ICES WGPDMO REPORT 2015

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

24–28 February 2015

Helsinki, Finland

International Council for the Exploration of the Sea
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Executive summary

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPD-MO) met 24–28 February 2015 at the Finnish Food Safety Authority (EVIRA) in Helsinki, Finland. The meeting, chaired by Neil Ruane (Ireland), was attended by 8 participants representing 8 ICES Member Countries. In order to consider the all Terms of Reference, intersessional work was done by WGPDMO members and several 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.

The group produced a report on new disease trends in wild and farmed fish and shellfish in the ICES area, based on national reports received from 14 member countries. Amoebic gill disease, which was reported as increasing in Norway in the 2014 report, has spread even further and the first cases of AGD were reported in Canada and the Faroe Islands. A new virus (virus Y) was reported in farmed rainbow trout in Norway with pathological lesions similar to those reported for heart & skeletal muscle inflammation (HSMI). A number of shellfish pathogens have expanded their range, with *Bonamia ostreae* reported in flat oysters in Denmark and the OsHV-1µVar in Pacific oysters in Norwegian and Swedish waters.

WGPDMO has completed a number of reports which are to be published including 'Parasites of marine finfish and shellfish posing a hazard to human health', 'Trends in important diseases affecting the culture of fish and molluscs' to be published as an ICES CRR and 'Disease management mitigates risk of pathogen transmission from maricultured salmonids' published in the journal "Aquaculture Environment Interactions".

A number of disease information leaflets have also been updated and details can found in Section 3 of this report.

WGPDMO has formulated a response to OSPAR Special request 03/2015 on the potential to integrate monitoring for plastic particles in current fish disease surveys. WGPDMO concludes that the feasibility of including monitoring for plastic particles in the current fish disease monitoring programmes should be further developed. In previous years, WGPDMO has highlighted the decline in fish disease monitoring carried out by ICES member states and hopes that the inclusion of plastics will provide new impetus into this monitoring. The group also recommends that ICES identifies other expert groups (EGs) with the potential to take samples for plastics monitoring.

WGPDMO acknowledges the ICES initiative to incorporate Aquaculture Science into its Strategic Plan and through the next three year reporting period will focus a number of ToRs on diseases of importance in aquaculture (see Annex 3). This will be overseen by the new Chair of WGPDMO, Ryan Carnegie of the Virginia Institute of Marine Science (VIMS).

1   Administrative details

Working Group name
Working Group on Pathology & Diseases of Aquatic Organisms (WGPDMO)
Year of Appointment
2013
Reporting year concluding the current three-year cycle
3
Chair
Neil Ruane, Ireland
Meeting venue(s) and dates
5–9 March 2013, Padova, Italy, (13 participants)
25–28 February 2014, Copenhagen, Denmark, (12 participants)
24–28 February 2015, Helsinki, Finland, (8 participants)

2   Terms of Reference a) – z)

ToR a) New disease trends in wild and cultured fish, molluscs, and crustaceans based on national reports.

ToR b) Parasites and other infectious agents in marine finfish and shellfish species posing a hazard to human health.

ToR c) Disease interactions between farmed and wild finfish.

ToR d) ICES publication 'Trends in important diseases affecting the culture of fish and molluscs in the ICES area 2003 – present'.

ToR e) Maps of fish and shellfish diseases (discontinued – see WGPDMO Report 2014 ICES CM 2014/SSGHIE:02).

ToR f) Development of the Fish Disease Index (FDI) in relation to results of FDI assessments of diseases of flounder and Baltic cod and liver histopathology and macroscopic liver lesions in the common dab.

ToR g) Disease associated population effects of commercial fish and shellfish species.

ToR h) ICES publications on pathology and diseases of marine organisms.

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

ToR j) Development of templates for the national reports from ICES Member Countries.

ToR k) OSPAR Special Request (OSPAR 4/2014) Interactions between wild and captive fish stocks.

ToR l) OSPAR Special Request (OSPAR 3/2015) Development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys.

3   Summary of Work plan

ToR a) New disease conditions and trends in diseases of wild and cultured marine organisms are reviewed. This is an annual, ongoing ToR for WGPDMO and will provide updated information for ToR d.

ToR b) A range of parasites and other infectious agents reported by WGPDMO have the potential to be harmful to human health if ingested in under-processed food. There is an upward trend in the consumption of raw fish and other seafood products which may increase this risk. A literature review of risk, prevention and mitigation strategies was prepared.

ToR c) WGPDMO has produced reports on disease interactions between farmed and wild finfish since 2010. A report was prepared in conjunction with OSPAR request 4/2014 (see ToR k).

ToR d) The previously published report (ICES CRR 265) requires updating with new information on diseases of importance for aquaculture, including new and emerging diseases. The current draft of the document was reviewed and final updates added during the meeting with a view to preparing the report for publication as an ICES Cooperative Research Report.

ToR f) The FDI has been validated for use in dab, flounder and cod. The preparation of data sets and an approach to calculate the FDI using the free software R has been completed and a user manual is currently in preparation.

ToR g) The potential risk to fish and shellfish populations due to disease is of considerable ecological and economical concern. A report providing information on diseases causing population effects was discussed.

ToR h) A number of ICES publications currently in preparation will be reviewed by WGPDMO. This is an ongoing, annual ToR.

ToR i) This is an annual ToR in compliance with a request from the ICES Data Centre.

ToR j) Variability exists within the National Reports for wild and farmed finfish and shellfish regarding disease occurrence and how it is reported. A generic reporting structure was presented and discussed, and its suitability for use in future meetings will now be tested.

ToR k) In response to a special request from OSPAR (4/2014) on interactions between wild and captive fish stocks, WGPDMO will discuss how the current ToR (C) can be utilised to provide a response to this request.

ToR l) In response to a special request from OSPAR (3/2015) WGPDMO is requested to define an appropriate common monitoring protocol for plastic particles in fish stomachs and selected shellfish species, to be applied across the OSPAR maritime area.

4   Summary of Achievements of the WG during 3–year term

ToR a) New disease trends in wild and cultured fish, molluscs, and crustaceans based on national reports.

- Summary trend report included in each WGPDMO annual report.

ToR b) Parasites and other infectious agents in marine finfish and shellfish species posing a hazard to human health.

- Final report to be submitted to a scientific journal in the field of zoonosis/food safety.

ToR c) Disease interactions between farmed and wild finfish.

- Jones, S. R. M., Bruno, D. W., Madsen, L. & Peeler, E. J. 2015. Disease management mitigates risk of pathogen transmission from maricultured salmonids. Aquaculture Environment Interactions **6**: 119-134.

ToR d) ICES publication 'Trends in important diseases affecting the culture of fish and molluscs in the ICES area 2003 – 2014'.

- Publication resolution 2013/1/SSGHIE06: the document is completed and ready for publication in the ICES Cooperative Research Report Series.

ToR f) The Fish Disease Index (FDI) in relation to results of FDI assessments of diseases of flounder and Baltic cod and liver histopathology and macroscopic liver lesions in the common dab.

- The FDI calculation is now available as an R package software from the authors.

ToR h) ICES publications on pathology and diseases of marine organisms.

- ICES Disease Leaflet Series (updated): No. 3 '*Ichthyophonus*, a systemic mesomycetozoan pathogen of fish' (S. R. M. Jones).
- ICES Disease Leaflet Series (updated): No. 7 '*Pseudoterranova* larvae ("codworm"; Nematoda) in fish' (M. Longshaw).
- ICES Disease Leaflet Series (updated): No. 8 '*Anisakis* larvae ("herringworm"; Nematoda) in fish' (M. Longshaw).
- ICES Disease Leaflet Series (updated): No. 19 'Marteiliosis of oysters caused by *Marteilia refringens*' (T. Renault & S. E. Ford).
- ICES Disease Leaflet Series (new): No. 58 'Heart and skeletal muscle inflammation (HSMI) of farmed Atlantic salmon (*Salmo salar* L.) and the associated *Piscine reovirus* (PRV)' (E. Biering & A. H. Garseth).
- ICES Disease Leaflet Series (new): No. 59 'Piscine myocarditis (cardiomyopathy syndrome' (D. Bruno).
- ICES Disease Leaflet Series (new): No. 60 'Amoebic gill disease (AGD) of farmed Atlantic salmon (*Salmo salar* L.' (N. M. Ruane & S. R. M. Jones).
- ICES Disease Leaflet Series (new): No. 61 'Liver tumours in flatfish' (S.W. Feist & T. Lang)

- ICES Disease Leaflet Series (new): No. 62 'Hyperpigmentation of common dab (*Limanda limanda* L.) (T. Lang, S.W. Feist, P. Noguera & D. Bruno)

- ICES Disease Leaflet Series (new): No. 63 'Pseudomoniasis in farmed fish' (P. Vennerström)

ToR k) OSPAR Special Request (OSPAR 4/2014) Interactions between wild and captive fish stocks.

- http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2014/Special%20Requests/OSPAR_%20Interactions_of_wild_and_captive_fish_stocks.pdf

ToR l) OSPAR Special Request (OSPAR 3/2015) Development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys.

- Report produced to be transferred to ICES Advice to OSPAR through ICES ACOM

5   Final report on ToRs, workplan and Science Implementation Plan

5.1   Produce an update of new disease trends in wild and cultured fish, molluscs and crustaceans based on national reports (ToR a)

The update in the following sections is based on national reports for 2014 submitted by Canada, Denmark, England & Wales, Finland, France, Germany, Ireland, The Netherlands, Norway, Poland, Russia, Scotland, Sweden and the USA. It documents significant observations and highlights the major trends in newly emerging diseases and in those identified as being important in previous years. The scientific names for each species mentioned can be found in Annex 5.

Wild Fish

Viruses

Piscine orthoreovirus **(PRV)** was detected in approximately 14% of returning Norwegian wild Atlantic salmon by qPCR, while the prevalence in hatchery-reared salmon was 24 % and 55 % in farm-origin (escapees) fish. As phylogenetic analyses of PRV from wild and farmed salmonids group together, the role of the virus in potential disease interactions between wild and farmed fish requires further study.

Infectious *haematopoietic necrosis* **virus (IHNV)** was isolated from anadromous rainbow trout originating from wild stock at a rearing facility in western Canada during an outbreak of IHN which resulted in 3% cumulative mortality. In the autumn of 2014, the virus was also isolated from 4 of 12 wild Chinook salmon ovarian fluid pools at the same facility.

Rhabdovirus, has been linked to reports of mass mortality among viviparous eelpout on the Swedish east coast. Three fish examined were lethargic, but had no visible external signs of disease or damage. All fish were positive for *Vibrio anguillarum* and virus cultures were positive for a *rhabdovirus*. Early indications are that this may be a novel *rhabdovirus* and further investigations are ongoing.

Lymphocystis, the prevalence of *lymphocysti*s was reported as increasing in both herring (0.3%) and flounder (2.8%) in the southern Baltic (ICES subdivisions 25 and 26).

Bacteria

Renibacterium salmoninarum was detected by qPCR in 4/404 Atlantic salmon brood-stock kidney samples in Norway. *Renibacterium salmoninarum* has been detected previously in wild salmon.

Yersinia ruckeri was recorded in two wild returning Atlantic salmon from the SE Sweden during wild fish surveillance (serotype II). In addition, serotype I was isolated from the eye and kidney of one wild cod from the Hanöbukten area.

Aeromonas sobria was linked to mortality of butterfish during the summer, on the SE coast of Sweden. The water temperature was 22°C. Three fish were autopsied, and two had external signs of skin lesions.

Ascomycetes

Sympoventuriaceae

Ochroconis globalis was isolated from a single returning Baltic salmon in 2011 From Sweden. Large granulomas in the kidney and liver were observed. Histological examination showed a fungal-like infection and further analysis isolated an *Ochroconis* spp. These results were verified as *Ochroconis globalis* by PCR and cultivation, representing the first report of this pathogen in fish in Europe.

Parasites

Protist

Sarcocystis sp. was tentatively identified in the cardiac and skeletal muscle of an Atlantic salmon from Mörrumsån, Sweden. Ventricular muscle cells were hypertrophic, with enlarged nuclei and pleomorphic appearances, lobulated or pseudo lobulated. Some cells had multiple nucleoli or vacuoles as well as mitotic activity and aligned nuclei. When stained with PAS and silver staining, intracellular vesicles varying in shape from round to oval, and lying in rows inside individual muscle cells. Although confirmation of the identification is pending, the structure is consistent with intracellular parasites belonging to the *Sarcocystidae* within the phylum *Apicomplexa*.

Myxozoa

Parvicapsula pseudobranchicola was detected from the kidney of 62 (37.6%) of 165 pink salmon collected north of Vancouver Island, Canada, by PCR amplification of small subunit rRNA gene. This was confirmed by sequencing and represents the first evidence of this salmon pathogen in this country.

Nematoda

Contracaecum osculatum - An increasing trend has been observed in recent years, in infected Baltic cod in the Polish and Russian EEZ in the Baltic Sea.

Anisakis simplex occurred at a prevalence of between 4–15 % in Baltic cod in Denmark, with a mean intensity between 1 and 4 parasites per fish. The prevalence of *A. simplex* in Baltic herring has decreased when compared with prevalence data over the past twenty years, in Poland and a decreasing prevalence has also been reported in the Russian EEZ. However, the prevalence of infected cod remains high in comparison to the results of studies conducted in earlier decades in Poland.

Pseudoterranova decipiens is an increasing problem in wild cod from the Baltic Sea. Parasitological investigations on cod sampled between March 2013 and April 2014, east of Bornholm, where grey seals are established indicated that cod worm was recorded in the musculature at prevalences up to 55 % and intensities of up to 56 worms per fish.

Other diseases

Hyperpigmentation has a generally low prevalence in dab within the Irish Sea and Severn regions (England & Wales), although there has been an increase at Liverpool Bay from 4.7% in 2006 to 20.5% in 2014 and in the Burbo Bight from 3.1% to 25%. Prevalence has increased in dab from the German Bight (compared to 2013), but has still not reached the high levels recorded approx. 10 years ago. Hyperpigmentation was recorded in dab from the Baltic Sea in 2014, but at a prevalence < 1.0 %.

Liver nodules in common dab from Red Wharf Bay in England had a prevalence of 12.1% in 2014 (n=72) whereas no liver nodules were observed during 2012 (n= 79). Historical prevalence at this site has generally been observed at ≈5 %.

Skin ulcers decreased in cod and flounder in the Baltic Sea (Poland EEZ). During the summer of 2014, several thousand migrating Atlantic salmon and sea trout died in the rivers of northern Finland and Sweden. There were several reports of fish with skin lesions and *Saprolegnia*-like infections. Ulcerative dermal necrosis (UDN) was suspected but not confirmed. The number of migrating fish was exceptionally high and water temperatures were above normal (reaching 23°C).

Nickel spill occurred on the west-coast of Finland in the river Kokemäenjoki where 66 tons of nickel was accidentally released into the river close to the estuary. No mortalities were recorded in large fish, but the effect of the release will be investigated to determine the potential effects on the survival of progeny of resident fish in the affected area.

Conclusions

- A novel rhabdovirus has been isolated, associated with high mortality in eelpout from the Swedish east coast.
- The first occurrence in Europe, of *Ochroconis globalis* is reported from Baltic salmon.
- A *Sarcocystis*-like organism has been found in an Atlantic salmon from Mörrumsån, Sweden.
- Wild Atlantic salmon broodstock in Norway tested positive for *Renibacterium salmoninarum* by real-time PCR.
- *Parvicapsula pseudobranchicola* was detected from pink salmon in western Canada and represents the first evidence of this salmon pathogen in Canada.

Farmed Fish

Viruses

Infectious pancreatic necrosis virus (IPNV) – In Norway, the number of cases has continued to decline, to 48 cases from 56 in 2013. The use of IPNV-resistant salmon has contributed to this decline.

Infectious salmon anaemia virus (ISAV) – In Norway, the virus was diagnosed in 10 farms, of which four were considered primary outbreaks and six due to spread from neighbouring farms. In western Canada, 2853 Atlantic salmon and 76 Chinook salmon were examined by RT-qPCR and the virus was not detected.

Heart and skeletal muscle inflammation (HSMI) – In Norway the increasing trend continued with 181 cases, up from 134 in 2013. A novel piscine orthoreovirus (PRV)-like agent (virus Y) was associated with mortality in rainbow trout. Histopathological changes associated with the novel agent resembled those of HSMI.

Salmon alphavirus (SAV) – The increasing trend in Norway continued with 142 outbreaks, up from 100 in 2013, and was due mainly to subtype SAV3 in western Norway. Subtype SAV2 was detected for the first time in areas previously occupied by SAV3, representing an increase in the historical range of SAV2 from mid Norway. In Scotland, there is a trend towards a subclinical/chronic form of PD. In Ireland, the number of cases declined from 8 to 3 in 2014.

Piscine myocarditis virus (PMCV) – In Norway, the increasing trend in the number of cardio-myopathy syndrome (CMS) outbreaks continued, with 107 in 2014, up from 100 in 2013. The Faroe Islands experienced the first outbreak of CMS in Atlantic salmon since the 1990s.

Bacteria

Aeromonas salmonicida **subsp.** *salmonicida* – In Norway, furunculosis was diagnosed in Atlantic salmon despite vaccination with a polyvalent, oil-adjuvated vaccine. Mortalities were low and a small number of fish displayed abscesses and/or haemorrhages in the musculature and peritoneum. Coincidentally, the bacterium was reported from nearby wild fish, probably contributing to an increased infection pressure to the farmed salmon.

Parasites

Crustacea

Lepeophtheirus salmonis – In Norway, a surveillance program for resistance against pharmaceuticals has been established, and reduced sensitivity in lice for the different pharmaceuticals is observed in all parts of the coast. However resistance against hydrogen peroxide remains rare. In the Faroes, salmon lice are the most important cause of disease in farmed salmon.

Paramoebida

Paramoeba perurans – In Norway, amoebic gill disease (AGD) continues to increase in importance in Atlantic salmon to 63 cases in 2014 and has tended to spread from Western Norway to Mid Norway. Most cases are diagnosed in autumn and early winter. AGD has also been diagnosed at five lumpsucker farms and one ballan wrasse farm in 2014 in Norway. In Ireland, AGD continues to be important in Atlantic salmon, with 14 clinical cases reported but with highly variable mortality. The majority of sites controlled the disease with freshwater baths. In Scotland, prevalence of AGD in Atlantic salmon shows interannual variability, from 3% to 39% between 2011 and 2014. There have been fewer cases of clinical disease in 2013 and 2014 compared with the previous 2 years. Most cases are recorded between June and February. Rising gill scores trigger hydrogen peroxide treatments and farms tend to treat when mean gill scores are still less than 1. Post-treatment losses are associated with acute

gill irritation caused by the treatment. Approximately half the marine farms treated with peroxide at least once in 2013/14 and treatments occurred more often in the first year of production. AGD was reported for the first time in Canada. Atlantic salmon at three production sites in British Columbia tested positive for the amoeba and showed clinical disease. In the Faroe Islands, the first outbreak of AGD was recorded. The amoeba had first been found in 2013 without disease. However, in 2014 a much higher prevalence of the amoeba has been found and salmon at several sites have AGD-like lesions, but without elevated mortality.

Microspora

Desmozoon lepeophtherii – In Scotland, the parasite was associated with a proliferative gill disease in Atlantic salmon. The disease occurred in late autumn and was characterized by necrotic and inflamed interlamellar tissue. In cases with a high number of spores in the gills, spores were also more likely to be recorded in kidney, spleen and hindgut.

Conclusions

- A novel piscine orthoreovirus -like agent (virus Y) in Norway was associated with mortality and HSMI-like pathology in rainbow trout.
- An increasing trend of subclinical or chronic pancreas disease is recognized in Scotland.
- SAV subtypes 2 and 3 now co-occur in western Norway.
- Amoebic gill disease (AGD) caused by *Paramoeba perurans*, was observed for the first time in Atlantic salmon in Canada and the Faroe Islands. The impact of AGD in Atlantic salmon continues to increase in Norway, and remains high in Ireland and Scotland.

Wild and farmed molluscs and crustaceans

Viruses

Ostreid herpesvirus 1 (OsHV-1) – Mortality in Pacific oysters in the River Crouch estuary, UK, adjacent to a purification centre was seen in July with a sea temperature at sampling of 23.9^{0}C. Initial sampling of affected oysters resulted in 13/30 positive for OsHV-1 µVar at the reference site in the Crouch, and 15/30 positive for OsHV-1µVar at a site to the east of the reference site; and the presence of OsHV-1 "wild-type" (but **not** the µVar) virus in 5/30 oysters from the River Roach, an adjacent estuary. Subsequent sampling in the wider estuary to map the extent of the infection identified no further positives for the microvariant, although "wild-type" OsHV-1 was identified in 6 oysters from two sites in the adjacent River Roach estuary. These wild-type positives have been described in oysters from the Roach previously, and do not appear to be associated with mortality events. The disease continues to be an important issue for oyster aquaculture in both Ireland and France.

In the autumn of 2014, several cases with high mortality among Pacific oysters were observed along the Swedish west coast as well as in the Oslo Fjord of Southern Norway. This is the first report of the disease in both these countries. Brackish water carried along the Swedish coast, decreased salinity from over 30 down to 24 per thousand, which may have induced stress in the oyster population. In the Oslofjord, water temperatures close to 27°C were registered by the end of July 2014 in an area where heavy mortalities were observed in September. OsHV-1 was identified by real-

time PCR in samples of live oysters, and sequencing confirmed the isolates to be OsHV-1 μvar.

Surveillance for OsHV-1 began in western Canada in 2013 and the country remains free of the disease to date.

Bacteria

Vibrio aestuarianus **in Pacific Oysters** – *V. aesturianus* was found by real-time PCR from 7/30 samples of Pacific oysters in the River Crouch estuary, UK. Retrospective screening of 2013 mortality samples (which had previously tested negative for OsHV-1) resulted in a number of positive results. In France, *Vibrio aestuarianus* DNA was reported in adult oysters (9/9 cases) in association with abnormal mortality. In Ireland a retrospective survey involving the testing of archive material from around the coast of Ireland was concluded in 2014. The survey consisted of 4 bays which were considered to have had at least one mortality event related to the presence of *V. aesturianus* prior to 2014 and three of the four bays showed multiple detections over a period of years. All mortalities associated with *V. aestuarianus* in 2014 occurred in adult and half grown oysters.

Vibrio splendidus **in blue mussels** – In France, bacteria identified as *V. splendidus* were detected in dying mussels and were shown to cause mortality in the laboratory. Unusual environmental conditions (large amounts of fresh water, re-suspension of sediments during a series of storms, and a high renewal rate of specific water masses in Brittany's Pertuis area) may be partially responsible for the mortalities.

Vibrio tapetis **in Manilla clam** – Brown Ring Disease was observed in cultured stocks in one bay in Ireland, where mortality had been occurring since 2011. *V. tapetis* was first detected in the bay in 1997 but it had not previously caused mortality. The possibility that high levels of RLOs observed in the affected stocks could be involved is being investigated.

Parasites

Bonamia ostreae **in European flat oysters** – Detected for the first time in Denmark in native oysters sampled in autumn as part of the regular screening programme in Limfjorden, performed twice a year since 2000. At the three sites sampled, *Bonamia*-like cells were observed in 2 of 31, 18 of 31 and 0 of 31 cultured oysters, respectively. The histological findings were confirmed by heart imprints of the same oysters and by PCR. RFLP and sequencing of the PCR products confirmed the parasite to be *Bonamia ostreae*.

Marteilia **sp. in blue mussels** – *Marteilia refringens* was detected in blue mussels in two batches collected in Brittany (Rade de Brest) in February and April, respectively, and another one collected in Normandy (Baie des Veys) in August in France. The pathogen was confirmed by histology and *in situ* hybridisation. Blue mussels from 4 locations along the Swedish west coast, all sampled from the county of Bohuslän, region of Västra Götaland, tested positive by PCR and confirmed by histology. Two of the sites were within the restriction area of previous years' findings, whereas the other two were new. In one of the new sites, only 1 (3.3%) of 30 investigated mussels were infected. The highest prevalence was in the other new site, where 8 (27%) of 30 mussels tested positive by PCR.

Haplosporidium nelsoni **in Pacific oyster** – A mortality event occurred in farmed Pacific oysters from the River Dart estuary (south west England) at the end of July 2014, and histological analysis revealed an intense infection with a haplosporidian parasite in one animal. This was later confirmed by PCR to be *H. nelsoni.* This case revealed an intense infection affecting the digestive gland and with all known developmental stages of the parasite including spore stages. Molecular screening subsequently revealed a high prevalence of positive animals in the absence of histological signs (26 out of 30 animals) suggesting that although Pacific oysters may be highly susceptible to infection, only a few animals may develop serious infection. Subsequent samples tested by PCR revealed 18/30 and 20/40 oysters positive by PCR with no signs of infection histologically. Archive samples from 2011, from the same location were also tested by PCR and 72/150 positive for *H. nelsoni*, again with no histological evidence of infection. *H. nelsoni* was detected at moderate prevalences of 3–27% in 21% of 2014 oyster survey samples from the Maryland part of Chesapeake Bay, USA. Both the mean prevalence and the geographic distribution for MSX disease in Maryland waters of Chesapeake Bay increased moderately from their very low levels of 2011–2013.

Perkinsus marinus **in eastern oyster** – *P. marinus* infections were detected at prevalences of 3–100% in 95% of 2014 oyster survey samples from Maryland waters of Chesapeake Bay, USA. The 2014 survey mean prevalence of 52% for perkinsosis decreased slightly from the moderate 2013 mean of 57%. *P. marinus* infections decreased more sharply in the higher-salinity, Virginia part of Chesapeake Bay, reaching levels not observed since 1998. *P. marinus* levels have been trending downward in recent years in the Gulf of Mexico as well. Reports from the northeastern USA indicate no change to *P. marinus* levels over previous years.

Other Diseases

Coccidiosis **in King scallop** - *Pecten maximus* sampled during 2013 from the English Channel were found to harbour an unidentified coccidian parasite infecting the adductor muscle in 23.4% of animals sampled (n=124). Various parasite stages were observed, predominantly zygote and developing oocyst containing multiple nuclei, but little host response was detected in most cases. Macroscopically, the scallops appeared healthy with normal appearance of the adductor muscle.

Haemic neoplasia **in hard clam** – A haemic neoplasia was noted in three experimental populations of hatchery produced hard clams in Rhode Island, USA. The prevalence of this condition was 3–10% in normally buried clams but 12–47% in weaker, surfacing clams. While the parasite QPX was present in the populations and could have contributed to the weak condition of some of the clams, the occurrence of neoplasia at such prevalences is unusual and warrants further investigation.

Conclusions

- First detection of *Haplosporidium nelsoni* in Pacific oysters in the UK.
- First detection of OsHV-1µVar in Pacific oysters in Norwegian and Swedish waters.
- *Vibrio aestuarianus* was associated with mortality in Pacific oysters in Ireland, England and France.
- First detection of *Bonamia ostreae* in Denmark, in native flat oysters from Limfjorden.
- *Marteilia refringens* was detected from blue mussels in Swedish waters from two areas outside the current restriction area.

5.2 Parasites and other infectious agents in marine finfish and shellfish species posing a hazard to human health (ToR b)

A. Alfjorden, M. Podolska, T. Karaseva, L. Madsen

Abstract

Several parasites and other infectious agents frequently reported by the WGPDMO in the annual update of disease trends (ICES WGPDMO reports 1999–2015) have the potential to be harmful to human health if ingested in unprocessed or inadequately/partly processed seafood. These include, but are not limited to, larval stages of the nematodes *Anisakis simplex* and *Pseudoterranova decipiens*. For them the risk to human health may occur in two ways: gastrointestinal infections with the possibility of subsequent peritonitis or the involvement of other sites in the body. Infections may also lead to allergic reactions to pathogen-related substances (e.g. secretory products) present in seafood. This review will identify reported zoonotic agents in ICES-member countries in the period 1999–2014 and summarize risks and mitigation strategies based on existing literature. It will also highlight issues that require further research and solutions.

Introduction

Enlarged world trade with fish products worldwide and an upward trend of travelling together with the global warming and its effects on zoonotic diseases have increased the hazard of these diseases. The movement and enlarged trade of farmed fish between the continents have also increased the interest for fresh non-heated fish products for consumption, which also will increase the risk to encounter the diseases for fish and shellfish consumers. The popularity of fish dishes with raw fish (e.g. sashimi, sushi, ceviche, carpaccio) has increased the consumption of raw fish worldwide. This growing interest has been linked to the growing market for fish and fish products as well as the strong development of aquaculture. In the same period the reported incidences of fish transmitted zoonoses have increased significantly (Keiser and Utzinger 2005; McCarthy and Moore 2000; Nawa *et al.* 2005; Robinson and Dalton 2009). The major zoonotic fish parasites belong to three groups of fish parasites: Digenic trematodes of *Opistorhorchiidae* and *Heterophyidae*, nematodes of the families *Anisakidae* and *Gnathostomatidae* and cestodes, especially from the family *Diphyllobothriidae*. Farmed fish can act as vectors for these zoonoses but almost no documented evidence of this has been published before 2000. Improvements in diagnostic techniques linked with new and more widely distributed epidemiological surveys have increased the documented disease cases and pointed out areas where risks are present and also suggested ways to lower these hazards (Lima dos Santos and Howgate 2011). Table 1 lists the reported zoonotic agents in ICES member countries over the last 15 years. Parasites most frequently reported are *Anisakis* sp. and *Pseudoterranovo* sp. both being nematodes. There is one report of *Diphyllobothrium*, a cestode, during the same period. Therefore this review will be focusing on those three agents. Based on existing literature risks and mitigation strategies for nematodes and cestodes will be summarized.

Table 1. Reported zoonotic agents in ICES member countries 1998-2014*.

