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International Council for the
Exploration of the Sea

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**REPORT OF THE WORKING GROUP ON PATHOLOGY AND DISEASES
OF MARINE ORGANISMS**

Copenhagen, 15-18 March 1993

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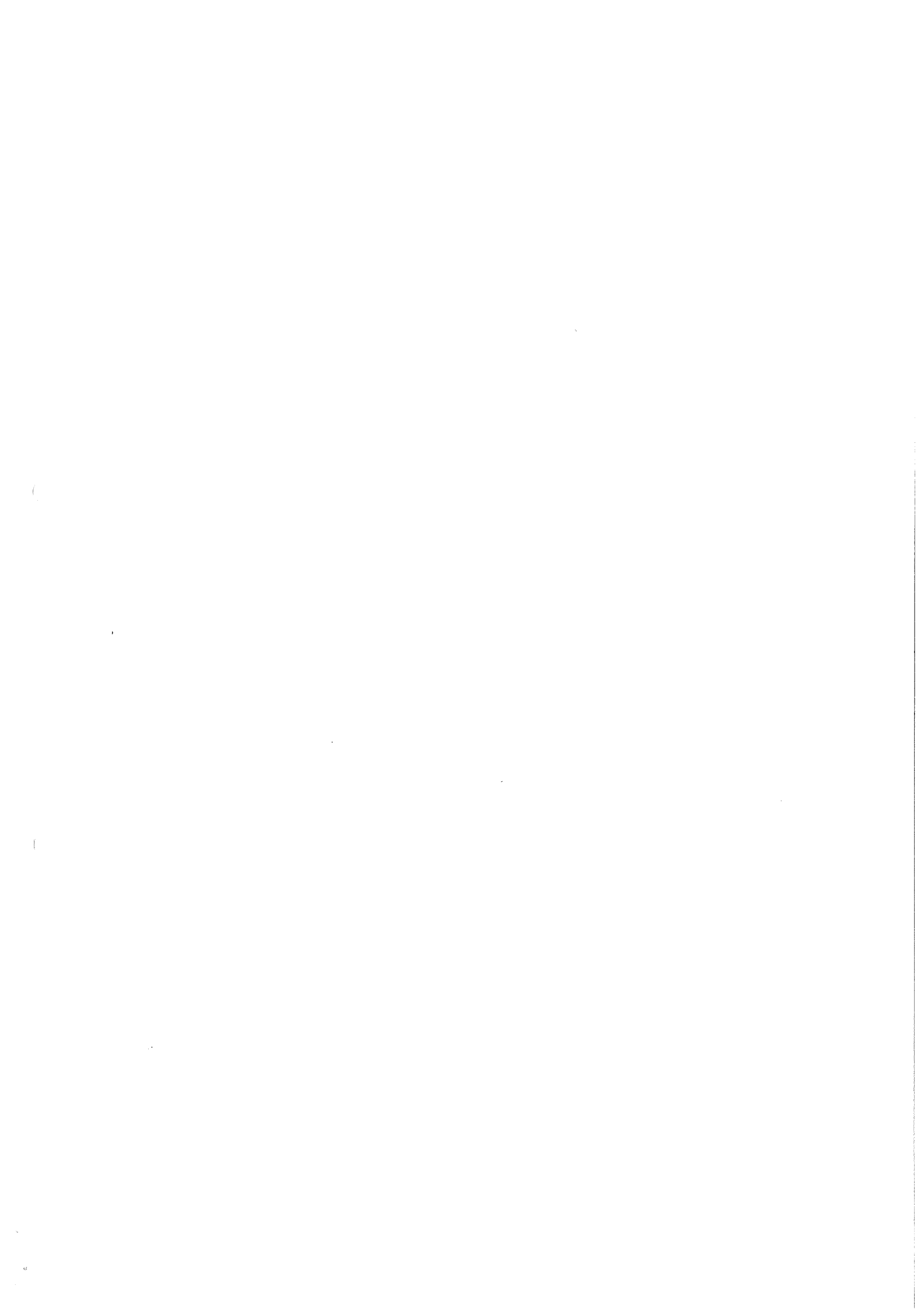


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REPORT OF THE WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

ICES, Copenhagen, 15- 18 March 1993.

1 INTRODUCTION

The Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) met at ICES in Copenhagen, with Dr A. H. McVicar presiding as Chairman (C.Res.1992/2:47). The participants were welcomed to the meeting by the ICES General Secretary, Dr Emory Anderson.

1.1 Opening of the Meeting

The meeting was opened at 10.00 hrs on Monday 15 March with the Chairman welcoming participants, particularly those new to the Working Group. It was unfortunate that one intended participant, F. Perkins from the USA, was unable to attend due to adverse weather conditions in the USA. A special welcome was extended to Dr Vello Kadakas from Estonia, who attended the Working Group as an observer. The list of participants is given in Annex 1.

The intention of asking special sub-groups to focus on detailed consideration of relevant agenda items, after an initial general outline of the area for discussion, was intimated. These sub-groups were asked to report back to the Working Group on the results of their discussions, i.e., conclusions and recommendations for consideration.

2 TERMS OF REFERENCE, ADOPTION OF AGENDA, SELECTION OF RAPORTEURS

The Terms of Reference as published in C.Res. 1992/2:47 were detailed. Particular attention was drawn to the new tasks added by the Mariculture Committee on chemotherapeutic resistance patterns of farmed fish disease and the efficacy of vaccines. This reflected a re-direction towards more detailed consideration of mariculture disease issues. The increased emphasis on mollusc diseases in the terms of reference was welcomed by the Chairman.

TERMS OF REFERENCE

- a) Evaluate disease prevalence data in marine fish stocks and related data on contaminants in sediments as recommended by the Sub-group indicated below;
- b) analyze national reports on new disease trends in wild fish, crustacean, and mollusc production;

- c) evaluate current research on mollusc diseases (e.g., epidemiological surveys, experimental pathology, diagnostic methods) to standardize approaches within ICES;
- d) analyze national reports on new disease trends in mariculture, and provide advice on preventive control measures;
- e) analyze and update information from studies in progress on disease interactions between farmed and wild fish populations;
- f) analyze available data on changes in the resistance profiles of fish diseases to chemotherapeutants currently in use in mariculture, and recommend improvements in strategies for their use;
- g) review available information on the efficacy of existing commercially available fish vaccines and the current status of vaccines under development;
- h) consider the usefulness of the ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish and make recommendations on the continuation of this series and on its content.

An agenda was agreed (Annex 2) and rapporteurs were appointed for individual tasks (Annex 3).

3 REPORT ON THE 1992 ICES STATUTORY MEETING

Items of relevance to WGPDMO from the 80th ICES Statutory Meeting held in Warnemünde, Germany on 24 September - 2 October 1992 were highlighted by the Chairman. These included the request to consider the usefulness of the disease diagnostic fiches, the need for more papers on mariculture to be submitted to the Mariculture Committee and the report on the Theme Session on "Diseases and Parasites in Wild Fish" convened by V. Dethlefsen. The extensive reference to shellfish disease issues in the report of the Study Group on Pollution Affecting Shellfish in Aquaculture and Natural Populations (Document C.M.1992/K:10), which indicated significant overlap with the activities of the WGPDMO, was noted and it was agreed that this would be further discussed under agenda item 4.

4 CONSIDERATION OF RELEVANT REPORTS

4.1 Report of the WGPDMO Sub-group Analysing Fish Disease Data Submitted to ICES

The Sub-group met in Copenhagen from 11-13 March 1993 under the chairmanship of A.D. Vethaak to:

- a) Analyse disease prevalence data sets already submitted to ICES for species other than dab, including data from the Baltic area, using logistic regression analysis and histological confirmation results.
- b) Compare fish disease prevalence data with the results arising from the assessment of data of contaminants in sediments as far as possible.

The Chairman presented the report of the Sub-group which is attached as Annex 4.

ICES fish disease data base: Most of the data submitted to ICES have been computerised. However, all countries still have to validate the ICES files against their original data. The details of data submitted to date are given in the Sub-group report.

A new format proposed by the ICES Secretariat was adopted by the Sub-group. Details will be sent to all Working Group participants by June 1993. A major improvement on the old format now makes it possible to submit the data on individual fish. As soon as the new format is available, each country should use it for submission of fish disease data. Historical data should be re-submitted in the new format. The deadline for submission to ICES of the data collected up to and including 1992, is 1 September 1993. ICES will send print-outs for validation by the end of December 1993 and each country will have a maximum of six weeks to put forward comments. Data should be submitted to ICES on computer diskette as far as possible.

Liver nodules: The Working Group adopted the definition for liver cell adenoma used by the Sub-group. The completion of the intersessional confirmation exercise on liver nodules $\geq 2\text{mm}$ in North Sea dab carried out by D. Bucke has resulted in data on nodules confirmed as neoplasia being available for analysis. Only data on confirmed cases of liver neoplasia should be submitted to ICES in the future. D. Bucke has agreed to histologically evaluate all liver nodule samples sent to him in the coming year.

Data analysis aspects: The Sub-group considerations on minimum sample size, the necessity of age/length

information on dab, and the existence of the Epi-Info computer program were reported to the WGPDMO.

Highlights of the standard methodology for the analysis of fish disease data proposed by the sub-group were presented. Logistic models defined by the GLIM software are used. In a general model for disease prevalence, length, sex, season, year and ICES rectangle were incorporated as explanatory factors. Disease odds ratios estimated by GLIM are given on maps, for each fish species and disease, produced by ICES. Odds ratios give a relative probability of a fish being affected that can be compared between ICES rectangles. A detailed example of the procedure is given in the Sub-group report.

Results of the data analysis using the standard methodology are illustrated by three maps for acute skin ulcerations in cod, lymphocystis in flounder and confirmed liver neoplasia in dab. Due to incomplete and unvalidated data currently at ICES, only a subset of the data was used to produce these preliminary maps. Final maps should include an indication of the scale used for the disease odds ratio, as well as a clearer indication of rectangles that were sampled but where disease is absent.

The Working Group agrees that some methodological problems remain to be solved as an intersessional task by S. des Clers, A.D. Vethaak and M. Carr.

In accordance with the terms of reference, some attempts were made to compare maps for sediment contamination levels of different pollutants, provided by ICES, with the preliminary disease maps. The Working Group pointed out that the maps were obviously not appropriate to establish correlations. Janet Pawlak, the ICES Environment Secretary, informed the Working Group of some major limitations regarding the maps, namely that sampling sites and dates for sediment data do not correspond to those for fish diseases, and that there is no information available on sediments in the Baltic Sea. Furthermore, there is a high intrinsic variability in the measurements of some sediment chemistry parameters, due to the use of analytical methods which were not intercalibrated. The Working Group strongly emphasizes that future collaboration with other relevant working groups is needed in order to study possible relationships between sediment contamination and the occurrence of fish disease. The Working Group recommends that A.D. Vethaak establish the necessary contacts.

Future activities of the Sub-group: The Working Group recommends that the Sub-group meet again before the 1994 Working Group meeting, to analyse the updated and validated ICES data for all species examined according to the standard protocol, in the Baltic and the North Sea.

Furthermore, the Sub-group should evaluate available information on factors which may have a possible impact on the prevalence and spatial distribution of fish diseases such as stock identity, stock density, recruitment, age-structure or fishing effort. The Working Group recommends that ICES should provide the necessary information to the extent possible. It is further recommended that available data for age-length keys in dab be compiled by T. Lang and S. Møllergaard and presented at the next Sub-group meeting.

The Sub-group should produce maps of relative disease odds to be considered by WGPDMO and to be used by other relevant working groups for comparison purposes.

4.2 Report from the meeting of the Coordinating Group of the Baltic Marine Biologists Working Group (BMB WG 25) "Fish Diseases and Fish Parasites of the Baltic Sea"

J. Thulin, Chairman of the BMB Working Group 25, gave a brief overview of the structure and activities of that Working Group which was established in 1989, with the main objective to develop a standardised methodology for fish disease studies in the Baltic Sea.

The Coordinating Group of the BMB WG 25 met at Hel Marine Laboratory, Poland, on 23-24 February 1993. The report of the meeting contains information about current research on fish diseases in the Baltic provided by representatives attending the meeting.

The discussion of the report presented to the WGPDMO focused on three main topics.

- liver tumours in Baltic flounder which appear to occur at high prevalences in Finnish waters, but at low prevalences in Estonian and western Baltic waters;
- the microscopic identification of *Ichthyophonus* spores which occurred in different fish species in Estonian waters without causing clinical signs of the disease;
- the M-74 syndrome, which causes high mortality rates of yolk-sac fry in salmon hatcheries using wild broodfish.

A BMB Symposium/Workshop on "Flounder Diseases and Parasites in the Baltic Sea" is scheduled to take place in Finland in 1994. WGPDMO welcomed the possibility of an ICES co-sponsorship.

The results of the BMB Sea-going Workshop on board R/V "ALKOR" in October 1991 on Methodological

Aspects of Fish Disease Studies in the Baltic Sea have been compiled and are ready for publication. A follow-up workshop is planned for 1994 which will focus on fish diseases and parasites in the eastern and northern parts of the Baltic Sea.

WGPDMO strongly emphasized the need to establish a regular communication between the BMB Working Group 25 and the ICES WGPDMO, in order to coordinate further activities and to exchange existing knowledge. Baltic countries are encouraged to submit fish disease data collected according to the standard methodology to ICES.

4.3 Quality Status Report of the North Sea Task Force

A group consisting of A. McVicar, T. Lang, D. Bucke and S. Møllergaard reviewed the item "5.5.1 Fish Diseases" in Section "5.5 Biological Effects" of the North Sea Task Force Quality Status Report. In agreement with ICES, some corrections were made and an amended version was submitted to the ICES Secretariat.

4.4 Report of the ICES Special Meeting on *Ichthyophonus*

The Working Group was informed about the main conclusions of the Second ICES Special Meeting on *Ichthyophonus* in herring held at the SOAFD Marine Laboratory in Aberdeen, Scotland on 21-22 January 1993. Relevant points of the meeting were discussed. The Working Group recommends that individual fish data collected on *Ichthyophonus* in herring be submitted to ICES by 1 September 1993 for inclusion in the fish disease data base using the new fish disease format.

4.5 Report of the Study Group on Pollution Affecting Shellfish in Aquaculture and Natural Populations

The report of M. Heral (ICES, Doc. CM 1992/K:10) was discussed. In order to coordinate future work, the Study Group can make contact with WGPDMO and take advantage of its long-standing expertise in the field of shellfish pathology and diseases.

Recommendations

The Working Group recommends that the Sub-group on Analysis of Fish Disease Data should meet again before the 1994 Working Group meeting, to analyse the updated and validated ICES data sets for all species examined according to the standard protocol, in the Baltic and the North Sea. Available information on factors with a

possible impact on the prevalence and spatial distribution of fish diseases such as stock identity, stock density, recruitment, age-structure or fishing effort for dab, cod and flounder should be considered.

The Working Group recommends that individual fish disease data collected, including *Ichthyophonus* in herring, be submitted to ICES by 1 September 1993 for inclusion in the fish disease data base, using the new fish disease reporting format. Data from previous years should be re-submitted.

5 RECENT TRENDS IN DISEASES IN WILD FISH, CRUSTACEANS AND MOLLUSCS

In all, eleven national reports on diseases in wild fish, crustaceans and molluscs were received.

The following trends were considered significant by the Working Group.

A. Fish

Ichthyophonus sp.: Limited additional data to those previously presented at the ICES Special Meeting on *Ichthyophonus* in Aberdeen, 1993, were available for analysis. The prevalence of this fungal disease in herring was still high, i.e., about 2-5%, outside the Shetland islands and off the Norwegian coast. In the eastern North Sea, the Skagerrak and the Kattegat, the prevalence was about 2%. In samples from the eastern Baltic, the southern and central parts of the North Sea and from the southern Icelandic waters, only occasional records were made. In the Kattegat and western Baltic, there is an indication of a decreasing trend.

Lymphocystis: On the Belgium shelf, the southeastern North Sea, the Skagerrak and the Kattegat, decreasing trends were observed for this disease in dab and flounder. A less pronounced decrease was noted in dab in the central and western North Sea. The disease is also reported to occur in Icelandic dab.

Infectious pancreatic necrosis (IPN): In Finland the IPN virus was isolated from wild grayling and whitefish used as brood fish.

Aeromonas spp.: Furunculosis was diagnosed in spawning Pacific salmon from the Canadian west coast. Atypical strains of *Aeromonas salmonicida* were isolated from wolffish and ocean pout in Canada, ulcerated Baltic flounders in Finland, and for the first time, from ulcerated dab, plaice and eels in Denmark.

Pseudomonas anguilliseptica: The bacteria is now consistently isolated in Finland from Baltic herring with eye lesions.

Vibriosis: In New Brunswick, Canada, *Vibrio anguillarum* was recovered from striped bass and tomcod.

Visceral granulomatosis: Off the Swedish west coast, the prevalence of this disease in mackerel was 33%.

Kudoa sp.: In sea trout from the southwest of France, *Kudoa* sp. did not reappear in 1992.

Glugea stephani: The prevalence of this protozoan disease in dab, plaice and flounder from the Belgian shelf has decreased.

Anguillicola crassus: The infection level of *A. crassus* in eel from the Baltic was still high, i.e., approximately 60%, outside two thermal discharge areas off the Swedish coast. However, the infection appeared to increase in other Swedish coastal areas as well as off the Polish coast.

Lepeophtheirus salmonis: In Norway, an increase in the reporting of Atlantic salmon and sea trout severely infected with *L. salmonis* was reported.

Epidermal hyperplasia/papilloma: There is an indication for a decreasing trend in dab from the southeastern North Sea and the Kattegat. Icelandic dab were only affected at a low prevalence as compared with North Sea dab.

Skeletal deformities: This abnormality showed an increase in dab, flounder and cod caught in the Belgian Shelf and in haddock near dump sites off the Scottish coast.

Liver nodules: Highest prevalences of liver nodules ≥ 2 mm were recorded in dab at the Dogger Bank and off the Humber. In the northern North Sea and southern Icelandic waters, the prevalences were found to be much lower. On the Belgian Shelf, the prevalences of this condition in dab and flounder showed an increasing trend.

Dermal melanisation: Dermal melanisation in North Sea dab has been observed for the past five years. There are indications for marked spatial trends of this condition with highest prevalences at stations off the northeast English coast. A single observation was also noted in plaice. A similar condition was also reported in dab from the Belgian shelf.

Ulcerations: Acute skin ulcerations in dab occurred at highest prevalences on the Dogger Bank and in the Firth of Forth area. In Icelandic dab, the average prevalence was comparable to the average prevalence observed in the North Sea.

B. Crustaceans and Molluscs

Haematodinium sp.: A low level of infection (1%) was noted in *Nephrops* from the Botney Gut-Silver Pit areas off the east coast of England. In the Skagerrak and Kattegat, the prevalence in undersized *Nephrops* was about 1%. The *Nephrops* population in the Clyde estuary (western Scotland) still showed high levels of infection.

Shell disease: In *Nephrops* from the Botney Gut-Silver Pit areas, the prevalence was still high, i.e., varying between 20 and 50%.

Bonamia ostreae: Populations of flat oysters from some coastal waters off southern England remained infected with *B. ostreae*. The disease situation in Ireland appears to be somewhat worsening with reports of increased mortalities in Galway Bay.

Conclusions:

- a) The apparent decreasing trend of *Ichthyophonus* in herring from the Kattegat and the western Baltic should be treated with caution due to the short time period this disease has been monitored.
- b) For both lymphocystis and epidermal hyperplasia/papilloma in North Sea dab, there is indication for a decreasing trend.
- c) On the Belgian Shelf, liver nodules in flatfish are showing an increasing trend.
- d) It is still difficult to evaluate the disease situation in some parts of the Baltic due to the limited information available.
- e) An increasing spread of *Anguillicola crassus* among eel populations in the Baltic is noted.
- f) A condition of unknown aetiology, dermal melanisation, is reported to occur in North Sea dab revealing a pronounced spatial distribution pattern.
- g) The prevalence of shell disease in *Nephrops* in certain areas of the southern North Sea remains high.
- h) In general, it is impossible to evaluate the disease situation of shellfish and molluscs due to limited information from wild populations.
- i) Studies in dab from southern Icelandic waters demonstrated the occurrence of the major diseases known from North Sea dab. Whereas the prevalence of epidermal hyperplasia/papilloma and liver nodules $\geq 2\text{mm}$ was comparatively low, the prevalence of

lymphocystis and skin ulcers was in the same average range as in the North Sea.

- j) There seems to be an increase in the isolation of atypical strains of *Aeromonas salmonicida* from marine fish species. The significance of this increase is not yet known.

6 RECENT TRENDS IN MARICULTURE DISEASES

Under the terms of ICES Council Resolution 1992/2:47 (d), the Working Group analyzed national reports on new disease trends in mariculture, and provided advice on preventive control measures. Written reports were received from eleven ICES member countries. The main trends and developments identified were as follows:

A. Fish

1 Atlantic Salmon

Infectious pancreatic necrosis (IPN): Although IPN is reported from Norway, Scotland, Sweden, Canada and the Faroes, its significance considerably decreased in 1992. A better control of the broodstock, separate incubation and exclusion of eggs from carriers, as well as disinfection of newly fertilised eggs could explain this trend.

Pancreas disease (PD): This disease is still occurring in Ireland, Scotland and Norway, though no significant trends were reported. Scotland reported some success with the containment of the disease by the fallowing of marine sites. Research in Scotland is directed at the relationships between IPN, PD and fading smolt syndrome.

Infectious Salmon Anaemia (ISA): This disease was only observed in Norway, where the number of farms with clinical ISA has been reduced from approximately 100 to 7, due to sanitary measures such as harvesting followed by fallowing.

Furunculosis (*Aeromonas salmonicida*) typical: This disease remains the principal problem in salmon farms (Canada, the Faroes, Norway and Scotland). Antibiotic resistance to drugs (oxytetracycline, oxolinic acid, tribriassin, amoxycillin) is an increasing problem. Vaccination programmes and management strategies appear to have some beneficial effects on the disease (the Faroes, Scotland, and Norway).

