has been used only for data on common dab, the concept of the FDI can be applied to disease data from other species. Because diseases, including parasites, to some extent are specific for each fish species, the set of conditions contributing to the FDI has to be selected for

each species. Also, the expert judgments on disease severity have to be specific for each species. Finally, data on disease prevalence for the other (new) fish species must be collected or made accessible in order to actually calculate FDI values.

To check the possibility of calculating FDI values for Baltic cod (*Gadus morhua*) and flounder (*Platichthys flesus*) from data currently available in the ICES Databank, an overview of the disease data for cod and flounder was presented. Details on the data used and the data flow are described in Annex 6 of this report. Tables 13.1 and 13.2 list those ICES statistical rectangles for which data from at least 10 sampling days is available. This number of sampling days is a minimum requirement in order to base the trend assessment on a sufficiently long time series of observations. Neither of the tables takes notice of the spectrum of parameters that were actually observed per sampling day. This spectrum varies over time and region. For further progress,

- the component diseases of a species-specific FDI for Baltic cod and flounder must be selected and
- severity judgments from relevant experts must be obtained.

With these prerequisites the calculation of FDIs for Baltic cod and flounder can be done for the areas listed in Table 13.1 and 13.2, following the same computational procedure as for common dab data. However, a lack of data for diseases that are considered essential for the new FDIs may reduce the number of ICES rectangles for which an FDI can actually be calculated.

It must be noted that presumably the data submitted to ICES in 3.2 format does not comprise all the data that has been submitted earlier to ICES in other formats than 3.2. This must be concluded from comparing the counts from earlier analyses to the counts presently found, as is discussed under ToR a (Section 5.2; Annex 6). If the presumption of more existing data is correct, there may be more areas for which an FDI can be calculated.

The WGPDMO discussed the general possibility of formulating an FDI-like index for other purposes, e.g. to summarise conditions in fish stock assessment. It was pointed out that the concept of the FDI can be applied in many more situations, as long as a set of relevant parameters and their relative importance is or can be defined (and the corresponding data is available).

13.1 Conclusions

1) In principle, a Fish Disease Index for Baltic cod and flounder can be calculated.

2) The specification of the diseases relevant for Baltic cod and flounder, as well as the assessment of disease severity, still need to be established.

13.2 Recommendations

The WGPDMO recommends that:

i) a group of experts be asked for their opinion on which diseases should contribute to an FDI for cod and flounder, respectively, and on the relative severity of the diseases. The procedure used for the common dab FDI should be adopted for this purpose. Suggested experts to be approached are D. Bruno, T. Lang, S. Feist, K.Trella, D.Vethaak, and T. Wiklund;

ii) corresponding FDI values and assessments for Baltic cod and flounder be established using data from the ICES Databank;

iii) ICES Databank request that ICES Member Countries submit cod and flounder disease data to the ICES Databank using the ICES Environmental Data Reporting Format 3.2.

Table 13.1. ICES statistical rectangles with data on Baltic cod (*Gadus morhua*) from at least 10 sampling days.

ICES RECTANGLE	NUMBER OF SAMPLING DAYS	NUMBER OF FISH
34F3	10	2248
36F1	10	3013
37F7	34	13577
37G1	26	18860
38F2	17	3940
38G0	29	20054
38G3	41	28988
38G4	38	17135
38G5	24	9331
39E9	15	6150
39G6	20	9530
39G8	19	9631
40F7	20	5828
41E7	11	2223
42F3	15	6849
44E8	12	3894
ALL		161251

Table13.2. ICES statistical rectangles with data on flounder (*Platichthys flesus*) from at least 10 sampling days.

ICES RECTANGLE	NUMBER OF SAMPLING DAYS	NUMBER OF FISH
32F3	18	6576
33F4	16	6795
34F4	11	3051
38G4	13	1163
38G5	12	819
ALL		18404

14 Provide an update on the status of ICES publications on pathology and diseases of marine organisms

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

S.W. Feist, Series Editor, gave an overview of progress made in the past year with regard to the Leaflets that provide diagnostic aids for identifying the most important diseases and parasites of fish and shellfish in the North Atlantic and adjacent seas. Fifty-six leaflets have been published in the period from 1984 to 1999 and are available on the ICES website as scanned documents (http://www.ices.dk/products/fiche/disease/2006/start.pdf). A number of leaflets are under revision and new ones are in preparation. A decision was made that these leaflets will be produced using a template prepared by ICES and the Series Editor. There are plans to install a SharePoint site on the ICES website specifically for the editorial subgroup and the authors of the Disease Leaflets.

14.2 Publication on the effects of climate change on marine fish and shellfish diseases

The WGPDMO agreed that the manuscript entitled 'Effects of climate change on marine fish and shellfish diseases' (authors: S. W. Feist, D. Bruno, S. Ford, S. R. M. Jones, T. Lang, M. Longshaw, A. Mansour and G. D. Stentiford) presented at the 2006 WGPDMO meeting should be published as soon as possible (preferably in the ICES Journal of Marine Science) without including results of the analysis of further datasets as originally planned.

14.3 Publications in the ICES Techniques in Marine Environmental Sciences Series (TIMES)

A manuscript entitled 'Histopathology of mussels *Mytilus* sp. for health assessment in biological effects monitoring' (authors: Feist, S. W., Bignell, J. P., Cajaraville, M., Marigomez, I., Villalba, A. and Lowe, D.) is in preparation and will be submitted to the TIMES Series Editor in September 2008.

A manuscript with guidelines for fish disease monitoring in the Baltic Sea was prepared as an outcome of the 2005 ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM) (authors: T. Lang and G. Rodjuk) and will be submitted to ICES for publication after review by selected WGPDMO members.

It was suggested to prepare a manuscript providing details on the construction of the Fish Disease Index (FDI; see Section 5 and Annex 6 of the present report) in the ICES TIMES Series (authors: W. Wosniok, T. Lang and possibly other WGPDMO members). Progress made will be reported to WGPDMO at its 2009 meeting.

14.4 Update of the ICES publication 'Trends in important diseases affecting the culture of fish and molluscs in the ICES area 1998–2002'

A suggestion was made to update the review prepared by WGPDMO members and published in 2004 in the ICES Cooperative Research Report Series (Vol. 265) with current information covering the period 2003–2007 and to make the updated document available on the ICES website. WGPDMO decided that the first step will be to conduct a feasibility study in order to assess the need for an update in light of (a) the availability of sufficient new information and (b) possible redundancies with other published documents. The feasibility study will be carried out by the WGPDMO

members N. Ruane, S. Jones and T. Renault and the results will be presented to WGPDMO.

14.5 Papers presented at the ICES Annual Science Conference 2008, Theme Session D: New trends in diseases of marine organisms: causes and effects

The WGPDMO suggested that, depending on the number and subject of papers submitted for the Theme Session (Co-Convened by S. MacLean, S. McGladdery, and T. Lang), publication of a set of papers in the ICES Journal of Marine Science (JMS) should be considered. Once the abstracts have been submitted (deadline: Monday 21 April 2008), the co-conveners will explore possibilities with the JMS Editor-in-Chief.

14.6 Conclusions

3) The WGPDMO emphasised that there are many topics related to pathology and diseases of marine organisms on the agenda of the WGPDMO that warrant a wider dissemination in the scientific community. A number of suggestions/recommendations regarding appropriate publications involving WGPDMO members were made.

14.7 Recommendations

The WGPDMO recommends that:

i) the manuscript entitled 'Effects of climate change on marine fish and shellfish diseases' (authors: S.W. Feist *et al*.) be published as soon as possible without including results of the analysis of further datasets;

ii) a manuscript with guidelines for fish disease monitoring in the Baltic Sea prepared as an outcome of the 2005 ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM) (authors: T. Lang and G. Rodjuk) will be submitted for publication in the ICES TIMES Series after review by selected WGPDMO members;

iii) a manuscript providing details on the construction of the Fish Disease Index (FDI) (co-authors: W. Wosniok and T. Lang and other WGPDMO members) be prepared for publication in the ICES TIMES Series and progress made will be reported to the WGPDMO at its 2009 meeting;

iv) a feasibilty study is carried out by WGPDMO members in order to assess the usefulness of producing an update of the WGPDMO publication 'Trends in important diseases affecting the culture of fish and molluscs in the ICES area 1998–2002' published in 2004 in the ICES Cooperative Research Report Series (Vol. 265). Results of the study will be presented to WGPDMO.

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

The process of fish disease data submission in the ICES Environmental Data Reporting Format 3.2 might benefit from further modification. General notes additional to the website based instruction from the ICES Databank have already been given in the WGPDMO 2006 report, p. 35/36. Some explanations of these notes are given below, and additional rules referring to the reporting of mixed graded/ungraded data are given.

15.1 Data identification

Reported fish and fish disease attributes (record type "10") must carry an identification that is unique within the entire data set submitted. The identification is contained in the CRUIS/STNNO/SMPNO/SUBNO combination, where typically "CRUIS" is the cruise identification, "STNNO" is the station number, "SMPNO" is the haul number. If individual fish data are reported, "SUBNO" is the number of fish, where numbering may start with 1 within each CRUIS/STNNO/SMPNO/SUBNO combination. If pooled data are reported, the contents of "SUBNO" differ for graded and ungraded data (see below).

15.2 Pooling of data

Data from several individuals may be pooled only for individuals that have all their reported attributes in common (CRUIS/STNNO/SMPNO/SUBNO combination, species, length, sex, set of diseases examined, and set of diseases present/absent).

15.3 Reporting pooled ungraded disease data

Ungraded disease data is characterized by MUNIT = AFNR in the type "10" records. A pool of fish is identified by "SUBNO". The number of fish in the pool is given in the field "NOINP". This is the number of fish examined in the CRUIS/STNNO/SMPNO/SUBNO data subset and reported about in the corresponding block of type "10" records with the same identification. For each pool there is one block of type 10 records. As a remark: for unpooled (=individual) data the field "NOINP" always contains a "1". The presence/ absence of a disease is indicated in the type "10" records by AFNR = 0 (disease absent for all individuals in the pool) or by AFNR = NOINP (disease present for all individuals). **An AFNR value other than 0 or NOINP is a mistake when reporting new data**. Note: historical data may have been pooled against this rule, i.e. with an AFNR value other than 0 or NOINP. This means that no calculation on the level of individual fish data is possible. The calculation of the Fish Disease Index from such data is still possible, but other uses of the data might not. Whenever individual data is originally available, it should be reported such that the "individual" level is maintained.

15.4 Reporting pooled graded disease data

Graded data is characterized by MUNIT = GRADE in the type "10" records. Here a pool is again a group of fish having all reported attributes (type "10" records) in common. Each pool has its own "SUBNO" identification. The "NOINP" field contains the number of fish in the pool, i.e. having the same set of attributes described in the corresponding type "10" records. However, different from ungraded data, the "Value" field in the type "10" records contains here the disease grade, not a count of observed responses.

15.5 Reporting ungraded and graded data jointly

There is no problem in reporting ungraded and graded disease attributes jointly for individual fish data. In this case, NOINP = 1 in all cases and the rules above can be followed, while indicating the ungraded/ graded nature of an attribute by setting MUNIT=AFNR or MUNIT=GRADE, respectively, per attribute.

The submission of ungraded and graded data jointly for pooled data is not recommended. The reason is that a proper reporting is only possible for certain combinations of attributes, where "proper reporting" means that the individual combination of attributes per fish is recognizable from the submitted file. If reporting data on

pools described by ungraded and graded attributes jointly, the ICES Data Centre should be contacted beforehand.

15.6 Additional check of submitted data

To improve the quality of fish disease data submissions in 3.2 format, the following global check is proposed as an addition to the formal default tests by DATSU:

Data submitters should be requested to report the total number of fish that was reported about in a submitted file, preferably by species. This number will then be compared with the number of fish found when reading the 3.2 data.

This procedure would allow a simple first test to detect the submission of a formally correct, but numerically incorrect file.

15.7 Conclusions

1) Submission of data with an aggregation level as low as possible is desirable. Therefore, reporting should avoid unnecessary data aggregation (pooling).

2) Contents of submitted data file should be verified.

15.8 Recommendations

The WGPDMO recommends that:

i) data submitters follow the proposal outlined in this text regarding style of data submission;

ii) the ICES Data Centre ask data submitters for total numbers of fish reported per file, preferably by species, and communicate these numbers to WGPDMO.

16 Other Business

16.1 Working group procedures

The WGPDMO continued to work in a paperless environment as adopted for the 2007 meeting. The presentation of the reports, subsequent discussion and modifications were carried out electronically.

16.2 Contact with other working groups

It was noted that a Term of Reference was given to WGCRAB for its 2009 meeting (ToR f: assess and report on the effects of disease on crab fisheries, and produce a manual for the fishing industry on *Hematodinium* infection of crabs including biosecurity). The WGPDMO emphasised that this ToR clearly falls under the purview of the WGPDMO and the WGCRAB should, therefore, make sure that WGPDMO expertise is involved when handling this and other similar ToRs.

To facilitate cooperation between ICES Expert Groups it was proposed that the WGPDMO Chair would make contact with the Chair of WGCRAB and other relevant Expert Groups (e.g. WGITMO and WGBOSV) to ensure they were aware of WGPDMO expertise on fish and shellfish disease.

16.3 Additional presentations

Simon Jones presented an update on sea lice in Canadian salmon aquaculture entitled: “Sea lice, salmon aquaculture and pink salmon in the Broughton Archipelago,

British Columbia, Canada". The presentation summarised laboratory and field evidence documenting the impact of *Lepeophtheirus salmonis* on juvenile pink salmon. Pink salmon as small as 0.7g were shown to have an innate resistance to the parasite. A threshold of lethal infection was estimated to be 10 lice/gram. Infections on approximately 6.8% of pink salmon exceeded the threshold in 2005, whereas less than 1% of infections exceeded the threshold in 2006 or 2007. The latter findings suggested a low level of risk to juvenile pink salmon.

17 Progress on tasks

Progress of tasks in the Terms of Reference was reviewed and it was concluded that all items had been dealt with in a satisfactory manner. Table 17.1 provides more information on items completed and those which require further action. Several intersessional tasks to be fulfilled prior to the 2009 WGPDMO meeting were identified.

Table 17.1. Progress on tasks of WGPDMO's Terms of Reference for 2008.

	TERM OF REFERENCE	STATUS
a	Provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible by testing the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties. (OSPAR request 13)	Preliminary task completed; will be revisited in 2009 as part of ToR i
b	Produce a report on new disease trends in wild and cultured fish, molluscs, and crustaceans based on national reports	On-going task; will be revisited in 2009 as part of ToR a
c	Review the status of proliferative kidney disease (PKD) epidemics caused by *Tetracapsuloides bryosalmonae* in wild salmonid populations	On-going task; new information on *T. bryosalmonae* will be presented in 2009 in national reports
d	Review the information on *Francisella* sp. and visceral granulomatosis in farmed cod and the potential for disease interaction between wild and farmed cod	Review completed; new information on *Francisella* will be presented in 2009 as part of ToR b
e	Provide a progress report on studies carried out on hyperpigmentation in common dab (*Limanda limanda*) from the North Sea with special emphasis on pathological findings and possible causes	On-going task; will be revisited in 2009 as part of ToR c and ToR d
f	Review the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants	Review completed; new information on sea lice will be presented in 2009 as part of ToR e
g	Provide an update of international collaborative actions involving fish and shellfish disease and pathology activities	On-going task; updates will be presented in 2009 as part of Other Relevant Projects
h	Provide an update on the validation and integration of molecular diagnostic and confirmatory techniques for pathogens of bivalves	Review completed
i	Provide an update on the use of the fish disease index for other fish species (e.g. Baltic cod and flounder) and other sets of available disease data (e.g. liver histopathology data)	On-going task; will be revisited in 2009 using Baltic cod and flounder data as part of ToR i
j	Provide an update on the status of ICES publications on pathology and diseases of marine organisms	On-going task; will be revisited in 2009 as part of ToR j
k	Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis	On-going task; will be revisited in 2009 as part of ToR k

18 Future activities of WGPDMO

There are several important issues in the field of pathology and diseases of marine organisms that require further consideration. It was agreed that a further meeting of WGPDMO is required in 2009 to consider the results of intersessional work, and to discuss new disease trends and new and outstanding items. The next meeting is planned for Riga, Latvia, during 3–7 March 2009.

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 new Terms of Reference for the 2009 WGPDMO meeting are appended in Annex 13.

20 Approval of the draft WGPDMO report

A rough draft of the 2008 WGPDMO report was approved before the end of the meeting and outstanding issues were identified and delegated to WGPDMO members. Information specifically sought by or provided to other ICES bodies will be extracted from the Terms of Reference conclusions and annexes and sent separately to the Chairs of the relevant ICES Working Groups.

21 Closure of the meeting

The Chair thanked the local host of the facility for providing excellent meeting facilities and arrangements and thanked the WGPDMO participants for their hard work and input during and in preparation of the meeting. The 2008 WGPDMO meeting was closed at 12:45 pm on 8 March 2008.

Annex 1: List of participants

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Annex 2: 2007 Terms of Reference

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: S. MacLean, USA) met 4–8 March 2008 at the Marine Institute in Oranmore, Galway, Ireland, to:

a) provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible by testing the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties. (OSPAR request 13)

b) The assessment should consider the prevalence of externally visible fish diseases, macroscopic liver neoplasms and liver histopathology in common dab (*Limanda limanda*).

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

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

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

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

g) review the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants.

h) provide an update of international collaborative actions involving fish and shellfish disease and pathology activities.

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

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

k) provide an update on the status of ICES publications on pathology and diseases of marine organisms.