YEAR	COUNTRY	PARASITES/BACTERIA & HOSTS
2014	Poland	*Contraceaecum osculatum* in Baltic cod
		Anisakis simplex in cod and herring
	Russia	*C. osculatum* in Baltic cod, *A. simplex* in herring
	Denmark	*Pseudoterranova decipiens* in Baltic cod
2013	Finland	*A. simplex* in Baltic herring and Atlantic Salmon brood fish
		P. decipiens in perch
	Poland	*A. simplex* and *C. osculatum* in cod
	Denmark	*P. decipiens* in Baltic cod
2012	-	
2011	Poland	*A. simplex*, *C. osculatum*, *P. decipiens*
		in the liver of Baltic cod
	Russia	*A. simplex* increasing trend in Baltic herring
		A. simplex in cod, capelin, haddock in Barents Sea
		P. decipiens in Barents Sea cod
		D. dendriticum (tapeworm) plerocercoid in smelt
		(abdominal cavity) in the Baltic
2010	Poland	*A. simplex* in herring
	Russia	*A. simplex* in Baltic Sea herring and Barents Sea cod
	Ireland	Red vent syndrome in Atlantic salmon
	Scotland	Red vent syndrome in Atlantic salmon
	Sweden	*P. decipiens* in cod, longspined bullhead, fourhourn sculpin
2009	Poland	*A. simplex* in Baltic herring
	Russia	*Anisakis simplex* in capelin
	Norway	Red vent syndrome in Atlantic salmon
	Scotland	Red vent syndrome in Atlantic salmon
2008	USA	*Mycobacterium pseudoshotsii* and *M. shotsii* in striped bass and bluefish
	Poland	*A. simplex* in Baltic herring
	Russia	*A. simplex* in haddock and capelin Barents Sea, *P. decipiens* in cod from Barents Sea
	Scotland	Red vent syndrome in Atlantic salmon
	Ireland	Red vent syndrome in Atlantic salmon
	Denmark	*M. marinum* in farmed turbot
2007	Russia	*A. simplex* in Baltic herring
	Poland	*A. simplex* in Baltic herring
	England & Wales	Red vent syndrome in Atlantic salmon
	Netherlands	*Vibrio vulnificus* in farmed barramundi
2006	USA	*Mycobacterium* spp. in striped bass
	Denmark	*V. vulnificus* in eels (wild)
	Russia	*A. simplex* in Baltic herring

	Poland	*A. simplex* in Baltic herring
	Spain	*V. vulnificus* in farmed eel
2005	Poland	*A. simplex* in Baltic herring
	Russia	*A. simplex* in Baltic herring, *P. decipiens* in Barents Sea cod
2004	USA	*Mycobacterium* spp. in striped bass
	Scotland	*A. simplex* in cod, haddock and whiting
	Russia	*A. simplex* in Barents Sea cod and in Barents Sea redfish, pink salmon and chum salmon, *P. decipiens* in Barents Sea cod
		A. simplex in Baltic herring
	Poland	*A. simplex* in Baltic herring
2003	-	
2002	USA	*Mycobacterium* spp. in striped bass
2001	USA	*Mycobacterium* spp. in striped bass
	Canada	*P. decipiens* in American plaice
	Russia	*A. simplex* in beaked redfish
	Spain	*A. simplex* in anchovies
	England & Wales	*A. simplex* in flounder
2000	Poland	*A. simplex* in herring
	Russia	*A. simplex* in Barents Sea cod, *P. decipiens* in Barents Sea cod
1999	Poland	*A. simplex* in Baltic herring and cod
	Russia	*A. simplex* in Barents Sea cod, pink salmon and keta salmon
	Spain	*V. vulnificus* in eels
1998	USA	*Mycobacterium* spp. in striped bass, *V. cholera* non-01 in striped bass and perch
	Europe	Mycobacterial granulomata in North Sea cod
	Russia	*A. simplex* in Barents Sea cod, red fish and long rough dab
		P. decipiens in Barents Sea cod

*Data originating from WGPDMO reports 1999 – 2015.

Nematodes

Anisakis sp. has continuously been reported from herring in the Baltic Sea over the years as well as from cod, haddock and other fish species from the Barents Sea (Table 1). Red vent syndrome (cause *Anisakis* sp.) in Atlantic salmon from the coast of Norway as well as the sea around the British Isles has been reported in the last 6 years (Table 1). Reports on *Pseudoterranova decipiens* also cover the screened period of years, primarily from Barents Sea cod as well as Baltic Sea cod (Table 1).

Anisakidosis is an infection due to larval stages belonging to the family *Anisakidae*, the genera *Anisakis* and *Pseudoterranova*, where *A. simplex* is the most prevalent pathogenic species. The life cycle involves small crustaceans as first intermediate hosts and fishes end cephalopods as second hosts and mammals as the definitive hosts. Nematodes belonging to the family *Anisakidae* (*Anisakis simplex*, *Pseudoterranova decipiens* and *Contracaecum osculatum*) represent a potential hazard for human health. A large number of marine fish and cephalopods can transmit the disease to humans (Sakanari and McKerrow 1989; Smith and Wotten 1978). *Anisakidosis* is a serious zoonotic dis-

ease and can be transmitted if eating raw as well as inadequately cooked, marinated, salted or smoked fish containing the parasite. Infection may lead to abdominal pain, nausea, vomiting and/or diarrhea. Viable, invasive larvae can migrate beyond the stomach, penetrating the intestine, liver and other internal organs (Sakanari and McKerrow 1989). Some patients can also develop syndromes of allergy following infection or consumption of dead larvae (Audicana and Kennedy 2008; Miranda da Silva 2008; Valero *et al.* 2003).

Anisakidosis has been reported almost all over the world with Japan as having the highest incidence (Ishikura and Namiki 1989; Ishikura and Kikuchi 1990). European countries have reported cases, too, among them Denmark (Andreassen and Jorring 1970), the Netherlands (van Thiel 1976), Germany (Möller and Schröder 1987), France (Bourree *et al.* 1995), Belgium (Vercammen *et al.* 1997), Spain (López-Serrano *et al.* 2000; Repiso Ortega *et al.* 2003), Norway (Eskesen *et al.* 2001), Iceland (Skirnisson 2006), Italy (Pampiglione 2002; Ugenti *et al.* 2007), the United Kingdom (Pozio 2008) and Austria (Kapral *et al.* 2009). The most common agent of human infection described in the literature is *A. simplex*, but other Anisakid species are known to represent a potential hazard for man, too. Based on results of multiple gene sequence analyses, Mattiucci *et al.* (2013) identified several new cases of gastric anisakiasis in Italy, which were caused by *A. pegreffii* nematodes. Cases of *Anisakidosis* caused by the presence of *Pseudoterranova decipiens* were reported in France (Pinel *et al.* 1996), Korea (Moon-Soo *et al.* 1999; Yu *et al.* 2001), Chile (Mercado *et al.* 1997, 2001; Jofré *et al.* 2008), Peru (Cabrera *et al.* 2003) and Iceland (Skirnisson 2006). A case of *Anisakidosis* caused by adult *Hysterothylacium aduncum* was noted in Japan (Yagi *et al.* 1996). First case of human *Anisakidosis* infected with *Contracaecum* sp. was reported in Australia (Shamsi *et al.* 2011). According to Takei and Powell (2007), the incidence of cases of *Anisakidosis* is expected to increase with the increasing popularity of sushi and sashimi.

Latest findings of Svanevik *et al.* (2013) revealed that the larvae of parasitic nematodes of the genus *Anisakis*, act as vector for bacteria during its migration from the intestine into the flesh of fish. The results indicated elevated levels of bacteria due to the presence *Anisakis* larvae in the flesh of blue whiting. The presence of *Anisakis* sp. has been reported in several species of farmed fish, e.g. rainbow trout *Oncorhynchus mykiss* (Bassleer *et al.* 1973; Wootten and Smith 1975) and *Salmo trutta* (Wootten and Smith 1975), salmon *Salmo salar* (Dick *et al.* 1987; Marty 2008), plaice *Pleuronectes platessa* (McKenzie *et al.* 1976) and cod *Gadus morrhua* (Hemmingsen *et al.* 1993) (for further information see Table 2, originating from Lima dos Santos and Howgate (2011).

Table 2. Lima Dos Santos & Howgate (2011).

Presence of Anisakidae larvae in cultivated fish in different countries.

Parasite	Fish	Country	References
Anisakis sp. *Contracaecum* sp.	Trout *Oncorhynchus mykiss*	Belgium	Bassleer et al. (1973)
Anisakis simplex	Trout *Oncorhynchus mykiss, Salmo trutta*)	Scotland	Wootten and Smith (1975)
Anisakis sp. *Contracaecum* sp.	Plaice (Pleuronectes platessa)	Scotland	McKenzie et al. (1976)
Contracaecum sp.	Salmon (Salmo salar)	USA	Dick et al. (1987)
Anisakis sp.	Cod (Gadus morrhua)	Norway	Hemmingsen et al. (1993)
Hysterothylacium aduncum	Salmonids	Chile	Gonzalez (1998), Carvajal and Gonzalez (1990), Carvajal et al. (1995), Sepúlveda et al. (2004), Torres et al. (2010)
Contracaecum sp.	Tilapia	Israel	Paperna (1996)
Contracaecum sp.	Walleye (*Sander vitreus*)	USA	Muzzall et al. (2006)
Anisakis spp. *Hysterothylacium*	Several marine species	China	Chen et al. (2008)
Anisakidae	Salmon (Salmo salar)	Canada	Marty (2008)
Anisakis simplex	Cobia (*Rachycentron canadum*)	Taiwan	Shih et al. (2010)

It has to be stressed that most studies on farmed salmonids fed with processed feeds report that the nematodes are not present (Skov *et al.* 2009; Skov *et al.* 2014). Skov *et al.* (2009) reported that none of the rainbow trout from either Danish freshwater culture (n=40) or Danish mariculture (n=166) harboured nematode larvae in the body cavity or the belly flap musculature. According to Skov *et al.* (2014) third stage larvae of anisakid nematodes were absent from the body cavity and musculature of 190 rainbow trout cultured in marine net-cages in Danish waters. Nevertheless, transmission of marine endoparasites was documented by findings of *H. aduncum* in the intestines. This is supported by Heuch *et al.* (2011) that reports on parasites frequently present in wild Norwegian cod were rarely found in farmed cod including food-borne helminthes. *A. simplex* was the most abundant at high prevalences in wild cod. Only about 1% of the hatchery-reared farmed fish harboured this worm. However, it is possible for smaller invertebrates and fish to enter into the net cages, and if they are infected, they may transfer the infection to the fish farm (Marty 2008; Skov *et al.* 2009). The source of fish infection with *Anisakis* in aquacultures is usually the feed (e.g. infected raw marine fish).

When it comes to *Pseudoterranova decipiens*, studies on the presence of *P. decipiens* in several species of fish, carried out along the Swedish east coast from 2004 to 2010, revealed a high prevalence in cod from the Southern Baltic. The presence of this nematode species was detected in the muscles of 74% cod examined in the Trelleborg area (ICES 2011). The dispersal of *P. decipiens* in the Baltic has become a growing problem in cod fisheries with the rapid increase in the number of the grey seals in the central and southern Baltic (Königson 2011). According to Hauksson (2011), the prevalence,

abundance and density of *Pseudoterranova* sp. larvae were the highest in Icelandic cod sampled closest to shore, which was also in closest proximity to grey seal colonies and in the shallowest waters. Buchmann and Kania (2012) suggested that an increasing seal population in the Baltic may impact the occurrence of *P. decipiens* in cod fillets. The occurrence of *P. decipiens* larvae has not previously been considered to be a problem for Baltic cod, because this nematode has not been frequently reported during the last 80 years. This phenomenon may apply to other anisakids, because the grey seal is also a final host for *C. osculatum*; therefore, an increasing seal population size may impact the dispersion of these nematode species in the Baltic. Most recent investigations of Nadolna and Podolska (2014), Haarder *et al.* (2014) and Mehrdana *et al.* (2014) indicated that the prevalence of cod infected with anisakid nematodes (especially *Contracaecum* sp.) increased in comparison to the results obtained in last three decades. Lunneryd *et al.* 2015 reported that up to 100% of the fish were infected with *Pseudoterranova decipiens* in some of the Southern Baltic areas. Sculpin were generally worse infected than cod both in abundance and prevalence of parasites. In the areas with salinity lower than 7‰ there was a sharp decrease of *P. decipiens* infected fish. In stomachs of grey seals the sealworm *P. decipiens* was only found in samples from the Central Baltic. Investigation of seals further north did only identify infections of *Contracaecum osculatum*. Statistical models showed a significant correlation between the number of seals in an area and the prevalence of sealworms in cod. The results indicate that seal presence drives the distribution in the Southern parts of the Baltic and that low salinity, or some other variable which correlates with salinity, limits the *P. decipiens* distribution in the Northern part.

Cestodes

Diphyllobothrium dendriticum has only been reported once in the ICES WGPDMO yearly reports over the last 15 years (Table 1) and in that case from a Baltic Sea smelt. Here it has to be remembered that the WGPDMO reports primarily are focusing on significant new findings. Several species of *Diphyllobothrium* are described as pathogenic to man with *D. latum* being the most important. There are two intermediate hosts: a copepod as the first intermediate host and a predatory freshwater, anadromous, or marine fish as the second intermediate host. The biology and geographical distribution of these cestodes are described by Dick (2007). Infections with the parasite are considered to be mild and persons infected do normally not show any disease symptoms, though infected persons may have diarrhoea, abdominal pain and anaemia (Dick 2007; Scholtz 2009). The incidences have increased largely in recent years, linked to the consumption of raw fish. Most often the infections are known to occur in connection with cold freshwater fish in northern parts of the world, but cases in South America and Asia have also been reported. Recent estimates indicate that approximately 20 million individuals might be affected (Scholtz *et al.* 2009). In the case of salmonids it seems that wild salmon is the main transmitter of human diphyllobothriasis (Scholz *et al.* 2009), but it might be that the cause for the recent increase could be due to the use of farmed salmon (Cabello 2007; Dick 2007). However, epidemiological studies performed during outbreaks in South America have not been able to demonstrate the presence of *Diphyllobothrium* larvae in imported salmon fillets. Extensive studies carried out in Chile established the presence of *Diphyllobothrium* larvae in cultivated salmonids, but the parasites were only reported in the fish viscera and never in muscle tissue.

The EFSA report 2011 states that there is limited reliable data on the occurrence of plerocercoids of *Diphyllobothrium* in strictly marine fish (Andersen 1977) from the Baltic Sea. Plerocercoids of *Diphyllobothrium* cestodes from salmonids have been identi-

fied as being *D. latum* but whitefish (subfamily: Coregoninae) do not harbour plero-cercoids of *D. latum*. Coegoneides are instead frequently infected with larvae of other *Diphyllobothrium* species, especially *D. dendriticum* and *D. ditremum* (Andersen 1977). According to Andersen and Valtonen (1992), plerocercoids of *D. latum* occur at low to moderate infection level in pike, turbot, perch and ruff, with the highest prevalence (~39%) and mean intensity (3.3) in turbot.

When it comes to the human cestode infections, Scholtz *et al.* (2009) concludes that human diphyllobothriosis have decreased radically in the endemic areas of Baltic Sea, but are still present in many of the countries that have border towards this brackish sea. In Finland there are still 20 cases reported/year (Scholz *et al.* 2009). This shows that Diphyllobothrioses still possess a human hazard in the Scandinavian and Baltic countries, especially when raw or poorly cooked fish are used. For example when fish used for consumption are marinated raw in sugar and salt, called "gravning". In some countries previously considered to be disease free (Austria, Czech Republic, Belgium, The Netherlands,and Spain), sporadic cases have been reported over the last 6 years, which were presumably linked to the consumption of raw imported fish (Wicht *et al.* unpublished). Most human infections in South America caused by *D. pacificum* and, allegedly, *D. latum* have been reported from Chile and Peru, with other cases in Argentina, Brazil, and Ecuador, including recent outbreaks of Diphylloboth-riosis in Rio de Janeiro and Saõ Paulo) (Scholz *et al.* 2009).

The EFSA report from 2011 concludes that water contamination with eggs of the tapeworms is increased by the ability of most *Diphyllobothrium* species to mature in nonhuman hosts. Because of their generally broad host specificity, their life cycles are maintained in nature, independently from humans. Therefore antihelminthic treat-ment of the human population does not necessarily eliminate the parasite from af-fected areas. Sylvatic cycles involving bears, foxes, seals, gulls, and other fish-eating birds and mammals probably play a crucial role in water contamination.

Prevention & Control

Cestodes

The report from Sholtz *et al.* (2009) states that the aim for preventing and controlling the disease is to break the life cycle of the parasite. Theoretically, any point of the life cycle can be attacked. In practice, measures should focus on the following three prin-cipal points: (i) prevention of water contamination (see above), (ii) treatment of peo-ple harbouring the parasite, and (iii) prevention of transmission of infective larvae from fish to humans. Sewage treatment plants and the use of sanitary facilities repre-sent the most effective sanitary measures to avoid water contamination. The human cases can effectively be treated with praziquantel. Niclosamide is an alternative anti-helminthic drug effective for diphyllobothriosis. To prevent the parasite from being transmitted by consumption of infected fish, the best prophylaxis is to avoid con-sumption of raw, smoked, or pickled fish. Fish should be well cooked; alternatively, kept at -18°C for 24 to 48 h to prevent the infection.

Nematodes

Evisceration immediately after the catch

This may reduce the post mortem migration of nematodes, however, this methodolo-gy does not eliminate parasites, which are already present in the flesh of the fish. The distribution of parasites in the body of the hosts depends on fish species and type of organ/tissue. *A. simplex* larvae migrate from the viscera into the flesh and internal

organs of fish, however, there is some controversy when the migration takes place (post mortem or in live fish). Smith (1984) reported that storage of un-gutted herring and mackerel on ice (3-5°C) results in post mortem migration of *A. simplex* larvae into the flesh, but no significant migration was seen in blue whiting, whiting, and walleye pollock. Wootten and Waddell (1977) presumed that larval *Anisakis* sp. could penetrate further into the musculature of smaller fish such as whiting and herring compared to larger cod. According to Stromnes and Andersen (1998), *A. simplex* larvae distributions within host tissues are possibly related to the availability of nutrients.

Generally, the examination of fish or fishery products for the presence of parasites is usually focused on detecting nematodes in fish flesh (fillets), and liver inspection is sometimes ignored. The liver was the most infected organ of cod (Szostakowska *et al.* 2005, Nadolna and Podolska 2013) and blue whiting, *Micromesistius poutassou* (Cruz *et al.* 2007). This organ is used for the production of cod-liver oil. The consumption of cod liver is popular in many European countries. Cod liver and fresh cod liver oil are also a part of the traditional north Norwegian fish dish "mølje". Canned cod liver is available on market in Island, Latvia, Lithuania, Russia, Poland and Norway.

Temperature treatments

Anisakid nematodes possess high tolerance to a wide range of temperature, due to the production of trehalose, acting as a cryoprotectant (Behm1997). *A. simplex* and *P. decipiens* larvae have the ability to survive freezing at high subzero temperatures (Adams *et al.* 2005, Stormo *et al.* 2009). Results indicated that 10% of *Anisakis* larvae may survive under microwave treatment (Lanfranchi and Sardella 2010) and remained alive after gamma-irradiation (Chai *et al.* 1991; Loaharanu and Murrell 1994; Padovani *et al.* 2005; Seo *et al.* 2006). Raw fish for consumption are required to be treated in certain ways. Within the EU the regulation is set by the Commission regulation (EU) No 1276/2011 (8 December 2011 - Annex III to Regulation (EC) No 853/2004), where it is set up that food business operators must ensure that the raw material or finished product undergo a freezing treatment in order to kill viable parasites that may be a risk to the health of the consumer. For parasites other than trematodes, the freezing treatment of the fish involves to lower the temperature in all parts of the product to at least –20°C for not less than 24 hours or –35°C for not less than 15 hours. In the USA, the FDA (Food and Drug Administration) requires that all fish and shellfish intended for raw or semi-raw (e.g. marinated or partly cooked) consumption should be blast frozen to -35°C or below for 15 hours, or be completely frozen to -20°C (-4°F) or below for 7 days (FDA, 1998). The same freezing treatment is required in Canada (Weir, 2005). Although adequate cooking, freezing or frozen storage may kill nematodes (Wharton & Aalders, 2002), the allergenic proteins of anisakids are thermo-stable (Audicana *et al.*, 2002; Moneo *et al.*, 2005) and resistant to freezing even in CO_2 modified-atmosphere packaging (MAP), commonly used to preserved fresh fish products (Pascual *et al.* 2010). Some allergens from *A. simplex* larvae are also highly resistant to heat and pepsine treatments (Caballero and Moneo 2004, Vidacek *et al.* (2009).

Sufficient monitoring

Several factors may influence on the prevalence of fish infection with anisakid nematodes: the presence of intermediate and final hosts, abiotic and biotic factors, salinity, oxygen availability and the presence of pollutants. According to European Food Safety Authority (EFSA), all wild caught fish must be considered at risk of containing any viable parasites of human health concern if these products are intended to be eaten raw or almost raw (EFSA 2010). For fishery products caught from fishing grounds in

the Baltic Sea, the following viable parasites: *A. simplex sensu stricto* (*s.s.*), *C. osculatum* (*s.s.*), *P. decipiens* (*s.s.*) and *Diphyllobothrium* spp. represent possible health risks (EFSA 2011). It must be emphasized, that in many counties there is no regular monitoring of commercially important fish species for the presence of parasites with zoonotic potential.

Application of methods other than visual examination to detect the presence of parasites

Several methods are applicable to detect the presence of Anisakid nematodes in fish and fish products. The most common method is visual examination and candling (examination on a light table). The effectiveness of this technique depends on the thickness of the fish fillet. According to Hafsteinsson *et al.* (1987), candling does not allow detection of nematodes embedded deeper than 6 mm in the fish muscle because of scattering and absorbance of visible light in the fish tissue. The employment of slicing techniques permit to improve the recovery rates of nematodes from 30% up to 70% (Karl and Leinmann 1993). Stormo *et al.* (2004) suggested the possibility of using target molecules (compounds absorbing light in the range of 300–600 nm) for the detection of nematodes in cod muscle. Heia *et al.* (2007) described application of imaging spectroscopy for detection Anisakid nematodes in cod fillets. The method is non-intrusive and should thus be feasible for industrial purposes. A promising new detection methodology, based on transillumination hyperspectral imaging has been described recently by Sivertsen *et al.* (2011).

Hyperbaric treatment

Brutti *et al.* (2009) evaluated the application of high hydrostatic pressure to inactivate *A. simplex* larvae. Hyperbaric treatment applied for 5 min at a pressure equal to 300 MPa was shown to be sufficient to devitalise all the larvae present in the fish. Since this method does not damage the outer cuticle of the parasite with the possible dispersion of antigenic material, it seems that high pressure treatments do not increase the risk of an allergenic reaction (Molina Garcia and Sanz 2002).

Larvicidal efficacy

Several authors have demonstrated significant effects of various natural products against *Anisakis simplex*, especially essential oils of different aromatic plants, e.g. *Matricaria chamomilla* and *Thymus vulgaris* (Hierro *et al.* 2004; Navarro *et al.* 2008; Barros *et al.* 2009; Romero *et al.* 2012; Giarratana *et al.* 2014).

Data analyses – estimating the infection rate

Data analyses including estimations of infection rates can be used to evaluate areas with high infection prevalence and intensity of infection in particular fish species and seasonal distribution of parasites. The usefulness of this method is rather limited, because Anisakids are widely distributed geographically and there are no anisakid free areas. It would be applicable for Baltic herring, because of the seasonality of *A. simplex* occurrence (peak of infection in spring, absence of parasites during the summer).

Aquaculture as a way to produce Anisakid free fish

In countries where traditional methods to control fishborne parasitic diseases have failed and where the disease is endemic, there are other ways to reduce the risk of eating hazard fish products, namely by producing fishes that are parasite free which can be used for the market of raw and lightly processed fish. In aquaculture you have this possibility and several reports from screening of many thousand farmed fish in Europe, North America and Japan have confirmed this as described earlier. However,

it must be clear that feeding farmed fish with pelleted food at just some stage or stages is not a guarantee to have this freedom. Hemmingsen *et al.* (1993) kept cod caught from wild stocks on uninfected feed for two years and in the end they were as infected as the wild cod sampled in the same period. This shows that cultivated fish still can become pyratized, if they have access to natural food or are fed with infected feed.

Conclusions

It is often difficult to associate an outbreak with a particular food item and furthermore, if the foodborne route is suspected, to identify how the food implicated became contaminated. Due to these difficulties, the acquisition of parasitic infections via the food transmitted route is almost certainly underdetected. It is clear that investigations from recent years, especially reports from the Baltic Sea region, are indicating a rapid increase in the presence of *Anisakidae* larvae infecting several species of fish in this area (Lunneryd *et al.* 2015; Haarder *et al.* 2014; Nadolna and Podolska 2013). These findings are associated with an increasing population of grey seals in the Southern Baltic Sea area. The ecological change and the increase in spread of this parasite are posing hazards for human consumption of these fish if fish are consumed e.g. raw. The Baltic Sea is surrounded by a large human population that are consuming fish products from this area. The quickly evolving interest for marine food resources and increasing focus on the freshness of fish products are changing the awareness of people concerning these risks. Rapid cooking and less frying, recommended by chefs/kitchen masters are setting new standards for risk of consumers to get *Anisakidosis*. A more widespread use of non-heated food products is also increasing the risk for serving food with still living parasites. If the consumers are not made aware of these problems and how the risks can be minimized, we will be facing an increase in prevalence of cases of human *Anisakidosis*. This awareness has to be changed and the risk factors made public for the food safety authorities as well for the human consumers.

Regarding risk handling, any part of the life cycle of the parasite can be attacked. In practice, (i) prevention of water contamination), (ii) treatment of people harbouring the parasite, and (iii) prevention of transmission of infective larvae from fish to humans are areas that should be focused on. Regarding the risk of human diphillybothriosis, sewage treatment plants and the use of sanitary facilities represent the most effective sanitary measures to avoid water contamination and this has made this problem less common during the last decades. Still there remains a risk for spread of this parasite via alternative hosts (canids, bears and other fish-eating animals) which can contribute to the infection establishing amongst fish as middle stage host of the parasite.

What is more of an issue during recent years are the problems attached to *Anisakidae*. How can we minimize these risk associated. In this review we have highlighted several possibilities: sufficient monitoring, evisceration immediately after the catch, visual examination and other methods to detect the presence of parasites, temperature treatments, hyperbaric treatment, larvicidal efficacy, seasonal infection rate estimations through data analyses and the use of aquaculture to control the infection. But many of these methods are not fully investigated or have several disadvantages, which means that parallel actions are needed in risk minimizing human behaviour. In conclusion we need both approaches put together in a series of practical actions. Risk decreasing actions together with a risk minimizing plan are needed to decrease the hazards for the human fish consumers. In the process of becoming aware of problems

in the marine ecosystem and revealing new trends in diseases and interactions, this ICES expert working group WGPDMO has an important role to play putting member countries data together on a yearly basis, elucidating areas that should be focused on in more detail as a term of reference.

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5.3 Disease interactions between wild and farmed finfish (ToR c and ToR k (OSPAR Request 2013/04))

S. R. M. Jones, D. W. Bruno, L. Madsen

Since 2010, WGPDMO has produced reports related to pathogens in mariculture. Prior to its meeting in 2014, WGPDMO along with WGAGFM, WGMME and WGAQUA, was tasked with providing written responses to a request from OSPAR (4/2014) related to disease interactions between farmed and wild fish. The ICES Advice Drafting Group ADGFISH met June 18–20 2014 in Copenhagen to formulate a response to the OSPAR request. ADGFISH was informed by the written contributions from the four ICES Expert Groups. Additionally, anonymous reviews of the Expert Group contributions were available. The written contribution from WGPDMO was subsequently peer-reviewed and has been published in the primary literature (http://www.int-res.com/abstracts/aei/v6/n2/p119-134/).

ADGFISH drafted an advice document which is available publically (http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2014/Special%20Requests/OSPAR_%20Interactions_of_wild_and_captive_fish_stocks.pdf). Regarding the interaction of diseases and parasites, the advice summary stated:

"Pathogen transmission and undesired genetic and ecological consequences of using antibiotics and other pharmaceuticals require additional mitigation measures. The mitigation of diseases and parasites in mariculture occurs at two scales: area-based (coordinated stocking, harvesting, and fallowing) and farm-based (vaccination, early pathogen detection, veterinary prescribed treatments, and depopulation or early harvest in the event of viral disease). Management zones, defined by local hydrography

(using circulation models) and biological properties of infectious agents should be established for each farm or farm cluster."

WGPDMO acknowledges the ICES initiative to incorporate Aquaculture Science into its Strategic Plan. The Expert Group has established competency in reporting significant trends in diseases and pathology associated with farmed finfish and shellfish species at the national level among ICES member countries. Furthermore, WGPDMO has the expertise to report and interpret information concerning pathogen interactions between wild and farmed aquatic species. Consequently the WGPDMO anticipates playing a lead role in the description and interpretation of disease and pathogen data in the ICES Strategic Plan.

5.4 Trends in important diseases affecting the culture of fish and molluscs in the ICES area 2003 – present (ToR d)

N. M. Ruane, S. R. M. Jones, L. Madsen, P. Vennerstrom, R. Carnegie, T. Renault

The ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) provides annual reviews of national reports on the disease status of wild and farmed fish and molluscs in the ICES area. In 2004, the group published a first report collating this information from 1998–2002. This second report aims to provide an update on the status of the major diseases described in the original report and also to provide an overview of new diseases which have emerged since the previous report was published. The table of contents for the new report is shown below and following Resolution 2013/1/SSGHIE06 the document will now be submitted to ICES for publication in the ICES Cooperative Research Report Series.

1   Background

2   Viral Diseases of Farmed Fish

     2.1 Infectious Salmon Anaemia

     2.2 Viral Haemorrhagic Septicaemia

     2.3 Pancreas Disease

     2.4 Infectious Pancreatic Necrosis

     2.5 Viral Nervous Necrosis/Viral Encephalopathy & Retinopathy

     2.6 Heart and Skeletal Muscle Inflammation

     2.7 Cardiomyopathy Syndrome

3   Bacterial Diseases of Farmed Fish

     3.1 Francisellosis

     3.2 Rainbow Trout Fry Syndrome

     3.3 Enteric Redmouth Disease/Yersiniosis

     3.4 Red Spot Disease/Pseudomoniasis

4   Parasitic Diseases of Farmed Fish

     4.1 Amoebic Gill Disease

5   Viral Diseases of Farmed Molluscs

     5.1 Ostreid herpesvirus 1 in bivalves

5.5   Developing the Fish Disease Index (FDI) (ToR f)

W. Wosniok, T. Lang

The present programme for FDI calculation is being prepared in R, which is a freely available software and more accessible to potential users than the packages used for previous versions of the FDI. In the course of developing the R version, a guiding document was prepared that will also serve as a user manual for the software. The document describes the purpose of the package, the intended output (descriptive statistics, FDI values, FDI assessments on individual and sampling location level, long-term trend analysis if the data allows) and the input required from the user (fish disease data and a control parameter file plus optional standardization parameters, BACs, EACs). It also addresses the default files for standardization and assessment that will be provided as part of the package, and the technical requirements for installing the package. The software package will be distributed as a zip file with a directory structure that can easily be copied by the user to obtain a suitable data processing environment. To facilitate interaction with the package, an Excel based interface will also be supplied.

5.6 Disease associated population effects of commercial fish and shellfish species (ToR g)

T. Lang, A. Alfjorden, S.W. Feist, S. Ford, S. Jones, S. MacLean, L. Madsen, V. Öresland, T. Renault, G. Stentiford

ABSTRACT

Pathogens are increasingly reported from populations of aquatic animals. Variations in the frequency and abundance of pathogens depend on spatial or temporal factors as well as those related to host effects such as age or condition. Similarly, the development of a disease is a function of the combined effects of the pathogen, host and environmental factors. Diseases may be one of several biological and non-biological factors regulating the abundance and composition of stocks or populations of aquatic organisms. Here we review evidence for population effects associated with diseases in wild marine gastropods, bivalves, crustaceans, and fishes. The report provides information on the types of diseases, together with data on diagnostic criteria, causative agents, geographical ranges and effects at the individual and population level. A discussion of the significance of the findings and references to key publications are provided. The overview shows that the effects of diseases may occur at various life stages of affected hosts. The extent to which these effects lead to measurable population changes (e.g., in growth, reproduction, mortality, recruitment, population demography, geographical distribution) is best evident in those populations already subject to long-term population level monitoring, such as in commercial species. However, quantitative evidence of population effects is so far scarce. From the information available, it may be concluded that some diseases play a much larger role in population performance and dynamics in marine fish and shellfish than previously recognised. Therefore, methods for incorporating disease data into population/stock assessment models should be further explored. In the first instance, this will be relevant for commercial species, for which most information is available. However, the development of realistic mathematic models, taking into account population effects of diseases, are becoming also more relevant for non-commercial species, e.g., as part of coastal zone management or assessments of general marine ecology in the context of a more holistic ecosystem approach to marine management.