Vibriosis (*Vibrio anguillarum*): Canada noted a shift in the main serotype being isolated from O2 to O1. Low mortalities were associated with the disease in Canada and Norway.

Cold water disease (*V. salmonicida*): This disease is still causing some losses in northern Norway despite the vaccination programme. In the Faroes, the vaccination programme limited the prevalence of the disease: all smolts transferred to sea are dip-vaccinated and also intraperitoneal injections are made with combined oil-based vaccines against vibriosis, cold water vibriosis and furunculosis. The disease was observed along the east coast of Canada in the early part of 1993.

Bacterial Kidney Disease (BKD): This disease is still of importance in Canada, Norway, and the Faroes. In the Faroes, the number of affected farms increased from 2 (in 1991) to 10 (in 1992). All broodfish were tested in 1992 for BKD (ELISA system) in a national programme, and only eggs and milt from fish found to be free from the bacteria were used for production.

Rickettsiosis (*Piscirickettsia salmonis*): This disease was isolated for the first time in Norway, in connection with an outbreak of necrotising hepatitis. The mortality due to the disease has been limited (a similar incidence has been reported on the west coast of Canada).

Sea lice: These parasites are still an important problem, particularly in Norway, Ireland and Scotland.

Hexamitosis: Hexamitosis was reported at a new farm site in Norway. No connection with previous outbreaks appears to have occurred.

Other conditions recorded

- Eye lesions associated with a pasteurilla-like organism have been described in Norway.
- In France, for the second year, a 10% mortality was associated with signs of meningo-encephalitis, the aetiology of which is unknown. In Ireland a similar clinico-pathology was reported associated with 30% mortalities in 1+ smolts.

Cardiomyopathy (CMS): This disease has been diagnosed on 80% of the farms in the Faroes, with varying mortalities.

2 Other salmonids

2.1 Rainbow trout

Viral haemorrhagic septicemia (VHS): In contrast to 1990 and 1991, no problems were recorded in Denmark.

Furunculosis (*Aeromonas salmonicida*) typical: This is the dominating disease problem in Finland, Sweden, and Denmark, despite varying results in vaccination programmes. Oxytetracycline-resistant bacterial strains occur frequently but, so far, strains resistant to oxolinic acid are rare.

Vibriosis (*Vibrio anguillarum*): This is the second most prevalent pathological problem in Finland. As in previous years, the success of vaccination is poor, most probably due to faulty vaccination procedures.

Infectious pancreatic necrosis (IPN) and bacterial kidney disease (BKD): These are recorded as in previous years, but without constituting any trend.

***Pseudomonas anguilliseptica*:** This was isolated in Finland, but did not cause serious outbreaks of the disease.

Sea lice (*L. salmonis*): In France, important outbreaks were more severe than during previous years. The condition was successfully treated by dichlorvos (immersion 7 min. in 15 ppm solution).

2.2 Brown Trout

Myxosporidiosis: In France, muscular infestation with *Kudoa* sp. (5% prevalence muscle) was demonstrated by a systematic survey, but in 1992 it was not associated with any postmortem lysis. During the summer, a sudden death syndrome appeared in large fish (2+) due to a rupture of the aortic bulbus.

3 Non-Salmonids

The **picorna-like virus** first reported in sea bass in 1990 continues to cause occasional increased mortality in sea bass larvae.

***Aeromonas salmonicida*:** An atypical *A. salmonicida* has caused disease problems in farmed turbot in Denmark.

Vibriosis: This has been a serious problem in unvaccinated juveniles of turbot and sea bass in France, most of them serotype 01, but for the first time in that country a serotype 02 was also observed; the serotype 03 identified in 1990 was not isolated again.

Pasteurellosis: Pasteurellosis was again noted in French Mediterranean farmed sea bass, but the disease appeared during the winter and was not as significant as the outbreak in 1990.

Mycobacteriosis (*Mycobacterium marinum*): This disease was recorded for the first time in sea bass in Denmark.

Flexibacteriosis: This systemic infection was observed for the second year in sea bream in the Mediterranean.

Myxosporidiosis: A high prevalence of encysted *Henneguya* was reported in *Coregonus* sp. in Finland.

Protozoa: A protozoan parasite associated with mortalities was found for the first time in the yolk sac of cod larvae in Denmark.

4 Molluscs

On the Atlantic coast of Canada a mass mortality of bay scallops (*Argopecten irradians*) occurred. This was linked to a **pseudoklossia-like parasite**. This parasite is believed to have been introduced during quarantine introduction of the bay scallops into Canadian waters.

On the Pacific coast of Canada a new protozoan occurred in cultured Japanese scallops (*Patinopecten yessoensis*) resulting in mortalities exceeding 90%.

On the Atlantic coast of the USA, the further spread of some diseases was noted:

1. *Perkinsus marinus* in Delaware Bay;
2. *Haplosporidium nelsoni* in Maine and in southern Florida;
3. *Bonamia ostreae* in Maine.

In addition, two new diseases have been reported: one on juveniles of *Crassostrea virginica*, characterized by the presence of abnormal growth of the left valve and brown ring; the second on black abalone (*Haliotis cracherodii*) in which a **coccidian** protistan has been found to be associated with high mortalities.

No major new trends were found in Europe for the existing protozoan diseases of *Ostrea edulis*, i.e., *Marteilia refringens* and *Bonamia ostreae*. However, in the Netherlands, there has been an increase in the prevalence of bonamiasis in young oyster stocks. This indicates that there is likely to be a further epizootic outbreak in 1993. This young oyster stock was produced from oysters surviving in 1990, which was the year of the first severe bonamiasis outbreak in Lake Grevelingen (NL). The anticipated natural build-up of a stock with genetic resistance to

bonamiasis is doubtful considering the recent increase of prevalence of the disease in the young stock.

Conclusions

- a) There is a trend toward decreasing problems of IPN- and PD-associated mortalities in Atlantic salmon. This may be due, in part, to management techniques such as the reduction of stocking density and/or the prevention of overlap of year classes.
- b) Furunculosis remains the main pathology in Atlantic salmon as in other salmonids, despite vaccination programmes. The resistance of *A. salmonicida* to the limited range of antibiotics available to fish farming is a matter for concern.
- c) The geographic extension or development of new diseases should be considered:
 - Cold water vibriosis was recently observed in Canada.
 - Rickettsiosis was reported in Norway. This was the first report in Europe for Atlantic salmon.
 - In non-salmonid species, diseases, even if they are not commonly observed, should be a matter for concern, e.g., encephalitis (picorna-like virus), Pasteurellosis, and Vibriosis (serotype O2) in sea bass.
- d) Parasites causing postmortem flesh liquefaction (e.g., *Kudoa* sp. in brown trout) could be potentially important in farmed fish.

Recommendations

Health management plans are a valuable strategy in the prevention of fish diseases and should be developed for all marine fish farms. The following basic principles ideally should be central to any health management plan on fish farms:

- a) the plan should be based on all-in/all-out policy;
- b) hygiene protocols and schedules should be documented in the plan;
- c) separate plans should be developed for separate species;
- d) plans should be developed to address problems of specific areas (e.g., stocking density).

7 EVALUATION OF NEW DATA ON DISEASE INTERACTIONS BETWEEN FARMED AND WILD FISH POPULATIONS

WGPDMO evaluated new data on disease interactions between farmed and wild fish populations (ICES C. Res. 1992/2:47d).

- a) It is evident that lessons can be learned from the interaction of transfers by man between wild and farmed animals. Such interactions have been responsible for effects of diseases on animal populations which vary from catastrophic epizootic to insidious chronic conditions which cause significant effects on population dynamics. It is important that efforts are made to control disease and manage the health of animal populations to avoid, where possible, detrimental effects of disease. In order to achieve this, it is necessary to improve knowledge about disease epidemiology in wild and farmed animals.
- b) It is evident that a limited number of investigations on interactions relating to fish are in progress. For example, in Norway studies into the interaction of furunculosis between farmed and wild fish have shown that small populations of migratory and non-migratory wild salmonids may be seriously affected by this disease. Furthermore, there have been increasing numbers of reports that wild salmonids are heavily affected with sea-lice. Investigations indicate that these infestations may result in early homing of these migratory fish. In other areas no correlation has been established between levels of infection (lice) and fish farms.

Conclusions

The Working Group recognizes that there is an interaction of infectious agents between wild and farmed fish populations. Fish farms may be the source for the spread of disease agents to wild fish and *visa versa*. There will be situations when disease outbreaks occur in farmed fish and the disease risk is also amplified for the wild fish. This risk is dependent on many variables, including the composition of the wild fish population in an area, ecological conditions and the nature of the farms. Such variables have to be quantified from area to area, and to completely eradicate the risk is difficult. The risk may, however, be lessened when health management plans are implemented which provide steps to prevent and/or to reduce disease where possible.

8 RESEARCH ON MOLLUSC DISEASES

Under the terms of ICES Council Resolution 1992/2:47, the WGPDMO was asked to evaluate current research on mollusc diseases. Five member countries responded with

written information. The following main points are highlights of the general review prepared by H. Grizel and presented in Annex 6.

a) Canada, Atlantic coast

Epizootiological surveys are being carried out following mortalities in mussels (*Mytilus edulis*), and giant sea scallops (*Placopecten magellanicus*). Experimental research into improved diagnostic methodologies, including monoclonal and polyclonal antibodies as well as DNA probes, are being developed for several mollusc species and pathogens. To date, monoclonal antibodies have been produced against *Perkinsus karlssoni*.

Canada, Pacific coast

Epizootiological surveys are being carried out following high mortalities in Japanese scallop (*Patinopecten yessoensis*) infected with an unknown parasite (SPX). Experimental research is conducted into improved diagnostic methodologies including monoclonal antibody production for the protozoan *Mikrocytos mackini* in Pacific oyster (*Crassostrea gigas*). Pathogenicity studies are being conducted in an attempt to understand the causal agent of SPX disease in Japanese scallops.

b) USA

Epizootiological studies involving standard techniques are used for the diagnosis of the protozoan *Perkinsus marinus* in *C. virginica* and *Perkinsus* sp. in other mollusc species. Several techniques have been developed including monoclonal and polyclonal antibody production. Research continues towards the development of an ELISA assay and flow cytometric methods for the detection of *P. marinus*. There is also ongoing research into a number of physiological parameters of molluscs to be used as possible indicators for measuring their health status.

c) Portugal

Epizootiological surveys were carried out to monitor the extent of mortalities in clams (*Ruditapes decussatus*), caused by *P. atlanticus*. There is also a monitoring programme for diseases in oysters, (*C. angulata* and *Ostrea edulis*). Standard diagnostic techniques are being used.

d) Spain

No report was received, but based on former information, it is known that epizootiological studies are being carried out to monitor the health status of mussels (*M. edulis* and *M. galloprovincialis*) and

oysters (*C. gigas* and *O. edulis*). Standard diagnostic techniques are being used. There is a research project on the role of mussels as carriers of several *Marteilia* sp.

e) France

Epizootiological surveys are routinely carried out to monitor the health status of several bivalve mollusc species involved in the extensive mariculture industry. Standard diagnostic methodologies are being used and at the same time there is a research programme into the use of monoclonal and polyclonal antibody production against the protozoan pathogens, *M. refringens*, *M. maurini*, *P. atlanticus*, *B. ostreae*, *Vibrio* sp. and *Rickettsia*. There is also a programme to develop DNA probes for some of the above pathogens. Further research to test the genetic resistance of *O. edulis* to *B. ostreae* is being undertaken.

f) Ireland

Epizootiological studies are carried out for monitoring the progress of *B. ostreae* and checking for the presence of *M. refringens* in *O. edulis*. Standard diagnostic techniques are used.

g) United Kingdom

Epizootiological studies are carried out for monitoring the progress of *B. ostreae*, and checking for the presence of *M. refringens* in *O. edulis*. Standard diagnostic techniques are used. A limited research programme involving the use of a number of physiological parameters and pathological methods to measure and describe the effects of environmental changes and resistance of *O. edulis* to *B. ostreae* have been completed.

h) Netherlands

Epizootiological studies are carried out to monitor the health status of stocks of *O. edulis* and *C. gigas* for protozoan diseases. Standard diagnostic techniques are used. A study into the resistance of selected stocks of *O. edulis* to *B. ostreae* was terminated due to a lack of funding.

i) Germany

Epizootiological studies are carried out on the occurrence of *M. intestinalis* in *Mytilus edulis*.

j) Norway

Epizootiological studies are carried out for monitoring the presence of *B. ostreae* and *M. refringens* in *O.*

edulis and in *Ruditaepea phillippinarum* for general disease.

k) Belgium

Epizootiological studies were carried out on the health status of mussels (*M. edulis*) and *Cardium edules*.

- 1) In Denmark, Sweden and Finland no epizootiological studies are carried out.

Conclusions

- 1) The most important need is to develop new, reliable and rapid techniques for diagnosing the most economically important diseases in marine molluscs. The Working Group identifies the important pathogens to be:

- *Bonamia ostreae* in *O. edulis* in Europe
- *Marteilia refringens* in *O. edulis* in Europe
- *Perkinsus marinus* in *C. virginica* in North America
- *Haplosporidium nelsoni* in *C. virginica* in North America.

- 2) When new diseases associated with high mortalities in molluscs occur, there is a need:

- to establish a research programme to define the characterization and pathogenesis of the disease;
- to purify the pathogen and to reproduce the disease by controlled laboratory experiments;
- to study the relationship between the development of the disease and husbandry conditions;
- to conduct transmission studies between other molluscan species in order to establish the role of carriers.

- 3) To promote a research project to culture and maintain stocks of the above-identified pathogens in order to develop basic research in immunology, as well as to have a better understanding of the organisms.

- 4) Control methods for the transfer of molluscs between countries require standardisation following the rules and guidelines of the OIE (Office International des Epizooties) and the EC (European Community).

9 ANTIBIOTIC RESISTANCE OF FISH PATHOGENS

Introduction

The current status of antibiotic resistance of fish pathogens was reviewed. A summary paper is presented in Annex 7.

The present situation in mariculture is that antibiotic resistance is almost exclusively associated with the pathogen *Aeromonas salmonicida* subsp. *salmonicida*, the causative agent of furunculosis in salmonids. An increased number of antibiotic resistant strains of *A. salmonicida* has been recovered from several countries, in addition to an increase in the number of multiresistant strains. There is also evidence that the same phenomenon occurs with *Vibrio salmonicida*.

It was noted that there was a general lack of standardisation of the methodology used to determine antibiotic resistance. It was decided that all member countries of ICES should provide their methodology for antibiogram determination during the next WGPDMO meeting in order to assess and, possibly, to standardise methodologies. In the meantime, several participants will exchange ten selected strains of *A. salmonicida* and perform antibiograms using their own methodologies. Results will be collated and analysed for the next meeting.

The lack of standardisation notwithstanding, there is an increase in the number of resistant, and multipleresistant strains of *A. salmonicida*. Possible causes of this increase were discussed. Hypothetical explanations include:

- a) fish are not treated individually with antibiotics, but as a group/population in a cage/tank/farm;
- b) treatment is performed in an aquatic environment;
- c) palatability of medicated fish food;
- d) dose and duration of treatment;
- e) a limited number of antibiotics are available.

Conclusions

- a) The main problem with antibiotic resistance appears to be associated with *A. salmonicida*. There is, however, no doubt that the resistance of this pathogen to several antibiotics is increasing. There is also an increase in the number of multiresistant strains. This is a cause for concern in several ICES member countries.

- b) There is an urgent need to standardise the methodology used for antibiogram determination of fish pathogens. The OIE should be contacted regarding this issue.
- c) During an outbreak of furunculosis, several samples from the affected fish population should be analysed and antibiograms performed on numerous isolates. The reason for this is that strains with different antibiotic resistance profiles can be recovered from the same hatchery or sea-cage site, the same tank or sea-pen, and/or even from the same fish.
- d) Chemotherapy represents only one aspect of an appropriate fish health management programme. Efforts should be made to reduce the use of chemotherapy by using complementary components of an appropriate fish health management programme.
- e) A need exists for better control of all aspects of medication once an antibiotic has been prescribed for treatment.
- f) Applications of the following rules will benefit fish health:
 - 1) accurate diagnosis;
 - 2) prompt application of treatment;
 - 3) appropriate dosage and duration of treatment.
- g) There is an urgent need to licence additional antibiotics as some countries are restricted to only two or three licenced products.

Recommendations

It is recommended that the antibiograms of 10 selected strains of *A. salmonicida* subsp. *salmonicida* be performed by several laboratories as a preliminary experiment to eventually standardise methodology. WGPDMO will consider the results at its next meeting.

It is recommended that the OIE be advised of the need to standardise the methodology used for antibiogram determination.

WGPDMO recommends to the Mariculture Committee that efforts should be directed towards the reduction of chemotherapy by using complementary components of an appropriate fish health management plan.

10 ANALYSIS OF EXISTING FISH VACCINES AND VACCINES UNDER DEVELOPMENT

The current status of existing fish vaccines and vaccines under development was presented. Summary papers on

these topics are provided in Annexes 8 and 9, respectively. Based on this information, the following conclusions were reached.

Conclusions

- a) The most important commercially available fish vaccines in aquaculture are against furunculosis, vibriosis, cold water vibriosis and yersiniosis. It is generally accepted that the efficacy of existing vaccines against yersiniosis, vibriosis and cold water vibriosis is good. There are, however, some reports of apparent failures. These could result from inadequate vaccination procedures or from differences between pathogenic strains in the affected fish and strains used in vaccine preparation.
- b) It is also accepted that, with current vaccines, injection is superior to immersion and to oral administration in inducing protection.
- c) The cost and manpower involved in vaccination with the current vaccines are particularly prohibitive for fish species in the low market range. There is a need to decrease vaccination costs and to improve the ease of vaccine application.
- d) There has recently been a notable improvement in the efficacy of furunculosis vaccines. However, the efficacy level is still inferior to current vaccines for other fish diseases mentioned above. Most antibiotics used in the farming of Atlantic salmon and rainbow trout are to control furunculosis.
- e) It is recognised by the WGPDMO that there is ongoing work to improve existing vaccines and to develop vaccines against new diseases. Much of this work is commercially-dependent and important aspects of the results are not yet available to the scientific community. Consequently, a discussion on this topic was not possible.
- f) The WGPDMO agreed that the proper use of vaccines, as part of a general fish health plan, has contributed significantly to improving fish health status and to reducing the use of antibiotics in fish mariculture. Appropriate use of vaccines should be encouraged.

11 THE USEFULNESS OF ICES DISEASE PUBLICATIONS

a) Training guide and video

There has been some delay in publishing the training guide. However, the Guide was sent to referees for

comments and the text had been revised accordingly, but now has to wait for the new ICES reporting format, which will not be available before June 1993.

Details regarding the submission of the publication were discussed with the ICES Publication Officer. S. Møller-gaard has accepted responsibility to revise the Guide and will maintain contact with the ICES Publication Officer. The expected date of final submission is late summer of 1993.

Considering the magnitude of the task, the Working Group proposed to abandon the video project.

Concern was expressed about the status of review papers initially prepared for a working group meeting to elucidate problems associated with the "Impact of the use of chemotherapeutics in aquaculture" and the "Glossary in Aquaculture". A. McVicar will contact H. Ackerfors, the Chairman of the Mariculture Committee, to determine the status of these publications.

Conclusion

The Training Guide will be finished by late summer of 1993.

The video project was abandoned.

b) Diagnostic fiches - recommendations on the continuation and content

G. Olivier, the editor of the fiches, presented lists of the proposed titles of fiches and the up-to-date (received and corrected) fiches. Working group members were asked to support this project by reminding colleagues who have been appointed as authors to complete their tasks.

In the future, fiches can be published in batches numbering ten or less, which should facilitate the procedure.

The fiches will be valuable as a reference tool, especially for undergraduate students. However, there are problems regarding advertising and sales. Working group members who have contacts with fish disease journals will investigate the possibility of advertisement of the fiches through these sources.

The future of the fiches was discussed and it was agreed not to seek regular contributions every year, but to finalize the submitted titles and to, eventually, revise some of the previously published fiches. Working group members identified fiches requiring revision and will inform the editor accordingly. Fiches dealing with new disease problems should be brought up as necessary.

Conclusion

Preparation of diagnostic fiches for publication will continue, but at a reduced frequency.

Recommendations

Preparation of the Training Guide for publication will proceed with a deadline of late summer of 1993.

A. McVicar will contact H. Ackefors, the Chairman of the Mariculture Committee, to establish the current status regarding the publication of papers on the "Impact of chemotherapeutics in aquaculture" and the "Glossary in Aquaculture".

12 ANALYSIS OF PROGRESS WITH TASKS

An analysis of progress regarding the tasks of the WGPDMO is presented in Annex 5.

13 OTHER BUSINESS

No other business was raised.

14 FUTURE ACTIVITIES

The future activities of the WGPDMO were discussed and members agreed on the proposed terms of reference for 1993, as stated in the recommendations (Annex 10).