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

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

Supporting information

Priority:	High. The development of the fish disease index has also increased the interest of HELCOM of developing the index into an indicator

Scientific justification and relation to action plan:	**Term of Reference a)** This is a response to OSPAR request no. 13. **Term of Reference b)** New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained. (all WGPDMO members) **Term of Reference c)** Epidemics of proliferative kidney disease with mass mortalities in wild salmonids are a major concern as a potential threat to the sustainability of anadromous populations. Information on the extent of fish mortalities, distribution of bryozoan hosts harbouring the infectious stage of the parasite, identification of salmonid populations at risk and improved understanding of the role of environmental factors, particularly temperature on the severity of epidemics, is urgently required. (T.A. Mo, S. Jones, and S. Feist) **Term of Reference d)** A systemic granulomatous disease in Atlantic cod (Gadus morhua) related to the presence of Francisella sp. has emerged and currently is one of the main bacterial diseases for farmed stock in Norway. This intracellular Gram negative bacterium has also been isolated from wild cod in Sweden. It is recommended that WPGDMO review the current status on control and diagnosis in farmed cod, the available literature regarding the potential for interaction between wild and farmed cod, and information on a visceral granulomatous condition in wild cod attributed to Francisella infection (T.A. Mo, A. Alfjorden, and D. Bruno) **Term of Reference e)** Hyperpigmentation has continued to increase in the common dab (Limanda limanda) populations in the North Sea. At the 2007 WGPDMO meeting a report was reviewed providing information on spatial and temporal trends in prevalence in North Sea areas, on histological and ultrastructural features of the condition, on host-specific effects on the prevalence and on effects of hyperpigmentation on the condition of the host. There still is a need for more information on pathology associated with hyperpigmentation, on possible causes of the condition (e.g. if pathogens are involved) and on similar conditions involving pigmentation anomalies in wild and farmed fish. Since more in depth studies on these issues are carried out at present, the results of which will be available for the next WGPDMO meeting, it is recommended to revisit the issue of hyperpigmentation at the 2008 WGPDMO meeting. (T. Lang, S.W. Feist, D. Bruno, W. Wosniok, and N. Ruane) **Term of Reference f)** The extensive use of chemotherapeutic agents against parasitic copepods in marine reared fish has led to a loss of susceptibility of sea lice to some of these agents. The limited availability of alternative medicines has raised a concern regarding the inevitability of increased tolerance. (S. Jones, T.A. Mo, and N. Ruane) **Term of Reference g)** WGPDMO is working collaboratively with other ICES and non-ICES groups in the field of diseases and pathology of marine organisms. It is always critical to keep WGPDMO members aware and able to review such international activities and report back to ICES. (T. Lang, S.W. Feist, N. Ruane, and A. Mansour)

Scientific justification and relation to action plan Continued:	**Term of Reference h)** The effective control of pathogens infecting molluscs requires diagnostic tests that are specific, reliable and sensitive, and that can discriminate between genera and species. Several methods are used to identify and characterise molluscan pathogens, among them, newly developed molecular methods appear very useful. Due to their specificity and sensitivity they allow species and strain identification. International standards proposed by the OIE now include such molecular techniques. Criteria used to identify molluscan pathogens should, however, include basic biological and ecological characteristics of pathogens as well as information on their genetic sequence. Thus, schemes for differential diagnosis incorporating molecular techniques have been developed. (T. Renault and S. Ford). **Term of Reference i)** This ToR might be of interest to HELCOM. For the 2007 meeting, the WGPDMO produced a report on the application of the Fish Disease Index (FDI) on externally visible diseases in common dab (Limanda limanda) and on the assessment of geographical areas by FDI-based criteria. It was recommended to extend this assessment to other species (e.g. Baltic cod and flounder) and to other sets of diseases (e.g. liver histopathology), for which empirical data is available, in order to assess the applicability of the FDI under more general conditions. (W. Wosniok and T. Lang). **Term of Reference j)** A number of ICES publications, either web-based or in ICES publication series, are being prepared or updated at present, the progress of which has to be reviewed by WGPDMO. It will be necessary to consider ways by which these can be linked to each other. New publications have to be considered. (S. Feist,T. Lang, and W. Wosniok) **Term of Reference k)** This is in compliance with a request from the ICES Data Centre. (W. Wosniok, T. Lang, S.W. Feist, D. Bruno)
Resource requirements:	None required, other than those provided by the host institute
Participants:	Represerntatives of all Member Countries and specialists invited by the Chair with expertise relevant to pathology and disease of wild and cultured finfish and shellfish. In total normally 20 participants.
Secretariat facilities:	Required to a limited extent, e.g. for data and publication issues
Financial:	None required
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	MCC, MHC, WGBEC
Linkages to other organizations:	BEQUALM, OIE, EU, OSPAR, HELCOM

Annex 3: Working Documents distributed prior to the Meeting

	2007 WGPDMO Terms of Reference	Working document (file)	Posted on SharePoint
b)	produce an update on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports;	WGPDMO2008_Canada_NatlReport WGPDMO2008_France_NatlReport WGPDMO2008_Iceland_NatlReport WGPDMO2008_Latvia_NatlReport WGPDMO2008_Netherlands_NatlReport WGPDMO2008_Norway_NatlReport WGPDMO2008_Poland_NatlReport WGPDMO2008_Denmark_NatlReport WGPDMO2008_Scotland_NatlReport WGPDMO2008_Sweden_NatlReport WGPDMO2008_Finland_NatlReport WGPDMO2008_Ireland_NatlReport WGPDMO2008_USA_NatlReport WGPDMO2008_England and Wales_NatlReport WGPDMO2008_Russia_NatlReport WGPDMO2008_Germany_NatlReport	2/15/08 2/15/08 2/15/08 2/15/08 2/15/08 2/15/08 2/15/08 2/20/08 2/20/08 2/20/08 2/21/08 2/21/08 2/21/08 2/25/08 2/27/08 2/28/08
c)	Review the status of proliferative kidney disease (PKD) epidemics caused by *Tetracapsuloides bryosalmonae* in wild salmonid populations (ToR c)		
d)	Review the information on *Francisella* sp. and visceral granulomatosis in farmed cod and the potential for disease interaction between wild and farmed cod.	WGPDMO_ToR_d_Francisella	2/29/08
e)	Provide a progress report on studies carried out on hyperpigmentation in common dab (*Limanda limanda*) from the North Sea with special reference to pathological findings and possible causes.	WGPDMO_ToR_e_hyperpigmentation	2/28/08
f)	Review the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants.	ToR f-reference-emamectin benzoate efficacy-Lees *et al.* 2008 WGPMDO_Tor_f-Sensitivity of sea lice	2/21/08 3/2/08
g)	Provide an update of international collaborative actions involving fish and shellfish disease and pathology activities.		
h)	Provide an update on the validation and integration of molecular diagnostic and confirmatory techniques for pathogens of bivalves.	WGPDMO 2008 Torh v2	3/2/08
i)	provide an update on the use of the fish disease index for other fish species (e.g. Baltic cod and flounder) and other sets of available disease daa (e.g. liver histopathology data).		
j)	Provide an update on the status of ICES publications on pathology and diseases of marine organisms.		
k)	Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre (possibly via sub-group) on a continuous basis.		

	2007 WGPDMO TERMS OF REFERENCE	WORKING DOCUMENT (FILE)	POSTED ON SHAREPOINT
	Terms of Reference	WGPDMO2008_TOR Wgpdmo recs 2007	 1/11/08
	Draft Agenda, Preliminary Attendee List, Draft Rapporteurs	WGPDMO2007Attendees-ToRs-Agenda-Rapporteurs	2/4/08
	Preliminary Meeting Information		12/7/07
	Additional Meeting Information-1		12/17/07
	Additional Meeting Information-2		1/18/08
	Additional Meeting Information -3		1/23/08
	Additional Meeting Information-4		2/5/08
	Additional Meeting Information-5		2/15/08
	Additional Meeting Information-6		2/21/08

Annex 4: Agenda

1) Opening of the meeting

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

3) ICES Annual Science Conferences 2007 and 2008 (input from members), and items of relevance to WGPDMO

- 3.1) 2007 ASC, MCC and ACOM meetings
- 3.2) 2008 ACS and Special Theme Session D on pathology and diseases of marine organisms
- 3.3) ICES Updated Strategic Plan in 2007
- 3.4) 2008 WGDEEP

4) Other relevant reports/activities for information

- 4.1) 2008 ICON meeting
- 4.2) NASCO *Gyrodactylus salaris* meeting

5) Provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible by testing the fish disease index developed by ICES and reported by WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties (OSPAR request 13) (ToR a)

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

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

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

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

10) Review the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants (ToR f)

11) Provide an update of international collaborative actions involving fish and shellfish disease and pathology activities (ToR g)

12) Provide an update on the validation and integration of molecular diagnostic and confirmatory techniques for pathogens of bivalves (ToR h)

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

14) Provide an update on the status of ICES publications on pathology and diseases of marine organisms (ToR j)

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

16) Any other business

- 16.1) Sea lice PPT presentation, S. Jones
- 16.2) WGPDMO members list

17) Analysis of progress with tasks

18) Future activities of WGPDMO

19) Approval of Recommendations

Annex 5: Rapporteurs

AGENDA ITEM(S)	2008 WGPDMO TERMS OF REFERENCE	RAPPORTEURS
1–4	Introductory session	S. MacLean
5	Provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible by testing the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties. (OSPAR request 13) (ToR a)	D. Bruno, N. Ruane, T. Lang
6	Produce a report on new disease trends in wild and cultured fish, molluscs and crustaceans, based on national reports (ToR b) • wild fish • farmed fish • wild and farmed shellfish	A. Alfjorden, D. Bruno, N. House, E. Bacevicius T.A. Mo, S. Jones, D. Cheslett, K. Kramer T. Renault, T. Lang, V. Öresland
7	Review the status of proliferative kidney disease (PKD) epidemics caused by *Tetracapsuloides bryosalmonae* in wild salmonid populations (ToR c)	S. Feist, M. Kirjuŝina, I. Briede
8	Review the information on *Francisella* sp. and visceral granulomatosis in farmed cod and the potential for disease interaction between wild and farmed cod (ToR d)	S. Jones, A. Alforden, L. Madsen
9	Provide a progress report on studies carried out on hyperpigmentation in common dab (*Limanda limanda*) from the North Sea with special reference to pathological findings and possible causes (ToR e)	N. Ruane, M. Podolska, K. Broeg, K. Kramer
10	Review the evidence for increased tolerance by *Lepeophtheirus salmonis* to chemotherapeutants (ToR f)	L. Madsen, D. Cheslett, S. MacLean
11	Provide an update of international collaborative actions involving fish and shellfish disease and pathology activities (ToR g)	I. Briede, M. Podolska, T. Lang
12	Provide an update on the validation and integration of molecular diagnostic and confirmatory techniques for pathogens of bivalves (ToR h)	K. Broeg, S. Feist, T. Renault
13	Provide an update on the use of the fish disease index for other fish species (e.g. Baltic cod and flounder) and other sets of available disease data (e.g. liver histopathology data) (ToR i)	T.A. Mo, N. Ruane, W.Wosniok
14	Provide an update on the status of ICES publications on pathology and diseases of marine organisms (ToR j)	T. Lang, V. Öresland, N. House
15	Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis (ToR k)	M. Kirjuŝina, S. MacLean, E. Bacevicius
16	Other business	D. Bruno, K. Broeg
17–21	Analysis of progress with tasks, future activities of WGPDMO, approval of recommendations, approval of draft report, closing of the meeting	S. MacLean

Annex 6: Assessment of ICES fish disease data for the OSPAR Quality Status Report 2010 using the Fish Disease Index (FDI) approach (ToR a)

T. Lang and W. Wosniok

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

Background – OSPAR Request 2008/13

WGPDMO 2008 Term of Reference a) provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible by testing the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties. **(OSPAR Request 13)**;

The assessment should consider the prevalence of externally visible fish diseases, macroscopic liver neoplasms and liver histopathology in common dab (*Limanda limanda*).

Introduction

At its meetings in 2006 and 2007, WGPDMO reviewed progress made in the development of a Fish Disease Index (FDI), a tool designed to be used for the assessment of data on diseases of wild fish in the ICES area (ICES 2006, 2007a).

The FDI was constructed mainly for data obtained from regular disease surveys in dab (*Limanda limanda*) on externally visible diseases, macroscopic liver neoplasms and liver histopathology. Such studies have been carried out by Denmark, Germany, The Netherlands and the UK in the North Sea and adjacent areas and data generated have been submitted to the ICES Data Centre. The FDI is also applicable to other species and geographical areas after modification. It consists of the following components.

- data on the presence or absence of a range of externally visible diseases and parasites, macroscopic liver neoplasms and liver histopathology;
- data on the severity of the diseases (disease grades);
- disease-specific weighting factors assigned by expert judgment on the basis of the effects of the diseases on the host;
- adjustment factors for confounding entities (length, sex and season).

The calculations involved result in scores for each of the diseases considered which are summarised into the final FDI for an individual fish. This score already contains adjustments for length and sex as well as a disease-specific weighting scheme. From the individual FDIs, mean values in a population in a given sampling area can be calculated and appropriate statistical analyses can be conducted.

In 2007, the FDI constructed was applied on empirical data derived from fish disease surveys carried out by Germany in the North Sea and adjacent areas. Using the common dab in the North Sea as a model, the externally visible lesions of the following diseases were used to illustrate the FDI: lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulceration, x-cell gill disease, hyperpigmentation, acute/healing fin rot/erosion, and the parasites *Stephanostomum baccatum*, *Acanthochondria cornuta*, and *Lepeophtheirus pectoralis*. Macroscopic liver neoplasms and histo-

pathological liver lesions were not considered at this stage but are intended to be added at a later stage.

For trend assessment, mean FDI values were adjusted for the season of data collection, additionally to the previous adjustment for sex and length. From these values an assessment statistic was calculated, which jointly accounts for the FDI level and trend. The level component of the statistic was obtained by dividing the FDI range into three equally sized intervals, using tertiles (the 33% and 66% percentile) as cut-points. FDI means were weighted by -1, 0, +1, according to their position in the lower, middle and upper interval, respectively. The sum of these weights, scaled to lie in the range (-1, +1), served as the level component of the test statistic. For the trend component of the statistic, a scaled version of the Mann-Kendall trend test statistic was used. The scaled version has values in the range (-1, +1), as the level component. Then the FDI assessment statistic was calculated as (0.5*level component + 0.5*trend component). The factor 0.5 was introduced only to arrive at a test statistic with values in the interval (-1, +1), i.e. for aesthetical reasons.

According to the resulting statistic, different "smiley faces" were assigned to individual geographical regions (ICES statistical rectangles) with a sufficient amount of disease data. A $p < 0.025$ resulted in a "green smiley face"; $0.025 < p < 0.975$ in a "yellow indifferent face"; and $p > 0.975$, in a "red frowny face". These faces, placed on a chart of the ICES statistical rectangles in the North Sea, provided a visual general assessment of levels and trends in overall disease status.

Relevance of the FDI for Regulatory Commissions (OSPAR, HELCOM)

The development of the FDI and related assessment criteria is of particular relevance for the international Regulatory Commissions since fish disease monitoring is part of the OSPAR Coordinated Environmental Monitoring Programme (CEMP) and since the FDI approach has been adopted in the ICES/OSPAR WKIMON process as component of integrated monitoring and assessment (ICES 2007b). Furthermore, HELCOM is developing indicators of ecological quality and related targets for monitoring and assessment in order to meet the goals of the Baltic Sea Action Plan and has requested to develop a fish disease indicator to be used in the Baltic Sea in relation to the assessment of effects of hazardous substances (HELCOM 2006). Therefore, ICES recommended that OSPAR, HELCOM and ICES Member Countries take note of the progress achieved in relation to the Fish Disease Index (FDI) and the related assessment criteria (ICES 2007c).

The current OSPAR CEMP encompasses, e.g. monitoring of contaminants and their general and contaminant-specific biological effects that should be carried out by OSPAR contracting parties either on a mandatory or on a voluntary basis. Fish disease studies are part of both the CEMP general biological effects monitoring and the PAH-specific biological effects monitoring (see Table 1) (OSPAR 2005).

Table 1. Fish disease monitoring requirements of the OSPAR Coordinated Environmental Monitoring Programme (CEMP).

CEMP – General biological effects monitoring	CEMP – PAH-specific biological effects monitoring	Priority fish species	Status within CEMP
Externally visible diseases	-	Dab (*L. Limanda*) Flounder (*P. flesus*) Cod (*G. morhua*) Whiting (*M. merlangius*)	I: voluntary (QA in place, assessment criteria not yet in place)
Macroscopic liver neoplasms	Macroscopic liver neoplasms	Dab (*L. Limanda*) Flounder (*P. flesus*) Dragonet (*Callionymus* spp.)	I: voluntary (QA in place, assessment criteria not yet in place)
Liver histopathology	Liver histopathology	Dab (*L. Limanda*) Flounder (*P. flesus*) Dragonet (*Callionymus* spp.)	I: voluntary (QA in place, assessment criteria not yet in place)

Since one of the requirements for fish disease monitoring becoming a mandatory part of the CEMP is the establishment of assessment criteria and since OSPAR is planning to include a section on fish diseases in its Quality Status Report (QSR) 2010, ICES – as part of its work programme for 2008 – was requested by OSPAR

> *…to provide an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible by testing the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties. (OSPAR request 13);*
>
> *The assessment should consider the prevalence of externally visible fish diseases, macroscopic liver neoplasms and liver histopathology in common dab (Limanda limanda).*

In order to meet the OSPAR request, ICES Member Countries running regular fish disease surveys in the OSPAR area or those that hold historic data generated according to the ICES standard guidelines were requested in 2007 to submit their complete set of fish disease data to the fish disease databank of the ICES Environmental Data Centre by using the new ICES Integrated Environmental Reporting Format Version 3.2.3 (http://www.ices.dk/env/repfor/ERF323.doc).

Diseases to be included in the construction of the Fish Disease Index (FDI)

According to its original construction (ICES, 2006), the Fish Disease Index includes the disease categories and key diseases shown in Table 2. Ideally, the data used should not only include information on the presence or absence of the key diseases but – if present – also on their severity (according to three defined grades). Such data, however, only exist for certain diseases (see below).

Table 2. Disease categories and key diseases to be used for calculating the Fish Disease Index for dab (*Limanda limanda*) (ICES, 2006).

Disease categories and key diseases for constructing the Fish Disease Index (FDI)		
Externally visible diseases	Macroscopic liver neoplasms	Liver histopathology
Lymphocystis Epidermal hyperplasia/papilloma Acute/healing ulceration, X-cell gill disease, Hyperpigmentation Acute/healing fin rot/erosion *Stephanostomum baccatum* *Acanthochondria cornuta* *Lepeophtheirus pectoralis*	Benign neoplasms Malignant neoplasms	Non-specific lesions Early non-neoplastic toxicopathic lesions Pre-neoplastic lesions (FCA) Benign neoplasms Malignant neoplasms

ICES fish disease data available for the assessment

In contrast to the previous fish disease assessment in 2007 that was done using only data on externally visible fish diseases from the German fish disease monitoring programme (ICES, 2007 a), the goal for the present assessment was to include data from all countries and, in addition to data on externally visible diseases, also on macroscopic liver neoplasms and on liver histopathology.

After all data had been submitted to ICES as requested (final submissions in mid January 2008), the fish disease databank was checked for data available for the assessment. The majority of information covers disease data generated in studies on dab from the North Sea and adjacent areas. The time span covered is from 1981 until 2007. Disease data are also available for flounder (*Platichthys flesus*), cod (*Gadus morhua*) and whiting (*Merlangius merlangus*), however, to a much lesser extent. These were not considered in the analysis and assessment.

Table 3 provides details on the total number of dab examined per year, submitting labs, the time spans covered by individual data sets, disease categories monitored (externally visible diseases, macroscopic liver neoplasms, liver histopathology) and as to whether the data submitted meet the original criteria defined for calculating the FDI and for the assessment (ICES, 2007a).

Table 3. Data on externally visible diseases (EVD), macroscopic liver neoplasms (MLN) and liver histopathology (LH) in dab (*Limanda limanda*) submitted by laboratories in ICES Member Countries to the ICES Data Centre (green: full set of diseases for FDI; yellow: only selected set of diseases).

Year	n ex.	Germany			UK									The Netherlands						Denmark		
		BFCG			ALUK			DOUK			BODC			DGWN			RIVO			DFHU		
		EVD	MLN	LH	EVD	MLN	LH	EVD	MLN	LH	EVD	MLN	LH	EVD	MLN	LH	EVD	MLN	LH	EVD	MLN	LH
1981	25590																					
1982	26810																					
1983	21305																					
1984	27750																					
1985	18652																					
1986	7893																					
1987	28906																					
1988	24634																					
1989	18522																					
1990	21822																					
1991	31935																					
1992	35211																					
1993	24362																					
1994	9361																					
1995	9510																					
1996	12152																					
1997	8692																					
1998	17527																					
1999	11485																					
2000	14211																					
2001	12920																					
2002	25449																					
2003	22915																					
2004	22506																					
2005	20508																					
2006	22276																					
2007	13304																					
n ex.	536208	310368			67.384			48.669			18.234			10.614			18.028			62.911		

BFCG: Federal Research Centre for Fisheries, Cuxhaven, Germany; **ALUK**: FRS Marine Laboratory, Aberdeen, UK; **DOUK**: Cefas, Weymouth, UK; **BODC:** British Oceanographic Data Centre**; DGWN:** ???, The Netherlands**; RIVO**: The Netherlands Institute for Fisheries Research**; DFHU**: Danish Institute for Fisheries and Marine Research

As can be seen from Table 3, the data are fragmented in that:

The time spans covered differ considerably and, thus, there is no period of time with data from all submitting labs.

- There is only a limited amount of most recent data (2006–2007).
- The majority of data submitted only concerns externally visible diseases. Only relatively few data on macroscopic liver neoplasms are available and data on liver histopathology are completely lacking in the ICES fish disease databank so far.
- The majority of data sets do not meet the original criteria defined for the FDI calculation, because either data do not cover all of the diseases/parasites meant to be included or data on severity grades are lacking.

Furthermore, there is indication that the data submitted to ICES using the Environmental Data Reporting Format 3.2.2 do not comprise all the data that had earlier been submitted in other formats. This must be concluded from comparing the counts from earlier analyses to the counts presently found (see Section 15 of the present WGPDMO report).