INTRODUCTION

Infectious agents such as viruses, bacteria and metazoan parasites are frequently documented within wild populations of aquatic organisms. Less well documented, however, are the risk factors and processes leading to disease states caused by these pathogens that are capable of eliciting measurable population-level changes. It is evident from studies in marine and freshwater organisms that some diseases affect growth, reproduction and survival of different life stages of fish and shellfish (…). Furthermore, there is concern that the number of disease outbreaks in marine organisms is increasing (Harvel *et al.* 1999, Lafferty *et al.* 2004, Bradley *et al.* 2005, Kim *et al.* 2005), leading to significant stock reductions in marine organisms (e.g., black abalone in California, sun stars in the Sea of Cortez, staghorn coral and long-spined sea urchin in the Caribbean, oysters at the east coast of the United Statess). Risk factors associated with disease may be anthropogenic or natural in origin and may include e.g. climate change, introduction of terrestrial pathogens or new host species, pollution, aquaculture, habitat destruction or changes in stock density through fishing or conservation (see citations in Harvell *et al.* 1999 and Lafferty *et al.* 2004).

Disease epidemics are the result of the complex interactions of pathogen, host and environmental factors and there are a number of well documented cases in which adverse disease effects have been documented at the population level in finfish and shellfish (bivalves, gastropods, crustaceans). However, while traditional stock assessment models include the growth, reproduction, recruitment, abundance, biomass and natural mortality within stocks or populations of marine organisms, quantitative disease data are rarely included in the traditional models. Thus, there exists an opportunity to better understand and model the dynamics and consequences of disease as an integral part of a comprehensive stock assessment process. An understanding of the role of disease in influencing populations of marine animals relies on the availability of systematically collected long-term datasets. When such disease data are available, they are mostly derived from studies of adult specimens and do not include young life stages that may be more susceptible. In addition, the tendency to emphasise mortality estimates as indicators of disease impacts may lead to an underestimation of population effects because in many cases, mortality is 'cryptic' and occurs without notice. Furthermore, many diseases do not kill the host but rather have sublethal effects, for example on growth and reproduction, resulting in more subtle population-level effects. Finally, diseases of non-infectious aetiology, such as those caused by exposure to contaminants, may occasionally have indirect or direct population-level effects. The contaminant can affect host susceptibility to an infectious agent, leading to a more acute disease process or may have direct toxic toxicological consequences.

The present review briefly summarises information on diseases associated with population effects in four taxonomic groups of marine animals: bivalves, gastropods, crustaceans and fish with an emphasis on species of commercial value. The review also presents information from cases where significant individual level effects were reported, leading to the valid assumption of likely population effects, even if these cases were not based on a true population study, e.g., by applying population dynamics models. In some instances, controlled laboratory studies are described where they have provided insight into the significance of infections observed in wild animals. For consistency, population effects considered in this review include declines in population size, effects on (natural) mortality, effects on recruitment, changes in size/age composition and changes in spatial distribution. Effects at the individual level including growth, reproduction, and survival are included if they are considered likely to lead to effects at the population level. As such, the review is aimed at an audience interested in factors having an impact on population dynamics of commercial species, such as stock assessment experts. However, the role of diseases in marine ecosystems may also be of interest to more general marine ecologists.

MATERIAL AND METHODS

A standard format is used to present information on diseases and associated population effects separately for bivalves, gastropods, crustaceans and fish. Within these four groups, information is grouped by species and by disease. For each named disease, information is provided on diagnostic criteria, causative agents, host and geographical ranges and effects at the individual and population level. Wherever possible, quantitative information, e.g., on disease-associated mortality, is presented. A discussion and conclusions on the significance of each disease is provided, also giving accompanying information, e.g., in case of parasitic diseases, on life cycles of parasites involving different host species etc.

RESULTS

Diseases of Gastropods

Abalone (*Haliotis spp.*)

Abalone Viral Ganglioneuritis (AVG) (Herpes–like virus infection)

Diagnosis: Infected abalone are easily removed off substrate. Clinical signs include enlarged mouth parts and protruding radula. The soft tissues surrounding the prolapsed mouth are turgid. No significant lesions are seen in other organs. The diagnostic features currently used to identify the disease are the histopathological finding of ganglioneuritis. Histological examination of infected abalone reveals lesions of nervous tissues including haemocyte infiltration and necrosis. Transmission electron microscopy (TEM) has been used to demonstrate the presence of herpes-like virus particles in nerves of affected abalone, though TEM is not used as a routine diagnosis method. The virus has been purified from infected animals and molecular diagnostic methods have been developed including conventional one-step PCR, TaqMan PCR and *in situ* hybridisation (OIE, 2009b).

Causative agent: Abalone herpes-like virus (AbHV) (Tan *et al.* 2008)

Host range: The first case of a previously unknown abalone disease was detected in a land-based farm located near Portland (Victoria, Australia) in December 2005 (Hardy-Smith, 2006a). Two other farms, one at Port Fairy, the other in Westernport Bay (Victoria, Australia) were secondly affected. Blacklip abalone (*Haliotis rubra*), greenlip abalone (*H. laevigata*) and their hybrids were demonstrated high mortality rates in affected farms (Hardy-Smith, 2006; Hooper *et al.*, 2007).

In May 2006, the disease was reported for the first time in a wild population from a coastal lagoon (Taylors Bay, Victoria, Australia) close from one of the abalone affected farms (Hardy-Smith, 2006b). The presence of the disease was then assessed on wild abalone stocks in more than 40 sites along the coast of western Victoria. In June 2006, the disease was detected in 19 sites.

Geographical range: Victoria, South Australia

Individual effects: Herpes-like virus infecting abalone is highly pathogenic and it may be assumed that it poses a significant mortality risk to its hosts. In affected abalone, the major lesions are confined to nervous tissue centred on the cerebral, pleuropedal and buccal ganglia. The lesions are primarily an increased cellularity and individual cell necrosis in the affected nerves.

Population effects: In Australian abalone farms, young stocks appeared more seriously affected than older animals. Cumulative mortality rates varied from 5% to 90%. Massive mortality occurred rapidly and a daily mortality rate at 5000 animals per day was recorded in one of the affected farms. The role of stress factors including high densities, spawning period and water temperature (up 17.5–18°C) was highly suspected as contributing factors to mortality.

The virus disease was first detected in May 2006 in the marine waters adjacent to one of the affected farms and led to mortalities in wild abalone stocks. It has been assumed that wild abalone in the vicinity of the affected farm became infected from contact with farm effluent and that the viral disease spread from this locality to affect other reefs along western Victoria coast. However, surveillance of the wild population was a result of the mortality events reported in abalone farms, not a result of un-

explained disease first observed in the ocean. In this context, it can be asked whether wild abalone has been affected before 2006 without attracting notice.

The disease rapidly spread among wild abalone in waters of western Victoria. In September 2006, the infected area of coast was approaching 200 km with significant impacts on abalone populations. When infections with the virus have moved through a reef area, mortalities of up to 95% have been noticed. The virus has shown itself to be capable of killing a majority of abalone on reefs, signifying that it is highly infectious, and capable of infectivity despite high dilution factors in seawater. The virus was reported most often where there were dense populations of abalone. Based on mortality and presence of lesions, abalone viral ganglioneuritis was no longer detectable in areas where it had previously been present.

Discussion: Although high mortality rates were reported in wild abalone associated with ganglioneuritis, the disease impact on wild stocks has been debated and reports on the extent of mortalities are contradictory (Stewarts and Edmunds, 2006). The behaviour of infectious disease in wild abalone stocks might be difficult to interpret, partly because of the numerous factors affecting abalone ecology.

The virus was first detected in land-based farms and subsequently reported in wild abalone. Although the disease was first reported in wild abalone close to an affected farm, the virus may have been present in wild abalone before this first report. It may be normally present in wild stocks, but only manifest as clinical disease under particular conditions. Abalone population has fluctuated over the years, although the causes of this fluctuation are not known and infection with the virus could be a contributing factor. On the other hand, although the virus might be dormant or unnoticed in Australian abalone before 2006, the spread of the disease among wild stocks is very much more like that of an epidemic in a naïve population. Finally, a recently conducted Australian national survey of over 3000 wild harvest abalone did not detect any evidence of the virus. The exact origin of the virus remains unknown.

Most of the currently recognized viruses of molluscs infect species that are farmed. Rearing conditions enhance the likelihood of their detection. The ecology of viruses infecting marine molluscs is often not well known and mechanisms that allow mollusc viruses to resist environmental conditions when outside their host need particular attention.

Herpes-like infection of abalone has been recently included in the Aquatic Animal Health Code (OIE, 2009a) and in the Manual of Diagnostic Tests for Aquatic Animals (OIE, 2009b). Related codes of practice, standard operating procedures (SOP) and educational leaflets have also been produced by Australian policy makers and proposed appropriate bio-security control measures to abalone farmers and divers. However, the infection caused by herpes-like virus in abalone is not listed at present as a notifiable disease by the EU legislation (Directive 2006/88/EC).

The virus disease sparked concern about possible long term consequences for the abalone industry not only in Victoria, but also in the rest of Australia. More knowledge on herpes-like viruses infecting molluscs is undoubtedly needed in order to develop suitable strategies to minimize their impact on shellfish.

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Black abalone (Haliotis cracherodii)

Withering syndrome

Diagnosis:

Causative agent: An intracellular bacterium, given the provisional status of '*Candidatus* Xenohaliotis californiensis', that infects the epithelial cells of the gastrointestinal tract of black abalone, has been identified as the causative agent of withering syndrome (Friedman *et al.* 2000).

Host range: Black abalone (*Haliotis cracherodii*)

Geographical range: the coast of California, USA

Individual effects: the foot of the abalone atrophies until it can no longer adhere to the substratum.

Population effects: mass mortalities

Discussion: Declines of abalone at 2 sites coincided with the strong 1997 to 1998 El Niño.

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Diseases of Bivalves

European flat oyster (*Ostrea edulis*)

Infection with *Bonamia ostreae*; Bonamiosis

Diagnosis: Clinical signs include dead or gaping oysters, but these clinical signs are not pathognomonic for this disease. Gross pathology can show yellow discoloration as well as extensive lesions including perforated ulcers in the connective tissues of the gills, mantle and digestive gland (Comps *et al.,* 1980), but most infected oysters appear normal. Microscopic detection methods are used, either heart imprints or histology (see OIE 2009). The latter implies sections of tissues that include gills, digestive

gland, mantle and gonad, that are stained with haematoxylin and eosin and observed in the microscope at increasing magnifications to x1000. A positive result in an imprint is small spherical or ovoid organisms (2–5 µm wide) within haemocytes, with basophilic cytoplasm and eosinophilic nucleus. Multinucleated cells can be observed. When it comes to histology a positive result is the presence of 2-5 µm wide cells within haemocytes or free in the connective tissue or sinuses of gills, gut and mantle epithelium, often in connection with intense inflammatory reaction. A positive diagnosis requires that the parasite is found inside haemocytes. Both techniques are not parasite species specific. A positive result outside the known geographical range of infection needs to be confirmed by the OIE Reference Laboratory. Confirmation of the parasite might be electron microscopy or molecular techniques like PCR. Parasite species-specific clarification can be done by PCR-RFLP which can differentiate between *B. ostreae* and *B. exitiosa*.

Causative agent: *Bonamia ostreae*: a haplosporidian protozoan infecting haemocytes of the European flat oyster, *Ostrea edulis*, which is the natural host of this pathogen (OIE 2009). Other flat oyster species like *O.chilensis*, *O. puelchana* and *O. angasi* can be infected when they are moved into infected zones. The parasite induces physiological disorders and eventually death of the oyster. Life cycle outside the host is unknown but transmission of the parasite directly from host to host by cohabitation or by inoculation of purified parasites is possible, suggesting that no intermediate host is needed (Hervio *et al.* 1995). The disease, Bonamiosis, was first reported from the coasts of France in 1979.

Species range: European flat oyster (*Ostrea edulis*) and other Ostrea species; *Crassostrea ariakensis* and *C. angulata*

Geographical range: The pathogen first gave rise to high mortalities in *O. edulis* in the summer 1979 in the oyster area I'lle de Tudy in Brittany, France (Comps *et al.* 1980). Oysters with gill perforations were seen. A study showed that the parasite rapidly spread to other parts of Brittany (Tigé *et al.* 1981). Bonamiosis occurred in Cornwall in 1982 and spreaded to Pool Harbour in 1986. Pattern of disease spread correlates with oyster movements. Wild stocks were almost completely wiped out in the Netherlands in 1991 (Stein 1997, van Banning).

B. ostreae infections have been found in Europe (France, Ireland, Italy, Netherlands, Portugal, Spain and the United Kingdom), Canada (British Columbia), and the United States of America (California, Maine and Washington States). Studies as well as reconstruction of historical records by Elston *et al.* (1986) trace the origin of the disease to California and possible an Atlantic North American site, followed by its spread within North America and to Europe.

Individual effects: *B. ostreae* induces physiological disorders and eventually death of the oyster. Oysters from disease-free areas can spend up to a year in infected waters before disease symptoms appear (Stein 1997). According to Montes (1991) it will take at least 3 months before the parasite is observed in a disease free batch that has been moved into an infected zone.

It seems that larger oysters are more affected than smaller oysters. The presence of Bonamia is more dependent on size than on age (Caceres-Martínez *et al.* 1995). In a study done by Caceres-Martinez *et al.* (1995), the parasite was first present when the oyster had a size of 4 cm, and from this length the prevalence of the parasite increased and at about 6 cm of length the mortality exceeded 10 %. The importance of size is also found in relation to gonadal development. Again it is rather size than age that matters.

Population effects: The introduction of the parasite of this disease is usually followed by high mortality rates of three year old oysters and therefore significantly affects recruitment (Stein 1997). Within 6 months of introduction to a dense population the parasite is associated with cumulative mortality rates of up to 80 % (Balouet *et al.* 1983).

The disease primarily affects mature oysters. The life cycle of the parasite seems to be connected to the maturation of oysters. Small oysters do not die because of the disease, but the mortalities among mature oysters are high.

France experienced a decline of flat oyster output from 20,000 mt in 1970 to 1,500 mt due to successive outbreaks of bonamiosis as well as marteiliosis (caused by *Marteilia refringens*) (Steins, 1997).

Studies done on flat oysters from natural beds in the Damariscotta river area in Maine, USA showed a relatively steady prevalence (between 20 and 45 %) from 1991 to 1995 and a low infection intensity (Zalabeta & Barber 1996). The reason for that was believed to be the low density of oysters in natural beds as well as the relatively cold winters. It was shown in this study that the highest prevalence and intensity of *B. ostreae* was recorded at the sampling point where oyster intensity and water temperature were the greatest.

Discussion

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Chilean oyster (*Ostrea chilensis*)

Infection with *Bonamia exitiosa*; Bonamiosis

Diagnosis: Clinical signs include dead or gaping oysters which might as well be indicative of other infections. Sometimes the gills can appear to be eroded. On the microscopic level lesions appear in the connective tissue of gill and mantle, and in the vascular sinuses around the stomach and intestine. There might be heavy haemocytic infiltration (OIE 2009). Diagnosis can be done by histology. In regions where *B. ostreae* and *B. exitiosa* both can be found, the histology needs to be confirmed by molecular characterisation, PCR-RFLP as well as sequencing (OIE 2009).

Causative agent: *Bonamia exitiosa*, a haplosporidian parasite infecting haemocytes of several oyster species and inducing physiological disorders and eventually death of the animal (Cranfield *et al.* 2005).

Species range: Chilean oyster (*Ostrea chilensis* (= *Tiostrea chilensis, Tiostrea lutaria*))

Geographical range: Infection is found in *O. chilensis* in locations around South Island, New Zealand, *O. angasi* in Australia and in *O. edulis* in Galicia, Spain (OIE 2009).

Individual effects:

Population effects: *Bonamia exitiosa* outbreaks in the *Ostrea chilensis* from Foveaux Strait, New Zealand have resulted in population reductions. The mortalities between 1985 and 1992 were evaluated by comparing dredge surveys done in 1990, 1992 and 1993 to a dredge survey done in 1975-76 (Doonan *et al.* 1994). The population had declined by 67 % in 1990, while it was 91 % in 1992 compared to the 1975 level. A dredge survey in 1993 showed that the population had increased slightly in comparison with the 1992 level (Doonan *et al.* 1994). Cranfield *et al.* (2004) proposed that stressors like mechanical disturbance of oysters by increasingly intense dredging as well as increasing scale of modification on benthic habitat by fishing increased the susceptibility of oysters to bonamiosis. This makes recovery of an oyster population after an epizootic closely linked to regeneration of the habitat.

Discussion

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Portuguese oyster (*Crassostrea angulata*)

Gill disease ('maladie des branchies', gill necrosis)

Diagnosis: The first gross sign of the gill disease consist of yellowish discolored zones of tissue associated with perforations of the gills and labial palps. Further development of these lesions results in large ulcerations and total destruction of affect-

ed gill filaments. Yellow or green pustules are also detectable on adductor muscle and mantle (Alderman and Gras, 1969; Comps, 1969 and 1970). In affected Portuguese oysters, transmission electron microscopy examination demonstrated hypertrophied cells containing virus particles (Comps *et al.*, 1976; Comps, 1978; Comps and Duthoit, 1979). Virus particles were icosahedral in shape, approximately 300 nm in diameter. Virus features, as well as their assemblage within the cytoplasm, characterised them as members of the *Iridoviridae* family. However, this affiliation remains uncertain as no molecular characterization has been carried out so far.

Causative agent: Irido-like virus, also termed gill necrosis virus (GNV)

Host range: Portuguese oyster (*Crassostrea angulata*), Pacific oyster (*Crassostrea gigas*)

Geographical range: Atlantic coast of France

Individual effects: The first gross sign of the disease are yellow spots on gills and labial palps. Gill lesions increase in size, inducing deep indentation and total destruction of gill filaments that might contribute to oyster death.

Population effects: Gill disease ('maladie des branchies') is regarded as the main cause of mass mortality outbreaks occurring in the late 60's among Portuguese oysters, *Crassostrea angulata*, along the Atlantic coast of France (Comps, 1970; Comps, 1972; Comps and Duthoit, 1976), and the French production of Portuguese oyster was drastically reduced related to gill disease in the late 60s. During certain years, the disease affected 70% of the oyster populations in Marennes Oleron area and Arcachon Bay. Maximum losses occurred in 1967 and losses subsequently declined. In 1968, survivors of previous outbreaks that had recovered from the disease presented cicatrisation of old lesions. Gill disease affected the Pacific oyster, *C. gigas*, to a lesser extent and the lesions were mostly limited suggesting resistance in this species (Comps, 1970; Marteil, 1976; Comps and Bonami, 1977).

Discussion: Different findings provided a presumptive link between the detection of GNV virus and the occurrence of gill disease. Although the presumptive link was substantial, the virus aetiology was not demonstrated by experimental transmission trials.

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Haemocyte disease

Diagnosis: Histological observations of affected Portuguese oysters showed an acute infiltration reaction consisting of virus-infected haemocytes. Transmission electron microscopy demonstrated atypical haemocytes containing virus particles in the affected Portuguese (Comps *et al.*, 1976; Comps, 1978; Comps and Duthoit, 1979). Virus particles were icosahedral in shape, approximately 350 nm in diameter. Virus features, as well as their assemblage within the cytoplasm, characterised them as members of the *Iridoviridae* family. However, this affiliation remains uncertain as no molecular characterization has been carried out. A virus was also observed in Pacific oysters (*C. gigas*) fom Arcachon Bay (Atlantic coast, France) (Comps and Bonami, 1977).

Causative agent: Irido-like virus, haemocyte infection virus (HIV)

Host range: Portuguese (cupped) oyster (*Crassostrea angulata*), Pacific oyster (*Crassostrea gigas*)

Geographical range: Atlantic coast of France

Individual effects: Affected oysters exhibited virtually no external signs of disease, except for a greyish discoloration of the visceral mass in some cases. Histological examination, however, revealed considerable degeneration of connective tissues and the presence of atypical cells interpreted as infected hemocytes. Affected oysters demonstrated high mortality rates.

Population effects: In 1970, massive mortality outbreaks were again reported among French *C. angulata* oysters. These mortality events were associated with the detection of an irido-like virus named haemocyte infection virus. The disease affected adult oysters and no distinctive clinical signs were noted (no gill lesions).

A similar disease associated with a morphologically similar virus was reported from Pacific oysters in France during an episode of summer mortality in the Bay of Arcachon in 1977 (Comps and Bonami, 1977). In 1977, a mortality of 15% was noticed in oysters kept in a purification plant in Arcachon.

Mortality was first observed in 1970 in the estuary of Marennes Oleron (Atlantic coast, France) and was paralleled by a similar event at Etel (Brittany, France). By 1971, the disease had also reached Arcachon Bay. The disease affected all oyster classes including adults from 1970 to 1973. A drastic reduction in spat recruitment and high mortality rates led to the almost total extinction of *C. angulata* in French waters in 1973.

Discussion: The first imports of the cupped oyster, *Crassostrea angulata*, to France from Portugal dated from 1860 and were carried out in order to palliate the lack of European flat oysters, *Ostrea edulis*. The Portuguese cupped oyster then spread all along the Atlantic French coast and its production increased rapidly reaching 90 000 tons per year during the 1950s.

However, the production decreased until the 'gill disease' outbreak in the late 1960s. The disease spread throughout culture areas and induced high mortalities. From 1970 to 1972, Portuguese cupped oysters were affected again by another syndrome characterized by massive haemocyte infiltration in connective tissues and high mortality rates. Both gill disease and haemocyte disease were associated with the detection of an irido-like virus and the Portuguese cupped oyster nearly disappeared from the French coast. Although no targeted studies have focused on the economic impacts of both diseases on *C. angulata* production, the annual loss of adult oysters reached 60

000 tons in the early 1970s. Gill disease and haemocyte disease were associated with massive mortalities among French *C. angulata* stocks and irido-like viruses were interpreted as the causative agents. Both virus diseases were assumed to lead to the total disappearence of *C. angulata* along the Atlantic French coast. It has been suggested that a period of massive production associated with high oyster densities might lead to a trophic unbalance with a decrease of defence mechanisms and enhanced susceptibility to diseases.

In this context of crisis, the massive introduction of Pacific cupped oyster, *C. gigas*, an exotic species, was officially decided and began in 1971 (Marteil, 1976; Bonami, 1977). Adult oysters originating from Canada (British Columbia) and spat imported from Japan were planted in different French locations all along the coastline.

The importation of *C. gigas*, which demonstrated resistance to irido-like virus diseases affecting *C. angulata*, allowed the persistence of oyster culture in France. Although the introduction of *C. gigas* in French waters may be considered as a success story at a commercial level, such animal introductions are risky with potential pathogen transfers and ecological impacts (invasive species). It is important to keep in mind that the question about the origin of irido-like viruses which affected *C. angulata* in France is still open. One of the hypotheses reported that these irido-like viruses were exotic pathogens introduced with *C. gigas*, a healthy appearing carrier species (Grizel & Héral, 1991). Indeed, the first *C. gigas* spat from Japan were probably planted in the Marennes-Oléron area in 1966 (Le Borgne *et al.*, 1973) concomitantly to the appearance of gill disease among *C. angulata*.

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Eastern oyster (Crassostrea virginica)

MSX Disease; Haplosporidiosis

Diagnosis: *H. nelsoni* infections must be diagnosed based on microscopic examination of tissues, or by molecular assays. Although gross signs of infection include lack of shell growth, pale digestive glad and emaciation (which can be seen only after removing the shell), these conditions are not exclusive to the disease and cannot be used as diagnostic criteria.

Causative agent: *Haplosporidium nelsoni* (Haskin *et al.*, 1966) is a member of the Phylum Haplosporidia, which is comprised of approximately 40 named species of mostly marine parasites, as well as a number of unnamed species (Burreson and Ford, 2004; Ford *et al.*, 2009). It is a highly lethal pathogen of the eastern (North America) oyster, *Crassostrea viginica*. It also infects the Pacific oyster, *C. gigas*, but at low prevalence and without reported mortality. Haplosporidians infect other molluscs and organisms such as polychaetes, crabs, trematodes and nematodes, and are found worldwide (Burreson and Ford, 2004).

Haplosporidian life stages include uni- and binucleated stages, and multinucleated plasmodia that develop into spores, although spores are not formed, or have not been described, for several species (Burreson and Ford, 2004). Although spores are assumed to be a stage involved in transmission, the complete life cycle and means of transmission are unknown for all haplosporidians, and alternate or intermediate hosts may exist for at least some species. *H. nelsoni* is inhibited by salinity < 15 and can be killed by salinity <10),

Species range: Eastern oyster (*Crassostrea virginica*)

Geographical range: In North America, *H. nelsoni* has been identified in tissue sections of eastern oysters from Nova Scotia, Canada to Florida, USA (Burreson and Ford, 2004). Reported identification by PCR in Gulf of Mexico oysters (Ulrich *et al.*, 2007) has not been confirmed by histology or additional PCR (Ford *et al.*, in press). The parasite has been found in Pacific oysters in China, Korea, Japan and the west coast of the United States, and probably, France (Burreson *et al.*, 2000; Renault *et al.*, 2000, Wang *et al.*, 2010).

Individual effects: The earliest stages of the parasite are small plasmodia found in the gill and inner palp epithelium (Farley, 1968; Ford and Tripp, 1996). They multiply intercellularly along the basal lamina, often stimulating intense hemocyte infiltration of the area and resulting in extensive sloughing of epithelial cells, parasites and hemocytes. Parasites eventually enter the circulation and are distributed to all tissues, where they continue to multiply. Histopathological effects include hemocyte infiltration, tissue disruption, and pycnotic nuclei. Highly susceptible individuals die within a few weeks of becoming infected and usually while they appear to be in good condition and growing well. More resistant individuals tolerate infections longer, but display clear sublethal effects of parasitism (Ford and Figueras, 1988). Feeding rates, enzyme activity, biochemical constituents, hemolymph components, fecundity and growth are all negatively affected, usually in proportion to infection intensity (Mengebier and Wood, 1969; Ford, 1986; Ford *et al.*, 1988; Matthiessen and Davis, 1992). The clearance rate, a measure of food acquisition potential, was ½ to 1/3 lower in infected oysters than in those without histologically detectable infections, but the oxygen consumption rate was not affected (Newell, 1985). Consequently, protein, lipid and glycogen contents were decreased by 36%, 37% and 51%, respectively, in systemically infected individuals compared to those without detectable infections (Barber *et al.*, 1988b). Tissue condition and fecundity were reduced by 31% and 81%, respectively (Barber *et al.*, 1988a).

Population effects: The first epizootic of MSX disease in the United States occurred in Delaware Bay between 1957 and 1959 (Ford and Haskin, 1982). An estimated 90–95% of the oysters in the high-salinity lower Bay died. Mortality declined in an up-bay direction but still ranged from 20 to 60%(Haskin and Ford, 1982). In 1959, epizootic mortalities began in the lower Chesapeake Bay, also resulting in losses in excess of 90% (Andrews, 1968). During a drought in the mid-1960s, mortalities associated

with *H. nelsoni* infections reached 45–55% in the middle portion of Chesapeake Bay (Farley, 1975). In the 1980s and 1990s epizootic outbreaks of MSX disease were recorded in the northeastern United States: Long Island Sound, on Cape Cod and in southern Maine (Matthiessen and Davis, 1992; Barber *et al.*, 1997; Sunila *et al.*, 1999), and in 2002, in Nova Scotia, Canada (Stephenson *et al.*, 2003). Where measured, associated mortality was estimated at up to 80–85%. The involvement of *H. nelsoni* in the mortalities is documented by examination of dead and moribund oysters. During epizootics, these individuals may have prevalences of 80 to 90% with the majority having advanced infections (Andrews, 1968; Ford and Haskin, 1982). The parasite has been reported south of Chesapeake Bay, but it has not been associated with oyster deaths in this region (Lewis *et al.*, 1992; Morrison *et al.*, 1992; Bobo *et al.*, 1997).

Although *H. nelsoni* clearly has an inhibitory effect on reproductive output of infected oysters, no measurable association was found on recruitment in Delaware Bay (Ford and Figueras, 1988). The likely reason is that most of the oysters remaining in the Bay after the initial epizootic mortalities were in the upper Bay where they were protected by low salinity from developing *H. nelsoni* infections that would have inhibited reproduction.

The economic consequences of MSX disease have been devastating (United States National Marine Fisheries Service; National Research Council, 2004). Harvests in Delaware Bay declined by 95%, and in Chesapeake Bays by 60%, in the years immediately following the initial epizootics of the late 1950s and early 1960s, and production has never recovered in either area. After an epizootic of MSX disease in Long Island Sound in 1996/97, harvests dropped 65 and have continued to decline since. To varying degrees, the parasite *Perkinsus marinus* (Dermo disease agent), has also contributed to the lack of recovery. During droughts when the parasite can develop infections in areas where oysters are normally protected by low salinity from harbouring severe infections, mortalities rise. Mortalities associated with drought and *H. nelsoni* and *P. marinus* infections in the Chesapeake Bay were so extensive between 1998 and 2002 that serious consideration was given to the introduction of a non native oyster (*C. ariakensis*) to replace the depleted and disease prone native species (National Research Council, 2004).

The development of resistance to MSX disease-caused mortality due to selective mortality has been documented in Delaware Bay and in portions of Chesapeake Bay. At present, the disease is no longer a population-level problem in Delaware Bay, although it continues to epizootics in other areas (Burreson and Ford, 2004; Carnegie and Burreson, 2010).

Discussion: Since the early 1800s, the eastern oyster has been the most economically valuable bivalve harvested on the east and Gulf coasts of the United States. It is consequently one of the most well studied marine species, both at the individual and the population level. Laboratories conducting molluscan studies, especially oysters, were present in the mid-Atlantic states when the first MSX disease outbreaks occurred and the impacts of the disease have been well documented in continuous datasets that date to the 1950s in some regions. The impact of disease on bivalves is much easier to document than it is on finfish or crustaceans, because they are sedentary and the persistence of shells post-mortem permits good estimates of mortality (Ford *et al.*, 2006).

In an example of the use of long-term data sets, Powell *et al.* (2008) examined a 54-year time series on changes in the stock size and distribution patterns of oysters (*Crassostrea virginica*) in Delaware Bay, USA. The data set showed not only the effect of the pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*, etiological agents of

MSX and Dermo diseases, respectively, on mortality, stock abundance and annual harvests, but also showed that the distribution of oysters contracted to low-salinity disease refuges during periods of intense infection pressure. In one particularly severe MSX disease epizootic associated with drought in the mid-1980s, atypically high salinity and infection levels caused the deaths of an estimated 70-75% of the oysters in the former low-salinity refuge area.

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Dermo disease; Perkinsiosis

Diagnosis: Diagnosis of Dermo and other diseases caused by *Perkinsus* spp. is usually made by detecting parasites in host tissues cultured in Ray's Fluid Thioglycollate

Medium (RFTM), in which the parasites enlarge, but do not replicated (Ray, 1966) or by molecular assays (Gauthier *et al.*, 2006; De Faveri *et al.*, 2009). Although the parasites are visible in histological section, this method is less sensitive than culture or RFTM (McLaughlin and Faisal, 1999). All recognized *Perkinsus* spp. enlarge in RFTM, so the method cannot differentiate among species and molecular assays are needed when it is believed that more than one species is present in an area. Although gross signs of *P. marinus* infection in eastern oysters include lack of shell growth and emaciation (which can be seen only after removing the shell), these conditions are not unique to the disease and cannot be used as diagnostic criteria.