ANNEX 1

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Copenhagen, 15-18 March 1993

| List of Participants | | | | |
|------------------------|--|----------------|----------------|---------------------------|
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List of Participants

| | | | | |
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| Dr A.D. Vethaak | Tidal Water Division Ecotoxicology Section P.O.Box 207 9750 AE Haren The Netherlands | | | |

ANNEX 2

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Copenhagen, 15-18 March 1993

AGENDA

1. Opening of the meeting. Structure of the meeting.
2. ICES Statutory Meeting 1992; items of relevance to the WGPDMO.
3. Terms of reference, adoption of agenda, selection of rapporteurs.
4. a) Evaluation of the report of the WGPDMO's Sub-group which met in Copenhagen from 11-13 March 1993 to analyze fish disease prevalence data and to compare disease data with contaminant data - Chairman, A. D. Vethaak (The Netherlands).
b) Other relevant reports for information - The BMB Working Group, the Study Group on Pollution Affecting Shellfish in Aquaculture and Natural Populations (ICES, Doc. 1992/K:10; submitted to the ICES Statutory Meeting 1992), and the report of the ICES Special Meeting on Ichthyophonus (ICES, Doc. C.M. 1993/F:9).
5. Analyze national reports on new disease trends in wild fish, crustaceans and molluscs.
6. Analyze and update information from studies in progress on disease interactions between farmed and wild fish populations.
7. Analyze national reports on new disease trends in mariculture, and provide advice on preventative control measures.
8. Evaluate current research on mollusc diseases to standardise approaches within ICES.
9. Analyze chemotherapeutant resistance profiles of fish diseases in mariculture.
10. Analyze data on existing fish vaccines and vaccines under development.
11. Consider the usefulness of ICES disease publications.
 - a) training guide and video.
 - b) diagnostic fiches - recommendations on continuation and content.
12. Analysis of progress with tasks.
13. Future activity of the WGPDMO.
14. Any other business.
15. Approval of recommendations.
16. Approval of draft WGPDMO report.
17. Closing of the meeting.

ANNEX 3

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

Copenhagen, 15-18 March 1993

RAPPORTEURS

| <u>Agenda item</u> | <u>Rapporteurs</u> |
|--------------------|----------------------------------|
| 1 | S. des Clers, T. Lang |
| 5 | D. De Clerc, J. Hoglund |
| 6 | F. Baudin Laurencin, F. Scullion |
| 7 | G. Bylund, D. Bucke |
| 8 | P. van Banning, H. Grizel |
| 9 | G. Olivier, D. Bucke |
| 10 | B. Hjeltnes, I. Dalsgaard |
| 11 | G. Olivier, S. Møllergaard |

ANNEX 4

REPORT OF ICES WGPDMO SUB-GROUP ON ANALYSIS OF DATA ON THE PREVALENCE OF DISEASES OF WILD MARINE FISH

Copenhagen, 15-18 March 1993

The Sub-group met in Copenhagen at ICES Headquarters with A.D. Vethaak presiding as Chairman. Apologies were received from J. Thulin who was unable to attend the meeting.

The agenda (Appendix 2) was adopted with minor revisions. All members present participated as rapporteurs.

1 OPENING OF THE MEETING

The meeting was opened at 9.15 hrs on Thursday 11 March by the Chairman, who welcomed the participants. The list of participants is given in Appendix 1.

2 TERMS OF REFERENCE, ADOPTION OF AGENDA, SELECTION OF RAPORTEURS

Participants were referred to the terms of reference given to the Sub-group by the ICES Council in C.Res. 1992/2:47:6.

- a) Analyse disease prevalence data sets already submitted to ICES for species other than dab including data from the Baltic area, using logistic regression analysis and histological confirmation results.
- b) Compare fish disease prevalence data with the results arising from the assessment of data of contaminants in sediments as far as possible.

3 DATA BASE

3.1 Status of the Existing ICES Fish Disease Data Base

J. R. Larsen overviewed the status of the ICES fish disease data base. Most of the data submitted by ICES member countries had been computerized, but data from some countries were submitted too late and could not be analyzed during the Sub-group meeting. It was stressed that the data, as entered into the ICES data base, had not yet been validated by all contributors. Consequently, results derived from the analysis of the ICES fish disease data are preliminary.

An overview of the total number of observations relating to fish diseases presently reported to ICES is tabulated in Tables 1-5 below. They are given for each species by country and by sampling year for the North Sea and the Baltic Sea. Data for 1992 are incomplete.

Table 1 Dab (*Limanda limanda*) North Sea.

| Country | Sampling year | | | | | | | | | | Total |
|-----------|---------------|------|------|------|------|------|------|------|------|------|-------|
| | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 | |
| Frequency | | | | | | | | | | | |
| Belgium | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 8 | 0 | 17 |
| Denmark | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 20 |
| Germany | 0 | 0 | 0 | 0 | 0 | 102 | 94 | 121 | 94 | 110 | 521 |
| Netherl. | 0 | 2 | 0 | 2 | 3 | 2 | 2 | 5 | 10 | 10 | 36 |
| UK | 0 | 0 | 0 | 0 | 0 | 33 | 44 | 75 | 57 | 15 | 224 |
| Total | 2 | 4 | 2 | 4 | 5 | 139 | 142 | 212 | 171 | 137 | 818 |

| Country | | Sampling year | | |
|-------------|------|---------------|------|-------|
| Frequency | 1988 | 1991 | 1992 | Total |
| Germany | 1 | 3 | 0 | 4 |
| Netherlands | 0 | 10 | 5 | 15 |
| Sweden | 0 | 2 | 0 | 2 |
| Total | 1 | 15 | 5 | 21 |

| Country | Sampling years | | Total |
|-----------|----------------|------|-------|
| Frequency | 1990 | 1991 | |
| Sweden | 26 | 145 | 171 |
| Total | 26 | 145 | 171 |

Table 4 Cod (*Gadus morhua*) North Sea.

| Country | | Sampling year | | | | |
|-----------|------|---------------|------|------|------|-------|
| Frequency | 1988 | 1989 | 1990 | 1991 | 1992 | Total |
| Germany | 46 | 45 | 48 | 54 | 72 | 265 |
| Sweden | 0 | 0 | 0 | 40 | 0 | 40 |
| UK | 0 | 5 | 0 | 0 | 0 | 5 |
| Total | 46 | 50 | 48 | 94 | 72 | 310 |

| Country | | Sampling year | |
|-----------|------|---------------|-------|
| Frequency | 1990 | 1991 | Total |
| Sweden | 27 | 211 | 238 |
| Total | 27 | 211 | 238 |

3.2 New Format

J. R. Larsen proposed a new, more flexible reporting format for submitting fish disease data to ICES. This will replace the format described in the report from the WGPDMO meeting in Ostende in 1991. The major difference is that the new format is based on fish disease data on individual fish rather than on pooled data. The Sub-group examined the proposed format and accepted it with minor amendments. ICES will provide national reporting laboratories with the new format and an instruction manual before 1 June 1993. In the future, data should be submitted on diskettes as paper forms will not generally be acceptable.

4 DATA ANALYSIS ASPECTS

4.1 Sample Size

S. des Clers overviewed some statistical definitions of minimum sample size used to detect disease and to estimate and compare prevalences (Appendix 3). Minimum sample sizes provide helpful guidelines for designing disease studies and interpreting observed disease prevalences. However, it is important to note that minimum samples sizes given by statistical theory refer to one sample of fish taken in isolation (e.g., one date, one station, one sex and one length group). In contrast, fish sampled for the ICES disease studies constitute multiple samples (e.g., two sexes, several dates, multiple length groups or stations by ICES rectangles). Therefore, although small samples may be collected, multiple samples guarantee a general consistency of the disease data base.

4.2 Age/Length Information for Dab

S. Mellergaard presented age/length data for dab which demonstrate temporal and spatial differences. The Sub-group emphasised that individual age/length data are necessary; for example, some diseases appear to be age-

related. It was recommended that T. Lang and S. Mellergaard liaise with the relevant working groups to compile available age/length data for different areas and to report back to the Sub-group next year. This information was previously requested from other working groups, but was not made available to the WGPDMO.

4.3 Presentation of the EPI-INFO Program

The EPI-INFO program is a freely available software program developed by WHO to handle epidemiological data. The program includes: a text editor, a data entry component for creating data bases, a data analysis component which includes a number of statistical tests, analyses of variances and a program for converting EPI-INFO data bases to other data base programs. A short presentation of the program was given and testing and discussion of the applicability of the program for analysing fish disease data was carried out. It was agreed that the EPI-INFO program is not suitable for ICES data analyses, since the program does not include logistic models. It may, however, be potentially useful for individual laboratories.

4.4 Standard Methodology

Definitions

The data analysed are the numbers of fish affected (NAF) and the total number of fish examined (NEX). In the analysis, the dependent variable is NAF/NEX and its variations are explained by a set of explanatory factors such as area, year, season, length-group and sex.

The odds of a fish from Area(1) being affected are given by a/b .

The odds ratio of a fish being affected in Area(1) relative to Area(2) is given by $(a/b)/(c/d)$ or $(a.d)/(b.c)$. For example, an odds ratio of 1.4 for Area(1) relative to Area(2) means that the probability of a fish being diseased in Area(1) is 1.4 times (40%) higher than that in Area(2). In the case of several areas, the odds ratios are usually given by reference to the first area which then has an odds ratio of 1 $((a/b)/(a/b))$.

Logistic models, which are particularly suitable for the analysis of multifactorial contingency tables, automatically transform the raw NAF/NEX data into the log of the disease odds (logistic or logit transformation).

Illustration of a logistic model analysis

For the analysis of ICES data, logistic models are defined using the GLIM software (Baker and Nelder, 1978:

The GLIM System Release 3, Numerical Algorithms Group, Oxford UK). The data are automatically transformed into logit by specifying a binomial error structure. This reflects the nature of the presence/absence of disease.

An example of a logistic model analysis in GLIM is given for a subset of ICES data for cryptocotyle in cod (German data only). This data set includes 11 areas, 2 years (1990 and 1991) and three length classes. In the case of cod, sex and season are omitted. The contingency table has $(11 \times 3 \times 2)$ 66 possible cells of which only 53 contain observations.

| | | Affected | | | |
|---------|--|----------|-------|---------------|--|
| | | YES | NO | | |
| Area(1) | | a | b | a + b | |
| Area(2) | | c | d | c + d | |
| | | a + c | b + d | a + b + c + d | |

Using the GLIM software

```

? $UNITS 53$           Declares the number of observations (units).
? $DATA RECT LEN YEAR NEX NAF$ Declares the names of factors and variables.
? $DINPUT 5$          To read the file (53 observations)

1 1 1 16 12           The data set gives the number observed NAF and NEX
1 2 1 31 27           for each combination of rectangle, length class and year.
1 3 1 99 96
2 1 1 7 1
2 2 1 94 0
2 3 1 46 0
3 1 1 10 0
3 2 1 69 1
...
11 2 2 32 16
11 3 2 22 8

?$FACTORS RECT 11 LEN 3 YEAR 2$ Specifies the number of levels for each factor (11 areas, 3 length
                                groups and 2 years).

?$ERROR BINOMIAL NEX$          Declares the error structure: Binomial with a logit link function
                                for the data transformation.

? $YVAR NAF$                   The number of affected fish is the dependent variable.

? $FIT$                        The null model fit provides the observed overall Log(disease
                                odds).

scaled deviance = 2085.3 d.f. = 52  The degrees of freedom are given by the number of observations
                                (units) minus the number of estimated parameters, here a unique
                                average.

? $DISPLAY ESTIM$
estimate      s.e.      parameter
1      -2.065      0.04628      1

```

All estimations and results are for the Log(disease odds). The overall disease odds is 0.13 ($\exp^{-2.065}$).

Irrespective of the potential significance of the different factors, a model containing all the factors is fitted to the data. Therefore, the data should always be interpreted in collaboration with a disease expert familiar with logistic models. In this example, the factors are the ICES rectangle, the length and the year of observation. The number of estimated Log(odds) is now $11 + (3-1) + (2-1) = 14$ and the number of degrees of freedom becomes $53 - 14 = 39$.

```

? $FIT RECT+LEN+YEAR$
scaled deviance = 42.265 at cycle 10  d.f. = 39

$DISPLAY ESTIM$

```

| | Estimate | s.e. | Parameter | Corresponding ICES rectangle n |
|----|----------|--------|-----------|--------------------------------|
| 1 | 0.7801 | 0.2510 | 1 | 38GO |
| 2 | -4.894 | 0.2648 | RECT(2) | 38G3 |
| 3 | -5.507 | 0.5390 | RECT(3) | 39G3 |
| 4 | -7.414 | 1.015 | RECT(4) | 38G4 |
| 5 | -7.896 | 1.020 | RECT(5) | 38G5 |
| 6 | -16.55 | 32.65 | RECT(6) | 39G8 |
| 7 | -14.49 | 102.6 | RECT(7) | 40G6 |
| 8 | -15.75 | 48.86 | RECT(8) | 39G6 |
| 9 | -15.03 | 55.10 | RECT(9) | 41G8 |
| 10 | -15.73 | 59.57 | RECT(10) | 40G5 |
| 11 | -1.512 | 0.2325 | RECT(11) | 37G1 |
| 12 | 1.103 | 0.1715 | LEN(2) | |
| 13 | 1.505 | 0.2042 | LEN(3) | |
| 14 | -1.047 | 0.2384 | YEAR(2) | |

Parameter estimates are given as Log(odds) for the constant term 1 which is for the first cell in the data table, i.e., RECT(1), LEN(1) and YEAR(1). All other parameters are estimated as Log(odds) ratios relative to the odds given by the parameter 1. The standard errors are given on a Log(odds) ratio scale.

The estimated disease odds for the different rectangles are calculated by taking the antilog (odds ratios). This gives an index of relative disease occurrence for each sampled rectangle, which is being used by the Sub-group for mapping purposes.

Interpretation of GLIM results

| Effect | Relative to | Estimated odds ratio | 95% CI |
|----------|-------------|----------------------|--------------------|
| RECT(2) | RECT(1) | 0.0075 | 1.30 |
| RECT(3) | " | 0.0040 | 1.71 |
| RECT(4) | " | 0.0006 | 2.76 |
| RECT(5) | " | 0.0004 | 2.77 |
| RECT(6) | " | 6.5e ⁻⁸ | 1.5e ¹⁴ |
| RECT(7) | " | 6.9e ⁻⁷ | 3.6e ⁴⁴ |
| RECT(8) | " | 1.4e ⁻⁷ | 1.6e ²¹ |
| RECT(9) | " | 2.9e ⁻⁷ | 8.5e ²³ |
| RECT(10) | " | 1.4e ⁻⁷ | 7.4e ²⁵ |
| RECT(11) | " | 0.22 | 1.26 |
| LEN(2) | LEN(1) | 3.04 | 1.19 |
| LEN(3) | LEN(1) | 4.05 | 1.23 |
| YEAR(2) | YEAR(1) | 0.35 | 1.27 |

At present, the analyses focus on differences between ICES rectangles. However, the estimated relative odds

ratios for the different rectangles are adjusted by GLIM for the other factors included in the analysis.

The odds of cryptococyle infections in cod are much higher in rectangles (1) and (11) than in other rectangles. There is an increase in the infection odds with length. The probabilities of infection are, in length class (2) 3.04 times and in length class (3) 4.05 times higher than in length class (1). The infection odds are lower for 1991 than for 1990 (0.35 vs. 1.00). A 95% confidence interval can be computed as 1.96 x s.e. on each side of the estimate. It is apparent in the above table that the standard errors of the relative odds ratios by ICES rectangle are very large and also include the value 1. There are no statistically significant differences between rectangles. Therefore, the predicted infection odds ratio should not be used for correlation with any variable and a map cannot be produced.

A fixed model structure

The necessary model structure is taken to include all the potential explanatory factors. The fixed model structure is RECTANGLE + YEAR + SEASON + SEX + LENGTH, or just RECTANGLE + YEAR + LENGTH, depending on the sampling design (e.g., all cruises in one season), and host species (e.g., the sex of cod is not noted). The advantage of a general model structure is that it can be used for all hosts and all diseases/infections and, therefore, provide a common basis for comparison. However, several methodological problems remain to be solved before a single model structure can be recommended. The sampling design can become unbalanced for data collected over several years by different countries. The necessity of a reference point in GLIM has to be investigated, and the robustness of relative disease odds needs to be evaluated for a variety of cases.

5 LIVER NODULES

5.1 Definition

The definition for liver cell adenoma used by the Subgroup is that of Myers *et al.*, 1991 (Environmental Health Perspectives 90, 7-15): "Liver cell adenomas are characterised by compression of the surrounding parenchyma, well defined separation of proliferative tissue from normal tissue, fairly normal architecture, and relative absence of other hepatic elements. Liver cell adenomas are usually basophilic, but eosinophilic and vacuolated variants do occur".

5.2 Evaluation of Samples Submitted for Histological Confirmation to D. Bucke

Following the above definition (Section 5.1), it was evident that not all nodules ≥ 2 mm in diameter evaluated in dab livers had a definitive neoplastic characteristic. Four categories were recognised: a) no obvious abnormalities; b) pathological changes - i.e., necroses, inflammatory reactions, melanomacrophage cell proliferations, cysts; c) hepatic cell foci - i.e., eosinophilic, basophilic and clear cell foci of cellular alteration; d) neoplastic lesions - i.e., hepatocellularadenoma, carcinoma and cholangioma. A. D. Vethaak demonstrated the relationship between maximum size of liver nodules and the length/age of individual flounder, showing that nodules with a size of ≥ 5 mm in diameter were mostly neoplasms.

The necessity for histological confirmation is for all nodules with a diameter ≥ 2 mm in diameter. It was agreed that D. Bucke will continue to evaluate liver nodules ≥ 2 mm in dab and flounder and will make the results available to be included in the ICES data base by the end of 1993.

6 ANALYSIS OF EXAMPLES

6.1 Introduction

According to the terms of reference, the analysis of the data focussed on cod and flounder data from both the North Sea and the Baltic Sea and on confirmed cases of liver nodules in North Sea dab. Data analysis was carried out using GLIM, following the procedure described in Section 4.4 on the data subsets listed below with subsequent plotting of the results in ICES rectangle maps. Estimates of relative disease odds for skin ulcer in cod based on the model RECT + YEAR + SEASON + LENGTH. Figures 1a and 1b, 2a and 2b, and 3a and 3b are presented below.

- Skin ulcers in cod (*Gadus morhua*), 1991/1992 data subset (Figures 1a and 1b).
- Lymphocystis in flounder (*Platichthys flesus*), 1991 data subset (Figures 2a and 2b).
- Hepatic neoplasia in dab (*Limanda limanda*), confirmed nodules ≥ 2 mm in diameter in female fish, ≥ 25 cm in length (Figures 3a and 3b).

Figure 1a Map indicating the sampled rectangles from which data were used for the logistic analyses of skin ulcers in cod.

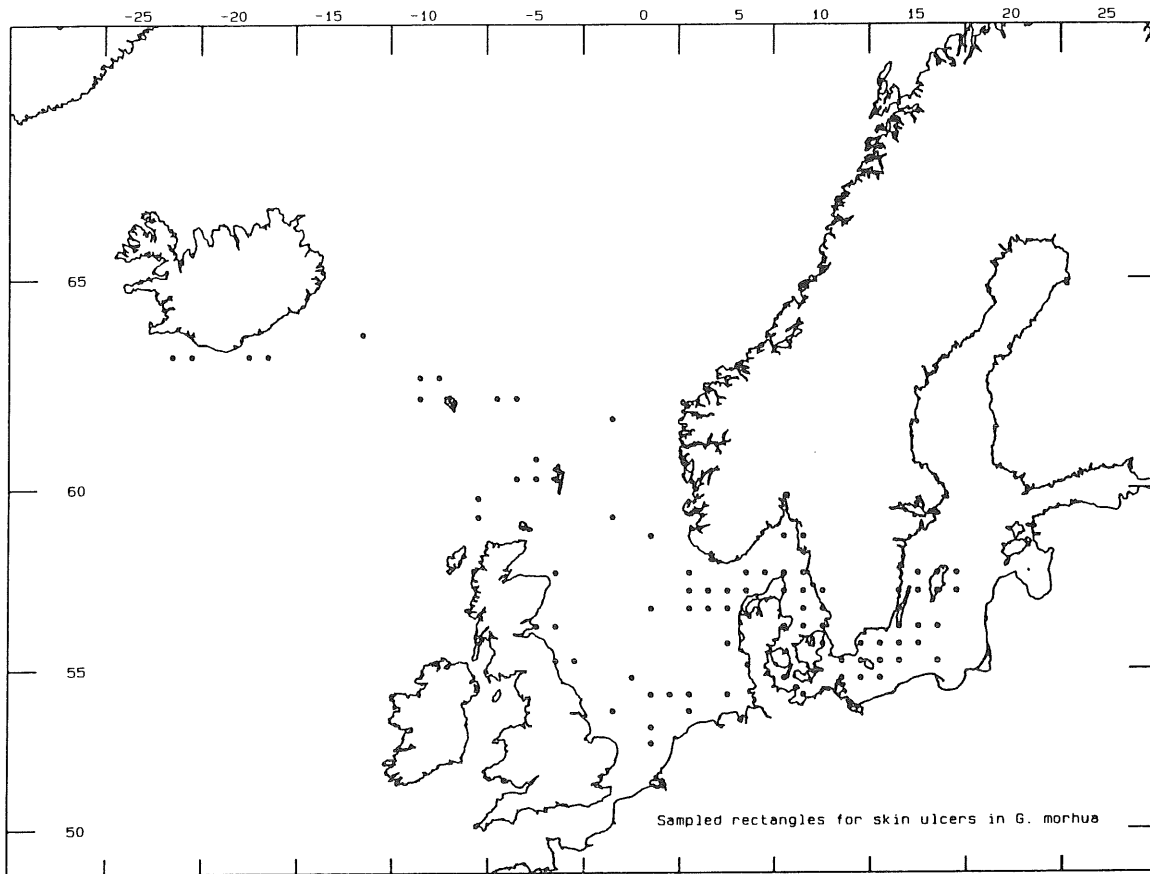


Figure 1b Estimates of relative disease odds for skin ulcer in cod based on the model RECT + YEAR + SEASON + LENGTH.