Modification of the Fish Disease Index construction

The findings from the screening of the ICES fish disease databank obviously have an impact on the assessment requested by OSPAR because it became clear that, because of the lack of data, the FDI as originally constructed could not be used for the data available. Based on this, the following modifications were made to the construction of the FDI in order to use as many fish disease data submitted as possible:

Three different FDIs based on different sets of diseases were calculated:

- FDI-EVD3, using the externally visible diseases (EVD) lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcers. This definition was chosen to allow an FDI calculation for as many ICES rectangles as possible.
- FDI-EVD9, using all 9 externally visible diseases given in Table 2. This is the set of diseases used in previous FDI calculations.
- FDI-EVD4, using the same externally visible diseases as FDI-EVD3 plus data on macroscopic liver neoplasms (MLN). This definition combined liver pathology data, as requested by Tor a), and the set of EVDs which otherwise allowed an FDI calculation for as many ICES rectangles as possible. These four diseases also represent the combination that allows an FDI calculation for as many rectangles as possible under the condition that macroscopic liver neoplasms are included.

All diseases entered the FDI calculation as ungraded (presence/ absence) data because of the lack of disease grades in a large part of the data. Data that had been reported as graded data was transformed to ungraded information.

Strategy for FDI calculation and assessment

The time frame for the inclusion and the necessary amount of data were set as follows:

- Only samplings between 1 January 1991 (starting point) and December 31, 2007 were included. A sampling is defined as "all catching done on one day in one ICES rectangle".
- For each FDI, only ICES rectangles with at least 10 samplings since the starting point and with determination of all the diseases used in that FDI were considered.

With these settings, FDI calculations and assessments were possible for 17 (FDI_EVD3), 10 (FDI_EVD9), and 6 (FDI_EVD4) ICES rectangles, respectively.

Disease-specific weighting factors were obtained from expert judgements on disease severity, using the Bradley-Terry approach to derive the weighting factors from the pairwise comparisons provided by the experts. Judgements from 5 experts were available and used for this calculation. The MLN data does not differentiate between benign and malignant tumours, but as the large majority of MLN cases consists of benign tumours, all reported MLN observations were considered as benign and associated with the corresponding disease weight. Adjustment factors for confounding entities (length, sex and season) where computed as reported previously (ICES 2007a, p.71–77). Also the p level for the assessment statistic and the resulting "smiley category" (green, yellow, red) were calculated as described there and summarized in the introduction above.

Results of the assessment of the ICES fish disease data using the Fish Disease Index (FDI) approach

The results of the application of the Fish Disease Index approach can be seen in Figures 1a-c and 2a-c.

Figures 1a-c illustrates the results regarding the temporal changes in the FDI as well as the assessment of the levels and changes recorded since 1 January 1991. Figure 1a shows the results for the approach including only data on the full set of 9 externally visible diseases (FDI_EVD9), Figure 1b for the set of only 3 externally visible diseases (FDI_EVD3), Figure 1c for the combination of 3 externally visible diseases and macroscopic liver neoplasms (FDI_EVD4).

Figures 2a-c provides the maps showing the assessment results for the 3 approaches above on a geographical basis.

Some major differences can be deduced from the results shown:

- By using the approach with only 3 externally visible diseases, an assessment could be made for 17 ICES statistical rectangles. Some major fluctuations in the FDI values were observed in single ICES rectangles but in most ICES rectangles (15 out of 17) no upward or downward trend occurred (Figure 1a). Only in two areas downward trends were observed. Consequently, yellow smileys indicating no trends were assigned to 15 and green smileys indicating a significant downward trend to 2 rectangles (Figure 2a). From this model, there is no indication for major regional differences or trends between ICES statistical rectangles.
- The approach using the full set of 9 externally visible diseases resulted in an assessment for 10 ICES statistical rectangles (Figure 1b). Again, some strong fluctuations were observed in single rectangles. In contrast to the approach with only 3 externally visible diseases, the majority of rectangles (7) was characterised by a significant upward trend in FDI, resulting in the assignment of red smileys (Figure 2b). From this model, there is indication for both regional differences between ICES statistical rectangles and for trends.
- The third approach with the same 3 externally visible diseases as above in combination with macroscopic liver neoplasms resulted in an assessment for only 6 rectangles (Figure 1c). Four out of these showed no trend (yellow smileys) and two a downward trend (green smileys) (Figure 2c). Due to the low number of rectangles no conclusions about regional or temporal patterns can be drawn.

Conclusions

From the application of the Fish Disease Index (FDI) to the ICES fish disease data there is evidence that the FDI approach is an appropriate tool for the analysis and assessment of fish disease data generated within monitoring programmes according to established ICES guidelines. It is considered ready for application in the OSPAR CEMP context.

There is evidence that a considerable amount of disease data available in national databanks is still missing in the ICES fish disease databank This is partly due to the fact that data have not yet been submitted (e.g. data on liver histopathology) or due to problems related to conversion or submission of data using the ICES Environmental Data Reporting Format 3.2.

The fish disease data submitted to the ICES Data Centre so far were not sufficient to conduct an overall assessment of levels and trends as planned by using the full FDI approach with all of its components. Reasons were: national data cover different time periods and different sets of diseases, lack of data on severity grades, patchy regional coverage. However, since the FDI can technically be modified in a way that still enables an assessment (i.e. by reducing the number of diseases considered, neglecting disease severity grades and considering the three disease categories (externally visible diseases, macroscopic liver neoplasms, liver histopathology) separately), an analysis and assessment was carried out with the data available, the results of which have to be considered as preliminary, however.

The results of the assessment using different approaches (set of diseases included in the construction of the FDI) clearly show that the temporal changes in the FDI and the resulting assessment of the disease status strongly depend on the set of diseases included in the construction of the FDI, because the diseases show a different behaviour in terms of levels and trends. Therefore, it is not sufficient to consider only few diseases. In contrast, it is advisable to include as many diseases as possible in the FDI because only then a comprehensive assessment of the overall disease status is possible.

A decision on the most appropriate FDI approach and a more comprehensive assessment of the fish disease data will only be possible once all data available in national databanks have been submitted to the ICES Data Centre and have been analysed. Ways will be explored with data originators and the ICES Data Centre as to how this can be achieved in a timely fashion in order to meet the OSPAR requirements for the QSR 2010. It is anticipated that WGPDMO will revisit this issue at its 2009 meeting.

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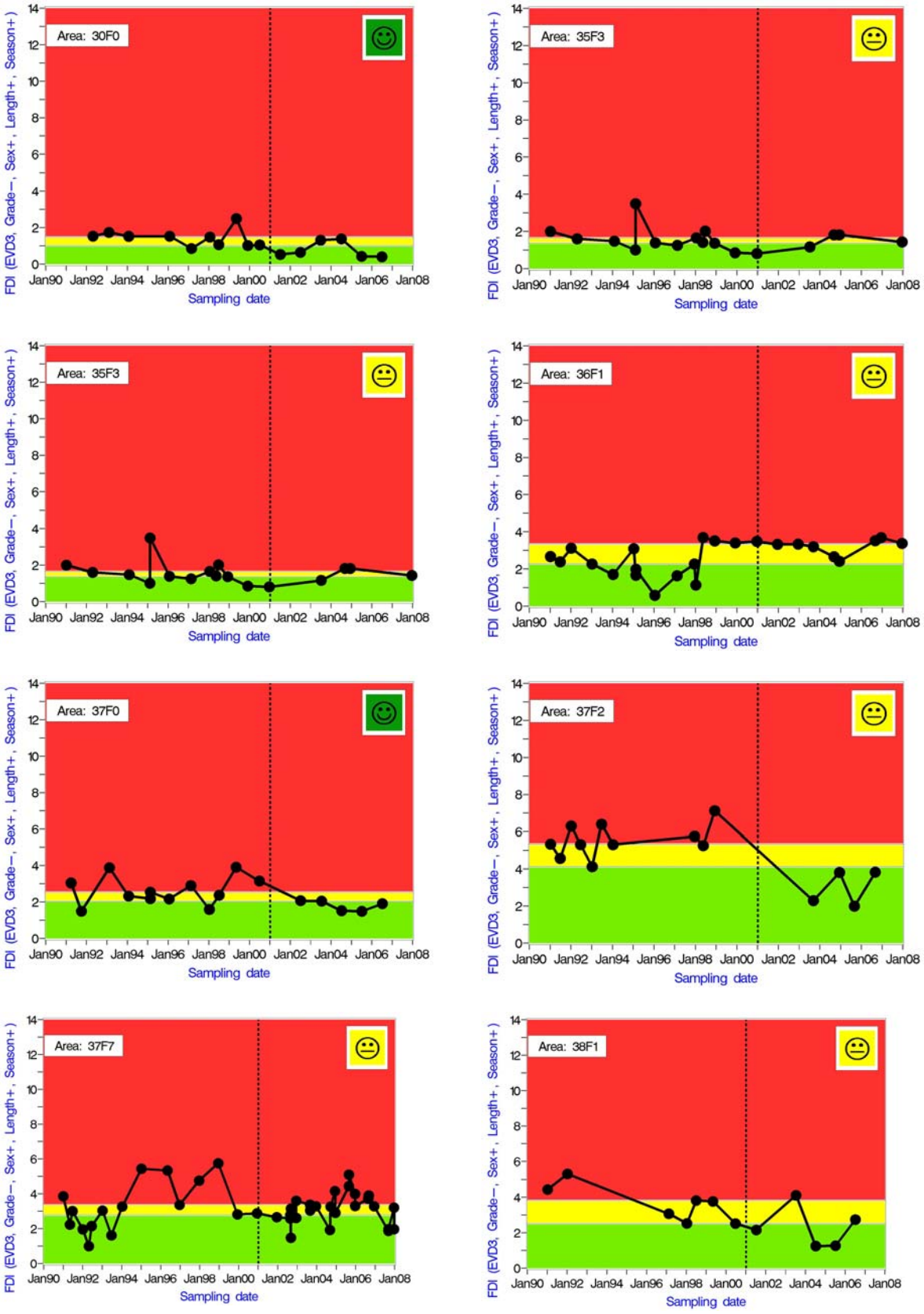

Figure 1a. Temporal changes in the FDI and assessment of levels and trends in ICES statistical rectangles (based on 3 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations)) Note: data set is incomplete!

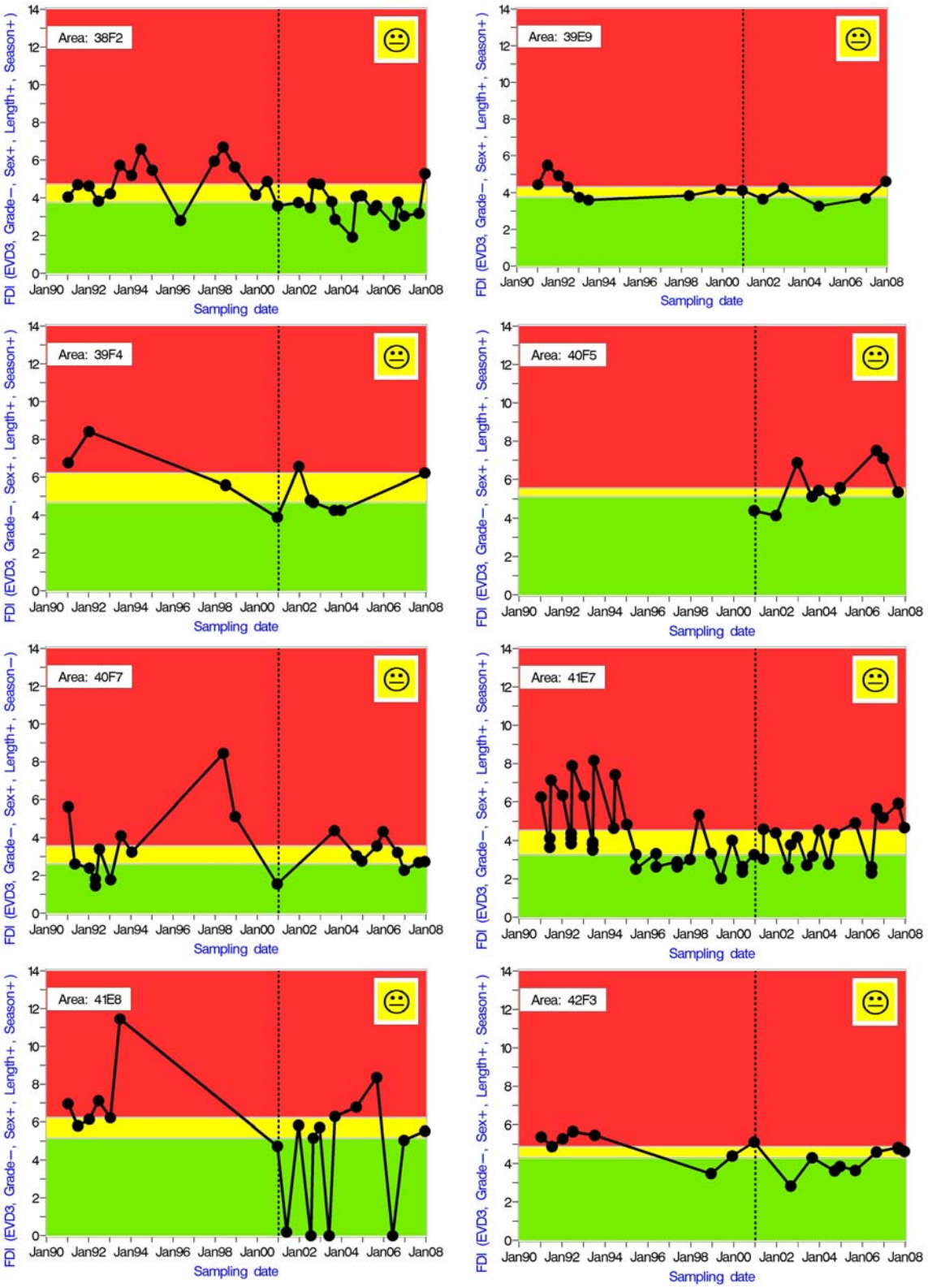

Figure 1a (cont.). Temporal changes in the FDI and assessment of levels and trends in ICES statistical rectangles (based on 3 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations)) Note: data set is incomplete!

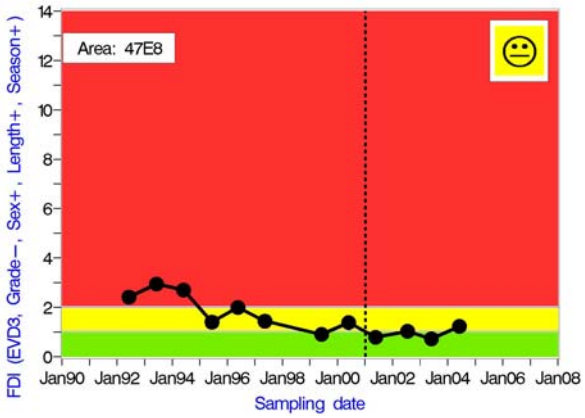

Figure 1a (cont.). Temporal changes in the FDI and assessment of levels and trends in ICES statistical rectangles (based on 3 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations)) Note: data set is incomplete!

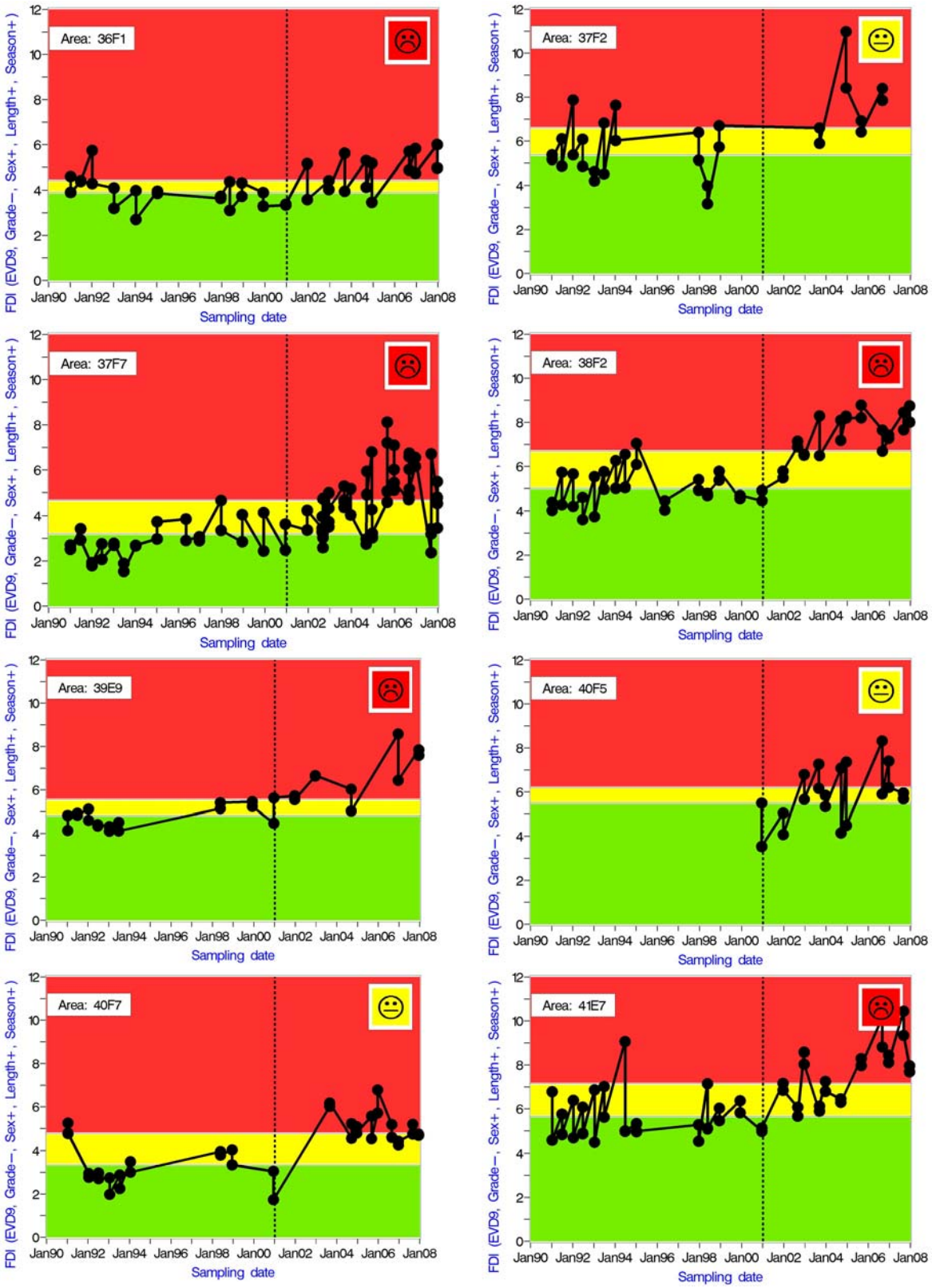

Figure 1b. Temporal changes in the FDI and assessment of levels and trends in ICES statistical rectangles (based on 9 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulceration, X-cell gill disease, hyperpigmentation, acute/healing fin rot/erosion, *Stephanostomum baccatum*, *Acanthochondria cornuta*, *Lepeophtheirus pectoralis*)) Note: data set is incomplete!