Causative agent: *Perkinsus* (= *Dermocystidium*, *Labyrinthomyxa*) *marinus* (Levine, 1978) is a one of several marine parasites in the genus Perkinsus (Villalba *et al.*, 2004). It is a highly destrustive pathogen of the eastern (North American) oyster, *Crassostrea virginia*. The life cycle of *P. marinus* inside the host begins with single celled trophozoites that undergo successive bipartitioning, resulting in multicellular bodies that rupture releasing small daughter trophozoites. Flagellated zoospores can be formed in culture, although they have not been reported *in vivo* and their role in the life cycle is uncertain. The parasite is transmitted directly between oysters and all *in vivo* stages are infective. *Perkinsus marinus* is inhibited in its development by salinities below about 10, although it can survive salinities at least as low as 3 (Chu *et al.*, 1993). It reproduces *in vivo* at temperatures above about 15°C and proliferates most rapidly at temperatures above 25°C (Chu and La Peyre, 1993).

Species range: Eastern oyster (*Crassostrea virginica*)

Geographical range: *Perkinsus marinus* has been detected in oysters from Maine in northeastern USA south along the Atlantic coast and into the Gulf of Mexico (Burreson *et al.*, 1994; Ford, 1996; Soniat, 1996; Gullian-Klanian *et al.*, 2008). It has also been reported on the Pacific coast of Mexico infecting the pleasure oyster, *C. corteziensis* (Caceres-Martinez *et al.*, 2008) and *Perkinsus*-like organisms were found *C. virginica* in Hawaii (Kern *et al.*, 1973).

Individual effects: Early infections occur in the epithelium of gills, mantle and digestive tract. Parasites, often inside hemocytes, subsequently spread throughout the oyster causing tissue lysis, probably caused by protease secretion (La Peyre *et al.*, 1996), hemocyte infiltration, embolisms, and a build-up of pigment (ceroid) cells (Mackin, 1951). The negative impact of *P. marinus* infection is typically in proportion to parasite burden, although not all studies have been able to document clear effects. For instance, one study found that heavily infected oysters produced less than half the quantity of faeces and pseudofaeces as did oysters in which no parasites were detected, implying that feeding rates were similarly depressed (Mackin, 1962), but another failed to find any effect of infection on respiration, feeding or assimilation, even at high intensities (Paynter, 1996). Shell growth may cease during the initial stages of infection (Paynter and Burreson, 1991), but does not necessarily stop altogether and can resume if food availability is high (Dittman *et al.*, 2001). As infection approach lethality, growth typically ceases (Menzel and Hopkins, 1955). The impact of *P. marinus* infection on soft tissue condition varies seasonally and isn't consistently measurable until infections have become advanced, at which time condition may be only 60 to 70% as great as in uninfected individuals (Dittman *et al.*, 2001; Ford and Smolowitz, 2007). A more severe negative impact has been measured on gametogenesis. Dittman *et al.* (2001) estimated from histological sections that heavily infected oysters had, on average, only 10% as many gametes as oysters in which no parasites were found, and in some heavily infected individuals, the gonad was totally devoid of

gametes. Kennedy *et al.* (1995) reported a similarly sharp drop in the number of eggs spawned by heavily infected oysters. Two additional physiological parameters have been associated with *P. marinus* infections: a decline in cellular free amino acid concentration (Paynter *et al.*, 1995), and a decrease in hemolymph pH (Dwyer and Burnett, 1996). The former may impair the ability of infected oysters to volume regulate when external salinity changes. The latter implies a loss of buffering capacity of the hemolymph during valve closure, which could affect a variety of metabolic functions. One consistent effect is that infected oysters are weakened and unable to maintain valve closure (Paynter, 1996).

Population effects: Dermo disease epizootics typically require two to three years to develop (Andrews and Hewatt, 1957; Bushek *et al.*, 1994). In enzootic waters, of the mid-Atlantic, spat (year class 0) develop only a few, light infections. Prevalence and intensity increase with time. Once infected, it is difficult for an oyster to rid itself of the parasite and in many sites here environmental conditions are favorable, it is likely that all the oysters are infected (Bushek *et al.*, 1994). In the Gulf of Mexico where *P. marinus* was first discovered, the parasite was associated with annual mortality rates of 50 to 100% (Mackin and Sparks, 1962; Quick and Mackin, 1971; Hofstetter, 1977), although no present-day mortality data are available. In the lower Chesapeake Bay, mortalities range from 25 to 60% annually (Andrews, 1988). In the lower Delaware Bay, Bushek *et al.* (1994) recorded cumulative mortality of nearly 60% over a two year period during the initial epizootic and annual rates range between 15% and 35% in the upper bay, where background death rate is considered to be about 10% per year (Powell *et al.*, 2008). Ford and Smolowitz (2006) reported two year cumulative mortalities ranging from 50% to 80% in New England sites. Curiously, mortality comparable to that reported in the Gulf of Mexico, mid-Atlantic and New England have not been reported in the southeastern United States where the parasite is also present, although intensities rarely become heavy (Bobo *et al.*, 1997). Most of the oysters in this region are intertidal and it is hypothesized that summer temperatures, which may exceed 50º C, may be lethal to the parasite (Dungan and Roberson, 1993; Bobo *et al.*, 1997).

Commerical harvests of eastern oysters in the mid-Atlantic estuaries have been seriously affected by *P. marinus*. Because the parasite can tolerate fairly low salinity and survives overwinter, it is widespread even during non-epizootic years and can quickly proliferate when environmental conditions become favourable. Harvests in the Chesapeake and Delaware Bay and in Long Island Sound have never recovered from the combined effects of Dermo and MSX diseases (United States National Marine Fisheries Service). In Delaware Bay, where the oysters have become resistant to the development of MSX disease, Dermo disease continues to limit harvests to less than 15% of what they were before the outbreak of MSX disease in 1957. In the upper and lower Chesapeake Bay, landings are about 10% of what they were in the 1950s. In Long Island Sound, they are about 5% of what they were before the combined MSX and Dermo disease outbreaks there in the early and mid-1990s.

Discussion: *Perkinsus marinus* was first discovered in the mid-1940s in the Gulf of Mexico and identified as the cause of oyster mortalities there, but it had probably been present, and causing oyster deaths, for decades at least (Ray, 1996). It was found shortly thereafter – as soon as searches were instituted - in sites from Florida to Virginia, suggesting that it was not new in the southeastern United States either. Regular monitoring and sporadic surveys indicated that for many years, the parasite, remained restricted largely to this region. Starting in the mid-1980s, however, it began to be detected in more northern areas. The northward spread of epizootics ac-

companied a warming trend that was especially pronounced in the winter and *P. marinus* is now well established in the north-eastern United States where infection and mortality patterns and levels are similar to those in more southern regions (Ford and Smolowitz, 2007). In contrast to the development of resistance to MSX disease by eastern oysters, only modest resistance to the development of Dermo disease has been reported in wild stocks, even after decades of selective mortality.

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Diseases of Crustaceans

American lobster (*Homarus americanus*)

Gaffkemia or Red tail disease in lobster

Diagnosis: Gaffkemia or Red tail disease caused by the bacterium *A.viridans* var. *homari* was first recorded by Snieszko & Taylor (1947). It is a serious disease, primarily occurring in holding facilities but it also occurs in natural populations (Stewart *et al.*, 1966, Keith *et al.*, 1992, Lavallée, *et al.*, 2001). The disease may lead to high mortality. Heavily infected specimens may have slightly reddish discolouration visible through the integument of the underside of the abdomen. Rapid and significant increase in mortality among impounded lobsters, particularly after stress. Haemolymph is thin and pinkish with a drastic reduction in number of circulating haemocytes and clotting time may be prolonged to absent in late infections. Gram stained haemolymph reveals numerous typical Gram positive tetrad-forming coccal bacteria (0.8–1.1 μm). Confirmation by Flourescent Antibody Test (FAT) using conjugated antiA. *viridans* var *homari* antiserum. Culture characteristics are non-motile, catalase negative, beta-haemolytic, facultative anaerobic, tetrad-forming coccal bacteria that often appear as grayish-white raised colonies on agar plates. Light infections can be detected by inoculating 0.5 ml haemolymph into phenethyl selective broth medium, incubate at 28 °C for 24 hr. Lobster haemolymph in vitro forms an excellent medium for growth. Confirm positive tubes by FAT. Electron microscopic examination shows …..

C**ausative agent:** The gram-positive bacterium *Aerococcus viridans* var. *homari* (formerly *Gaffkya homari*).

Host range: American lobster (*Homarus americanus*) and European lobster (*Homarus gammarus)*; other crustacean species are also susceptible to infection.

Geographical range: Both sides of the North Atlantic Ocean

Individual effects: The bacterium does not contain exoenzymes and can therefore only enter through breaks in the integument (Stewart *et al.*, 1969). This bacterium is unique in its ability to overcome the otherwise highly intrinsic defense mechanisms of the lobster (Cornick & Stewart, 1968). Less than ten *A.viridans* cells /kg body weight of lobster are sufficient to cause fatal bacteremia within a few weeks. The impaired clotting mechanism is a result of a drastic reduction in circulating haemocyte numbers (Stewart *et al.* 1969). Utilization of the lobster´s glucogen and ATP reserves by the bacterium lead to hepatopancreatic dysfunction and death (Stewart & Arie, 1973). The mean time to death for an infected lobster increases as the temperature is decreased (Stewart and Rabin 1970).

Population effects: *A. viridans* can cause epizootics in *Homarus* spp. although there are few reports on epizootics among wild populations. No major impact on fisheries has been reported. Haemolymph samples from 2035 American lobsters from five areas off the Canadian Atlantic coast revealed a 2 to 40% prevalence of *A. viridans* in wild stocks (…).Evidence suggested that a high natural prevalence of infection could lead to an epizootic ? during live-storage of captive lobsters (Stewart *et al.* 1966). There appears to be very few cases of Gaffkemia in wild populations of *H.gammarus* (…). Wiik *et al.* (1987) suggested that Gaffkemia was not enzootic in the south western part of the Norwegian coast. No epizootics have been reported from European waters and Gaffkemia in Europe may be a problem limited to holding tanks.

Discussion: Although *A.viridans* is widely distributed and can infect other species of crustacea, it has not been identified as the cause of epizootics in species other than *Homarus* spp. (Stewart and Rabin 1970). No epizootics have been reported from European. However, diseased individual may be underestimated in the wild if they are less likely to be trapped. The appearance *H.americanus* (van der Meeren, 2008) in European waters may pose a future problem.

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Epizootic shell disease (ESD)

Diagnosis:

Causative agent:

Host range:

Geographical range:

Individual effects:

Population effects:

Discussion:

Host: American lobster (*Homarus americanus*)

Disease: Epizootic shell disease (ESD). The term "shell disease" is a general one, used to describe a range of necrotic lesions, pits, and/or discolorations in the exoskeleton of crustaceans. Several types of shell diseases are found on *H. americanus.*

Causative agent: chitinovorous bacteria (some uncertainty about this)

Gross signs: the exoskeleton becomes pitted and marred at the site of infection. Melanization will normally occur and necrosis will be evident in advanced stages. Lobster gill filaments can also be infected by ESD.

Severity: varies from a few pits or a couple of small lesions on the carapace to deeply eroded, to melanized lesions covering nearly the whole body. The disease may cause death and reduced reproduction.

Prevalence: Highly variable, 20-30 % is not unusual, but > 90 % has been reported.

Diagnosis: Isolations of bacteria are tested for chitinolysis.

Geographical range of disease on *H. americanus*: East coast of USA with higher incidences in southern areas like Rhode Island. Four cases may have been detected in Norway in 2009. *H. americanus* is found between Newfoundland–Labrador to North Carolina.

Individual effects: minor to mortal, growth, reproduction and mortality rates are affected.

Population effects: Local fisheries have been drastically reduced (>90 percent reduction of landings has been reported that may be partly due to ESD). However, the magnitudes of population effects are unknown.

Discussion:

<u>Present knowledge</u>

History: Shell disease in *H. americanus* was first described by Hess (1937) who found the disease in individuals held in impoundments. Smolowitz *et al.* (2005b) mention four types of shell disease in H. *americanus*: impoundment shell disease, burntspot shell disease, and endemic and epizootic shell disease (ESD). The ESD was found in Rhode Island waters in 1997 (Castro and Angell, 2000) and because it spread quickly and was more severe than the endemic type known earlier, it was classified as epizootic. EDS have been spreading northwards along the U.S. east coast. The ESD was found in Long Island Sound, between New York and Connecticut in 1997

Individual effects: Shell disease is not contagious from lobster to lobster and the routes of transmission are not known. In advanced stages the bacteria penetrates the chitin layers to the epidermis (which does not contain chitin) exposing the lobster for secondary invaders which may be ultimately responsible for fatalities (Fisher, 1988). Although larval stages are most seriously affected by the disease but they discard their infections during moulting (Fisher, 1988). A slower growth rate, increased risk of death, and a disproportionate effect on ovigerous females (up to 70 to 80 percent have been found, Howell, 2005) translates into lower productivity, higher natural mortality, lower abundance, and decreased population egg production. While the onset and progression of ESD in American lobsters are undoubtedly multi-factorial, the direct causality of this disease is poorly understood (Tlusty *et al.* 2007).

Population effects: The disease has a major impact on the health and mortality of some lobster populations but the magnitudes of these effects are unknown. Time series analysis found that shell disease had a major effect on mortality in the late 1990s and early 2000s (Gibson and Wahle 2005). However, studies of larval mortality in the field are notoriously difficult and little is known of the possible effect to recruitment of infections in larvae. Shell disease is also found in other crustaceans, but population impact is little studied in general, including ESD on lobster. Mortalities of lobsters due to ESD are infrequent and occasionally severe. It is therefore important to further investigate the importance of the different factors potentially affecting this disease in order to improve population dynamical models.

Effects on fishery: The lobster fishery is locally important on the east coast of USA and Canada and the lobster fishery is the state of Maine's most valuable commercial fishery resource. However, local lobster populations have experienced severe declines in the southern part of the species distribution range. In 1999 the Long Island Sound population crashed and landings decreased by 92% (Balcom and Howell 2006). While various factors are implicated in these population declines, disease issues (including ESD) indicate that long-term health of the American lobster stock may be compromised (Tlusty *et al.* 2007).

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Norway lobster (Nephrops norvegicus)

Hematodinium infections

Diagnosis: Pathological alterations to host organs, tissues and haemolymph, often accompanied by a 'chalky' or 'cooked' appearance. External assessment of the opaquely discoloured carapace is common but pleopod staging will detect infected individuals more efficiently. Microscopic determination of the presence of parasites in organs like the haemolymph, PCR and ELISA based diagnostics.

Causative agent: Two Dinoflagellate species of the genus *Hematodinium*

Host range: Norway lobster, *Nephrops norvegicus*

Geographical range: Throughout the host species distribution range.

Individual effects: *Hematodinium* infections may have severe effects with dramatic pathological alterations to host organs, tissues and haemolymph that may result in death. *Hematodinium* can exhibit rapid logarithmic growth within a host (Shields & Squyars 2000) resulting in a fast disease development. The metabolic requirements of the parasites rapidly drain the protein and carbohydrate constituents of the host (Stentiford *et al.* 2000b, 2001a, Shields *et al.* 2003). The following physiological and biochemical disruptions to the muscles and other organs substantially alter the metabolism of infected hosts (Taylor *et al.* 1996, Stentiford *et al.* 2000a,b, 2001a,b, Shields *et al.* 2003). However, the processes leading to death of infected hosts are not well understood. Respiratory dysfunction is evident by the decline in hemocyanin levels (Field *et al.* 1992, Shields *et al.* 2003), the loss of oxygen binding capacity of the hemocyanin (Taylor *et al.* 1996), and the magnitude of parasitic congestion and disruption of the gills (Field *et al.* 1992, Field & Appleton 1995).). Sporulation is more or less synchronous and complete in *N. norvegicus* individuals. Dinospores have been shown to exit the infected host via the gills in *N. norvegicus* (Appleton & Vickerman 1998). The prevalence and abundance of *Hematodinium* infections may be correlated to size or age (Field *et al.* 1992, 1998, Messick 1994, Stentiford *et al.* 2001c), sex (Field *et al.* 1992, Stentiford *et al.* 2001c) and moult condition (Meyers *et al.* 1987, Field *et al.* 1992, Shields *et al.* 2005

Population effects: Annual seasonal epidemics varying between 20 and 70% in prevalence have been reported (Field *et al.* 1998, Stentiford *et al.* 2001c). The high prevalence during epizootics in combination with high risks of mortality has damaged commercial stocks of Norway lobster and fisheries. Comprehensive modelling studies have not been carried in relation to epizootic events. Mortalities are centred on the unfished juveniles and females, hosts not normally sampled by fisheries, with the potential for dramatic, but cryptic effects on host populations through reduced recruitment.

Discussion: There are only 2 described species of *Hematodinium* sofar is by no doubt a new species (Stentiford & Shields, 2005). Numerous questions remain unsolved regarding the life cycle, alternate hosts, host specificity, and how these parasites move through host populations (Stentiford & Shields, 2005). Long-term datasets from the Clyde Sea Area, Scotland, have shown that prevalence in *N. norvegicus* is highest in winter and spring in that area (Field *et al.* 1992, Field *et al.* 1998, Stentiford *et al.* 2001c, 2001d), reaching as high as 70% during epizootics (Field *et al.* 1992). In the Irish Sea prevalence has reached up to 35% (Briggs & McAliskey 2002), and showed seasonal peaks during summer and fall. Infections often have extremely high prevalence in juvenile and female hosts, with the potential for dramatic, but cryptic effects on host populations (Meyers *et al.* 1987, Stentiford *et al.* 2000a, 2001b, c, d). Outbreaks of these parasites have caused significant damage to stocks of Norway lobster and lobster fisheries. In Scotland, the *N. norvegicus* fishery suffered great economic losses to the *Hematodinium* parasite in the early 1990s (Field *et al.* 1992). Since then, this parasite has been responsible for annual seasonal epidemics of varying proportions of between 20 and 70% prevalence (Field *et al.* 1998, Stentiford *et al.* 2001c). The real cost of outbreaks of *Hematodinium* are hard to assess. Species of *Hematodinium* can reach high enough levels to regulate their host populations, but mortalities are also centred on the unfished juveniles and females, hosts not normally sampled by fisheries; hence impacts are often underreported (Stentiford and Shields, 2005). In addition, background mortalities due to the disease are often difficult to determine because dead hosts quickly become undiagnosable. Further, mortalities occur primarily in juveniles and females and often go unnoticed (Shields 2003, Shields *et al.* 2005). Few if any fisheries models make use of disease data (prevalence and distribution) to estimate effects or otherwise manage a fishery. The development of spatial models for systems with disease has great potential because such models can incorporate biological data and integrate it over different spatial scales. With such models, 'at risk' populations can be identified, especially where the structure (e.g. sex or sized biased) of that population makes it susceptible to epizootics (Stentiford and Shields, 2005).

Controlling diseases (may be parts of this could be put into the general discussion)

Disassembly of the catch at sea, re-baiting with infected animals, moving animals between locations (culling while underway), and using infected individuals as bait will contribute to the spread of disease to new locations. By understanding transmission and pathogenicity of a disease, the effect of such practices can be curtailed or minimized. Changes in fishing policies may also be warranted. Some evidence from the *Nephrops norvegicus* fishery in Scotland suggests that the population structure of a given fishery is related to the prevalence of *Hematodinium* infection within that fishery, with populations of small, size-matched individuals having the highest prevalence (Stentiford *et al.* 2001c). As such, fisheries management regimes to promote normal size distributions within the fishery have potential for averting epizootics.

Finally, with the advent of live shipping of crabs and lobsters to distant markets, there is an increased potential for the inadvertent introduction of pathogenic agents to new regions. However, to date, no definitive studies have modeled the impact of disease at the population level or on the status of the products harvested from these fisheries (Stentiford & Shields (2005).

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Caribbean spiny lobster (*Panulirus argus*)

Panulirus argus Virus 1 (PaV1) disease   OR   Spiny lobster viral disease

Diagnosis: Preliminary diagnosis of diseased lobsters is based on field observation of lethargic juveniles incapable of righting themselves that have opaque hemolymph which fails to clot. Confirmation of viral etiology is made through histology and electron microscopy (Shields and Behringer, 2004) and through molecular assays such as Polymerase Chain Reaction (PCR) (Montgomery-Fullerton *et al.*, 2007) and Fluorescent in-situ Hybridization (FISH) (Li *et al.*, 2006). Light microscopy of hemolymph of heavily infected lobsters shows destruction of nearly all hyaline and semigranular hemocytes; granular hemocytes are not infected. Infected cells have hypertrophic and irregularly shaped nuclei with emarginated chromatin and Cowdry Type-A inclusions. Electron microscopic examination shows unenveloped, icoahedral virions in the nuclei of hyalinocytes and semigranulocytes, and virions in the cytoplasm of heavily infected hemocytes.

Causative agent: *Panulirus argus* Virus 1 (PaV1), an unenveloped, icosahedral DNA virus, is pathogenic to juvenile Caribbean spiny lobster, *P. argus*. The virus is highly pathogenic to juveniles (50% to 90% mortality) and can be transmitted among spiny lobsters through natural means such as ingestion of infected tissue, by contact among lobsters, and via water over close distances (Butler *et al.* 2008).

Host range: The Caribbean spiny lobster (*P. argus*) is the only known host at this time. Spotted lobster (*Panulirus guttaus*), stone crab (*Menippe mercenaria*), and channel crab (*Mithrax spinosissimus*), that cohabit with spiny lobsters in the natural environment, did not become infected when inoculated with infected spiny lobster tissue although more than 90% of spiny lobsters did (Butler *et al.*, 2008). The prevalence of visibly diseased spiny lobsters is highest in algal and post-algal stages (Briones-Fourzan *et al.*, 2009). From experimental studies it was shown that the mode of exposure and juvenile size at infection appears to affect survival (Butler *et al.*, 2008). Although the disease most commonly affects juvenile spiny lobster, DNA with 95% similarity to PaV1 from Florida has been detected in muscle of market sized *P. argus* from Belize (Huichen-Mian *et al.*, 2009).

Geographic range: *Panulirus argus* virus 1 (PaV1) was first described by Shields and Behringer (2004) in juvenile spiny lobster from western Florida Bay (Gulf of Mexico, USA). Huchin-Mian, *et al.* (2008) later reported PaV1 from a reef lagoon on the Caribbean coast of Mexico, and the virus has been found in juvenile *P. argus* from Belize, and the US Virgin Islands (Butler, *et al.*, 2008). PaV1 induced mortalities have occurred in juvenile *P. argus* reared in Florida, the Bahamas and Belize and similar gross signs of disease have been observed in other locations suggesting a Caribbean-wide range of the virus (Butler *et al.* 2008).

Individual effects: Infected juvenile spiny lobsters are lethargic, become morbid and soon die. The primary cells affected by the virus are hyaline and semi-granular hemocytes while granular hemocytes are not infected (Shields and Behringer, 2004; Li and Shields, 2007). Histopathology of the disease includes nuclear hypertrophy, emarginated chromatin, and Cowdry Type-A bodies in nuclei of infected hemocytes. In heavily infected juveniles, other tissues will be infected. Hepatopancreas and con-

nective tissues show high grade infections and histopathology, including loss of re-serve inclusion (RI) cells, atrophy of hepatopancreas, and destruction of fixed phago-cytes (Huchin-Mian *et al.*, 2008; Shields and Behringer, 2004). Loss of glycogen-storing RI bodies from the spongy connective tissue is marked in infected lobsters, suggesting depletion of energy reserves in these animals (Shields and Behringer, 2004). Laboratory studies on the effect of PaV1 infection on physiological condition as measured by hemolymph refractive index, and field studies of relative hepatopan-creas weight show significantly lower refractive index (Behringer *et al.* 2008) and lower relative hepatopancreas weight (Briones-Fourzan *et al.*, 2009) in diseased than in healthy individuals, indicating an effect of infection on the nutritional status of diseased lobsters. Lethargy seen in advanced stages of infection likely is a result of the depressed physiological condition and altered metabolic state (Behringer *et al.* 2008).

Population effects: The mean prevalence is approximately 7% in most areas of Flori-da, however, the mortality rate is very high (50–90%) (Butler *et al.* 2008). Localized Florida areas with prevalence approaching 50% (Butler *et al.* 2008) could experience significant losses, however, no studies have been conducted to assess population im-pacts. A significant observable effect on the lobster is the altered social behavior of spiny lobsters. Caribbean spiny lobsters are social animals that commonly share dens for shelter from predation. As PaV1 is highly pathogenic to juvenile spiny lobster, and transmission occurs through physical contact with, or via water near, infected individuals (Butler *et al.* 2008), there is a significant potential for devastating impacts on the lobster population. However, field observations show that diseased lobsters are more likely to be alone in dens than uninfected lobsters, and laboratory experi-ments demonstrate that, even before infected lobsters show signs of disease, healthy lobsters avoid dens harboring diseased individuals when given a choice of cohabiting with healthy or infected conspecifics (Behringer *et al.* 2006). This behavioral response to infected individuals is not expressed when suitable habitat is limited. Field exper-iments in a naturally shelter-poor habitat show that cohabitation with infected con-specifics increases, as does prevalence of infection, when the availability of shelters is limited (Lozano-Alvarez *et al.*, 2008). Based on experimental and natural field obser-vations, prevalence of infection appears to be independent of population density (Lozano-Alvarez *et al.* 2008; Behringer *et al.* 2006).

Discussion: PaV1 is the first reported viral disease in any lobster species. The cur-rently reported distribution of PaV1 from the Florida Keys to Belize and the US Vir-gin Islands suggests a wider Caribbean range that may become apparent with more intense monitoring. Considering the high mortality rate and high prevalence (>20%) in some areas of Florida Bay, significant localized losses due to PaV1 infection are likely, however, such losses have not been documented. PaV1 induced mortalities in juvenile spiny lobster reared in experimental marine culture facilities in Florida, Be-lize and The Bahamas points to the seriousness of this disease to future stock en-hancement programs and mariculture ventures. The most significant documented impact on the population is the altered social behavior of the spiny lobster juveniles (see section above, Behringer *et al.* 2006). The field studies conducted by Lozano-Alvarez *et al.* (2008) indicates that the prevalence of PaV1 infection in juvenile spiny lobster will increase under conditions of decreasing available shelter. Management practices of providing artificial shelters in shelter poor environments may serve to limit the spread of the disease and increase survival of juvenile spiny lobsters.

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Snow crab (Chionoecetes opilio)

Bitter crab disease; Bitter crab syndrome

Diagnosis: Preliminary diagnosis of bitter crab syndrome is made by visual observation of limp, moribund crabs which exhibit alterations in the colour of the carapace from orange-tan to pink and the ventral exoskeleton of the snow crab from whitish-gray to opaque white and cream coloured heart and gill (Taylor and Kahn 1995; Shields *et al.* 2005). As in tanner crabs, the muscle tissue appears opaque and imparts a bitter flavor when cooked, hence the common name Bitter Crab Disease (BCD) (Meyers *et al.* 1987; Taylor and Kahn 1995). The hemolymph is opaque white in advanced stages of disease and confirmation of the infection, even in less advanced stages, is made by microscopic examination of hemolymph smears (Meyers *et al.*

1996; Pestal *et al.* 2003; Stentiford and Shields 2005). Immunologic and molecular based methods diagnostic for *Hematodinium* are available (Hudson and Adlard 1996; Field and Appleton, 1996; Small *et al.*, 2002; Small *et al.*, 2007a, b; Wheeler *et al.* 2007; Jensen *et al.* 2010).

Causative Agent: A parasitic dinoflagellate of the genus *Hematodinium* (see also Crustacean Section 3.2.). Recent examination of PCR amplified sequences of the 18S and ITS1 regions of ribosomal DNA found marked divergence in the ITS1 region, strongly suggesting two clades of *Hematodinium*; one infecting portunids (i.e., *Callinectes sapidus* and *Liocarcinus depurator*) from the North Atlantic and a second infecting all other assayed decapods from the north Atlantic and Pacific Oceans (Jensen *et al.* 2010), including *C. opilio*, *C. bairdi*, *C. tanneri* and *Nephrops norvegicus*.

Host range: This parasite has a very broad host range among decapod crustaceans (see review by Stentiford and Shields 2005). Less commercially but ecologically important crustaceans are infected as well (Johnson 1986).

Geographical range: The distribution of *Hematodinium* infections is essentially worldwide (see review of Stentiford and Shields, 2005). Published reports of *Hematodinium* infections in numerous crustacean species have been made from the North Atlantic and North Pacific Oceans and their adjacent seas, Arctic Ocean and the South Pacific Ocean. Higher prevalences have been reported from crabs residing in coastal areas with slower moving water masses (e.g., small embayments) than from more dynamic open ocean sites (Taylor and Kahn 1995); Meyers *et al.* 1996; Morado *et al.* 2002; Shields *et al.* 2007).

Individual effects: Hemolymph of infected crabs is milky in the chronic phase of infection due to an abundance of parasites and depletion of normal crab blood cells (hemocytes). Host response to the parasite seems to be lacking, although response to co-infecting pathogens is apparent (Johnson 1986; Stentiford *et al.* 2003). Decreases in carbohydrate, protein and glycogen reserves, loss of hemolymph clotting function and degeneration of muscle have been reported from crabs and lobsters with *Hematodinium* infections (Meyers *et al.* 1987; Field and Appleton 1995; Stentiford and Shields 2005). Histopathologic changes in naturally infected *C. opilio* have been documented and include dilation of hemal arterioles due to massive numbers of parasite, loss of connective tissue and reserve inclusion (RI) cells in gills, heart, hepatopancreas and muscle, loss of gill epithelium and trabecular cells, and distal swelling and fusion of gill lamellae associated with sporulation of the parasite (Wheeler *et al.* 2007).

Population effects: The prevalence of BCD in *C. opilio*, as in other crustacean hosts, varies widely spatially and temporally. Open water environments have rather low endemic levels of infection (<2%) whereas coastal embayments have the highest prevalence (26% to 80%) (Taylor and Kahn 1995; Meyers *et al.*1996; Morado *et al.* 2000; Dawe 2002). Small now crabs (i.e., <45 mm carapace width) of both sexes have greater prevalence than larger crabs (Morado *et al.* 2000; Pestal *et al.* 2003; Shields *et al.* 2005) and in the Conception Bay (Newfoundland) population, immature females had significantly greater prevalence than mature females (15.3% and 2.9% respectively); (Shields *et al.* 2005). However, long term monitoring of BCD in trawled and trapped snow crabs from Conception Bay demonstrated a shift in the epidemiology of the disease over time. The highest prevalence of BCD shifted from juvenile crabs in the 1997 to 2000 period to large legal-sized males in the 2003–2005 period with a prevalence of 34.6% in large clawed males in 2005. Examination of environmental and biotic data showed that crab density was not associated with the increase. However, increases in bottom temperature and related molting activity were highly correlated with this dis-

ease regime shift (Shields *et al.* 2007). The mortality rate of *Hematodinium* infections in *C. bairdi* is 100% (Meyers *et al.* 1987). With the general lack of response to the parasite by crustacean hosts, it is very likely the impact to local *C. opilio* populations with high prevalence of *Hematodinium*, such as Norton Sound, Alaska (50-80% infected) and Conception Bay, Newfoundland (up to 26% infected), is significant. Dawe (2002) examined the relation between *Hematodinium* prevalence and catch data from the Newfoundland Labrador continental shelf where prevalence is quite low, and although he found no supporting evidence of effects on catch rates, he acknowledged that annual sampling during one season only may not be appropriate for assessing the potential impact. As the median time to death of naturally infected snow crabs held in the laboratory was 61 days (Shields *et al.* 2005), many crabs may be infected and die before the next sampling event, as Dawe suggested.