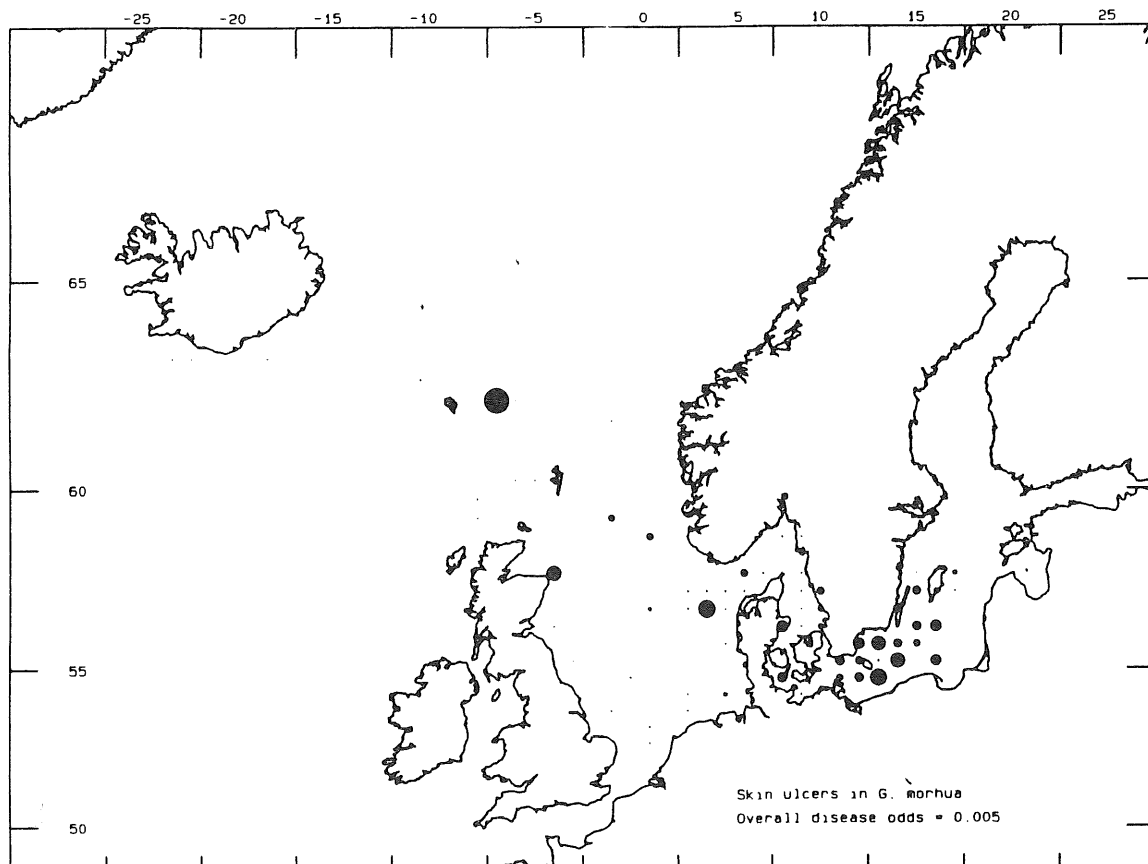


Figure 2a Map indicating the sampled rectangles from which data were used for the logistic analysis of lymphocystis in flounder.

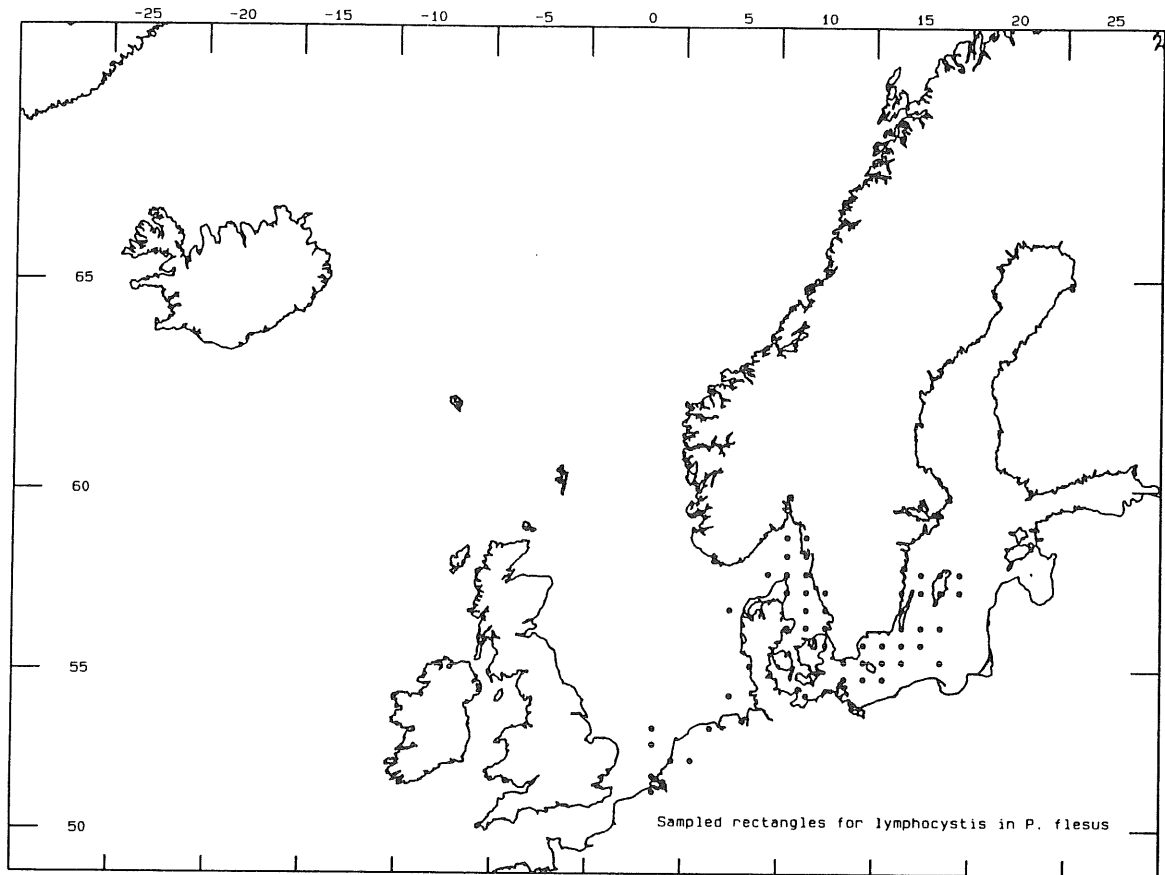


Figure 2b Estimates of relative disease odds for lymphocystis based on the model RECT + SEASON + LENGTH.

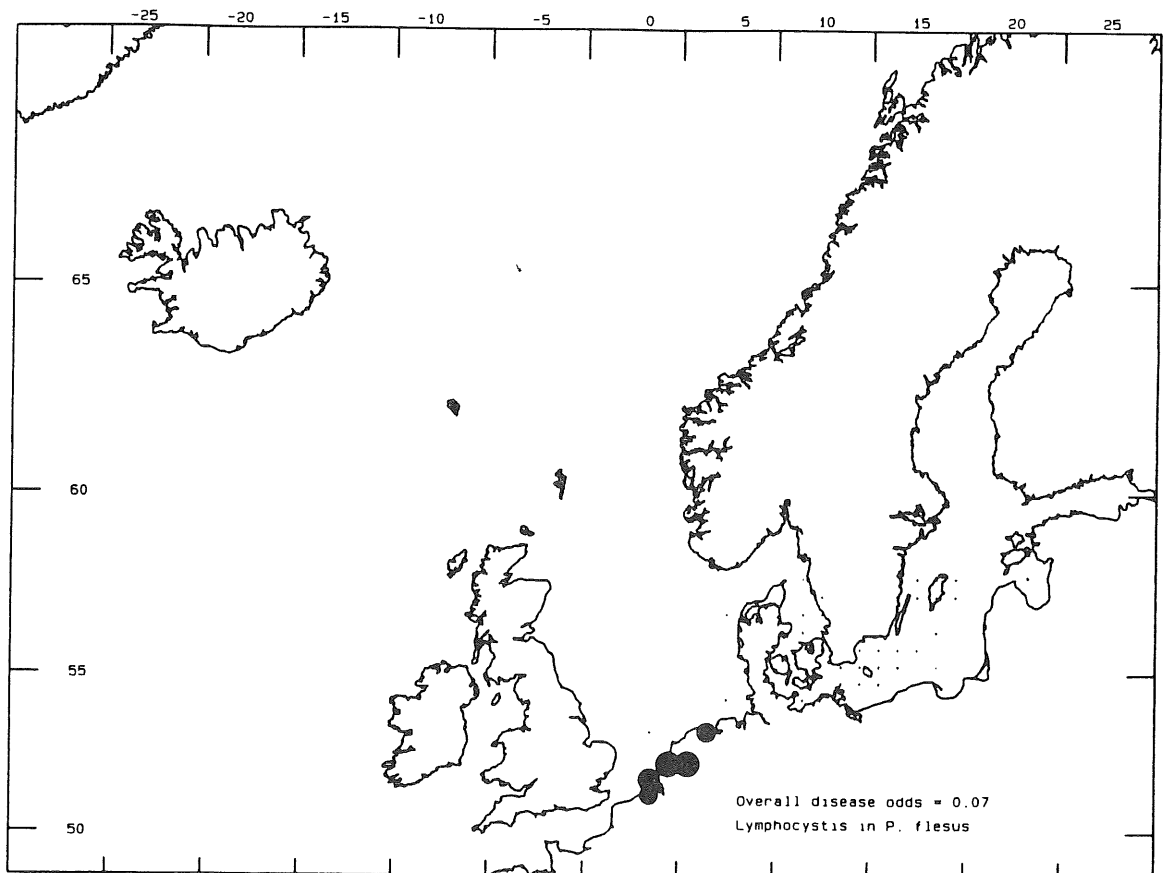


Figure 3a Map indicating the sampled rectangles from which data were used for the logistic analysis of hepatic neoplasia ≥ 2 mm in diameter in female dab ≥ 25 cm in length.

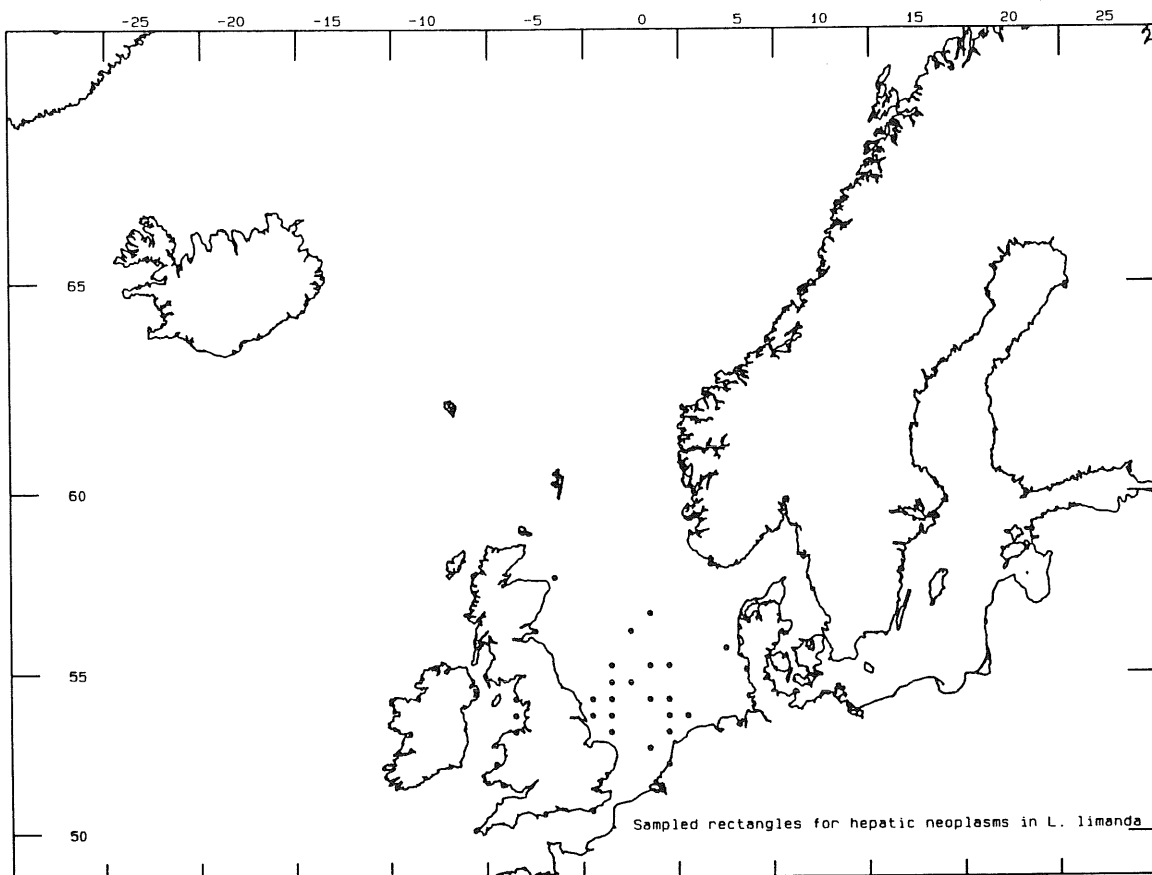
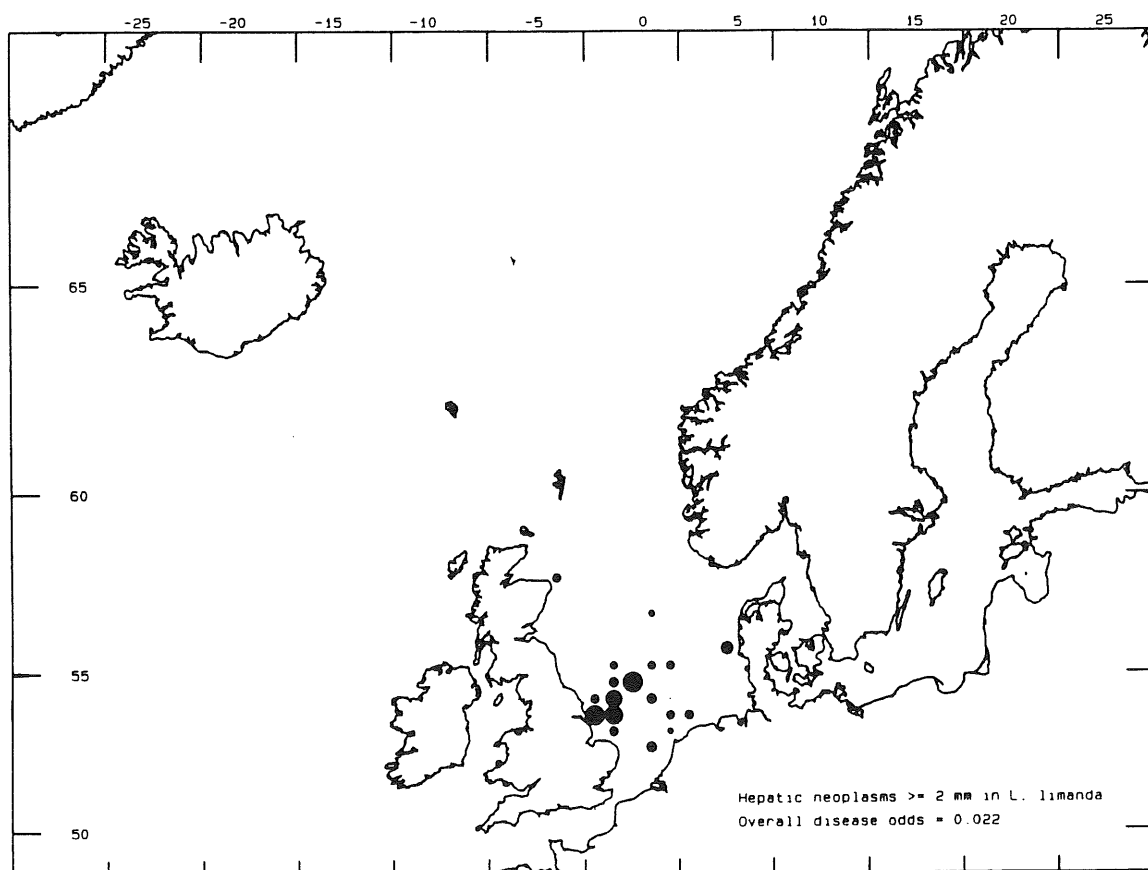


Figure 3b Estimates of relative disease odds for hepatic neoplasia based on the model RECT + SEASON + LENGTH.



Conclusions and Future Work

Using the above examples, several shortcomings in the available data sets were identified which required further attention, including: unbalanced design and choice of reference site in relation to relative disease odds ratio. Particularly, the problem of the large standard errors estimates due to unbalanced data design will require further investigation.

Due to unresolved methodological problems, a decision could not be made on a standard procedure for data analysis to be employed by ICES. Therefore, it is essential that a number of participants (des Clers, Vethaak and other experts, e.g., Carr) communicate intersessionally, preferably by e-mail to assist and guide ICES, no later than June 1993, in the analysis of the fish disease data in order to further facilitate the availability of summary data prior to the 1994 Sub-group meeting.

7 ICES SEDIMENT CONTAMINANT MAPS

J. R. Larsen presented examples of available maps showing the distribution of some contaminants in sediments within ICES rectangles and proposed that similar fish disease prevalence maps should be correlated. The group decided to focus on some contaminants, both organic and inorganic, for correlation purposes in this meeting. However, for definite analysis of the results, A. D. Vethaak will seek advice from the appropriate working groups regarding the most relevant contaminants in terms of bioactivity and bioavailability. He will report his findings at the next sub-group meeting.

8 CORRELATIONS OF DISEASE MAPS WITH SEDIMENT CONTAMINANT MAPS

The incompleteness of disease data maps and the apparent incompleteness of sediment data maps precluded attempts to make correlations between disease and pollution. Where contaminants are below detectable levels, the ICES rectangles that have been sampled should be indicated on all maps used for disease correlations.

9 ANY OTHER BUSINESS

9.1 ICES Fish Diseases Training Guide

It was reported that the ICES Fish Diseases Training Guide is in the final stages of preparation. This information was welcomed by the Sub-group as a

valuable aid to standardisation and quality assurance during data collection.

10 CONCLUSIONS/RECOMMENDATIONS

- 10.1 Disease data available for species other than dab (cod and flounder), including data from the Baltic area, were not in presentable form for analysis.
- 10.2 Due to the incompleteness of disease and sediment contaminant maps, it has not yet been possible to make comparisons between them.
- 10.3 Available age/length data should be collected and compiled by T. Lang and S. Mellergaard and presented at the next Sub-group meeting. Age/length keys are necessary for the interpretation of disease data. For liver neoplasms, it is necessary that affected dab are individually-aged.
- 10.4 It is recommended that the proposed new fish disease reporting format should replace previously used ICES disease reporting forms and that data should be submitted to ICES on diskettes.
- 10.5 Disease monitoring programmes must include data on liver nodules in dab and/or flounder because it has been reported that a strong association between the occurrence of liver nodules and contaminants in sediments may exist. Only those nodules ≥ 2 mm diameter should be categorised by histology and confirmed by D. Bucke. Insufficient information on the aetiology of liver neoplasms is available and further research is recommended.
- 10.6 A. D. Vethaak should consult appropriate working groups to determine the most relevant contaminant data maps to be used for comparison with fish disease data.
- 10.7 An intersessional advisory group should work by correspondence and e-mail to advise ICES no later than June 1993 on the methodology of data processing. ICES should prepare, to the extent possible, an analysis of fish disease data as described in the text of this report and make this available to the WGPDMO Sub-group by the end of February 1994.
- 10.8 Fully-validated disease data from previous years, whether previously submitted to ICES or not, should be incorporated into the new ICES disease reporting format and submitted to ICES by 1 September 1993.

10.9 The Sub-group should meet immediately prior to the WGPDMO meeting in 1994:

a) to evaluate disease data maps and tables compiled by ICES for species in the North Sea and Baltic Sea,

b) to further develop fish disease data validation and analytical methods,

c) to compare analysed fish disease data with available sediment contamination maps provided by ICES.

APPENDIX 1

Sub-group of Working Group on Pathology and Diseases of Marine Organisms

List of Participants

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|----------------------------|---|-----------------|-----------------|
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APPENDIX 2

Sub-group of Working Group on Pathology and Diseases of Marine Organisms

ICES Headquarters, Copenhagen 1993

AGENDA ADOPTED

1. Opening of the meeting. Background information.
2. Selection of rapporteurs, adoption of agenda, terms of reference.
3. EPI-INFO, a new statistics program for epidemiology on microcomputers: introduction, demonstration, possible use by ICES or sub-group participants.
4. Status of update by ICES of existing database. Presentation of disease data available for analysis.
5. Other methodological considerations.
6. Liver nodules: Review of current knowledge and histological confirmation of existing fixed material.
7. Data treatment within sub-groups.
8. General evaluation and discussion of results obtained.
9. Correlation of disease patterns with sediment contamination maps provided by ICES.
10. General evaluation and discussion of results obtained.
11. Any other business.
12. Conclusions and recommendations.
13. Approval of the draft sub-group report.
14. Closing of the meeting.

ANNEX C

Sample size determination
Sophie des Clers, Imperial College

1) Sample size to detect an infection (or disease)

The sample size for detection is determined from three quantities:

- the apparent prevalence expected in the population,
- the accepted level of risk α (alpha) attached to sampling, and
- the sensitivity of the diagnostic method used for detection.

For large populations and rare diseases, the exact binomial distribution for the number of diseased fish in the population is well approximated by the simpler Poisson distribution. This gives the chance of finding no infected fish in the sample of n fish as:

With *prev* the true proportion of affected fish in the population.

Detecting an infection is the opposite of missing it with the probability $p(0)$. The probability of detecting the infection is therefore $1 - p(0)$. Correcting the population prevalence *prev* for the sensitivity of the diagnostic method *sens* and accepting a risk α that 5% of all random samples taken have no infected fish when the population is indeed infected, implies that in 95% of the samples ($1-\alpha$) there will be at least one infected fish:

$$1 - \alpha = 1 - p(0) = 1 - e^{-n \cdot \text{prev} \cdot \text{sens}}$$

Therefore, the sample size necessary to obtain at least one infected fish depends only on the expected true population prevalence *prev* (a proportion) corrected for the diagnostic sensitivity *sens* and on the risk α and we have

$$n = - \frac{\text{Log}(\alpha)}{\text{prev} \cdot \text{sens}}$$

For a risk *alpha* of 0.05 (5%) gives $-\text{Log}(0.05)=2.99$. This has been called the "rule of three".