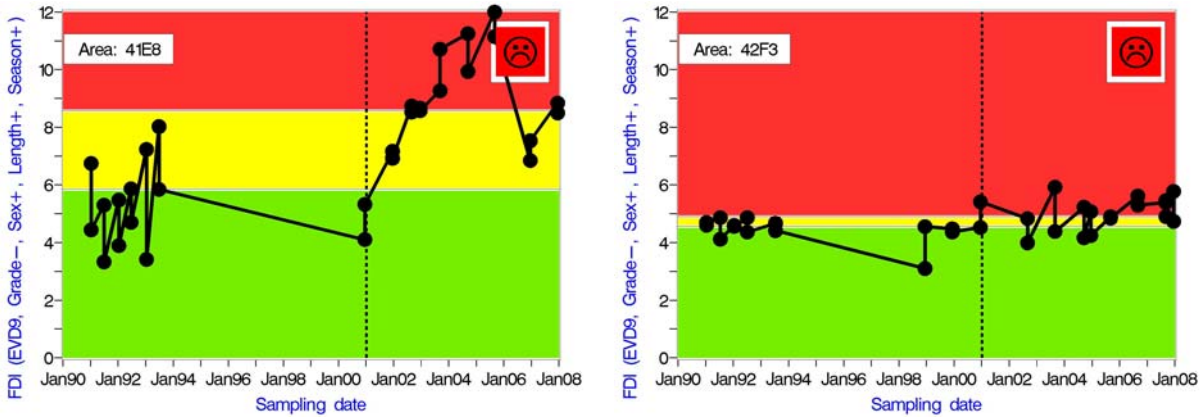

Figure 1b (cont.). Temporal changes in the FDI and assessment of levels and trends in ICES statistical rectangles (based on 9 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulceration, X-cell gill disease, hyperpigmentation, acute/healing fin rot/erosion, Stephanostomum baccatum, Acanthochondria cornuta, Lepeophtheirus pectoralis)) Note: data set is incomplete!

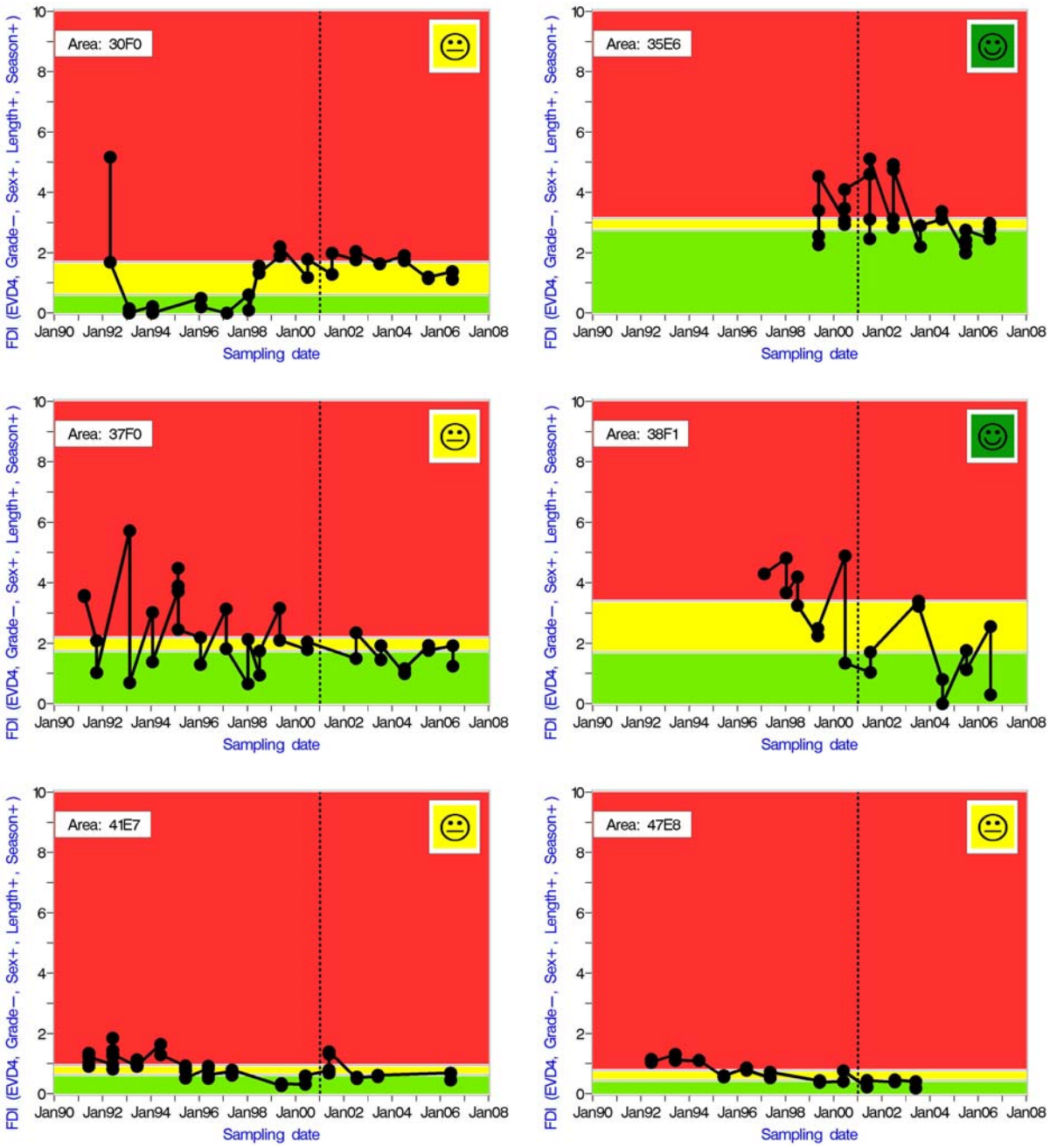

Figure 1c. Temporal changes in the FDI and assessment of levels and trends in ICES statistical rectangles (based on the combination of 3 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations) and macroscopic liver neoplasms) Note: data set is incomplete!

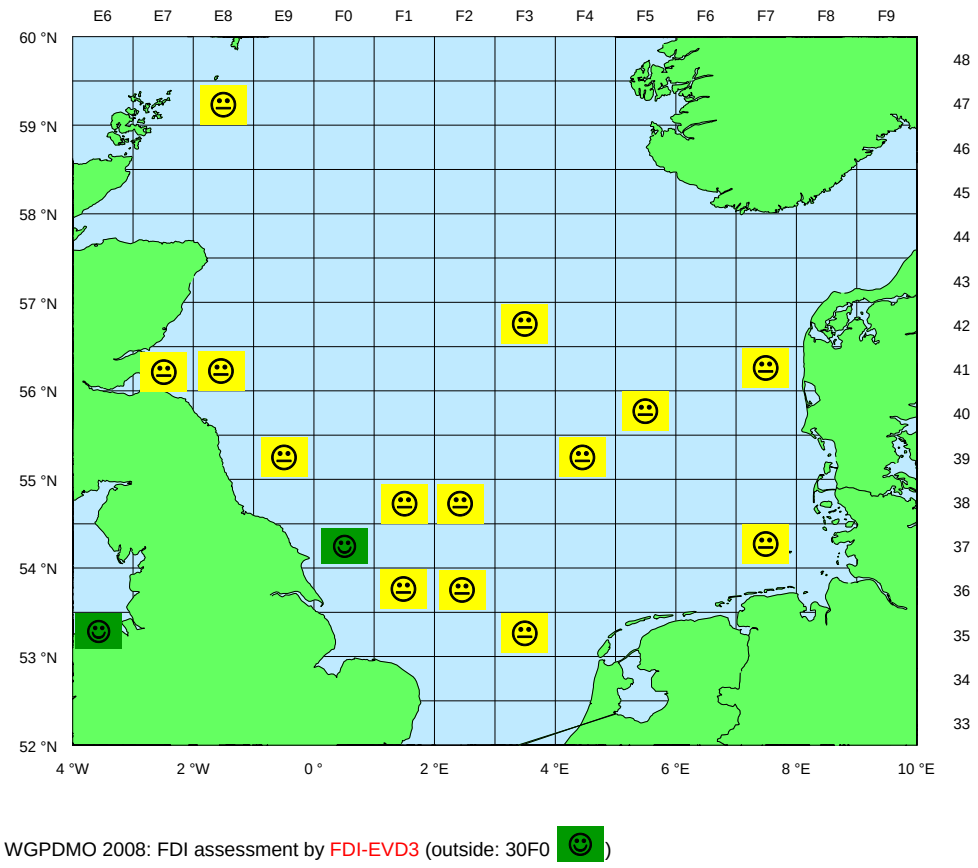

Figure 2a. Assessment of levels and trends in ICES statistical rectangles (based on 3 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations)) Note: data set is incomplete!

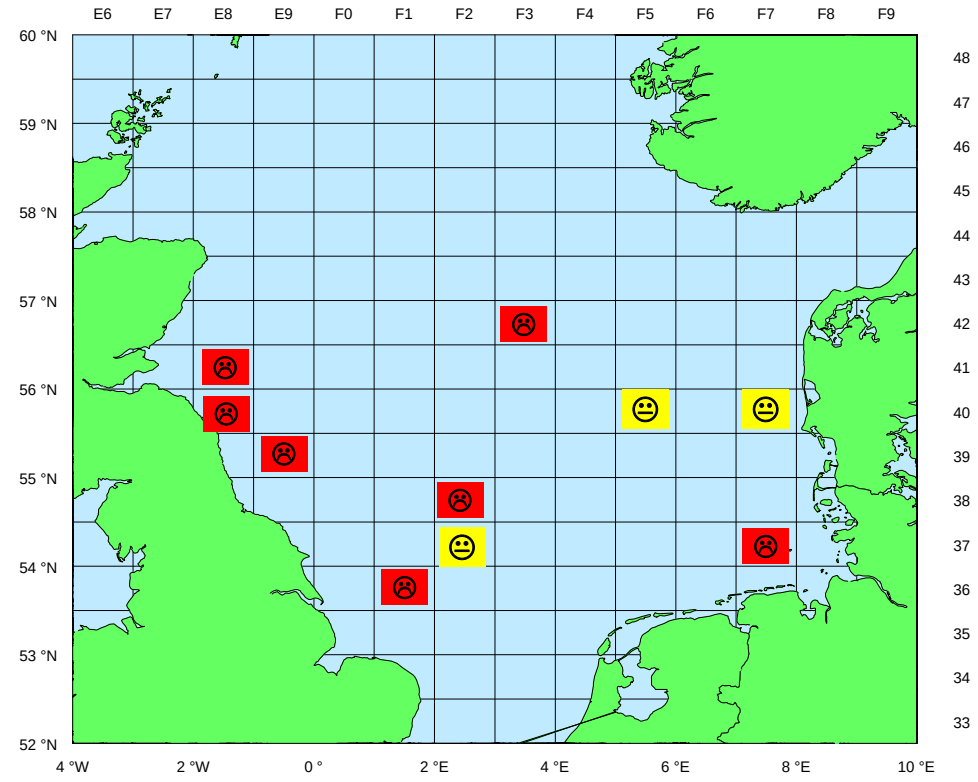

Figure 2b. Assessment of FDI levels and trends in ICES statistical rectangles (based on 9 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing ulceration, X-cell gill disease, hyperpigmentation, acute/healing fin rot/erosion, *Stephanostomum baccatum*, *Acanthochondria cornuta*, *Lepeophtheirus pectoralis*)) Note: data set is incomplete!

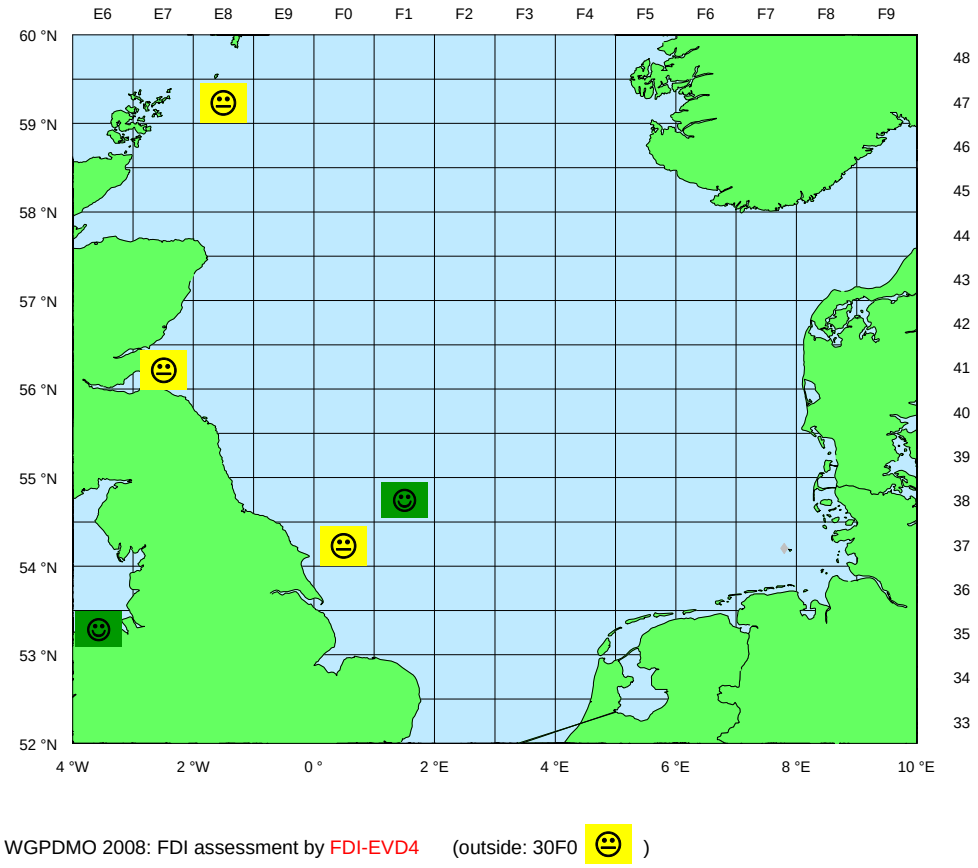

Figure 2c. Assessment of FDI levels and trends in ICES statistical rectangles (based on the combination of 3 externally visible diseases (lymphocystis, epidermal hyperplasia/papilloma, acute/healing skin ulcerations) and macroscopic liver neoplasms) Note: data set is incomplete!

Annex 7: A review of the status of proliferative kidney disease (PKD) epidemics caused by Tetracapsuloides bryosalmonae in wild salmonid populations (ToR c)

T.A. Mo, S. Jones, and S. Feist

Background

Proliferative kidney disease (PKD) caused by the malacosporean Tetracapsuloides bryosalmonae has long been recognized as an important disease of mainly salmonid fishes, particularly in aquaculture situations (Hedrick *et al*., 1993). In Europe, during the expansion of rainbow trout culture in the 1970s, the disease caused serious losses in hatcheries and several ongrowing sites and has remained a constraint to trout production to the present day. Phylogenetic analysis of the internal transcribed spacer 1 (ITS-1) rDNA region from samples collected from several European and North American locations indicates the existence of a North American clade with a subset of Italian and French sequences and a separate clade of isolates from the rest of Europe (Henderson and Okamura, 2004). This suggests that there was historic colonization from North America to southern Europe pre-dating fisheries movements. The parasite persists in bryozoan colonies throughout the year (Gay *et al*., 2001; Tops *et al*., 2004), proliferating as infected colonies increase growth with the rise in temperatures during the spring and summer (Tops *et al*., 2006). Infection of fish hosts occurs following contact with spores and the entry of the sporoplasms into the fish through the skin and gill epithelium, either interepithelially or intracellularly via mucous cells (Morris *et al*., 2000; Longshaw *et al*., 2002). Parasites are usually observed within renal interstitial tissues about 4 weeks (temperature dependent) post exposure with lesions developing shortly thereafter. Recent data has shown that spores produced in the renal tubules of infected fish are infective to bryozoans, thereby completing the life-cycle in the two hosts. This result has significant implications for decisions for stocking fish into areas thought to be free of PKD.

PKD has been observed in wild salmonids (Seagrave *et al*., 1981) but until recently the impact on wild populations was uncertain. The apparent correlation between observations of PKD and significant loss in brown trout populations in Switzerland is of great concern (Wahli *et al*., 2002, Burkhardt-Holm *et al*., 2005) and is the subject of ongoing research (Borsuk *et al*., 2006). In the United Kingdom, studies on the disease affecting brown trout have also demonstrated widespread occurrence and correlation with macroscopic disease signs and internal pathology (Feist *et al*., 2002; Peeler *et al*., 2008 (in press)), yet mortalities in wild populations have not been reported.

Outbreaks of PKD are positively correlated to water temperature. Temperatures above 15 deg C for more than 14 days seem to be necessary for the disease outbreak (Hedrick *et al*., 1993, Tops *et al*., 2006) and the outbreak is likely enhanced at higher temperatures. However, this probably could vary between geographical areas. The causative myxozoan parasite *Tetracapsuloides bryosalmonae* may be widely spread in European bryozoan (final host) and salmon populations (intermediate host) but water temperature may in general be so low that outbreaks are not observed at the salmonid population level. However, increased water temperatures due to climate changes may increase the occurrence of PKD.

In Norway, PKD outbreaks in fresh water farms producing brown trout and salmon for stocking in lakes and river have been observed for many decades. However, before 2006, PKD had only been demonstrated in one salmon river. Mortality among

salmon parr due to PKD was observed in the River Håelva in 1988 and 1990 but any effect on the salmon population was not studied. In 2006, severe mortality among yearlings of brown trout and Atlantic salmon was observed in the River Jølstra, western Norway and in the River Åelva, mid-Norway. PKD-induced mortality in the river Åelva has been estimated to be 50–85% (Sterud *et al*., 2007). In 2007, the mortality due to PKD among brown trout and salmon continued in the River Åbjøra and to lesser extent in the River Jølstra, probably because the summer was relatively cold and rainy.

In the autumn 2007, screening for the presence of *T. bryosalmonae* was performed in 16 Norwegian watersheds. Most of the selected rivers had experienced an unexplained reduction of the Atlantic salmon and/or sea trout populations and/or there was a lake with a large slow flowing section in the anadromous part of the watershed. The PKD parasite was found to be present in 15 of the selected watersheds (Forseth *et al*., 2007) but so far an eventual parasite-induced mortality to the salmonids has not been studied.

PKD remains a constraint to salmonid aquaculture and there is increasing evidence that the parasite can have a significant effect on wild populations. In particular, in situations where conditions are suitable for proliferation of the bryozoan hosts and increased temperatures facilitate multiplication of the parasite in the bryozoan hosts and subsequent release of infective stages to salmonid hosts.

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Annex 8: Review of *Francisella* sp. and visceral granulomatosis in farmed cod and the potential for disease interaction between wild and farmed cod (ToR d)

T. A. Mo, A. Alfjorden, D. Bruno and L. Madsen

A systemic granulomatous disease in Atlantic cod (*Gadus morhua*) related to the presence of *Francisella* sp. has emerged and currently is one of the main bacterial diseases for farmed stock in Norway. This intracellular Gram negative bacterium has also been isolated from wild cod in Sweden. It was recommended that the WPGDMO review the current status on control and diagnosis in farmed cod, the available literature regarding the potential for interaction between wild and farmed cod, and information on a visceral granulomatous condition in wild cod attributed to *Francisella* infection.

Background

Norway

In 2004 and 2005 a new bacterial disease was observed in several cod farms along the west coast of Norway. Granulomas were found in internal organs and even in somatic musculature of diseased cod. Mortalities were high for a long period. In 2005, two different research institutions identified the causative organisms to the genus *Francisella*. Later, the bacterium was described as *Francisella philomiragia* subsp. *noatunensis*. The numbers of confirmed francisellosis outbreaks in Norwegian cod farms recorded by the National Veterinary Institute were 4, 7, and 8 in the years 2005, 2006 and 2007, respectively. In addition, several cases with suspected francisellosis were found but not confirmed by bacterial identification (see below). It is also known there have been several suspected outbreaks that have not been reported, as the disease is not yet notifiable. Many farmers have chosen to slaughter their fish to avoid further losses and transmission of bacteria to wild fish and other cod farm localities. Experiments have shown that the bacterium is easily transmissible between individual fish and movements of sub-clinically infected fish create a great risk for spread of the bacterium. *Francisella* sp. has been isolated from whitish nodules in internal organs in wild cod from several localities in Norway.