Discussion: *Hematodinium* infections in crustaceans have been reported from around the world in commercially important species and less frequently from ecologically important crustaceans. Pestal *et al.* (2003) demonstrated that the use of only visual observation of carapace color and hemolymph appearance to determine infection can underestimate the true prevalence (as determined by microscopic examination) by 48%, so earlier reports of *Hematodinium* prevalence in snow crab populations (as in Dawe 2002) may have been severely underestimated. There is little definitive data on the effects of *Hematodinium* on commercially or ecologically important crustaceans. It is apparent, though, that this parasite is lethal to its crustacean hosts and that the prevalence in *C. opilio*, although variable, has increased markedly over time in certain coastal embayments where long term monitoring has taken place, e.g., Alaska and Bering Sea (Meyers *et al.* 1997; Morado *et al.* 2000) and Conception Bay, Newfoundland (Shields *et al.* 2007). Strong correlation of increasing bottom temperature with increasing prevalence of BCD and greater infection rates in legal-sized males in Conception Bay suggests that continued increase in local bottom temperature due to climate change will enhance the spread of the disease (Shields *et al.* 2007).

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Diseases of fish

Salmonids

Infectious haematopoietic necrosis (IHN)

Diagnosis: IHN is an acute, systemic disease, characterised by focal haemorrhage or petechiae in haematopoietic and other highly vascularised tissues. Virus titres in clinically affected fry are often in excess of 10^4 plaque-forming units per gram of whole fish. The virus may also be isolated from asymptomatic carrier fish. IHN virus replicates in cultured fish cells and infections are readily diagnosed from the presence of cytopathic effect (CPE), following isolation onto a suitable cell culture. Confirmation of the presence of IHNV is obtained by inhibition of CPE by using specific antisera. In addition, specific amplification of viral genomic RNA either from infected tissues or

from cell cultures is achieved by using reverse transcriptase polymerase chain reaction.

Causative agent: IHN is caused by infectious haematopoietic necrosis virus (IHNV), the type species within the genus Novirhabdovirus within the family Rhabdoviridae (Kurath *et al.* 2003). IHNV is a bullet-shaped virion with dimensions of 70 x 110 nm and a genome consisting of negative sense, non-segmented RNA encoding six viral peptides. The virus is enzootic through the Pacific Northwest of North America from Alaska to California and inland to Idaho (Kurath *et al.* 2003). IHN virus was first detected in Europe in 1987 in farmed rainbow trout. All European isolates appear to have originated from a single isolate of North American origin (Enzmann *et al.* 2005). The biology of the disease, the causative agent, and diagnostic methods have been reviewed by Bootland and Leong (1999).

Host range: The host range includes most species of Pacific salmon (*Oncorhynchus* spp.) and most other species of salmonids that naturally occur in the North Pacific as well as species that have been introduced, such as brook char (*Salvelinus fontinalis*), brown trout and Atlantic salmon (Bootland and Leong 1999). Sockeye salmon (*Oncorhynchus nerka*) are particularly susceptible whereas coho (*Oncorhynchus kisutch*) and pink salmon appear refractory to the infection. There are few reports of IHN virus in wild European fish suggesting a sporadic occurrence (Enzmann 2007).

Geographical range: North Pacific

Individual effects: IHN causes mortality mainly in fry, associated with significant decrease in egg-to-fry survival (see below).

Population effects: Epizootics associated with elevated mortality among fry have been observed in several populations of sockeye salmon. The egg-to-fry survival of sockeye salmon in Chilko Lake, British Columbia (BC), Canada was 3.8% following an IHN epizootic (Williams and Amend 1976). The historic egg-to-fry survival in this population had been 9.2%. Similarly, the egg-to-fry survival of sockeye salmon at Weaver Creek, BC, was 19.6% following an IHNV epizootic, compared with an average of 45% for the preceding 10 years (Traxler and Rankin 1989). In the latter case, approximately 50% of the 16.8 million migrating fry in the affected population died of IHN. Population effects were suggested from reports of IHN virus infections associated with dead and moribund sockeye smolts (Burke and Grischkowsky 1984) and in 2-year old sub-adult kokanee (sockeye) salmon (Traxler 1986).

Discussion: Despite the virulence of IHNV for young Pacific salmon, there are very few documented cases in which the virus has been isolated from adult salmon in the ocean (Traxler *et al.* 1997). Similarities in the genetic profile of IHN virus isolated from farmed Atlantic salmon in British Columbia provide a circumstantial link to infections in migratory sockeye salmon (Emmanegger *et al.* 2000). IHN is principally a disease of juvenile salmon and fish typically develop increased resistance with age and weight (Bootland and Leong 1999). There is no measurable evidence for population effects caused by the infection in Pacific salmon during their residence in the oceans.

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Proliferative Kidney Disease (PKD)

Diagnosis: The parasitic disease is characterised by a severe immunological response provoked by the presence of the parasite in the kidney (Chilmonczyk *et al.* 2002). Physiological manifestations include anaemia, ascites, and exophthalmia (Ferguson and Needham 1978). The kidney becomes grossly enlarged and splenomegaly often occurs (Ferguson 2006). The extent of anaemia will vary according to the severity of the infection and therefore may serve as a useful indicator of the disease (Hedrick *et al.* 1993). The infection is diagnosed from the presence of the parasite in stained kidney imprints or histological sections. Alternatively, amplification of DNA using parasite-specific oligonucleotide primers in a polymerase chain reaction provides evidence of infection.

Causative agent: Proliferative kidney disease (PKD) is caused by infection with the malacosporean parasite *Tetracapsuloides bryosalmonae* (Myxozoa). Fish become infected during exposure to freshwater containing infective spores. The infective spores are released from any one of several species of freshwater bryozoans that serve as the obligate alternate host for the parasite (Feist *et al.* 2001).

Host range: Most species of freshwater and anadromous salmonids belonging to the genera *Salvelinus*, *Salmo* and *Oncorhynchus* are susceptible to infection and to the development of PKD.

Geographical range: The distribution of PKD and its causative agent (*T. bryosalmonae*) is circumboreal, closely following that of its bryozoan and salmonid hosts.

Individual effects: PKD causes mortality in affected fish that can vary from 5% to 100% (Hedrick *et al.* 1993). It is a seasonal condition as peak infectivity occurs during increases of temperature in early summer (Hedrick 1985). Water temperatures greater than 15°C result in increased clinical signs of infection, faster disease progression, and

higher mortality rates (Ferguson 1981, Clifton-Hadley *et al.* 1984). Although water temperatures below 12°C have been suggested as a means of control on rainbow trout (*Oncorhynchus mykiss*) farms, infections have occurred at temperatures of 10 °C in wild populations (Ferguson 1981, Gay *et al.* 2001).

Population effects: There is evidence that PKD has contributed to the decline of certain salmonid populations (Feist *et al.* 2002; Tops *et al.* 2006; Sterud *et al.* 2007; Wahli *et al.* 2007, Kristmundsson *et al.* 2010). The apparent correlation between observations of PKD and significant loss in brown trout (*Salmo trutta*) populations in Switzerland is of great concern (Wahli *et al.* 2002, Burkhardt-Holm *et al.* 2005) and is the subject of on-going research (Borsuk *et al.*, 2006). Although studies in the United Kingdom have demonstrated widespread occurrence of the infection, correlation with macroscopic disease signs and internal pathology in brown trout (Feist *et al.*, 2002; Peeler *et al.*, 2008), mortalities in wild populations have not been reported. In western Canada, the prevalence of *T. bryosalmonae* in wild pink salmon (*Oncorhynchus gorbuscha*) in the Quinsam River between 2003 and 2010 ranged from 43% to 100%, raising the possibility of clinical PKD in a significant portion of the population when environmental conditions are conducive (Braden *et al.*, 2010). In Norway, PKD outbreaks in fresh water farms producing brown trout and salmon (*Salmo salar*) 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 western and mid-Norway in 2006, severe mortality among yearlings of brown trout and Atlantic salmon was observed in the River Jølstra and in the River Ålva. PKD-induced mortality in the river Ålva has been estimated to range from 50 to 85 % (Sterud *et al.* 2007). In 2007, 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.

Discussion: Although it is a freshwater disease, PKD is considered here because it affects anadromous salmonids and may become a problem during their marine phase. There is increasing evidence that PKD has a significant effect on the abundance wild salmon 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. Transmission to juvenile anadromous salmonids within freshwater indicates a risk of clinical infections following migration to the ocean.

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Ichthyophoniasis

Diagnosis: The disease is characterised by the presence of granulomas in spleen, kidney, liver, skeletal, and cardiac muscle. In severe infections, few organs remain unaffected. Ichthyophoniosis is readily diagnosed in histological preparations of affected tissues and, in severe cases, from the gross observation of punctuate white lesions on the surface of affected organs. Parasite spores will germinate in suitable culture me-

dia providing a further opportunity for detecting infections. Parasite-specific oligo-nucleotides primers provide the basis for highly sensitive polymerase chain reactions that will detect the presence of parasite DNA.

Causative agent: Ichthyophoniasis is caused by infection with the mesomycetozoean parasite *Ichthyophonus hoferi*. Transmission requires the ingestion of infected tissues followed by migration of the organism through the wall of the intestine (McVicar 1999). Recent evidence shows that infective spores can be released from the skin of infected fish and it has been hypothesised that infections in planktivorous fish result from ingesting spores free in the water column (Kocan *et al.* 2010).

Host range: The broad host range suggests widespread susceptibility among marine teleosts. McVicar (1999) commented on the scarcity of reports from elasmobranchs although the extent to which this reflects a reduced level of surveillance is not clear. There is evidence that Ichthyophonus can establish in freshwater salmonids (Schmidt-Posthaus & Wahli 2002, Hershberger *et al.* 2008).

Geographical range: The parasite appears to be ubiquitous in populations of marine finfish in all global waters north and south of the equator. Infections of freshwater fish have also been recorded.

Individual effects: Occurrence of granulomas in internal organs and disease-associated mortality have been reported.

Population effects: The overall prevalence of Ichthyophonus in adult Chinook salmon (*Oncorhynchus tshawytscha*) in the Yukon River (Alaska, USA and Yukon Territory, Canada) can exceed 40%. However, the prevalence drops significantly during the spawning migration as fish approach spawning grounds. This decline is attributed to pre-spawning mortality caused by the infection (Kocan *et al.* 2004). Mortality among Chinook salmon following exposure to Ichthyophonus spores had previously been demonstrated in controlled laboratory experiments (Jones and Dawe 2002).

Discussion: There are few examples of salmonid Ichthyophoniosis. The widespread occurrence of this disease in chinook salmon in the Yukon River is well documented. As Ichthyophoniosis was unreported in Pacific salmon in the Yukon River prior to 1985, Kocan *et al.* (2004) suggested it to be a recent phenomenon. *Ichthyophonus* occurs infrequently in sockeye salmon captured off the west coast of Vancouver Island, Canada (Tierney and Farrell 2004).

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Gyrodactylosis

Diagnosis: A diagnosis of gyrodactylosis is based on the microscopic identification of the parasites on the skin of affected fish. Specific identification to *G. salaris* is based on an examination of the morphology and morphometrics of the specimens, particularly of the hamuli, bars and marginal hooks of the opisthaptor. Alternatively, a suite of molecular markers, including those associated with ribosomal RNA (the internal transcribed spacer regions ITS1 and ITS2, 5.8S rDNA and the intergenic spacer IGS region) and the mitochondrial cytochrome oxidase 1 (COI) gene have proven useful as taxonomic tools. The applications, strengths and weaknesses of the morphometric, morphologic and molecular methods as diagnostic tools for *G. salaris* are discussed in Bakke *et al.* (2007).

Causative agent: The monogenean parasite *Gyrodactylus salaris* first reported from Atlantic salmon on the Baltic coast of Sweden (Malmberg 1957, cited in Bakke 2007).

Host range: *Gyrodactylus salaris* is a parasite of salmonids and has been reported on Atlantic salmon, rainbow trout and brown trout.

Geographical range: The parasite was observed in Norway for the first time in 1975 (Mo 2007) and, within 30 years, had become established in 45 Norwegian rivers (Mo and Norheim 2005). In addition to Sweden and Norway, the distribution of *G. salaris* appears to be widespread throughout much of northern and eastern Europe, including Russia, the Ukraine, and Georgia (McHugh *et al.* 2000; Bakke *et al.* 2007). Reports of the parasite in France and Portugal (McHugh *et al.* 2000) may be inaccurate and based on the misidentification of related parasites (Cunningham *et al.* 2001, Bakke *et al.* 2007). Bakke *et al.* (2007) provide a comprehensive review of the recent history of *G. salaris* throughout its known range.

Individual effects: The pathogenicity of *G. salaris* relates to damage to the integument of the fish associated with attachment and feeding. The damage associated with infection compromises the osmotic integrity of the epidermis and provides opportunities for secondary infections. Numerous factors associated both with the parasite and with the host contribute to the severity of pathology caused by *G. salaris*. Adaptation appears to have produced local variants of *G. salaris*, for example among Swedish Rivers (Hansen *et al.* 2003) or among hosts (Buchmann *et al.* 2000, cited in Bakke *et al.* 2007), possibly indicating a high potential for variation in pathogenicity. Some non-pathogenic strains of *G. salaris* have been identified on farmed rainbow trout in Denmark and on wild Arctic char in Norway (Mo 2007). In addition to local adaptations, the intensity of infection is an important determinant of pathogenicity and this appears to be related to the reproductive potential of the parasite (Bakke 2007). Host effects include age and species; there is good evidence for variation in susceptibility among infraspecific salmonid stocks. Historically, Baltic Sea stocks of Atlantic salmon were perceived to be resistant to *G. salaris* whereas stocks from the northeast Atlantic Ocean were susceptible (see Bakke *et al.* 2007 for several references). There is evidence that Baltic salmon stocks range from susceptible to resistant (Bakke *et al.* 2004).

Population effects: Population level effects of *G. salaris* have been well documented for Norwegian salmon. The following examples provide evidence of population impact. In 1984, losses due to the parasite were estimated at 520 tonnes, equivalent to 25% of the total salmon catch (Egidius *et al.* 1991). The average density of salmon parr and adults in 14 affected rivers was reduced by more than 85% (Johnsen *et al.* 1999, Bakke *et al.* 2007). *G. salaris* has caused the extinction of salmon in six rivers and threatens salmon populations in 34 others (Bakke *et al.* 2007). Economically, the total annual cost due to lost salmon fisheries, tourism and associated industries is estimated at US$34M. The annual costs of surveillance and eradication add a further US$23M (Bakke *et al.* 2007). Similarly among rivers on the Swedish west coast, the impact of *G. salaris* ranges from severe to negligible (Malmberg 1998). An epidemic of *G. salaris* with associated reduction in the density of salmon parr was reported from the River Keret, Russia, which drains into the White Sea (Johnsen *et al.* 1999, Bakke *et al.* 2007).

Discussion: Although being a freshwater disease, Gyrodactylosis is considered here because it affects anadromous salmonids. The population effects of *Gyrodactylus salaris* are arguably the most well documented among the diseases of salmon. Systematic surveillance of Norwegian rivers has provided insight into the origin, dissemination and impact of the parasite within the country. Furthermore, it has emphasised the importance of strict controls over fish movements and the need to have in place national infrastructure for the surveillance of health in wild fish populations.

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Salmon lice infestation

Diagnosis: A diagnosis of salmon lice is based on direct observation of the parasites on the surface of the fish. Larger adult and preadult parasitic stages are readily identifiable to species, whereas the identification of immature (*copepodid* and *chalimus*) stages requires microscopic examination. The absence of morphological keys for identifying the immature stages belonging to most species means that identification is only reliable to the genus level when multiple species occur. The use of molecular markers has permitted identification of all developmental stages to the species level (Jones *et al.*, 2006; Jones and Prosperi-Porta, 2011).

Causative agent: Sea lice are parasitic copepods belonging to the suborder Siphonostomatoida and the family Caligidae. The salmon louse, *Lepeophtheirus salmonis*, is considered the most important species affecting anadromous salmonids. Other caligid copepods affecting salmonids in the northern hemisphere include *Caligus elongatus* in the Atlantic Ocean and *Lepeophtheirus cuneifer*, and *Caligus clemensi* in the Pacific Ocean

Host range: The native range of anadromous salmonids includes the northern Atlantic and Pacific Oceans. Salmonid fishes have been introduced into the southern hemisphere to support sport fisheries and as farm stock.

Geographical range: While salmon introduced into the southern hemisphere have become infected with sea lice belonging to native species of *Lepeophtheirus* and *Caligus*, there are no records of *L. salmonis* from the southern hemisphere. There is evidence that the form of *L. salmonis* occurring in the Pacific Ocean is genetically distinct from that occurring in the Atlantic Ocean (Yazawa *et al.* 2008).

Individual effects: All parasitic stages of sea lice graze on cells, mucous and other fluids associated with the epidermis and dermis. The amount of tissue consumed and therefore the damage caused to the fish is proportional to the size the parasite and to the intensity of the infection. Thus damage caused by chalimus stages is limited due to the relatively small size of these stages and because they are tethered to one location by the frontal filament. The damage becomes greater in proportion to the increased size of the parasite as it moults to larger preadult and adult stages. In addition, preadult and adult stages are no longer tethered and may feed over a larger area of skin. Damage to the integument of marine fish disrupts the homeostasis of water and salts and may permit the entry of opportunistic pathogens (Finstad and Bjørn 2011). In addition, the saliva of salmon lice contains proteases and other compounds that may influence the immune capacity of the fish (Fast *et al.* 2004). Responses in Atlantic salmon and sea trout range from stress to mortality depending on the age of the fish and the severity of the infection (Wagner *et al.*, 2008). In contrast, inflammatory processes at the site of infection appear to limit infections on coho (*Oncorhynchus* kisutch), Chinook (*O. tshawytscha*) and pink salmon (*O. gorbuscha*) (Johnson and Albright, 1992; Fast *et al.*, 2002; Jones *et al.*, 2007).

Population effects: The direct effects of sea lice on individual salmon have been used as evidence for population-level effects, particularly in more susceptible species such as sea trout and Atlantic salmon. Furthermore, severe infestations with *L. salmonis* with the potential to cause a population level effect have been associated with salmon mariculture in several countries (Jones 2009). Estimates of the impacts of sea lice on salmon populations have been based on three lines of evidence: 1, the use of lethal thresholds derived from laboratory studies; 2, mathematical models and 3, comparing the relative numbers of returning adult salmon that were either untreated or treated for sea lice prior to release as smolts.

Theoretically, only infestations exceeding a lethal threshold intensity with a sufficiently large prevalence are expected to have a measurable population effect and these parameters have been determined for some species. Infestations with 30 chalimus *L. salmonis* stages were lethal for 40g salmon (0.75 per gram) and that 90 copepodids or 50 preadults were lethal for 60g sea trout (1.5 and 0.8 per gram, respectively) (Bjørn and Finstad 1997, Finstad *et al.* 2000). Wells *et al.* (2006) proposed a management of threshold of 13 *L. salmonis* fish^{-1} based on the physiological consequences of infestation on sea trout post-smolts ranging from 9g to 70g. Annual sampling at Vikbotten in northern Norway demonstrated infestations with *L. salmonis* on sea trout with intensities sufficiently high to have a potential for population-level effect (Finstad and Bjørn 2011). There is similar evidence that *L. salmonis* are causing population-level effects among Atlantic salmon postsmolts with in two Norwegian fjords (Finstad and Bjørn 2011). A lethal infestation threshold of 7.5 *L. salmonis* per gram was proposed for pink salmon (*Oncorhynchus gorbuscha*) weighing less than 0.7g (Jones and Hargreaves 2009). The number of these smallest pink salmon with infestations exceeding the threshold was estimated to not 7.8% in one monthly sample in 2005 and has not been greater than zero since 2008 (Jones and Hargreaves 2009). The latter study concluded that there was only evidence for a negligible effect of *L. salmonis* on juvenile pink salmon.

Mathematical models were used to estimate local extinctions of juvenile pink salmon as a result of infestations with *L. salmonis* (Krkošek *et al.* 2007). This study triggered scientific debate (Brooks and Jones 2008, Riddell *et al.* 2008) and left a number of unanswered questions. In contrast, a more recent model found no evidence of a relationship between the intensity of *L. salmonis* infestation on juvenile pink salmon and the population size of the same cohort that returned as adults (Marty *et al.* 2010). Mathematical models will become increasingly valuable as tools in predicting population-level effects of *L. salmonis* as the reliability of their biological parameters improves.

Studies in Ireland and Norway have estimated population impacts of *L. salmonis* by releasing tagged salmon smolts according to whether they had been treated for sea lice or not and comparing the number of returning adult salmon. While these studies were confounded by the low number of returning tagged salmon, in Norway higher numbers of treated compared with untreated fish returned fish were observed to return in multiple years and multiple rivers or fjords (Skilbrei & Vennevik 2006, Finstad and Bjørn 2011). Results from Irish studies indicated no consistent evidence of additional mortality among salmon postsmolts attributable to *L. salmonis* infestation (O'Donohoe *et al.* 2007).

Discussion: Numerous studies have documented the lethal and sublethal effects of *L. salmonis* on individual salmon. Population level impacts may be anticipated when a sufficiently large percent is infested to these levels. It is not known whether the effects of salmon lice are additive (in addition to other effects) or compensatory (affect salmon that would otherwise be affected by another process). Thus, there remains uncertainty in the extent to which salmon louse effects interact with the numerous other natural and anthropogenic factors influencing salmon population.

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M74 Syndrome, early life stage mortality, EMS (Early Mortality Syndrome)

Diagnosis: M-74 affects yolk sac fry from Atlantic salmon in the Baltic Sea. The disease is seen as clear neurological symptoms with disturbed swimming behaviour such as uncoordinated movements and cork-screw swimming towards the surface. Affected fishes can be dark in colouring; they have flared opercula and a white precipitate in the yolk sac. Functional disturbances are lowered heart rate and, in the viscera, a small and pale spleen can be seen. In the terminal stage, the fry are lethargic and have bradycardia. During the development of the disease, a decrease of pigmentation can be seen in the yolk sac oil droplet. Histopathological lesions are seen in the brain with large numbers of cells exhibiting pyknosis and karryorhexis. Other lesions are focal hemorrhages and periventricular hydropic degeneration. Low concentrations of thiamine (ca. 1.1–0.6 nmol/g roe) in roe are a clear indication whether a family group will develop the disease (Amcoff *et al.* 1999). Thiamine levels are also declining faster between fertilisation and the eyed egg stage in these groups, and a threshold value of 0.3nmol/g has been recorded where yolk sac fry below this concentration will develop M-74.

Early mortality syndrome (EMS) is another early life stage mortality resembling M-74. The disease is described from the Great Lakes of North America since 1968. Many disease pattern are similar such as it varies between family groups and that the fry show the same disease pattern with symptoms such as swim up, spiral swimming, lethargy, dark pigmentation, hyperaxcitability, tetanus, hydrocephalus and hemorrhages.

Causative agent: Thiamine deficiency (Fisher *et al.* 1995 and other publications) is the most important factor in the etiology of the disease but also low concentration of antioxidants, and nutritional factors such as females feed intake (prey containing thiaminases) can play a role. The cause of the deficiency is still unclear but a correlation between the amount of sprat in the Baltic Sea and levels of M74 have been reported (Karlsson *et al.* 1999)

Host range: Salmon (*Salmo salar*), Brown trout (*Salmo trutta*), Coho salmon (*Oncorhynchus kisutch*), Lake trout (*Salvelinus namaycush*), Steelhead (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tsawytscha*),

Geographical range: M-74: Baltic Sea, EMS: the Great Lakes of North America

Individual effects: The disease causes lethargy, darkning, loss of orientation and uncoordinated swimming, lethargy and finally total immobility and mortality due to severe brain damages.

Population effects: The mortality is normally 100% within family groups. The incidence varies between different river systems and from year to year.

Discussion:

Gadoids

Infestation with *Lernaeocera branchialis*

Diagnosis: The parasitic copepod is conspicuous in its adult stage. These stages are the adult females of which one to three individuals are usually present, but occasionally more, may be found in the gill cavity of individual fish. The distended body of the parasite is the most conspicuous feature, appearing red with ingested blood from the host. Convoluted egg strings are also apparent. The head region is embedded in the host tissues.

Causative agent: *Lernaeocera branchialis* (Linnaeus, 1767) is a parasitic copepod, within the family Pennellidae. This family includes the genera *Haemobaphes*, *Lernaeenicus*, and *Pennella*. Three species of *Lernaeocera* are known to occur, namely *L. branchialis, L. lusci* and *L. minuta.* The parasites have a two-host lifecycle with an intermediate fish host (usually a flatfish) and a definitive, or final fish host (usually a round fish).

The stages on the intermediate hosts are small, measuring around 2-3 mm in length. Flatfish can harbour several hundred parasites on the gills with little effect noted. The sexes are separate in the larval stage. Mating occurs on the gills of the flatfish host, after which the male dies. The female then undergoes a period of metamorphosis, detaches from the host and then seeks out the final, whitefish host. There is a limited amount of time in which the free swimming female must find a host, otherwise it dies. On contact with a suitable final host, the female then undergoes a more radical metamorphosis becoming firmly attached within the gadoid. The head end of the parasite becomes closely associated with a major blood vessel of the fish from which it derives its food. On reaching maturity, the female then produces eggs and the lifecycle then continues. Adult *L. branchialis* are around 3–4 cm in length. It has been estimated that *L. branchialis* has a lifespan of around 12–18 months on gadoids and whilst it is found mainly on juvenile and medium sized gadoids, it can be found on all age/size fish. The parasite cannot survive in salinities below 18 ‰.

Host range: *L. branchialis* has a wide host range using mainly cod (*Gadus morhua*), haddock (*Melanogrammus aeglifinus*), pollack (*Pollachius pollachius*) and whiting (*Merlangius merlangus*) as final hosts and flatfish such as flounder (*Platichthys flesus*), turbot (*Psetta maxima*), plaice (*Pleuronectes platessa*), dab (*Limanda limanda*), lemon sole (*Microstomus kitt*) and sole as intermediate hosts. It has been recorded to utilise at least 13 fish species as intermediate hosts and 21 fish species as final hosts. *Lernaeocera lusci* mainly uses bib (*Trisopterus luscus*) as a definitive host and sole (*Solea solea*) and dragonet (*Callionymus* spp.) as intermediate hosts. *L. minuta* uses sand goby (*Pomatoschistus minutus*) as a final host.

Geographical range: *L. branchialis* has a wide geographical distribution and is found throughout the North Atlantic and adjacent seas, from Norway to Morocco. In cod, the prevalence in the North Sea areas off the north-east coast, Dogger Bank, off the Humber and Rye Bay on the south coast varies between approximately 1% to 9%. Prevalence of infection appears to be higher in Irish Sea stations. Approximately 25% of cod examined at Red Wharf Bay (Anglesey) harboured the parasite in 2000 but in Liverpool Bay prevalence was approximately 4%. In haddock, almost 38% of fish sampled from Amble (off the north-east coast of England) were found to harbour the infection in 1997 and between 22% and 25% of fish examined off Flamborough were parasitised in 1999 and 2000 respectively. Of note, the parasite was not found in 100 haddock examined from the Firth of Forth or the Farne Deep in 2000. Between 1993 and 1996, the parasite had a wide distribution in whiting stocks but was present at

low prevalence. As with all susceptible species, some individuals were heavily infected.

Individual effects: It has long been recognised that *L. branchialis* exerts a pathological effect on the final host (Smith *et al.* 2007). The head of the parasite induces severe pathological changes in the attachment region and penetration may even reach the cardiac cavity and associated vessels. Pathological changes in this region have severe implication for the performance at the individual level. Infected fish are generally emaciated, and in poor condition. Clinical signs of parasitism range from no apparent effect to extreme emaciation and anaemia (Khan 1988); the latter being seen in only a small percentage of infected fish. Data from haddock caught in the North Sea would suggest that there is a reduction of around 20% in the fecundity of the fish host as a result of infections by the parasite. It has been estimated that there is a weight loss of between 10% and 30% in infected fish with a concomitant decrease in fat content.

Population effects: Population effects have long been suspected and the negative effects of parasitism on growth and fecundity have been reported (Kabata 1958). Recent occurrences of high prevalence of *L. branchialis* in juvenile gadoid populations in Scottish coastal waters have been recorded (…) but it is not known whether the presence of the infection had an effect on the strength of the adult population.

Discussion: Although population effects of the infestation with *Lernaeocera sp.* have long been suspected, no quantitative data on mortality or other population effects are available

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Clupeids

Viral Haemorrhagic Septicaemia (VHS)

Diagnosis: Clinical signs of VHS include haemorrhages in themeninges, serous surface, muscles, internal organs, the eyes and the skin (partly also ulcers), exophthalmia, darkening of the body, and pale gills. Heavily affected fish are lethargic, show abnormal swimming behaviour, and a dark discolouration.

Causative agent: VHS is a viral disease known for many years because of its severe impact on rainbow trout farms in Europe (Skall *et al.* 2005, Elston & Myers 2009). The VHS virus is negative-stranded RNA virus belonging to the Rhabdoviridae (Skall *et al.* 2005). The VHS virus has also been recorded in a wide range of wild marine fish species (Meyers *et al.* 1992, 1994, 1999, Dixon *et al.* 1997, 2003, Hedrick *et al.* 2003, Skall *et al.* 2005) including the Pacific herring (*Clupea pallasi*) in which VHSV was described for the first time by Myers *et al.* (1992) and is considered as a disease that mainly affects young fish (Elston and Myers 2009). At least four geographically distinct genotypes of the marine virus have been identified (I-IV) and the VHSV strain isolated

from Pacific herring belongs to the North American strain IVa (Elston and Myers 2009).

Host range: Besides *C. pallasi*, other clupeid species found to be infected are Atlantic herring (*Clupea harengus*), sprat (*Sprattus sprattus*) and pilchard (*Sardinops sagax*). Gadoid species known to be affected include Pacific hake (*Merluccius productus*), walleye pollock (*Theragra chalcogramma*) and haddock (Melanogrammus aeglifinus); (Myers *et al.* 1992, 1994, Smail 2000, Marty *et al.* 2003, Elston and Myers 2009) .

Geographical range: The VHS virus has been isolated from a variety of marine fish species in the Pacific, Atlantic and in the Baltic Sea. However, clinical signs attributed to the VHS virus were only reported from the Pacific (Myers *et al.* 1992, 1994, Marty *et al.* 2003) and the North Sea (Smail 2000).

Individual effects: VHS infection has been associated with haemorrhagic and ulcerative skin lesions in wild Pacific herring (Meyers *et al.* 1994, Marty *et al.* 2003), Pacific cod (*Gadus macrocephalus*) (Myers *et al.* 1992) as well as Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglifinus*); (Smail 2000).

Population effects: The collapse of the Pacific herring stock of Prince William Sound (PWS), Alaska, US, recorded in 1992/1993 and a mass mortality event in 1998 affecting small Pacific herring, Pacific hake (*Merluccius productus*) and walleye pollock (*Theragra chalcogramma*) from the Lisianski Inlet near Pelican, Alaska, US, were attributed to the VHS virus and were partly associated with haemorrhagic and ulcerative skin lesions (Meyers *et al.* 1992, 1994, 1999, Marty *et al.* 2003). For Pacific herring from the PWS, Marty *et al.* (1993) conclude from their model that the disease significantly affected both adult population abundance (in 1992-1993 and 1997-1998) and subsequent recruitment (in 1994 and 1999).

Discussion: There has been a strong debate as to the causes of the decline in the Pacific herring stock of Prince William Sound. Whilst Marty *et al.* (1993) and Meyers *et al.* (1994) suggested a direct causative link to VHSV infection, Pearson *et al.* (1999) and Elston & Myers (2009) concluded that VHSV was not the primary cause of the stock collapse but may have been only one of the factors contributing to the effects of increased herring biomass and associated starvation and poor body condition leading to the stock decline. An impact of the Exxon Valdez oil spill in 1989 on the population decline was disputed by Marty *et al.* (2003) and Elston and Myers (2009), whilst Thorne and Thomas (2008) argued that the decline was associated with the oil spill and Carls *et al.* (2002) did not rule out indirect effects of the spill.

