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REPORT OF

SEAGOING WORKSHOP ON METHODOLOGY OF FISH DISEASE SURVEYS

3-12 January 1984 R/V 'ANTON DOHRN'

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A - Introduction

Investigations on the disease status of fish populations can give important information on the possible mortality effects of diseases, and the differentiation of fish populations. In addition the potential of using diseases of marine organisms as a tool for monitoring the biological effects of pollution has been discussed within various ICES-bodies. In 1981 ICES recommended (Res. 1981/4:6) that as a first step in the realisation of a possible monitoring tool of biological effects the member countries should undertake observations on pathobiology in relation to the environment and report the results annually.

Based on first results the discussion of the possible relationship between diseases of marine fishes and pollution has been controversial. Much of the controversy seems to have been due to shortcomings in the methodology used in marine fish disease surveys. The Advisory Committee on Marine Pollution (ACMP) therefore developed the idea of holding a seagoing workshop to bring together scientists actively involved in the field of marine fish disease surveys to discuss the problems. At the 1982 Statutory Meeting of ICES in Copenhagen an invitation from the Federal Republic of Germany for a workshop on board the research vessel 'Anton Dohrn' in January 1984 was extended to ICES member countries. The invitation was discussed in the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) at its 1983 meeting in Lisbon and was gratefully accepted. It was decided that the final aim of the workshop would be to produce a paper including proposals for standardised methodologies which would be followed in forthcoming fish disease surveys.

B - Proceedings

The Seagoing Workshop on Methodology of Fish Disease Surveys took place on board RV 'Anton Dohrn' from 3-12 January 1984. Twelve scientists participated in addition to a research team from the Toxikologisches Laboratorium,

Cuxhaven, who performed routine sampling at all trawling stations (list of participants - Appendix I). A total of 29 positions were sampled in the North Sea, south of 56°N (chart of sampling positions - Appendix II). The opportunity was taken for workshop scientists to carry out practical tests in methodology, to present and discuss prepared papers on chosen topics and to consider, in detail, problem areas in disease sampling methodology. Detailed proceedings of the workshop are presented in Appendix III, procedures for diagnosing and recording dab and cod diseases as a model example in Appendix IV, and texts of contributed papers in Appendix V.

C - Identified sources of variability in disease sampling and proposed improvements

1. Objectives of fish disease surveys

It is essential to clearly define the main objectives of a disease study prior to commencement, as features of different fish species and disease conditions which are desirable for one type of study may be undesirable for another. The aims to be fulfilled by fish disease surveys can be grouped under the headings: studies of infectious agents, pathological conditions and monitoring disease frequency; studies on the effect of disease on fish populations; the use of biological tags as indicators of fish population parameters; the use of disease as indicators of changes in the quality of the environment.

a) Choice of host species

It was recognised that no one fish species would probably be appropriate for study in all parts of the ICES area. The criteria for selection depends largely on the aims to be fulfilled and baseline data on disease in an area are required before a suitable species can be recommended.

Commercially important species will clearly be most significant in studies on the impact of disease (decreasing aesthetic value, source of mortality) and in biological tag studies. If the aims are to consider disease in relation to environmental quality then commercial or non-commercial species may be used. In

such cases the choice of indicator species should be the one identified in baseline studies to be the most sensitive to suitable disease conditions.

It is of importance to identify fish populations and their geographical ranges so that results from different stocks are not mixed in disease surveys.

b) Choice of disease condition

Essential baseline data on disease conditions which are required prior to starting applied studies should include information on types of diseases present in the area and host for study and their seasonal fluctuations. Only then can particular target disorders be selected. It is not necessarily the most obviously observed, easily sampled or economically most important disease conditions which should be tackled first. Diseases should be considered in relation to study objectives and to known chemical and biological parameters. For example, certain types of disease, eg epidermal hyperplasia, ulcers and Lymphocystis which may have little apparent effect on the host because of healing or regression could be useful in environmental monitoring studies.

2. Planning of Cruises

a) Coordination with other fish disease investigations.