Sample size to obtain at least one affected fish

$$n = \frac{3}{\text{prev} \cdot \text{sens}}$$

Assuming a diagnostic sensitivity of 75% (*sens*=0.75), still with a sampling risk α of 5% gives a rule of 4.

$$n = \frac{4}{\text{prev}}$$

The link between sample size, true population prevalence and diagnostic sensitivity is given in Figure 1.

At high prevalences, an improved diagnostic does not make much difference on the sample size necessary. Resources may be saved by sampling more fish with a cheaper diagnostic. The decision depends on the cost of diagnosis per fish and the difference in relative costs of the two protocols which can easily be compared if the sensitivities of both methods are known.

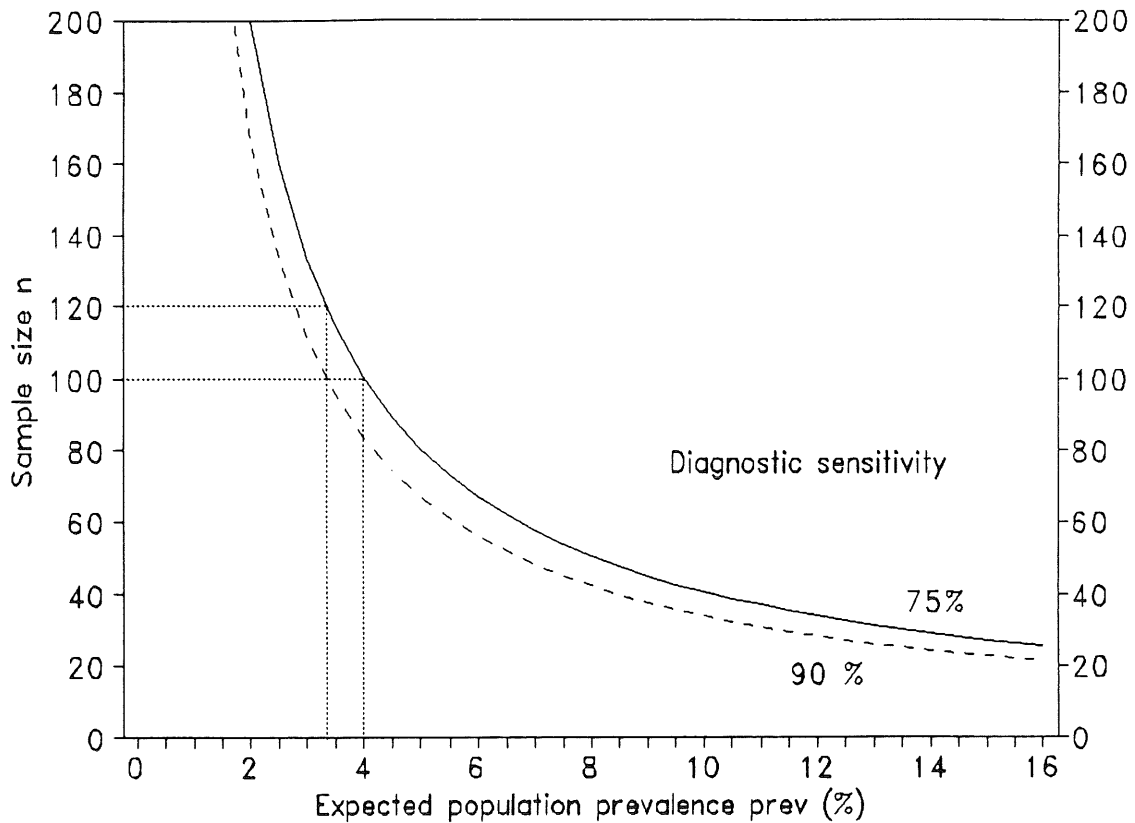


Figure 1. Sample sizes to detect rare infections in large populations for a diagnostic sensitivity of 75% and 90% ($\alpha = 0.05$)

2) Sample size to estimate prevalence

2.1) Precision of a sample value

The apparent prevalence of an infection in a population of N fish is given by

$$prev.sens = D.sens/N,$$

the true population prevalence $prev$ corrected for the sensitivity of the diagnostic method $sens$, with D the number of infected fish. The precision of an estimate from just one sample of fish increases with the number of fish sampled. In a large population, the apparent prevalence in a sample of n fish given by the hypergeometric probability distribution can be approximated by the binomial, with mean $prev.sens$ and standard error.

$$S.E. = \sqrt{\frac{prev.sens.(1-prev.sens)}{n}}$$

2.2) Confidence interval

When the sample is large enough, a confidence interval for the sample prevalence is obtained using the Normal approximation to the Binomial. The sampling risk of 5% corresponds to an absolute precision ($absprec$) of 1.96 the standard error on each side

of the sample prevalence value.

$$\text{prev.sens} \pm \text{absprec}, \quad \text{with } \text{absprec} = 1.96 \sqrt{\frac{\text{prev.sens} \cdot (1 - \text{prev.sens})}{n}}$$

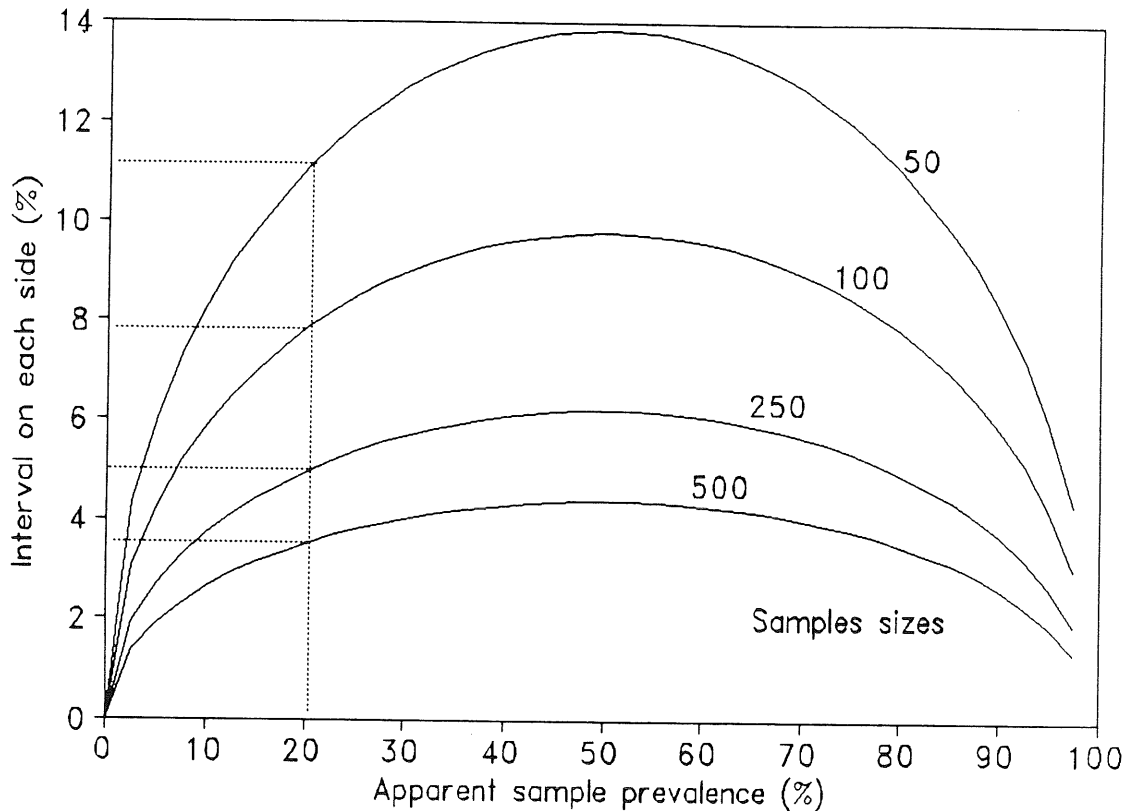


Figure 2 Precision around the apparent sample prevalence

The precision obviously deteriorates with decreasing sample sizes.

2.3) Sample size to achieve a precision

By rounding up 1.96 to 2 and taking the square of both sides in the expression for the confidence interval around the sample prevalence, the sample size n necessary to achieve an absolute precision absprec is

$$n \approx 4 \cdot \frac{\text{prev.sens} \cdot (1 - \text{prev.sens})}{\text{absprec}^2}$$

For example, accepting a sampling risk of 5%, the sample size necessary to estimate a suspected apparent prevalence of 6% for a *Dactylogyrus* infection in a large population of common carp with a confidence interval $\pm 4\%$ (i.e. this gives a 95% chance that the population prevalence is within 4% on each side of the sample value) is $n = 4 \cdot (0.06)(0.94) / ((0.04)^2) = 141$ fish. With 141 fish, an absolute precision of 4% means

that, 19 out of 20 samples ($\alpha=0.05$) would have a prevalence between 2% and 10%. This is as much precision as one can expect with more than twice the number of fish ($4/0.06=67$) needed for detection only.

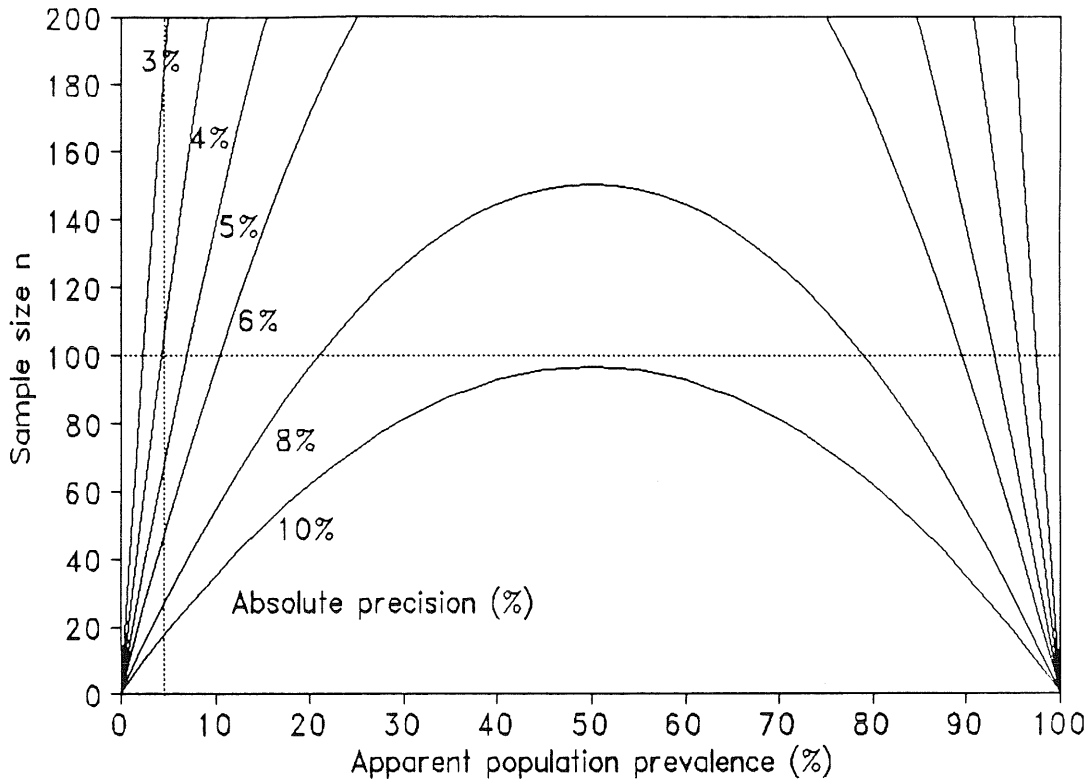


Figure 3 Sample sizes to achieve a given precision $\alpha = 0.05$

As the suspected population prevalence is higher, *absprec* becomes relatively higher, and more fish are necessary to obtain a confidence interval of the same width, for example, to estimate a suspected prevalence of 25%, with an absolute precision (*absprec*) of 4% on both sides of 25%, $n=4 \cdot (0.25)(0.75)/(0.04)^2 = 469$ fish are needed.

2.4) Testing the significance of a difference between prevalence values

In the case of a large population, and a large sample, we can use the normal approximation to binomial distribution to test the assumption that a sample value is consistent with a known, previously determined, population prevalence.

The normal statistic z_c is computed from the difference between the known population value *prev.sens* and a sample estimate *sprev.ssens*, divided by the population standard deviation can be calculated as:

The value of z_c , which would be zero if the sample estimate was equal to the population mean, is then checked against the table of a standardised normal deviate, to find the probability of a difference as large as the one observed. Accepting a sampling risk of 5%, one can either test that the sample prevalence is significantly smaller or larger than the

$$z_c = \frac{(|(sprev.ssens - prev.sens)|)}{\sqrt{\frac{prev.sens \cdot (1 - prev.sens)}{n}}}$$

known population value, a one-sided test, and the critical value for z_c is 1.96. Or one can test that the sample value is about the same (a double-sided test) as the population value, and the critical value for z_c is then 3.92. This is equivalent to test whether the prevalence value from the sample is inside the 5% confidence interval around the population value.

We proceed similarly to compare two sample prevalence values, or can use a χ^2 (Chi-square) test on the squared differences of the two sample values, which is strictly equivalent to the two-sided test given above. A χ^2 test with one degree of freedom has a 5% significance level of 3.84, which is simply the square of the value of 1.96 given above.

2.5) Sample size to detect a significant difference

It is possible, by using the Normal approximation given above, to estimate the sample size necessary to detect a difference between two sample prevalence values. In order to use published tables, one needs to collect samples of equal sizes and makes comparisons two by two. Alternatively, use the smallest size for the two samples to be compared. This may be particularly useful to assess the efficacy of a programme to control an infection. The minimum sample size (for both samples) needed to detect a difference of prevalence in two independent samples are computed from the improved approximation to the binomial case of Casagrande, Pike and Smith (1978) and given in Table 1. In comparing the two unknown population prevalence values now sampled, there are now two types of sampling risks. The sampling risk α (alpha) fixed at 5% to declare the two sample prevalence values different, when in fact the population values are the same. The sampling risk β fixed at 10%, to fail to detect a difference which is there. The proportions of infected fish in each sample p_1 and p_2 are apparent prevalence values, that is true prevalence values, corrected by the diagnostic sensitivities.

For example, 106 fish in each of the two samples make it possible to detect a 20% increase in prevalence from a value of 25%, but 1404 fish are necessary to find a significant difference between the two sample values when the increase is 5% from a value of 25%. Just as before, more fish are needed for prevalence values around 50%, to obtain the same absolute precision.

Most of all this table is useful to assess the detection power that corresponds to a feasible sample size or to a fixed budget. It can also be used, for example, to assess the magnitude of control needed to reduce an infection and obtain a significant effect, for different sampling efforts.

ANNEX 5

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

ANALYSIS OF PROGRESS WITH TASKS

- | | | | |
|------|--|-------|--|
| 1. | <u>Tasks completed.</u> | | An interessionally prepared report of the current situation is included with this report |
| i) | Analysis of recent trends of diseases in wild fish. Data submitted on new diseases and changes in the occurrence of previously known diseases in wild marine fish were evaluated and conclusions are presented in this report. | | and conclusions on trends and causes were made by WGPDMO. |
| ii) | Analyse national reports on disease trends in mariculture. Trends were considered, correlations between different countries investigated and advice offered on possible causes and control strategies. | vii) | Analyse data on existing fish vaccines and vaccines under development. Intersessionally prepared reports on (a) the efficacy of existing commercially available fish vaccines and (b) the current status of vaccines under development, are included with this report and their value in marine fish farming is assessed. |
| iii) | Analyse updated information on disease interactions between wild and farmed fish. Reports of studies in the area of possible disease transmission between farmed and wild fish, which are currently in progress in several countries, were assessed, but the data currently available did not permit WGPDMO to offer concrete advice. | viii) | Consideration of the usefulness of <i>ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish</i> . The status of the diagnostic fiches was discussed and it was agreed that the fiches remain a useful tool/or dissemination of toxic information. Due to the number of fiches already published, the need for regular publication of groups of ten fish has diminished. Fiches will continue to be published in smaller groups (3 or 4) with an emphasis on areas of interest from involved members. It was also agreed that older fiches will have to be revised to include new information that is now available. |
| iv) | Review the results of the sub-group who met to consider methods of statistical analysis of disease prevalence in wild marine fish stocks. The sub-group report was considered and endorsed by WGPDMO. | | |
| v) | Analyse current research on mollusc diseases in ICES member countries in order to standardise methodological approaches. The review of the current research on molluscs has indicated important requirements in mollusc disease research and proposals on these were made by the WGPDMO. | 2. | <u>Tasks to be continued.</u> |
| | | i) | Disease diagnostic fiches. |
| | | 3. | <u>Tasks on which progress has not been made.</u> None. |
| vi) | Analyse data on changes in the resistance profiles of fish disease to chemotherapeutants in mariculture. | 4. | <u>New tasks.</u> None. |

I C E S

**Working group on pathology and
diseases on Marine Organisms
Copenhagen 15 - 18 March 1993**

Current research on Molluscs diseases

General report prepared by H. Grizel.

With the participation :

Sharon E. Mc Gladdery from Canada (east part)

Suzan Bower from Canada (west part)

Frank Perkins from U.S.A.

Dave Bucke from U.K.

Francisco Ruano from Portugal.



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IFREMER
Laboratoire de pathologie et de génétique des Invertébrés marins
B.P. 133
17390, La Tremblade
France

January 19, 1993

Dear Dr. Grizel:

Please excuse the delay in replying to your request for information, dated December 3rd, 1992. We are currently in the process of drawing up protocols for the upcoming addition of shellfish to the Canadian Fish Health Protection Regulations (FHPR) and, since this may be relevant to the proposed one-day joint meeting with WGITMO under Recommendation 5, I will give you a brief summary of that as well.

Atlantic Coast of Canada

- 1) Research in epizootiological surveys:
 - a) Summer mussel (*Mytilus edulis*) mortality: Investigation of summer mussel mortality on the Magdalen Islands, includes genetic studies, mussel physiological studies as well as histopathology (in collaboration with Dr. A. Figueras). This investigation is being coordinated by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), Ministère des Pêches et Océans Canada (MPO) et l'Université du Québec à Rimouski (UQAR).
 - b) Giant sea scallop (*Placopecten magellanicus*) mortality: Abnormal haemocyte morphology was observed in tissue samples of scallops undergoing a mass mortality on the northern shore of the Gulf of St. Lawrence last year. No other obvious histopathology was observed. The exact aetiology of the haemocyte changes is still being investigated. Unfortunately no samples were preserved for electron microscopy.
- 2) Experimental pathology
 - a) Malpeque disease of eastern oyster (*Crassostrea virginica*): Transfers of Cape Breton ("susceptible" stocks) to PEI and NB will be carried out this year. Mortalities will be examined to determine whether or not Malpeque disease is still virulent in Gulf of St. Lawrence waters.
 - b) *Pseudoklossia*-like parasite of bay scallop (*Argopecten irradians*): This parasite was implicated in mass mortalities of bay scallops held for experimental purposes last year. The parasite showed abnormal systemic proliferation under the experimental conditions. The cause of this proliferation is under investigation (in collaboration with the Atlantic Veterinary College (AVC)).
 - c) *Chytrid*-like infection of quahaugs (*Mercenaria*): This infection was responsible for mass mortalities of broodstock quahaugs under hatchery conditions. The exact identity of the organism is being studied along with its distribution and pathogenicity in open water.



Pacific Biological Station
Nanaimo, B.C.
V9R 5K6

FAX: 506-851-773

Your file Votre référence

Our file Notre référence

8469-

January 15, 199

Dr. Sharon McGladdery
Department of Fisheries and Oceans
Gulf Fisheries Centre
P.O. Box 5030
Moncton, N.B. E1C 9B6

Dear Sharon:

As you requested, enclosed is a summary of the "current research on mollusc diseases" on the west coast of Canada that you can include in your submission to Dr. Henri Grizel for his report to the Working Group on Pathology and Diseases of Marine Organisms.

- 1) Research in epizootiological surveys:
 - a) Rickettsia-like infection of fixed macrophages of prawns (Pandalus platyceros) in Howe Sound, British Columbia. The cause of disease was originally determined from histology and electron microscopy. Experimentation indicated that the best method to assay for the prevalence of this disease was to look for gross signs in the digestive gland of freshly dissected living prawns and by using histology to verify the few questionable cases.
 - b) Hematodinium-like infection in the haemolymph of prawns (Pandalus platyceros) in Malaspina Strait, British Columbia. Histological examination of the heart and digestive gland is at least ten times more sensitive in detecting infection than by gross examination.
 - c) Unknown protozoan disease (SPX) of Japanese scallops (Patinopecten yessoensis) cultured in British Columbia. Although high mortalities are caused by this parasite, histology is required to confirm the presence of the pathogen.
- 2) Experimental pathology:

Laboratory experiments are used to identify the method of transmission, pathogenicity, and developmental morphology of the Rickettsia-like and Hematodinium-like infections of prawns and SPX in Japanese scallops. Laboratory studies are also being used to determine the pathogenicity and host specificity of Mikrocytos mackini the cause of Denman Island disease in Pacific oysters (Crassostrea gigas).
- 3) Diagnostic methods:

In conjunction with a visiting postdoctoral fellow (Dr. Dominique Hervio), we are attempting to produce monoclonal antibodies against Mikrocytos mackini which will be used for immunodiagnosis. Also, in collaboration with Dr. Bob Lester's laboratory (University of Queensland, Australia), a M. mackini fragment is being sequenced and this will eventually be used in diagnostic work.