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Ulcerative Mycosis

Diagnosis: Ulcerative mycosis and the associated skin lesions affecting Atlantic menhaden (*Brevoortia tyrannus*) are characterised by the presence of focal ulcers that penetrate deep beneath the basement membrane of the affected epithelial cell layers (Noga *et al.* 1988; cited in Johnson *et al.* 2007). Lesions are usually singular with a red margin and are most commonly found on the ventrum of affected fish near the anus (Noga and Dykstra 1986, Levine *et al.* 1990b; cited in Johnson *et al.* 2007), Histologically, fungal hyphae surrounded by severe granulomatous inflammation can be found (Dykstra *et al.* 1986, Noga and Dykstra 1986; cited in Johnson *et al.* 2007).

Causative agent: The lesions were termed 'ulcerative mycosis' because most contained invasive fungal (oomycete) hyphae of the genera *Aphanomyces* (confirmed bay Blazer *et al.* 2002) or *Saprolegnia.* Apart from the oomycetes, other pathogens such as dinoflagellates and myxosporeans (*Kudoa clupeidae*) have been suggested to play a role in the development of ulcerative lesions in Atlantic menhaden (Johnson *et al.* 2007, Reimschuessel *et al.* 2003; cited in Johnson *et al.* 2007). In the early 1990s, attention focused on the potential association of the dinoflagellate *Pfiesteria piscicida* with the development of lesions (Burkholder *et al.* 1992, Noga *et al.* 1996).

Host range: Atlantic menhaden (*Brevoortia tyrannus*). Experimental infections have shown that other species (mummichog, *Fundulus heteroclitus*; hogchocker *Trinectus*

maculatus; striped killifish, *Fundulus majalis*) are susceptible as well (Johnson *et al.* 2004).

Geographical range: Estuarine areas at the east coast of the USA, from Delaware to North Carolina

Individual effects: Individual effects consist of grossly visible skin ulcerations (see above) and the occurrence of granulomatous inflammatory lesions associated with the presence of fungal hyphae. Affected fish are characterised by higher liver somatic indices, neutrophil and monocyte percentages, and splenic mononuclear cell TGF-b mRNA levels than fish without lesions. Hematocrit values, plasma protein, and Ca concentrations are significantly lower in fish with ulcerative skin lesions than in those without (Johnson *et al.* 2007).

Population effects: Large fish kills of juvenile Atlantic menhaden with ulcerative skin lesions from the Pamlico River were observed in 1984 (Johnson *et al.* 2007). In 2001 and 2002, Atlantic menhaden from the same region displayed a prevalence of ulcerative skin lesions of 50 % for most sites (Johnson *et al.* 2007). However, the 2001 fish affected had more advanced stages of the disease. Healed stages of the disease were rare (< 1 % out of the fish with external lesions), possibly an indication of disease-associated mortality. Despite the high prevalence of skin ulcers, no mortalities were recorded.

Discussion: Due to the multifactorial etiology of skin ulcerations it has been difficult to discern single primary causes of the disease. Apart from the observation of regional fish kills and a high prevalence of associated skin ulcerations there is no quantitative information on population effects available.

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Ichthyophoniasis

Diagnosis: The disease infection is characterised by a chronic inflammatory response associated with the formation of granulomas in spleen, kidney, liver, skeletal muscle, cardiac muscle and other organs. In severe infections, few organs remain unaffected. Ichthyophoniosis is readily diagnosed in histological preparations of affected tissues. Parasite spores germinate in suitable culture media, providing a further opportunity for detecting infections. Parasite-specific oligonucleotides primers provide the basis for highly sensitive polymerase chain reactions that will detect the presence of parasite DNA. Severe cases of the disease in Atlantic and Pacific herring can easily be recognised by gross examination by the occurrence of whitish granulomas in the heart tissue of affected fish (Rahimian 1996, Kocan *et al.* 1999, Hershberger *et al.* 2002). In addition, external signs (nodules in the skin (= sandpaper effect), pigmented skin ulcers) have been reported (Sindermann 1990, Sindermann & Chenoweth 1993, Kocan *et al.* 1999, Hershberger *et al.* 2002, 2008). In Atlantic herring, a number of epizootics of the disease associated with mortalities have been reported (Sindermann 1990, Rahimian & Thulin 1996) that were characterised by severe infections and associated tissue responses of especially the cardiac muscle.

Causative agent: Ichthyophoniosis is caused by infection with mesomycetozoean parasites of the genus *Ichthyophonus* (Mendoza *et al.* 2002) According to Rand *et al.* (2000), at least two species (*I. hoferi* and *I. irregularis*) can be distinguished. However, in most cases infections recorded in fish have been attributed to *I. hoferi*.

Host range: The parasite appears to be ubiquitous in populations of marine finfish in all global waters north and south of the equator and has been recorded in from approx. 100 host species (Rand 1990, cited in Rahimian 1998). The broad host range suggests widespread susceptibility among marine teleosts and a low host specificity of the parasite. There is also evidence that Ichthyophonus has become established in populations of freshwater fishes (Schmidt-Posthaus & Wahli 2002, Hershberger *et al.* 2008) and also affects amphibians (Mikaelian *et al.* 2000). In Clupeids, Ichthyphoniasis has been reported to affect Atlantic herring (*Clupea harengus*); (Sindermann 1958, Sindermann 1990, Rahimian & Thulin 1996, Holst 1996, Rahimian 1998) Pacific herring (*Clupea pallasi*) (Marty *et al.* 1998, Kocan *et al.* 1999, Hershberger *et al.* 2002), sprat (*Sprattus sprattus*) (Rahimian 1998), alewife (*Alosa pseudoharengus*); (Sindermann & Chenoweth 1993, Hershberger *et al.* 2002) and Atlantic menhaden (Brevoortia tyrannus); (Johnson *et al.* 2008).

Geographical range: The parasite appears to be ubiquitous in populations of marine finfish in all global waters north and south of the equator.

Individual effects: Since experimental studies with rainbow trout (*Oncorhynchus mykiss*) revealed that a high intensity of cardiac infection in muscle may lead to cardi-

ac failure (Kocan *et al.* 2006), it is likely that cardiac failure may be a major cause of diseases-induced mortality that is considered the ultimate effect of the infection in herring (Sindermann 1990, Patterson 1996, Rahimian & Thulin 1996, Mallergaard & Spanggaard 1997, Rahimian 1998).

Population effects: Epizootics of Ichthyophoniosis associated with a high prevalence and significant mortalities have only be recorded in Atlantic herring from the western coast of the North Atlantic (Gulf of St. Lawrence, Gulf of Main) (Sindermann 1958, Tibbo & Graham 1963, Sindermann 1990, Sindermann & Chenoweth 1993) and its eastern coast (Skagerrak, Kattegat, North Sea) as well as in the Baltic Sea (Lang 1992, Rahimian & Thulin 1996, Mellergaard & Spanggaard 1997).

Ichthyophonus epizootics affecting Atlantic herring of the Gulf of St. Lawrence at a high prevalence were recorded in 1913/1914 and 1954/1955. In the 1954/1955 epizootic, a maximum prevalence of 78 % (average 27 %) and an associated mass mortality were recorded and it was estimated that at least 50 % of the mature herring in the Gulf were destroyed in the period 1954–1956 (Sindermann 1958, Tibbo & Graham 1963, Sindermann & Chenoweth 1993). In the Gulf of Maine, two major epizootics occurred in 1930/1931 and 1946/1947 and were noted mainly in coastal juvenile herring stocks (the target of the fisheries). Although a high prevalence was recorded (approx. 70 %), no mass mortalities were noted. However, there was evidence of a decline of the herring stock following the epizootic (Sindermann & Chenoweth 1993).

Rahimian & Tulin (1996) reported thousand of dead or dying Atlantic herring floating at the surface and washed on the shores of the Swedish coast in the period August-September 1991, all of which were found to be infected with *I. hoferi*. Based on a model developed by the ICES Working Group on Pathology and Diseases of Marine Organisms (ICES 1992) (assuming a survival time of 100 days after infection) and on prevalence data from a period of 3 years (3rd quarter 1991 to 4th quarter 1994), they estimated mortality rates ranging from 8.9 % (1st year) to 1.9 % (3rd year), resulting in a total mortality of more than 300 million herring, equalling 10 % of the entire population.

Based on data obtained in the Skagerrak, Kattegat, Baltic Sea and North Sea around Denmark during the same epizootic and a modified model, Mellergaard & Spanggard (1997) calculated annual mortality rates for 1991/1992 between 12.8 % and 36 %, depending on the geographical region. They concluded that the observed decline in the North Sea herring spawning stock biomass from 1.1 million tons in 1990 (pre-epizootic) to 0.5 million tons in 1995 (post-epizootic) may have been due to a combination of increased fishing intensity and the general effect of the *I. hoferi* epizootic.

Patterson (1996) was the first to apply a sophisticated model of disease dynamics to assess the impact of the *Ichthyophonus* outbreak of 1991 in North Sea herring. Rates of infection and mortality rates were estimated from field observations of prevalence and data on relative herring abundance and commercial catches. He concluded that the impact of the infection was significant but not catastrophic. Over the period 1991-1994, the disease was assessed as having caused a decline of approx. 10–20 % on catches or on population size, depending on assumptions about exploitation in the event that no disease has occurred.

In 2008, a high prevalence of Ichthyophonus was recorded in Icelandic summer spawning Atlantic herring (> 30 %) (http://www.fisheries.is/main-species/pelagic-fishes/atlantic-herring/) , considered to cause an additional mortality (ICES Advice

2009). However, the extent to which this may affect the stock has so far not been assessed.

In Pacific herring, the infection is ubiquitous among many wild populations (Marty *et al.* 1998, Kocan *et al.* 1999, Hershberger *et al.* 2002). However, no epizootics comparable to ones affecting Atlantic herring have been recorded. Natural populations of *C. pallasi* are known to be infected at sometimes high prevalences. For instance, in Puget Sound, Washington, US, prevalences ranged from 17 % to 55 % (calculated based on tissue culture of heart and liver tissue), depending on the sampling location (Hershberger *et al.* 2002). In spawning adults (3+ years), even a prevalence of > 70 % was noted (Kocan *et al.* 1999). It has further been documented from experimental studies that Ichthyophonus is highly pathogenic to immunologically naïve individuals, causing 80 % mortality in experimentally infected herring two month after exposure to the pathogen (Kocan *et al.* 1999). This high pathogenicity might explain the high prevalence recorded in the field.

To what extent populations of Pacific herring are affected by the infection remains unclear so far. However, Hershberger *et al.* (2002) describe an increase in annual mortality of Pacific herring in Puget Sound from 20 % in the late 1970s and early 1980s to 64–87 % during 1996–1999 and an accompanying decline in median and maximum age of herring. They suggest that these changes may have been due to a combination of infection with *I. hoferi* and an increased predation pressure caused by an increase in population size of harbour seals (*Phoca vitulina*) in that area. The authors speculate that predation may be targeted on larger herring that are more frequently affected by the Ichthyophonus infection and have a decreases swimming performance associated with cardiac pathology.

In 1993, approx. 80 000 tons (60 %) of spawning Pacific herring failed to return to Prince William Sound, Alaska, US, following which the prevalence of *I. hoferi* in survivors reached 27 %, more than double that seen in previous years (Marty *et al.* 1998) and it was proposed that the parasite might have been responsible for the heavy loss of herring (Kocan *et al.* 1999). Marty *et al.* (2003), however, concluded from a study carried out twice annually in the period 1994–2000 that changes in prevalence of Ichthyophonus were not related to changes in population abundance of Prince William Sound herring. Also Deriso *et al.* (2008) and Elston & Meyers (2009) reported that the *Ichthyophonus* infection was unlikely to be the cause of the herring collapse.

Discussion: Epizootics of *Ichthyophonus hoferi* and resulting mortalities certainly belong to the best-documented cases of disease-associated population effects in marine fish species. Although infections are widespread in terms of fish species and geographical areas affected, epizootics causing measurable effects on mortality and stock size have been scarce and were restricted to Atlantic herring from both sides of the Atlantic, where a number of epizootics followed by a decline in herring population were demonstrated. Also for Pacific herring, there is evidence that the infection is common in wild stocks and is highly pathogenic and it is, thus, likely that the parasite contribute to natural mortality. However, quantitative figures and clear evidence of population effects are lacking.

Other wild non-clupeid fish species for which Ichthyophonus epizootics have been recorded and associated mortalities or at least significant effects on the well-being have been suggested but that are not dealt with here are, e.g., haddock (*Melanogrammus aeglifinus*) (McVicar 1999) and mackerel (*Scomber scombrus*) (Sproston 1944). In aquaculture, *I. hoferi* caused significant problems in farmed fish, e.g. in yellowtail (*Se-*

riola quinqueradiata) in Japan, where the infection has been known as a chronic and usually fatal disease (Sindermann & Chenoweth 1993).

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Flatfish

Ichthyophoniasis

Diagnosis: see page 79

Causative agent: see page 79

Host range: Ichthyophoniosis has been recorded in yellowtail flounder (*Limanda fer-
ruginea*); (Ruggieri *et al.* 1970, Rand *et al.* 2000) and plaice (McVicar 1981, 1999, McVic-
ar & McLay 1985). Based on nuclear DNA sequence analysis, Rand *et al.* (2000)
reported two species of Ichthyophonus (*I. hoferi* and *I. irregularis*) in yellowtail floun-
der.

Geographical range: western North Atlantic (yellowtail flounder), North Sea (plaice)

Individual effects: Ruggieri *et al.* (1970) detected extensive lesions present in the
heart, liver, kidney, spleen, gastrointestinal tract, and body musculature as well as
mild lesions in the gills, gall bladder, brain and testes of infected yellowtail flounder
sampled in a geographically restricted area at the Sable Island Bank and the Western
Bank off Nova Scotia, Canada. Prevalences were 25 % and 57.4 %, respectively. In
other regions, including the Gulf of St. Lawrence, the disease either occurred at a
much lower prevalence or was absent. From their study on the pathology involved
and the finding of the absence of the disease from the ovary and only mild lesions in
the testes, the authors conclude that the disease may have very little effect on poten-

tial reproductive ability of the host. Although many organs were affected, the disease was characterised by an absence of classical inflammatory response but a great deal of necrosis, particularly in regions of parasite germination and 'hyphal' growth. In regions with a large amount of resting spores, the parasite was considered relatively inert and the spores were encapsulated in granulomas or by connective tissue.

Plaice is regarded as a highly susceptible species and the disease is considered lethal in approximately two months (McVicar 1990). The host cellular response was found to be low compared to other species (McVicar 1999).

Population effects: Despite the high prevalence of *Ichthyophonus* in yellowtail flounder and the severe pathological changes recorded, there was no indication of mass mortalities or fluctuations of the flounder population (Ruggieri *et al.* 1970). However, the authors state that the damage was so extensive that there can be no question that homeostasis is affected to the extent that many fish must succumb directly to the infection or are made so weak that they become easy prey or are readily killed by any drastic change in the physical and chemical characteristics of the environment. Thus, population effects cannot be excluded.

From studies in plaice from a wild population north of Scotland, McVicar (1981, 1990, cited in McVicar 1999) suspected a high mortality because he considered the disease to be lethal within a period of approx. 2 months. Because of this high mortality, the prevalence found in the infected population was consistently low (< 10 %). However, Mc Vicar (1990, cited in McVicar 1999) estimated that an infection rate of 10 % cause an annual mortality of 50 %. Although he, thus, considered the disease as an epizootic of significant proportions, he noted that no dead fish were observed in the area. However, this may have been due to a high predation pressure in the area that rapidly removes moribund or newly dead fish.

Discussion: Ichthyophonus epizootics in Atlantic herring have received most scientific and public attention, largely because of the conspicuous mortalities encountered. However, the geographically restricted study of Ichthyophoniosis in plaice provided sufficient evidence to assume that the disease may also cause population effects in other wild fish species.

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Infection with *Trypanoplasma sp.*

Diagnosis:

Causative agent:

Host range:

Geographical range:

Individual effects:

Population effects:

Literature cited:

Burreson EM, Zwerner DE (1984) Juvenile summer flounder, *Paralichthys dentatus*, mortalities in the western Atlantic Ocean caused by the hemoflagellate *Trypanoplasma bullocki*: evidence from field and experimental studies. Helgoländer Meeresuntersuchungen 37: 343-352.

Percids

Mycobacteriosis

Diagnosis: Fish mycobacteriosis, previously termed fish or piscine tuberculosis, is a chronic progressive disease in wild and captive fish worldwide. The primary pathological lesion associated with Mycobacteriosis is that of granulomatous inflammation (Roberts 2001). Granulomas are characterised by a central necrotic area surrounded by macrophages, epitheloid cells, and fibrous connective tissue. Skin lesions may or may not be present. If present, the severity of the lesions can vary from scale loss, small blisters to shallow ulcerations. Postmortem examination often reveals gray-to-white nodules (tubercules or granulomas) in most organs, including the kidney, spleen, and liver (Heckert *et al.* 2001). Diagnosis of mycobacteriosis and the specific causative agent is sometimes difficult due to the fact that clinical signs are largely non-specific, the organisms are not easy to culture because they are slow growing, and because there are many pathogen species potentially involved, requiring a relatively large array of laborious diagnostic techniques, e.g., HPLC, FAME analysis, DNA sequencing applying PCR techniques.

Causative agent: Fish mycobacteriosis is caused by several species of the genus Mycobacterium (Jacobs *et al.* 2009). Pathogens are known to cause serious diseases in most vertebrates, including humans, livestock, and both freshwater and marine fish. Most mycobacteria (except the ones in the *M.tuberculosis* complex and *M. leprae*) are aerobic, acid-fast, Gram-positive, non-spore-forming, non-motile, and free living saprophytes in soil and water. In a variety of marine fish species, a large range of intracellular pathogenic species has been identified, e.g., *Mycobacterium marinum*, *M. chelonae/salmoniphilum*, *M. abscessus*, *M. fortuitum*, *M. shottsii*, *M. pseudoshottsii* and 'M. chesapeaki' (Jacobs *et al.* 2009) and it can be expected that the list will grow. Some of these species are known to be pathogenic for humans. Transmission of the infection may occur through the water, contact with faeces of infected organisms, predation on infected material, cannibalism, transovarian transmission, vector organisms, and dermal wounds etc.

Host range: Mycobacteriosis was first diagnosed in cultured sea bass (*Dicentrarchus labrax*) from the Red Sea in 1990 (Colorni 1992). While Mycobacteriosis is common in aquaculture (sometimes associated with high mortalities) and in the aquaria fish trade, reports from wild fish have only received attention more recently (Decostere *et al.* 2004). According to Jacobs *et al.* (2009), more than 160 fish species (wild and cultured) have been shown to be susceptible to mycobacteriosis. The most conspicuous

cases of mycobacteriosis in wild fish have occurred in striped bass (*Morone saxatilis*) both from the US Pacific coast (Hedrick *et al.* 1987) and from the Atlantic coast in Chesapeake Bay, where the disease was first detected in 1997 (Heckert *et al.* 2001). Additionally, mycobacteria have been cultured from Atlantic menhaden (*Brevoortia tyrannus*) and white perch (*Morone Americana*) in the Chesapeake Bay as well as from ocean-caught Pacific juvenile coho salmon (*Oncorhynchus kisutsch*) and chinook salmon (*O. tshawytscha*) at low background rates (Jacobs *et al.* 2009). Presumptative Mycobacteriosis was also reported from Northeast Atlantic Mackerel (*Scomber scombrus*) (MacKenzie 1988).

Geographical range: worldwide

Individual effects: Mycobacteriosis is a chronic progressive disease that may take years to develop into a clinically noticeable illness and is considered to be ultimately fatal (Gauthier *et al.* 2008). Affected fish may lose their appetite, appear debilitated and emaciated, have impaired growth, and become more susceptible to infection by opportunistic bacteria. Skin lesions may or may not be present. If present, the severity of the lesions can vary from scale loss, small blisters to shallow ulcerations. Postmortem examination often reveals gray-to-white nodules (tubercles or granulomas) in most organs, including the kidney, spleen, and liver (Heckert *et al.* 2001). The primary pathological lesion associated with Mycobacteriosis is that of granulomatous inflammation (Roberts 2001). Advanced infection is usually accompanied by emaciation and has been suggested to be terminal (Heckert *et al.* 2001). Death occurs over a period of months to years (Decostere *et al.* 2004). However, this may be highly dependent on the pathogen and host species. Individual variability and host-pathogen interactions also limit the ability to generalise the specific impact of mycobacteriosis, including the pathology involved (Jacobs *et al.* 2009). A decreased condition factor has been recorded in Northeast Atlantic mackerel (*Scomber scombrus*) (MacKenzie 1988) associated with a high disease prevalence (up to 90 % in fish at age 3) but without any external pathological changes. High mortalities have been reported from aquaculture (Gauthier *et al.* 2008).

Population effects: Striped bass is a species of high economical importance and forms the basis of large recreational and commercial fisheries in the Chesapeake Bay, US. Beginning in 1997, striped bass exhibited poor body condition and ulcerative skin lesions, diagnosed as Microbacterium infection. Subsequently, a high prevalence (> 50 %) of dermal and visceral mycobacteriosis has been demonstrated (Vogelbein *et al.* 1999) and stock assessments have revealed an increase in mortality, possibly linked to the disease. Despite the fact that mycobacteriosis may occur at high prevalence in wild striped bass populations, significant mortalities have so far only been observed in captive fish (Hedrick *et al.* 1987, Bruno *et al.* 1998). This may be due to the chronic nature of the disease and that mortality is, thus, cryptic and therefore not directly measurable (Gauthier *et al.* 2008).

In order to elucidate the dynamics of the disease and possible population effects in striped bass from the Chesapeake Bay, Gauthier *et al.* (2008) analysed data on the occurrence of mycobacteriosis and population parameters (e.g., age) in the period 2003–2005. The prevalence of mycobacteriosis increased with age, reaching a prevalence of 80.0 % and 88.9 % in 5 years old male and female fish, respectively. In older male fish (up to age 11+), the prevalence was more or less at the same level, but in older females (up to age 11+), a decrease in prevalence was noted. This difference was attributed to possible differing migratory patterns, spawning stress, other life history differences or sex-specific disease-associated mortality (Gauthier *et al.* 2008). By ap-

plying a force-of infection modelling approach (Heisey *et al.* 2006) to the age-prevalence data, the authors were able to demonstrate a sex-specific disease hazard (females < males) and a significant disease-associated mortality. From the best-fitting model, a disease-associated mortality of 36 % was estimated, revealing a significant population effect of the disease.

Discussion: Besides Ichthyophoniosis in Atlantic herring, Mycobacteriosis in striped bass from Chesapeak eBay is one of the few examples where epidemiological models were applied successfully in order to estimate disease-associated effects on mortality in affected fish populations. In contrast to Ichthyophoniosis, mycobacteriosis is considered as a chronic disease that takes a comparatively long time to develop and to be lethal and displays, thus, more characteristics of an endemic rather than an epidemic disease. However, although timely restricted mass mortalities do apparently not occur, there is evidence from the model output that the disease may have a substantial impact on survival and, thus, effects at the population level.

Literature cited

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Gauthier DT, Latour RJ, Heisey DM, Bonzek CF, Gartland J, Burge EJ, Vogelbein WK (2008) Mycobacteriosis-associated mortality in wild striped bass (*Morone saxatilis*) from Chesapeake Bay, USA. Ecological Applications, 18(7): 1718–1727.

Heckert RA, Elankumaran S, Milani A, Baya A (2001) Detection of a new *Mycobacterium* species in wild striped bass in the Chesapeake Bay. Journal of Clinical Microbiology 39: 710-715.

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Eels

Eel Virus European X (EVEX)

Diagnosis:

Causative agent: The causative agent of the EVEX is a rhabdovirus that was first isolated in 1977 from European eel imported to Japan (Haenen *et al.* 2009). EVEX is a RNA virus with a bullet-like form and a size of 170-175 x 90-95 nm. It is closely related to the Eel Virus European (EVE).

Host range: EVEX and EVEX-like virus have been isolated from wild and farmed European eel (*Anguilla anguilla*), Japanese eel (*A. japonica*) and New Zealand eel (*A. dieffenbachia*) (Haenen *et al.* 2009).

Geographical range: EVEX was isolated from wild European eel in The Netherlands, Germany, Denmark, France, Sweden, UK, and Marocco, and wild adult eel (*A. dieffenbachia*) in New Zealand, however, without displaying clinical disease signs. These were only recorded in infected farmed European eels, e.g., in Italy

Individual effects: Vascular congestions of the abdominal surfaces and fins, anaemia, extensive haemorrhaging, skin ulcerations, necrosis of muscle, kidney, liver, and pancreas (Haenen *et al.* 2009). In eel farms, the mortality was below 20%

Population effects: Eel virus infections may adversely affect the spawning migration of eels, and could be a contributing factor to the worldwide decline of eel.

Discussion: We show that European eels infected with the rhabdovirus EVEX (Eel Virus European X) virus, developed hemorrhage and anemia during simulated migration in large swim tunnels, and died after 1000–1500 km. In contrast, virus-negative animals swam 5500 km, the estimated distance to the spawning ground of the European eel in the Sargasso Sea. Virus-positive eels showed a decline in hematocrit, which was related to the swim distance. Virus-negative eels showed a slightly increased hematocrit. Observed changes in plasma lactate dehydrogenase (LDH), total protein and aspartate aminotransferase (AAT) are indicative of a serious viral infection. Based on these observations, we conclude that eel virus infections may adversely affect the spawning migration of eels, and could be a contributing factor to the worldwide decline of eel.

Literature cited:

Haenen O, van Ginneken V, Engelsma M, van den Thillart G (2009) Impact of eel viruses on recruitment of European Eel. In: van der Thillart G, Dufour S, Rankin JC (eds.) Spawning Migration of the European Eel, Fish & Fisheries Series 30, Springer Science + Business Media B.V.: 387-400.

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Infestation with Anguillicoloides crassus

Diagnosis:

Causative agent:

Host range:

Geographical range:

Individual effects:

Population effects:

Discussion:

GENERAL DISCUSSION AND OUTLOOK

Many infectious diseases are well documented in marine organisms and the examples included in this review shed light on the possible role of diseases as determinants of population abundances among commercially valuable species. Unlike age- and predation-associated mortalities which are intrinsic characteristics of a species and therefore conducive to modelling, disease-associated mortality depends on interactions between host, pathogen and environmental variables for which data are often lacking. Therefore, despite the likelihood in some cases of significant effects on populations, disease may only be included within a poorly-defined stochastic component of an assessment model. This outlook will likely remain unchanged without the implementation of two key changes: the investment of sufficient long-term resources to collect the data necessary to draw conclusions about disease effects in populations and the close collaboration of disease and stock assessment experts.

Text fragments:

Due to their anadromous behaviour, salmon (and partly other salmonids) provide opportunities to monitor health that are rare in other marine finfish species: juvenile (embryo, parr, smolt) and late (spawning adult) life-history phases may be readily sampled during residence in the spawning river. Thus, while the examples of diseases used in this section are primarily based on samples collected in freshwater, population level effects of disease in salmon may be felt in both marine and freshwater habitats.

Even if clear population declines and diseases occur at the same time, it is difficult to identify the causes (see case of the Pacific herring, e.g., Deriso *et al.* 2008, Elston & Myers 2009 etc.)

Refer to epidemiological models attempting to quantify population level effects of diseases in fish, e.g., by Dobson & May (1986).

Interactions between wild and farmed shellfish/fish need to be highlighted

Develop a strategy for inclusion of epidemiology into stock assessment

Make destinction between chronic and acute diseases as to their pop effects.

M74: even if 50% of the adults cannot produce viable offspring, the remaining ones may be sufficient to sustain the stock.

Understanding population effects is a prerequisite of mitigation.

Literature cited:

Bradley MJ, Kutz SJ, Jenkins E, O'Hara TM (2005) The potential impact of climate change on infectious diseases of Arctic fauna. International Journal of Circumpolar Health 64: 468-477.

Edgerton *et al.* 2004: Understanding the causes of disease in European freshwater crayfish. Conservation Biology 18: 1466-1474

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, *et al.* (1999). Emerging marine diseases - climate links and anthropogenic factors. Science 285:1505–10.

Kim K, Dobson AP, Gulland FMD, Harvell CD (2005) Diseases and the conservation of marine biodiversity. In: Marine conservation biology: the science of maintaining the sea's biodiversity. Norse EA, Crowder LB (eds.), Island Press, Washington, Covelo, London, pp. 149-166.

Lafferty KD, Porter JW, Ford SE (2004) Are diseases increasing in the ocean? Annu. Rev. Ecol. Evol. Syst. 35:31–54.

Pearson,W. H., Elston, R. A., Bienert, R.W., Drum, A. S., and Antrim, L. D. 1999. Why did the Prince William Sound, Alaska, Pacific herring (Clupea pallasi) collapse in 1993 and 1994? Review of hypotheses. Canadian Journal of Fisheries and Aquatic Sciences, 56: 711–737.

Powell, E. N., Ashton-Alcox, K. A., Kraeuter, J. N., Ford, S. E., Bushek, D. (2008). Long-term trends in oyster population dynamics in Delaware Bay: regime shifts and response to disease. Journal of Shellfish Research 27 (4): 729–755.

5.7   ICES publications on pathology and diseases of marine organisms (ToR h)

S. W. Feist

Since the 2014 WGPDMO meeting, the ICES Secretariat has added the WGPDMO to the Disease Leaflets sharepoint site:

https://community.ices.dk/Committees/DISEASELEAFLETS/_layouts/15/start.aspx#/

in order to facilitate the production of new and revised leaflets. Intersessionally, it was agreed with ICES that some leaflets which WGPDMO did not regard as currently significant disease issues would be retained as part of the series reflecting the changing emphasis of disease issues over time.

A number of new leaflets proposed at the 2014 meeting were published.

- No. 61: Liver neoplasia in flatfish (Feist & Lang)
- No. 62: Hyperpigmentation in dab (Lang, Feist, Noguera & Bruno)
- No. 63: Pseudomoniasis in farmed fish (Vennerström)

In additional a revised leaflet has also been published.

- No. 21: Bacterial kidney disease (Bruno)

Further leaflets currently with the editor are as follows:

- Brown ring disease in clams (Paillard)
- Francisellosis in farmed cod (Alfjorden, Ruane)
- No. 37: Furunculosis (Bruno). Revised leaflet.

The four remaining leaflets listed in 2014 (Ostreid herpesvirus-1 in bivalves; *Bonamia exitiosa*; *Bonamia ostreae* and *Mycobacterium* spp. in wild fish) are still in preparation. It remains important for the WGPDMO to continue to propose titles of new leaflets and to suggest potential authors for these so that the series remains current with up to date and relevant information. In addition, the editor has contacted authors for production of further revised leaflets which are to be submitted during 2015.

- No. 42. *Exophiala* (Bruno).
- No. 24: *Mytilicola intestinalis* parasitism (Bignell)

Following discussions during the meeting WGPDMO decided that that the future production of disease leaflets should become a major focus of the group. As part of this the group has committed to continuously produce new leaflets and to also update previous versions in order to keep them relevant. The following list has been proposed for 2015/16.