Coordination of disease surveys, both nationally and internationally within particular geographical areas, is desirable and could be achieved by circulation of cruise plans. Members of Working Groups should take the opportunity of their annual meetings to exchange information on proposed sampling and consider the possibility of adjusting cruise programmes to prevent undue overlap. This would be of major importance when labour intensive investigations, such as age determination from otoliths, are being undertaken.

b) Integration with fish stock assessment surveys.

The advantages of integrating disease observations into fish stock assessment surveys, as outlined in ICES Resolution 1982/4:5, was again recognised by the Workshop, for economic reasons and to allow correlation

of data. However, the necessity for the presence of a disease specialist on board and/or suitably trained and motivated non-specialists was re-emphasised. Compatability of methods must be ensured with existing stock assessment methods, a major task which could be facilitated by using pre-existing fish recording sheets with minimal modification. The observation of disease conditions could be made in conjunction with the stock assessment samples being taken eg external conditions could be noted when taking lengths, otoliths and scales for ageing; internal anomalies could be identified when sex and maturity stages were determined or when stomach analysis was performed.

Established protocols should be used to systematically examine fish species for the selected lesions.

The incorporation of parameters routinely sampled on fish stock assessment surveys would also be of advantage to specialised fish disease surveys.

c) Training

For initial training, good practical guidance, demonstration, provision of samples and photographs, etc, are of primary importance. Certain well-defined fish disease symptoms such as Lymphocystis, skeletal malformations, pseudobranchial tumours, etc, and some parasites like Lernaeocera, Cryptocotyle, etc, can be easily recognised and recorded reliably. Before the first sample, an intensive briefing should be carried out illustrating the parameters to be included in the sampling and the types and use of protocols. The first samples from a cruise should be used for familiarisation and training the research personnel and the results should be treated with caution in the final analysis of data.

3. Selection of sampling stations

a) Routine cruises. If included in routine population dynamics cruises then selection of stations is dependent to some extent on established positioning but some attempt should be made to include areas of special disease interest.

b) Specific disease cruises. Selection of stations for specific disease cruises depends on disease patterns, distribution of fish species and environmental conditions.

Selection can be made on:

(i) a random basis

(ii) a grid network with higher density in areas of special interest

As a cautionary note, special care should be taken in selecting areas where marked environmental and fish population changes occur, and in choice of comparative areas. Knowledge of the physical parameters in both cases is essential, and particularly hydrographical parameters, such as circulation patterns should be included in the selection criteria of control areas. Both areas should be as similar as possible.

In areas with significant changes of depth in short distances, attention should be paid to depth contours within one sampling station.

4. Fishing methods

It was considered that fishing procedures used during disease surveys should be standardised when possible with established methods developed by fish stock assessment groups. It was recognised that changes in gear can substantially alter catch composition and consequently could influence the occurrence of diseased fish. Amongst others the following parameters could be of significance: (a) use of bobbins, rollers, chains; (b) influence of trawling speed; (c) types of codends; (d) duration of the trawl. This is an important area which has not been considered in the past in relation to disease studies and requires field investigation.

5. Sampling

a) Frequency of sampling

The degree of precision required by fish disease studies and the prevalence of the disease being investigated influences the number of samples required.

Since fish diseases may occur in relatively low numbers per sample or haul it is important to collect several samples from each fish population before any statistics are applied to the results from fish disease surveys. For naturally occurring diseases, changes in disease levels over a time period are considered to be of more significance than absolute levels.

Analysis of variabilities of results from single station sampling has indicated that repeated sampling may be required to reduce sampling errors to acceptable levels. In order to achieve acceptable variability with a given prevalence of disease, it is necessary to either (i) increase the sampling within the locality or (ii) by using tests of homogeneity, relate results from wider areas. In the latter case, the inclusion of data from fish populations and the biotic and physical environmental factors is essential.

b) Catch sampling and sub-sampling

Catch sampling, sub-sampling and processing procedures should follow established routines used by most fish stock assessment groups to facilitate the integration of information. Standardisation of procedures was identified as a key element in reducing the error factors in sampling variability.

The time phase between landing the catch and inspection of the samples should be as short as possible, otherwise small stages of disease, (eg pigment anomalies or the presence of ectoparasites will be difficult to recognise or missing.) Also any samples required for histological or other specialist examination must be processed as soon as possible after landing. When the catch is small every fish should be measured or counted and all the species of interest examined. When the catch is big, all large fish should first be removed before baskets in the total catch are counted or weighed. Fish should be sorted by species (sub-sample if necessary) and estimates obtained of total numbers. Count and/or measure uncommon species.