Sweden

During the summer of 2004 local fishermen reported an increased occurrence of Atlantic cod with skin ulcers from the archipelago of the west coast of Sweden, southern Skagerrak. Monitoring surveys organized by the Swedish Board of Fisheries during the end of summer and autumn reported up to 20% of small cod (one year class) caught in fish traps in the area with skin ulcers. Cod with ulcers caught in the archipelago of Lysekil at the west coast were investigated by the National Veterinary Institute of Sweden. The diseased fish showed both external lesions involving the skin and also white nodules in most of the internal organs: heart, kidney, liver and spleen. Histopathological investigations demonstrated a massive occurrence of granulomas of various sizes and stages. In 2005 samples were sent to Norway, where histopathological similarities with the granulomatous disease in farmed cod were noted. Molecular studies in association with the National Veterinary Institute, Norway, indicated the presence of a *Francisella*-like bacterium within tissue samples. Later in 2006 a small Gram-negative coccoid rod was isolated after initial inoculation of frozen tissue (heart, kidney or liver) on a CHSE-214 cell line. The isolated strain

(Ö391) showed high similarity values (99.9–100%) with the Norwegian *Francisella* strain 2005/50/F292. There are no cod farming activities ongoing in Sweden.

Scotland

Francisella has not been noted or described from farmed cod in Scotland, although in context there are very few farms.

Denmark

In 2006 a project was initiated with the purpose to find and validate the optimal growth conditions for cod in land-based recirculation systems in Denmark. Atlantic cod were imported from Norway at a weight of about 175 g (Batch 1) in March 2006. Another batch of cod was imported from Norway in August 2006 at a weight of about 5 g (Batch 2). Cod were sampled regularly and examined for the occurrence of pathogenic bacteria and viruses. The cod from Batch 1 examined in March 2007 had eye damages, ulcers and nodules in the internal organs, resembling pathological changes caused by *Francisella* sp. Analyses of tissue samples from the cod for the16S rRNA- and FopA-genes of *Francisella* sp. done by Are Nylund and Karl Ottem, University of Bergen, Norway, confirmed that the cod were infected with *Francisella* sp.. Cod from Batch 1 were euthanized with carbon dioxide and destroyed. *Francisella* sp. could also be found in Batch 2 cod sampled in June and July 2007. These cod had nodules in the internal organs.

Host susceptibility

Francisella spp. have been observed in several fish species; several tilapia species (*Oreochromis spp*.) (Hsieh, Tung, Tu, Chang and Tsai 2006), three-line grunt (*Parapristipoma trilineatum*)(Kamaishi, Fukuda, Nishiyama, Kawakami, Matsuyama, Yoshinaga and Oseko 2005), hybrid striped bass (*M. saxatilis x Morone chrysops*) (Ostland, Stannard, Creek, Hedrick, Ferguson,Carlberg and Westerman 2006) and Atlantic salmon (*Salmo salar*) (Birkbeck, Bordevik, Froystad and Baklien. 2007). Isolates from tilapia and three-line grunt are different from the isolates from cod and Atlantic salmon. The isolates that have caused disease outbreaks in cod and Atlantic salmon are very similar in the 16s rRNA gene (>99%) but may still represent two different *Francisella* species. In experiments, the cod *Francisella* isolate have been successfully transferred to Atlantic salmon but did not cause disease. Francisellosis in farmed Atlantic salmon has so far only been observed in fresh water (i.e. in salmon parr).

All post larvae stages of cod seem to susceptible to *Francisella philomiragia* subsp. *noatunensis*. In cohabitation experiments, transmission of *Francisella* has been documented by PCR after 37 days at low temperature (9°C) while development of macroscopic changes required more time.

Diagnosis and pathology

At autopsy, infected fish have moderate to massive occurrence of whitish nodules in internal organs, particularly in the spleen, liver and heart. Some fish may have eye lesions and haemorrhagic nodules under the skin. In histological sections, chronic inflammatory reactions often with massive occurrence of inflammatory nodules can be seen. These lesions are usually found in gills, heart, liver, spleen, kidney, eyes and somatic musculature. Within the granulomas many bacteria can be observed in vacuoles within host cells.

In typical cases, francisellosis has a chronic progression and the fish may have been infected for a long time when they eventually succumb to the disease. Mortality may be high during a short period or lower during a longer period. Reduced growth, mortality and downgrading/rejection at slaughter may result in great losses for the farmer. Even if the mortality may be lower during the winter, the infection seems to persist in the population.

Detection and identification

Light microscopic detection of intracellular bacteria indicates francisellosis, but demonstration of the bacterium is necessary for confirmation. *Francisella philomiragia* subsp. *noatunensis* is identified by cultivation and the use of molecular biological methods. The bacterium requires a specialised medium for growth (agar containing cysteine and blood) and can therefore be difficult to grow under field conditions. For early detection of infection, cultivation of samples from the cod spleen in CHAB at 22°C is recommended.

Efforts to grow isolates from frozen tissue in Sweden directly on this special agar have not been successful, but an initial growth step on a cell line (Chinook salmon embryo cell line (CHSE-214)) has resulted in isolation of the bacterium on the agar. Similarly, isolation of tularaemia (*Francisella tulariensis*) from dead animals often requires propagation by alternative methods (using animals for enrichment of the bacteria) before any isolation can be made on special medium.

Scotland

In Scotland a sheep polyclonal antiserum based on the Swedish strain (Ö 391: Eva Jansson, Sweden) of *Francisella* isolated from wild cod has been raised. The antiserum was found highly specific in a western blot to the same species of *Francisella* and did not cross-react with *Vibrio anguillarum*, *Aeromonas salmonicida* (typical and atypical strains), *Moritella viscosa* or *Renibacterium salmoninarum*. The working dilution of antibody has been optimized in the Western blot assay. Infection studies (strain Ö 391 and 2005/50/F292 D. Colquhoun, Norway) carried out in Scotland on 8, 20 and 700g cod established Koch's postulates in all groups. Fish showed sluggish movements, splenomegaly and a slightly swollen kidney. Scattered white nodular lesions were recorded. Histological examination showed an encapsulated visceral granuloma with small, pleomorphic Gram-negative bacteria within vacuolated cells. Lesions were representative of those described in wild cod.

Sweden

An immunohistochemical method for diagnosis of *Francisella* infection in Atlantic cod has been developed in Sweden (Eva Jansson). Antiserum was produced by immunization of New Zealand white rabbits (NZW/SVA) with O-antigens prepared from the Swedish isolate of *Francisella* sp. from wild cod (Strain 391) and the Norwegian isolate of *Francisella philomiragia* subsp. *noatunensis* (2005/50/F292; Duncan Colquhoun, NVI, Oslo). The antibodies produced in the rabbits gave a strong, specific response against the isolates when tested on ELISA. The antibodies did not react with *Francisella tulariensis.* An immunohistochemical method to demonstrate *Francisella sp.* in fixed tissue was developed. Strong immunoreactivity was detected in the histologic sections of organs with granulomas from affected cod collected from the Swedish west coast.

Denmark

In Denmark (Inger Dalsgaard) *Francisella* sp. was isolated on Cystine Heart Agar (Difco) with 2% haemoglobin, incubated at 20°C for 2 weeks. *Francisella* sp. grew with pale white, mucoid convex colonies. The Gram negative, short to coccoid cells were non-motile, aerobic, oxidase-variable, and glucose positive. H_2S was not produced in TSI and a unique fatty acid production was seen (API ZYM, rapid ID32A, ID32E). The Danish isolates were compared with a Norwegian isolate from cod (NVI 5330), supplied by Duncan Colquhoun, National Veterinary Institute, Oslo, Norway. The Danish isolates showed similar phenotypical reactions as the Norwegian isolate and based on this evidence, the Danish isolates were identified as *Francicella* sp.

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Annex 9: Progress report on studies carried out on hyperpigmentation in common dab (*Limanda limanda*) (ToR e)

T. Lang, F. Baumgart, D. Bruno, S.W. Feist, and P. Noguera

Background

Term of Reference e) provide a progress report on studies carried out on hyperpigmentation in common dab (*Limanda limanda*) from the North Sea with special reference to pathological findings and possible causes;

Introduction

At its 2007 meeting, the WGPDMO reviewed progress made regarding studies on hyperpigmentation in dab (*Limanda limanda*) from the North Sea and adjacent waters (Annex 6; ICES 2007). Results of the studies indicated the following:

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

Based on these results, the WGPDMO recommended in 2007 that:

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

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

New findings

In 2007, a Diploma Thesis on hyperpigmentation in dab (*Limanda limanda*) from the North Sea and Baltic Sea with data on regional and temporal patterns and possible causes was finalised (Baumgart, 2006). New findings are:

- The increase in prevalence of hyperpigmentation recorded in dab from almost all North See areas since the mid 1990s is largely caused by an increase in prevalence of the lowest severity grade 1. However, severity grades 2 and 3 have also increased, but to a lesser extent and with a temporal delay of some years.
- There are no strong seasonal effects on the prevalence. However, current data from areas with highest prevalences indicate higher prevalences in winter compared to summer.
- There is no indication of significant effects of sex on the prevalence of hyperpigmentation.
- The prevalence of hyperpigmentation depends on the age of fish: it increased from age groups 2 to 4 and decreases thereafter in age groups 5 to 8 to levels even lower than that recorded in age group 2. In young fish, severity grade 1 is dominating whilst there is a shift to a dominance of higher grades in older fish, possibly reflecting an ontogenetic progression of the condition.
- Dab with hyperpigmentation grade 3 grow significantly faster than control fish, i.e. their mean total length is higher than in non-affected fish of the same age.
- Changes recorded in population structure with a potential impact on prevalence: (1) more or less continuous decrease in mean total length of dab in the catches since 1988; (2) decrease in CPUE since the early/mid 1990s.
- A thorough virological and bacteriological study was carried out at the at FRS Aberdeen, UK, in spring 2007 using material collected on a cruise with RV Scotia off the Scottish east coast. The results did not reveal any involvement of pathogens in the aetiology of the condition.
- In dab with hyperpigmentation grade 3, the epidermis is thinner compared to non-affected controls whereas the melanocyte layer in the dermis is thicker.
- According to published literature, UV-B exposure causes an increase in melanin contents in the skin of fish (Fabacher *et al*., 1997; Fabacher and Little 1995) as well as an increased growth rate (Häkkiinen *et al*., 2002), thus supporting the hypothesis that hyperpigmentation in dab might be linked to increased UV-B exposure of early dab life stages.
- Pigment anomalies have been described to also occur in aquaculture flatfish (Venizelos and Benetti 1999, Bolker and Hill 2000, Ottesen *et al*., 1996, McEvoy *et al*., 1998) but the causes are still more or less unresolved. A number of potential impacting factors have been discussed, such as light intensity, food composition (e.g. fatty acid composition), neurological aspects and aquaculture-associated stress (Ottesen *et al*., 1996, Venizelos and Benetti 1999, Bolker and Hill 2000). However, in most cases there seem to be differences between the condition observed in aquaculture and hyperpigmentation. For instance, often aquaculture-related pigment anomalies

consist of a lack of pigmentation (albinism) instead of an excess of pigmentation.

Conclusions

- Spatial and temporal patterns of hyperpigmentation in dab, some effects of hyperpigmentation on the host, the impact of some host-specific factors (e.g. length, age, sex, population density) on the prevalence and the role of viral or bacterial infections have been described thoroughly in a Diploma Thesis (author: F. Baumgart, Univ. Rostock, Germany) released in 2007. The results are at present put together for publication in a scientific journal, involving F. Baumgart, SW Feist, D. Bruno, T. Lang *et al.* as co-authors.
- Histopathological studies on effects of hyperpigmentation on organs other than the integument recommended by WGPODMO at its 2007 meeting are still lacking. However, samples have been taken and processed and are will be analysed. Results will be published together with the results of the virological and bacteriological screening as well as the skin histopathology.
- A thorough comparison between hyperpigmentation and pigment anomalies known from aquaculture or pigment tumours in fish is still underway.
- Although the Diploma Thesis mentioned above discussed possible causes of hyperpigmentation, there is still no clear evidence on its aetiology. Work on this aspect will be continued, and funding is being sought for a PhD study.
- A compilation of relevant literature is provided below.

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Annex 10: Evidence of increased tolerance to the pesticides and medicines used to treat sea lice in salmon aquaculture (ToR f)

S. Jones, T.A. Mo, and N. Ruane

Introduction

The application of pesticides or medicines for the treatment of ectoparasitic copepods has a long tradition in the cultivation of freshwater fishes (Kabata, 1970). In the marine environment, outbreaks of sea lice, especially those of the salmon louse, *Lepeophtheirus salmonis*, have been associated with intensive cultivation of Atlantic salmon in open net pens since the mid-1960s (Pike, 1989). The ability of the parasite to cause disease in the confined culture environment led to significant losses and associated economic costs. These impacts triggered considerable research effort, spanning nearly four decades and focusing on the life history, epidemiology, host-parasite interactions and treatment of the parasite (Pike and Wadsworth, 1999; Johnson *et al*., 2004; Boxaspen, 2006; Costello, 2006). Thus, farmed salmon have been treated for infestations with sea lice virtually since the beginning of intensive aquaculture. There has been an evolution in sea lice management over the last 35 years, driven by the availability of new classes of sea lice therapeutants and by an awareness of the value of integrated pest management. Increased tolerance, or resistance, to pesticides and medicines is well documented among other arthropod pests, notably the insects (Denholm *et al*., 2002; IRAG UK, 2008), and the phenomenon is known from the aquatic environment. In the context of the present report, resistance of the freshwater parasite of carp, *Argulus* sp. to hexachloro-cyclohexane (lindane) was observed following only six treatments (Lahav *et al*., 1962). The purpose of this report is limited to the chemotherapeutants used to treat *L. salmonis* in salmon aquaculture, and will describe the evidence supporting increased tolerance to these compounds.

Classes of medicines and pesticides

Organophosphates: are synthetic acetylcholinesterase inhibitors typically administered by topical (bath) application. These compounds were among the first to be used to treat sea lice and are most effective against the mobile pre-adult and adult parasitic stages. Dichlorvos was used in Scotland and Ireland from the late 1970s. The use of metrophinate, a related compound, preceded the use of dichlorvos in Norway until the mid-1980s (Denholm *et al*., 2002). Application of a new-generation organophosphate compound, azamethiphos, began in Scotland and Norway in the mid-1990s. Azamethiphos has a wider therapeutic margin than dichlorvos and there are fewer human health concerns compared with earlier organophosphates (Rae, 2002; Grant, 2002). Although azamethiphos is authorised for use in Scotland, Norway, the Faeroes and Chile (O'Halloran and Hogans, 1996; Grant 2002; Burridge, 2003), its recent use has been limited.

Pyrethrum and Pyrethroids: pyrethrum, occurring in chrysanthemums and related plants and the semisynthetic pyrethroids cause a loss of function in neuronal sodium channels leading to paralysis (Burka *et al*., 1997). These compounds are broad-spectrum pesticides as they affect both attached and mobile sea lice stages following topical application. Pyrethrum was used against sea lice in experimental trials in Norway in 1989. However its applicability was limited by relatively high toxicity to fish and low solubility in water, restricting its application to an oil film on the water surface (Roth *et al*., 1993). From the mid-1990s the pyrethroids cypermethrin, high-cis-

cypermethrin and deltamethrin have been used as a treatment for sea lice. Cypermethrin is used in Scotland (Denholm *et al*., 2002) while the latter two compounds are used in Norway (Sevatdal *et al*., 2005). Deltamethrin was licensed for use in Chile in late 2007 (Lees *et al*., 2008).

Disinfectants: hydrogen peroxide has been used topically to treat sea lice infestations in Norway, Scotland and Canada (Roth *et al*., 1993, Burka *et al*., 1997, Grave *et al*., 2004). Its mode of action is not well understood but may be related to the formation of oxygen emboli in the haemolymph (Bruno and Raynard, 1994). The compound is only efficacious against mobile parasitic stages (Grant, 2002). The safety margin of H2O2 is narrow and not recommended for use above 14°C (Burka *et al*., 1997). Application of this compound was limited by difficulty of establishing an efficacious concentration in tarpaulin-lined net pens combined with the tendency of lice to recover shortly after treatment.

Insect Growth Regulators: diflubenzuron and teflubenzuron inhibit chitin synthesis in the copepod exoskeleton and have been used to treat sea lice since the mid-1990s. The compounds are delivered in-feed (Table 1). Teflubenzuron is authorised for use in salmon in Scotland, Norway, Ireland, Canada and the Faeroes (Grant, 2002, Westcott *et al*., 2008) and diflubenzuron has been used in Norway (Grave *et al*., 2004; Lunestad and Grave, 2005). These compounds target the actively moulting early developmental stages. Although the safety margin in fish is high (Grant, 2002), chitin inhibitors begin to lose efficacy 7 days after treatment (Branson *et al*., 2000).

Avermectins: ivermectin and emamectin benzoate (EB) are macrocyclic lactones which interfere with neuronal GABA- and glutamate-gated chloride channels (Burka *et al*., 1997). Limited off-label use of ivermectin as an in-feed treatment of sea lice has occurred in Ireland, Scotland and Canada (Palmer *et al*., 1987; O'Halloran and Coombs 1993; Johnson and Margolis, 1993, Denholm *et al*., 2002). In fish the safety margin of ivermectin is narrow (Burka *et al*., 1997; Johnson *et al*., 1993, Palmer *et al*., 1997). Emamectin benzoate is licensed for use in Norway, Scotland and Chile. The drug is available in the U.S.A. under the provisional status of an investigational new animal drug (Gustafson *et al*., 2006) and in Canada under the provisions of the emergency drug release process (Westcott *et al*., 2004). As a result of ease of administration, low toxicity (Roy *et al*., 2000) and efficacy against all lice stages which persists for up to 10 weeks (Stone *et al*., 2000), recent use of EB has increased in Scotland, Canada and the U.S.A. (Lees *et al*., 2008; Westcott *et al*., 2008; Gustafson *et al*., 2006) and presumably also in Norway and Ireland. Emamectin benzoate was used exclusively in Chile between 2000 and 2007 (Bravo *et al*., 2008; Lees *et al*., 2008). The use of EB in Chile is unique in that the target species is *Caligus rogercresseyi*. Additionally, EB in Chile is supplied by four manufacturers compared with one in the northern hemisphere (Bravo *et al*., 2008).

Evidence of Resistance

Over the last 15 years, treatment of sea lice in farmed salmon in Norway can be generally characterised by a sequence of preferred chemotheupeutic compounds: organophosphates preceded pyrethroids and most recently, emamectin benzoate is used. The chitin inhibitors, ivermectin and hydrogen peroxide have been less frequently used. A similar trend may be inferred from the scientific literature to have occurred in Scotland, Ireland, Canada and the U.S.A, although comprehensive data are lacking. These trends are related to the availability over time of products with improved margins of safety, efficacy and ease of delivery. Differences in treatment strategies (Grave *et al*., 2004) and in factors affecting the availability of chemothera-

peutants (Grant, 2002) also contributed to usage trends regionally and nationally. This pattern of repeated single usage has created theoretical opportunities for the development of increased tolerance. Indeed, changes in efficacy over time are suggested from references to anecdotal reports of increased tolerance among sea lice to organophosphate, hydrogen peroxide and pyrethroids in Norway, Scotland and Canada (Denholm *et al*., 2002). The anecdotes appear to be based on treatment failures and it is not clear whether alternative explanations such as inappropriate treatment concentration or exposure time were ruled out in these cases.

Confirmation of increased tolerance to medication requires the use of a bioassay or similar controlled sensitivity test (Sevatdal, 2005, SEARCH, 2006). The SEARCH report describes the use of field bioassays by local fish health services in several countries to screen for increased tolerance to organophosphates, pyrethroids and emamectin benzoate (EB). Unfortunately, the results of these routine assays do not appear to be public, and it is difficult to verify the accuracy of statements typified by the following:

> "In several areas in mid-Norway, the organophosphate dichlorvos totally lost its effect against sea lice in 1991–1992 and the same happened in mid– and southern Norway with azamethiphos in 1995–1996, rendering this compound virtually ineffective as an anti-sea lice agent." (Fallang *et al*., 2004).