1) *Mikrocytos* spp. (Carnegie)
2) *Bonamia exitiosa* (Carnegie)
3) *Bonamia ostreae* (Carnegie)
4) Ostreid herpesvirus (Renault)
5) ISA (Falk)
6) Pancreas Disease (Taksdal)
7) *Haematodinium* (Stentiford)
8) X-cell in dab (Feist)

9)   Vibriosis in oysters (Renault)

10) Tenacibaculosis in farmed fish (Jones)

11) Vibriosis in farmed salmonids (Lillehaug)

Finally, WGPDMO will discuss with the ICES Communication group about raising the profile of the leaflets outside the ICES community, including the possibility of making them more visible during internet searches.

5.8   Development of templates for the national reports from ICES Member Countries (ToR j)

P. Vennerstrom, S. R. Jones

Variability exists within the National Reports for wild and farmed finfish and shell-fish regarding disease occurrence (e.g. prevalence, number of animals, species infected). The purpose of this ToR is to develop templates for National Reports with the aim of standardizing the reporting structure. The templates will provide two main benefits. First, new members of WGPDMO need information on the activities and functions of the group and what the national report should contain. Second, new and established members will have a standardized set of tools and guidelines for use in the preparation of National Reports. An Excel spreadsheet would point out the minimal information that should be included in national reports. The group recognized the need to relate this information, where possible, to specific fish stocks, thereby facilitating transfer of disease information to stock assessment working groups. This will aid in the assessment of new disease conditions and trends assessed annually by the group in ToR a. Intersessional work will refine the Excel spreadsheet including definition of key terms. A beta-version of the spreadsheet will be shared among members before the end of 2015.

5.9 OSPAR Special Request (OSPAR 3/2015) Development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys (ToR l)

T. Lang

1 Introduction

1.1 OSPAR request

ICES is requested to define an appropriate common monitoring protocol to be applied across the OSPAR maritime area (taking into account work carried out by pilot projects, e.g. in Germany) as well as clearly articulating and defining the other steps that would be required in the practical work.

Background: The surveys for fish and shellfish diseases aim to gain data on the prevalence of diseases in wild fish submitted by ICES Member countries conducting this kind of monitoring. All steps involved in the practical work during fish disease surveys (sampling strategies, inspection of fish for target diseases, disease diagnosis) as well as reporting and validation of data submitted to the ICES Environmental Data Centre are done according to ICES standard quality assurance procedures. It is feasible, via these surveys to collect sampling material in sufficient amounts in order to conduct regular monitoring of plastic particles in fish stomachs and selected shellfish such as the common mussels.

1.2 Rational

The background of the OSPAR request is to explore possibilities to combine existing wild fish disease monitoring programmes in the OSPAR Region with monitoring of plastic litter ingested by fish, as a component for the assessment of the impact of litter on marine life required by the EU Marine Strategy Framework Directive (MSFD); (Descriptor 10). The main advantages of this synergistic approach are that (a) not only the ingestion of litter but also its harm to marine life (i.e., fishes) can be quantified and assessed and that (b) the existing infrastructures can be used for litter monitoring and assessment reducing costs of monitoring.

In the following, brief summaries of the existing programmes for fish diseases and marine litter are provided and the benefits of combining these programmes are described. More detailed methodological descriptions of procedures recommended are given in section 3.

2 Monitoring of fish diseases and marine litter: present status

2.1 Fish disease monitoring

Systematic fish disease monitoring in the OSPAR region started in the late 1970s and it is generally accepted that studies of externally visible fish diseases and contaminant-associated liver anomalies may provide information on the occurrence of environmental stress and are, therefore, considered as an important component of monitoring programmes (Lang 2002). Even if a scientific proof of cause-effect relationships between contaminants and diseases with a multifactorial aetiology is not easy to achieve, it is recommended that fish disease surveys should continue to be part of national and international environmental monitoring programmes since they can provide valuable information on changes in environmental health and may act as

an "alarm bell" indicator (Lang 2002). At present, a number of countries in the OSPAR and in the HELCOM region are carrying out regular fish disease surveys as part of their environmental monitoring and assessment programmes (Table 1). These studies are partly integrated in a multi-parameter monitoring approach, combining chemical (contaminants), biological (effects of contaminants) and hydrographical (physical/chemical characterisation of the habitat) measurements.

Studies on externally visible diseases, macroscopic liver neoplasms and liver histopathology are among the techniques recommended for biological effects monitoring under the OSPAR Coordinated Environmental Monitoring Programme (CEMP) (OSPAR 2005). They are also considered as components of monitoring programmes under the EU Marine Strategy Framework Directive (MSFD) for Descriptor 8 (*Concentrations of contaminants are at levels not giving rise to pollution effects*) of the qualitative descriptors for determining Good Environmental Status, addressing 8.2 (*Effects of contaminants*).

Largely through ICES activities, standard procedures for all steps involved in fish disease monitoring (e.g., sampling of fish, processing of samples, disease diagnosis as well as data reporting, analysis and assessment) have been developed and established, guidelines have been published (Bucke *et al.* 1996, Feist *et al.* 2004, www.bequalm.org) and quality assurance is in place (www.bequalm.org). Guidelines were transferred to technical guidelines under the OSPAR Joint Assessment and Monitoring Programme (JAMP) for general and contaminant-specific (PAH) biological effects monitoring (OSPAR 1997, 2008). Methodologies have repeatedly been intercalibrated during seagoing training exercises and workshops organized by ICES and other organisations (Anonymous 1989, Lang 2002, ICES 2006).

Table 1. Countries with fish disease monitoring programmes in the OSPAR and HELCOM regions.

Belgium	North Sea	EEZ
Germany	North Sea, Baltic Sea	EEZ and outside
UK	North Sea, Irish Sea, English Channel	EEZ
The Netherlands	North Sea	EEZ
Poland	Baltic Sea	EEZ
Russia	Baltic Sea	EEZ

Fish disease data are submitted on an annual basis to the ICES Environmental Data Centre (DOME) (http://ices.dk/marine-data/data-portals/Pages/DOME.aspx) using established ICES Environmental Data Reporting Formats and have repeatedly been used for environmental assessments, e. g. under OSPAR (OSPAR 2010).

2.2 Monitoring of marine litter

Monitoring of marine litter also has a tradition in the OSPAR context, and most efforts were so far placed on beach litter monitoring and monitoring of plastic particles in stomachs of seabirds. For both activities, guidelines and ecological quality objectives have been developed (Cheshire *et al.* 2009, OSPAR 2010b, van Franeker *et al.* 2011, Lippiat *et al.* 2013) and were summarised and modified according to the EU

Marine Strategy Framework Directive (MSFD) requirements by the MSFD Technical Subgroup on Marine Litter (TSG-ML) (EU 2011, 2013).

In order to meet the demands of EU MSFD Descriptor 10 (see text box), EU MS shall implement monitoring programmes for assessment, enabling the state of the marine waters with regard to marine litter to be evaluated on a regular basis. These should focus on two main aspects: the amount of litter in the environment and the impact of litter on marine life. In 2011, the TSG-ML pointed out that, although indicator 10.2.1 is based on assessing trends in the amount and composition of ingested litter, the MSFD also requests for the improvement of knowledge concerning the impacts of litter on marine life in general (EU 2011). This indicates that harm to marine life caused by litter has to be addressed.

EU Marine Strategy Framework Directive: Qualitative descriptors for determining good environmental status (Directive 2008/56/EC) with criteria and indicators (Directive 2010/477/EU) (EU 2008, 2010)

Descriptor 10: Properties and quantities of marine litter do not cause harm to the coastal and marine environment

10.1. Characteristics of litter in the marine and coastal environment

   –   *Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source (10.1.1)*

   –   *Trends in the amount of litter in the water column (including floating at the surface) and deposited on the sea- floor, including analysis of its composition, spatial distribution and, where possible, source (10.1.2)*

   –   *Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro-plastics) (10.1.3)*

10.2. Impacts of litter on marine life

   –   *Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis) (10.2.1).*

The TSG-ML identified the following characteristics of an indicator species to be used to monitor and assess trends in the amount and composition of ingested marine litter:

- an abundant species;
- easily attainable;
- foraging exclusively at sea;
- a species known to have a sufficiently high incidence of ingested litter to monitor change even in times or areas of lower pollution.

The same criteria are also relevant for the selection of suitable harm indicator species. In addition, the normal health conditions of the species should be known (baseline/background) so that deviations possibly caused by litter can be recorded.

Amongst other species, the TSG-ML addressed the use of fish for ingested litter monitoring and concluded that, from data available in 2011, it seems that many fish in the European areas are not very suitable as ingestion indicators, but available information is fragmentary and much more dedicated work is urgently needed (EU 2011). Therefore, the TSG-ML recommended that studies surveying the occurrence of litter in fish in the different marine regions are needed to evaluate the applicability of fish for ingested litter monitoring. EU MS were encouraged to assess the applicability of already ongoing fisheries analysis in their country also for litter monitoring purposes and/or the establishment of small-scale research projects focusing solely on this issue. Ultimately, MS should aim at a dedicated programme for analysis of litter ingested by fish, based on harmonised methods (EU 2011).

Since then, some studies on litter in fish have been carried out (EU 2011, 2013, Foekema *et al.* 2013, Lusher *et al.* 2013, Rochman *et al.* 2013, 2014a,b, Rummel 2014), but so far, such studies have not been incorporated in existing regular and coordinated fish surveys. Most of these studies focused on plastic ingestion, but not on harm to the fish. This could change if litter monitoring is integrated in fish disease monitoring.

In its 2011 report, the TSG-ML also considered invertebrates as bioindicator species for litter ingestion monitoring. In particular, Norway lobster (*Nephros norvegicus*) is addressed because of the frequent finding of plastic accumulation in their gastrointestinal tracts (EU 2011). However, other invertebrates were not considered because of a lack of information.

2.3 Combining monitoring of marine litter and fish diseases

The main benefit of integrating monitoring and assessment of marine litter into wild fish disease monitoring and assessment is that biological effects of litter on the target species can be studied at the individual and population level. Hence, by integrating these activities, one of the goals of the MSFD in relation to marine litter, namely to assess the impact of litter on marine life, can be met.

Other benefits are:

- Existing infrastructures (ship time for fish disease monitoring, coordinated and standardised sampling programmes, availability of data, ICES database) can be utilized, thereby, reducing costs of monitoring.
- Integrated studies on fish diseases and on ingested plastic litter can be combined with studies on other health effects (biomarkers) and fitness indicators (e.g., condition factor, liver somatic index, gonadosomatic index), enabling more firm conclusions about biological effects of ingested plastic on fish.
- Histopathology, so far focusing only on liver in existing fish disease monitoring programmes, can be extended to other organs that may aggregate litter particles (gills, intestine wall etc.) and/or show adverse pathological effects.
- If integrated biological and chemical programmes exist, contaminant data are useful also for understanding uptake mechanisms of litter and toxic effects.
- Seagoing fish diseases surveys can also be used for standardised monitoring of litter at the sea surface, in the water column and at the bottom (the latter is already implemented through the ICES International Bottom Trawl Survey, IBTS)

3 Protocol for integrated monitoring of fish diseases and ingested litter

A basic scheme describing an approach to combine litter monitoring with fish disease monitoring is provided in Fig. 1

3.1 Sampling and studies at sea

3.1.1 Fish sampling

Sampling of fish on board research vessels is preferably carried out with standard gears also used for stock assessment purposes, so that standardised data also useful

for other purposes can be obtained. For most fish disease surveys, bottom trawling is the method of choice since the main targets are demersal fish species (see 5.1.3). In the North Sea area, the GOV (Grande overture vertical) trawl in the configuration detailed in the ICES IBTS Manual (ICES 2012) is used by all countries taking part in the ICES International Bottom Trawl Survey (IBTS). Standard towing time is 30 min, but for fish disease surveys it may be extended to 60 min.

For pelagic trawling, no such internationally standardised nets exist. In the German fish disease programme, a PSN 205 trawl is used.

All trawls should be equipped with an inlay (codend) with a mesh size of 20 mm in order to be able to catch small fish specimens (and epibenthic invertebrates if they are to be examined for plastic litter ingestion, too).

3.1.2 Recording of litter in the nets

Independently of the fishing gear used for trawling, litter captured in the net should be recorded and categorized according to the ICES IBTS Manual (ICES 2012). It is not sufficient to record litter in the catch after the net has been emptied; the net material should also be carefully inspected because litter may be stuck in the meshes. Results of such studies underestimate the amount of litter particles on the sea floor or in the water column, since only macro litter is captured (the minimum size of the particles depends on the type of gear and the mesh sizes used).

3.1.3 Fish species for integrated monitoring

The key target fish species recommended for fish disease monitoring and the requirements according to ICES/OSPAR guidelines are summarized in Table 2.

In the Baltic Sea, dab is a target species only in the German fish disease monitoring programme in the western part (because its distribution is restricted to saline areas). Flounder, as a euryhaline species with a wide distribution pattern, is studied in the entire southern and central Baltic Sea by Germany, Poland and Russia. Cod also is a target species in the entire southern and central Baltic Sea and is part of the German, Polish and Russian fish disease monitoring programmes.

There are additional fish species that may be particularly useful for monitoring of ingested litter in the OSPAR region, such as the pelagic species Atlantic herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) that are widely distributed and may be appropriate bioindicator species to assess the impact of litter on marine life in the water column. As plankton feeders, these species very likely ingest smaller particles than demersal species (especially the large predators) that feed on larger food items. Among the demersal species, other flatfish species, e.g., plaice (*Pleuronectes platessa*) or long rough dab (*Hippoglossoides platessoides*), as well as gadoids, e.g., haddock (*Melanogrammus aeglefinus*) or saithe (*Pollachius virens*), may be useful. However, none of these species is among the main target species of present fish disease monitoring surveys. Therefore, fish disease programmes would need to be expanded in terms of species studied if litter and fish disease monitoring are integrated.

Table 2. Species and lesion categories recommended for fish disease monitoring according to ICES/OSPAR guidelines.

Species	Disease category	Size range	Sample size (n) examined per station (minimum)	Remarks
Common dab (*Limanda limanda*)	Externally visible diseases/parasites	15-19 cm 20-24 cm ≥25 cm	100 100 50	Size stratified examination has not always been used because stratified samples do not reflect the true stock composition as regards size/frequency distribution. If random sampling is applied, effects of the size distribution should, however, be accounted for in the data analysis.
	Macroscopic liver neoplasms	20-24 ≥25 cm	50 50	All liver nodules >2 mm are histologically confirmed
	Liver histopathology	20-24 cm	50	Random sampling of livers
European flounder (*Platichthys flesus*)	Externally visible diseases/parasites	20-24 cm 25-29 cm ≥30 cm	100 100 50	Size stratified examination is not always used because samples do not reflect the true stock composition. If random sampling is applied, effects of the size distribution should, however, be accounted for.
	Macroscopic liver neoplasms	25-29 cm ≥30 cm	50 50	All liver nodules >2 mm are histologically confirmed
	Liver histopathology	25-29 cm	50	Random sampling of livers
Atlantic cod (*Gadus morhua*)	Externally visible diseases/parasites	<29 cm 30-44 cm ≥45 cm	100 100 50	Size stratified examination has not always been used because stratified samples do not reflect the true stock composition as regards size/frequency distribution. If random sampling is applied, effects of the size distribution should, however, be accounted for in the data analysis.
Whiting (*Merlangius merlangus*)	Externally visible diseases/parasites	15-19 cm 20-29 cm ≥30 cm	100 100 50	At present, this species is not used for fish disease monitoring. Size stratified examination has not always been used because stratified samples do not reflect the true stock composition as regards size/frequency distribution. If random sampling is applied, effects of the size distribution should, however, be accounted for in the data analysis.
Dragonet (*Callionymus* spp.)	Macroscopic liver neoplasms	-	-	At present, this species is not used for fish disease monitoring
	Liver histopathology	10-15 cm	50	

3.1.4 Examination of fish for diseases

If possible, only live fish maintained in running seawater after sorting of the catch should be examined. The time span between catch and examination/dissection should

be recorded. This is also relevant for litter monitoring since regurgitation or defecation of litter particles may occur during maintenance as a function of time.

Based on established ICES and BEQUALM guidelines (Bucke *et al.* 1996, Feist *et al.* 2004, www.bequalm.org), target fish species are examined for the presence of three categories of diseases (Table 2). Externally visible diseases and macroscopic liver neoplasms are recorded by macroscopic inspection of the body surface and of the liver (with the naked eye). Additional organs can be inspected as required. Examination is carried out directly onboard the research vessel using fresh samples. Liver histopathology is studied in the lab, and for this purpose tissue samples are collected and fixed (10 % neutral buffered formalin) onboard the research vessel for subsequent analysis.

3.1.4.1 Externally visible diseases

The skin surface (including the fins) and the buccal and gill cavities of fish selected for external disease examination are inspected for the presence of a defined range of diseases and parasites (Bucke *et al.* 1996, www.bequalm.org). In Table 3, diseases and parasites used for regular monitoring and those for which limited datasets are available are listed.

Per species and sampling station/area, a minimum of 250 fish is examined. According to ICES/OSPAR guidelines, examination of fish is based on size-stratified samples (Table 1) selected from the catch. However, since this method does not provide data reflecting the actual size composition of the population, samples are alternatively taken randomly. However, since size is a factor well-known to have an impact on the prevalence of diseases, differences have to be accounted for by using adjustment factors calculated from empirical data (Lang and Wosniok 2008).

In addition, any entanglement in litter particles or mechanical damage clearly associated with previous contact to fishing gear should be recorded and, preferably, documented photographically.

3.1.4.2 Macroscopic liver neoplasms

Fish are inspected for the presence of liver lesions according to ICES guidelines (Feist *et al.* 2004). The presence of liver nodules >2 mm is recorded, often also other prominent lesions such as parasites (e.g., larval Nematoda, Acanthocephala, non-defined parasitic cysts), granulomas and discolourations are documented.

All nodules >2 mm found are fixed in 10 % neutral buffered formalin for later histological confirmation of the lesion. Only cases of confirmed neoplasms (benign or malignant tumours, excluding pre-stages) are reported for later data analysis (Feist *et al.* 2004). Information on histological techniques and diagnostic criteria are provided in Feist *et al.* (2004) and by BEQUALM (www.bequalm.org).

So far, the examination for the presence of macroscopic liver neoplasms according to ICES guidelines has been restricted to flatfish. However, it is recommended that the species range should be extended if more (or other) species are monitored for ingested litter particles in an integrated programme.

Based on ICES/OSPAR guidelines, 50 specimens per sampling station/area are inspected for the presence of macroscopic liver neoplasms. Size ranges have been defined for dab and flounder (see Table 2), but not yet for other species.

Table 3. Externally visible diseases and parasites in common fish species used for monitoring purposes in the OSPAR and HELCOM region.

Disease/parasite	Aetiology	Dab (*L. limanda*)	Flounder (*P. flesus*)	Cod (*G. morhua*)	Whiting (*M. merlangus*)	Herring (*C. harengus*)
Lymphocystis	Viral disease	+	+			+
Epidermal hyperplasia/ papilloma	Viral disease	+	+	+	+	
Acute/healing skin ulcerations	bacterial	+	+	+	+	+
Fin rot/erosion	bacterial	+	+	+	+	
X-cell gill disease	Protist, parasite	+	+			
Pseudobranchial swelling	Protist, parasite	+	+	+	+	
Hyperpigmentation	Causes unknown	+	+			
Skeletal deformities	Causes unknown	+	+	+	+	+
Stephanostomum baccatum	Digenea, parasite	+	+			
Cryptocotyle spp.	Digenea, parasite	+	+	+		+
Diclidophora merlangi	Monogenea, parasite				+	
Acanthochondria cornuta	Copepoda, parasite	+				
Lepeophtheirus pectoralis	Copepoda, parasite	+	+			
Lernaeocera branchialis	Copepoda, parasite			+	+	
Clavella adunca	Copepoda, parasite			+	+	
Caligus spp.	Copepoda, parasite			+	+	

3.1.4.3 Liver histopathology

Diagnostic criteria and methods applied for monitoring purposes are detailed in Feist *et al.* (2004). In brief, five categories of lesions are recorded:

- Non-specific lesions (mostly inflammatory, degenerative or regenerative)
- Early toxicopathic, non-neoplastic lesions
- Foci of cellular alteration (tumour pre-stages)

- Benign liver tumours
- Malignant liver tumours

Within each category, a number of typical lesions has been defined that should be considered for monitoring of biological effects of contaminants (Feist *et al.* 2004).

If fish disease monitoring is combined with litter monitoring and if resources permit, it may be useful to extend histopathological studies to include also other organs (gills, gastrointestinal tract, kidney) that may aggregate small litter particles (micro plastic) and may show adverse effects of litter, e.g., caused by mechanical damage, chemical additives or contaminant adhesion.

3.1.5 Examination of fish for ingested litter

3.1.5.1 Fish species

If monitoring and assessment of litter is to be combined with fish disease monitoring, the most practical approach would be to use fish from existing fish disease monitoring programmes to also study litter uptake and effects. This should preferably be done with fish that are internally inspected for macroscopic liver neoplasms and/or liver histopathology, since these fish are dissected anyway. Since flatfish (dab, flounder) are at present the target species for internal disease monitoring, a pilot study should be carried out to assess their potential for litter monitoring. If it turns out that they do not ingest litter in large enough amounts and prevalence for monitoring spatial and temporal patterns, alternative species also included in disease monitoring (cod, whiting, herring) or in other fish surveys (e.g., cod, saithe) should be used. An examination of fish stomach content databases for records of litter, such as http://www,cefas.defra.gov.uk/our-science/fisheries-information/fish-stomach-records.aspx could provide information as the suitability of certain fish species. Rummel (2014) showed that only 5.5 % of all fish he examined from the North Sea and Baltic Sea had ingested plastic particles (Table 4). About 75 % of the particles detected were classified as microplastic particles (<5 mm), indicating that the ingestion of microplastic occurs more frequently than that of macro particles. The highest prevalence (% of fish with ingested plastic particles in a sample) where recorded in mackerel in both seas, a species that so far has not been regularly studied for diseases.

3.1.5.2 Sampling for litter monitoring

If resources (time, staff, stability of the research platform) permit, part of the litter monitoring can directly be done onboard a research vessel, but this is only regarded as a practical and operational approach if the inspection is restricted to the identification and quantification of large particles (particles > 5 mm) in the gastrointestinal tracts. This could best be done using a dissection microscope, but it may also be possible just by the naked eye under stable and good light conditions. It should be noted that once opened and analysed for macro particles, further micro particle analysis may not be possible due to contamination issues (air-borne contamination of samples with e.g. micro fibres).

However, it is recommended to store biota samples onboard (freezer at -20 °C) for subsequent litter examination in the lab. In case of small fish specimens, they may be frozen whole; in case of larger fish, they should be dissected and the gastrointestinal tracts removed intact and frozen individually. Again, possible sample contamination needs to be avoided.

For this purpose, fish need to be sacrificed according to ethical requirements, and, after recording fish-specific supporting parameters and after inspection for externally visible diseases, are dissected in a clean and well illuminated environment using clean dissecting tools (scalpels, scissors, forceps).

Table 4. Plastic ingested by fish from the North Sea and Baltic Sea (Rummel 2014).

Species	North Sea (German Bight)		Baltic Sea	
	N examined	% affected	N examined	% affected
Dab (*L. limanda*)	74	5.4	15	0.0
Flounder (*P. flesus*)	16	0.0	20	10.0
Cod (*G. morhua*)	7	0.0	74	1.4
Herring (*C. harengus*)	13	0.0	20	0.0
Mackerel (*S. scombrus*)	38	13.2	13	30.8

After inspection of the inner organs for pathological changes (including macroscopic liver neoplasms) and taking tissue samples for histopathology of the liver and possibly other organs (see Tab. 2), the gut is removed by severing the intestine in the vent area and the oesophagus close to the mouth cavity. Organs attached to the gastrointestinal tract are removed, and the gut is placed in a suitable labelled (cruise, station, fish species, fish ID) container (avoiding plastic where possible) for freezing at -20 °C.

A sample size of at least 50 specimens per station should be envisaged. In case of dab and flounder, these could be the ones used for liver histopathology (see Tab. 2 and Fig. 1). The samples can be used for quantification of both (micro particles <5 mm) and macro particles (>5 mm). If size/age effects should be studied, 50 specimens from each of the size ranges defined should be taken in addition. Alternatively, due to the low occurrence of particles in stomachs it may be necessary to pool fish. An option is to dissolve pools (which may consist of gastrointestinal tracts of 100 fish or more) in acid and filter over a fine mesh to count particles. As contamination could be an issue in relation to micro particles, observations can be limited to a a certain particle size (e.g. 200 μm).

It is important during sampling to avoid contamination of the tissue sample with airborne particles (see above) or particles originating from clothing. In particular, plastic clothes (fleece wear) are known to emit fibres in high numbers that can later be misinterpreted as ingested plastic particles (Rummel 2014). Another contamination may occur if fish take up particles originating from the research vessel coating (colour flakes) or the gear while trawled in the net (Rummel 2014). Contamination of samples during processing in the lab is considered to be an even bigger problem and ways should be implemented to avoid contamination as far as possible (EU 2013). In order to quantify contamination, a blank control should be run continuously along with all samples to take into account contamination via air.

Further recommendations and protocols for processing fish to examine them for the presence of micro particles (size range 20 μm to 5 mm) and macro particles (>5 mm) have been developed by the TSG-ML and through various research projects (e.g., http://www.ilvo.vlaanderen.be/micro and De Witte *et al.*, 2014). These encompass

steps from sampling, identification and classification of particles, chemical analysis of polymer types, e.g. using Fourier transform infrared (FT-IR) or Raman spectroscopy, and data handling (EU 2013).

3.1.6 Data requirements and supporting parameters

Table 5 lists data requirements for a combined litter and fish disease monitoring. From all fish examined for externally visible diseases/parasites, macroscopic liver neoplasms and liver histopathology, sex and total length (to the nearest cm below) are recorded as minimum requirement. If equipment onboard research vessels and resources permits, age (subsequent reading of otoliths), total and gutted wet weight as well as weight of organs (liver, spleen, gonad) and stage of sexual maturation should be recorded. If onboard measurements are not possible, representative samples of fish can be frozen and later processed in the lab at land. Data generated can be used to assess effect on the general body fitness (condition factor, organossomatic indeces) and reproductive disturbances (gonadosomatic index, maturation index).

If fish disease monitoring is part of an integrated biological and chemical monitoring programme, additional supporting parameters may be measured, such as other biomarkers and contaminant concentrations in fish. These data are of course also valuable, since they may show more subtle biological effects and the role of contaminants (e.g., uptake of contaminants from ingested plastic particles). This would possibly imply to measure more substances than analysed presently as part of OSPAR/HELCOM/EU reporting requirements.

Data on marine litter in fish should be generated in a way that they can be reported to the ICES Environmental Database (DOME), preferably together with the fish disease data.

4 Discussion and conclusions

Trends in the amount and composition of litter ingested by marine fish and possible harm to the fish, the study of which is demanded by the EU Marine Strategy Framework Directive, can be monitored and assessed in combination with existing fish disease monitoring programmes. However, a number of points have to be considered:

- Pilot studies are required evaluating the usefulness of the fish species examined in present disease monitoring programmes for litter monitoring. First studies should focus on dab (*L. limanda*), flounder (*P. flesus*), saithe (*Pollachius virens*) and cod (*G. morhua*). Depending on the results, the suitability of other fish species should be assessed.

- OSPAR/EU Member States should continue running fish disease monitoring programmes as component of national and international monitoring requirements (e.g., under OSPAR and the EU) and countries that ceased their activities should be encouraged to implement new programmes. Sufficient know how is available through ICES activities and Expert Groups.

- Monitoring of marine litter is laborious and needs resources and sophisticated chemical analytical procedures, in particular when micro particles are to be analysed. The question if litter particles cause harm to fish cannot fully be answered by applying the methods established in fish disease monitoring programmes according to ICES/OSPAR guidelines. The programmes have to be extended to also cover other more litter-specific studies and methods.

- Monitoring of litter in combination with fish diseases should be part of a further integrated chemical and biological effects monitoring and assessment programme, such as the one developed by ICES and OSPAR in the WKIMON/SGIMC process (Davies and Vethaak 2012). This would meet the EU MSFD requirements with regards to Descriptor 8 (contaminants) and 10 (marine litter).

- The use of other types of fisheries surveys (e.g., fish stock assessment surveys coordinated by ICES) for monitoring of ingested marine litter should be explored.

- There are no existing monitoring programmes in OSPAR Member States focusing on diseases of wild shellfish in a way it is done for fish diseases. Therefore, this part of the request could not be addressed by WGPDMO.

Figure 1. Scheme illustrating a combined approach for monitoring of marine litter and diseases in wild fish.
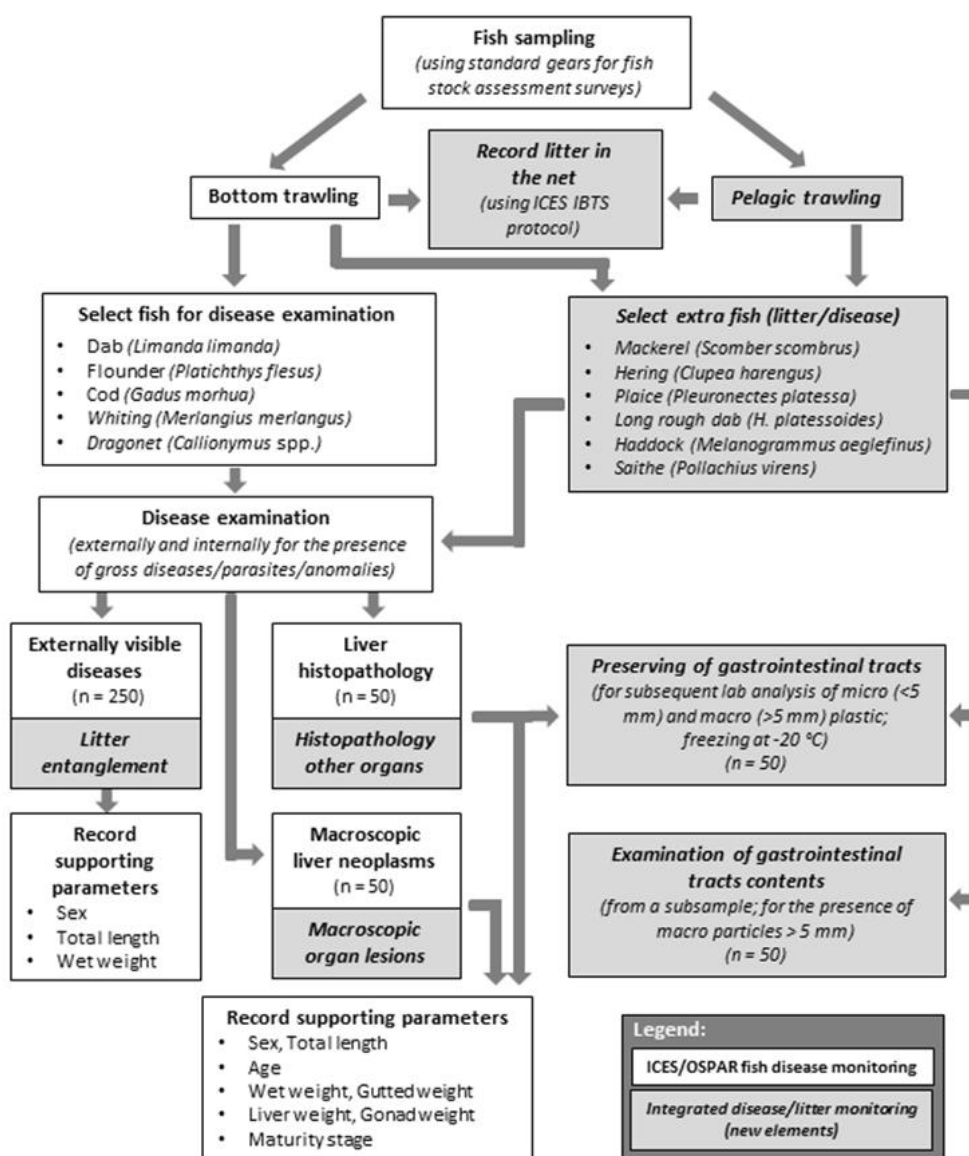
Table 5. Primary and secondary data to be recorded in combined litter and fish disease surveys.