.... / 2

- 3) Diagnostic methods
- a) Viral gametocytic hypertrophy of eastern oyster (*C. virginica*): Monoclonal and polyclonal antibodies are being developed for a papillomavirus of eastern oyster gametes and possible use for immunodiagnosis. In collaboration with IFREMER and the AVC.
 - b) Monoclonal antibodies have been produced to *Perkinsus karlssoni* of bay scallops and another *Perkinsus* parasite of Atlantic salmon (*Salmo salar*) by Dr. R.J. Cawthorn and Ms. B. Déspres (AVC). Polyclonal antibodies have also been produced for the *Chytrid*-like parasite of quahaugs. These researchers are also developing a DNA probe for the *Perkinsus* parasites. All techniques are being investigated for diagnostic applicability.

Pacific Coast of Canada

I am attaching information from Dr. Susan Bower, Pacific Biological Station, who is currently carrying out research on diagnostic tools for certain shellfish pathogens.

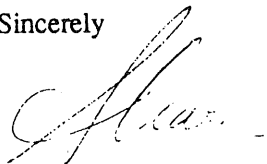
Canadian Fish Health Protection Regulations - Summary of suggested shellfish protocols

Disease-screening of molluscs being introduced into Canada or transferred within Canada is, to date, non-specific due to the number of different pathogens, uncertain pathogenicity, asymptomatic carriers and the continued observation of "new" parasites and pathogens. Specific diagnostic tools are being investigated for future application to disease-screening. To date, however, they are reserved for experimental use, either to enhance quantification in situations where a specific pathogen is known to occur (eg., *Mikrocytos mackini*) or provide additional screening to base-line histology (eg., thioglycollate for *Perkinsus* spp). Tissue samples collected before removal of histology sections may be used for bacterial and mycological culture. Virology is limited to histology detection and EM identification. We are not considering use of finfish cell-lines for detection of intra-cellular prokaryotes. Although the specification of histology is limited in sensitivity and application, we hope to redefine diagnostic protocols once we narrow down the list of disease agents which we need to diagnose.

Dr. Bower is also a member of the scientific advisory group for the shellfish portion of the Canadian Fish Health Protection Regulations. The head of this advisory group is Ms. Iola M. Price, Director of Aquaculture & Resource Development, Biological Sciences Directorate, DFO Ottawa.

I hope that these summaries are of use at the upcoming ICES meetings and am sorry that I am unable to attend.

Sincerely



Sharon E. McGladdery

c.c. Iola Price
Susan Bower

4) Immunology:

A Ph.D. student is currently work on stress response in the Pacific oyster. Some of this work examines the response of oyster haemocytes to pollution and changes in temperature and salinity. There are plans to expand the work to include studies on the effect of pathogens.

I am sending the original copy of this report by surface mail.

Yours sincerely,


Susan Bower

ICES - WGPDMO

Copenhagen 15-18 March 1993.

- Report -

MOLLUSCAN DISEASE RESEARCH IN FRANCE

1 - METHODOLOGY

Different diagnostic tools have been prepared during the last years against some pathogens. The most important are monoclonal and polyclonal antibodies against *Marteilia refringens*, *Marteilia maurini*, *Perkinsus atlanticus*, *Vibrio P₁*, a *Rickettsia* of *Pecten maximus* and *Bonamia ostreae*. Some DNA probes have also been prepared. But, instead, of the disponibility of these reagents, none is available for a current use in routine diagnostic. The tentative to obtain a performant method using monoclonal antibodies in a ELISA kit has failed. The reasons of the failure will be explained in the general analysis.

Consequently as, F. Perkins noticed, the standard techniques stay available in molluscan disease research, mainly when an abnormal mortality occurs.

On an other hand, research are still in progress in order to get mollusc cells line. Different complementary research ways are explored by a network. The main topics are :

- the role of non specific or specific growth factors (test and identification),
- the role of molecules such as cycline on cell multiplication,
- test of different medium,
- test of different tissus coming from larvae and adults,
- modification and definition of general methodology (dilaceration, digestion, antibodies, etc...),
- cells transformation using oncogenic genes coming from invertebrates or vertebrates.

2 - EPIDEMIOLOGY

Nothing really new occured during these last years. As mentioned last year, *Bonamia* and *Marteilia* situation seems stabilized, with some regression of *Marteilia* in some Britany rivers (e.g. Morlaix St-Brieuc and Cancale. The situation of *Vibrio P₁* is quite the same.

Experimental infections have been conduced with *Bonamia ostrea* in laboratory in order to know the eventual role of carrier of *Crassostrea gigas*. For the different cases (proximity, inoculation) we never founded a development of bonamiosis infection in *Crassostrea gigas* and we never founded transmission of Bonamiosis from *C. gigas* to the normal host, *Ostrea edulis*. All the data concerning the possible role of carrier of *C. gigas* for *Bonamia* and *Marteilia* diseases have been compiled in a special report fort the EEC authorities. Submitted to an european group for experts, *C. gigas* has been recognized officialy as non carrier for *Bonamia ostrea* and *Marteilia refringens*.

This kind of experiments should be utilized as reference for researchs in the same topics.

3 - LEGISLATION AND STANDARDS

In Europe, a new EEC Directive (91/67) defined the rules of transfert for the products of aquaculture inside the EEC countries and between EEC countries and the third countries. A standardization of the diagnostic methods has been adopted in order to define the agreement of zones for *Marteilia* and *Bonamia* diseases. The first disease must be check one time per year, during automn season after the infections period, and the second one must be check twice a year (spring and automn). According to the statistics data the minimum size of each sample must be 150 oysters.

The details of the rules are contained in annex. The same rules and recommendations have been adopted by the O.I.E. (Office International des Epizooties).

4 - GENETIC RESEARCH

At this time, according to the presence of Protozooans diseases in France, the main research in genetic concern selection technics. After the negative responses obtained (1) with different flat oysters species (2), with different geographical strains of *O. edulis*, we have tested for *O. edulis*, the intrapopulation variability of resistance to *Bonamia ostreae*.

Thje first results obtained with F_1 and F_2 generations have shown significant differences on the mortalities level and on the prevalence.between control and selected oysters.

In order to understand the mechanism of the eventual transmission of resistant genes, a genetic breeding plan have been prepared for this year. One of difficulties to realize the plan concern the mashership of the simultaneous maturation of a large quantity of flat oyster reared each one in a separated beaker.

La Tremblade, March 10th 1993.

H. GRIZEL.

a) THE EPIDEMIOLOGICAL SITUATION OF THE MOST IMPORTANT BIVALVES SPECIES IN PORTUGAL DURING 1992.

GROOVED CARPET SHELL CLAM *Ruditapes decussatus*:

Production of this specie is the most significant of portuguese aquaculture, and it has been strongly affected by the protozoan pathogen - *Perkinsus atlanticus*, directly involved in the drastic drops of production since 1984, 8 000 t to 3 000 t actually.

The prevalence of the agent, the most serious in portuguese Aquaculture, reach its maximum value in the year of 1986 - 70%, affecting the whole area of production in the south coasts of Portugal (Faro/Olhão and Alvor coastal lagoons in Algarve), decreasing to 50% actually.

Other agents also affects this specie, but with no significant pathogenic consequences on the populations (see attached table).

PORTUGUESE OYSTER *Crassostrea angulata*:

The production of this specie, 150 t/year, is actually confined to a few active wild beds, located in a area of approximately 500 ha in Sado river estuary. During the past 3 years and particularly in 1992, these natural beds shows signals of recovering, by the improvements observed on the internal and external shape, growth and seed settling rates.

The most serious pathogenic problems observed were:

- Necrosis of the abductor muscle, in its insertion on the shell, know as "Maladie du pie" or "Foot disease" - affecting less than 20% of the population. The causal agent were not yet clearly identified, several strains of bacteria has been isolated.

- Ciliates, such as *Ancistrum sp*, *Trichodina* and *Boveria sp*, the flagellate *Hexaminta inflata*, are also present in a very small prevalence, varying from 10 to 15% along the year, but with no evidence of pathogenic effects on the populations.

FLAT OYSTERS - *Ostrea edulis*.

Using the long line offshore system, flat oysters are raising in the Southeast coast of Portugal, reaching the production of 130 t in 1992.

This production is based on the importation of seed from french farms, certified by the IFREMER as free from *Martelia refringens* and *Bonaemia ostrea*.

In fact, no signs of this two diseases has been detected, during the production or in commercial size animals, in one year raising period in portuguese waters.

Any historical or present notice, of the presence of these two diseases, has been reported in portuguese territory, until now.

b) NATIONAL DISEASES CONTROL POLICIES

There exist several Laws in the portuguese legislation that rules aquatic animals disease control: - Law nº 39 209 of May 1953; nº 261/89 and 980 - A / 89.

The National Institute for Fisheries Research (INIP), as legal authority for this area of aquatic animals, is acting in collaboration with the National Animal Health Authority, to prevent the introduction of new pathogens and the spread of the existent, as well as the control of nosological situations.

Covered by that legal base, in order to carry on those tasks we adopt several procedures mentioned both in national legislation and in EEC, namely the Directive nº91/67 EEC of Feb.19.

c) DIAGNOSTIC METHODS

- Anatomic and histopathological studies
- Bacteriological analysis
- Virological "
- Parasitological "
- Mycological "

d) CONTROL METHODS

In shellfish diseases the only control method used is the prevention, based on the improvement of the raising conditions (low animal densities, improvement of the sediment, reducing grading and manipulation related operations etc.), showing to the shellfish farmers the risks involved with transfers and trading practices between farms as well as the introduction of animals without any sanitary control.

e) RESEARCH FINDINGS

The study of the clams disease, caused by *Perkinsus atlanticus*, has been carry on in our Institute since 1985, supported by national and international funds namely (NATO - Science for Stability Program ; FAR - 1; ATR).

| Agents | YEAR 1990 | | | | | | YEAR 1991 | | | | | |
|----------------|-----------|------|----------------|-------|------|-------|-----------|------|----------------|-------|------|-------|
| | Fk. | Mch. | Espo. +Cil. | Rick. | Bac. | Trem. | Fk. | Mch. | Espo. +Cil. | Rick. | Bac. | Trem. |
| Sampling Sites | | | | | | | | | | | | |
| R F I | 75.3 | 53.4 | 10.5 | 6.3 | 0 | 3.2 | 69.3 | 20.4 | 9.2 | 5.4 | 1.1 | 3.2 |
| i A II | 71.2 | 55.1 | 9.4 | 5.2 | 2 | 2.2 | 62.1 | 51.2 | 8.3 | 4.1 | 1.3 | 2.1 |
| a R III | 69.1 | 52.3 | 9.4 | 5.5 | 3 | 1.1 | 55.4 | 52.2 | 12.1 | 4.3 | 2.4 | 2.2 |
| O | | | | | | | | | | | | |
| R A | | | | | | | | | | | | |
| i L I | | | | | | | 76.2 | 61.4 | 4.2 | 10.3 | 0 | 1.9 |
| a V | | | | | | | | | | | | |
| O II | | | | | | | 75.1 | 61.1 | 4.4 | 10.1 | 0 | 1.6 |
| R | | | | | | | | | | | | |

P. atlanticus PK
M. topelis - Mch.
 Espor.Cil. - Espo+Cil
Ricketzias - Rick.
 Bac. - Bac.
 Tremat. - Trem.

Table I - Rates of Prevalence (%) of the diferent pathogens of Ruditapes decussatus on Faro and Alvor coastal lagoons

Highlights of Molluscan Disease Research in the United States

Methodology: Standard techniques of cytology, histology, and electron microscopy continue to be almost exclusively the methods being used to detect the presence of pathogens in epizootiological, host-parasite, and morphological studies of molluscan diseases. An exception is the Ray technique, used since 1952, wherein host tissues are incubated in fluid thioglycollate medium to help in the detection of *Perkinsus* spp. cells. However, the problems associated with 1) detecting pathogens in very light infections, 2) detecting pathogens when they are not morphologically distinct or are very small, 3) making judgements concerning taxonomic affinities, and 4) rapidly and inexpensively detecting the presence of the pathogens, all remain as important challenges worthy of addressing. Progress is being made.

C.F. Dungan and R.S. Roberson (1993. *Dis. Aquat. Org.* 15:9-22) have produced mono- and polyclonal antibodies quite specific to *Perkinsus marinus* found in *Crassostrea virginica* and capable of recognizing many *Perkinsus* sp. or spp. found in other molluscs from other regions of the world. The antibodies were generated using prezoosporangia as the source of immunogen. This newly developed technique should have considerable value in taxonomic and phylogenetic studies, in providing for a rapid assay method for use on smears, and for detecting very small numbers of *Perkinsus* spp. in host tissue. Their preparations are currently being used at the light microscope level as immunofluorescent markers.

In a personal communication Dungan has also reported developing a crude ELISA assay for diagnostic applications and the development of flow cytometric protocols for detection of *P. marinus* in tissue and environmental samples.

Susan Ford and co-workers are making progress toward developing a biochemical technique for detection of *Haplosporidium nelsoni* which is parasitic on *C. virginica*. They have obtained a nucleotide sequence for ribosomal RNA from *H. nelsoni* which they are attempting to incorporate into a probe for use at the light microscope level.

S. Kleinschuster and S.L. Swink (abstract from the 13th Milford Aquaculture Seminar and *Nautilus*-in press) and J. La Peyre, M. Faisal, and E.M. Burreson (*J. Eukaryotic Microbiol.*-in press) have both reported that they have obtained *P. marinus* in pure culture and have provided two different formulae for the culture media used. These contributions should lead to a much greater understanding of the biology of the *Perkinsea* found commonly in

molluscs in many of the temperate, subtropical and tropical marine waters of the world. In both laboratories the protist cells were first noticed in oyster organ and cell cultures. In a personal communication C.F. Dungan also reported in vitro propagation of *P. marinus* as well as cryopreservation of the cultured cells.

For most of the last 40 years the technique of choice for detecting *P. marinus* in oyster tissue was to place tissue explants in fluid thioglycollate medium (FTM) and incubate for several days permitting enlargement of meronts and merozoites so that the pathogen cells could be readily seen in fresh tissue squashes. In recent years a modification recommended by A. Farley as well as J.D. Gauthier and W.S. Fisher (1990. *J. Shellfish Res.* 9:367-371) is being used more frequently. It involves placing hemolymph in small volumes of FTM for induction of enlargement. The proponents of the technique argue that it is as sensitive as the tissue method and has the advantage of being more quantitative in assessments of infection intensities as well as in allowing individual oysters to be examined repeatedly.

In a recent paper entitled "Disseminated neoplasia of bivalve molluscs," R.A. Elston, J.D. Moore and K. Brooks (1992. *Rev. Aquatic Sci.* 6:405-466) reviewed the methods used for diagnosis and staging of the disorder. They concluded 1) when dealing with early stages there is a need for high sensitivity thus both hemocytologic (blood smears) and histologic techniques must be used, 2) phase constant observations of fresh smears is an inferior technique in most cases, and 3) antibody methods such as the immunoperoxidase technique of Smolowitz and Reinisch (1986. *J. Invert. Pathol.* 48:139-145) are the best overall because rapid scanning of large numbers of hemocytes at low magnifications is feasible and the significant problem of distinguishing neoplastic-appearing normal cells from the neoplastic ones is solved. Whereas the use of "flow cytometric measurement of DNA content provided nonsubjective, positive diagnosis," the authors (Elston, Moore, and Brooks, 1992) suggest that the relatively low sensitivity of the technique means that it is better suited for research rather than primary disease diagnosis as in epizootiological studies.

In Florida (Gulf of Mexico waters) W.S. Fisher and co-workers are engaged in attempts to develop techniques for determining oyster health which can be used as an indicator of estuarine habitat condition. This is being done with the realization that there is a high level of variability in bivalve physiology as a function of natural environmental fluctuations and seasonality with anthropogenic effects superimposed on those influences. More than fifteen biological characteristics are being recorded including the physiological measures of a) state of gonadal maturation, b) condition index, c) several tissue structure indices and d) hemolymph protein levels. Hemolymph measurements include hemocyte morphology and mobility, phagocytic capacity, superoxide production, hemolymph lectin levels and lysozyme content. Parasitic burdens and infection levels of *P. marinus* are also being quantified.

Two estuaries were selected for sampling. In Tampa Bay one clean and one polluted site which have similar salinities were chosen and the oysters sampled during the winter to minimize the influence of seasonal fluctuation. In Apalachicola Bay the research design resulted in sampling of two neighboring and similar, unpolluted sites over 12 months to determine the variability due to environmental fluctuations and temporal changes.

In a survey of individuals in the U.S. who are conducting epizootiological studies it was found that sample sizes ranged from 20 to 60 adult molluscs. The size often varies as a function of the incidences of infection anticipated, higher levels resulting in smaller sample sizes. For management decisions to be made concerning transport of molluscs from an estuary in one political jurisdiction to another, the sample size is most often 50 adults required by East Coast managers in the various state agencies. In California and Washington the managers require a sample size of 60 adults and 100 seed. The statistical value of these sample sizes has not been established. F. Kern of the U.S. National Marine Fisheries Service who manages evaluations of molluscan populations proposed for transport into the U.S., states that a minimum evaluation is 50 adults and 50 seed, sampled for histological examination 4 times during one year.

With respect to other methodology as it relates to management of diseases, R. Elston and R. Sizemore have prepared a manuscript in which objectives are stated for regulating transport of shellfish into waters of the State of Washington. Plans are underway to consider adoption of the objectives statement or some version of them, for the whole west coast of the U.S. and Canada. Thus, efforts are underway for standardizing regulations for movement of shellfish into the west coast region and between regions of the west coast with emphasis on preventing disease introductions. Yet to be established are the techniques to be used to detect molluscan diseases (particularly incipient cases), sample sizes, frequency of sampling, quarantine procedures, etc. Also to be determined is how an individual is to be certified as competent to evaluate molluscs for the presence of contagious diseases.

On the east coast of the U.S. each state manages its own waters with respect to shellfish transport with a staff member in the fisheries division of a department of natural or marine resources making judgements on a case-by-case basis, almost always as to whether to permit importations into the state's waters. Movements within state boundaries are not usually a concern. There is no standard procedure for selecting who is competent to make judgements as to whether a population of molluscs is diseased (i.e. there is no board of certification, state or national as in human or veterinary medicine). Selections are made on the basis of the reputation of the diagnostician as viewed by the state manager.

Primarily because there has been considerable concern over movement of the zebra mussel into fresh and oligohaline waters of

the East Coast from the Great Lakes, a committee within the Chesapeake Bay Program has been formed to establish regulations to control movements of exotic species, especially disease organisms, by researchers in pursuit of their studies in the Chesapeake Bay region. The rules have not been agreed upon but proposed protocols are under review. One can expect that researchers in the Chesapeake Bay region will be required to markedly improve holding and experimental facilities in the near future to prevent unwanted introductions such as diseases. For example, chlorination of effluent waters at 5 ppm total residual oxidant concentration for 24 hr at 40 C has been proposed. The cost of conducting research will undoubtedly increase.

Range Extensions

During the 1980's the mid- and northern-Atlantic coasts of the U.S. experienced warmer and drier years overall. It is suggested that this permitted *P. marinus* to express itself strongly in Delaware Bay oysters where it had rarely been observed, and as far north as Maine in low levels of incidence and intensity. It had not been observed north of the Delaware Bay prior to this time. It is not known whether the range extension resulted from transport of infected oysters from south to north (normal range is from Maryland to Texas) or the organism was transported by water currents. The former is the more likely scenario. These observations on range extensions were made by S. Ford (personal communication) and by Lewis, E.J. et al. (1993. U.S. Mar. Fish. Rev.-in press).

H. nelsoni has also been found as far north as Maine. Until recently the northern extent of its range was Massachusetts. It has also been found as far south as Florida (Hillman, unpublished data) thus the range has been extended below North Carolina in recent years (S. Ford and E.J. Lewis, ob cit.).

Recently *Ostrea edulis* set and grown at an aquaculture facility in Maine waters (Damariscotta River), were found by C. Friedmann and F. Perkins to be infected with *Bonamia ostreae*. The 3 samples of oysters which were examined were infected at the levels of 20 and 48% (1991) and 40% (1992). This is the first sighting of the pathogen from Maine waters. That region has been considered along with Connecticut as possible sites-of-origin for *B. ostreae* which was transmitted to California then to Europe (Elston et al., 1986. Dis. Aquatic Org. 2:49-54).

C.S. Friedman and R.P. Hedrick (1991. J. Shellfish Res. 10:515) reported that a haplosporidian protist closely resembling *Haplosporidium nelsoni* was found in *C. gigas* in two California embayments (Drakes Estero and Tomales Bay) as well as in a sample of oysters from a Matsushima Bay, Japan population. Drakes Estero is the bay where Japanese oysters have been planted in past years. Assuming that the organism in *C. gigas* can be proved to be *H. nelsoni*, the implications of these findings are that *C. gigas* was the carrier of *H. nelsoni* which appeared suddenly in Delaware Bay in 1957 (or was at least first recognized to be present). It is

unlikely that such an assertion can be proved since importations of *C. gigas* were few and not well documented.

New (?) Diseases

In the summer of 1990 an oyster hatchery on the north coast of Long Island, N.Y. (Oyster Bay) first experienced high (>54%) mortalities of *C. virginica* juveniles in the range of 15 to 24 mm long. The high mortalities have continued in the last two summers. The oysters are often characterized by overgrowth of the left valve, detachment of the adductor muscle and formation of a ring of abnormal conchiolin deposition most often beneath the free edge of the mantle. The ring is similar to that formed on the shell of Manila clams as part of the brown ring disease syndrome which has been described by Paillard and co-workers studying cultured clams from along the Brittany coast of France (1989. *Compt. Rend. Acad. Sci.* 309:235-241). The etiologic agent of juvenile oyster disease is unknown. Cytological and ultrastructural observations reveal no microbial suspects. The only entities associated with the disease are small (1-5 um diameter) coccoid bodies found most often between and within the mantle epithelial cells and in the connective tissue associated with mantle lesions. The bodies are oyster cell fragments, possibly autolysomes (Bricelj et al. 1992. *J. Shellfish Res.* 11:331-348). The search continues for the causative agent and techniques which can be adopted to correct the problem.