There are several published accounts of bioassay-confirmed, increased tolerance to organophosphates (Jones *et al*., 1992; Roth *et al*., 1996; Tully and McFadden, 2000) and pyrethroids (Sevatdal and Horsberg, 2003; Sevatdal *et al*., 2005). A report documenting resistance of sea lice to hydrogen peroxide, while not using a formal bioassay, described a controlled study using infected salmon from previously exposed and non-exposed sites (Treasurer *et al*., 2000). Westcott *et al*. (2008) found no bioassay evidence of increased tolerance to EB in sea lice in the Bay of Fundy, Canada, although seasonal or temperature associated trends were noted. Lees *et al*. (2008) used epidemiological modeling of pre- and post-treatment farm data to examine trends in the efficacy of EB in Scotland. The study found evidence that not all treatments were effective, that there was spatial variation in efficacy and that efficacy declined over time (Lees *et al*., 2008). A recent study used bioassay to describe increased tolerance to EB among *C. rogercresseyi* from 18 salmon farms in Chile (Bravo *et al*., 2008). The authors attribute this phenomenon to the exclusive use of this compound in Chile for greater than 7 years, preceded by the use of ivermectin for approximately 10 years. Higher doses and prolonged treatment regimes for EB were also reported (Bravo *et al*., 2008).

While more research is necessary to identify mechanisms of increased tolerance, molecular techniques such as gene expression studies may provide more sensitive tools for screening sea lice populations (Tribble *et al*., 2007; 2008).

Conclusions

Sea lice are serious pests of farmed Atlantic salmon. Treatment of sea lice can be effective but this is costly and access to efficacious medicines and pesticides is limited to a small number of available compounds and by regional or national local regulatory processes. The limited available information provides evidence of increased parasite tolerance to four classes of compounds: organophosphates, pyrethroids, hydrogen peroxide and avermectins.

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Annex 11: Validation and integration of molecular diagnostic and confirmatory techniques for pathogens of bivalves (ToR h)

T. Renault, S. Ford, and L. Madsen

The effective control of pathogens infecting molluscs requires diagnostic tests that are specific, reliable and sensitive, and that can discriminate between genera and species. Several methods are used to identify and characterise mollusc pathogens, among them, newly developed molecular methods appear very useful. Due to their specificity and sensitivity they allow species and strain identification. International standards proposed by the OIE now include such molecular techniques. Criteria used to identify mollusc pathogens should, however, include basic biological and ecological characteristics of pathogens as well as information on their genetic sequence. Thus, schemes for differential diagnosis incorporating molecular techniques have been developed.

Introduction

For many pathogens of molluscs, available diagnostic techniques have historically been based on histological and ultra-structural examinations. Thus, infectious agents can be diagnosed by applying stained tissue imprints. Histology examination provides also valuable information on the intensity and severity of infection at the individual level, co-infections with different noticeable pathogens as well as potential emerging pathogens and non-infectious conditions. However, the accuracy of diagnosis by means of the 'eye based' methods is highly linked to the experience and the training of the investigator, and the time allocated to the examination. Moreover, pathogens could be difficult to detect and recognise using these techniques, particularly when present in low numbers.

Although histology does not allow identification to the species level for most mollusc pathogens, this technique has extensively been used. In this context, in a large number of available reports and publications, pathogen speciation was based on host species and geographic range: i.e. a parasite presenting features characteristic of the genus *Bonamia* was identified as being *Bonamia ostreae* when detected in flat oysters, *Ostrea edulis*, in Europe. Some data must thus be considered with caution in terms of pathogen identification.

Recognising the need for diagnostic tools to discriminate between genera and species, efforts have been made to overcome limitations of microscopy. Molecular detection assays for pathogens infecting molluscs appear as suitable tools. They are being developed at an increasingly rate (Walker and Subasinghe, 2000; Renault *et al*., 2007). The routine use of molecular based diagnostic tools is however hampered by major concerns.

The DNA-based assays need formal validation. They must first be compared to traditional methods. Problems may arise when the new diagnostic test is assumed to be more sensitive and specific than the previous standard. Inter-laboratory assays need also to be performed in order to confirm reproducibility. Studies conducted in parallel with the same isolates in several laboratories would be ideal. It will also be necessary to identify regions of the pathogen genome that can be utilised for species differentiation. All molecular assays specific for a pathogen should be tested in parallel and validated, and further sensitive diagnostic assays that will clearly discriminate between all 'valid' species should be developed.

Taxonomy of mollusc pathogens is still unsettled. DNA sequencing has shed new light on viral, parasite and bacterial classification. The use of small sequences as probes for diagnostic purpose has been very rapid with usually little consideration for the lack of information about their true specificity. Also, DNA probes were most often designed from genes or clusters of genes of phylogenetic significance – such as the SSU rRNA gene for example – which frequently does not reflect the huge diversity in terms of virulence. There is a growing recognition of the need for strain differentiation in diagnostic procedures.

Finally, diagnostic tools are generally not standardized and differences could exist in reagents quality and preparation, in controls, as well as in the interpretation of results. Obviously, the use of a "standardised" diagnostic tool for routine analysis should allow the implementation of a calibrated and controlled process in laboratories but also in hatcheries. In this context, the development of commercial kits for the detection of mollusc pathogens appears as an interesting avenue to be explored. Such diagnostic tools may allow mollusc producers to stand sentinel and become a proactive player in the health management of their production. However, to date there are no commercial kits available for the detection of any mollusc pathogens.

Comparing diagnostic techniques for mollusc pathogen detection

Although several studies including some data on comparison of diagnostic techniques have been published in the last decade, only a few of them focused on the topic.

An in situ hybridisation technique was developed for the detection of *Marteilia refringens* (Le Roux *et al*., 1999) with particular emphasis on confirmation of suspected cases by means of histology. In 2005, Thébault *et al*. published a study focusing on evaluation of sensitivity and specificity values of in situ hybridisation and histology for the detection of *M. refringens*. They carried out a blind assay of 200 flat oysters (free or not of the pathogen) from three different populations using both techniques. Results were analysed using different methodological approaches. In a first step, histology was considered as the reference method ('gold standard') where sensitivity and specificity were assumed to be unity. The authors used also the maximum likelihood method based on the TAGS V.2.0 program (Pouillot *et al*., 2002) assuming that none of diagnostic techniques was the 'gold standard'. These approaches were completed by a third one using an iterative Markov Chain Monte Carlo (MCMC) technique (Bayesian method). Using this last approach, values of sensitivity and specificity for histology were 0.7 and 0.99, respectively, and 0.9 and 0.99, respectively for in situ hybridisation. This work was the first to provide such information for these diagnostic methods recommended by international standards. Moreover, the authors highlighted that the estimation of sensitivity and specificity for a newly developed diagnostic technique does not require a gold standard.

PCR detection of *Haplosporidium nelsoni* was evaluated using histology as a gold standard reference (Fegan, 2000). This author identified a lack of specificity of the PCR (0.7). However, such an evaluation should be carried out again using other methodological approaches including Bayesian methods in order to either confirm or reject these findings. Recently, a study focusing on detection of *Minchinia* sp. in rock oysters *Saccostrea cucullata* using DNA probes has been published (Bearham *et al*., 2008). The ability of PCR and ISH assays to diagnose infected individuals was compared to histological examination from a sample of 56 oysters. PCR and ISH assays appeared more sensitive with 26 and 29 positive individuals, respectively, versus 14 using histology.

Marty *et al*. (2006) reported an increased sensitivity of a new real-time PCR as confirmed with histopathology for detection of *Bonamia ostreae* in *Ostrea edulis* cultured in western Canada. Parasite DNA was confirmed in 4 oysters by real-time PCR on paraffin-embedded tissues that was not detected by histopathology.

Quahog Parasite Unknown (QPX) is a protistan parasite that causes disease and mortality in the hard clam *Mercenaria mercenaria*. PCR primers and DNA nucleotide probes were designed and evaluated for sensitivity and specificity for the QPX organism. A field validation was carried out by Stokes *et al*. in 2002. Two-hundred and twenty-four clams were collected over a 16 month period from a QPX endemic site in Virginia, USA. All individuals were analysed using PCR and histology. The authors demonstrated that the PCR assay was equivalent to histological detection, but only after the initially negative PCR products were reamplified. They pointed out that the failure of PCR to increase detection levels over histology was probably due to the patchy nature of QPX in clam tissues so that the chance of encountering QPX parasites (which are realitively easily detected in histological section due to the intense host response) is about equal in the tissue pieces collected for each assay.

'*Candidatus xenohaliotis californiensis*' is a Rickettsiales-like prokaryote responsible for withering syndrome, a fatal disease of wild and farmed eastern Pacific abalone, *Haliotis* spp. A method of rapidly detecting the pathogen in abalone gastrointestinal tissue has been developed based on the use of Hoechst fluorochrome (Moor *et al*., 2001). Comparison of this method with conventional histological examination was conducted on 109 samples. The fluorochrome method detected 90% of the infections detected by conventional histology with discrepancies due to false negative diagnosis of low-level infections.

PCR, traditional RFTM (Ray's Fluid Thioglycollate Medium), and body burden assays were compared in stout razor clams, *Tagelus plebius*, infected with *Perkinsus chesapeaki* (Bushek *et al*., 2008). In two samples, a species specific PCR assay detected no *P. chesapeaki*, whereas the RFTM assay detected 33% and 100%. The body burden assay provided a potential explanation for the discrepancy. In these two samples, the body burden assay estimated average parasite densities of only 0.26 and 0.03 parasites in a ~20 mg piece of tissue – the amount recommended for DNA extration. From this, approximately 50 ng is used as the template in the PCR reaction. In contrast, the amount of tissue used in the RFTM assay is about 200 mg. In another sample with much higher parasite densities, the two methods were comparable. Thus, the small amount of tissue, typically taken from a single tissue type, that is employed in a PCR reaction may limit its usefulness when infections are very light or localized, or both.

Molecular assays have also been developed and compared for Ostreid Herpesvius 1 (OsHV-1) in bivalve samples (Renault al., 2000). The methods include polymerase chain reaction (PCR) assays and in situ hybridization assays (ISH) (Lipart and Renault, 2002; Arzul *et al*., 2002). In a preliminary study, three techniques (PCR, ISH and immunochemistry) were used to detect OsHV-1 in 30 normal appearing Pacific cupped oysters (Arzul *et al*., 2002). Global agreement between techniques was determined using generalized Kappa (Fleiss, 1981). PCR appeared as the most sensitive method for detecting OsHV-1 in adults. In another study (Barbosa-Solomieu *et al*., 2004) using PCR and ISH, attempts were made to screen for OsHV-1 and in 200 fixed, paraffin-embedded oyster samples collected and processed in 1994. The results obtained through this molecular screening of OsHV-1 allowed comparison of the sensitivity of both techniques. Also, histological and TEM observations performed in 1994 were correlated with molecular diagnosis of the virus.

Interlaboratory evaluation

Lack of standardisation of tests and test protocols is a major impediment to the effective implementation of DNA-based methods. Standardisation requires international agreement and cooperation in test selection, practitioner training and laboratory accreditation. Improvements in the reproducibility, validity and comparability of data resulting from accreditation will also assist in assessing the suitability of DNA-based methods for detection of listed pathogens.

An important factor that needs to be addressed is the reproducibility between laboratories. The assay procedure not only consists of performing the diagnostic assay but also reproducing the same sensitivity, eliminating false interpretation and implementing contamination control procedures.

Ostreid herpes virus 1 (OsHV-1) Part of the EU funded project (VINO, FAIR-CT98-4334) was the organisation of a workshop in 2000 at the Ifremer laboratory in La Tremblade (Charente Maritime, France) in order to ensure that common protocols. PCR and ISH were used for OsHV-1 detection. VINO partners conducted trials using both techniques. The work involved with an evaluation of the reproducibility of both molecular detection techniques through an inter-laboratory analysis of a series of archived frozen and fixed samples. The ring trial involved the four laboratories routinely conducting molluscan pathogen diagnosis, including Ifremer (La Tremblade) which supplied the reference material (a series of previously analyzed reference samples). Supernatants of ground larvae (15) and seed (15) as well as histology slides (30 histological slides) were sent as positive and negative reference material to each participant of the inter-laboratory assay.

The 15 larval samples and 15 spat samples were analysed by PCR. Among the 18 samples considered as positive (laboratory A – Ifremer La Tremblade), only 14 samples were found to be positive using both primer pairs by each of the other laboratories. However, other samples appeared positive or negative depending on the laboratory (Table I). *In situ* hybridization analyses were done in the laboratory of Ifremer in La Tremblade (labelled A) on a series of samples collected in the years 1994–1995. Positive and negative material (histological sections from fixed spat) were selected and sent to three other European laboratories (B, C and D). Laboratories B, C and D found three "false negatives" and several "false positives". Laboratory B obtained seven "false positives" among ten negative samples. Consistent results were obtained in laboratories A and D but the latter failed to analyze seven samples (Table II).

The above described study appears to be the first step in a validation process. However, ring trials must be repeated in order to improve results.

Table I. PCR ring trial. Four laboratories were involved and two primer pairs were tested: C2/C6 and C5/C13.

N°	PCR							
	C2/C6				*C5/C13*			
	A	B	C	D	A	B	C	D
1	-	-	+	-	-	-	+	-
2	-	-	-	-	-	-	+	-
3	-	-	+	-	-	-	-	-
4	-	-	-	-	-	-	+	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	+	-
7	-	-	+?	-	-	-	+	-
8	-	-	+	-	-	-	+	-
9	-	-	+	-	-	-	+	-
10	-	-	-	-	-	-	+	-
11	-	-	-?	-	-	-	+	-
12	+	+	+	+	+	+	-	-
13	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	-	+
18	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+

N°	PCR							
	C2/C6				*C5/C13*			
	A	B	C	D	A	B	C	D
25	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+
27	+	-	+	+	+	+	+	+
28	+	-	-	-	+	-	-	-
29	+	+	+	+	+	+	+	+
30	-	+	+	+	+	+	+	+

PCR with C2/C6 gave consistent results considering laboratories A, B and D. Concerning the primer pair C5/C13, "false positives" were obtained in laboratories B, C and D. "False positives" were also observed in the laboratory C with both primer pairs.

Table II. ISH Ring trial. Four laboratories were involved. Positive results correspond to the detection of a staining on histological sections using a direct revelation method.

N°	ISH			
	C1/C6			
	A	B	C	D
VB1	-	?	-	-
VB2	-	+	-	-
VB3	-	+	-	-
VB4	-	+	-	-
VB5	-	+	-	-
VB6	-	+	+	-
VB7	-	ND	ND	-
VB8	-	-	+	-
VB9	-	+	+	-
VB10	-	+	?	-
VB11	+	+	+	-
VB12	+	+	+	-

N°	ISH			
	C1/C6			
	A	B	C	D
VB13	+	+	+	+
VB14	+	+	+	+
VB15	+	+	?	+
VB16	+	+	-	+
VB17	+	+	+	+
VB18	+	+	+	+
VB19	+	+	+	+
VB20	+	+	-	+
VB21	+	+	-	+
VB22	+	+	+	ND
VB23	+	+	?	ND
VB24	+	+	+	ND
VB25	+	+	-	ND
VB26	+	+	+	ND
VB27	+	-	+	ND
VB28	+	-	-	ND
VB29	+	+	+	ND
VB30	+	-	+	ND

Bonamia ostreae. A proficiency test for the detection of *Bonamia ostreae* by PCR is organised by the European Reference Laboratory for Mollusc Diseases in 2008. Seventeen laboratories will be involved. The test includes DNA extraction from gill tissues collected from 30 flat oysters, *Ostrea edulis*, fixed in ethanol and PCR analysis of the 30 samples based on a protocol previously published (Cochennec *et al*., 2000).

How to design valuable molecular tools?

The taxonomic history of various pathogenic agents infecting molluscs including viruses, bacteria and protozoan parasite has known controversy in the recent decades. However, in recent years molecular biology has lead to taxonomic clarification of some of the pathogens and may help in the design of more accurate molecular detection tools.

Molecular detection methods have recently been developed for the identification of different bacteria infecting molluscs including different *Vibrio* species. Thus, the aetiological agent of brown ring disease, *V. tapetis*, affecting Manila clam, *Ruditapes philippinarum*, can be identified using dot blot hybridisation and a species-specific primers (Paillard *et al*., 2001).

Although DNA molecular based techniques are useful for bacterial species identification, strains belonging to the same species may have different virulence levels (i.e. *V. splendidus*). Certain *Vibrio* strains ameliorate growth rates in mollusc species, other *Vibrio* strains may be pathogenic. *Vibrio splendidus*-related species have been reported in association with mortality outbreaks of oysters. Through epidemiological studies a high genetic diversity has been observed in this group suggesting a polyphyletic nature. Moreover, diagnostics based on biochemical characters gave poor results for species discrimination within this group. Although several strains clustered together, they could not be assigned to any known *Vibrio* species. In this context, genotyping appears a suitable tool in order to associate a specific strain to a disease. DNA typing may be carried out using different techniques targeting, intergenic rDNA spacer regions, individual genes (encoding virulence factors), a gene cluster and the whole genome. This approach needs to be completed by virulence characterisation based on experimental trials.

Taxonomic analysis of unidentified isolates based on a polyphasic approach including gene sequencing, fluorescent amplified-fragment length polymorphism (FAFLP) fingerprinting, DNA-DNA hybridisation and biochemical tests has successfully been used by several authors to define new species (Thompson *et al*., 2003a; 2003b; Faury *et al*., 2004;Le Roux *et al*., 2005). Although some discrepancies are observed between the different approaches, the results can be collectively used to identify bacterial isolates.

What about diagnostic kits?

In mollusc hatcheries different diseases are frequently reported in larvae and spat, causing mortalities. Most of these diseases are not notifiable diseases and therefore not subjected to specific control measures under EU or OIE legislation. However, pathogens associated with these mortalities, mainly viruses and bacteria, generate important economic loss and jeopardize the sustainable development of this important socio-economic activity in EU coastal regions.

The recent Council Directive 2006/88/EC "on animal health requirements for aquaculture animals and products thereof and on the prevention and control of certain diseases in aquatic animals", underlines the necessity for the development of aquaculture in the Community to increase the awareness and preparedness of the competent authorities and aquaculture production business operators with respect to the prevention, control and eradication of aquatic animal diseases. No doubt, an efficient management of the sanitary status of mollusc production implies a significant involvement of the farmers who –unavoidably– are in the front line in the fight against the diseases and can become key players in the control of the pathogens that threaten their livestock. If the latter should become reality then first the efforts of the farmers should be supported by the Authorities and the National laboratories involved in control of disease, and second the farmers should have validated, accurate, easy to use in the field and affordable screening tools at their disposal, which would allow an efficient monitoring of the sanitary status of their production. *However*, *to date there are no commercial kits available for the detection of any mollusc pathogens.*

Among microbial agents threatening European mollusc hatcheries and nurseries, herpes-like viruses have often been detected during mortalities outbreaks in several locations. Consequently, association of OsHV-1 with larval and spat mortalities has motivated the development of specific and sensitive diagnostic methods. According to the OIE International Aquatic Animal Health Code, OsHV-1 infection is not a notifiable disease and no diagnostic reference method or gold standard method is in force. No cell cultures from marine molluscs are available for virus detection. Detection of this pathogen is usually performed by PCR, real time PCR or in situ hybridization, which requires specialised and expensive laboratory equipment, highly qualified operators, or involves the use of carcinogenic reagents (PCR) and therefore cannot be performed routinely, easily, safely, efficiently and/or without generating elevated costs to the producers.

Recently, the collaborative work between Ifremer (La Tremblade, France) and a biotechnology company (SkuldTech, France) has resulted in the development of a mini-array method for OsHV-1 diagnostic as a kit prototype model. The aim of this tool is to allow the rapid, secure, cheap and handy screening of oyster samples and preliminary results have reported sufficient analytical and diagnostic specificity and sensitivity. A large scale validation process (inter-laboratory assay) will be necessary before the result could be *the first mollusc pathogen detection commercial kit that* will allow oyster producers to stand sentinel and become proactive players in the health management of their production.