6. Diagnosis of disease

Precision of diagnosis was identified as a major source of variability in fish disease surveys.

In the following section some information is given which participants of the workshop believed could improve diagnosis.

(a) General observations on the condition of the fish should be made at capture. In particular, reference should be made to any damaging materials and organisms in the trawl.

(b) Differences in the intensity of examination were identified as a major contributory factor to variability which emphasises the necessity for a clearly defined protocol in sub-sampling and diagnosis. Such a protocol, to be followed during the examination of fish for disease, is required to allow compatability of data between various sampling groups.

(c) The use of check lists for the important fish species would give a greater degree of precision in diagnosis. When familiar with check lists, investigators would retain a mental check list. Furthermore, the quality of the diagnosis could be improved if different investigators working as a team had responsibilities for different phenomena.

(d) It is suggested that negative results should always be recorded as an indication of search effort since incomplete data can be misleading.

(e) As a standard procedure, all conditions which cannot be readily identified in the field should be sampled for a more thorough analysis.

(f) The mere inspection of external phenomena is only a portion of the investigation of the health status of fish. At a certain stage of the project, information on other parameters should be obtained to gain an overview. This may include the histological examination of samples of fish not showing any external anomalies randomly taken from the catch. When internal organs are examined, eg the liver, a sub-sample of a representative size group of a species should be taken.

(g) Some gaps in the knowledge of the dynamics of development of diseases were identified. More information is required from experimental trials to indicate which conditions may be of limited pathogenic importance, progressive or lethal.

(h) If it is attempted to define severity of conditions, there must be definite criteria on which the sub-divisions are based and they should be clearly indicated and recorded, if possible, by numerical grading.

(i) General recommendations to improve observations of diseases were:-

(i) clean fish sample with water before investigation begins;

(ii) use bare hands to detect small skin anomalies;

(iii) use visual aids if necessary;

(iv) count parasites, until experienced, if a numerical grading system is used;

(v) do not record uncertain disease conditions, but take samples for further analysis.

7. Computer entry, and retrieval of disease data

a) Computer entry of data

Depending on the ultimate reasons for analysis, the following parameters are suggested for inclusion into a typical stock assessment computer entry form:

station and cruise numbers

area code

species name or code

total number of sample

total number observed

individual fish number

i) length of individual

ii) age of individual

iii) sex and gonad stage

iv) external condition observed

v) internal condition observed

vi) location of observation on or in fish

vii) intensity (number or grade)

This procedure ensures that negative data will be included as a quantifiable measure of effort. Also multiple external/internal conditions and parasite load can be identified on the same fish. Anomalies which are not clearly associated with disease should not be included under disease phenomenon tables eg mechanical fin damage.

b) Retrieval of data

Once the station and biological information has been keyed, retrieval of the data can be obtained in a variety of formats. Examples include inshore/offshore differences, all disease conditions occurring on one fish species, all fish species which exhibit one disease condition and sex/length/body location/intensity linked conditions.

These and other programmes can be combined on a cruise, season or yearly summary basis and procedures for statistical treatment of the data, can be developed depending on the sophistication required.

8. Evaluation and Presentation of Data

The physical and biotic environments, including fish populations were identified as containing factors which could substantially influence disease patterns and consequently data from investigations on these should be utilised whenever possible during the evaluation of disease data. Close collaboration with specialist groups working in these fields is essential to avoid heterogeneous disease data being used. By integration particularly with computerised stock-assessment data (information on distinct fish stocks, migration, condition factor, age, food habits etc) a better picture of a fish's general health can be provided. Although numbers of diseased fish collected in this way may be low, data from single samples not statistically viable and 'hot-spots' not immediately indicated, results can serve as a baseline and be a useful trend indicator of disease status if the study is made over an extended period (5-10 years).