Black abalone (*Haliotis cracherodii*) populations found near several of the California Channel Islands have been experiencing very high mortalities in recent years. A possible causative agent is a coccidian protist found in the nephridia. The disease is termed "withering syndrome" and is characterized by atrophied and flaccid foot muscle, discolored epipodium, lack of gonad development, weakness and decreased tactile responses. Some of this information has been published by Haaker et al. (1992. In: *Abalone of the World*, Shephard, S.A. et al., eds.; Blackwell Scientific, Oxford) and Friedman (1991. *J. Shellfish Res.* 10: 236). C. Friedman et al. are also preparing a manuscript entitled "Geographic distribution host specificity, and mortality of black abalone, *Haliotis cracherodii*, in California" which will provide more information on the syndrome.

Other:

In 1991 *Ostrea edulis*, wild stock obtained from Maine waters (Damariscotta River), were found by C. Friedmann and F. Perkins to be infected with *Bonamia* sp. The two samples of oysters which were examined were 20 and 48% infected. This is the first sighting of the pathogen from Maine waters. That region is a proposed location from which *B. ostreae* could have been transmitted to California then to Europe thus causing the extreme mortalities experienced in Europe (Elston et al., 1986. *Dis. Aquatic Org.* 2: 49-54).

Prepared by F.O. Perkins
March 2, 1993



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28 January 1993

Dear Henri

WG PDMO MEETING, 15-18 MARCH - "CURRENT RESEARCH ON MOLLUSC DISEASES"

This laboratory is involved in monitoring for evidence or prevalence changes of *Bonamia* in *Ostrea edulis* stocks. I will present the current status of this situation at the above meeting. We have not identified *Marteilia* in our bivalve stocks.

Regarding your request for data in research, we have completed a small programme, investigating resistance of *O. edulis* stocks to natural infections of *Bonamia* and there is a Government-funded project on "Environmental stress and disease in bivalve molluscs" at the University of Southampton.


The conclusions of FDL study (which was only preliminary) showed that *O. edulis* hatched in Scotland were infected to a lesser degree than stock from North Wales and the Solent. Most mortalities occurred in Solent oysters. We repeated this experiment twice and each time the same results were obtained. I will provide more details at the WG meeting.

A summary of the Southampton University work and results is attached. This was supplied by Lawrence Hawkins, who you know I believe.

I hope that our small contribution is sufficient.

Best wishes.

Yours sincerely


D BUCKE

Encs

ENVIRONMENTAL STRESS AND DISEASE IN MARINE BIVALVES

1. *Ostrea edulis* & *Crassostrea gigas*

The effects of natural environmental stressors on commercially exploited marine bivalves have been studied at the Department of Oceanography, University of Southampton using a suite of investigative techniques, focusing on changes in immunocompetence. Work is now in hand to study the interactions between natural and anthropogenic stressors on the immunocompetence of *Ostrea edulis* and *Crassostrea gigas*.

The use of this pluralist approach has made it possible to explain two aspects of the occurrence of bonamiasis in *Ostrea edulis* in terms of the effects of the environmental stressors on the oysters' ability to resist this (and other) pathogens. Firstly, the seasonal variation and prevalence of the disease in shallow waters, where mortalities become noticeable in spring and rise to peak in early summer. Secondly, the very much greater susceptibility of re-laid stock compared to any endemic population of the same species in the same area.

Following studies of the apparent linkage between disease susceptibility and variations in environmental conditions in one population of *O. edulis* investigation is in progress of anecdotal evidence of differential susceptibility to bonamiasis of *O. edulis* from different sites around the British Isles. In particular, animals from parts of Loch Fyne on the west coast of Scotland appeared to be immune despite ample opportunity for infection by stock movements into the area prior to the imposition of movement restrictions.

Comparisons were made of the physiological, metabolic and immunological responses of groups of oysters from three spatially separate populations: the Solent on the south coast of England, Conwy, North Wales and Loch Fyne, Scotland. Results to date show apparent differences in disease susceptibility that seem to be related to differences in energy partitioning; particularly with respect to population differences in size at gametogenesis.

2. *Mercenaria mercenaria*

In addition to the studies of *Ostrea edulis* and *Crassostrea gigas* the ecophysiology of the clam *Mercenaria mercenaria* has also been investigated. Clams subjected to chronic tidal exposure were found to have higher hydrogen peroxide concentrations on re-immersion, reduced lysozyme activities, lower rates of granulocyte locomotion and a greater number of small granulocytes when compared with control animals.

THE EFFECT OF TIDAL EXPOSURE ON ASPECTS OF METABOLIC AND IMMUNOLOGICAL ACTIVITY IN THE HARD CLAM *MERCENARIA MERCENARIA* (LINNAEUS)

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(Received 29 May 1992; accepted 1 July 1992)

Abstract—1. Variations in the immunological state of the marine bivalve *Mercenaria mercenaria* were followed by measurement of haemolymph lysozyme activity, haemocyte concentrations and haemocyte locomotion.

2. Animals subjected to chronic tidal exposure were found to have higher hydrogen peroxide concentrations on re-immersion, reduced lysozyme activities, lower rates of granulocyte locomotion and a greater number of small granulocytes when compared with control animals.

3. Cyclical variations could be linked to tidally related changes in metabolic activity quantified by measurement of hydrogen peroxide concentrations.

INTRODUCTION

The hard shell clam *Mercenaria mercenaria* (Linnaeus, 1758) inhabits soft substrates in coastal waters, often in areas with some degree of tidal exposure. The clams are normally buried in the sediment, but they have been shown to migrate vertically with a tidally related periodicity (Roberts *et al.*; 1989). Previous studies have shown that in other shallow water bivalve species, tidally related variations in metabolic activity correlate with changes in immune status (Conway, 1987; Hawkins and Hutchinson, 1990). It has also been shown that physiological stress, such as tidal exposure, can cause the diminution of marine invertebrate defence mechanisms (Cheng and Combes, 1990). The aims of the present study were to investigate any relationship between such variation and the immune state of *M. mercenaria*, and to measure the effect of tidal exposure on the immune state of this clam.

MATERIALS AND METHODS

Mercenaria mercenaria were collected at low tide from a shore site on Southampton Water, U.K. and acclimated for at least 30 days to an artificial tidal system. Animals were kept in atidal conditions for the first 10 days of this acclimation phase to suppress any existing endogenous rhythms before re-entrainment in the tidal system. The animals were divided into two groups of 10, kept on trays without sediment and subjected to a tidal rise and fall of 0.85 m with a period of 12 h 25 min. The control group remained covered by 0.01 m of water at the nadir of the cycle

whereas the experimental group were exposed to the air for 128 min per cycle, which approximated to the degree of aerial exposure at the collection site. Salinity was constant at 33‰, the water temperature remained in the range 13–15°C and the air temperature varied between 16 and 19°C for the duration of the experiment. The animals were provided with a daily microalgal ration of a mixture of *Isochrysis galbana*, *Tetraselmis suecica* and *Phaeodactylum tricorutum*.

Holes 0.5 mm in diameter were drilled through the shell so that haemolymph samples could be taken from the adductor muscle sinus by insertion of a sterile, hypodermic needle; the location of the sampling point was confirmed visually when animals were killed at the end of the experiment. Sampling from this part of the animal avoided possible contamination with lysozyme secreted into the digestive system for nutritive bacteriophagy (McHenry *et al.*, 1979). The holes were filled with sterilized plugs and animals were allowed to recover for at least 6 days between collection of samples. At each sampling the aliquots of haemolymph were examined under a light microscope to check for the presence of bacterial or protozoan infections; on the few occasions when an infection was detected the animal was removed from the experiment.

Metabolic activity was followed by measurement of haemolymph hydrogen peroxide concentrations rather than using oxygen respirometry which could not be readily determined where animals were exposed to air during the tidal cycle. The haemolymph samples were assayed using a series of colorimetric methods; hydrogen peroxide concentrations (mg/l) were determined by the method of Meattini (1984); peroxidase activity ($\mu\text{mol}/\text{sec}/\text{l}$) using the method

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Working Group on the Pathology
and Diseases of Marine Organisms

Antibiotic resistance profiles of fish pathogens: an emerging problem

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Upon confirmation of the presence of a bacterial fish pathogen which can cause significant mortalities in a hatchery or marine cage sites, management decisions are required and often include improvements or changes in husbandry conditions. Even if these measures are taken, it is often necessary to initiate chemotherapy in order to control a disease outbreak and limit losses. The choice of drug to be used is determined by the disease history of the site as well as the antibiotic resistance profile of the pathogen at the time of the outbreak. This is determined, in most cases, by the classical disc diffusion method.

Antimicrobial agents, their methods of application, and potential problems associated with drug therapy have been extensively reviewed (Michel, 1986; Aldeman, 1988; Austin and Austin, 1987). The major limitation of any drug therapy is certainly the development of antibiotic resistance due to plasmid carriage or chromosomal mutation. Antibiotic resistance, including multiple resistance, has been reported for several fish pathogens including Aeromonas salmonicida, Vibrio salmonicida, Vibrio anguillarum and Pasteurella piscicida (Inglis et al., 1991; Takashima et al., 1985; Zhao et al., 1992; Hjeltnes et al., 1987; Olivier 1992). Of special importance is the fact that multiple resistant strains are being reported at an alarming rate from several countries. The increase in antibiotic resistance of bacterial fish pathogens other than A. salmonicida, has received little attention. However, in Japan, several studies have described the increasing occurrence of antibiotic resistant strains of several fish pathogens, V. anguillarum, P. piscicida and Edwardsiella tarda (Takashima et al.,

1985; Zhao et al., 1992; Aoki, 1992). In Canada, V. anguillarum has been isolated yearly from salmon farms since the early 1980's. There has never been a change in antibiotic resistance profiles of these isolates until 1992 when strains from one site were partially resistant to oxytetracycline (zone of inhibition of 15 mm compared to a normal zone of 30-40 mm).

Tragically, the importance of antimicrobial resistance in fish pathogens may have been underestimated, although indication that it may cause significant problems was already available from the study of Aoki et al. (1983). These authors demonstrated that most strains of A. salmonicida isolated from wild salmonids in Japan were susceptible to all drugs used in the aquaculture industry whereas strains isolated from farmed fish showed a significant increase in antibiotic resistance and the emergence of multiresistant strains. The hypothesis suggested was that this increase in antibiotic resistance was linked to the amount of antibiotics used in fish farms. Since enormous quantities of antibiotics are used in aquaculture (Grave et al., 1990), this view is shared by Schlotfeldt et al. (1985), Tsoumas et al., (1989), Inglis et al. (1991a) and Olivier (1992) who have reported a significant increase in the level of resistance of A. salmonicida strains isolated from facilities in Germany, Scotland, and Canada.

Most of the literature on the antibiotic resistance of fish pathogens is centered on the problems associated with A. salmonicida subsp. salmonicida. Increase in resistance of A. salmonicida to several antibiotics has been on the rise in several countries including Spain, Switzerland, Scotland, Canada, and Norway in either fresh or seawater (Toranzo et al., 1991; Meir et al., 1992; Olivier, 1992; Richards et al., 1992; Hoie et al. 1992). In this particular species, antibiotic resistance has been linked to the presence of R factors (Hedges et al., 1985; Brazil et al., 1986; Bast et al., 1988). A conjugative R-plasmid was further characterized by Aoki et al. (1986), this plasmid encoded resistance to chloramphenicol, streptomycin, and sulphonamides.

In the last ten years, we have determined the antibiotic resistance patterns of over 700 A. salmonicida isolates. In Table 1, the antibiotic resistance profile of 140 strains received from 12 countries were analysed. At least 24 different profiles were identified based on 6 antibacterial agents with multiple resistant strains being more prominent in Scotland confirming results of others (Inglis et al., 1991; Richards et al., 1992; Sutherland and Inglis, 1992). The antibiotic resistance profiles of Canadian

isolates of A. salmonicida recovered from the east coast (Province of New Brunswick, where most of the salmon industry is located) are presented in Table 2. Eight antibiotic resistance profiles were recognized including several multiple resistant isolates. The variability in antibiotic resistance profiles was most prominent in freshwater whereas in marine sites only resistance to OTC was observed. The antibiotic resistance patterns of strains isolated from three other Canadian provinces are presented in Table 3. Ten different antibiotic resistance profiles were found indicating that, in Canada, strains carrying multiple antibiotic resistance are increasing.

Antibiotic resistance profiles of A. salmonicida isolates recovered from four separate facilities which have been plagued with recurrent furunculosis outbreaks for the last few years, are presented in Tables 4 and 5. What is most striking in all these hatcheries is the fact that at least four antibiotic resistance profiles were found in all cases ranging from completely sensitive strains to multiple resistant strains. In a few cases, strains with different antibiotic resistance profiles were also recovered from the same tank or the same pen. The presence of different antibiotic resistance profiles in a hatchery or a cage site was also recognized in the United States (Chapman et al., 1991) and Scotland (Inglis et al., 1991). The problem of various antibiotic resistance profiles in the same facility is compounded by the fact that, various antibiotic resistance profiles can be found from the same fish (Inglis et al., 1991). Similar results were obtained in Canada, antibiograms performed on isolated colonies obtained from original plates inoculated from either milt or kidney material of affected fish resulted in the presence of more than one distinct antibiotic resistance profile in at least four instances (Table 6). These results were further confirmed by our findings that strains of A. salmonicida received in our laboratory contained more than one antibiotic resistant profile (Table 7).

It is important to note that in addition to the problem of antibiotic resistance, there are several additional problems associated with chemotherapy. These include: antibiotic residues in marketable fish, the need for withdrawal periods (Jacobsen, 1989; Bjorklund et al., 1992), the possibility of contaminating wild fauna during treatment of fish farms (Samuelsen et al., 1992), immunomodulation (van der Heidjen et al., 1992), the lack of efficacy of a specific treatment due to incorrect mixing or problems of palatability in feed (Groman et al., 1992; Mitchell, 1992b) and the difference in treatment regimes when fish are medicated in fresh- or seawater

(O'Grady *et al.*, 1986). There is also the possibility of transferring R plasmids from wild bacteria to fish pathogens or vice versa, and finally the number of drugs licensed for use in aquaculture in different countries is variable and frighteningly small. In Canada only two drugs are approved, oxytetracycline and the potentiated sulphonamide "Romet" (Brackett, 1992); if more strains become resistant to both of these drugs chemotherapy will cease to be a treatment option.

It is also important to recognize that although chemotherapy represents the principal control measure for the majority of cases dealing with furunculosis there is yet no standardized methodology to monitor antibiotic resistance of most fish pathogens including *A. salmonicida*. For example, only three publications provide zone diameters of resistant and susceptible strains of *A. salmonicida* to oxolinic acid based on the disc diffusion method and the data is from a few isolates (Hastings and McKay, 1987; O'Grady *et al.*, 1987; Inglis *et al.*, 1991). For all bacterial fish pathogens, there is an urgent need to improve and standardize techniques to establish baselines of zone diameters which will allow a more rapid and precise determination of antibiotic resistance and allow comparisons between laboratories.

In summary, enough evidence has been presented to prove that the problem of antibiotic resistance, at least in the case of *A. salmonicida*, is very serious and of world wide concern. With the additional problems associated with chemotherapy, it becomes necessary to seriously improve our efforts into devising alternative methods of disease prevention. Results to date indicate that the problem is worsening and solutions are urgently required.

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Table 1. Antibiotic resistance profiles of *A. salmonicida* isolated from various countries

| | "n" | Sus | P | SSS | OTC | OA | S | OTC OA | OTC SSS | SSS P | SSS OA | SSS PS | S OA | S P | SSS OA P | SSS PS OTC | SSS PS S | SSS OTC S | SSS PS OA | OTC OA SSS | OTC PS SSS | OTC OA SSS PS | OTC OA SSS CL | OTC OA SSS PS S | OTC OA SSS S P | OTC OA SSS S CL | OTC CA SSS PS P S | |
|-------|-----|-----|---|-----|-----|----|---|-----------|------------|----------|-----------|-----------|---------|--------|----------------|------------------|----------------|-----------------|-----------------|------------------|------------------|------------------------|------------------------|-----------------------------|----------------------------|-----------------------------|----------------------------------|---|
| DEN. | 3 | | | 1 | | | | | | | 1 | 1 | | | | | | | | | | | | | | | | |
| SWE. | 1 | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| FIN. | 2 | | | 1 | | | | | | 1 | | | | | | | | | | | | | | | | | | |
| NOR. | 14 | 12 | | | | 1 | | | | | 1 | | | | | | | | | | | | | | | | | |
| WALES | 1 | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| ENG. | 2 | | | 2 | | | | | | | | | | | | | | | | | | | | | | | | |
| IRE. | 51 | 10 | | | 7 | 17 | | 1 | | | 13 | | 1 | | 1 | | | | | | 1 | | | | | | | |
| SCOT. | 28 | 16 | | 1 | 1 | 1 | 1 | | | | | | 1 | | | | | 1 | | | 1 | 2 | | 1 | | | | 2 |
| FRA. | 4 | | 2 | | | | | | | | | | | | | | | | | | | | 1 | | | 1 | | |
| SPAIN | 13 | 5 | | 5 | | 2 | | | | | 1 | | | | | | | | | | | | | | | | | |
| JAP. | 1 | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| USA | 20 | | 1 | 2 | 7 | | | | | 7 | | | | | | 2 | 1 | | | | | | | | | | | |
| CAN. | | + | + | + | + | + | | + | + | | | | | | | + | + | + | | + | + | + | | | + | | | |

+ indicates antibiotic resistance profile detected in Canada

Table 2. Antibiotic resistance profiles of *Aeromonas salmonicida* strains isolated from the province of New-Brunswick, Canada (1979 to 1992)

| Year | No. of sites affected | No. of isolates tested | Sen. | Antibiotic resistance profiles ^a | | | | | |
|------|-----------------------|------------------------|------|---|----|--------|---------|-----------|----------|
| | | | | OTC | OA | OTC OA | OTC SSS | OTC SSS S | SSS PS S |

Freshwater hatcheries

| | | | | | | | | | | |
|------|---|----|----|---|----|----|--|----|---|---|
| 1979 | 1 | 1 | 1 | | | | | | | |
| 1980 | 1 | 1 | 1 | | | | | | | |
| 1982 | 1 | 1 | 1 | | | | | | | |
| 1983 | 3 | 3 | | | | | | 3 | | |
| 1984 | 4 | 32 | 9 | 8 | | | | 15 | | |
| 1985 | 4 | 15 | 8 | 2 | 3 | | | 2 | | |
| 1986 | 1 | 2 | 2 | | | | | | | |
| 1987 | 1 | 2 | 2 | | | | | | | |
| 1989 | 4 | 17 | 5 | | 10 | 2 | | | | |
| 1990 | 5 | 91 | 18 | 2 | 56 | 15 | | | | |
| 1991 | 1 | 2 | | | | | | | | 2 |
| 1992 | 3 | 9 | 5 | | | | | | 2 | 2 |

Sea cage sites

| | | | | | | | | | | |
|------|---|----|---|---|--|--|--|--|--|--|
| 1984 | 2 | 6 | | 6 | | | | | | |
| 1985 | 5 | 11 | 3 | 8 | | | | | | |
| 1987 | 1 | 1 | | 1 | | | | | | |
| 1989 | 3 | 7 | 7 | | | | | | | |
| 1990 | 3 | 15 | 9 | 6 | | | | | | |
| 1991 | 3 | 4 | | 4 | | | | | | |
| 1992 | 1 | 2 | | 2 | | | | | | |

Isolates from rivers

| | | | | | | | | | | |
|------|--|---|---|--|--|---|--|---|--|--|
| 1979 | | 1 | 1 | | | | | | | |
| 1980 | | 1 | 1 | | | | | | | |
| 1986 | | 3 | 2 | | | | | 1 | | |
| 1987 | | 2 | 2 | | | | | | | |
| 1988 | | 1 | 1 | | | | | | | |
| 1989 | | 1 | 1 | | | | | | | |
| 1990 | | 1 | | | | 1 | | | | |
| 1991 | | 1 | 1 | | | | | | | |

a) Sen = sensitive to the six antibiotics tested. Antibiotic discs used included: OTC, Oxytetracycline 30 µg; S, streptomycin 10 µg; SSS, Triple sulfa 300 µg; P, penicillin 10 units; OA, oxolinic acid 2 µg and SxT (trimethoprim/sulfamethoxazole 1:20) 25 µg.

Table 3. Antibiotic resistance profiles of Aeromonas salmonicida strains isolated from the provinces of Quebec, Ontario, and British Columbia, Canada (1979 to 1992)

| Year | No. of isolates tested | Antibiotic resistance profiles ^a | | | | | | | | | |
|----------------|------------------------|---|----|-----|----|-----|------------|------------------|-------------------|-------------------------|----------------------------|
| | | Sen. | P | OTC | OA | SSS | OTC SSS | OTC SSS OA | OTC SSS SxT | OTC SSS SxT OA | OTC SSS OA S P |
| Quebec | | | | | | | | | | | |
| <1988 | 3 | 2 | | | | | 2 | | | | |
| 1989 | 13 | 1 | | | 3 | | 1 | 6 | | 2 | |
| 1990 | 194 | 6 | | | 28 | | | 1 | 1 | 158 | |
| 1992 | 7 | 2 | | | | | | 4 | | | 1 |
| Ontario | | | | | | | | | | | |
| <1990 | 11 | 7 | 1 | 1 | | 1 | 1 | | | | |
| 1991 | 11 | 1 | 10 | | | | | | | | |
| B.C. | | | | | | | | | | | |
| <1990 | 5 | 5 | | | | | | | | | |
| 1991 | 9 | 8 | 1 | | | | | | | | |
| 1992 | 87 | 49 | 1 | 14 | | 3 | 1 | | 19 | | |

a) Sen. = sensitive to the six antibiotics tested. Antibiotic discs used included: OTC, Oxytetracycline 30 µg; S, streptomycin 10 µg; SSS, Triple sulfa 300 µg; P, penicillin 10 units; OA, oxolinic acid 2 µg and SxT (trimethoprim/sulfamethoxazole 1:20) 25 µg.