In this context, a project has been prepared for the EU SME-call. The project expresses the will of oyster producing SMEs from different EU countries to address together an issue (i.e. get involved in the control of OsHV-1 outbreaks in their culture facilities). In this proposal all these requirements are fulfilled and subsequently, the key objectives of this project are the following:

i) transfer the new diagnostic method (mini-array) to different EU Laboratories involved in diagnostic test validation,

ii) perform an inter-calibration assay with these EU Laboratories in order to validate the mini-array diagnostic process (accuracy, reproducibility) according to the OIE recommendations (Manual of diagnostic tests for aquatic animals, 2006).

iii) transfer the validated mini-array diagnostic method to oyster hatcheries in different production locations within EU, and run comparative tests, in collaboration with National Laboratories involved in mollusc disease control in the first stage and autonomously in the final stage of the project.

The main objective of the project is to sustain and help hatcheries and laboratories involved in mollusc disease diagnosis to control OsHV-1 infections in oysters using the mini-array. Existing molecular tools, mainly PCR, are already used for routine detection of OsHV-1. Nevertheless, the already developed PCR techniques are not standardised in terms of reagents, controls and analysis interpretation. The mini-array was developed in order to offer a diagnostic tool -all in one-, as a kit. All reagents, controls and devices are disposable in one packaging. Moreover, this assay allows a naked eye reading of results. A “standardized” diagnostic tool for routine analysis can allow the use of a calibrated and controlled diagnostic tool in laboratories but also favour an easy transfer in shellfish hatcheries. After the validation step, it could be the first commercial diagnostic tool for a mollusc disease.

Conclusions

There are a number of mollusc pathogens for which DNA-based test methodologies are published. However, in general, further research is required before standardised and validated DNA-based test protocols can be implemented for disease diagnosis and pathogen detection in the mollusc aquaculture sector. Research needs vary for each pathogen depending on the existing knowledge base and state of the technology. Effective implementation will also be assisted by enhanced communication between aquatic animal health practitioners and scientists with expertise in disease diagnosis and pathogen detection.

Significant pathogens that require long, complex culture or histology-based confirmatory diagnosis are prime candidates for rapid, pathogen-specific diagnostic methods. This applies predominantly to microbial pathogens, but may be equally appropriate for protozoan parasites which are difficult to distinguish morphologically at the light microscope level or which have a diverse host-range. Rapid, pathogen-specific diagnostics would be particularly appropriate for disease management and control when diseases emerge in new geographic locations or host species. However, in the case of molluscs, histology provides a large amount of information and should be used initially, before and together with any other type of examination. It is particularly important because macroscopic examination usually gives no specific signs. Also, mortality may be due to several pathogens, or loss of condition following spawning, and this can only be determined by histology.

Where DNA-based tests are available and/or suitable, the most significant impediment to effective implementation is the lack of standardised methodologies that are validated for specific applications. There is a need for international agreement on methodologies that have been rigorously evaluated and accredited for specific applications in disease diagnosis and pathogen screening. There is also a need to ensure that tests are performed by trained staff with access to standardised reagents and suitably equipped laboratories.

The range of tools available and under development show different advantages and disadvantages for a range of different aquatic animal health applications. No one technique shows a replacement advantage over another, and none appear sufficient to merit "stand-alone" application, with the possible exception of pathogen-specific research.

For instance, in the case of Marteilia refringens, it is possible to recommend selected methods depending on the levels of investigation needed. The detection of *Marteilia refringens* by in situ hybridization could be used in addition to classical histological examination as a confirmatory method at genus level. Histology and in situ hybridization can thus be used as a two step diagnostic procedure, and could become a standard method for the assessment of a disease free status in a zone. Detection procedures that require presumptive methods for rapid detection of a suspected pathogen could be conducted by using digestive gland imprints. It is very important to keep in mind the multiple advantages of such a method (which can be applied in the course of sample preparation for histology) as it is cheap and provides an immediate answer. PCR tests, because of their specificity, could be proposed for the specific identification of encountered pathogens as a confirmatory procedure. However, standardization of protocols, including negative and positive controls, is required. Compared to transmission electron microscopy (the currently used confirmatory procedure), PCR provides a quick and specific answer.

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Annex 12: Recommendations

Report Section	Recommendation	For follow up by:
5	1. The Fish Disease Index (using incomplete data) should be submitted to ICES for review in May 2008 by independent experts, and following good reviews, ICES should submit the FDI to OSPAR for consideration.	ICES
5	2. WGPDMO should complete the FDI using the updated and complete disease data set and incorporating justifiable comments from the expert review, and discuss the project at the 2009 WGPDMO meeting.	WGPDMO
6.1	3. ICES Member Countries should continue to fund fish disease monitoring programmes to sustain fish health surveillance of wild stocks. Information obtained is of vital importance to integrated assessments of the health of marine ecosystems and will provide baseline data, e.g. to serve as a reference prior to establishing the culture of non-salmonid marine species. In addition, fish disease monitoring data will be useful in evaluating the effects of climate change on fish health and provide better understanding of pathogen interactions between wild and farmed fish.	ICES Member Countries
6.2	4. The status of red vent syndrome in wild Atlantic salmon should be reviewed at the 2009 WGPDMO meeting.	WGPDMO Members
6.2	5. Information on the aetiology of hyperpigmentation in common dab should be reviewed.	WGPDMO Members
6.2	6. Information on the abundance of *Anisakis simplex* in marine mammals, fish and other intermediate hosts (zooplankton and other invertebrates) should be reviewed.	WGPDMO Members
6.3	7. Information on new diagnostic methods for *Francisella*, and the impacts of francisellosis on populations of wild cod and other fish species, should be reviewed.	WGPDMO Members
6.3	8. ICES Member Countries should conduct further research to refine diagnostic tools and to develop treatments of vaccines for francisellosis in farmed cod.	ICES Member Countries
6.4	9. Studies on gill epithelial cell nuclear virus in soft clams (*Mya arenaria*) should be expanded.	ICES Member Countries
6.4	10. Investigations on *Bonamia exitiosa* in flat oysters (*Ostrea edulis*) in European countries should be initiated or continued in order to determine if the high prevalence (>50%) of *B. exitiosa* recently found in flat oysters in Spain, the first report of this pathogen in Europe, is a unique occurrence or if *B. exitiosa* is in other European locales.	ICES Member Countries
7	11. The distribution of *Tetracapsuloides bryosalmonae* in bryozoan populations in watersheds with salmonids should be determined.	ICES Member Countries
7	12. Field and laboratory studies on the growth of bryozoans, and on the life cycle of *T. bryosalmonae* under natural conditions in watersheds with and without salmonids, should be conducted. Because of recent changes in climatic conditions in the sub-arctic region, particular attention should be given to and by ICES Member countries of that region.	ICES Member Countries, particularly those in the sub-arctic region
7	13. Investigations should be conducted on *T.bryosalmonae*-infected smolts in the marine environment.	ICES Member Countries

8	14. Further surveillance and research should be conducted to document the distribution, severity and host specificity of francisellosis in wild and farmed fish.	ICES Member Countries
9	15. The WGPDMO should be updated on the results of ongoing histopathologic studies on the organs (e.g, liver, kidney, spleen, gonad, eye, thyroid gland, pineal gland, pituitary) in hyperpigmented fish	WGPDMO
9	16. A thorough review of the literature on malpigmentation in farmed flatfish and on fish pigment cell tumours should be conducted and reported to the WGPDMO.	WGPDMO
9	17. Potential causes of hyperpigmentation should be investigated, including analysis of food composition and pigment cell development/regulation in affected fish, the potential causative role of contaminants, and factors affecting skin hypersensitivity, e.g. UV-B irradiation.	ICES Member Countries
10	18. Research should be encouraged to identify and license new classes of sea lice medications and to develop alternative molecular tools for screening sea lice tolerance.	ICES Member Countries
10	19. Bioassay screens in all regions should be made public to permit an adequate assessment of local parasite populations.	ICES Member Countries
10	20. Member countries should encourage salmon aquaculture companies to practise integrated pest management (synchronise treatments within a management area, use alternating classses of sea lice medication, routinely monitor for sea lice) and coordinated use of bioassay techniques to screen for sea lice tolerance to medicines and pesticides.	ICES Member Countries
11	21. ICES Member Countries should note the existing international collaborative activities involving fish and shellfish disease and support the participation of the national institute in these activities.	ICES Member Countries
11	22. The workshop on methodologies for monitoring fish disease/parasites in coastal fish species from the Baltic Sea should be organised as part of the HELCOM FISH project with two WGPDMO members and a representative of the HELCOM coastal fish monitoring experts group serving as co-chairs.	WGPDMO, ICES Member Countries, HELCOM
11	23. Laboratories conducting fish disease monitoring programmes should participate in the BEQUALM programme to ensure quality assurrance and acceptance of data into international monitoring and assessment programmes.	ICES Member Countries
12	24. Communication networks of diagnostic practitioners and internationally recognised experts in aquatic animal health in Member Countries should be established and maintained in order to develop training and cooperative programmes for test validation and laboratory accreditation.	ICES Member Countries
13	25. A group of experts should be requested to provide suggestions of diseases to include in an FDI for cod (*Gadus morhua*) and flounder (*Platichthys flesus*) and on the relative severity of those diseases .	WGPDMO
13	26. ICES Data Centre should request that ICES Member Countries submit cod and flounder disease data to the ICES Databank using the ICES Environmental Data Reporting Format 3.2.	ICES Data Centre
13	27. A Fish Disease Index assessment for Baltic cod and flounder should be performed using data from the ICES Databank.	WGPDMO

14	28. Several manuscripts (concerning: disease and climate change; histopathology of mussels for health assessments; guidelines for fish disease monitoring in the Baltic Sea; construction of the Fish Disease Index) produced by the WGPDMO members should be submitted for publication in ICES TIMES or ICES Journal of Marine Science.	WGPDMO
15	29. ICES Data Centre should request that submitters of earlier fish disease data confirm the total numbers of fish reported per file, by species, and report back to the WGPDMO.	ICES Data Centre
15	30. Submitters of fish disease data should follow the format outlined in Report Section 15 for data submissions.	ICES Member Countries

Annex 13: WGPDMO Terms of Reference for the 2009 meeting

The **Working Group on Pathology and Diseases of Marine Organisms** [WGPDMO] (Chair: S. MacLean, USA) will meet in Riga, Latvia, from 3–7 March 2009 to:

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

b) provide a review of new diagnostic techniques for *Francisella* sp. and progress in vaccine development, and an update on the susceptibility of species other than cod to this pathogen;

c) provide an update on results of ongoing histopathological studies on organs other than the integument (e.g. eye, thyroid gland, pituitary) in hyperpigmented common dab (*Limanda limanda*) from the North Sea

d) provide a review of published literature on malpigmentation in farmed flatfish and fish pigment-cell tumours;

e) provide a review of available information on mathematical models describing the epidemiology and impacts of *Lepeophtheirus salmonis* on wild salmonid populations

f) provide an update on information available on the susceptibility of polyploid molluscs to pathogens;

g) review the information on trends in *Anisakis simplex* infection rates relevant to the red vent syndrome in Atlantic salmon, including information on marine mammal, fish and invertebrate hosts;

h) provide a review of available information on effects of diseases on recruitment and stock structure of commercial and non-commercial fish species;

i) provide an update on the OSPAR Request 13 (fish disease assessment inclusion in QSR 2010) and the fish disease index for other fish species (e.g. Baltic cod and flounder) and other sets of available disease data (e.g. liver histopathology data);

j) provide an update on the status of ICES publications on pathology and diseases of marine organisms;

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

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

Supporting Information

Priority:	WGPDMO is of fundamental importance to the ICES science and advisory process.

Scientific justification and relation to action plan:	Action Plan No: 1. **Term of Reference a)** New disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an assessment of these should be maintained (all WGPDMO members). **Term of Reference b)** The systemic granulomatous disease Francisellosis shows an increasing trend in farmed cod in Norway. An outbreak of Francisellosis has been observed in farmed Atlantic salmon in Chile. The isolates from cod and Atlantic salmon are almost identical (> 99% molecular marker similarity). It is recommended that WGPDMO review the current status of new diagnostic methods, host susceptibility and occurrence in wild and farmed marine fish species, as well as the status of progress in the vaccine development against the pathogen (A. Alfjorden, T.A. Mo, L. Madsen). **Term of Reference c)** Hyperpigmentation has continued to increase in the common dab (Limanda limanda) populations in the North Sea. At the 2008 WGPDMO meeting a report was reviewed providing new information on the involvement of pathogens as a primary cause and on epidemiological characteristics. There was consensus that there still is a need for further information on pathology associated with hyperpigmentation other than the integument (e.g. eye, thyroid gland, pituitary, liver, spleen, gonad) in order to assess potential causes or effects of the condition. Since more in depth studies on these issues are carried out at present, the results of which will be available for the next WGPDMO meeting, it is recommended to revisit the subject of hyperpigmentation at the 2009 WGPDMO meeting. (S.W. Feist, D. Bruno, T. Lang) **Term of Reference d)** Hyperpigmentation continues to increase in the common dab (Limanda limanda) populations in the North Sea. Previous studies have focussed on providing information on the spatial and temporal trends in prevalence in the North Sea, histological and ultrastructural features of the condition have been described and a recent virological and bacteriological study of hyperpigmented dab do not reveal any potential agents involved in the aetiology of the condition. Although a number of possible causes of the condition have been proposed (see 2008 report), further research is required. Malpigmentation (ranging from albinism to hyperpigmentation) commonly occurs in farmed flatfish and it is therefore recommended to conduct a literature review in order to propose new hypotheses regarding potential causes of hyperpigmentation in the common dab (N. Ruane, S.W. Feist, D. Bruno, T. Lang) **Term of Reference e)** Perceived effects of Lepeophtheirus salmonis on wild salmonid populations have led to the development of mathematical models which describe or predict parasite epidemiology and / or impacts. The epidemiology of sea lice in coastal ecosystems requires data from physical and biological oceanography, fisheries and those from adjacent salmon farms. These data may be limited in some coastal zones and additional empirical observations may be required to optimise models. The WGPDMO recommends that a review of epidemiological models and associated literature would permit assessment of knowledge gaps. The results of the review will be of interest to coastal zone managers and to other ICES Expert Groups within Mariculture. (S. Jones, T.A. Mo, W. Wosniok)

Scientific justification and relation to action plan continued:	**Term of Reference f)** Currently triploids constitute a large part of the oyster farm production and demonstrate a faster growth and a better taste (higher glycogen content). The use of such animals has also clearly emerged in recent decades as a possible way to deal with the impacts of infectious agents. Because triploid oysters grow rapidly, they reach market size before epizootic outbreaks occur. Moreover, reduced gametogenesis may be associated with a better use of energy for defence capabilities, thereby rendering triploids less susceptible to infectious diseases. Recent data demonstrated that triploid Pacific oysters showed higher haemocyte activities than diploids and were less susceptible to summer mortality after deployment in the field. Triploid Saccostrea glomerata showed reduced winter mortality due to QX (Marteilia sydneyi) disease relative to diploid oysters. However, triploid Crassostrea virginica did not demonstrate enhanced resistance to Perkinsus marinus. Finally, tetraploid animals can be used to produce triploid individuals. Tetraploid molluscs are mostly produced and maintained in hatchery nursery facilities. To date nothing is known about the susceptibility of tetraploids to infectious diseases. (T. Renault, S. Ford, D. Cheslett). **Term of Reference g)** Recent findings of 'red vent' associated with A. simplex in salmon in the UK and Iceland possibly is a manifestation of increased parasite population. The abundance of Anisakis simplex larvae seems to be increasing in many (economically) important fish species in the North Atlantic Ocean, e.g. larvae can be found in significant numbers in the flesh and internal organs of cod, herring, mackerel, Atlantic salmon and many more species. The complex life cycle of A. simplex involves zooplankton, fish, and whales as first and second intermediate and final hosts, respectively, and perceived recent changes in the abundance and distribution of these hosts may contribute to an increased abundance of A. simplex in the ecosystem. Information on the trends of A. simplex in Atlantic salmon and in key species of the intermediate and final hosts should may provide insight into the increased occurrence of the red vent condition in salmon. In addition, A. simplex is an EU listed zoonotic parasite, therefore, this ToR has relevance to human health concerns (humans consuming fish raw or under processed fish can become infected; A. simplex allergens remaining after proper cooking and processing have caused allergic reactions to humans in Italy, Spain, and Japan). (D. Bruno, T.A. Mo, M. Podolska, S. Feist) **Term of Reference h)** There is increasing information from studies in wild freshwater and marine fish species that diseases may affect growth, reproduction and survival of different life stages of fish and may thus have an impact on recruitment and stock structure. However, only in few cases have diseases been explicitly considered in stock assessment models estimating natural mortality and population dynamics. Since the potential risk to fish populations due to diseases is of considerable ecological and economical concern, the WGPDMO recognizes a need to conduct a literature review aimed at an assessment of the population effects of infectious and non-infectious diseases and associated pathology, as well as parasites. It is anticipated that the results of the review will be relevant for a range of ICES Expert Groups, including the stock assessment groups. (T. Lang, S.W. Feist, S. Jones, K. Broeg) **Term of Reference i)** Update on OSPAR request 2008/no.13 (W. Wosniok, T. Lang, M. Podolska) **Term of Reference j)** A number of ICES publications, either web-based or in ICES publication series, are being prepared or updated at present, the progress of which has to be reviewed by WGPDMO. It will be necessary to consider ways by which these can be linked to each other. New publications have to be considered. (S.W. Feist, L. Madsen, S. Ford, D. Bruno) **Term of Reference k)** This is in compliance with a request from the ICES Data Centre. (W. Wosniok, T. Lang)
Resource requirements:	None required other than those provided by the host institute.
Participants:	The Group is normally attended by 20–25 representatives of ICES Member Countries and guests with expertise in pathology and diseases of marine organisms.

Secretariat facilities:	Required to a limited extent for data and publication issues.
Financial:	No financial implications.
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	MCC, MHC, WGBEC
Linkages to other organizations:	BEQUALM, OIE, EU, OSPAR, HELCOM

Annex 14: Review Group Technical Minutes – RG/ADG MON1

RG/ADG MON1- Fish index review

Copenhagen, 19 – 21 May, 2008

1. Introduction

The Review Group was constituted to deal with the request from OSPAR for assessment of the proposed Fish Disease Index (FDI) and more importantly to undertake a thorough review of the technical details of the FDI. The RG/ADGMON1 was also to prepare the advice on the quality assurance of biological measurements but that Expert Group, due to lack of participation failed to meet. The BEWG did provide an update of the JAMP guidelines on benthic sampling which was subject to a separate review by Jorgen Nørrevang Jensen of the ICES secretariat.

The members of the RGMON1 were:

- Edmund Peeler, CEFAS, UK
- Mark Myers, NOAA, USA
- Jon Barry, UK
- Werner Wosniok, Germany
- Jørgen Nørrevang Jensen, DK
- Paul Keizer, Vice Chair ACOM
- Claus Hagebro, ICES Secretariat

The ADG, consisting of all of the members of the RG, met in Copenhagen 19–21 May 2008. It became apparent at the start of the meeting that additional documentation was needed to properly evaluate the FDI. A member of the WGPDMO, Thomas Lang, advised that this documentation was available in the 2006 and 2007 reports of the WGPDMO. These documents were subsequently made available to the RG members. Written reviewers provided before the beginning of the ADGMON meeting are attached as Annexes 1 and 2.

2. The Fish Disease Index (FDI)

2.1 Overview

The intent of the proposed FDI is to provide an "easy-to-understand indicator of ecosystem health to be used in the assessment of the environmental status of ecosystems" (WGPDMO, 2006). The WGPDMO has been working on this index for several years and has attracted the attention of OSPAR as a potential contribution to the QSR 2010. OSPAR 2007 requested that ICES undertake a further assessment of the FDI with a view to using it as a case study in the QSR 2010. Specifically the request was:

OSPAR (13-2008) An assessment of data on fish diseases in the OSPAR maritime area

"To trial the fish disease index developed by ICES and reported at WKIMON III through application in an evaluation of data collected by OSPAR Contracting Parties with a view to providing an assessment of fish disease in the OSPAR maritime area for inclusion in the QSR 2010 to the extent possible. The assessment should consider the prevalence of externally visible fish diseases, macroscopic liver neoplasms and liver histopathology in common dab (*Limanda limanda*)."