Data type reported	Parameter	Remarks
Primary sampling data	Geographical coordinates of sampling	**5.9.1.1** For instance in case of trawling, two coordinates per sample: net at ground and net off ground
	Dates and time of sampling	
	Duration of sampling	For instance in case of trawling, towing time (e.g., 30 Min. or 60 Min.)
	Type of sampling gear	
	Weather conditions	
	Water depth	
	Catch composition: species, number, weight, length/age structure	calculated from catch data
	Hydrography data, i.e. water temperature, salinity, oxygen, pH, turbidity	CTD data, chemical measurements
	Time span between sampling and examination/dissection of fish	
	Litter in the net	According to ICES IBTS Manual (ICES 2012)
Primary individual fish data (minimum requirements)	Fish ID	if tissue samples are taken for subsequent processing and analyses
	Total length (cm)	measured to the nearest 1.0 or 0.5 cm below
	Sex	female, male, unknown
	Type of disease	Specified in Bucke *et al.* (1996), Feist *et al.* (2004)
	Severity of disease	3 grades are common and have been defined for common diseases/parasites
	Litter entanglement, mechanical damage	Examination prior to dissecting; preferably photographic doumentation
	Litter in the gastrointestinal tract	Ship- and/or lab-based examination
Primary individual fish data (additional information)	Total wet weight (g)	specific balances are needed on board RVs
	Age (years)	from a sub-sample of fish covering all size groups; measurement by means of otolith reading; should be done by experts
	Stage of sexual maturity	keys are available for flounder, cod, and herring
	Organ wet weights (liver, gonad, spleen)	for calculation of somatic indices (LSI, GSI, SSI)
	Gutted fish weight	for calculation of condition factor and organosomatic indices
	Contaminants	concentrations of organic and inorganic contaminants in liver or muscle tissue (according to OSPAR JAMO/CEMP
	Biomarkers	e.g., HELCOM CORESET Core and Candidate indicators of effects of hazardous substances
Secondary fish data (calculated from primary data)	Condition factor (CF): $CF = weight (g)*100/length (cm)^3$	preferably based on gutted instead of total weight
	Liver somatic index (LSI): $LSI = liver\ weight\ (g)/fish\ weight(g)*100$	preferably based on gutted instead of total weight
	Spleen somatic index (SSI): $SSI = spleen\ weight\ (g)/fish\ weight(g)*100$	preferably based on gutted instead of total weight
	Gonadosomatic index (GSI): $GSI = gonad\ weight\ (g)/fish\ weight(g)*100$	preferably based on gutted instead of total weight
	Population density	calculated based on catch composition data and bottom area/water volume fished
	Population structure (age/length distribution)	calculated based on catch data

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6   Cooperation

- **Cooperation with other WG**
- **Cooperation with Advisory structures**
- **Cooperation with other IGOs**

- WGPDMO has strong links with the European Association of Fish Pathologists (EAFP) with members of WGPDMO serving on the EAFP Executive, as national Branch Officers and on the editorial board of the EAFP Bulletin.

- WGPDMO has strong links to the National Reference Laboratories of ICES member countries, with WGPDMO members employed by NRL's and/or EU Reference Laboratories for finfish, molluscan and crustacean diseases.

- WGPDMO has contributed to OSPAR Special Request 4/2014 on interactions between wild and captive fish stocks. The Group provided advice specifically in the area of '*transfer of disease and parasite interactions*'.

- WGPDMO is currently contributing to OSPAR Special Request 03/2015 on the development of a common monitoring protocol for plastic particles in fish stomach*s and selected shellfish on the basis of existing fish* disease surveys.

7   Summary of Working Group evaluation and conclusions

A copy of the Working Group self-evaluation can be found in Annex 4.

Annex 1: List of participants

Name	Address	Email
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Annex 2: Recommendations

Recommendation	Adressed to
1. WGPDMO acknowledges the ICES initiative to incorporate Aquaculture Science into its Strategic Plan. The Expert Group has established competency in reporting significant trends in diseases and pathology associated with farmed finfish and shellfish species at the national level among ICES member countries. Furthermore, WGPDMO has the expertise to report and interpret information concerning pathogen interactions between wild and farmed aquatic species. Consequently the WGPDMO recommends that, with respect to wild and farmed aquatic animals in the context of the ICES Strategic Plan, WGPDMO plays a lead role in the description and interpretation of disease and pathogen data (in cooperation with other relevant EGs).	SCICOM, WGAQUA
2. WGPDMO has undertaken to update the Disease Information Leaflet Series and would like to work with the communications group within the ICES Secretariat to increase the visibility of the leaflets in the broader community (e.g. generate more hits through internet searches).	ICES Secretariat (Communications group)
3. The feasibility of including monitoring for plastic particles in the current fish disease monitoring programmes should be developed. In previous years, WGPDMO has highlighted the decline in fish disease monitoring carried out by ICES member states and hopes that the inclusion of plastics will provide new impetus into this monitoring. A co-orinated response, into the feasibility of sampling for plastics, from the relevant ICES EGs involved in monitoring should be prepared.	SCICOM (to identify suitable EG) e.g. WGBEC, WGIAB, WGMG, WGMS, IBTSWG, WGNARS, WGSE, WGINOSE, WGEAWESS, WGBEAM, WGISUR, WGISDAA, WGIPS, WGBIFS
4. WGPDMO should focus on preparing disease specific reports describing the occurrence and spread of economically important diseases within the ICES area (in line with the new Science Plan for aquaculture) focussing on *Bonamia*, *Vibrio* species as primary causes of disease in molluscs and amoebic gill disease in finfish.	WGPDMO Chair (see Annex 3)

Annex 3: WGPDMO draft terms of reference 2016–2018

A **Working Group on Pathology and Diseases of Marine Organisms** (WGPDMO), chaired by Ryan Carnegie, USA, will work on ToRs and generate deliverables as listed in the Table below.

	Meeting dates	Venue	Reporting details	Comments (change in Chair, etc.)
Year 2016	16–20 February	Virginia, USA	Interim report by DATE April to SSGEPI	
Year 2017			Interim report by DATE to SSGEPI, SCICOM	
Year 2018			Final report by DATE to SSGEPI, SCICOM	

ToR descriptors

ToR	Description	Background	Science Plan topics addressed	Duration	Expected Deliverables
a	New and emerging disease trends in wild and cultured fish, molluscs and crustaceans based on national reports	New and emerging disease conditions and trends in diseases of wild and cultured marine organisms continue to appear and an annual assessment of these should be maintained.	Pillar 1 Goal 2	annual	Annual summary of trends reported in the WGPDMO annual report
b	ICES publications on pathology and diseases of marine organisms	WGPDMO aim to update all disease leaflets within the next reporting period (3 yrs) and also to increase the visibility and relevance of the leaflets	Pillar 1 Goal 2	annual	Updated identification leaflets on the ICES website
c	Prepare a report describing the spread and impact of *Bonamia ostrea* in flat oysters in ICES member states	Bonamiosis is a notifiable disease, both to the OIE and the EU (under Directive 2006/88/EC). WGPDMO has noted the recent spread of the disease and will undertake to prepare a report assessing the impact of the disease on flat oyster culture within the ICES area	Pillar 1 Goal 2	3 years	Report published in peer-reviewed journal/ICES publication
d	Summarise the role of *Vibrio* sp. pathogens contributing to	In recent years, WGPDMO has reported the	Pillar 1 Goal 2	3 years	Report published in peer-reviewed journal/ICES

	mortalities in shellfish aquaculture	increased concern related to *Vibrio* sp. pathogens and their effect on shellfish aquaculture. There is a need to fully investigate the current knowledge relating to these pathogens and identify areas for future research assessing their potential impact on shellfish aquaculture			publication
e	Prepare a report describing the occurrence and spread of AGD in marine salmonid farming in the ICES area	AGD has emerged as a sigificant issue for salmon farming in the Atlantic. This report will describe the spread, impact and current measures taken to mitigate effects of the disease and identify knowledge gaps and future areas for research	Pillar 1 Goal 2	3 years	Report published in peer-reviewed journal/ICES publication
f	Compile information on pathogen screening of wild salmonids in the ICES member states	Many ICES member countries screen wild broodstock used for restocking purposes for disease pathogens. This report will compile information on diseases and methods used in order to prepare a common approach to screening and assess the effectiveness of current practices	Pillar 1 Goal 2	3 years	Final report published as an ICES publication
g	Application of the Fish Disease Index using the R package following newly developed guidelines	The FDI approach has been developed for the analysis and assessment of data obtained by ICES Member Countries running regular fish disease surveys as part of their national environmental monitoring programmes. The application of the FDI as an assessment tool for environmental	Pillar 1 Goal 2		

		monitoring under OSPAR/HELCOM/EU MSFD will be assessed using the R package			
h	Provide expert knowledge and advice on fish disease and related data to the ICES Data Centre on a continuous basis	In compliance with a request from the ICES Data Centre	Pillar 1 Goal 2	annual	Provision of advice ICES Data Centre
i	Development of templates for the national reports from ICES Member Countries	Variability exists within the National Reports regarding disease occurrence. This ToR will aim to standardise the reporting structure adding value to ToR a	Pillar 1 Goal 2	1 year	Standard reporting template for WGPDMO

Summary of the Work Plan

Year 1
Year 2
Year 3

Supporting information

Priority	The current activities of this Group will lead ICES into issues related to the ecosystem effects of fisheries, especially with regard to the application of the Precautionary Approach. Consequently, these activities are considered to have a very high priority.
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants	The Group is normally attended by some 10–15 members and guests.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	There are no obvious direct linkages.
Linkages to other committees or groups	There is a very close working relationship with all the groups of SSGHIE. It is also very relevant to the Working Group on Aquaculture (WGAQUA) and will seek to form links with the Working Group on Socio-Economic Dimensions of Aquaculture (WGSDA).
Linkages to other organizations	OSPAR, HELCOM, EAFP

Annex 4: Copy of Working Group self-evaluation

1) **If applicable, please indicate to which of the research priorities (and sub priorities) of the Science Plan to which the WG make a significant contribution**

The group compiles new and emerging trends and occurrences of significant disease in farmed and wild aquatic animals. The information provided by national experts is communicated within ICES and the broader scientific community. Disease trend data are used by the group to define terms of reference which ensure issues relevant to ICES are discussed, researched and communicated as ICES identification leaflets and peer-reviewed publications.

A number of topical issues are presently being addressed by WGPDMO including pathogen interactions between wild and farmed fish, emerging diseases and parasites in fish and shellfish which pose a hazard to human health. WGPDMO contributes to the ICES Annual Science Conference and its members play an important role in both the OSPAR Coordinated Environmental Monitoring Programme for the North East Atlantic and HELCOM (Baltic Sea Environment Commission).

2) **In bullet form, list the main outcomes and achievements of the WG since their last evaluation. Outcomes including publications, advisory products, modelling outputs, methodological developments, etc.**

- Jones, S. R. M., Bruno, D. W., Madsen, L. & Peeler, E. J. 2015. Disease management mitigates risk of pathogen transmission from maricultured salmonids. *Aquaculture Environment Interactions* **6**: 119-134.

- ICES Disease Leaflet Series (updated): No. 3 '*Ichthyophonus*, a systemic mesomycetozoan pathogen of fish' (S. R. M. Jones).

- ICES Disease Leaflet Series (updated): No. 7 '*Pseudoterranova* larvae ("codworm"; Nematoda) in fish' (M. Longshaw).

- ICES Disease Leaflet Series (updated): No. 8 '*Anisakis* larvae ("herringworm"; Nematoda) in fish' (M. Longshaw).

- ICES Disease Leaflet Series (updated): No. 19 'Marteiliosis of oysters caused by *Marteilia refringens*' (T. Renault & S. E. Ford).

- ICES Disease Leaflet Series (new): No. 58 'Heart and skeletal muscle inflammation (HSMI) of farmed Atlantic salmon (*Salmo salar* L.) and the associated *Piscine reovirus* (PRV)' (E. Biering & A. H. Garseth).

- ICES Disease Leaflet Series (new): No. 59 'Piscine myocarditis (cardiomyopathy syndrome' (D. Bruno).

- ICES Disease Leaflet Series (new): No. 60 'Amoebic gill disease (AGD) of farmed Atlantic salmon (*Salmo salar* L.' (N. M. Ruane & S. R. M. Jones).

- ICES Disease Leaflet Series (new): No. 61 'Liver tumours in flatfish' (S.W. Feist & T. Lang)

- ICES Disease Leaflet Series (new): No. 62 'Hyperpigmentation of common dab (*Limanda limanda* L.) (T. Lang, S.W. Feist, P. Noguera & D. Bruno)

- ICES Disease Leaflet Series (new): No. 63 'Pseudomoniasis in farmed fish' (P. Vennerström)

- http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2014/Special%20Requests/OSPAR_%20Interactions_of_wild_and_captive_fish_stocks.pdf

3) Has the WG contributed to Advisory needs? If so, please list when, to whom, and what was the essence of the advice.

- WGPDMO has contributed to OSPAR Special Request 4/2014 on interactions between wild and captive fish stocks. The Group provided advice specifically in the area of '*transfer of disease and parasite interactions*'. The advice provided was summarised as follows:

  Open marine net pens facilitate virus and sea lice transfer, occasionally leading to infections and outbreaks of disease in farmed salmon. Epidemiological techniques and development of coastal circulation models permit the designation of areas of risk associated with sources of infection. Increased risk of exposure to neighbouring farms is inversely related to distance from and positively related to biomass at the source of infection. Susceptible wild or farmed fish occupying an area of risk may have an increased likelihood of exposure to pathogens, infection, and disease. There is evidence for elevated levels of sea lice on wild salmonids in several areas associated with salmon mariculture. Studies on the survival of salmon smolts treated prior to release with sea lice therapeutants compared with untreated smolts suggests that sea lice can increase mortality; however, links to mariculture are not conclusive. Risk of pathogen transmission from farmed to wild populations is estimated indirectly from epidemiological data. Further direct surveillance of wild populations is required.

  The link to the full document is:

  http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2014/Special%20Requests/OSPAR_%20Interactions_of_wild_and_captive_fish_stocks.pdf

- WGPDMO is currently contributing to OSPAR Special Request 03/2015 on the development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys. This request is currently ongoing.

4) Please list any specific outreach activities of the WG outside the ICES network (unless listed in question 7.2). For example, EC projects directly emanating from the WG discussions, representation of the WG in meetings of outside organizations, contributions to other agencies activities.

- WGPDMO has strong links with the European Association of Fish Pathologists (EAFP) with members of WGPDMO serving on the EAFP Executive, as national Branch Officers and on the editorial board of the EAFP Bulletin.
- WGPDMO has strong links to the National Reference Laboratories of ICES member countries, with WGPDMO members employed by NRL's and/or EU Reference Laboratories for finfish, molluscan and crustacean diseases.

5) Please indicate what difficulties, if any, have been encountered in achieving the workplan.

- Due to time limitations on each expert, WGPDMO needs to set focussed and realistic targets for future ToR's.

Future plans

6) Does the group think that a continuation of the WG beyond its current term is required? (If yes, please list the reasons)

- Yes. In the ICES Strategic Plan 2014-2018, ICES has made a number of strategic choices to further develop science, advisory and data work on aquaculture (see SCICOM September 2014 Doc 33). WGPDMO welcomes a greater emphasis on aquaculture and the importance of managing disease, to which this EG is ideally place to provide expert advice (see Q9 below).

7) If you are not requesting an extension, does the group consider that a new WG is required to further develop the science previously addressed by the existing WG. (If you answered YES to question 6 it is expected that a new Category 2 resolution will be submitted through the relevant SSG Chair or Secretariat.)

- See Category 2 resolution, Annex 2.

8) What additional expertise would improve the ability of the new (or in case of renewal, existing) WG to fulfil its ToR?

9) Which conclusions/or knowledge acquired of the WG do you think should be used in the Advisory process, if not already used? (please be specific)

WGPDMO acknowledges the ICES initiative to incorporate Aquaculture Science into its Strategic Plan. The Expert Group has established competency in reporting significant trends in diseases and pathology associated

with farmed finfish and shellfish species at the national level among ICES member countries. Furthermore, WGPDMO has the expertise to report and interpret information concerning pathogen interactions between wild and farmed aquatic species. Consequently the WGPDMO anticipates playing a lead role in the description and interpretation of disease and pathogen data in the ICES Strategic Plan.

Annex 5: Common and scientific names of host species in the report

bream	*Abramis brama*
butterfish	*Pholis gunnellus*
clam, hard	*Mercenaria mercenaria*
clam, Manilla	*Venerupis philippinarum*
cod, Atlantic	*Gadus morhua*
dab, common	*Limanda limanda*
eelpout, European	*Zoarces viviparous*
flounder, European	*Platichthys flesus*
herring, Baltic	*Clupea harengus membras*
mussel, blue	*Mytilus edulis*
oyster, eastern	*Crassostrea virginica*
oyster, European flat	*Ostrea edulis*
oyster, Pacific	*Crassostrea gigas*
salmon, Atlantic	*Salmo salar*
salmon, Chinook	*Oncorhynchus tshawytscha*
salmon, pink	*Oncorhynchus*
scallop, king	*Pecten maximus*
trout, rainbow	*Oncorhynchus mykiss*
trout, sea	*Salmo trutta*

Annex 6: Technical minutes by RGPLAST

Review group on RGPLAST for advice to ADGPLAST

Reviewers: Salud Deudero (Chair), Richard C. Thompson

Request

Development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys.

The surveys for fish and shellfish diseases aim to gain data on the prevalence of diseases in wild fish submitted by ICES Member countries conducting this kind of monitoring. All steps involved in the practical work during fish disease surveys (sampling strategies, inspection of fish for target diseases, disease diagnosis) as well as reporting and validation of data submitted to the ICES Environmental Data Centre are done according to ICES standard quality assurance procedures. It is feasible, via these surveys to collect sampling material in sufficient amounts in order to conduct regular monitoring of plastic particles in fish stomachs and selected shellfish such as the common mussels.

ICES is requested to define an appropriate common monitoring protocol to be applied across the OSPAR maritime area (taking into account work carried out by pilot projects, e.g. in Germany) as well as clearly articulating and defining the other steps that would be required in the practical work.

OSPAR Special Request (OSPAR 3/2015) Development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys (ToR l)

The reviewers suggest the outcomes of the expert group reports require a more specific description including protocols and sampling procedures regarding stomach contents, associated parameters and calculated indices in order to provide a scientific advice regarding a combined monitoring of marine litter and fish diseases.

The first point is that 'selected shellfish' are not included within the 'common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys'. Therefore, it should be noted that the reviewers have only focused their comments on fish stomachs in this draft revision report.

1. Introduction

Q1 A background and rationale of marine litter, especially plastics, is required along with fish species diseases.

Marine litter is mainly composed by 90% plastics derived debris. It is highly relevant to make a distinction between macro and microplastics. Thus, some paragraphs might be added, like:

Plastics ingested by marine species are categorized into microplastics (<5 mm), mesoplastics (1–25 mm) and macroplastics (>25 mm). More recently plastics smaller than (<1 microns) have been categorized as nanoplastics (GESAMP, 2015). In addition, microplastics found in marine organisms can derive either directly from pellets used in

products such as cosmetics, toothpastes, personal care products or textiles from clothes (primary microplastics) or can results from the degradation and fragmentation of larger items (secondary microplastics). Therefore, the protocol needs to establish why microplastics are important in the first place. What are the main questions they want to address? Without this information it is difficult to advice.

In addition, trophic level transfer of marine plastics across the food web (Farrell and Nelson, 2013) should be also considered by integrating wide range of fish feeding strategies (filter feeders, herbivores, fish predators, top predators, among others…).

2.2 Monitoring of marine litter

Q2 The reviewers disagree with the statement that many fish in the European areas are not very suitable as litter ingestion indicators (EU 2011). Indeed, information is fragmentary and much more dedicated work is urgently needed.

However, CEFAS database on fish stomach contents records reports plastic rubbish and strings on several fish species since 1972 (cod, saithe…) http://www.cefas.defra.gov.uk/our-science/fisheries-information/fish-stomach-records.aspx. Indeed, there is a problem of underreporting of plastic litter ingestion, especially with regard to microplastics ingestion (Deudero & Alomar, 2014).

2.3 Combining monitoring of marine litter and fish diseases

Q3 On page 96 it is indicated that this study is, at least in part, intended to monitor impacts of marine litter on marine life. There is nothing in the text about monitoring impacts, only presence of plastic in the fish. One cannot infer an impact form this alone. Even if it were possible to assess physical lesion in the fish, impacts are not limited to this and could include sub lethal effects, compromised feeding, behaviour etc. So it is not appropriate to consider impacts in this protocol. Of course any risk assessment involves data on a combination of severity and probability so knowing the frequency of encounter rate is valuable but on its own this protocol tells us nothing of impacts.

Q4 Regarding histopathology, as well as gills and intestine walls, monitoring programmes could be extended to gonads, for example. PCB and DDTs levels have been assessed in liver and gonads of swordfish (Porcelloni *et al.* 2012).

3 Protocol for integrated monitoring of fish diseases and ingested litter

3.1 Sampling and studies at sea

3.1.1 Fish sampling

Q5 Sampling of fish on board research vessels is preferably

Fish samples will be obtained on board research vessels, it would be also appropriate to complement data on plastic ingestion by obtaining fish samples from commercial vessels as they provide for a more extensive temporal survey.

Q6 All trawls should be equipped with an inlay (codend) with a mesh size of 20 mm in order to be able to catch small fish specimens (and epibenthic invertebrates if they are to be examined for plastic litter ingestion, too).

Regarding sampling gear it is important to ensure that the net used to catch fish will not catch litter of the same size range as the being counted. Fish are exposed to whatever accumulates in the cod end of the net and so there is a concern they might ingest

larger plastic that has also been captured by the net. Workers need to be aware of this issue. However, if sampling is done with trawl nets at the benthic and pelagic domain, most of these are designed to catch fish and mesh size at the cod end differ in size (i.e 70–75 mm (Genner *et al.*, 2010)). Unlike studies of fish collected from plankton nets (e.g. Boerger *et al.*, 2010) that are more likely to accumulate smaller plastics in the same range size of those being examined in fish, ingestion of plastics by fish whilst in the net itself is less probable. However, risk exists and a solution to this issue is to examine plastic used in the construction of the nets and ensure that fragments of the net were not a potential source of any of the material found in the fish.

Using a mesh size of 20mm will exclude sampling of microplastics in the water column or in the seafloor. Additional sampling data for marine litter can be included, such as coupling nets for microplastic identification to the trawl nets, as well as sampling microplastic in the sea surface with manta trawls (EU 2013, GESAMP 2015).

Moreover, it remains confuse if epibenthic invertebrates will be sampled and examined for plastic ingestion. There should be a specific protocol for those organisms including a wide array of taxa (sponges, cnidarians, ascidians, among others…)

3.1.3 Fish species for integrated monitoring

The key target fish species recommended for fish disease monitoring and the requirements according to ICES/OSPAR guidelines are summarized in Table 2.

Q7 Refer to the scientific name of fishes in the text, ex.:

> In the Baltic Sea, dab Limanda limanda is a target species only in the German fish disease monitoring programme in the western part (because its distribution is restricted to saline areas). Flounder Platichthys flesus, as a euryhaline species with a wide distribution pattern, is studied in the entire southern and central Baltic Sea by Germany, Poland and Russia. Cod Gadus morhua also is a target species in the entire southern and central Baltic Sea and is part of the German, Polish and Russian fish disease monitoring programmes.

Q8 It is remarkable that neither *Clupea harengus* nor *Scomber scombrus* are the main target species present in fish disease monitoring surveys. However, these species might be valid indicators for plastic ingestion in the pelagic compartment.

Q9 Not all are plankton feeders. Ontogenic shifts in diet composition should be taken into consideration, and no general statements should be made on trophic traits.

Q10 Table 2 on disease categories. It should be stated 'Externally visible diseases/parasites' if endo and ectoparasites are included in the examination.

3.1.4 Examination of fish for diseases

> **Q11** If possible, only live fish maintained in running seawater after sorting of the catch should be examined. The time span between catch and examination/dissection should be recorded. This is also relevant for litter monitoring since regurgitation or defecation of litter particles may occur during maintenance as a function of time.

Not only live fish should be sampled for plastic ingestion but dead fish as well. In this sense, it might be appropriate to perform first a pilot study to test adequacy of sampling only 'live fish maintained in running seawater after sorting of the catch

should be examined'. Moreover, running water could be contaminated with microplastics. Thus, QC/QA measures should be incorporated to this sampling procedure.

3.1.4.2 Microplastic liver neoplasms

Q12 We reinforce the statement in which the WG highlights that other fishes, apart from flatfish have to be integrated into the study for litter plastics.

3.1.4.3 Liver histopathology

Q13 Histopathological changes as consequences of microplastics ingestion by fish have been experimentally tested inducing hepatic stress resulting in glycogen-depleted liver (Rochman *et al.*, 2013). It is therefore very advisable to combine histopathology with plastic ingestion.

3.1.5.1 Sampling for litter monitoring

Q14 Indeed a pilot study should be carried out to assess the flatfish used for internal disease monitoring as potential species for litter monitoring. In addition, other species should be considered and included in this pilot study.

Q15 Further research could be conducted in assessing whether there is a correlation between high parasite infestation and high plastic ingestion.

3.1.5.2 Sampling for litter monitoring

> **Q16** However, it is recommended to store biota samples onboard (freezer at -20 °C) for subsequent litter examination in the lab. In case of small fish specimens, they may be frozen whole; in case of larger fish, they should be dissected and the gastrointestinal tracts removed intact and frozen individually. Again, possible sample contamination needs to be avoided.

If the fish is opened to the air then the probability of contamination from small airborne particles is very high. The protocol indicates further micro particle analysis (<5mm) 'may' not be possible. This needs to be changed. If the purpose is to look for large pieces of litter i.e. where airborne contamination is not feasible then the fish could be examined on the vessel. But if (as is the case with MSFD) and is implied here smaller particles are also of interest then ALL the examination needs to be done in clean conditions with procedural controls (blanks) and the items recovered need to be identified for example using Raman or FT-IR spectroscopy in order to confirm they are actually plastic.

A procedure for avoiding possible sample contamination when dissecting and removing gastrointestinal tracts from big fish aboard must be set. Therefore, fish-specific supporting parameters should be stated. Amongst others, material of the dissecting tools should also be clarified. It is recommended to use metal and glass to avoid plastic contamination (Woodall *et al.*, 2015).

Q17 Table 4- plastic ingestion refers only to macroplastic or microplastic as well? If possible it is advisable to add a measure of variation among samples (SD, SE…)

Q18 After inspection of the inner organs for pathological changes (including macroscopic liver neoplasms) and taking tissue samples for histopathology of the liver and possibly other organs (see Tab. 2), the gut is removed by severing the intestine in the vent area and the oesophagus close to the mouth cavity.

After gastrointestinal tract is removed, methods appropriate for plastic sorting and identification have to be applied (UE 2013). Basic biological parameters of fish species considered for the diseases monitoring (total length, fresh weight, eviscerated weight, gastrointestinal weight, etc...) will complement the stomach content data and will be needed for diet/trophic calculations.

Sorting for plastics in the gastrointestinal tract involves identification under stereomicroscopic at highest magnification. Estimated sorting time depending on expertise ranges from 1,5–2h time.

Some supporting parameters have to be calculated to assess plastic ingestion by fish species. Traditional stomach contents related indices include: vacuity index, frequency of occurrence (%), numerical abundance (%), among others (UE 2013).

- Vacuity Index = number of empty gastrointestinal tracts x100/total number of gastrointestinal tracts.
- Percentage Frequency of Occurrence = number of gastrointestinal tracts with the prey considered x100/total number of non-empty gastrointestinal tracts.
- Percentage of numerical abundance = number of each prey item x100/ total number of preys in all gastrointestinal tracts.

Q19 Alternatively, due to the low occurrence of particles in stomachs it may be necessary to pool fish. An option is to dissolve pools (which may consist of gastrointestinal tracts of 100 fish or more) in acid and filter over a fine mesh to count particles. As contamination could be an issue in relation to micro particles, observations can be limited to a certain particle size (e.g. 200 μm).

There are several constraints in the former paragraph. The issue of pooling samples has not yet been tested for plastic ingestion. Further research is needed to carry out this sampling procedure. Moreover, the fine mesh and acid must be defined. Several protocols are under consideration (UE 2013, Van Cauwenberghe *et al.,* 2012).

3.1.6 Data requirements and supporting parameters

Q20 Table 5: several parameters need to be further detailed in the primary and secondary data recorded in the combined surveys. For example,

Primary sampling data: Litter in the net and the biota catch composition (including fishes and invertebrates).

Primary individual fish data: Litter in the gastrointestinal tract. Add to the 'Remarks column': Identification under stereomicroscopic at highest magnification.

Primary individual fish data: Litter entanglement, mechanical damage. Also check for microplastics in the external parts of the body, gills and mouth.

In this table all info on stomach contents is lacking, see Q18 for more details.

Q21 If fish disease monitoring is part of an integrated biological and chemical monitoring programme, additional supporting parameters may be measured, such as other biomarkers and contaminant concentrations in fish.

Biomarkers are not defined in the text. Several oxidative stress biomarkers ROS might be applied to address plastic ingestion sub-lethal and lethal effects in selected fishes.

Q22 Data on marine litter in fish should be generated in a way that they can be reported to the ICES Environmental Database (DOME), preferably together with the fish disease data.

In the DOME database other parameters have to be added in order to report on stomach contents data regarding plastic litter. This part should be more developed, need to integrate and specify parameters regarding plastic characteristics: number of plastic items, plastic size, plastic type, plastic shape, plastic colour, plastic weight… (Anastosopoulou *et al.,* 2013; Lusher *et al.* 2013, Possatto 2011, UE 2013, GESAMP 2015)

Q23 Figure 1-modify accordingly to text changes, along with comments for supporting parameters Q18 and Table 5 (Q20). Moreover, for record supporting parameters other primary and secondary fish data has to be included (with data taken from Table 5). It is advisable to add cod *Gadus morhua* in the pelagic trawling surveys, since juveniles are within the pelagic realm.

4 Discussion and conclusions

Q 24 The question if litter particles cause harm to fish cannot fully be answered by applying the methods established in fish disease monitoring programmes according to ICES/OSPAR guidelines.

The harm to the fish is very complex to evaluate with the present knowledge and still much studies have to be performed. An advantage of a combined monitoring protocol for fish diseases and plastic ingestion is that further knowledge on harmful effects can be built up. However, there is a need to define harm exerted to fishes, establishing categories under the MSFD.

Q 25 There are no existing monitoring programmes in OSPAR Member States focusing on diseases of wild shellfish in a way it is done for fish diseases. Therefore, this part of the request could not be addressed by WGPDMO.

Indeed, the monitoring protocols are not addressing by ay means shellfish diseases nor shellfish plastic ingestion. Thus, important commercial shellfish species such as Mytilus, Ostraea… are excluded from the studies.

The reviewers think that valuable data from this combined protocol can be obtain with further clarification on the stated attempts.

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