Table 4. Multiple antibiotic resistance profiles of Aeromonas salmonicida strains isolated from Atlantic salmon in hatcheries experiencing recurrent outbreaks of furunculosis.

| Case # | Date | "n" | Antibiotic resistance profiles ^a | | | | | |
|-------------------|------------|-----|---|-----|------------|------------------|-------------------------|-------------------|
| | | | Sen. | OTC | OA | OTC OA | OTC SSS OA | OTC SSS SxT |
| Hatchery A | | | | | | | | |
| 89084 | March 89 | 1 | | 1 | | | | |
| 89560 | Oct. 89 | 1 | | | | | 1 | |
| 89630 | Oct. 89 | 1 | | | | | 1 | |
| 89674 | Nov. 89 | 1 | | | 1 | | | |
| 89708 | Nov. 89 | 1 | 1 | | | | | |
| 89712 | Dec. 89 | 1 | 1 | | | | | |
| 90034 | Feb. 90 | 2 | | 1 | 1 | | | |
| 90179 | March 90 | 46 | 1 | 1 | 29 | | 15 | |
| 90182 | March 90 | 22 | 4 | | 18 | | | |
| 90369 | May 90 | 55 | 4 | | 21 | | 30 | |
| Hatchery B | | | | | | | | |
| | | | Sen. | OA | OTC SSS | OTC SSS OA | OTC SSS OA SxT | |
| 89-9 | Sept. 89 | 2 | | | | 1 | 1 | |
| 89-10 | Oct. 89 | 9 | | 3 | | 5 | 1 | |
| 89-11 | Nov. 89 | 2 | 1 | | 1 | | | |
| 90-2 | July 90 | 6 | | | | 1 | 5 | |
| 90-3 | Sept. 90 | 56 | | 6 | | | 50 | |
| 90-4 | Nov. 90 | 13 | 1 | 5 | | | 7 | |
| 90-5 | No.-De.-90 | 30 | | 2 | | 1 | 27 | |
| 90-6 | Ja-May-91 | 7 | | 2 | | | 5 | |
| 90-7 | June 91 | 26 | | 5 | | | 21 | |
| 90-8 | Sept. 91 | 17 | | | | | 17 | |
| 90-9 | Oct. 91 | 39 | 5 | 8 | | | 26 | |

a) Sen. = sensitive to the six antibiotics tested. Antibiotic discs used included: OTC, Oxytetracycline 30 µg; S, streptomycin 10 µg; SSS, Triple sulfa 300 µg; P, penicillin 10 units; OA, oxolinic acid 2 µg and SxT (trimethoprim/sulfamethoxazole 1:20) 25 µg.

Table 5. Antibiotic resistance profiles of *Aeromonas salmonicida* strains isolated from one freshwater and three marine cage sites in 1991-2.

| Date | "n" | Antibiotic resistance profiles ^a | | | |
|------------------------|-----|---|-----|------------------|----------------------------|
| | | Sen. | OA | OTC SSS OA | SSS SxT OA S P |
| Hatchery C | | | | | |
| 1991 | 11 | 7 | 2 | 2 | |
| 1992 | 7 | 2 | | 4 | 1 |
| Sea cage sites: | | | | | |
| | | Sen. | OTC | SSS | OTC SSS SxT |
| Farm A | | | | | |
| Feb. | 2 | | 2 | | |
| May | 7 | 5 | | 1 | 1 |
| July | | | | | |
| Pen A | 11 | 7 | | | 4 |
| Pen B | 11 | 1 | 10 | | |
| Farm B | | | | | |
| July | | | | | |
| Pen A | 6 | | | | 6 |
| Pen B | 6 | 6 | | | |
| Pen C | 8 | 3 | | | 5 |
| Farm C | | | | | |
| Feb. | 2 | 2 | | | |
| July | 3 | | | | 3 |

a) Sen. = sensitive to the six antibiotics tested. Antibiotic discs used included: OTC, Oxytetracycline 30 µg; S, streptomycin 10 µg; SSS, Triple sulfa 300 µg; P, penicillin 10 units; OA, oxolinic acid 2 µg and SxT (trimethoprim/sulfamethoxazole 1:20) 25 µg.

Table 6. Antibiotic resistance profiles of single colonies of Aeromonas salmonicida recovered from original plates.

| Isolate number | number of colonies tested | antibiotic resistance profile ^a | | | | |
|-------------------|------------------------------|--|------|-----|-------------------|------------------------------|
| | | "n" | Sen. | SSS | SSS SxT OTC | SSS SxT S OTC OA |
| M04 (gonads) | 3 | 2 | | | 1 | |
| M00 (gonads) | 10 | 9 | | | 1 | |
| M40 (gonads) | 2 | 1 | | | 1 | |
| M21 (gonads) | 10 | | | | 10 | |
| M99 (gonads) | 10 | | | | 10 | |
| M80 (gonads) | 10 | | | | 10 | |
| 2A (Kidney) | 10 | | 8 | | 2 | |
| 13-1 (Kidney) | 8 | | | | 8 | |
| 13-6 (Kidney) | 5 | 5 | | | | |

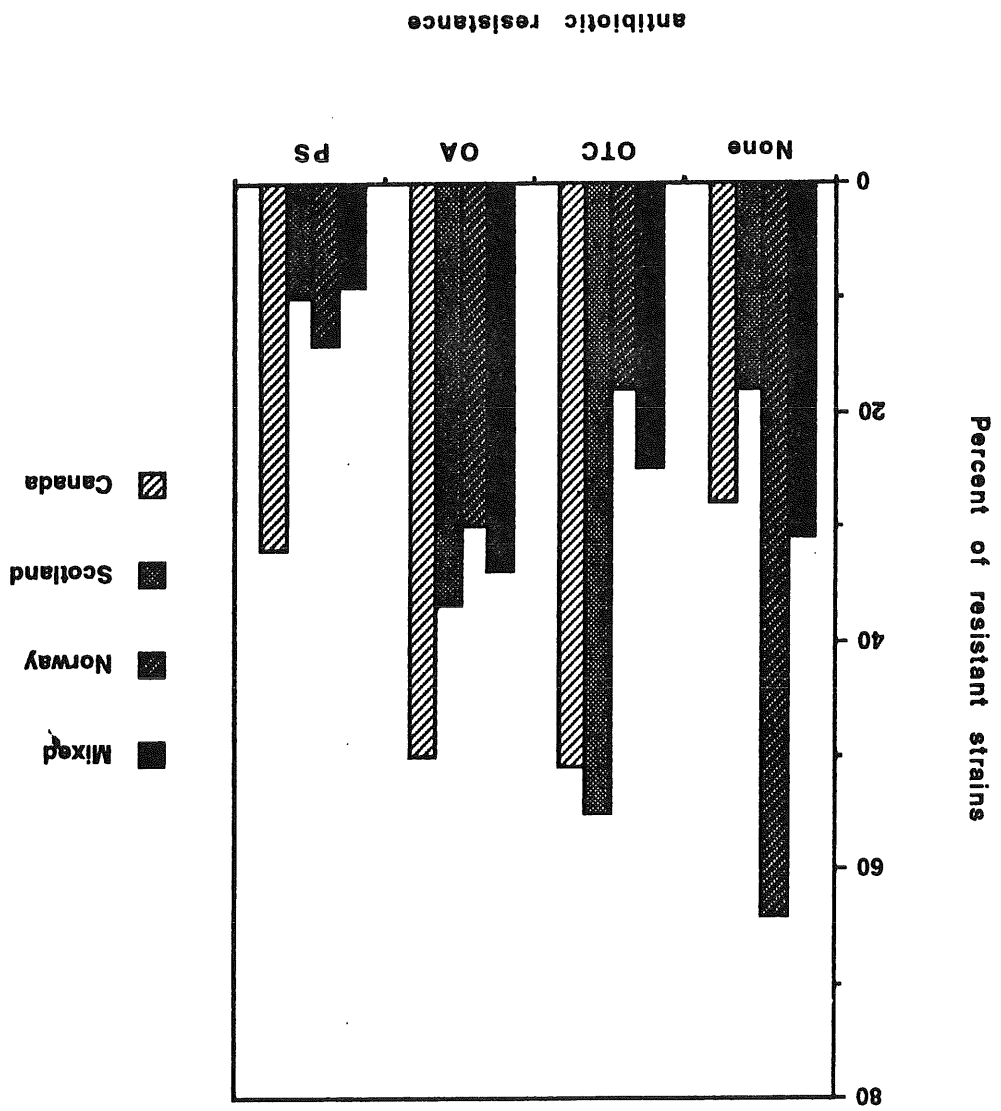
a) Sen. = sensitive to the six antibiotics tested. Antibiotic discs used included: OTC, Oxytetracycline 30 µg; S, streptomycin 10 µg; SSS, Triple sulfa 300 µg; P, penicillin 10 units; OA, oxolinic acid 2 µg and SxT (trimethoprim/sulfamethoxazole 1:20) 25 µg.

Table 7. Cultures of Aeromonas salmonicida that contained more than one strain with different antibiotic resistance profiles.

| Strain number | Source of inoculum for ATB ^a determination | Antibiotic resistance profile obtained ^b |
|---------------|---|---|
| M04 | TSA slant | Susceptible with several resistant mutants around OTC, SSS, O.A and SxT disks |
| | M04 (OTC res. mut.) | Res. to OTC, SSS, O.A and SxT |
| | M04 (SSS res. mut.) | Res. to OTC, SSS, O.A and SxT |
| | M04 (O.A res. mut.) | Res. to OTC, SSS, O.A and SxT |
| | M04 (SxT res. mut.) | Res. to OTC, SSS, O.A and SxT |
| 428 | TSA slant | Susceptible with several resistant mutants around OTC, SSS, O.A and SxT disks |
| | 428 (OTC res. mut.) | Res. to OTC, SSS, O.A and SxT |
| | 428 (SSS res. mut.) | Res. to OTC, SSS, O.A and SxT |
| | 428 (O.A res. mut.) | Res. to OTC, SSS, O.A and SxT |
| | 428 (SxT res. mut.) | Res. to OTC, SSS, O.A and SxT |
| 821,830 | TSA slants | Susceptible with several resistant mutants around OTC, SSS, O.A and SxT disks |
| | 821 (OTC res mut.) | Res. to OTC, SSS, O.A and SxT |
| | 830 (SSS res mut) | Res. to OTC, SSS, O.A and SxT |
| 851 | TSA slant | Susceptible with few resistant mutants around P and O.A. disks |
| | 851 (O.A res. mut.) | Resistant to P. SSS, OTC, O.A. |
| | 851 (P res. mut.) | Resistant to P. SSS, OTC, O.A. |

a) ATB = antibiogram

b) Sen. = sensitive to the six antibiotics tested. Antibiotic discs used included: OTC, Oxytetracycline 30 µg; S, streptomycin 10 µg; SSS, Triple sulfa 300 µg; P, penicillin 10 units; OA, oxolinic acid 2 µg and SxT (trimethoprim/sulfamethoxazole 1:20) 25 µg.



ICES Working Group on Pathology and Diseases in Marine Organisms
Copenhagen, Denmark 15-18 March 1993

The efficacy of existing commercially available fish vaccines.

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Yersiniosis

In 1965, Ross & Klontz published a on successful vaccination of RBT against yersiniosis. Commercial vaccines were taken into use in fish-farms in North America (Tebbit *et al.* 1981) and later on in Europe due to an increase in the isolation of *Y. ruckerii* and outbreaks of yersiniosis. Vaccines against yersiniosis are available from several producers. Different serotypes of *Y. ruckerii* have been described (Stevenson & Airdrie 1984). The serotypes included in the vaccine may differ depending on where the vaccine is used. In Norway, serotype I and III are included and serotype I and II in the vaccines sold in North America and in the rest of Europe. Recently, Erdal (1990) demonstrated cross-protection between serotype I and III. No cross-protection was found between serotype I and II. Based on laboratory trials and field tests, the RPP is reported to be > 80% (Hørlyck & Kehlet, 1985, Larsen, unpublished results, Vigneulle 1990) when vaccination is performed by injection. At present, oral vaccination does not give sufficient protection. The efficacy of the vaccines seems to be adequate as yersiniosis do not cause serious concern in the industry.

Vibriosis

There are several early reports of effective vaccination against vibriosis (Hayashi *et al.* 1964, Evelyn 1984) and commercial vaccines against this well known disease have been available for many years.

RBT is known to be highly susceptible against vibriosis and farming of RBT in seawater would not have been possible without effective vaccines. In the literature, good protection against vibriosis reported from laboratory and field trials with RBT (Egidius and Andersen 1979, Giorgetti *et al.* 1981, Rosenkvist-Jensen 1982). There are however unpublished reports of unadequate protection from Finland. However, based on the information from other countries (Håstein *et al.* 1986, Lillehaug, 1989, Dalsgaard, unpublished results), the

efficacy of vibrio vaccines for RBT seems to be sufficient.

Although vibriosis causes some losses in the farming of Atlantic, this species is far less susceptible and vibriosis is mainly regarded as a management disease. On Atlantic salmon, field and laboratory trials, are scarce or lacking. However, by the information released by the manufacturer, high RPP values are reported from laboratory trials.

The various commercial vaccines are based on isolates of different geographical origin. This may express a somewhat different antigenetic composition of the vaccines. *Vibrio anguillarum* serotype O1, O2 and *V. ordalii* may be included in the vaccine. The lack of efficacy previously reported from Canada, was probably a reflection of essential *Vibrio* isolates not being included in the commonly used vaccines.

Cold Water vibriosis

A vaccine against this disease was first introduced as an experimental vaccine in 1987 (Holm and Jørgensen, 1987). Both laboratory trials (Hjeltnes *et al.* 1987) and field trials (Lillehaug 1989, Lillehaug 1991) have demonstrated a very good efficacy of this vaccine (RPP>95%). This is supported by the fact that cold water vibriosis, which in the eighties, was a disease of great economical importance in Norwegian aquaculture, is controlled after the introduction of vaccine. Vaccines are produced by several companies, however, the basic concept is the same. So far, isolates of *V. salmonicida* have been very homogeneous with regard to biochemistry and serology (Egidius *et al.*, 1986; Holm, 1986; Espelid *et al.*, 1988). All isolates from Atlantic salmon belong to the same serotype (L1) while two serotypes have been isolated from Atlantic cod (T1 and T2). One of the cod serotypes (T1) is probably identical to the salmon serotype (Schrøder, 1990). In the last two years, there have been an increased in the severity of the outbreaks in northern Norway and high mortalities in Atlantic salmon are reported from the Faroe Islands. Whether this is due to inadequate vaccination or reflects a change in the antigenetic properties of *V. salmonicida*, are being investigated.

Furunculosis

In the literature, data concerning the vaccination of fish is contradictory. The first vaccination trials with fish were published by Duff (1942) who used an oral vaccine and reported consistently lower mortalities in vaccinated than in unvaccinated fish. However,

later work on vaccination against furunculosis, using oral or injectable vaccines based on whole killed cells, has produced conflicting results (see Hastings, 1988, for a review). Today, there are several commercial vaccines on the market as well as experimental vaccines with permits for field testing. Generally, the commercial vaccines are based on whole cells, ECP and adjuvance. As adjuvance are used mineral oil, certain oil emulsions (of confidential composition), glucan, aluminum phosphate, aluminum hydroxide. Although protection has been demonstrated (Lillehaug *et al.* 1992), at present, firm conclusions about the efficacy of these vaccines can not be drawn. However, protection seems to depend on the use of adjuvanted vaccines, which have to be administered by injection. There are information indicating that trippel vaccines (vibriosis/cold water vibriosis/furunculosis) induce better protection than mono furunculosis vaccines.

Furunculosis is regarded as one of the main disease problems by the fish farming industry. This is supported by the fact that most of the antibiotics used to day, are used in order to control furunculosis. This clearly indicates that the efficacy of the existing furunculosis vaccines is not satisfactory.

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VACCINES - Atlantic salmon/Rainbow trout

Vibriosis

Coldwater vibriosis

Yersiniosis

Furunculosis

Vibriosis/Coldwater vibriosis

Vibriosis/Coldwater vibriosis/furunculosis

VACCINES - Marine fish

Vibriosis - Cod

Vibriosis - Turbot

FISH VACCINES UNDER DEVELOPMENT

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Introduction

Due to pressure of time, this report is a brief overview of the state of development of certain vaccines against important fish diseases. The report is informal and largely based upon my own personal views in that a good deal of the information presented has not been published in the scientific literature. The report should not be taken as authoritative but simply as one to stimulate discussion on the development of vaccines and methods of their production and delivery.

Principal Vaccines Under Development

| | | | |
|------------|---|------------|--------------------|
| Bacterial: | Furunculosis <i>Edwardsiella ictaluri</i> Streptococcus Pasteurella BKD | Virus: | IHN IPN VHS? |
| | | Parasites: | Salmon lice |

Each of these vaccines will be addressed in turn with comments on methodologies of production and delivery.

Furunculosis

Injectable bacterins containing aluminium hydroxide or aluminium salt adjuvants have been used commercially for some years with moderate, but rather variable, efficacy (Lillehaug *et al.*, 1992). The mechanism of protection is not known. It is believed that the prolonged irritation caused by the adjuvant which results in formation of small

polymers which could be injected or fed. A second protective antigen is the extracellular LPS which differs antigenically from the cell wall LPS. However, high levels of antibodies are required for protection and high doses of antigen are required to elicit them. These doses are higher than can be produced in broth fermentation and economics restricts the commercial production of vaccines with effective protective doses of this antigen.

A newly patented method (Barrett and Leadbeater, 1992) of processing antigen for inclusion in the diet, has been used in small scale trials by us with the iron-restricted vaccine. This delivery led to production of high levels of anti-IROMP antibodies and high RPS values in fish challenged six weeks later. More extensive trials are in progress. Current data suggest that this delivery system is effective in raising antibodies to IROMP antigens but not to LPS antigens. Therefore the applicability of this method may be restricted to certain antigens, but we are currently optimistic in using it for delivery of the iron-restricted furunculosis vaccine.

In small scale trials, the iron-restricted vaccine with aluminium hydroxide adjuvant has been as effective as the Biomed vaccine. However, IROMP antigens do not appear to be very stable and the vaccine is recommended to be used fresh from the manufacturer.

It is likely that the IROMP vaccine coupled with a mineral oil adjuvant would provide longer protection. However, the granulomatous reaction to such adjuvants makes such vaccines unsuitable for fish which are sold complete with viscera. Some Scottish farmers supply such a market in France and for these fish the aluminium adjuvanted vaccine is recommended. Although Biomed claim that vaccinated fish do not have any growth penalty many Scottish farmers need further convincing of this.

al., 1992). Aqua health Ltd very interested in developing vaccine. Much work to be done on identifying protective antigens and how to produce them cheaply.

Renibacterium salmoninarum (BKD)

Still little progress in vaccine development. Groups in north America and UK concentrating on identifying virulence factors. Munn's group (Plymouth) have cloned several antigens and recombinant forms will be tested by the Marine Laboratory. Because *Renibacterium salmoninarum* is so slow growing a molecular biological approach to producing cheap antigen is necessary. However, there is far to go yet in identifying protective antigens which could then be cloned.

Viral and Parasite Vaccines

The future development of these vaccines will depend upon a molecular genetics approach to produce protective antigens by cloning their genes into a suitable expression system. For viruses this is necessary on commercial grounds as it is far too expensive to grow virus in tissue culture cells for vaccine production.

IHN

A recombinant vaccine produced in *E. coli* was developed by Leong's group (USA). A simple formalin inactivated bacterin administered by injection or immersion was reported as successful in experimental trials but failed in the field. One reason for this was apparently because of great antigenic variation in the IHNV field isolates. However, the published data on experimental trials did not provide convincing evidence that protection was due to specific immunity.

Our group is at stage c), having cloned four gut surface antigens which now require to be expressed in large quantities for immunisation trials.

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ANNEX 10

WORKING GROUP ON PATHOLOGY AND DISEASES OF MARINE ORGANISMS

ICES, Copenhagen, 15-18 March 1993

Recommendations:

- 1) The WGPDMO recommends that the Sub-group on Statistical Analysis of Fish Disease Data should meet before the 1994 WGPDMO under the Chairmanship of A.D. Vethaak:
 - a) to analyse the updated and validated ICES fish disease database for all species examined according to the new standard protocol, in the Baltic Sea and the North Sea;
 - b) to consider available information on factors with a possible impact on the prevalence and spatial distribution of fish diseases such as stock identity, stock density, recruitment, age-structure or fishing effort, for dab, cod and flounder.
- 2) The WGPDMO recommends that the diagnostic fiches continue to be published albeit at a lower frequency with an emphasis to revise older versions.
- 3) The WGPDMO recommends that it meet in Moncton, New Brunswick, Canada for four days during March 1994:
 - a) to analyse national reports on new disease trends in wild fish, crustacean and mollusc populations;
 - b) to analyse national reports on new disease trends in maricultured fish and shellfish;
 - c) to evaluate the WGPDMO Sub-group Report on the Analysis of Fish Disease Prevalence Data;
 - d) to assess the intersessional data presented on recent field trials and other relevant information on fish vaccines;
 - e) to compare antibiotic resistance profiles of *Aeromonas salmonicida* performed in laboratories of various participants;
 - f) to compare the European legislation, the OIE rules and the regulations under consideration in North America regarding the transfer of molluscs between countries. The WGPDMO should offer advice on the standardisation of the control methods used to monitor mollusc disease, based on the information coming from the above comparison and from data on current mollusc research.