In their report (WGPDMO 2008) WGPDMO notes that it was not possible to conduct the trial as requested due to the lack of suitable data in the ICES Database. They note that much more data exists in national laboratories and that this data should be uploaded to the ICES database. However as an interim measure the WGPDMO modified their recommended approach and applied the FDI using 3 different subsets of Externally Visible Diseases. While this application is instructive, the WGPDMO cautions that it is preliminary and that the results support the identified need to consider as broad a range of diseases as possible in the calculation of the index. The comments and recommendations in the following section apply to this trial assessment.

In addition to the direct response to the OSPAR request, ICES undertook an independent review of the FDI. Werner Wosniok, one of the main authors of the FDI, was present for the ADG meeting as the representative of WGPDMO. He gave a presentation of the development and the details of the FDI that provided clarification to the documentation available. During his presentation many if not all of the issues raised in the revised comments from two of the reviewers were addressed.

The following observations and recommendations are provided to WGPDMO for their consideration. Werner Wosniok, a member of WGPDMO and one of the main architects of the FDI agreed that it would be desirable to undertake much of this work prior to providing OSPAR with the final assessment to be included as a case study in the QSR 2010. There is however a timing issue. It appears that the deadline for information for the QSR will be March 2009 and the WGPDMO is not planning to meet until the first week in March. It was felt that every effort should be made to get this assessment into the QSR because it is an important development. Equally it was also important that WGPDMO has an opportunity to address many of the following recommendations.

ACTION: It was agreed that ICES should consult with OSPAR and the chair of WGPDMO to find a solution. Informal talks with OSPAR indicate that the material needs to be available for OSPAR ASMO 2009.

2.2 General Observations:

1) The Review Group was very supportive of the work to date and encouraged the continued development for specified application, i.e. assessment of fish heath, and potential for "indices" for other assessments. There is lot of inherent flexibility in the approach. A considerable amount of work has clearly been spent in developing a sophisticated and robust measure of disease occurrence and prevalence. The approach is clearly applicable to different types of assessments using various fish diseases as an indicator.

2) There is a need to prepare complete documentation for the index including detailed technical annexes. The "scattered" and incomplete documentation hampered the work of the Review Group which depended very heavily on input from Werner Wosniok for its deliberations.

   Among other points, a discussion of internal and external validity needs to be included. The external population is the wider population to which it may be possible to extrapolate the results. The target population is the immediate population to which the study results may be extrapolated. The target population may be the dab population within the sampling area (i.e. the ICES rectangle). The external population may be dab from a wide area, e.g. an OSPAR area. However, the external and target populations should be defined.

The external validity relates to the capacity to extrapolate results from a survey or study to the external population. The internal validity relates to the validity of the study to the target population. This raises questions about sampling. The FDI is calculated using a sample of fish. To reliably extrapolate the result to the target population (i.e. all dab in the square), the study population should be representative of this population (ideally all dab would have been equally likely to have been sampled). If this was not the case the results need to be interpreted with more caution. It appears that the sample design is quite robust but this needs to be documented.

a) There is some difficulty in understanding the present documentation due to the lack of clarity and sometimes apparent consistency in the use of various terms. In particular, the use of the term FDI does not appear to be consistent; it may require the use of a subscript to provide clarity. There seems to be some confusion in the way that the report is written in that the score for each fish is referred to as the FDI for an individual fish. However, the statistic that is calculated from all of these fish scores is also referred to as a Fish Disease Index. The documentation should be accompanied by a glossary of terms used.

b) Detailed equations should be included, formulas for the statistics, etc. For example in determining the 'Trend' Component of the FDI, the Mann-Kendall statistic is used. This is certainly a common non-parametric way to assess trend. It is presumed that the scaled version just scales the value by the maximum value possible if there was perfect positive trend. It would be helpful if the report actually gave the mathematical formula for what they have done.

3) Prepare a manuscript for publication. This will provide a strong primary citation for the method and potentially could stimulate further research into similar approaches that could advance the methods available for monitoring and assessing the effectiveness of management measures for human activities that impact marine ecosystems.

4) Update present assessment based on technical recommendations. While it was noted that the present trial assessment was informative, it would be preferable to address as many of these recommendations as possible prior to the QSR being finalized.

5) The level component of the FDI is dependent on the level during the assessment period and not on the actual level of the FDI. From the 2007 report, the level seems to be calculated by dividing the 'previous' results from a region into 33% chunks and assigning each one to a higher, middle and lower category. Then this surely means that the data you are assessing are conditional on being in that region. That is, if the 'previous' disease prevalences in that region are very high and if the new results are only fairly high, then the 'level' component will indicate that the new data are in the 'lower' category. That is, they may still be high compared to the rest of the world, but not high relative to previous results in that region.

2.3 Technical recommendations

1) It is recommended that the level and trend components should be presented separately. In the trial assessment the FDI statistic combines both the level and trend component. This has the advantage of producing a single measure. However, information is lost and the two statistics should be

presented simultaneously but separately, though ideally within the same figure. It is further noted that the trend statistic is non-parametric and does not necessarily reflect the magnitude of the trend, however it was concluded that there was no practical alternative to this approach.

2) An evaluation of the potential impact of sample clustering on the calculation of the index is needed. The sampling method used to capture dab needs to be discussed in the context of the FDI. Fish caught in a trawl (haul) can be considered as a cluster sample. Fish caught in the same trawl will be more similar than fish caught in separate trawls. The precision of an estimate of prevalence / index (i.e. the standard error - SE) is greater than the SE for a simple random sample because observations within one cluster are generally more similar than observations between clusters (observations on fish from the same cluster are not independent, thus failure to account the lack of independence will produce underestimates of the variance). The increase in SE resulting from cluster sampling is known as the design effect, D, which is determined by both the average cluster size and intra-cluster correlation co-efficient. An estimate of D is needed to calculate the sample size that will generate the required SE.

The SE and 95% confidence intervals adjusted for clustering can be calculated using a formula developed by Bennett et al (1991):

$$SE = \sqrt{\sum \frac{(pi - p)^2}{c(c-1)}}$$

Where pi = prevalence at the ith site, p = overall prevalence, c = number of sites

Consideration needs to be given as to whether this formula or another approach can be used to adjust the SE estimate of the FDI to take into account the clustered nature of the data.

In particular, the SE used in the simulation studies should be calculated taking into account the clustered nature of the data (however, if clustering does not appear to influence SE then it can be ignored).

3) The use of the raw data for assigning individual FDIs to a "tertile" needs to be investigated. Assigning a –1, 0 or +1 to the FDI values from the assessment period, depending on which "tertile" they are in, clearly loses information. Presumably, the scaling of the statistic to make it lie between 0 and 1 is based on dividing the final score by N (where N is the number of observations). Maybe an alternative to this that doesn't involve the artificial division into these categories would be to simply use the raw data and calculate:

$$L = \frac{1}{NM} \sum_{j=1}^{N} (F_j - mean)$$

where mean is the mean level of the previous data, N is the number of observations in the new data and M is the maximum possible value for the previous data (assumed to be the same for the higher and lower categories).

4) It is important to calculate the minimum change in either the level or trend statistic that can be detected given the available data; i.e. a power calcula-

tion. Similarly, the sensitivity of the FDI to the choice of diseases and the "expert" calculation of the severity index1 needs to be evaluated. Some simulations need to be run to investigate the sensitivity of the results to relative changes in the severity index (c.f., the disease-specific weighting factor) within the group of diseases being considered. Ultimately, what you want to know for these things is: how does a high, medium or low value of the FDI relate to the actual changes in disease on the ground? It should be possible to do some simulation studies to evaluate how changing the disease prevalence (say of each disease by 10%) or trend (say 10% over the study period) is reflected in a change of smiley face colour. This will also be a function of, for example, the length of the time series.

5) An obvious question for someone looking at the results will be the significance of the differences in levels between the reporting areas (ICES rectangles or OSPAR areas). The FDI is presently designed for comparisons within an ICES rectangle. It would also be useful to be able to compare the FDI between rectangles and analyses should be undertaken to allow such comparisons (spatial statistical approaches may be useful). These analyses will have to account for differences in sample size and dates.

6) Subsets of data could be used to investigate other outcomes, i.e. relating the FDI to direct measures of fish condition such as:

a) fish condition (a function of length and weight), or

b) growth rate (for fish with age data)

This would be one way of validating the ecological relevance of the FDI. This would help address the problem with the present approach that on the one hand, you may be detecting differences (red or green) that are not ecologically important and, on the other hand, not detecting differences that are ecologically important (i.e. you're power is not high enough to detect these important differences).

Future studies using the FDI could match changes over time (trend) in FDI with concurrently measured parameters in the same fish, such as age/length curves and condition index (weight/length relationship), when these data are available. This would be a reasonable way to assess the ecological relevance of the FDI and trends in FDI, and perhaps validate the disease-specific weighting factors established by the expert panel, e.g., do fish affected by lymphocystis exhibit reduced length at age or a reduced condition index.

7) It will be useful to explore the utility of FDIs for other assessments such as contaminant exposure or general ecosystem health. In the trial assessment the choice of diseases and the disease specific weighting is based on the estimated impact on the general health / well-being of the individual. The FDI is thus calculated to weight the score based on impact or importance of the diseases on fish health. Future applications could use FDI as a measure of environmental quality, by using diseases that are more strongly associated with chemical contaminant exposure, such as the grossly visible nodules and toxicopathic liver lesions as detected by histo-

[1] This is also referred to as the disease-specific weighting factor, so please be consistent—severity should refer to only the severity within the disease itself, as in mild, moderate or severe lymphcystis.

pathology. For example, for an FDI as a measure of environmental quality, the composite diseases could be assessed in terms of disease specific weighting by the strength of their association with contaminant exposure as demonstrated in the published, peer-reviewed literature, rather than by the expert panel method used in the present iteration of the FDI as a measure of fish health/condition.

8) It needs to be determined if there are any correlations structures in the data in the baseline period. If found this would impact the parameters for the Monte Carlo simulation.

3. Review comments to the JAMP Eutrophication Guidelines: Benthos

Jørgen Nørrevang Jensen reviewed these revised JAMP guidelines and provided the following feedback.

The updating of the Guideline text is well done and only a few minor comments can be added to the text.

1) Section 1 (Introduction) and 3 (Quantitative objectives): k-selected species should be changed to K-selected species in a few places which is the common notation.

2) Section 4 (Sampling strategy): The problem of obtaining representative samples is discussed in the text. The presence of spatial autocorrelation is very often overseen regardless that this can hamper the assumptions in statistical testing by increasing the occurrence or the Type I error. In order to clarify the issues one sentence could be added: "Contrary, the presence of autocorrelation can violate the basic assumptions in standard statistical analyses (Legendre *et. al.*, 2002)". The reference should be added to the reference list.

3) Section 9 (Reporting requirements): The Square bracket can be removed. The last sentence seems to be out of context referring to old actions and it is suggested to delete it.

4) Section 10 (References): Reference to be added (see point 2 above).

Annex 1: Fish index review by Dr Ed Peeler, Cefas

Revised 20 May following discussions with Dr Wosinok, and taking into comments from Jon Barry

1. Overview

A considerable amount of work has clearly been spent in developing a sophisticated and robust measure of disease prevalence.

From an epidemiological viewpoint the key issues are external and internal validity, sampling and variability.

2. Validity

A key issue in reviewing the FDI is that of validity – external and internal.

The external population is the wider population to which it may be possible to extrapolate the results. The target population is the immediate population to which the study results may be extrapolated. The target population may be the dab population within the sampling area (i.e. the ICES rectangle). The external population may be dab from a wide area. However, the external and target populations should be defined.

The external validity relates to the capacity to extrapolate results from a survey or study to the external population. The internal validity relates to the validity of the study to the target population. This raises questions about sampling. The FDI is calculated using a sample of fish. To reliably extrapolate the result to the target population (i.e. all dab in the square), the study population should be representative of this population (ideally all dab would have been equally likely to have been sampled). If this was not the case the results need to be interpreted with more caution.

A report explaining the FDI should discuss external and internal validity in the context of the use of the FDI.

3. Sampling

The sampling method used to capture dab needs to be discussed in the context of the FDI. Fish caught in a trawl (haul) can be considered as a cluster sample. Fish caught in the same trawl will be more similar than fish caught in separate trawls. The precision of an estimate of prevalence / index (i.e. the standard error - SE) is greater than the SE for a simple random sample because observations within one cluster are generally more similar than observations between clusters (observations on fish from the same cluster are not independent, thus failure to account the lack of independence will produce underestimates of the variance). The increase in SE resulting from cluster sampling is known as the design effect, D, which is determined by both the average cluster size and intra-cluster correlation co-efficient. An estimate of D is needed to calculate the sample size that will generate the required SE.

The SE and 95% confidence intervals adjusted for clustering can be calculated using a formula developed by Bennett et al (1991):

$$SE = \sqrt{\sum \frac{(pi - p)^2}{c(c-1)}}$$

Where pi = prevalence at the ith site, p = overall prevalence, c = number of sites

Statistical advice is needed to determine how this equation can be applied to the FDI.

In particular the SE used in the simulation studies should be calculated taking into account the clustered nature of the data (however, if clustering does not appear to influence SE then it can be ignored).

A correlation structure may still exist in the baseline data (e.g. due to temporal trends). If found, the correlation should be used in the stimulation study.

4. Using raw data (from Jon Barry)

Assigning a –1, 0 or +1 to the new values depending on which region they are in clearly loses information. Presumably, the scaling of the statistic to make it lie between 0 and 1 is based on dividing the final score by N (where N is the number of observations). Maybe an alternative to this that doesn't involve the artificial division into these categories would be to simply use the raw data and calculate something like:

$$L = \frac{1}{NM}\sum_{j=1}^{N}(F_j - mean)$$

where mean is the mean level of the previous data, N is the number of observations in the new data and M is the maximum possible value for the previous data (assumed to be the same for the higher and lower categories).

5. Power calculation

The minimum change in either the level or trend statistic that can be detected given the available data should be calculated.

6. Future studies

6.1 Comparisons in space

An important potential application of the FDI is to compare different regions and trends over time. The analyses to allow these comparisons to be made whilst accounting for differences in sample size and dates need to be developed (a similar approach to that used to consider

Spatial statistical approaches could be used to investigate spatial distribution of the FDI.

The FDI is designed for comparisons within an ICES rectangle. It would also be useful to be able to compare the FDI between rectangles and analyses should be undertaken to allow such comparisons (spatial statistical approaches may be useful).

6.2 Other outcomes

Subsets of data could be used to investigate other outcomes:

- fish condition (a function of length and weight), or

- growth rate (for fish with age data)

7. Severity score

The choice of disease specific weighting is based on the estimate impact on the general health / well-being of the individual. The FDI is thus calculated to weight the score based on impact on importance of disease with respect to fish health. Is this the most appropriate criteria? Are we not more interested in disease as indicators of environmental quality? In which case disease rankings may have been different. A terrestrial analogy may be a disease index for dairy cattle. The weighting for disease would be different depending on whether the index was being undertaken to give an indication of welfare of the cattle or the economic impact of disease.

Consideration should be given to formulating a second FDI using diseases and severity scores based on their value for environmental monitoring.

8. The FDI assessment statistic

The FDI statistic combines both the level and trend component. This has the advantage of producing a single measure. However, information is lost and the two statistics should be presented simultaneously but separately, though ideally within the same figure.

9. Summary of recommendations

- discuss external and internal validity of the FDI
- assess importance of clustered data on the SE of the mean FDI, adjust the estimate of the SE in the simulation studies
- investigate use of raw data to make within rectangle comparisons between baseline period and period of interest, and compare with current approach of 'binning' data into 3 categories.
- present trend statistic and level statistic separately
- assess correlation structure in the baseline period and if important take into account in the simulation studies
- power calculation: investigate minimum change in the FDI that can be detected given the available data
- revise use of colour for smiley faces
- prepare manuscript describing the FDI for publication in a peer-reviewed journal (e.g. Ecological Modelling).

10. References

Bennett, S., Woods, T., Liyanage, W.M., and Smith, D.L. 1991. A simplified general method for cluster-sample surveys of health in developing countries. World Health Statistics Quarterly, 44: 98-106.

Annex 2: Review from Jon Barry

I have had a second look at the Fish Disease Index using further information from the 2006 and the 2007 WGPCMO reports as well as Annex 6 of the WGPCMO report. I will be commenting chiefly on some of the statistical aspects of the Fish Disease Index (FDI).

I understand mostly how the adjustment was done for age and sex and am happy with this.

There seems to be some confusion in the way that the report is written in that the score for each fish is referred to as the FDI for an individual fish. However, the statistic that is calculated from all of these fish scores is also referred to as a Fish Disease Index. I'll refer to the fish one as a 'Fish Score'.

The FDI statistic is calculated as a weighted sum of a 'level' and a 'trend' component.

1. 'Level' component

From the 2007 report, this seems to be calculated by dividing the 'previous' results from a region into 33% chunks and assigning each one to a higher, middle and lower category. Maybe I'm missing something, but this surely means that the data you are assessing are conditional on being in that region. That is, if the 'previous' disease prevalences in that region are very high then if the new results are only fairly high, then the 'level' component will say that the new data are in the 'lower' category. That is, they may still be high compared to the rest of the world, but not high relative to previous results in that region. I also got confused by the fact that the diagrams use red, green and yellow colours for these regions – which are not the same as the red, green and yellow smiley faces used for the combined FDI statistic.

I haven't looked at where the previous data came from in terms of which surveys, times, sample designs and so on. Presumably, if these data are a mix of lots of surveys then there are all sorts of correlations between subsets of data and so on. Maybe it is best to just not go there!

Assigning a –1, 0 or +1 to the new values depending on which region they are in clearly loses information. Presumably, the scaling of the statistic to make it lie between 0 and 1 is based on dividing the final score by N (where N is the number of observations). Maybe an alternative to this that doesn't involve the artificial division into these categories would be to simply use the raw data and calculate something like:

$$L = \frac{1}{NM}\sum_{j=1}^{N}(F_j - mean)$$

where mean is the mean level of the previous data, N is the number of observations in the new data and M is the maximum possible value for the previous data (assumed to be the same for the higher and lower categories).

2. 'Trend' Component

The Mann-Kendall statistic is certainly a common non-parametric way to assess trend. I presume that the scaled version just scales the value by the maximum value possible if there was perfect positive trend. Again, it would be helpful if the report

actually gave the mathematical formula for what they have done. The other thing with Mann-Kendall is that its value does not specifically reflect the extent of the trend. So, assuming no random variation around the trend, if there was a constant 1% trend over ten years, this would yield the same value of the statistic as if there was a 50% trend over ten years. Of course, in practice, there will be random variation around the trend and so, for small trends at least, there probably would be some correlation between the value of the MK statistic and the underlying trend.

3. Further comments

I'm not sure that combining the trend and level components is a good idea. I'd like to see them assessed separately. For example, if you had a high area but a slightly decreasing trend then this area might not get picked up (i.e. you'd get an orange smiley face), even if the overall level is still high.

I still don't know how the monte-carlo simulation was done to assess the 2.5% and 97.5% end points. However, I could guess so I'm not too worried about this. What worries me is that whether a smiley case is red or green is based effectively on a randomisation under some null hypothesis of 'no change'. However, the problem with doing this is that, on the one hand, you may be detecting differences (red or green) that are ecologically unimportant and, on the other hand, not detecting differences that ARE ecologically important (i.e. you're power is not high enough to detect these important differences).

Ultimately, what you want to know for these things is: how does a high, medium or low value of this statistic relate to the actual changes in disease on the ground? It seems to me that you ought to be able to do some simulation studies to evaluate how changing the disease prevalence (say of each disease by 10%) or trend (say 10% over the study period) is reflected in a change of smiley face colour. This will also be a function of, for example, the length of the time series.

In summary, my major points are:

1) The level component seems to be conditional on past data and not on the actual level.

2) The trend statistic is non-parametric and does not necessarily reflect the magnitude of the trend.

3) I don't like combining the two components. Looks like adding apples and pears to me. Why not calculate the two components separately and then report the worst one?

4) Even if you do as I suggest in 3., I think you need to run some simulation studies to see how your indices reflect some disease-changing scenarios.

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