It is essential that the reporting format for disease should permit some evaluation of the data by including information on the intensity of investigation,

prevalence of disease, negative results and seasonal effects. The existing ICES reporting format does not require the inclusion of such types of data and thus needs improvement. Only clearly distinguishable conditions, preferably of known aetiology, should be included in reports; investigators should not attempt to assign a cause to all conditions of unknown aetiology but should seek expert advice when appropriate.

9. Target organs, pathology and parasites suitable for disease studies of fish in relation to environmental changes

The Workshop considered in detail the problems associated with the attempted use of fish diseases as indicators of changes in the quality of the environment.

a) If the aim of studying disease in relation to environmental changes is to examine the possible effects of these changes directly on fish, in an ideal situation similar techniques used in toxicological studies should be followed. This would mean sampling of a complete set of major organs from individual fish from a representative proportion of the population of each target species. However, under field conditions particular target organs may be selected although they should not be considered to be absolute. Prime candidates would be skin, fins and gills because of their direct contact with the environment and liver because of its detoxification role. As a standard procedure, all conditions which cannot be readily identified in the field should be sampled for a more thorough analysis.

b) Most disease studies in relation to environmental degradation are concerned with changes in naturally occurring conditions. It should be remembered that fish disease in the natural environment has a multi-factorial array of causes, in which pollution may or may not be included.

A list of criteria for disease conditions suitable for environment monitoring studies are:

- minimum processing for detection,
- easy identification,

- little effect on the host (mortality could mask the effect),
- transient disease conditions related to the changes in environmental quality,
- quantifiable.

Based on these criteria the following conditions can be identified as being suitable for study in dab and cod in the Southern North Sea.

I	General	: any gross condition which is repeatedly observed.
II	External conditions	: a ulcers* b hyperplasias/papillomas* (dab only) c Lymphocystis* (dab only) d skeletal deformities e fin rot f tumours* g pigment abnormalities h mechanical damage
III	Internal conditions	: a gill abnormalities* b pseudobranchial tumours (cod only) c liver pathology
IV	Parasites	: attached copepods, larval nematodes, digenean metacercariae, grossly observable protozoans.

* These conditions should have priority of sampling.

Such lists should not be inflexible as they could be misleading because of species and regional differences. As a model example of the problems associated with the diagnosis and recording of disease conditions in fish, more detailed information and recommendations on each of the conditions listed above is given in Appendix IV.

D - Gaps in knowledge

The Seagoing Workshop on Methodology of Fish Disease Surveys during its work became aware of serious gaps of knowledge in important fields related to such studies.

The workshop therefore urges the WGPDMO to look into the following items and to recommend both to its own members, and, through ICES, also to other scientific bodies to initiate research to cover the shortcomings.

1 There is a lack of information on the effect of pollution on parasites of marine fish. A literature review should be compiled.

2 There is insufficient knowledge on the behaviour of diseased fish which most probably will in some degree differ from the normal. The probability that the use of different fishing gear could influence the presence of different diseases was recognised.

3 Injuries due to fishing gear might have a certain impact on the health status of fish. To evaluate the importance of this impact, reference maps of fishing intensity should be collated with the distribution of disease.

4 Knowledge on the frequency and development of abnormal gill conditions in free-living marine fish species is poorly understood. Therefore, gill anomaly studies should be conducted.

5 Information on the dynamics of development of many diseases of marine fish is lacking. Experimental work is required to facilitate the overall understanding of disease processes.

6 Information is required on the possible connection of food composition, nutritional value and condition of fish with the health status of marine fish.

7 Present marine fish disease programmes largely concentrate on the monitoring of external diseases. It was felt essential that the investigation of internal organs and parasites should be included.

E - Recommendations to the WGPDMO

1 The Workshop report should be published as a Cooperative Research Report.

2 It was felt necessary that, after an appropriate time, seagoing workshops on the methodology of fish disease surveys should be repeated.

3 The participants of the workshop ask the WGPDMO to convey official thanks to Prof Dr Klaus Tiews, Bundesforschungsanstalt fur Fischerei, Institut fur Kusten-und Binnenfischeres, Hamburg, for having provided excellent facilities for this workshop.

4 The workshop asks to reconsider the proceu and format for disease reporting. It was felt essential WGPDMO should receive the original data for evaluation and then report back to ICES.

Appendix I

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Appendix II
Chart of sampling positions